Phylogenetic and population genetic studies in the genus *Streptocarpus* Lindl. (Gesneriaceae DC.)

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Streptocarpus Lindl. (Gesneriaceae DC.) is a genus of herbaceous plants containing approximately 160 species, of which the majority occur in Africa and Madagascar. They are largely restricted to shaded and moist habitats such as primary forest and rock outcrops. The genus contains considerable morphological variation, with subgenus *Streptocarpella* containing caulescent species and subgenus *Streptocarpus* mostly consisting of acaulescent growth forms, mainly the unifoliates, plurifoliates and rosulates. Preliminary molecular analyses conducted using nuclear ITS sequence data suggested that subgenus *Streptocarpus* evolved in tropical central Africa, before radiating in several independent waves into southern Africa. *Streptocarpus* has therefore only recently spread into South Africa. Amongst the South African species, 11 morphologically similar species were identified as being closely related, together forming the Cape primrose clade, based on the analysis of nuclear ITS sequence data. However, these analyses only contained a few South African species, and the ITS data did not provide enough resolution of relationships within this clade.

In this study nuclear and chloroplast sequence data as well as nuclear microsatellite data were therefore employed to unravel the complex relationships amongst the South African *Streptocarpus* species. The analyses indicate that 16 rosulate, palynologically similar species (*S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana*, the *S. cyaneus* complex [*S. cyaneus*, *S. parviflorus*, *S. fenestra-dei*, *S. kunhardtii* and *S. roseo-albus*], *S. floribundus*, *S. aylae* and *S. kentaniensis*), the core Cape primrose species, are closely related, while five unifoliate/plurifoliate, palynologically more variable species (*S. denticulatus*, *S. dunnii*, *S. pusillus*, *S. rimicola* and *S. bolusii*) consistently emerged as more distantly related to the core Cape primrose species. However, the positions of a further ten species (*S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus*, *S. longiflorus*, *S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*) were more complex in the analyses, indicating that hybridization has played a role in their evolution. Five of these species (*S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus* and *S. longiflorus*) are, however, palynologically homogenous and rosulates, and therefore are probably more closely related to the core Cape primrose species, while the other five (*S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*) are unifoliates/plurifoliates that are palynologically more heterogenous, and are probably more distantly related to the core Cape primrose species.

Amongst the core Cape primrose species, *S. primulifolius* emerged as being ancestral or having hybridized with many of the other species, while the *S. cyaneus* complex forms a geographically and genetically more isolated group. However, evolutionary relationships amongst these species were to a certain extent obscured by incomplete lineage sorting caused by limited interpopulation gene flow, frequent hybridization and rapid speciation. The analyses confirmed that the Pondoland Centre forests constitute important Pleistocene refugia, and revealed some of the historical migration routes along which the species had radiated.

Opsomming

Streptocarpus Lindl. (Gesneriaceae DC.) is ‘n genus van kruidagtige plante wat uit 160 spesies bestaan, waarvan die meerderheid in Afrika en Madagaskar voorkom. Hulle is grootliks beperk tot skadu en vogtige habitats soos primêre woude en klip-agtige dagsome. Die genus toon aansienlike morfologiese variasie, die subgenus *Streptocarpella* bevat gestamde spesies en subgenus *Streptocarpus* bevat stamlose groeivorms, hoofsaaklik enkelblariges, meerblariges en rosulate. Voorlopige molekulêre analises wat op nukleêre ITS geenopeenvolgingsdata deurgevoer is, dui daarop dat subgenus *Streptocarpus* in tropiese sentraal Afrika ontstaan het, voordat dit versprei het in verskeie onafhanklike vlae in suider Afrika. *Streptocarpus* het dus net onlangs versprei in Suid Afrika. Onder die Suid-Afrikaanse spesies is 11 morfologies gelyksoortige spesies geïdentifiseer om naby aan mekaar verwant te wees, en om saam die “Cape primrose clade” op grond van ITS geenopeenvolgings te vorm. Hierdie ontledings het egter min Suid-Afrikaanse spesies bevat en die ITS data het nie genoeg resolusie getoon om die verwantskappe binne hierdie klade te bepaal nie.

In hierdie studie is nukleêre en chloroplast geenopeenvolgings sowel as mikrosatelliet data gebruik om die komplekse verwantskappe tussen Suid-Afrikaanse *Streptocarpus* spesies te bepaal. Die analises toon dat 16 rosulate, palenologies soortgelyke spesies (*S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana*, die *S. cyaneus* kompleks [*S. cyaneus*, *S. parviflorus*, *S. fenestra-dei*, *S. kunhardtii* en *S. roseo-albus*], *S. floribundus*, *S. aylae* en *S. kentaniensis*), die kern “Cape primrose” spesies, naverwant is, terwyl vyf enkelblarige/meerblarige, palenologies meer uiteenlopende spesies (*S. denticulatus*, *S. dunnii*, *S. pusillus*, *S. rimicola* en *S. bolusii*) herhaaldelik blyk om vêrverwant te wees aan die “Cape primrose” spesies. Die posisies van ‘n verdere tien spesies (*S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus*, *S. longiflorus*, *S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* en *S. vandeleurii*) is bevind om meer kompleks te wees uit hierdie analises, wat daarop dui dat hibridisasie ‘n rol gespeel het in hulle evolusie. Vyf van hierdie spesies (*S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus* en *S. longiflorus*) is egter palenologies homoloog aan rosulate en daarom nader verwant aan die “Cape primrose” spesies, terwyl die ander vyf (*S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* en *S. vandeleurii*) wat enkelblariges of meerblariges is, palenologies meer heterogeen is, en waarskynlik vêrverwant aan die kern “Cape primrose” spesies is.

Onder die “Cape primrose” spesies, het *S. primulifolius* geblyk om die oerouer van baie ander spesies te wees of daarmee gehibridiseer het, terwyl die *S. cyaneus* kompleks ‘n geografies en geneties meer geïsoleerde groep vorm. Die evolusionêre verwantskappe tussen hierdie spesies is tot ‘n sekere mate vervaag deur onvolledige sortering van lyne wat deur beperkte interpopulasie gene vloei, dikwelse hibridisasie en versnelde spesieëring veroorsaak is. Die analises het bevestig dat die “Pondoland Centre” woude belangrike Pleistosene refugia vorm, en het party van die historiese migrasieroetes waarvolgens hierdie spesies versprei het, onthul.

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Symbols, abbreviations and formulae

Symbols

α: the arbitrary significance level that is used to determine whether there is enough statistical evidence to indicate that there is a real difference between the values being tested i.e. that the difference between the values is unlikely to be due to chance alone

ΔK: the rate of change of the posterior probabilities (Ln P(D)) produced from a given set of parameters over successive values of the number of clusters (K), divided by the variance amongst independent runs at a given K in the program Structure 2.2

σ: standard deviation

A: mean number of alleles/locus

***f*:** Weir & Cockerham's (1984) estimate of the inbreeding coefficient F_{IS}

F_{IS}: the inbreeding coefficient

H_E: expected heterozygosity

H_O: observed heterozygosity

K: number of clusters in the program Structure 2.2

n: mean number of individuals sampled/locus

P_L: number of polymorphic loci

Pr_A: number of private alleles in each population

Abbreviations

AC: admixed ancestry & correlated allele frequencies

Ac: acetate

***A.D.*:** *anno Domini*

aff.: *affinis* (having affinity with, but distinct from, the named taxon; usually applied to a taxon believed to be undescribed)

agg.: aggregate

AI: admixed ancestry & independent allele frequencies

AIC: Akaike Information Criterion

APG: Angiosperm Phylogeny Group

BI: Bayesian inference

bp: base pair

BP: before present

BS: bootstrap

BSA: bovine serum albumin

cf.: compare (used in binomial nomenclature before the species name to indicate that the species is not confirmed)

CI: consistency index

CTAB: hexadecyltrimethylammonium bromide

DNA: deoxyribonucleic acid

dNTP: deoxynucleotide-triphosphate

g: gravity

ILD: incongruence length difference

IPNI: International Plant Names Index

ITS: internal transcribed spacer region of the 18S-5.8S-26S nuclear ribosomal cistron

LGM: Last Glacial Maximum

MCMC: Markov chain Monte Carlo

MP: maximum parsimony

MPT: most parsimonious tree

mya: million years ago

NC: no admixture & correlated allele frequencies

NI: no admixture & independent allele frequencies

NJ: Neighbour-Joining

NN: Neighbour-Net

NOR: nucleolar organizer region

NS: not significant

PCo: principal co-ordinates analysis

PCR: polymerase chain reaction

PH: Partition-Homogeneity

PP: posterior probability

ppm: parts per millions

PSA: proportion of shared alleles

PVP: polyvinylpyrrolidone

RBGE: Royal Botanic Garden Edinburgh

rDNA: ribosomal deoxyribonucleic acid

Formulae

Ln P(D): an estimate of the posterior probability of the data given a specified model in the program Structure 2.2

RFLP: restriction fragment length polymorphism

RI: retention index

RNA: ribonucleic acid

***rpl*20-*rps*12:** *rpl*20-*rps*12 intergenic spacer

rRNA: ribosomal ribonucleic acid

SAM: shoot apical meristem

SE: standard error

spec. nov.: new species

s/s/y: substitutions/site/year

subg.: subgenus

subsp.: subspecies

Taq: Taq-Polymerase

TBR: tree-bisection-reconnection

***trn*C-D:** the three adjacent plastid intergenic spacers between the *trn*C and *ycf*6f genes, the *ycf*6f and *psb*M genes and the *psb*M and *trn*D genes

***trn*L-F:** *trn*L intron and *trn*L-*trn*F intergenic spacer region

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Chapter 1: Introduction

1.1. Background to this study

In 1818, the first *Streptocarpus* Lindl.[1] specimen, a sample of *S. rexii* Lindl. from the Knysna area, was collected by the Kew plant collector James Bowie. More discoveries were soon to follow, with *S. gardenii* Hook., *S. polyanthus* Hook., *S. saundersii* Hook., *S. parviflorus* Hook.f. and *S. dunnii* Hook.f. being discovered soon thereafter. The horticultural importance of this genus was quickly realized, and various cultivars, most descendents from *S. rexii*, were produced in close succession. By 1883, when Clarke revised *Streptocarpus*, he recognised 17 species. The unusual vegetative morphology of *Streptocarpus*, including the uneven development of the cotyledonary leaves and the growth form of a group of plants that produce only one foliage leaf during their life times, termed the "unifoliates", prompted much interest in the genus, and during most of the 20th century, genetic and physiological studies were conducted on *Streptocarpus* species. Then in 1971, Hilliard & Burtt published their revision of *Streptocarpus*, with an emphasis on the southern African taxa, recognizing 132 species. Hilliard & Burtt (1971) highlighted the morphological complexities of the genus, finding vegetative and floral morphology to be incongruent with each other in many cases. They upheld the division of *Streptocarpus* into two subgenera that was first proposed by Fritsch (1904), namely subgenus *Streptocarpus* and subgenus *Streptocarpella*, and suggested species groupings within subgenus *Streptocarpus* based on morphology. They also identified hybridization as an important factor in *Streptocarpus* evolution, suggesting a hybrid origin for species such as *S. johannis* L.L.Britten, *S. baudertii* L.L.Britten and *S. montigena* L.L.Britten, and proposing hybridization as the most likely origin of intermediate populations that they came across.

Towards the end of the 20th century, molecular techniques became increasingly popular as a means of reconstructing evolutionary relationships amongst taxa. A number of embryological (e.g. Holmqvist 1964), karyological (e.g. Skog 1984), palynological (Weigend & Edwards 1996), and molecular (e.g. Möller & Cronk 1997, 2001a; Smith *et al*. 1997, 1998; Mayer *et al*. 2003 & Smith *et al*. 2004b) studies revealed that *Streptocarpus* is a paraphyletic group, with the other African gesneriad genera nested within it. Möller & Cronk (1997) and later Möller & Cronk (2001a) reconstructed evolutionary relationships within *Streptocarpus* using ITS sequence data. The molecular phylogenies highlighted the complexity of evolution within *Streptocarpus*. Although the subgeneric division was largely confirmed by the molecular data, relationships below the subgeneric level indicated by the molecular data were in many cases

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[1]Different sources give different authors for some taxonomic names. In this thesis, the authors of names outside of Gesneriaceae were obtained from the "International Plant Names Index" web site (IPNI; http://www.ipni.org/index.html, accessed on 27 March 2008), and the "Angiosperm Phylogeny Website" (http://www.mobot.org/MOBOT/Research/APweb/welcome.html, accessed on 27 March 2008). If a taxonomic name was present on the IPNI website, then the author given there was used. Otherwise, authorship was obtained from the Angiosperm Phylogeny Website.

For taxonomic names within Gesneriaceae above the specific level, Burtt & Wiehler (1995), Burtt (1997), Weber (2004) and the IPNI web site were used. In each case, authorship was obtained from the most recent source containing the name. However, IPNI contains very few infrafamilial (between the family and genus ranks) Gesneriaceae names, and authorship as given in Weber (2004) was therefore used in these cases.

Authorship of specific and subspecific names within *Streptocarpus* was obtained from the "World Checklist of Gesneriaceae" (http://botany.si.edu/gesneriaceae/, accessed on 15 February 2008).

In the case of all taxonomic names, author names were written in their standard form given by the IPNI website.

incongruent with morphology. Both vegetative (Möller & Cronk 2001a) and floral (Harrison *et al*. 1999; Hughes *et al*. 2006) morphology appear to be plastic based on ITS phylogenies, suggesting that many of the growth forms and floral types have evolved independently several times and several reversals have occurred. The molecular data also provided insight into the biogeography of *Streptocarpus*. Möller & Cronk (2001b) inferred that subgenus *Streptocarpus* evolved towards central Africa, before spreading south in a series of independent waves. They suggest that *Streptocarpus* arrived in South Africa relatively recently, with the South African *Streptocarpus* taxa constituting a number of reasonably young, independent evolutionary lineages.

Amongst the South African taxa in their molecular phylogeny, Möller & Cronk (2001a, b) identified a relatively young, strongly supported South African *Streptocarpus* lineage with a geographical distribution from the Knysna area in the Western Cape to the Soutpansberg vicinity in Limpopo that they named the "Cape primrose clade". However, their ITS sequence phylogeny only contained a few South African *Streptocarpus* species, and the topology within this clade was not resolved and supported enough to determine relationships. The species emerging within this clade all possess the rosulate growth habit, all possess the open-tubed floral type except for two species, which both possess keyhole flowers, and all possess pollen type 13, except for one species each possessing pollen types 4 and 8. The vegetative and floral morphology and palynology are therefore only of limited use in unravelling relationships within this clade, probably due to the recent divergence of the species in this group.

The Cape primrose clade was therefore identified as one of the groups of research interest. In 2001, funding for research on evolution within *Streptocarpus*, including amongst the species of the Cape primrose clade, was granted by the Leverhulme Trust, UK, to be undertaken by Michael Möller (Royal Botanic Garden Edinburgh, RBGE), Dirk Bellstedt (University of Stellenbosch) and Trevor Edwards (then based at the University of Natal) as the principal applicants, and Mark Hughes (then also based at the RBGE) as a post-doctoral researcher. Additionally, funding was also granted from the Conservation and Management of Ecosystems and Biodiversity Thrust of the South African National Research Foundation to Dirk Bellstedt. Within this greater *Streptocarpus* project, a number of research priorities were identified. Although floral morphology provides some indication of potential pollinators, detailed field observations of the pollinators of *Streptocarpus* were lacking. The breeding systems of species have implications for gene flow and consequently modes of evolution within the genus, and pollination biology in the form of pollinator vigils, nectar composition analyses, etc. was therefore identified as one of the research priorities. Most *Streptocarpus* species only occur in small, fragmented forest patches, and the extent of gene flow at the population level through pollen and seed dispersal and its implications on evolution within *Streptocarpus* would therefore also be investigated through molecular techniques. Molecular techniques would also be applied to investigating the role of hybridization in the evolution of the genus. Speciation in *Streptocarpus* appears to have a strong geographical component. Forest, the main habitat in which *Streptocarpus* species occur, is likely to have been heavily influenced by past climatic cycles e.g. the Pleistocene glacial-interglacial cycles. Biogeography was therefore identified as another important focus area. These objectives culminated in a number of studies (Hughes *et al*. 2005, 2006, 2007), of which the present study, the reconstruction of relationships both amongst the species and populations in the Cape primrose clade, is one.

1.2. Objectives of the research

The main aims of this current study were consequently to:

- Determine the extent of the Cape primrose clade and how these taxa are related to the rest of *Streptocarpus* by incorporating many other South African taxa into the analysis.

- Determine relationships amongst the members of the Cape primrose clade, with an emphasis on the relationships of the two species possessing the so-called keyhole corolla morphology, *S. johannis* and *S. baudertii*.

- Determine how evolution has taken place in *Streptocarpus* species i.e. the role of hybridization on the evolution of the Cape primrose clade taxa, and identify geographical routes along which members of the Cape primrose clade might have radiated. A time scale for the evolution of *Streptocarpus* in South Africa was also estimated.

1.3. Contributions of co-authors and other collaborators in relation to my contribution

The data that I generated during this study were combined with data generated by many people over a number of years. The main contributors and their contributions in relation to my contribution are listed here:

- Persons that have collected specimens included in this present study include Dirk Bellstedt, Mark Hughes, Michael Möller, Trevor Edwards, Cameron McMaster, David Styles, Gavin McDonald, Ernst van Jaarsveld, Louise Badenhorst and Benny Bytebier.

- Michael Möller and Quentin Cronk have generated many nuclear internal transcribed spacer region of the 18S-5.8S-26S nuclear ribosomal cistron (hereafter called ITS) sequences of *Streptocarpus*, including many South African taxa, most of which were included in their 2001 papers (Möller & Cronk 2001a & b).

- Benny Bytebier and Dirk Bellstedt sequenced the ITS region of one plant/population for each of the seven populations of *S. johannis* analysed in this study.

- Mark Hughes did a post doctorate on the relationship between *S. primulifolius* Gand. and *S. rexii*, two widely distributed taxa in South Africa, at the Royal Botanic Garden Edinburgh (RBGE). He did, however, also work on some other taxa. He generated ITS and plastid sequences, plastid RFLP data and microsatellite data from 14 *S. primulifolius*, nine *S. rexii*, five *S. baudertii*, four *S. polyanthus* populations, and one *S. formosus* (Hilliard & B.L.Burtt) T.J.Edwards population. ITS and plastid sequences and plastid RFLP data were additionally generated from three *S. meyeri* B.L.Burtt populations, and ITS sequences and microsatellite data only were generated from 22 *S. cyaneus*-complex populations. In the case of the nuclear and plastid sequences, one plant was analysed from each population, while one to 32 plants/population were screened during the microsatellite analyses. Molecular data from the *S. primulifolius*, *S. rexii* and *S. formosus* populations were published in a paper that is included in the appendix of this thesis (Hughes *et al.* 2005).

- Zoe Goodwin was an MSc student based at the RBGE who concentrated on the *S. cyaneus* complex (including *S. cyaneus* S.Moore, *S. actinoflorus* T.J.Edwards & M.Hughes, *S. parviflorus*, *S. kunhardtii* T.J.Edwards, *S. fenestra-dei* Weigend & T.J.Edwards and *S. roseo-albus* Weigend & T.J.Edwards). She expanded on the ITS and plastid sequence data that had already been generated for this group by Mark Hughes. Photographs of the front and side views of flowers were included in her thesis, and she took morphometric measurements of plants belonging to the complex that were in

flower in the Royal Botanic Gardens Edinburgh greenhouses during the course of her project. Silica-dried leaf samples from some of the *S. cyaneus* complex populations were subsequently sent to me for further sequencing i.e. to fill in gaps in the sequences. However, for each of these samples, I detected discrepancies between the fragments that I had sequenced and the sequences that had been sent to me. These discrepancies between my sequences and the sequences sent to me from Edinburgh could either have resulted from differences in reaction conditions e.g. differences in the model of sequencer used in Stellenbosch vs Edinburgh, or sequencing a different individual from the same population, especially if there is a large amount of variation within populations of the *S. cyaneus* complex. For all the individuals in which I detected discrepancies, I resequenced all the nuclear and plastid regions for that individual to ensure that all the sequences inserted into the final analyses were from the same individual in each case.

- My task was to analyse the remaining South African rosulate taxa, as well as numerous outgroup species, by also using sequence and microsatellite data. In order to achieve this, I generated ITS and plastid sequence data (in each case one or two plants/population, but six plants for the *S. meyeri* population from Bastervoetpad) from an additional 19 South African species, as well as *S. montanus* Oliv. from Kenya and *S. papangae* Humbert from Madagascar. Included in these additional 21 species were five *S. dunnii* and three *S. gardenii* populations, two populations each of *S. caeruleus* Hilliard & B.L.Burtt, *S. grandis* N.E.Br., *S. lilliputana* Bellstedt & T.J.Edwards, *S. montigena* and *S. rimicola* Story, and one population each of *S. aylae* T.J.Edwards, *S. bolusii* C.B.Clarke, *S. cyaneus* subsp. *nigridens*, *S. denticulatus* Turrill, *S. fanniniae* Harv. ex. C.B.Clarke, *S. floribundus* Weigend & T.J.Edwards, *S. kentaniensis* Britten & Story, *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards, *S. modestus* L.L.Britten, *S. porphyrostachys* Hilliard, *S. pusillus* Harv. ex C.B.Clarke, *S. saundersii*, and *S. vandeleurii* Baker f. & S.Moore. Moreover, ITS and plastid sequence data were also collected from six more *S. meyeri* populations, two more *S. primulifolius* populations, and an extra population each from *S. formosus* and *S. parviflorus*. Plastid sequence data (two plants/population) and microsatellite data (18 to 30 plants/population) were generated from the seven *S. johannis* populations included in this study (the ITS region had already been sequenced by Benny Bytebier and Dirk Bellstedt), and ITS and plastid sequences (one plant/population), as well as microsatellite data (21 plants/population), were generated from two additional *S. primulifolius* populations. Furthermore, I also filled in gaps in sequences that had been produced at the RBGE. I completed the ITS sequences of ten *S. cyaneus*-complex, four *S. polyanthus*, four *S. rexii*, three *S. primulifolius*, and two *S. baudertii* populations, and one *S. meyeri* population. I also filled in gaps in the plastid sequences (which includes the three adjacent plastid intergenic spacers between the *trn*C and *ycf*6f genes, the *ycf*6f and *psb*M genes and the *psb*M and *trn*D genes [hereafter collectively referred to as *trn*C-D] and the plastid *rpl*20-*rps*12 intergenic spacer [hereafter called *rpl*20-*rps*12]) of 14 *S. primulifolius*, ten *S. cyaneus*-complex, eight *S. rexii*, six *S. baudertii*, four *S. polyanthus*, three *S. meyeri* populations and one *S. formosus* population. I did not detect any discrepancies between the fragments that I had produced and those that had been produced at the RBGE in any of the sequences for which I filled in gaps, except in the *S. cyaneus* complex, as explained above. I also amplified the plastid *trn*L intron and *trn*L-*trn*F intergenic spacer region (hereafter called *trn*L-F) for all the taxa included in the current analysis (both the populations of which these regions had already been sequenced, as well as the ones that I added).

- Precise details of sequence and microsatellite data for each species analysed in this thesis (and specifically indicating those data that I generated) are given in Tables 3.1 and 4.2 (the sequence data) and 4.1 (the microsatellite data).

1.4. Thesis layout

The rest of the thesis is presented in the following manner:

Chapter 2 contains the literature review, with discussions of the position of Gesneriaceae within Lamiales, the affiliations of *Streptocarpus* with the other gesneriad genera, including the other African genera, relationships within *Streptocarpus*, vegetative and floral diversity of *Streptocarpus*, and the informal morphological groupings proposed by Hilliard & Burtt (1971). The final section of Chapter 2 contains a discussion of the biogeography of *Streptocarpus* and subgenus *Streptocarpus* in the context of palaeoclimates and the vegetation history of southern Africa.

Chapter 3 describes the analyses conducted on the South African taxa in an attempt to identify the members of the Cape primrose clade and to unravel relationships within this group using ITS and plastid sequence data. This chapter has been written in the publication format to speed up publication after the completion of this thesis. However, many of the sections have been written in much greater detail than would be acceptable in a publication, but are given here fully within the context of the thesis, and will be shortened before publication.

Based on the sequence analyses of Chapter 3, a number of species within the Cape primrose clade were identified as being closely related to one another. Of these, five species were selected for finer-scale microsatellite analyses, the subject of **Chapter 4**, in an attempt to elucidate relationships further amongst these species. As is the case in chapter 3, this chapter was also written in publication format. However, the introduction is shorter seeing as most of the background has already been presented in previous chapters, and the rest of the sections are longer than they will be in the final publication.

Chapter 5 provides the general conclusions of the sequence and microsatellite analyses of Chapters 3 and 4.

Chapter 6 consists of a combined reference list for Chapters 1, 2, 3, 4 and 5 instead of showing an individual reference list at the end of each of these sections. This chapter is followed by appropriate appendices.

Chapter 2: *Streptocarpus*: an African genus in Gesneriaceae

2.1. An introduction to the genus

Streptocarpus is a largely African genus of herbaceous plants, mostly restricted to protected habitats amongst rocks, on overhangs or in evergreen forests. The genus is not only of interest to the botanist due to its fascinating seedling development, morphology and evolutionary patterns, but also captures the attention of the general public as an attractive indoor pot plant. It also serves as an effective indicator of environmental health as it is extremely sensitive to environmental disturbance.

The morphology of *Streptocarpus* is of particular interest due to its unusual ontogeny and diversity of bizarre growth forms. Seedling development is unusual in that, after germination, the two cotyledons develop unevenly, resulting in one (the macro-cotyledon) growing into a foliage leaf, while the development of the other one (the micro-cotyledon) is suppressed by the macro-cotyledon and withers away (Tsukaya 1997; Mantegazza *et al*. 2007). The adult plants are equally fascinating. The genus displays an array of unconventional vegetative morphologies, including a growth habit which consists of only one foliar organ (the macro-cotyledon). This continues to grow from the base of the organ throughout the plant's life time and produces inflorescences at the base of the lamina (Hilliard & Burtt 1971). Floral diversity within the genus is even more extensive, and closely related species sometimes possess diverse floral morphologies.

Streptocarpus is also complex from the evolutionary perspective. There are three main growth habits and six floral types present within the genus, each containing a considerable amount of variation, and many of these types appear to have evolved independently more than once (Harrison *et al*. 1999; Möller & Cronk 2001a; Hughes *et al*. 2006). The genus is therefore ideal for the study of the evolution of vegetative and floral morphology. Additionally, hybridization appears to have played a prominent role in the evolution of *Streptocarpus* (Hilliard & Burtt 1971; Hughes *et al*. 2005), often blurring species boundaries.

As is the case for many of the forest floor species in the Scarp Forests of the Eastern Cape and KwaZulu-Natal provinces of South Africa (Matolweni *et al*. 2000; Ernst van Jaarsveld 2006), a large number of *Streptocarpus* species are highly endemic. While the seeds of typical forest crown taxa such as *Podocarpus* Labill. (yellow wood; Podocarpaceae Endl.) are distributed by birds e.g. the Cape Parrot (*Poicephalus robustus* J.F.Gmel.), and, historically, also by large mammals, which spread the species over relatively large areas, *Streptocarpus* species have no specialised dispersal mechanisms and therefore often far more limited distributions. For example, *S. lilliputana* has only been found in three neighbouring river gorges in the Transkei (Eastern Cape Province), and *S. kentaniensis* is only known to occur within a small area around the town of Kentani in the Eastern Cape, and from the region close to the Kei River. Likewise, the distribution of *S. modestus* is also very limited, extending from Emboytji in the south to the mouth of the Mtentu River in the north. Many forest floor plants such as *Streptocarpus*, *Plectranthus* L.Hér. (Lamiaceae Lindl.), *Begonia* L. (Begoniaceae C.A. Agardh.) and Acanthaceae Juss. taxa produce very small seeds that do not attract seed carriers and are consequently not actively dispersed far from the mother plant. Individual taxa belonging to these plant groups therefore tend to be highly endemic to one or a limited number of neighbouring forest patches (Dirk U. Bellstedt, personal communication). This pattern is mirrored by the diversity of small animals in the Scarp Forests, since smaller animals also do not tend to travel very far. Amongst these are chameleons (Tolley & Burger 2007), slugs (Herbert 1997) and millipedes (Hamer & Slotow 2002).

Streptocarpus, as is the case with many other members of the family Gesneriaceae, serves as an important indicator of the health of the ecosystems in which it tends to grow. Members of the genus favour sheltered habitats such as forested gorges, overhangs and rocky outcrops, and in most cases require specific conditions in order to flourish. Most species like shady, humid habitats with a more or less constant source of water, but very good drainage. The majority of species are very sensitive to changes in their environment, including loss of forest cover and changes in water supply. *Streptocarpus* plants are also not very strong competitors, and quickly succumbs to the influx of invaders on the forest floor e.g. *Tradescantia* Rupp. ex L. (Commelinaceae Mirb.), as was observed during a field trip undertaken in "The Dargle" area of KwaZulu-Natal during this study, and also to tree invaders such as *Solanum mauritianum* Scopoli (bugweed; Solanaceae Adans.), *Acacia mearnsii* De Wild. (Black Wattle; Fabaceae Lindl.) and *Acacia dealbata* Link (Silver Wattle; Fabaceae), which are a major problem in KwaZulu-Natal and the Eastern Cape.

Thus, not only does *Streptocarpus* need to be conserved in its own right; it is also a valuable indicator of the status of the ecosystem it occupies, and an abundance of *Streptocarpus* species is indicative of undisturbed habitats that sustain numerous other plant and animal species requiring protection. Therefore, a knowledge of the *Streptocarpus* species composition of forest patches, as well as an understanding of the diversity distribution of each species i.e. whether a species is more or less uniform across its range, or whether each forest patch in which the species occurs harbours unique alleles, is valuable for identifying conservation priorities.

Finally, *Streptocarpus* is also important to horticulturalists and the general public. Known as the "Cape primrose" in horticulture, *Streptocarpus* and the closely related genus *Saintpaulia* H.Wendl., commonly known as the "African violet", are some of the most popular indoor ornamental plants in Europe and the USA. These plants are particularly suitable as house plants, because the indoor environment more closely mimics the shady, humid conditions under which they grow in nature (http://www.gesneriads.ca/Default.htm, accessed on 10 March 2008). *Streptocarpus* has been hybridised extensively since the early 19th century, and hybrid cultivars have been selected for horticultural purposes. *S. rexii* was the first species of the genus to be described (Lindley 1828), and was quickly introduced as a garden plant thereafter. It took less than a century for other species, including *S. gardenii*, *S. polyanthus, S. saundersii, S. parviflorus, S. dunnii, S. wendlandii* Spreng. and *S. fanniniae*, to be discovered and used in the production of new cultivars (Hilliard & Burtt 1971). *Streptocarpus* species possess a number of traits that make them especially suited to horticultural cultivation. Species readily hybridize with one another, even with relatively distantly related species within the subgenera, and an array of taxa can therefore be utilized in the search for desirable characteristics e.g. larger flowers, more flowers per inflorescence, longer flowering periods, and novel floral colours and patterns. Additionally, the vegetative propagation of plants is also easy. Little plantlets can be produced from leaf cuttings, and some plants e.g. *S. primulifolius*, produce large, branching rhizomes that separate over time, with each piece forming a new plant (Hilliard & Burtt 1971). Today, horticultural Cape primrose varieties are mainly rosulates, descended from South African taxa of subgenus *Streptocarpus* (Möller & Cronk 2001a), especially *S. rexii*, although some caulescent species of subgenus *Streptocarpella* are also grown e.g. *S. saxorum* Engl. (http://www.gesneriads.ca/Default.htm, accessed on 10 March 2008) or the hybrid "Good Hope" (*S. stomandrus* × *S. saxorum*).

The following sections of this chapter will describe the suprageneric position of *Streptocarpus* within Lamiales, the vegetative and floral morphology, and the biogeographic history of *Streptocarpus* as a whole in greater detail. In particular, the most up-to-date knowledge of the relationships within subgenus *Streptocarpus* and specifically the Cape primrose clade will be described. These sections will provide the necessary background for the subsequent chapters of

this thesis, in which the results of the phylogenetic and population genetic analyses of members of the Cape Primrose clade will be presented.

2.2. Suprageneric classification of *Streptocarpus*

2.2.1. Lamiales

Streptocarpus is currently classified within the tribe Didymocarpeae (D.Don) Endl., in the subfamily Didymocarpoideae (D.Don) Arn. of the family Gesneriaceae, which in turn forms part of the order **Lamiales** Bromhead. In higher-level phylogenetic analyses, which continue to contribute towards the ongoing Angiosperm Phylogeny Group (APG) classification, members of Lamiales emerge in a monophyletic clade within the euasterid I group of the angiosperms (Figure 2.1; Oxelman *et al*. 1999; Olmstead *et al*. 2000; Albach *et al*. 2001; Bremer *et al*. 2001, 2002; Soltis *et al*. 2007). Lamiales groups most closely with Gentianales Lindl., Solanales Dumort., Boraginaceae Juss. and Vahliaceae Dandy (the order(s) to which the latter two families belong within the euasterid I clade are still uncertain according to the current APG classification [http://www.mobot.org/MOBOT/Research/APweb/welcome.html, accessed on 25 March 2008]). Ever since the first cladistic analyses were carried out to investigate relationships within the asterids (Chase *et al*. 1993; Olmstead *et al*. 1993), it has been known that these five groups are closely related. However, the precise position of Lamiales relative to these taxa tends to vary from analysis to analysis (Oxelman *et al*. 1999; Olmstead *et al*. 2000; Bremer *et al*. 2001, 2002; Soltis *et al*. 2007), and its exact relationships are consequently still uncertain.

Lamiales emerges as a strongly supported group (Olmstead *et al*. 2000; Albach *et al*. 2001; Bremer *et al*. 2002; Wortley *et al*. 2007; Soltis *et al*. 2007) of 23 families, over 1 000 genera, and more than 23 000 species, many of which are of economic and horticultural importance (http://www.mobot.org/MOBOT/Research/APweb/welcome.html, accessed on 20 August 2007). The order is estimated to have originated 106 to 74 million years ago during the Cretaceous period (Bremer *et al*. 2004; Wikström *et al*. 2001, respectively). Members of Lamiales share a number of synapomorphies, including glandular hairs with a uniseriate stalk and vertical divisions in the head, leaves arranged in an opposite phylotaxy, immature anthers with "placentoid" tissue, fruit in the form of a capsule, an endosperm with micropylar haustoria, and the production of verbascosides, methyl- and oxygenated flavones, and iridoids or cornosides (Albach *et al*. 2001; http://www.mobot.org/MOBOT/Research/APweb/welcome.html, accessed on 20 August 2007).

Molecular analyses containing a representative sample of Lamiales have identified many well supported families (Olmstead & Reeves 1995; Oxelman *et al*. 1999; Olmstead *et al*. 2000, 2001; Albach *et al*. 2001; Bremer *et al*. 2002; Wortley *et al*. 2005, 2007; Soltis *et al*. 2007), and the deeper relationships within the order are relatively consistent amongst analyses (Figure 2.1). Thus, Plocospermataceae Hutch. resolves as sister to the rest of Lamiales (Oxelman *et al*. 1999; Bremer *et al*. 2002), followed by Oleaceae Hoffmanns. & Link (Oxelman *et al*. 1999; Olmstead *et al*. 2000, 2001; Albach *et al*. 2001; Bremer *et al*. 2001, 2002; Wortley *et al*. 2005, 2007; Andersson 2006; Soltis *et al*. 2007) and Carlemanniaceae Airy Shaw (Bremer *et al*. 2001), which both emerge in a polytomy along with a clade containing the rest of the families. Within this latter clade, Tetrachondraceae Skottsberg ex R.W.Sanders & P.D.Cantino resolves sister to the rest of the order (Oxelman *et al*. 1999; Bremer *et al*. 2001, 2002; Andersson 2006), followed by Calceolariaceae Raf. ex R.G.Olmstead (Olmstead *et al*. 2001; Wang *et al*. 2004; Wortley *et al*. 2005, 2007). Gesneriaceae then groups sister to the clade containing the other Lamiales families, either on its own (Olmstead *et al*. 2000, 2001; Albach *et al*. 2001), sister to *Peltanthera* Benth. (Oxelman *et al*. 1999; Bremer *et al*. 2001, 2002; Andersson 2006), a genus of uncertain status within Lamiales (http://www.ncbi.nlm.nih.gov/, accessed 11 June 2008), or

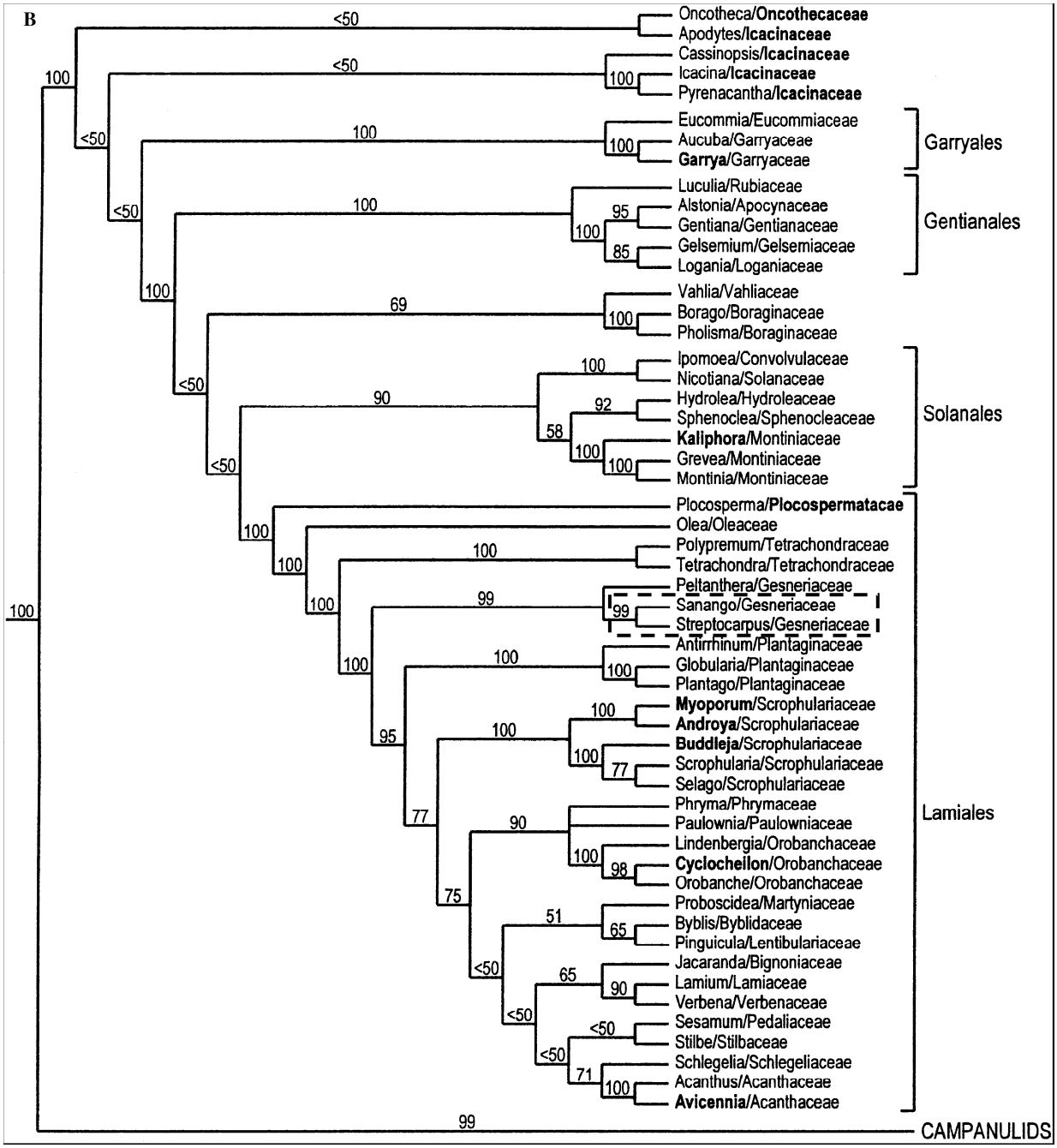

Figure 2.1: Part of the parsimony strict consensus tree of the asterids from Bremer *et al*. (2002) showing the relationships between Lamiales and its closest allies, as well as the position of Gesneriaceae within Lamiales. The tree was reconstructed from the three plastid protein-coding markers (1) *rbc*L, (2) *ndh*F and (3) *mat*K, and three non-coding markers, including (4) a region containing *trn*L exons, and the intron and intergenic spacers from *trn*T to *trn*F, (5) a region containing *trn*V exons and intron, *trn*M, and intergenic spacers from *trn*V to *atp*E, and (6) the *rps*16 intron. Jackknife values are indicated above the corresponding branches. The clade containing the representatives of Gesneriaceae is enclosed in a dashed rectangle (*Peltanthera* is no longer classified within Gesneriaceae, but has an uncertain status within Lamiales [http://www.ncbi.nlm.nih.gov/, accessed 11 June 2008]).

sister to or in an unresolved position along with Plantaginaceae Juss. (Wortley *et al*. 2005, 2007). The position of Plantaginaceae within Lamiales is variable amongst analyses. The family either emerges close to Gesneriaceae (Oxelman *et al*. 1999; Wortley *et al*. 2005, 2007), or along with the younger families of Lamiales (Olmstead *et al*. 2000, 2001; Bremer *et al*. 2001, 2002; Albach *et al*. 2001). Likewise, the position of Byblidaceae also tends to vary amongst analyses, grouping close to Gesneriaceae in Bremer *et al*. (2001), but amongst the younger families in Bremer *et al*. (2002). Calceolariaceae groups close to Gesneriaceae in Andersson (2006), but this analysis only contained a limited number of samples from across Lamiales and was only

based on one gene, the plastid *mat*K region. In analyses based on more data (Olmstead *et al*. 2001 used the three plastid regions *rbc*L, *ndh*F and *rps*2; Wortley *et al*. 2005 used the plastid regions *ndh*F and *rbc*L, and Wortley *et al*. 2007 also used *ndh*F and *rbc*L, as well as morphological, cytological and chemical data), Calceolariaceae emerges sister to a clade containing most of the Lamiales families, including Gesneriaceae.

In contrast, relationships amongst the more derived families within Lamiales (Acanthaceae, Bignoniaceae Juss., Lamiaceae, Lentibulariaceae Rich., Martyniaceae Stapf, Orobanchaceae Vent., Paulowniaceae Nakai, Pedaliaceae R.Br., Phrymaceae Schauer, Schlegeliaceae (A.H.Gentry) Reveal, Scrophulariaceae Juss., Stilbaceae Kunth, Thomandersiaceae Sreem., Verbenaceae J.St.-Hil., and, in some analyses, Plantaginaceae and Byblidaceae as well) are comparatively less clear (Olmstead & Reeves 1995; Olmstead *et al*. 2000; Albach *et al*. 2001; Bremer *et al*. 2001, 2002; Wortley *et al*. 2005), and the taxonomy within the order is very much in a state of flux (e.g. Cantino 1992; Cantino *et al*. 1992, 1997, 1998; Olmstead & Reeves 1995; Wagstaff *et al*. 1995, 1998; Wagstaff & Olmstead 1997; Steane *et al*. 1997; Oxelman *et al*. 1999; Reveal *et al*. 1999; Olmstead *et al*. 2001; Bremer *et al*. 2002; Schwarzbach & McDade 2002; Beardsley & Olmstead 2002; Wortley *et al*. 2007). Analyses of the order tend to recover strongly supported family clades, connected by short, unstable branches (Olmstead *et al*. 2001; Albach *et al*. 2001; Bremer *et al*. 2002; Wortley *et al*. 2005, 2007), and Wortley *et al*. (2005) estimated that at least 2 000 more parsimony-informative characters (corresponding to 10 000 more bases of sequence data from regions with similar substitution rates to those that have been employed so far) would be needed to fully resolve interfamilial relationships in Lamiales. A further complication to the taxonomy of the order is that not all of the constituent families form strongly supported monophyletic clades. Some of the families have been very difficult to delimit, and their circumscription is likely to change in the near future (http://www.mobot.org/MOBOT/Research/APweb/welcome.html, accessed on 20 August 2007). One such family is Scrophulariaceae. Its taxonomy has changed substantially in recent years (Olmstead *et al*. 2001; Bremer *et al*. 2002; Oxelman *et al.* 2005), and some taxa previously classified within Scrophulariaceae have subsequently been moved into Gesneriaceae e.g. *Titanotrichum* Solereder (Weber 2004; Wang *et al*. 2004).

Thus, while family delimitations within Lamiales are likely to undergo further revisions as more data are generated, the position of Gesneriaceae within Lamiales appears to be relatively stable. Gesneriaceae emerges sister to most of Lamiales in all of the analyses, and is consequently one of the older families within the order.

2.2.2. Gesneriaceae

Gesneriaceae itself consistently emerges as a strongly supported clade in all of the higher-level analyses done so far (Figure 2.1; Olmstead & Reeves 1995; Oxelman *et al*. 1999; Olmstead *et al*. 2000, 2001; Albach *et al*. 2001; Bremer *et al*. 2001, 2002; Wang *et al*. 2004; Wortley *et al*. 2005, 2007; Soltis *et al*. 2007). The family was estimated to have arisen about 78 million years ago by Bremer *et al*. (2004). However, Bremer *et al*. (2004) included *Peltanthera*, a genus of uncertain status within Lamiales (http://www.ncbi.nlm.nih.gov/, accessed 11 June 2008), in their Gesneriaceae clade, and Gesneriaceae therefore probably arose little after 78 million years ago. The family is broadly distinct from the rest of Lamiales taxa by possessing a combination of the following characters: a lack of iridoid compounds (Albach *et al*. 2001), opposite leaves (Smith 2000a), possession of anisocytic stomata (the stomata and guard cells are surrounded by two larger and one smaller subsidiary cells), a pair-flowered cyme inflorescence, intrusive parietal placentation, unilocular bicarpellate ovaries, capsular fruit and miniscule seeds (Cronquist 1981; Wiehler 1983; Smith *et al*. 1997b; http://www.mobot.org/MOBOT/Research/APweb/welcome.html, accessed on 28 March 2008), although there are many exceptions.

The most recent formal classification of Gesneriaceae, published by Burtt and Wiehler in 1995, recognised three subfamilies: Gesnerioideae, Coronantheroideae (Fritsch) Wiehler and Cyrtandroideae (Jack) Burnett. Members of subfamily **Gesnerioideae** are found in the neotropics, occurring in Mexico, Central America and northern South America (Weber 2004). Burtt and Wiehler (1995) recognised five tribes within Gesnerioideae, namely Gloxinieae (G.Don) G.Don, Episcieae Endl., Beslerieae Bartl., Napeantheae Wiehler and Gesnerieae Dumort. **Coronantheroideae** is also a predominantly New World group, with members occurring in south-western South America, eastern Australia and the south-western Pacific (Weber 2004), and are distinct from other members of Gesneriaceae in that their nectary is embedded in the ovary, and they also tend to possess the highest chromosome counts in the family (Wiehler 1983, Möller & Kiehn 2004). Burtt and Wiehler (1995) placed all taxa within subfamily Coronantheroideae into a single tribe: Coronanthereae Fritsch. Conversely, subfamily **Cyrtandroideae** is a predominantly Old World group, with its member taxa distributed across the tropics (Weber 2004). Taxa within Cyrtandroideae are different from the rest of the family in producing anisocotylous seedlings i.e. seedlings in which the cotyledons grow unequally after germination, and this character was used by Burtt (1963) to delimit this group. Members of this subfamily also lack endosperm in their seeds (Smith *et al*. 1997b). Subfamily Cyrtandroideae, now **Didymocarpoideae** due to priority reasons (Weber 2004), was further subdivided into five tribes: Klugieae Fritsch (now called Epithemateae (Meisn.) C.B.Clarke due to a rearrangement of genera amongst tribes, Burtt 1997), Didymocarpeae, Trichosporeae Nees, Cyrtandreae (Jack) Bartl., and Titanotricheae T.Yamaz. ex W.T.Wang.

Cladistic analyses using morphological (Boggan 1991; Smith 1997, 2000c; Roalson *et al*. 2005b; Clark *et al*. 2006) and molecular (Smith & Carroll 1997; Möller & Cronk 1997, 2001a; Samuel *et al*. 1997; Smith *et al*. 1997a, 1997b, 1998, 2004a, 2004b, 2006; Smith & Atkinson 1998; Smith 2000a, 2000b & 2000c; Zimmer *et al*. 2002; Clark & Zimmer 2003; Mayer *et al*. 2003; Perret *et al*. 2003, 2007; Wang *et al*. 2004; Roalson *et al*. 2005a, 2005b; Clark *et al*. 2006) data have provided valuable additional sources of information, especially regarding relationships amongst the New World taxa (Figure 2.2). These studies have led to adjustments in the taxonomy of Gesneriaceae at all levels by facilitating the identification of those groups that are based on morphological synapomorphies as opposed to those based on plesiomorphies or convergent characters.

Within Gesnerioideae, the historical tribe Sinningieae Fritsch has been resurrected (Smith & Carroll 1997; Smith *et al*. 1997b; Smith & Atkinson 1998; Zimmer *et al*. 2002; Roalson *et al*. 2005b), and a new tribe Sphaerorrhizeae Roalson & Boggan has been added (Zimmer *et al*. 2002; Roalson *et al*. 2005a), bringing the total number of tribes within Gesnerioideae to seven. These two newly added tribes, along with Episcieae emerge as a group (Zimmer *et al*. 2002; Roalson *et al*. 2005b), while Gesnerieae and Gloxinieae consistently group as sister to each other (Zimmer *et al*. 2002; Smith *et al*. 2004a; Roalson *et al*. 2005b). The tribes Beslerieae and Napeantheae share a strong mutual affinity in most analyses (Smith *et al*. 1997b; Smith & Carroll 1997; Smith & Atkinson 1998; Smith 2000b; Zimmer *et al*. 2002; Roalson *et al*. 2005b), and together group sister to the rest of the tribes within Gesnerioideae (Smith & Atkinson 1998; Smith 2000b; Zimmer *et al*. 2002; Roalson *et al*. 2005b). At the generic level, genera have been rearranged amongst tribes, new genera have been described, and other genera have been sunk into synonomy (Perret *et al*. 2003, 2007; Roalson *et al*. 2005a, 2005b; Clark *et al*. 2006). Molecular studies also show that, although the subfamily Coronantheroideae contains some Old World genera, it emerges along with members of the New World Gesnerioideae (Smith 1997;

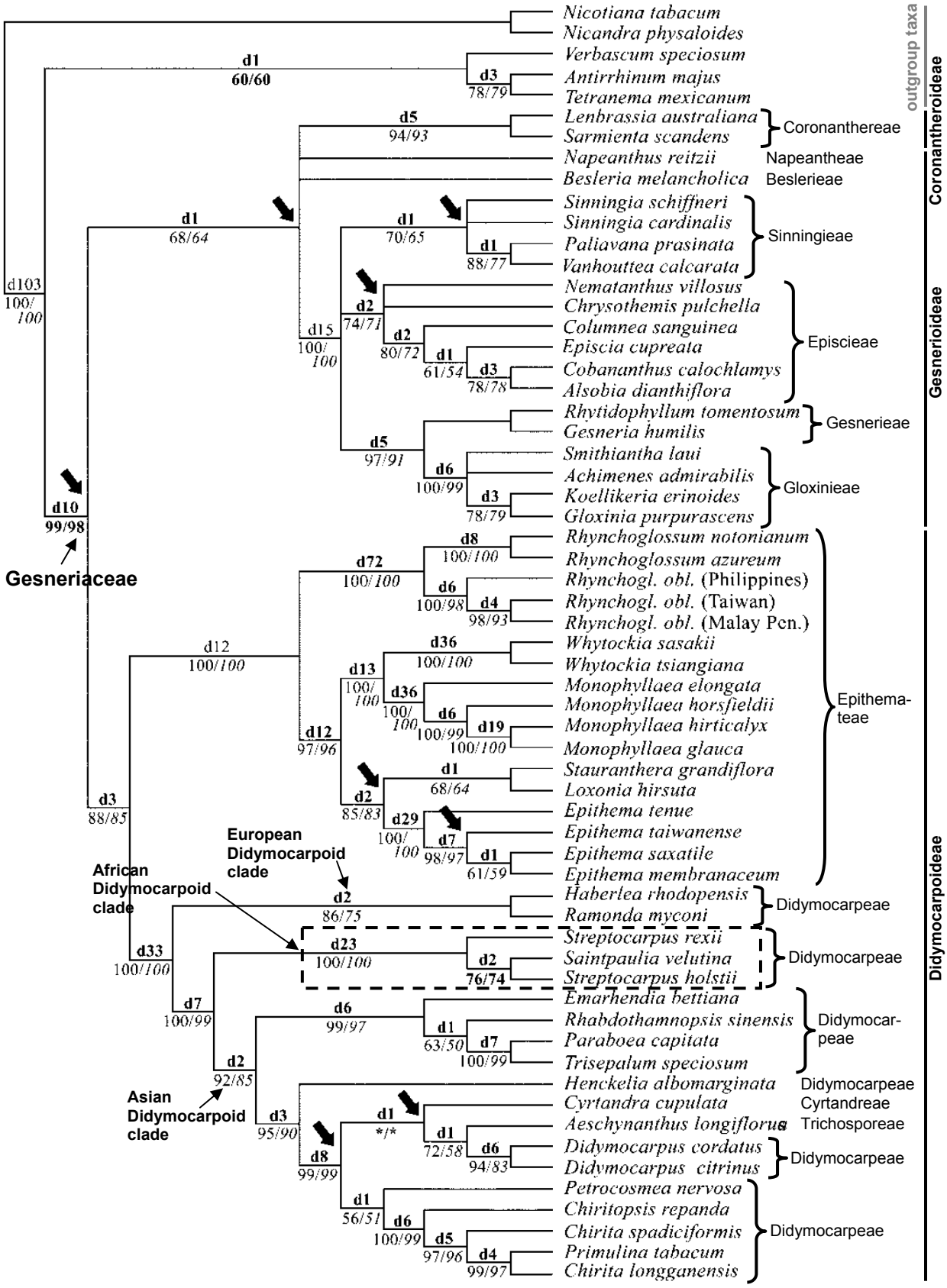

Figure 2.2: The parsimony strict consensus tree from Meyer *et al*. (2003) depicting the relationships within Gesneriaceae reconstructed using the plastid *atp*B-*rbc*L spacer and the *trn*L-F intron-spacer. Decay indices are given above the branches, while support values are given below the branches, bootstrap percentages in normal font to the left and jackknife percentages in italics to the right. The thick arrows indicate nodes that disagreed between the two strict consensus trees when Meyer *et al*. (2003) analysed the *atp*B-*rbc*L spacer and the *trn*L-F intron-

spacer separately. The *Streptocarpus* clade is enclosed in a dashed rectangle. Currently recognised tribes are given to the right, and subfamilies to the far right (Burtt & Wiehler 1995).

Smith *et al*. 1997b; Smith & Carroll 1997; Smith & Atkinson 1998; Mayer *et al*. 2003; Wang *et al*. 2004).

Sister to this New World Coronantheroideae-Gesnerioideae group is the Old World group Didymocarpoideae (Mayer *et al*. 2003; Wang *et al*. 2004). These Old World taxa constitute a notoriously difficult group to classify (Hilliard & Burtt 1971; Luegmayer 1993), and it is consequently not surprising that recent studies have largely refuted the older classification systems, which were based mostly on morphology. Within the Didymocarpoideae clade, members of the morphologically diverse tribe Epithemateae together group sister to the rest of the subfamily (Smith 2000c; Mayer *et al*. 2003; Wang *et al*. 2004). On the other hand, the Didymocarpoideae tribe Didymocarpeae does not emerge as monophyletic entity in the molecular and morphological analyses (Smith 1997; Smith *et al*. 1997b; Mayer *et al*. 2003), and Trichosporeae is possibly also not monophyletic (Smith *et al*. 1997b). Too few representatives of Cyrtandreae have been included in the analyses produced so far to assess its monophyly, and the remaining Didymocarpoideae tribe, Titanotricheae, is currently believed to be more closely related to Gesnerioideae and Coronantheroideae than to Didymocarpoideae (Wang *et al*. 2004). The evolutionary relationships revealed by some of these phylogenies led to the more recent, informal classification of Gesneriaceae by Weber in 2004.

Weber (2004) regarded relationships within Gesneriaceae as still rather uncertain, and therefore divided Gesneriaceae into four informal groups, rather than define formal taxonomic groups that might have to be changed again in the near future as relationships become clearer. These four groups are the **Gesnerioid Gesneriaceae**, which comprises over 50 genera (*ca.* 1 500 species) and holds the members of subfamily Gesnerioideae; the **Coronantheroid Gesneriaceae**, which consists of seven to nine genera (*ca.* 20 species) and contains the members of subfamily Coronantheroideae; the **Epithematoid Gesneriaceae**, which contains seven genera (over 80 species) and encompasses the members of the small Didymocarpoideae tribe Epithemateae; and the **Didymocarpoid Gesneriaceae**, which consists of over 70 genera (*ca.* 1 900 species) and holds the Didymocarpoideae tribes Didymocarpeae, Trichosporeae and Cyrtandreae (Weber (2004) excluded Titanotricheae from this group, believing it to rather belong in Scrophulariaceae). The Epithematoid Gesneriaceae are morphologically distinct within Gesneriaceae in having narrow medullary rays and seed coat cells with verrucate edges (Smith 1997). Members of the group occur mostly in south and south-eastern Asia down to New Guinea, although one species is found in West Africa, and another in Central America (Weber 2004). The Didymocarpoid Gesneriaceae shares a similar but far wider distribution. Its genera are concentrated around three major phytogeographical centres, forming a Eurasian group, a Sundaland group and an African group (Burtt 1998). Its taxa are distributed across south, east and south-east Asia, the Philippines, Indonesia, Polynesia, southern Europe, central and south-eastern Africa, Madagascar and the Comoro Islands (Weber 2004). It is within the Didymocarpoid Gesneriaceae that *Streptocarpus* and its closest relatives emerge (Smith 1997; Smith *et al*. 1997b, 1998, 2004b, 2006; Zimmer *et al*. 2002; Mayer *et al*. 2003; Wang *et al*. 2004; Roalson *et al*. 2005b).

2.2.3. The position of *Streptocarpus* within Gesneriaceae

Streptocarpus is morphologically one of the most diverse groups of plants within the Old World gesneriads. Its members have characteristics in common with both African and Asian taxa, and its position within Didymocarpeae is equivocal. *Streptocarpus* is similar to some of the Asian genera i.e. *Boea* Lam. and most of its allies (*Paraboea* (C.B.Clarke) Ridl., *Trisepalum* C.B.Clarke, *Rhabdothamnopsis* Hemsl., *Ornithoboea* C.B.Clarke, *Kaisupeea* B.L.Burtt,

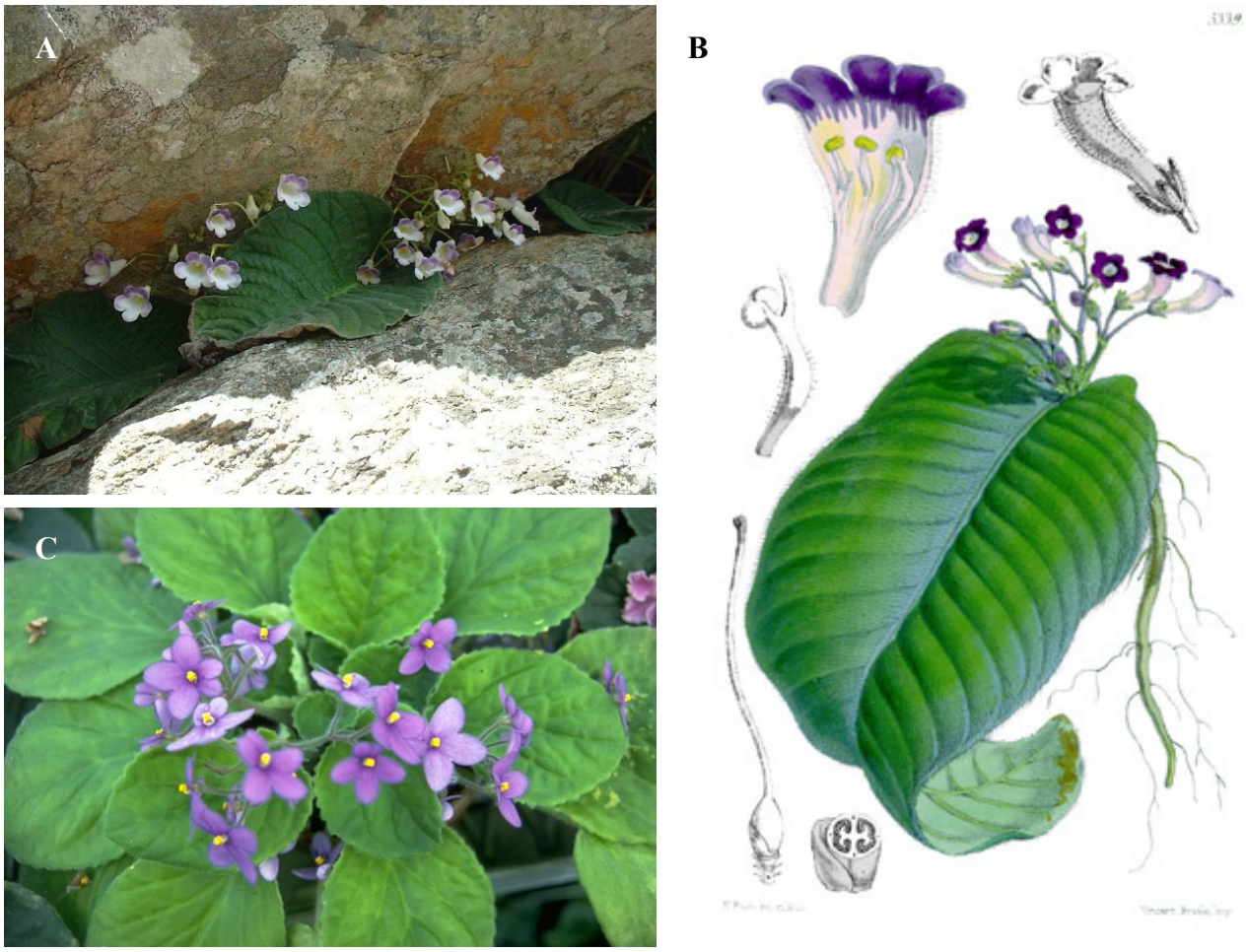

Figure 2.3: Representatives of some of the gesneriad genera occurring on the African continent. **A** a *Streptocarpus* species from Swaziland, **B** *Acanthonema strigosum*, and **C** *Saintpaulia ionantha* subsp. *grandifolia*. The *Saintpaulia* and *Streptocarpus* photographs come from http://www.gesneriads.ca/Default.htm, while the *Acanthonema* picture was copied from http://www.genera-gesneriaceae.at/.

Senyumia Kiew, A.Weber & B.L.Burtt and *Spelaeanthus* Kiew, A.Weber & B.L.Burtt), in that it also produces twisted fruits, a characteristic that it does not share with the other African genera (Weber 2004). On the other hand, these Asian genera together occur in southern, eastern and south-eastern Asia and Melanesia; in other words, far removed from the centre of diversity of *Streptocarpus*. *Streptocarpus* also shares morphological characteristics, in addition to a greater overlap in distribution range, with the African genera. Thus, relationships between *Streptocarpus* and the other African and Asian Didymocarpoid genera are rather complex.

Streptocarpus (Figure 2.3A) and the other gesneriad genera found on the African continent i.e. *Acanthonema* Hook.f. (Figure 2.3B), *Colpogyne* B.L.Burtt, *Epithema* Blume, *Hovanella* A.Weber & B.L.Burtt, *Nodonema* B.L.Burtt, *Saintpaulia* H.Wendl. (Figure 2.3C), *Schizoboea* (Fritsch) B.L.Burtt and *Trachystigma* C.B.Clarke (*Linnaeopsis* Engl. was sunk into *Streptocarpus* by Darbyshire in 2006), together constitute only nine of the over 150 genera, and *ca.* 160 of the *ca.* 3 500 species within Gesneriaceae (Weber 2004). Of these, *Nodonema* (1 species), *Acanthonema* (2 species), *Trachystigma* (1 species), *Schizoboea* (1 species) and *Saintpaulia* (6 species) are together distributed across central Africa, from Nigeria, Cameroon and Gabon in the west to Kenya and Tanzania in the east, while *Colpogyne* (1 species) and *Hovanella* (2 species) are endemic to Madagascar. *Streptocarpus* (160 species) is spread across central and southern Africa, with 115 species occurring on the African mainland, 40 species endemic to Madagascar, and two species occurring on both Madagascar and the Comoros Archipelago (*S. plantagineus* Vatke is only known from the type specimen, which was destroyed). However, an additional four *Streptocarpus* species have distribution ranges in Asia:

S. burmanicus Craib in Myanmar (Burma), *S. orientalis* Craib in Thailand and *S. sumatranus* B.L.Burtt in Sumatra, Indonesia. *Boea clarkeana* Hemsl., found in south-western China, was moved out of the Asian genus *Boea* Comm. ex Lam into *Streptocarpus* by Hilliard & Burtt in 1971 under the name *Streptocarpus clarkeanus* (Hemsl.) Hilliard & B.L.Burtt, but was subsequently placed back into *Boea* by Wang *et al*. in 1990. However, Wang *et al*. (1990) do not justify the replacement (Michael Möller, personal communication), and thus further studies are required to elucidate where this taxon is best placed. *Epithema tenue* C.B.Clarke is the only other Gesneriad species occurring on the African continent. Although *Epithema* (23 species) is a predominantly Asian genus, *Epithema tenue* occurs in Liberia, Cameroon, Gabon and Uganda (Darbyshire 2006; http://botany.si.edu/gesneriaceae/, accessed on 27 February 2008).

On the basis of the twisting of the fruit that occurs in *Streptocarpus* and *Boea* and its allies, Fritsch (1894) grouped these genera into a single tribe, Streptocarpeae Fritsch. However, *Streptocarpus* is very different from *Boea* and its allies in many other respects, which made Burtt (1963) suspect that twisted fruit had evolved several times independently in Gesneriaceae. He therefore dismantled Streptocarpeae, and amalgamated the genera bearing twisted fruits with all of the African, European and most of the Asian genera to form the much larger and more inclusive tribe, Didymocarpeae. He refrained from subdividing Didymocarpeae until relationships within this group became clearer. Subsequent molecular analyses have confirmed that *Boea* and its allies are not closely associated with *Streptocarpus* (Smith *et al*. 1997b; Mayer *et al*. 2003).

Hilliard & Burtt (1971) and previous authors considered the possibility that the African genera and *Streptocarpus* constitute a monophyletic group, but the evidence available to them was ambiguous, and they were unable to reach any firm conclusions. A characteristic shared amongst the predominantly African genera is the possession of only two fertile stamens (although *Acanthonema* produces four stamens, only two are usually fertile [http://www.genera-gesneriaceae.at/, accessed 09 June 2008]). The possession of two fertile stamens is, however, also found in about half of the predominantly Asian genera, while the other Asian genera are predominantly tetrandrous (Hilliard & Burtt 1971). The strongest case for a close affinity between two predominantly African genera was that between *Streptocarpus* and *Saintpaulia*, a link that was suspected after three *Streptocarpus* species were discovered on Madagascar that shared both vegetative and floral similarities with *Saintpaulia* (Humbert 1967). Additionally, *Streptocarpus* and *Saintpaulia* are embryologically similar, with both sharing a unicellular, uninucleate chalazal haustorium that degenerates very quickly, as opposed to the Asian genera *Chirita* and *Boea* in which the chalazal haustorium is large and binucleate (Holmqvist 1964). On the other hand, the other African genera were considered less similar to *Streptocarpus* by Hilliard & Burtt (1971): *Colpogyne* was regarded as too different from *Streptocarpus* to be closely related to it, *Acanthonema* was viewed to have enough of its own unique characters to maintain its generic status separate from *Streptocarpus*, and there was not enough known about *Trachystigma* at the time for inferences to be made regarding its relationships relative to the other genera occurring in Africa. Thus, based on the morphological characters that had been examined at the time, it was difficult to determine the position of *Streptocarpus* relative to the other African genera in Didymocarpoideae.

In comparison, Hilliard & Burtt (1971) were sceptical about the placement of the four Asian species (*S. burmanicus*, *S. orientalis*, *S. sumatranus* and *Boea clarkeana* [then *Streptocarpus clarkeanus*]) in *Streptocarpus*. *S. burmanicus* and *S. orientalis* possess two characteristics which were not found in the rest of *Streptocarpus*. These are the possession of two flaps on the roof of their corolla tube into which their styles fit, and stigmas with an aborted dorsal lobe and a bilobed ventral lobe. These two characteristics are, however, both found in the Asian genus *Chirita*, and their placement in *Streptocarpus* or in *Chirita* is equivocal based on morphological

data. *S. sumatranus* and *B. clarkeana* are both dissimilar to each other and to the rest of *Streptocarpus*. Although Hilliard & Burtt (1971) moved *B. clarkeana* from *Boea* into *Streptocarpus* because it superficially resembles the latter genus, they had their doubts even then. *S. sumatranus* shares a similar calyx structure with *S. daviesii* N.E.Br. ex C.B.Clarke, and a similar stigma with *S. johannis*. It is, on the other hand, very different to these two species and the rest of the genus in many other respects. Thus, these taxa were placed into *Streptocarpus*, not because they were especially similar to the rest of the genus, but because they were isolated entities with uncertain affinities that happen to share the possession of twisted fruit with *Streptocarpus*, a characteristic that is shared with other Asian taxa i.e. *Boea* and its allies.

Recent palynological and molecular studies have shed more light on relationships amongst the African and Asian Gesneriads, showing that the predominantly African taxa share closer affinities than was previously thought. Weigend & Edwards (1996) carried out a palynological investigation of many of the African and Madagascan species (Table 2.1), including all the species in *Trachystigma*, *Schizoboea* and *Hovanella* (labelled as *Didymocarpus madagascariensus* [*sic*] C.B.Clarke and *D. vestitus* Baker in the paper due to this group previously forming a section within the Asian genus *Didymocarpus* Wall.). Also included were one of the two *Acanthonema* species, five of the six current *Saintpaulia* species (in 2006, Darbyshire sank 15 *Saintpaulia* species into subspecies and varieties of *Saintpaulia ionantha* H.Wendl, retaining only six of the previous 21 species) and 131 *Streptocarpus* species (including *Streptocarpus heckmannianus* (Engl.) I.Darbysh., previously classified within *Linnaeopsis* Engl.). Two Asian species were also included, namely the Thai species *Streptocarpus orientalis*, and the south-western Chinese species *Boea clarkeana* (previously *Streptocarpus clarkeanus*). Weigend & Edwards (1996) found that, although the African Gesneriads are palynologically very diverse, their pollen possesses characteristics that indicate a closer relationship with the Asian taxa (Luegmayer 1993) than with the New World taxa (Fritze & Williams 1988). Within the predominantly African Gesneriads, many of the genera appear to be related. *Hovanella* was found to be palynologically indistinguishable from *Streptocarpus*, and the pollen of *Schizoboea* was typical of the pollen found in *Streptocarpus*. Moreover, the pollen of *Trachystigma* and the one *Acanthonema* species investigated were found to be identical, and this pollen type was also found in *Streptocarpus*. *Saintpaulia* also shows a linkage to *Streptocarpus*. Although most of the *Saintpaulia* species possess their own unique pollen types, two of the *Saintpaulia* species included, *S. ionantha* and *S. orbicularis* (now both classified within *S. ionantha*) possess a similar pollen type to that common within *Streptocarpus*, and also share many morphological characteristics with some of the Madagascan *Streptocarpus* species. In contrast to this, Weigend & Edwards (1996) found no palynological evidence of a close relationship between the two Asian *Streptocarpus* species *S. orientalis* and *B. clarkeana* and the other African species. Rather, the pollen of these two Asian species was found to be identical to that of some of the Asian *Chirita* and *Didymocarpus* taxa. *Linnaeopsis* was also found to possess its own pollen type, but has been sunk into *Streptocarpus* by Darbyshire (2006). Thus, all of the current African genera display palynological links to *Streptocarpus*, while the Asian *Streptocarpus* species analysed were found to be very different. Weigend & Edwards (1996) deduced from this that the African and Madagascan taxa probably constitute a monophyletic group within the Old World Gesneriads, separate from the Asian taxa.

Table 2.1: The African gesneriad species examined by Weigend & Edwards (1996), grouped according to their pollen type(s)[1]. Within each pollen type, species outside *Streptocarpus* are listed first, followed by the *Streptocarpus* species arranged according to Hilliard & Burtt's (1971) classification, with members of subgenus *Streptocarpella* given first, followed by the Madagascan and Comorian members of subgenus *Streptocarpus*, and finally the African mainland members of subgenus *Streptocarpus*. The mainland members of subgenus *Streptocarpus* species are arranged according to Hilliard & Burtt's (1971) informal morphological groupings, with the putative hybrid origins of *S. montigena*, *S. johannis* and *S. baudertii* given, as well as the hypothesised closest relative of *S. caeruleus*. In cases where the binomial used by Weigend & Edwards (1996) differs from the currently accepted taxon name, the older name is placed in parentheses. Species analysed by Weigend & Edwards (1996) that were reinstated or raised to specific rank after Hilliard and Burtt (1971) are placed next to the species in which they classified them, and newly described species are placed next to the species with which they are believed to share the closest relationships by their authors if they were unknown to Hilliard and Burtt (1971). Distribution data, obtained from Hilliard & Burtt (1971) and http://persoon.si.edu/Gesneriaceae/, are given in parentheses and are abbreviated as follows: Ang for Angola, Bur for Burundi, Cam for Cameroon, CAR for the Central African Republic, DRC for the Democratic Republic of the Congo, Eth for Ethiopia, Gab for Gabon, Gam for The Gambia, Ken for Kenya, Mad for Madagascar, Mal for Malawi, Moz for Mozambique, Rwa for Rwanda, SA for South Africa, Sud for Sudan, Swa for Swaziland, Tan for Tanzania, Uga for Uganda, Zam for Zambia and Zim for Zimbabwe. Other abbreviations are: agg. for aggregate; all. for alliance, subg. for subgenus and subsp. for subspecies. An * marks groups in which all the members analysed possess the same pollen type.

Pollen type	Taxa
1	**subg. *Streptocarpus*, Group D:** *Streptocarpus daviesii* (SA)
2	**subg. *Streptocarpus*, Group B, Bb:** *Streptocarpus nimbicola* (Mal)
3	*Streptocarpus heckmannianus* (*Linnaeopsis heckmanniana*; Tan)
4	*Acanthonema strigosum* (Gab, Cam); *Boea clarkenana* (*Streptocarpus clarkeanus*; China); *Trachystigma mannii* (Gabon); **subg. *Streptocarpella:*** *Streptocarpus orientalis* (Thailand), *S. campanulatus* (Mad), *S. glabrifolius* (Mad), *S. papangae* (Mad) and *S. suffruticosus* (Mad); **subg. *Streptocarpus*, Group A, Aa:** *S. wendlandii* (SA); **Group B, Bb:** *S. pogonites* (SA); **Bc:** *S. micranthus* (SA); *S. baudertii* (**the *S. meyeri* all. × D**; SA)
5	**subg. *Streptocarpus*, Group A, Ae:** *Streptocarpus trabeculatus* (SA)
6	**subg. *Streptocarpella:*** *Streptocarpus euanthus* (Tan), *S. bambuseti* (Tan), *S. kirkii* (Tan, Ken), *S. saxorum* (Tan, Ken), *S. hirsutissimus* (Tan), *S. holstii* (Tan) and *S. inflatus* (Tan)
7	**subg. *Streptocarpella:*** *Streptocarpus pallidiflorus* (Tan), *S. glandulosissimus* (DRC, Rwa, Bur, Uga, Tan, Ken) and *S. nobilis* (from Gam, through Cam to the CAR)
8	**subg. *Streptocarpus*:** *Streptocarpus johannis* (**the *S. rexii* agg. × D**; SA); **Group D:** *S. polyanthus* subsp. *polyanthus* (SA), *S. polyanthus* subsp. *verecundus* (SA), *S. polyanthus* subsp. *dracomontanus* (SA), *S. polyanthus* subsp. *comptonii* (SA), *S. prolixus* (SA), *S. silvaticus* (SA) and *S. haygarthii* (SA)
9	**subg. *Streptocarpus*, B, Ba:** *Streptocarpus pole-evansii* (SA) and *S. dunnii* (SA)
10	**subg. *Streptocarpus*, Group A, Ac*:** *Streptocarpus goetzei* (Mal, Moz, Tan) and *S. compressus* (Tan); **Ae:** *S. kungwensis* (Tan); *S. bindseilii* (Rwa)
11	*Hovanella madagascarica* (*Didymocarpus madagascariensis*; Mad) and *H. vestita* (*D. vestitus*; Mad); *Schizoboea kamerunensis* (Uga, Tan); **subg. *Streptocarpella:*** *Streptocarpus thysanotus* (Tan); **subg. *Streptocarpus*, Madagascan:** *S. tsimihetorum*; **subg. *Streptocarpus*, Group B, Bb:** *S. montanus* (Tan, Ken), *S. milanijianus* (Mal) and *S. hirtinervis* (Mal); **Bc:** *S. brachynema* (Moz); **Bf*:** *S. davyi* (SA), *S. pusillus* (SA) and *S. rimicola* (SA); **Bh*:** *S. cyanandrus* (Zim), *S. pumilus* (Zim) and *S. hirticapsa* (Zim); **Bi*:** *S. bolusii* (SA), *S. latens* (SA), *S. leptopus* (Mal, Moz) and *S. rhodesianus* (Ang, Zam, DRC)
12	**subg. *Streptocarpella:*** *Streptocarpus schliebenii* (Tan), *S. stomandrus* (Tan), *S. kimbozanus* (Tan), *S. elongatus* (Cam, São Tomé Island, Sud), *S. hilsenbergii* (Mad), *S. thompsonii* (Mad, the Comoro Islands), *S. muscosus* (Mad), *S. leandrii* (Mad) and *S. andohahelensis* (Mad); **subg. *Streptocarpus*, Madagascan:** *S. lokohensis*, *S. itremensis*, *S. mangindranensis*, *S. velutinus*, *S. perrieri* and *S. tsimihetorum*; **subg. *Streptocarpus*, Group A, Aa:** *S. porphyrostachys* (SA), *S. molweniensis* (SA) and *S. saundersii* (SA); **Ab (the**

[1] Although *Saintpaulia difficilis*, *S. goetzeana*, *S. grandifolia*, *S. pendula*, *S. pusilla*, *S. shumensis*, *S. teitensis*, *S. tongwensis*, *S. velutina*, *Streptocarpus beampingaratrensis*, *S. caulescence*, *S. dolichanthus*, *S. erubescens*, *S. fasciatus*, *S. galpinii*, *S. hildebrandtii*, *S. integrifolius*, *S. longiflorus*, *S. pentherianus*, *S. polyphyllus* and *S. stellulifer* were also included in Weigend & Edwards' (1996) list of species analysed, the paper does not give their pollen type, and these taxa are therefore not included in this table.

	***S. cooperi* agg.):** *S. cooperi* (SA), *S. michelmorei* (Mal, Moz, Zam, Zim) and *S. solenanthus* (Mal, Tan, Zam, Zim); **Ad (the *S. monophyllus* agg.):** *S. monophyllus* (Ang), *S. arcuatus* (Mal) and *S. vandeleurii* (SA); **Group B, Bc:** *S. umtaliensis* (Zim) and *S. bullatus* (Tan); **Bd*:** *S. burundianus* (Bur) and *S. masisensis* (DRC); **Bg*:** *S. exsertus* (Ken); *S. burttianus* (Tan)
13	**subg. *Streptocarpus*, Madagascan:** *S. ibityensis*; **Madagascan and Comoroian:** *S. variabilis*; **subg. *Streptocarpus*, Group A, Ab (the *S. cooperi* agg.):** *Streptocarpus grandis* (SA, Zim); **Ae:** *S. cooksonii* (SA); **Group B, Ba:** *S. denticulatus* (SA); **Be*:** *S. fanniniae* (SA), *S. candidus* (SA) and *S. wilmsii* (SA); **the *S. meyeri* all.*:** *S. meyeri* (SA), *S. kentaniensis* (SA) and *S. modestus* (SA); *S. montigena* (***S. meyeri* × *S. rexii***; SA); *S. caeruleus* (**distantly related to *S. cyaneus***; SA); **Group C*, the *S. rexii* agg.*:** *S. rexii* (SA), *S. primulifolius* (SA), *S. formosus* (SA), *S. floribundus* (SA), *S. cyaneus* subsp. *cyaneus* (SA, Swa), *S. cyaneus* subsp. *polackii* (SA), *S. cyaneus* subsp. *longi-tommi* (SA), *S. cyaneus* subsp. *nigridens* (SA), *S. fenestra-dei* (SA), *S. roseo-albus* (SA), *S. parviflorus* subsp. *parviflorus* (SA) and *S. parviflorus* subsp. *soutpansbergensis* (SA); **C:** *S. gardenii* (SA)
14	*Saintpaulia ionantha* (Tan) and *S. orbicularis* (Tan)
15	**subg. *Streptocarpella:*** *Streptocarpus tsaratananensis* (Mad) and *S. macropodus* (Mad)
16	**subg. *Streptocarpus*, Group A, Ad (the *S. monophyllus* agg.):** *Streptocarpus eylesii* subsp. *eylesii* (Mal, Moz, Zam, Zim), *S. eylesii* subsp. *brevistylis* (Mal) and *S. wittei* (DRC, Mal, Zam)

In 1997, Möller & Cronk carried out a molecular analysis to investigate the relationship between *Streptocarpus* and *Saintpaulia* more closely (Figure 2.4). They sequenced the ITS region of 19 species of *Streptocarpus* and eight *Saintpaulia* species (but see Darbyshire 2006). The *Streptocarpus* taxa emerged in two sister clades, with the species from subgenus *Streptocarpus* (occurring from Malawi down to South Africa) grouping sister to the subgenus *Streptocarpella* species (found in Madagascar, Tanzania and Kenya). Within the subgenus *Streptocarpella* clade, the *Saintpaulia* taxa emerged as a monophyletic group sister to the eastern central African *Streptocarpus* taxa (Tanzanian and Kenyan), which together grouped sister to the Madagascan *Streptocarpus* species. An analysis of the nuclear floral symmetry gene *CYCLOIDEA* by Smith *et al*. (2004b) also resulted in *Saintpaulia* representatives emerging within *Streptocarpus*. *Saintpaulia* samples also emerged within *Streptocarpus* in analyses using plastid *ndh*F sequences (Smith *et al*. 1997b, 1998) and plastid *rbc*L/*atp*B-spacer and *trn*L-F intron-spacer sequences (Mayer *et al*. 2003). The molecular evidence is supported by karyological evidence: subgenus *Streptocarpella* species (with the exception of *S. papangae*, *S. schliebenii* Mansf. and *S. suffruticosus* Humbert) share a basic chromosome number of x = 15 with *Saintpaulia*, a basic chromosome number that is not shared with subgenus *Streptocarpus* species (Jong & Möller

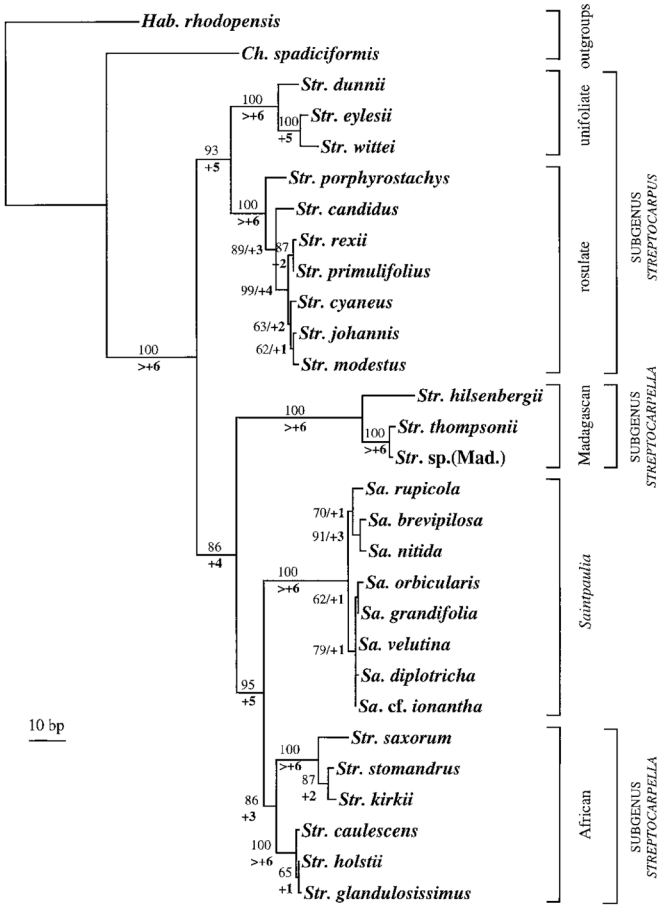

Figure 2.4: The single most parsimonious tree from Möller & Cronk (1997) based on ITS sequence data and built using the parsimony algorithm showing reconstructed relationships within *Streptocarpus* and the position of *Saintpaulia* within *Streptocapus* subgenus *Streptocarpella*. Bootstrap values are above the branches, and decay indices are below the branches. Genus names are abbreviated as follows: *Ch*. for *Chirita*, *Hab*. for *Haberlea*, *Sa*. for *Saintpaulia*, and *Str*. for *Streptocarpus*. The classification of the included samples, as well as the distribution of members of subgenus *Streptocarpella* and the growth forms of members of subgenus *Streptocarpus*, are given to the right.

2000; Möller & Cronk 2001a). The two genera also have palynological and embryological characteristics in common, as discussed previously.

Möller & Cronk's (2001a) subsequent molecular analysis of numerous African taxa was not only in agreement with these findings, but also shed more light on relationships amongst *Streptocarpus* and other African Gesneriaceae genera (Figure 2.5). They expanded their ITS data set to include 77 species from across *Streptocarpus* (representing a little over half of the species in the genus), four *Saintpaulia* samples representing two of the current six *Saintpaulia* species, two samples from the former genus *Linnaeopsis* (now sunk into *Streptocarpus*), and one each from *Haberlea*, *Didymocarpus*, *Chirita* and *Schizoboea*. *Haberlea* (a European genus), *Didymocarpus* and *Chirita* (both Asian genera) were inserted as outgroup taxa, and consequently emerged outside the ingroup clade. Just as in Möller & Cronk (1997), the *Streptocarpus* taxa emerged in two main sister clades, although these two clades were not as geographically distinct from each other as in Möller & Cronk (1997). However, as in Möller & Cronk (1997), all the representatives of the other African genera (*Saintpaulia*, *Linnaeopsis* [which is now part of *Streptocarpus*] and *Schizoboea*) emerged as coherent groups nested within the *Streptocarpus* clade. Möller & Cronk (2001a) also generated nuclear (ITS) and plastid (*trn*L-F) sequence data from the Asian *Streptocarpus* species *S. orientalis*. Its sequences were, however, so divergent from the other African taxa, and more similar to Asian taxa, that this sample was not included in the final analysis. This study therefore indicated that the Asian *Streptocarpus* species *S. orientalis* was only distantly related to the rest of the genus, but that *Saintpaulia*, the former *Linnaeopsis* and *Schizoboea* evolved from within *Streptocarpus*. Based on nuclear (ITS) and chloroplast (*trn*LF) data the Madagascan *Hovanella* and *Colpogyne* were also found nested within the genus *Streptocarpus*, the former in subgenus *Streptocarpella*, the latter in subgenus *Streptocarpus* (Möller 2003), which is in line with the basic chromosome number in *Colpogyne* of x = 16 (Jong & Möller 2000). *Hovanella madagascarica* was counted with x = 14 (Kiehn *et al*. 1998), a unique number in *Streptocarpus*, but this count awaits confirmation. *Acanthonema* also resides within *Streptocarpus*, but with as yet uncertain affiliation (Möller, unpublished). Thus only *Nodonema* and *Trachystigma* have not been investigated molecularly.

A morphological characteristic shared by many of the African gesneriads, including *Schizoboea*, *Hovanella*, *Saintpaulia*, the former *Linnaeopsis* and most of *Streptocarpus* subgenus *Streptocarpella* and some of *Streptocarpus* subgenus *Streptocarpus* is the possession of verruculose seeds, with each seed coat cell producing a dome-shaped protrusion in its centre (Beaufort-Murphy 1983). In contrast, most species of subgenus *Streptocarpus* tend to produce reticulate seeds, a characteristic that probably evolved amongst the more derived taxa of subgenus *Streptocarpus*. *Hovanella* and *Trachystigma* are linked by the possession of similar fruit structures. The horizontally held fruit opens only along its upper surface, forming a type of rain-splash capsule that enables the seeds to be washed away by the rain (Weber & Burtt 1998).

Thus, the African mainland (*Acanthonema*, *Saintpaulia*, *Schizoboea* and *Trachystigma*) and Madagascan (*Colpogyne* and *Hovanella*) genera that have been sampled so far have all been found to emerge within *Streptocarpus*, indicating that the African genera of subfamily Didymocarpoideae probably constitute a monophyletic assemblage. In contrast, the Asian species classified as *Streptocarpus* by Hillard & Burtt (1971) probably do not belong to the *Streptocarpus* lineage, and are better placed with the other Asian Didymocarpoid taxa.

Morphological and molecular analyses including more representatives from across Didymocarpoideae have revealed the position of the African genera within the Didymocarpoid Gesneriaceae. Smith *et al*. (1997b) and Smith (2000c) included genera from across Didymocarpoideae in their analyses, and provided preliminary evidence from cladistic analyses of relationships within this group. The most recent molecular analysis was done by Mayer *et al*.

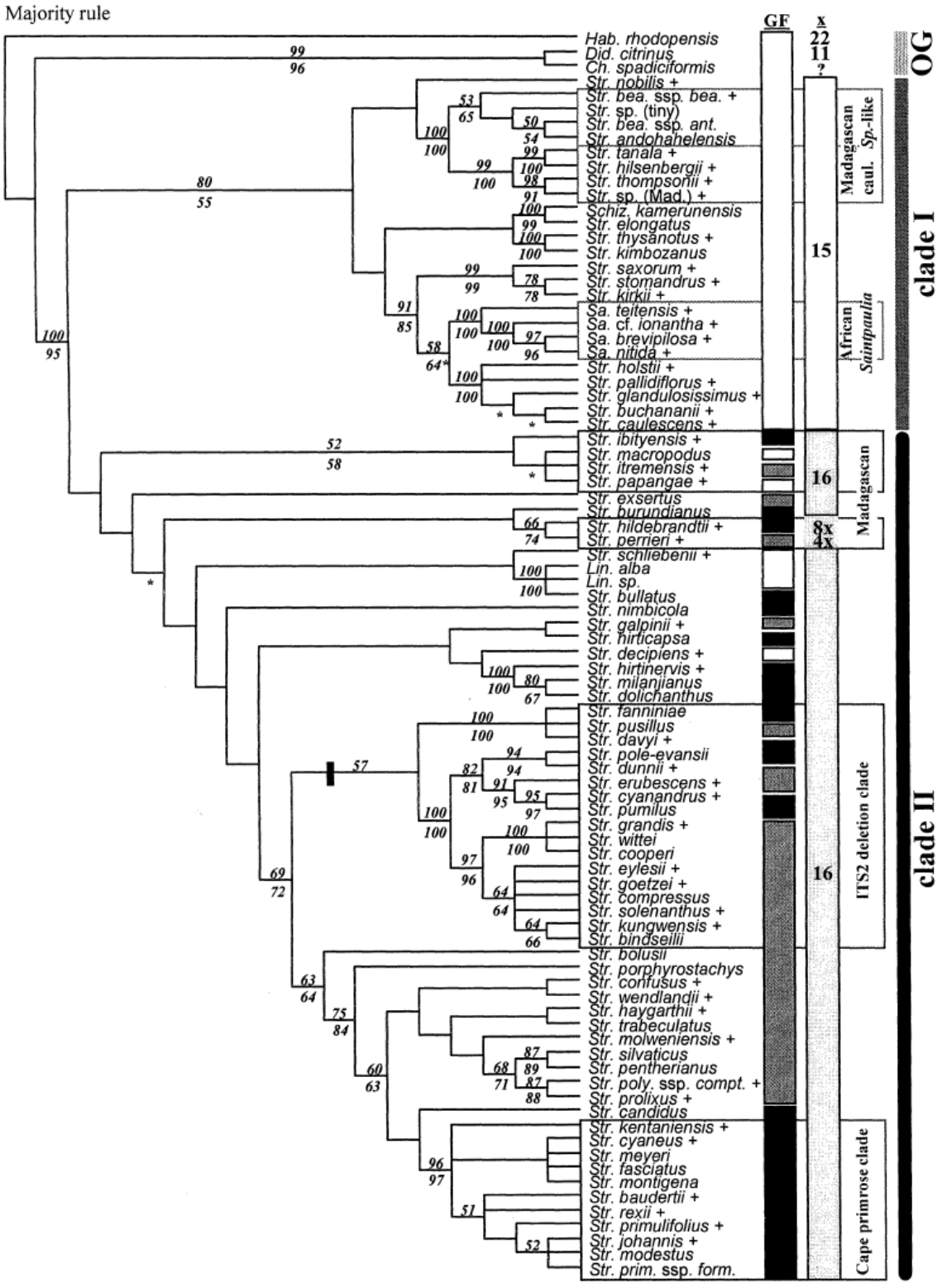

Figure 2.5: Parsimony majority-rule consensus tree from Möller & Cronk (2001a) showing relationships within *Streptocarpus* reconstructed using sequences from the nuclear ITS region. Numbers above the branches indicate bootstrap support, whereas numbers below the branches give jackknife support. GF gives the growth form of the species: white indicates caulescent taxa, gray unifoliate and plurifoliate taxa, and black rosulate taxa; x gives the basic chromosome number (or ploidy level in the case of *S. hildebrandtii* and *S. perrieri*) for the species for which counts have been made (marked by a + sign); the black vertical bar in the tree denotes a shared *ca*. 40 bp deletion; OG indicates the outgroup taxa; * signifies nodes that collapsed in the strict consensus tree. Genus abbreviations are as follows: *Ch*. for *Chirita*, *Did*. for *Didymocarpus*, *Hab*. for *Haberlea*, *Lin*. for *Linnaeopsis*, *Sa*. for *Saintpaulia*, *Schiz*. for *Schizoboea*, and *Str*. for *Streptocarpus*. The Cape Primrose clade is in a box in the bottom right-hand corner.

in 2003 (Figure 2.2). Although this analysis was primarily conducted to investigate the Didymocarpoideae tribe Epithemateae, Mayer *et al*. (2003) also included multiple samples from across the rest of Didymocarpoideae. These Didymocarpoid samples formed a monophyletic clade, but relationships within this clade appear to reflect geography more closely than tribal boundaries. The Asian genera emerged in a monophyletic group, with those genera producing straight capsular fruits (*Henckelia*, *Aeschynanthus*, *Didymocarpus*, *Petrocosmea*, *Chiritopsis*, *Chirita* and *Primulina*) and those with indehiscent fruit (*Cyrtandra*) together emerging sister to those with twisted capsular fruits and their allies (*Emarhendia*, *Rhabdothamnopsis*, *Paraboea* and *Trisepalum*). Sister to this Asian clade was a monophyletic group containing the two African genera included in the analysis i.e. *Streptocarpus* and *Saintpaulia*. This Asiatic-African clade grouped sister to the two European genera added (*Haberlea* and *Ramonda*). The patterns uncovered in Smith *et al*. (1997b) and Smith (2000c) roughly agree with those found in Mayer *et al*. (2003).

Thus, the comparison of data from several independent sources has helped to elucidate relationships amongst the African genera. Holmqvist (1964), Skog (1984), Weigend & Edwards (1996), Möller & Cronk (1997, 2001a), Smith *et al*. (1997, 1998), Mayer *et al*. (2003) and Smith *et al*. (2004b) not only indicate that *Streptocarpus* forms a paraphyletic group with *Saintpaulia* nested within it, but also furnish information concerning the affiliations of *Saintpaulia* within *Streptocarpus* i.e. that its closest linkages lie with the *Streptocarpus* species occupying the same areas (east-central Africa) rather than with the Madagascan *Streptocarpus* species. The palynological work of Weigend & Edwards (1996) and the expanded analysis of Möller & Cronk (2001a) additionally show that the Asian species *S. orientalis* and *B. clarkeana* are probably unrelated to the rest of *Streptocarpus*, but that the other African genera i.e. *Schizoboea*, *Hovanella*, *Trachystigma*, *Acanthonema* and *Linnaeopsis*, probably evolved from progenitors of the central African *Streptocarpus* species. Darbyshire (2006) has subsequently sunk *Linnaeopsis* into *Streptocarpus*. Smith *et al*. (1997b), Smith (2000c) and Mayer *et al*. (2003) pinpointed the position of the African genera within Didymocarpoideae using *Streptocarpus* and *Saintpaulia* as representatives. These studies found that African genera form a monophyletic group sister to most of the Asian genera, including those with twisted fruit, with the European genera sister to this combined Africa-Asia clade.

2.3. *Streptocarpus*

Streptocarpus is one of the larger genera within Gesneriaceae, currently containing about 160 species. It is surpassed only by *Cyrtandra* Forst. (644 species), *Columnea* L. (197 species), *Aeschynanthus* Jack (184 species) and *Chirita* Buch.-Ham. (180 species), while *Henckelia* Spreng. (155 species) and *Besleria* L. (153 species) have similar species numbers (http://botany.si.edu/gesneriaceae/, accessed on 12 May 2008; Darbyshire 2006). The genus extends across the eastern half and tropical areas on the African mainland, and on to some of the Comoro Islands and Madagascar (Figure 2.6). The Asian *Streptocarpus* species have been dealt with earlier and are not likely related to the African-Madagascan lineage.

S. rexii, the species with the most southern distribution in *Streptocarpus*, extending from the Knysna vicinity in the south in a broad band across the Eastern Cape to the Kokstad vicinity in the north, was also the first species of the genus to be discovered and described, and is consequently the type species of the genus. It was first found near Knysna in 1818 by James Bowie, a plant collector from Kew, and was named after George Rex, the founder of Knysna who also played a key role in the town's initial development (Figure 2.7). Hooker (1827) originally placed the species into the Asian genus *Didymocarpus*, but it was removed to a genus of its own, *Streptocarpus*, soon thereafter by Lindley (1828). By the time Clarke carried out a revision in 1883, a number of additional taxa had been discovered, which he divided into 17

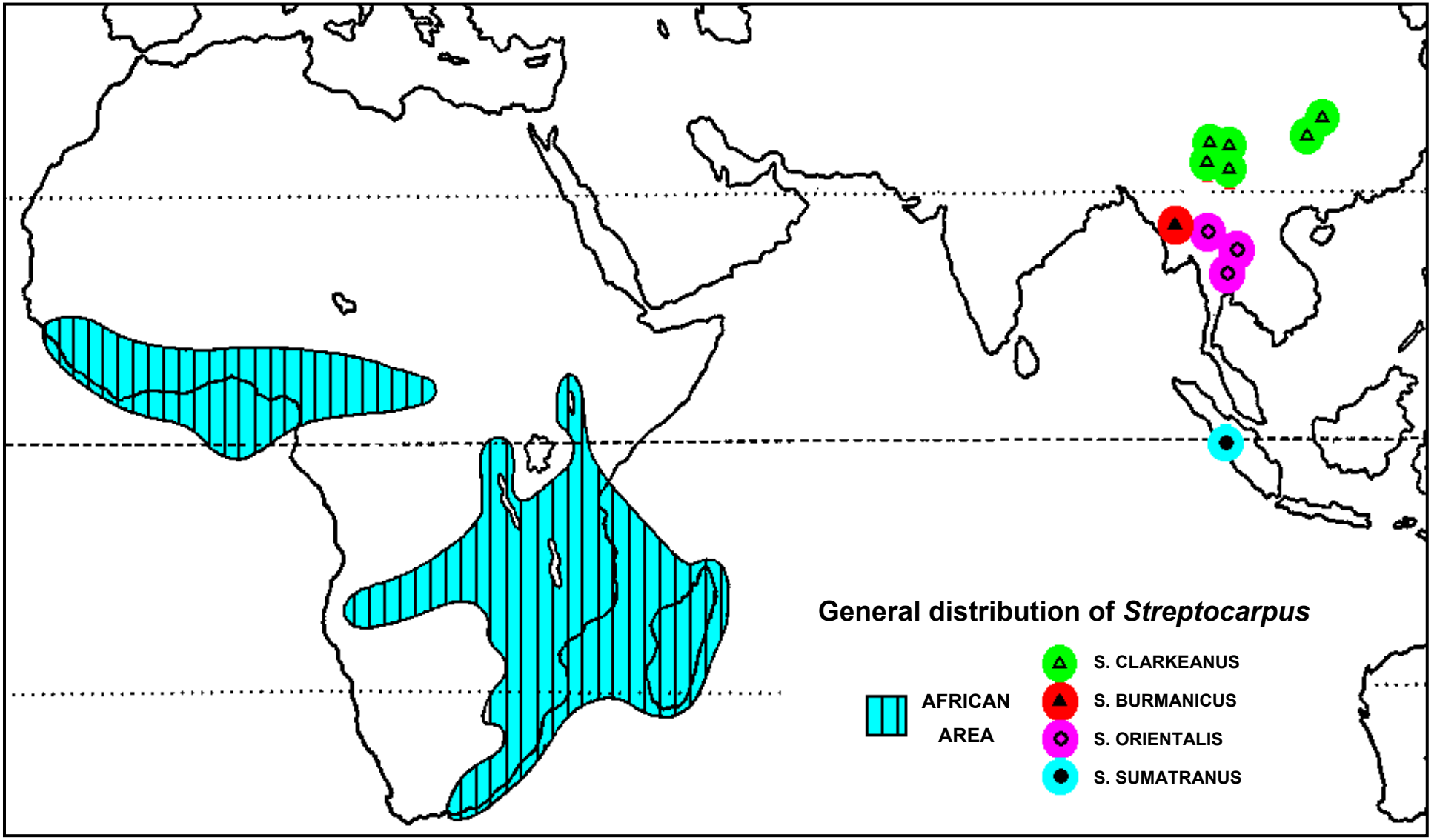

Figure 2.6: Distribution of *Streptocarpus*, with the localities of the four Asian species placed in the genus by Hilliard & Burtt (1971) also indicated. Map adapted from Hilliard & Burtt (1971).

Figure 2.7: Photograph taken by Dirk Bellstedt at the Royal Botanic Garden Edinburgh of the original *S. rexii* herbarium specimen, the first and type specimen of *Streptocarpus*. The specimen was made from an *S. rexii* plant growing at Kew that had been collected by the Kew plant collector, James Bowie (comments by Mr Bill Burtt).

species. Fritsch subsequently divided the species between two subgenera, *Streptocarpus* subgenus *Streptocarpus* and *Streptocarpus* subgenus *Streptocarpella* in his 1894 account of the whole family. The most recent revision of the genus was completed in 1971 by Hilliard and Burtt, in which they recognised 132 species.

Streptocarpus displays a number of unusual characteristics amongst the Old World gesneriads. A distinctive morphological characteristic, and the one giving the genus its name, is its twisted fruit capsules. The twisting takes place as the fruit matures after pollination and fertilization, and appears to enable the vast number of seeds to be released slowly over long periods rather than all at once. Most *Streptocarpus* species in subgenus *Streptocarpella* possess a basic chromosome number (x = 15) that is relatively rare in Gesneriaceae (see also 'WebCyte' at http://elmer.rbge.org.uk/webcyte/webcyteintro.php), but that is also found in *Saintpaulia* and many Asian *Aeschynanthus* (Rashid *et al*. 2001). Another characteristic of interest within *Streptocarpus* is the presence of the unifoliate growth form in some of its species, in which the macrocotyledon develops into the only foliage organ that the plant will possess. In spite of this, this growth habit is also not restricted to *Streptocarpus*, occurring in *Monophyllaea*, *Acanthonema* and *Trachystigma* as well. A characteristic that is unique to African *Streptocarpus* in subgenus *Streptocarpus* is the formation of abscission lines across the lamina i.e. the ability to abscise the distal part of their lamina during unfavourable periods of the year. This has possibly evolved in response to the seasonal environments in which these species grow, and has not been recorded anywhere else in Gesneriaceae (http://www.gesneriads.ca/default.htm, accessed 07 April 2008). Thus, as is the case with most plant groups, no single, unique characteristic unites the whole of *Streptocarpus*; rather the genus is distinguished from the rest of Gesneriaceae by a suit of morphological characters.

Members of *Streptocarpus* are predominantly herbs, rarely shrubs (as in Madagascan caulescent species), of variable growth forms bearing opposite leaves with determinate growth in the caulescent growth forms (subgenus *Streptocarpella*), or leaves arranged in a rosette or solitary with a basal meristem that grows longer in the acaulescent taxa (subgenus *Streptocarpus*). Their pedunculate cymes are axillary, either being borne from the base of the lamina or from the petiolode (leaf stalk). The sepals are usually free at the base. The corolla is typically noticeably zygomorphic, but otherwise variable in form. There are only two stamens (the anterior pair), and their anthers usually adhere to each other face to face (except in the woody Madagascan species such as *S. papangae* and *S. suffruticosus*). Nevertheless, lateral staminodes are usually also present, with the posterior one usually missing. The nectary is either annular or shortly cupular, and the ovary is ovoid to cylindrical. The ovules are restricted to the recurved tip of the placentae. The fruit is a twisted cylindrical capsule, and the seeds are numerous and small, with no or very little endosperm, and either have a reticulate or verruculose seed coat (Hilliard and Burtt 1971; Weber 2004). Some of these characteristics will be discussed in more detail below.

2.3.1. Vegetative morphology within *Streptocarpus*

The ontogeny and morphology of the members of the genus *Streptocarpus* (and of some of the Didymocarpoid Gesneriaceae) are rather unusual among the angiosperms. After germination, the two cotyledons are equal in size, but soon one starts growing larger than the other one, a phenomenon known as **anisocotyly** (Jong 1970). The **macrocotyledon** continues to grow from a **basal meristem** into a mature foliage leaf, while the other one, the **microcotyledon**, does not develop further and eventually withers away. The cotyledons become spatially separated from the roots by a short axis, the hypocotyl, and, in some species, the macrocotyledon becomes vertically raised above the microcotyledon through the development of a mesocotyl, the part of the axis between the two cotyledons (Hilliard & Burtt 1971), which later become the **petiolode**, a structure with a dual function of petiole and stem (Jong 1970). Anisocotyly is genetically fixed. By contrast, which cotyledon will develop into the macrocotyledon appears to be

determined by environmental stimuli (Saueregger & Weber 2004). Tsukaya (1997), working with *Monophyllaea*, another anisocotylous Gesneriaceae genus, found that each cotyledon has a meristematic region at its base, the basal meristem, and that both consequently have the potential for continuous growth after germination. This was elegantly demonstrated by the exogenous application of cytokinin that removes the inhibitory block imposed by the macrocotyledon over the microcotyledon and results in seedlings forming two macrocotyledons (Rosenblum & Basile 1984; Mantegazza *et al*. 2007). Saueregger & Weber (2004) discovered that it is the relative exposure of the cotyledons to light that determines which of the two cotyledons would develop into the macrocotyledon. By exposing *S. rexii* seedlings to light from various angles, they were able to show that the cotyledon receiving the most light is the one that expands into an adult foliage leaf. Exposure to light provides a physiological advantage in that light stimulates photosynthesis, growth and the synthesis of plant hormones, possibly including those that suppress the growth of the microcotyledon. This suppression ultimately leads to anisocotyly (Saueregger & Weber 2004; Mantegazza *et al*. 2007). Both Tsukaya (1997) and Saueregger & Weber (2004) concluded that gravity has no influence on determining which cotyledon becomes the macrocotyledon, but that it may affect the orientation of the macrocotyledon in some Gesneriaceae plants with long hypocotyls, such as *Monophyllaea* or *Chirita*.

While the early development of the seedling is more or less uniform across the genus, *Streptocarpus* plants can assume a variety of growth forms as they mature (Figure 2.8 depicts some of these). The growth forms on the African mainland can be classified into three main categories—caulescent, unifoliate and rosulate—although there are many variations on the theme. The **caulescent** taxa (Figure 2.8A) have the most "conventional" growth habit in the genus. Growth of the macrocotyledon in the caulescent taxa is determinate due to the cessation of activity of its basal meristem during development. Following the enlargement of the macrocotyledon, the shoot apical meristem (SAM) arises between the cotyledons in the seedling (Imaichi *et al*. 2007) and results in the growth of an elongated stem with opposite decussate leaves (leaves borne in opposite pairs, with successive pairs at right angles to each other) and axillary inflorescences (Hilliard & Burtt 1971). Thus, a conventional central post-embryonic SAM develops culminating in a plant with a main stem and leaves (Mantegazza *et al*. 2007). The macrocotyledon usually develops into a petiolate leaf at the base of the stem, sometimes becoming the largest leaf on the mature plant (Hilliard & Burtt 1971).

The other two main growth forms, the unifoliates and rosulates, do not develop normal SAMs, and have therefore been termed **acaulescent**. The **unifoliates** (Figure 2.8B) constitute the group of plants with the most reduced vegetative architecture in the genus. Growth in the macrocotyledon of the seedling in the unifoliates continues from the basal region of the cotyledon until the induction of the inflorescence meristem to form the sole foliar organ that the plant will possess (Hilliard & Burtt 1971), and can reach a length of 75 cm and more in some species (Möller & Cronk 2001a). The hypocotyl separates the cotyledons from the radicle, and in due course thickens to form a stalk linking the cotyledons to the mass of adventitious roots that collectively constitute the root system of the plant. As the plant matures, inflorescence buds start to form at the base of the lamina (Hilliard & Burtt 1971). The structure containing the lamina, the petiole (situated between the lamina and the inflorescence(s)), when present, and the stalk below the inflorescence are collectively referred to as the **phyllomorph** (Jong 1970), while the stalk below the inflorescence alone is termed the **petiolode** (Jong 1970). Thus, the first inflorescence meristem forms near the base of the lamina, followed by a succession of additional acropetal inflorescence stalks that arise progressively up the petiole and midrib. Flowering and fruiting is followed by the death of the entire plant. Plants that flower and fruit only once are termed **monocarpic**, irrespective of their lifespan (phyllomorphs often take more than one flowering season to reach maturity and flower e.g. *S. trabeculatus* Hilliard takes more

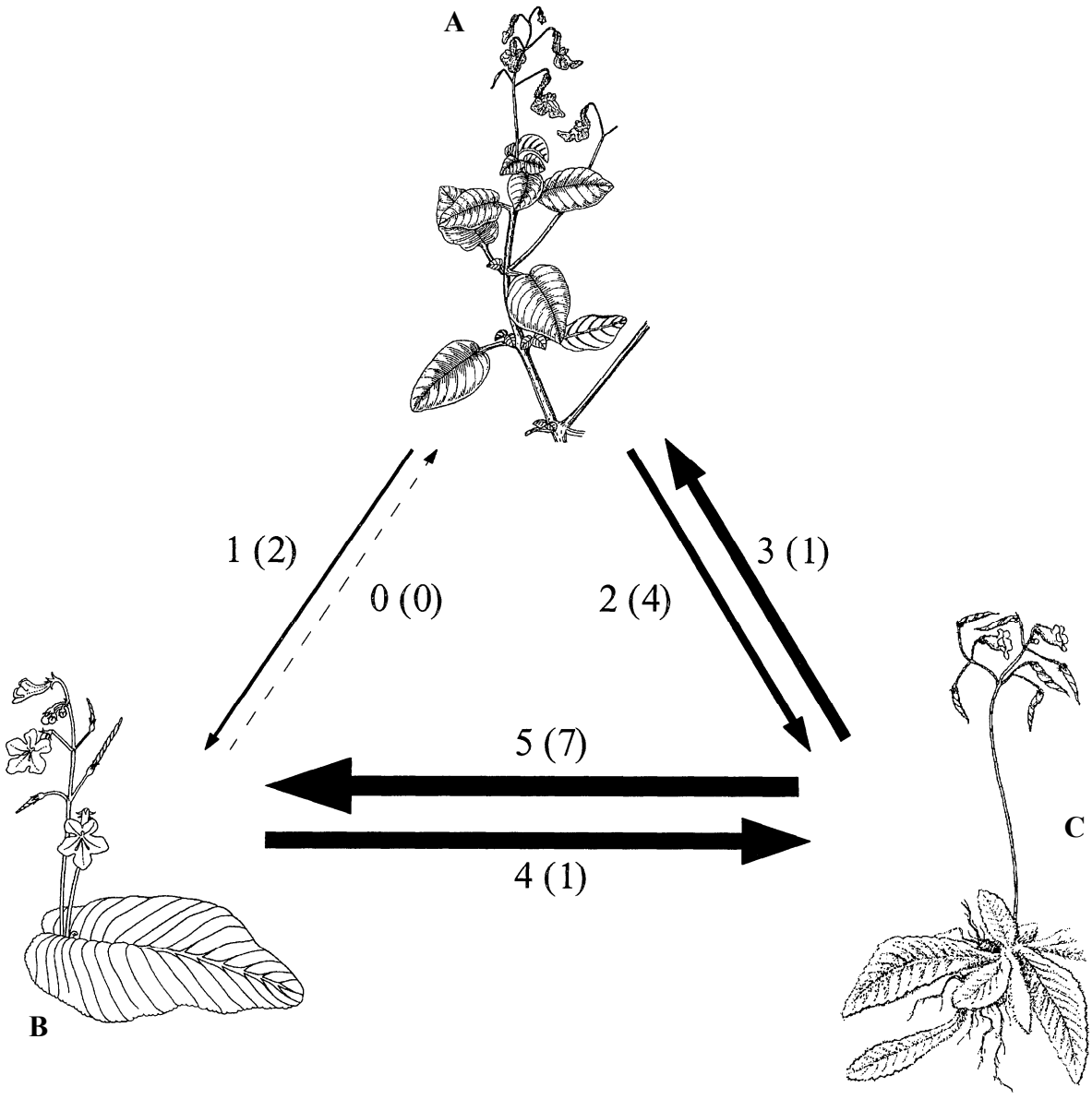

Figure 2.8: Diagram from Möller & Cronk (2001a) depicting three of the main growth forms found in *Streptocarpus* (**A** caulescent, **B** unifoliate, but also representing the plurifoliates, and **C** rosulate), and the estimated number and direction of transitions between them. This was determined by parsimony character optimizations performed on the genus-wide ITS tree in Figure 2.5 by Möller & Cronk (2001a). The width of the arrows linking the growth forms is proportional to the frequency of transitions between them. The numbers on the left give the number of transitions estimated using the ACCTRAN optimization, and the numbers in parentheses to the right give the number of transitions enforcing the DELTRAN optimization.

than four years, Hilliard & Burtt 1971). About 30 species, including *S. polyanthus*, *S. porphyrostachys*, *S. bolusii*, *S. dunnii*, *S. rimicola*, *S. saundersii*, *S. vandeleurii*, *S. denticulatus*, *S. grandis* and *S. pusillus*, are unifoliates (Hilliard & Burtt 1971).

A group of longer lasting *Streptocarpus* taxa also only possesses one phyllomorph at a time, but this phyllomorph is replaced after each flowering season. In these plants, the first phyllomorph is the enlarged macrocotyledon. As this phyllomorph begins to flower, a second phyllomorph develops towards the base of the first one. This second one continues to increase in size as the first one nears the end of its life, and eventually replaces the first one. This process is repeated

each flowering season until the whole plant dies. This growth form is found in *S. daviesii* (Hilliard & Burtt 1971).

Still another group of plants, the **plurifoliates**, link the unifoliate and rosulate forms. They initially develop in the same way as in the unifoliates, but one to three additional phyllomorphs form on the petiolode of the original one. Even so, plants rarely possess more than three phyllomorphs at any one time, and one phyllomorph is usually larger and temporarily dominant over the others. The bases of older phyllomorphs tend to persist after the rest of each phyllomorph has died, and these bases grow together to form a sort of stock. Phyllomorphs are irregularly arranged on the stock, and usually more than one phyllomorph is flowering at a time. Although each phyllomorph is monocarpic, the plant as a whole is **polycarpic** i.e. flowers and fruits over several seasons. Taxa that display this growth habit include *S. porphyrostachys* and two of the four subspecies of *S. polyanthus*, *S. polyanthus* subsp. *polyanthus* and *S. polyanthus* subsp. *dracomontanus* Hilliard (Hilliard & Burtt 1971).

Plants that possess many phyllomorphs at a time arranged more or less in a rosette, none of them tending to be dominant over the others, are termed **rosulates** (Figure 2.8C; Hilliard & Burtt 1971). Rosulates are described as **centric** if the phyllomorphs are arranged in a regular spiral around a vertical axis, as in *S. gardenii*, *S. baudertii*, *S. montigena*, *S. kentaniensis*, *S. lilliputana* and *S. meyeri*, and **excentric** if they arise in ranks along the upper surface of a horizontal axis (Jong 1978), as in *S. primulifolius*, *S. formosus*, *S. rexii*, *S. johannis*, *S. cyaneus*, *S. modestus*, *S. aylae*, *S. caeruleus*, *S. parviflorus* and *S. montanus*. This horizontal axis superficially resembles a rhizome, but is actually formed from the compaction of the persistent bases of phyllomorphs that have already flowered and died. The currently alive phyllomorphs are clustered in a tuft at the one end of this horizontal axis (Hilliard & Burtt 1971).

A variation of the rosulate growth form is characterised by one (or occasionally more than one) phyllomorph becoming dominant over the rest during flowering. Its lamina increases in size, and its petiolode lengthens to raise it above the level of the phyllomorphs that are not flowering yet. This growth form is found in a few *Streptocarpus* taxa, but is at its most elaborate in *S. fanniniae*, where the phyllomorphs additionally branch. In the seedling of *S. fanniniae*, the macrocotyledon expands, and its petiolode lengthens considerably. This allows the phyllomorph to creep along the ground, developing adventitious roots from the ventral surface of its petiolode as it lengthens. Its tip bends sharply upwards to hold its developing lamina half-erect. Phyllomorph primordia soon begin to develop from its petiolode, usually in pairs, a short distance away from the junction between the lamina and petiolode. These give rise to new phyllomorphs, which in turn creep along the ground due to the elongation of their petiolodes, sinking adventitious roots into the substrate from their ventral surface and bending at the tip to hold their expanding lamina in a raised position. These in turn give rise to further phyllomorph primordia, causing the plant to expand laterally (Hilliard & Burtt 1971; Jong & Burtt 1975). The oldest phyllomorph is the first to reach maturity. It produces an inflorescence primordium at the junction between the lamina and petiolode, followed by the development of a phyllomorph primordium just below the developing inflorescence. This gives rise to a new phyllomorph. However, this phyllomorph does not creep along the ground and root, but grows vertically and itself produces its own inflorescence and a new phyllomorph. This one in turn elongates vertically and produces an inflorescence and new phyllomorph at the meristematic zone at the base of its lamina. Up to five phyllomorphs can develop one on top of the other in this fashion, and the whole plant can reach up to one metre in height as a result of this erect flowering shoot system. Consequently, the vertical "stem" of these plants consists of a series of individual phyllomorphs, each arising from the previous one. The oldest phyllomorph flowers first, followed by the newer phyllomorphs that have progressively expanded laterally from this one as they reach maturity (Hilliard & Burtt 1971).

Madagascar and the Comoro Islands together contain species displaying a wider diversity of growth forms compared to those found on the African mainland. Some are the same while others are unique. Thus, the islands also possess caulescents e.g. *S. thompsonii* R.Br. and *S. leandrii* Humbert ex B.L.Burtt, unifoliates e.g. *S. semijunctus* B.L.Burtt, *S. capuronii* Humbert and *S. lokohensis* Humbert, plurifoliates e.g. *S. itremensis* B.L.Burtt, and rosulates e.g. *S. ibityensis* Humbert, *S. revivescens* Humbert ex B.L.Burtt, *S. levis* B.L.Burtt and *S. mandrerensis* Humbert, although these differ to varying degrees from their African-mainland counterparts. On the other hand, no *rexii*-type rosulates occur on these islands (although *S. ibityensis* is quite similar), and there are also vegetative characteristics unique to Madagascar, such as the possession of an indumentum of mixed brown and white hairs (*S. lokohensis*, *S. sambiranensis* Humbert, *S. velutinus* B.L.Burtt, *S. suborbicularis* B.L.Burtt, *S. boinensis* Humbert and *S. polyphyllus* Humbert).

In addition to the more familiar growth forms, there are also growth forms present on Madagascar and the Comoro Islands that are absent amongst the African mainland *Streptocarpus* species, but are nevertheless similar enough to suggest evolutionary links to the mainland taxa. These fall into three main types. The first one consists of three species (*S. beampingaratrensis* Humbert, *S. andohahelensis* Humbert and *S. mandrerensis* Humbert) that are morphologically strikingly similar to *Saintpaulia*. They possess leaves arranged in a basal rosette, elongated petioles with sharply demarcated and orbicular laminas. Florally they are also similar to *Saintpaulia*, having axillary inflorescences with long peduncles and short-tubed, rather wide corollas and verruculose seeds, but twisted fruit capsules that are rather short compared to the rest of *Streptocarpus*. The second growth form is characterised by plantain-like leaves, and is found in five species (*Streptocarpus boinensis*, *S. polyphyllus*, *S. variabilis* Humbert, *S. perrieri* Humbert and *S. hildebrandtii* Vatke). Their leaves are also arranged in a basal, regular rosette (rarely solitary), but with petioles that gradually widen into the lamina and leaf veins that mostly ascend from the base and branch. This growth form only occurs on Madagascar, but its morphological characteristics suggest that it belongs amongst the mainland subgenus *Streptocarpus* species (Hilliard & Burtt 1971). The third group contains seven Madagascan species (*S. coursii* Humbert, *S. tsaratananensis* Humbert ex B.L.Burtt, *S. campanulatus* B.L.Burtt, *S. macropodus* B.L.Burtt, *S. suffruticosus*, *S. glabrifolius* Humbert, *S. papangae*) that develop into shrubs. Also taking its floral characteristics into account i.e. short filaments arising at the base of the corolla, and large, elongated seeds with pointed ends, this last group shares different characteristics with different mainland taxa, and its affiliations are therefore equivocal. Finally, there are species e.g. *S. tsimihetorum* Humbert, *S. cordifolius* Humbert and *S. sambiranensis*, that are so different from the mainland taxa that recent evolutionary links are unlikely (Hilliard & Burtt 1971). Thus, Madagascar and the Comoro Islands harbour growth forms that resemble those on the African mainland as well as many unique growth forms.

The growth form of all adult *Streptocarpus* plants consequently becomes evident after the initial stages of seedling development. In species where a central SAM forms, the plant develops a caulescent growth form. Species without a central SAM produce a series of phyllomorphs from the meristematic region below the lamina, the **groove meristem** (Jong 1978), of previous phyllomorphs that together make up the body of the plant. Amongst the acaulescent growth forms, the phyllomorph is therefore the basic unit, and it is the number of phyllomorphs present at any given time (and during the whole life of the plant) and the arrangement of these phyllomorphs in relation to one another that determines the growth form. Lamina and petiolode growth, as well as the formation of new phyllomorphs and inflorescences, all originate at the base of the lamina midrib from three meristematic regions. The **basal meristem** is responsible for the growth of the lamina, the **petiolode meristem** causes the growth of the lamina midrib and the petiolode, and new phyllomorphs and inflorescence

shoots arise from the **groove meristem**. These three intercalary meristematic regions are located at the junction of the lamina and petiolode, with the groove meristem located on the adaxial side (Jong 1978; Mantegazza *et al*. 2007). Each phyllomorph produces adventitious roots and is usually monocarpic, although a single phyllomorph occasionally flowers in two successive seasons (e.g. *S. bolusii*). Consequently, if the species only produce one phyllomorph during its lifetime, as in the unifoliates, then it usually only lasts for one flowering season after maturity. On the other hand, if the plant produces a succession of phyllomorphs, then it is capable of surviving for many flowering seasons.

A trait common amongst the acaulescent growth habits is the formation of the abscission line. Many *Streptocarpus* plants tend to occur in areas receiving predominantly summer rainfall, and the unfavourable period is consequently during the cold, dry winter months. A drop in temperature and shorter day lengths appear to be the triggers of abscission, seeing as greenhouse plants that are kept well watered and only experience a relatively small decrease in temperature also abscise their leaf tips. Thus, abscission seems to follow a general slowing down of growth rather than just a lack of moisture (Hilliard & Burtt 1971). This phenomenon has probably evolved along with the range expansion of the genus from the northern, more tropical regions to the southern, more seasonal regions (Möller & Cronk 2001b) as a general strategy for reducing the total size of the plant, thereby increasing its chances of survival by reducing water loss (Hilliard & Burtt 1971). Phyllomorphs that have, however, flowered and are due to die soon do not abscise (Hilliard & Burtt 1971).

Although abscission is a common occurrence amongst the acaulescent growth forms, it has been observed in neither wild nor greenhouse plants of *S. lilliputana*, a species recently discovered along the Pondoland coast. This species has a very limited distribution, having so far only been found in three neighbouring gorges, the Lupatana River Gorge, the Myokane River Gorge, and along the Mkozi River above Fraser Falls in Fraser Gorge. The plant grows in deep shade on rock seepages (Bellstedt & Edwards 2004), and this highly protected microhabitat has possibly resulted in the species not needing to retain its ability to abscise its lamina tips (Dirk U. Bellstedt, personal communication). Leaf abscission also does not occur in the caulescent taxa or in the acaulescent taxa from Madagascar, and leaf abscission probably evolved after the split of the African mainland from Madagascan plants. This characteristic has thus evolved in the acaulescent lineage only on the African mainland in response to the more seasonal environment that these plants inhabit (Hilliard & Burtt 1971).

Growth habit is, however, not always uniform within a species. For example, *S. polyanthus* and *S. dunnii* contain both monocarpic unifoliate and perennial plurifoliate variants. *S. bolusii* usually develops into a unifoliate, but its original phyllomorph occasionally flowers in two successive flowering seasons (Hilliard & Burtt 1971). Furthermore, the distinction between the various growth forms is not always clear-cut, with many intermediate taxa existing. *S. schliebenii* and *S. decipiens* Hilliard & B.L.Burtt, for example, have growth habits intermediate between the caulescent and acaulescent growth forms, showing a phyllomorphic pattern in their juvenile stages, followed by the development of a leafy stem upon maturity (Möller & Cronk 2001a). *S. nobilis* C.B.Clarke is usually caulescent, but develops into a unifoliate under certain conditions, such as short day length (Lawrence 1943). *S. bullatus* Mansf. possesses a rhizome that produces a succession of flowering shoots. These shoots have very elongated petiolodes reminiscent of shoots (Hilliard & Burtt 1971). The *Saintpaulia*-like *Streptocarpus* species in Madagascar produce leaves in the form of a rosette (Hilliard & Burtt 1971), but because they possess a SAM (their stem axis is just reduced), these plants are classified as caulescents rather than acaulescents (Möller & Cronk 2001a). Thus, the evolution of vegetative patterns appears to be rather complex in some cases.

Suggestions have been made over the years concerning the cause and order of the evolution of the various growth forms in *Streptocarpus*. Burtt (1970) proposed that the evolution of persistent growth in one of the cotyledons after germination in Old World Gesneriaceae taxa could have been driven by the tendency for the plants to grow in forest habitats, and the paucity of endosperm in their dust-like seeds. These factors together make the rapid growth of photosynthetic structures more advantageous than first investing energy in stem production. The acaulescent taxa may have taken this even further, by completely replacing the growth of a shoot system with the production of one or a series of photosynthetic organs (the phyllomorphs), perhaps in response to growing under more extreme conditions. Photosynthesis is at its most efficient in the unifoliates, which do not have the problem of self-shading. On the other hand, unifoliates usually die after their first flowering season, whereas rosulates can reproduce over a longer period. Thus, the specific environment in which the plant grows (e.g. on slopes, which would favour unifoliates) determines which growth form is most advantageous, and has therefore probably driven the evolution of the diverse morphological types present in *Streptocarpus* today (Möller & Cronk 2001a).

Oehlkers (1964) found that the unifoliate growth form is caused by the presence of recessive alleles at two independent loci. He proposed that the unifoliates might have arisen through mutations rendering the genes dysfunctional from a rosulate progenitor with dominant alleles. This would enable unifoliates to arise from rosulates reasonably easily, but would make the reverse transition (the genes becoming functional again) much more difficult, therefore suggesting that the rosulate growth form preceded the unifoliate form. Möller & Cronk (2001a), however, suggested that the transition from unifoliates to rosulates could have been achieved by introgression with rosulate taxa. Hilliard & Burtt (1971), in contrast, suggested that the unifoliate form preceded the rosulate form. They proposed that the caulescent growth form came first, and reasoned that, through paedomorphosis (possession in the adult stage of features typical of the juvenile stage of an organism's ancestor), the transition from the caulescent to the unifoliate form is more likely than the transition from the caulescent to the rosulate form. In agreement with this, Möller & Cronk (2001a) suggested that the ability of all the acaulescent growth forms to abscise distal parts of their lamina implies that the unifoliates preceded the rosulates, because this mechanism is much more likely to have evolved in the unifoliates, which cannot regulate leaf area by abscising whole leaves. However, their phylogenetic data are inconclusive in this respect.

The study conducted by Möller & Cronk in 1997 investigating the relationship between *Streptocarpus* and *Saintpaulia* provided some initial hints regarding relationships amongst the growth forms of *Streptocarpus* (Figure 2.4). The 19 species of *Streptocarpus* which they included in the analysis represent all the main growth forms in the genus. The *Streptocarpus* taxa grouped according to growth form, the caulescent taxa (subgenus *Streptocarpella*) emerging separately from the acaulescent taxa (subgenus *Streptocarpus*). Additionally, amongst the acaulescent representatives, the unifoliates and rosulates separated into two clades. Furthermore, members of the unifoliate clade shared a *ca.* 40 bp deletion in ITS2 not found in any of the other taxa. Consequently, based on this study, growth habit appears to be a conservative character, each growth type having evolved only once.

However, the expanded analysis of Möller & Cronk (2001a) uncovered more complicated patterns (Figure 2.5). The 77 *Streptocarpus* taxa analysed emerged in two main sister clades, referred to as **clade I** and **clade II**. In this analysis *Streptocarpus* taxa mostly did not group strictly in accordance with the growth form displayed by the individual taxa. All but three of the caulescent taxa emerged in clade I, the remaining three (*S. papangae*, *S. schliebenii* and *S. macropodus*) emerging together with the acaulescent taxa in clade II. Furthermore, although the acaulescent growth forms (unifoliates / plurifoliates and rosulates) all emerged within clade

II, the individual growth forms did not consistently form separate groups. For example, both unifoliates and rosulates emerged within the ITS2 deletion clade, which was only occupied by unifoliates in their previous analysis (Möller & Cronk 1997). Constraining these individual growth forms separately, as well as all the growth forms together to emerge as monophyletic groups resulted in significantly longer trees, and it was therefore concluded that the taxa possessing each growth form do not constitute monophyletic clades. However, two of the other Madagascan growth forms—the *Saintpaulia*-like taxa represented by *S. beampingaratrensis* and *S. andohahelensis*, and the group with plantain-like leaves represented by *S. hildebrandtii* and *S. perrieri*—did, emerge in separate, monophyletic clades.

Parsimony character optimizations on this phylogeny (Möller & Cronk 2001a) suggested that the caulescent form is the most primitive, and gave estimates of the number of times that each growth form has evolved from every other growth form during the evolution of *Streptocarpus* (Figure 2.8). Evolutionary transitions between the unifoliate / plurifoliate and rosulate growth forms appear to have occurred most frequently, with the unifoliate / plurifoliate form estimated to have arisen from the rosulate form five to seven times, and the rosulate form one to four times from the unifoliate / plurifoliate form. The rosulate form appears to have evolved from the caulescent form two to four times, while the caulescent form has probably evolved from the rosulate form one to three times. Evolutionary transitions between the unifoliate and caulescent forms appear to have been the least common, with the unifoliate form having evolved from the caulescent form once or twice, and the caulescent form never having evolved from the unifoliate form. Thus, each growth form appears to have evolved several times during the evolution of the genus, and there is no clear signal to indicate the order in which the different growth forms evolved. However, investigations into the floral morphology of *Streptocarpus* were to uncover even more complex patterns.

2.3.2. Floral morphology within *Streptocarpus*

Although the inflorescences of Gesneriaceae are very diverse, Weber (1973, 1982) and Wiehler (1983) realised that the various types are all composed of one to many basic building blocks, the so-called 'pair-flowered cyme' (Figure 2.9). **The pair-flowered cyme** is an unusual type of cyme in that it consists of a hypopodium terminating in two lateral bracteoles (the α- and β-bracteoles), above which are carried a terminal flower and an axillary front flower with its own subtending bracteole (the γ-bracteole). Having stated this, the γ-bracteole is more often than not suppressed or aborted, giving the front flower the appearance of being adventitious, and one or both of the α and β bracteoles also do not develop in some instances (Weber 1995; 2004). The presence of bracts subtending flowers is usually conservative within the angiosperms, making it an important diagnostic character for inflorescence identification (Coen & Nugent 1994). However, the number and position of the bracteoles within *Streptocarpus* and *Saintpaulia* are highly variable (Harrison *et al.* 1999).

Subsequent cyme units arise from the axils of the bracteoles of the previous units. Although the pair-flowered cyme unit is determinate, the inflorescence as a whole is indeterminate in that it can proliferate through successive development of more pair-flowered cyme units from the axils of the bracteoles of previous units. The resulting branching pattern can be dichasial (opposite branching of units below the flower that terminates each axis, each branch in turn terminating in a flower), monochasial (the next unit only arising from one of the bracteoles of each unit, forming a cincinnus), dichasial followed by monochasial (forming a double cincinnus), or unbranched (the inflorescence has reduced to a single unit with or without the accompanying front flower (Figure 2.9). Inflorescences are consequently variable with regards to branching patterns, the number of flowers contained in the inflorescence, bracteole presence/absence, displacement, form, size and colouration, and length of the peduncle, hypopodia and pedicels. The number of cyme units per leaf axil and the size and arrangement

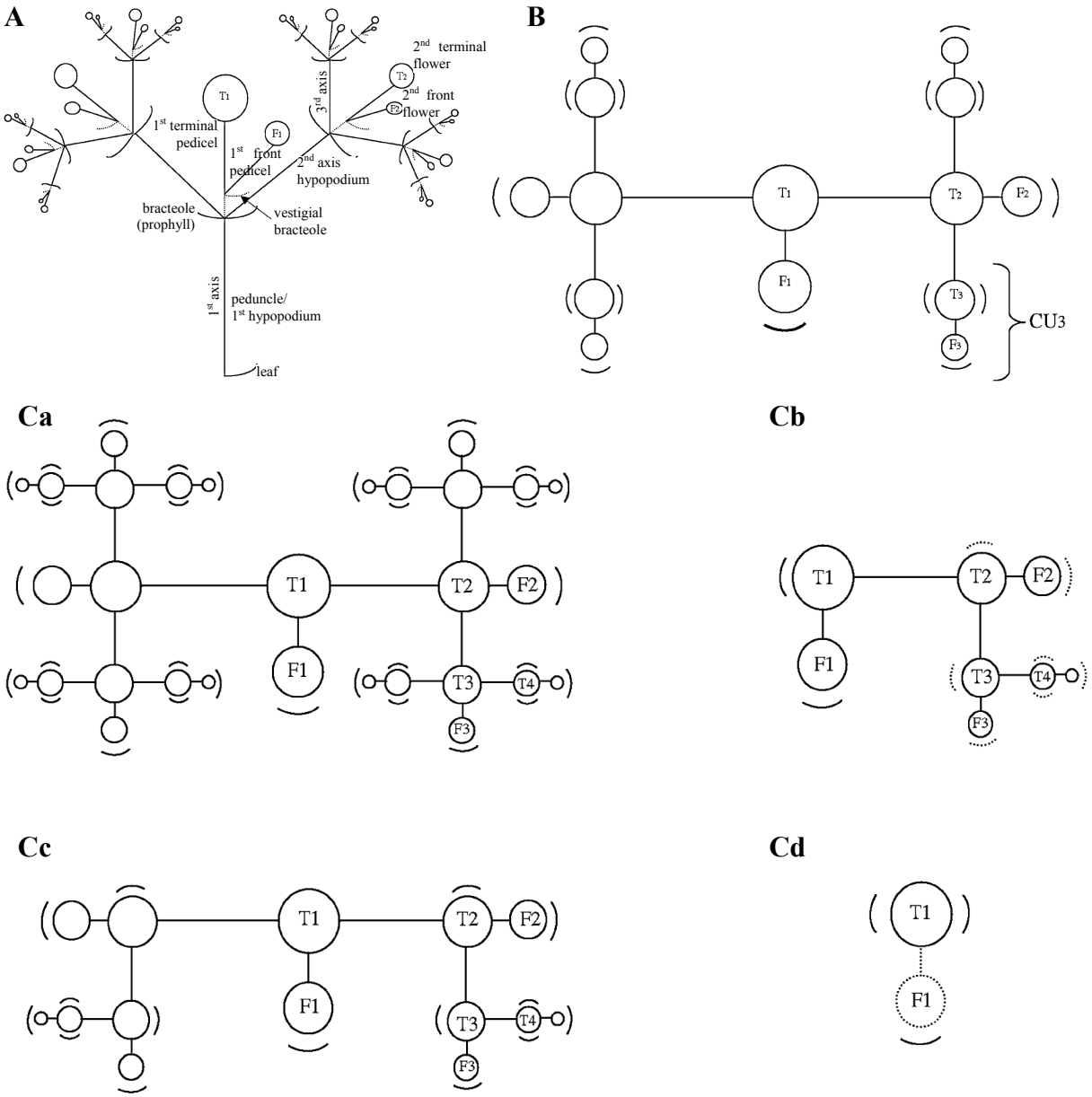

Figure 2.9: Inflorescence diagrams copied from Haston & Ronse De Craene (2007) illustrating the pair-flowered cyme inflorescences of Gesneriaceae. **A**: Lateral view of a three-levelled dichasial branching inflorescence; **B**: Overhead view of a two-levelled dichasial branching inflorescence; C: Some of the branching patterns present in Gesneriaceae. **Ca** illustrates dichasial branching, **Cb** monochasial branching, **Cc** dichasial to monochasial branching, and **Cd** represents an unbranched inflorescence. Structures illustrated with a dotted line are often compressed or suppressed on the plant and therefore are not usually visible. T denotes terminal flowers, F front flowers, and CU a single cyme unit consisting of a hypopodium (the cyme unit peduncle) terminating in a pair of lateral bracteoles, above which the terminal and front flowers are borne. The numbers give the iteration number of the pair-flowered cyme unit.

of leaves subtending inflorescences versus the other foliage leaves, and the distances between these leaves also differs within Gesneriaceae (Weber 1995, 2004; Haston & Ronse De Craene 2007).

In *Streptocarpus*, the flowers are borne in axillary inflorescences in the caulescent taxa (Hilliard & Burtt 1971), and from the groove meristem at the base of the lamina in the acaulescent taxa (Jong & Burtt 1975). Flowers are either solitary e.g. *S. andohahelensis*, or borne in cymose inflorescences in a dichasial, as in *S. grandis*, or double cincinnus, as in *S. polyanthus*,

arrangement (Hilliard & Burtt 1971). In fact, *Streptocarpus* displays a large range of the total variation in inflorescence branching patterns present in Gesneriaceae as a whole (Haston & Ronse De Craene 2007). However, branching pattern is not necessarily species-specific, with some species displaying a wide range of branching patterns, either amongst individuals or even on the same individual (Michael Möller, pers comm.).

The flowers of *Streptocarpus* are sympetalous (the five petals are united by their margins along most of their length to form a long tube), zygomorphic and nectiferous. The androecium consists of five stamens, the two anterior ones being fertile and the three posterior ones forming staminodes, although the central staminode is often missing (Hilliard & Burtt 1971). The anthers of the two fertile stamens are positioned face to face in most species, held together by their epidermal cells forming papillae upon contact with each other, which then interlock (Oehlkers 1962).

However, apart from these similarities, *Streptocarpus* flowers are highly variable in shape, size, colour and scent, leading to the description of six floral types based on the outer appearance of the flower alone (Hilliard & Burtt 1971; Harrison *et al*. 1999): pouch, personate, *Saintpaulia*, *S. dunnii*, open-tubed and keyhole (Figure 2.10). In the **pouch type** (Figure 2.10Aa–b), the flowers are very small compared to the other types (corollas less than 10 mm long), and possess a small yet wide, curved floral tube. This type occurs in both subgenera, although it is more prevalent in subgenus *Streptocarpella* and predominant in the Madagascan species (Harrison *et al*. 1999; Hughes *et al*. 2006), including *S. papangae*. The **personate type** (Figure 2.10Ba–b), on the other hand, is only found in a few closely related caulescent African species. It is strongly zygomorphic, with a lower lip that is spread out, presumably forming a landing platform for insects, and possesses raised ridges that mostly conceal the floral mouth (Hilliard & Burtt 1971). The flowers are enantiostylous, the style and stamens reciprocally displaced (although only the style is displaced in *S. pallidiflorus* C.B.Clarke) to opposite sides of the flower (Harrison *et al*. 1999). Presumably the pollinator gains access to the flower by landing on the lower lip, thereby depressing it and exposing the floral mouth (Hilliard & Burtt 1971). ***Saintpaulia*** (Figure 2.10Ca & Figure 2.3C) has flowers very different from that of *Streptocarpus*. The flowers of *Saintpaulia* are characterised by rotate corollas (possessing a very short floral tube and spreading lobes), bright yellow protruding anthers that are conspicuous against the violet corolla (Harrison *et al*. 1999), and enantiostyly of the style only (Willis 1973). In addition, *Saintpaulia* flowers are buzz-pollinated (Harrison *et al*. 1999), rewarding their pollinator with pollen, not with nectar, as is the tendency in *Streptocarpus* (Hilliard & Burtt 1971). The flower of ***Streptocarpus dunnii*** (Figure 2.10Da–b) is unique in many ways. It is one of the few *Streptocarpus* species to have a red, rather robust corolla, and the tube-like shape of the flower is also somewhat different from the other *Streptocarpus* species (the only other red-flowered *Streptocarpus* being the northern Mozambican species, *S. myoporoides* Hilliard & B.L.Burtt, which possesses a similar flower shape as well). The proximal part of its floral tube is straight, before becoming arcuate (bow-shaped) towards the distal end, and its lobes are small and almost regular (Hilliard & Burtt 1971). More widespread, but almost entirely restricted to the acaulescent species, is the **open-tubed type** (Figure 2.10Ea–b). Although this is a very diverse form, the flowers are generally characterised by a wide, relatively straight, infundibular (more or less gradually widening from the base to the more or less spreading lobes, resembling a funnel) floral tube, terminating in an open, usually circular floral mouth. This type is mostly restricted to subgenus *Streptocarpus*, and occurs in the following species pertinent to this study: *S. montanus*, *S. bolusii*, *S. denticulatus*, *S. porphyrostachys*, *S. grandis*, *S. rimicola*, *S. pusillus*, *S. saundersii*, *S. vandeleurii*, *S. fanniniae*, *S. meyeri*, *S. modestus*, *S. kentaniensis*, *S. caeruleus*, *S. montigena*, *S. rexii*, *S. primulifolius*, *S. formosus*, *S. floribundus*, *S. cyaneus*, *S. roseo-albus*, *S. fenestra-dei*,

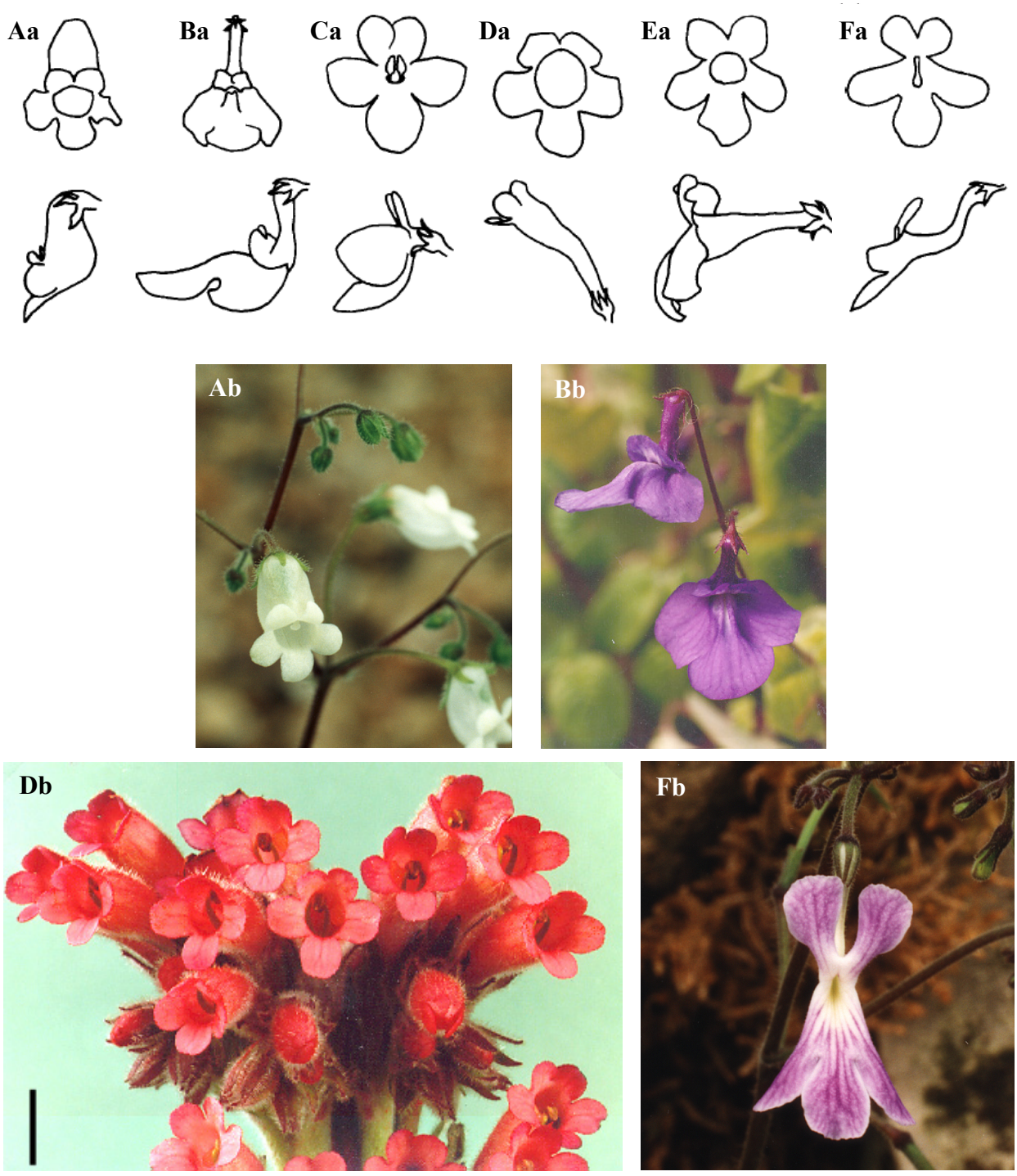

Figure 2.10: Front and side views of the six floral types recognised by Harrison *et al*. (1999) and Hughes *et al*. (2006) with photographs of representative species. **Aa** shows the pouch type with **Ab** *Streptocarpus muscosus* as an example, **Ba** the personate type with **Bb** *Streptocarpus holstii*, **Ca** the *Saintpaulia* type, **Da** the *S. dunnii* type with **Db** a photograph of *Streptocarpus dunnii*, **Ea** the open-tubed type, and **Fa** the keyhole floral type with **Fb** *Streptocarpus haygarthii* as an example. Line drawings copied from Harrison *et al*. (1999) and photographs taken by Michael Möller at the Royal Botanic Garden Edinburgh.

S. kunhardtii, *S. longiflorus*, *S. parviflorus* and *S. gardenii*. In contrast, the **keyhole type** (Figure 2.10Fa–b) possesses a narrow, bent floral tube and a strongly laterally compressed mouth, the latter often being slightly narrower in the centre, resembling a keyhole. This type is only present in about 12 species (Michael Möller, personal communication), all but one

(*S. saxorum*) amongst the acaulescent taxa, and includes *S. johannis*, *S. baudertii* and *S. polyanthus* (Hilliard & Burtt 1971).

However, as is the case with the vegetative morphology, although floral diversity has been divided into six floral types for convenience, the boundaries are not always clear. For example, it is difficult to distinguish between some of the smaller-flowered pouch and small open-tubed types. The open-tubed type is very diverse, not only containing flowers that approach the pouch form, but also containing species with dorsoventrally compressed floral mouths e.g. *S. gardenii*, species with more or less strongly dilated distal parts of the floral tube e.g. *S. primulifolius*, and species with strictly tube-shaped corollas e.g. *S. candidus* Hilliard (Michael Möller, personal communication). The great variation within the open-tubed type is probably indicative of different pollinators e.g. long-tongued flies and short-tongued bees (Potgieter & Edwards 2005). The keyhole floral type serves as another example. The Madagascan *S. semijunctus* possesses the constricted floral mouth and wide-spreading limb of other keyhole species, but its floral tube is more or less straight and slightly inflated compared to the narrow, strongly curved floral tubes of *S. polyanthus*, *S. johannis* and *S. baudertii* (Hilliard & Burtt 1971). Thus, floral morphology is complex, and there are variations within each of the floral types.

The evolution of the diversity of floral types within *Streptocarpus* has probably been driven by pollinators and differences in breeding systems. Pollinator data are largely lacking for almost all species, but the pollination syndromes of the various floral types have been largely hypothesized based on floral morphology: it has been suggested that the pouch type is pollinated by small flies (Harrison *et al*. 1999); the personate and open-tubed types by bees (Harrison *et al*. 1999); *Saintpaulia* by means of buzz pollination by bees (Vogel 1978; Dafni 1992; Harrison *et al*. 1999); *S. dunnii* by birds (Vogel 1954); and the keyhole type by a long-tongued insect (Hilliard & Burtt 1971) e.g. butterflies or moths (Harrison *et al*. 1999).

Parsimony character optimizations performed on reconstructed nuclear ITS phylogenies by Harrison *et al*. (1999) and Hughes *et al*. (2006) suggest that the small pouch type, which is present in both subgenera, is the plesiomorphic type in *Streptocarpus*, the other types present in the genus having evolved either directly from the pouch type or through the open-tubed type (Figure 2.11). The personate, *S. dunnii* and *Saintpaulia* types appear to have evolved only once, whereas the pouch, open-tubed and keyhole types have each evolved several times (two, seven and eight times, respectively; Hughes *et al*. 2006). Although corolla size is quite variable within some species e.g. *S. polyanthus* and *S. haygarthii* N.E.Br. ex C.B.Clarke, suggesting that it has been evolutionary quite labile (Hilliard & Burtt 1971), the character optimizations also revealed that the general evolutionary trend within *Streptocarpus* seems to be from smaller to larger flower sizes, with the large, open-tubed form predominating in the areas more recently occupied by *Streptocarpus*, as in southern Africa. Probably linked to this (because of the tendency for larger flowers to provide greater rewards to pollinators, thereby making outcrossing more likely) is the unusual evolutionary trend from a predominantly inbreeding breeding system towards more outcrossing taxa within the *Streptocarpus* lineage (Harrison *et al*. 1999; Hughes *et al*. 2006). However, this trend is reversed in the Cape, where *Streptocarpus* has reverted from the outbreeding *S. primulifolius* to the inbreeding *S. rexii* (Hughes *et al*. 2005).

Molecular work and field observations have led to the discovery of the pollination vector for some of the taxa. Hughes *et al*. (2006) investigated the breeding system of three species possessing the plesiomorphic pouch floral type. They generated microsatellite data from four loci for four to seven populations each of *S. micranthus* C.B.Clarke, *S. lanatus* MacMaster and *S. ibityensis*. Hughes *et al*. (2006) found significant deviations from Hardy-Weinberg equilibrium towards inbreeding for all three species, especially in *S. micranthus*. These species have very small, hanging flowers with no scent, and it is not very likely that they attract

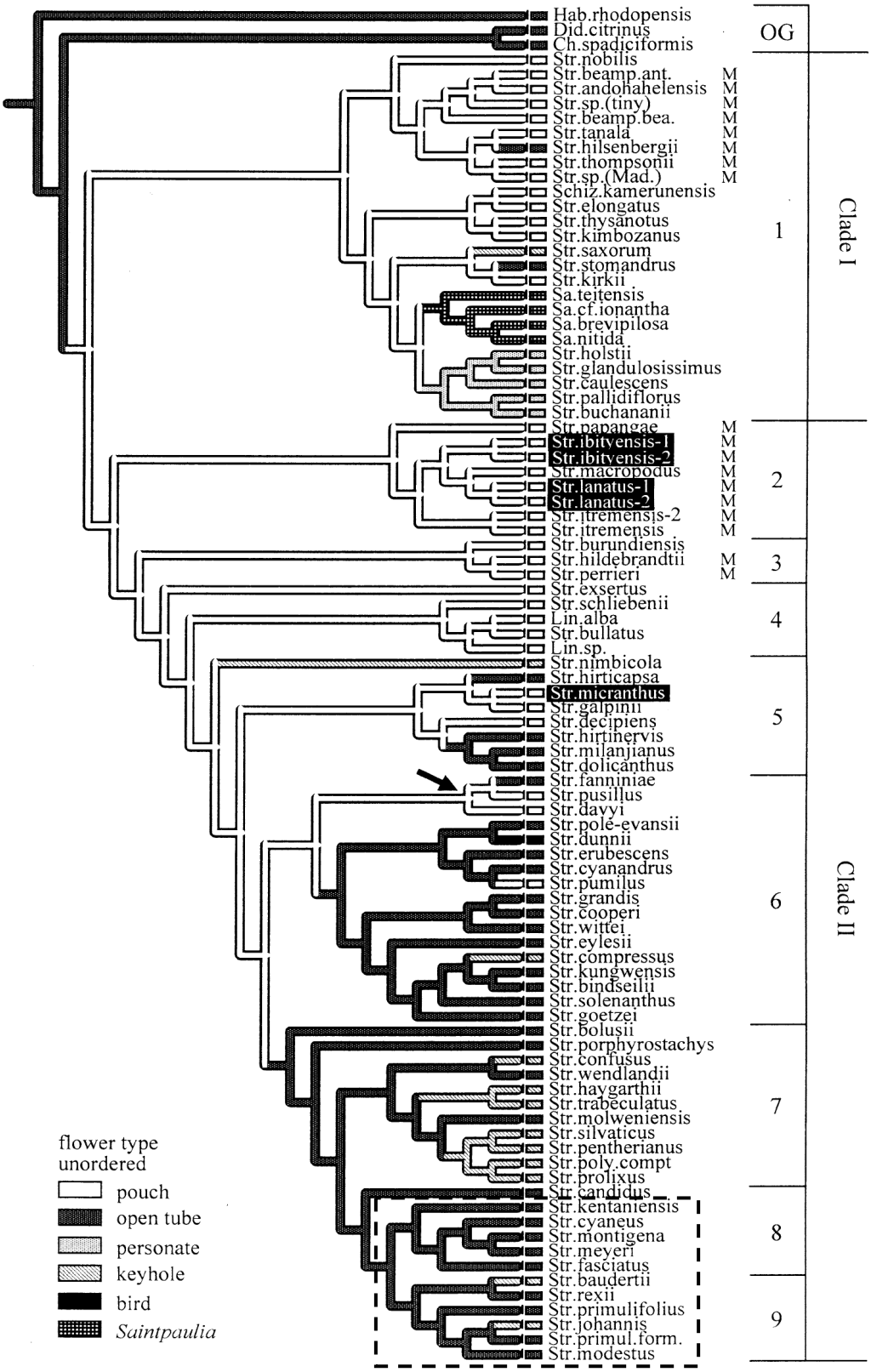

Figure 2.11: Parsimony majority-rule consensus tree from Hughes *et al*. (2006) reconstructed using the ITS sequences used by Möller & Cronk (2001a, b) showing the most parsimonious evolution of floral types in *Streptocarpus*. The taxon samples highlighted in black, representing *Streptocarpus ibityensis*, *S. lanatus* and *S. micranthus*, indicate samples that have been added to the sequence matrix used to build the ITS tree since Möller & Cronk published their tree in 2001. Optimizations for the evolution of floral type were carried out in MacClade with the DELTRAN option enforced. Although polytomies were randomly resolved for the optimizations, the character state at only one node (indicated by an arrow) varied depending on the topology used. OG points to the outgroup taxa, and M marks species endemic to Madagascar (and the Comoro Islands). Genus abbreviations are the same as in Figure 2.5. The Cape Primrose clade is enclosed in a dashed box.

pollinators. Their insignificant flowers, together with the inbreeding signal evident from the microsatellite data, indicate that these species are predominantly inbreeders. The flowers of other species possessing the pouch type sometimes do not open at all, and are cleistogamous in these species (Hilliard & Burtt 1971; Michael Möller, personal communication). Thus, at least some of the taxa possessing the pouch floral type do not attract pollinators. While pollination data are lacking for the personate and *Saintpaulia* floral types, observations have been made for the *S. dunnii* type. Francois Krige and Michael Möller observed malachite sunbirds, *Nectarinia famosa* L., pollinating *S. dunnii* at the Verloren Vallei Nature Reserve, Dullstroom, Mpumalanga province, South Africa. This is probably a case of pollination guild, as the malachite sunbird pollinates other red-flowered species occurring in the same area, including the red and orange flowered genus *Crocosmia* Planch (Iridaceae Juss.) and *Protea* L. species (Dirk Bellstedt, personal communication). The pollination of an open-tubed species has also been documented. Dirk U. Bellstedt, Michael Möller and Mark Hughes photographed and captured the nemestrinid fly, *Stenobasipteron wiedemanni* Lichtwardt, pollinating the open-tubed species *S. primulifolius* in Silaka in the Eastern Cape. However, the open-tubed type is prevalent throughout much of subgenus *Streptocarpus*, occurring in species well out of the distribution range of *Stenobasipteron wiedemanni*, and this floral type also is quite diverse (see above). It is therefore unlikely that *Stenobasipteron wiedemanni* is the only pollinator of the open-tubed type (and perhaps of *S. primulifolius* as well). Another open-tubed species, the recently described *S. lilliputana* (Bellstedt & Edwards 2004), possesses a rather distinct flower, with a narrow, downwardly bent, arcuate floral tube that widens suddenly towards its distal end. The floral mouth is somewhat dorsoventrally compressed, resembling *S. gardenii* (another open-tubed species) in this respect. The flower of *S. lilliputana* superficially resembles that of certain Acanthaceae species that co-occur with it e.g. *Asystasia varia* N.E.Br., *Mackaya bella* Harv. and *Salpinctium natalensis* (Clarke) T.J.Edwards. The resemblance is probably the result of Dodsonian mimicry (Dirk U. Bellstedt, personal communication) or a pollination guild (Michael Möller, personal communication). Unfortunately, the pollinator(s) of *S. lilliputana*, as well as of most of the other *Streptocarpus* species, including those with the keyhole floral type, are still unknown.

In summary, the evolution of the floral morphology in *Streptocarpus*, as is the case with the vegetative morphology, is by no means straightforward. The pouch floral type appears to be the plesiomorphic state, but the genus contains at least five, sometimes highly variable floral types, most of which have seemingly evolved more than once. Furthermore, the breeding systems of only a handful of taxa are known, and floral-type boundaries might have to be adjusted as more information becomes available. The interplay between vegetative and floral morphology is also unclear. Although floral types are not evenly distributed between the two subgenera (which are defined in terms of growth form), suggesting that there is some correlation between floral type and growth habit (Harrison *et al*. 1999), this correlation is not very strong, which further highlights the complexity in the evolution of both these characteristics.

2.3.3. Infrageneric classification of *Streptocarpus*

The variable morphology provided the impetus for the first formal generic subdivision of *Streptocarpus*, which was proposed by Fritsch in 1894. Based on the different growth habits found within the genus, he divided *Streptocarpus* into sections ***Caulescentes***, ***Unifoliati*** and ***Rosulati***. However, he soon realised that the caulescent taxa should be more separated from the unifoliates and rosulates, and he therefore raised *Caulescentes* to the subgeneric level under the name **subgenus *Streptocarpella*** Fritsch, and grouped sections *Unifoliati* and *Rosulati* into subgenus *Eustreptocarpus* (Fritsch 1904). Apart from the renaming of subgenus *Eustreptocarpus* to **subgenus *Streptocarpus*** in accordance with the International Code of Botanical Nomenclature of 1966, the subgeneric classification has remained unchanged.

However, due to the existence of many intermediates between the unifoliate and rosulate growth forms and the existence of more than one growth form within some species, Hilliard & Burtt (1971) discarded sections *Unifoliati* and *Rosulati* within subgenus *Streptocarpus*. Within subgenus *Streptocarpella*, Engler (1921) used the concept of "Gruppe" to divide the species into smaller groups, a rank that Hilliard & Burtt (1971) considered to be equivalent to the taxonomic rank of series. Although Hilliard & Burtt (1971) considered these groups to reflect evolutionary affinities reasonably well, some of the more recently discovered taxa could not be accommodated within these existing series, and many more would have to be created to cover the morphological diversity occurring within the subgenus. They therefore did not uphold this classification, and only listed it for the sake of completeness. Consequently, *Streptocarpus* is currently divided into two subgenera, subgenus *Streptocarpus* and subgenus *Streptocarpella*, with no further formal divisions between the subgeneric and specific levels.

Subgenus *Streptocarpella* is characterised by the caulescent growth form, in which the macrocotyledon looses its basal meristem early and consequently develops into a leaf of determinate size, and a vertical shoot system develops with cauline (borne on an aerial stem, separated by elongated internodes), usually petiolated leaves (Hilliard & Burtt 1971). The subgenus contains a few taxa displaying a rosette-like habit, but because these taxa possess a SAM, they are considered more similar to the other caulescent taxa in subgenus *Streptocarpella* than to the acaulescent rosulates. The leaf tips do not abscise, and inflorescences are always axillary. Members of this subgenus possess verruculose seeds (Möller & Cronk 1997), and have a basic chromosome count of x = 15 (Hilliard & Burtt 1971), except in *S. schliebenii* and the Madagascan woody caulescents *S. papangae* and *S. suffruticosus* (Jong & Möller 2000). The subgenus extends from Sierra Leone in the west across the tropical African mainland to the Comoro Islands and Madagascar in the east. Subgenus *Streptocarpella* does not occur further south than Malawi on the African mainland (Hilliard & Burtt 1971).

The acaulescent growth forms (unifoliates, plurifoliates and rosulates) all make up subgenus *Streptocarpus*, which possesses a basic chromosome count of x = 16 (Hilliard & Burtt 1971; Jong & Möller 2000) and mostly reticulate seeds (Möller & Cronk 1997). Their vegetative plant bodies are made up of one or more phyllomorphs, subsequent phyllomorphs being similar to the first one, and these phyllomorphs can abscise the distal parts of their laminas during unfavourable periods (Hilliard & Burtt 1971). Inflorescences arise from the groove meristem at the base of the lamina of the phyllomorphs (Jong & Burtt 1975). The subgenus occurs over a larger area than does subgenus *Streptocarpella*, extending from the George and Knysna vicinities in the Western Cape (South Africa) in the south, up through KwaZulu-Natal (South Africa), Mpumalanga (South Africa) and Swaziland, Limpopo (South Africa), Zimbabwe, Zambia, Malawi, Tanzania and The Democratic Republic of the Congo into southern Ethiopia in the north, and from Angola in the west to Mozambique and Madagascar in the east (Hilliard & Burtt 1971).

The ITS study carried out by Möller & Cronk in 1997 (Figure 2.4) was in perfect accordance with the present subgeneric classification. In contrast, Möller & Cronk's (2001a) subsequent ITS analysis containing more taxa revealed some complications regarding infrageneric relationships (Figure 2.5). The 77 species of *Streptocarpus* separated into two main clades that were not entirely congruent with the subgeneric classification, but fully congruent with basic chromosome numbers. All but three members of subgenus *Streptocarpella* emerged in clade I, which only contained taxa known to possess a basic chromosome number of x = 15. All of the representatives of subgenus *Streptocarpus* emerged within clade II, along with the remaining three members of subgenus *Streptocarpella* (the African *S. schliebenii* and the Madagascan woody caulescent *S. papangae* and *S. suffruticosus*). Clade II contained all taxa known to have x = 16 chromosomes (or polyploid multiples thereof) in the basic state. It therefore appears that

chromosome number is a better indication of relationships within the genus than is growth form.

2.3.4. Informal species groupings within subgenus *Streptocarpus* of the African mainland

Within subgenus *Streptocarpus*, Hilliard & Burtt (1971) divided the African mainland species into four main groups (A, B, C and D) that were intended to reflect natural affinities within the subgenus. They were, however, based on the groups established by Hilliard (1966a, 1966b, 1966c) for the species of KwaZulu-Natal, and their expanded size, and hence the greater diversity present within these groups rendered them less distinct from one another and consequently more difficult to define. Moreover, some species i.e. *S. caeruleus* and the members of the *S. meyeri* alliance, only appear to be distantly allied to Group C and Group B, respectively, and Hilliard & Burtt (1971) suspected *S. johannis*, *S. baudertii* and *S. montigena* of possibly being the result of hybridization events between members of different groups. They therefore had to define a few "satellite" groupings in addition to the four main groups in order to accommodate these "anomalous" species. The great diversity within the main groups and the existence of atypical and intermediate species resulted in Hilliard & Burtt (1971) not going as far as to classify their groups as formal taxonomic assemblages.

In defining these groups, both floral and vegetative characters played significant roles. Some of the groups are almost uniform for floral type and growth form, while other groups are much more of a mixture. Within each group, the species are often arranged in a gradation series for certain characters. Therefore, the order of the species is important i.e. a species is in most cases more similar to the species on either side of it than to the other species in the group, and the first and last members of a group may be very different from each other.

They also speculated on the relationships between the African mainland and Madagascan and Comorian species belonging to subgenus *Streptocarpus*, associating the latter with Groups A, B and D of the mainland taxa, but these relationships are tentative, and are therefore not discussed here.

2.3.4.1. Subgenus *Streptocarpus,* Group A

Members of this group are typically monocarpic unifoliates, rarely plurifoliate perennials (species that are sometimes or always plurifoliates include *S. compressus* B.L.Burtt, *S. eylesii* S.Moore and *S. galpinii* Hook.f.), of medium to large size. Most of the species possess the open-tubed floral type, although the pouch (*S. galpinii*) and keyhole (*S. compressus* and *S. trabeculatus*) types are also represented in this group to a very limited extent. The flowers are blue, violet, or more rarely white or cream, but never pink or red, and the corolla mouth is usually wide open. Patterning on the floor of the corolla tube or on the lower lip is in the form of white wedges or white or darker patches, but never spots or distinct lines. The stigma is usually stomatomorphic (mouth-shaped, with a short upper and lower lip), and the fruit is characteristically over 50 mm long, except in the case of *S. trabeculatus*, *S. cooksonii* B.L.Burtt and *S. galpinii*, in which the fruit is considerably shorter (Hilliard & Burtt 1971).

This group contains 18 species, and is further divided into five subgroups, namely Aa, Ab, Ac, Ad and Ae. Species belonging to Group A include[2]:

[2] Species distributions were obtained from Hilliard & Burtt (1971) and http://persoon.si.edu/Gesneriaceae/. Country abbreviations in this section are as follows: **Ang** for Angola, **Bur** for Burundi, **Cam** for Cameroon, **CAR** for the Central African Republic, **DRC** for the Democratic Republic of the Congo, **Eth** for Ethiopia, **Gab** for

Aa: *S. porphyrostachys* (SA), *S. wendlandii* (SA), *S. molweniensis* Hilliard (SA) and *S. saundersii* (SA);

Ab (*S.* agg. *cooperi*): *S. cooperi* C.B.Clarke (SA), *S. grandis* (SA, Zim), *S. michelmorei* B.L.Burtt (Mal, Moz, Zam, Zim) and *S. solenanthus* Mansf. (Mal, Tan, Zam, Zim);

Ac: *S. goetzei* Engl. (Mal, Moz, Tan) and *S. compressus* (Tan);

Ad (*S.* agg. *monophyllus*): *S. monophyllus* Welw. (Ang), *S. eylesii* (Mal, Moz, Zam, Zim) *S. wittei* De Wild. (DRC, Mal, Zam) and *S. vandeleurii* (SA); and

Ae: *S. kungwensis* Hilliard & B.L.Burtt (Tan), *S. trabeculatus* (SA), *S. cooksonii* (SA) and *S. galpinii* (SA).

2.3.4.2. Subgenus *Streptocarpus*, Group B

Group B is both vegetatively and florally much more variable than Group A. The group displays the widest array of growth forms of any of the groups, containing unifoliates, plurifoliates and rosulates, and each subgroup contains at least one species that bears its flowers on an aerial stem structure, usually an elongated petiolode. The leaf margins are often coarsely and irregularly jagged and toothed. The corolla is also more variable in form, with the pouch and open-tubed floral types being prominent. However, *S. nimbicola* Hilliard & B.L.Burtt produces keyhole flowers, and the flowers of *S. dunnii* fall into their own unique class. The flowers are mostly white, pinkish, or red, but occasionally violet, and the inside of the tube frequently contains unicellular trichomes. The corolla is usually marked with strips in the tube, or heavier spots or broken markings in the throat and on the lower lip. Typically, the lateral staminodes are conspicuous and the style is as long as or longer than the ovary. The stigma is also stomatotrophic or unequally two-lipped, but the capsule is mostly short, rarely exceeding 50 mm (Hilliard & Burtt 1971).

The main part of this group consists of 31 species, which Hilliard and Burtt (1971) further divided into subgroups Ba–Bi:

Ba: *S. denticulatus* (SA), *S. pole-evansii* Verd. (SA), *S. dunnii* (SA) and *S. myoporoides* (Moz);

Bb: *S. pogonites* Hilliard & B.L.Burtt (SA), *S. montanus* (Tan, Ken), *S. hirtinervis* C.B.Clarke (Mal) and *S. nimbicola* (Mal);

Bc: *S. brachynema* Hilliard & B.L.Burtt (Moz), *S. umtaliensis* B.L.Burtt (Zim), *S. micranthus* (SA), *S. bullatus* (Tan) and *S. decipiens* (SA);

Bd: *S. burundianus* Hilliard & B.L.Burtt (Bur) and *S. masisiensis* De Wild. (DRC);

Be: *S. fanniniae* (SA), *S. candidus* (SA) and *S. wilmsii* Engl. (SA);

Bf: *S. davyi* S.Moore (SA), *S. pusillus* (SA) and *S. rimicola* (SA);

Bg: *S. exsertus* Hilliard & B.L.Burtt (Ken) and *S. phaeotrichus* B.L.Burtt (Eth);

Bh: *S. erubescens* Hilliard & B.L.Burtt (Mal), *S. cyanandrus* B.L.Burtt (Zim), *S. pumilus* B.L.Burtt (Zim) and *S. hirticapsa* B.L.Burtt (Zim); and

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Gabon, **Gam** for The Gambia, **Ken** for Kenya, **Mad** for Madagascar, **Mal** for Malawi, **Moz** for Mozambique, **Rwa** for Rwanda, **SA** for South Africa, **Sud** for Sudan, **Swa** for Swaziland, **Tan** for Tanzania, **Uga** for Uganda, **Zam** for Zambia, and **Zim** for Zimbabwe.

Bi: *S. bolusii* (SA), *S. latens* Hilliard & B.L.Burtt (SA), *S. leptopus* Hilliard & B.L.Burtt (Mal, Moz) and *S. rhodesianus* S.Moore (Ang, Zam, DRC).

In addition to these subgroups, **the *S. meyeri* alliance**, comprising *S. meyeri* (SA), *S. kentaniensis* (SA) and *S. modestus* (SA), forms an additional, more distant subgroup within Group B. The alliance is characterised by plants with a centric rosulate growth habit, although *S. modestus* is an excentric rosulate and is the species that links the alliance most strongly to Group C. Within Group B, *S. meyeri*, and to a lesser extent *S. kentaniensis*, is similar to *S. rhodesianus* (Subgroup Bi) in possessing corollas with coloured tubes, but white lobes. This characteristic is not found anywhere else on the mainland, but is common amongst the Madagascan species. Of these Madagascan species, *S. ibityensis* and *S. revivescens* share certain characteristics with both *S. meyeri* and *S. rhodesianus*, leading Hilliard and Burtt (1971) to suspect that these species constitute the remnants of a historically larger species complex with a wider distribution. *S. meyeri* and *S. rhodesianus* are also similar in other ways. They are both rosulate plants that grow in open rocky places, and both possess unicellular hairs and spots in the corolla tube. However, they differ in that *S. meyeri* produces far longer and more robust fruit than does *S. rhodesianus*. Members of the *S. meyeri* alliance are also unlike the other members of Group B in not producing elongated stems or petiolodes in association with flowering, a characteristic that plays a key role in Group B, and their styles are also not much longer than their ovaries (Hilliard & Burtt 1971).

Within the *S. meyeri* alliance, *S. meyeri* is most strongly allied to *S. kentaniensis*, with *S. modestus* forming a more distant relative. *S. meyeri* and *S. kentaniensis* share many floral characteristics, including similar corolla shapes and colouration, both in terms of the contrast between the coloured corolla tube and white limbs, as well as the yellow colouring of the corolla tube floor, which is overlaid with violet lines and spots. They also possess similar styles and stigmas. However, their leaves are very different, although the foliage of *S. kentaniensis* is unique within *Streptocarpus*, and is consequently dissimilar to that of all the other species (Hilliard & Burtt 1971).

S. modestus constitutes a more distant member of the *S. meyeri* alliance, and links the alliance to Group C to a certain extent. The species is similar to *S. meyeri* in terms of corolla shape, as well as the coloured lines on the corolla tube floor that barely extend beyond the floral mouth. On the other hand, its violet flowers and excentric rosulate growth habit are more reminiscent of *S. rexii*, which is in Group C (Hilliard & Burtt 1971).

2.3.4.3. Subgenus *Streptocarpus,* Group C

This group contains centric and excentric rosulate perennials with leaves that are much longer than they are broad. Corolla colour is variable, but corolla shape and markings are quite uniform. All of the species possess the open-tubed floral type (which is, however, also widespread in Group A, and to a lesser extent in Group B), and the throat and lower lip are characteristically marked with three to nine (usually seven) purple lines. The style is somewhat flattened, and the stigma is unequally two-lipped. The fruit is typically long, frequently over 100 mm (Hilliard & Burtt 1971).

This group consists of the *S.* agg. *rexii* species i.e. *S. rexii* (SA), *S. primulifolius* (SA), *S. cyaneus* (SA) and *S. parviflorus* (SA), as well as *S. gardenii* (SA). Geographically, Group C extends in a crescent from the George-Knysna vicinity in the Western Cape province of South Africa in a gradually widening, coastal band up to Port St. Johns in the Eastern Cape, from where it begins to follow a more inland route in an arc through the Natal Midlands, Swaziland and up as far north as the Soutpansberg in Limpopo province of South Africa. *S. rexii*, *S. primulifolius*, *S. cyaneus* and *S. parviflorus* have been grouped together into *S.* agg. *rexii* on

the grounds that the areas between their distribution ranges tend to contain populations that are morphologically intermediate between the neighbouring species. Thus, the East London vicinity in the Eastern Cape is characterised by morphologically intermediate populations between *S. rexii* and *S. primulifolius*, Kranskop in KwaZulu-Natal contains a population intermediate between *S. primulifolius* and *S. cyaneus*, and it is difficult to distinguish between *S. cyaneus* and *S. parviflorus* in the Soutpansberg vicinity. *S. gardenii*, although considered to be closely related to the *S.* agg. *rexii* species, is nevertheless morphologically more distinct, and intermediates between *S. gardenii* and *S. rexii* appear to be more definitely of hybrid origin. The species constituting this group have always been believed to be closely related, and this group forms a well defined, but somewhat isolated entity within subgenus *Streptocarpus* with very few obvious linkages to the other groups (Hilliard & Burtt 1971).

There are a number of species that are morphologically intermediate between Groups B and C. The *S. meyeri* alliance shows some connections with Group C, although it appears to be more strongly affiliated with Group B. On the other hand, *S. montigena* (SA) is morphologically more intermediate between Groups B and C, and *S. caeruleus* (SA) appears to be a distant relative of Group C. Hilliard & Burtt (1971) hypothesised that the origins of *S. montigena* lie in a hybridization event between *S. meyeri* and *S. rexii*, as the species has the centric rosulate growth habit and vertical axis of *S. meyeri*, but the flower shape of *S. rexii*. In contrast, the affinities of *S. caeruleus* are less clear. Hilliard & Burtt (1971) included two forms under this species name, one with a short and one with a long floral tube. However, Edwards *et al*. (1992) has subsequently raised these two forms to specific level under the names *S. caeruleus* and *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards, respectively. The blue flowers and double yellow marks in the throat separate *S. caeruleus* from Group C, as *S. rexii* and allies typically have purple lines along the floral floor. These purple lines are, however, absent in one form of *S. cyaneus* (part of the *S. rexii* aggregate) and Hilliard & Burtt (1971) suspected that this is where the affinities of *S. caeruleus* lie.

2.3.4.4. Subgenus *Streptocarpus,* Group D

Members of this group are monocarpic unifoliates or plurifoliate perennials, with leaves no longer than twice their width. All of the members possess the keyhole floral type (this floral type is, however, present in one or two species in both Group A and Group B), with a narrow, curved floral tube and a mouth that is typically noticeably compressed from either side. The floral limb is held obliquely, and the lower lip sometimes possesses darker patches, but there is never a clear pattern of lines or spots. The style is shorter than the ovary, and the stigma possesses a gelatinous tip, and is mostly undivided, but rarely bilobed (Hilliard & Burtt 1971).

Group D contains no further subdivisions, and consists of *S. polyanthus* (SA)*, S. prolixus* C.B.Clarke (SA)*, S. silvaticus* Hilliard (SA)*, S. daviesii* (SA)*, S. pentherianus* Fritsch (SA)*, S. haygarthii* (SA) and *S. confusus* Hilliard (SA). This group also has no obvious linkages to the rest of subgenus *Streptocarpus* (Hilliard & Burtt 1971).

Two species, *S. johannis* (SA) and *S. baudertii* (SA), appear to be intermediates between Groups C and D. Both species have the keyhole floral type most typical of Group D, but they are both rosulates, and are more similar to the other rosulates of subgenus *Streptocarpus* in this respect. It has been observed that *S. johannis* resembles hybrids between *S. rexii* (Group C) and *S. polyanthus* (Group D). *S. johannis* has a similar corolla shape to Group D, but the coloured lines on its floral limb, its rosulate growth habit, strap-like leaves, and conspicuous horizontal rhizome are more similar to that of the *S. rexii* aggregate. In comparison, its stigma is intermediate between the two groups. The stigma of the *S. rexii* aggregate is unequally two-lipped, while that of Group D is subcapitate or, more rarely, bilobed. In *S. johannis*, the stigma is equally two-lipped. Thus, Hilliard & Burtt (1971) proposed that *S. johannis* arose as a hybrid

between Groups C and D, with *S. polyanthus* and *S. rexii* as the most likely parents. However, within *S. johannis*, they observed quite a difference between the plants occurring in its northern range compared to those in its southern range. The northern plants around Mt. Ngeli in southern KwaZulu-Natal are relatively small and less robust in stature, and their flowers are quite pale with relatively narrow floral lobes. In contrast, their southern counterparts around Port St. Johns are more robust, and their flowers are suffused with a rich purple colouring similar to the *S. primulifolius* plants that grow sympatrically with them. Based on these differences, Hilliard & Burtt (1971) suggested that *S. johannis* might have arisen more than once from separate hybridization events between the same parent species.

S. baudertii also has the keyhole floral morphology characteristic of Group D, but its growth habit is so similar to that of *S. meyeri* that non-flowering plants of the two species cannot be told apart. In both species, the leaves arise from a short, thick, vertical axis, and the leaves themselves are broadly elliptic with a dense indumentum. The striking similarities with Group D and *S. meyeri* led Hilliard & Burtt (1971) to propose that *S. baudertii* formed as the result of hybridization between *S. meyeri* and one of the members of Group D, perhaps *S. polyanthus*.

A number of observations can be made regarding the above groups. Madagascan species appear in all of Hilliard & Burtt's (1971) main groups except for Group C. Thus, from the morphological data it appears that there are many independent links between subgenus *Streptocarpus* on the African mainland and on Madagascar, implying either multiple dispersal events, or a prolonged linkage via a historical land bridge (Möller & Cronk 2001b) between the two landmasses.

A number of observations can be made regarding the above groups. Group B is the most variable of the four main groups in terms of growth habit and floral type, whereas Groups A, C and D are comparatively more uniform. Group A predominantly contains unifoliate, open-tubed species, members of Group C are all rosulates with open-tubed flowers, and all of the members of Group D possess keyhole flowers, either with a unifoliate or plurifoliate growth habit. In contrast, Group B contains all of the acaulescent growth forms (unifoliate, plurifoliate and rosulate), and all of the floral types found within subgenus *Streptocarpus* (open-tubed, pouch, keyhole [*S. nimbicola*], and the floral type produced by *S. dunnii*). Thus, while the open-tubed floral type is widely spread amongst Groups A, B, and C, the pouch, keyhole and *S. dunnii* types are much more group-specific. With regards to growth form, unifoliates and plurifoliates occur in Groups A, B and D, while the rosulate form is only found within Groups B and C, and the caulescent form only within Group B.

These differences in morphological variability are mirrored by the geographical range of the groups. Group B has the widest distribution, extending across the entire range of subgenus *Streptocarpus* from Ethiopia in the north down into South Africa in the south, and from the Democratic Republic of the Congo and Angola into Mozambique. Group A also has a large distribution range, extending from Angola, the Democratic Republic of the Congo and Tanzania down to South Africa. In contrast, Groups C and D have far more limited distributions, only occurring in South African and Swaziland. Group D extends from Soutpansberg in northern Limpopo down through Mpumalanga, Swaziland, KwaZulu-Natal and into the northern extremities of the Eastern Cape, while Group C has larger distribution range, in addition to these areas also occurring throughout most of the Eastern Cape and into the eastern-most part of the Western Cape.

Since Hilliard & Burtt (1971), 20 new species have been added (reinstated, raised to specific level or newly described) to *Streptocarpus* in Gilli (1973), Hilliard & Burtt (1975, 1986, 1990), Fischer (1988), Pócs (1991), Edwards *et al*. (1992), Hilliard (1992), Weigend & Edwards (1994a, 1994b), Burtt (1999), Edwards (2003), Bellstedt & Edwards (2004) and MacMaster *et*

al. (2005). Amongst these, Edwards *et al*. (1992) added another species, *S. fasciatus* T.J.Edwards & Kunhardt, to the *S. rexii* aggregate, and raised *S. caeruleus* subsp. *caeruleus* and *S. caeruleus* subsp. *longiflorus* Hilliard & B.L.Burtt to specific level (*S. caeruleus* Hilliard & B.L.Burtt and *S. longiflorus* (Hilliard & B.L.Burtt) T.J.Edwards). Weigend & Edwards (1994a) raised *S. primulifolius* subsp. *primulifolius* Gand. and *S. primulifolius* subsp. *formosus* Hilliard & B.L.Burtt to specific level (*S. primulifolius* Gand. and *S. formosus* (Hilliard & B.L.Burtt) T.J.Edwards, respectively), and described *S. floribundus* Weigend & T.J.Edwards, a group of plants that Hilliard & Burtt (1971) classified under *S. primulifolius*, but that Weigend & Edwards (1994a) considered intermediate between *S. primulifolius* and *S. cyaneus*. Weigend & Edwards (1994b) defined two new species, *S. roseo-albus* Weigend & T.J.Edwards and *S. fenestra-dei* Weigend & T.J.Edwards. They considered *S. roseo-albus* as very close to *S. cyaneus*, while *S. fenestra-dei* appeared to be intermediate between *S. cyaneus* and *S. parviflorus*. Edwards (2003) described two new species, one of which was *S. kunhardtii* T.J.Edwards, a species apparently closely allied with *S. roseo-albus*. Bellstedt & Edwards (2004) described *S. lilliputana* Bellstedt & T.J.Edwards, a species whose affinities were not obvious, but which appeared to be closely related to Group C (*S. gardenii* and its relatives). Additionally, *S. actinoflorus* T.J.Edwards & M.Hughes and *S. aylae* T.J.Edwards are still awaiting description (Edwards *et al*. 2008, in press). Thus, Group C has subsequently been expanded to accommodate another eight species, with two more soon to follow.

Analyses conducted after Hilliard & Burtt (1971), including Weigend & Edwards (1996) and Möller & Cronk (2001a), have however produced mixed results regarding species relationships in subgenus *Streptocarpus*. In Weigend & Edwards' (1996) palynological investigation of *Streptocarpus*, Madagascan and Comorian species possessed pollen types 11, 12 and 13; species from Group A were found to possess pollen types 4, 5, 10, 12, 13 and 16; Group B types 2, 4, 9, 11, 12 and 13, with the *S. meyeri* alliance having pollen type 13; *S. montigena*, *S. caeruleus* and Group C also possessed type 13; *S. johannis* was found to possess type 8; *S. baudertii* type 4; and Group D types 1 and 8 (the pollen types of Weigend & Edwards (1996) are listed in Table 2.1, and the pollen type of taxa included in Figure 2.12 are indicated to the right of the figure). Thus, Groups A and B are the most diverse, while the *S. meyeri* alliance and Group C were found to be palynologically homogenous. Pollen type 13 was mostly found in South African rosulate taxa, including Group C, the *S. meyeri* alliance, *S. montigena*, *S. caeruleus*, four members of Group B (amongst others *S. denticulatus* [Subgroup Ba] and *S. fanniniae* [Be]) and two members of Group A (including *S. grandis* [Ab]), but also in the Madagascan and Comorian species *S. variabilis*. This is congruent with the close relationship between the *S. meyeri* alliance and the *S. variabilis* that Hilliard & Burtt (1971) suspected, and also shows a recent divergence between the *S. meyeri* alliance and Group C. Weigend & Edwards (1996) proposed that the *S. meyeri* alliance, Group C and the *S. grandis* alliance, which all possess pollen type 13, form a coherent group based on palynology and phytogeography. Evolution amongst the South African rosulates appears to have happened recently and rapidly, not allowing enough time for palynological divergence to occur amongst the lineages.

Weigend & Edwards (1996) viewed pollen type 12 as close to type 13. Type 12 is found in a number of species from Groups A, amongst others *S. porphyrostachys* and *S. saundersii* from Subgroup Aa and *S. vandeleurii* from Ad, and five Group B species. In addition, ten species from subgenus *Streptocarpella* also possess pollen type 12. Weigend & Edwards (1996) regarded pollen types 10, 11, 15 and 16 as extreme variants of types 12 and 13, with these types differing from one another in the size and density of supratectate structures. They therefore viewed the *S. grandis* alliance, which ranges from East Africa to South Africa, as a focal point from which extensive radiation has taken place, linking many of the South African taxa to

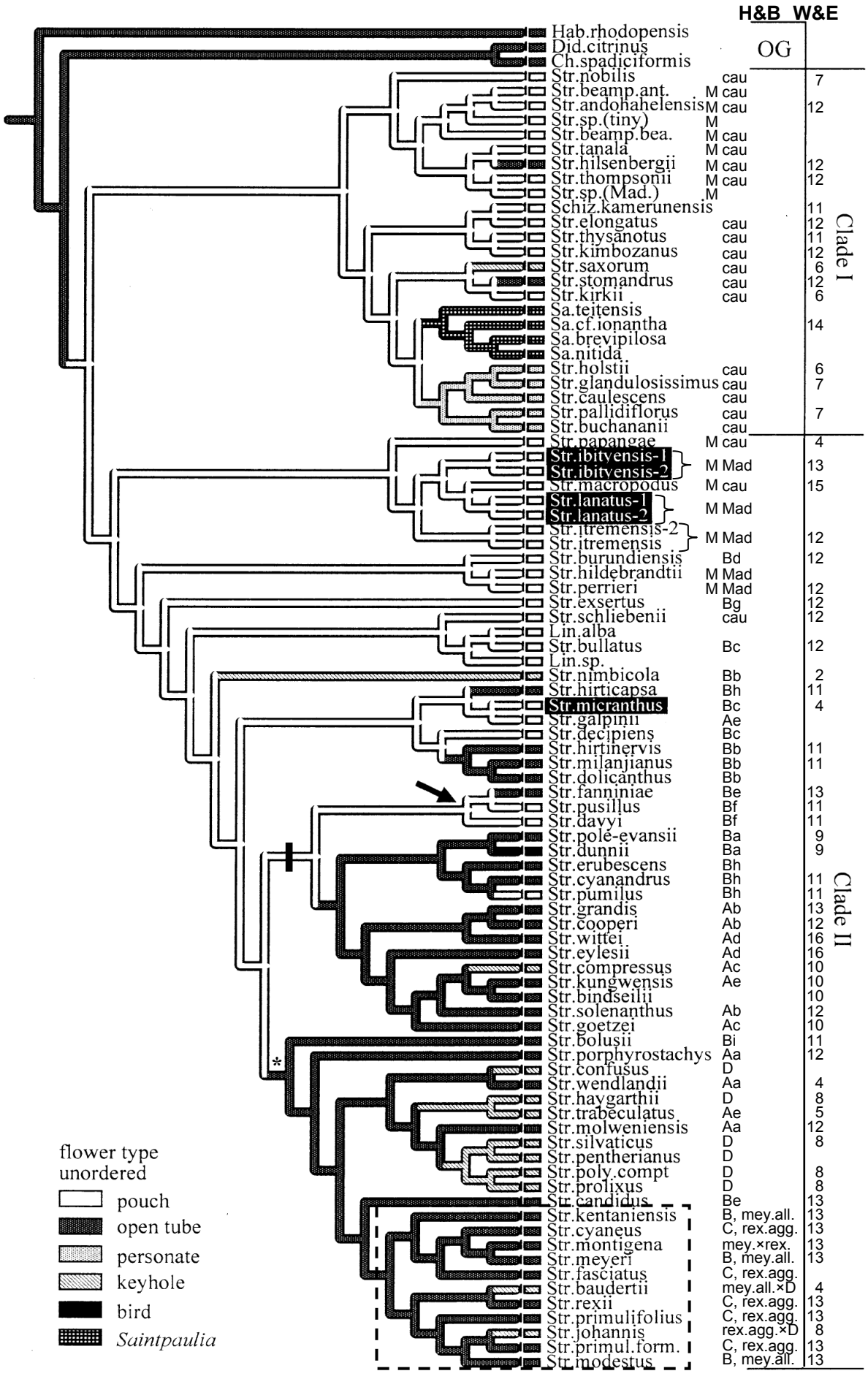
H&B W&E
OG
Hab.rhodopensis
Did.citrinus
Ch.spadiciformis
Str.nobilis cau 7
Str.beamp.ant. M cau
Str.andohahelensis M cau 12
Str.sp.(tiny) M
Str.beamp.bea. M cau
Str.tanala M cau
Str.hilsenbergii M cau 12
Str.thompsonii M cau 12
Str.sp.(Mad.) M
Schiz.kamerunensis 11
Str.elongatus cau 12
Str.thysanotus cau 11
Str.kimbozanus cau 12
Str.saxorum cau 6
Str.stomandrus cau 12
Str.kirkii cau 6
Sa.tejtensis
Sa.cf.ionantha 14
Sa.brevipilosa
Sa.nitida
Str.holstii cau 6
Str.glandulosissimus cau 7
Str.caulescens cau
Str.pallidiflorus cau 7
Str.buchananii cau
Str.papangae M cau 4
Str.ibityensis-1
Str.ibityensis-2 M Mad 13
Str.macropodus M cau 15
Str.lanatus-1
Str.lanatus-2 M Mad
Str.itremensis-2
Str.itremensis M Mad 12
Str.burundiensis Bd 12
Str.hildebrandtii M Mad
Str.perrieri M Mad 12
Str.exsertus Bg 12
Str.schliebenii cau 12
Lin.alba
Str.bullatus Bc 12
Lin.sp.
Str.nimbicola Bb 2
Str.hirticapsa Bh 11
Str.micranthus Bc 4
Str.galpinii Ae
Str.decipiens Bc
Str.hirtinervis Bb 11
Str.milanjianus Bb 11
Str.dolicanthus Bb
Str.fanniniae Be 13
Str.pusillus Bf 11
Str.davyi Bf 11
Str.pole-evansii Ba 9
Str.dunnii Ba 9
Str.erubescens Bh
Str.cyanandrus Bh 11
Str.pumilus Bh 11
Str.grandis Ab 13
Str.cooperi Ab 12
Str.wittei Ad 16
Str.eylesii Ad 16
Str.compressus Ac 10
Str.kungwensis Ae 10
Str.bindseilii 10
Str.solenanthus Ab 12
Str.goetzei Ac 10
Str.bolusii Bi 11
Str.porphyrostachys Aa 12
Str.confusus D
Str.wendlandii Aa 4
Str.haygarthii D 8
Str.trabeculatus Ae 5
Str.molweniensis Aa 12
Str.silvaticus D 8
Str.pentherianus D
Str.poly.compt D 8
Str.prolixus D 8
Str.candidus Be 13
Str.kentaniensis B, mey.all. 13
Str.cyaneus C, rex.agg. 13
Str.montigena mey.×rex. 13
Str.meyeri B, mey.all. 13
Str.fasciatus C, rex.agg.
Str.baudertii mey.all.×D 4
Str.rexii C, rex.agg. 13
Str.primulifolius C, rex.agg. 13
Str.johannis rex.agg.×D 8
Str.primul.form. C, rex.agg. 13
Str.modestus B, mey.all. 13
Clade I
Clade II
flower type
unordered
pouch
open tube
personate
keyhole
bird
Saintpaulia

Figure 2.12: The tree from Figure 2.11, showing the evolution of floral types in *Streptocarpus*, with the formal and informal morphological groupings and hypothesised hybrid origins proposed by Hilliard & Burtt (1971) given under the heading H&B, and the pollen types of Weigend & Edwards (1996) provided under W&E. Species that were raised to specific level or reinstated after Hilliard and Burtt (1971) are assigned to the same morphological group as the species in which they were classified by Hilliard and Burtt (1971), and species that have been newly described since Hilliard and Burtt (1971) are assigned to the same group as the species with which they are believed to share the closest relationships by their respective authors. Within the tree, the black, vertical bar denotes the "ITS2 deletion clade", a group of taxa sharing a *ca*. 40 bp deletion, while the * marks a monophyletic clade containing only South African species. OG points to the outgroup taxa, M marks species endemic to Madagascar (and the Comoro Islands) with Mad indicating Madagascan species classified under subgenus *Streptocarpus* by Hilliard & Burtt (1971), and cau indicates taxa placed in subgenus *Streptocarpella* by Hilliard & Burtt (1971). Other abbreviations are as follows: mey. for *S. meyeri*, rex. for *S. rexii*, all. for alliance and agg. for aggregate. Genus abbreviations are the same as in Figure 2.5. The Cape Primrose clade is enclosed in a dashed box.

species from further north into a single, coherent group. Pollen type 10 occurs in three species from Group A; 11 was found in the two *Hovanella* species, *Schizoboea kamerunensis*, *Streptocarpus tsimihetorum* from Madagascar and 14 species from Group B, including *S. montanus* (Bb), *S. pusillus* (Bf), *S. rimicola* (Bf) and *S. bolusii* (Bi); pollen type 15 occurs in two subgenus *Streptocarpella* species; and 16 in two species from Group A. Thus, pollen types 10, 11, 12, 13, 15 and 16 together encompass most of Group A (except for *S. wendlandii* [Aa] and *S. trabeculatus* [Ae]), most of Group B (except for *S. pole-evansii* [Ba], *S. pogonites* and *S. nimbicola* [Bb] and *S. micranthus* [Bc]), the whole *S. meyeri* alliance, *S. montigena*, *S. caeruleus*, and the whole of Group C, as well as all of the Madagascan and Comorian subgenus *Streptocarpus* species and some species from subgenus *Streptocarpella*.

In contrast, most of Group D, including *S. polyanthus, S. prolixus, S. silvaticus* and *S. haygarthii* formed a distinct group of species possessing pollen type 8, confirming the morphological data. On the other hand, *S. daviesii* possesses its own unique pollen type (type 1) forming tetrads. The only other species with type 8 pollen is *S. johannis*, congruent with Hilliard & Burtt's (1971) belief that a member of Group D was one of its hybrid parents. In contrast, *S. baudertii* possesses pollen (type 4) unlike any of its suspected relatives, sharing the same type with *Acanthonema strigosum*, *Trachystigma mannii*, the two Asian species *S. orientalis* and *Boea clarkeana*, five members of subgenus *Streptocarpella*, including *S. papangae*, one species from Group A and two from Group B. In spite of this, the pollen of *S. baudertii* (type 4) and *S. johannis* (type 8) only differ in lumina size (Weigend & Edwards 1996), and are therefore similar even though they possess pollen of different types.

Thus, the palynological revision of Weigend & Edwards (1996) confirmed some of Hilliard & Burtt's (1971) groupings, while being in conflict with others. Group C and D appear to be reasonably coherent groups, although the pollen characteristics are not evolving fast enough to determine relationships between Group C and other closely related species. In contrast, Groups A and B, which are morphologically the most diverse and have the largest geographical ranges, are also palynologically the most heterogeneous.

The nuclear ITS sequence analysis of Möller & Cronk (2001a) was also not entirely congruent with the morphological groups of Hilliard & Burtt (1971). Nevertheless, although none of the groups formed monophyletic clades, representatives from the same group did tend to emerge together (Figure 2.5, shows the ITS tree from Möller & Cronk (2001a), and Figure 2.12, shows a slightly expanded ITS tree from Hughes *et al*. (2006) with the morphological groupings of Hilliard & Burtt (1971) indicated to the right for the included taxa). Thus, the Madagascan subgenus *Streptocarpus* species and members of Groups A and B arose intermingled towards the base of Clade II as a paraphyletic group constituting the more northerly members of subgenus *Streptocarpus*. Within this part of the tree is the ITS2 deletion clade (marked by a vertical bar in the Figure 2.12), which contains a paraphyletic Group B and a monophyletic

Group A clade. In contrast, all the representatives of the *S. meyeri* alliance and Groups C and D arose along with a few members of Group A (*S. porphyrostachys*, *S. wendlandii*, *S. trabeculatus*, *S. molweniensis*) and Group B (*S. bolusii* and *S. candidus*) in the monophyletic clade containing only South African members of the subgenus. This latter clade (indicated by an * in Figure 2.12) contains the strongly supported Cape primrose clade, within which the representatives of the *S. meyeri* alliance are interspersed amongst all of the members of Group C included in the analysis i.e. *S. cyaneus*, *S. rexii*, *S. primulifolius* and *S. formosus*, as well as *S. montigena* (suspected of having originated as a hybrid between *S. meyeri* and *S. rexii* by Hilliard & Burtt 1971), *S. baudertii* (suspected of being of hybrid origin between *S. meyeri* and a member of Group D by Hilliard & Burtt 1971), and *S. johannis* (believed by Hilliard & Burtt 1971 of being the result of hybridization event(s) between the *S. rexii* aggregate and Group D). Thus, from this analysis it appears that the Madagascan species lineage and Groups A and B diverged first within subgenus *Streptocarpus*, followed by Group D, which evolved from within these groups. The *S. meyeri* alliance and Group C also evolved from within one of these groups, perhaps from *S. candidus* (Subgroup Be).

The Cape primrose clade thus contains members of Hilliard & Burtt's (1971) *S. meyeri* alliance, Group C, as well as the intermediate species that were believed by them to be of hybrid origin with members from one of these two groups as a parent (*S. montigena*, *S. johannis* and *S. baudertii*). These species occupy the southernmost extreme of the geographical range of *Streptocarpus*, and possess a centric or excentric rosulate growth habit with rounded (*S. meyeri* and *S. baudertii*) to strap-like leaves (at it most extreme in *S. kentaniensis*, but members of Group C also tend to have leaves that are much longer than they are broad) and open-tubed or keyhole flowers. Although this clade is mostly compatible with morphological (Hilliard & Burtt 1971) and palynological (Weigend & Edwards 1996) data, and is strongly supported in the ITS analysis carried out by Möller & Cronk (2001a), relationships within this clade were not resolved, and are therefore unclear.

Although the morphological groupings of Hilliard & Burtt (1971), the pollen types of Weigend & Edwards (1996) and the ITS phylogeny of Möller & Cronk (2001a) are not in fully congruent with one another, these independent lines of evidence caused these three studies to draw many of the same conclusions regarding the evolution of *Streptocarpus*, as is evident in the following and last section.

2.3.5. Biogeography of *Streptocarpus*

2.3.5.1 Origin and early radiation of *Streptocarpus*

Streptocarpus has in some respects a puzzling distribution. The genus is not only widespread on the African mainland; close to a third of its species are endemic to Madagascar, a further two species are found both on Madagascar and the Comoros Archipelago, and three Asian species are also currently classified within the genus (although their relationship(s) to the rest of the genus are more tenuous). Both the caulescent and acaulescent taxa occur in central Africa and Madagascar, while the acaulescent taxa also extend from Ethiopia down through eastern Africa and into southern Africa (Hilliard and Burtt 1971). The species found on Madagascar are vegetatively highly diverse. Not only are both caulescent and acaulescent growth forms found on the island, but some Madagascan taxa possess growth forms not found on the African mainland (Hilliard & Burtt 1971; Möller & Cronk 2001a). In contrast, floral diversity amongst the Madagascan taxa is minimal, with the pouch floral type predominating (Hughes *et al*. 2006; Figure 2.11). This paucity of floral diversity amongst the Madagascan species has been attributed to a lack of pollinating insects on Madagascar by Brian L. Burtt (personal communication to Mark Hughes, as referred to in Hughes *et al*. 2006). While the species on

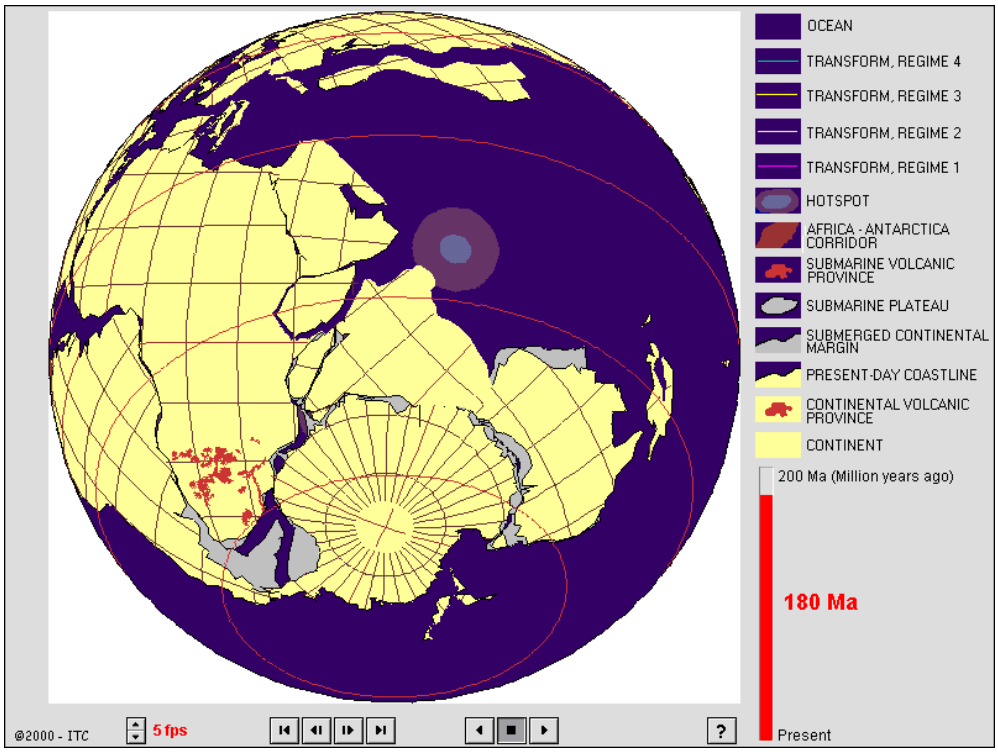

Figure 2.13: The position of the present-day continents within Gondwana. Figure copied from the website of Prof Marten de Wit, University of Cape Town.

Madagascar share a few similarities with some of the mainland species, especially in subgenus *Streptocarpella*, exact affinities are often uncertain or ambiguous. Amongst the acaulescent species on the African mainland, there is a steady increase in species density from north to south, with the highest species densities occurring in the Eastern Cape and KwaZulu-Natal in South Africa (Hilliard & Burtt 1971). These distribution patterns, however, make more sense when the geological, climatological and vegetation history of the African continent is taken into account.

Madagascar and Africa were once part of the southern supercontinent Gondwana, which formed 700 to 500 million years ago (mya) during the late Precambrian period (Figure 2.13). The continent extended from the South Pole to the equator, and alternated between having a reasonably mild climate during warmer periods, and being largely covered in ice sheets during colder periods. The supercontinent also consisted of the land masses forming present-day Arabia and India, which are now both located in the Northern Hemisphere, as well as the predominantly Southern Hemisphere landmasses South America, Antarctica, Australia, New Guinea and New Zealand. About 300 mya, Gondwana merged with Laurentia, Baltica, Siberia and other land masses, and by approximately 170 mya, all of the continents formed a huge, single landmass, Pangaea, which reached almost from the North to the South Pole. Within Pangaea, Madagascar was nestled between present-day Somalia, Kenya and Tanzania on the east coast of the African mainland, and present-day India. The Pangaea breakup began during the early Jurassic period, and this was shortly followed by the fragmentation of the re-formed Gondwana as a result of a huge mantle plume during the mid Jurassic period. This was accompanied by a steady northward movement of Gondwana during the Jurassic and Cretaceous periods. The fragmentation of Gondwana was initiated when Madagascar-India-Antarctica-Australia-New Zealand broke away from Africa-South America and started to move in a southerly direction, in the process forming the beginnings of the Indian Ocean about 140 mya. Next came the simultaneous separation of Africa from South America to form the early Atlantic Ocean, and Antarctica-Australia from India-Madagascar during the early

Cretaceous period, 135–120 mya (McCarthy & Rubidge 2005). During the late Cretaceous (95–80 mya), New Zealand detached from Australia-Antarctica, Australia broke away from Antarctica, and India split off from Madagascar, slowly drifting northwards to collide with Asia approximately 50 mya during the Eocene epoch (McCarthy & Rubidge 2005). Africa moved progressively northwards to reach its current position about 30 mya during the Oligocene epoch (McCarthy & Rubidge 2005). Thus, Madagascar detached from the African mainland during the earliest phases of the breakup of Gondwana. In contrast, the Comoro Islands are much younger, having progressively arisen as a result of volcanic eruptions between 8 mya and 100 000 years ago (Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al*. 1986).

The incredible morphological diversity, including the presence of both the caulescent and acaulescent growth forms of *Streptocarpus*, evident on both the African mainland and on Madagascar led Hilliard & Burtt (1971) to suggest that the genus as a whole, as well as the two lineages possessing these two growth forms, evolved before the breakup of Gondwana. Additionally, the previous, more northerly position of Madagascar within Gondwana i.e. attached to present-day Somalia, Kenya and Tanzania, would place the extant Madagascan species closer to the current localities of their closest allies within *Streptocarpus* i.e. *Saintpaulia*, members of subgenus *Streptocarpella*, and some of the northern species of subgenus *Streptocarpus* (Hilliard & Burtt 1971). Weigend & Edwards (1996) narrowed down the origin of the genus *Streptocarpus* to northern Malawi, Tanzania and Madagascar, due to the presence here of both subgenera and most of the other African genera (*Schizoboea*, *Saintpaulia*, *Colpogyne* and *Hovanella*), and the palynological coherence of subgenus *Streptocarpella* and subgenus *Streptocarpus* in this area. A good candidate for the area of origin of *Streptocarpus* within this region is the Eastern Arc Mountains. These mountains and hills together form a diagonal chain through Tanzania and into southern Kenya, and are mostly covered in mesic forests. Evidence from various sources (Lovett & Friis 1996; Emberton *et al*. 1997; Hochkirch 1998; Wilkinson *et al*. 2002; Fuchs *et al*. 2005) suggests that these mountain blocks have been climatically stable since the mid Tertiary period, and perhaps even earlier, and they are believed to be the sites of extraordinarily high levels of speciation of many taxa, including *Impatiens* L., *Begonia* L. and *Saintpaulia* (Burgess *et al*. 2007). This area would therefore have provided an ideal habitat for the origin and early radiation of *Streptocarpus*. The Madagascan and African taxa are each both morphologically (Hilliard & Burtt 1971) and partly palynologically (Weigend & Edwards 1996) distinct from each other, further supporting an ancient separation of the species occurring on these two landmasses. Members of both subgenera are present in central Africa and Madagascar, whereas southern Africa only contains members of subgenus *Streptocarpus*. Based on this, Hilliard & Burtt (1971) deduced that *Streptocarpus* arose towards the northern end of its distribution range, before radiating southwards.

After the divergence of *Streptocarpus* to form the caulescents and acaulescents, the caulescent taxa radiated in an east-west direction, remaining in tropical central Africa and Madagascar. In contrast, the acaulescent taxa have evolved the ability to survive unfavourable periods of the year by abscising the distal parts of their leaves, and were consequently able to radiate into regions with warm, wet summers, but cold, dry winters (Hilliard & Burtt 1971). The distribution of morphological characters within groups of closely related species of the acaulescent taxa, and the presence of what appear to be only a few, relictual acaulescent species in central Africa caused Hilliard & Burtt (1971) to propose that subgenus *Streptocarpus* evolved in the north, and radiated steadily southwards, before reaching South Africa (Hilliard & Burtt 1971). Hilliard & Burtt (1971) quoted Briden (1967) who postulated that the equator extended obliquely across Africa from Ghana to the Red Sea during the late Cretaceous. Hilliard & Burtt (1971) proposed that subgenus *Streptocarpus* migrated progressively southwards in response to the southerly movement of the equator in Africa caused by

continental drift, leaving behind it a few relict species in the north. More recent evidence, however, shows that the position of the equator after the Cretaceous remained largely unchanged (Anderson 2001), indicating that this is not the reason for the southerly migration. Hilliard & Burtt (1971) further postulated that KwaZulu-Natal forms a recent centre of diversification of the genus rather than the area of origin of subgenus *Streptocarpus* (which might be suspected due to the incredible diversity of species in South Africa). Weigend & Edwards (1996), however, cautioned that the South African *Streptocarpus* taxa should not be considered to be very young, as they are palynologically already quite diverse. The Northern and Eastern Transvaal provinces (presently called the Limpopo and Mpumalanga provinces, respectively) contain many disjunctions in the distribution of closely related taxa, leading Hilliard & Burtt (1971) to infer that this area had undergone relatively dramatic climatic fluctuations in the past, probably during the Quaternary period. Thus Hilliard & Burtt (1971) speculated that *Streptocarpus* arose in the north and diverged into the caulescent and acaulescent forms before the Gondwana brakeup. They further postulated that subgenus *Streptocarpus* then migrated southwards, undergoing some extinctions in the north of South Africa, and diversifying within KwaZulu-Natal. Unfortunately, Hilliard & Burtt (1971) had no way of placing an age on the genus, and were therefore not able to evaluate the validity of their proposals.

Möller & Cronk (2001b) used the ITS phylogeny from their previous study (Figure 2.5; Möller & Cronk 2001a) to reconstruct the past biogeographical patterns of *Streptocarpus*. Unfortunately, similarly to Hilliard & Burtt (1971), they were unable to establish unequivocally the place of origin of the genus i.e. whether *Streptocarpus* evolved in Central Africa or on Madagascar. However, by comparing the branch lengths from dated ITS phylogenies of other taxa (Suh *et al*. 1993; Sang *et al*. 1994; Wendel *et al*. 1995) with the branch lengths in their own analysis, Möller & Cronk (2001b) estimated that *Streptocarpus* probably did not evolve earlier than 50 mya. This age is more realistic when taking into account that Lamiales and Gesneriaceae are both believed to have arisen during the Cretaceous period, Lamiales 106–74 mya (Bremer *et al*. 2004; Wikström *et al*. 2001, respectively), and Gesneriaceae a little after 78 mya (Bremer *et al*. 2004). This estimated age of *Streptocarpus*, however, places the origin of the genus long after the breakup of Gondwana, raising the problem of how the genus spread between Africa and Madagascar. Möller & Cronk (2001b) ruled long-distance dispersal out, seeing as *Streptocarpus* displays no special adaptations for dispersing its seeds over long distances. They argued that plants grow in habitats sheltered from strong winds, and *Streptocarpus* seeds, although very small, are not fine enough to be carried far by the wind, and do not possess any special adaptations to animal dispersal. The seed coat is also not strong enough to survive the passage through the gut of an animal. However, evidence has been uncovered that a land bridge might have existed between Madagascar and Africa 45–26 mya (McCall 1997), a time range that correlates well with the estimated age of *Streptocarpus* based on the average ITS substitution rates. The two subgenera likely also evolved before the land-bridge connection between the African mainland and Madagascar disappeared (Möller & Cronk 2001b). Post-Gondwana dispersal events between Africa and Madagascar are not uncommon, having already been recorded in animals such as frogs (Vences *et al*. 2003, 2004), chameleons (Raxworthy *et al*. 2002), snakes (Nagy *et al*. 2003) and lemurs (Yoder & Yang 2004), and in plants such as Asclepiadoideae Burnett (Apocynaceae Juss.; Liede & Meve 2002), Melastomataceae Juss. (Renner 2004) and the orchid genus *Disa* P.J. Bergius (Orchidaceae Juss.; Linder & Kurzweil 1999), and *Streptocarpus* may be another example of such dispersal events. It is, however, unclear as to whether *Streptocarpus* travelled between the two landmasses once, or a few times. Madagascan species emerged in three different places in Möller & Cronk's (2001b) ITS tree. There was, however, no significant difference in tree length between the unconstrained analysis and the tree constructed forcing the Madagascan and Comoros Archipelago taxa to emerge as a monophyletic group relative to the rest of the genus.

In spite of this, the presence of both growth forms on both landmasses, and the shortest trees showing three separate colonisations, one for the caulescents (the clade receiving 100% bootstrap support) and two for the acaulescents (neither clade very strongly supported), do indicate that dispersals between the two landmasses probably occurred two or three times, or that independent lineages migrated over a land bridge at more or less the same time. Having stated this, the fact that no species are shared between the two landmasses indicates that these events are extremely rare, and have not happened recently (Möller & Cronk 2001b).

Möller & Cronk (2001b) unfortunately did not include *Streptocarpus* samples from the Comoro Islands in their analysis, and they were therefore not able to deduce the affinities of the Comoros Archipelago taxa to the rest of *Streptocarpus*. They did, however, include a Madagascan sample of one of the two species (*S. thompsonii* R.Br.) that are spread over both Madagascar and the Comoro Islands, and in their previous study (Möller & Cronk 2001a) they also generated a partial sequence of the other species occurring in both places, *S. variabilis* Humbert. *S. thompsonii* emerged within the caulescent Madagascan clade, whereas the partial sequence of *S. variabilis* grouped together with acaulescent Madagascan samples, suggesting that *Streptocarpus* also spread to the Comoros Archipelago on at least two separate occasions, probably from Madagascar. The oldest island, Mayotte, is no more than 10 million years old (Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al*. 1986), by which time the land bridge had disappeared, and long-distance dispersal therefore seems to be the only mechanism by which the two Comoro Islands species could have reached the archipelago (Möller & Cronk 2001b). This would entail dispersal of about 500 km from Madagascar, or 450 km from the African mainland.

Möller & Cronk (2001b) also provided molecular data in support of the progressive southerly radiation of subgenus *Streptocarpus*, with more recently evolved clades in their tree having progressively more southerly distribution ranges (Figure 2.14). The ability of most of the acaulescent taxa to abscise their leaf tips has enabled subgenus *Streptocarpus* to occupy habitats not suitable for subgenus *Streptocarpella*. Möller & Cronk (2001b), however, attributed the complex distribution patterns evident in subgenus *Streptocarpus* to changes in forest cover caused by climatic fluctuations during the Miocene and Pleistocene epochs (Coetzee 1978). Warm periods would promote the expansion of forest patches, thereby enabling the accompanying subgenus *Streptocarpus* taxa to expand their ranges and radiate, while colder periods would induce the fragmentation of the forests, causing extinction and allowing allopatric speciation to take place. Evidence assembled from various sources does indeed show that Africa has been exposed to variable climatic conditions during the Earth's recent history, which probably had dramatic effects on forest cover and therefore also *Streptocarpus*.

2.3.5.2. Climate and vegetation over the last 65 million years in eastern and southern Africa

Little is known about past climates, but evidence gleaned from various sources has uncovered general trends. Inferred sea-surface temperatures, which reflect global trends in temperature, indicate a progressive reduction since the end of the Cretaceous period 65 mya (Figure 2.15). Within the Cenozoic era (65 mya to present), temperatures rose slightly to peak at about 55 mya, before falling again. The opening of the Drake Passage between South America and Antarctica about 35 mya initiated the Antarctic Circumpolar Current. This isolated Antarctica from the warmer northern waters causing the growth of the Antarctic Ice Sheet 32–25 mya, which in turn caused the southern oceans to cool from about 23 mya onwards. The last 20 million years have seen the uplift of southern Africa (the African Super Swell), predominantly the eastern side, as a result of a mantle plume, and most of southern Africa consequently lies at heights in excess of 1 000 m today (Figure 2.16). The uplift is believed to have occurred in two

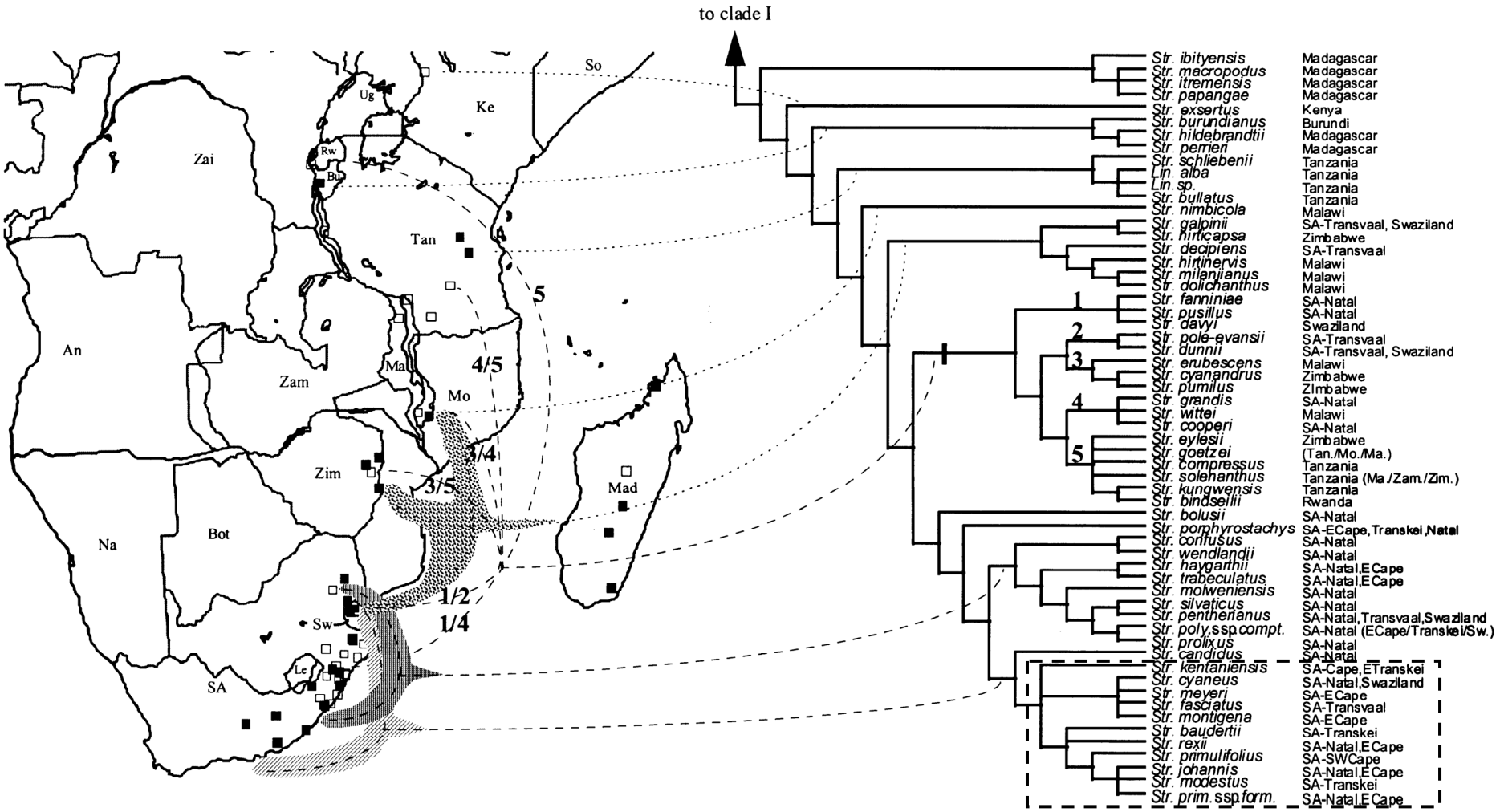

Figure 2.14: Clade II from Figure 2.5, with the geographical range of the clades indicated to illustrate the progressive southerly migration of subgenus *Streptocarpus* down the east coast of Africa into South Africa. Genus abbreviations are the same as for Figure 2.5. Locality abbreviations are as follows: ECape for the Eastern Cape Province, ETranskei for eastern Transkei, Ma. for Malawi, Mo. for Mozambique, SA for South Africa, Sw. for Swaziland, SWCape for the ?, Tan. for Tanzania, Zam. for Zambia, and Zim. for Zimbabwe. The Cape Primrose clade is enclosed in a dashed box. Diagram copied from Möller & Cronk (2001b).

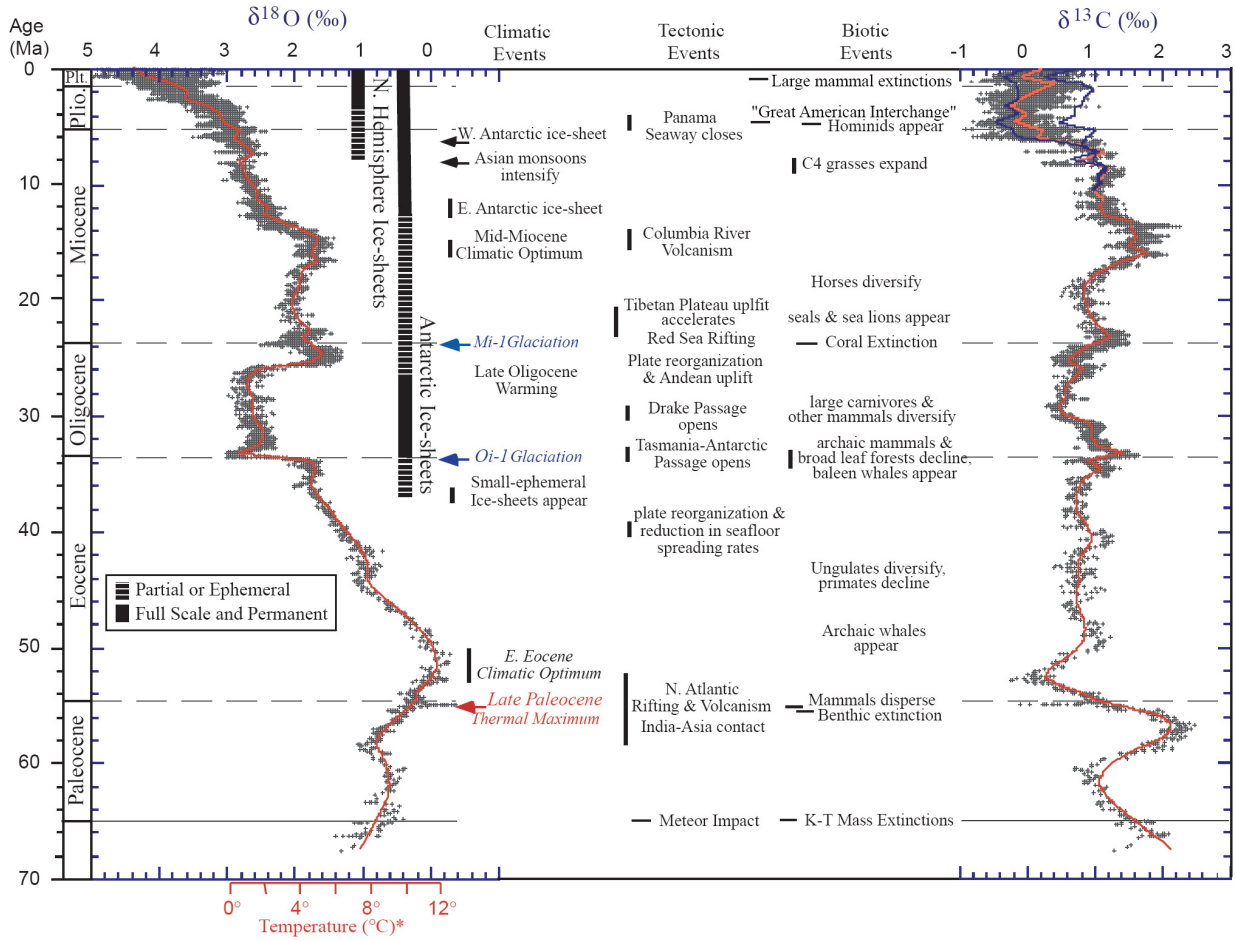

Figure 2.15: Sea-surface temperatures and the growth of ice sheets over the past 70 million years inferred from the ratio of oxygen isotopes (δ ^{18}O) found in deep-ocean cores. The duration and intensity (solid bars for >50% of present ice cover and broken bars for <50% of present ice cover) of ice sheets in each hemisphere is indicated by bars. The duration of major climatic, tectonic and biotic events are given in the middle. The curves to the right showing the ratios of carbon isotopes (δ ^{13}C) in the Pacific (red curve) and Atlantic (blue curve) Oceans are an indication of changes in ocean productivity and deep-sea circulation patterns caused by continental drift. δ ^{13}C in the Pacific and Atlantic Oceans only started to deviate from each other from the mid-Miocene onwards. Figure copied from Zachos *et al*. (2001).

main stages, one 20 mya and the other 5 mya. These uplifts substantially increased the height of the eastern escarpment, resulting in less rain reaching the interior from the Indian Ocean, but more rain falling along the East Coast. Another factor also changed precipitation patterns in southern Africa. For most of the Cenozoic era, warm, humid air was flowing into southern Africa from both the Indian and Atlantic Oceans, providing relatively moist conditions to both sides of the subcontinent. However, about14 mya, a strong high-pressure system settled over the South Atlantic Ocean, establishing the Benguela Upwelling System. Global temperatures also took another dip at around this time (McCarthy & Rubidge 2005). The Benguela Upwelling System steadily intensified and Atlantic sea-surface temperatures declined markedly after about 3.2 mya, with periods of noticeable intensification and even cooler sea-surface temperatures at about 2 mya and 0.6 mya (Marlow *et al*. 2000). The cooling of the South Atlantic Ocean and the uneven uplift of southern Africa led to an east-west rainfall gradient, causing the aridification of the interior and the West Coast. Global temperatures took their final, dramatic dip towards recent temperature ranges from 2 mya onwards. The temperature of the last one million years has been cooler than at any time since the dinosaurs. The Quaternary period (1.64 mya to present) has been characterized by frequent and regular glacials punctuated by interglacials. Thus colder periods have been the norm during this time, interspersed with

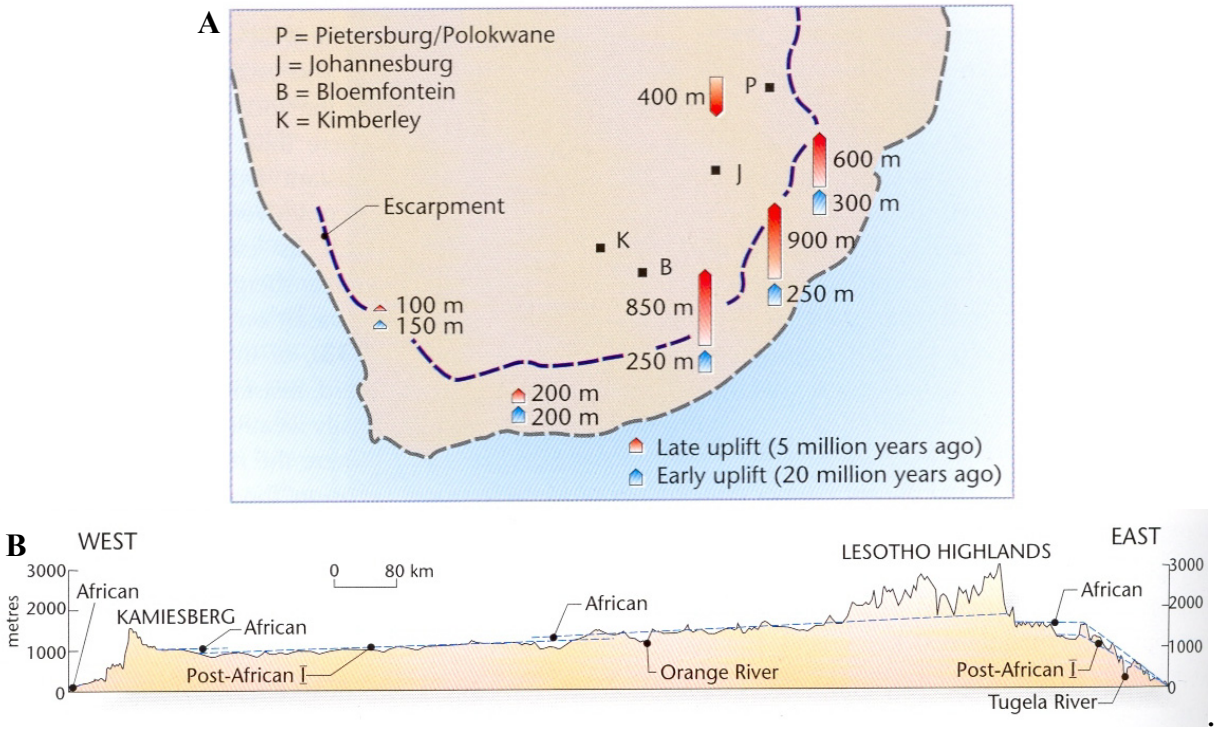

Figure 2.16: The results of the African Super Swell that mainly occurred 20 and 5 million years ago on the elevation of **A** the South African land surface and on **B** a cross-section through the subcontinent. Figures copied from McCarthy & Rubidge (2005).

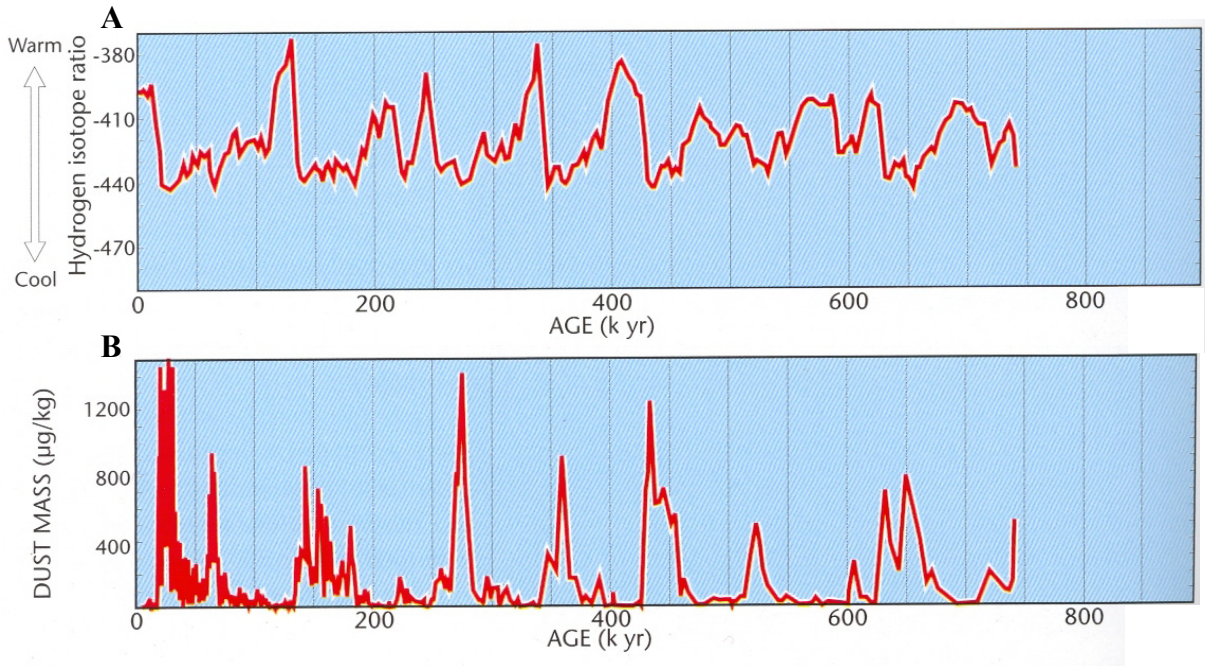

Figure 2.17: Fluctuations in **A** temperature and **B** aridity over the past 740 000 years in the southern hemisphere inferred from hydrogen isotope ratios and dust levels in an Antarctic ice core. Figures copied from McCarthy & Rubidge (2005).

brief warmer interludes (Figure 2.17). The ice ages led to lower rainfall, which caused more arid conditions in southern Africa, resulting in large deserts in the interior spreading as far north as the Congo (Figure 2.17). The most recent ice age peaked at about 18 000 years ago, with ice

sheets finally retracting from the northern hemisphere continents only 10 000 years ago (McCarthy & Rubidge 2005). The current interglacial has therefore seen the brief return of relatively warm, moist conditions to a usually colder, more arid southern Africa (McCarthy & Rubidge 2005).

DeMenocal (2004) investigated African climatic trends over the past five million years (the Pliocene, Pleistocene and Holocene epochs) in greater detail, and the effects that these have had on mammalian evolution. He found that the climatic variability in subtropical Africa has been increasing in abrupt step-like stages over this period, and that Africa has been becoming steadily more arid since about 2.8 mya. This pattern seems to be correlated with the onset and amplification of the glacial cycles of the higher latitudes, with cooler, drier conditions prevailing in Africa during glacial maxima. Before 3 mya, conditions were more mesic, and the climate in subtropical Africa was alternating between wetter and drier periods in reasonably mild 19 000 to 23 000 year cycles (Figure 2.18). These cycles were mainly driven by orbital precession (the gradual, circular change in the orientation of the Earth's axis of rotation relative to inertial space due to gravitational forces acting on the Earth's equatorial bulge from the Sun and the Moon, and to a much lesser extent from Jupiter and Saturn), which completes a cycle every 21 000 years. However, terrestrial events were soon to alter these climatic patterns. The closure of the Isthmus of Panama finished about 3 mya, cutting off interchange between the Pacific and Atlantic Oceans. This redirected ocean currents, and consequently heat distribution and precipitation patterns, and probably contributed towards the gradual onset of glacial cycles about 2.8 mya. The period between 2.8 and 1.7 mya was characterised by modest 41 000 year cycles and a gradual increase in aridity in Africa. However, conditions became cooler about 1.7 mya, and the 41 000 year cycles increased in amplitude i.e. the climate alternated between more extreme periods. Since about 1 mya, African climatic cycles have mainly been driven by the Northern Hemisphere glacials and interglacials (a full astronomical theory of which was first compiled by Milankovitch for the Pleistocene in 1920 and 1941 (Berger 1988)), and have therefore been occurring in 100 000 year cycles. Superimposed upon these prevailing 19 000–23 000, 41 000 and 100 000 year cycles have been 10 000–100 000 year changes in the amplitude of African palaeoclimatic variability. These changes in the extent of variability are driven by the cyclical change in the Earth's orbital eccentricity i.e. the changing shape of the Earth's orbit around the Sun from an almost circular path to a more elongated path caused by gravitational interactions of the Earth with Jupiter and Saturn. Orbital eccentricity determines seasonal solar insolation (solar radiation received by the Earth's surface). In summary, Africa had a less variable, more mesic climate that was paced by orbital precession before the high-latitude ice sheets became large enough to sustain the glacial cycles. Since then, the African climate has chiefly changed in response to the glacial cycles, and has been becoming progressively more variable and arid (DeMenocal 2004).

Examination of the fossil record of mammalian lineages reveals a reasonably close correlation between the climatic variability patterns detected by DeMenocal (2004) and accelerated evolutionary rates. Vrba (1995) compared appearances and disappearances of antelope taxa in the fossil record with what one would expect under a null hypothesis of uniform rates of turnover (rates of speciation and extinction), and detected a noticeable deviation from the null hypothesis 2.7–2.5 mya, with lesser deviations at 1.8 and 0.7 mya (Figure 2.19). Additionally, many of the antelope species that first appeared 2.7 mya were adapted to more arid environments than those from before. Increased adaptations to arid environments were also detected in rodent lineages from 2.3 mya compared to those from 3 mya (Wesselman 1985). In the human lineage, the changes in climatic variability 2.8, 1.7 and 1 mya roughly correspond with changes in species morphology, novelty and diversity (Vrba *et al*. 1989; Wood 1992; Kimbel 1995; Vrba 1995). Thus, the main periods of climatic change (2.8, 1.7 and 1 mya)

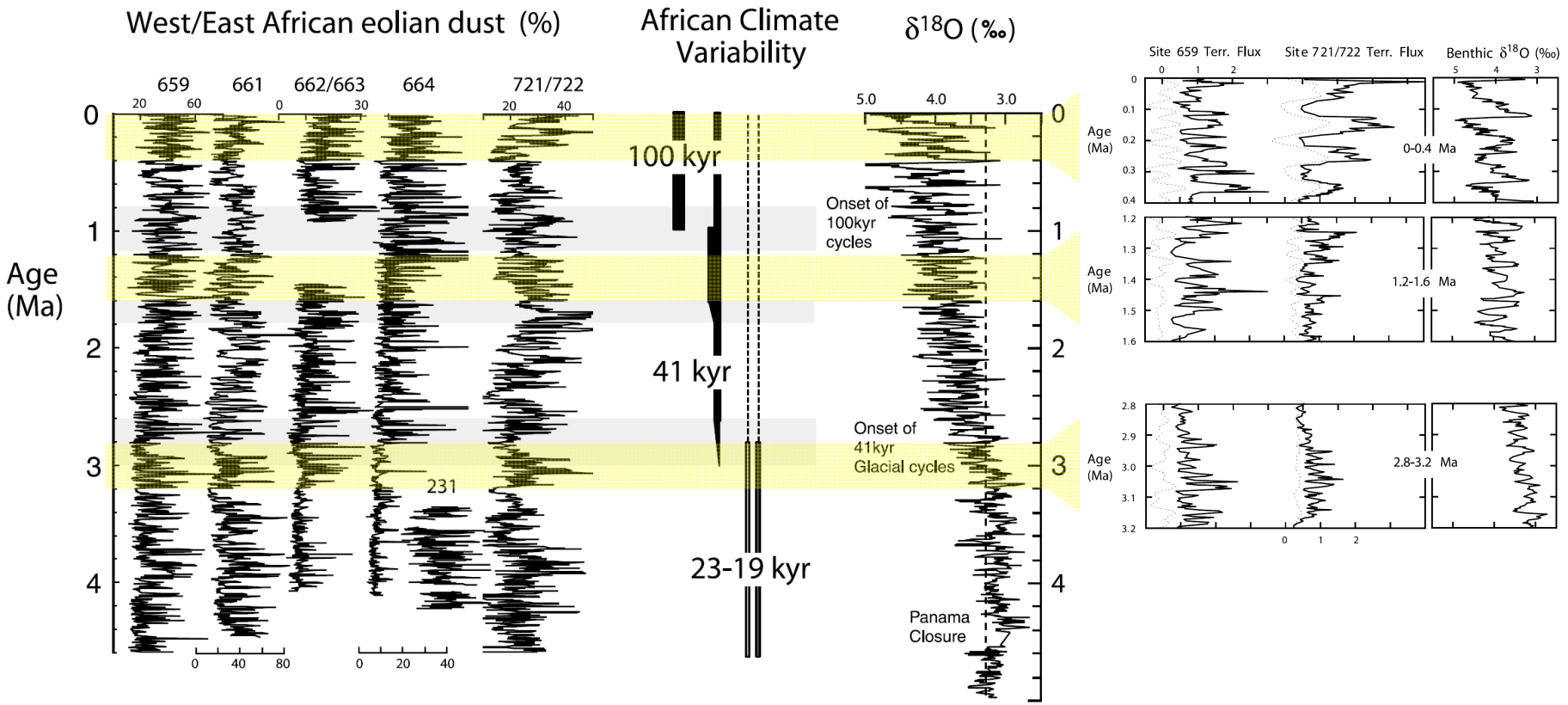

Figure 2.18: Climatic cycles in subtropical Africa over the last 5 million years inferred by DeMenocal (2004) from quantities of eolian sediment (more sediment indicates more arid conditions) and the ratio (δ ^{18}O) of the stable rare oxygen isotope ^{18}O to the most abundant stable isotope ^{16}O (a lower ratio indicates hotter temperatures) at drill sites in the Atlantic Ocean off West Africa (659, 661, 662, 663 and 664) and in the Arabian Sea off East Africa (231, 721 and 722).

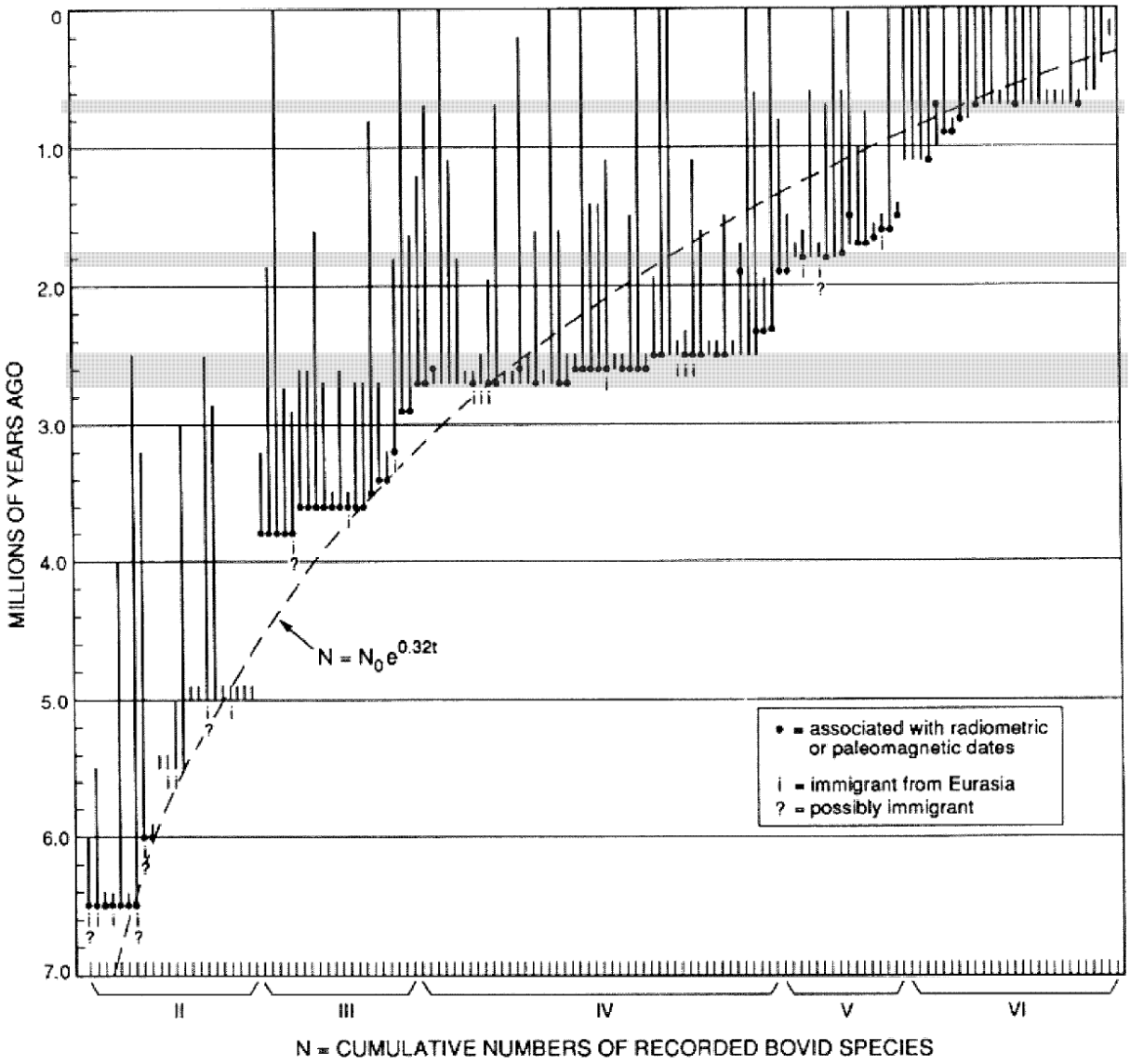

Figure 2.19: The duration (the vertical lines) of antelope taxa over the last seven million years as observed in the fossil records by Vrba (1995) compared with a null hypothesis of uniform rates of speciation and extinction (the dashed curve). A considerable departure from this assumption of uniform rates of turnover was detected 2.7 - 2.5 mya, with more minor deviations 1.8 mya and 0.7 mya. These intervals are highlighted in gray in the figure.

tended to be associated with accelerated species turnovers in antelope and human evolution, and morphological changes in antelope, rodent and human lineages, suggesting that environmental stress provides the impetus for genetic variance, natural selection and morphological novelty (DeMenocal 2004).

These climatic shifts and oscillations have also had considerable effects on vegetation distribution, including that of forests, throughout the Cenozoic era and into modern times. Today forest occurs in the form of fragmented patches that only occupy about 0.1% of the South African land surface area (Geldenhuys 2000), and is found in the more mesic regions in the southern and eastern parts of South Africa (Figure 2.20). Along the South Coast, a region that receives winter to all-year rainfall, forest patches occur in a thin band from the Cape Peninsula to Port Elizabeth, before broadening inland into the mostly summer-rainfall areas along the East Coast (Mucina & Geldenhuys 2006). In Mpumalanga and Limpopo provinces, the forest patches are able to occur further away from the coast as their lower latitude and the influence from the warm Indian Ocean result in rain-bearing air penetrating further inland

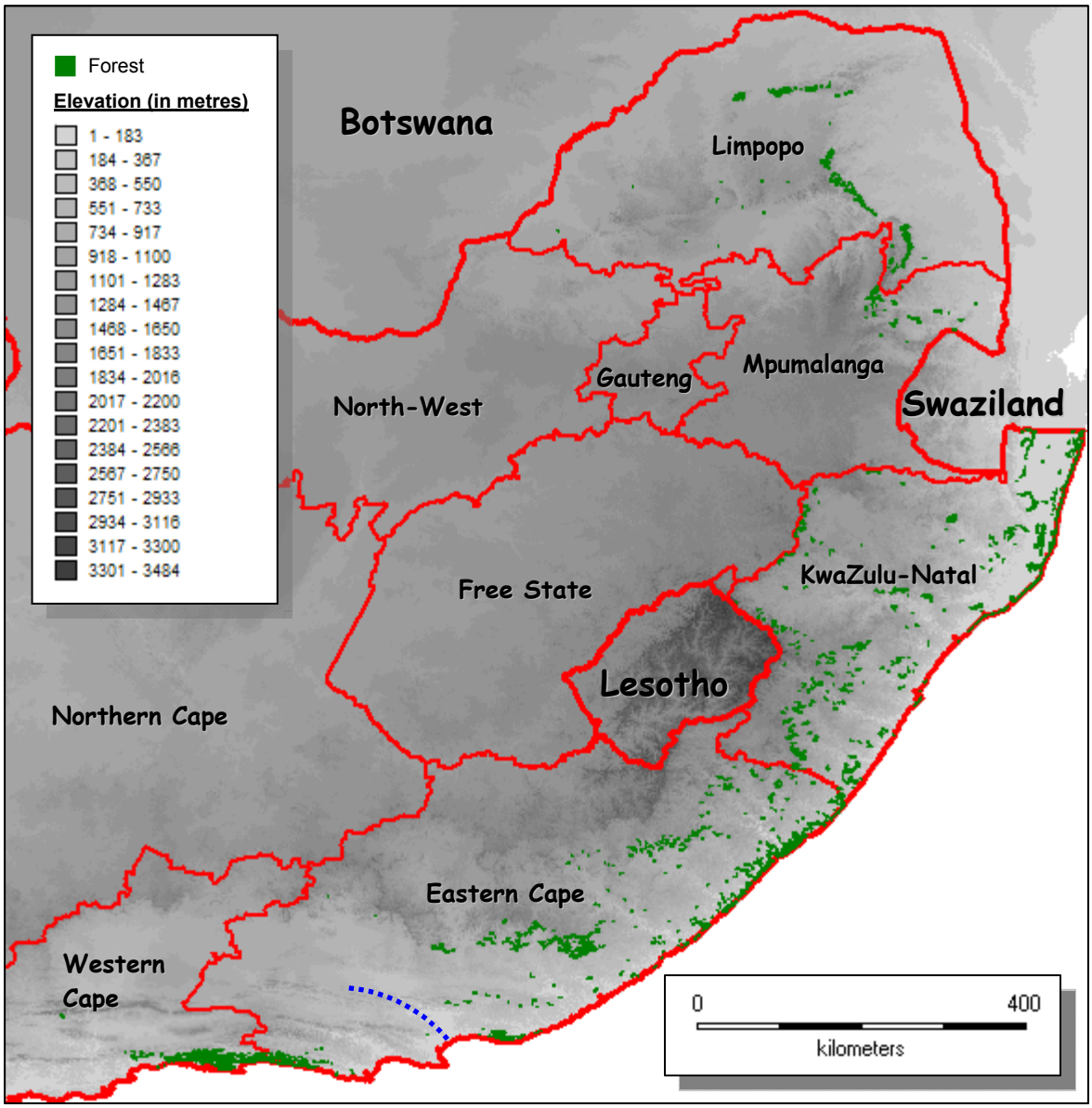

Figure 2.20: Current forest cover in eastern South Africa (CSIR 1996) with the approximate position of the Bedford Gap shown by a blue stippled line.

(Mucina & Geldenhuys 2006). In eastern and northern South Africa, forests mostly occurs in the form of fragmented patches that are surrounded by all-year rainfall fynbos and Albany Thicket in the south of the Eastern Cape, and summer-rainfall grasslands or savanna throughout the rest of its range (Mucina & Geldenhuys 2006). Cooper (1985) divided South African forests into two main types: afromontane forest and Indian Ocean coastal belt forests. He subdivided afromontane forest into two subtypes: montane *Podocarpus* forest and mist belt mixed *Podocarpus* forest, and Indian Ocean coastal belt forests into six subtypes: dune forest, swamp forest, sand forest, riverine forest, coast lowland forest and scarp forest. However, Mucina & Geldenhuys (2006) has subsequently divided South African forests into 12 vegetation units: Southern Afrotemperate, Northern Afrotemperate, Southern Mistbelt, Northern Mistbelt, Scarp, Southern Coastal, Northern Coastal, Sand, Ironwood Dry, Lowveld Riverine, Swamp and Mangrove.

Most of the forest types present in South Africa today constitute extensions of larger African forest zones (Chapman & White 1970; White 1983; Timberlake & Shaw 1994), which in turn form part of two main African floristic regions i.e. the Afromontane Region and the Tongaland-Pondoland Regional Mosaic. The Afromontane Region is also found in Zimbabwe, Malawi,

and along the East African mountain ranges into Ethiopia in the north and in Cameroon and northern Angola in the west, a range that resembles that of *Streptocarpus* on the African mainland. The coastal forests form part of the Tongaland-Pondoland Regional Mosaic, which shares some elements with the Zanzibar-Inhambane Regional Mosaic (Mucina & Geldenhuys 2006). Patterns of taxa shared amongst extant forest patches today provide clues regarding the biogeographic history of the forest patches. In particular, forest patches tend to share more taxa with neighbouring patches to their north and east than with forest patches to their south and west (Geldenhuys 1992a). Furthermore, the southern Cape forests constitute the geographical distribution limit of many taxa that have far greater distribution ranges (Geldenhuys 1992b). These patterns suggest that many taxa emerged in the mountains and coastal areas of east Africa before migrating steadily southwards (McKenzie 1978; Tinley 1985; Cawe 1986; Geldenhuys 1992b). *Streptocarpus* therefore shares a more northerly origin and southerly migration with many other taxa. Afromontane forests are believed to have been present in southern Africa since before the Last Glacial Maximum (LGM) ca. 18 000 years ago, while the Indian Ocean coastal belt forests are only believed to have migrated down the coast from Mozambique ca. 8 000 years ago after the establishment of modern sea levels and the stabilization of the dunes on which they occur. The Scarp Forests are believed to have originated from the afrotemperate forests (Lawes 1990).

Information is slowly accumulating on the past distributions of vegetation types in response to palaeoclimatic patterns. The current distribution and the potential impact that palaeoclimates had on forest distribution in KwaZulu-Natal were investigated by Eeley *et al*. (1999). Using the present distribution of the forests (Figure 2.21) and current environmental data to determine the main climatic (temperature and rainfall) and geological correlates of Cooper's (1985) eight forest subtypes, Eeley *et al*. (1999) constructed a model to infer potential present forest cover, as well as palaeoclimatic forest distribution during the Holocene altithermal ca. 7 000 years ago and the Last Glacial Maximum (LGM) ca. 18 000 years ago in KwaZulu-Natal. The LGM was characterised by a generally colder, drier climate worldwide, while temperatures were warmer than at present and precipitation increased in eastern South Africa during the Holocene altithermal. Using their palaeoclimatic model to estimate current forest cover, Eeley *et al*. (1999) recovered far more extensive forest cover (Figure 2.22A) than is presently the case (Figure 2.21). They attributed the less extensive actual forest cover to the influence of local climatic variation, and to anthropogenic influences. However, the current view is that the shrinkage of forests predates agriculture in southern Africa (Ellery & Mentis 1992; Meadows & Linder 1993; O'Connor & Bredenkamp 1997), and West *et al*. (2000) and Foord (1999; 2001) have detected recent expansions of forest in certain areas of the Transkei and KwaZulu-Natal rather than recent expansions of grasslands. Another explanation accounting for the lower current forest cover compared to simulated current forest cover is that their map of actual forest cover (Figure 2.21) only shows forests greater than 0.5 km^2 in size, although many forest fragments are smaller than this. Modelling of forest cover during the LGM ca. 18 000 years ago (Figure 2.22B) revealed a much reduced and more fragmented forest cover than at present, as well as a shift of afromontane forest to the lower altitude areas currently occupied by Scarp Forest, in response to the colder and drier climate of the time. The Holocene altithermal ca. 7 000 years ago was warmer and wetter, and the climate was consequently able to support far more extensive forest cover. Additionally, the modelling inferred that afromontane and Indian Ocean coastal belt forests interdigitated in the areas currently inhabited by Scarp Forest (Figure 2.22C). KwaZulu-Natal forests were affected less by Quaternary climatic oscillations than the forests of other areas e.g. in Limpopo, and the present-day Scarp Forest areas therefore constitute important refugia that have sustained various forest types during past forest shrinkage events (Lawes 1990). Both the shrinkage and the shifting positions of forest subtypes were

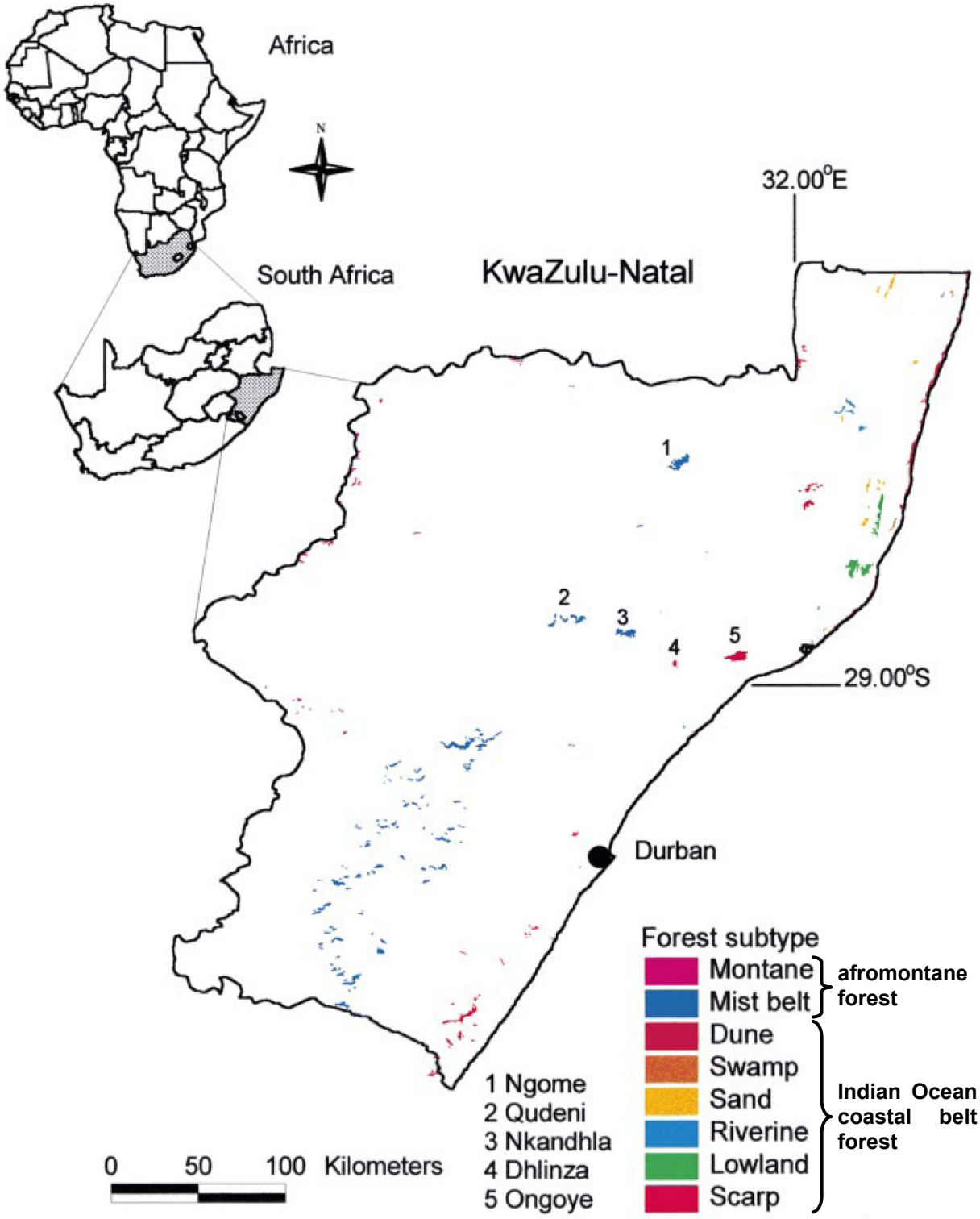

Figure 2.21: Current distribution of indigenous forest patches over 0.5 km^2 in size in KwaZulu-Natal (South Africa) from Eeley *et al*. (1999). Forest classification is according to Cooper (1985).

probably detrimental to certain species, and have probably led to many extinctions during the most climatically variable periods. Although Eeley *et al*. (1999) only focused on forest distribution in KwaZulu-Natal, this study demonstrates the possible effects that palaeoclimatic variations had on forests in eastern South Africa as a whole. Additionally, the Quaternary period overall is characterised by alternations between glacials and interglacials of which the LGM and the present time are only the most recent. The forest distribution modelling of Eeley

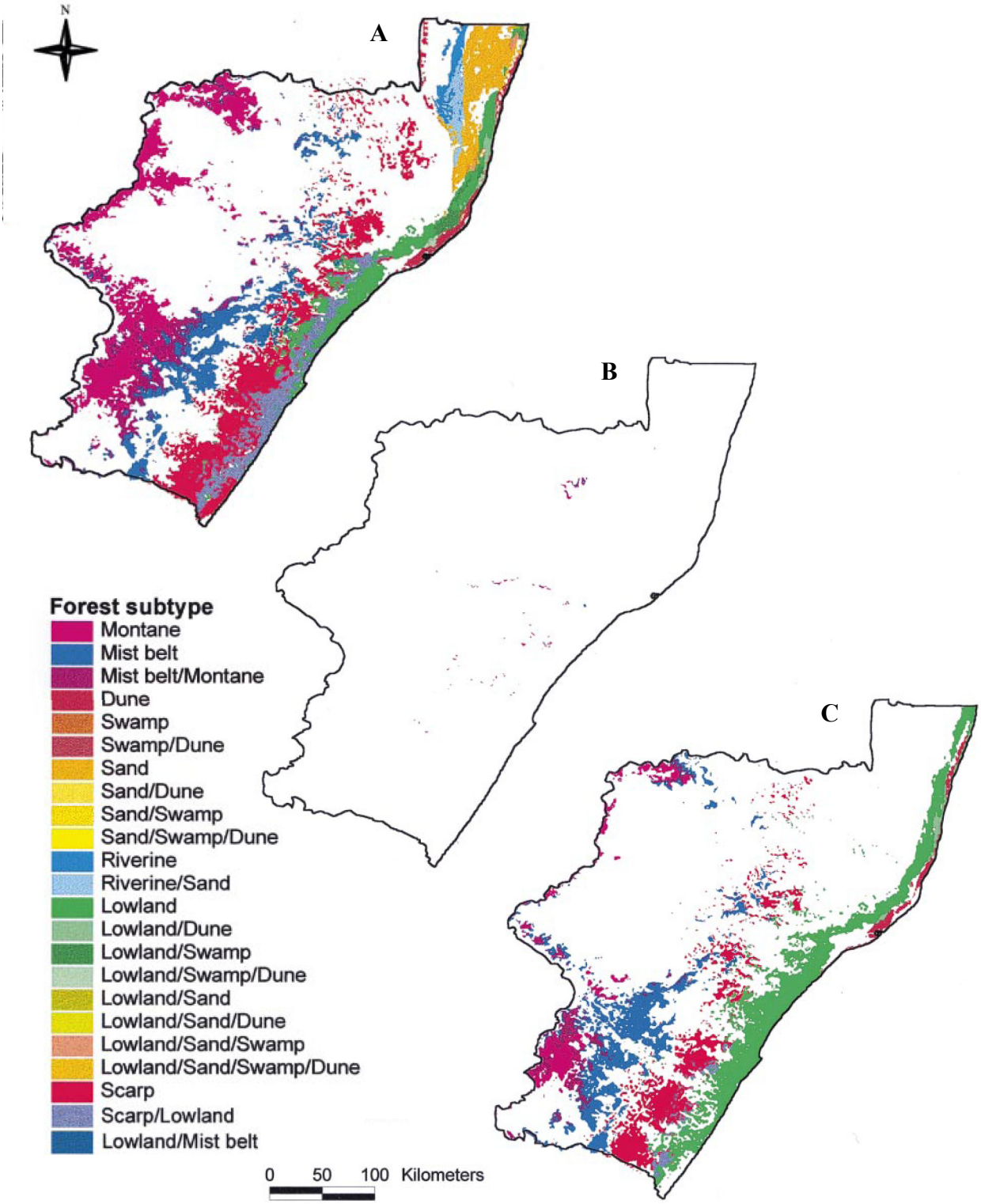

Figure 2.22: Inferred distribution of indigenous forest patches in KwaZulu-Natal by the model constructed by Eeley *et al*. (1999) to infer palaeoclimatic distributions. **A** shows estimated present forest cover, **B** during the Last Glacial Maximum (ca. 18 000 years ago), and **C** during the Holocene altithermal (ca. 7 000 years ago). Forest classification is according to Cooper (1985).

et al. (1999) can therefore probably be assumed to reflect changes in forest distribution during the previous glacial-interglacial cycles at a frequency of 100 000 years for the past million years (DeMenocal 2004) as well.

Bond *et al*. (2003a, 2003b) studied the relative effects of climate, fire and atmospheric CO_2 concentrations on current forest distribution throughout South Africa. Instead of using a correlative method (Eeley *et al*. 1999), Bond *et al*. (2003a, 2003b) estimated tree cover using a mechanistic model that simulates the response of vegetation types to climate, substrate and atmospheric CO_2 levels according to established physiological principles (Woodward *et al*. 1995; Cramer *et al*. 2001). This model also contains a fire module that simulates fire using climatic data (used to estimate moisture content of plant litter) and the number of years between successive burns. Their simulations revealed that in the absence of fire the more mesic parts of South Africa could sustain far greater stem biomass than is currently the case. For example, the more mesic areas along a latitudinal transect through southern Mpumalanga (26.75°S) showed significantly greater stem biomass, both at the present atmospheric CO_2 concentration (Figure 2.23a), as well as at that of the LGM (Figure 2.23b) in the absence of fire in their simulations. However, the most dramatic change is evident at the present atmospheric CO_2 concentration, representing an increase in stem biomass that would result in a shift from grassland to forest. Bond *et al*. (2003a, 2003b) concluded that fire is an important determinant of tree density in mesic areas, and that forests in eastern South Africa would be far more widespread without fire in areas receiving an annual rainfall greater then 650 mm. Their findings are supported by numerous fire-exclusion experiments carried out in eastern South Africa, including Everson & Breen (1983), Westfall *et al*. (1983), Skowno *et al*. (1998), Hoffmann & O'Connor (1999) and Titshall *et al*. (2000), which all detected an increase in woody species in mesic areas in the absence of fire. Fires in the summer-rainfall areas are chiefly fuelled by fire-dependent grasslands and savannas, and the frequency of burns is therefore determined by the extent and productivity of grassland communities relative to forests. The extent and productivity of the summer-rainfall grasslands is in turn linked to atmospheric CO_2 levels (Bond *et al*. 2003a, 2003b), as these grassland communities are dominated by C_4 plants (Vogel *et al*. 1978; Schulze *et al*. 1996) that have a photosynthetic advantage over C_3 plants in hot growing seasons at lower CO_2 concentrations (Ehleringer *et al*. 1997). The discrepancies between current forest cover in KwaZulu-Natal (Figure 2.22A) predicted by Eeley *et al*. (1999) based on climatic correlates and actual forest cover (Figure 2.21) is therefore probably the result of the omission of fire and atmospheric CO_2 concentrations from their modelling. Fire and CO_2 levels also probably played a major role during the LGM and the Holocene altithermal (and during previous glacial-interglacial cycles) in limiting forest cover. Forest cover was therefore most likely even more limited and fragmented than the modelling of Eeley *et al*. (1999) shows during these periods (Figure 2.22B & C).

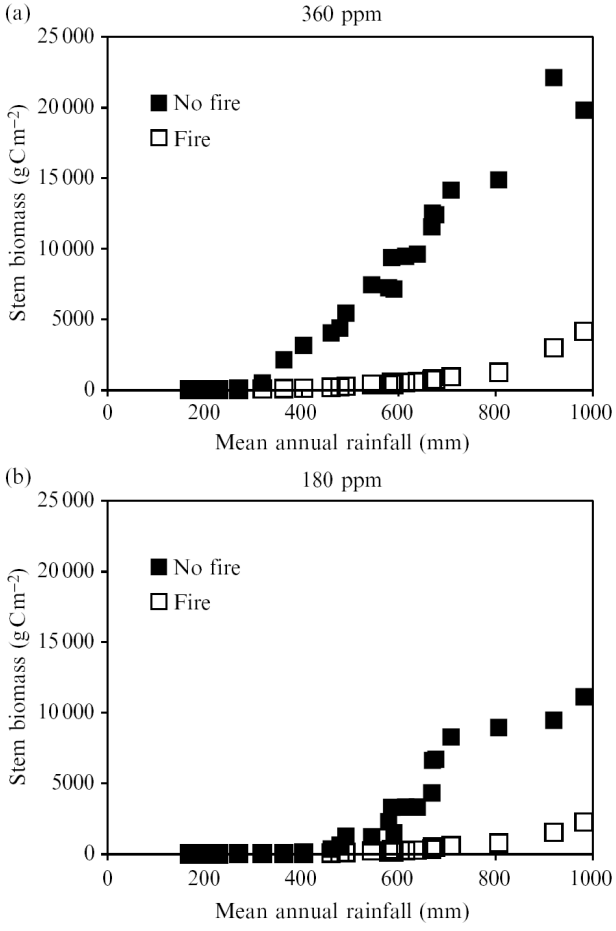

Figure 2.23: Influence of fire and atmospheric CO_2 levels on stem biomass in the summer-rainfall areas of South Africa along a transect at latitude 26.75°S (which runs through southern Mpumalanga) in increasingly mesic environments in the absence and presence of fire at **(a)** the current atmospheric CO_2 concentration and **(b)** CO_2 concentration during the Last Glacial maximum, ca. 18 000 years ago, simulated by Bond *et al*. (2003b).

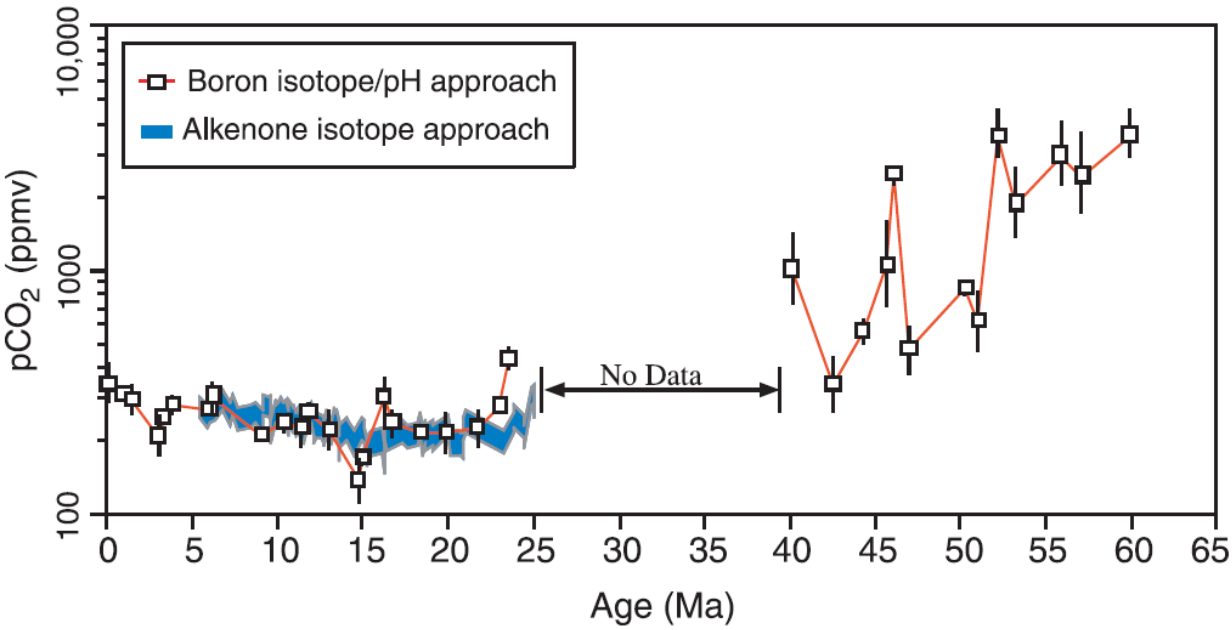

Figure 2.24: Atmospheric CO_2 concentrations during the Cenozoic era inferred from both boron isotope ratios (which vary according to the pH of the water, which in turn is affected by the atmospheric CO_2 concentration) in marine formanifers, and from carbon isotope ratios present in the alkenones of phytoplankton from deep-sea sediment cores from the Pacific Ocean. Figure copied from Zachos *et al*. (2001).

CO_2 levels in the atmosphere have been changing throughout the Earth's recent history (Figure 2.24). At the beginning of the Cenozoic era, CO_2 levels stood at about 3 500 parts per million (ppm) (McCarthy & Rubidge 2005). However, levels fell through much of the Tertiary period (Berner 1997), dropping below 500 ppm, the concentration at which C_4 plants would have started to gain a photosynthetic advantage over C_3 plants in the tropics, about 7 mya during the Miocene epoch (Ehleringer *et al*. 1997). The Quaternary period (1.64 mya to present) saw the alternation between glacials and interglacials, with CO_2 levels ranging from about 270 ppm during the interglacials (including the present one), and about 180 ppm during glacials (Petit *et al*. 1999).

These climatic shifts and oscillations, as well as changing atmospheric CO_2 levels, have determined the relative distributions of the vegetation types in Africa. During the Cretaceous period (146–65 mya), southern Africa was surrounded by warm seas resulting in a moist climate that supported extensive subtropical and warm-temperate forests (McCarthy & Rubidge 2005). These widespread forests are believed to have persisted through much of the Tertiary period (Coetzee 1982; Scott *et al*. 1997) until the cooling of the Atlantic Ocean and the establishment of the Benguela Upwelling System brought colder water to the West Coast (McCarthy & Rubidge 2005). This led to the replacement of the warm, mesic climate with colder, more arid conditions (Udeze & Oboh-Ikuenobe 2005), especially in the western part of southern Africa. Aridification of the West Coast and interior was exacerbated by the greater uplift of eastern southern Africa, which caused warm, humid air flowing in from the Indian Ocean to lose most of its moisture before it had surmounted the eastern escarpment en route to the interior. The increasing aridity led to a contraction of forests, especially in the interior and along the West Coast. These more arid regions were occupied by desert scrub and dry woodlands before the arrival of grassland communities (McCarthy & Rubidge 2005). Grasses first emerged over 65 mya (Jardiné & Magloire 1965; Muller 1981; Scott & Srivastava 1984; Grass Phylogeny Working Group 2001). However, dentition adapted to grazing only started appearing worldwide in fossils of animals from the mid Miocene, about 15 mya (MacFadden 2000), and C_4 grasslands seem to have made an abrupt appearance in Africa only 8–6 mya (Cerling *et al*. 1997), suggesting that grasses took a long time to become widely established.

Ehleringer *et al*. (1997) proposed that the fall of CO_2 concentrations ca. 7 mya below 500 ppm, the concentration below which C_4 plants begin to have a photosynthetic advantage over C_3 plants in the tropics, prompted the rapid spread of C_4 plants detected at this time. As CO_2 concentrations fell further, C_4 plants would have been able to outcompete C_3 in a wider range of growing conditions. Vrba (1995) detected a remarkable increase in the rates of speciation and extinction of antelope in the fossil records between 3 mya and 2.5 mya (Figure 2.19), with many of the new species being grazers, and these changes in turnover rates and morphology have been linked to the expansion of grasslands in eastern Africa (Vrba 1995; Bobe & Eck 2001; Bobe *et al*. 2002). However, an examination of eastern and southern African fossil mammals only revealed a considerable increase in grazers, suggesting the widespread establishment of grasslands and savanna, after 1.8 mya (Reed 1997). Thus grassland communities, and therefore also the regular fires that restrict forests, probably only arrived in South Africa during the Pliocene or Pleistocene epochs (Bond *et al*. 2003a). During the Pleistocene (1.64 mya to 10 000 BP), forest distribution probably alternated in response to the alternating CO_2 levels and temperatures that accompanied the oscillations between the glacials and interglacials. During the glacials, CO_2 concentrations were very low, and this together with the colder, drier climate is probably the reason for the exceedingly limited distribution of forests during the LGM in KwaZulu-Natal found by Eeley *et al*. (1999; Figure 2.22B). Forest was able to re-expand during the interglacial, including the present one (Figure 2.22C), but in some areas faster than in others (Lawes 1990). For example, forests were only able to expand into Limpopo province 6 000 years ago due to persistent arid conditions as a result of close proximity to an arid corridor that stretched between north-western southern Africa and East Africa (Deacon & Lancaster 1988; Scott *et al*. 1997). Even within the present interglacial, palynological and charcoal records reveal that forests expanded and contracted in response to warmer, more mesic periods (14 000–12 000 years ago, 4 000–2 500 years ago, and 1 400 years ago to present) and more arid periods in the southern Cape (Scholtz 1986). In summary, forests were widespread in southern Africa for much of the Cenozoic era. However, with falling atmospheric CO_2 levels, the advent of colder, drier conditions and the ensuing arrival of grasslands, vegetation in eastern and southern Africa alternated between warmer, wetter periods that would have been conducive to the invasion of forests into grassland communities, and cooler, drier periods that would have favoured grasslands and the attendant frequent fires that would have restricted forests.

Lawes *et al*. (2007) inferred the historical dispersal routes of forest-dwelling birds, non-volant (not capable of flying) mammals and frogs amongst southern African forest patches after the LGM from present-day species compositions (Figure 2.25). A faunal similarity between the Mozambican and Zimbabwean forest patches and the more southern South African Indian Ocean Coastal Belt (coastal) forests, as well as a southward decrease in species richness along the coast, indicate that the coastal forest fauna arrived in South Africa via the Mozambican coast after the LGM. Furthermore, numerous faunal similarities indicate that there has been extensive interchange between the KwaZulu-Natal coastal and Scarp Forests. Scarp Forest fauna show relatively little evidence of suffering major losses during the LGM, as they contain on average fewer generalists than either afrotemperate or coastal forests. In contrast, faunal patterns in modern afrotemperate forests (here referring to the higher-altitude forests including montane and mistbelt forests) suggest large-scale extinctions during the LGM, and extant birds and mammals are mainly the result of post-LGM recolonizations from Scarp Forest refugia. Lawes *et al*. (2007) inferred that the coastal faunal lineages dispersed from tropical East African refugia down through Mozambique and into the South African coastal forests after the LGM, subsequently undergoing considerable interchange with Scarp Forests. The Scarp Forests were less affected by the LGM than the afrotemperate forests (Lawes 1990), and many Scarp faunal lineages would therefore have been able to survive the forest shrinkages of LGM. After the LGM, faunal Scarp lineages dispersed into adjacent afrotemperate forests, the northern

Scarp lineages dispersing along the Mpumalanga escarpment into the northern afrotemperate forests, while the central and southern afrotemperate forests were recolonized by the central and southern Scarp Forests, respectively. On the other hand, the Bedford Gap (Figure 2.20), an arid corridor in the vicinity of the Sundays and Great Fish river valleys, hampered recolonizations from Eastern Cape refugia southwards, and the southern and western afrotemperate forests show only limited evidence of post-LGM faunal dispersals (Lawes *et al*. 2007). Because this study inferred similar migration routes for diverse animals, these dispersal routes probably also reflect the post-LGM expansion of southern African forests, and therefore possibly the concurrent recolonization of *Streptocarpus* as well. Moreover, if the previous glacials also affected forest distribution in similar ways, then these recolonization patterns could be applicable to previous glacials, not just to the most recent one.

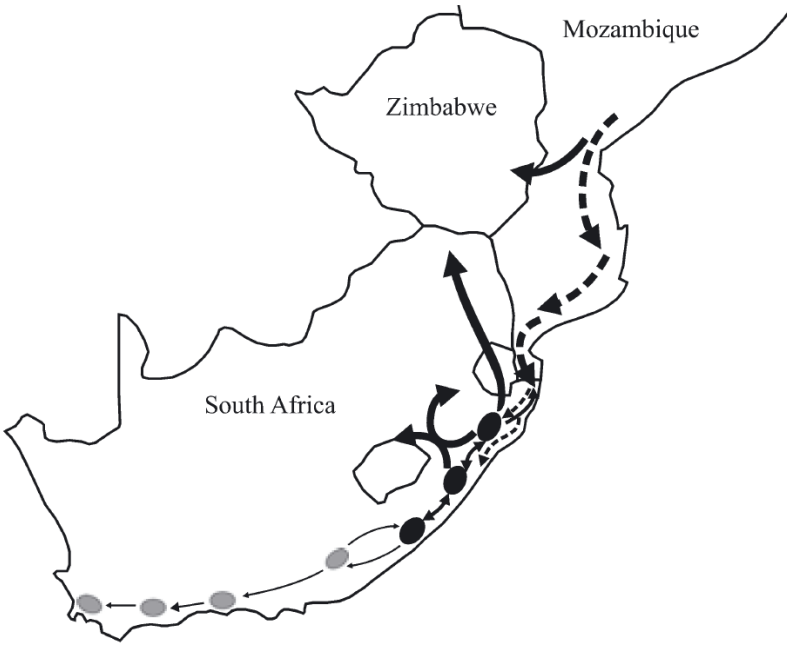

Figure 2.25: Dispersal routes followed by forest-dependent and forest-associated birds, non-volant mammals and frogs after the Last Glacial Maximum (LGM), inferred by Lawes *et al*. (2007) from current forest patch faunal assemblages. The width of the arrows indicates the relative inferred frequency of the dispersal routes; solid arrows indicate the movements of Afrotemperate fauna, while broken arrows indicate that of Afrotropical fauna. The black circles mark major LGM refugia, while the grey circles mark minor ones.

In summary, forest cover has changed considerably throughout the Cenozoic era in response to global temperature trends, changing rainfall patterns and changing atmospheric CO_2 concentrations. These factors determine the relative distribution of grassland communities versus forests, which in turn affects the frequency and intensity of fires. The fire regime again influences the ability of forests to colonize grasslands, and therefore controls the size of forest patches. Many of the South African forests form part of the greater Afromontane Region, an African floristic region whose range is similar to that of *Streptocarpus* on the mainland. *Streptocarpus* has therefore had an extensive area in which to radiate from its more northern birthplace. Forests, and therefore also the forest-dwelling *Streptocarpus* taxa, have likely undergone periods of considerable contraction and expansion during the Quaternary period in response to the glacial-interglacial cycles. Evidence from various mammal lineages suggests that the periods of climate and habitat change 2.9–2.4 mya, 1.8–1.6 mya and 1.2–0.8 mya during the last 5 million years led to increased speciation and extinction rates in lineages, and these periods of change (and previous ones as well) probably also led to radiations and the evolution of novel morphological characters in the *Streptocarpus* lineage. The similar patterns of recolonizations after the LGM detected in different faunal groups possibly also reflects the patterns of forest expansion after the LGM (and perhaps previous glacials as well), and these dispersal routes could therefore probably also have been followed by *Streptocarpus* in its colonization of newly formed forests after the glacials.

2.3.5.3. Radiation of *Streptocarpus* in South Africa

The progressive southerly radiation of subgenus *Streptocarpus* evident from Möller & Cronk's (2001b) study culminated in the evolution of the South African taxa, most of which fall into two sister clades, a unifoliate clade and a rosulate clade, the latter referred to as the "Cape Primrose clade". Although the ranges of these two clades overlap considerably, the unifoliates and rosulates occupy different ecological niches, and could consequently have evolved sympatrically. The presence of a number of highly variable species within these two clades implies that speciation is actively occurring within these taxa (Möller & Cronk 2001b).

Hughes *et al*. (2005) investigated the relationship between *S. primulifolius* and *S. rexii*, two rosulate species constituting the southern limb of Hilliard & Burtt's (1971) *Streptocarpus* agg. *rexii*, and emerging in the Cape primrose clade in Möller & Cronk's (2001a) analyses. *S. rexii* extends from the George-Knysna vicinity in the Western Cape along the coast to the East London area in the Eastern Cape, from where it follows a more inland route parallel to the coast up to Mt. Ngeli in southern KwaZulu-Natal. *S. primulifolius* follows the coast from where *S. rexii* veers inland, extending from East London in the south up along the coast parallel to *S. rexii* to Port Edward, from where it also adopts a more inland route parallel to the coast that terminates at Inchanga Hill in the Natal Midlands, KwaZulu-Natal. The East London vicinity where the ranges of these two species overlap contains populations (referred to as *S.* cf. *primulifolius* in Hughes *et al*. 2005) that are to varying extents morphologically intermediates between *S. primulifolius* and *S. rexii* (Hilliard & Burtt 1971). Hilliard & Burtt (1971) hypothesised that, because these two species form the southern extremity of *Streptocarpus*, they probably each arose independently in the north and spread southwards in parallel, before meeting and hybridising around East London. Using nuclear and chloroplast sequence data and nuclear microsatellite data, Hughes *et al*. (2005) showed that the East London populations in fact constitute evolutionary intermediates between *S. primulifolius* and *S. rexii* (Figure 2.26). Their analyses revealed that *S. primulifolius* constitutes a polyphyletic group, with *S. rexii* and the intermediate populations (referred to as *S.* cf. *primulifolius* in the paper) nested within it. Moreover, neither *S. rexii* nor *S.* cf. *primulifolius* constitute monophyletic groups. Rather, the northern *S. rexii* populations group with some of the *S.* cf. *primulifolius* populations, while the rest of *S.* cf. *primulifolius* groups with the southern *S. rexii* populations. They also included a population of *S. formosus* from the Umtamvuna river gorge (Port Edward, KwaZulu-Natal), which also emerged within *S. primulifolius*. By applying previously published ITS substitution rates to their ITS phylogeny, they estimated that *S. primulifolius* probably arose during the Pleistocene, and that *S. rexii* diverged from *S. primulifolius* 0–0.44 mya, reaching its current range during the Holocene. In addition, they found that the highest levels of genetic diversity occur in the *S. primulifolius* populations, *S.* cf. *primulifolius* contains intermediates levels of diversity, while the northern and southern *S. rexii* populations contain very little or no diversity (Figure 2.27). Hughes *et al*. (2005) therefore concluded that *S. primulifolius* evolved in the north, spread down the coast as far as East London, where it gradually evolved into *S. rexii* as it spread progressively more inland. *S. rexii* then spread northwards along a more inland, high altitude route and southwards along the coast.

In summary, Möller & Cronk (2001b) concluded that *Streptocarpus* arose far more recently than before the breakup of Gondwana, and was able to disperse between the African mainland and Madagascar as a result of a land bridge 45–26 mya. The dispersal of the genus to the Comoros Archipelago occurred after the disappearance of the land bridge, and must therefore have happened through long-distance dispersal. Subgenus *Streptocarpus* on the African mainland spread progressively southwards in a series of southerly waves, probably influenced by climatic fluctuations during the Miocene and Pleistocene epochs. The most southerly members of *Streptocarpus*, *S. primulifolius* and *S. rexii*, constitute a reasonably young lineage

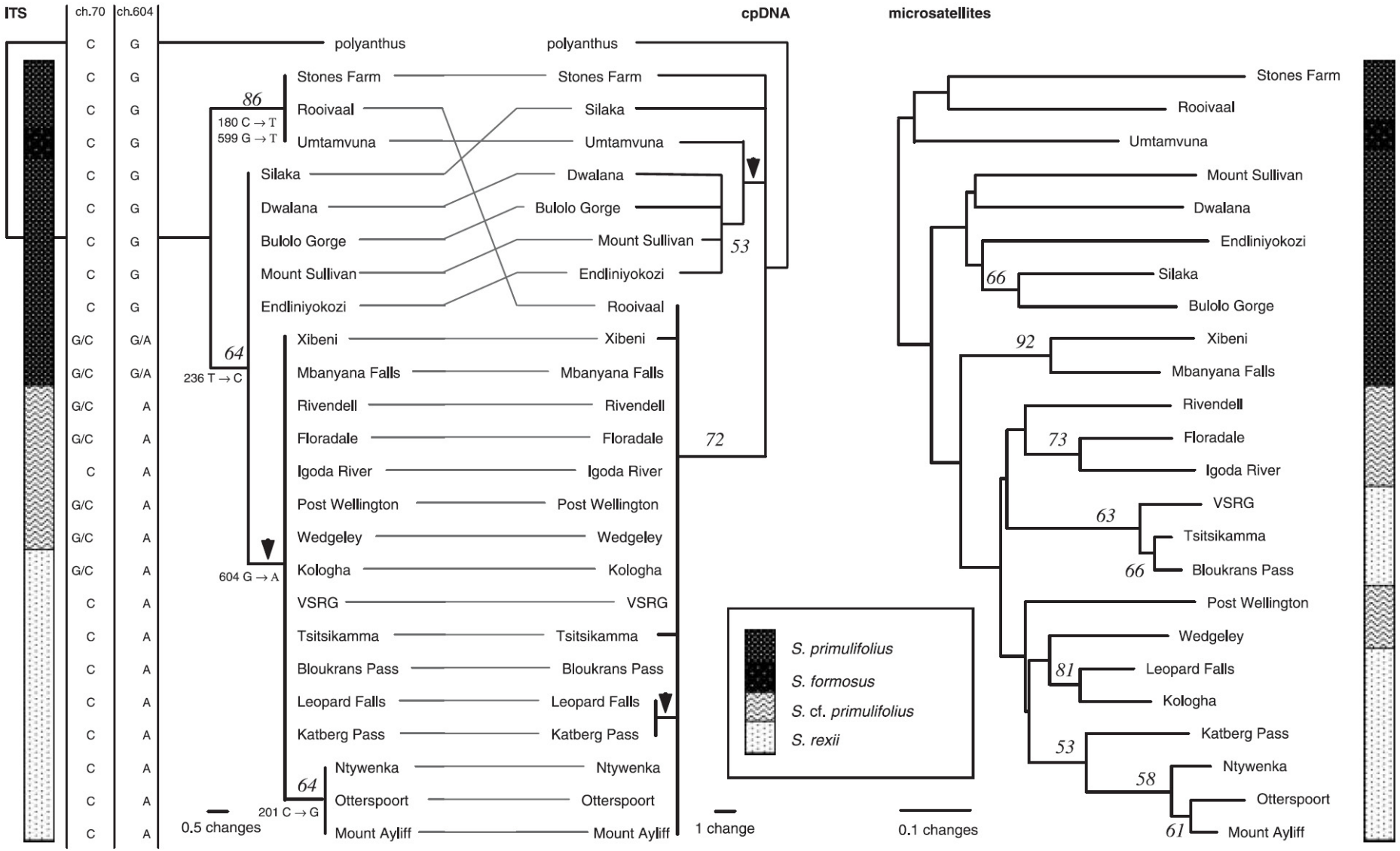

Figure 2.26: Equally parsimonius tree reconstructed from nuclear ribosomal ITS and chloroplast (cpDNA) sequence data, respectively, rooted using *S. polyanthus*, and a neighbour-joining tree reconstructed from nuclear microsatellite data showing relationships amongst *S. primulifolius* and *S. rexii* populations, and the populations morphologically intermediate between the two species (referred to as *S.* cf. *primulifolius*). The two columns of letters to the left show the character states at two polymorphic sites within ITS for each population. Italic numbers give the bootstrap support of the associated clade and arrows indicate clades that collapse in the strict consensus trees.

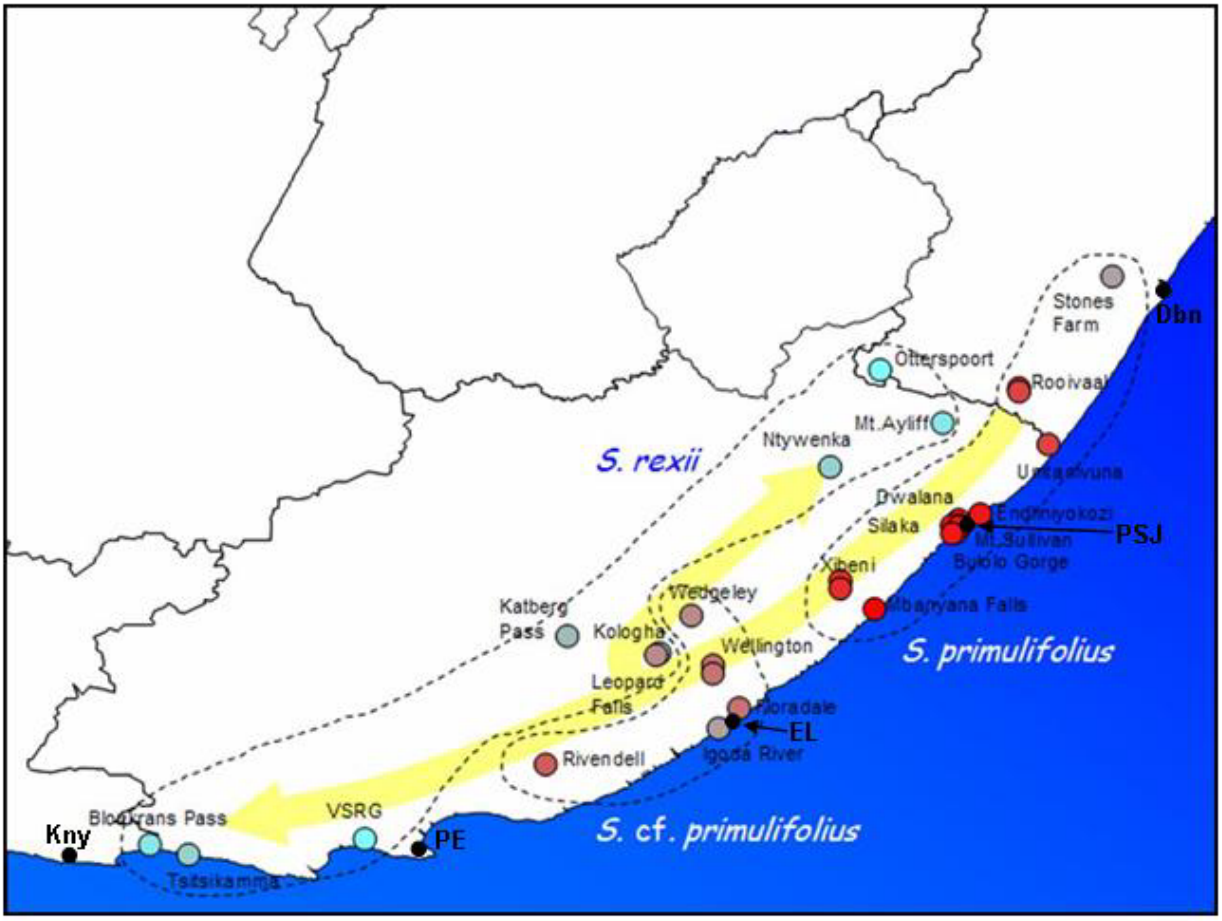

Figure 2.27: The evolutionary history of *S. primulifolius*, *S. rexii* and the populations morphologically intermediate between the two species (referred to as *S.* cf. *primulifolius*) inferred by Hughes *et al.* (2005). The yellow arrow indicates that *S. primufolius* arose in southern KwaZulu-Natal, spread down the coast through the Eastern Cape towards East London, where it gave rise to *S. rexii*, which in turn spread north along an inland route and south along the coast. The dashed lines demarcate the ranges of *S. primulifolius*, *S.* cf. *primulifolius* and *S. rexii*. The circles mark the localities of the populations sampled, and their colour indicates gene diversity found within each population, red for highest diversity and blue for lowest diversity. For orientation, some coastal towns are shown: Kny = Knysna, PE = Port Elizabeth, EL = East London, PSJ = Port St Johns, Dbn = Durban.

that probably arose towards the beginning of the Quaternary period. By this time, the glacial-interglacial cycles had already started, and it is therefore likely that the forest patches were as fragmented or even more fragmented than they are today. Thus, the only way for *Streptocarpus* could have spread from forest patch to forest patch would have been by rare long-distance dispersal events. However, whether dispersal is effected by the wind or by animal dispersal is unknown.

Chapter 3: Phylogenetic studies in South African *Streptocarpus* taxa

3.1. Introduction

Streptocarpus (Gesneriaceae) is a herbaceous plant genus ranging across the tropical and subtropical, forested parts of Madagascar and the African mainland, including eastern South Africa. The genus name comes from its twisted fruit, a ubiquitous characteristic in the genus except amongst a few of the Madagascan species in which hardly any twisting occurs (Hilliard & Burtt 1971). However, twisted fruit does not appear to be an all-encompassing characteristic of this African lineage. Although the fruit of the other eight African Gesneriaceae genera are not twisted (Weber 2004), pollen (Weigend & Edwards 1996) and molecular (Smith *et al*. 1997b, 1998; Möller & Cronk 1997; Möller & Cronk 2001a; Mayer *et al*. 2003; Möller 2003; Smith *et al*. 2004b) analyses suggest that the other African Gesneriaceae genera analysed so far are actually nested within *Streptocarpus*, suggesting that twisting of the fruit capsule has been lost independently on a number of occasions. The African Gesneriaceae therefore appear to constitute a monophyletic group within Gesneriaceae, a relationship that is also supported by chromosome counts and seed coat characters.

Streptocarpus, in its current taxonomic circumscription, is divided into two subgenera i.e. subgenus *Streptocarpella* and subgenus *Streptocarpus*. Although the boundaries of these two subgenera are reasonably clear based on growth habit, chromosome numbers and ITS sequence data (Hilliard & Burtt 1971; Möller & Cronk 2001a), relationships within the taxonomically complex subgenus *Streptocarpus* are far less clear. Based on vegetative and floral morphology, Hilliard & Burtt (1971) suggested relationships within subgenus *Streptocarpus*, but remarked on the incongruences between vegetative and floral morphology. They based some of their groups on shared growth habit, with floral characters being variable in these groups, while they based other groups on shared floral morphology, with growth habit being variable. Pollen data were collected by Weigend & Edwards in 1996, supporting some of Hilliard & Burtt's (1971) morphological groupings while contradicting others. The first large-scale molecular analysis of the genus *Streptocarpus* was conducted in 2001, in which evolutionary relationships were reconstructed based on nuclear ITS sequence data by Möller & Cronk (2001a). Based on this phylogeny, each of the growth forms present in subgenus *Streptocarpus* were found to have evolved more than once (Möller & Cronk 2001a). Harrison *et al*. (1999) and later Hughes *et al*. (2006) optimized floral type onto ITS phylogenies, and also found floral type to be plastic with regards to relationships reconstructed using ITS data. Thus growth habit, floral type, pollen type and ITS sequence data are inconsistent with one another within subgenus *Streptocarpus*. Despite these incongruences, Möller & Cronk (2001a) identified a group of South African species that formed a strongly supported clade in their ITS phylogeny, which they called the 'Cape primrose clade'. Growth habit was found to be uniform amongst these species, and floral and pollen type showed little variability. However, only a few of the South African *Streptocarpus* species currently recognised were included in this analysis, and the full extent of the Cape primrose clade could therefore not be determined. Moreover, the ITS data were not variable enough to provide resolution within this clade, and relationships are therefore unclear.

Hughes *et al*. (2005) took the first step in resolving relationships at the finer scale in the Cape primrose clade. They investigated the relationship between the morphologically similar species *S. primulifolius* and *S. rexii* and some morphologically intermediate populations using nuclear ITS and plastid sequence data and nuclear microsatellite data. These intermediate populations are also geographically intermediate between the two species. *S. primulifolius* has a coastal

range extending from the Durban vicinity in KwaZulu-Natal to East London in the Eastern Cape, the intermediate populations are located in the East London vicinity, and *S. rexii* occurs from the Knysna area in the Western Cape in a long, thin, initially coastal but then inland arc up to around Matatiele in KwaZulu-Natal. The morphological and geographic intermediacy of these populations led Hilliard & Burtt (1971) to suggest that they had arisen as a result of hybridization between *S. primulifolius* and *S. rexii*. However, the molecular data told a different story. Hughes *et al*. (2005) concluded that *S. primulifolius* evolved towards the north of its range, spreading down the coast towards the East London vicinity, where *S. rexii* evolved from *S. primulifolius* three (or more) times. *S. rexii* then spread north and south to occupy its current range. The intermediate populations therefore constitute evolutionary intermediates rather than hybrids between *S. primulifolius* and *S. rexii*. These intermediate populations were together referred to as *S.* cf. *primulifolius* in Hughes *et al*. (2005). However, the type specimen of *S. primulifolius* was collected at Floradale near East London, and these populations are therefore rather referred to as the "southern *S. primulifolius* populations" in this study.

The microsatellite data of Hughes *et al*. (2005) also revealed the breeding systems of the taxa analysed. *S. primulifolius* was found to be an outbreeder. However, in the process of evolving into *S. rexii*, this lineage shifted to being chiefly an inbreeder, choosing an autogamous breeding system in the new ranges into which it migrated in a manner analogous to Baker's law (Baker 1955; Stebbins 1957). Pollen limitation presents a problem in that *Streptocarpus* ovaries contain hundreds of ovules, and a certain minimum number of these ovules have to be fertilised in order for the ovary to develop into a fruit capsule, as is the case in other plant lineages that produce multi-seeded fruit. Insect pollination in *S. primulifolius* provides sufficient pollen deposition to ensure adequate seed set and hence the development of an adequate number of fruit capsules to ensure the species' survival, but the change in breeding system of *S. rexii* to autogamy allowed it to overcome the limitation of pollen availability that the species would otherwise have experienced when occupying new territory (Trevor Edwards, personal communication).

Hybridization has frequently been reported in *Streptocarpus* (Hilliard & Burtt 1971) and is likely to have played a role in the evolution of the genus, since very few barriers to hybridization apparently exist. In this context, autogamous species such as *S. rexii* could play a profound role. The abscission of the flower and consequently the prevention of seed capsule development that occurs if too few ovules in the ovary are fertilized means that the chance deposition (by insect, wind or rain) of low numbers of heterologous pollen grains onto the stigma of a *Streptocarpus* plant is highly unlikely to result in seed development. However, in autogamous species such as *S. rexii*, where self-fertilization with copious pollen grains would follow a chance heterologous pollen deposition, this could lead to successful hybrid establishment in the F_1 and even successive generations (Trevor Edwards, personal communication). Thus, evolution of autogamy could play a pivotal role in hybridization in *Streptocarpus* and increase the frequency of chloroplast capture and lineage mixing with considerable evolutionary consequences.

Another factor that has probably influenced the course of evolution in *Streptocarpus* is habitat availability. Most *Streptocarpus* species grow only in forest habitats. A number of recent publications have added to our knowledge of palaeoclimates, vegetation history and historical biogeographical patterns of lineages in southern Africa where the Cape primrose clade of *Streptocarpus* occurs. DeMenocal (2004) determined that the African climate has been becoming progressively more arid, particularly over the past 2.8 million years. Climatic cycles have been becoming increasingly more variable over the past 1.2 million years, with severe glacial periods occurring every 100 000 years. Eeley *et al*. (1999) modelled forest cover during the Last Glacial Maximum and Holocene altithermal in KwaZulu-Natal, and inferred that forest

was far more restricted during the Last Glacial Maximum, but more extensive during the Holocene altithermal, a pattern that would have followed the 100 000 year climatic cycles of the late Pleistocene. Bond *et al*. (2003a, 2003b) identified further environmental factors restricting the extent of forest cover, and concluded that forest cover was even more restricted than Eeley *et al*. (1999) predicted during the Last Glacial Maximum. Lawes *et al*. (2007) inferred migration routes that certain forest-associated and forest-dependent faunal groups followed in recolonising forests after the Last Glacial Maximum. The members of the Cape primrose clade are mostly distributed in the forests of eastern South Africa, and this group is therefore well suited for studying historical biogeographic patterns e.g. detecting Pleistocene refugia (Hughes *et al*. 2005) and previous floral migration routes, in the region.

Although the relationship between *S. primulifolius* and *S. rexii* has been unravelled (Hughes *et al*. 2005), relationships amongst the rest of the species constituting the Cape primrose clade remain unclear. This current study was therefore undertaken to investigate evolutionary patterns in the South African *Streptocarpus*, to evaluate the circumscription of the Cape primrose clade according to independent data and to determine the extent of this group, and to assess Hilliard & Burtt's (1971) hypotheses regarding the origin and relationships of the species included. An attempt was also made to uncover historical biogeographic patterns in South African *Streptocarpus*, but especially in the Cape primrose clade, and to attach a time frame to the evolution of *Streptocarpus* in South Africa.

3.2. Materials and Methods

3.2.1. Taxon sampling

Based on the relationships detected by Möller & Cronk (2001a) from their nuclear ITS sequence data, nuclear and plastid sequence data were initially collected from representatives of almost all the species constituting the Cape primrose clade, using *S. polyanthus* as the outgroup. However, as the study progressed, it became clear that relationships were more complex, in that taxa that emerged outside the Cape primrose clade in the ITS analysis did not necessarily also emerge amongst Cape primrose clade taxa in the plastid analyses and vice versa. An increasing number of additional taxa indigenous to South Africa were therefore included in order to clarify relationships amongst the South African taxa occurring in KwaZulu-Natal and the Eastern Cape today. This culminated in using the Madagascan endemic *S. papangae*, a member of subgenus *Streptocarpella* (Hilliard & Burtt 1971), as the root for the phylogenetic analyses based on relationships reconstructed by Möller & Cronk (2001a, 2001b). In total, 102 populations representing 31 out of the 52 South African species described to date were included in the sequence analyses, with multiple populations included for widely distributed species. Table 3.1 lists all the taxa analysed in this study, and Figures 3.1–3.3 show the distributions of the taxa sampled.

3.2.2. DNA extraction

Leaf tips (approximately 1 cm × 1 cm) were collected from plants in the field and preserved in silica gel, or, in the minority of cases, leaf samples were taken directly from plants growing in cultivation that had been collected from the field. Total genomic DNA was extracted from dried leaf squares weighing 2–5 mg using the hexadecyltrimethylammonium bromide (CTAB) extraction method (Doyle & Doyle 1987), with modifications. These modifications involved adding ca. 10 mg of polyvinylpyrrolidone (PVP) to eliminate polyphenols (Maliyakal 1992) and doubling the concentration of β-mercaptoethanol in the extraction buffer of each sample,

Table 3.1: Specimens used to generate the nuclear and plastid sequences for the phylogenetic analyses. Abbreviations are as follows: BB = Benny Bytebier; DUB = Dirk Bellstedt; MH = Mark Hughes; MMO = Micheal Möller; TJE = Trevor Edwards; Mt. = Mountain; S.A. = South Africa. Each specimen that amplified successfully is marked with a ✓, and those that were unsuccessful are indicated with a ×. The letter in superscript next to the ✓ indicates who sequenced the sample: a = I generated the sequences; b = samples were sequenced at the Royal Botanic Gardens Edinburgh (RBGE); c = I supplemented sequences that had already been amplified at the RBGE; d = Benny Bytebier and Dirk Bellstedt generated the sequences. For the samples in which I supplemented already-existing sequences, the number of base pairs that I contributed to the parts of the sequences that were included in the analyses is indicated in parentheses after the letters. Images of representative taxa are included in Appendix B.

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
S. aylae T.J.Edwards	David Styles s.n.	Msikaba, Eastern Cape, S.A.	aylMsikaba	1	-31.2833	29.9233	✓[a]	✓[a]	✓[a]	✓[a]
S. baudertii L.L.Britten	MH 1065	Harmony, KwaZulu-Natal, S.A.	bauHarmony	1	-30.3818	28.8935	✓[a]	✓[a]	✓[b]	✓[b + c(108)]
S. baudertii	MH 1067	Otterspoort, KwaZulu-Natal, S.A.	bauOtterspoort	1	-30.4583	28.9454	✓[b]	✓[a]	✓[b]	✓[b + c(981)]
S. baudertii	MH 1095	Otterspoort, KwaZulu-Natal, S.A.	bauOtterspoort	1	-30.4583	28.9454	✓[a]	✓[a]	✓[b]	✓[b + c(816)]
S. baudertii	MH 1080	Ntywenka, Eastern Cape, S.A.	bauNtywenka	1	-31.1623	28.5729	✓[b]	✓[a]	✓[b + c(608)]	✓[b + c(659)]
S. baudertii	Ernst van Jaarsveld s.n.	Collywobbles, Eastern Cape, S.A.	bauCollywobbles	1	-32.0500	28.5833	✓[b]	✓[a]	✓[b + c(229)]	✓[b + c(135)]
S. baudertii	MH 1156	Hillsdrift Farm, Eastern Cape, S.A.	bauHillsdrift	1	-32.8821	28.0418	✓[b]	✓[a]	✓[b]	✓[b + c(764)]
S. bolusii C.B.Clarke	Louise Badenhorst s.n.	Ngcobo, Eastern Cape, S.A.	bolNgcobo	1	-31.5917	27.9344	✓[a]	✓[a]	✓[a]	✓[a]
S. bolusii	Louise Badenhorst 60	Satansnek, Eastern Cape, S.A.	bolSatansnek	1	-31.6023	27.9433	✓[a]	✓[a]	✓[a]	✓[a]
S. caeruleus Hilliard & B.L.Burtt	DUB 1011a	Lajuma, Limpopo, S.A.	caeLajuma	1	-23.0235	29.4309	✓[a]	✓[a]	✓[a]	✓[a]
S. caeruleus	DUB 1065	Blouberg, Limpopo, S.A.	caeBlouberg	1	-23.0778	28.9931	✓[a]	✓[a]	✓[a]	✓[a]
S. cyaneus S.Moore	MH 1297	Georges Valley, Limpopo, S.A.	cyaGeorgesValley	1	-24.0041	29.9741	✓[a]	✓[a]	✓[b + c(270)]	✓[a]
S. cyaneus	MH 1329	Mariepskop, Limpopo, S.A.	cyaMariepskop	1	-24.5391	30.8699	✓[a]	✓[a]	✓[a]	✓[a]
S. cyaneus	MH 1258	The Bonnet, Limpopo, S.A.	cyaTheBonnet	1	-24.9326	30.8089	✓[a]	✓[a]	✓[b + c(250)]	✓[a]
S. cyaneus	MH 1243	Kaapsehoop, Mpumalanga, S.A.	cyaKaapsehoop	1	-25.6070	30.7667	✓[a]	✓[a]	✓[a]	✓[a]
S. cyaneus	MH 1376	Shiyalongubu Dam, Mpumalanga, S.A.	cya ShiyalongubuDam	1	-25.7618	31.2680	×	✓[a]	✓[a]	✓[a]
S. cyaneus	MH 1355	Josephsdal, Mpumalanga, S.A.	cyaJosephsdal	1	-25.9439	31.1157	✓[a]	✓[a]	half missing[a]	✓[a]
S. cyaneus ssp. *longi-tommii* Weigend & T.J.Edwards	MH 1232	Die Geut, Limpopo, S.A.	cyaSspLonDie Geut	1	-25.1616	30.6247	✓[a]	✓[a]	✓[b]	✓[b & c(315)]
S. cyaneus ssp. *nigridens* Weigend & T.J.Edwards	DUB 1077	Soutpansberg, Limpopo, S.A.	cyaSspNig Soutpansberg	1	-23.0027	30.2383	✓[a]	✓[a]	✓[a]	✓[a]
S. denticulatus Turrill	DUB 1096	Belfast, Mpumalanga, S.A.	denBelfast	2	-25.6252	29.9956	✓[a]	✓[a]	✓[a]	✓[a]
S. dunnii Hook.f.	MH 1210	Sabie, Limpopo, S.A.	dunSabie	2	-25.1684	30.6425	✓[a]	✓[a]	✓[a]	✓[a]

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
S. dunnii	MH 1239	Steenkampsbergen, Mpumalanga, S.A.	dun Steenkampsbergen	2	-25.2281	30.1567	✓[a]	✓[a]	✓[a]	✓[a]
S. dunnii	MH 1268	Uitvlugt Farm, Mpumalanga, S.A.	dunUitvlugt	1	-25.4599	30.0371	✓[a]	✓[a]	✓[a]	✓[a]
S. dunnii	MH 1273	Slaaihoek, Mpumalanga, S.A.	dunSlaaihoek	1	-25.7244	30.4789	✓[a]	✓[a]	✓[a]	✓[a]
S. dunnii	MH 1371	Angle Station, Mpumalanga, S.A.	dunAngleSt	1	-25.8691	31.0882	✓[a]	✓[a]	✓[a]	✓[a]
S. fanniniae [Harvey ex] C.B.Clarke	DUB 1025	Mount Gilboa, KwaZulu-Natal, S.A.	fanMtGilboa	1	-29.2897	30.2904	✓[a]	✓[a]	✓[a]	✓[a]
S. fenestra-dei Weigend & T.J.Edwards	MH 1305	God's Window, Limpopo, S.A.	fenGodsWindow	1	-24.8760	30.8889	✓[a]	✓[a]	✓[b]	✓[b & c(720)]
S. floribundus Weigend & T.J.Edwards	TJE s.n.	Kranskop, KwaZulu-Natal, S.A.	floKranskop	1	-28.9167	30.9500	✓[a]	✓[a]	✓[a]	✓[a]
S. formosus (Hilliard & B.L.Burtt) T.J.Edwards	TJE 2167	Umtamvuna, KwaZulu-Natal, S.A.	forUmtamvuna	1	-31.0020	30.1730	✓[b]	✓[a]	✓[b]	✓[b & c(749)]
S. formosus	Jan Burring 27	Mzamba, Eastern Cape, S.A.	forMzamba	2	-31.0833	30.1333	✓[a]	✓[a]	✓[a]	✓[a]
S. gardenii Hook.	DUB 1032	Injasuti, KwaZulu-Natal, S.A.	garInjasuti	2	-29.1200	29.4365	✓[a]	✓[a]	✓[a]	✓[a]
S. gardenii	DUB 1027	Mount Gilboa, KwaZulu-Natal, S.A.	garMtGilboa	2	-29.2897	30.2904	✓[a]	✓[a]	✓[a]	✓[a]
S. gardenii	DUB 0560	Hoha Forest, KwaZulu-Natal, S.A.	garHohaForest	2	-30.1333	29.5833	✓[a]	✓[a]	✓[a]	✓[a]
S. grandis N.E.Br.	DGA Styles 3019	Kingscliff Farm, KwaZulu-Natal, S.A.	granKingscliff	1	-29.4167	30.8167	✓[a]	✓[a]	✓[a]	✓[a]
S. grandis	DGA Styles 3007	north-west of Inanda Mt., KwaZulu-Natal, S.A.	granNWInandaMt.	1	-29.6333	30.8500	✓[a]	✓[a]	✓[a]	✓[a]
hybrid *S. meyeri* × southern *S. primulifolius*	MH 1160	Wedgeley, Eastern Cape, S.A.	hybWedgeley	1	-32.2554	27.5704	✓[a]	✓[a]	✓[b]	✓[b + c(792)]
S. johannis L.L.Britten	DUB 0546	Nsikeni, KwaZulu-Natal, S.A.	johNNsikeni	1 (ITS); 2 (plastid)	-30.1351	29.5495	✓[d]	✓[a]	✓[a]	✓[a]
S. johannis	DUB 0714	Hebron Road, KwaZulu-Natal, S.A.	johNHebronRd	1 (ITS); 2 (plastid)	-30.4006	29.5751	✓[d]	✓[a]	✓[a]	✓[a]
S. johannis	DUB 0226	Manzimnyama River, KwaZulu-Natal, S.A.	joh NManzimnyama	1 (ITS); 2 (plastid)	-30.6116	29.6292	✓[d]	✓[a]	✓[a]	✓[a]
S. johannis	DUB 0915	Myokane, Eastern Cape, S.A.	johSMyokane	1 (ITS); 2 (plastid)	-31.4432	29.7632	✓[d]	✓[a]	✓[a]	✓[a]
S. johannis	DUB 0593	Magwa Falls, Eastern Cape, S.A.	johSMagwaFalls	1 (ITS); 2 (plastid)	-31.4478	29.6394	✓[d]	✓[a]	✓[a]	✓[a]
S. johannis	DUB 0840	Embotyi, Eastern Cape, S.A.	johSEmbotyi	1 (ITS); 2 (plastid)	-31.4500	29.7250	✓[a]	✓[a]	✓[a]	✓[a]
S. johannis	DUB 0637	Mount Sullivan, Eastern Cape, S.A.	johSMtSullivan	1 (ITS); 2 (plastid)	-31.6038	29.5370	✓[d]	✓[a]	✓[a]	✓[a]
S. kentaniensis L.L.Britten	J. Joannou 6	Kentani, Eastern Cape, S.A.	kenKentani	1	-32.6250	28.1250	✓[a]	✓[a]	✓[a]	✓[a]

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
& Story										
S. kunhardtii T.J.Edwards	TJE s.n.	Itala Nature Reserve, KwaZulu-Natal, S.A.	kunItala	1	-27.4500	31.2167	✓[a]	✓[a]	✓[a]	✓[a]
S. lilliputana Bellstedt & T.J.Edwards	DUB 0611	Lupatana Gorge, Eastern Cape, S.A.	lilLupatana	1	-31.3908	29.8304	✓[a]	✓[a]	✓[a]	✓[a]
S. lilliputana	DUB 0627	Upper Fraser Falls, Eastern Cape, S.A.	lilUFraserFalls	1	-31.4007	29.7167	✓[a]	✓[a]	✓[a]	✓[a]
S. longiflorus (Hilliard & B.L.Burtt) T.J.Edwards	DUB 1064	Blouberg, Limpopo, S.A.	lonBlouberg	1	-23.0778	28.9931	✓[a]	✓[a]	✓[a]	✓[a]
S. meyeri B.L.Burtt	MH 1327	Mariepskop, Limpopo, S.A.	meyMariepskop	2	-24.5391	30.8699	✓[a]	✓[a]	✓[a]	✓[a]
S. meyeri	DUB 0913	Bastervoetpad, Eastern Cape, S.A.	meyBastervoetpad	6	-31.1807	28.0473	✓[a]	✓[a]	✓[a]	✓[a]
S. meyeri	MH 1168	Cathcart, Eastern Cape, S.A.	meyCathcart	1	-32.3334	27.0975	✓[b + c(237)]	✓[a]	✓[b]	✓[b + c(811)]
S. meyeri	MH 1175	Howieson's Poort, Eastern Cape, S.A.	mey HowiesonsPoort	1	-33.3692	26.4757	✓[b]	✓[a]	✓[b]	✓[b + c(626)]
S. meyeri	DUB 0844	Zuurberg, Eastern Cape S.A.	meyZuurberg	2	-33.2743	25.7752	✓[a]	✓[a]	✓[a]	✓[a]
S. meyeri	MH 1165	Charlton Farm, Somerset East, Eastern Cape, S.A.	meySECharlton Farm	1	-32.6310	25.5090	✓[b]	✓[a]	✓[b]	✓[b + c(959)]
S. meyeri	DUB 0896	Glen Craig Farm, Somerset East, Eastern Cape, S.A.	meySEGlenCraig Farm	2	-32.6481	25.6559	✓[a]	✓[a]	✓[a]	✓[a]
S. meyeri	DUB 0776	Graaff-Reinet, Eastern Cape, S.A.	meyGraaffReinet	2	-32.2633	24.4954	✓[a]	✓[a]	✓[a]	✓[a]
S. meyeri	R. Jamieson s.n.	Baviaanskloof, Eastern Cape, S.A.	meyBaviaanskloof	1	-33.3750	24.2250	✓[a]	✓[a]	✓[a]	✓[a]
S. modestus L.L.Britten	DUB 0624	Upper Fraser Falls, Eastern Cape, S.A.	modUFraserFalls	2	-31.4000	29.7333	✓[a]	✓[a]	✓[a]	✓[a]
S. montanus Oliv.	BB 1937	Mbololo Forest, Taita Taveta, Kenya	montanKenya	1	-3.3333	38.4500	✓[a]	×	✓[a]	✓[a]
S. montigena L.L.Britten	DUB 0897	Katberg Pass, Eastern Cape, S.A.	montig KatbergPass	2	-32.4366	26.6547	✓[a]	✓[a]	✓[a]	✓[a]
S. montigena	Cameron McMaster s.n.	Elandsberg, Eastern Cape, S.A.	montigElandsberg	2	-32.4833	26.8833	✓[a]	✓[a]	✓[a]	✓[a]
S. papangae Humbert	MMO 9718	Col de Beampingaratra, Tuléar Province, Madagascar	papMadagascar	1	-24.4500	46.8500	✓[a]	✓[a]	✓[a]	✓[a]
S. parviflorus Hook.f. ssp. *parviflorus*	DUB 1075	Soutpansberg, Limpopo, S.A.	parSspPar Soutpansberg	1	-23.0208	30.2410	✓[a]	✓[a]	✓[a]	✓[a]
S. parviflorus	MH 1292	Magoebaskloof Hotel, Limpopo, S.A.	parMagoebaskloof Hotel	1	-23.8874	30.0014	✓[b]	✓[a]	✓[a]	✓[a]
S. polyanthus Hook.	MH 1025	Karkloof Falls, KwaZulu-Natal, S.A.	polKarkloofFalls	1	-29.3952	30.2795	✓[a]	✓[a]	✓[b & c(99)]	✓[b & c(515)]
S. polyanthus	MH 1022	Shelter Falls, KwaZulu-Natal, S.A.	polShelterFalls	1	-29.4791	30.2461	✓[a]	✓[a]	✓[b & c(680)]	✓[b & c(623)]

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
S. polyanthus	MH 1031	Ferncliff, KwaZulu-Natal, S.A.	polFerncliff	1	-29.5498	30.3412	✓[a]	✓[a]	✓[b & c(55)]	✓[b & c(681)]
S. polyanthus	MH 1048	Inanda, KwaZulu-Natal, S.A.	polInanda	1	-29.6414	30.8544	✓[a]	✓[a]	✓[b]	✓[b & c(1129)]
S. porphyrostachys Hilliard	DUB 0984	Mtentu Gorge, Eastern Cape, S.A.	porMtentuGorge	1	-31.2408	30.0333	✓[a]	✓[a]	✓[a]	✓[a]
S. primulifolius Gand.	TJE s.n.	Table Mt. , KwaZulu-Natal, S.A.	nPriTableMt	1	-29.6000	30.5833	✓[a]	✓[a]	✓[a]	✓[a]
S. primulifolius	TJE s.n.	Camperdown, KwaZulu-Natal, S.A.	nPriCamperdown	1	-29.7167	30.5333	✓[a]	✓[a]	✓[a]	✓[a]
S. primulifolius	TJE 3137	Monteseel, KwaZulu-Natal, S.A.	nPriMonteseel	1	-29.7333	30.6833	✓[a]	✓[a]	✓[a]	✓[a]
S. primulifolius	MH 1052	Stone's Farm, KwaZulu-Natal, S.A.	nPriStonesFarm	1	-29.7654	30.6402	✓[b]	✓[a]	✓[b]	✓[b & c(74)]
S. primulifolius	MH 1088	Rooivaal Farm, KwaZulu-Natal, S.A.	cPriRooivaal	1	-30.5876	29.9549	✓[b]	✓[a]	✓[b]	✓[b & c(770)]
S. primulifolius	DUB 0965	Msikaba, Eastern Cape, S.A.	cPriMsikaba	1	-31.2917	29.8050	✓[a]	✓[a]	✓[a]	✓[a]
S. primulifolius	DUB 0969	Msikaba, Eastern Cape, S.A.	cPriMsikaba	1	-31.2917	29.8383	✓[a]	✓[a]	✓[a]	✓[a]
S. primulifolius	MH 1126	Endliniyokozi, Eastern Cape, S.A.	cPriEndliniyokozi	1	-31.5198	29.6731	✓[b]	✓[a]	✓[b]	✓[b & c(650)]
S. primulifolius	MH 1135	Bulolo Gorge, Eastern Cape, S.A.	cPriBululoGorge	1	-31.5573	29.5143	✓[b]	✓[a]	✓[b & c(24)]	✓[b & c(624)]
S. primulifolius	DUB 0635	Mount Sullivan, Eastern Cape, S.A.	cPriMtSullivan	1	-31.5971	29.5298	✓[b]	✓[a]	✓[b]	✓[b & c(84)]
S. primulifolius	DUB 0587	Dwalana Forest, Eastern Cape, S.A.	cPriDwalana	1	-31.6046	29.4658	✓[b]	✓[a]	✓[b]	✓[b & c(849)]
S. primulifolius	MH 1134	Silaka, Eastern Cape, S.A.	cPriSilaka	1	-31.6493	29.5055	✓[b]	✓[a]	✓[b]	✓[b & c(718)]
S. primulifolius	MH 1139	Xibeni, Eastern Cape, S.A.	cPriXibeni	1	-32.0063	28.6558	✓[b]	✓[a]	✓[b & c(195)]	✓[b & c(792)]
S. primulifolius	MH 1140	Mbanyana Falls, Eastern Cape, S.A.	cPriMbanyanaFalls	1	-32.2181	28.9048	✓[b]	✓[a]	✓[b & c(204)]	✓[b & c(817)]
S. primulifolius	MH 1161 & 1162	Moonstone Forest, Wedgeley, Eastern Cape, S.A.	sPriWedgeley[1]	1	-32.2673	27.5690	✓[a]	✓[a]	✓[b & c(30)]	✓[b & c(761)]
S. primulifolius	MH 1143	Post Wellington Farm, Eastern Cape, S.A.	sPriPostWellington[1]	1	-32.6288	27.7256	✓[b]	✓[a]	✓[b & c(102)]	✓[b & c(741)]
S. primulifolius	MH 1157	Floradale Nursery, Eastern Cape, S.A.	sPriFloradale[1]	1	-32.9406	27.9222	✓[a]	✓[a]	✓[b & c(79)]	✓[b & c(847)]
S. primulifolius	MH 1155	Igoda River Mouth, Eastern Cape, S.A.	sPriIgoda[1]	1	-33.0931	27.7704	✓[a]	✓[a]	✓[b]	✓[b & c(109)]
S. primulifolius	MH 1174	Rivendell Farm, Eastern Cape, S.A.	sPriRivendell[1]	1	-33.3560	26.5055	✓[b]	✓[a]	✓[b]	✓[b & c(757)]
S. pusillus [Harvey ex]	DUB 1029a	Mount Gilboa, KwaZulu-Natal,	pusMtGilboa	1	-29.2867	30.2927	✓[a]	✓[a]	✓[a]	✓[a]

[1] Based on the intermediate morphology and geographical position between *S. primulifolius* and *S. rexii* of the populations in the East London area, these intermediate populations were together referred to as *S.* cf. *primulifolius* in Hughes *et al*. (2005). However, the type specimen of *S. primulifolius* was collected at Floradale near East London, and these populations are therefore rather referred to as the "southern *S. primulifolius* populations" in this study.

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
C.B.Clarke		S.A.								
S. rexii (Hook.) Lindl.	MH 1066	Otterspoort, KwaZulu-Natal, S.A.	rexOtterspoort	1	-30.4583	28.9454	✓[b]	✓[a]	✓[b & c(580)]	✓[b & c(557)]
S. rexii	MH 1100	Mount Ayliff, Eastern Cape, S.A.	rexMtAyliff	1	-30.8467	29.4045	✓[a]	✓[a]	✓[a]	✓[b & c(1155)]
S. rexii	MH 1075 & 1081	Ntywenka, Eastern Cape, S.A.	rexNtywenka	1	-31.1702	28.5810	✓[b]	✓[a]	✓[b & c(610)]	✓[b & c(828)]
S. rexii	MH 1170 & 1171	Katberg Pass, Eastern Cape, S.A.	rexKatbergPass	1	-32.4619	26.6584	✓[b]	✓[a]	✓[b]	✓[b & c(702)]
S. rexii	MH 1149	Kologha, Eastern Cape, S.A.	rexKologha	1	-32.5377	27.3414	✓[a]	✓[a]	✓[b]	✓[b & c(471)]
S. rexii	DUB 0521	Leopard Falls, Stutterheim, Eastern Cape, S.A.	rexLeopardFalls	1	-32.5586	27.3152	✓[a]	✓[a]	✓[b & c(81)]	✓[b & c(704)]
S. rexii	MH 1176	Van Stadens River Gorge, Eastern Cape, S.A.	rexVSRG	1	-33.9100	25.1939	✓[a]	✓[a]	✓[b & c(279)]	✓[b & c(678)]
S. rexii	MH 1181	Bloukrans Pass, Western Cape, S.A.	rexBloukransPass	1	-33.9480	23.6267	✓[b]	✓[a]	✓[b & c(120)]	✓[b & c(555)]
S. rimicola Story	DUB 1047	Thabazimbi, Limpopo, S.A.	rimThabazimbi	1	-24.4713	27.6168	✓[a]	✓[a]	✓[a]	✓[a]
S. rimicola	DUB 1048	Thabazimbi, Limpopo, S.A.	rimThabazimbi	1	-24.4713	27.6168	✓[a]	✓[a]	✓[a]	✓[a]
S. roseo-albus Weigend & T.J.Edwards	MH 1353	Agnes Mine, Mpumalanga, S.A.	rosAgnesMine	1	-25.8314	30.9738	✓[a]	✓[a]	✓[a]	✓[a]
S. saundersii Hook.	DGA Styles 3013	Inanda, KwaZulu-Natal, S.A.	sauInanda	1	-29.7167	30.9167	✓[a]	✓[a]	✓[a]	✓[a]
S. vandeleurii Baker f. & S.Moore	DUB 1055	Thabazimbi, Limpopo, S.A.	vanThabazimbi	2	-24.4682	27.6125	✓[a]	✓[a]	✓[a]	✓[a]

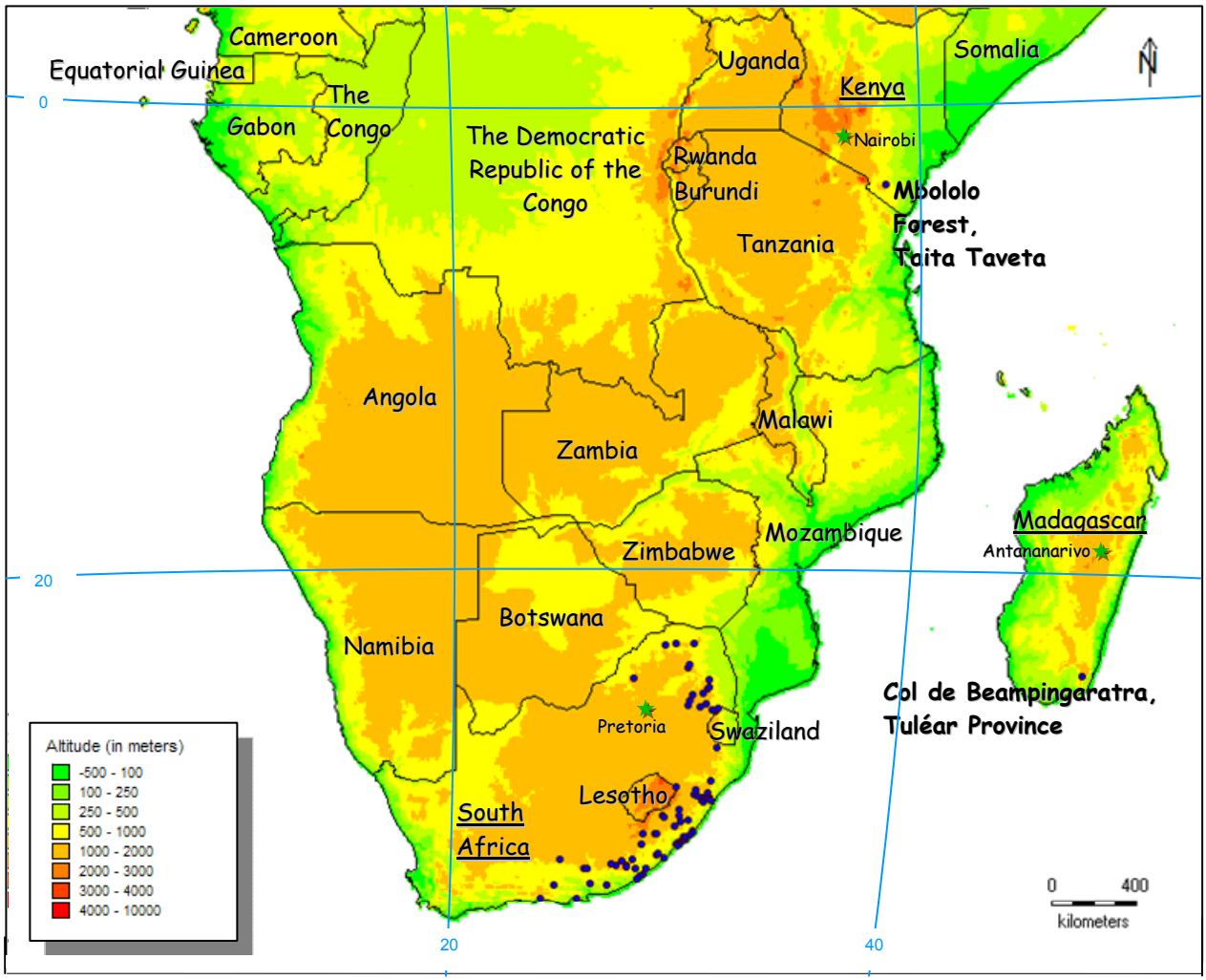

Figure 3.1: Map showing the distribution of the samples analysed during this study. Localities are marked with blue circles, and countries from which samples were collected are underlined. The political map layer was obtained from http://www.diva-gis.org/data.htm.

homogenizing the leaf tissue using a tissue lyser (Southern Cross Biotechnology (Pty) Ltd, Cape Town, South Africa), and leaving the DNA to precipitate at -18°C for at least 30 minutes after adding 2.5 × volumes of ice-cold isopropanol. The protocol is given in full in Appendix 1.

3.2.3. PCR Amplification and Sequencing

Sequences from four regions were collected for examining relationships amongst taxa. These include the nuclear ITS region, and the plastid regions *trn*L-F, *rpl*20-*rps*12 and *trn*C-D.

All these regions were amplified in a P×2 Thermal Cycler (Thermo Electron Corporation, Waltham, Massachusetts, USA) or a MultiGene II Personal Thermal Cycler (Labnet International Inc.,Woodbridge, New Jersey, USA). PCR amplifications for all markers were performed using final concentrations of 1× JMR-455 buffer, 2.5 mM $MgCl_2$, 200 µM dNTPs, 40 µg/ml BSA (bovine serum albumin), 0.5 µM of forward and reverse primer, 0.025 U/µl Taq-polymerase, and *ca*. 2.4 ng DNA in 100 µl volumes.

The following primers were used to amplify each of the regions: AB101 (Douzery *et al*. 1999) and ITS 8P (Möller & Cronk 1997) for the ITS region, c and f (Taberlet *et al*. 1991) for the *trn*L-F region and *rpl*20 and 5'-*rps*12 (Hamilton 1999) for the *rpl*20-*rps*12 region. For the amplification of the *trn*C-D region, primer pairs *trn*C-f & *trn*D-M (Demesure *et al*. 1995) and *ycf*6f & *psb*Mr (http://bfw.ac.at/200/1859.html, accessed on 01 November 2007) were used.

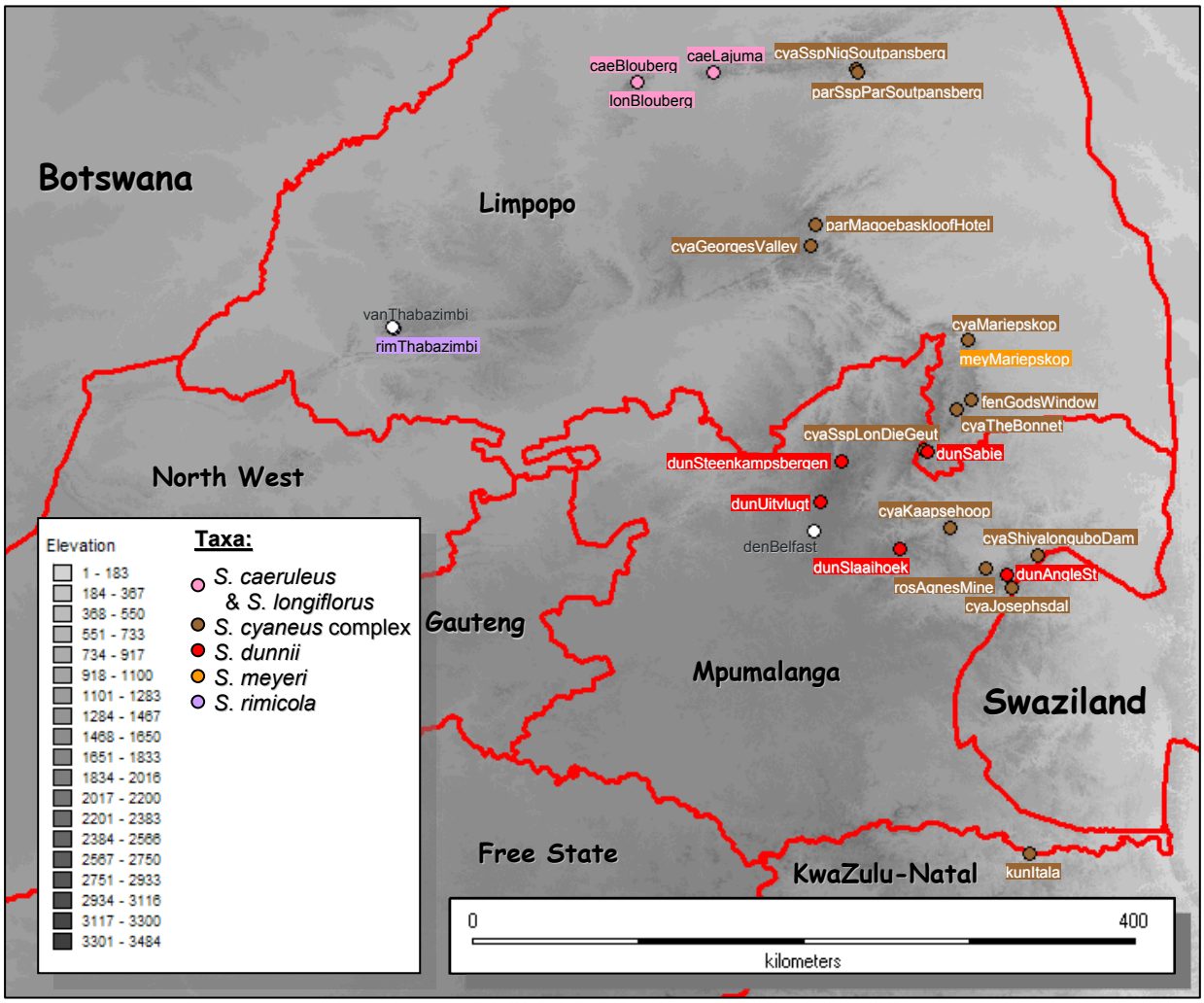

Figure 3.2: Distribution of the samples collected in northern South Africa. Taxon label abbreviations are given in Table 3.1. The political map layer was obtained from http://biogeo.berkeley.edu/bgm/gdata.php.

Additionally, primers *trn*Cf1 (5' ACATGGCCGAGTGGTAAG 3'), *trn*CDf4 (5' GCTAAGAGGCGGAAGTTG 3'), *trn*CDr1 (5' GAAAGAGTCTGATTCATATGATAGA 3'), *trn*CDf3 (5' AGAATAAGAGATCGATAGTATGG 3'), and *trn*CDr4 (5' TACTATTCAAGTCTCGACTACG 3'), designed on the *trn*C-D sequences of the *Streptocarpus* taxa sequenced during the course of this work, were used in various combinations to obtain sequences spanning the entire region (Figure 3.4).

The PCR programs for the regions are as follows: for ITS, an initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C, 55°C and 72°C for 1 minute each, and a final extension for 10 minutes at 72°C; for *trn*L-F, 35 cycles of 45 seconds each at 94°C, 54°C and 72°C, respectively, and a final extension for 6 minutes at 72°C; for *rpl*20-*rps*12, an initial denaturation at 95°C for 1 minute, followed by 35 cycles at 95°C for 30 seconds, and 53°C and 72°C for 45 seconds each, and a final extension for 10 minutes at 72°C; and for *trn*C-D, an initial denaturation at 95°C for 1 minute, followed by 35 cycles at 95°C for 30 seconds, 57°C for 30 seconds and 72°C for 2 minutes, and a final extension for 10 minutes at 72°C. PCR products were checked on 2% agarose gels and cleaned using the Wizard® SV Gel and PCR Clean-up System (Promega Corporation, Madison, Wisconsin, USA). After cleanup, the amount of PCR product was estimated by band intensity on a 2% agarose gel in comparison to a standard amount of pGEM plasmid (Promega).

Cycle sequencing was carried out in 10 µl reactions containing 1.3 µl Terminator Ready

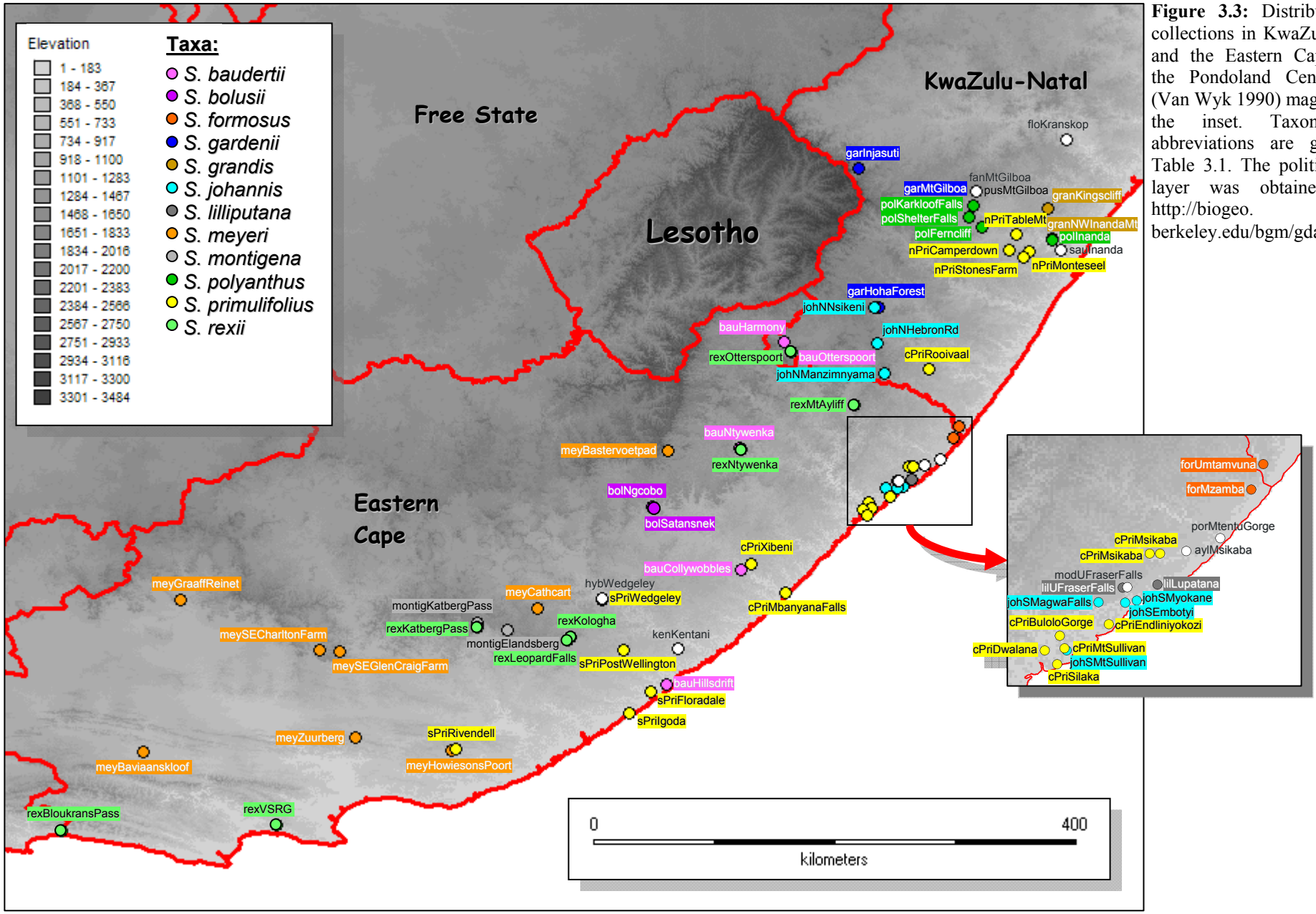

Figure 3.3: Distribution of collections in KwaZulu-Natal and the Eastern Cape, with the Pondoland Centre area (Van Wyk 1990) magnified in the inset. Taxon label abbreviations are given in Table 3.1. The political map layer was obtained from http://biogeo.berkeley.edu/bgm/gdata.php.

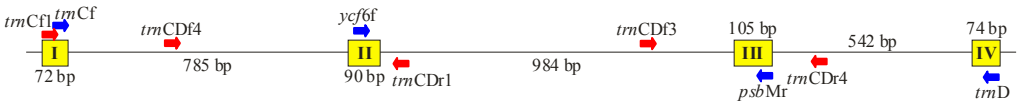

Figure 3.4: The plastid *trn*C-D region, with genes and intergenic spacers drawn to scale. Genes are labelled as follows: I = tRNA-Cys (GCA); II = *pet*N; III = *psb*M and IV = tRNA-Asp (GUC). The blue arrows represent previously published primers (refer to the text for details), and the red arrows represent primers that were designed during the course of this study. The length, measured in base pairs (bp), is indicated for the genes and intergenic spacers constituting the *trn*C-D region.

Reaction Mix (Applied Biosystems, Foster City, USA), 2.7 µl Half-Dye™ Mix (Bioline Ltd, London, UK), 0.32 pmol/µl of each primer, and approximately 100 ng of DNA. The primers used for sequencing are the same as listed for amplification above, with the additional use of the internal ITS 2G (Möller & Cronk 1997) for the sequencing of ITS, when needed. All regions were cycle sequenced in a P×2 Thermal Cycler (Thermo Electron Corporation, USA) or a MultiGene II Personal Thermal Cycler (Labnet International, Inc., USA) using 35 cycles, each of which consisted of 10 seconds at 96°C, 30 seconds at 52°C, and 4 minutes at 60°C.

Cycle sequencing products were cleaned by gel filtration through Centri-Sep 96 Multi-well Filter plates (Whitehead Scientific, Cape Town, South Africa) and run on an ABI Prism 3100 or 3130 XL 16-capillary Genetic Analyser (Applied Biosystems, Foster City, USA) at the Central Analytical Facility, University of Stellenbosch. Sequence electropherograms were edited in Chromas 2.13 (Technelysium Pty., Tewantin, Australia), and sequences were aligned visually in BioEdit 7 (Hall 1999).

3.2.4. Analysis of sequence data

Two data sets were assembled, one containing sequences from the nuclear ITS region and the other consisting of the concatenated sequences from the three plastid regions. Because of the differing inheritance patterns of the nuclear and plastid genomes, congruence of the resulting two data sets was assessed in PAUP*4.0b10 (Swofford 2003) using the incongruence length difference (ILD) test (Farris *et al.* 1995). This method, known in PAUP* as the Partition-Homogeneity (PH) test, compares the length of the most parsimonious trees (MPTs) constructed from the original separate matrices with the length of the MPTs constructed from several pseudomatrices produced by randomly shuffling characters amongst the original data sets. A significant incongruence amongst data sets results in a *P*-value < 0.05, which indicates that the matrices should not be combined. Heuristic search settings for the PH test were set as follows: 1 000 partition homogeneity replicates; stepwise addition to get starting trees; a simple addition sequence; tree-bisection-reconnection (TBR) branch-swapping and the MulTrees option in effect.

Four different techniques were applied to the sequence data to reconstruct evolutionary relationships amongst the taxa. Both maximum parsimony (MP) and Bayesian inference (BI) methods were applied to the nuclear and plastid data sets. Additionally, Neighbour-Net (NN) and Neighbour-Joining (NJ) networks of both all of the taxa and of only those taxa emerging in the clades containing most of the Cape primrose species in the nuclear and plastid trees were also constructed to investigate relationships amongst the taxa further. In all analyses, the characters were unordered and given equal weights, and gaps were treated as missing data.

The MP analyses were performed in PAUP*4.0b10 (Swofford 2003). The most parsimonious topologies were sought using the heuristic search strategy: initial trees were constructed using stepwise addition, adding taxa randomly to the growing trees; these trees were then subjected to

TBR branch swapping in the search for shorter trees. This process was repeated 1 000 times in an attempt to locate the global optimum, retaining a maximum of 10 trees per replicate, with MulTrees in effect. Support for clades was estimated by performing 1 000 bootstrap (BS) pseudoreplicates with random taxon addition and TBR branch swapping. Branches with BS values of 50–74% were considered to be moderately supported, whereas clades with BS values ≥ 75% (written in bold typeface in the text and figures) were considered to be strongly supported.

The monophyly of the species was assessed further by determining the extent to which the results of the MP analyses would differ if the topologies of the trees were constrained so that all species and species complexes were forced to emerge as monophyletic entities. Species that are represented by more than one individual (refer to Table 3.1), and were therefore each constrained to be monophyletic, are *S. bolusii*, *S. montigena*, *S. meyeri*, *S. dunnii*, *S. denticulatus*, *S. rimicola*, *S. grandis*, *S. vandeleurii*, *S. johannis*, *S. modestus*, *S. lilliputana*, *S. formosus*, *S. polyanthus*, *S. gardenii*, *S. primulifolius*, *S. rexii* and *S. baudertii*. Additionally, *S. caeruleus* and *S. longiflorus*, species that are considered to be closely related to each other by Hilliard & Burtt (1971), and members of the *S. cyaneus* complex (*S. cyaneus*, *S. parviflorus*, *S. fenestra-dei*, *S. roseo-albus* and *S. kunhardtii*) were also each forced to form monophyletic clades. The same heuristic search options as described above were applied to these analyses, and the difference between the unconstrained versus the constrained analyses for the each of the data sets was assessed for significance using the Templeton (Wilcoxon signed-rank) test (Templeton 1983) in PAUP*4.0b10.

BI was used as an additional optimality algorithm to assess similarity of topologies constructed using different evolutionary assumptions. The optimum model of character evolution for each of the three plastid regions and the nuclear region was individually determined by the Akaike Information Criterion (AIC; Akaike 1974) test as implemented in MrModeltest 2.2 (Nylander 2004). Separate BI analyses were then performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) on the ITS data matrix and a combination of the three plastid regions, in the latter case treating each region as a separate partition so that optimal DNA substitution models could be applied to each region. The two separate analyses were run three times for 150 000 generations each to monitor levels of mixing amongst chains under different chain-heating regimes, and to determine the similarity of topologies across runs. The temperature had to be reduced to 0.1 for both data sets to improve mixing amongst chains. Runs of 1 500 000 generations were then conducted on the two separate data sets, using two independent runs of four chains each to determine the difference in results obtained from independent runs (given as the average standard deviation of split frequencies in MrBayes), and sampling every 100 generations. The number of generations constituting the burn-in was determined visually by plotting the log likelihood values of the cold chain across the generations of each run, and only trees from the generations after the log likelihood had reached stationarity were retained. A 50% majority-rule consensus tree was constructed in PAUP*4.0b10 for each of the data sets after having discarded the trees generated during the burn-ins. Clades with posterior probability values (PP) ≥ 0.95 (written in bold typeface in the text and figures) were deemed to be strongly supported.

The relationship between support values from the MP and BI analyses for the two data sets was investigated by plotting BS against PP values for branches in the trees that were supported by both BS and PP values of over 50% and 0.50, respectively.

In addition to investigating relationships amongst the taxa using bifurcating phylogenetic tree methods, networks were also constructed to assess relationships. When dealing with relationships within species, and amongst closely related species, networks provide an alternative, and perhaps more realistic, way of visualising the signal within data sets. Because one is dealing with recent evolutionary events in *Streptocarpus*, it is highly likely that ancestral

characteristics will be sampled along with more derived ones. While molecular phylogenetic trees only allow sampled taxa to emerge at terminal nodes, network algorithms allow taxa to emerge in deeper (ancestral) positions within the network (Posada & Crandall 2001). Thus, networks can give a better idea of which taxa are more ancestral and which are more derived based on the number of other taxa connected to the taxon, and its position and abundance in the network (Castelloe & Templeton 1994). Networks can also provide an indication of the extent of conflict within the data. Network algorithms insert cycles into the parts of the topology where the course of evolution is equivocal based on the data (whether due to homoplasy or hybridization), rather than forcing taxa into strictly bifurcating relationships (Makarenkov *et al*. 2006). Therefore, depending on the type of network constructed, cycles can be interpreted as showing incongruence amongst the characters constituting the data set, possibly indicating alternative pathways along which evolution could have occurred.

Conflict within the nuclear and plastid data sets was explored by computing Neighbour-Net (NN) networks in SplitsTree 4.8 (Huson & Bryant 2006). Distances in the form of the proportion of positions at which each sequence pair differs was calculated with the UncorrectedP method (due to low divergence across the data sets) with ambiguous states treated as missing data, the NN algorithm was applied to the resulting distance matrix to compute a set of incompatible splits, and the EqualAngle algorithm was used to construct a planar split network of the resulting splits. Neighbour-Joining (NJ) networks were also constructed in SplitsTree 4.8, using the UncorrectedP method with ambiguous states treated as missing data and EqualAngle algorithms.

3.2.5. Dating of clades in the phylogenetic trees

Finally, in order to obtain a rough idea of the time frame during which the South African *Streptocarpus* species have been evolving, the age of the clade containing all of the South African samples—a clade that was supported by both the nuclear and plastid data—was estimated. Fossils and calibration points within *Streptocarpus* are unfortunately lacking, and dating was therefore performed by applying previously published, independently calibrated rates of ITS (Kay *et al*. 2006) and *trn*L-F (Richardson *et al*. 2001) evolution from other angiosperm groups to the current phylogenies.

Kay *et al*. (2006) collected and compared 29 ITS substitution rates from studies on both woody and herbaceous plants from 21 different angiosperms families. These rates ranged from 0.38×10^{-9} substitutions/site/year (s/s/y) in *Hamamelis* L. (Hamamelidaceae R.Br.) to 19.00×10^{-9} s/s/y in *Gentiana* L. Section *Ciminalis* (Adans.) Dumort (Gentianaceae Juss.). The fastest ITS substitution rate (that of *Gentiana* Section *Ciminalis*) was rather different from the other rates. This rate was, however, calculated from a single base substitution. This, together with the fact that the calculated rate is more than twice as fast as the next fastest rate (8.34×10^{-9} s/s/y in *Soldanella* L. [Primulaceae Borkh.]) caused Kay *et al*. (2006) to exclude this rate from most of their analyses, and this rate was therefore also not used in the current analysis. Amongst the remaining rates, Kay *et al*. (2006) found no significant correlation between detected ITS evolutionary rates and phylogenetic relatedness. It is therefore invalid to assume that the ITS substitution rate of the lineage that is the most closely related to subgenus *Streptocarpus* is probably a more accurate estimate of the ITS substitution rate in subgenus *Streptocarpus* than those of more distantly related lineages. A good example of this can be found in *Streptocarpus*, in which Möller & Cronk (2001a) found that the rates of evolution of ITS are 2.44 times faster in subgenus *Streptocarpella* than in subgenus *Streptocarpus*. Kay *et al*. (2006) did, however, find a correlation between rates and growth form. The ITS regions in the annual and perennial herbaceous lineages analysed evolve on average at almost twice the rate (4.13×10^{-9} s/s/y) of ITS in the woody lineages (2.15×10^{-9} s/s/y), reflecting the higher rates detected in herbs compared with woody trees in previous studies. The increased rate of molecular evolution in

herbaceous lineages compared with woody lineages has been attributed to the shorter life cycles of herbs compared with trees, although there are confounding factors. Thus, rather than using rates of related lineages for calibrating trees, Kay *et al*. (2006) recommended using the rates calculated from all the lineages with the appropriate growth form. Because subgenus *Streptocarpus* consists of herbaceous perennials, the ten substitution rates from the herbaceous groups (excluding the divergent rate of the herbaceous *Gentiana* Section *Ciminalis*) were included in the present calculations. Thus, the rates used in the current study ranged from 1.72×10^{-9} s/s/y in *Saxifraga* L. (Saxifragaceae Juss.) to 8.34×10^{-9} s/s/y in *Soldanella*.

For calculating the age of the corresponding clade in the plastid tree, evolutionary rates of *trn*L-F published by Richardson *et al*. (2001) were used. Richardson *et al*. (2001) listed three rates of substitution for *trn*L-F, namely 4.87×10^{-10} s/s/y in *Phylica* L. (Rhamnaceae Juss.), 1.30×10^{-9} s/s/y in *Inga* Mill. (Fabaceae Lindl.), and 8.24×10^{-9} s/s/y in *Aichryson* Webb & Berthel. (Crassulaceae J.St.-Hil.). The former two genera—*Phylica* and *Inga*—consist of trees and shrubs; only *Aichryson* consists of annuals and perennials. Nevertheless, *Inga* taxa have very short generation times for trees. Almost half of the species are fast-growing, many of the remaining species are small- to medium-sized, and many of the canopy species are capable of producing seed 2–3 years after germination (Richardson *et al*. 2001). Due to the lack of data (the limited number of published rates for *trn*L-F), the age of the clade in the present study was calculated using the rates from all three genera, despite the diversity of growth forms.

These previously published ITS and *trn*L-F rates were used to estimate the average ages of the clade in the nuclear and plastid phylogenies by, for each region, calculating the average distance (in branch lengths) of the unique sequences from the present time to the base of the clade containing all of the South African samples. The branch lengths of the unique sequences in the ITS tree were determined directly from the ITS phylogram constructed during the phylogenetic analyses. For the *trn*L-F dating, the branch lengths were determined by visualizing only the *trn*L-F data on the topology reconstructed using all three plastid regions. For each of the two data sets, the average calculated distance was then divided by the total length of its corresponding sequence matrix to get the substitutions/site. This was in turn divided by each of the published substitution rates found in the other angiosperm lineages to get the age of the clade given each rate. Finally, these age estimates were averaged to get a mean approximate age for the South African clade, along with its standard error (SE), for each data set.

3.3. Results

3.3.1. Congruence of the nuclear and plastid data sets

The PH test, conducted to determine the extent of congruence between the nuclear and plastid data sets, yielded a *P*-value of 0.001. Results of the test therefore indicate that the two data sets contain conflicting phylogenetic signals, and should not be combined. Although in previous publications (e.g. Barker & Lutzoni 2002; Hipp *et al*. 2004) the ILD test has been shown to be a poor test of the combinability of separate data sets, the data sets were nevertheless analyzed separately in all subsequent analyses.

3.3.2. Analyses of the nuclear ITS sequence data using bifurcating phylogenetic tree methods

The ITS alignment contained a high level of variability (Table 3.2). Of the 749 characters included in the final alignment, 181 (24.2%) were variable, and 127 (70.2%) of the variable characters were parsimony informative. However, most of the variability was contained at

Table 3.2: Statistics of the nuclear ITS matrix and of the separate and combined plastid DNA matrices analysed under parsimony criteria.

	Nuclear data	Plastid data			
	ITS	***trn*C-D**	***trn*L-F**	***rpl*20-*rps*12**	**Combined plastid data**
Total number of characters analysed	**749**	2 275	836	769	**3 881**
Number of variable characters	**181 (24.17%)**	176 (7.74%)	54 (6.46%)	72 (9.36%)	**302 (7.78%)**
Number of parsimony-informative characters	**127 (16.96%)**	93 (4.09%)	25 (2.99%)	37 (4.81%)	**155 (3.99%)**

deeper levels within the tree (Figure 3.5) i.e. amongst the main clades and within the more poorly sampled clades, and the number of parsimony-informative characters amongst the taxa in the more extensively sampled clade (clade VII in Figure 3.5) was only 21, 2.8% of all the characters. Another factor leading to a lack of resolution in the nuclear trees was the prevalence of polymorphic peaks in the ITS sequences (Figure 3.6). This was particularly common in the taxa emerging in the more extensively sampled clade, and caused less resolved topologies in the ensuing analyses. The lack of resolution in the ITS trees is therefore due to a lack of informative characters and the presence of ambiguities in the existing informative characters.

In the MP analysis of all the characters, the ITS data yielded 9 890 equally parsimonious trees of 267 steps, with a consistency index (CI) of 0.7715, a retention index (RI) of 0.9368, and an average number of steps per character of 0.356 (267 steps/749 characters), indicating a low level of substitution saturation (Table 3.3). The BS analysis revealed that 37 internal branches had more than 50% BS support, of which 23 (62.2%) were strongly supported (had a BS≥**75**%).

Imposing topological constraints in order to test the effect of making all the species and the two species complexes monophyletic resulted in trees that were significantly different in length from trees produced by the unconstrained analysis in the Templeton (Wilcoxon signed-ranks) test. The resulting MPTs were 109.4% longer (P = 0.0006) than the equivalent analysis run without topological constraints.

For the BI analysis, the SYM+G model was selected for the ITS data by MrModeltest. After 1 500 000 generations, the average standard deviation of split frequencies was 0.010804. This indicates that the sets of trees sampled by the two independent runs are similar, and were therefore probably sampled from similar areas of the tree space. Trees from the first 50 000 generations were discarded as the burn-in. The BI analysis yielded more clades in the 50% majority-rule consensus tree than in the strict consensus tree of the MP analysis, and contained a greater proportion of strongly supported clades (with a PP ≥ **0.95**).

The MP and BI analyses of the ITS data resulted in a phylogeny that was well resolved amongst the deeper clades. The two samples collected outside of South Africa, *S. papangae* from Madagascar and *S. montanus* from Kenya, emerged distant to the rest of the taxa, which collectively formed the strongly supported clade I (PP=**1.00**; BS=**99**%). The South African taxa separated into six main clades, labelled II–VII in Figure 3.5. Clade II (PP=0.72; BS=**83**%) contains all the samples of *S. vandeleurii*, *S. grandis*, *S. rimicola*, *S. denticulatus* and *S. dunnii*, each forming moderately to strongly supported monophyletic clades (PP=0.78–**1.00**; BS=67–**100**%), as well as the individual samples of *S. pusillus* and *S. fanniniae*. The geographic range of this clade extends from Thabazimbi in Limpopo through Mpumalanga to Inanda in KwaZulu-Natal. Clade III (PP=**1.00**; BS=**100**%) contains the two *S. bolusii* representatives and has a narrow distribution towards the northern part of the Eastern Cape. Clade IV has a very wide distribution: the Mtentu Gorge is located in the northern part of the Eastern Cape along the

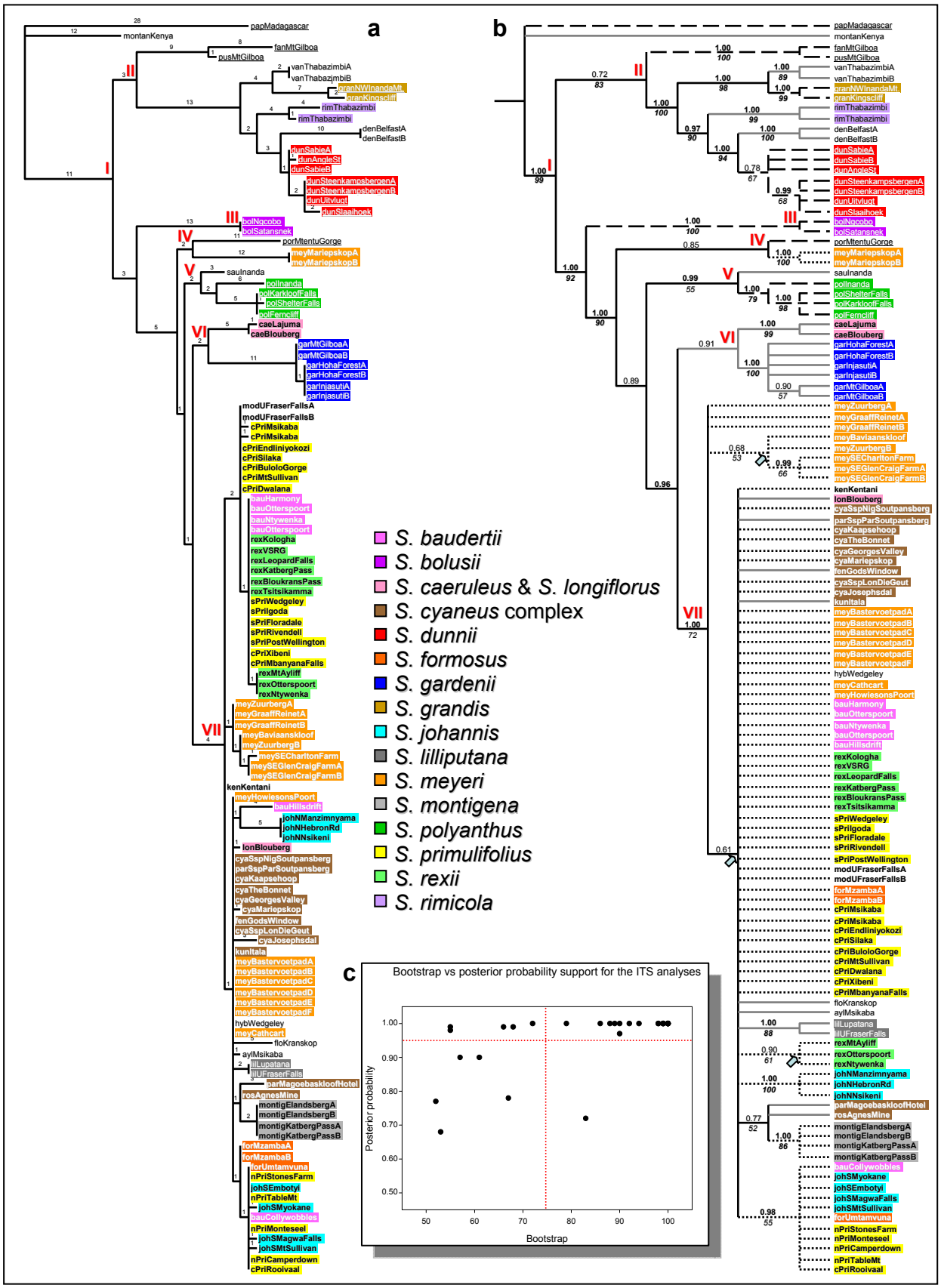

Figure 3.5: Maximum parsimony (MP) and Bayesian inference (BI) trees resulting from the phylogenetic analyses of the nuclear ITS data set with taxa represented by individuals from more than one population colour-coded. **a:** One of 9 890 MP trees of 267 steps, with branch lengths shown. **b:** The BI 50% majority-rule consensus tree with posterior probabilities (PP) given above the branches in normal type, and MP bootstrap (BS) percentages of the equivalent clade from the parsimony analysis given below the branches in italics. BS ≥ 75% and PP ≥ 0.95 are considered to indicate strong support and are written in bold type. Branches that collapse in the strict consensus

tree in the MP analysis are indicated with arrows on the BI tree. Stippled branches indicate samples of species that emerged within the Cape primrose clade in Möller & Cronk's (2001a) ITS analysis, species that fell outside the Cape primrose clade in the same analysis are indicated by dashed branches and underlined taxon labels, and grey branches indicate species that were not analysed by Möller & Cronk (2001a). **c:** A plot of BS versus PP for those clades with both ≥ 50% BS and ≥ 0.50 PP support in the MP and BI analyses, respectively. A red stippled line separates moderate support values from strong support values for both measures.

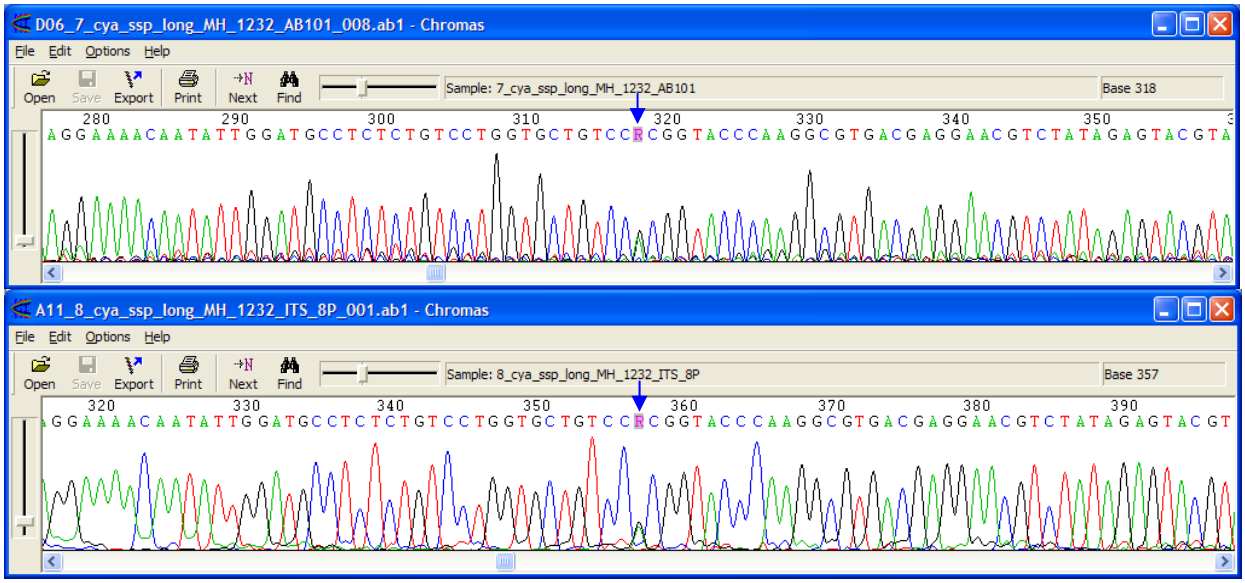

Figure 3.6: A polymorphic site indicated by blue arrows within the ITS sequence of *S. cyaneus* ssp. *longi-tommii* from Die Geut, Limpopo Province, South Africa, evident in the chromatograms of sequences generated from both the 5' and 3' directions.

Table 3.3: Tree statistics for the nuclear ITS and the combined plastid DNA analyses conducted under parsimony and Bayesian criteria. CI = consistency index; RI = retention index; BS = bootstrap support; PP = posterior probability.

	Nuclear data	Plastid data
Parsimony analyses with unconstrained topologies:		
Number of trees found	9 890	8 512
Tree length	267	358
CI	0.7715	0.8855
RI	0.9368	0.9509
Average number of steps/character	0.356	0.090
Number of clades with BS ≥ 50%	37	46
Number of clades with BS ≥ 75%	23 (62.16%)	28 (60.87%)
Parsimony analyses with topological constraints in effect:		
Number of trees found	9 890	9 880
Tree length	292	409
CI	0.705	0.775
RI	0.911	0.890
Bayesian analyses:		
Number of clades present in over 50% the most likely trees	37	55
Proportion of clades with PP ≥ 0.95	27 (72.97%)	44 (80.00%)

coast, whereas Mariepskop is much further north, in the southern part of the Limpopo Province. However, this clade may be the result of long-branch attraction as is indicated by its low BI and MP support (PP=0.85; BS<50%) and the long branch lengths leading to the *S. porphyrostachys* (11 steps) and to the *S. meyeri* (12 steps) samples compared to the length of the branch (2 steps) linking them together. Clade V (PP=**0.99**; BS=55%), which contains the *S. saundersii* representative and all the members of *S. polyanthus* sampled for this study, also has a very

narrow geographical distribution, with all the localities situated towards the middle of KwaZulu-Natal. However, this very narrow distribution is possibly a sampling artefact, seeing as *S. polyanthus* has a far wider distribution than is covered by its representative samples in this analysis. Clade VI, on the contrary, again extends over a very broad area, from two of the most northerly South African localities (Lajuma and Blouberg) in Limpopo to the Hoha Forest in southern KwaZulu-Natal, and comprises all of the *S. caeruleus* and *S. gardenii* populations, each forming well-supported monophyletic clades (PP=**1.00**; BS=**99–100**%). However, the entire Clade VI is poorly supported, with a PP of only 0.91 and a BS of less than 50%. Clade VII contains the majority of the samples and covers the entire geographical range of the South African taxa. This clade has a high PP value of **1.00**, but is only moderately supported in the parsimony analysis (BS=72%).

Consequently, it is apparent from the ITS trees that the main clades that tend to have more limited geographical distributions also tend to receive stronger branch support (clades II [PP=0.72; BS=**83**%], III [PP=**1.00**; BS=**100**%] and V [PP=**0.99**; BS=55%]), whereas the main clades with wider distributions tend to be only moderately supported (clades IV [PP=0.85; BS<50%], VI [PP=0.91; BS<50%]), with exceptions (Clade VII [PP=**1.00**; BS=72%]).

Within the more extensively sampled clade, clade VII, most of the samples emerge in unresolved positions. The majority of the southernmost *S. meyeri* samples (meyBaviaanskloof, meySECharltonFarm, meySEGlenCraigFarm and meyZuurbergB) group together (PP=0.68; BS=53%), with the three samples collected from the Somerset East area (one from meySECharltonFarm and two from meySEGlenCraigFarm) forming a more strongly supported subclade (PP=**0.99**; BS=66%) within this clade. The remaining southernmost *S. meyeri* samples (meyGraaffReinet and meyZuurbergB) emerge in unresolved positions sister to this clade and a clade (PP=0.61; BS<50%) containing the remaining members of clade VII. This latter clade only contains a few subclades, the vast majority of the samples emerging in unresolved positions. However, the two *S. lilliputana* populations group together strongly (PP=**1.00**; BS=**88**%), the three northernmost *S. rexii* populations (rexOtterspoort, rexMtAyliff and rexNtywenka) constitute another clade (PP=0.90; BS=61%), likewise the three northern *S. johannis* populations (johNNsikeni, johNHebronRd and johNManzimnyama) form a strongly supported clade (PP=**1.00**; BS=**100**%), while the four southern *S. johannis* populations (johSMagwaFalls, johSEmbotyi, johSMyokane and johSMtSullivan) emerge in a separate clade along with the four northern *S. primulifolius* populations (nPriTableMt, nPriCamperdown, nPriMonteseel and nPriStonesFarm), the *S. primulifolius* population from Rooivaal, the *S. formosus* sample from Umtamvuna, and the *S. baudertii* sample from Collywobbles (PP=**0.98**; BS=55%). The only other clade in clade VII is one containing the four *S. montigena* samples (PP=**1.00**; BS=**86**%), which group together with two populuations of the *S. cyaneus* complex (PP=0.77; BS=52%).

The graph comparing MP and BI support values for those clades in the ITS trees receiving both a BS ≥ 50% and a PP ≥ 0.50 (Figure 3.5c) displays a positive correlation between the two support measures at lower values of BS and PP i.e. towards the bottom left-hand corner of the graph. However, PP reaches its maximum long before BS, resulting in a levelling out of PP at its maximum at higher BS measures. Consequently, all clades that were supported in the MP analysis (BS≥50%) were also supported in the BI analysis (PP≥0.50), and all those that were strongly supported in the MP analysis (BS≥75%) were also strongly supported in the BI analysis (PP≥0.95), with one exception: clade II has a BS of **83**%, but a PP of only 0.72. Conversely, clades that received support in the BI analysis did not necessarily also receive support in the MP analysis. Indeed, the clade in the tree containing clades VI and VII possesses a PP of **0.96**, but a BS<50%. Thus, PP support tended to be higher than BS support, and, with

the exception of six clades, clades that were supported in the BS analysis were strongly supported in the BI analysis.

3.3.3. Analyses of the combined plastid sequence data using bifurcating phylogenetic tree methods

In contrast to the ITS data, the plastid data yielded more resolved trees. Although the plastid alignment contained a lower proportion of variability than did the ITS alignment, with 7.8% variable characters, about half of which were parsimony-informative (Table 3.2), the concatenated plastid regions amounted to more than five times the number of characters compared with the ITS alignment. Consequently, the plastid matrix contained 155 parsimony-informative characters compared to 127 in the ITS data. Additionally, variability was more evenly spread amongst taxa in the plastid trees (Figure 3.7) than in the ITS trees (Figure 3.5), providing a certain amount of resolution amongst the taxa in the more extensively sampled clade (57 [1.5%] of all the characters were parsimony informative amongst the taxa in clade F in Figure 3.7), while being limited enough to enable the unambiguous alignment of the more distantly related taxa.

MP analysis of the plastid matrix using all the characters resulted in 8 512 equally parsimonious trees of 358 steps, with a CI of 0.8855, an RI of 0.9509, and a very low average number of steps per character of 0.09 (Table 3.3). The BS branch support analysis supported 46 internal branches, of which 28 (60.9%) received ≥ 75% support. Thus, the plastid alignment was more informative than the ITS alignment, yielding trees that were somewhat better resolved and contained a larger number of strongly supported clades. The CI and RI were also higher than in the ITS analysis.

The MPTs resulting from the plastid analysis with constraints imposed on the tree searches were 114.3% longer ($P < 0.0001$) than was the case in the unconstrained analysis. Thus, forcing the species and two species complexes to emerge as monophyletic groups had a noticeably detrimental effect on the results.

For the BI analysis, the AIC tests conducted in MrModeltest chose GTR+I+G as the optimum model of character evolution for the *trn*C-D region, and GTR+I for both the *rpl*20-*rps*12 and *trn*L-F regions. At the completion of the plastid BI analysis, the average standard deviation of split frequencies was 0.013613, signifying that the two tree samples are similar to each other. Trees from the first 150 000 generations had to be discarded as burn-in. The BI analysis of the plastid data also yielded trees that were more resolved, and had a greater number of strongly supported clades than the MP analysis.

Both the MP and BI phylogenetic analyses of the plastid data yielded more resolved trees (Figure 3.7) than the ITS analyses. Like in the ITS analyses, the South African samples emerged as a distinct clade, clade A (PP=**1.00**; BS=**96**%), separate from the non–South African taxa. The South African samples segregated into five main clades, labelled B–F in Figure 3.7. Clade B (PP=**0.98**; BS=**89**%) contains the *S. pusillus* and two *S. bolusii* representatives, with a geographical distribution from towards the middle of KwaZulu-Natal to the northern part of the Eastern Cape. Clade C (PP=**1.00**; BS=**100**%) contains all the representatives of *S. montigena*, which were collected from two localities situated very close to each other in the middle of the Eastern Cape. Clade D (PP=**1.00**; BS=**100**%), also a purely Eastern Cape clade, contains all the members of *S. meyeri*, except for those from Mariepskop, Bastervoetpad and Cathcart i.e. all of the southernmost *S. meyeri* populations. Two individuals of *S. meyeri* were analysed from Mariepskop, which is located much further north (approximately 800 km) in the Limpopo

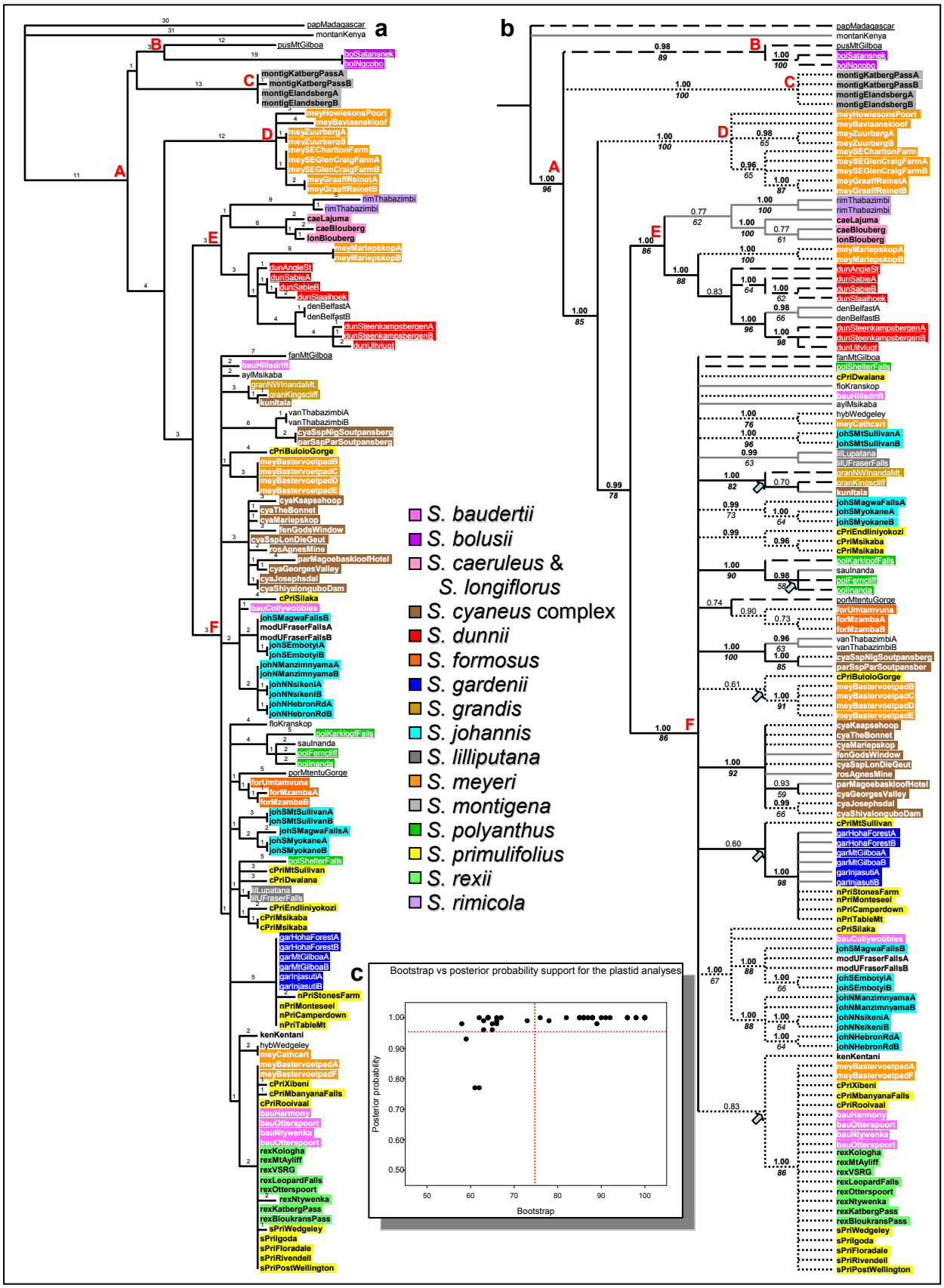

Figure 3.7: Topologies retrieved from maximum parsimony (MP) and Bayesian inference (BI) analyses of the combined plastid data set with taxa represented by individuals from more than one population colour-coded. **a:** One of 8 512 MP trees of 358 steps, with branch lengths shown. **b:** The BI 50% majority-rule consensus tree with posterior probabilities (PP) given above the branches in normal type, and MP bootstrap (BS) percentages of the equivalent clade from the parsimony analysis given below the branches in italics. BS ≥ 75% and PP ≥ 0.95, considered to indicate strong support, are given in bold type. Branches that collapse in the strict consensus tree in

the MP analysis are indicated with an arrow on the BI tree. Stippled branches indicate samples of species that emerged within the Cape primrose clade in Möller & Cronk's (2001a) ITS analysis; species that fell outside the Cape primrose clade in the same analysis are indicated by dashed branches and underlined taxon labels, and grey branches indicate species that were not analysed by Möller & Cronk (2001a). **c:** A plot of BS vs PP support for those clades with BS ≥ 50% and PP ≥ 0.50 support in both the MP and BI analyses, respectively. A red stippled line separates moderate support values from strong support values for both measures.

Province. These two samples emerged in clade E (PP=**1.00**; BS=**86**%), together with the *S. rimicola*, *S. caeruleus*, *S. longiflorus*, *S. dunnii* and *S. denticulatus* samples, which were collected in the Limpopo province and northern Mpumalanga.

As is the case in the ITS trees, the largest clade in the plastid tree, clade F (PP=**1.00**; BS=**86**%), spans the entire geographic range of the South African taxa sampled. At its deepest level, this clade is a large polytomy, but does contain 14 subclades, of which 10 (*ca.* 71%) are strongly supported (PP≥**0.95**; BS≥**75**%), either just in the BI analysis, or in both the BI and MP analyses. Of the 11 species and species complexes represented by samples from more than one population emerging in this more extensively sampled clade, only *S. lilliputana* and *S. formosus* form monophyletic groups. The two *S. lilliputana* representatives form a strongly supported clade (PP=**0.99**; BS=63%). The three *S. formosus* representatives group together (PP=0.90; BS<50%), forming a weak link with *S. porphyrostachys* (PP=0.74; BS<50%), the population geographically closest to the *S. formosus* populations. The other species represented by more than one population are more dispersed in this clade. Three of the four *S. polyanthus* populations emerge with *S. saundersii* in a strongly supported clade (PP=**1.00**; BS=**90**%). The *S. johannis* populations emerge in three separate clades, one containing the samples from Mount Sullivan (PP=**1.00**; BS=**96**%), another the samples from Myokane and one of the Magwa Falls representatives (PP=**0.99**; BS=73%), and the rest together with with *S. baudertii* from Collywobbles, *S. modestus* from Fraser Falls and *S. primulifolius* from Silaka also included (PP=**1.00**; BS=67%). Although the three northern populations of *S. johannis* (johNNsikeni, johNHebronRd and johNManzimnyama) form a strongly supported group (PP=**1.00**; BS=**88**%) within this clade, the southern populations (johSMagwaFalls, johSEmbotyi, johSMyokane and johSMtSullivan) are more dispersed. Another strongly supported clade (PP=**1.00**; BS=**92**%) contains all the members of the *S. cyaneus* complex except for *S. cyaneus* subsp. *nigridens* and *S. parviflorus* subsp. *parviflorus* from Soutpansberg, and *S. kunhardtii* from Itala. The two Soutpansberg samples together emerge sister to *S. vandeleurii* from Thabazimbi (PP=**1.00**; BS=**100**%), which is also situated in the Limpopo Province. *S. kunhardtii* from Itala groups with the two *S. grandis* representatives (PP=**1.00**; BS=**82**%), all of which are found in KwaZulu-Natal. The northern *S. primulifolius* populations (nPriTableMt, nPriCamperdown, nPriMonteseel and nPriStonesFarm) group with the *S. gardenii* individuals (PP=**1.00**; BS=**98**%), which in turn groups weakly with the central *S. primulifolius* population from Mount Sullivan (PP=**0.60**; BS<50%). Three of the other central *S. primulifolius* populations also group together, namely the two Msikaba and the Endliniyokozi populations (PP=**0.99**; BS<50%). Four of the six *S. meyeri* individuals from Bastervoetpad form a strongly supported group (PP=**1.00**; BS=**91**%), which together group weakly with the *S. primulifolius* population from Bulolo Gorge (PP=0.61; BS<50%). The two remaining Bastervoetpad representatives (individuals A and F) emerge in a large group containing most of the rest of the taxa (PP=**1.00**; BS=**86**%). These include representatives from the three northernmost *S. baudertii* populations (two from Otterspoort, and one each from Harmony and Ntywenka), three of the central *S. primulifolius* populations (cPriRooivaal, cPriXibeni and cPriMbanyanaFalls), all the southern *S. primulifolius* populations (sPriWedgeley, sPriPostWellington, sPriFloradale, sPriIgoda and sPriRivendell), and all the *S. rexii* populations. This clade weakly links with the *S. kentaniensis* sample (PP=0.83; BS<50%). The only other *S. meyeri* population to emerge within clade VII is meyCathcart, which groups strongly (PP=**1.00**; BS=**76**%) with the hybrid population (hybWedgeley).

Thus, most of the species represented by more than one population, including *S. formosus*, *S. lilliputana*, *S. gardenii*, *S. rexii*, *S. grandis*, *S. polyanthus*, *S. johannis* and the *S. cyaneus* complex, either form isolated groups or tend to group together. In contrast, the members of *S. meyeri*, *S. baudertii* and *S. primulifolius* are more scattered.

The support measures from the MP and BI analyses of the plastid data show an even more predictable relationship than in the ITS analyses, forming a distinctly upside-down L shape in the plot (Figure 3.7c). All clades with strong PP support also received BS≥50%, and, with the exception of three clades, all clades that received BS≥50% were strongly supported in the BI analysis. Thus, PP appears to need less evidence to reach its maximum value than is the case for the BS measure.

A comparison of the topologies resulting from the separate phylogenetic analyses of the nuclear and plastid data in this study reveal several incongruences between the two data sets (Figure 3.8), confirming the results of the PH test that the two data sets are too different to be combined. The youngest (most terminal) clades in the more poorly sampled parts of the phylogenies tend to maintain their internal structure between the two trees. However, relationships among these clades were not congruent between the two trees, as indicated by the pink lines in Figure 3.8. Moreover, not only do the topologies of the two trees disagree with regards to relationships amongst the taxa emerging in the more poorly sampled parts of the phylogenies, but some of the taxa that group in the poorly sampled parts (outside of clade VII) in the ITS tree emerge in the more extensively sampled parts (within clade F) in the plastid tree (indicated by green lines) and vice versa (blue lines). Relationships within the more extensively sampled clades of the ITS (clade VII) and plastid (clade F) analyses also do not appear to be strictly congruent. The relative positions of the samples in the ITS and plastid trees that emerge in resolved positions in the clade VII of the ITS tree are indicated by orange lines. There is, however, not enough resolution within clade VII of the ITS analysis to conclude much.

3.3.4. Analyses of the nuclear and plastid data sets using network approaches

In order to gain additional insight into relationships, network approaches were employed. Neighbour-Net (NN; Figure 3.9) and Neighbour-Joining (NJ; Figure 3.10) networks constructed from the entire ITS (Figures 3.9a & 3.10a) and plastid (Figures 3.9b & 3.10b) data sets were largely congruent with each other and with their corresponding phylogenetic trees (Figures 3.5 & 3.7). Thus, most of the main clades in the nuclear phylogenetic trees (clades I, III, V, VI and VII) are represented in the nuclear networks, and all of the main clades in the plastid phylogenetic trees (clades A–F) are represented in the plastid networks.

The NN algorithm yielded networks that are largely tree-like (Figure 3.9). In other words, despite the presence of parallelograms in both the nuclear and plastid NN networks, which signifies conflict in the data, the parallelograms tend to be long and thin, especially in the nuclear NN network (Figures 3.9a), indicating that most of the groupings are supported by many characters, while only a few characters oppose these same groupings. Furthermore, the members of strongly supported clades in the phylogenetic trees emerged on longer, thinner parallelograms than the members of less well-supported clades, indicating a greater number of characters in support of and fewer in conflict with these groupings. The NJ networks built from the nuclear (Figure 3.10a) and plastid (Figure 3.10b) data contain all of the strongly supported (PP≥**0.95**; BS≥**75%**) groupings present in the phylogenetic trees, mostly on very long branches. Thus, the main plastid, and to a lesser extent, nuclear groupings tend to be supported by many characters, and conflict amongst these main groupings, especially in the nuclear data, is minimal.

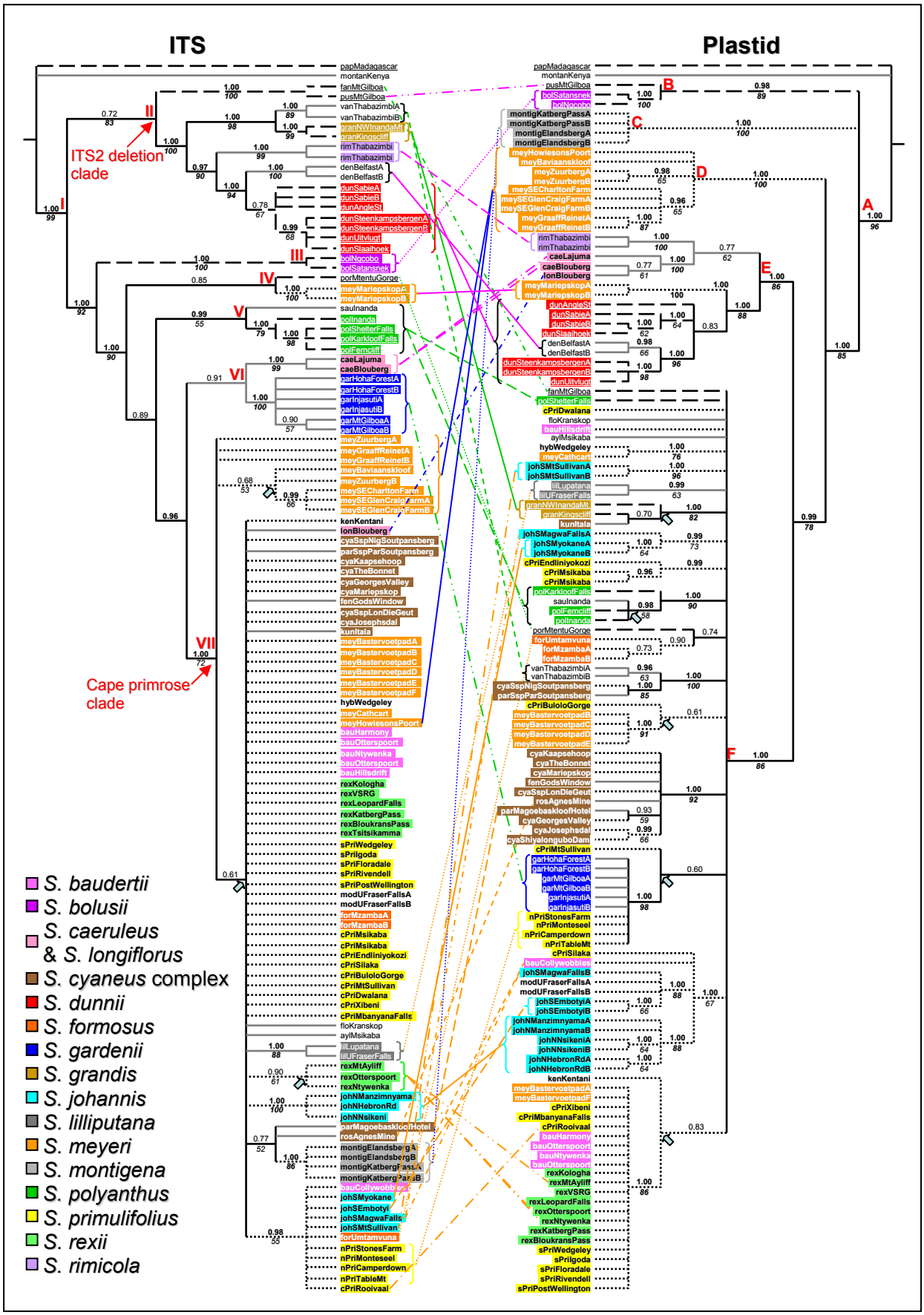

Figure 3.8: Bayesian 50% majority-rule consensus trees from the nuclear and plastid analyses (Figures 3.5b & 3.7b). The "ITS2 deletion clade" and "Cape primrose clade" from Möller & Cronk (2001a, 2001b) are indicated on the ITS tree, and correspond to clades II and VII, respectively. Disagreement between the trees caused by taxa emerging in the more extensively sampled clade (clade VII) in the ITS tree, but in the more poorly sampled parts

of the tree (outside clade F) in the plastid tree are indicated by blue lines; taxa that emerge within clade F in the plastid tree, but in the more poorly sampled parts of the ITS tree are indicated by green lines; and pink lines point to the relative positions of the taxa that fall in the more poorly sampled parts of both trees. Orange lines indicate the positions in the ITS and plastid trees of the taxa that emerged within subclades of clade VII in the ITS tree and clade F in the plastid tree.

Because the taxa in the more poorly sampled parts of the topologies emerged on such long branches in the networks, relationships amongst the taxa in the more extensively sampled groups (samples that emerged in clade VII in the ITS trees and in clade F in the plastid trees) are difficult to see in Figures 3.9 and 3.10, and additional networks were therefore constructed including only the taxa emerging in the more extensively sampled groups (Figures 3.11–3.14).

3.3.4.1. Network analyses of the samples emerging in the more extensively sampled group based on the nuclear data set

As is the case for the networks including all of the taxa, the relationships amongst the samples in the ITS networks including only the taxa from the more extensively sampled group (Figures 3.11 & 3.12) are congruent with those amongst the samples in clade VII of the nuclear phylogenetic trees (Figure 3.5), with all of the clades present in the trees represented by equivalent groupings in the networks.

The centre of the NN network (Figure 3.11) contains broad parallelograms, indicating that a large percentage of the characters are in conflict with one another concerning deeper relationships amongst these samples. This conflict is also evident in the phylogenetic trees (Figure 3.5), in which most of the members of clade VII emerge in unresolved positions. The conflict is at least in part caused by the ambiguous state of some of the characters in the ITS sequence matrix resulting from polymorphic peaks in the ITS chromatograms. However, there are also very few characters that are phylogenetically informative amongst these samples in the ITS sequence matrix (21 characters, 2.8% of all the characters), and noise therefore probably has contributed to the conflict depicted by the parallelograms. Homoplasy, and possibly also characters sharing different histories, might also have contributed to the conflict. Thus, conflict within the ITS data probably has several causes. Conflict amongst characters is consequently the reason for the lack of resolution in the nuclear phylogenetic trees, where most of the members of clade VII emerge in a large polytomy. In contrast, there is far less conflict amongst characters within the individual groupings in the NN network, evident from the long, thin parallelograms or single lines leading to these groupings in the NN network.

As is the case in clade VII of the nuclear phylogenetic trees (Figure 3.5), of the nine species and species complexes represented by more than one population in the extensively sampled group, only *S. lilliputana* and *S. montigena* form isolated groups in the nuclear NJ network (Figure 3.12). Nevertheless, geographically proximate, conspecific samples do tend to group together. Thus, the northern *S. johannis* populations (johNNsikeni, johNHebronRd and johNManzimnyama) group together as they do in the nuclear phylogenetic trees, while the northernmost *S. rexii* populations (rexOtterspoort, rexMtAyliff and rexNtywenka) also group together in the NJ network and nuclear phylogenetic trees. The southernmost *S. meyeri* populations (meyBaviaanskloof, meyGraaffReinet, the two Somerset East populations [meySECharltonFarm and meySEGlenCraigFarm] and meyZuurberg) also cluster in the NJ network. The six *S. meyeri* samples from Bastervoetpad emerge individually in the NJ network, suggesting that they are different from one another, but emerge on zero-length branches in the same polytomy in the parsimony phylogram (Figure 3.5a), suggesting that they are identical to one another. These six sequences are identical, except for a single nucleotide indel that is present in the meyBastervoetpadB sample, but absent in the rest, and five polymorphic sites. When constructing the networks, the "HandleAmbiguousStates: Ignore" option was specified.

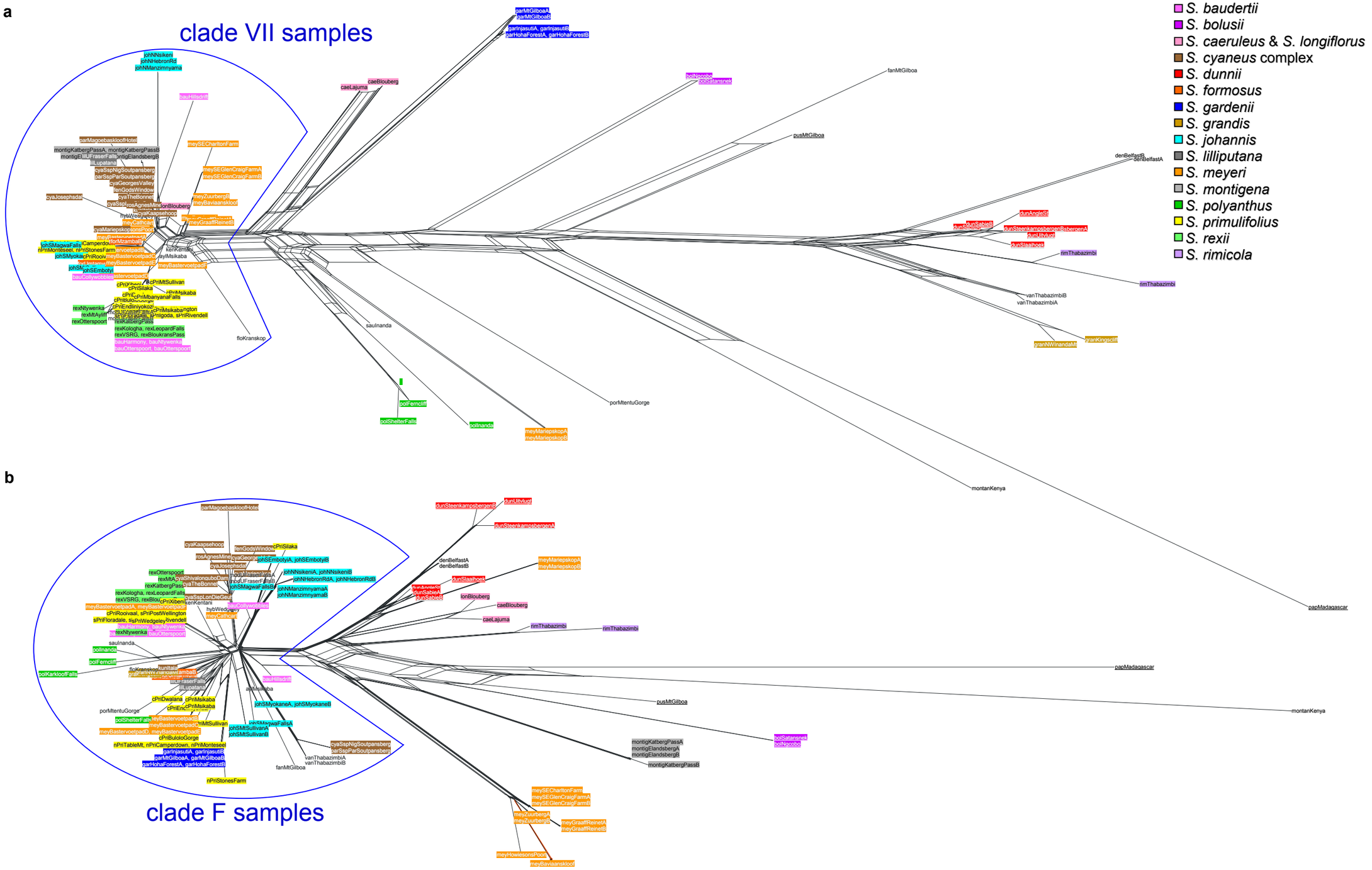

Figure 3.9: Neighbour-Net (NN) networks of the **a** ITS and **b** plastid data. The taxa emerging in the more extensively sampled clades of the phylogenetic trees (clade VII in the ITS trees [Figure 3.5] and clade F in the plastid trees [Figure 3.7]) are circumscribed in blue in each network. Due to the close relationships amongst the samples in these more extensively sampled clades, separate networks including only these samples are shown in Figures 3.11 (the ITS NN network of samples emerging in clade VII) and 3.13 (the plastid NN network of samples emerging in clade F).

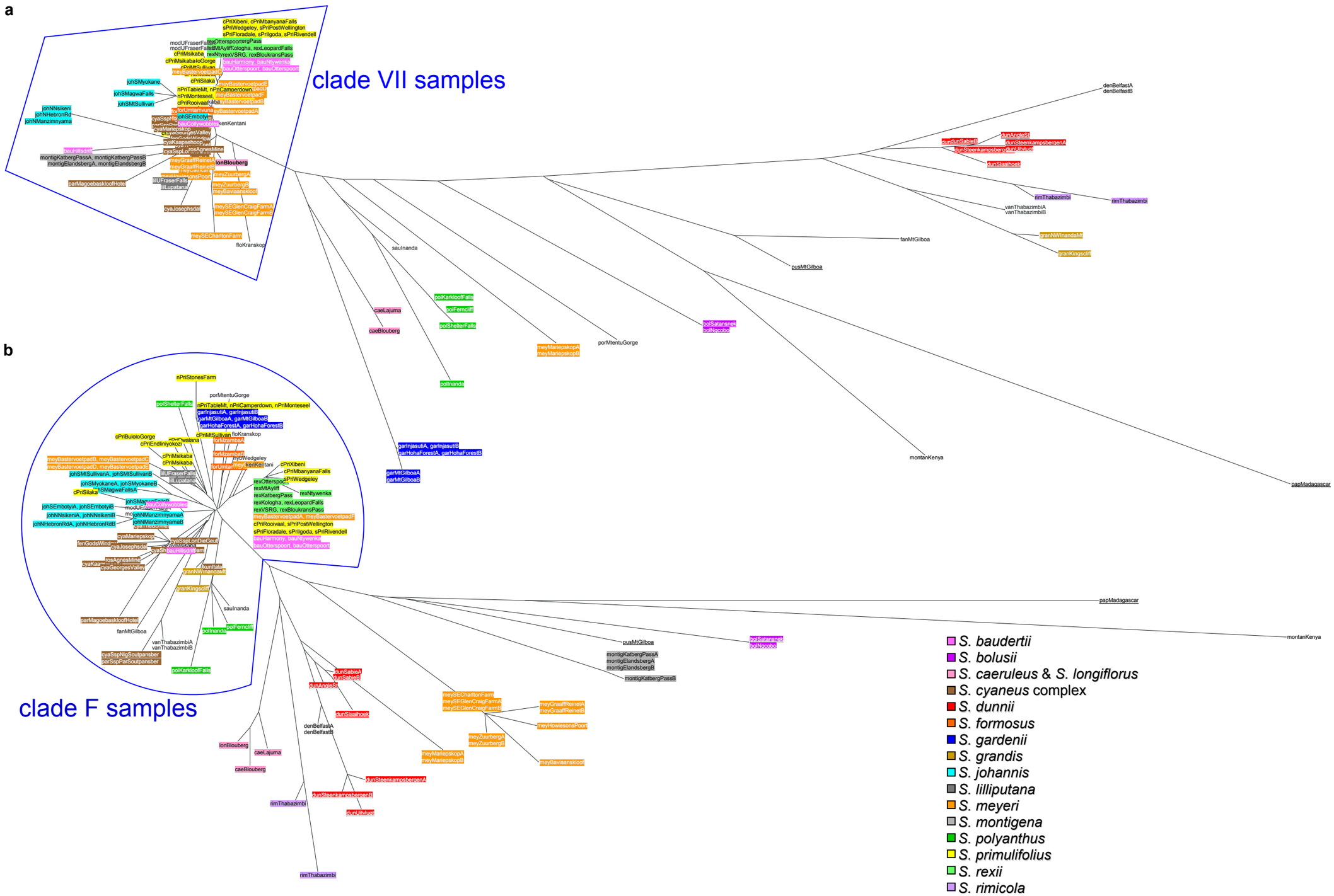

Figure 3.10: Neighbour-Joining (NJ) networks of the **a** ITS and **b** plastid data. The taxa emerging in the more extensively sampled clades of the phylogenetic trees (clade VII in the ITS trees [Figure 3.5] and clade F in the plastid trees [Figure 3.7]) are circumscribed in blue in each network. Due to the close relationships amongst the samples in these more extensively sampled clades, separate networks including only these samples are shown in Figures 3.12 (the ITS NJ network of samples emerging in clade VII) and 3.14 (the plastid NJ network of samples emerging in clade F).

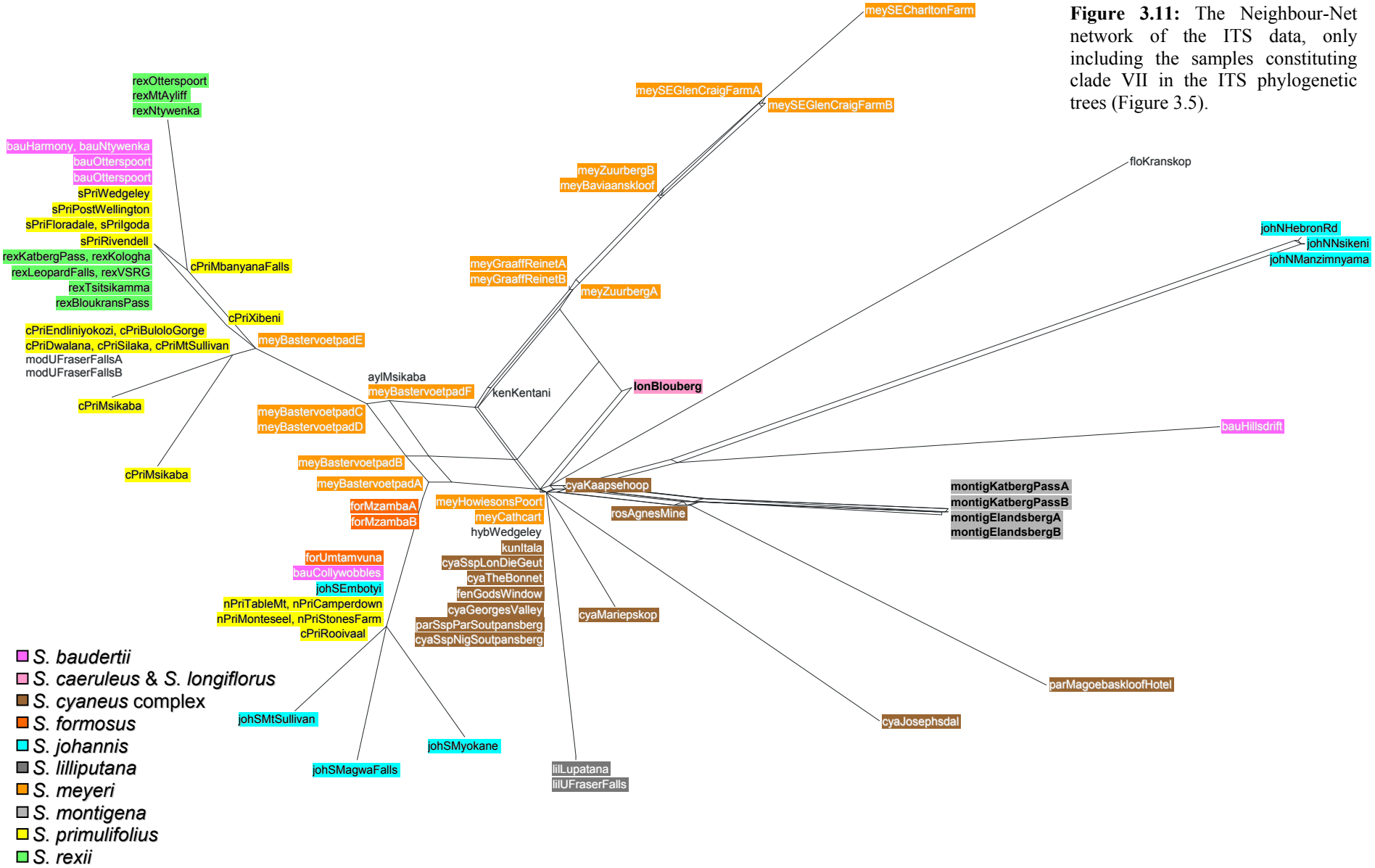

Figure 3.11: The Neighbour-Net network of the ITS data, only including the samples constituting clade VII in the ITS phylogenetic trees (Figure 3.5).

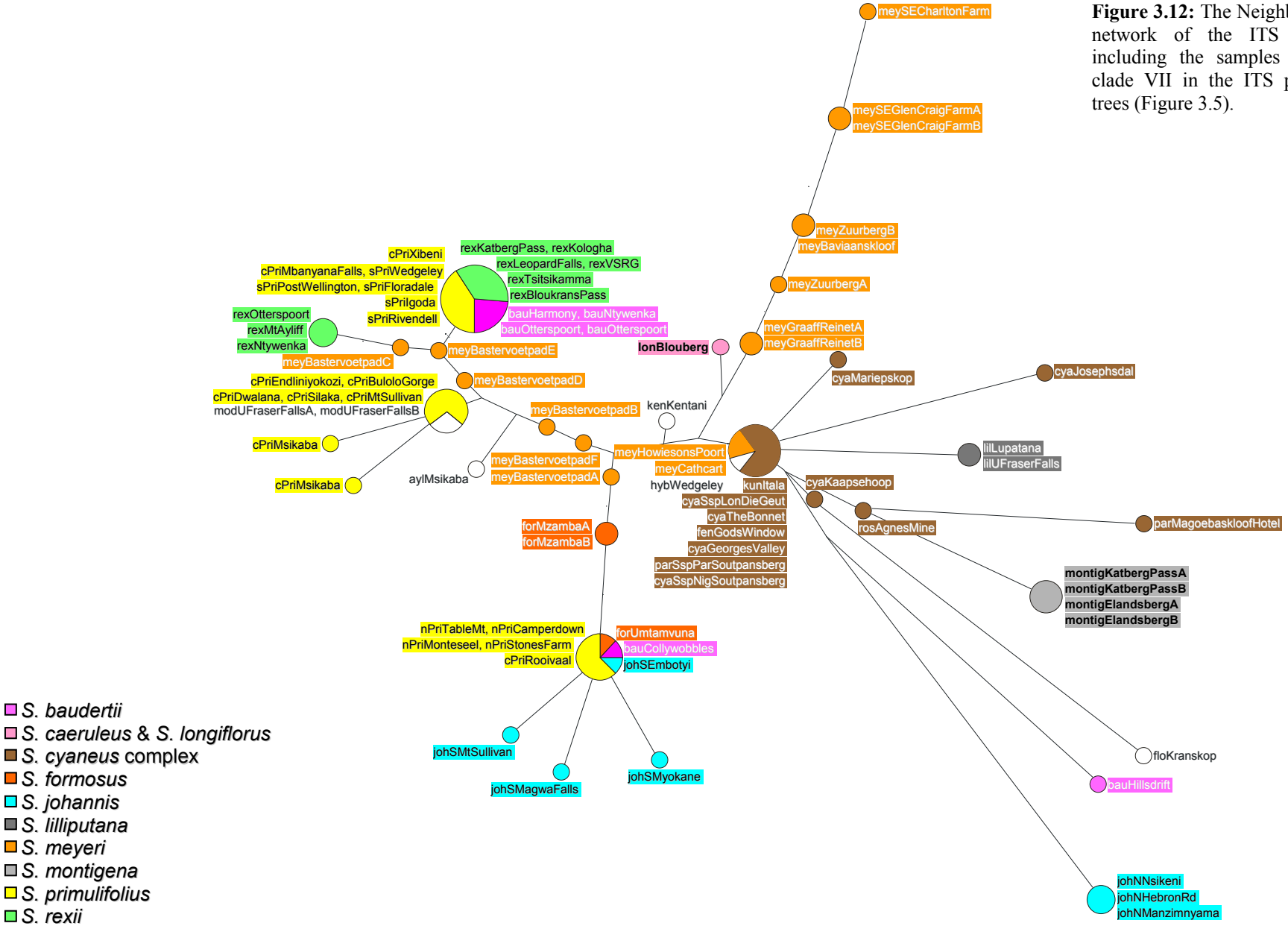

Figure 3.12: The Neighbour-Joining network of the ITS data, only including the samples constituting clade VII in the ITS phylogenetic trees (Figure 3.5).

However, this option and the "HandleAmbiguousStates: AverageStates" option were found to produced networks with identical topologies, and it therefore appears that there is a bug in SplitsTree 4.8. The separate positions of the *S. meyeri* Bastervoetpad samples therefore reflect the assignment of average states to their polymorphic sites rather than definite differences amongst these six samples.

Samples from other species and conspecific geographical clusters also group together, but along with samples from other species. Thus, the northernmost *S. primulifolius* populations (nPriTableMt, nPriCamperdown, nPriMonteseel, nPriStonesFarm and cPriRooivaal) cluster together with the southern *S. johannis* populations (johSMyokane, johSEmbotyi, johSMagwaFalls and johSMtSullivan), the *S. formosus* populations (forMzamba and forUmtamvuna) and *S. baudertii* from Collywobbles. This grouping also emerges in the nuclear phylogenetic trees, excluding the forMzamba samples. The rest of the *S. primulifolius* samples group into two separate clusters, the populations in the southern part of the Pondoland Centre of Endemism (Van Wyk 1990) area (cPriMsikaba, cPriEndliniyokozi, cPriBululoGorge, cPriMtSullivan, cPriDwalana and cPriSilaka) grouping together with the *S. modestus* samples (modUFraserFallsA and modUFraserFallsB). The rest of the *S. primulifolius* populations (cPriXibeni, cPriMbanyanaFalls, sPriWedgeley, sPriPostWeillington, sPriFloradale, sPriIgoda and sPriRivendell) cluster along with the southernmost *S. rexii* (rexKatbergPass, rexKologha, rexLeopardFalls, rexVSRG, rexTsitsikamma and rexBloukransPass) and the northernmost *S. baudertii* (bauHarmony, bauOtterspoort and bauNtywenka) populations. However, all of these samples emerge in unresolved positions in the phylogenetic trees. Likewise, the *S. cyaneus*–complex samples also emerge in unresolved positions in the phylogenetic trees, but are more resolved in the NJ network. Most of the *S. cyaneus*–complex samples share identical sequences (except for indels and polymorphic sites) along with the two *S. meyeri* populations from Howison's Poort and Cathcart and the hybrid population from Wedgeley, although some of the *S. cyaneus*–complex samples are quite different.

In summary, while *S. lilliputana*, *S. montigena*, *S. formosus* and *S. rexii* appear to be rather uniform species *S. meyeri*, the *S. cyaneus* complex, *S. primulifolius*, *S. johannis* and *S. baudertii* are more heterogenous. Although most of the species and species complexes do not form isolated groups in the analyses, conspecific populations tend to group together, indicating that there is some taxonomic structure in the nuclear networks and phylogenetic trees.

3.3.4.2. Network analyses of the samples from the more extensively sampled group based on the plastid data set

The plastid networks only including taxa emerging in clade F of the plastid phylogenetic trees (Figures 3.13 & 3.14) are also in strong agreement with each other, as well as with relationships within clade F of the plastid phylogenetic trees (Figure 3.7).

The centre of the plastid NN network (Figure 3.13) is distinctly net-like. However, unlike in the NN network of the nuclear data (Figure 3.11), in the NN network of the plastid data (Figure 3.13) most of the parallelograms are at the bases of the individual subgroups rather than amongst these subgroups, and there is therefore very little conflict amongst the characters concerning relationships amongst the constituent subgroups. Thus, while the large polytomy in the plastid phylogenetic trees (clade F; Figure 3.7) is to a small extent due to conflict amongst the characters, the lack of resolution in clade F is mostly due to a lack of parsimony-informative characters at this level to resolve relationships amongst the 14 subclades. The conflict evident amongst the characters at the bases of the individual subgroups cannot be caused by the characters having different histories, seeing as plastid genetic material is inherited as a single unit, and must therefore be the result of homoplasy.

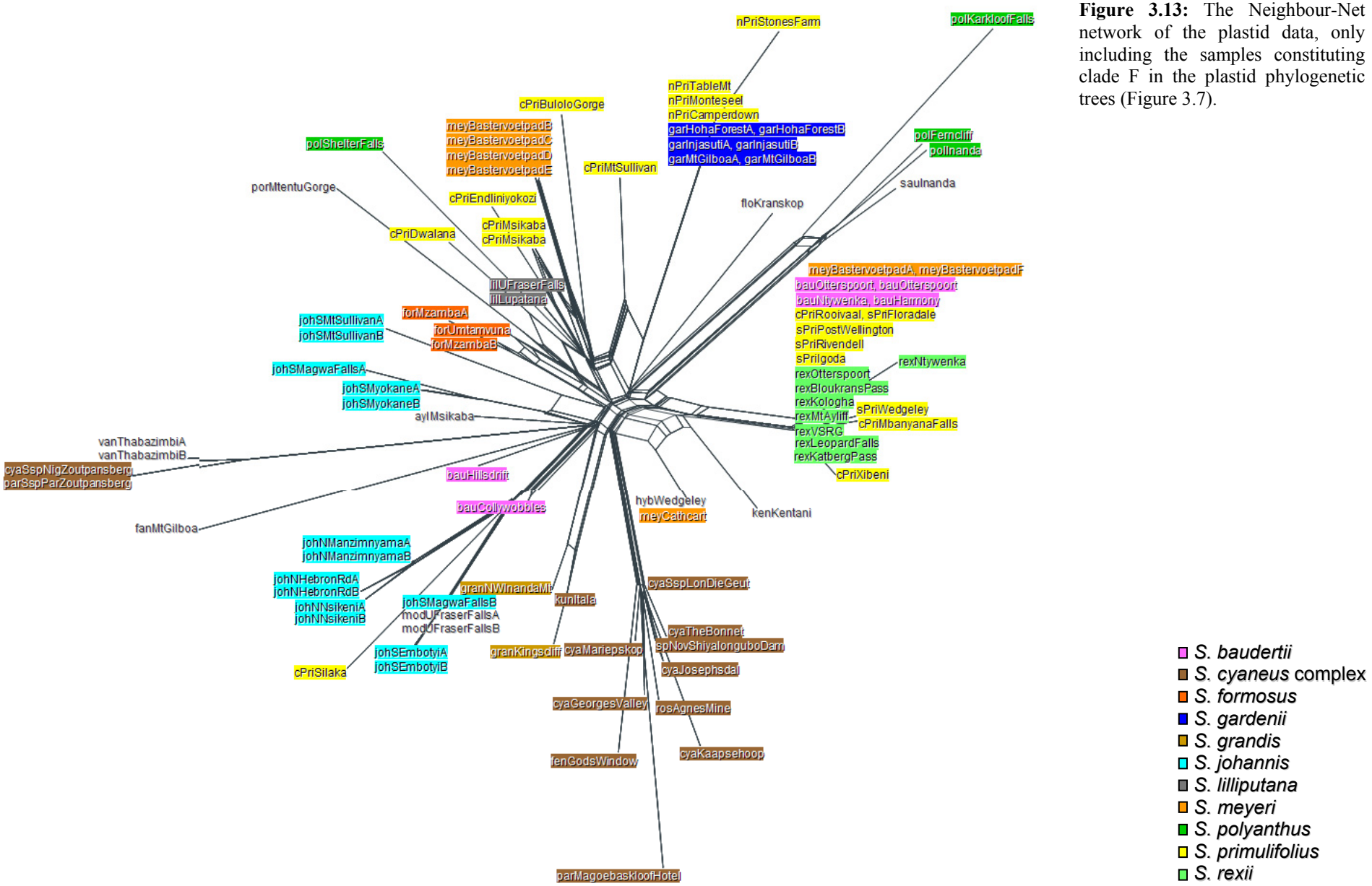

Figure 3.13: The Neighbour-Net network of the plastid data, only including the samples constituting clade F in the plastid phylogenetic trees (Figure 3.7).

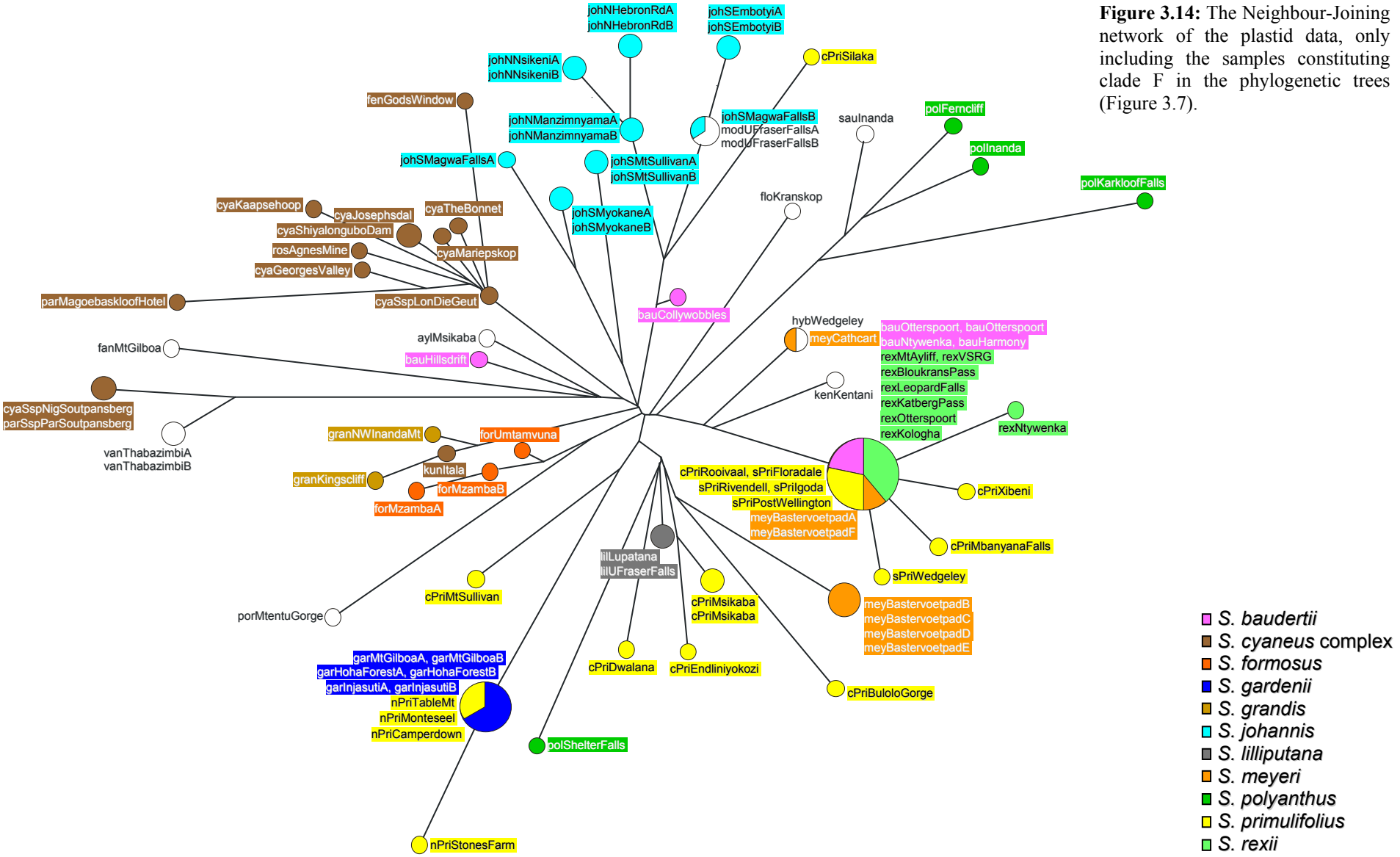

Figure 3.14: The Neighbour-Joining network of the plastid data, only including the samples constituting clade F in the phylogenetic trees (Figure 3.7).

The unresolved relationships amongst the subgroups are also reflected in the plastid NJ network (Figure 3.14), in which the branch lengths in the centre are noticeably shorter than those within the individual groups. The plastid NJ network contains all of the groupings depicted in the plastid phylogenetic trees, which were described earlier on in section 3.3.3., but with some additional resolution. More specifically, some of the samples that emerged in unresolved positions in clade F of the plastid phylogenetic trees emerge within larger groupings in the NJ network, and many of the 14 subclades in the phylogenetic trees emerge together in larger groupings in the NJ network. Additionally, the NJ network identifies potentially more ancestral samples in many of the groupings. Thus, the two representatives from the *S. johannis* Manzimnyama population group ancestral to the other two northern *S. johannis* populations (johNHebronRd and johNNsikeni), and the *S. cyaneus* subsp. *longi-tommii* sample from Die Geut emerges ancestral to the group containing most of the remaining samples of the *S. cyaneus* complex. The networks are therefore largely in agreement with their corresponding phylogenetic trees, but also provide additional information regarding relationships i.e. the extent to which conflict amongst characters has contributed to the polytomies in the phylogenetic trees and potential ancestors to some of the groupings, that is not portrayed in the phylogenetic trees.

3.3.5. Dating of clades in the nuclear and plastid phylogenetic trees

A comparison of the nuclear (Figure 3.5) and plastid (Figure 3.7) phylogenetic trees reveals that the only high-level clade (containing representatives from more than two species) in agreement between the two analyses is the one containing all of the South African species included i.e. clade I in the nuclear analyses, and the corresponding clade A in the plastid analyses.

The average distance of the unique sequences in the nuclear phylogram (Figure 3.5a) from the base of clade I to the present time is 19.29 steps. Using this number of substitutions and the published rates of ITS substitution found in other herbaceous angiosperm lineages collected by Kay *et al*. (2006), the clade containing the South African samples was estimated to have started diverging 7.3 million years ago (mya) (± 1.0 SE).

The average depth of unique haplotypes of the corresponding clade in the plastid analysis, clade A, is 3.00 steps based on the *trn*L-F data alone (Figure 3.15). This clade depth and the *trn*L-F rates from Richardson *et al*. (2001) resulted in the estimation of 3.5 mya (± 2.0 SE) as the time when clade A started to diverge.

The South African taxa included in this study therefore started diverging approximately 7.3 or 3.5 mya i.e. sometime during the Pliocene or during the late Miocene epochs. However, due to the method used to calculate these dates, they cannot be seen as much more than gross approximations of the true date of divergence of these South African taxa.

3.4. Discussion

3.4.1. Evolution of ITS in South African *Streptocarpus*

Despite the more limited sampling in some parts of the trees, the phylogenetic analyses of the ITS data included in this study yielded phylogenies (Figure 3.5) congruent with the ITS analysis carried out by Möller & Cronk (2001a) throughout the tree, the only exception being relationships within the Cape primrose clade (clade VII in Figure 3.5). However, relationships in this clade in the present analyses, as well as in Möller & Cronk (2001a), are unsupported or only weakly supported, and the topology is therefore uncertain.

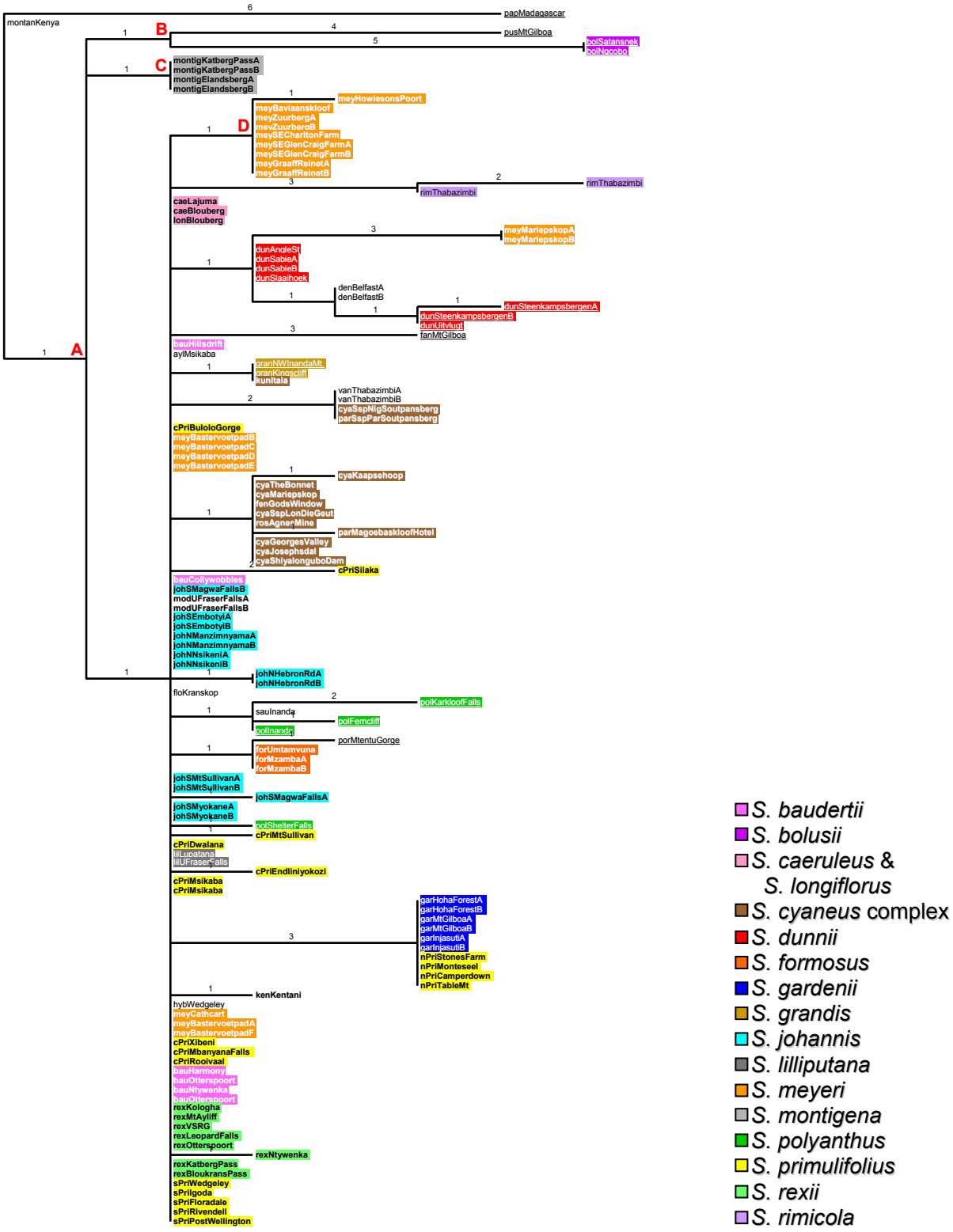

Figure 3.15: A phylogram of the plastid topology (shown in Figure 3.7) with all but the *trn*L-F data excluded. Numbers above branches give branch lengths. Clades A–D from Figure 3.7 are labelled, whereas clades E and F are not supported by the *trn*L-F data.

The tree and network topologies (Figures 3.5, 3.9a, 3.10a, 3.11 & 3.12) of the current study are well resolved and mostly strongly supported in the more poorly sampled parts, but show very little resolution in the more extensively sampled parts i.e. amongst the ITS lineages emerging in clade VII in the phylogenetic trees (Figure 3.5). ITS in *Streptocarpus* therefore evolves fast enough to provide a strong signal for relationships amongst the major ITS lineages within the genus and two subgenera, but a comparison of sequences obtained by direct sequencing does not provide enough resolution of relationships at the specific level, especially amongst closely related species, and at the population level.

The lack of divergence amongst closely related ITS lineages is due to the lack of time during which mutations can accumulate and become fixed within evolutionary units. ITS divergence is further hampered by molecular drive i.e. the homogenization of the tandem ITS copies within and between the nucleolar organizer region(s) (NOR) (Brown *et al*. 1972). Thus, the ITS copies in a group of individuals that frequently cross with one another will tend towards sharing a single sequence over time (if concerted evolution tends to occur at a faster rate than mutation; see also Hughes *et al*. 2005), swamping all but one version of the evolutionary signal at each nucleotide base site. Therefore, populations and species (if there is frequent gene flow amongst conspecific populations; Hughes *et al*. 2007) tend towards becoming fixed at each site in the ITS sequence for a particular characteristic. However, if hybridization plays a noticeable role in a group, then concerted evolution would also be operational at the supraspecific level, homogenising ITS sequences amongst hybridising species as well. Thus, the lack of resolution amongst the Cape primrose clade taxa is probably due to the species constituting this clade being too young for a significant amount of variation to have accumulated and become fixed within each species. The Cape primrose clade is a reasonably young clade in *Streptocarpus* (Möller & Cronk 2001b), and Hughes *et al*. (2005) estimated an age of 1.11 million years for the *S. rexii–S. primulifolius* clade. However, hybridization has probably also contributed to the lack of resolution within this clade to a certain extent, eroding differences and leading to superficially similar sequences amongst species that hybridize with each other.

Hybridization is a well-known occurrence in *Streptocarpus*. Many instances of hybridization were detected by Hilliard & Burtt (1971) based on morphological intermediacy and the close proximity of the putative parents to the suspected hybrid population. Furthermore, hybridization has been performed with ease within the subgenera throughout the horticultural history of *Streptocarpus* to introduce desirable characteristics e.g. red flower colour, into cultivars. Based on the number of haploid chromosomes found in the *Streptocarpus* species that have been analysed so far (http://elmer.rbge.org.uk/Webcyte/webcyteintro.php, accessed on 18 July 2008), chromosome number appears to be largely fixed at the subgeneric level, with only a few exceptions. Polyploidy is very rare and occurs exclusively in the Madagascan and Comoro Islands species. Most of the species in subgenus *Streptocarpella* possess a haploid chromosome number of 15, while most of the members of subgenus *Streptocarpus* possess a haploid chromosome number of 16 (Möller & Cronk 2001a). Thus, in terms of chromosome numbers, species are hypothetically largely compatible with one another within the subgenera. There is also considerable circumstantial evidence that hybridization has occurred amongst the taxa included in this study: the ITS and plastid topologies are largely incongruent with each other, and the ITS sequences from many of the taxa, especially those of the *S. meyeri* samples from the Bastervoetpad population, include a number of sequence polymorphisms.

The way in which the ITS region evolves and is inherited does not only cause misleading phylogenetic reconstructions amongst closely related taxa, but also amongst distantly related species, potentially affecting larger parts of reconstructed topologies. The ITS region forms part of the 45S cluster. Numerous 45S clusters are arranged in tandem along the chromosome, and these together constitute a NOR. Although concerted evolution tends to homogenise these

repeats, it does not always keep pace with events that introduce variation amongst the repeats e.g. mutation and interbreeding, whether within or between species. Variation amongst the different repeats results in polymorphic peaks and/or length variations in consensus ITS sequences. A further complication potentially hampering the fixation of characters is variability in the number and location of NORs in the genome amongst species (Álvarez & Wendel 2003). Denduangboripant *et al*. (2007) examined the distribution, size and inheritance of the NORs in the genomes of two species in subgenus *Streptocarpus*, *S. dunnii* and *S. rexii*, as well as in their F_1 hybrid offspring and subsequent backcrosses between these F_1 hybrids and each of the original species. They found NORs at two different loci in the genomes of both *S. dunnii* and *S. rexii*. However, the number and location of NORs tends to be highly variable amongst species (Schubert & Wobus 1985; Dubcovsky & Dvořák 1995; Thomas *et al*. 2001; Murray 2002), and other *Streptocarpus* species might therefore possess fewer or more NOR loci; e.g. *S. johannis*, *S. baudertii* and *S. kentaniensis*, amongst others, possess one locus (Möller *et al*. 2008). Generating sequences from more than one locus potentially means that one is comparing paralogous rather than orthologous genes (Álvarez & Wendel 2003). This was probably the case for *Aeschynanthus* (Gesneriaceae) where direct sequencing was not possible due to high intraindividual length variations and diversity of up >5% between cloned ITS sequences (Denduangboripant & Cronk 2000). This was shown to probably be the result of ancient locus duplication rather than hybridization (Möller *et al*. 2008). However, Denduangboripant *et al*. (2007) found that the ITS sequences of *S. dunnii* and *S. rexii* were highly homogenous, perhaps due to recent duplication events in each of the species (Möller *et al*. 2008). If hybridization has occurred amongst taxa possessing distantly related ITS lineages in the ITS topologies, then concerted evolution or lineage sorting events (as NORs are inherited in a Mendelian fashion; Denduangboripant *et al*. 2007) might result in a topology that does not accurately reflect evolutionary history, depending on which characteristic happens to become fixed at each ITS site. The ITS of the offspring of the hybridization event might retain the ITS sequences from both parents at different loci within the genome, might become fixed for the ITS sequence from one or the other parent, or concerted evolution might result in a chimera ITS sequence due to the fixation of some characters for the state present in the one parent, while the remaining characters become fixed for the state present in the other parent (chimera ITS sequences have been found in *Amelanchier* Medik. (Rosaceae Juss.), Campbell *et al*. 1997, *Begonia*, Chiang *et al*. 2001 and *Ilex* L. (Aquifoliaceae A.Rich.), Manen 2004, for example). Which of these scenarios occurs will determine the position of the hybrid lineage in phylogenetic analyses relative to its parent species. The last of the three scenarios listed above would result in a sequence that behaves erratically in a phylogenetic analysis, potentially leading to more poorly resolved trees (Álvarez & Wendel 2003). Denduangboripant *et al*. (2007) found no cases of the third scenario, as they detected no intermediate ITS types between those of *S. dunnii* and *S. rexii* in any of their descendents. They did, however, find, after a cross between *S. dunnii* and *S. rexii*, followed by a backcross to *S. rexii*, some cases in which both the *S. dunnii* and *S. rexii* types were retained, and other cases in which the *S. dunnii* type was completely replaced by the *S. rexii* type within the two generations. Thus, the traces of a hybridization event can be erased astonishingly quickly in *Streptocarpus*. Phylogenetic reconstruction can also be hampered if some of the ITS copies become pseudogenes. Because they are no longer functional, they will not be subject to functional constraints, and will therefore accumulate mutations much faster than their functional counterparts. These can cause further complications if they recombine with functional ITS copies (Álvarez & Wendel 2003). Pseudogenes are, however, unlikely to have been sequenced in the present study seeing as the conserved ITS1 angiosperm motif GGCRY-(4 to 7n)-GYGYCAAGGAA (Liu & Schardl 1994) was found to be present and reasonably conserved in the ITS *Streptocarpus* alignment. Furthermore, pseudogenes are usually only detected and a problem when cloning techniques are employed, and here a direct sequencing approach was used. Also, universal primers were used, and there are no unusually long branch

lengths in the ITS phylogram and GC content of the sequences were found to be consistently high, further refuting the possible inclusion of pseudogenes in this study. Finally, most algorithms for reconstructing evolutionary history assume that the characters are evolving independently of one another. However, ITS is a region that violates this assumption (Álvarez & Wendel 2003), since certain parts of the ITS regions are involved in forming hairpin structures that serve as critical processing signals during the enzymatic processing of the RNA transcript (Torres *et al*. 1990; Liu & Schardl 1994; Schlötterer *et al*. 1994; Mai & Coleman 1997), and regions of ITS are therefore under functional constraints. Denduangboripant and Cronk (2001) even found compensatory mutations in length variants in *Aeschynanthus* (Gesneriaceae).

Thus, ITS is certainly informative in reconstructing evolutionary relationships, but reconstructions should always be treated with caution (Álvarez & Wendel 2003). The ITS topologies retrieved in this study reflect the close relationships and/or frequency of hybridization amongst the species in the Cape primrose clade, and the more distant relationships amongst the ITS lineages of the taxa in the less well-sampled clades, with factors such as hybridization, incomplete homogenization, and interdependence of some of the nuclear bases potentially influencing the relationships retrieved during the analyses.

3.4.2. Evolution of the plastid genome in South African *Streptocarpus*

In comparison to nuclear DNA, which is biparentally inherited, the plastid genome is only inherited through the maternal line in most angiosperms, including *Streptocarpus* (Möller *et al*. 2004), and does not undergo recombination. While the matrilineal inheritance of the plastid genome only allows the maternal history of a lineage to be reconstructed, it allows this to be done in a clear way. Whereas reconstructions of the evolutionary history of nuclear loci are confounded by continuous pooling and recombination between the alleles from the different parents, which can be especially problematic when hybridization of distantly-related species is involved, mother and offspring share the same plastid lineage, enabling the maternal history of each sample to be traced in a tree comparatively easily. Analysing markers with different inheritance patterns enables the detection of potential hybridization events due to cyto-nuclear disequilibria (Freeland 2005).

The plastid topologies in the current study (Figures 3.7, 3.9b, 3.10b, 3.13 & 3.14) are slightly less resolved amongst the more distantly related lineages than are the ITS topologies. This is in accordance with the generally slower mutation rate observed in the plastid genome—the rate of synonymous substitutions, for example, has been found to be on average four to five times slower in the plastid genome than in the plant nuclear genome (Wolfe *et al*. 1987), also for Gesneriaceae in particular (Möller *et al*. 1999). The more closely related lineages i.e. those in clade F of the phylogenetic trees (Figure 3.7), are mostly separated into different subclades, many of them strongly supported. There is, however, no resolution amongst these subclades.

This lack of resolution amongst the subclades in clade F is puzzling. An inspection of the equally parsimonious trees reveals that this polytomy is both due to zero branch lengths and minor conflict amongst the equally parsimonious trees regarding relationships amongst the subclades. The fact that relationships on either side of this node i.e. both amongst the less well-sampled lineages and in the individual subclades in clade F, are mostly strongly supported indicates that this polytomy is probably due to rapid speciation in a short period of time. The reason for this rapid divergence possibly lies in the climatic history of the region.

Clade F is reasonably young compared to the South African clade as a whole, which was estimated to have started diverging 7.3 mya (± 1.0 SE) from the ITS data and 3.5 mya (± 2.0 SE) according to the plastid data. Before about 3 mya i.e. during the late Miocene and most of

the Pliocene, the African climate was generally more mesic, with reasonably mild climatic cycles of about 19 000–23 000 years in length. However, the last 2.8 million years have been characterised by the progressive onset and intensification of glacial cycles, along with progressive decreases in temperature and increases in aridity. The period between 2.8 and 1.7 mya saw a gradual increase in aridity in Africa, and the lengthening of the climatic cycles to 41 000 years. Temperatures dropped noticeably at about 1.7 mya, along with an intensification of the 41 000 year cycles. Since then, the amplitude of the climatic cycles has increased, and the period since 1 mya has been characterised by recurring glacial-interglacial periods of about 100 000 years in length, during which conditions have fluctuated between warmer, more mesic periods during the interglacials and colder, more arid periods during the glacials (DeMenocal 2004). Atmospheric CO_2 levels also fell steadily through much of the Tertiary period (Berner 1997), and have been alternating between about 270 parts per million (ppm) during the interglacials and about 180 ppm during glacials (Petit *et al*. 1999). Low atmospheric CO_2 levels confer a photosynthetic advantage to C_4 plants such as certain grasses relative to C_3 plants, which include most trees (Ehleringer *et al*. 1997). Thus, atmospheric CO_2 levels fell through much of the Pliocene, and Africa has been becoming progressively more arid, with considerable increases of climatic variability and aridity occurring 2.8, 1.7 and 1 mya.

These changes in temperature, aridity and CO_2 levels have in turn affected forest cover in South Africa. Much of southern Africa is believed to have been covered in forests throughout most of the Quaternary period (Coetzee 1982; Scott *et al*. 1997). However, with the progressive intensification of cooler temperatures and more arid conditions, and the falling atmospheric CO_2 levels bestowing greater photosynthetic advantages to C_4 plants relative to C_3 plants, forests would have become progressively more fragmented (McCarthy & Rubidge 2005). *Streptocarpus* is a predominantly forest-dwelling group, and would have been similarly affected by the changing environmental conditions. The increasingly fragmented forests would probably have resulted in substantial extinctions of *Streptocarpus* taxa. However, several studies have also linked increases in environmental stress and variability to morphological innovations and bursts of speciation (e.g. Wesselman 1985; Vrba *et al*. 1989; Wood 1992; Vrba 1995; Kimbel 1995; Potts 1998). Thus, a scenario can be envisaged in which many of the older, forest-dependent lineages would have perished—although some would have survived in forest refugia (Hughes *et al*. 2005)—while more drought-adapted lineages e.g. *S. meyeri*, would probably have survived the increasingly more arid conditions. Subsequent re-expansions of forest habitats would have opened up new niches in which newly evolved *Streptocarpus* species could have arisen and become established. This scenario could produce a polytomy like the one in the current plastid phylogeny. Furthermore, these periodic shrinkages and expansions of the forest habitats would also have been conducive to reasonably frequent hybridization events. Species that had arisen allopatrically, and therefore were not necessarily isolated from one another by prezygotic or postzygotic reproductive barriers, would have been brought together during the mesic interglacials, increasing the chances of hybridizations occurring.

Within the large polytomy, many of the subclades are strongly supported, and are themselves partially resolved with varying amounts of support. Thus, although variation amongst the more distantly-related samples is slightly less in the plastid (clades B–E) than in the ITS (clades II–VI) topologies, the reverse appears to be the case amongst the more closely related species and at the population level, where the plastid topologies are much more resolved than the ITS topologies. This apparently faster rate of ITS evolution at higher taxonomic levels, but slower rate at lower levels was also found by Möller *et al*. (1999). They attributed this phenomenon to the method used to collect ITS data. The consensus sequence generated from the ITS copies of an individual only records characteristics that are fixed or nearly so within the individual; mutations that have recently arisen, and are therefore only present within some of the hundreds of copies of 45S clusters in the genome, will either be coded as ambiguities or will be

indistinguishable from the background noise. Therefore, if one compares ITS consensus sequences of distantly-related taxa, then one is likely to detect most of the variation amongst these taxa. In contrast, if one compares consensus ITS sequences from closely related taxa or populations, variation that has arisen since their divergence is likely not to be fixed yet, and will therefore not be resolved as definite differences. These polymorphisms have been observed in population of *S. rexii* and *S. primulifolius* in South Africa (Hughes *et al*. 2005). Branch lengths across the plastid tree are consequently probably more representative of relative lineage ages, both because of the lack of detection of recent, unfixed mutations in the ITS regions, and because the branch lengths in the plastid topologies are based on variation in three different regions together containing 3 881 characters rather than just on the 749 characters from the ITS region in the nuclear analyses.

In summary, the plastid phylogenies reflect the maternal history of the samples. The large polytomy in the plastid phylogeny suggest a sudden appearance of many of the extant *Streptocarpus* taxa occurring in eastern South Africa. This is possibly the result of palaeoclimatic patterns, which have resulted in a general trend towards more arid conditions over the past few million years, but also greater climatic variability. The drier periods are likely to have had devastating effects on forest-dwelling taxa such as *Streptocarpus*, leading to widespread extinctions. However, subsequent re-expansions of forest habitats would have opened up new, unoccupied territory for the rapid expansion and radiation of *Streptocarpus*.

3.4.3. A comparison of the nuclear and plastid topologies and analytical methods applied

A tendency evident in both the nuclear and plastid phylogenies is for the posterior probabilities (PP) of the clades to be higher than the corresponding bootstrap (BS) support values. This reflects a general pattern that has been found in many phylogenetic studies comparing BS and PP support assigned to clades (e.g. Rannala & Yang 1996; Yang & Rannala 1997; Larget & Simon 1999), and has led to much controversy as to the merits of using PP as a measure of clade support (Alfaro & Holder 2006). Alfaro *et al*. (2003) simulated data sets from 18 different trees representing evolution under a number of different conditions (pectinate [asymmetric] and symmetric topologies, assuming and not assuming a molecular clock, high and low rates of character evolution, and decreasing the internode length in different parts of the tree relative to the rest of the tree) to determine the accuracy of different support measures, including PP and BS, in supporting the correct clades under different evolutionary scenarios. They found that the PP value assigned to a clade tends to be roughly equivalent to the probability of that clade being correct e.g. 70% of nodes supported by a PP of 0.70 are probably correct, whereas *ca*. 95% of nodes supported by a BS of 70% are likely to be correct. They also found that PP tends to assign far higher support values to short branches than does BP, an indication that PP needs less evidence to provide strong support. This is advantageous for resolving relationships based on very few characters. However, the trade-off for this increased sensitivity is that PP could be more likely to assign high support to incorrect nodes where there are very few characters supporting a node. BS needs more characters to assign equivalent support to a node, and is therefore less likely to provide strong support to incorrect relationships. However, maximum parsimony BS is more susceptible to long-branch attraction than is PP, and under these conditions could provide high support to incorrect relationships. Thus, PP and BS measure different features of the data, and should therefore be interpreted differently. PP is more useful if one wants to assess support of nodes based on the data that have been collected and the model imposed, and can be directly interpreted as the probability that the clade to which it has been assigned is correct, conditional on the data and the model. BS, on the other hand, is more useful if one wants to assess the sensitivity of observed results to sampling error i.e. how likely it is that the node will still be present in the tree if more data are gathered.

A comparison of the ITS and plastid topologies reveals the lack of congruence between the two data sources (indicated in Figure 3.8), and the result from the ILD test confirms this. Although the ILD test is widely used as a character congruence measure, there are concerns about its actual usefulness in detecting incongruence amongst data sets resulting from differences in evolutionary history (e.g. Yoder *et al*. 2001; Barker & Lutzoni 2002). The main concern centres around the effect of noise on the analysis, which is believed to increase the probability of the test falsely concluding that data sets are incongruent. However, the striking differences between the trees built from the two separate matrices in the current study supports the conclusion of the test that the data should not be combined. The incongruences are probably caused by a combination of hybridization and incomplete lineage sorting. As mentioned previously, hybridization is believed to be an important aspect of *Streptocarpus* evolution, and therefore certainly cannot be ruled out as a cause of some of the incongruences. However, the analyses include many closely-related taxa, and incomplete lineage sorting will therefore also be responsible for some of the incongruences. Hybridization and incomplete lineage sorting are probably also responsible for many of the taxa not emerging as monophyletic entities, especially in the more extensively sampled parts of the nuclear and plastid topologies.

The incongruences between the two topologies, and the fact that hardly any of the species emerge as monophyletic entities is therefore due to the young age of many of the species, and the prevalence of hybridization within *Streptocarpus*. Thus, the ITS and plastid data each only reveal certain aspects of the evolutionary history of the South African *Streptocarpus* species, and a more complete history of the species can therefore only be inferred by taking all evidence, including morphology and present-day distribution, into account.

3.4.4. *Streptocarpus* in South Africa

One of the few clades to be shared between the ITS and plastid topologies is the clade containing all of the South African samples (clade I in the ITS phylogeny and clade A in the plastid phylogeny). This does not, however, mean that the South African *Streptocarpus* species constitute a single lineage, since only two species from outside of South Africa were included in the current analyses i.e. the Madagascan *S. papangae* and the Kenyan *S. montanus*. The biogeographic consideration (Möller & Cronk 2001b) of the phylogenetic reconstruction of evolutionary relationships containing representatives from across *Streptocarpus* based on ITS data (Möller & Cronk 2001a) identified a relatively young, purely South African clade comprising most of the included South African species. However, *S. grandis*, *S. dunnii*, *S. pusillus*, *S. fanniniae* and other South African species (e.g. *S. galpinii*, *S. decipiens*, *S. pole-evansii* and *S. cooperi*) emerged along with species collected from Swaziland (*S. davyi*), Mozambique (*S. goetzei*), Zimbabwe (*S. hirticapsa*, *S. cyanandrus*, *S. pumilus* and *S. eylesii*), Malawi (*S. hirtinervis*, *S. milanjianus*, *S. dolichanthus*, *S. erubescens*, *S. wittei* and *S. goetzei*), Tanzania (*S. goetzei*, *S. compressus*, *S. solenanthus* and *S. kungwensis*) and Rwanda (*S. bindseilii*), outside of this main South African clade in its sister ITS2 deletion clade and in the more ancestral sister clade of the ITS2 deletion–main South African clade. Thus, rather than the South African taxa forming a clade separate from the rest of the subgenus, patterns reconstructed by Möller & Cronk (2001b) suggest that the subgenus gradually spread southwards. All of the earliest bifurcations within subgenus *Streptocarpus* occurred more towards central eastern Africa and northern southern Africa based on the current distribution ranges of the species, whilst the major clades containing the South African species and other southern African species appear to have arisen more recently. The main South African clade in Möller & Cronk (2001a, 2001b) is one of the products of the most recent major bifurcation in the subgenus. The patterns detected in the current study are therefore in accordance with the historical biogeographic patterns of *Streptocarpus* revealed by Möller & Cronk (2001b) i.e. a

progressive southerly spread of subgenus *Streptocarpus* from central eastern Africa to South Africa.

The South African clade was estimated to be about 7.3 million years old (± 1.0 SE) based on the ITS data, and 3.5 million years old (± 2.0 SE) based on the plastid data. These ages have, however, not been calculated using all of the South African species, but rather only 56% of the currently recognised species occurring in South Africa. The species included are probably nevertheless representative of *Streptocarpus* in South Africa, since members from all four of Hilliard & Burtt's (1971) main morphological groups of subgenus *Streptocarpus* (groups A, B, C and D), and 8 (subgroups Aa, Ab, Ad, Ba, Be, Bf, Bi and the *S. meyeri* alliance) out of the 11 (representatives from subgroups Ae, Bb and Bc not included) subgroups of groups A and B (groups C and D were not subdivided) containing South African species were included. Thus, although only a little over half of the South African species are included in the topologies, these together represent most of the morphological (and therefore presumably also most of the evolutionary) diversity present in South Africa today. The diversity of the samples included in the current study is also evident from the ITS phylogeny of Möller & Cronk (2001a). The current study includes representatives from all of the clades in which South African species emerged in Möller & Cronk (2001a), except for the more ancestral clade (containing *S. galpinii* and *S. decipiens*) that emerges sister to the clade containing both the ITS2 deletion clade and the main South African clade. Thus, according to both ITS reconstructions (Möller & Cronk 2001a) and morphological groupings (Hilliard & Burtt 1971), the South African clade of the current analyses is largely representative of *Streptocarpus* in South Africa, and the calculated dates can therefore be interpreted as the approximate length of time that *Streptocarpus* has been diversifying in South Africa. Estimating the ages of clades using rates of sequence evolution calculated from other lineages is a more inaccurate method than using an internal calibration point e.g. a dated fossil, and this is also evident from the divergent dates obtained from the two independent data sources (7.3 million years old (± 1.0 SE) based on the ITS data, and 3.5 million years old (± 2.0 SE) based on the *trn*L-F data). However, the dating analyses nevertheless show that the clade to which the sampled South African species belong is probably not older than 10 million years. *Streptocarpus* consequently probably started arriving in South Africa in independent waves from the late Miocene or during the Pliocene onwards, in the period soon before forest habitats started to become more limited in extent and fragmented and climatic variability began to increase.

3.4.5. The Cape primrose clade

Although relationships in the South African clades of the nuclear and plastid topologies are largely incongruent with each other, the ITS South African clade topology is largely congruent with that reconstructed from ITS data by Möller & Cronk (2001a). Thus, clade II in the current ITS phylogeny is congruent with the ITS2 deletion clade of Möller & Cronk (2001a), the clade containing clades III–VII in the current phylogeny is congruent with the main South African clade of Möller & Cronk (2001a), and the species constituting the Cape primrose clade in Möller & Cronk (2001a) that were represented in the current study i.e. all species except for *S. fasciatus*, also group together in the current ITS topologies, together forming clade VII in the ITS phylogeny. These include *S. kentaniensis*, *S. cyaneus*, most of the *S. meyeri* populations, *S. montigena*, *S. baudertii*, *S. rexii*, *S. primulifolius*, *S. johannis*, *S. modestus* and *S. formosus*. Furthermore, some additional species that were included in the current study also emerged in this clade, including other members of the *S. cyaneus* complex (*S. parviflorus*, *S. fenestra-dei*, *S. kunhardtii* and *S. roseo-albus*), *S. longiflorus*, *S. floribundus*, *S. aylae* and *S. lilliputana*. This grouping is supported by morphology, in that all of these species possess the rosulate growth habit, while most of the species emerging in the less well-sampled clades are unifoliates, with some also containing plurifoliate forms. However, the rosulate growth habit is also found in

S. gardenii and *S. caeruleus*, two species that were also not included in Möller & Cronk (2001a). These two species together constitute clade VI of the current ITS phylogeny, which emerges sister to clade VII. Thus clades VI and VII together form a group that is morphologically coherent in terms of growth habit in the ITS phylogeny.

The large polytomy in the plastid topologies (clade F) constitutes a more inclusive clade containing more taxa than does clade VII in the ITS topologies. Thus, in addition to many of the species listed above, clade F in the plastid topologies also contains the *S. gardenii*, *S. polyanthus*, *S. saundersii* and *S. porphyrostachys* populations. This clade is therefore perhaps more comparable with clades IV–VII of the nuclear topologies than just with clade VII, with the differing positions of some of the *S. meyeri* populations, *S. caeruleus*, *S. longiflorus*, *S. montigena*, *S. fanniniae*, *S. grandis* and *S. vandeleurii* being due to hybridization and/or incomplete lineage sorting. Thus, clade F possibly does not only contain the members of the Cape primrose clade, but also its closest relatives, with the lack of resolution amongst these lineages being caused by the rapid speciation and slower evolution of the plastid genome compared to the ITS region at higher taxonomic levels. Alternatively, clade F does represent the Cape primrose clade, with the extra taxa arising in this clade as a result of having captured the chloroplast of members of the Cape primrose clade.

Despite the numerous incongruences between the ITS and plastid topologies of the current study, many of the species in clades VI–VII of the ITS trees also emerge in the large polytomy of the plastid phylogeny. The species shared between clades VI–VII of the ITS trees and clade F of the plastid trees—referred to from now on as the "core Cape primrose clade species"—include *S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana*, all the members of the *S. cyaneus* complex (*S. cyaneus*, *S. parviflorus*, *S. fenestra-dei*, *S. kunhardtii* and *S. roseo-albus*), *S. floribundus*, *S. aylae* and *S. kentaniensis*.

These species share a number of morphological characteristics. They are perennial, acaulescent, rosulate herbs. Their laminas are much longer than broad, being lanceolate, strap-shaped or oblong, and are decurrent on the petiole in most species. Their laminas possess a cuneate base and crenate or crenulate margin. The plants bear one to several inflorescences arising at the apex of the petiolode. Their peduncles and pedicels have a pilose indumentum containing both glandular and eglandular hairs in most species, and most species also produce bracts. Their sepals are linear to lanceolate. Most of the species possess the open-tubed floral type, except for *S. johannis* and *S. baudertii* which each possess the keyhole type (Hilliard & Burtt 1971; Harrison *et al*. 1999; Hughes *et al*. 2006). Thus, most species have straight floral tubes, round corolla mouths and slightly oblique corolla limbs, characteristic of the open-tubed floral type. However, the flowers of *S. johannis* and *S. baudertii* are typical of the keyhole type, with S-shaped floral tubes, laterally compressed corolla mouths and strongly oblique corolla limbs. *S. lilliputana* also possesses the open-tubed floral type, but it differs from the other open-tubed species in that its floral tube is curved downwards. The corolla tube of all the species is whitish or pale or medium violet outside, but the insides of corolla tubes are variable amongst species. The corolla throat in most species is glabrous, except for hairs on the roof and/or floor of the tube. The floor of the tube is also white or pale or medium violet, often possessing a yellow line, which is streaked with violet in *S. kentaniensis* and *S. formosus*. The floor of most species is marked with 3–7 violet stripes that either extend all along the tube or just into the throat. The corolla limb is bilabiate, white, rose-pink or pale or medium violet, and is also glabrous on the inside in most species. The five corolla lobes are elliptic, suborbicular or oblong. The stamens arise one third to two thirds up the corolla tube. The anther lobes are 1–3 mm long, and either white or violet in colour. The staminodes in most of the species are much shorter than the stamens and are inconspicuous, but the lateral staminodes are conspicuous in *S. modestus* and *S. primulifolius*. The ovary is greenish. The style is white in colour, and terete or dorsoventrally

compressed. The stigma is equally or unequally bilobed (Hilliard & Burtt 1971; Bellstedt & Edwards 2004). These species are also palynologically largely homogenous, with most of the species possessing Weigend & Edwards' (1996) pollen type 4. However, *S. baudertii* and *S. johannis* are exceptions, possessing types 4 and 8 respectively. On the whole, these core species are morphologically similar in many respects.

3.4.5.1. *S. primulifolius*

Amongst these core species, *S. primulifolius* has one of the largest distribution ranges, found in forest patches occurring in a long, thin line of approximately 450 km that extends along the northern Eastern Cape and southern KwaZulu-Natal coasts. The northern *S. primulifolius* populations sampled in this study (nPriTableMt, nPriCamperDown, nPriMonteseel and nPriStonesFarm) group together mostly separated from the rest of the *S. primulifolius* populations by relatively long branches in both the nuclear and plastid topologies. In contrast, relationships amongst the central populations sampled (cPriRooivaal, cPriMsikaba, cPriEndliniyokozi, cPriBulololoGorge, cPriMtSullivan, cPriDwalana, cPriSilaka, cPriXibeni and cPriMbanyanaFalls) are mostly unresolved in the nuclear tree, and emerge dispersed amongst representatives of other species in the plastid topologies. Relationships amongst the southern populations (sPriWedgeley, sPriPostWellington, sPriFloradale, sPriIgoda and sPriRivendell) are also unresolved in the ITS tree, but they group together along with three of the central *S. primulifolius* populations in the plastid topologies. Thus, the northern *S. primulifolius* populations constitute the most genetically coherent group in all the topologies, while the central populations are phylogenetically the most dispersed.

The northern *S. primulifolius* populations are also morphologically distinct within the species. They are characterised by large plants, with their phyllomorphs reaching up to 700 × 150 mm in size, an indumentum on the underside of the lamina that consists of strigose, eglandular trichomes interspersed with stalked, glandular trichomes; deep blue corollas with dark purple corolla floors, dark stripes (Trevor Edwards, unpublished data), a red flush at the base of the lower lip (Hilliard & Burtt 1971) and an ovary indumentum of stalked glandular trichomes with purple heads. In contrast, the central and southern populations are characterised by plants that are comparatively smaller, with their phyllomorphs only reaching 460 × 120 mm in size, the indumentum on the underside of their laminas contains only eglandular trichomes; their corollas are pale with whitish throats, blue to lilac lobes and stripes that are deep violet on the floral lobes, but red-purple in the throat and a pubescent ovary indumentum with stalked glandular trichomes only occurring on their styles (Trevor Edwards, unpublished data). Thus, the northern populations are both genetically and morphologically the most distinct group within *S. primulifolius*, while the central and southern populations are morphologically more similar to each other (Trevor Edwards, personal communication) and also share some plastid haplotypes.

The diversity of plastid haplotypes present in the central, Pondoland Centre populations and their scattered distribution in the plastid phylogenies, compared to that of the northern and southern populations either implies that they constitute the most ancestral part of *S. primulifolius* from which the northern and southern populations have evolved, or that this is the area in which the most hybridization has been occurring. Hughes *et al*. (2005) detected large amounts of genetic diversity in these central populations, and ascribed this to the presence of large populations having existed at these sites over long periods of time. These forest patches occur along a coast that is bathed by the warm Mozambique current and that consists of numerous deep gorges, and it is therefore likely that forests in this area would have been able to survive previous glacial-interglacial cycles reasonably intact in these relatively protected habitats. This area is also rich in a surprising number of endemics of other plant groups, but perhaps particularly pertinent is the fact that many other forest-floor endemics, such as *Plectranthus*, also occur in this area. This area is therefore viewed as an important Pleistocene

refugium (Van Wyk & Smith 2001), and it is likely to have served as such for *S. primulifolius* (Hughes *et al*. 2005). The *S. primulifolius* population from Rooivaal, whose plants are morphologically indistinguishable from those of the northern populations, groups along with the northern populations in the nuclear topologies, but with a number of other populations in the plastid topologies, including two central and all of the southern *S. primulifolius* populations, as well as with the *S. rexii* populations. Geographically, the closest population in this small plastid polytomy to cPriRooivaal is rexMtAyliff, and Hughes *et al*. (2005) therefore suggested that the differing positions of cPriRooivaal in the nuclear and plastid phylogenies is caused by a chloroplast capture event with *S. rexii*. In order for the Rooivaal plants to have captured the maternally inherited plastid (Möller *et al*. 2004), seeds of *S. rexii* must have been dispersed near Rooivaal, probably along a river. This is a plausible explanation, since *S. primulifolius* and *S. rexii* share the open-tubed floral type and have similar floral shapes and markings, and it is not inconceivable that the pollinator of *S. primulifolius* i.e. the long-proboscid nemestrinid fly *Stenobasipteron wiedemanni* (Trevor Edwards, unpublished data; Dirk Bellstedt, Michael Möller and Mark Hughes, personal observation), would also visit an *S. rexii* flower if this were to occur in its range. The flowering times of *S. primulifolius* and *S. rexii* (Hilliard & Burtt 1971) also overlap.

Hughes *et al*. (2005) did not sample the northern *S. primulifolius* populations as extensively as was done in the current study, and was therefore not able to make many deductions regarding this group and its relationships to the rest of the species. In contrast to the central populations, there are no or very few differences amongst the ITS and plastid sequences of the northern populations, but the northern populations are together separated from the other *S. primulifolius* populations (except for cPriRooivaal) by quite a few differences, both genetic and morphological. Assuming that pollination and seed dispersal are mediated by the same agents in the northern and central *S. primulifolius* populations, the relative homogeneity of the northern populations is unlikely to be due to frequent gene flow amongst the populations, since the central populations, which are genetically very different from one another, are geographically far closer to one another than are the northern populations. Rather, the homogeneity of the northern populations probably indicates that the individual populations have diversified from a common ancestor relatively recently. Furthermore, the long branch lengths between the northern population group and the rest of the species implies that northern *S. primulifolius* has been separated from the rest of the species for quite a long time. However, whether the reasonably isolated northern *S. primulifolius* existed as a single population before diversifying or fragmenting recently, or whether it diversified early on, but subsequently underwent population crashes due to the considerable forest shrinkages believed to have occurred in this area (Eeley *et al*. 1999) leading to the extinction of most of the original populations before diversifying again, is open to speculation. Thus, the genetic and morphological distinctiveness and the homogeneity of the northern populations within the species suggest a long isolation period, but only a recent diversification into the separate populations.

In comparison, the patterns retrieved in this study indicate that the southern populations are a younger offshoot in *S. primulifolius*, in accordance with the findings of Hughes *et al*. (2005) in which these populations are collectively referred to as *S.* cf. *primulifolius*. The morphological similarities between the central and southern populations, the declining genetic diversity in the microsatellite data of Hughes *et al*. (2005) from the central to the southern populations, the similarities of the ITS and plastid sequences amongst the southern populations, and the lack of any differences between the ITS and plastid sequences of the southern populations and those of some of the central populations supports the relatively recent establishment of the southern populations from the central populations further up the coast.

Amongst the core species that are represented by samples from more than one population, *S. primulifolius* is the most dispersed in the nuclear and plastid topologies. Samples of *S. primulifolius* are spread widely in the well-sampled clades in both the ITS networks and plastid analyses, suggesting that this species is the ancestor of many of these species, and that these species have evolved from different *S. primulifolius* populations, or that they have captured genetic material from *S. primulifolius* as a result of hybridization. The species that group most closely with *S. primulifolius* in the ITS and/or plastid topologies are *S. rexii*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana* and *S. johannis*. Of these, *S. rexii*, *S. johannis* and *S. baudertii* are closely associated with *S. primulifolius* in both the ITS and plastid analyses.

In summary, *S. primulifolius* probably first arose in the middle of its range in the Pondoland Centre, from where it spread both north, and later also south, to cover its present range. The species associates with many other taxa in the ITS and/or plastid topologies, and is therefore ancestral to or has undergone hybridization with these taxa.

3.4.5.2. *S. rexii*

S. rexii is another species with a long, thin distribution that stretches in an arc of about 800 km from the Matatiele vicinity in southern KwaZulu-Natal to the Knysna in the Western Cape (Hilliard & Burtt 1971). Hughes *et al*. (2005) data suggested that *S. rexii* evolved more than once from the southern *S. primulifolius* populations (*S.* cf. *primulifolius* in Hughes *et al*. 2005) based on relationships inferred from their genetic data and morphological observations made by Hilliard & Burtt (1971) and later by themselves in the field.

The patterns obtained in the current study are compatible with, or at least do not contradict, this proposed close affinity between *S. rexii* and the southern *S. primulifolius* populations and a polyphyletic origin of *S. rexii*. The southern *S. primulifolius* and most of the *S. rexii* populations emerge in unresolved positions in clade VII in the ITS phylogenies, but group together in the ITS networks. In all of the ITS topologies, the three northernmost *S. rexii* populations group together. In the plastid topologies, all of the southern *S. primulifolius* and *S. rexii* populations group together (although along with representatives from other species as well). Thus, although the current study is not resolved enough to show the exact origin(s) of *S. rexii*, the topologies are consistent with a close relationship between the southern *S. primulifolius* populations and *S. rexii* and the monophyletic origin of the three northernmost *S. rexii* populations detected by Hughes *et al*. (2005). The evolution of *S. rexii* from the southern *S. primulifolius* populations is also supported by morphology, since Hilliard & Burtt (1971) described a typical plant from the southern *S. primulifolius* populations as “a very large and deeply coloured *S. rexii*”.

3.4.5.3. *S. johannis*

S. johannis also shows linkages to *S. primulifolius* in both the nuclear and plastid topologies. Based on its intermediate morphology and geographical proximity, Hilliard & Burtt (1971) proposed that *S. johannis* came into being as a result of hybridization between members of their Groups C and D. *S. johannis* possesses the keyhole floral type characteristic of species in their Group D. However, plants in their Group D also possess the unifoliate and/or plurifoliate growth habit. The horizontal rhizome, rosulate growth habit, strap-like leaves and corolla markings of *S. johannis* are more characteristic of the species in their Group C, even though these plants also possess the open-tubed floral type. From Group C Hilliard & Burtt (1971) suggested *S. rexii*, *S. primulifolius* and *S. gardenii*, and from their Group D *S. haygarthii* and *S. polyanthus*, as possible hybrid parents for *S. johannis*.

Although *S. haygarthii* was not sampled in the current study, the representative of this species in the ITS analysis of Möller & Cronk (2001a) emerged in the clade predominantly containing

the other included species of Hilliard & Burtt's (1971) Group D, far away from *S. johannis*. However, representatives of *S. haygarthii* have not been included in any analyses performed on plastid data, and its position relative to the other species according to this marker is therefore uncertain. *S. rexii*, *S. primulifolius*, *S. gardenii* and *S. polyanthus* were, however, all included in the current analyses. Of these latter four species, only *S. primulifolius* shows any tendency to group with members of *S. johannis* in the ITS and plastid topologies.

S. primulifolius is similar to *S. johannis* in many respects, so much so that it is difficult to distinguish between non-flowering plants of the two species in the forests around the base of Mount Sullivan where they co-occur (Dirk Bellstedt, personal communication). They are both perennial rosulates with horizontal rhizomes. Their leaves are also very similar, both species possessing pilose laminas with cuneate bases that are decurrent on the petiole (Hilliard & Burtt 1971). In the areas in which their distribution ranges overlap, both species produce grey-green leaves of more or less equal sizes (Dirk Bellstedt, personal communication). Inflorescences are borne from the tops of the petiolodes, and the peduncles and pedicles are pilose with glandular and eglandular hairs (Hilliard & Burtt 1971).

However, *S. primulifolius* and *S. johannis* have different floral types. While *S. johannis* bears keyhole flowers, *S. primulifolius* produces open-tubed flowers. Floral type, however, appears to be rather plastic in *Streptocarpus* (Harrison *et al*. 1999; Hughes *et al*. 2006). For example, the keyhole type has been found in 12 other species in the genus. Based on reconstructions of the evolutionary relationships of *Streptocarpus* based on ITS sequence data, many of these species are only distantly related to one another. *S. saxorum* is a keyhole species that is currently classified within subgenus *Streptocarpella*, while the other 11 keyhole species that are in subgenus *Streptocarpus* emerge in four to seven separate positions in the subgenus *Streptocarpus* clade (Hughes *et al*. 2006). Likewise, the open-tubed type is believed to have evolved more than once in *Streptocarpus* (Harrison *et al*. 1999; Hughes *et al*. 2006). However, even more compelling evidence of the plasticity of floral type has recently come to light (Trevor Edwards and Dirk Bellstedt, personal observations). A form of *S. porphyrostachys*—an open-tubed species occupying a coastal range of about 35 km straddling the Eastern Cape–KwaZulu-Natal border (Hilliard & Burtt 1971)—has recently been discovered in the Msikaba river gorge that produces keyhole flowers. Distinctive intermediate forms between the open-tubed and keyhole forms were also detected in the same river gorge. No keyhole species occur in the same gorge, and this form did not deviate from *S. porphyrostachys* in any way other than in floral form, and it is therefore unlikely to be a hybrid, but perhaps rather the case of incipient pollinator switch (Michael Möller, personal communication). Harrison *et al*. (1999) came to the conclusion that small changes early on in floral development lead to dramatic differences in the mature flower, and floral type is therefore possibly only controlled by a few major genes (although other floral characters such as floral tube length are highly variable in *Streptocarpus* and are therefore probably controlled by many modifying genes; Michael Möller, personal communication). This would make the evolution of one floral type from another reasonably easy, and therefore a reasonably frequent occurrence. This is one of the more astonishing, but not the only example of floral diversity within *Streptocarpus* species. *S. polyanthus*, *S. haygarthii* and *S. cyaneus* are examples of other species that have variable flowers, although the variability lies in the size, shape and markings of the flowers (Hilliard & Burtt 1971) rather than in floral type. Thus, floral morphology appears to be plastic, not only at the infrageneric level, but even within species in some cases. *S. johannis* could consequently have evolved from *S. primulifolius* and subsequently acquired the keyhole floral type independently. While the floral type of *S. johannis* differs from that of *S. primulifolius*, geographic, genetic and vegetative morphological evidence indicates that *S. primulifolius* is the ancestor of *S. johannis*. Alternatively, the origins of *S. johannis* could lie in a cross between *S. primulifolius* and

S. haygarthii, the former species providing the vegetative morphology and the latter species the floral morphology of *S. johannis*. This is a hypothesis still to be tested in the future.

The genetic data also provide some indication of relationships amongst the population clusters of *S. johannis*. The species has a disjunct distribution, with its populations clustering towards the north and south of its distribution range. The southern cluster is made up of coastal populations (johSMtSullivan, johSMagwaFalls, johSEmbotyi and johSMyokane) spread over approximately 30 km from Port St. Johns to Embotyi in the northern Eastern Cape. These southern *S. johannis* populations fall within the distribution range of the central *S. primulifolius* populations and co-occur in the same forest patches around Mount Sullivan at Port St. Johns. However, they occupy different microhabitats—*S. johannis* grows in moister, seepage habitats in gorges, whilst *S. primulifolius* occurs on slightly drier earth banks and on cliffs in gorges in these areas (Dirk Bellstedt, personal communication). The northern cluster of *S. johannis* contains inland populations (johNManzimnyama, johNHebronRd and johNNsikeni) occurring from about the Kokstad to the Kingscote vicinities in southern KwaZulu-Natal. These populations extend over about 60 km, and are located further apart from one another than are the southern populations. In between the northern and southern clusters is a gap of a little less than 100 km in which no *S. johannis* populations have so far been found. Although there are no forest patches directly between the northern and southern clusters, most of the more peripheral forests have been searched without success, and this disjunction is therefore possibly a natural one (Dirk Bellstedt, personal communication).

In many respects, *S. johannis* follows the same molecular patterns observed in the central and northern populations of *S. primulifolius*. The southern *S. johannis* populations are genetically quite diverse. In the nuclear topologies they group together, but with minor differences between them, while they emerge in two (in the plastid networks) or three (in the plastid phylogenies) separate groups in the plastid topologies. In comparison, the northern populations are far more similar to one another. Their ITS sequences are identical, and they form a strongly supported group in the plastid topologies with the johNManzimnyama representatives together occupying the internal position and the representatives of the other two northern populations the terminal positions in the plastid networks.

Similar patterns can be found in the morphology of the population clusters. The southern cluster consists of far more morphological variation, especially in terms of corolla pigmentation, markings and the colouring in the floral throat. The *S. johannis* plants at Mount Sullivan in the southern cluster have white flowers with a pale yellow throat, the Magwa plants have mauve flowers, and the Myokane and Embotyi plants have pale mauve flowers with yellow throats. Compared to the southern populations, the northern populations consist of plants that are smaller and less robust, with smaller, dark-green leaves as opposed to the larger, grey-green leaves found in the southern populations. The flowers of the northern populations are also smaller, typically reaching only 60–80% of the size of those in the southern populations, and have narrower corolla lobes. The Nsekeni population typically bears mauve flowers, the flowers in the Hebron population are lighter mauve with each corolla lobe possessing a distinct line that extends into the floral mouth, and the Manzimnyama flowers are white, but also with lines on the corolla lobes (Hilliard & Burtt 1971; Dirk Bellstedt, personal communication).

The high genetic diversity of the southern populations relative to the northern populations is probably indicative of the southern populations being older, and the forest patches in the southern Pondoland Centre are therefore probably the birthplace of *S. primulifolius* as well as *S. johannis*. The northern and southern populations group separately in the ITS topologies, which would suggest independent origins of the two population clusters. However, the northern populations group with some of the southern populations in the plastid analyses, indicating that the northern cluster probably arose from within the southern cluster. Current distribution ranges

of many plant species suggest that a migration route existed from the Pondoland Centre to the KwaZulu-Natal Drakensberg mountains via the Ngeli range (Van Wyk & Smith 2001), which is the site of the present-day Manzimnyama River population (situated at the base of Mount Ngeli) of *S. johannis*, and *S. johannis* therefore probably also spread along this path. The monophyly and relative uniformity of the northern populations in both analyses probably means that the northern populations share a relatively recent, common origin from one of the southern populations. The fact that the other two northern populations radiate out from the Manzimnyama population in the plastid networks probably indicates that Manzimnyama is ancestral to the other two. This also makes geographical sense, seeing as the Manzimnyama population is the most southern population in the northern cluster. Thus, *S. johannis* probably arose in the Port St. Johns–Embotyi forests, where it spread in this reasonably protected area. The species would probably have been able to follow the historical migration path between the Pondoland Centre and the Drakensberg mountains during a time when forest cover was more extensive, becoming established in the region currently occupied by the Manzimnyama population. The other northern *S. johannis* populations were probably established soon afterwards, seeing as the Manzimnyama population is not very different from the other two populations. Subsequent forest shrinkages probably resulted in *S. johannis* becoming extinct in the centre of its range.

The genetic data also indicate that there has not been much gene flow amongst the populations. Although ITS sequences were only generated from one individual per *S. johannis* population, two individuals were analysed from each population for the more variable plastid markers. The plastid sequences are different amongst all of the populations, but are identical within populations in all but one (johSMagwaFalls). This is indicative of plastid fixation within most of the populations (considering the small sample size of two), with very little gene flow occurring amongst them.

In summary, *S. johannis* quite possibly evolved from the central *S. primulifolius* populations in the forest patches of the southern Pondoland Centre (with possible genetic input from *S. haygarthii*), where it diversified. The northern populations of *S. johannis* appear to be a more recent offshoot of the southern populations, probably reaching their current localities via the same migration routes that many other plant taxa appear to have followed.

3.4.5.4. *S. baudertii*

The *S. baudertii* populations are more scattered in the nuclear and plastid topologies. The five populations analysed emerge in three different places according to both markers. While the three northernmost populations (bauHarmony, bauOtterspoort and bauNtywenka) group together along with *S. rexii* and the southern *S. primulifolius* populations in the ITS networks and in the plastid topologies, the two southernmost populations (bauCollywobbles and bauHillsdrift) each consistently emerge separately from their conspecific populations. The Collywobbles population emerges rather closely to the southern *S. johannis* populations, especially those collected at Embotyi and Magwa Falls, in both the ITS and plastid topologies. The Hillsdrift population, in comparison, assumes comparatively isolated positions according to both markers. Geographically, the species also has a rather scattered distribution, with only a few populations occurring in a long, thin band over about 144 km from the area around Matatiele in the north to East London in the south (Hilliard & Burtt 1971).

Hilliard & Burtt (1971) proposed a hybrid origin for *S. baudertii*. Its intermediate geographical distribution and morphology led Hilliard & Burtt (1971) to propose that it originated as a hybrid between *S. johannis* and *S. meyeri*, since it possesses the keyhole floral type of *S. johannis*, but a vegetative morphology that is so similar to that of *S. meyeri*, that non-flowering plants of the two species are difficult to tell apart (Hilliard & Burtt 1971; Dirk Bellstedt, personal

communication). However, the genetic analyses show little evidence for this origin. The only *S. meyeri* samples to group with any of the *S. baudertii* populations are two of the individuals collected at Bastervoetpad. The Bastervoetpad samples, however, group separately from the other *S. meyeri* populations, and their genetic signals most likely reflect a recent hybridization event with *S. rexii*, which also occurs at Bastervoetpad. *S. rexii* is autogamous. The close proximity of the anthers and stigma (Hughes *et al*. 2005) and the loose coherence of the anthers to each other (Michael Möller, personal communication) enables *S. rexii* flowers to self-pollinate when the corolla tube falls off the flower. This not only enable *S. rexii* to establish itself more easily in new habitats (Hughes *et al*. 2005), but also makes *S. rexii* more likely to hybridize. This is because *S. rexii* is not pollen-limited, and stray pollen grains from other species that land on the stigma of an *S. rexii* flower will therefore probably result in offspring, as long as no other reproductive barriers exist (Trevor Edwards, personal communication). The similarity between *S. meyeri* and *S. baudertii* might be due to independent, parallel adaptations to the environments in which they grow. Both species occupy dry habitats on rocky outcrops and cliff faces, and their vegetative morphology shows adaptations to these dry conditions (Hilliard & Burtt 1971). The small, round, prostrate, densely hairy leaves of *S. meyeri* and *S. baudertii* compared to the large, strap-shaped, erect or suberect leaves of the other members of the Cape primrose clade, and the stout rhizomes of *S. meyeri* and *S. baudertii* (Hilliard & Burtt 1971) afford them greater protection against water loss. Similarly, there is little evidence for *S. johannis* being one of its parents. The only *S. baudertii* population to group with *S. johannis* is the Collywobbles population, possibly the result of gene flow between the two keyhole species or a shared origin. There is therefore little support for *S. baudertii* having originated from hybridization between *S. johannis* and *S. meyeri*.

The genetic discontinuities within the species according to both markers are indicative of one of two alternatives. Either *S. baudertii* is a monophyletic species that has undergone extensive hybridization with different species, or the species is polyphyletic, having arisen two or more times from different species.

The distinctive vegetative and floral morphologies of *S. baudertii* would suggest that it is a monophyletic species, and that it emerges in disjunct positions in the topologies as a result of subsequent hybridizations with other species. Hybridization between *S. baudertii* (a keyhole species) and the bordering open-tubed species is not as unlikely as it may seem. The *S. baudertii* populations lie very close to populations of other species—the three northernmost populations lie close to *S. rexii* populations, and the two southernmost populations are very close to *S. primulifolius* populations. Moreover, open-tubed and keyhole species appear to be capable of hybridising. For example, in the areas in which *S. johannis* (a keyhole species) and *S. gardenii* (an open-tubed species) overlap i.e. the forest patches occupied by the northern *S. johannis* populations, *S. johannis* is found in the higher altitude areas, while *S. gardenii* occupies lower altitudes. In between populations of these two species there is often a hybrid zone, consisting of individuals with flowers intermediate in form between these two species (Hilliard & Burtt 1971; Dirk Bellstedt, personal observations). The isolated positions of most of the *S. baudertii* populations together with the different populations hybridizing with the species into whose ranges they fall could have contributed to the apparently polyphyletic origin of the species in the topologies.

Alternatively, the scattered pattern of the *S. baudertii* populations in the analyses could be caused by the species being polyphyletic. There is variation amongst all of the populations, especially in terms of floral morphology i.e. in the number of flowers per inflorescence, in the size of the flowers, and in the shape of the corollas (Hilliard & Burtt 1971; Dirk Bellstedt, personal observation). In addition, the Hillsdrift population is also vegetatively quite distinct. While *S. baudertii* plants in the other populations possess very flat rosettes, the leaves are held

far more erect in the Hillsdrift plants (Michael Möller and Dirk Bellstedt, personal observations). As discussed under *S. johannis*, floral type is quite plastic in the genus, and the keyhole floral type appears to have several independent origins (Harrison *et al*. 1999; Hughes *et al*. 2006). The distinctive vegetative morphology of *S. baudertii* is perhaps also not necessarily indicative of monophyly of the species, but perhaps due to independent, parallel adaptations to the environments in which they grow. Another oddity of the species is in the scattered distribution of its populations. *Streptocarpus* seeds have no special morphological adaptations to long-distance dispersal, and no seed dispersal agents have been identified for *Streptocarpus* (Möller & Cronk 2001b). Moreover, *S. baudertii* grows in dry habitats in crevices or on cliff faces (Hilliard & Burtt 1971), and there are habitats in between the known populations where *S. baudertii* could occur, but does not (Dirk Bellstedt, personal communication). The disjunct distribution of the populations must therefore either be due to the species having arisen and spread before southern Africa became more arid, subsequently being wiped out in the less protected habitats, or each of the populations constitute independent lineages. It is therefore also possible that *S. baudertii* is polyphyletic.

Thus, the monophyly of *S. baudertii* is uncertain. The Hillsdrift population is morphologically and genetically different from the rest, and could therefore have a separate origin. However, whether the remaining populations emerge in two separate positions because of independent origins or due to subsequent hybridization is more difficult to say. The Collywobbles population groups with *S. johannis* populations according to both markers, and this population could therefore have evolved from *S. johannis*, acquiring its floral type in the process, or have subsequently hybridized with it. The three northernmost populations share the same ITS and plastid sequences, and therefore probably also the same origin. However, they group most closely with *S. rexii* and the southern *S. primulifolius* populations, which together constitute a relatively young lineage (Hughes *et al*. 2005). It is therefore difficult to imagine that *S. baudertii* evolved from one of this lineage, but has had enough time in which to evolve its distinctive vegetative and floral morphologies. *S. johannis* may also have been part of the parentage of the northern and Hillsdrift *S. baudertii* populations, but the signal might have been lost due to subsequent hybridization with *S. rexii* and the southern *S. primulifolius* populations. Thus, whether *S. baudertii* as a whole is monophyletic or polyphyletic, the close relationship between the northernmost *S. baudertii* populations and *S. rexii* and the southern *S. primulifolius* populations is probably due to recent hybridization rather than being indicative of evolutionary origins.

3.4.5.5. *S. modestus*, *S. formosus*, *S. gardenii* and *S. lilliputana*

The other species that show tendencies to group with *S. primulifolius* in the analyses i.e. *S. modestus*, *S. formosus*, *S. gardenii* and *S. lilliputana*, mostly have far more limited distributions. *S. modestus* and *S. lilliputana* are only found in the Pondoland Centre, and their ranges overlap. *S. modestus* occurs in forest patches at Magwa Falls, Fraser Falls, on the ridges of the Msikaba River Gorge and in the Umtentu River Gorge in the southern Pondoland Centre (Hilliard & Burtt 1971), and *S. lilliputana* has only been found in three neighbouring gorges: the Lupatana River Gorge, the Myokane River Gorge, and along the Mkozi River above Fraser Falls in Fraser Gorge (Bellstedt & Edwards 2004). *S. formosus* occurs a little to the north in the Nyameni, Mzamba, Oribi and Umtamvuna River Gorges (Weigend & Edwards 1994a). *S. gardenii*, in comparison, has a far larger distribution. The Drakensberg forms its western boundary, while in the east it is found from Tabankulu in northern Eastern Cape to Mount Ngwibi near Vryheid in northern KwaZulu-Natal (Hilliard & Burtt 1971).

Although these four species group with *S. primulifolius* in some of the analyses, they also show links to other species, and their origins and affiliations are therefore more uncertain. The *S. modestus* population analysed shows strong links to *S. johannis* in the plastid topologies, but

groups with the central *S. primulifolius* populations from the southern Pondoland Centre in the nuclear networks. *S. formosus* is genetically closest to the group containing the northern *S. primulifolius* populations in the ITS networks, but groups loosely with *S. porphyrostachys* in the plastid analyses. The close relationships between *S. formosus* and the northern *S. primulifolius* populations makes the most morphological sense, seeing as Hilliard & Burtt (1971) viewed *S. formosus* as a subspecies of *S. primulifolius*. However, the *S. porphyrostachys* population is geographically the closest *Streptocarpus* population included in this study, and this could be yet another case of hybridization obscuring relationships. *S. gardenii* groups with the northern *S. primulifolius* populations in the plastid topologies, but is most closely related to *S. caeruleus* in the ITS topologies. However, in a larger ITS analysis run with a representative of *S. candidus* included (not shown here), the *S. gardenii* populations group most closely with this species. *S. gardenii* and *S. candidus* have abutting distribution ranges. Mount Ngwibi near Vryheid constitutes the north-eastern boundary of *S. gardenii*, while *S. candidus* has a distribution range a little north-east of *S. gardenii* between Vryheid and the Ngome forest in northern KwaZulu-Natal. The close relationship between *S. gardenii* and *S. candidus* according to ITS data could therefore either be the result of a shared origin or of subsequent hybridization. Finally, *S. lilliputana* appears to have radiated out from the *S. cyaneus* complex in the ITS networks, but shows loose relationships with some of the central *S. primulifolius* populations in the plastid networks. Its unique corolla shape makes is likely due to a very localised pollinator adaptation (Michael Möller, personal communication).

All of these grouping therefore make geographical sense, and it is difficult to distinguish the origin of each of the species from genetic signal that might have been caused by incomplete lineage sorting or subsequent gene flow.

The remaining species in the core Cape primulifolius group i.e. the species belonging to the *S. cyaneus* complex, *S. floribundus*, *S. aylae* and *S. kentaniensis*, do not show very strong ties with any of the other included species.

3.4.5.6. The *S. cyaneus* complex

Another group that was reasonably well sampled is that of the *S. cyaneus* complex. Members of this complex are found from Itala Nature Reserve (*S. kunhardtii*, Edwards 2003) on the KwaZulu-Natal–Mpumalanga border, all along the eastern escarpment of the Drakensberg to a little north of the Soutpansberg in Limpopo (Hilliard & Burtt 1971). Hilliard & Burtt (1971) recognised two species: *S. cyaneus* and *S. parviflorus*. However, these are highly variable species that show some morphological structure at the population level, and Weigend & Edwards (1994b) subsequently subdivided them to include two more species i.e. *S. roseo-albus* and *S. fenestra-dei*, at the same time dividing the remaining populations of *S. cyaneus* into four subspecies and those of *S. parviflorus* into two. In addition, Edwards *et al*. (1992) and Edwards (2003) described two newly discovered species, *S. fasciatus* and *S. kunhardtii* respectively, which they believed show strong ties to the complex. *S. actinoflorus*, previously part of *S. parviflorus*, awaits description.

The genetic data mostly support the close relationships of the species belonging to this complex. Most of the samples form a strongly supported group in the plastid topologies, with only the *S. cyaneus* and *S. parviflorus* representatives from Soutpansberg and *S. kunhardtii* emerging outside of this group. Representatives of this complex emerge in unresolved positions in the ITS phylogenies, but tend to emerge in the same part of the ITS networks (although with members of some other species). The close relationship of *S. kunhardtii* with *S. grandis* in the plastid topologies is unexpected. These species are morphologically very different from each other. They differ in most characters, including growth habit, shape of the lamina base, number of flowers in their inflorescences, corolla shape and floral markings (Hilliard & Burtt 1971;

Edwards 2003). Likewise, the grouping of the *S. cyaneus* and *S. parviflorus* from Soutpansberg with *S. vandeleurii* in the plastid topologies does not make much morphological sense. *S. vandeleurii* is a unifoliate with a cordate leaf base and villous leaf veins and stalks. Its inflorescence carries about 36 flowers strongly scented on a stout peduncle. The floral tube is curved strongly downwards. The flower is white with a pronounced yellow blotch at the base of the lower lip, which is sometimes also patterned with three linked V-shaped reddish-purple markings in the mouth. In comparison, *S. cyaneus* and *S. parviflorus* are rosulates with cuneate leaf bases. They only carry up to 20 flowers at a time, but usually less, and their flowers are not scented. Their floral tubes are straight and the floor is marked with a yellow stripe usually patterned with violet streaks that extend out onto the lower lip (Hilliard & Burtt 1971). The range of *S. vandeleurii* overlaps with those of *S. cyaneus* and *S. parviflorus*, including in the Soutpansberg area (Hilliard & Burtt 1971), and these species could therefore have hybridized. The *S. cyaneus* and *S. parviflorus* representatives from Soutpansberg and *S. kunhardtii* share the same ITS sequences with many other members of the *S. cyaneus* complex, and their divergent positions in the plastid topologies are therefore probably the result of hybridizations or incomplete lineage sorting rather than real disjunctions in the complex.

Relationships within the complex are, however, less clear, both due to limited sampling of the taxa and a lack of resolution. The two species that are represented by more than one population i.e. *S. cyaneus* (eight populations) and *S. parviflorus* (two populations), both do not form monophyletic groups according to either of the markers. *S. roseo-albus*, *S. fenestra-dei* and *S. kunhardtii* were each only represented by one sample, and each of these samples emerged amongst the *S. cyaneus* populations (although *S. kunhardtii* emerges outside of the complex in the plastid topologies). The paraphyly of *S. cyaneus* is, however, not surprising. The distribution range of *S. cyaneus* encompasses the range of almost the entire complex, and Weigend & Edwards (1994b) considered *S. cyaneus* as "central to the group". The other species have therefore probably evolved from different populations of *S. cyaneus*. The *S. cyaneus* subsp. *longi-tommii* sample from Die Geut, a locality that lies on the border between Limpopo and Mpumalanga provinces, emerges ancestral to the group containing most of the *S. cyaneus* complex representatives in the plastid networks, and emerges as one of the many samples at the base of the looser group in the ITS networks. Hilliard & Burtt (1971) observed that most of the morphological variation of *S. cyaneus* occurs towards the centre of its range, which is where Die Geut is located, and the complex might therefore have first arisen in the centre, before spreading outwards to cover its present range.

The *S. cyaneus* complex is therefore a comparatively isolated but reasonably coherent group in *Streptocarpus*. *S. cyaneus* constitutes the core of the group, from which the other species appear to have evolved.

3.4.5.7. *S. floribundus*

S. floribundus is a geographically isolated species that is only known from one population on Kranskop in central KwaZulu-Natal. Before being raised to specific level by Weigend & Edwards (1994a), it was tentatively recognised under *S. primulifolius* by Hilliard & Burtt (1971), who believed that it also showed strong links to *S. cyaneus*. Hilliard & Burtt (1971) proposed two alternatives—that it either came into being as the result of hybridization between *S. primulifolius* and *S. cyaneus*, or that it is the remnant of the historical link between these two species.

In the current analyses, *S. floribundus* emerges on relatively long branches according to both markers, and is therefore one of the older core Cape primrose clade taxa. However, its affinities are difficult to unravel. In the ITS networks it shows loose linkages with *S. cyaneus*, but occupies an unresolved position in the plastid topologies. It therefore perhaps evolved from

S. cyaneus. However, whether it morphologically only superficially resembles *S. primulifolius*, or whether *S. primulifolius* did indeed evolve from the *S. cyaneus* complex, with *S. floribundus* constituting a remnant of the link between the two species, is difficult to discern. If *S. floribundus* is a remnant of a *S. cyaneus* lineage that spread southwards to form *S. primulifolius*, then one would expect *S. floribundus* to possess genetic linkages with *S. cyaneus*, but nevertheless emerge as sister to the *S. primulifolius* populations. However, the lack of resolution at the base of the well-sampled clades in the nuclear and plastid topologies prevents the inference of the older relationships in these clades.

3.4.5.8. *S. aylae* and *S. kentaniensis*

Similarly to *S. floribundus*, *S. aylae* and *S. kentaniensis* are genetically reasonably isolated from other members of the core Cape primrose taxa.

Hilliard & Burtt (1971) suggested *S. meyeri* as the closest relative of *S. kentaniensis*. However, there is no support for this in any of the topologies. *S. kentaniensis* occurs in dry forests in the area around Kentani and the Kei River Mouth. It is a distinct species in this group, possessing long, thin, comparatively succulent leaves with prominent venation on the underside of the leaves and long, thick, circular petiolodes (Hilliard & Burtt 1971). In fact, its leaves are unlike those of any other *Streptocarpus* species (Hilliard & Burtt 1971), let alone any other members of the core Cape primrose group. Its flowers are also very different from most other core Cape primrose taxa, and are far more akin to those of *S. meyeri* (Hilliard & Burtt 1971). These morphological differences are at least in part an adaptation to the hotter, drier environments in which *S. kentaniensis* occurs, but could also be indicative of a more distant relationship to many of the other members of the core Cape primrose clade. Additionally, the flowering time of *S. kentaniensis* (July–September) is very unusual in the South African *Streptocarpus*. Most species, including all the species that are also found in the Kentani area, flower in the South African summer (October–April). The only South African species whose flowering times overlap with that of *S. kentaniensis*—*S. modestus* flowers from September to October, *S. polyanthus* from July to February and *S. haygarthii* from September to April—are geographically far removed, occurring close to the Eastern Cape–KwaZulu-Natal border or in KwaZulu-Natal (Hilliard & Burtt 1971). *S. kentaniensis* has therefore had very little opportunity to hybridize with other species.

In terms of vegetative morphology, *S. aylae* is far more similar to the other members of the core Cape primrose group. However, its flowers are rather different from the other open-tubed core Cape primrose taxa. The corolla is campanulate, the floral tube being much shorter and the mouth being far wider than in the other species (Trevor Edwards, unpublished data). Its atypical floral morphology is perhaps also indicative of a more distant relationship to other members in the core Cape primrose group, or simply a local adaptation to pollinators. The species' aberrant floral morphology probably indicates that it does not share the same pollinator with the other species e.g. *Stenobasipteron wiedemanni*, the pollinator of *S. primulifolius* (Trevor Edwards, unpublished data; Dirk Bellstedt, personal observation), and the species has therefore not hybridized with any of the other species. *S. aylae* also has a very restricted distribution, only being found in the Msikaba River Gorge so far (Trevor Edwards, unpublished data), and its highly restricted distribution has probably exposed it to fewer species with which it could potentially hybridize.

S. kentaniensis and *S. aylae* are therefore both morphologically distinctive plants and are possibly also reasonably old members of this group with origins that are difficult to unravel due to the lack of resolution in the phylogenetic trees further back in time.

3.4.6. Species of uncertain affiliations

The relationships of a number of species included in this study are less clear, since they emerge in the well-sampled clade reconstructed from one of the markers, but outside of the well-sampled clade reconstructed from the other. These include *S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus*, *S. longiflorus*, *S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*. Some of these species are rosulates (*S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus* and *S. longiflorus*), while the remainder are unifoliates and/or plurifoliates (*S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*). Furthermore, the rosulates whose pollen was analysed by Weigend & Edwards (1996) i.e. *S. meyeri*, *S. montigena*, *S. fanniniae* and *S. caeruleus*, all possess pollen type 13, the same type found in most of the core Cape primrose species. In contrast, the pollen of these unifoliate/plurifoliate species is more variable, with *S. polyanthus* producing pollen type 8, *S. saundersii*, *S. porphyrostachys* and *S. vandeleurii* type 12 and *S. grandis* type 13. Therefore, both morphology and pollen type seem to suggest that these five rosulates are probably members or close relatives of the Cape primrose clade, while the five unifoliates/plurifoliates are more distantly related to the Cape primrose taxa. The discrepancies between the nuclear and plastid topologies could be the results of gene capture events between distantly related species.

3.4.6.1. *S. meyeri*

Amongst these rosulate species, *S. meyeri* is represented by the greatest number of populations. The species has a very large, but disjunct distribution. The bulk of the populations are concentrated across most of the Eastern Cape, occurring in rocky habitats within the area circumscribed by Graaff-Reinet, Quagga (both near the border with the Western Cape), Ugie and Kei Cuttings. However, isolated populations occur about 800 km away from the main area of the species at Pilgrims Rest and on Mariepskop on the Mpumalanga-Limpopo border. This disjunction appears to be a real one, since the intervening areas have been explored quite extensively without finding any sign of the species (Hilliard & Burtt 1971).

The *S. meyeri* populations are widely scattered in the analyses. The southernmost populations (meyBaviaanskloof, meyGraaffReinet, meySECharltonFarm, meySEGlenCraigFarm, meyZuurberg) group together in the ITS networks and in the plastid topologies. However, the affiliations of the central populations (meyHowiesonsPoort, meyCathcart and meyBastervoetpad) are more variable. meyHowiesonsPoort emerges with the southernmost populations in the plastid topologies, but in the well-sampled clade (clade VII) in the nuclear topologies. Both meyCathcart and the Bastervoetpad samples emerge in the well-sampled clade according to both markers. In contrast, the meyMariepskop population consistently emerges outside of the well-sampled clade in both the ITS and plastid topologies.

There is therefore a definite geographical pattern to the positions of the populations in the analyses. The southernmost populations, which occur in an area in which no other *Streptocarpus* species are found, tend to emerge together and separately from all the other species. However, the *S. meyeri* populations that co-occur with other *Streptocarpus* species tend to group separately from each other and from the southernmost populations. Geographically, the Howieson's Poort population is situated close to a *S. primulifolius* population, the Cathcart population is close to populations of *S. montigena*, *S. rexii* and *S. primulifolius*, and the Bastervoetpad population co-occurs with a *S. rexii* population that was not included in this study. However, the locality where *S. meyeri* is exposed to the most other *Streptocarpus* species is on Mariepskop, where *S. parviflorus*, *S. cyaneus*, *S. micranthus*, *S. wilmsii* and *S. confusus* also occur (Hilliard & Burtt 1971), the latter three of which were not sampled in this study. *S. meyeri* therefore seems to hold its integrity in the absence of other species, but tends to

cluster with geographically proximal species rather than with conspecific populations in areas where it co-occurs with other species.

Morphologically, *S. meyeri* is rather a uniform species. However, the geographically isolated Mariepskop population is morphologically quite different. The leaves tend to grow out from one side of the rosette in this population, giving an uneven appearance. In contrast, the rosettes in the other populations are far neater and more regular (Hilliard & Burtt 1971). Hilliard & Burtt (1971) also suspected some of the phyllomorphs in the Mariepskop population of occasionally perennating. The Mariepskop population is therefore both genetically and morphologically quite different from the rest of the species, and is either an ancient population of *S. meyeri* that has hybridized with some other species, in the process acquiring its aberrant morphology, or this could be a case of convergent evolution. The population occurs at the top of Mariepskop, a high mountain, and the exposure of this population to frost may have led it to evolve characteristics similar to those of the drought tolerant *S. meyeri*.

The rest of the species, however, probably constitutes a single lineage. The southernmost populations appear to have diverged from one another quite a long time ago, based on the relatively long branches present amongst them, especially in the plastid topologies, with only limited subsequent gene flow being evident. The tendency for the central populations to emerge separately from the rest of *S. meyeri* is most likely due to subsequent hybridization. These plants are morphologically similar to the southern populations, and there is no reason to suspect that they arose independently. No place is hybridization more evident than in the Bastervoetpad population. This population was represented by six individuals in the analyses. A comparison of their ITS sequences reveals six polymorphic characters where the sequences either have one base or another base or both of these bases in their consensus ITS sequences. Furthermore, these six individuals are fixed for one of two very different plastid haplotypes. The large number of differences between these two haplotypes and the lack of intermediate haplotypes between them (although only six individuals were assayed) indicates that it is highly unlikely that both of these two haplotypes would have evolved from within the Bastervoetpad population. Neither of these haplotypes group with any of the other *S. meyeri* plastid haplotypes, and they therefore both probably arrived in the population as a result of hybridization with one or more other species. The one haplotype groups weakly with the *S. primulifolius* population from Bulolo Gorge, while the other is identical or very similat to the plastid lineages of the southern *S. primulifolius* populations, the northernmost *S. baudertii* populations and the *S. rexii* populations. *S. rexii* also occurs at Bastervoetpad. As discussed under *S. baudertii*, pollination of an *S. rexii* flower by pollen from another species is likely to result in successful fertilization (in the absence of reproductive barriers) since *S. rexii* is not pollen limited (Trevor Edwards, personal communication), and this Bastervoetpad haplotype therefore originated in *S. rexii*.

S. meyeri therefore appears to be an old South African *Streptocarpus* lineage (although the Mariepskop population might be an evolutionary independent entity) that either evolved before the Cape primrose clade diverged and has subsequently captured a more recent ITS lineage, or evolved more recently and has captured the plastid lineage of an older species. Many of its populations have subsequently hybridized with other species, in the process obscuring the original affinities of the species.

3.4.6.2. *S. montigena*

S. montigena is one of the rosulate species that is represented by more than one population in the present analysis. The species is confined to a single range of mountains between Queenstown and King Williams Town in the middle of the Eastern Cape (Hilliard & Burtt 1971). In all of the analyses, the four samples representing the species (two representatives

from each population) consistently form a strongly supported clade, indicating that *S. montigena* is monophyletic. Furthermore, the sequences generated from the four samples are very similar to one another in both analyses (the ITS sequences are identical, and only one of the samples has a slightly different plastid sequence to the other three samples, which are identical to one another), suggesting that the two populations have only recently diverged from each other. Thus, *S. montigena* is a reasonably homogenous, monophyletic species. However this is as far as agreement between the reconstructions based on the two markers goes, since the *S. montigena* clade emerges within the well-sampled clade in the nuclear trees, but in an unresolved position right at the base of the South African clade in the plastid phylogenies.

Hilliard & Burtt (1971) proposed that *S. montigena* originated as a result of hybridization between *S. meyeri* and *S. rexii*, since it shares a similar leaf rosette and vertical axis with *S. meyeri*, but its flowers are far more similar to that of *S. rexii*. Although *S. montigena* does not group closely with either of these species in any of the analyses, its position in the ITS analyses suggests close links with one of the core Cape primrose taxa, while its position in the less well-sampled parts of the tree in the plastid analyses suggests links outside of the core Cape primrose group. The long length of the branch leading to the *S. montigena* clade in the plastid analyses suggests that the closest relatives of its plastid lineage were not sampled in this study. However, what these closest relative might be is more difficult to say. Only *S. rexii* and *S. meyeri* currently occur in the vicinity (Hilliard & Burtt 1971), and the plastid lineage of *S. montigena* would either have to be from a geographically proximate species that is now extinct, or arrived at its present locality through long-distance seed dispersal.

Thus, the analyses show that *S. montigena* either arose as the result of hybridization between a member of the Cape primrose clade and an as yet unsampled species, or it evolved from the unsampled species, arriving at its current locality through long-distance dispersal, subsequently hybridizing with one of the members of the Cape primrose clade, in the process capturing its ITS and perhaps also acquiring its present floral morphology. The third option is that *S. montigena* evolved from within the Cape primrose clade, subsequently capturing the chloroplast of a more distantly related unsampled species.

3.4.6.3. *S. fanniniae*

Although *S. fanniniae* is classified as a rosulate (Möller & Cronk 2001a), it has a bizarre growth habit. The "typical" rosulate species in the core Cape primrose group carry a number of phyllomorphs arranged in a neat (centric) or rather untidy (excentric) rosette, with no phyllomorph being more dominant than the rest. Their inflorescences are carried on a peduncle that arises from the groove meristem at the base of the lamina. In contrast, *S. fanniniae* produces phyllomorphs that creep along the ground, developing adventitious roots from the underside of their petiolode as they elongate. New phyllomorphs are produced a little below the junction of the petiolode and midrib, which in turn creep along the substrate and give rise to further phyllomorphs. The inflorescences are borne on a series of erect phyllomorphs, and this erect flowering shoot system can reach up to a metre high (Hilliard & Burtt 1971).

Hilliard & Burtt (1971) classified *S. fanniniae* into their subgroup Be along with *S. candidus* and *S. wilmsii* based on their similar floral morphology. These three species bear scented flowers (which is an unusual characteristic in *Streptocarpus*) of more or less the same size and shape, possess similar markings in their flowers and all have appendaged filaments and similar capsules. However, their growth habits are very different—*S. candidus* is a more typical rosulate and *S. wilmsii* is usually a unifoliate. In growth habit, *S. fanniniae* is more similar to species such as *S. bullatus*, *S. masisiensis* and *S. davyi*, which also produce leafy flowering axes (Hilliard & Burtt 1971). However, none of these species, except for *S. fanniniae*, were included in this study, and no more can therefore be said about this species, other than that the nuclear

topologies and its unusual morphology both indicate that it probably is not very closely related to the core Cape primrose clade taxa. *S. fanniniae* occurs from Vryheid and Ngome down to Ixopo in KwaZulu-Natal (Hilliard & Burtt 1971), and therefore overlaps with members of the core Cape primrose group, such as *S. primulifolius*. *S. fanniniae* could therefore have perhaps captured the chloroplast of one of the core species.

3.4.6.4. *S. caeruleus* and *S. longiflorus*

Although these two rosulate species emerge separately in the nuclear tree—*S. caeruleus* sister to *S. gardenii* and *S. longiflorus* within the well-sampled clade—they group together in the plastid phylogeny. Their close relationship according to the plastid marker is more in line with their morphology. They are similar both with regards to vegetative and floral morphology, differing only in number of leaves, the size of their flowers (the length of their peduncles, sepals, filaments, ovaries, styles, and in the length and width of their corollas), floral patterning (two yellow spots or short bars flanking the lower medial lobe in *S. caeruleus* as opposed to a yellow bar that forks in the throat in *S. longiflorus*), in the position of stamen attachment in their flowers, and in the symmetry of their stigmas (Hilliard & Burtt 1971; Edwards *et al*. 1992), and Hilliard & Burtt (1971) regarded them as subspecies within *S. caeruleus*.

3.4.6.5. *S. polyanthus* and *S. saundersii*

S. polyanthus and *S. saundersii* are two of the non-rosulate species included in the analyses. *S. saundersii* is a strict unifoliate, while *S. polyanthus* produces one to three leaves, depending on subspecies (Hilliard & Burtt 1971). These two species tend to group together in both of the analyses, the single *S. saundersii* representative emerging sister to all four *S. polyanthus* populations in the nuclear topologies, but amongst three of the four *S. polyanthus* populations (polShelterFalls emerges in an unresolved position in the well-sampled clade) in the plastid analyses.

Although these two species have different floral and pollen types, *S. polyanthus* producing keyhole flowers and Weigend & Edwards' (1996) type 8 pollen, while *S. saundersii* possesses the open-tubed floral type and pollen type 12, they share a number of other morphological characteristics. Their laminas have a cordate base, crenate margins and a pilose indumentum, with the lower surface of the leaf frequently being reddish in colour. Their peduncles and pedicles are pilose. Their stamens arise half-way up the corolla tube, and their filaments and anthers are usually white. Their styles are terete and pubescent (Hilliard & Burtt 1971). These species appear to belong together according to the topologies reconstructed from both markers, and some morphological characteristics. Furthermore, both their morphology and their position in the nuclear topologies suggest that they do not belong amongst the core Cape primrose taxa, and their emergence within the well-sampled clade in the plastid topologies is consequently either the result of chloroplast capture or the lack of resolution not adequately separating the core Cape primrose taxa from close relatives.

Thus, the boundaries of the Cape primrose clade are not clearly evident from the analyses, since there are a number of taxa that emerge in different groupings depending on the marker used. In the cases in which the molecular markers are incongruent with each other, morphology is not always indicative of relationships, since both floral type and growth habit appear to be rather plastic within the genus. The multiple origins of many of the floral types and growth habits are evident both from mapping these traits onto phylogenetic trees reconstructed from ITS sequence data (Harrison *et al*. 1999; Möller & Cronk 2001a; Hughes *et al*. 2006), and from the fact that a morphological assessment of the genus highlights the lack of a strict correlation between growth habit and floral type (Hilliard & Burtt 1971). The lack of congruence between genetic markers and the plasticity of morphological characters are possibly caused by the same

thing, since hybridization would both confound phylogenetic reconstructions, and would also result in the transfer of morphological characteristics amongst distantly related lineages.

3.4.7. Species not belonging to the Cape primrose clade

There are, however, some South African species included in the analyses that consistently emerged outside of the well-sampled clade, and are therefore not members of the Cape primrose clade. These are *S. dunnii*, *S. denticulatus*, *S. pusillus*, *S. rimicola* and *S. bolusii*. These more distant relatives of the core Cape primrose taxa are all unifoliates and/or plurifoliates and possess a variety of pollen types: *S. dunnii* type 9, *S. pusillus*, *S. rimicola* and *S. bolusii* type 11 and *S. denticulatus* type 13 (Weigend & Edwards 1996). Apart from the fact that *S. dunnii* and *S. denticulatus* group together according to both markers, relationships amongst these species are also largely incongruent between the nuclear and plastid topologies, indicating that hybridization and possibly also incomplete lineage sorting have also played major roles in the evolution of groups in other parts of the genus.

3.4.8. Evolutionary patterns amongst the South African *Streptocarpus* species

The obvious and widespread incongruences between the nuclear and plastid topologies indicate complex evolutionary patterns in the history of *Streptocarpus*. Many of these have been ascribed to hybridization, and with good reason. Hybridization is easily accomplished between many of the species in subgenus *Streptocarpus* under greenhouse conditions. Additionally, *Streptocarpus* species have overlapping ranges, with most forest patches containing multiple species, and individuals that are morphologically intermediate between geographically proximate species have been found in some instances. However, the most convincing evidence of hybridization uncovered in this study is in the Bastervoetpad population of *S. meyeri*. ITS sequences generated from the six individuals analysed contain multiple polymorphic sites, and two highly divergent plastid haplotypes were detected that are unlikely to have arisen in a single population, especially since intermediate haplotypes were also not detected. The frequency of hybridization has probably increased as a result of the evolution of autogamous species such as *S. rexii* (Hughes *et al*. 2005) and *S. caeruleus* (Edwards *et al*. 1992). These species are not pollen-limited, and pollen from other species landing on their stigmas is consequently likely to result in successful hybridization (Trevor Edwards, personal communication).

However, not all of the incongruences have necessarily been caused by hybridization; many are probably the result of incomplete lineage sorting. Incomplete lineage sorting is more commonly cited as a reason for taxa not emerging as monophyletic entities amongst young, closely related species. However, restricted gene flow in many *Streptocarpus* species may prevent lineage sorting from ever running to completion. Lineage sorting starts when a new species arises from a population of an extant species. This population is likely to contain a number of alleles for many of its loci, with the alleles of each locus not necessarily possessing the same history i.e. not all sharing the same most recent common ancestor. Over time, a large number of the alleles in both the more ancestral and in the more recent species will disappear from the respective entities as a result of genetic drift (stochastic lineage sorting) and selection, while the remaining alleles will survive and diversify. These newly diversified alleles will be spread amongst the conspecific populations as a result of a gene flow, while the lack of or very limited amount of genetic exchange between the two species will result in the set of alleles at each locus in the one species diverging from those in the other species over time resulting in complete lineage sorting. Thus, as time passes, the alleles of any given locus in each species are more likely to share a common ancestor that arose after the split between the two species, and conspecific individuals will therefore be genetically more similar to one another than any are to members of the sister species.

However, *Streptocarpus* presents two problems that hinder lineage sorting. Its tendency to hybridize, even amongst distantly related species, results in the introduction of distantly related genetic material into some of the populations. Additionally, most *Streptocarpus* species have highly fragmented distributions caused by the restricted nature of the forest patches in which they occur. If these species do not have agents that disperse their pollen and seeds beyond the boundaries of their own forest patches, then conspecific populations will be more or less isolated from one another, and will consequently not evolve in parallel i.e. the newly arising alleles will not be spread amongst the populations. This has been found to be the case in *S. primulifolius* (Hughes *et al*. 2007), and appears to be the case for many of the other *Streptocarpus* species included in these analyses. If lineage sorting did not run to completion within these species before their populations became isolated from one another, then gene flow will be insufficient to complete lineage sorting once their populations become isolated from one another. However, incomplete lineage sorting is less likely to be a problem for species that have arisen from only one or a few individuals, as is the case in *S. montigena* (that is, if this species did indeed arrive in its present locality via long distance dispersal).

Related to the isolated nature of the populations constituting most of the species is the issue of how indicative are long branches between the sequences generated from conspecific populations of the age of a species. In many places in this discussion the presence of long branches between conspecific populations has been used as an indication that the species is quite old. However, not all of the species can be compared to one another in this way. In species such as *S. primulifolius*, where gene flow amongst populations has been found to be extremely limited due to the limited between-population foraging of its pollinator, the long-tongued nemestrinid fly (Hughes *et al*. 2007), conspecific populations will diverge from one another faster, and the species will therefore appear to be old after a relatively short period of time. In comparison, in species such as *S. dunnii*, which is pollinated by the more mobile malachite sunbird (Vogel 1954; Francois Krige and Michael Möller, personal observation; Hughes *et al*. 2007), gene flow amongst populations is much more frequent, so populations will take comparatively longer to diverge from one another. Thus, in the topologies the branch lengths amongst the *S. dunnii* populations are a little longer than those amongst the *S. primulifolius* populations, but this probably means that *S. dunnii* is much older than *S. primulifolius*. Pollinator data are lacking for almost all *Streptocarpus* species, and seed dispersal data are even more limited, so the degree of gene flow in most species can only be guessed.

Thus, incomplete lineage sorting is likely to present a problem in the reconstruction of evolutionary relationships, not only amongst closely related species, but also potentially at higher taxonomic levels in *Streptocarpus*, and the incongruences between the topologies of this study are probably caused by both hybridization and incomplete lineage sorting.

3.4.9. Historical biogeography of *Streptocarpus* in South Africa

The radiation of *Streptocarpus* in southern Africa must be interpreted in light of the palaeoclimate and historical biogeography of the region in general and also in the context of previous deductions that have been made from phylogenetic reconstructions of evolutionary relationships in *Streptocarpus* in particular.

The historical biogeographical patterns of the flora and fauna of eastern southern Africa were heavily influenced by the increasingly more extreme Pleistocene climatic fluctuations outlined by DeMenocal (2004). One of the most important factors dictating distribution ranges and migration routes of taxa is the distribution of suitable habitat, and forest cover has been found to have fluctuated dramatically in response to climate change. Eeley *et al*. (1999) modelled the effect of the prevailing palaeoclimatic conditions of the Last Glacial Maximum and the

Holocene altithermal on forest cover in KwaZulu-Natal, and came to the conclusion that forest was far more restricted during the colder, drier period of the Last Glacial Maximum, but more extensive during the warmer, wetter Holocene altithermal. Bond *et al*. (2003a, 2003b) highlighted the importance of atmospheric CO_2 concentrations and fire regimes on the extent of forest cover—two factors that were not included in the models used by Eeley *et al*. (1999)—and inferred a lower tree density during the lower CO_2 concentrations of the Last Glacial Maximum. Lawes *et al*. (2007) analysed the historical dispersal routes of certain forest-associated and forest-dependent faunal groups. They uncovered migration routes from tropical East African refugia down the east African coast into the coastal forests of eastern South Africa (an area extending southwards from the Mozambique/KwaZulu-Natal border to the Tugela River mouth, and 200 km inland to the west), from these coastal forests into the neighbouring scarp forests and then into the afrotemperate forests of northern, eastern and southern South Africa after the Last Glacial Maximum. These patterns of faunal migration are probably to a certain extent indicative of forest expansion patterns of the time. Although these studies concentrated on conditions during and following the Last Glacial Maximum, similar conditions predominated during the glacial-interglacial cycles of the past 1 million years (DeMenocal 2004), and these patterns of fluctuating forest cover and faunal migration routes can probably be extrapolated to the previous glacial cycles as well.

Historical biogeographic inferences of *Streptocarpus* as a whole were made by Möller and Cronk (2001b) based on their ITS phylogeny (Möller and Cronk 2001a) and on current distribution ranges of the sampled taxa. They concluded that subgenus *Streptocarpus* originated in eastern central Africa, from where it spread south into southern Africa and South Africa. However, this southward radiation does not appear to have involved a single lineage, but rather occurred in a series of waves, with every wave representing a different phylogenetic unit or clade in the larger phylogeny. On the finer scale, the Hughes *et al*. (2005) analysis of the wide-ranging Pondoland *Streptocarpus* species, *S. primulifolius*, indicated that the deep gorges along the Pondoland coast have served as Pleistocene refugia against the effects of aridification during the dry glacial cycles of this epoch, and allowed the taxa occurring in these gorges to survive and thereafter to radiate during the intervening interglacials.

This background leads us to the question of how these new phylogenetic data on *Streptocarpus* can be interpreted. The incongruences between the reconstructions based on each marker make definite deductions regarding deeper evolutionary relationships and potential historical biogeographical patterns very difficult. However, a comparison of the present-day distributions (Figures 3.1–3.3) of the taxa that group together in the well-sampled clades according to both markers i.e. the core Cape primrose taxa, and the distributions of those taxa that consistently emerge outside of this group i.e. the outgroup taxa, reveals some interesting patterns. The distribution ranges of these outgroup taxa (*S. dunnii*, *S. denticulatus*, *S. pusillus*, *S. rimicola* and *S. bolusii*) range from Engcobo in the northern Eastern Cape to southern Mpumalanga and west to Thabazimbi in the North-West Province. Members of the core Cape primrose group (*S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana*, the *S. cyaneus* complex, *S. floribundus*, *S. aylae* and *S. kentaniensis*) together have a far wider distribution and extend into more southern localities, occurring from the Soutpansberg vicinity in a long arc down through Limpopo, Mpumalanga, Swaziland, KwaZulu-Natal, along the coastal half of the Eastern Cape and into the easternmost extremes of the Western Cape at Knysna.

The lineage containing the core Cape primrose taxa has therefore arisen from amongst the northern South African (and perhaps other southern African) *Streptocarpus* species, and constitutes the most recent southerly radiation of subgenus *Streptocarpus*. However, the birthplace of the group containing these species is more difficult to ascertain. The distribution

ranges of the core Cape primrose taxa with more northern distributions overlap extensively with those of the northern South African species, and this group could therefore have arisen in any of a number of localities. However, nowhere are a larger number of core Cape primrose taxa found than in the Pondoland Centre. Not only is the largest number of species found in this area, but four of the species—*S. modestus*, *S. formosus*, *S. lilliputana* and *S. aylae*—are narrow endemics within this region. Additionally, *S. primulifolius* and *S. johannis*, species with populations occurring both inside and outside of the Pondoland Centre, attain their highest diversity in this area. This area harbours a vast number of endemics (Van Wyk & Smith 2001), not just those in *Streptocarpus*. The gorges of the Pondoland Centre have provided relatively protected habitats during past climatic fluctuations in which species have been able to survive and speciate over long periods of time while species growing in other localities were probably much more prone to extinctions during drier periods. The species that survived the glacial maxima in the protected gorges of the Pondoland Centre would have been able to radiate outwards to occupy the newly formed habitats during more mesic periods. Thus, whether the high species density in the Pondoland Centre indicates that the group containing the core Cape primrose taxa arose in this area, or whether this area contains the highest species numbers due to the protection that it has provided during drier periods when species would have become extinct in other areas, is unclear. The high endemicity of many independent plant groups in the Pondoland Centre indicates that this area is probably an important Pleistocene refugium, a conclusion that has also been reached by other authors (Van Wyk & Smith 2001; Hughes *et al*. 2005), from which many taxa have radiated.

While the birthplace of this group is uncertain, its current geographical extent indicates that it radiated along many of the previous migration routes inferred for faunal and floral lineages. The *S. cyaneus* complex is currently spread across northern KwaZulu-Natal, Swaziland, Mpumalanga and Limpopo. This distribution range closely matches the route followed by faunal lineages studied by Lawes *et al*. (2007), which extended from the coastal forests of South Africa into northern South Africa. However, while Lawes *et al*. (2007) inferred a northerly migration along this route for the faunal lineages that they studied, it is unclear whether *Streptocarpus* species spread north, south or both north and south along this route. *S. primulifolius* appears to have spread from the Pondoland Centre both northwards and southwards along the eastern South African coast, a migration corridor also found in the faunal groups investigated by Lawes *et al*. (2007). The present-day disjunct distribution of *S. johannis* and the evolution of the northern *S. johannis* population cluster from the southern *S. johannis* populations suggest that this species probably followed the migration routes traversed by many other plant taxa between the Pondoland Centre and the KwaZulu-Natal Drakensberg mentioned in Van Wyk & Smith (2001). The evolution of *S. rexii* from the southern *S. primulifolius* populations indicates yet another migration route followed by *Streptocarpus*. Due to the limited dispersal capabilities of some of the species e.g. *S. primulifolius* (Hughes *et al*. 2007), most of these migrations and radiations probably occurred during periods when forest patches were much more continuous e.g. during the Holocene altithermal and similar periods.

However, the evolution of the core Cape primrose taxa group and of other *Streptocarpus* groups has not been entirely dependent on the availability of forest habitats. Some *Streptocarpus* species appear to have shed their dependence on forests and evolved more drought-tolerant characteristics. A good example of this is *S. meyeri*, which occurs in dry habitats amongst rocks. This species has evolved smaller, hairier leaves and a thick axis that enables it to withstand drier conditions. Similarly, *S. baudertii* and *S. montigena* are also more drought-tolerant then their forest-dependent counterparts.

The lineage containing the core Cape primrose taxa therefore appears to constitute the most recent wave in the progressive southerly spread of subgenus *Streptocarpus* inferred by Möller

& Cronk (2001b). The radiation patterns of this group inferred from this study are congruent with historical migration routes followed by other biota. The extensive geographical overlap of this group with more distantly related *Streptocarpus* species has probably provided the opportunity for the prolific hybridization to occur that is probably responsible for the widespread incongruences between the nuclear and plastid topologies.

3.5. Conclusions

This study has contributed to unravelling relationships amongst the South African species of the evolutionary complex and therefore also taxonomically complex plant genus *Streptocarpus*.

Both ITS and plastid sequence data matrices were generated, and these markers were found to be useful at different taxonomic levels. The ITS region appears to have evolved at a faster rate at deeper taxonomic levels, but provides almost no resolution at the specific and population levels, especially within the more extensively sampled clade, due to the delay of gene homogenization (Möller *et al*. 1999). In contrast, the plastid regions have together evolved faster than ITS at lower taxonomic levels, and were therefore more useful in unravelling more recent relationships.

In both the nuclear and plastid topologies, the South African samples formed a clade separate from the two samples collected from outside of the southern African mainland. The dating analyses performed on the ITS and *trn*L-F sequence matrices to estimate the age of this South African clade produced rather divergent dates, but neither of these analyses yielded ages of more than a few million years, indicating that *Streptocarpus* has probably not been present in South Africa for longer than 10 million years.

Although the nuclear and plastid topologies were largely incongruent with each other, many of the species emerging in the Cape primrose clade of Möller & Cronk (2001a) also grouped together in the ITS and plastid analyses of this study, confirming their close relationships. These include *S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. cyaneus* and *S. kentaniensis*, all rosulates. Moreover, a number of rosulate species that were not sampled by Möller & Cronk (2001a) also emerged in the extensively sampled clades in all of the analyses, including *S. gardenii*, *S. lilliputana*, *S. parviflorus*, *S. fenestra-dei*, *S. kunhardtii*, *S. roseo-albus*, *S. floribundus* and *S. aylae*. These species are also palynologically largely homogenous. Weigend & Edwards (1996) classified the pollen of all of these species that they analysed into their type 13, except for that of *S. baudertii* and *S. johannis*, which were assigned pollen types 4 and 8, respectively.

Within this core Cape primrose clade, *S. primulifolius* appears to have played a pivotal role, either being ancestral to many of the species, or having subsequently hybridized with them. The other large group amongst the core taxa is the *S. cyaneus* complex, which appears to be a more isolated group. However, relationships amongst the core Cape primrose taxa remain largely unresolved, since the more extensively sampled clades in both the nuclear and plastid topologies form large polytomies. In the nuclear topology, this is probably in part due to the way in which ITS evolves and the way in which the ITS data were generated, but the fact that the plastid topology also contains a large polytomy suggests that many of the members of the core Cape primrose taxa arose as a result of a sudden burst of speciation, possibly in response to historical patterns of climatic variability. This, along with possible incomplete lineage sorting and hybridization, has hindered the reconstruction of relationships within this group.

Some of the species that emerged amongst the core Cape primrose taxa in the ITS topologies grouped with more distantly related species in the plastid topologies and vice versa, and their positions within the Cape primrose clade are therefore questionable. These are the rosulates

S. meyeri, *S. montigena*, *S. fanniniae*, *S. caeruleus* and *S. longiflorus* and the unifoliates/plurifoliates *S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*. However, pollen type is reasonably consistent with these two morphological groups. The rosulates that have been analysed (*S. meyeri*, *S. montigena*, *S. fanniniae* and *S. caeruleus*) possess Weigend & Edwards' (1996) pollen type 13, as do most of the core Cape primrose taxa, while pollen type amongst these unifoliates/plurifoliates is more diverse, with the species producing types 8 (*S. polyanthus*), 12 (*S. saundersii*, *S. porphyrostachys* and *S. vandeleurii*) and 13 (*S. grandis*). Thus, although neither ITS genetic signal, plastid genetic signal, growth form nor pollen type can be used alone to determine relationships (since many incongruences exist amongst these four data sets), taking all of these character sets into account seems to indicate that most of the above rosulates are probably close relatives of the core Cape primrose taxa, while the unifoliates/plurifoliates (perhaps with the exception of *S. grandis*) are probably more distant relatives of the core Cape primrose taxa. However, the position of *S. grandis* is especially unclear, since it is a unifoliate that possesses pollen type 13. The incongruences amongst ITS, plastid, growth form and pollen type are probably largely due to the lack of reproductive barriers amongst many of the species, which has probably led to many occurrences of distantly related species exchanging genetic material, in the process also capturing morphological characteristics.

A number of the unifoliate/plurifoliate species were, however, found to group outside of the well-sampled clades in both the nuclear and plastid analyses, and are therefore not members of the Cape primrose clade. These are *S. denticulatus*, *S. dunnii*, *S. pusillus*, *S. rimicola* and *S. bolusii*. Pollen type also largely confirms the more distant relationships of these taxa from the core Cape primrose species, with *S. dunnii* producing pollen type 9, *S. pusillus*, *S. rimicola* and *S. bolusii* type 11 and *S. denticulatus* type 13 (Weigend & Edwards 1996).

A comparison of the distribution ranges of the core Cape primrose taxa with those of the species that consistently emerge outside of the well-sampled clades shows that the lineage containing the core Cape primrose taxa constitutes the most recent southerly wave in the progressive southerly radiation of subgenus *Streptocarpus*. The birthplace of this group is uncertain, but its current distribution suggests that it spread along many of the migration routes followed by other floral and faunal taxa, perhaps radiating from areas in the Pondoland Centre of Endemism.

Thus, a number of the South African species appear to be closely related. However, due to the lack of resolution amongst the members of the core Cape primrose group, relationships amongst these species were more difficult to unravel. Additionally, only one or two individuals were sampled per population, so levels of intrapopulation variation were not assessed. These problems could be overcome by sampling more taxa and more individuals per taxon and comparing a larger number of independent data sets, approaches that have been shown to lead to an increase in resolution in many phylogenetic analyses. Nuclear microsatellite markers have been found to be of phylogenetic utility in the *S. primulifolius–S. rexii* lineage (Hughes *et al*. 2005), and an expansion of the use of these markers to include more members of the Cape primrose clade may clarify relationships in this evolutionary young group. This is the approach followed in the next chapter.

Chapter 4: Population genetic studies of selected South African *Streptocarpus* taxa

4.1. Introduction

The sequence analyses of the previous chapter identified 16 species, the core Cape primrose taxa, that are closely related according to both ITS and plastid sequence data. These are *S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana*, the members of the *S. cyaneus* complex (*S. cyaneus*, *S. parviflorus*, *S. fenestradei*, *S. kunhardtii* and *S. roseo-albus*), *S. floribundus*, *S. aylae* and *S. kentaniensis*. However, while these sequence analyses provided some indication of relationships amongst these species, many of these species appear to be the result of recent, rapid radiation, and sequence regions do not tend to evolve at a rapid enough rate within such short time-scales to resolve relationships between them.

Microsatellite regions, on the other hand, tend to evolve at a faster rate than do most other genetic regions, and these markers are very often used in unravelling relationships both within species and amongst closely related species. Hughes *et al*. (2005) investigated the relationships between *S. primulifolius*, *S. formosus* (although only one population of this species was analysed) and *S. rexii* using both sequence and microsatellite data, and found that these species together constitute a single lineage that evolved along the South African coast in the form of *S. primulifolius*, which then spread south, giving rise to *S. rexii* on a number of independent occasions in the East London area before spreading north along an inland route and south along the coast. Additional species that share the most recent evolutionary links with the *S. primulifolius–S. rexii* lineage according to the sequence analyses of the previous chapter were selected for further analyses to provide a fuller picture of evolution within the Cape primrose clade in the Eastern Cape and southern KwaZulu-Natal provinces of South Africa. *S. johannis* and *S. baudertii* populations showed linkages to *S. primulifolius* in both the nuclear and plastid sequence analyses of the previous chapter, and were therefore selected for these finer-scale analyses. In addition, relationships within *S. primulifolius* were investigated further by adding two more populations from the species' northern range. The microsatellite data of Hughes *et al*. (2005) revealed that genetic diversity within *S. primulifolius* tends to decrease in a north to south direction, leading them to the conclusion that *S. primulifolius* evolved towards the north of its range. However, Hughes *et al*. (2005) only included one population from the northern extremities of *S. primulifolius*—the population collected at Stone's Farm—and this population appeared to contain considerably less genetic diversity than the *S. primulifolius* populations a little to the south in the forested gorges of the southern Pondoland Centre (Van Wyk 1990). Microsatellite data from two further populations from the northern extremities of the species' range were therefore generated to investigate this anomaly further. Finally, a population that is suspected of being the result of hybridization was also included to shed more light on its affinities.

The aims of this current microsatellite study were therefore to verify the relationships retrieved by the sequence analyses of the previous chapter and the microsatellite analyses of Hughes *et al*. (2005) with the addition of further populations, to provide further resolution amongst these closely related species, and to investigate relationships within these species using various microsatellite analytical techniques and sequence phylogenies.

4.2. Materials and Methods

4.2.1. DNA extraction and amplification of the microsatellite regions

Seven populations of *S. johannis* representing seven different localities, as well as two additional populations of *S. primulifolius* from the northern extremities of the species' range, were added to the populations already genotyped at the Royal Botanic Gardens Edinburgh (RBGE; Table 4.1 lists population details, and Figure 4.1 shows a map of sample localities). The *S. johannis* populations analysed in the current study include three from the northern part of the species' range (jNsikeni, jHebronRd and jManzimnyama), and four from its southern localities (jMyokane, jEmbotyi, jMagwaFalls, jMtSullivan01). An additional *S. johannis* population from Mount Sullivan, jMtSullivan02, was analysed previously at the RBGE.

For the nine populations genotyped in the current study, DNA was extracted from 18–30 individuals per population as described in Appendix 1. Nine nuclear microsatellite regions described in Hughes *et al*. (2004) were used to screen these populations. These are StrepDN110, StrepCtg16, StrepD14, StrepJH448, StrepPR239, StrepB22, StrepJH432, StrepPR241 and StrepK17. Fluorescently-labelled primers, reagent concentrations and thermal cycling steps were the same as in Hughes *et al*. (2004). Amplified microsatellite products were run on an ABI Prism 3100 36 cm capillary Genetic Analyser (Applied Biosystems, Foster City, USA) with POP 4 polymer. Peak data were analysed in GeneMapper 3.7 (Applied Biosystems, Foster City, USA) and formatted for further analysis using the Microsatellite Toolkit (Park 2001).

4.2.2. Analysis of the microsatellite data

Population structure in the microsatellite data amongst all of the populations was investigated in a number of ways. Population statistics and diversity indices were calculated for all the populations as well as only within taxon groups (the northern *S. primulifolius*, the central *S. primulifolius*, the southern *S. primulifolius*, the *S. rexii*, the *S. johannis* and the *S. baudertii* populations). Genetic distances amongst the populations and amongst the individuals were computed in the form of both chord (Cavalli-Sforza & Edwards 1967) and the proportion of shared alleles (PSA; Bowcock *et al*. 1994) distances, and these distance matrices were used to construct population-level microsatellite trees, and also served as the basis for Principal Co-ordinate (PCo) analyses (Gower 1966). Structure amongst the populations was also investigated using a Bayesian approach.

Genetic Data Analysis (GDA) 1.0 (Lewis & Zaykin 2001) was used to calculate most of the population statistics, including the mean number of individuals sampled/locus (n), the number of polymorphic loci (P_L), mean number of alleles/locus (A), expected heterozygosity (H_E) and observed heterozygosity (H_O). The number of private alleles in each population (Pr_A) set against the data set as a whole, as well as just within each population group was computed in GenExAl 6 (Peakall & Smouse 2006), and Weir & Cockerham's (1984) estimate of the inbreeding coefficient F_{IS} (*f*) was obtained using FSTAT 2.9.3.2 (Goudet 2002). The F_{IS} values were subsequently tested for significance at the 5%, 1% and 0.1% levels while correcting for false positives by means of the Holm's method (Holm 1979), a type of sequential Bonferroni correction.

Genetic distances amongst populations and amongst individuals were calculated in Microsat 2 (Minch *et al*. 1995). Kalinowsky (2002) emphasized the careful selection of the genetic distances used to analyse data, as each one has its own statistical and evolutionary properties that make it more or less appropriate, depending on the circumstances. Two distance measures,

Table 4.1: Populations sampled during the course of this microsatellite study, along with their descriptive statistics. Superscript numbers next to the collection numbers indicate the following: 1: populations that were analysed in the current study; 2: populations analysed at the Royal Botanic Gardens Edinburgh (RBGE) and included in Hughes *et al*. (2005); 3: populations analysed at the RBGE, but which have not yet been included in any publications. n = mean number of individuals sampled/locus; P_L = number of polymorphic loci; A = mean number of alleles/locus; Pr_A = number of private alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; *f* = estimate of the inbreeding coefficient F_{IS}. The number of private alleles was calculated across all the populations (the top value in black font), as well as within each population group (the bottom value in colour). NS = not significant, * = significant at the 5% level and ** = significant at the 1% level. None of the F_{IS} values were found to be significant at the 0.1% level.

Taxon name	Collection number	Locality	Latitude	Longitude	Abbreviated population names	Number sampled	n	P_L	A	Pr_A	H_E	H_O	*f*
S. primulifolius Gand.	TJE s.n.[1]	Table Mt. , KwaZulu-Natal, S.A.	-29.6000	30.5833	npTableMountain	21	20.38	8 (89%)	3.75	1 (3%) 14 (41%)	0.38	0.28	0.265 **
S. primulifolius	TJE 3137[1]	Monteseel, KwaZulu-Natal, S.A.	-29.7333	30.6833	npMonteseel	21	20.38	5 (56%)	3.00	1 (4%) 6 (22%)	0.39	0.18	0.536 **
S. primulifolius	MH 1052[2]	Stone's Farm, KwaZulu-Natal, S.A.	-29.7654	30.6402	npStonesFarm	28	24.78	5 (56%)	2.67	2 (8%) 9 (37%)	0.27	0.21	0.236 **
Mean						**23.3**	**21.84**	**6.0 (66.7%)**	**3.14**	**1.3 (5%) 9.7 (34%)**	**0.35**	**0.22**	**0.346**
S. primulifolius	MH 1088[2]	Rooivaal Farm, KwaZulu-Natal, S.A.	-30.5876	29.9549	cpRooivaal	23	19.00	9 (100%)	3.89	0 (0%) 13 (37%)	0.45	0.49	-0.079 NS
S. primulifolius	MH 1126[2]	Endliniyokozi, Eastern Cape, S.A.	-31.5198	29.6731	cpEndliniyokozi	20	18.00	9 (100%)	4.67	5 (12%) 8 (19%)	0.56	0.47	0.160 **
S. primulifolius	MH 1135[2]	Bulolo Gorge, Eastern Cape, S.A.	-31.5573	29.5143	cpBululoGorge	28	21.56	9 (100%)	5.00	4 (9%) 4 (9%)	0.56	0.43	0.238 **
S. primulifolius	DUB 0635[2]	Mount Sullivan, Eastern Cape, S.A.	-31.5971	29.5298	cpMtSullivan	32	30.67	9 (100%)	4.89	5 (11%) 11 (25%)	0.61	0.57	0.067 NS
S. primulifolius	DUB 0587[2]	Dwalana Forest, Eastern Cape, S.A.	-31.6046	29.4658	cpDwalana	22	18.22	9 (100%)	4.56	4 (10%) 8 (19%)	0.55	0.40	0.283 **
S. primulifolius	MH 1134[2]	Silaka, Eastern Cape, S.A.	-31.6493	29.5055	cpSilaka	15	13.56	8 (89%)	3.67	3 (9%) 5 (15%)	0.44	0.36	0.192 *
S. primulifolius	MH 1139[2]	Xibeni, Eastern Cape, S.A.	-32.0063	28.6558	cpXibeni	24	20.56	9 (100%)	6.00	6 (11%) 16 (30%)	0.52	0.47	0.110 *
S. primulifolius	MH 1140[2]	Mbanyana Falls, Eastern Cape, S.A.	-32.2181	28.9048	cpMbanyana	24	21.89	8 (89%)	5.67	2 (4%) 13 (25%)	0.57	0.49	0.135 *
Mean						**23.5**	**20.43**	**8.8 (97.8%)**	**4.79**	**3.6 (8%) 9.8 (23%)**	**0.53**	**0.46**	**0.138**
S. primulifolius	MH 1145[3]	Reservoir Kloof, Wedgeley, Eastern	-32.2673	27.5690	spResKloof[1]	24	22.11	7 (78%)	2.56	0 (0%) 3 (13%)	0.36	0.25	0.298 **

[1] Based on the intermediate morphology and geographical position between *S. primulifolius* and *S. rexii* of the populations in the East London area, these intermediate populations were together referred to as *S.* cf. *primulifolius* in Hughes *et al*. (2005). However, the type specimen of *S. primulifolius* was collected at Floradale near East London, and these populations are therefore rather referred to as the "southern *S. primulifolius* populations" in this study.

Taxon name	Collection number	Locality	Latitude	Longitude	Abbreviated population names	Number sampled	n	P_L	A	Pr_A	H_E	H_O	*f*
		Cape, S.A.											
S. primulifolius	MH 1161[2]	Moonstone Forest, Wedgeley, Eastern Cape, S.A.	-32.2673	27.5690	spWedgeley[1]	15	14.13	6 (67%)	2.63	1 (4%) 3 (13%)	0.29	0.29	0.016 NS
S. primulifolius	MH 1143[2]	Post Wellington Farm, Eastern Cape, S.A.	-32.6288	27.7256	spPostWellington[1]	28	24.44	8 (89%)	2.67	1 (4%) 4 (17%)	0.33	0.20	0.399 **
S. primulifolius	MH 1157[2]	Floradale Nursery, Eastern Cape, S.A.	-32.9406	27.9222	spFloradale[1]	15	14.56	8 (89%)	2.67	1 (4%) 2 (8%)	0.36	0.24	0.361 **
S. primulifolius	MH 1155[2]	Igoda River mounth, Eastern Cape, S.A.	-33.0931	27.7704	spIgoda[1]	16	15.56	6 (67%)	2.11	0 (0%) 1 (5%)	0.24	0.21	0.145 NS
S. primulifolius	MH 1174[2]	Rivendell Farm, Eastern Cape, S.A.	-33.3560	26.5055	spRivendell[1]	20	18.67	8 (89%)	3.44	1 (3%) 9 (29%)	0.43	0.29	0.335 **
Mean						**19.7**	**18.24**	**7.2 (80.0%)**	**2.68**	**0.7 (3%) 3.7 (15%)**	**0.34**	**0.25**	**0.259**
S. rexii (Hook.) Lindl.	MH 1066[2]	Otterspoort, KwaZulu-Natal, S.A.	-30.4583	28.9454	rOtterspoort	13	11.89	0 (0%)	1.00	0 (0%) 0 (0%)	0.00	0.00	NA
S. rexii	MH 1100[2]	Mount Ayliff, Eastern Cape, S.A.	-30.8467	29.4045	rMtAyliff	16	15.22	1 (11%)	1.11	0 (0%) 1 (10%)	0.01	0.01	0.000 NS
S. rexii	MH 1081[2]	Ntywenka, Eastern Cape, S.A.	-31.1702	28.5810	rNtywenka	16	15.89	3 (33%)	1.44	0 (0%) 1 (8%)	0.12	0.06	0.472 *
S. rexii	MH 1171[2]	Katberg Pass, Eastern Cape, S.A.	-32.4619	26.6584	rKatberg	19	18.00	3 (33%)	1.44	0 (0%) 3 (23%)	0.14	0.07	0.496 **
S. rexii	MH 1149[2]	Kologha, Eastern Cape, S.A.	-32.5377	27.3414	rKologha	24	21.33	4 (44%)	1.56	0 (0%) 1 (7%)	0.14	0.09	0.417 *
S. rexii	DUB 0521[2]	Leopard Falls, Stutterheim, Eastern Cape, S.A.	-32.5586	27.3152	rLeopardFalls	24	21.56	7 (78%)	2.44	0 (0%) 6 (27%)	0.28	0.24	0.145 NS
S. rexii	MH 1176[2]	Van Stadens River Gorge, Eastern Cape, S.A.	-33.9100	25.1939	rVSRG	16	14.78	0 (0%)	1.00	1 (11%) 1 (11%)	0.00	0.00	NA
S. rexii	MH 1180[2]	Tsitsikamma, Eastern Cape, S.A.	-34.0202	23.9049	rTsitsikamma	6	5.25	2 (22%)	1.38	0 (0%) 2 (16%)	0.11	0.05	0.560 NS
S. rexii	MH 1181[2]	Bloukrans Pass, Western Cape, S.A.	-33.9480	23.6267	rBloukrans	12	9.75	1 (11%)	1.13	0 (0%) 1 (10%)	0.02	0.00	1.000 NS
S. rexii	MH 1186[3]	Wilderness National Park, Western Cape, S.A.	-33.9833	22.6106	rWilderness	1	1.00	0 (0%)	1.00	0 (0%) 0 (0%)	0.00	0.00	NA
Mean						**14.7**	**13.47**	**2.1 (23.3%)**	**1.35**	**0.1 (1%) 1.6 (13%)**	**0.08**	**0.05**	**0.441**
S. formosus	TJE 2167[2]	Umtamvuna, KwaZulu-	-31.0020	30.1730	fUmtamvuna	22	17.89	8	5.22	6 (13%)	0.46	0.40	0.130 *

Taxon name	Collection number	Locality	Latitude	Longitude	Abbreviated population names	Number sampled	n	P_L	A	Pr_A	H_E	H_O	*f*
(Hilliard & B.L.Burtt) T.J.Edwards		Natal, S.A.						(89%)					
hybrid *S. meyeri* × southern *S. primulifolius*	MH 1160[3]	Wedgeley, Eastern Cape, S.A.	-32.2554	27.5704	hWedgeley	6	5.38	8 (89%)	4.50	4 (10%)	0.75	0.49	0.367 **
S. johannis L.L.Britten	DUB 0546[1]	Nsikeni, KwaZulu-Natal, S.A.	-30.1351	29.5495	jNsikeni	24	19.33	7 (78%)	2.22	2 (10%) 4 (20%)	0.28	0.14	0.825 **
S. johannis	DUB 0714[1]	Hebron Road, KwaZulu-Natal, S.A.	-30.4006	29.5751	jHebronRd	23	21.63	5 (56%)	2.00	1 (6%) 3 (17%)	0.23	0.12	0.480 **
S. johannis	DUB 0226[1]	Manzimnyama River, KwaZulu-Natal, S.A.	-30.6116	29.6292	jManzimnyama	25	21.56	2 (22%)	1.22	0 (0%) 1 (9%)	0.04	0.01	0.674 **
Mean						**24.0**	**20.84**	**4.7 (52.2%)**	**1.81**	**1.0 (6%)** **2.7 (16%)**	**0.18**	**0.09**	**0.660**
S. johannis	DUB 0139, 0836 & 0915[1]	Myokane, Eastern Cape, S.A.	-31.4432	29.7632	jMyokane	30	26.50	7 (78%)	4.38	0 (0%) 9 (23%)	0.36	0.14	0.623 **
S. johannis	DUB 0593[1]	Magwa Falls, Eastern Cape, S.A.	-31.4478	29.6394	jMagwaFalls	19	16.78	7 (78%)	3.33	2 (7%) 8 (27%)	0.44	0.36	0.183 **
S. johannis	DUB 0840[1]	Embotyi, Eastern Cape, S.A.	-31.4500	29.7250	jEmbotyi	22	18.33	8 (89%)	4.33	4 (10%) 13 (33%)	0.48	0.32	0.337 **
S. johannis	DUB 0637[1]	Mount Sullivan, Eastern Cape, S.A.	-31.6038	29.5370	jMtSullivan01	18	13.78	7 (78%)	3.44	2 (6%) 3 (10%)	0.51	0.30	0.430 **
S. johannis	DUB 0633[3]	Mount Sullivan, Eastern Cape, S.A.	-31.5988	29.5409	jMtSullivan02	22	20.88	8 (89%)	4.88	4 (9%) 13 (30%)	0.55	0.39	0.301 **
Mean						**22.2**	**19.25**	**7.4 (82.2%)**	**4.07**	**2.4 (7%)** **9.2 (25%)**	**0.47**	**0.30**	**0.375**
S. baudertii L.L.Britten	MH 1065[3]	Harmony, KwaZulu-Natal, S.A.	-30.3818	28.8935	bHarmony	24	22.57	5 (56%)	2.71	2 (8%) 8 (33%)	0.32	0.30	0.073 NS
S. baudertii	MH 1067[3]	Otterspoort, KwaZulu-Natal, S.A.	-30.4583	28.9454	bOtterspoort	16	15.13	6 (67%)	2.38	2 (9%) 7 (33%)	0.30	0.28	0.056 NS
S. baudertii	MH 1080[3]	Ntywenka, Eastern Cape, S.A.	-31.1623	28.5729	bNtywenka	9	9.00	5 (56%)	2.00	3 (17%) 9 (50%)	0.28	0.25	0.117 NS
S. baudertii	Ernst van Jaarsveld s.n.[3]	Collywobbles, Eastern Cape, S.A.	-32.0500	28.5833	bCollywobbles	6	6.00	7 (78%)	3.29	2 (7%) 12 (41%)	0.47	0.45	0.031 NS
S. baudertii	MH 1156[3]	Hillsdrift Farm, Eastern Cape, S.A.	-32.8821	28.0418	bHillsdrift	11	10.13	8 (89%)	4.50	8 (20%) 26 (64%)	0.55	0.37	0.345 **
Mean						**13.2**	**12.56**	**6.2 (68.9%)**	**2.98**	**3.4 (13%)** **12.4 (46%)**	**0.38**	**0.33**	**0.124**

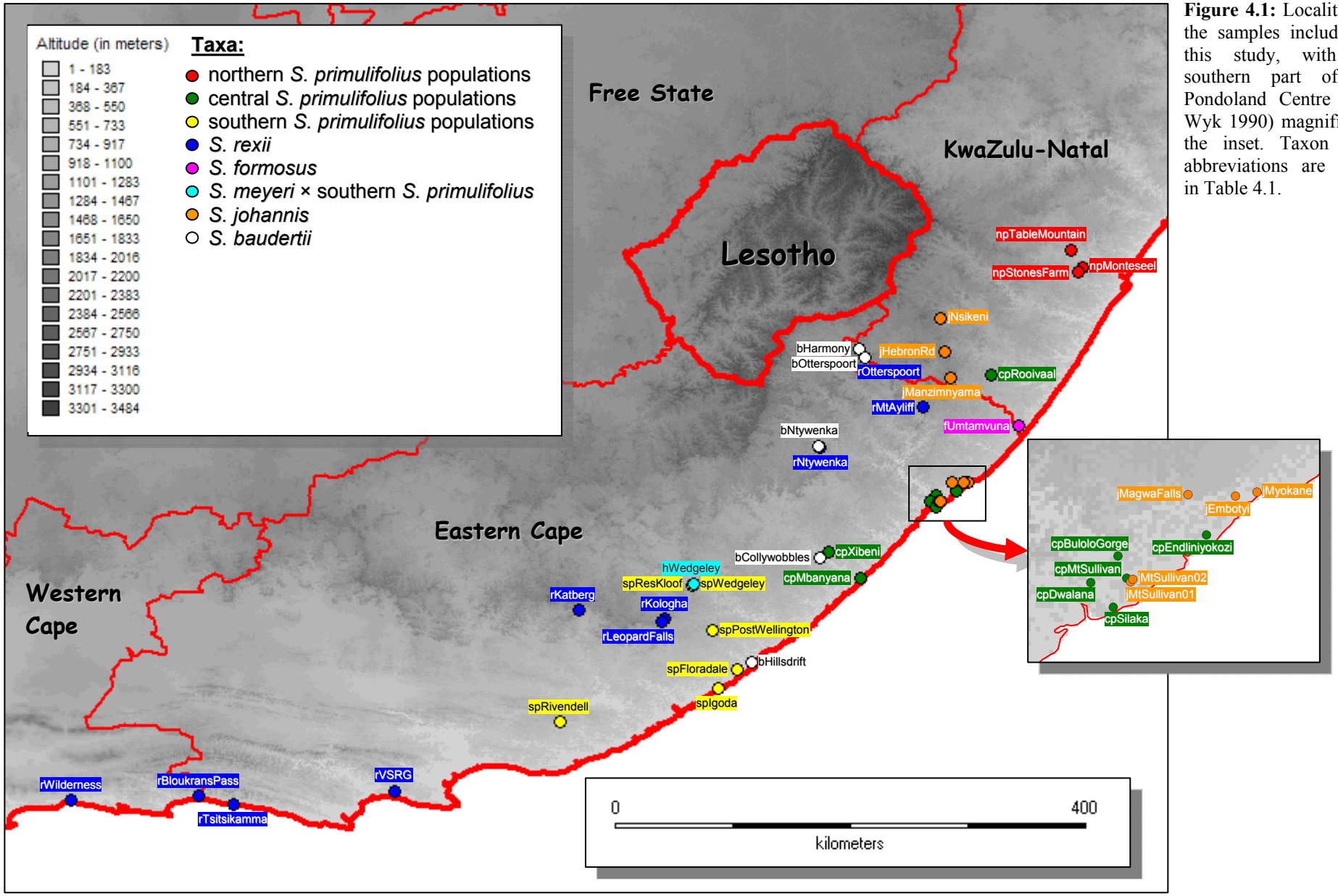

Figure 4.1: Localities of the samples included in this study, with the southern part of the Pondoland Centre (Van Wyk 1990) magnified in the inset. Taxon label abbreviations are given in Table 4.1.

which were also used by Hughes *et al*. (2005)—chord and PSA—were therefore calculated to see the effect of different distance measures on the results and so that the results from this study could be compared with those of Hughes *et al*. (2005). Chord distance is a measure that determines the straight-line distances amongst entities (populations or individuals) if the entities are converted to theoretical points on the surface of a hypersphere (the higher dimensional analogue of a sphere). The position of each entity on the hypersphere is determined by vectors representing the alleles present and relative allele frequencies of that entity. For diploid organisms, PSA is calculated as the number of shared alleles across all the loci divided by 2n, where n is the number of loci included in the analysis. This amount is then subtracted from 1 to give a genetic distance. The resulting population-level distance matrices were used to construct neighbour-joining distance trees of the populations, while the population- and individual-level distance matrices were input into PCo analyses.

The neighbour-joining population trees and majority-rule bootstrap consensus trees were built from the population-level distance matrices of chord and PSA distances calculated in Microsat. The trees were constructed and edited with the aid of Neighbor, Drawgram, Drawtree and Consense, programs distributed as part of the Phylip package (version 3.67; Felsenstein 2004). For the bootstrap (BS) analyses, 100 BS replicates were performed in each case. A BS value of less than 50% was considered as weak support, BS values from 50% to 74% as moderate support, and values equal to and greater than 75% as strong support.

Structure in the data was also investigated by PCo analyses of the population- and individual-level distance matrices. PCo analysis is a method for reducing the dimensionality of data, while preserving as much of the original information as possible. Consequently, plotting the results of a PCo analysis presents a convenient way of visualising more of the total variance contained in the data on fewer axes than would be possible if one were to try to plot the original data, thereby facilitating the identification of groups of similar entities. PCo analyses were performed on the four distance matrices i.e. chord and PSA distances amongst populations and individuals, using the PCOORD module of the R Package 3 (Legendre & Vaudor 1991). For each of the four analyses, graphs of the eigenvalues of successive axes were built to visualise the proportion of the total variance displayed on successive axes, and three-dimensional graphs were then constructed showing the first three axes i.e. those axes that contain the largest proportion of the total variance of the data.

Population structure, both amongst all of the populations and only amongst the eight *S. johannis* populations, was investigated further in Structure 2.2 (Falush *et al*. 2007), a program for identifying clusters (K) of related individuals within a data set using a model-based Bayesian approach. Data are analysed in Structure under sets of explicit parameter specifications, and the program then reports the posterior probability of the data being produced by populations that have evolved in accordance with the parameters specified. The parameter specifications of runs yielding the highest posterior probabilities are therefore assumed to most closely reflect the actual evolutionary history of the populations included in the analysis.

Parameter specifications come in the form of alternative models that specify the extent of gene flow that has occurred in the past amongst the extant populations, and the degree of consistency of allele frequencies across the populations. Ancestry models include the admixture model and the no-admixture model. The admixture model makes allowances for individuals with mixed ancestry by assuming that each individual has inherited a portion of its genome from the ancestors of other clusters. The program outputs posterior mean estimates of these proportions for each individual. The no-admixture model assumes that each individual is entirely from only one of the clusters i.e. not of mixed ancestry. The program output therefore reports the probability of each individual belonging to each cluster (Pritchard *et al*. 2007).

Two alternative allele frequency models are available. The correlated-allele-frequencies model estimates the allele frequencies of a hypothetical ancestral population, and assumes that the K clusters containing the individuals in the data set have each undergone independent genetic drift from these ancestral allele frequencies. The allele frequencies for the K clusters are therefore considered likely to be quite similar to one another under this model. Conversely, the independent-allele-frequencies model assumes that allele frequencies of the clusters are completely independent of one another (Pritchard *et al*. 2007).

All four combinations of these models—admixed ancestry & correlated allele frequencies (AC), admixed ancestry & independent allele frequencies (AI), no admixture & correlated allele frequencies (NC) and no admixture & independent allele frequencies (NI)—were applied to the data sets run in the program. Runs of 100 000 generations (a burn-in period of 50 000 generations and 50 000 Markov chain Monte Carlo (MCMC) repetitions after the burn-in period) were conducted, with proposals for the number of clusters ranging from 1 to the number of populations included in the runs. Fifteen runs were executed for each proposed K, so that the variance amongst runs at each K could be assessed. Further runs were conducted on data belonging to clusters identified by Structure that were consistent across runs i.e. clusters that were always assigned the same populations, in order to identify substructure amongst the populations (Structure only detects structure at one hierarchical level at a time).

A key application of the Structure algorithms is to identify the number of clusters contained in the data. The number of clusters is often chosen by selecting the K that is assigned the highest estimate of the posterior probability (called Ln P(D) in Structure) by the program. However, by testing the ability of Structure to identify the correct number of clusters using simulated data, Evanno *et al*. (2005) found that this method of determining the number of clusters from the program's output does not always produce the correct answer. Evanno *et al*. (2005) found that once the Structure algorithm passes the true value of K, the Ln P(D)s of successively larger values of K begin to plateau i.e. they either level out or continue to increase at a reduced rate. Additionally, they found that the variance of Ln P(D) amongst runs for each value of K increases beyond the true value. This led them to develop a method for producing a more accurate estimation of K by taking the double derivative of the average Ln P(D) with respect to K (this measures the rate of the rate of change [acceleration] of Ln P(D) for successive values of K), and dividing this by the standard deviation of Ln P(D) across runs for each K. This produces a value, ΔK, for all but the first and last Ks. The K possessing the largest ΔK value represents the smallest number of clusters that accounts for most of the structure in the data, and was found by Evanno *et al*. (2005) to be an accurate method of estimating the true K. This method was therefore used in the current analyses to identify the number of clusters constituting the data sets.

4.2.3. Amplification and analysis of the sequence data

In addition to these microsatellite analyses, nuclear ITS and plastid sequence phylogenies were constructed including only sequences from the populations assayed in the microsatellite analyses (Table 4.2 provides details of the samples used). *S. dunnii* emerged outside of the clade into which all of these samples emerged in both the ITS (Figures 3.5, 3.9a & 3.10a) and plastid (Figures 3.7, 3.9b & 3.10b) topologies of the previous chapter, and one of the representatives of this species was therefore used to root the current phylogenetic trees. PCR amplification, sequencing, sequence alignment, maximum parsimony analyses and BS analyses were carried out as described in section 3.2 of the previous chapter. A BS value of less than 50% was considered as weak support, BS values from 50% to 74% as moderate support, and values equal to and greater than 75% as strong support.

Table 4.2: Specimens included in the phylogenetic analyses of the nuclear and plastid sequence data. Abbreviations are as follows: DUB = Dirk Bellstedt; MH = Mark Hughes; TJE = Trevor Edwards; Mt. = Mountain; S.A. = South Africa. A ✓ indicates successful amplification, and the letter in superscript next to the ✓ indicates who sequenced the sample: a = I generated the sequences; b = samples were sequenced at the Royal Botanic Gardens Edinburgh (RBGE); c = I supplemented sequences that had already been amplified at the RBGE; d = Benny Bytebier and Dirk Bellstedt generated the sequences. For the samples in which I supplemented already-existing sequences, the number of base pairs that I contributed to the parts of the sequences that were included in the analyses is indicated in parentheses after the letters. Images of representative taxa are included in Appendix B.

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
S. baudertii L.L.Britten	MH 1065	Harmony, KwaZulu-Natal, S.A.	bHarmony	1	-30.3818	28.8935	✓a	✓a	✓b	✓b + c(108)
S. baudertii	MH 1067	Otterspoort, KwaZulu-Natal, S.A.	bOtterspoort	1	-30.4583	28.9454	✓b	✓a	✓b	✓b + c(981)
S. baudertii	MH 1080	Ntywenka, Eastern Cape, S.A.	bNtywenka	1	-31.1623	28.5729	✓b	✓a	✓b + c(608)	✓b + c(659)
S. baudertii	Ernst van Jaarsveld s.n.	Collywobbles, Eastern Cape, S.A.	bCollywobbles	1	-32.0500	28.5833	✓b	✓a	✓b + c(229)	✓b + c(135)
S. baudertii	MH 1156	Hillsdrift Farm, Eastern Cape, S.A.	bHillsdrift	1	-32.8821	28.0418	✓b	✓a	✓b	✓b + c(764)
S. dunnii	MH 1371	Angle Station, Mpumalanga, S.A.	dunAngleSt	1	-25.8691	31.0882	✓a	✓a	✓a	✓a
S. formosus (Hilliard & B.L.Burtt) T.J.Edwards	TJE 2167	Umtamvuna, KwaZulu-Natal, S.A.	fUmtamvuna	1	-31.0020	30.1730	✓b	✓a	✓b	✓b & c(749)
hybrid *S. meyeri* × southern *S. primulifolius*	MH 1160	Wedgeley, Eastern Cape, S.A.	hWedgeley	1	-32.2554	27.5704	✓a	✓a	✓b	✓b + c(792)
S. johannis L.L.Britten	DUB 0546	Nsikeni, KwaZulu-Natal, S.A.	jNsikeni	1 (ITS); 2 (plastid)	-30.1351	29.5495	✓d	✓a	✓a	✓a
S. johannis	DUB 0714	Hebron Road, KwaZulu-Natal, S.A.	jHebronRd	1 (ITS); 2 (plastid)	-30.4006	29.5751	✓d	✓a	✓a	✓a
S. johannis	DUB 0226	Manzimnyama River, KwaZulu-Natal, S.A.	jManzimnyama	1 (ITS); 2 (plastid)	-30.6116	29.6292	✓d	✓a	✓a	✓a
S. johannis	DUB 0915	Myokane, Eastern Cape, S.A.	jMyokane	1 (ITS); 2 (plastid)	-31.4432	29.7632	✓d	✓a	✓a	✓a
S. johannis	DUB 0593	Magwa Falls, Eastern Cape, S.A.	jMagwaFalls	1 (ITS); 2 (plastid)	-31.4478	29.6394	✓d	✓a	✓a	✓a
S. johannis	DUB 0840	Embotyi, Eastern Cape, S.A.	jEmbotyi	1 (ITS); 2 (plastid)	-31.4500	29.7250	✓a	✓a	✓a	✓a
S. johannis	DUB 0637	Mount Sullivan, Eastern Cape, S.A.	jMtSullivan01	1 (ITS); 2 (plastid)	-31.6038	29.5370	✓d	✓a	✓a	✓a
S. primulifolius Gand.	TJE s.n.	Table Mt. , KwaZulu-Natal, S.A.	npTableMountain	1	-29.6000	30.5833	✓a	✓a	✓a	✓a
S. primulifolius	TJE 3137	Monteseel, KwaZulu-Natal, S.A.	npMonteseel	1	-29.7333	30.6833	✓a	✓a	✓a	✓a
S. primulifolius	MH 1052	Stone's Farm, KwaZulu-Natal, S.A.	npStonesFarm	1	-29.7654	30.6402	✓b	✓a	✓b	✓b & c(74)
S. primulifolius	MH 1088	Rooivaal Farm, KwaZulu-Natal, S.A.	cpRooivaal	1	-30.5876	29.9549	✓b	✓a	✓b	✓b & c(770)
S. primulifolius	MH 1126	Endliniyokozi, Eastern Cape, S.A.	cpEndliniyokozi	1	-31.5198	29.6731	✓b	✓a	✓b	✓b & c(650)
S. primulifolius	MH 1135	Bulolo Gorge, Eastern Cape, S.A.	cpBulooGorge	1	-31.5573	29.5143	✓b	✓a	✓b & c(24)	✓b & c(624)

Taxon name	Collection number	Locality	Abbreviated population names	Number sampled	Latitude	Longitude	ITS	*trn*L-F	*rpl*20-*rps*12	*trn*C-D
S. primulifolius	DUB 0635	Mount Sullivan, Eastern Cape, S.A.	cpMtSullivan	1	-31.5971	29.5298	✓[b]	✓[a]	✓[b]	✓[b] & c(84)
S. primulifolius	DUB 0587	Dwalana Forest, Eastern Cape, S.A.	cpDwalana	1	-31.6046	29.4658	✓[b]	✓[a]	✓[b]	✓[b] & c(849)
S. primulifolius	MH 1134	Silaka, Eastern Cape, S.A.	cpSilaka	1	-31.6493	29.5055	✓[b]	✓[a]	✓[b]	✓[b] & c(718)
S. primulifolius	MH 1139	Xibeni, Eastern Cape, S.A.	cpXibeni	1	-32.0063	28.6558	✓[b]	✓[a]	✓[b] & c(195)	✓[b] & c(792)
S. primulifolius	MH 1140	Mbanyana Falls, Eastern Cape, S.A.	cpMbanyana	1	-32.2181	28.9048	✓[b]	✓[a]	✓[b] & c(204)	✓[b] & c(817)
S. primulifolius	MH 1161 & 1162	Moonstone Forest, Wedgeley, Eastern Cape, S.A.	spWedgeley[2]	1	-32.2673	27.5690	✓[a]	✓[a]	✓[b] & c(30)	✓[b] & c(761)
S. primulifolius	MH 1143	Post Wellington Farm, Eastern Cape, S.A.	spPostWellington[2]	1	-32.6288	27.7256	✓[b]	✓[a]	✓[b] & c(102)	✓[b] & c(741)
S. primulifolius	MH 1157	Floradale Nursery, Eastern Cape, S.A.	spFloradale[2]	1	-32.9406	27.9222	✓[a]	✓[a]	✓[b] & c(79)	✓[b] & c(847)
S. primulifolius	MH 1155	Igoda River Mouth, Eastern Cape, S.A.	spIgoda[2]	1	-33.0931	27.7704	✓[a]	✓[a]	✓[b]	✓[b] & c(109)
S. primulifolius	MH 1174	Rivendell Farm, Eastern Cape, S.A.	spRivendell[2]	1	-33.3560	26.5055	✓[b]	✓[a]	✓[b]	✓[b] & c(757)
S. rexii (Hook.) Lindl.	MH 1066	Otterspoort, KwaZulu-Natal, S.A.	rOtterspoort	1	-30.4583	28.9454	✓[b]	✓[a]	✓[b] & c(580)	✓[b] & c(557)
S. rexii	MH 1100	Mount Ayliff, Eastern Cape, S.A.	rMtAyliff	1	-30.8467	29.4045	✓[a]	✓[a]	✓[a]	✓[b] & c(1155)
S. rexii	MH 1075 & 1081	Ntywenka, Eastern Cape, S.A.	rNtywenka	1	-31.1702	28.5810	✓[b]	✓[a]	✓[b] & c(610)	✓[b] & c(828)
S. rexii	MH 1170 & 1171	Katberg Pass, Eastern Cape, S.A.	rKatberg	1	-32.4619	26.6584	✓[b]	✓[a]	✓[b]	✓[b] & c(702)
S. rexii	MH 1149	Kologha, Eastern Cape, S.A.	rKologha	1	-32.5377	27.3414	✓[a]	✓[a]	✓[b]	✓[b] & c(471)
S. rexii	DUB 0521	Leopard Falls, Stutterheim, Eastern Cape, S.A.	rLeopardFalls	1	-32.5586	27.3152	✓[a]	✓[a]	✓[b] & c(81)	✓[b] & c(704)
S. rexii	MH 1176	Van Stadens River Gorge, Eastern Cape, S.A.	rVSRG	1	-33.9100	25.1939	✓[a]	✓[a]	✓[b] & c(279)	✓[b] & c(678)
S. rexii	MH 1181	Bloukrans Pass, Western Cape, S.A.	rBloukransPass	1	-33.9480	23.6267	✓[b]	✓[a]	✓[b] & c(120)	✓[b] & c(555)

[2] Based on the intermediate morphology and geographical position between *S. primulifolius* and *S. rexii* of the populations in the East London area, these intermediate populations were together referred to as *S.* cf. *primulifolius* in Hughes *et al*. (2005). However, the type specimen of *S. primulifolius* was collected at Floradale near East London, and these populations are therefore rather referred to as the "southern *S. primulifolius* populations" in this study.

4.3. Results

4.3.1. Amplification of the sequence and microsatellite regions

The nuclear ITS and three plastid regions amplified successfully in all of the samples included in these sequence analyses. In contrast, amplification of the microsatellite regions in the populations added to the ones genotyped at RBGE i.e. the seven *S. johannis* populations and two additional *S. primulifolius* populations from the northern part of the species' range, was more variable. Of the nine loci originally amplified, three were problematic within *S. johannis*. StrepPR239 and StrepB22 tended to be more successful in individuals from the northern *S. johannis* populations than in individuals from the southern *S. johannis* populations. Conversely, StrepPR241 worked well in the southern populations, but failed to amplify in almost all of the samples from the northern populations. StrepPR241 also did not work in the two northern *S. primulifolius* populations analysed in the current study. The selective amplification of the primer pairs amongst the populations is probably the result of mutations in the primer recognition regions, in itself hinting at certain relationships amongst the populations.

4.3.2. Descriptive statistics of the microsatellite data

The population statistics and diversity indices calculated from all nine microsatellite loci for the populations are given in Table 4.1. The statistics calculated are similar to those calculated by Hughes *et al*. (2005), taking into account that the addition of extra populations to the data set has affected the number of private alleles values.

The central *S. primulifolius* populations (cpRooivaal–cpMbanyana) constitute the population group with the greatest genetic diversity. It possesses the highest mean number of polymorphic loci (8.8; all the loci are polymorphic in six out of the eight populations in this group), the highest mean number of alleles/locus (4.79), the largest mean number of private alleles per population set against the rest of the data set (3.6), and the highest mean expected (0.53) and observed (0.46) heterozygosities compared with the means of the other population groups (the northern *S. primulifolius*, the southern *S. primulifolius*, the *S. rexii*, the *S. johannis* and the *S. baudertii* populations). The number of private alleles per population within the central *S. primulifolius* populations versus the number of private alleles per population set against all the populations in the data set are much more divergent from each other for the peripheral populations (cpRooivaal to the north and cpXibeni and cpMbanyana to the south) than is the case for the populations in the southern Pondoland Centre (cpEndliniyokozi–cpSilaka). This indicates that the peripheral populations share many more alleles with populations from the other groups than do the Pondoland Centre populations. *S. formosus* also appears to possess high levels of genetic diversity, although this species is only represented by one population (fUmtamvuna).

In contrast, the northern (npTableMountain–npStonesFarm) and southern (spResKloof–spRivendell) *S. primulifolius* population groups each harbour comparatively lower levels of genetic diversity, possessing more or less average levels compared with those of the other population groups of the mean number of polymorphic loci (6.0 and 7.2, respectively), mean alleles/locus (3.14 and 2.68), and mean expected (0.35 and 0.34) and observed (0.22 and 0.25) heterozygosities, and lower than average mean numbers of private alleles (1.3 and 0.7) set against the whole data set. Their mean levels of inbreeding (0.346 and 0.259) are also higher than that of the central *S. primulifolius* population group (0.138), although they are more or less average compared with the means of the other population groups.

The population that is thought to be the result of hybridization between *S. meyeri* and southern *S. primulifolius* (hWedgeley) is also genetically highly diverse. All eight microsatellite loci that

amplified successfully in this population are polymorphic, it has one of the highest values of alleles/locus (4.50), a high number of private alleles set against the rest of the populations (4), and the highest expected heterozygosity (0.75). It also has a high observed heterozygosity (0.49), but demonstrates a significant divergence from panmixia.

S. johannis shows a large difference in diversity statistics between the northern and southern populations. The northern populations (jNsikeni–jManzimnyama) possess a mean number of polymorphic loci of 4.7, a mean number of alleles per locus of 1.81, a mean number of private alleles within the species of 2.7, and expected and observed heterozygosity values of 0.18 and 0.09, respectively. In contrast, the southern populations (jMyokane–jMtSullivan02) possess 7.4, 4.07, 9.2, 0.47 and 0.30 for these same statistics respectively. This signifies a far lower genetic diversity within the northern populations compared with the southern populations. All of the *S. johannis* populations display significant levels of inbreeding. Furthermore, the difference between observed and expected heterozygosities in the northern populations is more pronounced than in the southern populations—the mean observed heterozygosity is half of the mean expected heterozygosity (0.09 vs. 0.18) in the northern *S. johannis* populations, but over 60% that of the mean expected heterozygosity in the southern populations (0.30 vs 0.47). However, levels of inbreeding in the northern and southern populations were not found to be significantly different from each other at α=0.05 using a Student's t-test.

The *S. baudertii* populations (bHarmony–bHillsdrift) also harbour higher than average genetic diversity compared to the other population groups. The two southernmost *S. baudertii* populations (bCollywobbles and bHillsdrift), which are geographically quite distant from each other and the other *S. baudertii* populations, possess the highest levels of genetic diversity within the species, having the largest number of polymorphic loci (7 in bCollywobbles and 8 in bHillsdrift), alleles/locus (3.29 and 4.50), private alleles within the species (12 and 26), and expected (0.47 and 0.55) and observed (0.45 and 0.37) heterozygosities within *S. baudertii*. The most striking feature of this species, however, is that it possesses the highest mean number of private alleles within the population groups (12.4) and the second highest mean number of private alleles per population set against the whole data set (3.4). These statistics highlight the large genetic differences amongst the populations constituting this species.

The species displaying the lowest genetic diversity is *S. rexii*. The populations sampled for this species (rOtterspoort–rWilderness) possess the lowest mean number of polymorphic loci in the data set (2.1), with the northernmost (rOtterspoort and rMtAyliff) and most of the southernmost (rVSRG, rBloukrans and rWilderness) populations tending to be more genetically uniform than the more central populations. Likewise the mean number of alleles/locus is at its lowest in the *S. rexii* populations (1.35). The populations also have the lowest mean number of private alleles, set against both the rest of the data set (0.1), as well as to the other member of *S. rexii* (1.6), and the lowest mean expected (0.08) and observed (0.05) heterozygosities. Gene diversity and the difference between the number of private alleles/population set against the rest of the data set vs. the number of private alleles/population within *S. rexii* are at their greatest towards the centre of the species' range (especially in rLeopardFalls), indicating that it is these populations that both possess the highest diversity and share the most alleles with populations from other species.

4.3.3. Population- and individual-level distance matrices calculated from the microsatellite data

Chord and PSA genetic distances amongst populations and amongst individuals were calculated from the data set containing all of the loci as well as from a data set excluding the microsatellite loci that amplified less consistently in some of the *S. johannis* and northern *S. primulifolius* populations (StrepPR241, StrepPR239 and StrepB22) to see the effect of missing data on the

analyses. The population-level trees constructed from distance matrices excluding the three problematic loci were better supported and more similar to each other than those constructed including all the loci, and the three problematic loci were therefore excluded when constructing the distance trees and PCo plots shown here. These three loci were also excluded from the analyses run in Structure, since the user manual (Pritchard *et al*. 2007) warns against the effects that data that are missing in a systematic way e.g. due to null alleles, has on the results.

4.3.4. Population-level trees built from the microsatellite data and the nuclear and plastid sequence data

4.3.4.1. Neighbour-joining trees built from the microsatellite distance matrices

The population-level trees constructed from the chord (Figure 4.2a) and PSA (Figure 4.2b) distance matrices calculated from six of the nine loci are similar to each other. Specifically, they share six major groups, labelled from I–VI in Figure 4.2. Group I constitutes a group containing the *S. primulifolius* populations collected from the southern Pondoland Centre and a little to the south (cpEndliniyokozi, cpBululoGorge, cpSilaka, cpMtSullivan, cpXibeni, cpMbanyana and cpDwalana), although with BS<50%. There is some disagreement between the two trees regarding the relationships amongst cpEndliniyokozi, cpSilaka and cpBululoGorge, although these alternative arrangements are not very strongly supported (BS<60%) in either of the trees. All of the *S. primulifolius* populations from the northern part of the species' range (npTableMountain, npMonteseel and npStonesFarm) emerge together in group II (BS of 69% in the chord tree and 66% in the PSA tree). Sister to group II is group III (BS<50%), which contains the two southernmost *S. baudertii* populations (bCollywobbles and bHillsdrift). These two southernmost *S. baudertii* populations therefore emerge separately from the three northernmost *S. baudertii* populations (bHarmony, bOtterspoort and bNtywenka), which cluster together in the strongly supported group V (BS of **94**% and **96**%). All of the *S. johannis* populations cluster together along with cpRooivaal (geographically, the *S. primulifolius* population situated closest to the northern *S. johannis* populations) and the *S. formosus* population from Untamvuna in group IV (BS<50%). Most of the relationships are rather strongly supported within this group: cpRooivaal and fUmtavuna emerge together with **93**% and **79**% BS support in the two trees, respectively; the three northernmost southern *S. johannis* populations (jMagwaFalls, jEmbotyi and jMyokane) group together with **89**% and **99**% BS support, and the three northern *S. johannis* populations (jNsikeni, jHebronRd and jManzimnyama) are supported by **100**% BS values in both trees. There is, however, disagreement within this group between the two trees regarding whether the two MtSullivan populations are more similar to the northern *S. johannis* populations, or whether they belong with the cpRooivaal, fUmtamvuna and the other southern *S. johannis* populations. However, the alternative relationships for these two populations are very weakly supported (BS<53% in both cases), and no firm deductions can therefore be made either way. Most of the rest of the populations i.e. the southern *S. primulifolius* and the *S. rexii* populations, all emerge in group VI (BS<50%) in both trees. Within this group, the northernmost *S. rexii* populations (rOtterspoort, rMtAyliff and rNtywenka) and rKatberg consistently emerge in the same group (BS<50%), the southernmost *S. rexii* populations (rWilderness, rBloukrans, rTsitsikamma and rVSRG) as well as rKologha and rLeopardFalls group together (BS<50%), while the southern *S. primulifolius* populations are dispersed throughout group VI. Although disagreement regarding the affiliations of the spRivendell population exists between the two trees, in both topologies it groups with the more southern *S. primulifolius* and *S. rexii* populations. The only other disagreement between the two trees is the relationship of the putative hybrid population, hWedgeley, to the rest of the taxa. This population emerges sister to group VI (the southern *S. primulifolius* and *S. rexii* populations group) in the tree constructed from chord distances, but

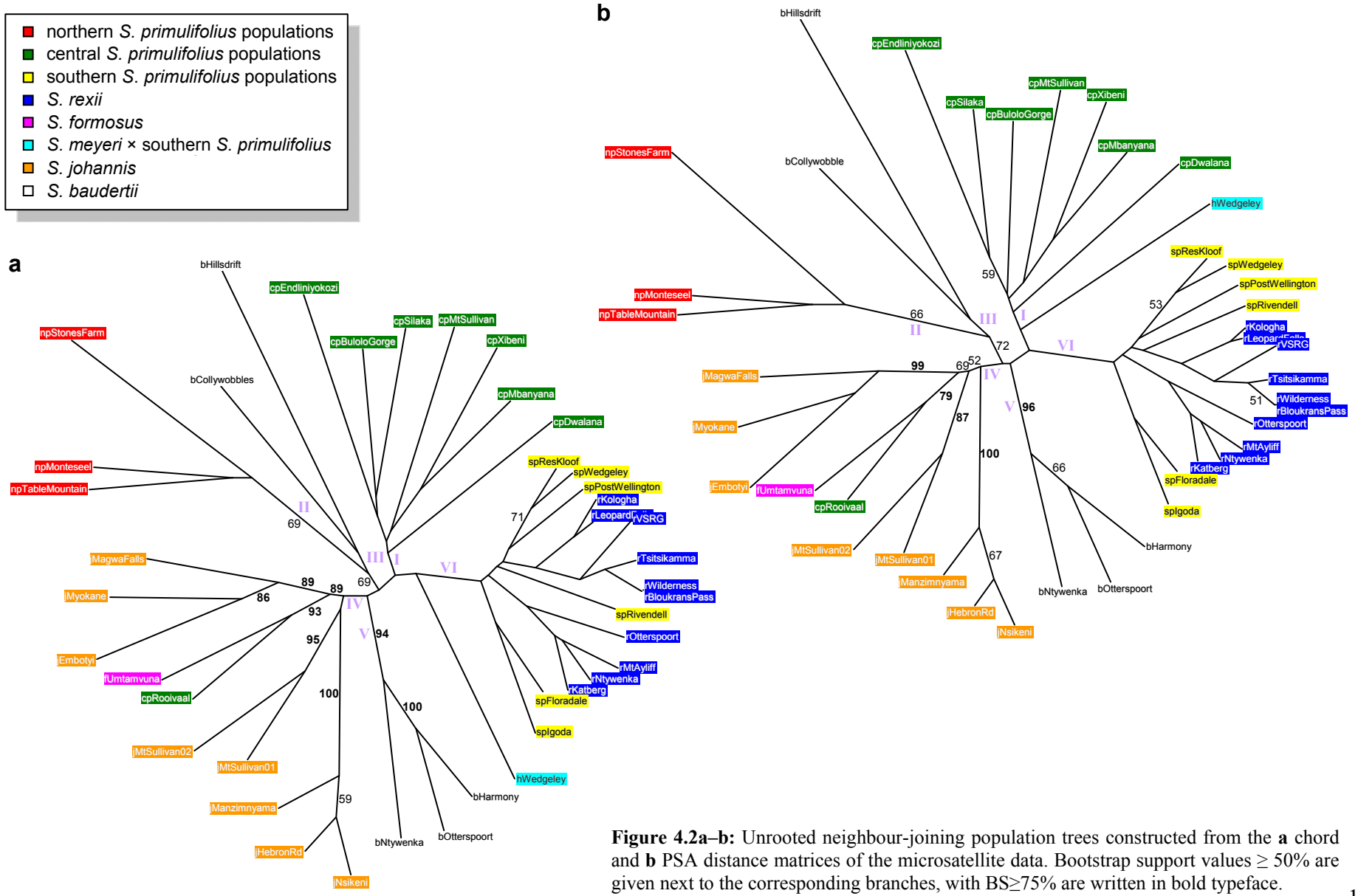

Figure 4.2a–b: Unrooted neighbour-joining population trees constructed from the **a** chord and **b** PSA distance matrices of the microsatellite data. Bootstrap support values ≥ 50% are given next to the corresponding branches, with BS≥75% are written in bold typeface.

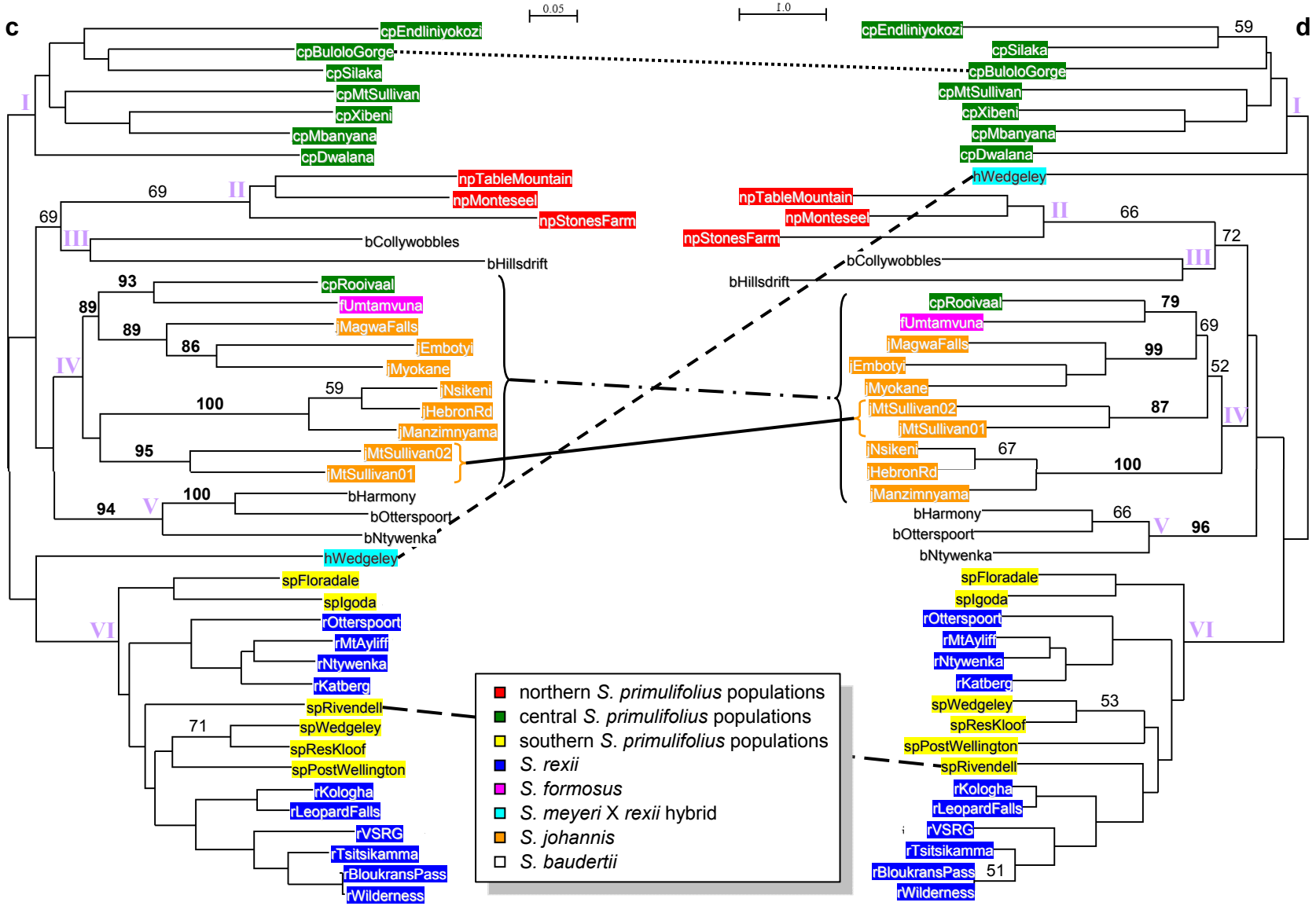

Figure 4.2c–d: Neighbour-joining population trees constructed from **c** chord and **d** PSA distances calculated from the microsatellite data and rooted on central *S. primulifolius* populations. Bootstrap support values ≥ 50% are given above the branches, with BS≥75% written in bold typeface. Lines indicate disagreements between the trees.

closer to group I (the *S. primulifolius* populations from the Pondoland Centre and a little to the south) in the tree constructed with PSA distances. The affiliations of this population are therefore unclear.

The unrooted chord and PSA distance microsatellite trees (Figure 4.2 a & b) were rooted on group I (Figure 4.2 c & d), the group containing the *S. primulifolius* populations collected from the Pondoland Centre (cpEndliniyokozi, cpBululoGorge, cpMtSullivan, cpDwalana and cpSilaka) and the populations a little to the south (cpXibeni and cpMbanyana). More distantly related *Streptocarpus* species could not be used to root the microsatellite trees, since genetic distances amongst some of the ingroup populations were found to be equal to or close to 1, and it is therefore unlikely that reliable shared alleles (as opposed to homoplasies) would be found in more distantly related taxa. Members of *S. primulifolius* were selected for rooting, since the more scattered positions of the *S. primulifolius* samples in the topologies built from the nuclear and plastid sequence data of the previous chapter (Figures 3.5, 3.7 and 3.9–3.14) and the close association of its samples with those of the other species included in these microsatellite analyses i.e. *S. rexii*, *S. formosus*, *S. johannis* and *S. baudertii*, suggest that *S. primulifolius* is the ancestor of these species. Within *S. primulifolius*, the group containing the Pondoland Centre, cpXibeni and cpMbanyana populations was chosen as the root for the current microsatellite trees, since the analyses of the nuclear and plastid sequence topologies in the previous chapter and the microsatellite analyses of Hughes *et al*. (2005) revealed that these *S. primulifolius* populations harbour the highest levels of genetic diversity within the species, and are therefore probably ancestral to the rest of *S. primulifolius*.

With the microsatellite trees rooted on the *S. primulifolius* populations in group I, the rest of the populations form two sister groups, one containing the northern *S. primulifolius* (group II), northernmost *S. baudertii* (group III), the *S. johannis*–cpRooivaal–fUmtamvuna (group IV) and southernmost *S. baudertii* (group V) groups, and the other containing the southern *S. primulifolius* –*S. rexii* (group VI) group, with the hWedgeley population emerging sister to group VI (the southern *S. primulifolius* and *S. rexii* populations group) in the tree constructed from chord distances, but in an unresolved position sister to group I (the *S. primulifolius* populations from the Pondoland Centre and a little to the south) and the clade containing groups II–VI in the tree constructed with PSA distances.

The inclusion of additional populations and the exclusion of three of the nine microsatellite markers have resulted in some rearrangements in the relationships amongst the populations reconstructed in the current study compared with those recovered in the PSA tree of the microsatellite data shown in Hughes *et al*. (2005). These include different relationships amongst the *S. primulifolius* populations of the Pondoland Centre, the inclusion of cpXibeni and cpMbanyana amongst these Pondoland Centre *S. primulifolus* populations, and different relationships amongst the southern *S. primulifolius* (called *S.* cf. *primulifolius* in Hughes *et al*. 2005) and *S. rexii* populations in Hughes *et al*. (2005). However, the population trees of the current study still reflect the main evolutionary patterns of *S. primulifolius* and *S. rexii* evident in the PSA tree of the microsatellite data shown in Hughes *et al*. (2005). The trees built from different distance matrices in this current study are also in minor conflict with each other (indicated by lines in Figure 4.2 c & d), as discussed above. However, the alternative relationships of these populations are each only weakly (BS<50%) or moderately (50%≤BS<75%) supported.

4.3.4.2 Maximum parsimony analysis of the nuclear ITS sequence data

The ITS sequence analysis containing only representatives of the populations from which microsatellite data were also generated (Figure 4.3) yielded topologies that are congruent with

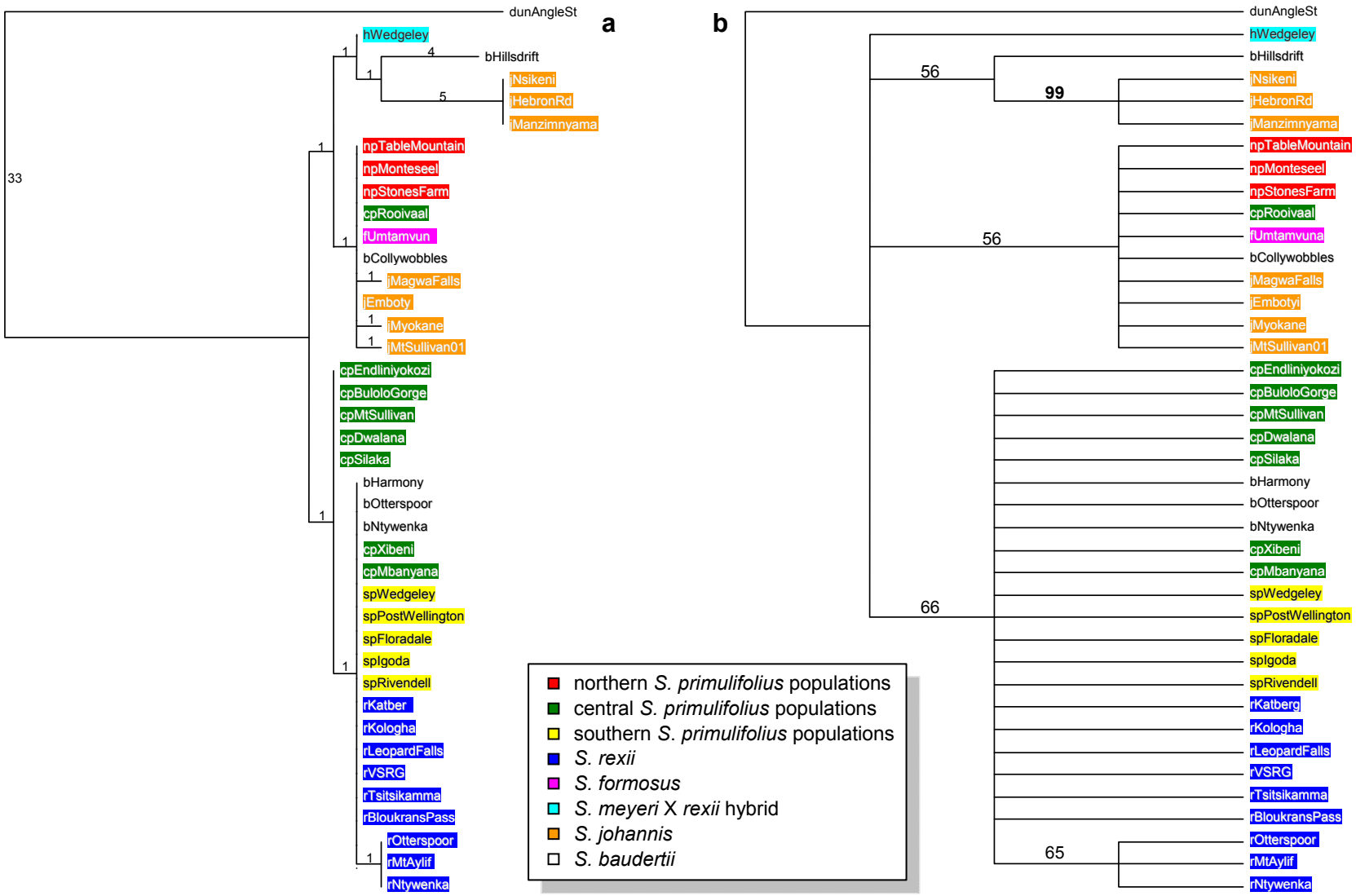

Figure 4.3: Maximum parsimony trees resulting from the phylogenetic analyses of the nuclear ITS data set. **a:** One of 8 920 most parsimonious trees of 52 steps (CI = 0.962, RI = 0.973) with branch lengths shown. **b:** The strict consensus tree with bootstrap percentages given above the branches. BS ≥ 75% are considered to indicate strong support and are written in bold type.

the larger ITS topologies of the previous chapter (Figures 3.5, 3.9a, 3.10a, 3.11 & 3.12) and with the ITS tree in Hughes *et al*. (2005).

The current, smaller ITS analysis included 749 characters, 50 (6.7%) of which were variable. Of these variable characters, 38 (76.0%) were parsimony informative. The analysis yielded 8 920 equally parsimonious trees of 52 steps with an average number of steps per character of 0.069 (52 steps/749 characters), a consistency index (CI) of 0.962 and a retention index (RI) of 0.973. All five of the internal branches present in the strict consensus tree received bootstrap (BS) support values of greater than 50%, one of which was strongly supported (had a BS≥75%).

This smaller ITS analysis resulted in a topology (Figure 4.3) that is more resolved than the ITS sequence analysis including all of the taxa (Figure 3.5), and is in strong agreement with the ITS networks (Figures 3.9a, 3.10a, 3.11 & 3.12) of the previous chapter. The *S. dunnii* sample from Angle Station was specified as the outgroup, and therefore emerged outside of the clade containing the rest of the samples (the ingroup) in this current topology (Figure 4.3). Within this clade, the hybrid population emerged in an unresolved position sister to the three main clades into which the remaining population samples grouped in all of the most parsimonious trees. The first main clade contained the northern *S. johannis* populations (jNsikeni, jHebronRd and jManzimnyama), which formed the only strongly supported group in the topology (BS=**99**%), together emerging sister to the *S. baudertii* sample from Hillsdrift (BS=56%). The northernmost *S. primulifolius* populations (npTableMountain, npMonteseel, npStonesFarm, cpRooivaal) grouped in the second main clade along with the geographically proximate *S. formosus* population (fUmtamvuna), *S. baudertii* from Collywobbles (bCollywobbles) and the four southern *S. johannis* populations for which sequence data were available (jMagwaFalls, jEmbotyi, jMyokane and jMtSullivan01) with a BS of 56%. The rest of the samples (the central *S. primulifolius* populations cpEndliniyokozi, cpBulo loGorge, cpMtSullivan, cpDwalana, cpSilaka, cpXibeni and cpMbanyana, the northernmost *S. baudertii* populations bHarmony, bOtterspoort and bNtywenka and all of the southern *S. primulifolius* and *S. rexii* populations) emerged together in the third main clade (BS=66%), with the three northernmost *S. rexii* populations (rOtterspoort, rMtAyliff and rNtywenka) together forming a subclade (BS=65%) within this clade.

4.3.4.3. Maximum parsimony analysis of the plastid sequence data

The current, smaller plastid sequence phylogenetic analysis resulted in trees (Figure 4.4) that are more resolved and better supported than the ITS phylogeny (Figure 4.3). Of the 3 881 plastid sequence characters analysed, 66 (1.7%) were variable, of which 41 (62.1%) were parsimony informative. The analysis resulted in 1 950 trees of 71 steps each (0.036 steps/character) with a CI of 0.930 and an RI of 0.962.

The current plastid analysis yielded a topology (Figure 4.4) in agreement with the plastid topologies of the previous chapter containing a larger number of samples (Figures 3.7, 3.9b, 3.10b, 3.13 & 3.14) and the plastid tree in Hughes *et al*. (2005), but to a certain extent in conflict with the current ITS phylogeny (Figure 4.3). In the current plastid phylogenetic tree (Figure 4.4), the two *S. johannis* samples from Mount Sullivan formed a strongly supported clade (BS=**96**%). The two *S. johannis* Myokane samples emerged together (BS=62%) along with one of the *S. johannis* samples from Magwa Falls (BS=**75**%). The rest of the *S. johannis* samples emerged together with the *S. primulifolius* representative from Silaka and *S. baudertii* from Collywobbles (BS=67%). This latter clade contains two strongly supported subclades. The two *S. johannis* Embotyi representatives (BS=65%) emerged together with the other *S. johannis* Magwa Falls sample (BS=**85**%) in one clade. The second clade contains the northern

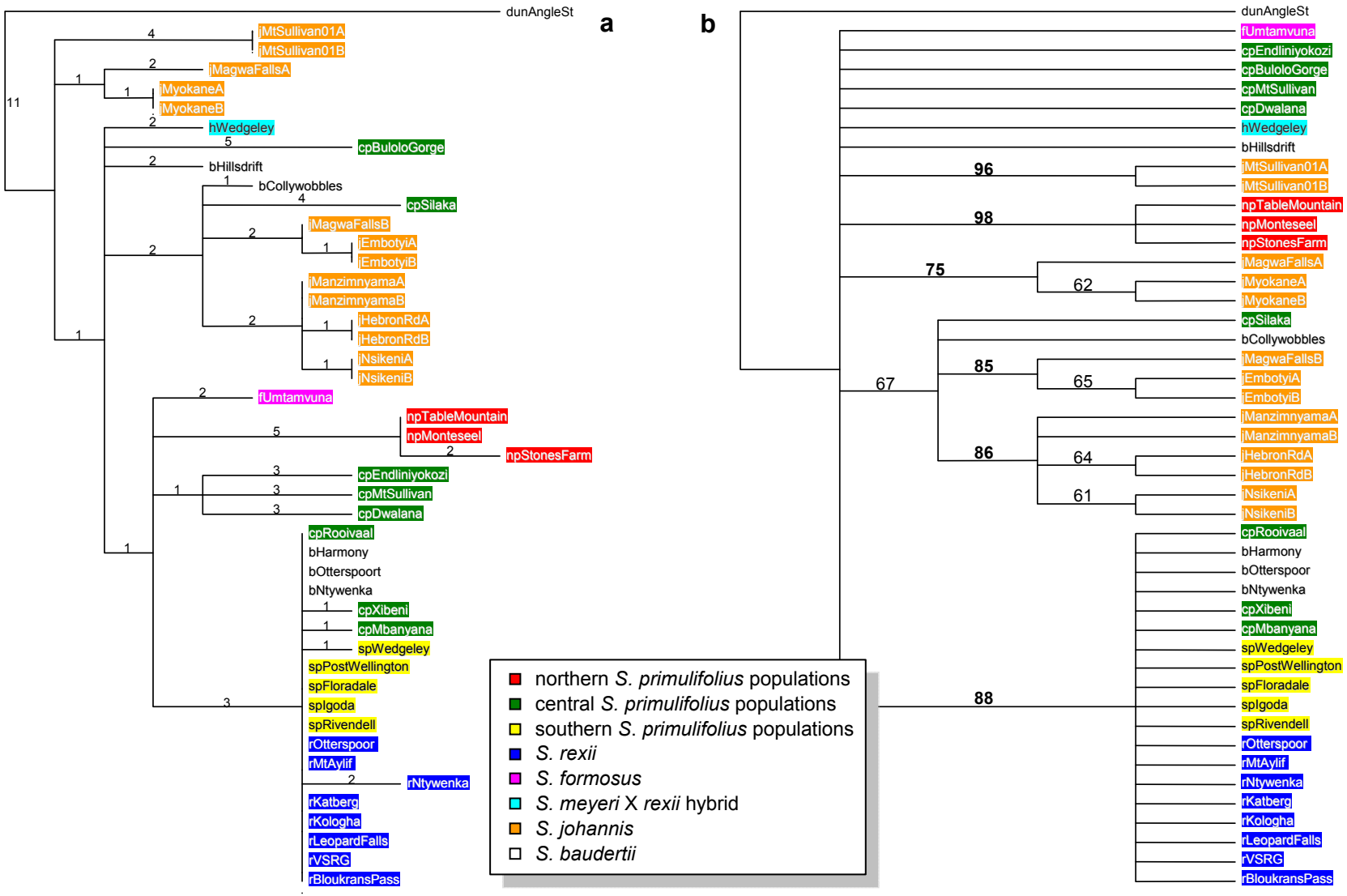

Figure 4.4: Maximum parsimony trees resulting from the phylogenetic analyses of the plastid data set. **a:** One of 1 950 most parsimonious trees of 71 steps (CI = 0.930, RI = 0.962) with branch lengths shown. **b:** The strict consensus tree with bootstrap percentages given above the branches. BS ≥ 75% are considered to indicate strong support and are written in bold type.

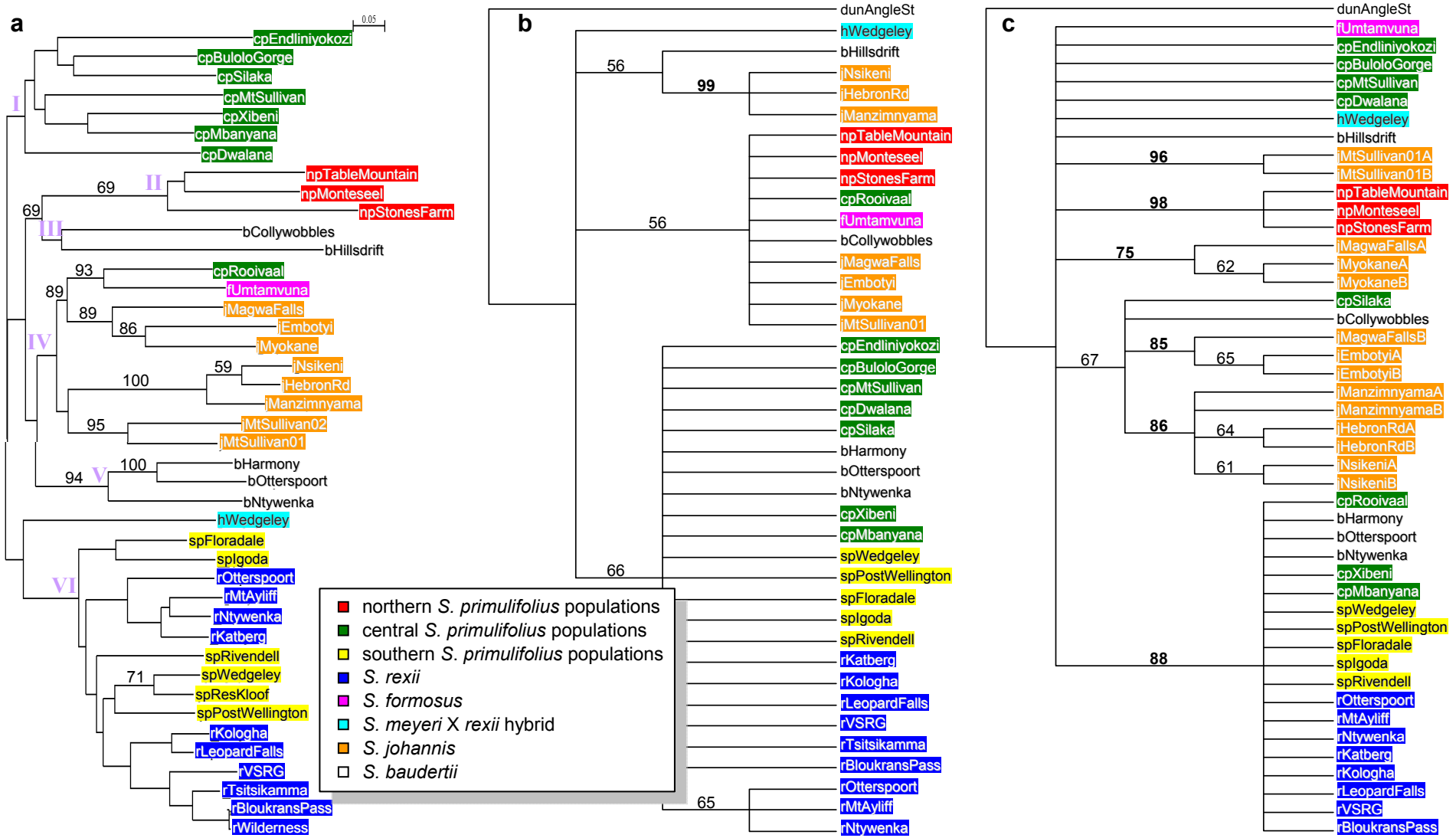

Figure 4.5: The population-level trees constructed from the nuclear microsatellite data and the nuclear and plastid sequence data. To the left is **a** the population-level neighbour-joining distance tree constructed from the chord distance matrix calculated from the microsatellite data, and in the middle and to the right are the maximum parsimony strict consensus trees built using the **b** ITS and **c** plastid sequence data. Bootstrap support values are shown above the branches in each tree.

S. johannis samples (BS=**86**%), with the Nsikeni samples forming one clade (BS=61%), the Hebron Road samples another clade (BS=64%), and the Manzimnyama samples emerging in unresolved positions in the northern *S. johannis* clade. The three northern *S. primulifolius* populations (npTableMountain, npMonteseel and npStonesFarm) grouped together strongly (BS=**98**%), while most of the central *S. primulifolius* (cpEndliniyokozi, cpBululoGorge, cpMtSullivan and cpDwalana), the *S. formosus* (fUmtamvuna), the hybrid (hWedgeley) and the *S. baudertii* Hillsdrift populations emerged in unresolved positions in the ingroup clade. The rest of the samples (the *S. primulifolius* samples cpRooivaal, cpXibeni and cpMbanyana, the northernmost *S. baudertii* populations bHarmony, bOtterspoort and bNtywenka and all of the southern *S. primulifolius* and *S. rexii* populations) grouped together in a strongly supported clade (BS=**88**%).

A comparison of the chord (Figures 4.2a, 4.2c & 4.5a) and PSA (Figures 4.2b, 4.2d) microsatellite neighbour-joining trees and the ITS (Figures 4.3 & 4.5b) and plastid (Figures 4.4 & 4.5c) sequence phylogenies allows the identification of the relationships that are congruent across the topologies, as well as those that are in conflict. The northern *S. johannis* populations (jNsikeni, jHebronRd and jManzimnyama) emerge as a strongly supported group in all of the topologies, receiving BS support values of **86**–**100**%. The northern *S. primulifolius* populations (npTableMountain, npMonteseel and npStonesFarm) group together in the microsatellite and plastid topologies (BS of 66–**98**%), and emerge in unresolved positions in a larger polytomy in the ITS phylogeny. The northernmost *S. baudertii* populations (bHarmony, bOtterspoort and bNtywenka) also emerge as a group in the microsatellite analyses (BS of **94**% and **96**%), and in unresolved positions in larger polytomies in the ITS and plastid phylogenies. Likewise, the southern *S. primulifolius* (spResKloof, spWedgeley, spPostWellington, spFloradale, spIgoda and spRivendell) and the *S. rexii* populations group together in the microsatellite trees (BS<50%), but emerge in unresolved positions in larger polytomies in the ITS and plastid phylogenies.

However, other relationships are more variable across the topologies. The cpRooivaal and fUmtamvuna populations group along with all of the *S. johannis* populations in the microsatellite trees (BS<50%) and with the southern *S. johannis* populations (jMagwaFalls, jEmbotyi, jMyokane and jMtSullivan) in a larger polytomy in the ITS phylogeny. However, the plastid topology shows no links amongst the cpRooivaal, fUmtamvuna and *S. johannis* populations, with cpRooivaal grouping in a large polytomy also containing the northern *S. baudertii*, the southern *S. primulifolius* and the *S. rexii* populations and cpXibeni and cpMbanyana, fUmtamvuna emerging in an unresolved position in the ingroup clade, and the samples collected from the southern *S. johannis* populations emerging in three different clades. The microsatellite trees also contain a group (BS<50%) containing the Pondoland Centre *S. primulifolius* populations (cpEndliniyokozi, cpBululoGorge, cpMtSullivan, cpDwalana and cpSilaka) and the *S. primulifolius* populations situated a little further south (cpXibeni and cpMbanyana). These *S. primulifolius* populations emerge in a larger polytomy in the ITS phylogeny, but either in unresolved positions in the ingroup or closer to other populations in the plastid phylogeny. The two southernmost *S. baudertii* populations (bCollywobbles and Hillsdrift) emerge together in the microsatellite trees (BS<50%), but separately in both the ITS and plastid phylogenies. The hybrid population (hWedgeley) is the most erratic population in the topologies, emerging in different positions in the two microsatellite trees and in unresolved positions in the ingroups in both the ITS and plastid phylogenies.

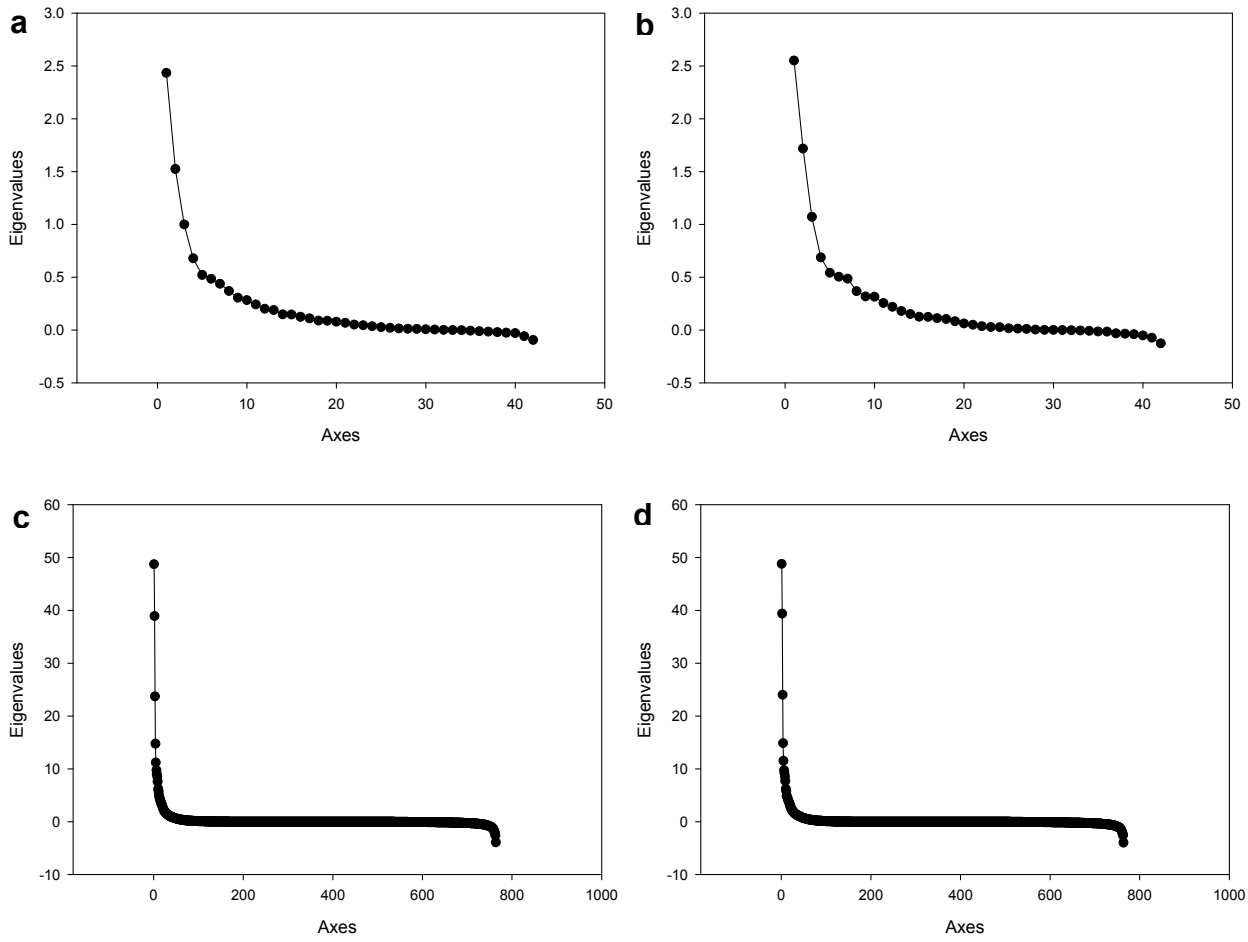

Figure 4.6: Plots of the eigenvalues of successive axes from the PCo analyses performed on **a** chord and **b** PSA distances amongst populations, and on **c** chord and **d** PSA distances amongst individuals.

4.3.5. Principal Co-ordinate analyses of the distances amongst populations and individuals based on the microsatellite data

Structure amongst and within the taxa was also investigated by performing Principal Co-ordinate (PCo) analyses on distances calculated amongst populations and individuals within the data set from six of the nine loci. Popular ways of determining the number of axes of a PCo analysis that should be plotted in order to see a representative proportion of the total variance of the data include the scree test (Cattell 1966) and the Guttman-Kaiser criterion (Guttman 1954; Kaiser 1960, 1961). The scree test states that the important axes are those whose eigenvalues fall above where the eigenvalues of successive axes plateau in a graph depicting the eigenvalues of the axes, because these axes together contain the bulk of the total variance in the data. According to the Guttman-Kaiser criterion, only those axes with eigenvalues > 1 display a significant proportion of the variance contained in the data. The plots of the eigenvalues of the axes from the two population-level analyses (Figure 4.6 a & b) indicate that visualising the first three axes is enough, as these axes satisfy both the scree test and the Guttman-Kaiser criterion. These first three axes contain 39.22% of the total variance of the data in the chord analysis (axis 1: 18.92%, axis 2: 12.11% and axis 3: 8.19%), and 38.26% in the PSA analysis (axis 1: 17.91%, axis 2: 12.33% and axis 3: 8.01%). However, the same is not true for the individual-level analyses (Figure 4.6 c & d). It is difficult to see the exact number of significant axes above the plateau in these two graphs that would be considered significant by the scree test, but according to the Guttman-Kaiser criterion, one would have to plot the first 37 axes of the individual-level analysis of the chord distances, and the first 40 axes of the analysis using PSA distances in

order to capture a representative proportion of the total variance in the original data. Indeed, the first three axes of the individual-level chord and PSA analyses only account for 3.86% (axis 1: 1.65%, axis 2: 1.34% and axis 3: 0.87%) and 3.84% (axis 1: 1.63%, axis 2: 1.34% and axis 3: 0.87%) of the variance in the data, respectively. However, it is difficult to visualise more than three axes at a time, and only the first three axes are therefore shown in the individual-level PCo plots.

The resulting two three-dimensional population-level and individual-level PCo graphs are shown in Figure 4.7 a–d and are also included in the slide show on the CD accompanying the thesis. These two population-level PCo graphs (Figure 4.7 a & b) are very similar to each other, as are the individual-level graphs (Figure 4.7 c & d). This indicates that the signal in the original data is strong enough to display similar patterns despite the use of different distance measures. In the population-level analyses (Figure 4.7 a & b), most of the population groups form clusters. The northern *S. primulifolius* populations together constitute the most isolated group in the plots. Most of the central *S. primulifolius* populations cluster together, with the exception of cpRooivaal and cpEndliniyokozi, which are both a little more separated from the other central *S. primulifolius* populations. The sole *S. formosus* population, fUmtamvuna, emerges between the northern and southern *S. johannis* populations and the central *S. primulifolius* populations. The southern *S. primulifolius* and *S. rexii* populations overlap with each other, and together segregate from the rest of the populations. The hWedgeley population, which is believed to be a hybrid between southern *S. primulifolius* and *S. meyeri*, does not show specific affinities to any of the other populations. However, *S. meyeri* is not included in these plots, so it is difficult to draw any conclusions concerning the affinities of this population. Within *S. johannis*, the northern populations cluster separately from the southern populations, the latter being a little more spread out. This is probably the result of the greater genetic diversity found in the southern populations compared with the northern populations. The most dispersed population group is, however, *S. baudertii*, whose populations emerge interspersed amongst most of the other population groups. This is also the species that possesses the greatest mean number of private alleles per population amongst conspecific populations, further evidence that the populations of *S. baudertii* are very different from one another.

The plots of the individual-level PCo analyses (Figure 4.7 c & d) display much less segregation of the population groups than do the population-level plots. Even the members of the northern *S. primulifolius* populations, which are isolated from the other populations in the population-level plots, cluster amongst members of the other populations. This lack of distinction of the population groups in the individual-level PCo plots is probably the result of visualising only the first three of the 764 axes that were generated by the individual-level PCo analyses. However, despite extensive overlap, positioning of members of population groups relative to members of other population groups is similar to that found in the population-level analyses. Thus representatives of the northern *S. primulifolius* populations also emerge towards the top right-hand corner of the graphs, the central *S. primulifolius* populations towards the bottom left-hand corner, the southern *S. primulifolius* and *S. rexii* populations towards the top left-hand corner, and *S. formosus* and *S. johannis* towards the right. Individuals belonging to the suspected hybrid population constitute the most dispersed population in these graphs, highlighting how different the genotypes of the six individuals collected from this population are from one another. However, in light of the fact that the first three axes of each of the individual-level analyses contain less that 5% of the total variance of the data, the individual-level PCo analyses failed and therefore should in actual fact be discarded.

4.3.6. Bayesian genetic cluster analyses of the microsatellite data

Two data sets containing six of the nine microsatellite loci were analysed in Structure 2.2: one

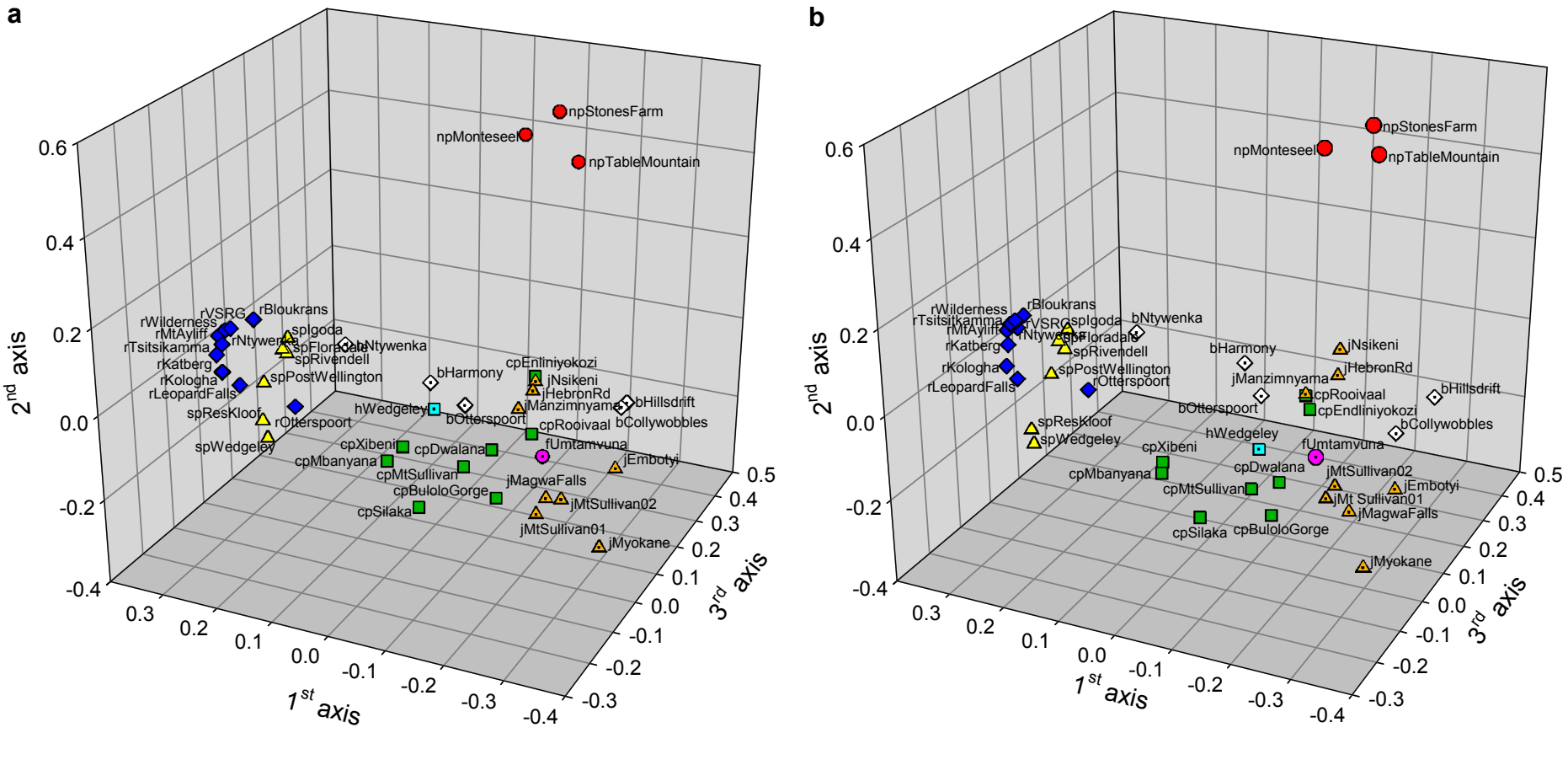

Figure 4.7a–b: 3-D plots of the population-level PCo analyses conducted on the **a** chord and **b** PSA distance matrices.

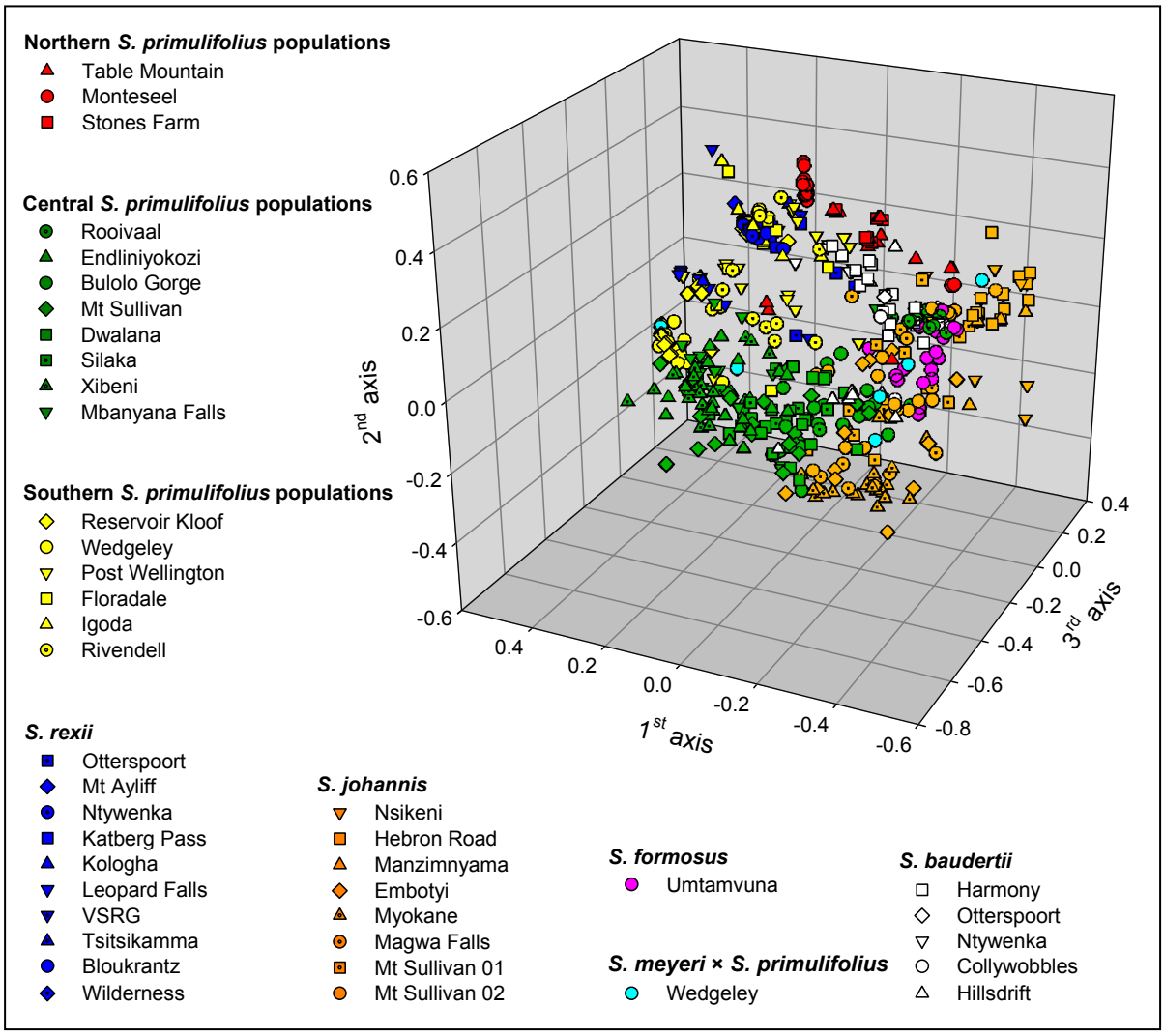

Figure 4.7c: 3-D plot of the PCo analysis carried out on the chord distances calculated amongst individuals.

containing the microsatellite data from all of the populations and the other containing only the microsatellite data from the eight *S. johannis* populations. However, the analyses including all the populations yielded ambiguous results. The calculations performed to identify the number of clusters (K) constituting the data were optimized by more than one value of K, and at each of these Ks, populations segregated into different clusters across runs. Falush *et al*. (2003) also found cases of inconsistent clustering in some of their Structure investigations of geographic structure in *Helicobacter pylori*, a chronic gastric pathogen of human beings. Falush *et al*. (2003) attributed these results to the complex genetic history of this species, with several independent waves of human migration resulting in genetic mixing of distantly related lineages of the pathogen. Based on the phylogenetic analyses of the ITS and plastid sequence data of the previous chapter of the current study, a similar scenario also seems to be the case amongst the *Streptocarpus* taxa. Falush *et al*. (2003) were able to circumvent this problem in their study by identifying clusters using the linkage model in Structure. However, the linkage model requires genetic distances amongst loci to be input in order to measure linkage disequilibrium amongst loci. As the chromosomal positions of the microsatellite loci and hence the genetic distances amongst loci used to study *Streptocarpus* are unknown, this approach could not be used here and the results are therefore not reported here. The inability of Structure to identify structure within the *Streptocarpus* data set as a whole probably means that evolution amongst these species does not conform to the assumptions made by the algorithm. This highlights the complexity of the data, and reflects the high proportion of shared alleles amongst population

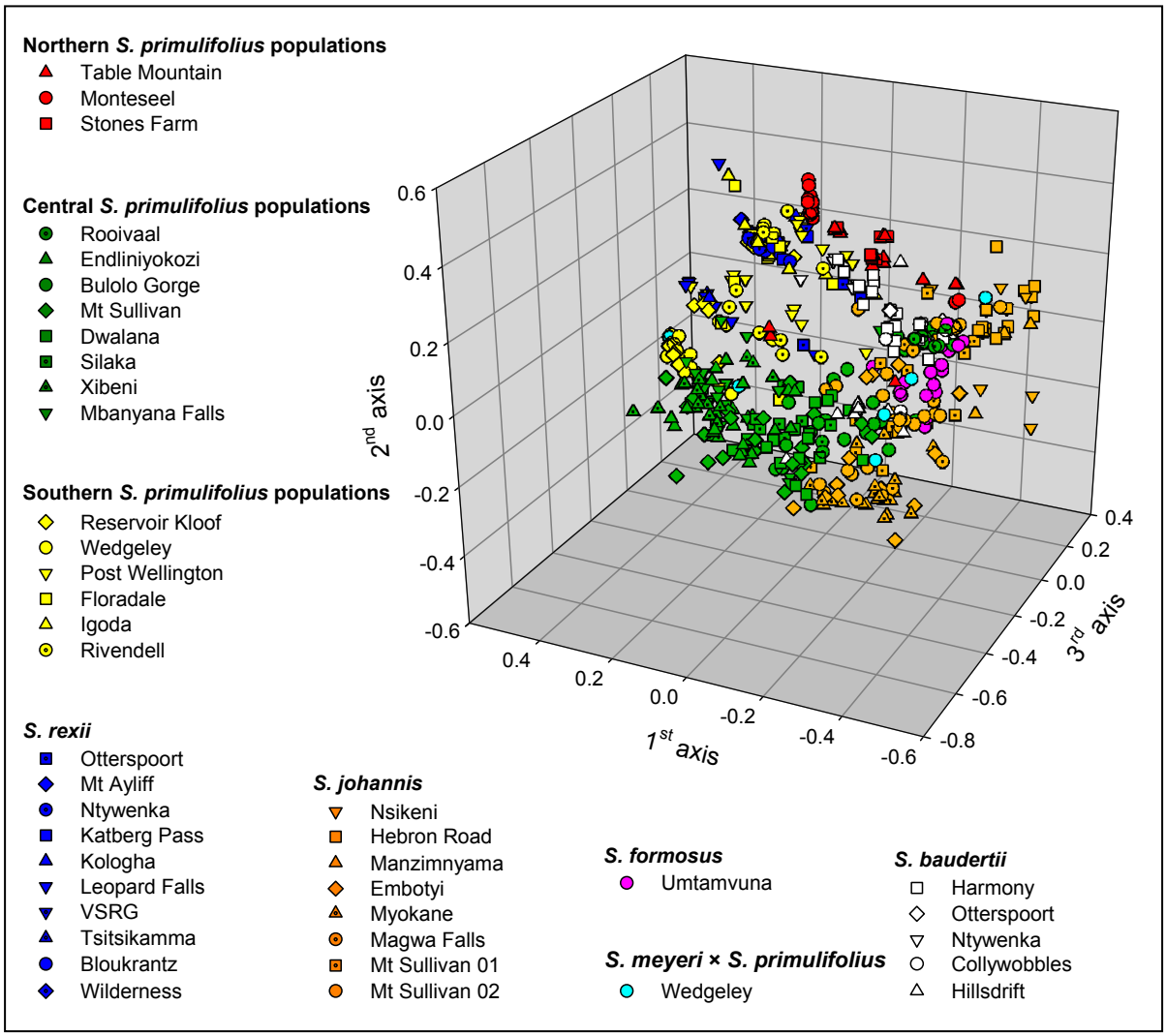

Figure 4.7d: 3-D plot of the PCo analysis carried out on the PSA distances calculated amongst individuals.

groups, the lack of bootstrap support for groups in the population trees, and the continuity amongst clusters i.e. the lack of distinct clusters, most evident in the individual-level PCo analyses, all of which may be the result of hybridization.

In contrast, the analyses of the *S. johannis* populations in Structure yielded more clear-cut results. Table 4.3 shows results from all the runs conducted on the *S. johannis* data, Figures 4.8 and 4.9 show graphs of the results of some of the analyses, and Figure 4.10 depicts the identified clusters on a map of the population localities.

Analysis of all the *S. johannis* populations produced the highest Ln P(D) at K=8 for each of the four models—admixture & correlated allele frequencies (AC), admixture & independent allele frequencies (AI), no admixture & correlated allele frequencies (NC) and no admixture & independent allele frequencies (NI)—and Ln P(D) would probably have continued to increase if the data had been run at higher values of K. However, after applying the calculations proposed by Evanno *et al*. (2005) to the results of the analyses, the K that optimises the calculations appears to be smaller. Under the AC model, the data produced ambiguous results, possessing a high ΔK value at both K=2 and K=3 (Table 4.3). However, ΔK was much higher for K=2 than at any other value in the other three models (Figure 4.8b shows ΔK vs K for the *S. johannis* data run under the NC model), implying that the number of main clusters in *S. johannis* is probably two. In all runs at K=2, the *S. johannis* populations consistently separated into the northern (jNsikeni, jHebronRd and jManzimnyama) and the southern (jMagwaFalls, jEmbotyi,

Table 4.3: Results of the analysis of the *S. johannis* populations in Structure 2.2. The smallest number of clusters accounting for most of the variance in the data is highlighted in light yellow for each data set analysed, and the model that produced significantly higher posterior probability estimates for these proposed number of clusters is highlighted in yellow. K: number of proposed clusters; Ln P(D): an estimate of the posterior probability of the data under the specified model parameters; σ: standard deviation; ΔK: a measure of the rate of change of the average estimated posterior probability of successive K values divided by the standard deviation across runs at each K; AC: admixed ancestry & correlated allele frequencies; AI: admixed ancestry & independent allele frequencies; NC: no admixture & correlated allele frequencies; NI: no admixture & independent allele frequencies.

K	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK
	***S. johannis*: jNsikeni, jHebronRd, jManzimnyama, jMyokane, jMagwaFalls, jEmbotyi, jMtSullivan01 & jMtSullivan02**															
	AC				**AI**				**NC**				**NI**			
1	-2581.6 to -2581.4	-2581.51	0.08		-2580.2 to -2579.8	-2580.07	0.12		-2581.7 to -2581.3	-2581.51	0.12		-2580.2 to -2579.8	-2580.05	0.11	
2	-1947.9 to -1934.3	-1939.11	3.21	109.7	-1961.7 to -1958.0	-1960.26	1.14	288.6	-1940.3 to -1932.9	-1936.31	2.04	173.6	-1968.5 to -1966.3	-1967.39	0.61	508.5
3	-1652.0 to -1646.3	-1649.24	1.66	123.7	-1671.6 to -1666.5	-1668.93	1.51	144.0	-1648.8 to -1642.8	-1645.66	1.93	105.3	-1664.9 to -1660.7	-1662.77	1.48	153.2
4	-1584.6 to -1551.6	-1564.30	10.26	3.1	-1611.5 to -1567.6	-1595.47	18.04	0.6	-1591.0 to -1548.8	-1558.69	12.34	2.7	-1606.2 to -1568.5	-1584.64	15.40	0.3
5	-1529.9 to -1484.9	-1510.77	14.77	0.1	-1531.5 to -1498.1	-1511.25	9.96	1.2	-1555.0 to -1470.4	-1505.36	22.94	0.5	-1565.1 to -1489.1	-1511.63	16.24	0.3
6	-1505.2 to -1422.2	-1458.65	27.80	0.1	-1492.3 to -1410.9	-1438.69	22.33	0.5	-1579.2 to -1418.7	-1462.40	50.06	0.0	-1456.2 to -1413.4	-1433.63	17.18	2.2
7	-1580.0 to -1364.2	-1408.27	51.02	0.4	-1398.4 to -1341.3	-1377.39	19.99	0.3	-1631.4 to -1339.0	-1421.24	71.86	0.1	-1525.9 to -1354.3	-1393.15	44.45	0.9
8	-1388.0 to -1316.2	-1339.69	25.70		-1375.1 to -1287.1	-1310.55	28.88		-1581.9 to -1303.2	-1371.75	101.02		-1328.1 to -1306.8	-1313.76	6.79	
	Cluster 1 (the northern *S. johannis* populations): jNsikeni, jHebronRd & jManzimnyama															
	AC				**AI**				**NC**				**NI**			
1	-277.0 to -276.6	-276.71	0.11		-276.4 to -276.3	-276.31	0.04		-276.8 to -276.6	-276.69	0.06		-276.4 to -276.3	-276.31	0.03	
2	-215.8 to -212.1	-213.75	0.93	52.1	-219.5 to -216.6	-218.05	0.98	19.9	-205.1 to -201.4	-203.49	1.07	51.42	-207.5 to -206.7	-207.10	0.25	132.7
3	-212.0 to -191.2	-199.40	6.00		-182.9 to -177.5	-179.24	1.33		-194.6 to -179.9	-185.35	3.71		-172.2 to -170.4	-171.15	0.44	
	Cluster 2 (the southern *S. johannis* populations): jMyokane, jMagwaFalls, jEmbotyi, jMtSullivan01 & jMtSullivan02															
	AC				**AI**				**NC**				**NI**			
1	-1623.5 to -1621.1	-1622.67	0.55		-1620.7 to -1620.4	-1620.59	0.08		-1623.5 to -1622.3	-1622.70	0.34		-1620.7 to -1620.4	-1620.56	0.09	
2	-1330.0 to	-1329.37	0.58	399.0	-1335.9 to	-1334.61	0.62	334.8	-1327.2 to	-1326.13	0.58	390.7	-1333.3 to	-1332.47	0.48	442.2

K	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK	Range of Ln P(D)	Mean of Ln P(D)	σ of Ln P(D)	ΔK
	-1328.0				-1333.7				-1325.3				-1331.9			
3	-1465.9 to -1237.9	-1269.46	60.65	0.4	-1273.7 to -1248.2	-1254.61	8.08	1.9	-1474.2 to -1235.5	-1256.29	60.73	0.0	-1292.5 to -1247.7	-1258.19	15.67	0.9
4	-1195.2 to -1166.4	-1183.78	10.41	2.6	-1206.4 to -1174.5	-1189.90	10.61	0.5	-1218.4 to -1162.2	-1186.38	14.78	0.4	-1240.6 to -1175.9	-1198.41	17.24	0.3
5	-1157.0 to -1101.8	-1124.88	22.76		-1154.3 to -1113.0	-1120.07	13.24		-1152.1 to -1102.9	-1122.23	21.17		-1245.3 to -1121.2	-1132.84	31.21	
	Cluster 2.1 (the three northernmost southern *S. johannis* populations): jMyokane, jMagwaFalls & jEmbotyi															
	AC				**AI**				**NC**				**NI**			
1	-720.9 to -719.4	-720.29	0.36		-718.7 to -718.5	-718.55	0.06		-721.5 to -720.0	-720.57	0.49		-718.8 to -718.4	-718.56	0.09	
2	-739.1 to -635.9	-651.87	28.40	0.4	-644.4 to -631.7	-633.77	3.21	4.8	-626.8 to -624.6	-625.41	0.63	45.0	-627.6 to -625.8	-626.60	0.49	51.8
3	-578.5 to -568.0	-570.80	3.34		-566.1 to -563.3	-564.30	0.76		-562.1 to -557.3	-558.64	1.17		-561.1 to -559.0	-560.11	0.73	
	Cluster 2.2 (the two Mount Sullivan *S. johannis* populations): jMtSullivan01 & jMtSullivan02															
	AC				**AI**				**NC**				**NI**			
1	-564.7 to -562.4	-563.11	0.56		-563.6 to -563.3	-563.42	0.09		-563.3 to -562.4	-562.89	0.29		-563.5 to -563.4	-563.41	0.04	
2	-513.9 to -509.5	-510.95	1.13		-540.8 to -529.8	-534.77	3.40		-511.3 to -508.0	-509.22	0.85		-523.2 to -522.1	-522.49	0.30	

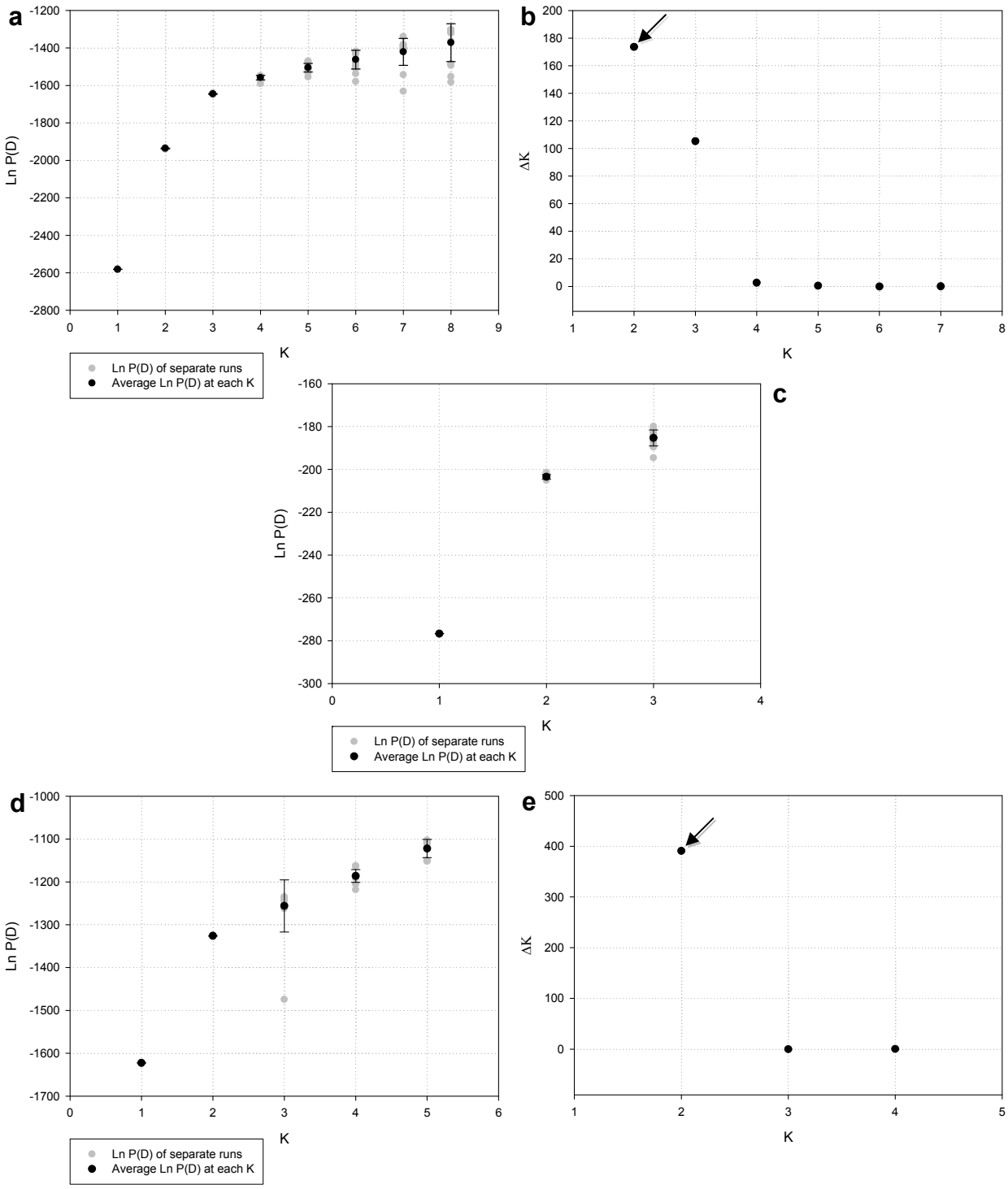

Figure 4.8a–e: The results of the Structure analyses of all the *S. johannis* populations (**a** & **b**), of all the northern *S. johannis* populations (**c**), and of all the southern *S. johannis* populations (**d** & **e**). **a**, **c** and **d** are plots of the posterior probability estimates (Ln P(D)) of the runs conducted under the no-admixture & correlated-allele-frequencies (NC) model, showing the Ln P(D) of each run at each K (the grey circles), the average Ln P(D) at each K (the black circles), and the standard deviation across runs at each K (the error bars). **b** & **e** depict ΔK for runs performed on the *S. johannis* data set as a whole, and for runs conducted on the southern populations only, respectively. ΔK represents the rate in change of Ln P(D) across successive values of K, and the K which possesses the highest ΔK (indicated by an arrow in each case) is therefore identified as the most likely number of clusters, as this is the smallest value of K that captures most of the structure in the data.

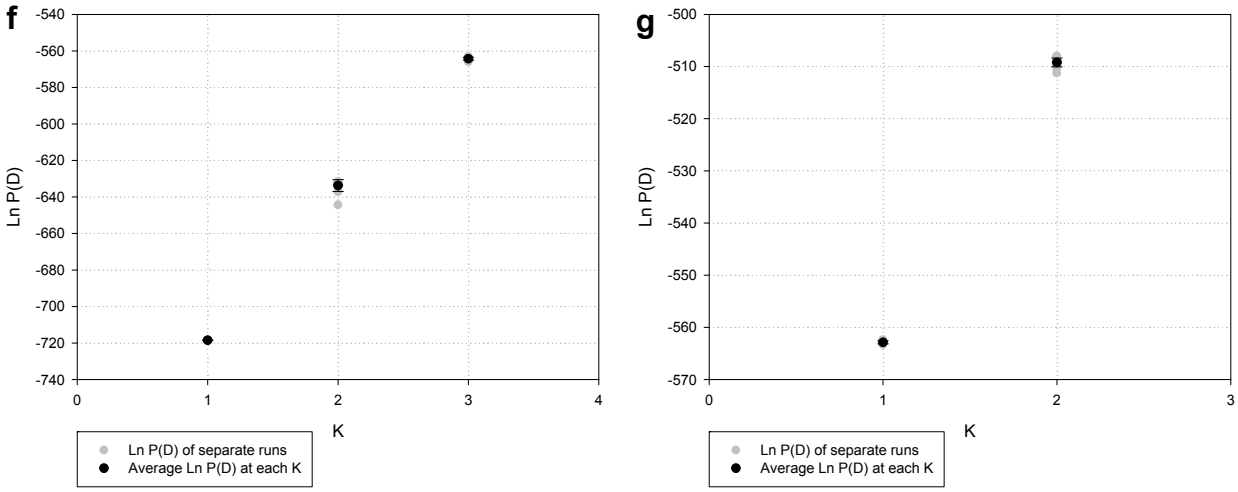

Figure 4.8f–g: Posterior probabilities (Ln P(D)) of successive numbers of clusters (K) for **f** the three northermmost southern *S. johannis* populations (jMyokane, jEmbotyi and jMagwaFalls) and **g** the two Mount Sullivan populations conducted under the no-admixture & correlated-allele-frequencies (NC) model, showing the Ln P(D) of each run at each K (the grey circles), the average Ln P(D) at each K (the black circles), and the standard deviation across runs at each K (the error bars).

jMyokane and the two MtSullivan populations) populations, and all of the eight populations were assigned to their corresponding clusters with a membership coefficient of > 90% (Figure 4.9a).

The analyses run under the NC model tended to produce the highest posterior probabilities (Table 4.3). Although there was some overlap between the Ln P(D)s produced by the AC and NC runs, the average Ln P(D) at K=2 for the NC model is significantly greater i.e. more than 2 log likelihoods greater, than the average for any of the other three models. The NC model therefore probably reflects the evolutionary history of *S. johannis* at its highest hierarchical level more accurately than do any of the other three models.

Consequently, based on the results of the Structure analyses, *S. johannis* appears to contain two main clusters, one comprising the northern populations (Cluster 1) and the other containing the southern populations (Cluster 2). The NC model produced the highest average posterior probabilities, implying that allele frequencies amongst the *S. johannis* populations are quite similar (correlated allele frequencies), and that there has been very little or no gene flow between the two clusters (no admixture). The similarity of allele frequencies suggests that these two clusters share a recent common ancestor (seeing as the NC model precludes the possibility that their similar allele frequencies could be the result of extensive gene flow between the two clusters).

In order to investigate substructure within *S. johannis*, data from the northern and southern clusters were then analysed separately in Structure. Analyses of the northern populations produced similar results to those of *S. johannis* as a whole, also optimising at K=2 and producing significantly higher Ln P(D) values using the NC model. Although the calculations of Evanno *et al*. (2005) cannot be applied to runs of three or fewer K proposals (because ΔK measures rate of change between successive proposals of K), an inspection of the results reveals that Ln P(D) increases to a much greater extent between K=1 and K=2 than between K=2 and K=3 (Figure 4.8c). Additionally, the standard deviation remains reasonably low at K=1 and K=2, but increases dramatically at K=3. The greater rate of change between K=1 and K=2, and the dramatic increase in variance from K=2 to K=3 indicates that the northern populations probably also contain two clusters. However, across all runs with K=2 and the NC model

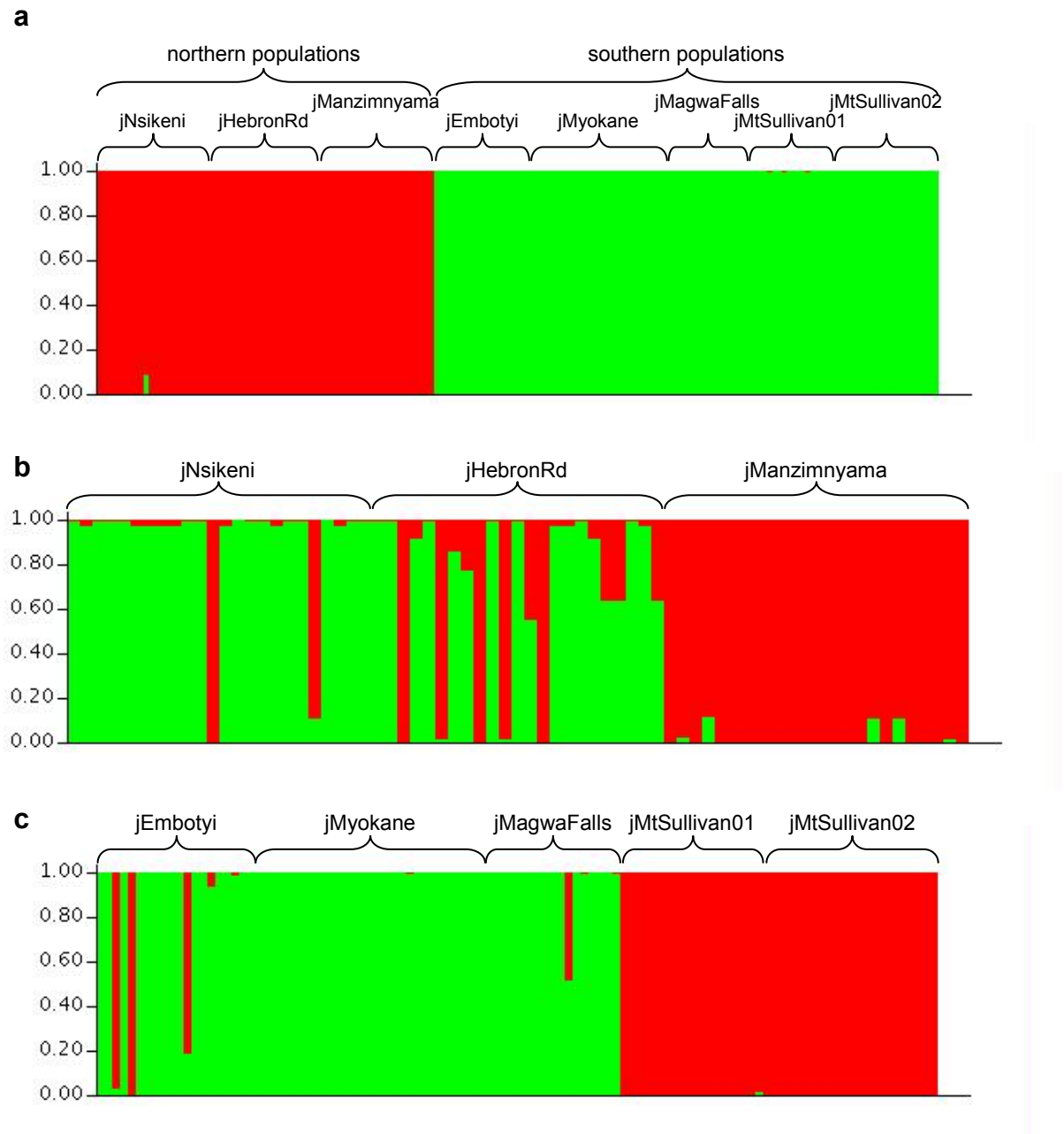

Figure 4.9: Plots of the membership coefficients of each individual in each cluster for the analysis of **a** all of the *S. johannis* populations, **b** the northern *S. johannis* populations, and **c** the southern *S. johannis* populations. Vertical lines represent individuals, and each vertical line is partitioned amongst the clusters, depending on the individual's estimated membership coefficient in each cluster. The two clusters identified amongst the southern *S. johannis* populations (Clusters 2.1 and 2.2) were identified as most likely each containing only one cluster, and bar plots for these two clusters are therefore not shown.

chosen, the individuals assigned to the two clusters do not correspond to present population boundaries (Figure 4.9b). jNsikeni and jManzimnyama (the most northern and most southern of the northern populations, respectively) consistently segregated into separate clusters (Clusters 1.1 & 1.2) with a membership coefficient of > 90%. However, the middle population, jHebronRd, was partitioned between the two clusters with 60% of the individuals being assigned to the cluster containing individuals from jNsikeni (Cluster 1.1), and about 30% of the individuals being assigned to the cluster containing jManzimnyama (Cluster 1.2). Therefore, this seems to imply that the jHebronRd population is a mixture of individuals with different genetic makeups. However, the relationship of these two northern clusters to each other i.e.

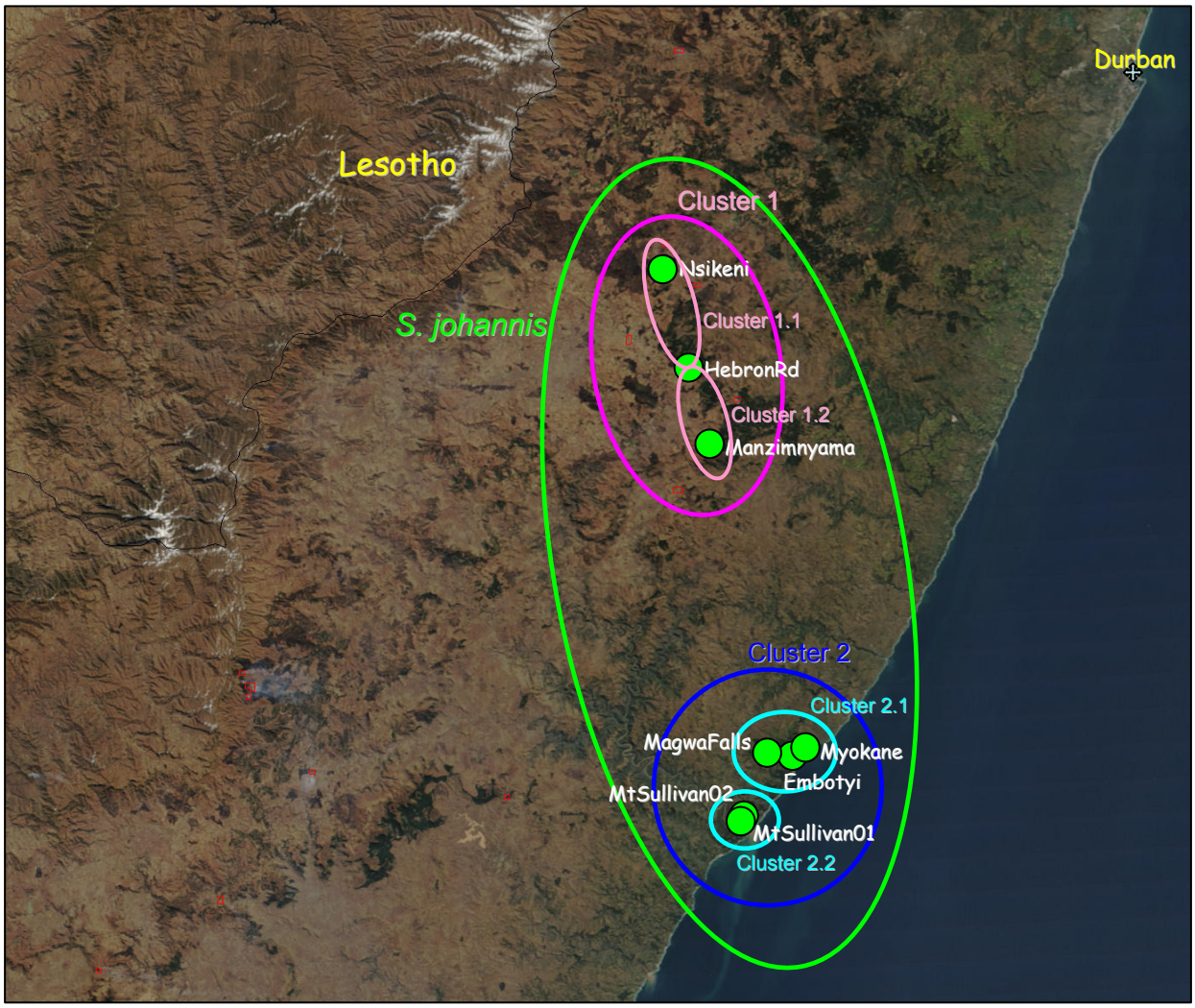

Figure 4.10: A map of the *S. johannis* population localities showing the populations contained in each of the clusters inferred from the Structure analyses. The background map was obtained from MODIS Rapid Response System at http://rapidfire.sci.gsfc.nasa.gov/.

whether the two clusters represent independent migrations from the north and south, respectively, or whether the one cluster spread from the north, branching into the southern cluster that in turn spread south or whether the one cluster spread from the south, branching into the northern cluster that in turn spread north, is unclear.

The southern populations also appear to consist of two clusters (Figure 4.8 d & e), one containing jEmbotyi, jMagwaFalls and jMyokane (Cluster 2.1), and the other containing the two MtSullivan populations (Cluster 2.2), with each population possessing membership coefficients of > 85% in their respective clusters (Figure 4.9c). This makes geographical sense, seeing as the two MtSullivan populations are together located much further away from the other southern populations. The NC model also produced significantly higher Ln P(D) values in these analyses.

These two southern population clusters (Clusters 2.1 and 2.2) were also each analysed in Structure, and appear to each constitute one cluster (Figure 4.8 f & g). However, the model most accurately reflecting the evolutionary history of each of these clusters could not be identified, as the Ln P(D) of runs conducted using different models produced similar results for each of these data sets (Table 4.3).

4.4. Discussion

Evolutionary patterns yielded by the microsatellite analyses are not only largely in accordance with the findings from the larger ITS and plastid sequence analyses of the previous chapter, but also provide additional resolution of relationships amongst the members of the Cape primrose clade included in the microsatellite analyses.

4.4.1. *S. primulifolius*

Of the species included in these microsatellite analyses, the *S. primulifolius* populations are the most scattered, interspersed amongst the populations of the other species in the population-level PCo plots, arising in four different clades in the population-level microsatellite trees, in two of the three main clades in the ITS sequence phylogeny and in three of the five main clades in the plastid sequence phylogeny. The analyses therefore suggest that *S. primulifolius* is the ancestor of the other species represented in the analyses.

Within *S. primulifolius*, the northern (npTableMountain, npStonesFarm and npMonteseel), central (cpRooivaal, cpEndliniyokozi, cpBulolоGorge, cpMtSullivan, cpDwalana, cpSilaka, cpXibeni and cpMbanyana) and southern (spWedgeley, spResKloof, spPostWellington, spFloradale, spIgoda and spRivendell) population groups tend to emerge separately in the analyses, with the central populations constituting the most diverse population group in the species, as was the case in Hughes *et al*. (2005). In comparison to the northern and southern population groups, the central populations contain the highest levels of genetic diversity, the highest average number of private alleles compared to the rest of the populations, are more scattered in the population-level PCo plots and plastid sequence phylogeny, and form a deeper group (are separated from one another by the longest branch lengths) in the population-level microsatellite trees, revealing their relatively old age relative to the northern and southern population groups. A comparison of the number of private alleles in each of the central *S. primulifolius* populations compared to the other central *S. primulifolius* populations and the number of private alleles of each of the central *S. primulifolius* populations compared to all of the populations included in the microsatellite analyses reveals that the peripheral populations of the central *S. primulifolius* population group (cpRooivaal to the north and cpXibeni and cpMbanyama to the south) share a greater number of alleles with other populations included in the microsatellite analyses than do the other, more central populations within this group (cpEndliniyokozi, cpBulolоGorge, cpMtSullivan, cpDwalana and cpSilaka). These peripheral central *S. primulifolius* populations therefore probably share closer links to other population groups than do the more central populations.

Although levels of genetic diversity between the northern and southern *S. primulifolius* groups (the southern population group referred to as *S.* cf. *primulifolius* in Hughes *et al*. 2005) are similar according to the diversity statistics, the northern populations harbour on average a higher number of private alleles per population, branch lengths amongst the northern populations tend to be longer in the population-level microsatellite trees and they together form a deeper group than the group containing the southern populations in these trees, indicating that the northern *S. primulifolius* populations probably diverged from one another after the central populations, but before the southern populations. The northern populations together constitute the most isolated cluster in the population-level PCo plots and emerge together in a strongly supported clade in the plastid sequence phylogeny, and separately from the central and southern *S. primulifolius* populations in the ITS phylogeny, highlighting the distinctiveness of this population group within *S. primulifolius*.

In accordance with the findings of Hughes *et al*. (2005), the southern *S. primulifolius* populations constitute the youngest group within *S. primulifolius*, clustering closely in the

population-level PCo plots and being separated by the shortest branches in the population-level microsatellite trees and the plastid sequence phylogeny.

The northern, central and southern *S. primulifolius* population groups also show different levels of inbreeding, with the northern populations showing an average inbreeding coefficient of 0.346, the southern populations 0.259, and the central populations possessing the lowest average levels of inbreeding (0.134). This is quite possibly due to the relative population densities of the different population groups and the effect this has on population numbers of the pollinator. Most of the central populations are concentrated in a very small area in the forested gorges of the Pondoland Centre. This relatively high concentration of populations probably can support larger pollinator population sizes than can the relatively sparsely distributed populations of the northern and southern *S. primulifolius* population groups. Higher pollinator population numbers would result in the *S. primulifolius* plants in the central populations being visited by pollinators more frequently than the plants in the northern and southern population clusters, resulting in the plants in the central populations selfing less frequently.

In summary, in keeping with the findings of the sequence analyses of the previous chapter, *S. primulifolius* shares links with many of the other core Cape primrose species. Within *S. primulifolius*, the greatest sequence and microsatellite genetic diversity is found in the central populations. These high levels of genetic diversity suggest that the species probably evolved towards the centre of its range before radiating towards the north, and later towards the south to form its extant populations.

4.4.2. *S. rexii*

Consistent with the findings of Hughes *et al*. (2005) and the ITS and plastid sequence analyses of the previous chapter, the *S. rexii* populations (rOtterspoort, rMtAyliff, rNtywenka, rKatberg, rKologha, rLeopardFalls, rVSRG, rTsitsikamma, rBloukransPass, rWilderness) emerge amongst the southern *S. primulifolius* populations in the population-level PCo plots and population-level microsatellite trees and in the ITS and plastid sequence phylogenies of this chapter, suggesting that *S. rexii* evolved from within the southern populations of *S. primulifolius*. Although the relationships in the present population-level microsatellite trees do not entirely agree with those in the microsatellite tree shown in Hughes *et al*. (2005), probably as a result of the addition of two extra populations (spResKloof and rWilderness) and the exclusion of three of the microsatellite loci from the analyses, both analyses show the same evolutionary patterns, with the northern and southern arms of *S. rexii* constituting separate lineages. All of the *S. rexii* populations show very low levels of genetic diversity, in accordance with the postulated young age of the species (Hughes *et al*. 2005). Furthermore, the microsatellite data show a tendency for a decrease in genetic diversity of the populations towards the north and south of the species' range, indicating that the populations towards the centre of *S. rexii*'s range are probably older than those towards the species' northern and southern extremities. Most of the populations in which the inbreeding coefficient could be estimated i.e. all populations in which all the individuals are not identical at all loci, showed significant deviations from Hardy-Weinberg equilibrium, consistent with the species being chiefly autogamous (Hughes *et al*. 2005).

4.4.3. *S. formosus*

S. formosus was viewed as a subspecies of *S. primulifolius* by Hilliard & Burtt (1971). Although Weigend & Edwards (1994a) raised it to specific level, they also viewed it as a very close relative of *S. primulifolius*. Morphologically, it differs from *S. primulifolius* only in floral characters. *S. formosus* possesses a pale violet corolla with bright yellow pigmentation on the floor of the corolla tube and a dark-stippled throat (Weigend & Edwards 1994a). In contrast,

S. primulifolius produces a pale bluish-violet corolla with a deep violet floral tube floor and five reddish-purple stripes lining the floral throat (Hilliard & Burtt 1971).

S. formosus is only represented by one population in these microsatellite analyses (fUmtanvuna), and not much can therefore be inferred regarding the affinities of this species as a whole. This population emerged in an unresolved position in the plastid sequence phylogeny of the present chapter and between many of the population groups in the population-level PCo plots, but sister to the Rooivaal population of *S. primulifolius* in the population-level microsatellite trees reconstructed from both distance matrices and in an unresolved position in the same clade with cpRooivaal in the ITS phylogeny of this chapter. The two *S. formosus* populations included in the sequence analyses of the previous chapter also emerged close to *S. primulifolius* populations, including Rooivaal, in the ITS sequence networks (Figures 3.9a, 3.10a, 3.11 & 3.12), but closest to *S. porphyrostachys*, a species not included in the microsatellite analyses, in the plastid sequence topologies (Figures 3.7, 3.9b, 3.10b, 3.13 & 3.14) of the previous chapter. Although *S. porphyrostachys* was not included in these microsatellite analyses, *S. formosus* and *S. porphyrostachys* are morphologically very dissimilar, and they are therefore probably not closely related to each other. The microsatellite and ITS sequence analyses and morphology therefore offer some support for *S. formosus* having arisen from *S. primulifolius*, but having subsequently perhaps hybridized with the geographically proximate *S. porphyrostachys* (both *S. formosus* and *S. porphyrostachys* occur in the Umtamvuna River Gorge, for example; Hilliard & Burtt 1971), in the process capturing its chloroplast.

4.4.4. *S. baudertii*

Of all the individual population groups analysed, the *S. baudertii* populations harbour the highest average number of private alleles (12.4) compared to their conspecific populations, indicating the uniqueness of the populations from one another within this species, with the Hillsdrift population being especially distinct within *S. baudertii*. In the population-level PCo plots, and especially in the population-level microsatellite trees, the *S. baudertii* populations form two separate groups, the one containing the three northernmost populations (bHarmony, bOtterspoort and bNtywenka) and the other the two southernmost populations (bCollywobbles and bHillsdrift). However, these two southernmost populations are each on long branches joined together by very short branches in both population-level microsatellite trees, and therefore possibly group together due to the absence of other taxa in these analyses rather than because they share a common ancestor. The three northernmost populations also emerge in the same polytomy in the ITS and plastid sequence analyses. However, the two southernmost populations consistently emerge separately from each other and the rest of *S. baudertii* according to both sequence markers. *S. baudertii* therefore emerges as a polyphyletic species in both the sequence and microsatellite analyses, with the three northernmost populations constituting a monophyletic group, and the two southernmost populations probably constituting two further evolutionary entities. The fact that the two keyhole species, *S. baudertii* and *S. johannis*, mostly emerge separately in the sequence and microsatellite analyses suggests that this is yet another case of the independent evolution of the keyhole floral type from the open-tubed type (based on their ITS sequence phylogenies, Harrison *et al*. 1999 and Hughes *et al*. 2006 detected four and eight independent origins of the keyhole floral type from the open-tubed and pouch types).

4.4.5. The hybrid population

According to the microsatellite population statistics, the hybrid population (hWedgeley) is the most diverse population included in the analyses. A high diversity is, however, to be expected in a population containing genetic material from different sources. The population also shows

significant deviations from Hardy-Weinberg equilibrium. The discrepancy between the observed and expected heterozygosities, which causes the significant deviation from Hardy-Weinberg equilibrium, is the result of an uneven distribution of alleles in the population as a result of a larger number of individuals being homozygous for their respective alleles than would be expected under random mating. This can be the result of individuals within the same population having different ancestral origins, and their alleles not having been dispersed enough by random mating to cause the alleles to be more evenly distributed within the population. The fact that the six members of the hybrid population are scattered throughout the individual-level PCo plots also suggests that its members have different histories. The different genetic affiliations of the members of this population are probably also the cause of the erratic position of this population in relation to the other populations in the PCo plots, the population-level microsatellite trees and in ITS and plastid phylogenies. It emerges in between many of the other population groups in the population-level PCo plots, in unresolved positions within the ingroups of both the ITS and plastid phylogenies, sister to the southern *S. primulifolius* and the *S. rexii* populations in the chord population tree, but sister to the central *S. primulifolius* populations in the PSA tree.

This population is believed to be the result of hybridization between *S. meyeri* and southern *S. primulifolius*. The microsatellite data of one of the putative hybrid individuals are indistinguishable at all but one locus from members of the spResKloof and spWedgeley (Mark Hughes, personal communication). These two latter populations are geographically situated very close to the hybrid population. The hWedgeley samples were collected from the upper edge of a cliff, while the spWedgeley samples were collected from a population situated 5–10 m below (Dirk Bellstedt, personal communication). The other individuals sampled from the hWedgeley population are, however, genetically quite different from those from spResKloof and spWedgeley and the other *Streptocarpus* populations genotyped so far, and possibly originate from a species that is not included in these microsatellite analyses. This species is most likely *S. meyeri*. Although microsatellite data from *S. meyeri* were not generated, the hWedgeley population shows a close relationship to the *S. meyeri* population collected at Cathcart, geographically the closest *S. meyeri* population to it, in both the ITS (Figures 3.5, 3.9a, 3.10a, 3.11 & 3.12) and plastid (Figures 3.7, 3.9b, 3.10b, 3.13 & 3.14) sequence analyses of the previous chapter. Although the *S. meyeri* Cathcart population is geographically not as close to the hWedgeley population as is the spWedgeley population, *S. meyeri* Cathcart nevertheless is found in the same drainage system as hWedgeley, situated further upstream of the Kei. However, the *S. meyeri* genetic material might have come from closer at hand. The environment around the hWedgeley population is suitable for *S. meyeri*, and there are quite possibly unsampled *S. meyeri* populations growing close by (Dirk Bellstedt, personal communication).

The hWedgeley population is also suspected of being a hybrid between *S. meyeri* and southern *S. primulifolius* due to its morphological intermediacy. Based on vegetative morphology, the members of the hybrid population are indistinguishable from *S. meyeri*, also possessing the small, round, hairy leaves that are probably adaptations to growing in drier environments. The hybrid's flowers are, however, more intermediate between the two species in both size and patterning (Dirk Bellstedt, personal communication). *S. primulifolius* flowers are large with pale bluish-violet floral lobes. The floral tube is deep violet and marked with five reddish-purple stripes that extend along the floor of the corolla tube and out onto the base of the floral lobes. *S. meyeri* flowers, in contrast, are much smaller than those of *S. primulifolius*, and possess white floral lobes and a violet floral tube marked with a yellow stripe (Hilliard & Burtt 1971). The flowers in the hybrid population are smaller than those of southern *S. primulifolius*, but larger than those of *S. meyeri*. The colour of the floral lobes of the hybrid flowers is also intermediate, being very pale. The floral tube floor of the hybrid population is also marked with

the stripes found in southern *S. primulifolius*, but, whereas in *S. primulifolius* these stripes extend out to a certain extent onto the floral lobes, the stripes are largely restricted to the floral throat in the hybrid population. The corolla floor is, however, also marked with a yellow stripe, similar to that found in *S. meyeri* (Dirk Bellstedt, personal communication).

The hybrid population therefore shows both genetic and morphological links to both *S. primulifolius* and *S. meyeri*, and this is probably the reason for its erratic behaviour in the microsatellite analyses.

4.4.6. *S. johannis*

Of all the species included in these analyses, *S. johannis* was analysed the most extensively. Deeper relationships amongst the *S. johannis* populations are unresolved in the ITS and plastid sequence phylogenies, but the microsatellite population-level trees and Structure analyses both indicate that the *S. johannis* populations share a common origin. However, *S. johannis* forms a paraphyletic group in the microsatellite population trees, with the *S. primulifolius* population from Rooivaal and the *S. formosus* population from Untamvuna emerging in the same group. This is similar to relationships portrayed in the ITS phylogeny, in which the southern *S. johannis* populations group with a number of other samples, including cPriRooivaal and forUmtamvuna. Geographically, these latter two populations are quite close to *S. johannis*, and these patterns might therefore suggest some genetic transfer.

The genetic data also give some indications of relationships within *S. johannis*. The diversity statistics show that the northern populations of *S. johannis* (jNsikeni, jHebronRd and jManzimnyama) are relatively homogenous. According to the ITS and plastid phylogenies, and both methods of calculating the distances amongst populations from the microsatellite data, the northern populations form a reasonably compact group in the population-level PCo plots and consistently group together in the population-level microsatellite and sequence trees, separated by zero-length or short branches. This is consistent with the northern populations constituting a reasonably young, monophyletic group. The Structure analyses including only the northern populations, however, produced unexpected results, with the northern populations consisting of two clusters, one consisting largely of the Nsikeni samples and most of the Hebron Road samples, and the other the Manzimnyama samples and the remaining Hebron Road samples.

The southern populations (jMagwaFalls, jEmbotyi, jMyokane, jMtSullivan01 and jMtSullivan02), in comparison, contain higher levels of genetic diversity, and together form a more spread out group in the population-level PCo plots and in the population-level microsatellite trees and plastid sequence phylogeny. The southern cluster therefore appears to be the older of the two clusters. Within the southern cluster, the Structure analyses identified two main clusters, one containing the geographically proximate populations collected from Magwa Falls, Embotyi and Myokane, and the other the two populations from Mount Sullivan. These clusters also emerge as strongly supported groups in the population-level microsatellite trees. These two clusters make geographical sense, seeing as the Mount Sullivan populations are quite far away (about 20 km) from the other three southern populations, which are about 7 km apart from each other.

There are, however, inconsistencies between the population-level microsatellite trees and the ITS and plastid sequence phylogenies concerning the relationship of the northern cluster to the southern populations. In the population-level tree constructed from chord distances, the northern populations emerge from amongst the southern populations, similar to the pattern in the plastid sequence phylogeny, while in the microsatellite population-level tree constructed from the PSA distance matrix, the northern populations group sister to the southern populations. This is more similar to the groupings in the poorly resolved ITS sequence phylogeny, in which

the northern cluster groups separately from the polytomy containing the southern populations. Although the genetic data are therefore not entirely clear concerning the relationships between the northern and southern clusters, the higher genetic diversity of the southern cluster, evident from both the plastid sequence and microsatellite analyses, suggests that the southern cluster is the older of the two, and therefore probably diversified first, while the northern cluster is much younger. Two alternative scenarios can consequently be envisaged. The first scenario is that ancestors of the southern and northern *S. johannis* clusters diverged from one another early on in the history of the species, each diversifying into populations. The northern populations largely died out during climatically more stressful periods e.g. during the Pleistocene glacials, with only a few individuals managing to hold on in a few more protected areas, while the southern populations, which occur in the reasonably protected gorges of the Pondoland Centre, survived these more arid periods reasonably intact. The northern populations could then have diversified again during wetter periods. This would produce the patterns portrayed in the PSA population-level tree, in which the two clusters appear to have formed at the same time, but the northern populations diversified from one another later on than did the southern populations. The alternative scenario is that the southern cluster evolved first and diversified before subsequently giving rise to the northern cluster, perhaps when the floral corridor between the Pondoland Centre and the KwaZulu-Natal Drakensberg mountains described in Van Wyk & Smith (2001) formed. This scenario is more in line with the patterns in the chord population-level tree and in the plastid sequence phylogeny.

Nevertheless, whatever the evolutionary relationship between the northern and southern clusters, the patterns depicted in the population-level PCo plots and the results of the Structure analyses both indicate that the northern and southern populations are currently genetically distinct from each other, and that there has therefore been very little subsequent gene flow between as well as within the two population clusters. In the population-level PCo plots, the two population groups cluster separately from each other, and the Structure analyses of all the *S. johannis* populations identified two main clusters, one containing all the northern populations and the other containing all the southern populations. Moreover, the model producing the highest likelihood values in the Structure analyses including all of the *S. johannis* populations, only the northern populations, and only the southern populations, was one specifying no admixture, indicating that there has been very little subsequent gene flow between and within these two clusters since their divergence.

Another facet of evolution in *S. johannis* revealed by these microsatellite analyses is that all of the populations in both clusters show significant deviations from Hardy-Weinberg equilibrium towards inbreeding. This can be explained in terms of the development of the flowers of *S. johannis*. *S. johannis* possesses the keyhole floral type i.e. its flower is characterised by a narrow, strongly S-shaped floral tube and a laterally compressed floral mouth, the mouth resembling a keyhole since it is slightly narrower at the centre (Hilliard & Burtt 1971). The stamens arise halfway up the floral tube (Hilliard & Burtt 1971), and are positioned towards the top of the floral tube at maturity (Trevor Edwards, personal communication). As the flower develops, the stamens mature faster than the pistil, and are therefore carrying mature pollen grains while the style is still quite short. The stigma also matures quite early, becoming receptive to pollination before the style has reached its full length. As the flower matures, the style elongates down the floral tube. When the style has almost reached its full length, the stigmatic surface is forced into contact with the anthers, thereby effecting self-pollination. This is therefore a mechanism to ensure that pollination still occurs in the absence of a pollination vector. The fact that the stigma becomes receptive quite early on means that there is an opportunity for outcrossing to occur. However, if outcrossing does not occur, then the stigma will still be receptive when it reaches the anthers, enabling self-pollination to take place (Trevor Edwards, personal communication). The autogamy of *S. johannis* is also evident in greenhouse

plants, which show a high seed set in the absence of pollinators. Plants growing in nature also show excellent seed set (Trevor Edwards and Dirk Bellstedt, personal communication). *S. johannis* is therefore another example of an autogamous species, as is the case with *S. rexii*.

The precocious development of the stigma on an immature gynoecium is characteristic of all keyhole flowers that have been examined (*S. johannis*, *S. polyanthus*, *S. prolixus*, *S. silvaticus*, *S. wendlandii*, *S. daviesii*, *S. pole-evansii*, *S. trabeculatus*, and the keyhole form of *S. porphyrostachys*). However, most of the populations of *S. baudertii*, the other keyhole species included in these analyses, do not show significant levels of inbreeding. Although *S. baudertii* is capable of selfing (Dirk Bellstedt, personal communication), this species is a grassland plant, and its distribution overlaps with that of the common tabanid fly *Philoliche aethiopica* Thunberg. The overlapping distributions as well as the floral tube length of *S. baudertii* make this fly a good candidate as the pollinator of *S. baudertii*, and *Philoliche aethiopica* possibly visits *S. baudertii* plants frequently enough to ensure that self-pollination does not occur very often (Trevor Edwards, personal communication).

4.5. Conclusions

The results of the microsatellite analyses are largely congruent with those of the sequence analyses, but provide additional resolution amongst these closely related taxa due to the more rapid pace of evolution of microsatellites.

S. primulifolius is the most scattered species in the population-level PCo plots, and emerges throughout the population-level microsatellite and sequence trees, indicating that it is quite possibly the ancestor of the other species included in the analyses. However, a cautionary note should be added here. Due to the nature of microsatellite regions, they evolve at a much more rapid rate than do other regions e.g. the nuclear ITS region and plastid regions, and the signal quickly becomes saturated as one uses microsatellite data to reconstruct relationships further back in time. For this reason, only species that appeared to share the closest relationships based on the larger sequence topologies of the previous chapter were included in the microsatellite analyses. However, despite this many of the genetic distances calculated amongst the more distantly related groups in these microsatellite analyses were approaching 1, and the deeper relationships should therefore be regarded with more caution. Having stated this, populations of all of the species included in these microsatellite analyses group closely with *S. primulifolius* populations in the larger ITS and plastid sequence topologies of the previous chapter (except, that is, for the hybrid population, which groups most closely with *S. meyeri* populations in the sequence topologies), and a close linkage with *S. primulifolius*, whether it is as the result of evolution from *S. primulifolius* or due to subsequent gene flow with *S. primulifolius*, is therefore believable. The data indicate that *S. primulifolius* evolved in the Pondoland Centre, later spreading towards the north and then towards the south.

The *S. rexii* populations contain low levels of genetic diversity, and emerge amongst the southern *S. primulifolius* populations, forming two groups in the population-level microsatellite trees, one mostly containing the northernmost populations, and the other mostly containing the southernmost populations. The results of the current analyses are therefore consistent with the findings of Hughes *et al*. (2005), who inferred that *S. rexii* evolved three or more times from the southern *S. primulifolius* populations before spreading north and south.

The *S. formosus* population included shares its closest links with the *S. primulifolius* population collected from Rooivaal. *S. formosus* therefore also appears to have evolved from within *S. primulifolius* in the northern Pondoland Centre, which is also the current geographical locality of *S. formosus*.

The *S. baudertii* populations are genetically quite distinct from one another and cluster into separate groups in the microsatellite and sequence analyses, and *S. baudertii* is therefore probably a polyphyletic species.

These analyses support the suspected hybrid origin of the hWedgeley population to a certain extent. One of the six samples is very similar to the southern *S. primulifolius* populations collected from the same area, but the other five samples are rather different from the other samples included in these analyses, possibly coming from *S. meyeri*, the species that this population groups mostly closely with in the larger sequence topologies of the previous chapter.

S. johannis samples emerge together based on the microsatellite analyses (although with the fUmtamvuna and cpRooivaal also emerging in this group). Within this group the *S. johannis* samples group into two main genetic clusters: the younger northern population cluster and the older southern populations. The analyses indicate that since the divergence of these two clusters, there has been very little or no subsequent gene flow between them. All of the *S. johannis* populations show significant levels of inbreeding, which is probably due to the way in which *S. johannis* flowers develop.

Thus, the microsatellite data provided additional resolution of relationships amongst the populations and shed more light on the breeding systems in the species analysed, but due to the fast evolution of microsatellite loci, some of the deeper relationships might be the results of homoplasy rather than synapomorphies. More analyses, perhaps involving the generation of sequence data from a number of quickly evolving, unlinked nuclear regions would provide both suitable amounts of resolution of relationships amongst the populations without the signal being saturated, and would also help to detect hybridization events.

Chapter 5: General conclusions and future work

The objectives of this study, which were to establish the extent of the Cape primrose clade and determine the evolutionary position of the Cape primrose clade within South African *Streptocarpus*, to determine relationships amongst the members of the Cape primrose clade, to investigate modes of evolution in the South African *Streptocarpus* species, to identify geographical routes along which members of the Cape primrose clade have radiated, and to attach a time scale to evolution of *Streptocarpus* in South Africa, were largely met.

The analyses of the plastid and nuclear sequence data in chapter 3 contributed to determining the boundaries of the Cape primrose clade within *Streptocarpus* of South Africa. The topologies reconstructed from the sequence data from both genomes indicate that 16 species, the core Cape primrose taxa, are closely related. These are *S. primulifolius*, *S. rexii*, *S. johannis*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana*, the members of the *S. cyaneus* complex (*S. cyaneus*, *S. parviflorus*, *S. fenestra-dei*, *S. kunhardtii* and *S. roseo-albus*), *S. floribundus*, *S. aylae* and *S. kentaniensis*. In contrast, five species (*S. denticulatus*, *S. dunnii*, *S. pusillus*, *S. rimicola* and *S. bolusii*) consistently emerged outside of the clade into which the core Cape primrose species emerged according to the data from both genomes, indicating that they are only distantly related to the core Cape primrose species. However, incongruences between the topologies of the different genomes hindered the determination of the affiliation of the ten remaining species included in the sequence analyses, namely *S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus*, *S. longiflorus*, *S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*. These species emerged amongst the core Cape primrose species according to the sequence data from one of the genomes, but outside of the clade containing the core Cape primrose species in the topologies reconstructed from the sequence data from the other genome, and the molecular data therefore provide conflicting signals regarding the relationships of these ten species relative to the other taxa included in the analyses. However, a consideration of vegetative morphology (Hilliard & Burtt 1971; Weigend & Edwards 1994a, 1994b; Edwards 2003; Bellstedt & Edwards 2004; Edwards *et al*. 2008, in press) and palynology (Weigend & Edwards 1996) might indicate the relationships of these ten species. The core Cape primrose species are all rosulates, and most possess pollen type 13 (although *S. johannis* possesses type 8 and *S. baudertii* type 4). In contrast, the five species that consistently emerged outside the core Cape primrose species clade are all unifoliates/plurifoliates that possess different pollen types: *S. dunnii* with pollen type 9, *S. pusillus*, *S. rimicola* and *S. bolusii* type 11 and *S. denticulatus* type 13. Five of the ten species with uncertain affiliations (*S. meyeri*, *S. montigena*, *S. fanniniae*, *S. caeruleus* and *S. longiflorus*) are rosulates possessing pollen type 13 (although *S. longiflorus* was not palynologically assessed by Weigend & Edwards 1996), and are therefore morphologically and palynologically more similar to the core Cape primrose species. In contrast, the other five species of uncertain affiliations (*S. polyanthus*, *S. saundersii*, *S. porphyrostachys*, *S. grandis* and *S. vandeleurii*) are more similar to the five species consistently emerging in more distant positions relative to the core Cape primrose species in being unifoliates/plurifoliates and possessing a variety of pollen types (*S. polyanthus* with type 8, *S. saundersii*, *S. porphyrostachys* and *S. vandeleurii* type 12, and *S. grandis* type 13). A consideration of ITS sequence data, plastid sequence data, vegetative growth type and pollen type suggests that most of the South African rosulate taxa probably share a common origin amongst the South African *Streptocarpus* species, which evolved from amongst the palynologically more variable unifoliates/plurifoliates species.

The molecular analyses also resolved relationships amongst the core Cape primrose species to a certain extent. *S. primulifolius* is a species with a large, reasonably central geographical

distribution range within the core Cape primrose species, extending from the Durban area in KwaZulu-Natal south along the South African coast to the East London area in the Eastern Cape. The populations from the northern, central and southern parts of *S. primulifolius*' range included in the analyses emerged scattered amongst populations of many other Cape primrose species in the nuclear and/or plastid sequence analyses, including *S. rexii*, *S. baudertii*, *S. modestus*, *S. formosus*, *S. gardenii*, *S. lilliputana* and *S. johannis*. The sequence analyses therefore indicate that *S. primulifolius* is either ancestral to or has hybridized with many of the other KwaZulu-Natal–Eastern Cape *Streptocarpus* species. Of these species, populations of *S. rexii*, *S. johannis* and *S. baudertii* grouped closely with *S. primulifolius* populations in both the nuclear and plastid sequence analyses, and were therefore selected for the finer scale microsatellite analyses presented in chapter 4. The sequence and microsatellite analyses confirmed the findings of Hughes *et al*. (2005) that *S. rexii* is a polyphyletic species that evolved more than once from amongst the southern *S. primulifolius* populations. The genetic data also indicate that *S. baudertii* is polyphyletic, with the three northernmost populations constituting an evolutionary entity separate from the two southernmost populations according to all of the genetic data. The two southernmost *S. baudertii* populations appear to constitute two further separate evolutionary entities. Geographically, *S. johannis* has a disjunct distribution, with the northern populations separated by a little less than 100 km from the southern populations. The genetic data largely reflect this geographical disjunction, with the younger northern population cluster either having arisen from within the older southern population cluster, or being sister to it. The species belonging to the *S. cyaneus* complex form a more isolated entity within the Cape primrose clade, showing far fewer links with the other core Cape primrose species.

The incongruences between the nuclear and plastid topologies and the tendency for populations to group with geographically proximate populations of other species rather than with conspecific populations in the genetic analyses highlights the predominance of hybridization amongst proximate *Streptocarpus* species. However, more direct evidence of the occurrence of hybridization was also found in the *S. meyeri* population from Bastervoetpad and in the hybrid population from Wedgeley. The ITS sequences generated from the six *S. meyeri* plants collected from Bastervoetpad shared six polymorphic sites, and the same six individuals possessed one of two distantly related plastid haplotypes. The single representative of the hybrid population from Wedgeley consistently grouped with the *S. meyeri* sample from Cathcart in both the ITS and plastid sequence analyses. However, the hybrid population was morphologically intermediate between *S. meyeri* and southern *S. primulifolius*, and the microsatellite data generated from the six plants showed that the microsatellite profile of one of the individuals is indistinguishable from those of southern *S. primulifolius* individuals collected close by, while the microsatellite profiles of the other five individuals are more distinct, probably originating from *S. meyeri* (which was not included in the microsatellite analyses). Another facet of evolution in *Streptocarpus* appears to be the evolution of autogamy, documented in *S. rexii* by Hughes *et al*. (2005) and detected in *S. johannis* in this current study. Autogamy enabled *S. rexii* to spread into new territory (Hughes *et al*. 2005), and was possibly also what enabled *S. johannis* to spread inland to occupy the northern part of its current range. A third facet of evolution in *Streptocarpus* confirmed by the genetic patterns obtained in this study is the very limited or lack of gene flow amongst conspecific populations. In most of the species, conspecific populations did not form monophyletic groups, probably as a result of a combination of the young age of the species and hybridization. Recent evolution would mean that lineage sorting has not run to completion, and hybridization would result in certain populations capturing distantly related genetic material. These two factors combined with a lack of gene flow amongst conspecific populations would result in patterns observed in the genetic topologies, with populations of a species remaining genetically distinct rather than being swamped by genetic material from other parts of the same species, which is what would happen

if gene flow were more frequent. The limited gene flow evident in many of the species analysed in the current study and in Hughes *et al*. (2007) is due to both the paucity of pollinators and habitat fragmentation. Despite many hours of pollinator vigils in the field, pollinator data are lacking for most species. Forest is currently a very fragmented vegetation type, covering only about 0.56% of the South African land surface area (Low & Rebelo 1996). Futhermore, forest has probably been even more fragmented during recent historical periods (Eeley *et al*. 1999; Bond *et al*. 2003a, 2003b).

Relationships amongst and within the species also indicated possible historical geographical routes along which *Streptocarpus* might have spread. The forest patches of the Pondoland Centre contain both the greatest density of core Cape primrose species and the highest levels of genetic diversity in species that also occur elsewhere (e.g. *S. primulifolius* and *S. johannis*), indicating that this area has provided a protected habitat in which *Streptocarpus* populations could persist during extreme palaeoclimatic periods such as the Pleistocene glacial maxima (Hughes *et al*. 2005). The current geographical distribution of the *S. cyaneus* complex closely resembles the historical migration routes followed by faunal lineages after the Last Glacial Maximum (and probably also after previous glacial maxima during the last one million years) inferred by Lawes *et al*. (2007), and the *S. cyaneus* complex probably also spread north and/or south along this route. The older, southern *S. johannis* populations form an older cluster that is geographically separate from the younger, northern cluster of *S. johannis* populations, and *S. johannis* quite possibly spread from the south to the north via the floral migration route between the Pondoland Centre and the KwaZulu-Natal Drakensberg mentioned in Van Wyk & Smith (2001). *S. rexii* followed yet another migration route in the process of spreading into the areas that it currently occupies.

Thus, while these genetic analyses have elucidated evolutionary relationships and evolutionary processes in *Streptocarpus*, many questions remain unanswered. Within the Cape primrose clade, incomplete lineage sorting, hybridization and the lack of resolution within the extensively sampled clades into which the core Cape primrose species emerged in the nuclear and plastid sequence analyses obscured the relationships amongst many of the core Cape primrose species. Some of these species do not share close enough relationships to enable relationships to be unravelled with rapidly evolving marker types such as microsatellites, and these relationships would have to be determined by rapidly evolving sequence markers. The analysis of several unlinked sequence regions would help in unravelling relationships, even in the presence of incomplete lineage sorting and hybridization. Another aspect that was not explored in many of the species is intrapopulation variation. While up to 32 plants/population were analysed in the species included in the microsatellite analyses (*S. primulifolius*, *S. rexii*, *S. baudertii*, *S. johannis*, one population of *S. formosus* and one hybrid population), only one or two plants/population were included in the sequence analyses, and intrapopulation variation for these species are therefore largely undetermined. Analyses such as microsatellites and restriction fragment length polymorphisms would provide insight into intrapopulation variability. Biogeographical aspects have also not been fully resolved. Möller & Cronk (2001b) inferred that *Streptocarpus* arrived in South Africa from further north. However, only two *Streptocarpus* species from outside of South Africa were included in these current analyses, and the routes that *Streptocarpus* followed to reach South Africa and the different southern African lineages to which the South African species belong could therefore not be exactly ascertained. This would require the inclusion of species from Mozambique, Zimbabwe, and from further north in future analyses. Moreover, amongst the South African species only 31 of the 52 *Streptocarpus* species described to date were included in these analyses, and the exact position of the Cape primrose clade amongst the South African species could therefore not be determined. The addition of more South African species to these analyses would clarify relationships.

This current study has therefore not only highlighted some unresolved issues, but has also laid a firm foundation for future studies of evolution in southern African *Streptocarpus*.

Chapter 6: References

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Appendix A: DNA extraction protocol

Total genomic DNA was extracted using the following modified version of Doyle & Doyle's (1987) 2 × hexadecyltrimethylammonium bromide (CTAB) extraction method:

1. Silica-dried or fresh leaf tips measuring *ca*. 1 cm × 1 cm were ground using a tissue lyser (Southern Cross Biotechnology (Pty) Ltd, Cape Town, South Africa) in 500 µl of 2 × CTAB extraction buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA, 2% CTAB), 0.4% β-mercaptoethanol and *ca*. 10 mg of polyvinylpyrrolidone (PVP). The homogenised samples were then incubated at 60°C for 45 minutes, inverting the tubes every 15 minutes to mix the contents.

2. To each sample, 500 µl of chloroform-isoamyl alcohol (24:1, v/v) was then added and mixed for 10 minutes.

3. This was followed by a centrifugation step at 7 000 × gravity (g) for five minutes to separate the lighter aqueous phase containing the DNA from the heavier organic phase.

4. The supernatant containing the DNA was then retrieved and transferred into a new Eppendorf tube, to which *ca*. 2.5 × the volume of the supernatant of cold isopropanol was added. The samples were then refrigerated at -18°C for at least 30 minutes to aid precipitation of the DNA.

5. Following refrigeration, the DNA was centrifuged at a low speed (3 000 × g) for 5 minutes. The supernatant was carefully pipetted off before adding 1.5 ml of wash buffer (1 part 40 mM NH_4Ac [ammonium acetate] to 3 parts 100% ethanol by volume), dislodging the DNA pellet from the side of the tube, and leaving the tubes to lie on their sides for 20 minutes to give the wash buffer time to wash the DNA pellet thoroughly.

6. The samples were then centrifuged at 3 000 × g for two minutes, the supernatant was again poured off, and the samples were allowed to dry before redissolving the DNA in 100 µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA).

7. The samples were left to dissolve overnight in the fridge at about 4°C before undergoing a further precipitation step. This entailed adding 200 µl of deionised (Milli-Q) water, 150 µl of 7.5 M NH_4Ac and 1.125 ml of cold ethanol to the 100 µl DNA sample. The samples were centrifuged at 10 000 × g for 10 minutes and the supernatant was then removed. The samples were allowed to air-dry before redissolving them in 100 µl of TE buffer overnight.

Appendix B: Images of key *Streptocarpus* taxa

Figure B.1: *S. primulifolius* Endliniyokozi, a typical representative of the Pondoland central *S. primulifolius* populations.

Figure B.2: *S. rexii* Leopard Falls, showing many seed capsules, a result of the species being autogamous.

Figure B.3: *S. primulifolius* Rivendell, a representative of the southern *S. primulifolius* populations.

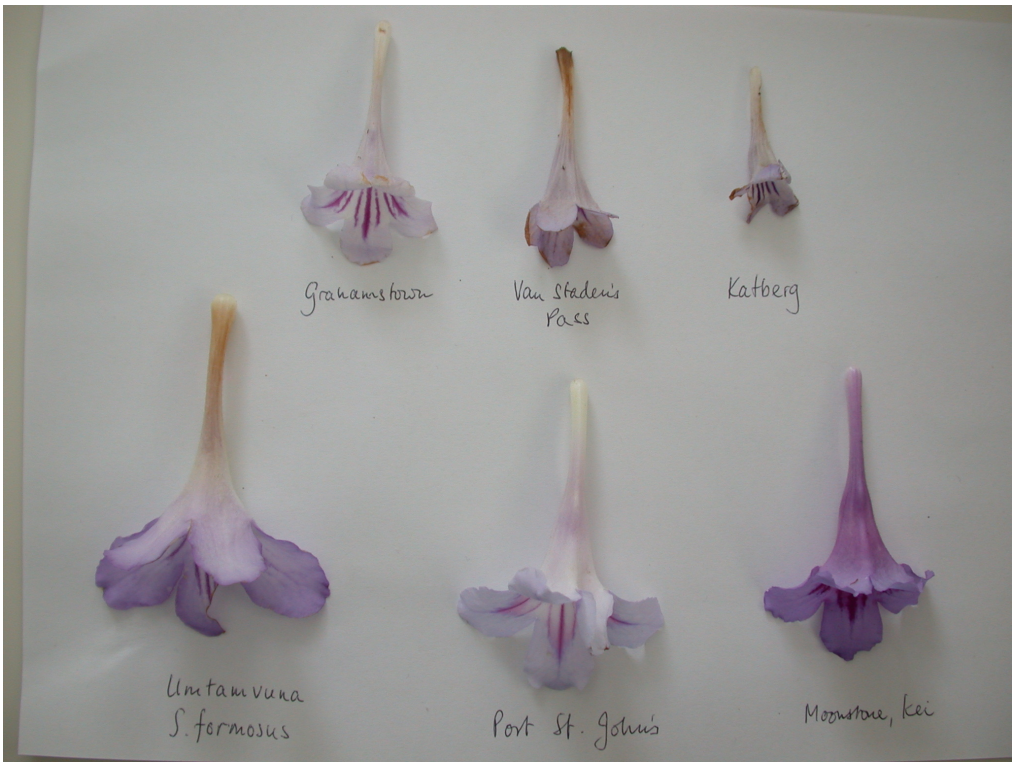

Figure B.4: A comparison of the corollas of *S. primulifolius* Dwalana (central populations indicated as "Port St. John's"), *S. primulifolius* Wedgeley (southern populations indicated as "Moonstone, Kei"), *S. primulifolius* Rivendell (southern populations indicated as "Grahamstown"), *S. rexii* Van Staden's Pass (southern *S. rexii*) and *S. rexii* Katberg Pass (northern *S. rexii*).

Figure B.5: The enlarged corolla of *S. johannis* Hebron Road, a representative of the northern *S. johannis* populations, illustrating the keyhole floral morphology.

Figure B.6: *S. johannis* Hebron Road, a representative of the northern *S. johannis* populations, showing it growing in nature.

Figure B.7: The enlarged corolla of *S. johannis* Nsikeni, a representative of the northern *S. johannis* populations.

Figure B.8: *S. johannis* Nsikeni, a representative of the northern *S. johannis* populations, showing it growing in nature.

Figure B.9: The enlarged corolla of *S. johannis* Mt. Sullivan, a representative of the southern *S. johannis* populations.

Figure B.10: *S. johannis* Mt. Sullivan, a representative of the southern *S. johannis* populations, showing it growing in nature.

Figure B.11: The enlarged corolla of *S. johannis* Magwa Falls, a representative of the southern *S. johannis* populations.

Figure B.12: *S. johannis* Magwa Falls, a representative of the southern *S. johannis* populations, showing it growing in pot culture.

Figure B.13: The enlarged corolla of *S. johannis* Myokane, a representative of the southern *S. johannis* populations.

Figure B.14: *S. johannis* Myokane, a representative of the southern *S. johannis* populations, showing it growing in nature.

Figure B.15: The unique corolla morphology of *S. lilliputana*.

Figure B.16: *S. modestus*

Figure B.17: *S. baudertii* Harmony, the most northerly *S. baudertii* population, illustrating its morphology and seed set.

Figure B.18: *S. baudertii* Otterspoort, another northern *S. baudertii* population.

Figure B.19: *S. baudertii* Hillsdrift, the most southerly *S. baudertii* population.

Figure B.20: *S. baudertii* Collywobbles, a central *S. baudertii* population.

Figure B.21: *S. meyeri* Graaff-Reinet, illustrating its morphology, which is uniform throughout its southerly range.

Figure B.22: *S. modestus* Katberg Pass, illustrating its morphology in high-altitude cliff grassland.

Figure B.23: *S. cyaneus* subsp. *longi-tommii* Mt. Formosa, illustrating the morphology of *S. cyaneus* in general, although considerable corolla length and colour variation occurs in this species and its allies.

Figure B.24: *S. caeruleus* Lajuma, illustrating its morphology and growth habit in a cliff crevice.