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The Evolution of Annuality in Association with a Shift to More Arid Environments in the Daisy Genera *Ifloga* and *Trichogyne*.

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Abstract

An annual life history is often viewed as a model adaptation to arid environments. Annuality is predicted to have evolved in response to low adult survival and high seedling survival. In this study I evaluated the idea that increases in aridity should be associated with the evolution of an annual life history. I also investigated the correlated evolution of annuality and growth form. Ancestral character staes for life history characters and climate variables were mapped onto a molecular phylogeny (obtained using plastid *trn*L-*trn*F and *psb*A-*trn*H and nuclear ETS sequences) of the genera *Trichogyne* and *Ifloga* (Asteraceae). Bayesian methods were used for phylogeny inference and maximum likelihhood methods for ancestral state reconstructions. Only two phylogentically independent contrasts were obtained and so the association between changes from annuality to perenniality and increases in aridity along branches of the tree were recorded and evaluated using Fisher's exact test. In order to account for ancestral character state reconstruction uncertainty, four different possible scenarios suggested by the maximum likelihood methods for the evolution of annuality were examined. This is the first molecular phylogeny of the group. Bayesian analysis of the sequence data places the *Trichogyne*+*Ifloga* clade within the Gnaphileae. The genus *Ifloga* is shown to be paraphyletic. *Trichogyne ambigua*, as currently described, is polyphyletic and may contain two species. The origin of the *Trichogyne*+*Ifloga* clade is within southern Africa and a northwards migration via the arid corridor is suggested to explain the disjunct distribution of the two Northern Hemisphere species. There is an association between the duration of the moisture growing season and the evolution of annuality. This is consistent with the idea that annuality is favoured by long drought periods making perennation difficult. The evolution of annuality was correlated with a non-woody, tufted, growth form. Amphi-basicarpy was discovered for *T. polycnemoides*, making it only the second known example of this reproductive strategy within Asteraceae.

Introduction

The genus *Ifloga* Cass. comprises six annual species, four of which occur in South Africa, one being a Canary Islands endemic and another occurring around the Mediterranean basin and into N.W. India (Hilliard, 1981). *Trichogyne* Less. comprises nine species, all of which occur within the winter rainfall region of South Africa, with one perennial species extending into the summer rainfall zone (Hilliard, 1981). The southern African annuals within *Ifloga*, occur within the arid regions of the summer rainfall region, from the Karoo to the Kalahari and into southern Namibia (Hilliard, 1981). The annual species of *Trichogyne*, with the exception of *T. verticillata*, have ranges within the more arid zone of the South African winter rainfall region, while the perennials of the genus occupy more mesic ranges (Hilliard, 1981).

Annual plants are widespread throughout the world and are particularly prevalent in desert ecosystems, with an annual life history often being held to be a model example of adaptation within arid systems (Van Rooyen, 1999). In the main this is because the same characters occur repeatedly in unrelated annual desert taxa worldwide (Gutterman, 1982) and owing to the high representation of annual species in arid floras, compared with those of more mesic environments (Van Rooyen, 1999). There is a large literature describing the characteristics of annual plants in arid systems and especially their seed ecology (e.g. Clauss and Venable, 2000; Gutterman, 2000; Gutterman, 2002). Previous research, however, has generally stopped short of investigating what characters are selected to bring about an annual life history and what biological and environmental factors are the key drivers in the evolution of these characters.

Demographic models predict that low adult survival and high seedling survival should favour the evolution of annuality (Stearns, 1992; Bell, 1976). Schaffer and Gadgil (1975) and Van Rooyen (1999) suggest that annuality in deserts is a drought avoidance strategy, selected for by high aridity making perennation difficult in these environments. Evans et al. (2005), evaluated these predictions in a phylogenetic framework and suggested that annual life histories may have evolved in response to an increase in dry season maximum temperature and increased aridity in the growing season, but not dry season aridity. Verboom et al. (2004) suggest that the evolution of higher relative growth rates (RGR), allowing for rapid maturation and flowering, is a key step in the evolution of annuality and that it is associated with the onset of a

shorter wet season. It is therefore unclear as to whether an increase in the level of aridity, duration or both of the dry or wet season is important in driving the evolution of annuality.

It is uncertain as to whether annuality evolves more readily from perenniality or *vice versa*. It is likely that the ancestral character state in the angiosperms is perenniality and that annuality is derived (Taylor et al. 2003). As many plant genera contain both annual and perennial species, this implies that annuality has evolved frequently within the angiosperms and that shifts in life history can take place relatively quickly. One could therefore expect the transition rate from perenniality to annuality along a phylogenetic tree to be greater than that from annuality to perenniality. Bena et al. (1998), however, infer a perennial ancestor for some perennial genera and an annual ancestor for others, suggesting that annuality has also been lost a number of times. This observation led Thomas et al. (2000) to propose that switches from perenniality to annuality and back again could be effected by small changes in developmental processes and without dramatic genetic change. This suggests that, for a given amount of molecular change, the transition rate between the two life histories may be similar.

High relative growth rates are necessary in order for annuals to be able to utilise resources, which are only intermittently available for plant growth. Verboom et al. (unpubl. data) supported this by showing the RGRs of facultative annual individuals of the grass *Ehrharta calycina* to be higher than those of the perennial individuals of the species. High RGRs are correlated with high specific leaf areas (SLA) (Poorter and Remkes, 1990), with a high SLA providing the photosynthetic capacity necessary for rapid growth. High investment in leaf growth by annuals produces a large photosynthetic area, essential in providing for rapid development (Van Rooyen, 1992) and a large reproductive effort. Allocation of resources away from leaves to the building of non-photosynthetic, support or storage structures may therefore be viewed as maladaptive in annuals, annuality being generally associated with herbaceous taxa that have little or no woody support structure. In Namaqualand many of the annual members of the Asteraceae family have photosynthetic stems (pers. obs.) and annuality is common within the grasses (Chapman, 1996), which share this characteristic. The role of phylogenetic origin in influencing particular evolutionary outcomes is well recognised (e.g. Price and Carr, 2000) and therefore a woody phylogenetic background is expected to constrain the evolution of annuality.

During field collecting it was observed that there is variation in growth forms amongst the annual species of *Ifloga* and *Trichogyne*. The majority of the annual species in both genera have a tight basal rosette of leaves, which I term a tuft (fig. 1a). In contrast, *T. verticillata* has a woody upright stem (which I term a pole; fig. 1b), which may branch at the base later in the growing season to form a candelabra-like structure (fig. 1c). *T. polycnemoides* has both growth forms, with individuals initially forming tufts and later in the season developing poles (fig. 1d).

Figure 1. The different growth forms: (a) tuft, (b) pole (c) branched and (d) tuft and pole, exhibited by the annuals within *Trichogyne and Ifloga*. All figures are of *T. polycnemoides*.

I predict that the evolution of the tufted growth form will be correlated with the evolution of annuality in more arid environments because the need to grow and flower quickly becomes more pressing as the period of resource availability decreases, increasing the cost relative to the benefit of investing in non-photosynthetic structures. Flower heads are present both within tufts and at the nodes on poles. There is, however, no previous record of flower heads within the tufts of *T. polycnemoides*. Amphi-geocarpy is the production of flowers and fruit below and above ground level, while amphi-basicarpy is the production of flowers and fruit at and above ground level (Barker, 2005). The presence of flower heads within the tufts of *T. polcynemoides* would make the species amphi-basicarpic (Barker, 2005). Amphicarpic species are often capable of self-fertilisation and there is little to no dispersal of seeds produced at or below ground level (Barker, 2005). Amphicarpy has been associated with plants from arid regions (Mandak, 1997) and with annual species (Kaul et al. 2000).

The genus *Ifloga* was erected by Cassini in 1819 and contained a group of annual or perennial species with inconspicuous flower heads borne in the axils of dense tufts of leaves. Merxmuller et al. (1977) placed *Ifloga* in an extended Gnaphalieae within the *Gnaphalium-Helichrysum* group of the Asteraceae. The Gnaphalieae (everlastings) tribe is cosmopolitan, but has its greatest diversity within southern Africa, Australia and South America (Bayer et al., 2000). Based on floral characters, Hilliard (1981), described the subgeneric sections *Trichogyne* and *Ifloga* within the genus *Ifloga*. Using a morpholgy-based cladistic analysis, Anderberg (1991), however, found both subgenera to be monophyletic and elevated *Trichogyne* to the generic level, placing it as sister to *Ifloga* within the Gnaphalieae. Hilliard (1981) and Anderberg (1991) used the same floral characters for their classifications. In a molecular-based phylogeny reconstruction of the South African Gnaphalieae, Bayer et al. (2000) placed *Trichogyne* as the earliest diverging group within the Gnaphalieae and suggested that it may actually be closer to a clade containing the genus *Metalasia* in the subtribe Relhaniinae. They did not, however, sample *Ifloga* and support for this placement of *Trichogyne* was weak.

This study aims to produce a phylogeny for the species of *Ifloga* and *Trichogyne* and in tandem with ecological analysis use this to address the following questions: (1) Are *Ifloga* and *Trichogyne* monophyletic and where do they fit within the Gnaphalieae? (2) Given that *Ifloga* is widely distributed, does the group have its origin in southern Africa or elsewhere? (3) Is the rate of transition from perennial to annual higher than that from annual to perennial? (4) Is the evolution of annuality associated with a shift to more arid environments? (5) Amongst annuals, is the tufted or the pole growth form ancestral? (6) Is the evolution of the tufted growth form correlated with the evolution of annuality and associated with increased aridity? (7) What is the adaptive significance of the tufted or pole growth form? (8) Is *T. polycnemoides* amphi-basicarpic?

Method

Taxon Sampling

In order to assess generic delimitation in *Trichogyne* and *Ifloga,* as many species as possible were included for the ingroup (see appendix). I extracted DNA from herbarium specimens for species that were unable to be collected in the field. Unfortunately, I was unable to get samples either from the wild or from herbarium sheets of *I. obovata*, the Canary Islands endemic, and *I. thellungiana*, which is only known from the type locality. We did, however, find the very infrequently collected *T. candida* from within 10km of the type locality. *Ifloga molluginoides* had at one time been placed in the genus *Comptonanthus* (Nordenstam, 1964) together with several members of *Lasiopogon* Cass. To assess monophyly and to examine relationships with other members of the Gnaphalieae, four species of *Lasiopogon* were included in the analysis as well as members of all the other gnaphaloid groups (Table 1). These groups were identified from the phylogenetic analyses of Bayer et al. (2000) and Bergh et al. (unpubl.). Many of the annual species within *Trichogyne* and *Ifloga* have been very poorly collected and our sampling led to a better understanding of the species' ranges. In the field it was noted as exceptional, in comparison with the distributions of the other species within the genera, that the range of *T. ambigua* extended across a large altitudinal gradient from sea level to 800m and so a specimen from both a coastal and montane habitat was included in the phylogeny reconstruction.

DNA extraction, PCR, sequencing and sequence alignment

DNA was extracted from silica-dried leaf samples collected in the field or from herbarium specimen leaf samples using the protocol of Doyle and Doyle (1987). Grinding of leaf samples was done in liquid nitrogen with a small amount of sterile sand, using a mortar and pestle. Extracted DNA was resuspended in water and diluted to 10^{-1} or 10^{-2} prior to amplification. Three gene regions were amplified. The plastid regions were the *trn*L intron and the *trn*l-F intergenic spacer, amplified together using the "c" and "f" primers from Taberlet et al. (1991) and the *psb*A-*trn*H intergenic spacer using the primers of Sang et al. (1997). The 3' portion of the external transcribed spacer (ETS) of nuclear ribosomal DNA was amplified using the primers AST1 (Markos & Baldwin, 2001) and 18S-ETS (Baldwin and Markos, 1998). PCR reactions were prepared on ice in 25µl, volumes each containing 13.2 µl of sterile water, 2.5 µl of 10xDNA polymerase buffer, 1.5µl $MgCl_2$ (25mM), 1.0 µl dNTP (10mM), 1.25 µl of each primer and 0.2 µl of Taq DNA polymerase (Bioline Ltd, London, U.K.). 0.5 µl of DMSO (100%) were added to reactions for the ETS nuclear region and the volume of sterile water reduced by this amount. DNA amplification was done using the following program: an initial denaturation of two minutes at 95°C; 30 cycles of 1 min. at 94°C. 1 min. at 52°C and 2 min. at 72°C; and a final extension of 8 min. at 72°C. Gel checks of the PCR products were done using a 1% agarose gel. Cleaning of the PCR products and cycle sequencing were done by Macrogen cc., Seoul, South Korea. Cycle sequencing used the same primers as were used for PCR. Forward and reverse sequences were assembled, edited and aligned using CodonCode Aligner version 2.0.1 (CodonCode Corp.,Delaware, U.S.A). Final visual alignment was done in MacClade 4.07 (Maddison and Maddison, 2000). Sections of the psbA-trnH region that were unalignable, due to indels, were excluded from the analysis.

Phylogenetic analysis

The two plastid gene regions were combined as they are from the same non-recombining locus. The plastid and nuclear partitions were first analysed separately using both parsimony and Bayesian inference. Parsimony analyses were performed using PAUP* version 4.0b10 (Swofford, 2002), with 10 000 random addition replicates, TBR branch swapping, MULTREES in effect and retaining all the shortest trees. Bayesian inference was performed using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001). With the exception of one clade within *Trichogyne*, there was no supported (PP>0.90) conflict and so the two data partitions were grouped for further

analyses. To avoid under-parameterisation separate GTR+I+Γ models were applied to each gene region (Huelsenbeck and Rannala, 2004). Model parameters and tree topologies were estimated simultaneously. Model parameters were treated as unknown with uniform priors. Bayesian topology estimation used two independent runs, each with one cold and three heated Markov chains and having a random starting tree. The incrementally heated chains had a temperature parameter value of 0.1. The analysis was run for 1.5 x 10^6 generations and trees were sampled every 100^{th} generation. The ASDF score in MrBayes and plots of the sampled -log likelihoods against generation time in Tracer version 1.3 (Rambaut and Drummond, 2005) were used to assess convergence. The first 7000 trees were discarded as burn-in, before calculating the posterior probabilities.

Climate variables

Species ranges for the southern African members of *Trichogyne* and *Ifloga* were mapped using herbarium record localities. The Eurasian species, *I. spicata, was* excluded due to difficulty in accessing herbarium and climatic records for the species. Values for climate variables were obtained from Schulze et al. (1997). The coefficient of variation (CV) in mean annual rainfall, duration of the moisture-growing season, potential evapotranspiration (PET) during the dry season and precipitation (P) during the dry season were obtained from Schulze et al. (1997). The dry season was defined as the four consecutive months of the year with the lowest precipitation. The moisture growing season (MGS) is the number of days in the wet season for which precipitation exceeds one third of potential evaporation and is used as a measure of the duration for which there is sufficient soil water availability to allow for sustained plant growth (Schulze et al., 1997). PET is the amount of water that would be transpired by a green lawn, completely shading the ground and never short of water (Penman, 1956) and is a measure of the "drying power" (Evans et al., 2005) at a particular location over a particular period, the dry season in this study. Following Evans et al. (2005), two indices of aridity were calculated from PET. PET divided by precipitation was calculated as a measure of aridity due to drought (Budyko 1974 in Evans et al. 2005), and PET minus precipitation was calculated as a measure of aridity due to high daily temperatures. Taken together, these variables provide measures of the duration of the water resource pulse, sufficient for growth (MGS duration), the harshness of the period between pulses (PET/P and PET-P) and the likelihood of the occurrence of the resource pulse in any one season (CV rainfall).

Character and climate reconstructions

Due to sparse taxon sampling within the outgroup genera, only the section of the tree containing *Ifloga* and *Trichogyne* was used for ancestral trait reconstruction. Annuality and perenniality as well as the presence or absence of a tufted growth form was scored as discrete characters for species of *Trichogyne* and *Ifloga*. Maximum likelihood was used for discrete character reconstructions because it is able to give a measure of the uncertainty generated by the mapping of character states onto a given tree. Bayesian methods were not used because there is relatively little phylogenetic uncertainty within the ingroup and so Bayesian analysis would not significantly alter the uncertainty of any one state at an internal node (Ronquist, 2004). Maximum likelihood character reconstructions were done in Mesquite version 2.0b.i69 (Maddison and Maddison, 2007) using the maximum posterior probability tree from the MCMC posterior sample derived from the combined sequence data. A Markov k-state one parameter (Mk-1) model, using branch length data and with equal forward and reverse character state transition rates, was used (Lewis, 2001). An asymmetrical Mk model, with unequal transition rates, was tested for each data set, but did not improve the –log likelihood scores by more than two units for annual/perennial or growth form reconstructions and so does not add significant explanatory power over the Mk-1 model (Pagel, 1999). Character state reconstruction was also done using an Mk-1 model with equal branch lengths to provide a cladogenetic as opposed to anagenetic model of character state evolution.

To test for any influence of the conflicting topology within *Trichogyne* between the nuclear and plastid consensus trees on the character state reconstructions, annual/perennial and growth form character state reconstructions were also done on the nuclear maximum posterior probability tree. Since this ancestral state reconstruction for *Trichogyne* did not differ from that based on the combined data set, the latter was used for further analyses. Tests for the independent evolution of annuality and the tufted growth form were done using the correlation analysis function in Mesquite, which follows the method of Pagel (1994). One thousand simulation runs, each with ten likelihood iterations, were done. Reconstruction of the continuous climate variables was done using least squares parsimony. Since only two phylogenetically independent contrasts in life-cycle duration were present within the ingroup, the association between climate and the transitions between annuality and

perenniality were evaluated by noting whether there had been an increase or decrease in the measures of aridity with each annual/perennial state transition. This allowed the climate data to be coded discretely as an increase or decrease and Fisher's exact test was used to test for significance of the association (Maddison, 1990). To account for uncertainty in mapping ancestral character states, four different scenarios of the evolution on life cycle duration were examined in this way.

Growth form ecological analyses

Individuals of *T. polycnemoides* were examined in the field for the presence of flower heads within the tuft. Field observations suggested that plants of *T. polycnemoides*, with the tufted growth form, were more clumped in their distribution at fine scales than those of *T. verticillata*, which only has the pole growth form. This clumping, possibly as result of low dispersal due to amphi-basicarpy, was tested for by marking and photographing three 50 cm x 50 cm grids for both *T. polycnemoides* and *T. verticillata*. All individuals within a grid were dug up to make the counting of the individuals possible. The number of individuals was plotted onto the scaled photograph and the frequency of individuals within a grid was sampled by throwing random quadrats of 5 cm x 5 cm. The coefficient of dispersion (C.D.) was calculated for each species by dividing the variance in the number of plants per quadrat by the mean number of plants per quadrat. A C.D. of greater than one is associated with a clumped distribution. It was also observed that individuals of *T. polycnemoides* often grew out of a dead tuft, now below ground level, from the previous season. So to further test for amphicarpy, a Chi-square test was used to test for an association between the presence of living individuals and dead tufts from last season at the same site. To test for self-fertilisation within the tufts of *T. polycnemoides*, twelve immature individuals were transplanted from the wild into separate pots and grown in a common garden isolated from potential pollination vectors. To test for a difference in seed length between seeds from tufts and those from poles for the common garden individuals a simple t-test was used.

Results

Phylogeny

The sequence data set included 1543 aligned characters, compiled from ETS (447 bp), trnL-trnF (727 bp) and psbA-trnH (369 bp). The parsimony analysis results were broadly consistent with those from the Bayesian analyses. The consensus topologies obtained from separate Bayesian analyses of the plastid (fig. 2a) and nuclear (fig. 2b) molecular data show similar levels of resolution and there is a high level of congruence between the trees from the two data sets. The maximum posterior probability tree from the combined Bayesian analysis of the two data partitions shows higher levels of support than were present in either of the topologies obtained separately with each data partition (fig. 3).

Low node support (PP < 0.90) prevents the confident identification of the sister clade of *Ifloga* + *Trichogyne* being possible, though the maximum posterior probability tree (fig. 3) suggests that the *Ifloga* + *Trichogyne* clade is sister to a clade containing the stoeboid alliance + *Helichrysum* and its allies. *Ifloga* and *Trichogyne* form a clade (PP = 1.00; fig. 3) that is strongly supported by both the nuclear and plastid data partitions. *Trichogyne* is monophyletic (PP =1.00) and embedded within *Ifloga* (PP = 0.91). *Ifloga* is not monophyletic, with *I. molluginoides* diverging earlier than the rest of the *Ifloga* species (PP = 0.91).

Within *Trichogyne* there are two well-supported clades, one perennial and the other mostly annual (fig. 3). There is conflict between the nuclear and plastid consensus trees on the topology of the annual clade (fig. 2a and 2b), with the plastid and nuclear tree placing *T. paronychiodes* and *T. polycnemoides* as sister to *T. candida* (PP=1.00 in both cases) respectively. There is further conflict in this clade over the placement of *T. decumbens* with the plastid consensus tree placing it as sister to *T. polycnemoides* (PP=1.00) and the nuclear consensus tree placing it as sister either to the *T. candida-T. paronychiodes* clade or the *T. lerouxiae-T.verticillata* clade. Within the perennial clade, the coastal and montane forms of *T. ambigua* are not sister to each other, the coastal form being placed as sister to *T. repens* (PP = 1.00; fig. 3). This resolution is supported by both nuclear and plastid sequence data (fig. 2a and 2b). Within *Ifloga,* the Mediterranean *I. spicata*, is sister to *I. glomerata* from the summer rainfall region of South Africa (PP = 1.00; fig. 3).

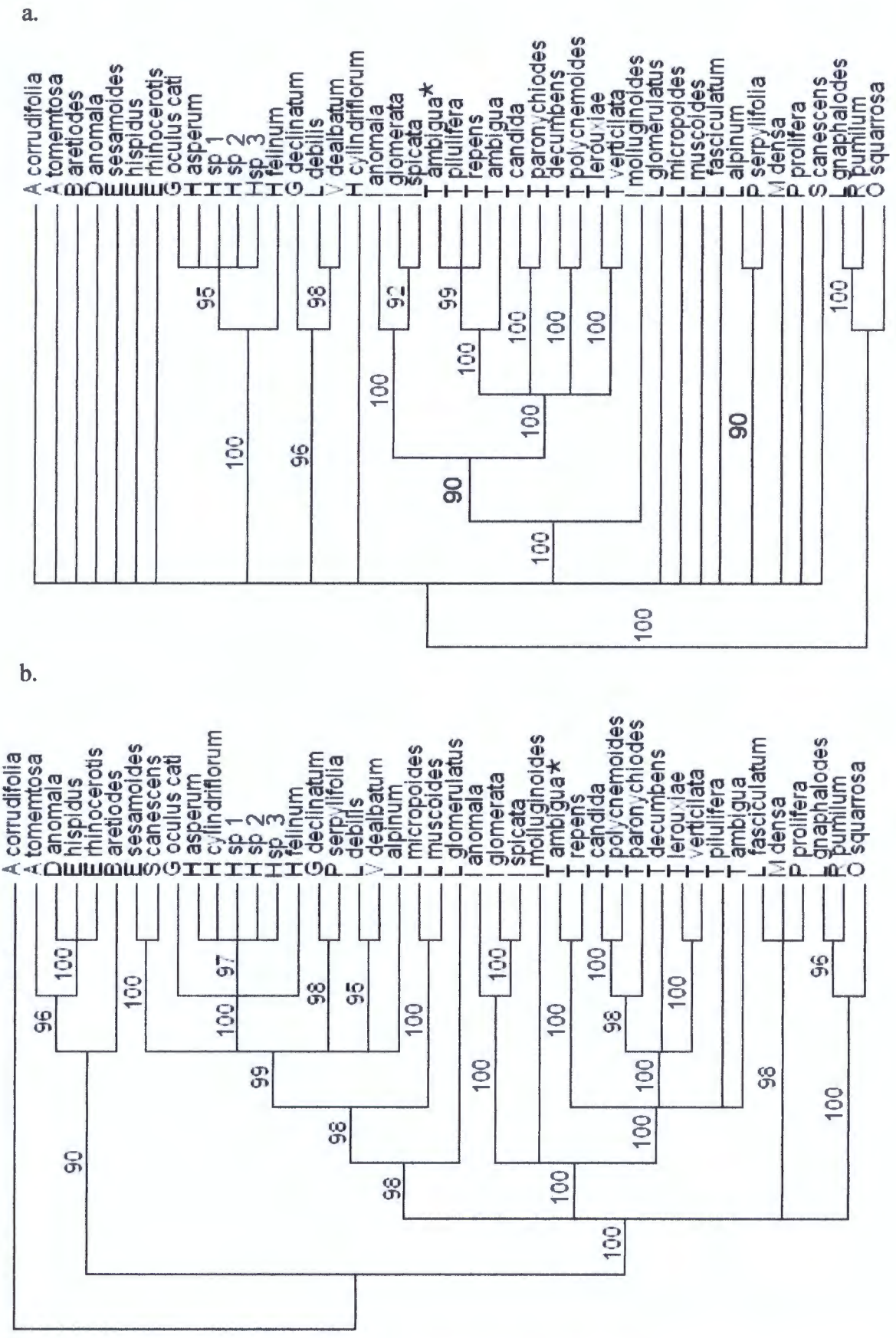

Figure 2. Majority rule consensus topology based on the Bayesian analysis of (a) plastid sequence data and (b) nuclear sequence data. Posterior probabilities are represented as percentages to the left of a node. * indicates the coastal form of *T. ambigua* .

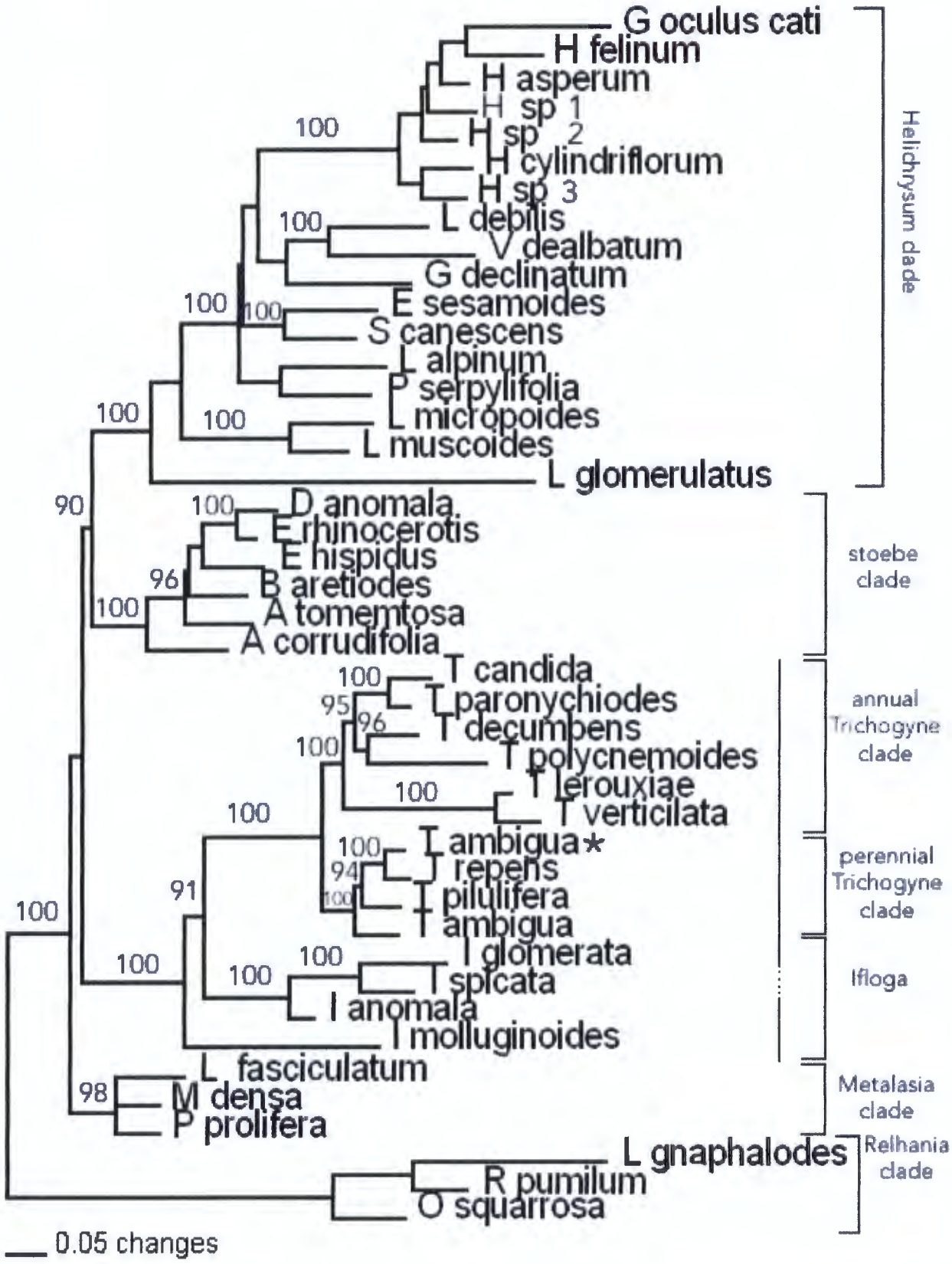

Figure 3. Combined sequence data maximum posterior probability phylogram. Posterior probabilities from the Bayesian analysis of the combined sequence data are represented as percentages above the nodes. A solid line indicates a southern African distribution, while a dotted line indicates a Northern Hemisphere distribution. * indicates the coastal *T. ambigua.*

Annual/perennial character state reconstructions

The ancestral life history state of the *Ifloga* + *Trichogyne* clade (node A) is uncertain, with only moderate support (proportional likelihood (PL) = 0.70; fig. 4) for annuality being the ancestral state. Annuality is strongly supported as the ancestral state of the *I. anomala – I. spicata* clade at node C (PL = 1.00). There is moderate support for the most recent common ancestor of the *Trichogyne* clade (node D) being perennial (PL= 0.74).

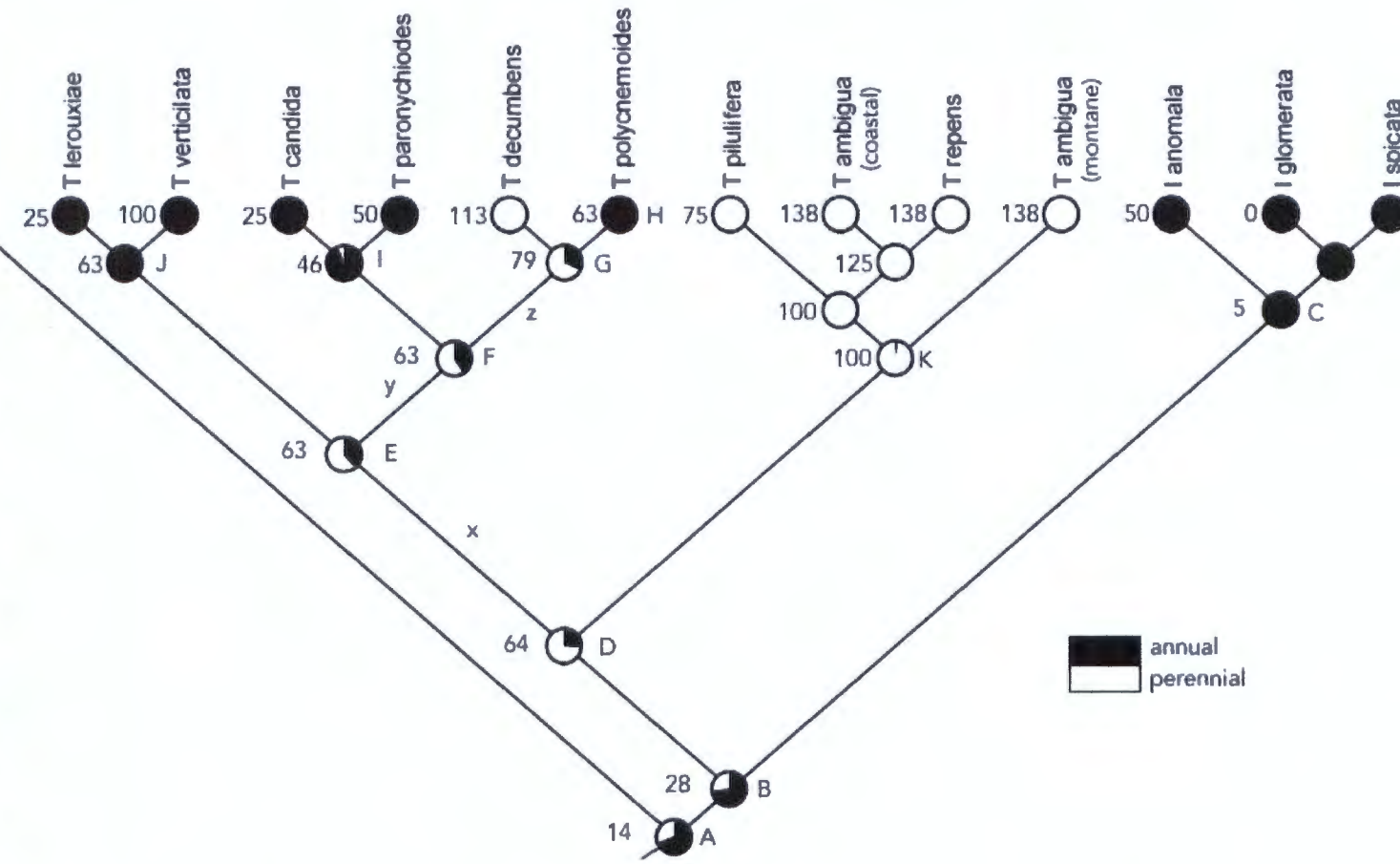

Figure 4. Reconstructions of annuality and perenniality (node pie charts show proportional likelihood of the state at that node) and duration (days) of the moisture growing season (numbers to the left of each node, least squares parsimony) on the maximum posterior probability tree.

A high level of uncertainty is associated with the ancestral state at nodes E and F. This is because the short lengths of branches x and y (also see fig. 3), favour a perennial state, even though the majority of the tip states are annual. It is therefore unclear how many times annuality evolved within the entire clade. The maximum likelihood reconstruction supports, albeit weakly, annuality being lost at D and then

evolving independently three times at H, J and I. The character state reconstruction using equal branch lengths is the same as that presented in scenario two (fig. 6).

Annual growth form character state reconstruction

There is a high degree of uncertainty regarding the ancestral growth form (node A) in *Trichogyne* + *Ifloga* (fig. 3). There is moderate support (PL = 0.79) for the ancestor of the *Trichogyne* clade (node D) having a non-tufted growth form and strong support (PL= 0.98) for the most recent common ancestor (node K) of the perennial clade within *Trichogyne* also having a non-tufted growth form. There is thus some support for the tufted growth form arising independently three times, at H, J, and I within the predominantly annual clade of *Trichogyne*. With the exception of T. *verticillata* all the annual species within the ingroup have the tufted growth form. The evolution of growth form and life cycle duration is correlated (p = 0.001).

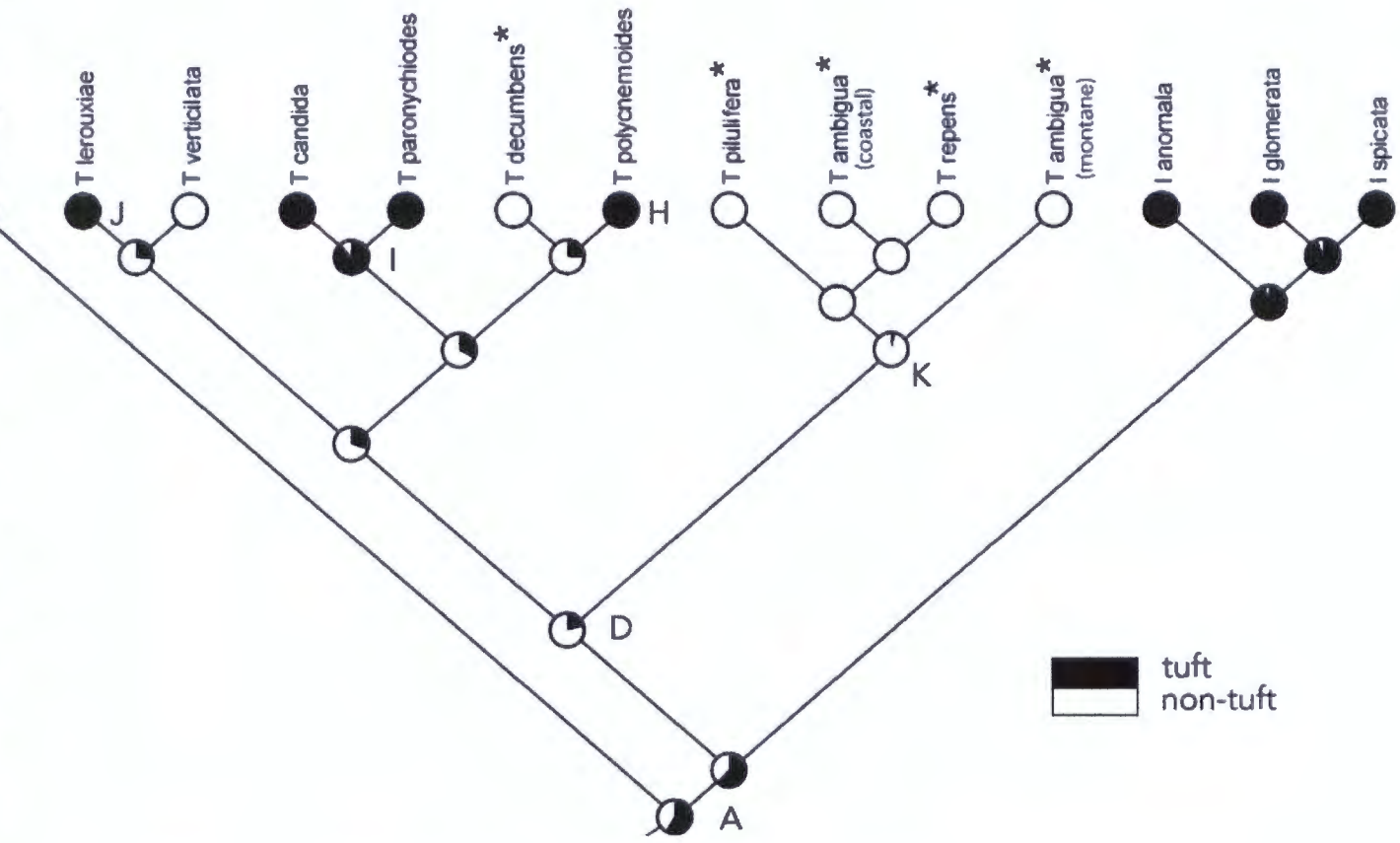

Figure 5. Ancestral growth form reconstruction on the maximum posterior probability tree. Pie charts at the nodes represent the proportional likelihood of the character state at that node. * indicates a perennial.

Climate and life history evolution

Figure six shows the four different scenarios for the evolution of annuality that were examined. The results for each of these scenarios are presented in table two. The switch to an annual life history coincides with a decrease in the duration of the moisture growing season (MGS) in all four scenarios while the opposite is true for the switch to perenniality (table 2). Fisher probability values could not be calculated for some of the scenarios due to a switch in life history state only occurring in one direction. These scenarios do however, qualitatively support an association a between shorter MGS and the transition to annuality. The non-significant results for an association between the evolution of annuality and a decrease in MGS duration are due to a small sample size leading to a lack of power. The association between a switch in life cycle duration and dry season aridity due to drought or high temperatures is not as clear as is the case for MGS. There is also no clear association between an increase in the CV of mean annual rainfall and the switch to an annual life history. The evolution of the tufted growth form is associated with a decrease in MGS duration because of its correlation with the evolution of annuality in the group. *T. verticillata* is the exception amongst the annual species in being associated with a relatively long moisture growing season and having the pole growth form.

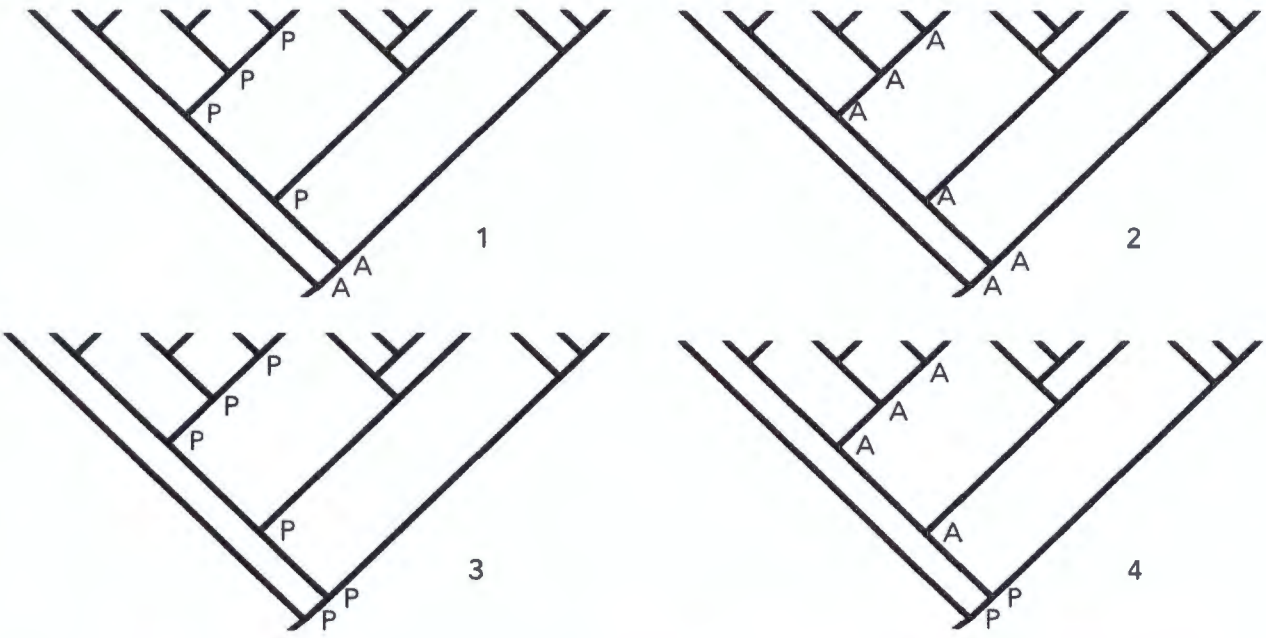

Figure 6. The four scenarios used to evaluate the association between switches between the evolution of annuality and aridity. Tip states on the tree are the same as in figure 4.

Table 1: The association between the evolution of life cycle duration (annuality (A) and perenniality (P)) and changes in moisture growing season (MGS), dry season aridity due to drought (PET/P), dry season aridity due to high temperatures (PET-P) and CV of mean annual rainfall for the four scenarios presented in figure 6. Ns=not significant.

Scenario:	(1)		(2)		(3)		(4)	
direction:	A→P	P→A	A→P	P→A	A→P	P→A	A→P	P→A
MGS								
↑	1	0	2	0	0	0	2	0
= or ↓	0	3	0	0	0	5	0	3
Fisher p:		Ns						0.1
PET/P								
↑	1	3	0	0	0	4	0	2
= or ↓	0	0	2	0	0	1	2	1
Fisher p:								Ns
PET-P								
↑	1	3	0	0	0	3	1	1
= or ↓	0	0	2	0	0	2	1	2
Fisher p:								Ns
CV rainfall								
↑	0	2	0	0	0	4	1	2
= or ↓	1	1	2	0	0	1	1	1
Fisher p:		Ns						Ns

Growth form ecology

Flower heads with seed were found within the tufts of *T. polycnemoides*, making the species amphi-basicarpic (fig. 7). In the field *T. polycnemoides* has a clumped distribution (C.D. = 2.5), whilst the non-tufted annual, *T. verticillata,* is regularly distributed (C.D. = 0.6) In *T. polycnemoides* the presence of clumps of alive individuals was significantly associated with the presence of the previous seasons dead tufts below the soil surface in *T. polycnemoides* ($X^2 = 5.55$ d.f. = 1 $p<0.05$). Male and female flowers within the same inflorescence in *T. polycnemoides* tufts matured simultaneously (fig. 8) and tufts in the common garden produced seeds, demonstrating ability to self-fertilise. In *T. polycnemoides* the seeds from pole flower heads were significantly shorter in length ($t = 4.02$ d.f = 8 $p<0.05$) than those from

tuft flower heads. One seed was produced per flower head at each node on the poles, whereas there were two seeds per flower head in the tufts of *T. polycnemoides*.

Figure 7. Flower head within the tuft of a *T. polycnemoides* individual

Figure 8. Simultaneous maturation of male and female flowers within a flower head of a *T. polycnemoides* tuft.

Discussion

Under a strict monophyly criterion, the paraphyly of *Ifloga* necessitates some nomenclatural changes. Donoghue and Cantino (1998) state that paraphyletic groups should not be recognised formally as they are likely to confound the study of evolution. *Ifloga molluginoides* is alone in the genus *Ifloga* in having all the female flowers lie inside the main involucre (Hilliard and Burt, 1981). *Ifloga thellungiana* has female flowers both within and occasionally outside the main involucre and was identified by Hilliard and Burt (1981) as the species linking *I. molluginoides* to *Ifloga.* One option is to erect a monotypic genus for *I. molluginoides.* This approach is, however, argued against by Schrire and Lewis (1994), as only synapomorphies with other species are informative about relationships with the result that monotypic genera create taxonomic redundancy. Schrire and Lewis (1994), however, do also state that if a species is well supported near the base of the cladogram, then it may validly be represented a monotypic genus. There is only moderate support for the paraphyly of *Ifloga* (fig. 3) and Backlund and Bremer (1998) state that only clades that are the best supported should be preferred for formal classification. Any nomenclatural changes at the generic level should therefore be postponed until the support for the node making *Ifloga* paraphyletic is increased and there is a complete sampling of the species within *Ifloga.* If *I. thellungiana* were to form a clade with *I. molluginoides* that was paraphyletic to the rest of *Ifloga* then there would be no need for concerns of taxonomic redundancy in placing the two in a new genus.

The species *T. ambigua* L. (Druce) is the type for the genus *Trichogyne* (Hilliard 1981). It occurs both in coastal duneveld and inland in arid montane fynbos across a wide altitudinal gradient from sea level to c. 850m. The polyphyly of the montane and the coastal samples (fig. 3) could be explained by incomplete lineage sorting as described by Tosi et al. (2003). Evidence against incomplete lineage sorting is that the polyphyly of the coastal and montane forms is supported by both the plastid and nuclear loci with strong node support for the paraphyly of the two forms (fig. 2). Moreover, preliminary examination of morphological characters (data not shown) suggests that the two forms are morphologically divergent. Montane specimens had flower heads that were 2 mm - 3 mm in length, while coastal specimens had flower heads of 3 mm - 4 mm in length. Coastal specimens had involucral bracts that were not more than half the length of the flower head, while the involucral bracts on the

montane specimens were up to ¾ of the length of the flower head. Flower heads on the montane specimens were at intervals of 1 mm - 3 mm along the stem, while in coastal specimens this distance was 1 mm or less. The stems of the non-flowering branches are maroon in the montane specimens, while the coastal specimens did not have this pigmentation. The montane form is also the earliest diverging within the perennial clade in *Trichogyne* (fig. 3), with all the species in the *T. ambigua* (coastal) – *T. pilulifera* occurring at low altitude at the coast or on the coastal plane. It is unusual for species within this genus to have broad montane to coastal distributions and the coastal form of *T. ambigua* is sister to *T. repens*, which also occurs on the coast. This evidence suggests that *T. ambigua*, as currently described, may comprise two species. More specimens, however, would need to be examined in order to confirm and improve the morphological characters for classification before any nomenclatural changes were made.

The phylogenetic reconstruction suggests a southern African origin for *Ifloga* and *Trichogyne* (fig. 3) with the Northern Hemisphere species arising later. Disjunctive distributions in the African arid flora support the hypothesis of a large, fragmented arid belt reaching from Southwest Africa to the "Western Sahara (including arid parts of the Cape Verde and Canary Islands) via Eastern Africa" and into the Arabian peninsula at some time in the past (Jurgens, 1997). This "arid corridor" (Verdcourt, 1969) is suggested to have reached its greatest extent during the glacial periods of the Pleistocene (van Zinderen Bakker, 1975; Goldblatt, 1978). The origin of I. *spicata* within the arid, summer rainfall *Ifloga* clade and its being sister to *I. glomerata*, which occurs throughout the arid rainfall Karoo and Kalahari region of South Africa (Hilliard, 1981), supports a northwards migration and speciation of *Ifloga* via the arid corridor. Morphologically *I. spicata* resembles *I. glomerata* more than *I. obovata* (Canary Islands endemic). This suggests that *I. obovata* is derived from *I.* spicata, which is ubiquitous along the Mediterranean in North Africa and into the Arabian Peninsula, Pakistan and India. A molecular dating analysis would be useful in further elucidating the timing and potential route of the northwards migration of *Ifloga*.

Key to understanding the evolution of an annual life history is knowing which genes when expressed result in completion of the life cycle within one year, and what amount of molecular change differentiates them from those present in perennial individuals. Lammer et al. (2004) found that the addition of a single alien

chromosome resulted in a switch from annuality to perenniality in Chinese Spring wheat, but did not identify which genes on the chromosome coded for post reproductive growth and perennation. The rejection of a maximum likelihood reconstruction model with unequal transition rate parameters, meaning that for a given amount of molecular change there is an equal probability of transition between annaulity and perenniality, and annuality being ancestral and then lost (fig. 4) suggests that the switch between annuality and perenniality is not biased in a given direction for the *Trichogyne+Ifloga* clade. The model's rejection, however, could also be the effect of a small sample size not justifying the fitting of an unequal transition rate model due to the number of transitions between the character states being too small (Schluter et al., 1997). The likelihood best estimates of the forward and reverse transition rates between two discrete characters are estimated given the branch lengths and the tip states on the tree (Shluter et al., 1997). Therefore without controlling for the tip states on the tree it is not possible, using ancestral state reconstructions, to directly evaluate the suggestion by Thomas et al. (2003) that the change from annuality to perenniality or *vice versa* can take place with relatively little genetic change. A high transition rate estimate would support Thomas et al. (2000) by allowing for a switch in life history state over short branch lengths, representing little molecular change.

As well as knowing the genetic basis for annuality, recognising what environmental characteristics are associated with the evolution of annuality is important in elucidating the mechanisms that drive selection for an annual life history. Demographic models (e.g. Bell, 1976) predict that selection for annuality will be favoured in environments where adult survival is low and seedling survival is high, but do not say what environmental factors may cause this. Evans et al. (2005) states that the evaluation of climatic factors associated with annuality must be done on a seasonal time scale as it allows a focus to be placed on periods of the year when one life history state is expected to be more negatively effected than the other. My data indicate that it is a shortening in the length of the pulse in water availability that is associated with the evolution of annuality in the *Trichogyne+Ifloga* clade (table 2). Evans et al. (2005) also found the evolution of annuality to be associated with an increase in aridity during the wet season. This is consistent with Schwinning and Ehleringer (2001), finding that the duration of soil moisture pulses is important in selecting for different life history traits in arid environments. Annual plants are able to

set seed within a short period of resource availability and avoid drought, whereas perennials that germinate during the wet season have to survive the following dry season as seedlings or young adults. This requires the initial pulse in soil moisture to be long enough to allow for investment in storage structures.

The lack of a clear association between the evolution of annuality and either measure of dry season aridity (table 1) may be due to the low sample sizes from the low number of state transitions on the tree. It may also, however, be because it is not the intensity of aridity, but the length of the dry season above a certain threshold intensity that selects for annuality. If dry season aridity due to drought or high temperatures regularly reaches levels intense enough to, if sustained, kill perennial plants, and then it is the length for which this intensity is maintained that becomes important. Drought induced dormancy in perennials may also lessen the effect of high dry season aridity. Schenk and Jackson (2002) found the timing of a resource pulse to be important in favouring different life history states. It was expected that a pulse in soil moisture availability once a year would exert weaker selection for annuality than a pulse once every ten years. Finding no clear association between CV of mean annual rainfall, measuring the probability of a pulse in rainfall of greater than or equal to the average in rainfall occurring, and the evolution of annuality may be explained by Namaqualand having a low CV of mean annual rainfall in comparison with other deserts systems (Cowling et al., 1999) and therefore, although short in duration, soil moisture pulses are relatively regular and therefore have not been a strong selective force for annuality in Namaqualand. The evolution of annuality being associated with the length of the resource pulse in the system needs to be further tested for Namaqualand annuals. This association may also be tested more generally for annuals that may have evolved in response to different resource pulses such as pulses in light generated by disturbance in forested environments.

The need for annuals to grow quickly to set seed during the short period of soil moisture availability, makes investment in non-photosynthetic structures unattractive as this brings with it the cost of slowing growth (Pate et al. 1990), delaying flowering (Verburg and Grava, 1998) and/or reducing reproductive effort (Bonser and Aarsen, 2006). In contrast, perennials employing a drought tolerance strategy need to invest in reserve storage to persist through drought and then regrow (Kausch et al. 1981; Wyka, 1999). There is too much uncertainty is to know which growth form character

state the ancestor of the *Ifloga+Trichogyne* clade possessed (fig. 5) and so any constraint imposed by a non-photosynthetic phylogenetic background on the evolution of annuality can not be properly evaluated. Figure five does, however, show with moderate support annuality arising three times from a non-tufted ancestor suggesting that contrary to expectation a woody phylogentic background has not constrained the evolution of the annual habit. The correlation in the evolution of annuality and the tufted growth form does, however, fit with observations of high investment in photosynthetic over non-photosynthetic structures in annuals.

The discovery of amphi-basicarpy in *T. polycnemoides* makes it the first known example of this reproductive strategy within the African Asteraceae and one of only five known amphicarpic species within this group (Barker, 2005). Many of the species listed as amphicarpic are from arid habitats and occupy areas of low and strongly seasonal rainfall (Barker, 2005). The amphi-basicarpic daisy *Catananche lutea* produces flowers at ground level that are subsequently retracted below the soil surface by contractile roots (De Clavijo 1995). The presence of dead *T. polycnemoides* tufts below the soil surface may therefore be the result of root contraction or passive burial by wind blown sand. The low dispersal of tuft seeds is shown by their germination from within the dead tuft (fig. 8) and the resultant clumped distribution of *T. polycnemoides.* The pole seeds, being smaller, would be expected to have a greater potential for dispersal (Fenner, 1985) and individuals germinating from the bigger basal seeds that germinate *in situ* have been shown to be better competitors and have higher survival in low soil moisture conditions (Cheplick, 1987 and references therein) for other amphicarpic species. Flower heads on the poles of *T. polycnemoides* are also more likely to be capable of cross-pollinating than those buried within tufts. *T. polycnemoides* flowers within the tuft before investing in growing a pole. If, once the tuft flower heads are mature, soil moisture availability decreases jeopardising the continued survival of the individual, there is a rapid production of seed within the tuft flower heads and the pole flower heads are aborted (pers. obs.). Cheplick (1987) suggests that basal seeds are more important to individual fitness and that this is why they are produced first.

The presence of amphi-basicarpy in *T. polycnemoides* may represent a bet-hedging strategy, where in an arid environment, survival to the end of the growing season is uncertain and so the ability to produce seed, by self-fertilisation and flowering within

the tuft, and ensure a passage into the next generation is developed as soon as possible. If resource availability remains high then a branched candelabra-like growth from can be produced, allowing for out-crossing and the production of many aerial seeds for dispersal. Schnee and Waller (1986), also suggest this bet-hedging strategy in an amphicarpic annual species. Zohary (1937 in Cheplick, 1987) suggested that amphicarpy might be a strategy to hold the "mother site", where seeds experience the same favourable conditions as the parent. Reciprocal transplant experiments, however, showed basal seeds that were planted in different habitats from the parent site were not outperformed by seeds left to establish at the parent site (Cheplick, 1987). Barker (2005), suggested that the burial of seeds might be a strategy to prevent seeds being washed away by high water run-off. *Trichogyne polycnemoides* occurs on soils with a hard crust where run-off rates would be high and so this strategy is an alternative explanation for the evolution of amphi-basicarpy within the species. The majority of the tufted annuals within the *Trichogyne+Ifloga* clade appear to have clumped distributions (pers. obs.). The presence of both the tufted and the pole growth form in *T. candida* and *I. glomerata* suggests that these species may too be amphi-basicarpic. I suggest that the tufted growth form is an adaptation to arid environments with short moisture growing seasons, where there is the need to be able to produce seed quickly and early within the season due to uncertainty in the duration of resource availability. In contrast *T. verticillata*, occurring in more mesic habitat with a longer MGS, does not have to flower early within the season to ensure a spot in the next generation and so can defer flowering to the end of the season, first investing in a relatively large branched structure to hold many flower heads and seeds.

Transitions in life history state within *Trichogyne+Ifloga* are associated with transitions to more arid wet season environments. This also agrees with the northwards migration of the genus via the arid corridor occurring within an annual clade. The tufted growth form is appears to be an adaptation to an arid envionment, by facilitating early seed set and therefore a greater chance of being represented in the next generation.

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Samplig table: * denotes sequencing that was done as part of this research project

Species name	**Collecting Locality**	**psbA-trnH**	**trnL-trnF**	**ETS**
Amphiglossa corrudifolia DC.	South Africa	M. Koekemoer 1291	0	M. Koekemoer 1291
Amphiglossa tomentosa (Thunb.) Harv.	South Africa	N. G. B. 1332	N. G. B. 1332	N. G. B. 1332
Bryomorphe lycopodioides (Sch. Bip.) Levyns	South Africa	N. G. B. 1155	AF098820, AF100483	N. G. B. 1155
Disparago anomala Schlechter ex Levyns	South Africa	N. G. B. 1224	N. G. B. 1224	N. G. B. 1224
Edmondia sesamoides (L.) Hilliard	South Africa	N. G. B. 1130	AF098844, AF100507	N. G. B. 1130
Elytropappus hispidus (L.f.) Druce	South Africa	N. G. B. 1043	N. G. B. 1043	N. G. B. 1043
Elytropappus rhinocerotis (L.f) Less.	South Africa	N. G. B. 3.5	N. G. B. 3.5	N. G. B. M1
Gnaphalium declinatum L.f.	South Africa	N. G. B. 1073	N. G. B. 1073	N. G. B. 1073
Galleoma oculus cati *	Calvinia	NGB 1703	NGB 1703	NGB 1703
Helichrysum asperum (Thunb.) Hilliard & Burtt.	South Africa	N. G. B. 1165	N. G. B. 1165	N. G. B. 1165
Helichrysum cylindriflorum (L.) Hilliard & Burtt.	South Africa	0	AF098839, AF098839	N. G. B. 1063
Helichrysum felinum Less.	South Africa	N. G. B. 1194	N. G. B. 1194	N. G. B. 1194
Helichrysum sp. 1*	South Africa	N. G. B. 1640	N. G. B. 1640	N. G. B. 1640
Helichrysum sp. 2*	South Africa	N. G. B. 1683	N. G. B. 1683	N. G. B. 1683
Helichrysum sp. 3*	South Africa	N. G. B. 1678	N. G. B. 1678	N. G. B. 1678
Ifloga Spicata (Sch. Bip.) Webb*	Morocco	JL 17590	JL 17590	JL 17590
Ifloga glomerata (Harvey) Schltr.*	Sasolburg	NVK 15040	NVK 15040	NVK 15040
Ifloga molluginoides (D.C.) Hilliard*	Port Nolloth	NBG 1690	NBG 1690	NBG 1690
Ifloga anomola Hilliard*	Karoopoort	CHT 27	CHT 27	CHT 27
Lasiopogon debilis *	Sout River bridge	NGB 1646	NGB 1646	NGB 1646
Lasiopogon glomerulatus *	Sout River bridge	NGB 1645	NGB 1645	NGB 1645
Lasiopogon micropoides *	Port Nolloth	CHT 13	CHT 13	CHT 13
Lasiopogon muscoides *	Leliefontein	NGB 1692	NGB 1692	NGB 1692
Lachnospermum fasciculatum Baill.	South Africa	N. G. B. 1105	N. G. B. 1105	N. G. B. 1105
Leontopodium alpinum Cass	Zurich, Switzerland.	N. G. B. Z1	AF141733, AF141821	N. G. B. Z1
Leysera gnaphalodes (L.) L.*	South Africa	N. G. B. 1441	AF098810, AF100473	N. G. B. 1441
Metalasia densa (Lam.) Karis	South Africa	NGB1266	AF098848, AF100511	NGB1266
Oedera squarrosa (L.) Anderb. & Bremer	South Africa	0	AF098812, AF100475	N. G. B. 1065
Phaenocoma prolifera (L.) D. Don.	South Africa	N. G. B. 1206	AF098825, AF100488	N. G. B. 1206
Plechostachys serpyllifolia *	South Africa	N. G. B. 1271	AF098849, AF100512	N. G. B. 1271
Rhynchopsidium pumilum L.f. (DC)*	South Africa	P. O. Karis 770	AF098811, AF100474	P. O. Karis 770
Syncarpha canescens (L.) B. Nord.	South Africa	N. G. B. 1222	N. G. B. 1222	N. G. B. 1222

Trichogyne ambigua Druce coastal*	Koeberg	N.G.B. 1627	N.G.B. 1627	N.G.B. 1627
Trichogyne ambigua Druce montane*	Niewoudtville	N. G. B. 1328	AF098847, AF100510	N. G. B. 1328
Trichogyne verticillata (L.f.) Less.*	Piketberg	N.G.B. 1621	N.G.B. 1621	N.G.B. 1621
Trichogyne pilulifera (Schltr) Anderb. *	Kotzesrus	C.H.T. 1	C.H.T. 1	C.H.T. 1
Trichogyne paronychiodes (D.C.) Fenzl.*	Goegap	C.H.T. 18	C.H.T. 18	C.H.T. 18
Trichogyne polycnemoides (Fenzl.) Anderb.*	Kammieskoon	C.H.T. 3	C.H.T. 3	C.H.T. 3
Trichogyne candida (Hilliard) Anderb.*	Trekkersdraai, S. of Garies	C.H.T. 24	C.H.T. 24	C.H.T. 24
Trichogyne decumbens (Thunb.) Less.*	Katbakkies pass	CHT 26	CHT 26	CHT 26
Trichogyne repens (L.) A. Anderb. *	Elands Bay	DGE	DGE	DGE
*Trichogyne lerouxiae**	Soebatsfontein	CHT 9	CHT 9	CHT 9
Vellereophyton dealbatum (Thunb.) Hilliard & Burtt.	South Africa	0	AF098808, AF100471	NGB 1256