

**MOLECULAR SYSTEMATIC STUDY OF  
SOUTHERN AFRICAN *OXALIS*  
(OXALIDACEAE)**

by

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## **Declaration**

By submitting this thesis 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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## SUMMARY

The genus *Oxalis* forms a major part of the flora of southern Africa, in particular the Cape Floristic Region (CFR) at the southwestern tip of the continent, but the current taxonomy is outdated and ecological knowledge of the lineage is sadly incomplete. In this thesis I set out to address several aspects of *Oxalis* systematics that urgently require attention.

Firstly, the current macro-morphological taxonomy requires phylogenetic testing, as it is acknowledged to be incomplete and artificial. I address this need by providing a DNA sequence-based phylogeny of three markers, using three different inference methods, for nearly three quarters of the indigenous species. This phylogeny confirmed both the monophyly of the southern African taxa, and the artificiality of the current classification system. It is congruent with previous sequence-based reconstructions of smaller groups of southern African *Oxalis* species, and with the palynological classification proposed for the genus.

Secondly, previous phylogenetic work on the southern African members could not resolve basal relationships within the southern African clade. I attempt to address this problem by sequencing three extra chloroplast markers for a select group of taxa, followed by separate and combined (total evidence) molecular phylogenetic analyses. This approach did increase resolution at the base of the southern African lineage, but many clades still showed poor resolution and support despite the use of more than 7 000 bases of sequence data. Resolving these clades within the southern African *Oxalis* phylogeny remains a challenge, and should prove a fertile field for future research.

Thirdly, the ages (and thus duration of presence) of many Cape plant lineages within the CFR are of major interest, given that the CFR represents a global biodiversity hotspot. The age of the genus in the Cape is estimated by analyzing combined sequence data for all sampled taxa under both a Bayesian Relaxed Clock and a semi-parametric Penalised Likelihood method, using calibration points inferred from Relaxed Clock analyses of the entire order Oxalidales, for which fossil data are available. In an attempt to account for known problems with divergence time estimation, I explored the potential bias introduced by method used, marker genome source and different calibrations on the root. The results indicate substantial variation in the age of crown southern African *Oxalis* over a nearly twenty million year period, varying according to source data, calibration estimate and methodology employed in the reconstruction. Despite this major variability, all average estimates are older than

18 million years, which agrees with a growing body of evidence that there has been a gradual accumulation of floristic diversity in the CFR, rather than a rapid, recent burst of speciation.

Fourthly, as the produced phylogenies conclusively show the artificial nature of the current taxonomy, I propose a new, almost completely different classification for southern African *Oxalis* taxa. Although a significant improvement, this classification is considered informal due to the complete disagreement between the old and proposed new taxonomies, poor resolution in some of the proposed lineages, and a need to confirm proposed groups (clades) with the identification of morphological synapomorphies. Potential synapomorphies for various clades are proposed and discussed, which should guide future research.

Fifthly, the presence of bulbs in this genus is of great interest as a potential pre-adaptation for seasonally arid climates. The evolution of the bulbous habit in *Oxalis* is here explored for the first time. I address the sequence of major morphological character state changes leading to the suite of characters corresponding to the bulbous habit. The homology of basal leaf petioles, fleshy leaf scales and tunics is discussed, and it is shown that many bulb characters present in the southern African lineage are also found in the close relatives of this lineage, and are thus older than this lineage. The ecological and evolutionary implications of bulb geophytism in the CFR *Oxalis* are also discussed.

Finally, I address certain taxonomic issues that arose during the course of this study. Co-authors and I describe the new species *O. saltusbelli* and *O. ericifolia*. We also clarify issues surrounding the tremendously variable group species *O. flava* and propose some nomenclatural changes and synonyms for related taxa. We also address the taxonomic position of the rare species *O. purpurata*, which was located too late in the course of this study to include in the main analyses.

## OPSOMMING

Die genus *Oxalis* vorm a belangrike komponent van die flora van Suider-Afrika, veral van die Kaapse Floristiese Streek (KFS) aan die suid-westelike punt van die kontinent. Ongelukkig is die mees onlangse taksonomiese klassifikasie verouderd en is ekologiese inligting vir hierdie groep beperk. In hierdie studie adresseer ek verskeie aspekte van *Oxalis* sistematiek wat dringend aandag benodig.

Eerstens moes die huidige makromorfologiese taksonomie onderwerp word aan filogenetiese toetsing, aangesien dit erken word as onvolledig en onnatuurlik. Ek spreek dit aan deur 'n DNS basisvolgorde-gebaseerde filogenie te verskaf wat op drie merkers gebaseer is en met die gebruik van drie verskillende deduksie-metodes verkry is, vir ongeveer driekwart van alle inheemse spesies. Hierdie filogenie bevestig beide die monofilie van die Suider-Afrikaanse taxa en die kunsmatigheid van die huidige klassifikasie sisteem. Dit stem ooreen met vorige basisvolgorde-gebaseerde rekonstruksies van kleiner groepies suider-Afrikaanse *Oxalis* spesies, en met die palinologiese klassifikasie wat vir die genus voorgestel is.

Tweedens kon vorige filogenetiese studies van die Suider-Afrikaanse lede van *Oxalis* nie die basale verwantskappe tussen die Suider-Afrikaanse groep ("clade") verklaar nie. Ek probeer hierdie probleem aanspreek deur die basisvolgordes van drie addisionele chloroplas merkers van 'n geselekteerde groep taksa te bepaal, en dit deur beide aparte en gekombineerde (totale bewys) molekulêre filogenetiese analyses te ondersoek. Hierdie benadering het die basale resolusie en steun binne die Suider-Afrikaanse groep ("clade") verhoog, maar verskeie interne groepe ("clades") het steeds swak resolusie en ondersteuning ten spyte van die gebruik van meer as 7 000 basispare data.. Resolusie van hierdie groepe ("clades") binne die Suider-Afrikaanse *Oxalis* filogenie bly 'n uitdaging, en behoort 'n interessante veld te bied vir toekomstige navorsing.

Derdens is die ouderdom (en dus duur van aanwesigheid) van baie plant ontwikkelingslyne binne die KFS van groot belang, gegewe dat die KFS 'n belangrike biodiversiteits brandpunt verteenwoordig. Die ouderdom van *Oxalis* in die KFS is beraam deur analyses van gekombineerde data vir alle versamelde spesies met gebruik van beide die "Bayesian Relaxed Clock" en semi-parametriese Gepenaliseerde Waarskynlikheids metodes. Kalibrasiepunte is afgelei uit "Relaxed Clock" analyses van die hele orde Oxalidales, waarvoor fossiele data beskikbaar is. In 'n poging om bekende probleme van afstammelingstydskatting te adresseer, ondersoek ek potensiële

vooroordele wat ingevoeg word deur die metodes gebruik, die genoombron van die merker en verskillende kalibrasies van die basis van die boom. Resultate dui op wesenlike variasie in die ouderdom van die kroon Suider-Afrikaanse *Oxalis* groep (“clade”) oor ‘n periode van amper 20 miljoen jaar, afhangende van die databron, kalibrasie skattings en metodiek gevolg in die rekonstruksie. Ten spyte van hierdie wesenlike variasie, is alle gemiddelde ouderdomme ouer as 18 miljoen jaar, wat ooreenstem met ‘n toenemende kern van bewyse dat daar ‘n geleidelike opbou van floristiese diversiteit in die KFS plaasgevind het, eerder as ‘n vinnige, onlangse ontploffing in spesiasie.

Aangesien die geproduseerde filogenieë deurslaggewend wys dat die huidige taksonomie kunsmatig is, word daar vierdens ‘n nuwe, feitlik totaal verskillende klassifikasie vir Suider-Afrikaanse *Oxalis* taksa voorgestel. Alhoewel dit ‘n beduidend verbeterde sisteem voorstel, word die nuwe klassifikasie as informeel beskou op grond van die totale teenstrydigheid tussen ou en nuwe sisteme, swak resolusie in sommige voorgestelde ontwikkelingslyne en die behoefte om die voorgestelde groepe (“clades”) te bevestig deur die identifikasie van morfologies gedeelde afgeleide kenmerke. Potensiële gedeelde afgeleide kenmerke vir verskeie groepe (“clades”) word bespreek, en dit behoort toekomstige navorsing verder te lei.

Vyfdens is die aanwesigheid van volle in die genus van groot belang as potensiële voor-aanpassing vir seisoenale-droë klate. Die ewolusie van die bol-groeivorm in *Oxalis* word hier vir die eerste keer verken. Ek bespreek die volgorde van groot morfologiese kenmerk-staat veranderinge wat lei tot ‘n stel kenmerke wat korrespondeer met die bol-groeivorm. Die homologie van basale blaas-petiole, vlesige blaas skubbe en tunika word bespreek, en daar word gewys dat baie bol-kenmerke aanwesig in die suider Afrikaanse ontwikkelingslyn ook in naverwante lede van hierdie ontwikkelingslyn aangetref word, en dus ouer is as die suider Afrikaanse lyn. Die ekologiese en ewolusionêre implikasies van bol geofitisme in die CFR lede van *Oxalis* word ook bespreek.

Ten slotte spreek ek sekere taksonomiese probleme aan wat gedurende die verloop van die studie na vore gekom het. Medewerkers en ek beskryf die nuwe spesies *O. saltusbelli* en *O. ericifolia*. Ons klaar ook aspekte rondom die geweldig variërende groep-spesie *O. flava* op, en stel sekere nomenklatoriese veranderinge en sinonieme vir verwante taksa voor. Ons ondersoek die taksonomiese posisie van die skaars spesie *O. purpurata*, wat te laat opgespoor is om in die hoof-analises van hierdie studie in te sluit.

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## AIMS AND OBJECTIVES OF THIS STUDY

The major objective of this study is to provide a phylogenetic hypothesis of relationships between the *Oxalis* species of the southern African region using DNA sequence data. Subsidiary objectives include dating the southern African radiation and increasing basal resolution and support for the major clades involved. Another objective is to propose an urgently-required updated classification system for all indigenous taxa. Further objectives include exploring the evolution of bulbs within *Oxalis* as a whole, and discussing the implications of this habit for the southern African taxa. Finally, various smaller taxonomic updates and species descriptions are presented.

The layout of this thesis is as follows:

Ch 1. **Literature Review.** This introductory chapter has been written in the format required for the journal Molecular Phylogenetics and Evolution so as to maintain a degree of consistency, as two of the main chapters have been submitted to this journal.

Ch 2. **A phylogenetic structure and age estimates of southern African *Oxalis* (Oxalidaceae)** will be submitted to Molecular Phylogenetics and Evolution, and is formatted according to the requirements of this journal.

Ch 3. **An updated classification scheme for southern African *Oxalis* based on DNA sequence data** will be submitted to Taxon, and was therefore formatted according to the requirements of this journal.

Ch 4. **A model of bulb evolution in the eudicot genus *Oxalis* (Oxalidaceae)** has been accepted for publication in a special Cape Biota issue of Molecular Phylogenetics and Evolution, and is formatted according to the requirements of this journal. All of this work is my own, with commentary by my three co-authors, who acted as co-supervisors of this chapter.

Ch 5. ***Oxalis saltusbelli*: a new *Oxalis* (Oxalidaceae) species from the Oorlogskloof Nature Reserve, Nieuwoudtville, South Africa** has been accepted for publication in South African Journal of Botany, and is formatted according to the requirements of this journal. The three authors contributed equally to the preparation of this manuscript.

Ch 6. **An unusual new species of *Oxalis* (Oxalidaceae) from the Knersvlakte, South Africa** has been accepted for publication in South African Journal of Botany, and is formatted according to the requirements of this journal. Seventy percent of this work is my own, with the remainder shared equally by my two co-authors.

Ch 7. **Reassessment of the taxonomic status of *Oxalis fabaefolia* (Oxalidaceae) and the description of a unique form of *O. flava* from the Northern Cape Province of South Africa** has been prepared with the aim of submission to South African Journal of Botany, and is formatted according to the requirements of this journal.

Ch 8. **Taxonomic position of *Oxalis purpurata* Jacq. (Oxalidaceae)** has been written up with the aim of submission as a short taxonomic note to Bothalia, and is formatted according to the requirements of this journal.

Ch 9. **Concluding chapter.** In this chapter I summarise the objectives achieved in the course of this work, and make some suggestions on promising future research opportunities.

## Chapter 1: Literature Review

### *Oxalis*, the Cape Flora and molecular systematics

This introduction is divided into three main sections. In the first, the impact that modern methods of phylogenetic inference has had on our understanding of the evolution of the Cape Flora are examined in the context of the genus *Oxalis* as the largest geophytic genus of the region. This is followed by a general introduction to the genus *Oxalis*, and insights into its systematics. Thirdly, some background is given on the molecular markers utilized in this study.

#### ***1) The impact of molecular phylogenetics on the Cape Flora***

The dawn of sequence-based phylogenetics has been lauded on various fronts. Arguments have been advanced emphasizing the utility of protein or DNA sequences over traditional morphology-based analyses, claiming greater amounts of data to analyse, lesser levels of homoplasy, less ambiguity in character interpretation and greater ease of modelling as major advantages (Hillis et al., 1996). The course of the last two decades has shown differently; molecular sequences are manifestly prone to convergence, modelling sequence evolution has proven challenging (Kelchner and Thomas, 2007), and horizontal transfer, paralogy and long-branch attraction have all added to the list of problems encountered by molecular systematists.

Of all potential advantages, only the sheer bulk of sequence data available at the DNA or protein level has held out as a useful advantage over morphological characters. Despite changes in the taxonomy of some groups, sequence data have generally supported previous classification systems. Conflicts between morphology-based classification and DNA-derived evidence have mostly been confined to groups that were taxonomically questionable or controversial to begin with. These have generally been resolved in favour of DNA due to the wealth of data provided (APG II, 2003). Very often subsequent revisions of such classifications have highlighted previously-overlooked morphological and karyological characters as potential synapomorphies for these newly-proposed clades (Palmer et al., 2004).

The Cape Floristic Region, also known as the Cape Floral Kingdom (Takhtajan 1986), is globally recognized as a major centre of plant species richness and endemism (reviewed in Linder, 2003). Occasionally recognized in conjunction with the Succulent Karoo flora as the Greater Cape Floristic Region (Born et al., 2006), the current indigenous diversity

tally in the region is in excess of 9 000 vascular plant species, of which 68% are endemic (Germishuizen and Meyer, 2003; Linder, 2003). The flora is dominated by an unusual assemblage of families, including Proteaceae, Ericaceae and Restionaceae. It also includes a very high percentage of geophytes and relatively few annuals for a Mediterranean region (Goldblatt and Manning, 2000; Linder, 2003).

Historically, the boundaries of the CFR have been consistently demarcated by various authors (Bolus, 1886; Marloth, 1908; Goldblatt, 1978), culminating in the presently held definition of Goldblatt and Manning (2000). Geographically, the CFR is confined to the extreme south-western corner of the African continent, with isolated outliers to the north and east. Vegetation remarkably similar to the CFR Fynbos Biome is found on the Kamiesberg in Namaqualand, the Roggeveld mountains in the Grahamstown region (arguably part of the eastern CFR), the Drakensberg and extending up the eastern escarpment to the Chimanimani mountains of Zimbabwe (Levyns, 1964; Galley and Linder, 2006). Several CFR taxa extend further along the Great Rift Valley into southern Ethiopia (*Protea*, *Erica* and *Disa*). In contrast, taxa typical of the Succulent Karoo Biome tend to extend up the west coast of southern Africa into Namibia, tracking the winter rainfall distribution area. Examples include *Moraea*, whose distribution range extends from the CFR northward to near the Orange-Fish River confluence in southern Namibia (Goldblatt, 1986), and *Zygophyllum*, which extends from the CFR to the arid areas in the Horn of Africa and Asia (Bellstedt et al., 2008).

Although the CFR is recognized as a global hotspot of endemism, diversity and extinction risk (Myers et al., 2000), the history of the flora is very poorly known. This results both from a poor fossil record and the uncertain relationships of many elements of the flora. Molecular techniques, in general, have proven useful in exploring historical patterns and processes. More specific to the CFR, phylogenetic methods have provided radically new ways of observing and describing the flora, and testing hypotheses on its origins and patterns of radiation. The methodologies of phylogenetics, and the sheer amount of data generated from DNA sequencing, have allowed explicit testing of relationships, more objective ways of estimating biogeographical patterns, taxon divergence times and processes driving speciation in a speciose and diverse flora.

Biogeography - A number of previous authors (Levyns, 1964; Axelrod and Raven, 1978) assumed the primary source of CFR taxa to be either Gondwana or tropical Africa. Levyns (1964) cited the Proteaceae, the conifers *Widdringtonia* and *Podocarpus* and the

Restionaceae as prime examples of taxa most probably derived from ancient Gondwanan vicariance or tropical African dispersal. Other authors (Adamson, 1958) favoured vicariance between tropical Africa and the CFR to explain the current distribution of CFR taxa.

The impact of molecular phylogenies has shed light on these hypotheses. Growing bodies of molecular evidence from a number of key CFR clades have shown them to be of Cape origin, with subsequent migration out of the CFR to the enrichment of tropical African floras (Galley and Linder, 2006). Examples include the genus *Protea*, where tropical African species are shown to be deeply embedded within the Cape clade (Barracough and Reeves, 2005) and *Phyllica*, where dispersals from the CFR also colonized surrounding African islands (Richardson et al., 2001). Galley et al. (2007) extended this general pattern to four CFR monocot lineages (*Disa*, the Irideae *pro parte*, the *Pentaschistis* clade and the African Restionaceae). Preliminary data suggest that even the mega-genus *Erica*, a Cape taxon with tentatively European roots (McGuire and Kron, 2005; Galley and Linder, 2006), shows back-migrations from the CFR onto the Drakensberg (N. Lester, unpublished data). Other clades exhibiting the same trends include the stapeliads (Bruyns 2005), *Leucadendron* (Barker et al., 2004) and the Bruniaceae (Quint and Classen-Bockhoff, 2006). There is thus evidence that CFR clades serve as a source of diversity for tropical Africa, contrary to the hypotheses of Levyns (1964) and Axelrod and Raven (1978).

In terms of the biogeographical origins of the flora, the potential Gondwanan origins of many Cape taxa with representation on other southern continents have been largely refuted. In these taxa, ancestral vicariance was postulated as having given rise to present day distributions as the continents separated. Examples of Cape families with potential Gondwanan distributions include the Proteaceae, Restionaceae, Cunoniaceae and Iridaceae. In the Proteaceae, it is clear that both the origins of the Cape genera and their position within the global Proteaceae phylogeny are incongruent with ancient Gondwanan vicariance (Barker et al., 2007; Sauquet et al., 2008). Warren and Hawkins (2006) estimated ages for fourteen small Cape lineages (paleoendemics, or relicts) and in all cases found ages inconsistent with a Gondwanan vicariance scenario.

Taxon divergence times – Since the term was coined in the 1960's (Zuckerkandl and Pauling, 1965), molecular clock methods have been both useful and contentious. There is still substantial controversy relating to aspects of molecular clock models, such as the

model of DNA substitution used (including rate heterogeneity amongst sites; Arbogast et al., 2002), rate heterogeneity amongst markers (Magallón and Sanderson, 2005), calibration type and placement, accommodation of error (Graur and Martin, 2004) and rate variation amongst lineages (Sanderson, 1997). Recent developments have turned more and more towards using Bayesian approaches (Thorne et al., 1998; Huelsenbeck et al., 2000; Drummond and Rambaut, 2007) to estimate divergence times. One of the more recent additions to the Bayesian arsenal is BEAST (Drummond and Rambaut, 2007). This program collectively offers several advantages over other methods such as NPRS (Sanderson, 1997) and Penalized Likelihood (Sanderson, 2002), such as co-estimation of model parameters, topologies and divergence times, estimation of confidence intervals on ages, accommodations for violations of rate constancy, the capacity to place realistic priors on calibration points and the relaxation of the assumption of auto-correlation of rates from parent to daughter branch. Although the program suffers from the general criticisms leveled against Bayesian techniques (such as relevance of the prior; Huelsenbeck et al., 2001; Felsenstein 2004), BEAST's flexibility and ease of use, in addition to the advantages listed above, are certain to make this program a valuable addition to modern phylogenetics.

The age and timing of radiation within Cape clades is a subject of much interest, not least because age estimation of multiple Cape clades can help to discriminate between hypotheses of rapid, recent additions to the flora, or a more gradual accumulation of species over time (Linder, 2003). The competing hypotheses of sudden and recent speciation (often linked to the establishment of the Benguela current and Mediterranean climate; Axelrod and Raven, 1978; Marlow et al., 2000) and a more gradual establishment of the current flora can be tested with the use of divergence time methods as outlined above. Initial results from phylogenies of *Phyllica* (Richardson et al., 2001) supported a recent origin and radiation for the genus in the CFR. This was followed by age estimates for a number of Cape clades such as the Iridaceae (Goldblatt et al., 2002), Restionaceae (Linder and Hardy, 2004) and *Pelargonium* (Bakker et al., 2005). All of these provided substantially older Oligocene-Miocene ages for initial colonization of the Cape. In contrast, studies of the Cape *Heliophila* (Mummenhof et al., 2005), the grass genus *Ehrharta* (Verboom et al., 2003), and the huge subfamily Ruschioideae of the Aizoaceae (Klak et al., 2004) yielded very young ages consistent with recent radiation and the climate-driven speciation hypothesis. Linder (2005) summarized the spread of age estimates known for Cape clades at the time, which showed no clear single speciation trigger. Since then, published age estimates have been supplied for *Muraltia* (Forest et

al., 2007), the species-poor genus *Melianthus* (Linder et al., 2006), fourteen unrelated relict lineages (Warren and Hawkins, 2006), the genera *Disa* and *Pentaschistis* (Galley et al., 2007) and the legume tribes Podalyriaceae and Crotonaceae (Edwards and Hawkins, 2007; Boatwright et al., 2008). These have reinforced the pattern of gradual radiation of the current species flocks. Although most phylogenies do not support the hypothesis of a single trigger for massive speciation, it is possible that such an event could indeed be responsible for the radiation of Succulent Karoo lineages (Verboom et al., 2008). Age estimates for more exclusively Succulent Karoo clades, as well as clades with both Succulent Karoo and Fynbos representatives, would be needed to confirm this. *Pelargonium*, which is well-represented in both biomes, does have a secondary radiation of Succulent Karoo taxa that is estimated to have begun approximately 8 mya (Bakker et al., 1999).

Taxonomy – Despite the utility of DNA in a wide variety of systematic aims, one of the major uses remains the testing of current taxonomic systems as hypotheses of relationships. Sequence data has proven useful at every level of the taxonomic hierarchy, from the origin of life (Brown and Doolittle, 1999; Cicarelli et al., 2006) to the detailed demographic histories of populations (Kingman, 1982). Plant systematics has experienced a revolution in the establishment of a large-scale, well-supported classification of higher-order angiosperm relationships based primarily on DNA sequence data (APG, 1998; APG II, 2003). This has generally been lauded as a substantially improved hypothesis of angiosperm relationships. Despite this, aspects of many previously proposed classification systems have been strongly supported by the APG system, with differences mostly confined to previously problematic taxa.

The CFR flora is taxonomically relatively well-documented compared to the remainder of Africa (Linder, 2003). However, new species are described from the area on a regular basis, and the potential diversity of many groups, such as *Oxalis*, remains underexplored (Salter, 1944; Oberlander et al., 2002).

Most molecular phylogenetic studies of the Cape Flora have focused, at least at some level, on taxonomic implications. Quite often these have resulted in classifications that support previous work, such as confirming the paleo-endemism of the woody Iridaceae (Reeves et al., 2001), the monophyly of the African Restionaceae (Linder et al., 2003), the taxonomically isolated positions of Geissolomataceae and Grubbiaceae (APG II, 2003), and the non-monophyly of the African tribe Proteae of the Proteaceae (Johnson

and Briggs, 1975). In other instances, such studies have radically altered the understanding of certain Cape groups. Examples of this include the expansion of the Stilbaceae to include the genera *Retzia*, *Halleria* and *Nuxia* (amongst others; Bremer et al., 1994; Olmstead et al., 2001). This decreased the number of endemic families within the Cape Flora by two: the monotypic Retziaceae were sunk into Stilbaceae, and the inclusion of *Nuxia* generalizes the distribution of the family to sub-Saharan Africa. Another unexpected conjunction involving paleo-endemics links the forest tree *Curtisia dentata* with the Fynbos paleoendemic family Grubbiaceae (Fan and Xiang, 2003).

Many genera within the Cape Flora have been reduced to synonymy as a direct result of molecular phylogenetic analyses. Examples of this are the genera *Homeria* and *Galaxia* of the Iridaceae (into *Moraea*; Goldblatt et al., 2002), the genus *Nylandtia* into *Muraltia* (Forest et al., 2007) and the genus *Schizodium* into *Disa* (Bytebier et al., 2007). In other cases, such as the synonymising of *Herschelianthe* and *Monadenia* under *Disa* (Kurzweil et al., 1995; Johnson et al., 1998) and the sinking of the minor genera of the CFR Ericaceae into *Erica* (Oliver, 1993; Oliver, 1994), DNA sequence data are corroborating the findings of previous morphology-based work (Bellstedt et al., 2001; Douzery et al., 1999; McGuire and Kron, 2005). In contrast to these taxonomic simplifications, sequence data have also pointed out the isolated phylogenetic (and hence taxonomic) status of taxa currently not recognized as such (i.e. *Trichocephalus stipularis*; Richardson et al., 2001). The end result of these taxonomic reshufflings is a more stable, more predictive, more inclusive classification of the Cape Flora as a whole.

Processes – a recent trend in studies of the Cape Flora has seen a greater emphasis on using phylogeny to also infer processes of speciation. Several authors have postulated key innovations as responsible for the radiation of Cape clades. Klak et al. (2004) proposed tracheid anatomy, hygrochastic capsules and leaf shape as potential key innovations for the speciose subfamily Ruschioideae. Bakker (1999) proposed a key innovation associated with cambial differentiation of stem tissues in *Pelargonium* that allow for the evolution of succulence. Verboom et al. (2003) discussed several possible characters in the grass *Ehrharta* that could lead to radiation of the genus, including adaptation to different soil types. Forest et al. (2007) found a shift in diversification rate after the development of certain elaiosome characters, suggesting a role for myrmecochory in speciation.

The role of hybridization in the Cape Flora is understudied, although it is estimated to be small (Linder, 2003). Despite this, several phylogenies have shown patterns of strong incongruence between plastid and nuclear topologies that are indicative of potential hybridization. Barraclough and Reeves (2005) produced a phylogeny of the genus *Protea* that is drastically different from all established morphological classification systems (Rourke, 1980; Rebelo, 2001). Although other mechanisms can account for such incongruence, hybridization between *Protea* species seems the most likely explanation, given that morphologically-identified hybrids are often encountered naturally. The plastid and nuclear topologies of the Rosaceae genus *Cliffortia* also differ substantially (Whitehouse, 2002). Given that the genus is largely wind-pollinated, extensive hybridization is a potentially good explanation for the observed incongruences. Speciation through autopolyploidy has also not been assessed in the Cape Flora, although it does not seem to have played a role in *Leucadendron* (Liu et al., 2006). Recent assessments of genomic C-values (Doležel, 1997) in *Oxalis* indicate that ploidy levels vary substantially within this genus. In fact, diploid to hexaploid series have been observed within the boundaries of single currently-recognized species such as *O. flava* L., *O. obtusa* Jacq. and *O. hirta* L. (J. Suda, pers. comm.). Aneuploidy and dispolyploidy also seem to have been significant in the radiation of various other lineages including the monocot families Amaryllidaceae (Snijman, 2004; Strydom and Spies, 2007), Hyacinthaceae (Johnson and Brandham, 1997) and Iridaceae (Goldblatt et al., 1984), and dicot families such as Geraniaceae (*Pelargonium*) (Gibby et al., 1996) and Scrophulariaceae (Jong, 1993; Steiner, 1996). These results suggest that the role of chromosome evolution in driving radiation in the CFR has been underestimated.

## **2) The genus *Oxalis*.**

The family Oxalidaceae has been taxonomically problematic for many reasons. Originally placed in or close to the order Geraniales by several authors (Cronquist, 1981, Takhtajan, 1980), more current hypotheses utilising DNA sequence data have convincingly refuted this relationship (Price and Palmer, 1993; Hilu et al., 2003). Instead the Oxalidaceae is now placed in an unexpected clade including the previously unrelated families Brunelliaceae, Cephalotaceae, Connaraceae, Cunoniaceae and Elaeocarpaceae (including Tremandraceae; Crayn et al., 2006). This clade, the order Oxalidales, is morphologically variable, including trees, shrubs, lianas, annuals, carnivorous pitcher plants, drought-adapted xerophytes and geophytes. The order is mostly confined to the southern continents (with the notable exception of *Oxalis*), particularly Australasia and South America. Although originally proposed purely on the basis of sequence data, recent

floral morphological findings have corroborated the patterns of relationships inferred from DNA (Matthews and Endress, 2002). The closest relative to the family Oxalidaceae is the Connaraceae, which shares pinnately compound leaves with articulated leaflets, heterostyly, a connate androecium and similar floral ontogeny (APG website; <http://www.mobot.org/MOBOT/research/APweb/welcome.html>).

The Oxalidaceae currently includes five genera, *Averrhoa*, *Biophytum*, *Dapania*, *Oxalis* and *Sarcotheca*. *Hypseocharis* has often been linked to the Oxalidaceae, but recent sequence-based work has placed this genus in its own family closely related to the Geraniaceae (Price and Palmer, 1993). The phylogenetic relationships between the various oxalidaceous genera has not been addressed phylogenetically, but analyses of all oxalidaceous *rbcL*, *matK* and *ndhF* plastid sequences from Genbank produce congruent trees that support *Oxalis* as sister to the other four genera.

*Oxalis* is by far the largest genus in the Oxalidaceae. The most recent global taxonomic revision of the genus was published by Knuth (1930). He grouped the southern African species into five sections based on general morphological characters such as leaflet breadth and the arrangement of leaves on the stem. These sections were artificial, and subsequent authors have proposed very different classifications (Salter, 1944; Lourteig, 1994; Lourteig, 2000).

Research on American *Oxalis* has been extensive over the last few decades. The reproductive ecology of the often weedy section *Corniculatae* and several species of section *Ionoxalis* have been well-studied (Ornduff, 1972; Weller, 1976; Weller and Denton, 1976). The mechanism of tristylly inheritance has also been studied in several South American *Oxalis* taxa (Lewis and Jones, 1992; Trognitz and Hermann, 2001). All non-southern African *Oxalis* were taxonomically revised by Alicia Lourteig, particularly in her treatments of *Oxalis* subgenus *Thamnoxys* (Lourteig, 1994) and the other three subgenera *Monoxalis*, *Oxalis* and *Trifidus* (Lourteig, 2000). Her proposed taxonomies reflect natural affinities between groups of species better than those of Knuth (1930), at least in terms of the *O. tuberosa* alliance (Emshwiller, 2002). Lourteig (2000) subdivided the huge *Oxalis* subgenus *Oxalis* into three major groups: taxa with above-ground stems, taxa with rhizomes and other non-bulbous geophytes, and a bulbous group. She placed all of the southern African taxa within the latter group, despite not having studied them in any detail. The global weed *O. pes-caprae* represents the only southern African species in her treatment.

*Oxalis* is well-represented in southern Africa, with over 200 species currently recognized, and many potential new taxa hidden in the *incertae* shelves of herbaria in the region. The seminal morphological monograph of southern African *Oxalis* is that of Salter (1944). The introductory chapters cover the author's extensive knowledge of the morphology of the genus, as well as cursory notes on ecology, distribution, modes of reproduction and relationship. The work did not set out to provide an entirely natural classification for *Oxalis*. Nevertheless it is of considerable importance due to its comprehensive assessment of local herbarium specimens, a great number of necessary synonymisations, and as a summary of the many new species and varieties published by Salter over the previous decade.

Salter (1944) divided southern African *Oxalis* into nine sections on the basis of one or more distinctive characters. These sections vary in size, from the three species in section *Stictophyllae* and the five species each of sections *Sagittatae* and *Campanulatae*, to the huge section *Angustatae*, which contains almost half of all indigenous species (Oberlander et al., 2004). Several sections are further divided into subsections and series of informally grouped taxa. Salter (1944) considered section *Cernuae* to be the most “primitive” group based on the presence of pseudo-umbellate inflorescences (compared to all other southern African *Oxalis*, which have a single flower per inflorescence). Apart from this one shared character, the groups of related taxa placed within this section are very dissimilar. The remaining taxa were divided into four mostly broad-leafleted, acaulescent sections (*Oppositae*, *Foveolatae*, *Sagittatae* and *Stictophyllae*), the aquatic section *Campanulatae*, a semi-succulent section *Crassulae*, a range of miscellaneous, unrelated taxa in the “dustbin” section *Latifoliolatae*, and the huge, mostly linear-leafleted, caulescent section *Angustatae*.

Despite many well-known and easily recognized species, the current taxonomy is plagued by problems. Although Salter (1944) is a monumental work of data collation and nomenclatural corrections, it suffers from being over sixty years old and is thus out of date. Species in the genus itself are notoriously hard to identify and subject to great epharmonic variation. Many recently-collected herbarium specimens are misidentified, adding to the taxonomic confusion. The very limited flowering time of most species, and the sporadic nature of appearance above-ground in drier years, confound collection efforts. A large number of informative characters are confined to the bulbs and inflorescences. As the flowering period of *Oxalis* is generally restricted, and the bulbs of

many species are difficult to collect, many available herbarium specimens are too incomplete to identify correctly. Moreover, Salter's (1944) species concepts are inconsistent, with considerable morphological variation lumped into highly variable "group" species in some cases, whilst other groups with comparable variation are divided into many species and varieties. Such difficulties have led to the genus being understudied for half a century, a trend that has only recently been addressed.

Studies on southern African *Oxalis* over the period 1944-1996 were limited in scope, and contributed very little to understanding the general biology of South African taxa. Ornduff (1973) compared the northern and southern populations of the aquatic species *O. disticha* and concluded that they represent separate species, based on morphological and karyological evidence. He separated the northern populations from *O. disticha* under the name *O. dines*. Some cursory reports on mole-rat predation were reported from invasive populations of *O. pes-caprae* by Galil (1967) in Israel. Huynh (1969) undertook extensive light-microscopic palynological analyses of *Oxalis*, including many South African species. Her findings revealed many discrepancies between her pollen types and the classification of Salter (1944). Bayer (1992) emphasised the difficulty in finding tangible demarcations between *Oxalis* species, and proposed that the eleven species of section *Angustatae* subsection *Pardales* and the three species of section *Cernuae* subsection *Lividae* be synonymised under *O. pardales* and *O. livida*, respectively. Oliver (1993) described the unusual species *O. oculifera* from the Gifberg plateau.

Recent work on the genus resulted in greater taxonomic conflict. After Salter (1944), the next major work on southern African *Oxalis* is that of Dreyer (1996). She presented a detailed, near-complete review of the palynology of the genus in southern Africa, and identified four major pollen types. Based mainly on tectum structure, she identified micro-reticulate rugulate (type A), micro-rugulate spinate (type B), reticulate (type C) and supra-areolate (type D) pollen types. Reticulate pollen (type C) was found to be most common, and was inferred to be the primitive condition, as it is present in New World *Oxalis* taxa and in the sections considered most "primitive" by Salter (1944). Although these pollen types are well-defined and easily recognizable, they did not correspond to the taxonomic groupings proposed by Salter (1944). Dreyer (1996) argued that due to the constraints imposed by pollination and fertilization, pollen characters are often taxonomically more reliable than macro-morphological characters. She concluded that the scattered distribution of these pollen types across the classification argued against the naturalness of the Salter (1944) taxonomy.

During the period 1996-2004 several new species and minor taxonomic changes were proposed. Dreyer and van Wyk (1996) proposed the synonymy of *O. henrici* under *O. engleriana* based on palynological data. Williamson (1999) described the new species *O. psammophila* from the Richtersveld. Dreyer and Johnson (2000) summarised previous karyological work on southern African *Oxalis*, and published new chromosome counts for several taxa. Oberlander et al. (2002) attempted an initial biogeographical analysis of southern African *Oxalis* species, and identified three major centres of diversity and endemism in the Cape Peninsula/Boland region, the Cederberg/Knersvlakte, and the Kamiesberg. Kumwenda et al. (2004) revised the small section *Sagittatae* and rediscovered the locality of *O. minuta* var. *callosa*. They confirmed that this taxon does not belong to section *Sagittatae* and that it is distinctly different to the typical variety. They thus raised it to specific rank as *O. hygrophila* Dreyer, and included it in section *Latifoliolatae*. Oberlander et al. (2004) presented a phylogenetic reconstruction of relationships within the large, loosely-defined section *Angustatae* subsection *Lineares* based on DNA sequence data. Analysis of non-coding plastid *trnL* intron and *trnL-trnF* spacer markers (hereafter referred to as the *trnL-F* region) conclusively showed the artificiality of this subsection. Their newly-proposed relationships showed substantial congruence with the distribution of pollen types identified by Dreyer (1996).

#### Economic and ecological importance:

Several *Oxalis* species (i.e. *O. triangularis* St. Hil. and *O. oregana* Nutt.) have served as model systems for the investigation of circadian nastic leaf movements in plants (Johnnson *et al.*, 1981; Rinnan and Johnnson, 1986). The tuber-bearing *O. tuberosa* Molina (oca) is an important Andean crop plant that has also become a common market vegetable in other parts of the world such as New Zealand (Emshwiller and Doyle, 1998). In South Africa, the ubiquitous weed *O. pes-caprae* is used as a garnish in waterblommetjie bredie (A stew made with the endemic aquatic herb *Aponogeton distachyos*; Watt and Breyer-Brandwijk, 1962).

The genus exhibits immense horticultural potential. A number of garden varieties of American taxa such as *O. triangularis* and *O. deppei* are already commercially available in South Africa. African species have only recently started entering the horticultural market, and local nurseries often stock species such as *O. purpurea* and *O. glabra*. *Oxalis* plants are easy to grow, and advances in callus and plantlet initiation have increased the

potential for commercialization of southern African taxa (Crouch et al., 1993; M. Jooste, unpublished data).

Ecologically, *Oxalis* is perhaps most well-known as a notorious weed. New World species that have become established in South Africa include *O. corniculata* and *O. latifolia* (Salter, 1944). The former is an annual species capable of self-fertilization that has become a globally-established garden pest. The spread of *O. latifolia* is made easier by a bulbous reproductive mechanism similar to that of the southern African taxa. In turn, South Africa has been the native source of numerous weedy *Oxalis* species. *Oxalis pes-caprae* is the most widespread weed, and has become invasive in regions of Mediterranean climate such as Australia, Spain, Israel and California. The species is a particular menace in Australia, where it is responsible for oxalic acid poisoning of stock (Peirce, 1997). Studies in Spain and in the eastern Mediterranean have shown that these invasions are spreading exclusively via bulbs, without viable seed set (Rottenburg and Parker, 2004; Castro et al., 2007). These Mediterranean invasions belong to two main strains: a sterile, highly invasive pentaploid form, and a tetraploid form of more limited distribution (Castro et al., 2007). *O. purpurea* has, to a lesser extent, also become invasive in Australia (Peirce, 1990). Interestingly, in the case of both species these invasions are confined to regions of Mediterranean climate.

### **3) DNA Markers**

A number of DNA markers were assessed for phylogenetic utility in this study. The following section provides a brief overview of the markers that were eventually chosen as being the most informative.

#### **ITS**

The Internal Transcribed Spacer marker of the nuclear ribosomal 18S-5.8S-26S cistron is unquestionably the nuclear marker of choice for plant molecular systematists. First utilised for phylogenetic purposes by Baldwin (1992), usage of the marker has grown tremendously for a variety of reasons, as summarised by Alvarez and Wendel (2003).

The ITS is part of the array of multiple-repeat coding regions responsible for RNA components of the ribosome. Bounded by 18S and by 26S, the marker covers various functional regions of the cistron, including the RNA-coding and highly conserved 5.8S region, which is isolated by the two highly variable spacers that give the marker its name. Although transcribed as a unit together with the 18S, 5.8S and 26S genes, the spacers

appear to function in the ribosomal maturation process, where they are spliced out of the RNA molecule (Baldwin et al., 1995). There are apparent constraints on certain regions of the spacers, as shown by domains conserved across a wide variety of plant taxa (Mai and Coleman, 1997). The entire cistron region is thought to undergo concerted evolution, whereby differing copies are homogenised so that all genomic copies are theoretically identical. ITS is generally considered one of the most variable markers available to plant molecular systematists (Alvarez and Wendel, 2003), and is thus of particular use at lower taxonomic levels.

It is ironic, considering the extensive usage of ITS, that most of the original advantages accredited to ITS as a source of phylogenetic inference have now been shown to be problematic. The presence of concerted evolution has led to the assumption that paralogous loci and pseudogenes are deleted in favour of functional ITS transcripts, leading to ease of sequencing and interpretation. In recent years concerted evolution and pseudogenisation of ITS have been shown to plague many taxonomic groups (Franzke and Mummenhof, 1999; Mayol and Rosello, 2001; Harpke and Peterson, 2006; but see Razafimandimbison *et al.*, 2004 for an example where pseudogenes did not violate phylogenetic inference). Furthermore, rapid rates of both substitution and indel evolution sometimes make sequence alignment very difficult. This leads to problematic primary homology assessments and increased chance of multiple hits along a single branch (Simmons and Freudenstein, 2003). Even the often-used advantage of ITS in highlighting incongruences with respect to plastid data is not guaranteed to provide evidence of hybridisation, particularly if the retrieved sequences have undergone concerted evolution towards the maternal ITS haplotype.

Given the large number of studies based solely on ITS sequence data (34% of papers surveyed by Alvarez and Wendell, 2003), problems caused by the heterogeneous behaviour of this marker should be a sign for caution. Despite these and other problems, however, ITS has proven very useful in many taxa, particularly at the species-level and below. Nevertheless a major drive has been implemented to find alternative loci to supplement or supplant ITS as the nuclear marker of choice in plant molecular systematics. It is uncertain whether any particular marker will enjoy the domination achieved by ITS over the last few decades, but quite a number of nuclear loci have now been favourably assessed for phylogenetic signal, including malate synthase in Arecaceae (Lewis and Doyle, 2001), chalcone synthase in Brassicaceae (Lihová et al., 2006), *RPB2*-

*I* in Ericaceae (Goetsch et al., 2006) and glutamine synthetase in Oxalidaceae (Emshwiller and Doyle, 1999) and Passifloraceae (Yocktang and Nadot, 2004).

#### *ndhF*

This plastid protein-coding marker has been in use since the early days of plant phylogenetic inference (Olmstead and Sweere, 1994; Clark, Zhang and Wendel, 1995; Smith et al., 1997). The gene codes for one of the subunits of the nicotinamide dehydrogenase complex and is estimated to have twice the mutation rate of the more widely-used marker *rbcL* (Sugira, 1989). The 3' end of the marker, in particular, has much higher levels of nonsynonymous base substitution and transversion bias than the 5' end due to different constraints on the function of various parts of the molecule (Kim and Jansen, 1995). This overall higher mutation rate makes it valuable for phylogenetic inference at the family level. It has been used in conjunction with other plastid markers in the families Malpighiaceae (Davis et al., 2001) and Phyllanthaceae (Kathriarachchi et al., 2005) as well as at higher taxonomic ranks, for example within the asterids (Bremer et al., 2002).

#### *trnK* including *matK*

The popular marker *trnK* was initially targeted more for the *matK* ORF embedded within it than for the entire intron. The demand for *matK*, in particular, was fuelled by findings suggesting that it was potentially one of the most variable coding regions in the plastid genome (Johnson and Soltis, 1994). Another attractive aspect of *matK* was that the observed variation was not predominantly confined to 3<sup>rd</sup> codon positions, but is more evenly spread (Hilu et al., 2003). This results in a high frequency of non-synonymous amino acid mutations, and greater utility of 1<sup>st</sup>- and 2<sup>nd</sup> codon positions for phylogenetic inference.

The *matK* ORF is considered to be the only maturase more or less universally present in the plastids of higher plants (Hilu et al., 2003). The *trnK* intron is thought to have arisen as a bacterial (or fungal) transposon, capable of self-splicing and mobility, that invaded the plastid genome relatively early in streptophyte evolution (*matK* is present in the plastids of charophyte algae). Subsequent evolution caused degradation of reverse transcriptase function, leading to loss of mobility for the intron. Hausner et al. (2006) provided an elegant model of *trnK* evolution, and proposed that *matK* is solely responsible for splicing all other group II introns in the plastid genome; hence its retention in the reduced plastome of the parasite *Epifagus virginiana* (Wolfe et al., 1992).

Although *matK* has generally lived up to phylogenetic expectations, the marker is not without its problems. High rates of mutation have made universal primer design problematic in some cases (Hilu et al., 2003). Although of value in yielding large numbers of characters, the length of the region generally precludes a single amplification, which has cost implications.

The popularity of *matK* has not been carried over to the *trnK* region in general. Considering that *matK* comprises almost 65% ( $\pm 1600$  bp of 2500 bp) of total intron length, and thus contains the potential majority of variable sites, this is perhaps understandable. Shaw et al. (2005) found the non-coding parts of the intron to be relatively invariant across seed plants; the region ranked 18th in terms of average potentially informative characters out of 21 sequenced. Nevertheless *trnK*, mostly in conjunction with various parts of *matK*, has proven useful in several studies outlined in Shaw et al. (2005). The presence of highly conserved flanking regions in the *trnK* coding region provides universal primers, which in turn allow the design of internal PCR (or sequencing) primers for *matK*.

#### *trnL-F*

This region was one of the first available, and still is one of the most commonly-used, non-coding plastid markers in plant systematic studies (Taberlet et al., 1991). The entire marker, or the constituent *trnL* intron, 3' *trnL* exon and *trnL-trnF* spacer, has been used to address a range of phylogenetic questions across a wide spectrum of the taxonomic hierarchy, from closely related species (Oberlander et al., 2004) to higher-level studies within the angiosperms (Borsch et al., 2003). Reasons for the tremendous popularity of this marker include the early publication of protocols and primer sets (Taberlet et al., 1991), the universality of the primers, and, arguably, impetus (everyone else is doing it).

Despite the immense amounts of data gathered for this marker, *trnL-F* has been shown to be of limited use at the species-level. Shaw et al. (2005, and references therein) found the overall variability of *trnL-F*, with respect to twenty other plastid non-coding markers, to be very limited at the species-level. In accord with this, other recent analyses have used *trnL-F* at ordinal or supra-ordinal level, and even across the angiosperms, with great success (Borsch et al., 2003). In general, the *trnL-trnF* spacer has been shown to be more variable than the intron (Shaw et al., 2005), which can be attributed to more stringent constraints on secondary structure in the latter marker.

### *trnS-G*

Initially described from Hamilton (1999), numerous studies have shown this marker to be substantially more variable than the *trnL-F* region (Xu et al., 2000; Olson, 2002; Perret et al., 2003). The region is between 700 and 1000 bp long (Shaw et al., 2005) and the primers are anchored in the transfer RNA genes for serine and glycine. Shaw et al. (2005) showed the region to be of far greater utility than many other well-known markers. It is the most variable of the non-coding plastid markers cited by Shaw et al. (2005) to be used in this study.

### *trnT-L*

This region lies directly upstream of *trnL-F* and was introduced in the same paper (Taberlet *et al.*, 1991). Consisting entirely of a spacer between the genes for transfer RNA's of threonine and leucine, this region has generally received less attention than its contiguous downstream markers, and is seldom used independently from *trnL-F*. Shaw et al. (2005) attributed this to difficulty with the 5' primer (Taberlet a), which causes amplification problems in many taxa. Despite this problem, *trnT-L* is a variable and useful marker at many levels. It has been used to resolve both relationships amongst early-diverging angiosperm clades (with *trnL-F*; Borsch *et al.*, 2003) as well as population-level studies in Japanese beeches at the other end of the taxonomic hierarchy (again, including *trnL-F*; Okaura and Harada, 2002).

Shaw *et al.* (2005) found *trnT-L* to be among the more variable markers of their 21-marker analyses, which corroborates the findings of various other studies cited in their work, namely that *trnT-L* is in general more variable than *trnL-F*. Combined with better designed upstream primers, this region is now an attractive candidate for future studies.

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Chapter 2: A phylogenetic structure and age estimates of southern African *Oxalis*  
(Oxalidaceae)

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Abstract

The plant genus *Oxalis* is a large and important part of the Cape Floristic Region at the southern tip of Africa, yet research on this genus has been hampered by a lack of knowledge of its phylogenetic history. Here we present a phylogeny for SA *Oxalis* taxa covering three quarters of known indigenous species, and attempt to place an age upon the crown southern African clade. In general, parsimony, likelihood and Bayesian Inference analysis methods yield topologies that are largely congruent with one another. Despite the application of more than 7 000 base pairs of sequence data in focused analyses, large portions of the basal topology of SA *Oxalis* remain uncertain. The basic structure of *Oxalis* in southern Africa nevertheless yields three major clades, one of which contains the vast majority of species. The closest relatives of SA *Oxalis* species appear to be bulbous and pseudo-bulbous New World taxa. Due to a lack of fossil data for Oxalidaceae, ages were inferred using a two-step relaxed clock approach, with posterior ages from analyses of the order Oxalidales used as priors on more focused *Oxalis* phylogenies. Estimates of the age of crown SA *Oxalis* are very ambiguous, and differ substantially according to analysis method used, constraints on root age and on data partition. Despite substantial variation in age, the consensus of these analyses is that *Oxalis* has been present in the southern African region for more than 18 million years. This agrees with many recent studies that have found the flora of the region to have evolved far less explosively and recently than previously thought.

Key words: Bayesian Relaxed Clock, ITS, *ndhF*, Oxalidales, Penalized Likelihood, *trnK*, *trnL-F*, *trnS-G*, *trnT-L*

## Introduction:

The Cape Floristic Region (CFR) at the southern tip of Africa is of considerable value as a laboratory for testing theories of plant evolution and diversification. This is due to high levels of diversity and endemism within the CFR, and a sound taxonomic understanding of the region (Goldblatt and Manning, 2000). Phylogenetic interest in the Cape Flora increased markedly after the publication of a dated phylogenetic analysis of *Phylica* (Rhamnaceae), which used volcanic island ages to estimate an age for this Cape-centered genus (Richardson et al., 2001). This heralded an increased focus on DNA-based phylogenies for Cape groups. Some preliminary progress was presented in an overview of the Cape Flora by Linder (2003), and he lobbied for more research into neglected CFR groups in order to confirm the emerging patterns. Since then, research on the CFR has progressed beyond the mere reconstruction of phylogenies towards attempts to use these phylogenies to infer processes of speciation and extinction (Van der Niet et al., 2006), the role of key innovations (Klak et al., 2004), shifts in pollinator (Bakker et al., 2005), substrate and habitat preferences (Verboom et al., 2004; Linder and Hardy, 2005; Verboom et al., 2008), migration patterns (Galley and Linder, 2006; Galley et al., 2007) and ages of Cape clades within the Cape (Edwards and Hawkins, 2007; Forest et al., 2007).

Despite this major increase in interest, the phylogenetic investigation of Cape lineages has been somewhat uneven. Although several major lineages such as the Proteaceae (Rourke 1998; Barker et al. 2004; Barraclough and Reeves 2005; Barker et al. 2007, Sauquet et al., 2008) and the Restionaceae (Eldenas and Linder, 2000; Linder et al., 2003; Linder et al., 2005; Hardy et al., 2008) have been well-studied, with substantial increases in ecological (Protea Atlas Project) and phylogenetic knowledge, other taxonomic groups have not been analysed. These include some of the largest genera within the flora, such as *Erica* and *Oxalis*.

*Oxalis* (ca. 500 spp.) constitutes a major component of both the New World and the CFR floras (Lourteig, 1994; 2000; Salter, 1944). The centre of morphological diversity and putative place of origin of the genus is in South America, within which it exhibits a range of habits (from semi-succulent shrubs to vines to geophytes). Southern African taxa (hereafter referred to as SA *Oxalis*) constitute roughly 40% of the genus as currently recognized, and share a bulbous habit, with above-ground plant parts borne on a seasonal stem. The current taxonomy (Salter, 1944) is clearly artificial and in need of major

revision (Dreyer, 1996; Oberlander et al., 2004). Previous researchers assumed that all the southern African taxa are closely related (Knuth, 1930), but the relation of SA *Oxalis* to the remainder of the genus is unknown. Virtually nothing is known about the age of the genus, the origin of SA *Oxalis*, the age of the southern African radiation, and indeed how they arrived on the African continent. It also remains conjectural whether the southern African taxa are indeed monophyletic.

Contextually the position of *Oxalis* among the major plant groups of the CFR remains uncertain. Linder (2003) recognized 33 Cape Clades in the CFR. He used two criteria for determining the status of a Cape Clade: more than half of extant diversity of the clade had to be present in the region, and the clade must have originated within the CFR. *Oxalis* is the seventh largest genus in the CFR (Goldblatt and Manning, 2000) and the largest geophytic genus in the CFR (Procheş et al., 2006), yet despite being such a significant component of the Succulent Karoo and Fynbos floras, no information was available for Linder (2003) to determine whether *Oxalis* was a Cape Clade or not.

Major studies on SA *Oxalis* include Salter (1944), who established the currently-recognized classification system, and the palynological monograph of Dreyer (1996), which often contradicts the Salter (1944) taxonomy. Very high levels of epharmonic plasticity and morphological variation, inadequately documented species diversity and distribution, and poor herbarium specimens have complicated taxonomic research. Ecological data for most species, even such basic knowledge as substrate and pollinator preferences, are unknown. More recent systematic publications on SA *Oxalis* only address minor classification issues (Bayer, 1992; Dreyer and Van Wyk, 1996) or describe new species (Ornduff, 1973; Oliver, 1993; Williamson, 1999; Kumwenda et al., 2004; Manning and Goldblatt, 2008). In a recent study, Oberlander et al. (2004) began to address systematic questions using plastid non-coding DNA sequence data. These studies raised substantial new questions, as sequence data conflicted with the Salter (1944) classification, but were largely congruent with palynological data (Dreyer, 1996). This study included less than 40 % of southern African species, had poor outgroup sampling and revealed the presence of a large polytomy of the southern African taxa and some *Oxalis* outgroups. A comprehensive systematic analysis of the genus in southern Africa is thus still lacking.

Our aims in this paper are threefold: to provide a well-sampled phylogenetic reconstruction of *Oxalis* in southern Africa, to address the poor resolution of major

clades, and to estimate the time that *Oxalis* has had to diversify in South Africa. We address our first aim by sequencing one nuclear (ITS) and two plastid loci (*trnL*-F and *trnS*-G) for three quarters of recognized southern African species. We attempt to improve support values for major poorly-resolved clades by sequencing three extra plastid markers (*trnK*, *ndhF* and *trnT*-L) for a select group of 24 taxa. Finally, we address the age of SA *Oxalis* by analyzing the large-scale, three marker data set, using both the Bayesian relaxed clock method of Drummond and Rambaut (2007) and a semi-parametric Penalized Likelihood method (Sanderson, 2002). In order to address major issues relating to divergence time estimation that have been recently highlighted in the literature (Graur and Martin, 2004; Magallón and Sanderson, 2005; Pulquério and Nichols, 2007), we compare age estimates for the SA *Oxalis* lineage between partition (plastid vs nuclear vs combined), method used (Bayesian Relaxed Clock vs Penalized Likelihood with two smoothing values) and calibration error.

## Materials and Methods

### *a) Species collection and sampling*

DNA samples of most species were collected in the field during the *Oxalis* flowering season (generally the rainy season: the austral winter in the west, the austral summer in the east). These were supplemented by material from herbarium accessions housed in the Bolus Herbarium (BOL, University of Cape Town, Cape Town), and living specimens from Stellenbosch University and Karoo National Botanical Gardens. Genus-level outgroup DNA for the Oxalidaceae was provided by B. Gravendeel. Voucher specimens containing locality and other data are housed at the Stellenbosch University Herbarium (STEU, Stellenbosch University, South Africa). Species were identified through comparison to the Salter collections at Bolus (BOL) and Compton (NBG, Kirstenbosch, Cape Town) Herbaria.

Outgroups were used to root all trees in both parsimony and model-based approaches. For the 24-taxon data set, *O. corniculata* was considered the most distantly related to the southern African clade. For all 171-taxon analyses, a clade consisting of members of the genera *Averrhoa*, *Biophytum*, *Dapania* and *Sarcotheca* was placed as monophyletic sister to all sampled *Oxalis*. All outgroup taxa have strong evidence for their positions given their respective sampling (Oberlander et al. 2009; Emshwiller *et al.*, preliminary data).

The lack of structure at the base of the southern African clade was addressed using a two-fold approach: separate taxon-rich and character-rich data sets. To explore the effects of taxon and character sampling more fully, two major data sets were compiled. All currently recognized and collected species were included in a 171-taxon data set (150 southern African species and 21 outgroups) for the markers *trnL-F*, *trnS-G* and ITS. A 24-taxon data set was also constructed that spanned three outgroup *Oxalis* species and selected taxa representing the major clades within the southern African clade. This data set consisted of the above-mentioned markers, as well as three additional plastid markers: the *trnK* intron, including *matK*; the 3' end of the plastid coding gene *ndhF*; and the *trnT-L* spacer region directly upstream of *trnL-F*. These markers were ultimately chosen from a preliminary screen of nine markers (seven plastid and two nuclear) sampled for high levels of variability or ease of amplification. The plastid gene *rbcL* and intergenic spacer *rps12-rpl20* proved too invariant for phylogenetic purposes in southern African *Oxalis*, and were consequently not pursued further. Although primers for the nuclear-encoded but chloroplast-expressed glutamine synthetase amplicon have been designed specifically for *Oxalis*, (Emshwiller and Doyle, 2002), this marker proved too difficult to amplify.

For calibration purposes, *trnL-F* sequences of five of the six families currently recognized in Oxalidales were downloaded from Genbank (provided by the following studies: Connaraceae: Bruneau et al., 2001; Zhang and Simmons, 2006; Brunelliaceae, Cunoniaceae, Cephalotaceae: Bradford and Barnes, 2001; Elaeocarpaceae: Crayn et al., 2006), and supplemented by *Oxalis* taxa sequenced in this study, to form the Oxalidales data set. The *Oxalis* taxa were chosen to represent successively closer outgroups to the southern African clade, as well as to make the crown Oxalidaceae and crown *Oxalis* nodes representative between analyses. Although taxon sampling for Cunoniaceae (including *Hooglandia*, McPherson and Lowry, 2004), Elaeocarpaceae and Oxalidaceae was relatively representative, only three sequences for the Connaraceae were available. Brunelliaceae and the monospecific Cephalotaceae were represented by two and one sequences respectively. Although sequences for more taxa in the Elaeocarpaceae were available, these all had zero-length terminal branches and were removed from further analyses.

#### *b) DNA extraction and sequencing*

DNA extraction of most silica-dried samples followed a modified 2X CTAB procedure (Doyle and Doyle, 1987), described in Oberlander et al. (2004). Although this extraction procedure provided mostly clean DNA for successful PCR, secondary metabolites

prevented PCR amplification in a few samples, and a dilution series was required. Quite a few accessions, particularly older herbarium samples, had degraded DNA. In most cases re-extraction from extra silica gel-preserved material or from our own herbarium of living collections provided good quality DNA. Only five samples had DNA of such poor quality that markers in excess of  $\pm 450$  bp could not be amplified.

Attempts to increase the number of sampled species through the use of herbarium material (from BOL) were unsuccessful except in two cases. The two exceptions (MO653, *O. dines*; MO664, *O. fourcadei*) were the youngest of the BOL specimens, both under ten years old. All BOL herbarium samples were extracted using a Qiagen DNeasy DNA extraction kit, according to the manufacturer's protocols.

Amplification of *trnL*-F proceeded according to the protocol outlined in Oberlander et al. (2004), although quarter-reactions were used in all subsequent PCR's. Amplification of *trnL*-F and *trnT*-L proceeded using the Taberlet et al. (1991) primers. Amplification of *trnS*-G used the Hamilton (1999) primers. Amplification of the *trnK* intron used the 3914F and 2R primers of Johnson and Soltis (1995). ITS amplification used the AB101 and AB102 primers of Douzery et al. (1999). Amplification of *ndhF* used the forward primer of Olmstead and Sweere (1994), 972F, but a modified reverse primer was designed for use in *Oxalis* (2110R-Ox: CCT ACA TAT TTG ATA CCT TCT CC). Cocktails for all markers contained the following reactants: 0.2 mM each dNTP, 2.5 mM Supertherm buffer and MgCl<sub>2</sub>, 12.5 pmol of forward and reverse primers, 0.25 U of Supertherm Taq polymerase, and 20-50 ng of template DNA, ultra-distilled H<sub>2</sub>O to final volume of 25  $\mu$ l. PCR temperature protocols all involved a 94 °C denaturation step for 60 s, a 60 s annealing step, and a 90 s extension step at 72 °C repeated between 30 and 35 times. In all cases a seven minute final extension step finished off the PRC protocol. Due to the length of the *trnK* intron, this region was amplified in two pieces, with roughly 290 bp of overlap (primers 3914F and 1470R, and 1176F and 2R; Johnson and Soltis, 1995).

PCR products were run through a 2% agarose gel to check band size and for potential multiple bands. Correctly-sized, clean bands were excised and purified using Wizard® DNA Prep purification kits (Promega, U.S.A.), according to the manufacturer's protocols. In the case of ITS, which is multiple copy, bands that were too bright, too smeary or obviously multi-banded were discarded, and the samples were submitted to a new PCR run with higher annealing temperatures.

All sequencing reactions were made up to a total of 10 µl, and consisted of 1 µl Big Dye™ Terminator Mix RR (Applied Biosystems, U.S.A.), 3 µl sequence dilution buffer, a final primer concentration of 0.3 pmol/µl and 5 µl of purified PCR product. Primers used for sequencing were the same as the PCR primers in all cases. Both forward and reverse reads were sequenced for all markers except *trnS-G*, which is short enough to confidently read through in one direction. Sequencing protocols were as follows for all markers, except for ITS: 10 s at 96 °C, 30 s at 52 °C, 4 min at 60 °C, for 35 cycles. For ITS the annealing temperature was set at 55 °C, but the sequencing protocol was otherwise identical. All sequencing products were run on an ABI 377 sequencer (Applied Biosystems, U.S.A.) at the Stellenbosch University Central Analytical Facility.

Base calling in the sequence chromatograms of forward and reverse primers for each taxon was confirmed or corrected in Chromas v2.3 (<http://www.technelysium.com.au>). Corrected sequences were imported into BioEdit v7.0.0 (Hall, 1999) where contigs were assembled. The embedded ClustalW function (Thompson *et al.*, 1994) within BioEdit was used for automatic alignment. All alignments were manually checked. Where obvious, the alignment in areas surrounding insertions was optimized to reflect the nature of the repeat. For the protein-coding markers (*ndhF* and *matK* within *trnK*), sequences were converted into amino acids within BioEdit in order to aid alignment and to check for potential pseudogenisation. Sequences were checked against Genbank (NCBI) submissions through BLAST searches, as a preliminary screen for potential contamination.

### *c) Phylogenetic analysis*

Each data set was analysed using both parsimony and model-based approaches. Parsimony analyses were performed in PAUP\* v4.0 beta 10 (Swofford, 2003) on a G5 Apple PowerPC running Mac OS 10.3. Likelihood analyses used GARLI v0.951 and v0.96 (Zwickl, 2006). Bayesian Inference utilized the program MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) run in both Mac OS 10.3 and Windows XP environments. Gaps were not coded, as these tended to support already well-supported clades in preliminary analyses.

For parsimony analyses, exported NEXUS files from BioEdit were run under heuristic searches to find multiple most parsimonious trees, with all characters coded as equally-weighted and unordered. Heuristic searches employed 1000 replicates using random-addition generated starting trees and TBR branch swapping. All other options for the

parsimony criterion were kept as default. Up to a maximum of ten best trees found per replicate were saved in order to more efficiently explore tree space. Parsimony bootstrap (1000 replicates, starting trees generated by simple addition) was implemented as a measure of support for clades.

For Maximum Likelihood (ML) analyses, an optimal model of sequence evolution was chosen using Modeltest v3.6 (Posada and Crandall, 1998), using likelihood values obtained by PAUP\*. Likelihood searches used a genetic algorithm procedure implemented under the optimal model in the program GARLI v0.951 and v0.96 (Zwickl, 2006), using default parameters. Non-parametric bootstrap support measures were constructed using the bootstrap function of the same program. The final trees from 100 bootstrap replicates were compiled into a majority-rule consensus tree using PAUP\*.

For Bayesian Inference, each data set was run through MrModeltest v2.2 (Nylander, 2004) to determine the optimal model of DNA evolution for the data using both a Likelihood Ratio Test (LRT, Felsenstein, 1988) and the Akaike Information Criterion (AIC, Posada and Crandall, 1998). Where the models nominated by these tests differed, the model suggested by the AIC was preferred. All analyses for MrBayes v3.1.2 were run from a batch file instead of command line driven. Two concurrent analyses were run for  $5 \times 10^6$  generations for each data set, utilizing Metropolis-coupled Markov Chain Monte Carlo (MCMCMC), sampling every 500 generations. Apart from the implemented model and generation time, all other settings were kept at their default values. Visual inspection of the posterior distribution, together with MrBayes' own convergence diagnostics, were used to judge whether stationarity had been reached, and the extent of the burnin (10 % of total run length in all cases). Consensus topology and branch lengths (with burnin removed) were calculated using the sumt command. Posterior probability values of clades were used as estimates of clade support. Where possible, differing partitions of the data were assessed using Bayes Factors (BF; Kass and Raftery, 1995) to determine the partitioning scheme most favoured by the data.

The congruence of signal between the various data sets was assessed using the Incongruence Length Difference test (ILD test, Farris et al., 1995), implemented as the Partition Homogeneity Test in PAUP\*. This was also judged visually by inspection for incongruent nodes based on bootstrap values. Nodes were judged strongly incongruent if they supported different bipartitions of taxa with parsimony/likelihood bootstrap values greater than 70% in each segregate data set.

#### *d) Divergence time estimation*

Due to a lack of convincing fossils for Oxalidaceae, the basic methodology of divergence time estimation comprised two major components. Firstly, an initial estimate of ages for the family Oxalidaceae and the genus *Oxalis* within the larger context of the order Oxalidales was implemented using a relaxed-clock approach in BEAST (Drummond and Rambaut, 2007). This was followed by estimation of ages for crown SA *Oxalis*, using different, more focused data sets, and the BEAST-inferred ages for family and genus. The latter component utilized both a semi-parametric approach (Penalized Likelihood implemented in r8s; PL; Sanderson, 2002) and the Relaxed-Clock approach without assumed autocorrelation of rates from ancestral to descendant branches (RCM; BEAST).

#### Sequence data

Outgroups included two taxa each from the Malpighiales and Celastrales, orders considered close relatives of Oxalidales (APGII, 2003; Soltis et al., 2000). *Zygophyllum* was chosen as the most distantly related outgroup. Data from *Cassine schinoides* (Spreng.) R.H.Archer, Cunoniaceae, Cephalotaceae and Brunelliaceae were compiled from separate intron and spacer sequences, and consequently are incomplete for parts of the 3' end of the *trnL* exon and the *trnL-trnF* spacer. Two species of the Connaraceae, *Connarus championii* Thwaites and *Byrsocarpus coccineus* Schumach., do not have data for the *trnL* intron and *trnL-trnF* spacer, respectively.

The 171-taxon data set was used to calculate ages for crown SA *Oxalis*. Although plastid and ITS trees derived from these data were incongruent at several deeply-embedded nodes, we followed a total evidence approach that yielded greater resolution and support, including several nodes not supported in any individual analysis. In addition, the combined trees were congruent with several morphological characters that define clades of interest. Although we consider the combined data as most representative of relationships, independent analyses of each data partition were performed in both PL and RCM. The successive sister relationship of clades to the southern African taxa was mirrored by representative species in both analyses, so as to provide anchorage points for secondary calibration attempts.

#### Calibration nodes

Fossil data convincingly identified as oxalidaceous are scarce. The sole possible exception, *Averrhoites affinis* (Newberry) Hickey, is only dubiously assignable to the Oxalidaceae (Hickey, 1977). Consequently secondary calibration points for the family

were inferred from a larger-scale phylogenetic analysis of the Oxalidales. The order includes six families (APGII) of which three besides Oxalidaceae have documented fossil records; Cunoniaceae (Barnes et al., 2001; Schönenberger et al., 2001), Elaeocarpaceae (Dettmann and Clifford, 2001) and Connaraceae (Retallack, 1992). The Cunoniaceae, in particular, has a rich fossil record, with extant crown-group genera already present in the late Paleocene (*Eucryphia*) and Eocene (*Ceratopetalum*, *Codia*) of Australia.

Fossil constraints utilized in this study were both primary and secondary calibrations from the literature. Primary constraints consisted mostly of fossil data from Cunoniaceae (summarized in Barnes et al., 2001). In addition, a minimum age constraint of 78 mya was placed on the split between Cunoniaceae and Elaeocarpaceae + Brunelliaceae + Cephalotaceae, as inferred by Crepet et al. (2004) using fossil cunoniaceous flowers from the Late Santonian to Early Campanian of Sweden (Schönenberger et al., 2001). Although Crepet et al. (2004) placed this constraint on a Cunoniaceae excluding *Eucryphia*, this genus is currently considered to be deeply embedded within Cunoniaceae and the above date is thus more appropriately applied as mentioned. Fossil calibrations from the Connaraceae could not be used due to the limited sequences available.

Secondary constraints were placed upon the tree using data from Crayn et al. (2006) for Elaeocarpaceae. Available fossil data for *Elaeocarpus* could not be used directly due to the non-monophyly of the genus in *trnL*-F-based trees. The two chosen secondary calibration points (the crown group old Tremandraceae, and *Vallea* + *Aristotelia*) were chosen due to the strongly-supported monophyly of these two clades (Crayn et al., 2006).

When analyses were run without constraining the age of the root of the Oxalidales, this age was inferred to be Carboniferous or older (>300 mya). This is clearly highly unlikely for a deeply-embedded angiosperm lineage; consequently constraints were placed upon this node. Uncertainty in the root age of the Oxalidales data set was accounted for by assigning four different maximum possible ages to the root (from 120 to 90 mya, in increments of 10 my). Given an inferred age of the eurosid clade of at most 117 mya (Wikström et al., 2003), an age of 120 mya would be an extreme maximum age for the combined Oxalidales, Malpighiales and Celastrales. Due to the deeply embedded nature of the order Oxalidales within the eudicots, more recent ages of 100 and 90 mya can be considered more sensible estimates for the age of this clade. Internal calibrations using fossils were implemented as uninformative uniform priors in BEAST. Secondary

calibrations within the Elaeocarpaceae were given normal priors using the provided means and standard deviations in Crayn *et al.* (2006).

Although the broad pattern of relationships within *Oxalis* was congruent with previous studies, *trnL-F* did not resolve a monophyletic southern African clade. This appears to be a feature of this marker for this clade, and is probably a combination of slow evolution over the *trnL-F* region combined with secondary structure constraints on the intron that enforce complementary base mutations in RNA stem regions. In order to bypass this, ages were determined for the demonstrably monophyletic *Oxalis* and Oxalidaceae, and not for the age of the southern African radiation.

The Oxalidales data set:

Oxalidales *trnL-F* data were imported into BEAUti v1.4.6 to create an XML file for analysis by BEAST v.1.4.6. Internal calibration used fossil dating points as uniform priors with the date of the fossil equal to the minimum age of the node. A uniform prior was placed on the root, such that the minimum value was placed at the present, and the maximum age varying between 120 and 90 million years, in increments of 10 million years. This served to account for uncertainty in the maximum age of the order. All BEAST analyses ran for a total of  $5 \times 10^6$  generations, and were repeated twice in order to check for convergence. Upon completion, independent analyses were visually inspected in Tracer v1.4. Upon removal of burnin, posterior distributions of parameters for each node were summarized and collated into single files (using LogCombiner v.1.4.6) in order to increase the estimated sample size (ESS) of the parameters.

The Oxalidaceae data set:

Mean ages and standard deviations for crown group Oxalidaceae and crown group *Oxalis* were derived from the posterior distributions of all four analyses that had different maximum ages. These estimates were then used to date the southern African clade using Penalized Likelihood and Bayesian relaxed clock approaches.

Penalized likelihood (PL) – All penalized likelihood analyses were implemented in r8s (Sanderson, 2002), which does not utilize a data matrix to infer phylogenies, but requires an input topology (with branch lengths) inferred from some other source. For PL analyses the Oxalidaceae input topologies were inferred using a model-based approach in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) run on the Computational Biology Service Unit cluster (<http://cbsuapps.tc.cornell.edu/mrbayes.aspx>) housed at Cornell

University, USA. Convergence was assessed using MrBayes v3.1.2 as well as the Tracer v1.4 software package. In addition, analyses using parsimony implemented in PAUP\* v4.0b10 (Swofford, 2003) were also run to assess the effect of optimality criterion on topology and branch lengths.

In order to assess confidence limits surrounding divergence times for the southern African clade, a sample of 100 trees was randomly drawn from the posterior distribution of trees derived from MrBayes analyses of the 171-taxon data sets. A set of 100 randomly chosen ages from the Oxalidales analyses was assigned to these trees as calibration points for r8s. As suggested by the r8s manual, a cross-validation procedure was implemented to find a roughly optimal level of smoothing for all trees. Cross-validation gave equivocal and sometimes incomplete results, with most analyses providing a best smoothing value of either 1 or 3.2. Consequently all PL analyses were repeated for both smoothing values (hereafter referred to as the r8s<sub>1</sub> and r8s<sub>3.2</sub> analyses). All PL analyses used the truncated-Newton algorithm. The genera *Sarcotheca*, *Dapania*, *Averrhoa* and *Biophytum* were pruned from the trees prior to analysis, so as to correctly infer branch lengths on either side of the new root. Batch files for r8s analyses were created using BBEdit Lite v.6.1.2 (Bare Bones Software, Bedford, Massachusetts, USA), and r8s outputs were logged to separate files instead of the Terminal window. A mean and standard deviation for the southern African clade was calculated by filtering the output file data in Microsoft Excel.

RCM – Relevant XML files for the Oxalidaceae data set was prepared using BEAUti v1.4.6, but with a normal prior set on the `treemodel.rootheight` parameter (which estimates the age of the root of the tree) with mean and standard deviation equal to those of the posterior distributions of crown Oxalidaceae from the Oxalidales analyses. These four priors were individually the only calibration point for each data set. *Oxalis* and oxalidaceous genera were respectively forced to monophyly as the two most basal sister clades. This was done to minimize the effect of the long branches leading to *Biophytum*, *O. barrelieri*, and *Oxalis* section *Corniculatae*. All other settings were identical to the Oxalidales BEAST analyses. Means and standard deviations for the southern African clade were calculated directly from Tracer v.1.4. As partitioned data sets failed to reliably achieve convergence between independent runs, all analyses were unpartitioned.

Differences in crown age estimates of the *Oxalis* and SA *Oxalis* nodes, based on data partition, age of the root Oxalidales, and analysis method, were assessed using a factorial ANOVA in the program Statistica v8.0 (StatSoft Inc., Tulsa, USA). One hundred

replicates from analyses using RCM in Beast, for the root node = 120 mya and root node = 90 mya, for the plastid, ITS and combined data sets, were randomly drawn from the posterior distributions. All one hundred ages retrieved in each PL analysis, at smoothing values of 1 and 3.2, were also used for the same data partitions and root ages. Each individual set of 100 dates was checked for normality using the Shapiro-Wilks test. Overall homogeneity of variances was tested using a Levene test in Statistica v8.0.

## Results

### *a) Species collection and sampling*

A total of 150 species, comprising *ca.* 75% of currently recognized southern African diversity, were collected over the period 2001-2007 (Table 2). With the exception of section *Cernuae* subsection *Goetzea*, all sections and subsections *sensu* Salter (1944) were sampled. Although the genus is under-collected and many potential new species await description or discovery, we are confident that the broad taxonomic spread and sampling percentage ensured adequate coverage of all major clades. In the case of the many species complexes, multiple exemplars of each species from a broad geographical or morphological range were sequenced to provide a preliminary test of monophyly. Due to computation time constraints, however, these species complexes are represented by only single accessions here.

### *b) DNA extraction and sequencing*

For the 171-taxon data set, all but six species (*O. burkei*, *O. dines*, *O. fourcadei*, *O. lanata*, *O. livida* and *O. minuta*,) were sequenced for all three chosen markers. These five taxa lacked data for the *trnS*-G spacer. A portion of the ITS1 spacer could not be sequenced for *O. copiosa* due to length polymorphisms. The 24-taxon data set was complete for all taxa and markers with two exceptions. The 3' end of the *trnK* exon could not be amplified for twelve taxa, so that this data set had substantial missing data (22%). Secondly, a putative *ndhF* pseudogene was amplified for *Oxalis oculifera*, and was excluded. Although the ITS marker for this species was also unusual (many indels, many substitutions, substitutions in highly conserved areas), this sequence was retained in both 171- and 24-taxon data sets as the sole nuclear marker for this species, and because analyses of ITS with and without *O. oculifera* showed very similar results (data not shown).

Basic properties of the ITS and plastid data sets show expected patterns (Table 3). Average ( $\pm$  s.d.) sequence length for the 171-taxon data set are: ITS: 772.2 ( $\pm$  11.0); *trnL-F*: 917.7 ( $\pm$  12.9); *trnS-G*: 716.3 ( $\pm$  23.3; based on 165 taxa). Average ( $\pm$  s.d.) sequence length for the 24-taxon data set are: ITS: 770.5 ( $\pm$  12.6); *ndhF*: 1078.7 ( $\pm$  1.5); *trnK*: 2474.4 ( $\pm$  17.9; based on 11 taxa complete for the intron); *trnL-F*: 920.4 ( $\pm$  4.1); *trnS-G*: 920.4 ( $\pm$  4.1); *trnT-L*: 800.5 ( $\pm$  12.1). Average % GC-contents ( $\pm$  s.d.) for the 171-taxon data set are: ITS: 56.7 ( $\pm$  2.0); *trnL-F*: 32.7 ( $\pm$  0.5); *trnS-G*: 25.2 ( $\pm$  0.6; based on 165 taxa). Average % GC-contents ( $\pm$  s.d.) for the 24-taxon data set are: ITS: 56.6 ( $\pm$  1.2); *ndhF*: 29.1 ( $\pm$  0.3); *trnK*: 31.0 ( $\pm$  0.4; based on 11 taxa complete for the intron); *trnL-F*: 32.7 ( $\pm$  0.4); *trnS-G*: 25.2 ( $\pm$  0.7); *trnT-L*: 22.8 ( $\pm$  0.7). As expected, plastid data are less GC-rich than ITS data. The very high length standard deviations on the two *trnS-G* data sets indicate the large number of indels present in this marker. The large difference in standard deviations between the two *trnL-F* matrices is due to considerable outgroup length variation and a very large deletion in the *trnL-trnF* spacer in *O. virginica* in the 171-taxon data set. The average length of the *trnS-G* spacer was somewhat longer in the 24-taxon matrix due to ambiguous data on the 5' end of the spacer that was excluded from the larger *trnS-G* matrix.

### c) Phylogenetic analysis

Alignment of *ndhF*, *trnK*, *trnL-F* and *trnT-L* proved unproblematic in all cases. The *trnS-G* spacer was relatively easily alignable across *Oxalis* in both 171-taxon and 24-taxon data sets, but outgroup alignment was more intractable. ITS proved extremely variable, to the point where portions of the ITS1 spacer were almost unalignable with any confidence across ingroup and outgroup taxa. We tried various different alignments of this marker, using a variety of gap-opening procedures and manual alterations. In all cases, the basic topology of produced trees was very similar. This suggests that the signal present in less variable parts of the matrix was strong enough to overwhelm the more noisy aspects of this marker.

The ILD test implemented in PAUP\* to test for incongruence between data sets yielded substantial disagreement between the nuclear and plastid data partitions in the 171-taxon data set. However, visual scrutiny of produced trees showed that most strongly incongruent nodes (judged as being strongly supported in alternative positions by different partitions) were located near the tips of the trees. Excluding these taxa did not increase congruence between partitions, even with more than a third of taxa excluded (62 of 171 taxa,  $P < 0.05$ ). The *trnL-F* and *trnS-G* partitions showed no significant

incongruence ( $P = 0.31$ , 100 replicates), thus justifying their combination into a single plastid data set. The 24-taxon data set also exhibited strong incongruence between plastid and nuclear partitions ( $P = 0.009$ ), but the incongruence could be localized to just one taxon, namely *O. commutata*. The exclusion of this species created barely congruent data sets ( $P = 0.054$ ). All individual plastid markers were congruent with ITS and with each other.

Comparison of trees produced by parsimony, maximum likelihood and Bayesian inference of the 171-taxon data set yield interesting data (Table 3). Plastid trees are generally better-resolved and have higher numbers of well-supported nodes than ITS-based trees. This can most probably be attributed to the higher levels of homoplasy in the latter data set. Trees produced by combined data are superior to either individual data set in terms of resolution and support. Parsimony and likelihood have comparable levels of resolved nodes and well-supported nodes, but Bayesian Inference trees have substantially greater resolution and support. Generally, model-based methods resolve the spine of the topology with better support, and are in agreement with one another and with the parsimony analyses of the plastid and combined data sets. A summary topology and informal names for clades mentioned in the discussion are presented in Fig. 1.

The 24-taxon data set yielded trees that are generally congruent with the 171-taxon data set. In all cases (except *trnL-F*), Bayesian Inference produced much better supported trees than parsimony. The ITS partition had the lowest CI/RI levels of all partitions in the 24-taxon data set, indicating substantial homoplasy. Mirroring the findings of the 171-taxon data set, the combined data approach provided the best-resolved and best-supported trees. Using Bayes Factors, a six partition model, with all model parameters unlinked between partitions, was favoured over single partition ( $2\ln BF_{6\text{partition}/1\text{partition}} = 606.46$ ) and plastid/nuclear partitions ( $2\ln BF_{6\text{partition}/2\text{partition}} = 89.48$ ) of the data.

The basic topology of the outgroups to SA *Oxalis* is relatively consistent (Figs 2-4). Section *Corniculatae* and *O. barrelieri* are always among the first lineages to separate within *Oxalis*, followed by successive branches leading to *O. acetosella*, the clade containing the *O. tuberosa* alliance (de Azkue and Martinez, 1990; Emshwiller, 2002) and section *Ionoxalis*, which is generally resolved as the sister lineage to a monophyletic SA *Oxalis*.

Within the southern African clade, three major clades are strongly supported: the three species of section *Cernuæ* subsection *Lividae* (the Livida clade), the species-poor clade containing the widespread weed *O. pes-caprae* (the Pes-caprae clade) and a Core southern African clade, containing the vast majority of species. Trees from the combined 24-taxon data set strongly support a sister relationship between the Livida and Pes-caprae clades, but the various partitions and optimality criteria give equivocal results for the 171-taxon data set (Figs 2-7). All summer-rainfall African taxa, thus taxa outside the CFR region, are deeply embedded within SA *Oxalis*.

The core southern African clade is poorly resolved despite strong support as a whole. Major clades include an unexpected, weakly-supported clade containing *O. commutata* and *O. orbicularis*, the Stellata clade, a mostly Acaulescent clade and a mostly Caulescent clade. Various clades within the Acaulescent (the Flava and Purpurea clades) and the Caulescent (the Hirta and Glabra clades) are generally strongly supported.

The 24-taxon analyses yield trees that are very similar to the 171-taxon study (Figs 5-7). The Livida and Pes-caprae clades are well-supported as sister lineages. The Core southern African clade is divided into two main units corresponding to the Caulescent clade and a combined Stellata + (Commutata-orbicularis) + Acaulescent clade that is strongly supported by Bayesian Inference but not parsimony.

#### *d) Divergence time estimation*

Four independent Bayesian analyses of the Oxalidales, run without a clock implementation in MrBayes 3.1.2, were judged to have reached convergence after the first 1000 sampled generations. Post-burnin consensus trees from these analyses were strongly congruent with one another, with parsimony-based trees and with currently accepted phylogenies of Oxalidales. Topologies within the Cunoniaceae and Elaeocarpaceae are similar to the analyses of Bradford and Barnes (2001) and Crayn et al. (2006), respectively. Higher-scale relationships between families agree with most recent phylogeny-based classifications. The overall agreement between the various trees, and similar distributions of parameters between independent runs as judged in Tracer v1.3, provide confidence that the posterior distribution was adequately sampled.

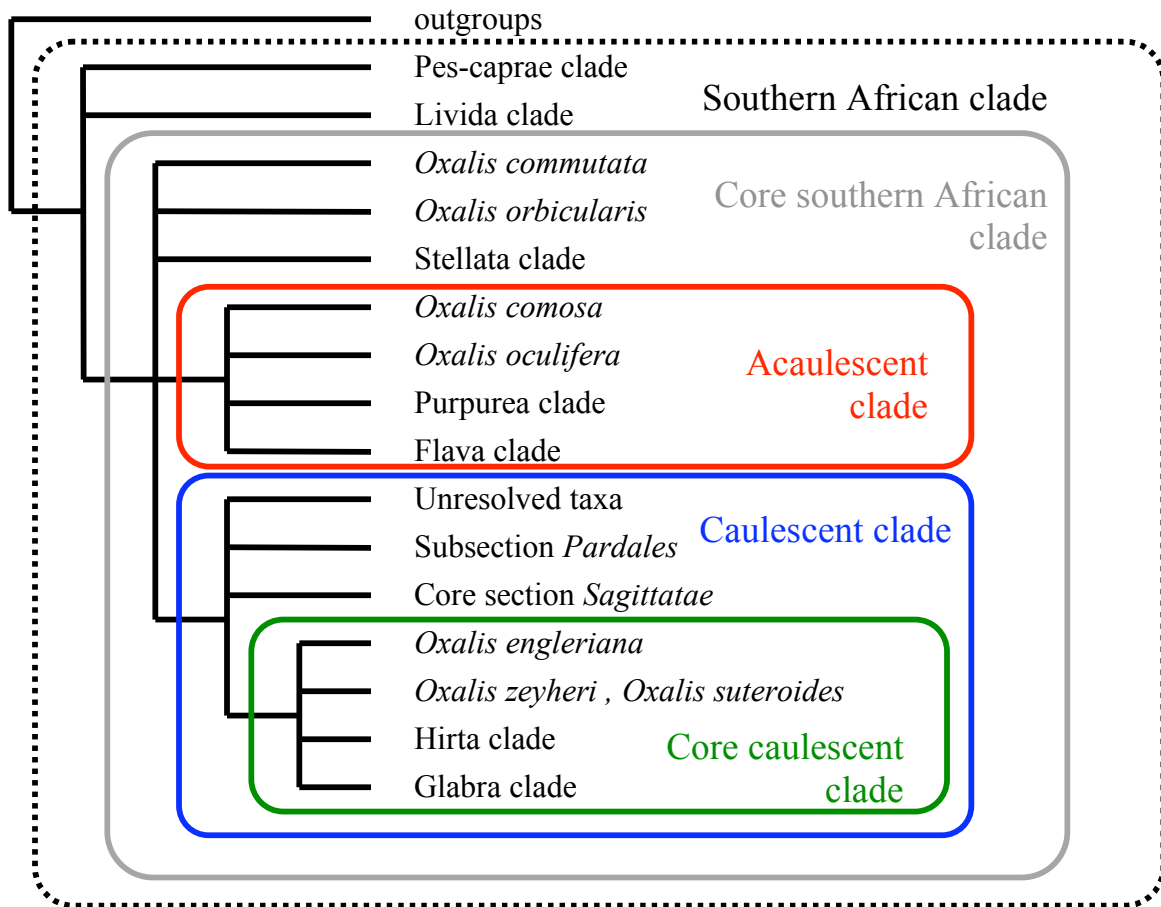


Fig. 1 Summary of current phylogenetic relationships between the major groups of southern African *Oxalis*. Names of clades correspond with those in the text.

When utilizing mostly flat priors on nodes, most BEAST analyses did not run due to unrealistic tree starting parameters, particularly tree topology. This resulted from the nature of the starting trees produced by BEAST. Poor starting trees often violate *a priori* either the hard constraints placed by uniform priors on divergence times, or the monophyletic status of enforced clades. Both violations cause BEAST to crash. Due to this neither coalescent nor UPGMA-generated trees provided a good starting approximation for these analyses. Consequently, randomly chosen Newick trees (with ultrametric branch lengths) from previous analyses were edited into the XML file as the starting tree for each independent run.

Preliminary RCM analyses yielded trees strongly congruent with other analysis methods and with previously published phylogenies for families within the Oxalidales (Bradford and Barnes, 2001; Crayn et al., 2006), except for the placement of *Zygophyllum* as sister to the Celastrales. This placement has no support in current phylogenies and is most probably an artifact of long-branch attraction; consequently *Zygophyllum* was excluded from subsequent RCM analyses. Other differences included the strong support for a monophyletic *Elaeocarpus* + *Sericolea* + *Aceratium* clade, which was not retrieved by either parsimony or non-clock constrained Bayesian analyses. This clade is supported, if weakly, by Crayn *et al.* 2007. Mean ages varying from  $73.8 \pm 10.0$  mya to  $61.0 \pm 8.0$  mya for the family Oxalidaceae were recorded depending on the age of root Oxalidales.

Bayesian estimates of ages for crown SA *Oxalis* in combined analyses range from  $23.1 \pm 4.3$  mya to  $19.4 \pm 3.6$  mya in order of decreasing root node age (Fig. 8a). The topologies and support values of these analyses are very similar to those of unrooted Bayesian analyses of the same data set (Fig. 9). Penalized likelihood estimates with a smoothing factor of 3.2 for combined data (Fig. 8b) yielded similar mean ages, but with broader variance:  $24.4 \pm 7.7$  mya to  $18.6 \pm 5.6$  mya. In contrast, a smoothing rate of 1 yielded substantially older estimates ranging from  $29.0 \pm 6.6$  mya to  $24.1 \pm 4.9$  mya (Fig. 8c). Different partitions of the combined data set also yielded very different results. Plastid data sets provided uniformly higher ages, whilst ITS ages and those of both partitions combined were very similar. In BEAST, the co-efficient of variation parameter significantly rejected a strict molecular clock for all data sets (i.e. zero lay outside the 95 % confidence intervals of the parameter). All analyses included zero in the 95 % confidence intervals of the covariance parameter; this implies no significant autocorrelation of rates across produced phylogenies.



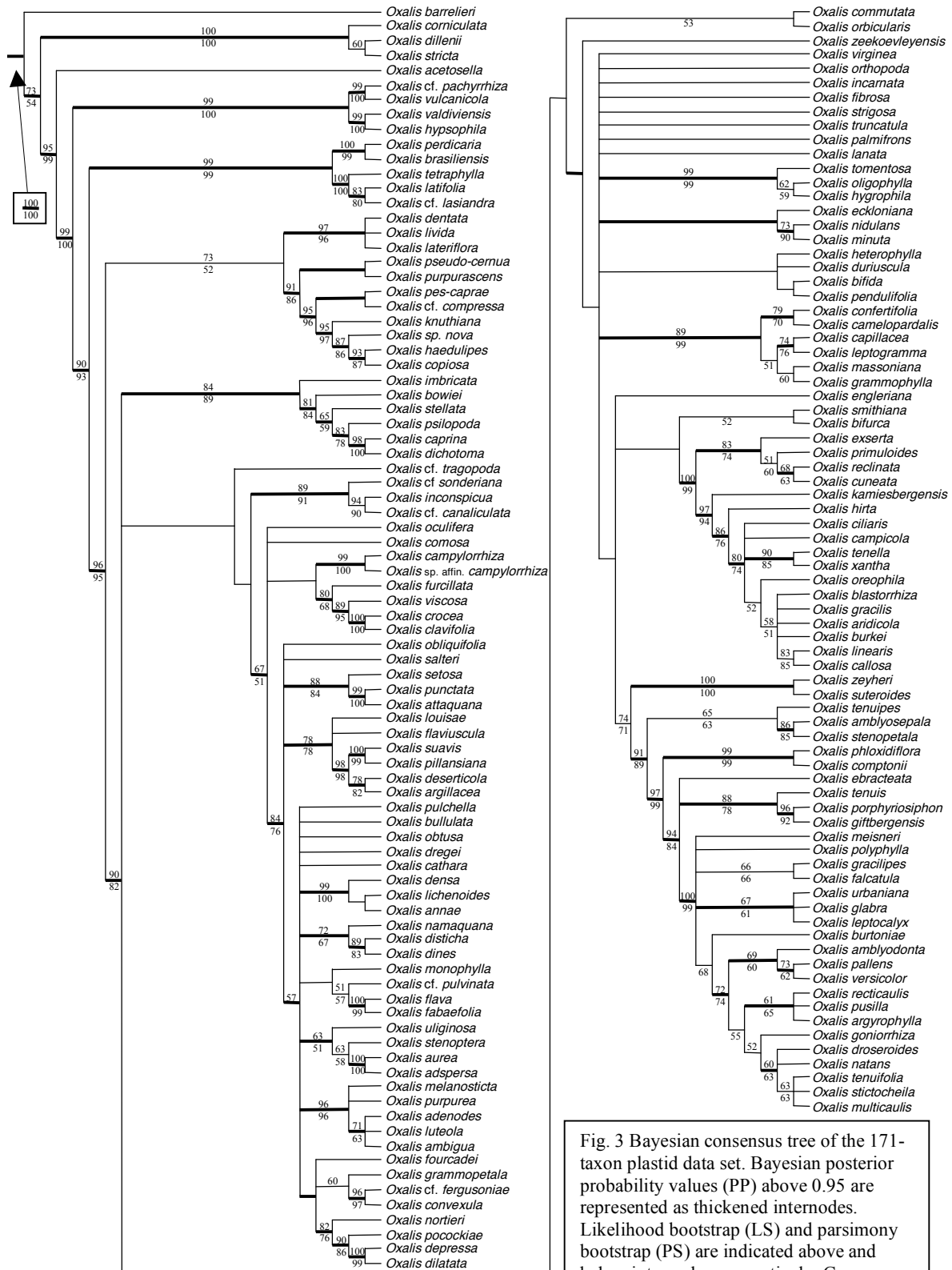


Fig. 3 Bayesian consensus tree of the 171-taxon plastid data set. Bayesian posterior probability values (PP) above 0.95 are represented as thickened internodes. Likelihood bootstrap (LS) and parsimony bootstrap (PS) are indicated above and below internodes, respectively. Genus-level oxalidaceous outgroups are excluded from the figure.

Thirteen of the eighteen separate samples (2 x Oxalidales root age by 3 x method by 3 x partition type) were normally distributed, with three of the remaining five barely significant at the  $p = 0.05$  level. Although homogeneity of variance was rejected at the  $p = 0.05$  level, ANOVA is generally robust to violations of this nature. Results from the factorial ANOVA showed highly significant differences between partitions ( $F(2, 1782) = 548.09$ ,  $p > 0.0000$ ), Oxalidales root node ages ( $F(1, 1782) = 318.04$ ,  $p > 0.0000$ ) and analysis methods ( $F(2, 1782) = 199.86$ ,  $p > 0.0000$ ). Highly significant interaction effects between variables were encountered for method and root age ( $F(2, 1782) = 7.0123$ ,  $p > 0.00093$ ) and partition and method ( $F(4, 1782) = 30.051$ ,  $p > 0.0000$ ). Interactions between partition and root age were non-significant ( $F(2, 1782) = 0.22055$ ,  $p > 0.80210$ ).

Plastid age estimates for crown SA *Oxalis* are significantly older than ITS ages for the same clade, no matter what method or root age is used. Also of interest is the inconsistent behaviour of the  $r8s_{3,2}$  analyses, where ITS and combined ages agree with those of BEAST. Plastid ages, using the same smoothing value, however, are more in agreement with the plastid ages from the  $r8s_1$  analyses.

## Discussion

### *Species collection and sampling*

A total of 150 species or species complexes out of *ca.* 200 southern African species were located and sequenced for both nuclear and plastid markers for this study. Although it is almost certain that new species await discovery and description (Bayer, 1992), we are confident that the potential phylogenetic spectrum encompassed in the current taxonomy has been covered, and that the major clades have been identified. It is possible but unlikely that any currently-recognized species would upset the basic division of SA *Oxalis* into three main clades. Our outgroup sampling does not definitively answer which is the sister clade to SA *Oxalis*, although the New World bulb-bearing section *Ionoxalis* is undoubtedly a close relative.



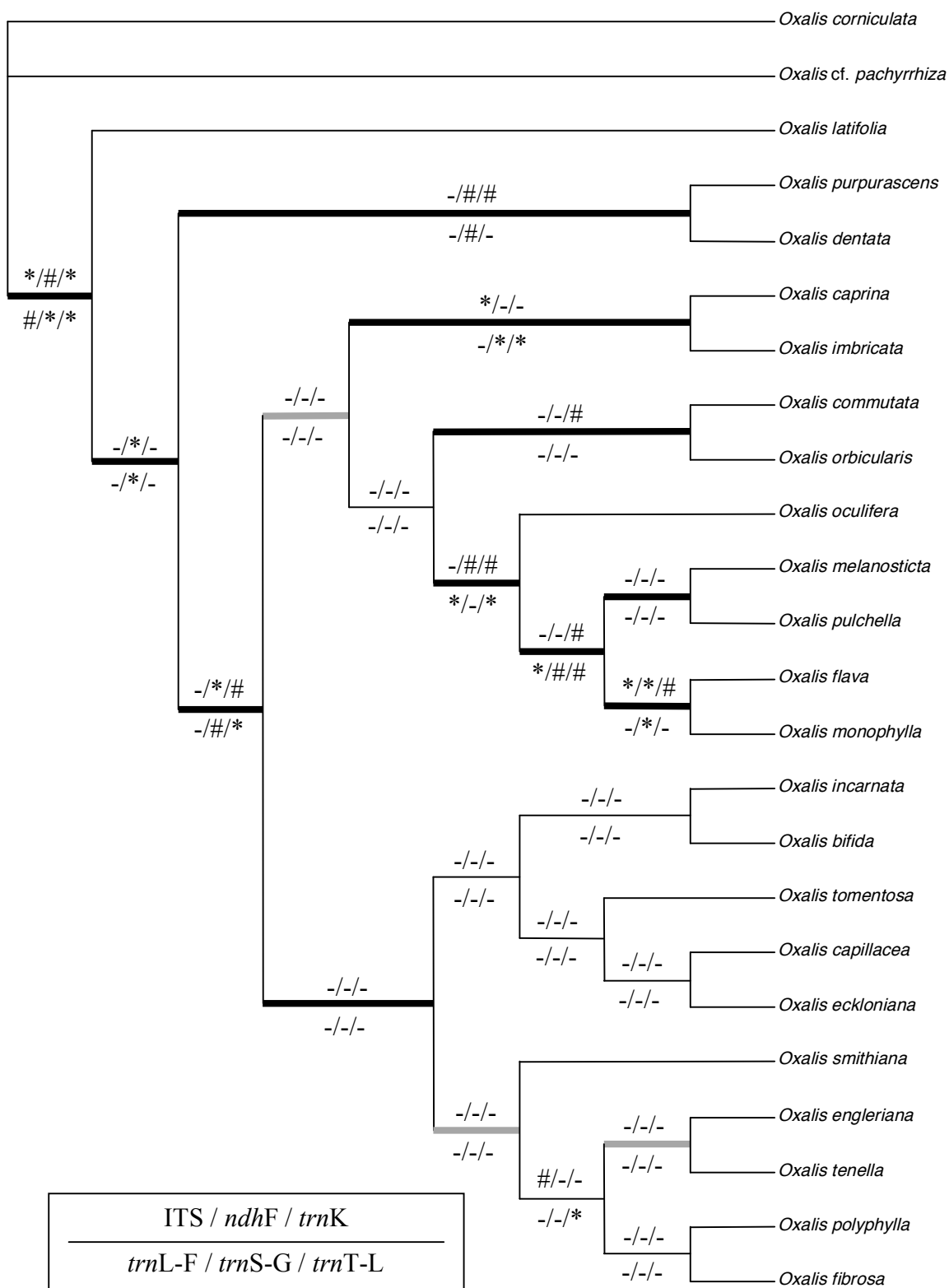


Fig. 5 The single topology found by parsimony analysis of the combined 6-partition data set for 24 taxa, with parsimony bootstrap (PB) values. This topology is one of the four trees found when gaps were coded and included in parsimony analyses. Thickened grey branches indicate clades present with 50% < PB < 70%. Thickened black branches indicate clades present with PB > 70%. Support values are summarised for individual partitions as indicated in the legend. Hyphens (-) indicate lack of support at the 50% PB level. Number signs (#) indicate support with 50% < PB < 70%. Asterisks (\*) indicate clades present with PB > 70%.

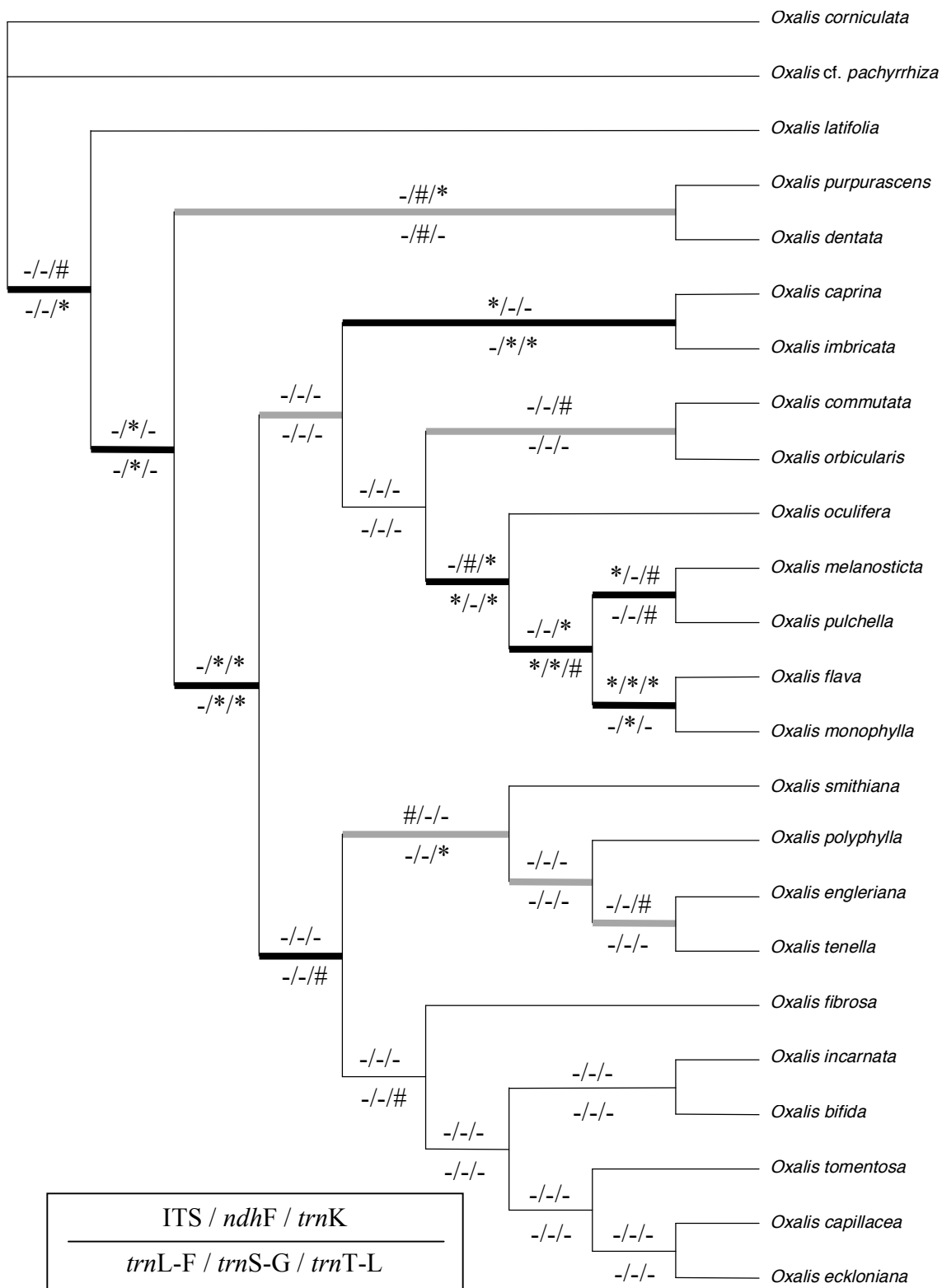


Fig. 6 Likelihood topology and Likelihood Bootstrap values (LB) of the combined 6-partition data set of 24 taxa. Thickened black branches indicate clades present at LB > 70%. Thickened grey branches indicate clades present with 50% < LB < 70%. Thickened black branches indicate clades present with LB > 70%. Support values are summarised for individual partitions as indicated in the legend. Hyphens (-) indicate lack of support at the 50% LB level. Number signs (#) indicate support with 50% < LB < 70%. Asterisks (\*) indicate clades present with LB > 70%.

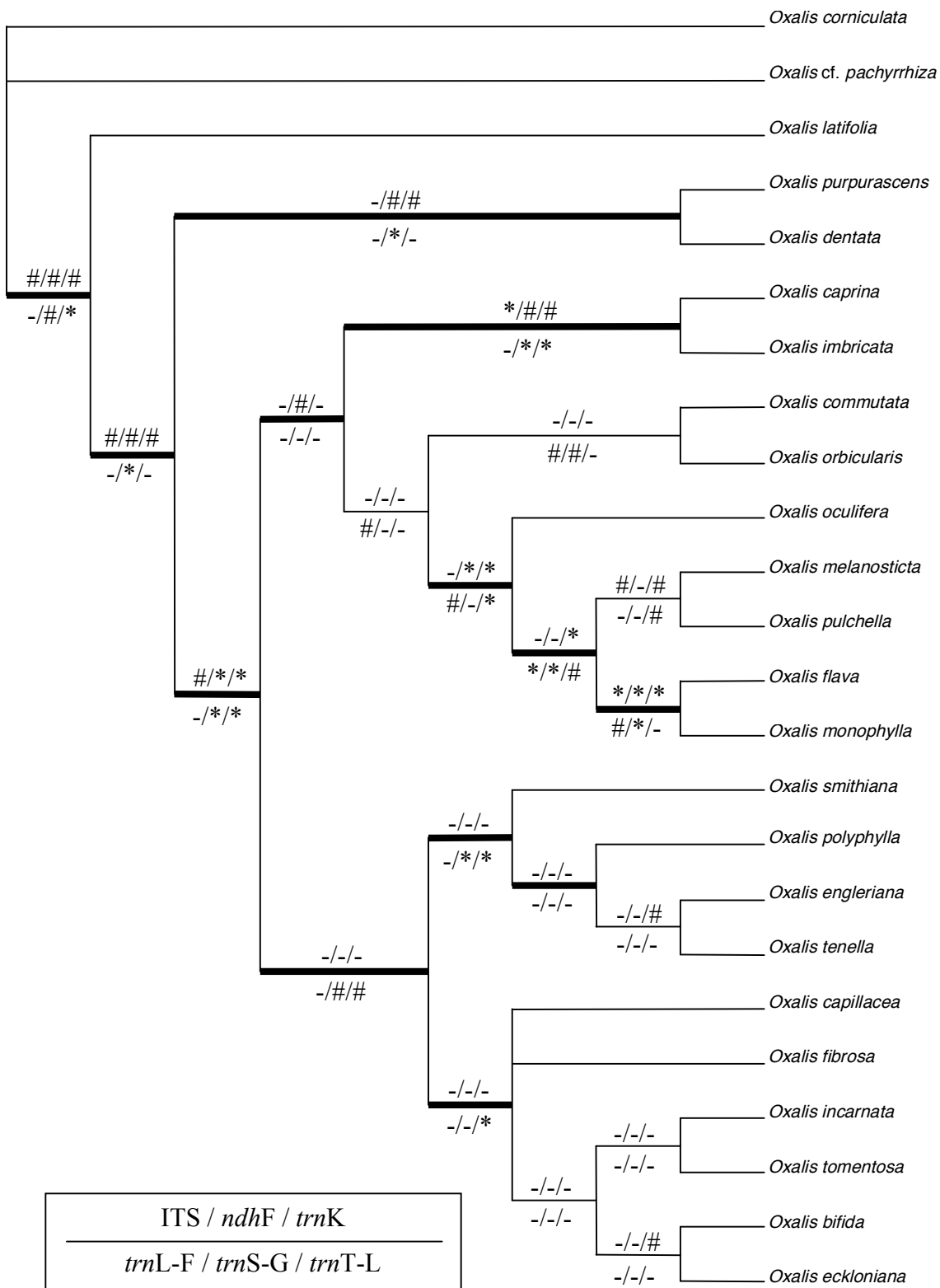


Fig. 7 Bayesian topology and Posterior Probabilities (PP) of the combined 6-partition data set of 24 taxa. Thickened black branches indicate clades present at PP > 0.95. Support values for individual partitions are summarised as indicated in the legend. Hyphens (-) indicate lack of support at the 0.5 PP level. Number signs (#) indicate support 0.5 < PP < 0.95. Asterisks (\*) indicate clades present at PP > 0.95.

### *The order Oxalidales*

Despite not being the focus of this study, the basic topology of the order Oxalidales is of interest, as this study is the most comprehensive to date for this lineage (Chronograms with confidence intervals are available on request from KCO). The order is clearly monophyletic based on *trnL*-F evidence, as are the six constituent families and the genus *Oxalis*, the largest genus in the order. The relationships of Cunoniaceae, Elaeocarpaceae, Cephalotaceae and Brunelliaceae are generally consistent with those proposed by previous authors (Bradford and Barnes, 2001; Crayn et al., 2006; Nandi et al., 1998; Savolainen et al., 2000). The sister relationship between the Connaraceae and Oxalidaceae is convincing, and is now supported by a variety of morphological evidence. This includes high levels of oxalic acid in plant tissues, floral ontogeny and the presence of heterostyly in both families, particularly tristily (summarized in Matthews and Endress, 2002).

### *Phylogenetic analysis*

Despite the high levels of incongruence between plastid and ITS gene tree, almost all strongly incongruent nodes involve terminal or small groups of taxa deeply embedded within strongly supported clades, and not basal nodes. In these cases, the species involved are clearly correctly placed, on morphological or palynological grounds, in the larger context of SA *Oxalis*. It is unclear whether the localised incongruence involving these taxa is a result of hybridization, ancestral character polymorphism or some other mechanism. Several studies have reported considerable variation in ploidy levels within the southern African clade (summarized in Dreyer and Johnson, 2000). This is corroborated by preliminary research into *Oxalis* genomic C-value studies that suggest that polyploidy is very common in southern African taxa (Doležal, 1997; J. Suda, unpublished data), particularly within species complexes. It is uncertain whether this is caused by allopolyploidy or whole genome duplication. Across the southern African clade, the most strong incongruence occurs within the Hirta and Glabra clades, where the basal resolution of taxa is different between ITS and plastid partitions. In terms of likelihood analyses, the most significant incongruence involves the outgroup taxon *Oxalis barrelieri*. The very long branch subtending this species could indicate long-branch attraction.

Overall, the phylogenetic utility of the various markers used in this study varies. In general, plastid markers were easy to amplify and sequence, and the spacer regions in particular showed moderate levels of variability that could be useful in future studies. The

plastid genes *ndhF* and *matK* would be very useful at larger family or order-scale analyses. The latter, in particular, is well-sampled for Oxalidales (45 sequences from all six families in the Oxalidales on Genbank). Of the three sampled chloroplast spacer regions, *trnS-G* and *trnT-L* are both more variable and more useful than the widely-used *trnL-F* region. In the case of *trnL-F*, it appears the data for this marker are just too invariant to be able to strongly discriminate against different trees. This is in accordance with the findings of Shaw et al. (2005), which found both *trnS-G* and *trnT-L* to be more variable than *trnL-F*.

The very high number of parsimony-informative sites in ITS would make this marker ideal for work at or below the species level, whilst the substantial homoplasy would discredit it for work above family level in the Oxalidales. However, although the ITS sequences generated here make sense in terms of the groups of morphologically similar species that they support, it is stressed that the effects of concerted evolution and of hybridization have not been tested in SA *Oxalis*. ITS is known to suffer from incomplete lineage sorting, pseudogenisation and concerted evolution (Mayol and Rosello, 2001; Alvarez and Wendell, 2003; Razafimandimbison et al., 2004), all processes that can confuse phylogenetic inference. This is particularly important when considering the high levels of ploidy variation in SA *Oxalis*. If this is the result of hybridization, then a single- or low-copy nuclear gene (Kim et al., 2008) would be a better candidate than ITS for species-level analyses. A promising marker would be nuclear encoded (but chloroplast expressed) glutamine synthetase (*ncpGS*; Emshwiller and Doyle, 2002). The initial primers for this locus were designed specifically for use in *Oxalis*, and phylogenetic work on this marker has now been successfully extended to unrelated families, such as Passifloraceae (Yockteng and Nadot, 2004) and Euphorbiaceae (Kulju et al., 2007). Although *ncpGS* was investigated as part of this study, PCR amplification was difficult and sequencing reads were poor, possibly due to mutations in primer binding sites.

In general, both the 171- and 24-taxon analyses agree on the basic topology of SA *Oxalis*. This reassuring picture is complicated by the potential problem of hybridization. Although Salter (1944) found no examples of hybridisation between *Oxalis* in the field, he did acknowledge a single potential hybrid between *O. macra* and *O. creaseyii* in his garden collection (neither species was sampled for this study, but both can confidently be placed in the Hirta clade based on their supra-areolate pollen). No other collection of SA *Oxalis* has been identified as being of hybrid origin, although this most probably reflects lack of knowledge rather than evidence of absence. The octoploid Andean crop species

*O. tuberosa* is known to have arisen through allopolyploid hybridization between *O. picchensis* and an unknown species (Emshwiller and Doyle, 2002; Emshwiller, 2002), so *Oxalis* is definitely capable of such hybridization. Very little is known of the role of hybridisation in the CFR flora as whole.

In addition to the potential conflicting effects of hybridization, the basic topology of SA *Oxalis* is still very poorly resolved. Several major clades (i.e. the Caulescent clade) are little more than large polytomies that not even 7 000 base pairs of data could resolve. Other clades (the Flava and Purpurea clades) received varying support from different optimality criteria, despite several strong potential morphological and palynological synapomorphies. Although the produced phylogenies make sense in light of what is known of *Oxalis* macro-morphology and palynology (Oberlander et al., 2004), there is still room for improvement in terms of resolution and support for many of the major groups. Future researchers should address these potential problems as well as the role of hybridization in the evolution of the genus.

In terms of classification, this study agrees with and reinforces the view of Oberlander et al. (2004) that the Salter (1944) taxonomy requires major revision. No single section *sensu* Salter (1944) is monophyletic. All but three subsections (*Cernuae* subsections *Lividae* and *Costatae* and *Angustatae* subsection *Pardales*) are artificial based on DNA evidence. Amongst the most surprising results are the convincing non-monophyly of the aquatic section *Campanulatae* and the close relationship between taxa with umbellate-inflorescences and taxa with single-flowered inflorescences in the Stellata clade. The aquatic species, in particular, would provide an ideal basis to study convergence of morphological characters as a result of adaptation to similar habitats.

#### *Divergence time analyses*

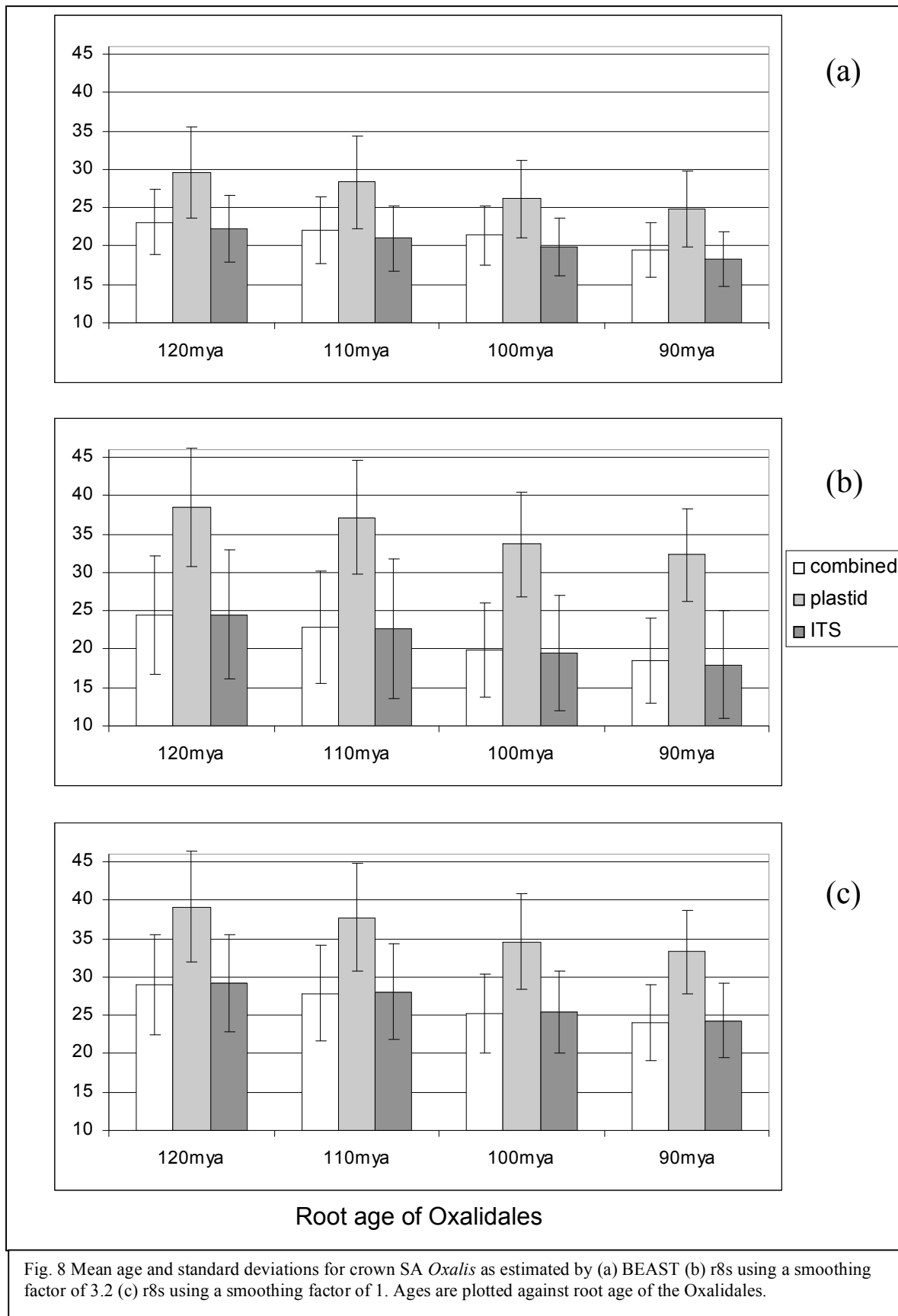
Methods that explicitly model distributions of rates and times have become popular methods of dating phylogenies, with the Multidivtime package (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002) in extensive use (Rutschmann, 2006). This program, much like PL, explicitly models autocorrelation of rates across nodes. Multidivtime assigns a rate to daughter nodes that is drawn from a distribution with the mean of this distribution equal to the rate on the parent node. This allows a fully parametric approach to the problem of divergence time estimation in the face of non-clock like data, in contrast to NPRS or PL. Advantages to this relaxed clock approach, apart from the explicit modeling of variable rates, include the utilization of Bayesian

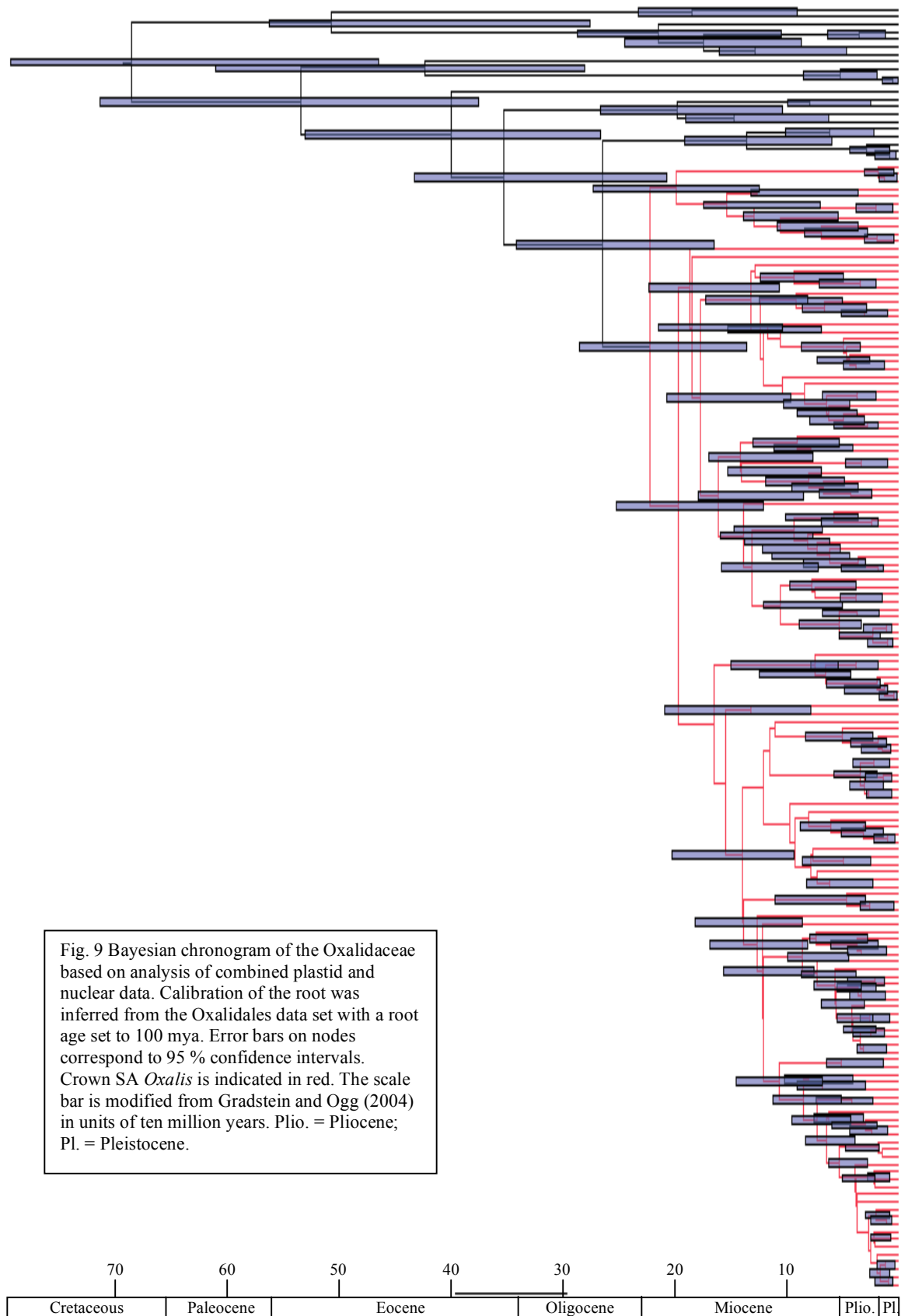
MCMC methods to co-estimate substitution rate parameters and divergence times for a given data set.

The BEAST family of programs (Drummond and Rambaut, 2007) is a more recent addition to relaxed clock methodologies. This program differs from previous relaxed clock approaches by abandoning autocorrelation of rates between parent and daughter nodes. The explicitly uncorrelated set of rates across the tree can be modeled by a variety of different distributions as implemented in BEAST itself. Due to this BEAST can be used to explore the degree of autocorrelation present in a data set. Calibration points and uncertainty are analogously assigned a distribution on the prior for the relevant node, which aids in calibrating the tree. A further advantage to BEAST is that it simultaneously infers topology and model parameters together with divergence times. This makes it essentially a relaxed-clock version of the well-known Bayesian phylogenetics program MrBayes (Ronquist and Huelsenbeck, 2003), with all the attendant advantages and disadvantages of Bayesian inference (Felsenstein, 2004).

The results of the factorial ANOVA clearly indicate that there is great uncertainty regarding the age of the SA *Oxalis* radiation (Fig 10). Uncertainty regarding calibration times, different methods of analysis, and different DNA markers all yield substantially different estimates of the crown southern African clade. Several aspects of age estimation are of interest here. Firstly, age estimates from plastid data sets are significantly older than estimates from ITS. This is consistent across method and root age assumptions used, although the degree of difference between ITS and plastid ages differs across methods. The latter explains the significant result of interactions between partition and method. Although coalescence times between independently segregating loci are not expected to be identical, a gap of 6 to 14 million years between ages seems worryingly high.

Secondly, although BEAST and  $r8s_{3,2}$  estimates for SA *Oxalis* in the combined data sets are in rough agreement, the ages inferred in  $r8s_1$  are generally older by 4-5 million years. All in all, interactions between major variables are inconsistent and influence one another in dissimilar ways. In general, the BEAST analyses have the least (but still significant) variation in age between partition and root node age used.





Thirdly, it is unclear precisely what is causing these large differences between analyses. We cannot explain why age estimates vary so much depending on partition or method used. Violation of autocorrelation of rates (as judged by the covariance parameter in BEAST analyses) does not explain the agreement between the r8s<sub>3.2</sub> and BEAST analyses. Non-clocklike behaviour in the data set is a possible explanation: larger smoothing values in r8s force more clocklike behaviour onto trees. However, it is the trees with the smaller smoothing value (r8s<sub>1</sub>; and hence less clocklike) that are substantially older than other analyses, whilst the more clocklike r8s analyses agree with the non-clocklike BEAST estimates. Under-sampling bias (Linder et al., 2005) is not a factor, as all analyses used the same data matrix. Simply taking into account the uncertainty in root age of the Oxalidales yields mean estimates for crown SA *Oxalis* ranging from 29 to 19 mya (combined data analyses). Excluding the older ages of the r8s<sub>1</sub> analysis narrows the total range in mean estimates to a period between 19 and 24 million years ago, but there is no *a priori* reason to discard this analysis, nor the substantially older estimates of the plastid analyses. Given the uncertainty in the root age for the Oxalidales as a whole, we advocate a conservative approach. Mean age estimates for the SA *Oxalis* lineage in the region range from  $39 \pm 7$  mya (PL of plastid, r8s<sub>1</sub>, root age 120 mya) to  $18 \pm 7$  mya (from PL of ITS, r8s<sub>3.2</sub>, root age 90 mya), a 20 million year bracket. Even excluding the ages inferred using a 120 mya maximum constraint on the Oxalidales (which can be argued are unrealistically old) would barely change this figure.

If the uncertainty regarding age estimates between data partitions and methods are general features of age inference outside of our data sets, then estimates for Cape clades, and indeed many other plant groups, will have to be re-evaluated. Of the previous age estimates of Cape clades, three (Goldblatt et al., 2002; Klak et al., 2004; Linder et al., 2005) used only plastid data, two (Mummenhof et al., 2005; Edwards and Hawkins, 2007) used ITS, and six (Richardson et al., 2001; Verboom et al., 2003; Bakker et al., 2005; Barraclough and Reeves, 2005; Forest et al., 2007 and Boatwright et al., 2008 ) used data from more than one genome. As summarized by Edwards and Hawkins (2007), different techniques of age estimation can result in large differences in age from the same data set. If partitions from different loci also produce inconsistent ages, then single-locus studies (here including all plastid markers) should perhaps be accepted with caution. It is of interest that the two studies yielding the youngest ages for Cape clades (Klak et al., 2004; Mummenhof et al., 2005) both used data from a single locus. It is also interesting that the sole example of independent estimates for the same Cape clade (the Podalyrieae *sensu stricto* of the Fabaceae) yielded mean ages that differed from one another by 15

million years (Edwards and Hawkins, 2007; Boatwright et al., 2008), even though both studies used the same fossil calibration point.

In this study we have attempted to account for many major sources of misleading divergence time estimates, such as rate variation across lineages and autocorrelation of rates, data source, calibration error (albeit just of one node) and methodology. Ages for many Cape clades have been estimated under circumstances that have not assessed the impact of these factors. In particular, calibration points for Cape clades have proven problematic given the poor fossil record for Cape plants (Linder, 2003). It would be interesting to compare ages for Cape clades derived from Relaxed Clock Methods such as BEAST with previous estimates, and to assess any measurable bias in light of the method used. With the advent of Relaxed Clock approaches using Bayesian Inference, it should become easier to attempt a more systematic approach to these problems by constructing higher-order phylogenies with good calibration points, and then using posterior estimates of age as priors for focal groups of interest. Such an approach would lead to the prior distributions on age dominating over the likelihood function in the posterior distributions of the groups of interest. In the absence of internal calibration points, however, this is perhaps a desirable property, as it properly transfers uncertainty regarding clade ages from well-calibrated phylogenies onto particular clades of interest.

Although *Oxalis* age estimates vary greatly over a 20 million year period, all average estimates from all analyses are more than 18 million years old. This is substantially older than several other predominantly Succulent Karoo clades such as *Heliophila* (Mummenhof et al., 2005) or the Aizoaceae subfamily Ruschiodeae (Klak et al., 2004), which are reconstructed as being very recent introductions to the Cape Flora. Unlike the many older Cape clades with predominantly Fynbos distributions, such as the Restionaceae (Linder et al., 2005), the tribes Crotalariae *pro parte* and Podalyrieae of the Fabaceae (Edwards and Hawkins, 2007; Boatwright et al., 2008) and the major groups of Cape Proteaceae (Barracough and Reeves, 2004; Barker et al., 2007), *Oxalis* straddles the boundary of the Fynbos and Succulent Karoo biomes, as it includes both many Fynbos and Succulent Karoo taxa. Due to the poor resolution of relationships between several major clades, particularly the poor resolution of the Core southern African clade, the original biome preference of SA *Oxalis* cannot be confidently inferred. Nevertheless some general comments can be made. The few summer rainfall taxa within SA *Oxalis* are

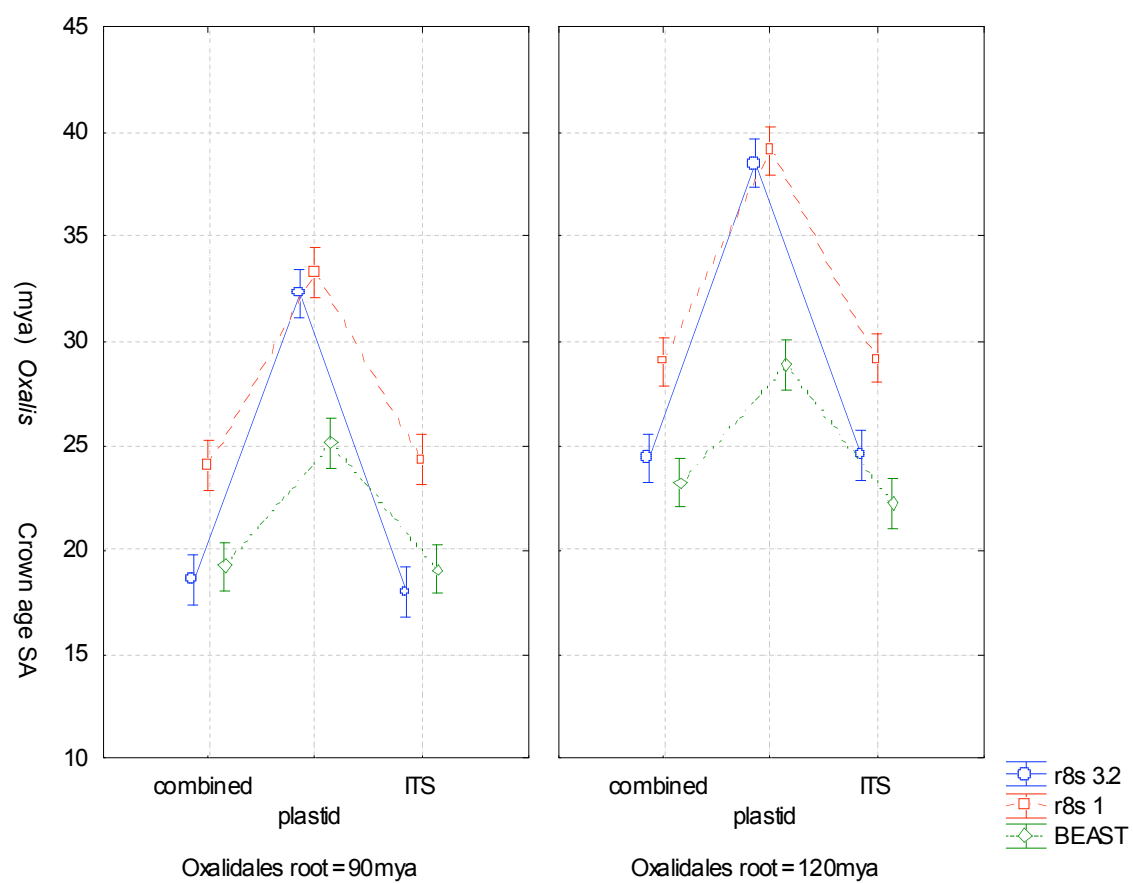


Fig. 10 Mean ages of factors investigated in the factorial ANOVA. Error bars indicate 95 % confidence intervals for mean ages.

all deeply embedded within otherwise CFR lineages, thus making them escapees from the CFR. This mirrors many other CFR clades, such as the genera *Protea* (Barracough and Reeves, 2005) and *Leucadendron* (Barker et al., 2004) of the Proteaceae, *Cliffortia* (Whitehouse, 2002), *Disa*, Irideae *pro parte*, *Pentaschistis* and the African Restionaceae (Galley et al., 2007). This indicates that the common ancestor of crown SA *Oxalis* probably inhabited the South-Western Cape, in the region now covered by the CFR. Of particular interest is the placement of the Livida clade. This predominantly Fynbos and Renosterveld lineage is constructed in one of two positions: as sister to the Namibian/South African Pes-caprae clade, or as sister to the Core southern African clade. Either position would strengthen reconstructions of a Fynbos ancestry for either SA *Oxalis* as a whole, or the segregate core southern African clade.

Given that the oldest inferred date still falls well short of the opening of the South Atlantic (McLoughlin, 2001), and that the southern African taxa are monophyletic and deeply nested within a predominantly New World clade, the most likely scenario for the origin of the ancestral southern African taxon is one of dispersal from the South American continent. The most likely dispersal propagule was seed, although dispersal by bulbils is also possible. The ancestral seed morphology of *Oxalis* is endospermous, with a long dormancy period, which could enable long-distance dispersal (although endospermy and dormancy have subsequently been lost in several derived SA lineages, such as the Flava, Glabra and Hirta clades). *Oxalis* is one of the few CFR taxa that share a trans-South Atlantic Ocean relationship, together with *Prionium*, *Drosera* and Haemadoraceae, and possibly Bruniaceae (summarized in Galley and Linder, 2006). Furthermore, *Oxalis* is the second genus after *Drosera* for which directionality of dispersal can be inferred (from South America to Africa). The actual dispersal agent remains conjectural.

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Table 1 Fossil calibration points utilized for analyses of the Oxalidales. Numerical ages were derived for period/epoch estimates using the geologic time scale of Gradstein and Ogg (2004).

| Node                                 | Fossil                      | Reference                   | Age                  | Age used |
|--------------------------------------|-----------------------------|-----------------------------|----------------------|----------|
| 1° calibration points                |                             |                             |                      |          |
| Crown clade                          |                             |                             |                      | 58.7-    |
| <i>Eucryphia</i>                     | <i>Eucryphia falcata</i>    | Barnes et al. (2001)        | Late Paleocene       | 55.8     |
|                                      |                             |                             | Middle Eocene-Early  | 48.6-    |
| <i>Codia</i> and <i>Callicoma</i>    | <i>Codia australiensis</i>  | Barnes et al. (2001)        | Oligocene            | 40.4     |
| <i>Acsmithia</i> and                 | <i>Spiraeanthemum</i> /     |                             |                      | 44-      |
| <i>Spiraeanthemum</i>                | <i>Acsmithia</i>            | Barnes et al. (2001)        | Late Middle Eocene   | 40.4     |
|                                      |                             | Schonenberger et al. (2001) | Late Santonian-Early |          |
| Crown Cunoniaceae                    | <i>Platydiscus peltatus</i> |                             | Campanian            | 84-78    |
| 2° calibration points                |                             |                             |                      |          |
|                                      |                             |                             |                      | 38 ±     |
| Crown Tremandraceae                  | -                           | Crayn et al. (2007)         | -                    | 3        |
|                                      |                             |                             |                      | 56 ±     |
| <i>Vallea</i> and <i>Aristotelia</i> | -                           | Crayn et al. (2007)         | -                    | 4        |

Table 2 Species used in this study, with Genbank accession numbers, voucher specimens and classification sensu Salter (1944).

| Species   | Current section       | Current subsection     | Reference number | Voucher specimen   | Accession no.: ITS       | Accession no.: trnL-F    | Accession no.: trnS-G    |
|---|-----------------------|------------------------|------------------|--------------------|--------------------------|--------------------------|--------------------------|
| <i>Averrhoa bilimbi</i> L.                                  | -                     | -                      | AVCAR            | -                  | <a href="#">EU436862</a> | <a href="#">AJ582291</a> | <a href="#">EU437151</a> |
| <i>Averrhoa carambola</i> L.                                | -                     | -                      | AVBIL            | -                  | <a href="#">EU436863</a> | <a href="#">EU437032</a> | <a href="#">EU437152</a> |
| <i>Biophytum abyssinicum</i> Steud. ex. A. Rich.            | -                     | -                      | BIOAB            | -                  | <a href="#">EU436864</a> | <a href="#">AJ582292</a> | <a href="#">EU437153</a> |
| <i>Biophytum</i> sp.  | -                     | -                      | BIOPHIL          | -                  | <a href="#">EU436865</a> | <a href="#">AJ582293</a> | <a href="#">EU437154</a> |
| <i>Dapania pentandra</i> Capuron                            | -                     | -                      | DAPEN            | -                  | <a href="#">EU436861</a> | <a href="#">EU437031</a> | <a href="#">EU437150</a> |
| O. sp. nova   | unplaced              | -                      | MO620            | Mannheimer 2591    | <a href="#">EU436885</a> | <a href="#">EU437047</a> | <a href="#">EU437174</a> |
| <i>Oxalis</i> "canaliculata" (manuscript name, M. B. Bayer) | unplaced              | -                      | MO583            | Ex Hort.           | <a href="#">EU436946</a> | <a href="#">EU437088</a> | <a href="#">EU437232</a> |
| <i>Oxalis acetosella</i> L.                                 | -                     | -                      | MO665            | J. Walter 7182     | <a href="#">EU436870</a> | <a href="#">EU437036</a> | <a href="#">EU437159</a> |
| <i>Oxalis adenodes</i> Sond.                                | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO147            | L. Dreyer 716      | <a href="#">EU436903</a> | <a href="#">EU437060</a> | <a href="#">EU437191</a> |
| <i>Oxalis adspersa</i> E. & Z.                              | <i>Angustatae</i>     | <i>Xanthotrichae</i>   | MO66             | L. Dreyer 635      | <a href="#">EU436936</a> | <a href="#">AJ582290</a> | <a href="#">EU437223</a> |
| <i>Oxalis ambigua</i> Jacq.                                 | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO555            | K. Oberlander 0140 | <a href="#">EU436905</a> | <a href="#">EU437061</a> | <a href="#">EU437193</a> |
| <i>Oxalis amblyodonta</i> Salter                            | <i>Angustatae</i>     | <i>Multifoliolatae</i> | MO597            | M. B. Bayer 7397   | <a href="#">EU437007</a> | <a href="#">EU437116</a> | <a href="#">EU437293</a> |
| <i>Oxalis amblyosepala</i> Schltr.                          | <i>Latifoliolatae</i> | -                      | MO721            | K. Oberlander 0168 | <a href="#">EU436982</a> | <a href="#">EU437105</a> | <a href="#">EU437268</a> |
| <i>Oxalis annae</i> Bolus f.                                | <i>Foveolatae</i>     | -                      | MO436            | L. Mucina 170903/3 | <a href="#">EU436920</a> | <a href="#">EU437072</a> | <a href="#">EU437208</a> |
| <i>Oxalis argillacea</i> Bolus f.                           | <i>Angustatae</i>     | <i>Xanthotrichae</i>   | MO282            | L. Dreyer 786      | <a href="#">EU436940</a> | <a href="#">AJ582294</a> | <a href="#">EU437227</a> |
| <i>Oxalis argyrophylla</i> Salter                           | <i>Angustatae</i>     | <i>Lineares</i>        | MO313            | K. Oberlander 0040 | <a href="#">EU437005</a> | <a href="#">AJ582295</a> | <a href="#">EU437291</a> |
| <i>Oxalis aridicola</i> Salter                              | <i>Latifoliolatae</i> | -                      | MO103            | L. Dreyer 672      | <a href="#">EU436977</a> | <a href="#">AJ582296</a> | <a href="#">EU437263</a> |
| <i>Oxalis attenuata</i> Salter                              | <i>Foveolatae</i>     | -                      | MO45             | M. B. Bayer 7007   | <a href="#">EU436918</a> | <a href="#">EU437071</a> | <a href="#">EU437206</a> |
| <i>Oxalis aurea</i> Schltr.                                 | <i>Latifoliolatae</i> | -                      | MO267            | L. Dreyer 771      | <a href="#">EU436935</a> | <a href="#">EU437080</a> | <a href="#">EU437222</a> |
| <i>Oxalis barrelieri</i> L.                                 | -                     | -                      | MO454            | DUB 809            | <a href="#">EU436866</a> | <a href="#">EU437033</a> | <a href="#">EU437155</a> |
| <i>Oxalis bifida</i> Thunb.                                 | <i>Oppositae</i>      | <i>Bifurcatae</i>      | MO19             | L. Dreyer 608      | <a href="#">EU437017</a> | <a href="#">AJ582297</a> | <a href="#">EU437303</a> |
| <i>Oxalis bifurca</i> Lodd.                                 | <i>Oppositae</i>      | <i>Bifurcatae</i>      | MO324            | DUB 764            | <a href="#">EU436965</a> | <a href="#">EU437100</a> | <a href="#">EU437251</a> |
| <i>Oxalis blastorrhiza</i> Salter                           | <i>Angustatae</i>     | <i>Lineares</i>        | MO284            | L. Dreyer 788      | <a href="#">EU436974</a> | <a href="#">AJ582298</a> | <a href="#">EU437260</a> |
| <i>Oxalis bowiei</i> Lindl.                                 | <i>Cernuae</i>        | <i>Stellatae</i>       | MO502            | Ex. Hort.          | <a href="#">EU436895</a> | <a href="#">EU437055</a> | <a href="#">EU437183</a> |
| <i>Oxalis brasiliensis</i> Lodd.                            | -                     | -                      | MO391            | J. Holmes 0008     | <a href="#">EU436879</a> | <a href="#">EU437044</a> | <a href="#">EU437168</a> |
| <i>Oxalis bullulata</i> Salter                              | <i>Foveolatae</i>     | -                      | MO288            | L. Dreyer 792      | <a href="#">EU436908</a> | <a href="#">EU437063</a> | <a href="#">EU437196</a> |
| <i>Oxalis burkei</i> Sond.                                  | <i>Angustatae</i>     | <i>Lineares</i>        | MO29             | L. Dreyer 618      | <a href="#">EU437029</a> | <a href="#">AJ582299</a> | -                        |
| <i>Oxalis burtoniae</i> Salter                              | <i>Angustatae</i>     | <i>Lineares</i>        | MO304            | K. Oberlander 0031 | <a href="#">EU437008</a> | <a href="#">AJ582300</a> | <a href="#">EU437294</a> |
| <i>Oxalis callosa</i> R. Knuth                              | <i>Latifoliolatae</i> | -                      | MO532            | K. Oberlander 0120 | <a href="#">EU436973</a> | <a href="#">EU437103</a> | <a href="#">EU437259</a> |
| <i>Oxalis camelopardalis</i> Salter                         | <i>Angustatae</i>     | <i>Pardales</i>        | MO371            | K. Oberlander 0071 | <a href="#">EU437012</a> | <a href="#">EU437119</a> | <a href="#">EU437298</a> |
| <i>Oxalis campicola</i> Salter                              | <i>Angustatae</i>     | <i>Lineares</i>        | MO276            | L. Dreyer 780      | <a href="#">EU436981</a> | <a href="#">AJ582301</a> | <a href="#">EU437267</a> |

|  |                       |                        |       |   |                          |                          |                          |
|--|-----------------------|------------------------|-------|---|--------------------------|--------------------------|--------------------------|
| <i>Oxalis campylorrhiza</i> Salter               | <i>Latifoliolatae</i> | -                      | MO127 | L. Dreyer 696                                     | <a href="#">EU436947</a> | <a href="#">AJ582302</a> | <a href="#">EU437233</a> |
| <i>Oxalis capillacea</i> E. Mey. ex Sond.        | <i>Angustatae</i>     | <i>Pardales</i>        | MO36  | L. Dreyer 625<br>L. Dreyer and K. Oberlander 0001 | <a href="#">EU437013</a> | <a href="#">AJ582304</a> | <a href="#">EU437299</a> |
| <i>Oxalis caprina</i> L.                         | <i>Cernuae</i>        | <i>Stellatae</i>       | MO7   |   | <a href="#">EU436892</a> | <a href="#">AJ582305</a> | <a href="#">EU437180</a> |
| <i>Oxalis cathara</i> Salter                     | <i>Crassulae</i>      | -                      | MO582 | Ex. Hort.   | <a href="#">EU436931</a> | <a href="#">EU437077</a> | <a href="#">EU437218</a> |
| <i>Oxalis cf. pulvinata</i> Salter               | <i>Crassulae</i>      | -                      | MO576 | Ex. Hort.   | <a href="#">EU436926</a> | <a href="#">EU437076</a> | <a href="#">EU437213</a> |
| <i>Oxalis cf. tragopoda</i> Salter               | <i>Cernuae</i>        | <i>Stellatae</i>       | MO490 | Ex. Hort.   | <a href="#">EU436897</a> | <a href="#">EU437057</a> | <a href="#">EU437185</a> |
| <i>Oxalis ciliaris</i> Jacq.                     | <i>Angustatae</i>     | <i>Lineares</i>        | MO24  | L. Dreyer 613                                     | <a href="#">EU436979</a> | <a href="#">AJ582307</a> | <a href="#">EU437265</a> |
| <i>Oxalis clavifolia</i> Sond.                   | <i>Angustatae</i>     | <i>Glandulosae</i>     | MO556 | K. Oberlander 0141                                | <a href="#">EU436951</a> | <a href="#">EU437090</a> | <a href="#">EU437237</a> |
| <i>Oxalis commutata</i> Sond.                    | <i>Latifoliolatae</i> | -                      | MO17  | L. Dreyer 606                                     | <a href="#">EU436898</a> | <a href="#">AJ582308</a> | <a href="#">EU437186</a> |
| <i>Oxalis comosa</i> E. Mey. ex Sond.            | <i>Oppositae</i>      | <i>Bifurcatae</i>      | MO238 | L. Mucina 220901/30A<br>Bayer and Oberlander 0009 | <a href="#">EU436954</a> | <a href="#">EU437091</a> | <a href="#">EU437240</a> |
| <i>Oxalis compressa</i> L. f.                    | <i>Cernuae</i>        | <i>Eu-cernuae</i>      | MO519 |   | <a href="#">EU436883</a> | <a href="#">EU437046</a> | <a href="#">EU437172</a> |
| <i>Oxalis comptonii</i> Salter                   | <i>Angustatae</i>     | <i>Lineares</i>        | MO298 | L. Dreyer 802                                     | <a href="#">EU436985</a> | <a href="#">AJ582309</a> | <a href="#">EU437272</a> |
| <i>Oxalis confertifolia</i> (O. Kuntze) R. Knuth | <i>Angustatae</i>     | <i>Pardales</i>        | MO358 | K. Oberlander 0075                                | <a href="#">EU437011</a> | <a href="#">EU437118</a> | <a href="#">EU437297</a> |
| <i>Oxalis convexula</i> Jacq.                    | <i>Foveolatae</i>     | -                      | MO209 | K. Oberlander 0014                                | <a href="#">EU436913</a> | <a href="#">EU437067</a> | <a href="#">EU437201</a> |
| <i>Oxalis copiosa</i> Bolus f.                   | <i>Cernuae</i>        | <i>Eu-cernuae</i>      | MO227 | K. Oberlander 0021                                | <a href="#">EU436887</a> | <a href="#">EU437049</a> | <a href="#">EU437176</a> |
| <i>Oxalis corniculata</i> L.                     | -                     | -                      | MO256 | K. Oberlander 0025                                | <a href="#">EU436867</a> | <a href="#">AJ582310</a> | <a href="#">EU437156</a> |
| <i>Oxalis crocea</i> Salter                      | <i>Angustatae</i>     | <i>Sessilifoliatae</i> | MO124 | L. Dreyer 693                                     | <a href="#">EU436950</a> | <a href="#">AJ582311</a> | <a href="#">EU437236</a> |
| <i>Oxalis cuneata</i> Jacq.                      | <i>Angustatae</i>     | <i>Lineares</i>        | MO287 | L. Dreyer 791                                     | <a href="#">EU436969</a> | <a href="#">EU437101</a> | <a href="#">EU437255</a> |
| <i>Oxalis densa</i> N.E. Br.                     | <i>Foveolatae</i>     | -                      | MO783 | K. Oberlander 0169                                | <a href="#">EU436921</a> | <a href="#">EU437073</a> | <a href="#">EU437209</a> |
| <i>Oxalis dentata</i> Jacq.                      | <i>Cernuae</i>        | <i>Lividae</i>         | MO49  | C. Cilliers 0004                                  | <a href="#">EU436888</a> | <a href="#">AJ582312</a> | <a href="#">EU437177</a> |
| <i>Oxalis depressa</i> E. & Z.                   | <i>Foveolatae</i>     | -                      | MO464 | K. Oberlander 0085                                | <a href="#">EU436909</a> | <a href="#">EU437064</a> | <a href="#">EU437197</a> |
| <i>Oxalis deserticola</i> Salter                 | <i>Angustatae</i>     | <i>Glandulosae</i>     | MO526 | K. Oberlander 0114                                | <a href="#">EU436939</a> | <a href="#">EU437083</a> | <a href="#">EU437226</a> |
| <i>Oxalis dichotoma</i> Salter                   | <i>Cernuae</i>        | <i>Stellatae</i>       | MO333 | K. Oberlander 0052<br>Bayer and Oberlander 0014   | <a href="#">EU436893</a> | <a href="#">EU437053</a> | <a href="#">EU437181</a> |
| <i>Oxalis dilatata</i> L. Bolus                  | <i>Foveolatae</i>     | -                      | MO524 |   | <a href="#">EU436910</a> | <a href="#">EU437065</a> | <a href="#">EU437198</a> |
| <i>Oxalis dillenii</i> Jacq.                     | -                     | -                      | MO667 | J. Walter 6565                                    | <a href="#">EU436868</a> | <a href="#">EU437034</a> | <a href="#">EU437157</a> |
| <i>Oxalis dines</i> Ornduff                      | <i>Campanulatae</i>   | -                      | MO653 | N. A. Helme 1355                                  | <a href="#">EU436943</a> | <a href="#">EU437085</a> | -                        |
| <i>Oxalis disticha</i> Jacq.                     | <i>Campanulatae</i>   | -                      | MO596 | K. Oberlander 0159                                | <a href="#">EU436942</a> | <a href="#">EU437084</a> | <a href="#">EU437229</a> |
| <i>Oxalis dregei</i> Sond.                       | <i>Campanulatae</i>   | -                      | MO447 | L. Mucina 210903/11                               | <a href="#">EU436930</a> | <a href="#">AJ582313</a> | <a href="#">EU437217</a> |
| <i>Oxalis droseroides</i> E. Mey. ex Sond.       | <i>Angustatae</i>     | <i>Lineares</i>        | MO362 | K. Oberlander 0079                                | <a href="#">EU436994</a> | <a href="#">EU437111</a> | <a href="#">EU437280</a> |
| <i>Oxalis duriuscula</i> Schltr.                 | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO461 | K. Oberlander 0082                                | <a href="#">EU437020</a> | <a href="#">EU437123</a> | <a href="#">EU437306</a> |
| <i>Oxalis ebracteata</i> Savign.                 | <i>Angustatae</i>     | <i>Lineares</i>        | MO75  | L. Dreyer 644                                     | <a href="#">EU436990</a> | <a href="#">AJ582314</a> | <a href="#">EU437276</a> |
| <i>Oxalis eckloniana</i> Presl.                  | <i>Sagittatae</i>     | -                      | MO39  | L. Dreyer 628                                     | <a href="#">EU437025</a> | <a href="#">AJ582315</a> | <a href="#">EU437311</a> |
| <i>Oxalis engleriana</i> Schltr.                 | <i>Angustatae</i>     | <i>Multifoliolatae</i> | MO195 | K. Oberlander 0010                                | <a href="#">EU436961</a> | <a href="#">AJ582316</a> | <a href="#">EU437247</a> |

|  |                       |                    |       |                              |                          |                          |                          |
|--|-----------------------|--------------------|-------|------------------------------|--------------------------|--------------------------|--------------------------|
| <i>Oxalis exserta</i> Salter               | <i>Angustatae</i>     | <i>Lineares</i>    | MO117 | L. Dreyer 686                | <a href="#">EU436967</a> | <a href="#">AJ582317</a> | <a href="#">EU437253</a> |
| <i>Oxalis fabaeifolia</i> Jacq.            | <i>Crassulaceae</i>   | -                  | MO152 | L. Dreyer 721/A              | <a href="#">EU436925</a> | <a href="#">EU437075</a> | <a href="#">EU437212</a> |
| <i>Oxalis falcata</i> Salter               | <i>Angustatae</i>     | <i>Lineares</i>    | MO476 | K. Oberlander 0096           | <a href="#">EU436992</a> | <a href="#">EU437109</a> | <a href="#">EU437278</a> |
| <i>Oxalis fergusoniae</i> Salter           | <i>Foveolatae</i>     | -                  | MO501 | K. Oberlander 0111           | <a href="#">EU436912</a> | <a href="#">EU437066</a> | <a href="#">EU437200</a> |
| <i>Oxalis fibrosa</i> Bolus f.             | <i>Sagittatae</i>     | -                  | MO332 | K. Oberlander 0051           | <a href="#">EU436958</a> | <a href="#">EU437095</a> | <a href="#">EU437244</a> |
| <i>Oxalis flava</i> L.                     | <i>Crassulaceae</i>   | -                  | MO25  | L. Dreyer 614                | <a href="#">EU436924</a> | <a href="#">AJ582318</a> | <a href="#">EU437211</a> |
| <i>Oxalis flaviuscula</i> Salter           | <i>Crassulaceae</i>   | -                  | MO132 | L. Dreyer 701                | <a href="#">EU436929</a> | <a href="#">AJ582319</a> | <a href="#">EU437216</a> |
| <i>Oxalis fourcadei</i> Salter             | <i>Foveolatae</i>     | -                  | MO664 | P. A. Bean & J. H. Vlok 2046 | <a href="#">EU436923</a> | <a href="#">EU437074</a> | -                        |
| <i>Oxalis furcillata</i> Salter            | <i>Foveolatae</i>     | -                  | MO228 | K. Oberlander 0022           | <a href="#">EU436952</a> | <a href="#">AJ582320</a> | <a href="#">EU437238</a> |
| <i>Oxalis giftbergensis</i> Salter         | <i>Angustatae</i>     | <i>Lineares</i>    | MO292 | L. Dreyer 796                | <a href="#">EU436988</a> | <a href="#">AJ582321</a> | <a href="#">EU437274</a> |
| <i>Oxalis glabra</i> Th.                   | <i>Angustatae</i>     | <i>Lineares</i>    | MO155 | L. Mucina 170601/2           | <a href="#">EU437000</a> | <a href="#">AJ582322</a> | <a href="#">EU437286</a> |
| <i>Oxalis goniorrhiza</i> E. & Z.          | <i>Angustatae</i>     | <i>Lineares</i>    | MO594 | K. Oberlander 0157           | <a href="#">EU437002</a> | <a href="#">EU437114</a> | <a href="#">EU437288</a> |
| <i>Oxalis gracilipes</i> Schltr.           | <i>Angustatae</i>     | <i>Lineares</i>    | MO76  | L. Dreyer 645                | <a href="#">EU436991</a> | <a href="#">EU437108</a> | <a href="#">EU437277</a> |
| <i>Oxalis gracilis</i> Jacq.               | <i>Angustatae</i>     | <i>Lineares</i>    | MO535 | K. Oberlander 123            | <a href="#">EU436976</a> | <a href="#">EU437104</a> | <a href="#">EU437262</a> |
| <i>Oxalis grammopetala</i> Sond.           | <i>Foveolatae</i>     | -                  | MO571 | K. Oberlander 0156           | <a href="#">EU436906</a> | <a href="#">EU437062</a> | <a href="#">EU437194</a> |
| <i>Oxalis grammophylla</i> Salter          | <i>Angustatae</i>     | <i>Pardales</i>    | MO101 | L. Dreyer 670                | <a href="#">EU437016</a> | <a href="#">AJ582323</a> | <a href="#">EU437302</a> |
| <i>Oxalis haedulipes</i> Salter            | <i>Cernuae</i>        | <i>Eu-cernuae</i>  | MO434 | L. Mucina 180903/9           | <a href="#">EU436886</a> | <a href="#">EU437048</a> | <a href="#">EU437175</a> |
| <i>Oxalis heterophylla</i> D.C.            | <i>Oppositae</i>      | <i>Bifurcatae</i>  | MO518 | Bayer and Oberlander 0008    | <a href="#">EU437018</a> | <a href="#">EU437121</a> | <a href="#">EU437304</a> |
| <i>Oxalis hirta</i> L.                     | <i>Angustatae</i>     | <i>Lineares</i>    | MO77  | L. Dreyer 646                | <a href="#">EU436971</a> | <a href="#">AJ582324</a> | <a href="#">EU437257</a> |
| <i>Oxalis hygrophila</i> Kumwenda & Dreyer | <i>Latifoliolatae</i> | -                  | MO230 | Kumwenda and Dreyer 0001     | <a href="#">EU437024</a> | <a href="#">AJ582333</a> | <a href="#">EU437310</a> |
| <i>Oxalis hypsophila</i> Phil.             | -                     | -                  | MO456 | L. Dreyer 805                | <a href="#">EU436874</a> | <a href="#">EU437040</a> | <a href="#">EU437163</a> |
| <i>Oxalis imbricata</i> E. & Z.            | <i>Oppositae</i>      | <i>Subintegrae</i> | MO345 | K. Oberlander 0062           | <a href="#">EU436896</a> | <a href="#">EU437056</a> | <a href="#">EU437184</a> |
| <i>Oxalis incarnata</i> L.                 | <i>Oppositae</i>      | <i>Subintegrae</i> | MO346 | K. Oberlander 0063           | <a href="#">EU436957</a> | <a href="#">EU437094</a> | <a href="#">EU437243</a> |
| <i>Oxalis inconspicua</i> Salter           | <i>Latifoliolatae</i> | -                  | MO138 | L. Dreyer 707                | <a href="#">EU436944</a> | <a href="#">EU437086</a> | <a href="#">EU437230</a> |
| <i>Oxalis kamiesbergensis</i> Salter       | <i>Angustatae</i>     | <i>Lineares</i>    | MO592 | Ex. Hort.                    | <a href="#">EU436970</a> | <a href="#">EU437102</a> | <a href="#">EU437256</a> |
| <i>Oxalis knuthiana</i> Salter             | <i>Cernuae</i>        | <i>Eu-cernuae</i>  | MO153 | L. Dreyer 721                | <a href="#">EU436884</a> | <a href="#">AJ582325</a> | <a href="#">EU437173</a> |
| <i>Oxalis lanata</i> L. f.                 | <i>Oppositae</i>      | <i>Subintegrae</i> | MO211 | L. Dreyer 751                | <a href="#">EU437027</a> | <a href="#">EU437125</a> | -                        |
| <i>Oxalis lasiandra</i> Zucc.              | -                     | -                  | MO455 | Ex. Hort.                    | <a href="#">EU436876</a> | <a href="#">EU437041</a> | <a href="#">EU437165</a> |
| <i>Oxalis lateriflora</i> Jacq.            | <i>Cernuae</i>        | <i>Lividae</i>     | MO82  | L. Dreyer 651                | <a href="#">EU436890</a> | <a href="#">EU437051</a> | -                        |
| <i>Oxalis lateriflora</i> Jacq.            | <i>Cernuae</i>        | <i>Lividae</i>     | MO83  | L. Dreyer 652                | -                        | -                        | <a href="#">EU437178</a> |
| <i>Oxalis latifolia</i> Knuth              | -                     | -                  | MO254 | K. Oberlander 0023           | <a href="#">EU436875</a> | <a href="#">AJ582326</a> | <a href="#">EU437164</a> |
| <i>Oxalis leptocalyx</i> Sond.             | <i>Angustatae</i>     | <i>Lineares</i>    | MO386 | K. Oberlander 0076           | <a href="#">EU437001</a> | <a href="#">EU437113</a> | <a href="#">EU437287</a> |
| <i>Oxalis leptogramma</i> Salter           | <i>Angustatae</i>     | <i>Pardales</i>    | MO28  | L. Dreyer 617                | <a href="#">EU437014</a> | <a href="#">AJ582327</a> | <a href="#">EU437300</a> |

|  |                       |                        |       |   |                          |                          |                          |
|--|-----------------------|------------------------|-------|---|--------------------------|--------------------------|--------------------------|
| <i>Oxalis lichenoides</i> Salter           | <i>Foveolatae</i>     | -                      | MO281 | L. Dreyer 785                                     | <a href="#">EU436919</a> | <a href="#">AJ582328</a> | <a href="#">EU437207</a> |
| <i>Oxalis linearis</i> Jacq.               | <i>Angustatae</i>     | <i>Lineares</i>        | MO110 | L. Dreyer 679<br>K. Oberlander 0078               | <a href="#">EU436972</a> | <a href="#">AJ582329</a> | <a href="#">EU437258</a> |
| <i>Oxalis livida</i> Jacq.                 | <i>Cernuae</i>        | <i>Lividae</i>         | MO361 |   | <a href="#">EU436889</a> | <a href="#">EU437050</a> | -                        |
| <i>Oxalis louisae</i> Salter               | <i>Crassulae</i>      | -                      | MO139 | L. Dreyer 708<br>K. Oberlander 0026               | <a href="#">EU436928</a> | <a href="#">AJ582330</a> | <a href="#">EU437215</a> |
| <i>Oxalis luteola</i> Jacq.                | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO257 |   | <a href="#">EU436904</a> | <a href="#">AJ582331</a> | <a href="#">EU437192</a> |
| <i>Oxalis massoniana</i> Salter            | <i>Angustatae</i>     | <i>Pardales</i>        | MO399 | J. Holmes 0016<br>K. Oberlander 0088              | <a href="#">EU437015</a> | <a href="#">EU437120</a> | <a href="#">EU437301</a> |
| <i>Oxalis meisneri</i> Sond.               | <i>Angustatae</i>     | <i>Lineares</i>        | MO468 |   | <a href="#">EU436993</a> | <a href="#">EU437110</a> | <a href="#">EU437279</a> |
| <i>Oxalis melanosticta</i> Sond.           | <i>Stictophyllae</i>  | -                      | MO33  | L. Dreyer 622                                     | <a href="#">EU436901</a> | <a href="#">AJ582332</a> | <a href="#">EU437189</a> |
| <i>Oxalis minuta</i> Thunb.                | <i>Sagittatae</i>     | -                      | MO163 | L. Dreyer 727<br>L. Dreyer and K. Oberlander 0003 | <a href="#">EU437028</a> | <a href="#">AJ582334</a> | -                        |
| <i>Oxalis monophylla</i> L. f.             | unplaced              | -                      | MO9   | K. Oberlander 0080                                | <a href="#">EU436927</a> | <a href="#">AJ582335</a> | <a href="#">EU437214</a> |
| <i>Oxalis multicaulis</i> E. & Z.          | <i>Angustatae</i>     | <i>Lineares</i>        | MO390 |   | <a href="#">EU436998</a> | <a href="#">EU437112</a> | <a href="#">EU437284</a> |
| <i>Oxalis namaquana</i> Sond.              | <i>Crassulae</i>      | -                      | MO144 | L. Dreyer 713<br>K. Oberlander 0160               | <a href="#">EU436941</a> | <a href="#">AJ582336</a> | <a href="#">EU437228</a> |
| <i>Oxalis natans</i> L. f.                 | <i>Campanulatae</i>   | -                      | MO604 |   | <a href="#">EU437009</a> | <a href="#">EU437117</a> | <a href="#">EU437295</a> |
| <i>Oxalis nidulans</i> E. & Z.             | <i>Sagittatae</i>     | -                      | MO212 | L. Dreyer 752<br>K. Oberlander 0112               | <a href="#">EU437026</a> | <a href="#">AJ582337</a> | <a href="#">EU437312</a> |
| <i>Oxalis nortieri</i> Salter              | <i>Foveolatae</i>     | -                      | MO503 |   | <a href="#">EU436914</a> | <a href="#">EU437068</a> | <a href="#">EU437202</a> |
| <i>Oxalis obliquifolia</i> Steud. ex Rich. | <i>Foveolatae</i>     | -                      | MO323 | DUB 711   | <a href="#">EU436915</a> | <a href="#">EU437069</a> | <a href="#">EU437203</a> |
| <i>Oxalis obtusa</i> Jacq.                 | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO122 | L. Dreyer 691                                     | <a href="#">EU436922</a> | <a href="#">AJ582338</a> | <a href="#">EU437210</a> |
| <i>Oxalis oculifera</i> E.G.H Oliver       | <i>Latifoliolatae</i> | -                      | MO295 | L. Dreyer 799                                     | <a href="#">EU436953</a> | <a href="#">AJ582339</a> | <a href="#">EU437239</a> |
| <i>Oxalis oligophylla</i> Salter           | <i>Angustatae</i>     | <i>Lineares</i>        | MO293 | L. Dreyer 797<br>K. Oberlander 0066               | <a href="#">EU437023</a> | <a href="#">AJ582340</a> | <a href="#">EU437309</a> |
| <i>Oxalis orbicularis</i> Salter           | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO366 |   | <a href="#">EU436899</a> | <a href="#">EU437058</a> | <a href="#">EU437187</a> |
| <i>Oxalis oreophila</i> Salter             | <i>Angustatae</i>     | <i>Lineares</i>        | MO97  | L. Dreyer 666<br>K. Oberlander 0068               | <a href="#">EU436975</a> | <a href="#">AJ582341</a> | <a href="#">EU437261</a> |
| <i>Oxalis orthopoda</i> Salter             | <i>Oppositae</i>      | <i>Bifurcatae</i>      | MO351 |   | <a href="#">EU436956</a> | <a href="#">EU437093</a> | <a href="#">EU437242</a> |
| <i>Oxalis pachyrrhiza</i> Weddell          | -                     | -                      | MO460 | L. Dreyer 809                                     | <a href="#">EU436871</a> | <a href="#">EU437037</a> | <a href="#">EU437160</a> |
| <i>Oxalis pallens</i> E. & Z.              | <i>Angustatae</i>     | <i>Lineares</i>        | MO85  | L. Dreyer 654                                     | <a href="#">EU436997</a> | <a href="#">AJ582342</a> | <a href="#">EU437283</a> |
| <i>Oxalis palmifrons</i> Salter            | <i>Angustatae</i>     | <i>Multifoliolatae</i> | MO403 | J. Holmes 0019<br>K. Oberlander 0067              | <a href="#">EU437021</a> | <a href="#">EU437124</a> | <a href="#">EU437307</a> |
| <i>Oxalis pendulifolia</i> Salter          | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO350 | Till Botrand 0002                                 | <a href="#">EU437019</a> | <a href="#">EU437122</a> | <a href="#">EU437305</a> |
| <i>Oxalis perdicaria</i> (Molina) Bertero  | -                     | -                      | MO647 |   | <a href="#">EU436878</a> | <a href="#">EU437043</a> | <a href="#">EU437167</a> |
| <i>Oxalis pes-caprae</i> L.                | <i>Cernuae</i>        | <i>Eu-cernuae</i>      | MO93  | L. Dreyer 662                                     | <a href="#">EU436882</a> | <a href="#">AJ582343</a> | <a href="#">EU437171</a> |
| <i>Oxalis phloxidiflora</i> Schltr.        | <i>Angustatae</i>     | <i>Lineares</i>        | MO109 | L. Dreyer 678<br>K. Oberlander 0135               | <a href="#">EU436984</a> | <a href="#">EU437106</a> | <a href="#">EU437270</a> |
| <i>Oxalis pillansiana</i> Salter & Exell   | <i>Angustatae</i>     | <i>Xanthotrichae</i>   | MO549 |   | <a href="#">EU436938</a> | <a href="#">EU437082</a> | <a href="#">EU437225</a> |
| <i>Oxalis pocockiae</i> L. Bolus           | <i>Foveolatae</i>     | -                      | MO37  | L. Dreyer 626                                     | <a href="#">EU436911</a> | <a href="#">AJ582344</a> | <a href="#">EU437199</a> |
| <i>Oxalis polyphylla</i> Jacq.             | <i>Angustatae</i>     | <i>Lineares</i>        | MO47  | C. Cilliers 0002                                  | <a href="#">EU437010</a> | <a href="#">AJ582345</a> | <a href="#">EU437296</a> |
| <i>Oxalis porphyriosiphon</i> Salter       | <i>Angustatae</i>     | <i>Lineares</i>        | MO715 | K. Oberlander 0167                                | <a href="#">EU436987</a> | <a href="#">EU437107</a> | <a href="#">EU437273</a> |
| <i>Oxalis primuloides</i> R. Knuth         | <i>Angustatae</i>     | <i>Lineares</i>        | MO142 | L. Dreyer 711                                     | <a href="#">EU436966</a> | <a href="#">AJ582346</a> | <a href="#">EU437252</a> |

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|---|-----------------------|------------------------|-------|--------------------|--------------------------|--------------------------|--------------------------|
| <i>Oxalis pseudo-cernua</i> R. Knuth          | <i>Cernuae</i>        | <i>Purpuratae</i>      | MO614 | P. Craven 4781     | <a href="#">EU436880</a> | <a href="#">EU437045</a> | <a href="#">EU437169</a> |
| <i>Oxalis psilopoda</i> Turcz.                | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO347 | K. Oberlander 0064 | <a href="#">EU436891</a> | <a href="#">EU437052</a> | <a href="#">EU437179</a> |
| <i>Oxalis pulchella</i> Jacq.                 | <i>Foveolatae</i>     | -                      | MO23  | L. Dreyer 612      | <a href="#">EU436907</a> | <a href="#">AJ582347</a> | <a href="#">EU437195</a> |
| <i>Oxalis punctata</i> L. f.                  | <i>Foveolatae</i>     | -                      | MO50  | C. Cilliers 0005   | <a href="#">EU436917</a> | <a href="#">AJ582367</a> | <a href="#">EU437205</a> |
| <i>Oxalis purpurascens</i> Salter             | <i>Cernuae</i>        | <i>Purpuratae</i>      | MO51  | L. Dreyer 634      | <a href="#">EU436881</a> | <a href="#">AJ582348</a> | <a href="#">EU437170</a> |
|   |                       |                        |       | K. Oberlander 0024 | <a href="#">EU436902</a> | <a href="#">AJ582349</a> | <a href="#">EU437190</a> |
| <i>Oxalis purpurea</i> L.                     | <i>Stictophyllae</i>  | -                      | MO255 | L. Dreyer 746      | <a href="#">EU437004</a> | <a href="#">AJ582350</a> | <a href="#">EU437290</a> |
| <i>Oxalis pusilla</i> Jacq.                   | <i>Angustatae</i>     | <i>Lineares</i>        | MO182 | L. Dreyer 718      | <a href="#">EU436968</a> | <a href="#">AJ582351</a> | <a href="#">EU437254</a> |
| <i>Oxalis reclinata</i> Jacq.                 | <i>Angustatae</i>     | <i>Lineares</i>        | MO149 | K. Oberlander 0170 | <a href="#">EU437003</a> | <a href="#">EU437115</a> | <a href="#">EU437289</a> |
| <i>Oxalis recticaulis</i> Sond.               | <i>Angustatae</i>     | <i>Lineares</i>        | MO792 | L. Dreyer 784      | <a href="#">EU436932</a> | <a href="#">AJ582352</a> | <a href="#">EU437219</a> |
| <i>Oxalis salteri</i> L. Bolus                | <i>Crassulae</i>      | -                      | MO280 | DUB 770            | <a href="#">EU436916</a> | <a href="#">EU437070</a> | <a href="#">EU437204</a> |
| <i>Oxalis setosa</i> E. Mey. ex Sond.         | <i>Foveolatae</i>     | -                      | MO325 | DUB 699            | <a href="#">EU436964</a> | <a href="#">EU437099</a> | <a href="#">EU437250</a> |
| <i>Oxalis smithiana</i> E. & Z.               | <i>Oppositae</i>      | <i>Bifurcatae</i>      | MO322 | L. Dreyer 694      | <a href="#">EU436945</a> | <a href="#">EU437087</a> | <a href="#">EU437231</a> |
| <i>Oxalis sonderiana</i> (O. Kuntze) Salter   | <i>Latifoliolatae</i> | -                      | MO125 | L. Dreyer 689      | <a href="#">EU436948</a> | <a href="#">EU437089</a> | <a href="#">EU437234</a> |
| <i>Oxalis sp. affin. campylorrhiza</i> Salter | <i>unplaced</i>       | -                      | MO120 | K. Oberlander 0086 | <a href="#">EU436894</a> | <a href="#">EU437054</a> | <a href="#">EU437182</a> |
| <i>Oxalis stellata</i> E. & Z.                | <i>Cernuae</i>        | <i>Stellatae</i>       | MO465 | L. Dreyer 675      | <a href="#">EU436983</a> | <a href="#">AJ582353</a> | <a href="#">EU437269</a> |
| <i>Oxalis stenopetala</i> Salter              | <i>Angustatae</i>     | <i>Lineares</i>        | MO106 | K. Oberlander 0138 | <a href="#">EU436934</a> | <a href="#">EU437079</a> | <a href="#">EU437221</a> |
| <i>Oxalis stenoptera</i> Turcz.               | <i>Latifoliolatae</i> | -                      | MO553 | L. Dreyer 749      | <a href="#">EU436996</a> | <a href="#">AJ582355</a> | <a href="#">EU437282</a> |
| <i>Oxalis stictocheila</i> Salter             | <i>Angustatae</i>     | <i>Lineares</i>        | MO185 | J. Walter 6518     | <a href="#">EU436869</a> | <a href="#">EU437035</a> | <a href="#">EU437158</a> |
| <i>Oxalis stricta</i> L.                      | -                     | -                      | MO669 | K. Oberlander 0093 | <a href="#">EU436959</a> | <a href="#">EU437096</a> | <a href="#">EU437245</a> |
| <i>Oxalis strigosa</i> Salter                 | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO473 | K. Oberlander 0075 | <a href="#">EU436937</a> | <a href="#">EU437081</a> | <a href="#">EU437224</a> |
| <i>Oxalis suavis</i> R. Knuth                 | <i>Latifoliolatae</i> | -                      | MO385 | K. Oberlander 0115 | <a href="#">EU436963</a> | <a href="#">EU437098</a> | <a href="#">EU437249</a> |
| <i>Oxalis suteroides</i> Salter               | <i>Angustatae</i>     | <i>Glandulosae</i>     | MO527 | L. Dreyer 639      | <a href="#">EU436978</a> | <a href="#">AJ582356</a> | <a href="#">EU437264</a> |
| <i>Oxalis tenella</i> Jacq.                   | <i>Latifoliolatae</i> | -                      | MO70  | K. Oberlander 0027 | <a href="#">EU436995</a> | <a href="#">EU437112</a> | <a href="#">EU437281</a> |
| <i>Oxalis tenuifolia</i> Jacq.                | <i>Angustatae</i>     | <i>Lineares</i>        | MO258 | L. Dreyer 800      | <a href="#">EU436986</a> | <a href="#">AJ582358</a> | <a href="#">EU437271</a> |
| <i>Oxalis tenuipes</i> Salter                 | <i>Angustatae</i>     | <i>Lineares</i>        | MO296 | L. Dreyer 793      | <a href="#">EU436989</a> | <a href="#">AJ582359</a> | <a href="#">EU437275</a> |
| <i>Oxalis tenuis</i> Salter                   | <i>Angustatae</i>     | <i>Lineares</i>        | MO289 | J. Holmes 0009     | <a href="#">EU436877</a> | <a href="#">EU437042</a> | <a href="#">EU437166</a> |
| <i>Oxalis tetraphylla</i> Cav.                | -                     | -                      | MO392 | Te Roller 0006     | <a href="#">EU437022</a> | <a href="#">AJ582360</a> | <a href="#">EU437308</a> |
| <i>Oxalis tomentosa</i> L. f.                 | <i>Angustatae</i>     | <i>Multifoliolatae</i> | MO62  | L. Dreyer 605      | <a href="#">EU436960</a> | <a href="#">AJ582361</a> | <a href="#">EU437246</a> |
| <i>Oxalis truncatula</i> Jacq.                | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO16  | J. Holmes 0011     | <a href="#">EU436933</a> | <a href="#">EU437078</a> | <a href="#">EU437220</a> |
| <i>Oxalis uliginosa</i> Schltr.               | <i>Campanulatae</i>   | -                      | MO394 | Bytebier 2015      | <a href="#">EU436999</a> | <a href="#">AJ582362</a> | <a href="#">EU437285</a> |
| <i>Oxalis urbaniana</i> Schltr.               | <i>Angustatae</i>     | <i>Lineares</i>        | MO229 | Till Botrand 0001  | <a href="#">EU436873</a> | <a href="#">EU437039</a> | <a href="#">EU437162</a> |
| <i>Oxalis valdiviensis</i> Barnéoud           | -                     | -                      | MO646 | K. Oberlander 0034 | <a href="#">EU437006</a> | <a href="#">AJ582363</a> | <a href="#">EU437292</a> |
| <i>Oxalis versicolor</i> L.                   | <i>Angustatae</i>     | <i>Lineares</i>        | MO307 | Ex. Hort.          | <a href="#">EU436955</a> | <a href="#">EU437092</a> | <a href="#">EU437241</a> |
| <i>Oxalis virginea</i> Jacq.                  | <i>Oppositae</i>      | <i>Subintegrae</i>     | MO580 | L. Dreyer 642      | <a href="#">EU436949</a> | <a href="#">AJ582364</a> | <a href="#">EU437235</a> |
| <i>Oxalis viscosa</i> E. Mey. ex Sond.        | <i>Angustatae</i>     | <i>Sessilifoliatae</i> | MO73  |                    |                          |                          |                          |

|  |                   |                        |        |                       |                                 |                                 |                                 |
|--|-------------------|------------------------|--------|-----------------------|---------------------------------|---------------------------------|---------------------------------|
| <i>Oxalis vulcanicola</i><br>(Donn.-Sm.) Lourt.                            | -                 | -                      | MO702  | Ex. Hort.             | <a href="#"><u>EU436872</u></a> | <a href="#"><u>EU437038</u></a> | <a href="#"><u>EU437161</u></a> |
| <i>Oxalis xantha</i><br>Salter   | <i>Angustatae</i> | <i>Lineares</i>        | MO102  | L. Dreyer 671         | <a href="#"><u>EU436980</u></a> | <a href="#"><u>AJ582365</u></a> | <a href="#"><u>EU437266</u></a> |
| <i>Oxalis</i><br><i>zeekoevleyensis</i> R.<br>Knuth                        | <i>Oppositae</i>  | <i>Subintegrae</i>     | MO197  | K. Oberlander<br>0012 | <a href="#"><u>EU436900</u></a> | <a href="#"><u>EU437059</u></a> | <a href="#"><u>EU437188</u></a> |
| <i>Oxalis zeyheri</i><br>Sond.   | <i>Angustatae</i> | <i>Multifoliolatae</i> | MO590  | Ex. Hort.             | <a href="#"><u>EU436962</u></a> | <a href="#"><u>EU437097</u></a> | <a href="#"><u>EU437248</u></a> |
| <i>Sarcotheca laxa</i><br>Knuth  | -                 | -                      | SARLAX | -                     | <a href="#"><u>EU436859</u></a> | <a href="#"><u>AJ582366</u></a> | <a href="#"><u>EU437148</u></a> |
| <i>Sarcotheca</i><br><i>monophylla</i><br>(Planch. ex Hk.f.)<br>Hallier.f. | -                 | -                      | SARMON | -                     | <a href="#"><u>EU436860</u></a> | <a href="#"><u>EU437030</u></a> | <a href="#"><u>EU437149</u></a> |

|                           | <i>ndhF</i>                     | <i>trnK</i>                     | <i>trnT-L</i>                   |
|---------------------------|---------------------------------|---------------------------------|---------------------------------|
| <i>O. corniculata</i>     | <a href="#"><u>EU437313</u></a> | <a href="#"><u>EU437337</u></a> | <a href="#"><u>EU437361</u></a> |
| <i>O. cf. pachyrrhiza</i> | <a href="#"><u>EU437314</u></a> | <a href="#"><u>EU437338</u></a> | <a href="#"><u>EU437362</u></a> |
| <i>O. latifolia</i>       | <a href="#"><u>EU437315</u></a> | <a href="#"><u>EU437339</u></a> | <a href="#"><u>EU437363</u></a> |
| <i>O. purpurascens</i>    | <a href="#"><u>EU437316</u></a> | <a href="#"><u>EU437340</u></a> | <a href="#"><u>EU437364</u></a> |
| <i>O. dentata</i>         | <a href="#"><u>EU437317</u></a> | <a href="#"><u>EU437341</u></a> | <a href="#"><u>EU437365</u></a> |
| <i>O. caprina</i>         | <a href="#"><u>EU437318</u></a> | <a href="#"><u>EU437342</u></a> | <a href="#"><u>EU437366</u></a> |
| <i>O. imbricata</i>       | <a href="#"><u>EU437319</u></a> | <a href="#"><u>EU437343</u></a> | <a href="#"><u>EU437367</u></a> |
| <i>O. commutata</i>       | <a href="#"><u>EU437320</u></a> | <a href="#"><u>EU437344</u></a> | <a href="#"><u>EU437368</u></a> |
| <i>O. orbicularis</i>     | <a href="#"><u>EU437321</u></a> | <a href="#"><u>EU437345</u></a> | <a href="#"><u>EU437369</u></a> |
| <i>O. melanosticta</i>    | <a href="#"><u>EU437322</u></a> | <a href="#"><u>EU437346</u></a> | <a href="#"><u>EU437370</u></a> |
| <i>O. pulchella</i>       | <a href="#"><u>EU437323</u></a> | <a href="#"><u>EU437347</u></a> | <a href="#"><u>EU437371</u></a> |
| <i>O. flava</i>           | <a href="#"><u>EU437324</u></a> | <a href="#"><u>EU437348</u></a> | <a href="#"><u>EU437372</u></a> |
| <i>O. monophylla</i>      | <a href="#"><u>EU437325</u></a> | <a href="#"><u>EU437349</u></a> | <a href="#"><u>EU437373</u></a> |
| <i>O. oculifera</i>       | <a href="#"><u>EU437326</u></a> | <a href="#"><u>EU437350</u></a> | <a href="#"><u>EU437374</u></a> |
| <i>O. incarnata</i>       | <a href="#"><u>EU437327</u></a> | <a href="#"><u>EU437351</u></a> | <a href="#"><u>EU437375</u></a> |
| <i>O. engleriana</i>      | <a href="#"><u>EU437328</u></a> | <a href="#"><u>EU437352</u></a> | <a href="#"><u>EU437376</u></a> |
| <i>O. smithiana</i>       | <a href="#"><u>EU437329</u></a> | <a href="#"><u>EU437353</u></a> | <a href="#"><u>EU437377</u></a> |
| <i>O. tenella</i>         | <a href="#"><u>EU437330</u></a> | <a href="#"><u>EU437354</u></a> | <a href="#"><u>EU437378</u></a> |
| <i>O. polyphylla</i>      | <a href="#"><u>EU437331</u></a> | <a href="#"><u>EU437355</u></a> | <a href="#"><u>EU437379</u></a> |
| <i>O. capillacea</i>      | <a href="#"><u>EU437332</u></a> | <a href="#"><u>EU437356</u></a> | <a href="#"><u>EU437380</u></a> |
| <i>O. bifida</i>          | <a href="#"><u>EU437333</u></a> | <a href="#"><u>EU437357</u></a> | <a href="#"><u>EU437381</u></a> |
| <i>O. tomentosa</i>       | <a href="#"><u>EU437334</u></a> | <a href="#"><u>EU437358</u></a> | <a href="#"><u>EU437382</u></a> |
| <i>O. eckloniana</i>      | <a href="#"><u>EU437335</u></a> | <a href="#"><u>EU437359</u></a> | <a href="#"><u>EU437383</u></a> |
| <i>O. fibrosa</i>         | <a href="#"><u>EU437336</u></a> | <a href="#"><u>EU437360</u></a> | <a href="#"><u>EU437384</u></a> |

Table 3 Properties of marker data sets and trees for the 171-taxon data set and the 24-taxon data set. Node resolution is represented by number of nodes present in the parsimony or likelihood bootstrap consensus tree, or in the 50 % majority rule consensus Bayesian tree.

**171-TAXON DATA SET**

|                       |          | aligned<br>characters | %<br>missing<br>data | %<br>pars.<br>inform. | No.<br>trees | tree<br>length | CI/RI       | Model                    | no.<br>internal<br>nodes<br>resolved | %<br>internal<br>nodes<br>resolved | nodes ><br>80 %<br>Bootstrap,<br>0.95 PP | %<br>strong<br>support<br>nodes |
|-----------------------|----------|-----------------------|----------------------|-----------------------|--------------|----------------|-------------|--------------------------|--------------------------------------|------------------------------------|--|---------------------------------|
| parsimony             | combined | 3516                  | 1.43%                | 27.1                  | 230          | 4504           | 0.499/0.733 | -                        | 109                                  | 64.5                               | 72                                       |                                 |
|                       | plastid  | 2634                  | 1.90%                | 22.5                  | 250          | 2124           | 0.663/0.813 | -                        | 108                                  | 63.9                               | 60                                       |                                 |
|                       | ITS      | 882                   | 0.00%                | 41                    | 20           | 2218           | 0.379/0.703 | -                        | 78                                   | 46.2                               | 46                                       |                                 |
| likelihood            | combined | -                     | -                    | -                     | -            | -              | -           | GTR<br>+ I +<br>□<br>TVM | 113                                  | 66.9                               | 75                                       |                                 |
|                       | plastid  | -                     | -                    | -                     | -            | -              | -           | + I +<br>□<br>GTR        | 102                                  | 60.4                               | 66                                       |                                 |
|                       | ITS      | -                     | -                    | -                     | -            | -              | -           | + I +<br>□<br>GTR        | 90                                   | 53.3                               | 53                                       |                                 |
|                       | combined | -                     | -                    | -                     | -            | -              | -           | + I +<br>□<br>GTR        | 142                                  | 84.0                               | 101                                      |                                 |
| Bayesian<br>Inference | plastid  | -                     | -                    | -                     | -            | -              | -           | + I +<br>□<br>GTR        | 122                                  | 72.2                               | 89                                       |                                 |
|                       | ITS      | -                     | -                    | -                     | -            | -              | -           | + I +<br>□               | 110                                  | 65.1                               | 73                                       |                                 |

**24-TAXON DATA SET**

|                       |          |      |        |      |       |      |             |                   |    |      |    |  |
|-----------------------|----------|------|--------|------|-------|------|-------------|-------------------|----|------|----|--|
| parsimony             | combined | 7359 | 8.27%  | 5.5  | 1     | 2145 | 0.781/0.478 | -                 | 15 | 68.2 | 9  |  |
|                       | plastid  | 6540 | 9.30%  | 4.2  | 436   | 1527 | 0.830/0.549 | -                 | 15 | 68.2 | 9  |  |
|                       | ITS      | 819  | -      | 15.9 | 42    | 604  | 0.677/0.394 | -                 | 7  | 31.8 | 3  |  |
|                       | ndhF     | 1082 | 4.16%  | 5.6  | 684   | 308  | 0.808/0.543 | -                 | 7  | 31.8 | 3  |  |
|                       | trnK     | 2564 | 21.97% | 3    | 49226 | 521  | 0.858/0.475 | -                 | 7  | 31.8 | 0  |  |
|                       | trnL-F   | 1000 | -      | 3.1  | 941   | 176  | 0.864/0.667 | -                 | 6  | 27.3 | 2  |  |
|                       | trnS-G   | 973  | -      | 5.9  | 87440 | 287  | 0.794/0.563 | -                 | 7  | 31.8 | 1  |  |
|                       | trnT-L   | 921  | -      | 4.9  | 439   | 222  | 0.860/0.687 | -                 | 8  | 36.4 | 1  |  |
| Bayesian<br>Inference | combined | -    | -      | -    | -     | -    | -           | GTR<br>+ I +<br>□ | 21 | 95.5 | 14 |  |
|                       | plastid  | -    | -      | -    | -     | -    | -           | GTR<br>+ I +<br>□ | 19 | 86.4 | 12 |  |
|                       | ITS      | -    | -      | -    | -     | -    | -           | GTR<br>+ I +<br>□ | 15 | 68.2 | 4  |  |
|                       | ndhF     | -    | -      | -    | -     | -    | -           | GTR<br>+ □<br>GTR | 11 | 50.0 | 3  |  |
|                       | trnK     | -    | -      | -    | -     | -    | -           | + I +<br>□<br>GTR | 11 | 50.0 | 4  |  |
|                       | trnL-F   | -    | -      | -    | -     | -    | -           | + □<br>GTR        | 6  | 27.3 | 2  |  |
|                       | trnS-G   | -    | -      | -    | -     | -    | -           | + □<br>GTR        | 12 | 54.5 | 8  |  |
|                       | trnT-L   | -    | -      | -    | -     | -    | -           | + □               | 13 | 59.1 | 6  |  |

|                       |          |   |   |   |   |   |   |                   |    |      |    |
|-----------------------|----------|---|---|---|---|---|---|-------------------|----|------|----|
| Likelihood<br>- GARLI |          | - | - | - | - | - | - | GTR<br>+ I +<br>□ |    |      |    |
|                       | combined | - | - | - | - | - | - | TVM<br>+ I +<br>□ | 15 | 68.2 | 9  |
|                       | plastid  | - | - | - | - | - | - | GTR<br>+ I +<br>□ | 15 | 68.2 | 10 |
|                       | ITS      | - | - | - | - | - | - | TVM<br>+ I +<br>□ | 6  | 27.3 | 2  |
|                       | ndhF     | - | - | - | - | - | - | TVM<br>+ I +<br>□ | 6  | 27.3 | 2  |
|                       | trnK     | - | - | - | - | - | - | TIM +<br>I + □    | 10 | 45.5 | 1  |
|                       | trnL-F   | - | - | - | - | - | - | TIM +<br>□        | 4  | 18.2 | 2  |
|                       | trnS-G   | - | - | - | - | - | - | GTR<br>+ □        | 6  | 27.3 | 2  |
|                       | trnT-L   | - | - | - | - | - | - | GTR<br>+ □        | 10 | 45.5 | 3  |

### Chapter 3: An updated classification scheme for southern African *Oxalis* based on DNA sequence data

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#### Abstract

The current macro-morphology-based taxonomy of *Oxalis* in southern Africa is artificial, and requires revision. Previous palynological and DNA sequence-based studies, while congruent with one another, show marked departures from the most recent morphological treatment. Here we present a primarily sequence-based classification for the genus. DNA data emphasize the artificial nature of the current classification and highlight the difficulty of finding morphological characters that can demarcate sensible taxonomic units in *Oxalis*. An informal naming system for clades is proposed, following the precedent of the Angiosperm Phylogeny Group. These temporary designations for taxa are justified because of the near-complete disagreement between current and proposed classification systems and the need to find synapomorphic morphological characters or stronger confidence levels for many clades. Nomenclatural changes evident from the phylogeny, and putative macro-morphological and palynological synapomorphies for strongly-supported clades, are suggested.

#### Introduction

The genus *Oxalis* is well-represented in southern Africa, with over 200 species currently recognized, and many potential new taxa hidden in the *incertae* shelves of herbaria in the

region. Despite many well-known and easily recognized species, the current taxonomy is plagued by problems. The most recent morphological treatment of the genus (Salter, 1944) is a monumental work of data collation and nomenclatural corrections, but is over sixty years out of date. The genus itself is morphologically plastic and notoriously hard to identify, and a significant fraction of recently-collected herbarium specimens are misidentified, adding to the taxonomic confusion. Most species only appear above-ground in the austral winter months, and often skip an entire season if rainfall is not sufficient, confounding collection efforts. Many informative characters are confined to the bulbs and inflorescences, and as the flowering period of *Oxalis* is generally short, and the bulbs of many species are difficult to collect, many specimens are simply too poor to identify correctly. Moreover, the species concepts of Salter (1944) are inconsistent; with large amounts of morphological variation packed into highly variable “group” species, whilst other groups with comparable variation are divided into many species and/or varieties. Such difficulties have led to the genus being understudied and ignored for half a century.

Recent work on the genus initially resulted in more conflict. Apart from Salter (1944), the other major work on southern African *Oxalis* is that of Dreyer (1996), which resulted in a detailed, near-complete review of the palynology of the genus in the region. She identified four major pollen types among southern African species: micro-reticulate regulate (A), micro-rugulate spinate (B), reticulate (C) and supra-areolate (D), with the reticulate type the most common. She also inferred reticulate pollen to be the ancestral type in southern African taxa. Although these pollen types are well-defined and easily recognized, they do not correspond to any of the taxonomic groupings proposed by Salter (1996). Dreyer (1996) argued that since pollen evolution is considered more conservative due to the constraints imposed by pollination and fertilization, the current scattered distribution of pollen types across the classification provides evidence for the artificial nature of this taxonomy.

Oberlander & *al.* (2004) attempted a reconstruction of relationships within the large, loosely-defined section *Angustatae* subsection *Lineares* based on DNA sequence data. Analysis of non-coding plastid *trnL* intron and *trnL-trnF* spacer markers (hereafter

referred to as the *trnL*-F region) showed the artificiality of this subsection. Moreover, newly proposed relationships did show substantial congruence with the distribution of pollen types proposed by Dreyer (1996). Thus independent data sources both argued for the need to reclassify the genus.

Here we present a new classification for *Oxalis* in the southern African region based on morphological and DNA sequence data. Potential macro-morphological, anatomical and palynological synapomorphies are suggested for clades, where such data are available. Informal names for clades are suggested, following the precedent of the Angiosperm Phylogeny Group (APG, 1998; APGII, 2003).

#### Materials and methods

The proposed classification is based on phylogenies built from DNA sequence data (Oberlander & al. 2004; Oberlander & al. 2008; Ch2). Morphological characters were obtained from Salter (1944), and supplemented by examination of living specimens obtained from extensive field collections from across the entire range of southern African *Oxalis* over the past eight years. Herbarium specimens of most species were collected, and are housed in the Stellenbosch University Herbarium (STEU). Reference samples of most species have also been added to the *Oxalis* living collection maintained in the Stellenbosch University Botanical Gardens. Palynological data was obtained from Dreyer (1996). All *Oxalis* specimens collected or acquired from other collectors over this period were assigned a project number beginning with MO, which is generally referenced in the text.

#### Results:

Species are placed in groups of associated taxa within each named clade (Fig. 1). In the following sections, brief descriptions of individual clades are presented, followed by lists of included taxa as sampled by Oberlander et al. (2008). Species not sampled by Oberlander et al. (2008), but which can reliably be placed to a clade via palynological or macro-morphological evidence are followed by an asterisk (\*), or a superscripted number sign (<sup>#</sup>), respectively.

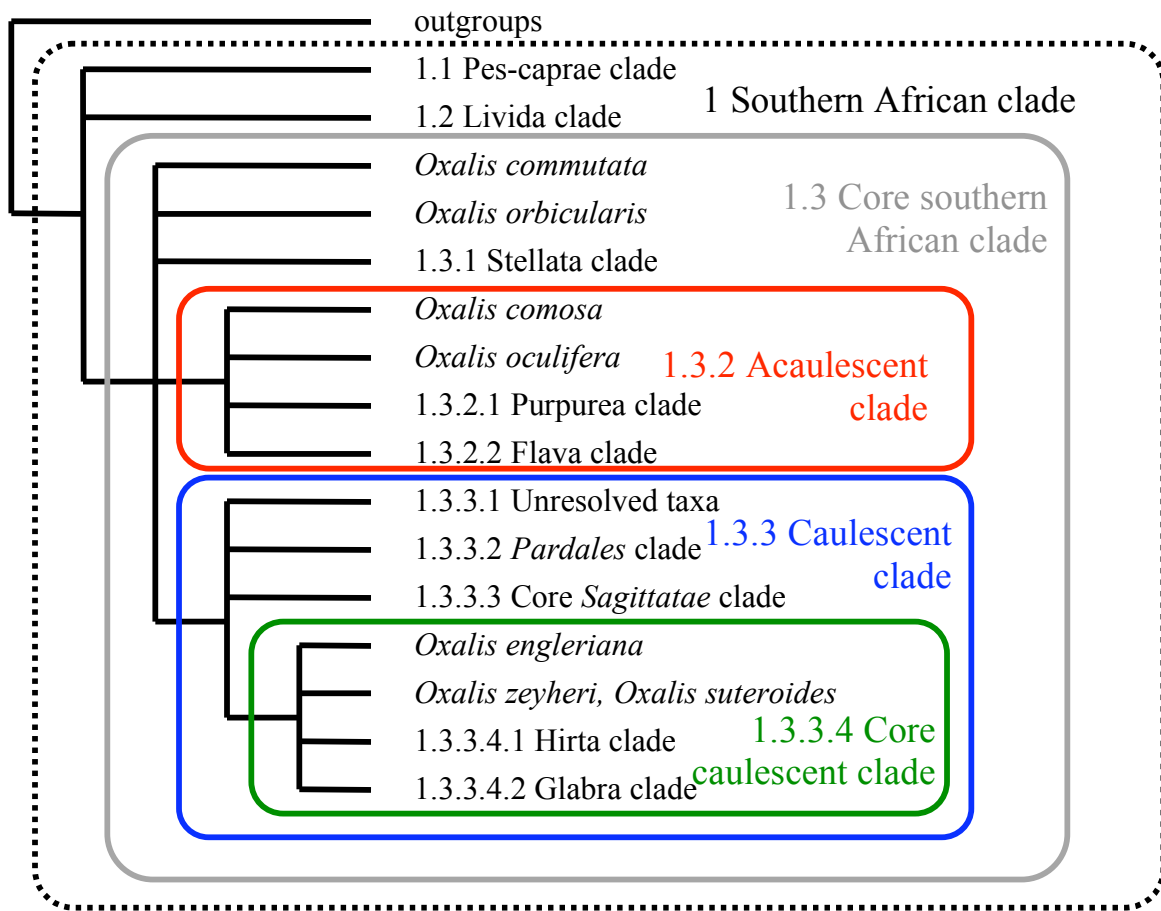


Fig.1 Summary of current phylogenetic relationships between the major groups of southern African *Oxalis*. Names of clades correspond with those in the text.

## 1) Main clades:

### Southern African clade:

Although outgroup sampling was sparse, outgroup taxa spanned the range of current taxonomy and growth form diversity in the genus (Oberlander & al. 2008, Chapter 2). Although there are several uncertainties in topology with regard to the placement of subgenus *Thamnoxys* (*O. barrelieri* L.) and section *Corniculatae*, all inference methods and all major data sets agreed on a close relationship between section *Ionoxalis* and the southern African taxa. The presence of true bulbs, as well as callose structures on the leaves and sepals in both taxa, supports this relationship.

All analyses, and all markers except *trnL-F*, agreed on a monophyletic southern African clade. The *trnL-F* region has been shown to be less variable (slower-evolving) than the other sampled non-coding plastid regions, and the non-monophyly of the southern African clade for this marker is presumed to be the result of an insufficient number of informative characters (Oberlander & al. 2008, Chapter 2).

The southern African clade comprised three well-supported clades. The species-poor *Pes-caprae* and *Livida* clades are contrasted with the large Core southern African clade.

### 1.1 *Pes-caprae* clade

This small clade contained many of the species of section *Cernuae* subsection *Eucernuae* (Salter, 1944), as well as the two members of subsection *Costatae* and *Oxalis purpurata* of *Cernuae* subsection *Purpuratae* (Oberlander, unpublished data, Chapter 8). A taxon that does not conform to any of the currently recognised species (MO620) was also included in phylogenetic analyses. Several unsampled and little-known species, such as *O. laxicaulis* Knuth from Namibia (possibly a form of *O. haedulipes* Salter), might also belong to this clade. The included species have mostly (but not universally) cymose umbellate inflorescences, and flower colour is either white or yellow. In most species the bulbs are distinctly longitudinally ridged. None of these characters, however, are confined to this clade and are possibly symplesiomorphic for the southern African clade as a whole.

This is one of the few clades that includes representatives from outside of the CFR and Namaqualand region, with *O. purpurascens* and *O. pseudo-cernua* recorded from central Namibia. It is possible that several species are indigenous to both Namibia and South Africa, but are known under different names in the two countries. This broad distribution and the isolated status of the clade as one of the three main lineages within southern African *Oxalis*, raises interesting biogeographical questions, as the other clades are predominantly or entirely South African. It is possible that *Oxalis* first entered the southern African region through the arid corridor in the west, and this clade might contain members that still cling to their ancestral distribution range in Namibia. In the context of other possible biogeographical migration routes into the CFR for *Oxalis*, it is clear that no current summer-rainfall clade is ancestral. This makes an entry through the summer-rainfall region less likely than through the west, although evidence for either is still equivocal.

Included species: *Oxalis compressa* L. f., *Oxalis copiosa* Bolus f., *Oxalis haedulipes* Salter, *Oxalis knuthiana* Salter, *Oxalis pes-caprae* L., *Oxalis purpurata* Jacq., *Oxalis schaeferi* R. Knuth<sup>#</sup>, *Oxalis luederitzii* Schinz.<sup>#</sup>, *Oxalis* potential *sp. nova* (MO620), *Oxalis pseudo-cernua* R. Knuth, *Oxalis purpurascens* Salter

### 1.2 *Livida* clade

The *Livida* clade, corresponding to *Cernuae* subsection *Lividae sensu* Salter (1944) consists of three species (*Oxalis livida*, *Oxalis dentata* and *Oxalis lateriflora*). Subsection *Lividae* is taxonomically isolated within section *Cernuae*. As in all members of *Cernuae* the inflorescence is a cymose umbel, but Salter (1944) did not propose affinities to any other members of the section. The species share several morphological similarities, including pale, longitudinally-ridged bulb tunics and cauline peduncles, which support their close affinity. However, species boundaries within the group are vague, with diagnostic *O. dentata* characters fading progressively north and east of the Cape Peninsula. Salter (1944) separated these three species purely on the presence and orientation of a contractile root, but these characters are known to be morphologically

plastic. Bayer (1992) studied all available specimens of subsection *Lividae*, and concluded that the three species merely represent forms of *O. livida*. Dreyer (1996) found that all three species have micro-reticulate pollen (pollen type C1), and no palynological conclusions regarding relationships of and within this group could thus be proposed.

All analyses strongly support the monophyly of this subsection; however, placement of this clade is ambiguous. ITS sequence data suggested that subsection *Lividae* was sister to the Core southern African clade; whilst *trnS*-G and *trnL*-F data weakly supported a sister relationship to the Pes-caprae clade. More taxon-restricted analyses utilising substantially more plastid data strongly supported the latter topology (Oberlander & al. 2008, Chapter 2). Morphologically, the members of the Livida clade are more similar to the Pes-caprae clade than to the Core southern African clade. It is unclear how many of these similarities are symplesiomorphic, however. Either sister relationship raises interesting biogeographical questions, as the earliest-diverging lineages of the Pes-caprae clade include Namibian, Namaqualand and CFR endemics, whilst the Livida clade is exclusively a CFR taxon. The latter relationship would strengthen evidence for a Cape origin of all southern African *Oxalis*, but more data are needed to resolve this question.

The specific or subspecific status of the three taxa sunk by Bayer (1992) remains debatable. Personal experience with the group revealed no clear characters that demarcate the separate species, but morphologically very different populations of plants corresponding to *O. livida* and *O. lateriflora* do grow virtually sympatrically in Elandsberg Private Nature Reserve. A more intensive, population-level study is required for further understanding of relationships within this group.

Included species: *Oxalis dentata* Jacq., *Oxalis lateriflora* Jacq., *Oxalis livida* Jacq.

### 1.3. The Core southern African clade

The Core southern African clade is supported by both plastid and nuclear data (Oberlander & al. 2008, Chapter 2). This clade contained the vast majority of morphological and taxonomic diversity present among indigenous *Oxalis* taxa. Although well-supported, the clade itself was not well-resolved. The Core southern African clade

diversity was divided into five main lineages, namely the species *O. commutata* and *O. orbicularis*, as well as three large clades, the Stellata clade, the Acaulescent clade and the Caulescent clade.

*Oxalis commutata* and *O. orbicularis* are confined to the CFR region, with *Oxalis commutata* restricted to Fynbos and *O. orbicularis* to cool south-facing slopes on the borders of the Little Karoo. Despite both species being acaulescent and bearing broad leaflets, they are morphologically relatively dissimilar. Salter (1944) placed the former species in the completely artificial section *Latifoliolatae* and the latter in the equally questionable section *Oppositae*. Neither species can be conclusively placed based on sequence data (Oberlander & al. 2008, Chapter 2). *Oxalis commutata* groups in an isolated position within the Acaulescent clade using ITS data alone. Plastid evidence, however, disagreed with such a placement and suggests a sister relationship with *O. orbicularis* instead. Palynologically, *O. commutata* would be better placed in the Flava clade, but neither plastid data nor combined data approaches support this.

### 1.3.1 Stellata clade

For its size, this clade is remarkably variable. Taxa with both cymose and single-flowered inflorescences are included, and species can be caulescent or acaulescent. This clade is novel, but not entirely unexpected, as some groups of included taxa from different sections are morphologically very similar. For example, the cymose summer-rainfall taxa have traditionally been included in *Cernuae* subsections *Stellatae* and *Purpuratae*. Species boundaries within these subsections are, however, unclear. Historically *Oxalis bowiei* and *O. semiloba* have often been confused with one another and with the unrelated weed *O. latifolia*. Although *O. semiloba* and *O. bowiei* are very similar, Salter (1944) retained their separate specific status based on several morphological characters; the most important of these being the exclusive presence of rust-coloured apical calli on the sepals of *O. semiloba*. Forms of the extremely variable and widespread *O. stellata* from the Eastern Cape appear to gradually merge into *O. tragopoda*, which in turn is superficially very similar to *O. bowiei*. Sequence data conclusively showed a strong relationship between these three very similar summer-rainfall species. All three were

strongly retrieved in the Stellata clade. Unfortunately DNA samples of *O. semiloba* (MO770) were degraded, and only the *trnL-trnF* spacer could be amplified (K. C. Oberlander, unpublished data). Although this was not analysed, it shares two potential sequence synapomorphies with *O. bowiei*. The morphological similarity between *O. semiloba* and *O. bowiei*, the gradual merging of forms of *O. stellata* with *O. tragopoda*, and the strongly-supported relationship between *O. tragopoda* and *O. bowiei* all suggest that this group of species is indeed closely related. It thus appears that the summer-rainfall species of the Stellata clade are monophyletic.

Potential morphological characters supporting this clade include greenish-yellow corolla tubes and an unusual form of endospermy whereby the seeds germinate soon after release from the capsule, but have undeveloped plumules. Salter (1944) noted that this germination pattern occurs in several species in this clade, including *O. bowiei*, *O. dichotoma*, *O. imbricata* and *O. psilopoda*, but did not include information on other species. Our own germination studies have shown *O. caprina* to have the same suite of characters (unpublished data). However, both Salter (1944) and our own germination studies found other, unrelated species also display this unusual germination pattern. It is clear that the endospermous/exendospermous germination pattern is not as clear a divide as previously thought (Salter, 1944), and more comprehensive seed and seedling character sampling is needed.

Included species: *Oxalis anomala* Salter<sup>#</sup>, *Oxalis caprina* L., *Oxalis dichotoma* Salter, *Oxalis imbricata* E. & Z., *Oxalis ioeides* Salter<sup>#</sup>, *Oxalis psilopoda* Turcz., *Oxalis stellata* E. & Z., *Oxalis bowiei* Lindl., *Oxalis semiloba* Sond.<sup>#</sup>, *Oxalis stenorrhyncha* Salter<sup>#</sup>, *Oxalis tragopoda* Salter

### 1.3.2 The Acaulescent clade.

Morphologically the retrieval of this large clade was unexpected. Although Oberlander & al. (2004) did retrieve a large clade corresponding to this one, the internal arrangement of taxa in their study was not well-resolved or supported. The Acaulescent clade contains two species in isolated positions and two variably supported clades. Members of the

Purpurea clade are mostly acaulescent, broad-leaved plants with endospermous seeds and mostly micro-reticulate to reticulate pollen (Dreyer, 1996). The Flava clade includes a heterogeneous assemblage of plants with exendospermous seeds, mostly with reticulate pollen with clustered intraluminary bacules (a potential synapomorphy; Dreyer, 1996), and a wide array of growth forms and habitat preferences.

Support for the Acaulescent clade (consisting of *O. comosa*, *O. oculifera*, the Purpurea clade and the Flava clade) is strong (Oberlander & al. 2008, Chapter 2). However, different analysis methods, and different partitions of the data, suggested different resolutions of these taxa. Bayesian Inference (BI) provided the most parsimonious distribution of morphological characters by segregating all of the endospermous species (except *O. comosa*) into the Purpurea clade with strong support, and all but *O. oculifera* of the exendospermous species into the Flava clade. Both of these clades are supported by putative morphological synapomorphies. However, parsimony either did not retrieve Flava or Purpurea clades, or supported them only weakly. It is possible, indeed likely, that BI with appropriate models better accounted for rate variation and homoplasy within the sampled data sets, which parsimony is inherently poorer at dealing with. Topologies obtained through Bayesian Inference can thus be considered a better representation of the true topology, especially as they are supported by these morphological characters. Maximum likelihood analyses executed in PAUP\* produced the same basal topology as BI (data not shown). We thus uphold monophyletic Flava and Purpurea clades, because it is supported by aspects of the morphology of the included species. In recognition of alternative topologies, however, we refrain from formally naming these two clades until more data are available (Oberlander & al., 2008, Chapter 2)

The sister group relationship between the Flava and Purpurea clades is tentative, based on the above-mentioned topological uncertainty. Bayesian Inference generally showed strong statistical support for both the Flava and Purpurea clades, as well as for their sister relationship. These three clades were only weakly supported by parsimony. No morphological characters could be identified as potential synapomorphies for the combined Flava and Purpurea clades. A potential anatomical character (mature tunics

consisting of multiple layers of macro-sclereids) is possibly synapomorphic, but the state of this character still needs to be determined for many more of the included taxa (Gebregziabher, 2004). This particular character might very well be synapomorphic for the entire Acaulescent clade.

Salter (1944) placed the species *O. comosa* in section *Oppositae* subsection *Bifurcatae*, despite it being geographically isolated from other members of this subsection. It is common on granite outcrops in Namaqualand, and can attain well over 0,5 m in height, rendering it amongst the tallest of all southern African *Oxalis*. All other members of the subsection are found in the CFR or the coastal areas of the summer-rainfall region. Although superficially similar to other members of *Bifurcatae*, especially the variable and widespread *O. heterophylla*, the precise affinities of this species are unclear.

The combined and plastid data sets placed *O. comosa* strongly within the acaulescent clade (Oberlander & al. 2008, Chapter 2), although there is very little morphological evidence to support such a relationship. Barring *O. comosa*, almost all other members of this clade are acaulescent or, if caulescent, clearly related to stemless species. This species lacks the swollen leaflet epidermal cells of members of the Purpurea clade and the pollen morphology and uniseriate hairs of the Flava clade. There is also very little similarity between this species and *O. oculifera*. Further investigation would require a more in-depth search for putative synapomorphies between this species and these other taxa.

The recently discovered species *O. oculifera* is an enigmatic member of *Oxalis* (Oliver, 1993). It is geographically isolated to the Gifberg massif, and bears no obvious morphological resemblance to any other species. The species has a campanulate corolla similar to members of section *Campanulatae*, but the petals are tricoloured. The bulb tunics resemble those of *O. grammopetala* in section *Foveolatae*, and the leaflets are subpeltate, a character state otherwise not found in southern African taxa. Hairs are of mixed glandular and uniseriately multicellular types, but the latter are curled into short spirals, a character thus far unique in the genus. Its habitat preference is also unusual,

with plants growing in extremely shallow soils over sandstone rock, in association with mosses and Iridaceae. These microhabitats become waterlogged in winter, and dry out completely in the summer months. *O. oculifera* produces unusual white pollen in its long and mid anther whorls. The pollen is reticulate, of the subtype C10, which suggests a relationship with the Flava clade. However, no other member of the Flava clade can be considered remotely similar on a macro-morphological basis.

*Oxalis oculifera* is no less unusual at the molecular level (Oberlander & al. 2008, Chapter 2). Two of the six markers sampled for this species appeared to be paralogous: amplified ITS sequences were very divergent from those of other southern African taxa, and *ndhF* primers targeted a pseudo-gene of this marker. Analyses of four other plastid markers clearly placed *O. oculifera* within the Caulescent clade. Model-based methods placed *O. oculifera* as sister to the sampled members of the Purpurea and Flava clades with relative confidence. Morphologically, such characters as reticulate pollen of the type C10 (Dreyer, 1996) and the presence of uniseriate multicellular hairs suggest the strongest affinity of this species with the Flava clade. Due to this uncertainty, *O. oculifera* remains unplaced within the Acaulescent clade until further data can be gathered.

#### 1.3.2.1 *Purpurea* clade

This clade included members of the very small section *Stictophyllae*, several species currently placed in section *Oppositae*, and the bulk of section *Foveolatae*. Although support for this clade was mixed, included members exhibit a number of morphological similarities. Most taxa are acaulescent with broad leaflets and a generally fleshy habit. All taxa are endospermous and produce unremarkable reticulate pollen (Dreyer, 1996). The leaflets of almost all taxa produce massively swollen epidermal cells that result in deeply sunken stomata. When dried, these cells collapse into an impresso-punctate state resembling an inverted cobblestone sidewalk. The few taxa that lack this character (*O. luteola*, *O. adenodes* and forms of *O. ambigua*) have nigro-punctate leaflets instead, which is somewhat similar to the impresso-punctate condition, but differs in the degree of cellular enlargement. Although many *Oxalis* species have enlarged abaxial epidermal cells, massively swollen cells covering both leaflet surfaces constitute a character unique

to the *Purpurea* clade. Bulbs of species in this clade also often display outer tunic elaborations, giving the bulbs a wrinkled, winged or bullulate appearance. The mechanism of formation of these tunic structures has only recently become apparent (Gebregziabher, 2004), and this too might constitute a synapomorphy for the entire *Purpurea* clade.

Included species: *Oxalis adenodes* Sond., *Oxalis algoensis* E. & Z.<sup>#</sup>, *Oxalis ambigua* Jacq., *Oxalis annae* Bolus f., *Oxalis attackuana* Salter, *Oxalis bullulata* Salter, *Oxalis convexula* Jacq., *Oxalis crispula* Sond.<sup>#</sup>, *Oxalis densa* N.E. Br., *Oxalis depressa* E. & Z., *Oxalis dilatata* L. Bolus, *Oxalis fergusoniae* Salter, *Oxalis fourcadei* Salter, *Oxalis foveolata* Turcz.<sup>#</sup>, *Oxalis grammopetala* Sond., *Oxalis inaequalis* Weintraub, *Oxalis lawsonii* Bolus f.<sup>#</sup>, *Oxalis lichenoides* Salter, *Oxalis luteola* Jacq., *Oxalis melanosticta* Sond., *Oxalis nortieri* Salter, *Oxalis obliquifolia* Steud. ex Rich., *Oxalis obtusa* Jacq., *Oxalis oreithala* Salter<sup>#</sup>, *Oxalis pocockiae* L. Bolus, *Oxalis pulchella* Jacq., *Oxalis punctata* L. f., *Oxalis purpurea* L., *Oxalis reflexa* Salter<sup>#</sup>, *Oxalis rubro-punctata* Salter<sup>#</sup>, *Oxalis senecta* Salter<sup>#</sup>, *Oxalis setosa* E. Mey. ex Sond.

#### 1.3.2.2 *Flava* clade

This clade has never before been taxonomically recognized, due to the extreme morphological diversity of its constituent species. Included species can be stemless or caulescent, broad- or linear-leaved, glabrous or covered in a dense indumentum. Leaflets can vary in number from one to twenty per petiole, and a wide variety of bulb types are exhibited, ranging from densely fibrous, deeply buried bulbs to tiny structures borne just beneath the soil surface. The clade includes the morphologically unusual new species *O. ericifolia* Oberlander & Dreyer (Oberlander & al., 2008, Chapter 6). This diversity is reflected in the scattered taxonomic placement of included species in four different sections and, in one case, a species considered unassignable to any existing section (*O. monophylla*). All the included species are exendospermous. Potential synapomorphies include reticulate pollen of the subtypes C10-C11 (otherwise rare in the genus; Dreyer, 1996), and a specialized mode of vegetative reproduction wherein a modified root contributes to the formation of the new bulb (Salter, 1944). Uniseriate, multicellular, non-

glandular hairs are present in many species of the clade, while other species are completely glabrous at reproductive maturity. Significantly, this unusual trichome type is present on the embryo and cotyledons of the otherwise glabrous *O. flava* (pers. obs.), and certain forms of this species retain a multicellular indumentum into adulthood (Form B; Salter 1944). This suggests that this species, and perhaps others, have secondarily lost this character.

Included species: *Oxalis adspersa* E. & Z., *Oxalis argillacea* Bolus f., *Oxalis aurea* Schltr., *Oxalis campylorrhiza* Salter, *Oxalis* “*canaliculata*” (manuscript name, M. B. Bayer), *Oxalis cathara* Salter, *Oxalis clavifolia* Sond., *Oxalis crocea* Salter, *Oxalis deserticola* Salter, *Oxalis dines* Ornduff, *Oxalis disticha* Jacq., *Oxalis dregei* Sond., *Oxalis ericifolia* Oberlander, Dreyer and Curran, *Oxalis fabaefolia* Jacq., *Oxalis flava* L., *Oxalis flaviuscula* Salter, *Oxalis furcillata* Salter, *Oxalis inconspicua* Salter, *Oxalis louisae* Salter, *Oxalis monophylla* L. f., *Oxalis namaquana* Sond., *Oxalis pillansiana* Salter & Exell, *Oxalis pulvinata* Salter, *Oxalis salteri* L. Bolus, *Oxalis simplex* Salter<sup>\*,#</sup>, *Oxalis sonderiana* (O. Kuntze) Salter, *Oxalis stenoptera* Turcz., *Oxalis suavis* R. Knuth, *Oxalis uliginosa* Schltr., *Oxalis viscosa* E. Mey. ex Sond.

### 1.3.3 The Caulescent clade

This Caulescent clade is currently not supported by any obvious morphological characters. Both plastid and nuclear data supported similar but not identical groupings (Oberlander & al. 2008, Chapter 2). ITS topologies supported a clade similar, but not identical, to the Caulescent clade by including the aberrant *O. oculifera* sequence (almost certainly a misplacement based on palynological data) and excluding the subsection *Pardales*, core section *Sagitatae* and *O. heterophylla* clades. Plastid data was thus dominating phylogenetic inference for this clade. A potential plastid indel synapomorphy was found in *trnS-G* (a 10 bp deletion located at positions 529-550 of the aligned *trnS-G* data set), but this inference was complicated by some substitutional variation in the adjacent regions.

The clade includes nearly half of the currently recognised species, including the majority of section *Angustatae* and parts of sections *Oppositae*, *Latifoliolatae* and *Sagittatae*. Although not all taxa have above-ground stems, most are caulescent to varying degrees. This clade had very little internal structure, with a large basal polytomy containing a miscellany of species. These included a well-supported Core caulescent clade and a monophyletic *Pardales* clade. Several smaller clades were also retrieved with significant support, including a clade consisting of *O. tomentosa*, *O. oligophylla* and the recently described *O. hygrophila*. The unusual species *Oxalis palmifrons* was also associated with this lineage, but the pedigree is somewhat complicated by the contrasting signal provided by plastid and nuclear compartments for this species (Oberlander & al. 2008, Chapter 2). Another newly-described species, *Oxalis saltusbelli*, clarifies the situation surrounding *O. palmifrons* (Dreyer & al., 2008; Chapter 5). Additional clades included a pairing of the summer-rainfall species *O. smithiana* and *O. bifurca*, the *O. heterophylla* complex, the forest endemic *O. incarnata*, the Namaqualand species *O. virginea*, and a polytomy of CFR taxa. All members of section *Sagittatae* were also included in the Caulescent clade.

#### 1.3.3.1 Unresolved taxa

These species and small clades were not associated with any of the larger groupings in the Caulescent clade. They are morphologically highly variable and no obvious affinities can be proposed.

*Oxalis zeekoevleyensis* is a locally-abundant species of the Caledon and Bredasdorp regions. Although placed in section *Oppositae* by Salter (1944), this species lacks the opposite bracts on the peduncle that define this section. Despite superficial resemblance to *O. purpurea*, *Oxalis zeekoevleyensis* is entirely unrelated, and no other species in *Oxalis* is an obvious close relative. Similarly, the extreme southwestern CFR endemics *O. lanata*, *O. truncatula* and *O. strigosa* also superficially resemble one another, but their sequence data suggested that these similarities could possibly represent retained symplesiomorphic characters.

*Oxalis virginea* is an interesting species of the Namaqualand region. Salter (1944) considered this species a close relative of *O. ambigua*, but sequence data did not support such a placement. Although *O. virginea* was strongly supported as a member of the Acaulescent clade based on ITS data, plastid sequences overrode the nuclear signal in the combined data sets to place this species in the Caulescent clade instead (Oberlander & al. 2008, Chapter 2). More data are needed to resolve this situation. This species is apparently very local and the plastid-ITS conflict might be the result of recent hybridisation.

The only shade-loving member of southern African *Oxalis*, *O. incarnata*, is confined to forest patches near running water in the southwestern and southern Cape regions from the Cape Peninsula to Uitenhage. This species is unusual in more than just habitat preference. It is one of the few documented southern African *Oxalis* in which populations can lack one or more tristylous floral morphs. Although populations in the southern Cape exhibit all three morphs, most populations on the Cape Peninsula consist entirely of long-styled plants. It is uncertain why the mid and short morphs are so rare on the Peninsula: southern Cape populations have healthy representations of all three morphs. The geographically closest populations from the Kogelberg and Stellenbosch regions are only known to contain long morphs (S. Siqueira, unpublished data; K. Oberlander, unpublished data). Plants from Jonkershoek populations are capable of selfing (Oberlander, pers. obs.). *Oxalis incarnata* also has an unusual form of above-ground vegetative reproduction through terminal bulbils. Its naturally fragmented distribution range, unusual means of reproduction and apparent loss of tristylous make *O. incarnata* an ideal candidate for biogeographical and reproductive studies. Neither sequence- nor morphology-based data suggested any close relatives for this species.

*Oxalis smithiana* and *O. bifurca* are summer-rainfall species with unusually pronounced bifurcate leaflets. These species differ in terms of the presence of an above-ground stem. *O. bifurca* is strongly caulescent, whilst *O. smithiana* is always acaulescent. Salter (1944) placed both species in *Oppositae* subsection *Bifurcatae*, but did not explicitly state a close relationship between them (in fact, he questioned the inclusion of *O. smithiana* in

this subsection). Both plastid and nuclear data only weakly supported a sister relationship between these two species, but the combined analysis provided substantially greater support. Biogeographically these two species and *O. tysonii* (probably a form of *O. bifurca*) represent the only species with bifurcate leaflets and single-flowered inflorescences in the summer-rainfall region of southern Africa.

A strongly-supported clade containing *O. tomentosa*, *O. oligophylla* and the recently described *O. hygrophila* was retrieved in all analyses. A similar strongly-supported relationship was found by Oberlander & al. (2004) and subsequently confirmed by Zietsman & al. (2009). *Oxalis tomentosa* is a common shale-loving, lowland species of the southwestern CFR, while the other two are confined to cool sandstone mountain slopes of the northern CFR. Recent findings of a plant similar to *O. hygrophila* in Bain's Kloof Pass await confirmation, but would extend the distribution of this species more than 160 km southward. The plants are morphologically similar in having pure white corolla lobes and acaulescent habits. *Oxalis palmifrons* is an enigmatic taxon from the Ceres and Sutherland regions that grouped strongly with *O. tomentosa* on ITS evidence, but whose plastid sequence was not correspondingly placed. This incongruence might indicate hybridisation or incomplete lineage sorting, but needs to be corroborated by further studies. The newly-described *Oxalis saltusbelli*, (Dreyer & al., 2008; Chapter 5) from the Oorlogskloof Nature Reserve on the northern limits of the CFR, shares many similarities to both *O. tomentosa* and *O. palmifrons*.

The aptly named *Oxalis heterophylla* of section *Oppositae* subsection *Bifurcatae* is a widespread species that has a very late flowering season (August to October). This taxon is an incredibly variable group species that has received little scientific attention, and it is possible that a number of distinct taxonomic entities are currently lumped under this name. Salter (1944) considered the pendulous-leafleted species *O. duriuscula* and *O. pendulifolia* to be very close relatives, but did not include them in the subsection *Bifurcatae*. Sequence data placed these three species in a strongly-supported clade together with the partial shade-lover *O. bifida*. This latter species is very similar to *O. heterophylla*, but shares the early flowering time of the pendulous-leafleted taxa.

Unassigned Caulescent clade species: *Oxalis bifida* Thunb., *Oxalis duriuscula* Schltr., *Oxalis bifurca* Lodd., *Oxalis fibrosa* Bolus f., *Oxalis heterophylla* D.C., *Oxalis hygrophila* Kumwenda & Dreyer, *Oxalis incarnata* L., *Oxalis lanata* L. f., *Oxalis oligophylla* Salter, *Oxalis orthopoda* Salter, *Oxalis palmifrons* Salter, *Oxalis pendulifolia* Salter, *Oxalis saltusbelli* Dreyer and Roets, *Oxalis smithiana* E. & Z., *Oxalis strigosa* Salter, *Oxalis tomentosa* L. f., *Oxalis truncatula* Jacq., *Oxalis tysonii* Phillips<sup>#</sup>, *Oxalis virginea* Jacq., *Oxalis zeekoevleyensis* R. Knuth

#### 1.3.3.2 Core *Sagittatae* clade

The five species in section *Sagittatae* are easily recognised by their sagittate anthers, narrow floral tube and the spreading mid- and short-level reproductive whorls in the flower. Within this morphologically distinct group a tremendous amount of variation is found, notably within the *O. eckloniana* complex and the closely related *O. minuta* and *O. nidulans*. Two other species are included in this section, namely *O. microdonta* from the Montagu area and *O. fibrosa* from the Little Karoo. These latter species have seldom been collected. *Oxalis fibrosa* is distinct from the rest of *Sagittatae* in bearing multicellular glandular hairs, a very different bulb and micro-rugulate spinate pollen (type B) instead of rugulate reticulate pollen (type A) (Dreyer, 1996). Dreyer (1996) considered pollen types A and B to be related, but could not rule out affinities to other major pollen types. *Oxalis fibrosa* is also the only member of the section with a Succulent Karoo distribution, while the other species are predominantly Fynbos and Renosterveld taxa.

Salter (1944) considered this group to be a distinctive natural entity. The only taxonomic change since Salter (1944) has been the removal of *Oxalis minuta* var. *callosa* Salter to section *Latifoliolatae* as *Oxalis hygrophila*. This reclassification was supported by palynological, morphological and sequence-based evidence (Kumwenda and Dreyer, 2004, Oberlander & al., 2004).

Sequence-based phylogenies conclusively supported the monophyly of all taxa with pollen type A (BS 100 %, PP 1.00, here referred to as the core *Sagittatae* clade) (Oberlander & al. 2008, Chapter 2), but yielded no relationship with *O. fibrosa*. This species remained unresolved in the caulescent clade and was variously placed by different optimality criteria and data partitions, but was never associated with the core *Sagittatae* clade. This suggests a more distant relationship between *Oxalis fibrosa* and the pollen type-A taxa. The shared reproductive characters are quite distinct, and it does seem unlikely that such characters could be convergent. Dreyer (1996) did suggest that *O. fibrosa* pollen could have arisen from a pollen type A ancestor via adaptation to a new biome and (presumably) new pollinators, but this needs support from studies of the pollination biology of these species. If *O. fibrosa* is closely related to the pollen type A taxa, and the shared morphological characters are truly synapomorphic, then this species is most probably sister to this group. If not, there are sufficient macro-morphological and ecological characters to segregate *O. fibrosa* from section *Sagittatae*, but not to suggest alternative close relatives.

Included species: *Oxalis eckloniana* Presl., *Oxalis microdonta* Salter<sup>#</sup>, *Oxalis minuta* Thunb., *Oxalis nidulans* E. & Z.

#### 1.3.3.3 *Pardales* clade

The relations of subsection *Pardales* with other *Oxalis* taxa were found to be ambiguous (Oberlander & al., 2008, Chapter 2). The combined topologies and plastid analyses supported a relationship with the Caulescent clade, but did not provide any other conclusive evidence of affinities. ITS data were found to be even more inconclusive in supporting a weak sister relationship with the morphologically dissimilar *O. orbicularis*. Despite superficial morphological resemblance to members of the Hirta and Glabra clades (caulescent habit and linear leaflets), such an affinity was never supported.

This subsection is somewhat morphologically similar to other members of section *Angustatae*, but also displays several unique characters, such as retrorse hairs on the outer bulb tunics, and distinctive pellucid streaks on the leaflets and sepals that turn black on

drying. Although these characters make members of *Pardales* easily identifiable, species identification within the subsection is problematic. Several species are described on the basis of very limited collections (e.g. *Oxalis melanograptia* and *Oxalis lineolata*), although this is a common problem throughout *Oxalis*. Other species are widely collected and the taxonomic boundaries better understood. Bayer (1993) reduced all the species in this subsection to synonymy under *O. pardalis* due to the poorly defined species boundaries and the presence of many morphological intermediates. Dreyer (1996) upheld the original circumscription of the species due to the extreme morphological variability evident in the section and in order to assess as much potential palynological variation as possible. All species in subsection *Pardales* produce reticulate pollen. Geographically, species are divided into a northern (three species found on the Niewoudtville escarpment and Knersvlakte and south along the eastern edge of the Cedarberg) and a southern group, (widespread south of the Ceres Karoo in the east and Clanwilliam in the west; Bayer 1992).

Six of the eleven species *sensu* Salter (1944) were sampled by Oberlander & al. 2008, Chapter 2). Sequence data of both plastid and nuclear origins strongly supported the monophyly of subsection *Pardales* (BS 100%, PP 1.00). Within this clade, the northern and southern clades were also strongly retrieved. Although not sampled, morphological similarity and geographical proximity support a placement of the Knersvlakte endemic *O. melanograptia* with the Niewoudtville/eastern Cedarberg endemics *O. grammophylla* and *O. massoniana* in this northern clade. The southern clade is more variable and widespread.

The molecular results of Oberlander & al. (2008, Chapter 2) did not support Bayer's (1992) proposal to sink all members of subsection *Pardales* into a single, highly variable group species. At least two geographically-supported clades were retrieved, separated by the Cedarberg and the Olifant's River valley. Whether these two clades represent two distinct species or complexes of more strictly delimited taxa is not clear, but further population level analyses focussed on this problem are currently in progress (Krige pers. com.). Subsection *Pardales* presents an ideal case for studying potential drivers of

speciation in *Oxalis*, including pollinator-induced speciation (forms from the Ceres Karoo are night-flowering and show other signs of potential moth-pollination, Bayer 1992) and potential genetic drift through founder effects (geographically close populations are often morphologically dissimilar). A more intensive study to address species boundaries and morphological variation in this clade is also required.

Included species: *Oxalis camelopardalis* Salter, *Oxalis capillacea* E. Mey. ex Sond., *Oxalis confertifolia* (O. Kuntze) R. Knuth, *Oxalis heidelbergensis* Salter<sup>#</sup>, *Oxalis grammophylla* Salter, *Oxalis leptogramma* Salter, *Oxalis lineolata* Salter<sup>#</sup>, *Oxalis massoniana* Salter, *Oxalis melanograptia* Salter<sup>#</sup>, *Oxalis pardalis* Sond.<sup>#</sup>, *Oxalis robinsonii* Salter & Exell<sup>#</sup>

#### 1.3.3.4. The Core Caulescent clade.

Virtually all of the included species the Core caulescent clade have well-developed above-ground stems. Most species tend to have apically congested leaves with leaflets that are longer than broad. The clade is palynologically very variable, including a range of reticulate pollen types, as well as the distinctive supra-areolate D-type pollen.

Oberlander & al. (2008, Chapter 2) found that plastid and nuclear representations of this clade differed somewhat. Separately, neither received substantial support, whilst combined analyses provided strong evidence for the monophyly of this lineage. ITS data included *O. fibrosa* in a poorly-supported core caulescent clade, whilst plastid data included the sister taxon relationship of *O. smithiana* and *O. bifurca*. The clade as retrieved in combined analyses contained the single species *O. engleriana*, the sister pair *O. suteroides* and *O. zeyheri*, and the Hirta and Glabra clades.

*O. engleriana* is the only species in southern Africa with reticulate pollen of the subtype C6 (Dreyer, 1996). This multifoliolate species is found in the Caledon region and extends northwards to the Breede River valley at Worcester and southwards to Bredasdorp. Despite superficial resemblance to an inland species, *O. zeyheri*, these two are not sister species. Instead, *O. zeyheri* and *O. suteroides* from the Niewoudtville escarpment

resolved as sister taxa. Some populations of *O. zeyheri* occur further inland on the Niewoudtville plateau, but this species extends southwards on the drier inland side of the Cedarberg to Ceres and Montagu. The bulb systems of these two species are very similar.

#### 1.3.3.4.1. *Hirta* clade

This clade corresponds closely with the similarly named clade of Oberlander & al. (2004). The first suggestion of the existence of this clade resulted from the palynological review of Dreyer (1996), who found a variety of taxa, scattered across the current classification, that share a unique pollen type. The tectum in these taxa is drastically reduced, and distinctive supra-tectal structures have developed into a new layer above the tectum. Dreyer (1996) considered this pollen type to be so distinct that the multiple origins presumed by the classification were highly unlikely. The phylogenetic results of Oberlander & al. (2004) strongly supported the monophyly of this clade. Our recent molecular results, based on both nuclear and additional plastid data, strengthen this hypothesis of monophyly (Oberlander & al., 2008, Chapter 2). All markers supported a monophyletic *Hirta* clade, with the unique pollen type serving as a distinctive morphological synapomorphy. Additional macro-morphological characters, such as fluid-filled channels or bulges in the bulb tunics (Gebregziabher, 2004), loss or reduction of glandular hairs, and fused, sessile cotyledons, may constitute additional synapomorphies, but these still need to be confirmed for all members of this clade.

Although all markers agreed on the natural status of the *Hirta* clade, different partitions of the data yielded different internal topologies. This may be the result of either hybridisation or incomplete lineage sorting. It is interesting that most of the aberrant pollen types identified by Dreyer (1996) belong to this lineage. Also, the only example of confirmed hybridisation in southern African *Oxalis* is between two species of the *Hirta* clade, namely *O. macra* and *O. creaseyi* (Salter, 1944).

Included species: *Oxalis albiuscula* Salter\*, *Oxalis aridicola* Salter, *Oxalis blastorrhiza* Salter, *Oxalis burkei* Sond., *Oxalis callosa* R. Knuth, *Oxalis campicola* Salter, *Oxalis ciliaris* Jacq., *Oxalis creaseyi* Salter\*, *Oxalis cuneata* Jacq., *Oxalis exserta* Salter, *Oxalis*

*gracilis* Jacq., *Oxalis helicoides* Salter\*<sup>#</sup>, *Oxalis hirta* L., *Oxalis kamiesbergensis* Salter, *Oxalis linearis* Jacq., *Oxalis macra* Schltr.\*, *Oxalis oreophila* Salter, *Oxalis petiolulata* Bolus f.\*, *Oxalis primuloides* R. Knuth, *Oxalis pseudo-hirta* Salter\*, *Oxalis reclinata* Jacq., *Oxalis stokoei* Weintraub\*, *Oxalis subsessilis* L. Bolus\*, *Oxalis tenella* Jacq., *Oxalis xantha* Salter

#### 1.3.3.4.2. *Glabra* clade

This clade contained the bulk of section *Angustatae* subsection *Lineares*, as well as species from subsections *Sessilifoliatae*, *Glandulosae* and *Multifoliolatae*, and sections *Latifoliolatae* and *Campanulatae*. Despite poorly-supported and polytomous relationships within this clade, the included taxa are morphologically similar. Leaflets of all species are much longer than broad, except in the species *O. amblyosepala*, and most taxa have two or four large calli at the apex of the leaflets. These characters could be synapomorphic for this clade. Pollen morphology provided little information on the monophyly of this lineage, as most species have the reticulate pollen common to many other southern African taxa. Palynological exceptions include *O. tenuipes* var. *biapiculata*, with reticulate pollen of the type C12, *O. droseroides* and *O. phloxidiflora* with reticulate pollen of the type C14-C15, and *O. levis* with reticulate pollen of the type C5.

The clade is entirely confined to the CFR and its inland environs: no species of this clade are found in Namaqualand. This is one of the few large clades in *Oxalis* to have fewer species in Succulent Karoo than in Fynbos: it is an almost entirely Renosterveld/Fynbos lineage. The clade includes one aquatic species, *O. natans*, which Salter (1944) included in section *Campanulatae*. This species is clearly unrelated to the other superficially similar aquatic taxa, and a position in this clade was confirmed by multiple accessions from two separate populations (Oberlander, data not shown). Palynological data also support this placement.

Included species: *Oxalis amblyodonta* Salter, *Oxalis amblyosepala* Schltr., *Oxalis argyrophylla* Salter, *Oxalis burtoniae* Salter, *Oxalis callimarginata* Weintraub<sup>#</sup>, *Oxalis comptonii* Salter, *Oxalis droseroides* E. Mey. ex Sond., *Oxalis ebracteata* Savign., *Oxalis*

*falcatula* Salter, *Oxalis fragilis* Salter<sup>#</sup>, *Oxalis giftbergensis* Salter, *Oxalis glabra* Th., *Oxalis goniorrhiza* E. & Z., *Oxalis gracilipes* Schltr., *Oxalis involuta* Salter<sup>#</sup>, *Oxalis leptocalyx* Sond., *Oxalis levis* Salter<sup>#</sup>, *Oxalis meisneri* Sond., *Oxalis multicaulis* E. & Z., *Oxalis natans* L. f., *Oxalis pallens* E. & Z., *Oxalis perineson* Salter & Exell<sup>#</sup>, *Oxalis phloxidiflora* Schltr., *Oxalis polyphylla* Jacq., *Oxalis porphyriosiphon* Salter, *Oxalis pusilla* Jacq., *Oxalis recticaulis* Sond., *Oxalis stenopetala* Salter, *Oxalis tenuipes* Salter, *Oxalis tenuis* Salter, *Oxalis stictocheila* Salter, *Oxalis tenuifolia* Jacq., *Oxalis urbaniana* Schltr., *Oxalis variifolia* Steud.<sup>#</sup>, *Oxalis versicolor* L.

## 2. Unassigned taxa

These species are accepted as valid, but they lack evidence on which to base a convincing placement in any particular clade in the current system. All of these taxa are poorly known from just a few herbarium specimens, often from isolated localities.

*Oxalis ausensis* R. Knuth is potentially related to or conspecific with *O. sonderiana* on morphological grounds, but natural populations of this taxon must be relocated in order to confirm this. Pollen of this species differs from that of *O. sonderiana* (Dreyer, 1996).

*Oxalis calvinensis* R. Knuth was considered to be a close relative of *O. imbricata* by Salter (1944). Although this may be possible, *O. imbricata* and other members of the Stellata clade are biogeographically distant from *O. calvinensis*. Until this species is relocated, no conclusive statement can be made about its natural affinities.

*Oxalis crispula* Sond. is similar in almost all respects to *O. ambigua*, differing principally in the undulation of the leaflets. Populations of *O. ambigua* do occasionally have plants with variously undulating leaflets. The single known locality at the extreme southern end of *O. ambigua*'s distribution range, and the otherwise close morphological resemblance suggests that this is not a good species.

*Oxalis davyana* R. Knuth has not been rediscovered since the original collections by Galpin. Salter (1944) did not have access to living material. It is impossible to state close

affinities beyond a general resemblance to some of the broad-leaved members of the Stellata clade.

*Oxalis extensa* Salter is only known from one herbarium record well outside the distribution range of any morphologically similar species. Attempting to judge affinities for such a poorly-known species is impossible.

*Oxalis hirsuta* Sond. may be a member of the Hirta clade. The species has an uncommon distribution range for this clade, however. No palynological data are available for this species.

*Oxalis lasiorrhiza* Salter is most probably a member of the Pes-caprae clade, which does include a few species with single-flowered inflorescences. This is supported by the yellow flowers and longitudinal ridges on the tunics. Recent collections from Niewoudtville have not yet been confirmed.

*Oxalis lindaviana* Schlechter is an enigmatic species that has not yet been rediscovered in its type locality (Piekenier's Kloof). Salter (1944) described a similar but possibly unrelated plant from the Worcester region, with very distinctive bulbils, which he considered to be this same species. The green-coloured corolla tube of the latter description could link it with the Stellata clade, as could the locality.

*Oxalis marlothii* Schltr. ex R. Knuth is thought to be merely a variety of *O. obtusa*, but this can only be confirmed once living material of this taxon is located.

*Oxalis petraea* Salter could be an affinity of (or even conspecific with) *O. sonderiana*, on morphological and palynological grounds. Like *O. petraea*, *Oxalis sonderiana* also occurs on the Knersvlakte.

*Oxalis psammophila* G. Will. is a recently-described species from the Richtersveld. It does appear to be a good species based on the description. This species is not particularly

similar to *O. strigosa*, as suggested by Williamson (1999). Nothing is known of the pollen of this species, and no affinities can be suggested at present.

*Oxalis rhomboidea* Salter is probably a member of the *Purpurea* clade based on its morphological similarity to *O. adenodes*.

### 3. *Dubious taxa and incertae*

Several of the species retained by Salter (1944) are described on the basis of particularly poor specimens, often lacking bulbs, flowers and locality details. Several appear to have been grazed or otherwise damaged forms of more common species. In all cases Salter (1944) stated grave doubts about the taxonomic status of these species.

*Oxalis viscidula* Schltr. appears to be a form of *O. imbricata*. The sole specimen lacks bulbs and Salter (1944) upheld this species on the basis of dentate filaments and apparently opposite bracts. This is confusing, because Salter's (1944) description of *Oxalis imbricata* describes the filaments as toothed. Close examination of the specimen cannot determine whether the bracts are sub-opposite or clearly opposite. On this basis we propose that *O. viscidula* be considered as a poor specimen of *O. imbricata*.

*Oxalis quinata* Savign.

*Oxalis incerta* R. Knuth

*Oxalis linoides* R. Knuth

*Oxalis neglecta* R. Knuth

*Oxalis leipoldtii* Schlechter

### Discussion

It is noteworthy that, despite the recognised artificiality of Salter's (1944) system, the major divisions of our classification correspond broadly to his. There is a mostly clear division between taxa with many-flowered vs. single-flowered inflorescences, and the latter group is subdivided into (mostly) acaulescent and caulescent groups.

We argue for a preliminary, informal classification for three main reasons:

1) Nomenclatural uncertainty and conflict

There is currently little consistency in nomenclatural rank between the major classification systems of Lourteig (2000) and Salter (1944). The southern African taxa have not been placed in a nomenclatural system with the rest of *Oxalis* since Knuth's (1930) revision of the genus. His southern African sections are decidedly unnatural, and have since been replaced by the nine-section system of Salter (1944). There are hierarchically-induced conflicts as well as historical problems in both classifications. Close relatives of the southern African taxa include the bulb-bearing New World taxa belonging to section *Ionoxalis*, a section recently revised on morphological grounds (Lourteig, 2000). Other close relatives belong to sections *Pseudobulbosae* (which DNA evidence places within *Ionoxalis*, Oberlander & al., submitted) and section *Articulatae*. If the sister-taxon status of *Ionoxalis* (and these other closely-related taxa) and southern African taxa is to be upheld (which seems clear from our preliminary data, Oberlander & al. 2008), then the southern African clade as a whole should be awarded the taxonomic rank of section. Alternatively, the southern African clade could consist of three sections corresponding to the *Pes-caprae*, *Livida* and core southern African clades. Either solution would invalidate Salter's (1944) sectional system.

Furthermore, DNA-based data show that every single section *sensu* Salter (1944) is non-monophyletic. The two systems can only be reconciled with regard to sections *Sagittatae* and *Stictophyllae*. The monophyly of even these two sections is problematic, despite their small size.

2) Lack of data

As argued by Bayer (1992), current information on *Oxalis* morphology and anatomy are limited. A substantial number of species have been described on the basis of very limited herbarium material. In the majority of species almost nothing is known about within-

species variation, structure and means of vegetative propagation of the underground organs, edaphic, climatic and pollinator preferences, and even distribution ranges. This makes the identification of potential synapomorphies for clades that are strongly supported by sequence data challenging.

### 3) Uncertainties within phylogeny

Despite a large number of clades that received strong support based on DNA evidence, topologies derived from different markers and different sub-cellular compartments do show some level of conflict. Moreover, different inference methods show sometimes radically different support values for clades (Oberlander 2008, Chapter 2). In the interests of taxonomic stability, and to avoid future confusion, an approach was followed in which clades that did not receive support from all data sources and analysis types were not given a provisional name.

Although all the major clades reported here did receive some level of support, very little internal structure to these clades was consistently retrieved. This was particularly evident within the *Purpurea*, *Flava* and *Glabra* clades, where parsimony and model-based topologies, and plastid and ITS data sets, supported very different resolutions of taxa. Zietsman & al. (2009) reported the maintenance of ancestral *O. tomentosa* haplotypes in *O. oligophylla*, a putative recent neo-endemic of the Giftberg. Ancestral polymorphisms and incomplete lineage sorting would explain the incongruent trees as products of separate evolutionary histories. Given that the inheritance mechanisms of plastid and nuclear DNA are separate, and the increasing evidence for substantial polyploidy in the genus, (Suda and Dreyer, pers. comm.), the incongruence between the various data sets can most probably be explained as either incomplete lineage sorting or hybridisation. Consequently, a conservative approach is followed here in delimiting groups of species within the major clades.

## Conclusions

In this paper we present a new, substantially different, phylogenetically-based classification of the southern African members of *Oxalis*. Although higher support values for significant nodes are required before this classification can be taxonomically formalized, we highlight palynological and macro-morphological characters that can be tested as synapomorphies for these clades in future analyses. The proposed classification thus presents a much improved, more natural summary of relationships among southern African members of *Oxalis*.

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Chapter 4: Towards a model of bulb evolution in the eudicot genus *Oxalis* (Oxalidaceae)

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#### Abstract:

The origins and monophyly of the bulbous habit in the eudicot genus *Oxalis* are uncertain, but key character state transitions in the evolution of true bulbs are currently thought to be reflected in extant pseudobulbous and other geophytic taxa. We test the relationships between the two major groups of bulbous *Oxalis* taxa, namely the southern African lineage which is centered in the speciose Cape Floristic Region (CFR), and the New World section *Ionoxalis*, by including the rhizomatous geophyte *O. acetosella*, the caudiciform stem succulent *O. articulata*, and the rhizomiform pseudobulbous *O. triangularis*, in combined phylogenetic analyses of nrITS and *trnL-F* sequence data. We optimize several key bulbous characters in ancestral state reconstructions on produced phylogenies. Results of our analyses indicate that the evolution of bulbous characters in the genus is more complex than previously thought. Although the two major bulb types are homologous, the rhizomiform pseudobulbous habit arises from within true bulbs, and in most reconstructions the caudiciform stem succulent *O. articulata* is inferred to have secondarily lost several distinctive bulbous characters. *Oxalis acetosella* is not as closely related to the bulbous lineage as previously thought. More sampling from other key taxa are needed before the order in which key bulbous characters were acquired can be verified. We discuss these results in terms of the taxonomic and ecological implications for the CFR *Oxalis* taxa.

#### Keywords:

bulb morphology; *Ionoxalis*; *Oxalis articulata*; *Oxalis incarnata*; *Oxalis pes-caprae*; *Oxalis triangularis*

#### Introduction:

The Cape Floristic Region (CFR) of southern Africa is globally unparalleled in geophyte diversity with roughly 25% (*ca.* 2100 spp.) of the total flora constituting plants with this habit (Procheş et al., 2005; Procheş et al., 2006). The geophytic habit has been an important adaptive strategy in several large Cape lineages, including the Geraniaceae (Bakker et al., 2005), Iridaceae (Goldblatt et al., 2002) Hyacinthaceae (Goldblatt and Manning, 2000) and the Orchidaceae (Linder and Kurzweil, 1999). Most hypotheses on the generation of this remarkable CFR geophyte diversity posit connection to the climate of the CFR, including long-term climatic stability (Procheş et al., 2005) and the establishment and maintenance of a Mediterranean-type climate (Linder, 2003). Several authors have proposed key innovations in certain lineages as being primarily responsible for enabling adaptive radiations in the CFR (Klak et al., 2004; Verboom et al., 2004), but few have discussed the evolution of geophytism per se as a potential key innovation in light of the Mediterranean

climate. For example, Bakker et al. (2005) found that the acquisition of certain tuber characters in *Pelargonium* section *Hoarea* led to subsequent species increase and posed this as a possible key innovation (Bakker et al., 2005).

Bulbs, one of the better known geophytic structures, are particularly prominent among the monocotyledons. Monocotyledons acquired bulbs many times during their evolutionary history, as they are present in multiple unrelated families (e.g. Alliaceae, Hyacinthaceae, Liliaceae and Amaryllidaceae, Goldblatt and Manning, 2000). A bulb is generally defined as a vertically compressed stem, bearing swollen leaves or leaf bases (Raunkaier, 1934; Bold et al., 1980; Manning et al., 2002). The bulk of the bulb volume is leafy in origin, mostly as storage tissue, whilst the associated stem has limited storage function. This contrasts to superficially similar underground storage organs such as corms or rhizomes, where storage function is confined to stem tissues.

*Oxalis* is one of very few non-monocot angiosperm bulbous taxa. The genus is morphologically extremely variable, with South America housing most of this diversity (Lourteig, 1994; Lourteig, 2000). Only  $\pm 15\%$  (ca. 35 spp. confined to section *Ionoxalis*) of native New World *Oxalis* taxa have true bulbs (Lourteig, 2000), while all indigenous southern African taxa (>200 spp) are bulbous. Most southern African species (hereafter referred to as SA taxa) are indigenous to the CFR, making *Oxalis* the largest single genus-level contributor to the geophyte diversity in this region (181 spp. in the Greater CFR region; Procheş et al., 2005). Although Linder (2003) did not include *Oxalis* as one of his Cape clades, the genus boasts very high levels of endemism in the CFR (92%; Procheş et al., 2006) and is thus important to consider in phylogenetic analyses of the Cape Flora as a whole. In particular, such high levels of endemism in the only bulbous dicot lineage in the CFR require explanation.

With few modifications *Oxalis* plant architecture conforms to general vascular plant architecture as defined by internal structure (Esau, 1963; Mauseth, 1980). Most *Oxalis* leaves have a semi-amplexicaul basal region, a petiole and a lamina. The lamina is divided into one or more (usually three) leaflets. The three main regions of the leaf are separated by articulations that allow nastic movements of the petiole and leaflets (Salter, 1944; Robb, 1963; Levy and Moore, 1993). The leaf base often extends into membranous, stipule-like structures that sometimes protrude beyond the basal articulation. The leaf base can also be variously succulent, depending on the species, and the basal articulation between the leaf base and the terete part of the petiole often serves as an abscission zone.

The bulbs of both SA and New World taxa are very similar. Both bear a flattened vertical axis (basal plate) bearing fleshy leaf scales, imbricated by several layers of papery or gummy tunics (Denton, 1973; Gebregziaber, 2004). In SA taxa the above-ground plant parts are borne on a seasonal underground stem that forms at nodes on the basal plate (thus confirming the stem status of the basal plate). The bulb itself does not anchor any photosynthetic leaves. In section *Ionoxalis* the basal plate is surrounded by many spirally arranged scale-like leaf bases or fleshy bulb scales, which do not enclose the bulb. In addition to serving as storage organs, bulbs in section *Ionoxalis* also act as anchorage point for the photosynthetic leaves (i.e. the plants are acaulescent; Lourteig, 2000). Although tunics are formed, these only form with the new growth flush and are often lost as the bulb matures (Denton, 1973).

Compared to the New World taxa that have been taxonomically well-studied (Lourteig, 1994; Lourteig, 2000), SA *Oxalis* has received limited focus since the Knuth (1930) and Salter (1944) revisions. This has resulted in uncertainty in the relationships between the South American and African taxa, although the latter were assumed to be relatively derived (Knuth, 1930). In her treatment of section *Ionoxalis*, Denton (1973) considered the bulb types of the two continents to be non-homologous, resulting from independent origins. She considered the South American and southern African bulb types to be different based on the arrangement of the leaf bases, fleshy bulb scales and protective scales. Although Denton (1973) considered these differences sufficient to argue in favour of a polyphyletic origin of bulbs in *Oxalis*, this ignored the many similarities shared by these two bulb types, as well as the subterranean structures of some non-bulbous taxa.

Estelita-Teixeira (1982) compared the bulbs of the American taxa *O. latifolia* Knuth. (section *Ionoxalis*), *O. debilis* Knuth (= *O. corymbosa* DC.; section *Ionoxalis*) and *O. triangularis* subsp. *papilionacea* (Hoffmanns. ex Zucc.) Lourteig (= *O. oxyptera* Prog.; section *Pseudobulbosae*) and found many anatomical similarities. She considered the elongated rhizomiform bulb (as defined by her) of *O. triangularis* as primitive in *Oxalis*, with bulbs of *O. latifolia* and *O. debilis* being successively more derived. Unfortunately no southern African species were included in her study.

Lourteig (2000) grouped the SA taxa and section *Ionoxalis* together in an unnamed bulbous group. She also concluded that taxa with subterranean stems in the sections *Articulatae*, *Oxalis*, *Palmatifoliae* and *Pseudobulbosae* were potential close relatives of these

bulbous species based on several morphological characters. A shared base chromosome number of  $x = 7$  (Dreyer and Johnson, 2000; De Azkue, 2000) supports this contention.

A number of character state transitions are important in the evolution of *Oxalis* bulbs, including the acquisition of succulence, geophytism, the origins of fleshy bulb scales and tunics, internodal compression, and spatial separation of storage and photosynthetic functions onto different stem axes. However, very little is known about the order in which these characters were acquired, or even whether they have independent origins in different branches of *Oxalis* phylogeny. In a first attempt at exploring patterns of character evolution leading to bulbs, we provide a phylogenetic hypothesis of relationships within the genus *Oxalis* as based on two independent DNA markers, namely the multiple-copy Internal Transcribed Spacer of nuclear ribosomal DNA (ITS) and the *trnL* intron and associated *trnL-trnF* spacer (*trnL-F*) from the plastid genome. We explore the series of changes as outlined above by ancestral state reconstruction on the produced phylogeny. On the basis of these results, we discuss the current morphological diversity observed in CFR *Oxalis*.

## Materials and Methods

Taxon sampling for phylogenetic analysis followed previous studies (Emshwiller, 2002; Oberlander et al., 2004). Sequences of the geophytic non-bulbous species *O. acetosella* L. (section *Oxalis*) and *O. articulata* Savigny (section *Articulatae*), and the rhizomiform bulbous *Oxalis triangularis* were included as potential close relatives of the bulbous taxa based on morphology. Seventy two sequences (51 ITS and 21 *trnL-F*) were newly generated for this study, supplemented by sequences retrieved from GenBank, for a total of 57 taxa across the family Oxalidaceae. Several species were represented by independent accessions (Table 1). This is the most comprehensive sampling for *Oxalis* phylogeny to date, with nine extra-African and all nine southern African sections represented.

Data were aligned in Bioedit v7.0.0 (Hall, 1999) using default values of the embedded ClustalW alignment algorithm (Thompson et al., 1994) and manually adjusted. Gaps were not scored, as these were present in homopolymer regions of dubious alignment, autapomorphic or supported already well-supported clades.

ITS, *trnL-F* and combined matrices were analysed using parsimony, likelihood and Bayesian Inference (BI) criteria. Parsimony analyses had the following settings: 1000 random-addition-sequence heuristic searches, using TBR branch-swapping, saving the ten shortest trees per replicate. Confidence levels for nodes were constructed using 1000

bootstrap replicates using simple addition. All parsimony analyses were conducted in PAUP\* 4.0b10 (Swofford, 2002). Likelihood trees were inferred using the program GARLI (v0.951 and v0.96; Zwickl, 2006), with an appropriate model as selected by Modeltest v3.7 (Posada and Crandall, 1998). Bootstrap support measures were constructed using the same program. Bayesian Inference was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Relevant models for BI of all data sets were selected using MrModeltest v2.2 (Nylander, 2004). All analyses were run twice, for  $5 \times 10^6$  generations, saving trees every 500 generations to avoid autocorrelation of samples. The first 1000 samples (500 000 generations) were discarded as burnin for all data sets. Combined analyses were conducted using both a global model and a partitioned approach. Tests for incongruence between the ITS and *trnL*-F data were conducted using the partition homogeneity test in PAUP\* (ILD test; Farris et al., 1995). One thousand bootstrap replicates were conducted using the same settings as heuristic parsimony searches.

Given the results of our combined analyses, we tested whether the two differing placements of *O. articulata* (topology 1: sister to section *Ionoxalis* + *O. triangularis*; topology 2: sister to the SA clade) could be rejected by using the SH-test (Shimodaira and Hasegawa, 1999) of topology in PAUP\*, as well as Bayes Factors in a Bayesian context (Kass and Raftery, 1995). We also tested whether *O. articulata* sister to the rest of the bulbous clade could be rejected (topology 3). Although no tree in any analysis retrieved this topology, this species is the only non-bulbous taxon in the bulbous clade, and such a basal position would make sense from a parsimonious view of morphology. SH-tests were conducted under the relevant models chosen by Modeltest using 1 000 bootstrap replicates with the Re-Estimated Log Likelihood (RELL) approximation. Bayes Factors for the relevant positions of *O. articulata* were calculated using the constraint command in MrBayes, which forces monophyly of selected taxa. Analyses under each constraint were run using the same parameters as the combined 2-partition data set.

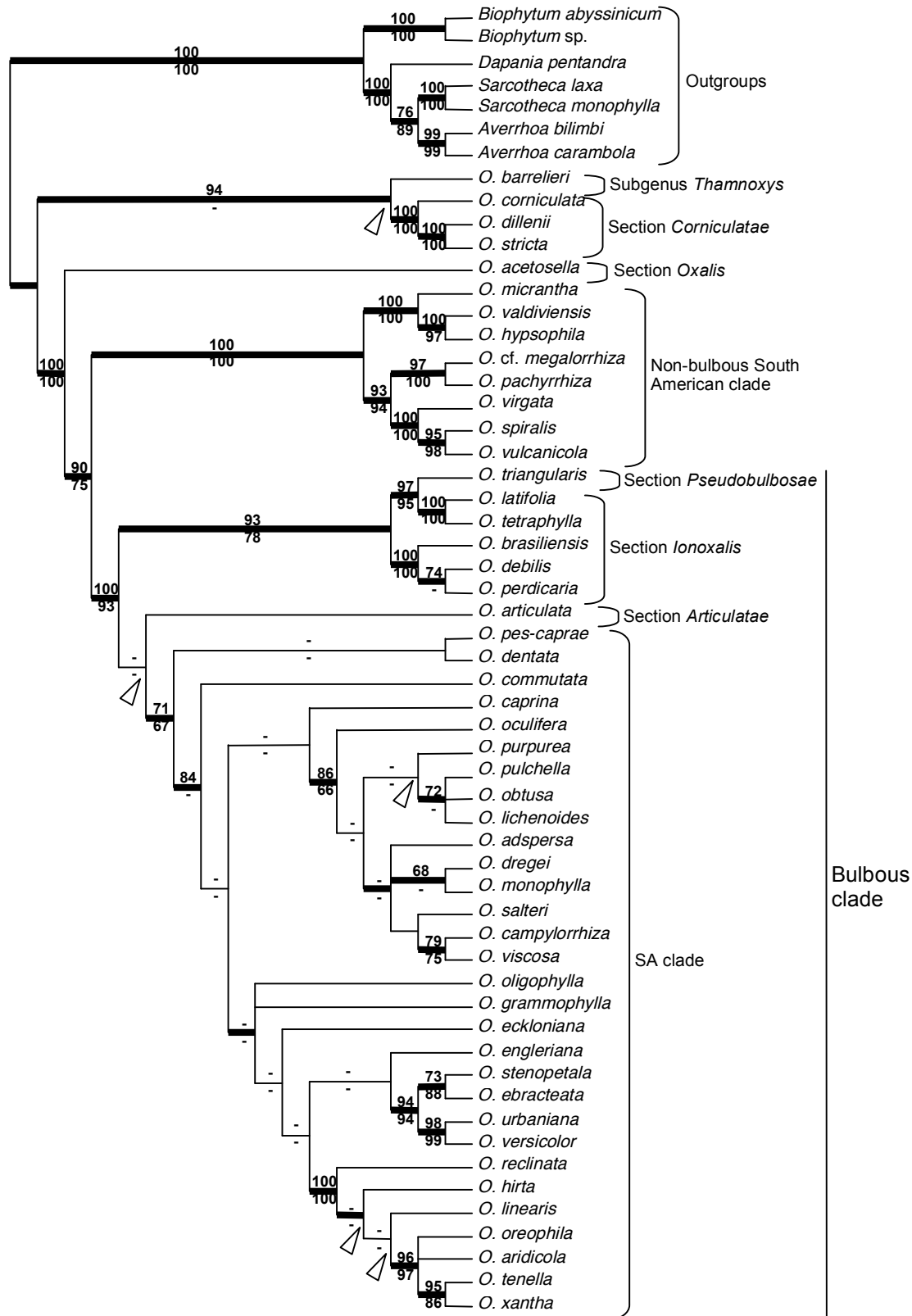


Fig. 1 Consensus tree topology obtained through Bayesian Inference of the combined 57 taxon data set. Thickened branches indicate nodes with greater than 0.95 posterior probability. Numbers above branches are likelihood nonparametric bootstrap values, numbers below the nodes are parsimony bootstrap values. Hyphens indicate values of less than 65 %. White arrows indicate nodes that collapse in the parsimony strict consensus tree. Clades of interest mentioned in the text are labeled.

In order to evaluate the evolution of bulbous characters across the phylogeny, a morphological data matrix of the major characters of interest was constructed in Mesquite v1.12 (Maddison and Maddison, 2006). Character scoring for non-SA taxa was taken from Denton (1973), Lourteig (1994) and Lourteig (2000). Data from SA taxa was extracted from Gebregziabher (2004), Salter (1944) and our own observations (Mesquite character matrix available on request from KCO). The best likelihood trees produced by the GARLI searches were used as best inferences of phylogeny from our DNA data. We tested both parsimony and likelihood character reconstruction on these trees. Parsimony reconstruction used default parameters, while likelihood reconstruction used the 1-parameter, symmetrical model of character evolution (Mk1).

#### Results:

The aligned ITS and *trnL*-F data sets were 881 (36.3 % parsimony informative) and 1115 characters (15.1 % parsimony informative) long, respectively. Modeltest selected the GTR + I +  $\Gamma$  model of sequence evolution for the ITS data set, and the GTR +  $\Gamma$  model for *trnL*-F. MrModeltest identified identical models. General topologies from ITS and *trnL*-F were very similar to one another. In addition, the partition homogeneity test did not support incongruence of the two data sets ( $P = 0.138$ ), so only the results of the combined analyses are presented here (Fig. 1).

The combined 1196-character data set (24.4 % parsimony informative) produced 66 most parsimonious trees of length 1850 (CI: 0.576; RI: 0.760). Modeltest selected the TIM + I +  $\Gamma$  model for the combined, single partition data, whilst MrModeltest selected GTR + I +  $\Gamma$ . Bayes Factors strongly supported a 2-partition, unlinked analysis (using the models selected by MrModeltest) of the combined data over a single partition ( $2\ln BF_{2\text{partition}/1\text{partition}} = 182.26$ ).

The oxalidaceous genera *Averrhoa*, *Biophytum*, *Dapania* and *Sarcotheca* are sister to a monophyletic *Oxalis* with parsimony bootstrap support (PB) of 100 %, likelihood bootstrap support (LB) of 100 % and posterior probability (PP) of 1.0. We rooted these taxa as sister based on data from more inclusive *trnL*-F and *rbcL* analyses (data not shown). Both model-based methods strongly support the position of *O. barrelieri* (subgenus *Thamnoxys*) as sister to section *Corniculatae*. This is not supported by parsimony. Of the remaining taxa (which form a well-supported clade), *O. acetosella* is sister to two large clades, one containing only American non-bulbous taxa (including the *O. tuberosa* alliance; de Azkue and Martinez,

1990; Emshwiller, 2002), the other a clade containing all bulbous and pseudo-bulbous taxa as well as *O. articulata* (henceforth called the bulbous clade; Fig. 1).

The bulbous clade is very strongly supported by all three analysis methods. Many of the species in this clade have callose structures on their leaves and flowers, and a basic chromosome number of  $x = 7$  is common amongst members. The bulbous clade is divided into three major lineages: the moderately-to strongly-supported SA lineage, the strongly-supported section *Ionoxalis* + *O. triangularis*, and *O. articulata*. *Oxalis triangularis* is strongly supported in an embedded position within section *Ionoxalis*. Likelihood bootstrap analyses support a weak relationship between the *Ionoxalis/O. triangularis* clade and *O. articulata* (topology 1: LB 56 %), while Bayesian Inference weakly links this species to the SA clade (topology 2: PP 0.70). Parsimony is equivocal.

SH-tests of the combined data set could not reject any of the three tested positions for *O. articulata* (topology 1:  $P = 0.780$ ; topology 2: best; topology 3:  $P = 0.628$ ). Bayes Factors favoured all three positions over unconstrained analyses, with topology 2 receiving the strongest support ( $2\ln BF_{\text{topology 1/unconstrained}} = 3.96$ ;  $2\ln BF_{\text{topology 2/unconstrained}} = 8.4$ ;  $2\ln BF_{\text{topology 3/unconstrained}} = 6.78$ ). Consequently we cautiously consider topology 2 (*O. articulata* sister to the southern African clade) as the optimal topology. However, due to the non-rejection of other positions for this taxon, we performed all character optimizations on all three different trees. The three trees used for character optimization in Mesquite v1.12 (Maddison and Maddison, 2006) were the best likelihood trees derived from GARLI analyses using constraints on the position of *O. articulata*.

All analyses are broadly congruent with previous phylogenetic studies of *Oxalis* (Emshwiller and Doyle, 1998; Emshwiller and Doyle, 2002; Emshwiller, 2002; Oberlander et al., 2004). The congruence between these analyses suggests that they present a reasonable approximation of *Oxalis* phylogeny.

## Discussion:

### Model of *Oxalis* bulb evolution

The most basal relationships in *Oxalis* remain uncertain, but are cautiously inferred to reside in the pinnate-leaved subgenus *Thamnoxys* (Lourteig, 1994). The close relationship between section *Corniculatae* and *O. barrelieri* L. (subgenus *Thamnoxys*) in our phylogenies

is unexpected and may result from long-branch attraction. However, it is the methods that most account for long-branch attraction bias (Likelihood and Bayesian Inference) that most strongly support this node. Given that *O. barrelieri* is the only member of the subgenus that is sampled, more thorough coverage from the subgenus *Thamnoxys* is expected to solve this.

Our initial expectations of bulb evolution in *Oxalis* were that geophytic non-succulent taxa such as *O. acetosella*, caudiciform taxa such as *O. articulata* and rhizomiform bulbous taxa such as *O. triangularis* would be successive sister taxa to a clade containing section *Ionoxalis* and the SA taxa. Such a scenario would provide a parsimonious model of character changes that would also enumerate the sequence in which certain typical bulbous characteristics were acquired. As is seen from our produced phylogenies, such a scenario is not retrieved. Instead, *O. acetosella* is not as closely related to the bulbous lineage as indicated by Lourteig (2000), and *Oxalis triangularis* is deeply embedded within a paraphyletic *Ionoxalis*. Similarly, although a basal split between *O. articulata* and all other bulbous taxa could not be ruled out on our data, this species is more comfortably housed as sister to either the SA clade, or the *Ionoxalis* clade. All of these unexpected positions alter the inference of bulbous character acquisition.

For example, the distribution of succulence throughout *Oxalis* is of interest as a potential starting point towards the evolution of true bulbs. The closest relatives of the Oxalidaceae are woody shrubs, trees, or lianas (APGII, 2003), a habit shared by most oxalidaceous genera. *Oxalis* itself is variable in growth form. Subgenus *Thamnoxys* includes annuals, shrubs and subshrubs, while *O. pachyrrhiza* Weddell and relatives are stem succulents. Some members of the *Oxalis tuberosa* alliance (represented in our study by *O. spiralis* and *O. vulcanicola*) produce tubers as storage structures, while rhizomatous taxa and taxa with rhizomiform bulbs are found in the American sections *Oxalis*, *Articulatae*, *Palmatifoliae* and *Pseudobulbosae*. Section *Articulatae* is wholly subterranean stem-succulent (caudiciform) in habit, while stem and leaf bases in sections *Palmatifoliae* and *Pseudobulbosae* contribute variously to storage. Species in section *Ionoxalis* and all SA taxa are bulbous, with storage capacity being leafy in origin. Several oxalidaceous lineages thus show a reduction in woodiness and an increase in development of storage organs, which can be either of stem origin (tubers or rhizomes) or leaf origin (bulbs or pseudobulbs). Our results indicate multiple origins for stem storage organs in *Oxalis*, at least once in the South American non-bulbous clade and once in *O. articulata*. This awaits confirmation through more densely taxon-sampled analyses. The origins of leaf storage, however, are more ambiguous, with this character equivocally reconstructed over several internal nodes.

However, the ancestor to the bulbous clade is confidently inferred to have leafy storage tissue in all likelihood reconstructions and parsimony reconstructions of topologies 1 and (Fig. 2).

Another character change of major interest is the move to geophytism. *Oxalis* displays different types of geophytism, including tubers (*Oxalis tuberosa* alliance), rhizomes (sections *Oxalis* and *Articulatae*) and bulbs (section *Ionoxalis* and all SA taxa). Storage organs in sections *Pseudobulbosae* and *Palmatifoliae* can be interpreted as elongated bulbs or as rhizomes with succulent leaf bases. Our optimizations for this character are ambiguous. Although the ancestor of the bulbous clade is confidently inferred to be geophytic, the precise origins of this character might be much more ancient, as the nodes subtending the bulbous clade are equivocally resolved. This is due to the placement of the rhizomatous species *O. acetosella*.

Fleshy leaf scales and tunics constitute important components of *Oxalis* bulbs. Fleshy leaf scales serve as the primary storage units of the bulb. Tunics differ primarily from fleshy leaf scales in being thinner in transverse section, heavily sclerified, and (in the SA taxa, at least), dead at maturity. The leafy nature of both of these structures has been confirmed by comparative anatomy and positional homology (Esau, 1960; Mauseth, 1988; Oberlander, unpublished data). Moreover, it has become increasingly certain that the precise homologue of the fleshy leaf scale and of the tunic is the basal foot portion of the photosynthetic leaf. The anatomy (particularly the vasculature) of fleshy bulb scales, immature tunics and the basal region of photosynthetic leaves are very similar. Many SA taxa show a morphological continuum between these structures, where photosynthetic leaves gradually reduce their leaflets and terete parts of their petioles as one moves down the seasonal stem. Once underground, the remaining basal structures are very similar to small fleshy leaf scales. Occasional above-ground bulbs have been observed to form with underdeveloped leaflets at the apex of an otherwise normal fleshy leaf scale or young tunic (Oberlander, pers. obs.).

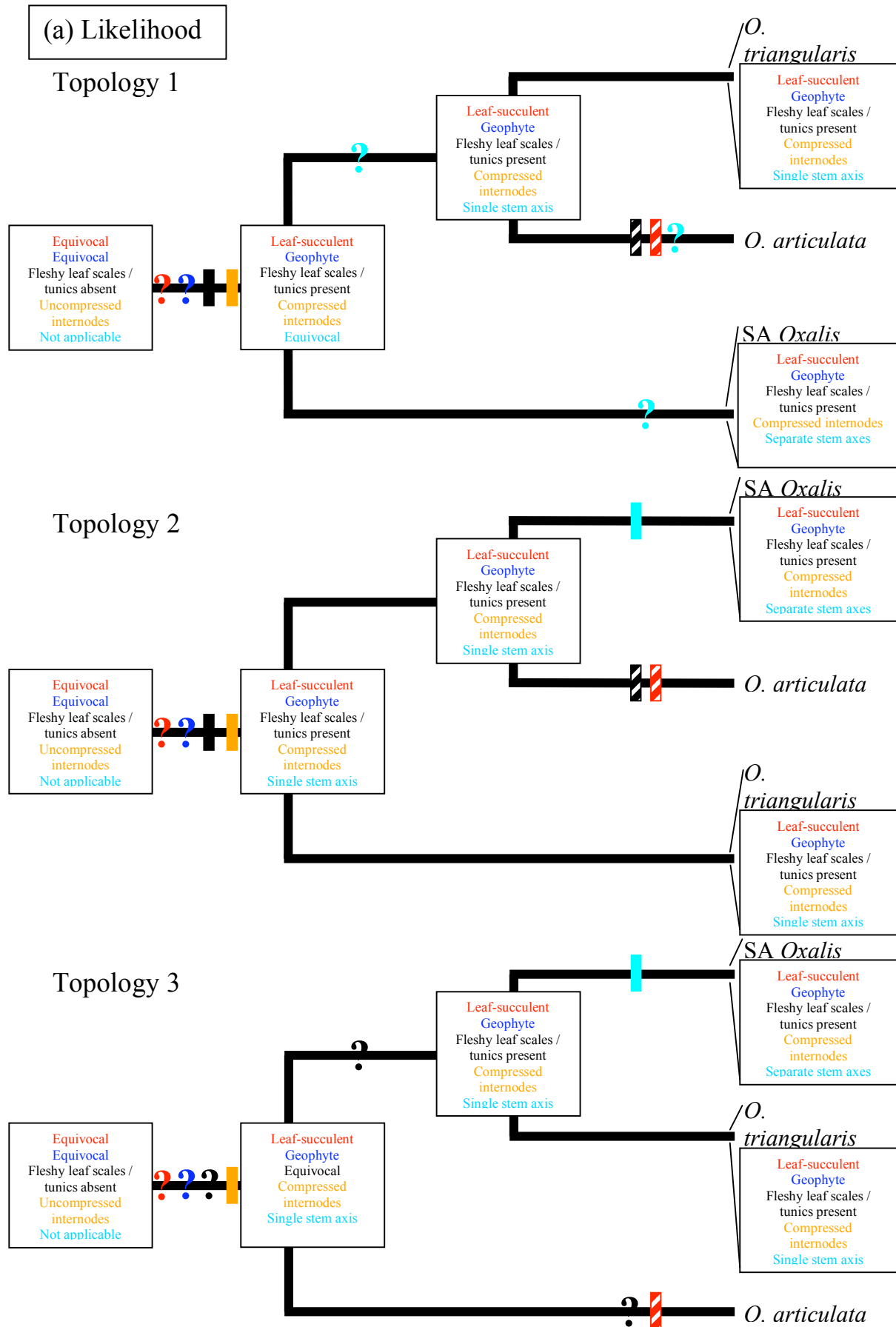
Although the leafy nature of leaf scales and tunics is now understood, the evolution of these structures is more uncertain. All SA taxa, members of section *Ionoxalis* and *O. triangularis* are reported to have both of these leaf-derivative structures. The caudiciform *O. articulata* has neither. Due to identical distributions of fleshy leaf scales and tunics in our sampled taxa, all optimizations of these two characters produce identical results (Fig. 2). Thus it remains unclear whether tunics are a modification of fleshy leaves or a new structure derived from the base of the photosynthetic leaf. In topologies 1 and 2 likelihood optimizations unequivocally retrieve tunics and fleshy leaf scales at the base of the bulbous

clade, with losses of these characters in the lineage leading to *O. articulata*. Topology 3 is equivocal for these characters. Parsimony is equivocal for all three topologies.

Another defining characteristic of bulbs is the presence of a compressed stem anchorage for the storage leaves. Two types of subterranean stems are present in both the SA taxa and section *Ionoxalis*, namely the compressed main stem axis itself and normal-length, axillary, seasonal stolons or runners that produce new bulbs or above-ground shoots. In section *Ionoxalis* these ephemeral stems are responsible for clonal reproduction of the bulbs (Denton, 1973; Estelita-Teixeira, 1982), whilst in SA taxa they mostly produce the above-ground plant parts, allowing for photosynthesis and sexual reproduction. All lineages of the bulbous clade can produce stems of both types, although the precise homology of the compressed stems in *O. articulata* is obscured by the fleshy storage nature of the stem in this species. This character is optimized to the base of the bulbous clade in all analyses.

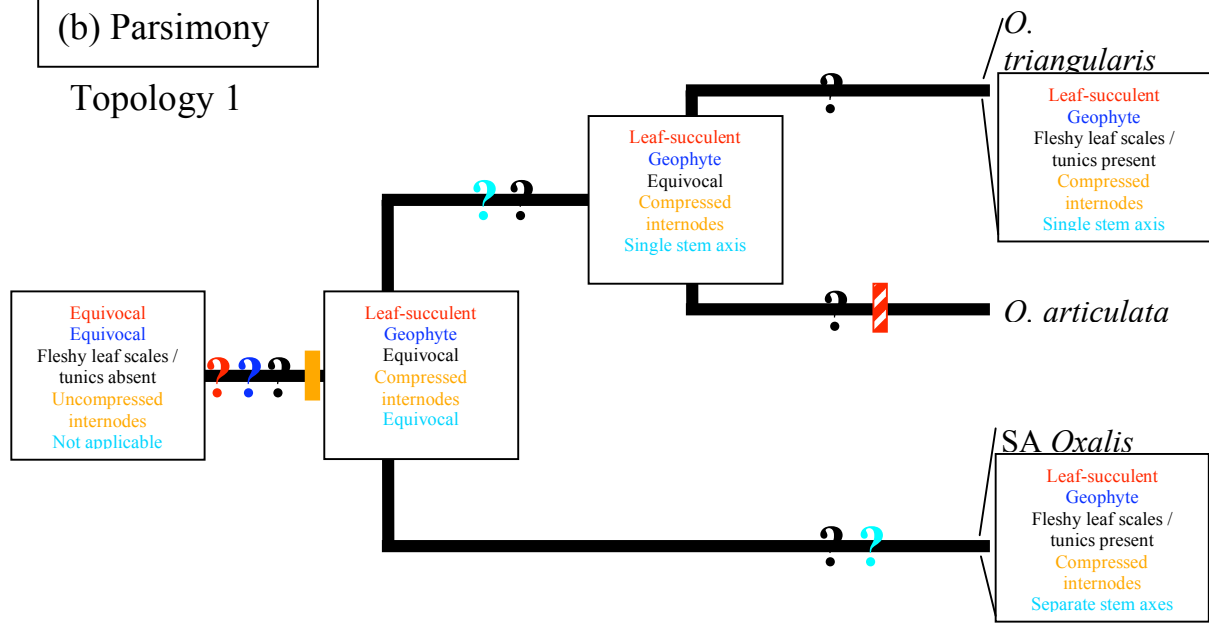
A distinctive character of the SA clade is the separation of photosynthetic leaves from the main bulb axis, so that the bulb itself consists only of fleshy leaf scales and tunics emerging from a compressed main axis stem. Seasonal axillary stems emerge from the main axis to produce an annual above-ground shoot that only bears normal photosynthetic leaves. This stem can also secondarily produce new axillary underground shoots that transform into new bulbs. In most constructions this character is resolved as being unique to the SA lineage.

It is clear that, no matter the resolution of the major components of the bulbous clade, section *Ionoxalis* and the SA taxa are closely related, and this forces reinterpretation of Denton's (1973) view that the bulbs of New World and SA taxa evolved separately. However, at present, our reconstructions do not provide a definitive answer to whether the bulbs are truly homologous. Likelihood optimizations on the best topology unambiguously reconstruct several of the major characters that define a bulb, such as compressed stems, fleshy leaf storage organs and geophytism, to the base of the bulbous clade, with losses of some characters in *O. articulata*. Other topologies (which we cannot reject) and parsimony methods of reconstruction are less clear. It is also clear that elongated rhizomiform bulbs such as those found in *O. triangularis* are not primitive, as postulated by Estelita-Teixeira (1982), but derived from more typical bulbs of the section *Ionoxalis*, in which this species is deeply embedded. This suggests that other pseudo-bulbous taxa of sections *Pseudobulbosae* and *Palmatifoliae* may show similar patterns.

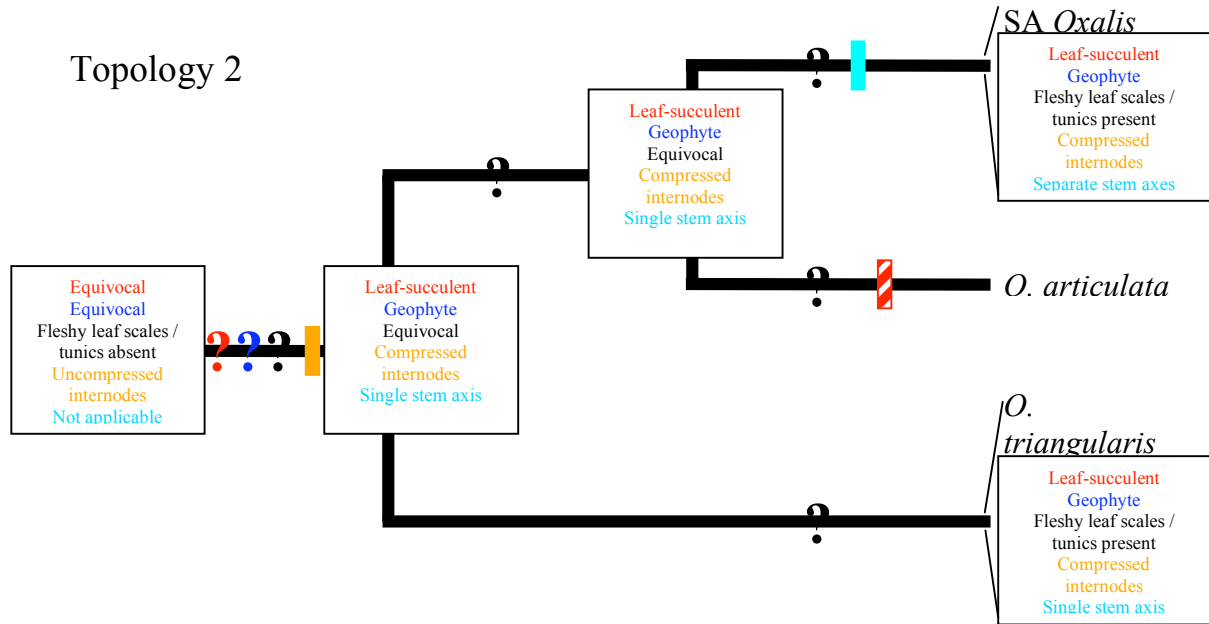


(b) Parsimony

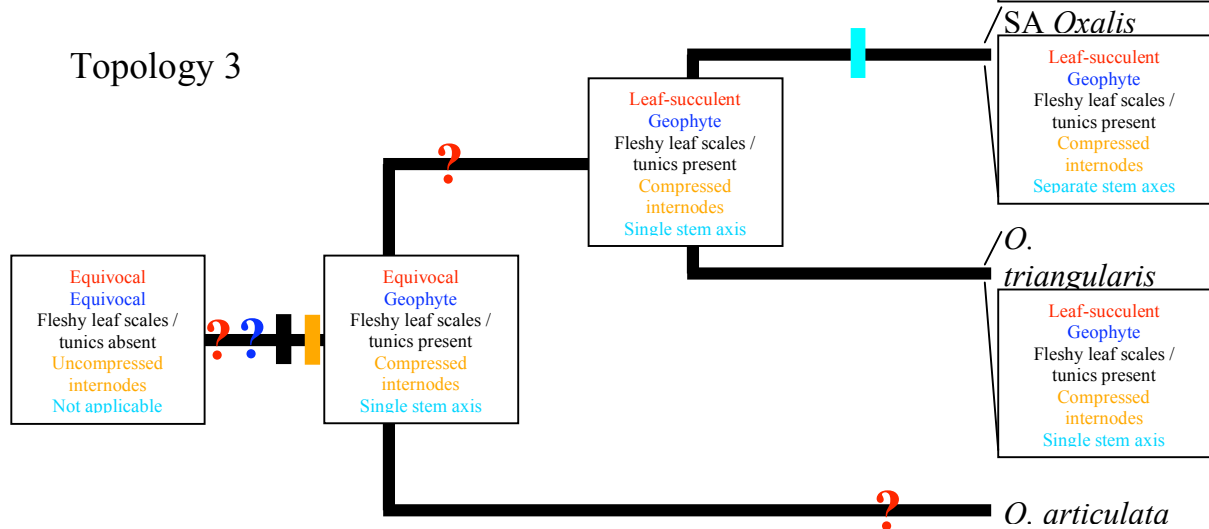
Topology 1



Topology 2



Topology 3



Since most characters optimize to the base of the bulbous clade, the sequence of transitions of these characters remains unclear. It is uncertain whether leaf succulence evolved before stem compression, for example. However, we trust that improved sampling will localize these characters to different branches of the phylogeny leading to bulbs. Other characters need to be explored as well, including the contractile root mechanism common to both *Ionoxalis* and the SA taxa.

With regards to *O. acetosella*, this species has leaf bases dispersed along the stem axis rather like members of section *Pseudobulbosae*. However, in the case of *O. acetosella* the stem is elongated, thin, and a true rhizome (i.e. not rhizomiform pseudobulbous), and the leaf bases are the remains of photosynthetic leaves whose leaflets and petioles have abscised. Our phylogenies argue against the placement of *O. acetosella* as a close relative of the bulbous clade. It also differs from the bulbous clade in terms of basic chromosome number ( $x = 11$  instead of  $x = 7$ ; Marks, 1955). It further lacks calli, a character shared by members of the bulbous clade. Other members of section *Oxalis*, to which *O. acetosella* belongs, are needed to confirm this placement. At least one, *O. dimidiata* Donn.-Sm., has both succulent stems and calli.

#### Implications for *Oxalis* in the CFR

The presence of a seasonal stem dedicated to photosynthesis and sexual reproduction in SA taxa releases the bulb from growth close to or just above the soil surface. This provides obvious water retention, drought avoidance and anti-predator advantages. This character may be considered a potential key innovation for the SA clade. In many southern African species, layers of old tunics sheathe (and presumably protect) the entire length of each year's new seasonal stem. This tunic arrangement is easily explained in conjunction with the action of the contractile root. As the annual bulb is formed, the contractile root pulls this new bulb deeper into the ground, leaving the old tunics behind (Salter, 1944). After several years the old tunics will naturally form a tube through which the new seasonal stem can grow. In contrast, many other southern African species (e.g. *O. polyphylla*) do not produce a

Fig. 2 preceding page. Reconstructions of various bulbous characters on the three topologies of the bulbous clade using (a) likelihood and (b) parsimony. The remainder of the tree is not presented. Blocks of text refer to reconstructions at internal nodes of the phylogeny (note that the SA clade and section *Ionoxalis* + *O. triangularis* are represented as terminals, as reconstruction of characters in these clades was identical in all cases). Colours refer to the characters of interest: red to type of succulence (stem/leaf/none), blue to geophytism (present/absent), black to fleshy leaf scales and tunics (present/absent), ochre to degree of internode compression (compressed/uncompressed) and cyan to separation of bulbous and photosynthetic stem axes (Single stem axis/separate stem axes). Unequivocal likelihood reconstructions of characters were assigned to nodes when one character was favoured above the others by more than the decision threshold of 2 log likelihood units. State transitions (represented as coloured rectangles) were reconstructed on branches when either reconstruction method unequivocally favoured two different states at adjacent nodes. Unequivocal reversals of character states are indicated by hatched rectangles of the relevant colour. Question marks indicate equivocal results for reconstruction of a state transition to one particular branch. The root node in all cases is the node leading to the bulbous clade and South American non-bulbous clade.

contractile root at maturity, and the new bulb forms within the remains of the old, at the same depth. The capability to produce two different stem-types and three different leaf-types has and *O. compressa*) and species complexes (the *O. heterophylla* complex; the *O. stellata* complex) display radically different above-ground architectures. Much of this variation may be explained by combining different stem and leaf structures.

It is tempting to compare closely related species that differ in the placement of their photosynthetic leaves. Southern African *Oxalis* taxa display two types of leaf arrangement, namely leaves loosely arranged along the stem axis, or leaves congested into a dense apical cluster. The latter arrangement is very similar to that of fleshy bulb scales around a basal plate. Moreover, several unrelated southern African species with apically congested leaves also form aerial bulbils on the above-ground plant parts. *O. incarnata* is the only indigenous forest-endemic southern African species. Leaves of this species are produced in pseudo-whorls that often terminate in an above-ground bulbil. At the end of the growing season all above-ground plant parts die back, except for the terminal bulbils, which are released into the surrounding environment. Other species, such as *O. pocockiae* and *O. inaequalis*, produce bulbils in the axils of their leaves. These aerial bulbils are structurally identical, if somewhat underdeveloped, to those of bulbils produced underground. The production of aerial bulbils always occurs in apically congested parts of the stem. This lends credence to the idea that species characterized by apically congested leaf arrangement have utilized the inherent genetic ability to create a basal plate in an above-ground context. Similarities between apically congested leaf arrangements and the basal plate/fleshy bulb scale system include: a) both have compressed stems, with very short internodes, surrounded by closely packed leaf structures; b) both are capable of producing bulbils (responsible for vegetative reproduction) in axillary buds on the compressed stem and c) both occur at the apex of stoloniferous or rhizomatous stems.

The current classification of southern African *Oxalis*, which segregates species on the basis of leaf arrangement, has been recognized as artificial (Salter, 1944; Bayer, 1992; Dreyer, 1996). Species with apically congested leaves are separated from cauline-leaved taxa with little regard to overall affinity (Salter, 1944). If such a radical rearrangement of organs can be construed as the independent co-opting of basal plate morphology onto above-ground plant parts in many unrelated species, then the artificiality of a system based on this character is obvious. Similarly, such a mechanism makes closely related or even sister species capable of very different overall morphologies. This highlights the tremendous plasticity of southern African *Oxalis*, and the caution with which morphological characters must be approached. It

might also have predisposed the genus to extensive speciation in the wide array of microhabitats available in the CFR.

Unlike many other CFR geophytes, the vegetative parts of *Oxalis* display the most variation. Apart from flower colour, *Oxalis* flowers are generally uniform, making pollinator-driven radiation an unlikely explanation of the massive diversity of species within the CFR. This contrasts with many other CFR geophytes (Johnson, 1995; Goldblatt and Manning, 1996; Johnson et al., 1998; Manning and Goldblatt, 2005; Van der Niet et al., 2006). The tristylous breeding system, also expressed in *Oxalis*, is thought to fix many floral traits (Barrett, 1990). Thus most diversity (reflected in species numbers) in CFR *Oxalis* is a result of variation in the vegetative organs, particularly of the leaves and bulbs (Salter, 1944). We do not know what the drivers of bulb morphological differences are in the SA clade. Molerat-mediated bulb dispersal has been recorded (Galil, 1967) in *O. pes-caprae*, as has baboon predation on *O. polyphylla* bulbs (Oberlander, pers. obs.), but the effect of such interactions on speciation remains unknown. Procheş et al. (2005) found that *Oxalis* species with larger bulb sizes tend to occur in the northern and eastern areas of the CFR, and argue that this is linked with the reliability of winter rainfall in the extreme south west of the CFR. Unrelated *Oxalis* species from the drier regions of the CFR produce masses of linear, interwoven, undulate tunics that may serve as insulation and aid water retention during hot summer months (Salter, 1944). Similarly, many unrelated species develop sticky outer bulb tunics, which may represent adaptations against drought or predation. Other characters that could also be influenced by climatic factors such as summer aridity include the average depth of the bulb, the presence/absence of a contractile root, size and number of fleshy leaves and the nature of the tunics.

Although evidence for the homology of the bulbous condition in *Oxalis* can still not be considered fully tested, it is apparent that the members of section *Ionoxalis*, the SA taxa, *O. articulata* and *O. triangularis* are closely related. If the bulbous condition is plesiomorphic for these taxa, then the SA clade presents an interesting case for comparing speciation rates between closely related groups. *Oxalis* has evidently undergone explosive speciation (over 200 species) in a region globally considered a centre of bulb diversity (Goldblatt and Manning, 2000; Procheş et al., 2005; Procheş et al., 2006), whereas continental South America (the putative home of the ancestral bulbous taxon in the genus) only boasts about 50 bulbous and pseudo-bulbous species (Lourteig, 2000). It is possible that the same evolutionary pressures prompting speciation in other bulbous taxa are responsible for the *Oxalis* radiation within the CFR.

Our data show that although a basic understanding of relationships in *Oxalis* has now been gained, the phylogenetic placement of much of the genus is still uncertain. In light of the importance of *Oxalis* as a horticultural asset, as a major weed and as a genus of great size and systematic value within the CFR, a representative, well-sampled global phylogeny is still required. Not least, such a phylogeny would allow refinement of the tentative model of bulb evolution represented here, and allow for more thorough testing of the various steps involved.

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Table 1 Taxonomy, reference numbers and ITS and *trnL*-F representation for taxa included in this study. The taxonomy follows Lourteig (1994) and Lourteig (2000) for all non-southern African *Oxalis* accessions, except for *O. dillenii* (Eiten, 1963; Watson, 1989), and *O. vulcanicola* (Emshwiller and Doyle, 1998). The classification of the southern African taxa follows Salter (1944). Markers not sequenced for an accession are marked by a capital X. Locality details for taxa are to be found in Emshwiller and Doyle (1998) and Oberlander et al. (2004).

| Name   | Subgenus/section             | Specimen voucher   | ITS             | <i>trnL</i> -F  |
|--|------------------------------|--------------------|-----------------|-----------------|
| <b>Generic outgroups</b>                                       |                              |                    |                 |                 |
| <i>Averrhoa bilimbi</i> L.                                     | -                            | AVBIL              | <u>EU436862</u> | <u>AJ582291</u> |
| <i>Averrhoa carambola</i> L.                                   | -                            | AVCAR              | <u>EU436863</u> | <u>EU437032</u> |
| <i>Biophytum abyssinicum</i> Steud. ex. A. Rich.               | -                            | BIOAB              | <u>EU436864</u> | <u>AJ582292</u> |
| <i>Biophytum</i> sp.   | -                            | BIOPHIL            | <u>EU436865</u> | <u>AJ582293</u> |
| <i>Dapania pentandra</i> Capuron                               | -                            | DAPEN              | <u>EU436861</u> | <u>EU437031</u> |
| <i>Sarcotheca laxa</i> Knuth                                   | -                            | SARLAX             | <u>EU436859</u> | <u>AJ582366</u> |
| <i>Sarcotheca monophylla</i> (Planch. ex Hk.f.) Hallier.f.     | -                            | SARMON             | <u>EU436860</u> | <u>EU437030</u> |
| <b>Non-southern African <i>Oxalis</i></b>                      |                              |                    |                 |                 |
| <i>Oxalis acetosella</i> L.                                    | <i>Oxalis/Oxalis</i>         | J. Walter 7182     | <u>EU436870</u> | <u>EU437036</u> |
| <i>Oxalis articulata</i> forma. <i>crassipes</i> (Urb.) Lourt. | <i>Oxalis/Articulatae</i>    | E. Emshwiller 146  | X               | <u>EU437146</u> |
| <i>Oxalis articulata</i> Savigny                               | <i>Oxalis/Articulatae</i>    | Tosto & Hopp, 2000 | <u>Z66545</u>   | X               |
| <i>Oxalis barrelieri</i> L.                                    | <i>Thamnoxyis/Thamnoxyis</i> | DUB 809            | <u>EU436866</u> | <u>EU437033</u> |
| <i>Oxalis</i>  | <i>Oxalis/Ionoxalis</i>      | J. Holmes 0008     | <u>EU436879</u> | <u>EU437044</u> |

|   |                            |                    |                        |                        |
|---|----------------------------|--------------------|------------------------|------------------------|
| <i>brasiliensis</i><br>Lodd.<br><i>Oxalis</i> cf.<br><i>megalorrhiza</i><br>[Feuillée]<br>Jacq. | <i>Oxalis/Carnosae</i>     | L. Dreyer 809      | <b><u>EU436871</u></b> | <b><u>EU437037</u></b> |
| <i>Oxalis</i><br><i>corniculata</i> L.  | <i>Oxalis/Corniculatae</i> | E. Emshwiller 793  | X                      | <b><u>EU437126</u></b> |
| <i>Oxalis</i><br><i>corniculata</i> L.  | <i>Oxalis/Corniculatae</i> | K. Oberlander 0025 | <b><u>EU436867</u></b> | X                      |
| <i>Oxalis debilis</i><br>Knuth  | <i>Oxalis/Ionoxalis</i>    | E. Emshwiller 171  | X                      | <b><u>EU437144</u></b> |
| <i>Oxalis debilis</i><br>var.<br><i>corymbosa</i><br>(DC.) Lourt.                               | <i>Oxalis/Ionoxalis</i>    | E. Emshwiller 445  | <b><u>U74290</u></b>   | X                      |
| <i>Oxalis dillenii</i><br>Jacq.   | <i>Oxalis/Corniculatae</i> | J. Walter 6565     | <b><u>EU436868</u></b> | X                      |
| <i>Oxalis dillenii</i><br>Jacq.   | <i>Oxalis/Corniculatae</i> | E. Emshwiller 1160 | X                      | <b><u>AJ582310</u></b> |
| <i>Oxalis</i><br><i>hypsochila</i><br>Phil.   | <i>Oxalis/Alpinae</i>      | L. Dreyer 805      | <b><u>EU436874</u></b> | <b><u>EU437040</u></b> |
| <i>Oxalis</i><br><i>latifolia</i><br>Knuth  | <i>Oxalis/Ionoxalis</i>    | K. Oberlander 0023 | <b><u>EU436875</u></b> | <b><u>AJ582326</u></b> |
| <i>Oxalis</i><br><i>micrantha</i><br>Bert. ex Colla   | <i>Oxalis/Alpinae</i>      | E. Emshwiller 744  | <b><u>AF470234</u></b> | <b><u>EU437140</u></b> |
| <i>Oxalis</i><br><i>pachyrrhiza</i><br>Weddell  | <i>Oxalis/Carnosae</i>     | E. Emshwiller 387  | <b><u>U74275</u></b>   | <b><u>EU437138</u></b> |
| <i>Oxalis</i><br><i>perdicaria</i><br>(Molina)<br>Bertero                                       | <i>Oxalis/Ionoxalis</i>    | Till Botrand 0002  | <b><u>EU436878</u></b> | <b><u>EU437043</u></b> |
| <i>Oxalis</i><br><i>spiralis</i> Ruiz<br>& Pav. ex G.<br>Don                                    | <i>Oxalis/Lotoideae</i>    | E. Emshwiller 590  | X                      | <b><u>EU437130</u></b> |
| <i>Oxalis</i><br><i>spiralis</i> Ruiz<br>& Pav. ex G.<br>Don                                    | <i>Oxalis/Lotoideae</i>    | E. Emshwiller 187  | <b><u>AF098977</u></b> | X                      |
| <i>Oxalis stricta</i><br>L.   | <i>Oxalis/Corniculatae</i> | J. Walter 6518     | <b><u>EU436869</u></b> | <b><u>EU437035</u></b> |

|  |                              |                                |                 |                 |
|--|------------------------------|--------------------------------|-----------------|-----------------|
| <i>Oxalis tetraphylla</i> Cav.   | <i>Oxalis/Ionoxalis</i>      | J. Holmes 0009                 | <u>EU436877</u> | <u>EU437042</u> |
| <i>Oxalis triangularis</i> ssp. <i>papilionacea</i> (Hoffmanns. ex Zucc.) Lourteig | <i>Oxalis/Pseudobulbosae</i> | E. Emshwiller 456              | X               | <u>EU437141</u> |
| <i>Oxalis triangularis</i> St. Hilaire   | <i>Oxalis/Pseudobulbosae</i> | MO1165                         | <u>FJ410328</u> | X               |
| <i>Oxalis valdiviensis</i> Barnéoud  | <i>Oxalis/Alpinae</i>        | Till Botrand 0001              | <u>EU436873</u> | <u>EU437039</u> |
| <i>Oxalis virgata</i> Rusby  | <i>Oxalis/Lotoideae</i>      | E. Emshwiller 292              | <u>U74267</u>   | <u>EU437131</u> |
| <i>Oxalis vulcanicola</i> (Donn.-Sm.) Lourt.                                       | <i>Oxalis/Lotoideae</i>      | MO702                          | <u>EU436872</u> | <u>EU437038</u> |
| <b>Southern African <i>Oxalis</i></b>  |                              |                                |                 |                 |
| <i>Oxalis adspersa</i> E. & Z.   | <i>Oxalis/Angustatae</i>     | L. Dreyer 635                  | <u>EU436936</u> | <u>AJ582290</u> |
| <i>Oxalis aridicola</i> Salter   | <i>Oxalis/Latifoliolatae</i> | L. Dreyer 672                  | <u>EU436977</u> | <u>AJ582296</u> |
| <i>Oxalis campylorrhiza</i> Salter   | <i>Oxalis/Latifoliolatae</i> | L. Dreyer 696                  | <u>EU436947</u> | <u>AJ582302</u> |
| <i>Oxalis caprina</i> L.   | <i>Oxalis/Cernuae</i>        | L. Dreyer & K. Oberlander 0001 | <u>EU436892</u> | <u>AJ582305</u> |
| <i>Oxalis commutata</i> Sond.  | <i>Oxalis/Latifoliolatae</i> | L. Dreyer 606                  | <u>EU436898</u> | <u>AJ582308</u> |
| <i>Oxalis dentata</i> Jacq.  | <i>Oxalis/Cernuae</i>        | C. Cilliers 0004               | <u>EU436888</u> | <u>AJ582312</u> |
| <i>Oxalis dregei</i> Sond.   | <i>Oxalis/Campanulatae</i>   | Mucina 210903/11               | <u>EU436930</u> | <u>AJ582313</u> |
| <i>Oxalis ebracteata</i> Savign.   | <i>Oxalis/Angustatae</i>     | L. Dreyer 644                  | <u>EU436990</u> | <u>AJ582314</u> |

|   |                              |                                |                        |                        |
|---|------------------------------|--------------------------------|------------------------|------------------------|
| <i>Oxalis eckloniana</i> Presl.         | <i>Oxalis/Sagittatae</i>     | L. Dreyer 628                  | <b><u>EU437025</u></b> | <b><u>AJ582315</u></b> |
| <i>Oxalis engleriana</i> Schltr.        | <i>Oxalis/Angustatae</i>     | K. Oberlander 0010             | <b><u>EU436961</u></b> | <b><u>AJ582316</u></b> |
| <i>Oxalis grammophylla</i> Salter       | <i>Oxalis/Angustatae</i>     | L. Dreyer 670                  | <b><u>EU437016</u></b> | <b><u>AJ582323</u></b> |
| <i>Oxalis hirta</i> L.                  | <i>Oxalis/Angustatae</i>     | L. Dreyer 646                  | <b><u>EU436971</u></b> | <b><u>AJ582324</u></b> |
| <i>Oxalis lichenoides</i> Salter        | <i>Oxalis/Foveolatae</i>     | L. Dreyer 785                  | <b><u>EU436919</u></b> | <b><u>AJ582328</u></b> |
| <i>Oxalis linearis</i> Jacq.            | <i>Oxalis/Angustatae</i>     | L. Dreyer 679                  | <b><u>EU436972</u></b> | <b><u>AJ582329</u></b> |
| <i>Oxalis monophylla</i> L.             | <i>Oxalis/unplaced</i>       | L. Dreyer & K. Oberlander 0003 | <b><u>EU436927</u></b> | <b><u>AJ582335</u></b> |
| <i>Oxalis obtusa</i> Jacq.              | <i>Oxalis/Oppositae</i>      | L. Dreyer 691                  | <b><u>EU436922</u></b> | <b><u>AJ582338</u></b> |
| <i>Oxalis oculifera</i> E. G. H. Oliver | <i>Oxalis/Latifoliolatae</i> | L. Dreyer 799                  | <b><u>EU436953</u></b> | <b><u>AJ582339</u></b> |
| <i>Oxalis oligophylla</i> Salter        | <i>Oxalis/Angustatae</i>     | L. Dreyer 797                  | <b><u>EU437023</u></b> | <b><u>AJ582340</u></b> |
| <i>Oxalis oreophila</i> Salter          | <i>Oxalis/Angustatae</i>     | L. Dreyer 666                  | <b><u>EU436975</u></b> | <b><u>AJ582341</u></b> |
| <i>Oxalis pes-caprae</i> L.             | <i>Oxalis/Cernuae</i>        | L. Dreyer 662                  | <b><u>EU436882</u></b> | <b><u>AJ582343</u></b> |
| <i>Oxalis pulchella</i> Jacq.           | <i>Oxalis/Foveolatae</i>     | L. Dreyer 612                  | <b><u>EU436907</u></b> | <b><u>AJ582347</u></b> |
| <i>Oxalis purpurea</i> L.               | <i>Oxalis/Stictophyllae</i>  | K. Oberlander 0024             | <b><u>EU436902</u></b> | <b><u>AJ582349</u></b> |
| <i>Oxalis reclinata</i> Jacq.           | <i>Oxalis/Angustatae</i>     | L. Dreyer 688                  | <b><u>FJ410329</u></b> | <b><u>FJ410330</u></b> |
| <i>Oxalis salteri</i> L. Bolus          | <i>Oxalis/Crassulae</i>      | L. Dreyer 784                  | <b><u>EU436932</u></b> | <b><u>AJ582352</u></b> |
| <i>Oxalis stenopetala</i> Salter        | <i>Oxalis/Angustatae</i>     | L. Dreyer 675                  | <b><u>EU436983</u></b> | <b><u>AJ582353</u></b> |
| <i>Oxalis tenella</i> Jacq.             | <i>Oxalis/Latifoliolatae</i> | L. Dreyer 639                  | <b><u>EU436978</u></b> | <b><u>AJ582356</u></b> |

|  |                          |                    |                        |                        |
|--|--------------------------|--------------------|------------------------|------------------------|
| <i>Oxalis urbaniana</i><br>Schltr.           | <i>Oxalis/Angustatae</i> | Bytebier 2015      | <b><u>EU436999</u></b> | <b><u>AJ582362</u></b> |
| <i>Oxalis versicolor</i> L.                  | <i>Oxalis/Angustatae</i> | K. Oberlander 0034 | <b><u>EU437006</u></b> | <b><u>AJ582363</u></b> |
| <i>Oxalis viscosa</i><br>E. Mey. ex<br>Sond. | <i>Oxalis/Angustatae</i> | L. Dreyer 642      | <b><u>EU436949</u></b> | <b><u>AJ582364</u></b> |
| <i>Oxalis xantha</i><br>Salter               | <i>Oxalis/Angustatae</i> | L. Dreyer 671      | <b><u>EU436980</u></b> | <b><u>AJ582365</u></b> |

Chapter 5: *Oxalis saltusbelli*: a new *Oxalis* (Oxalidaceae) species from the Oorlogskloof Nature Reserve, Nieuwoudtville, South Africa

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**Abstract**

In South Africa, the genus *Oxalis* includes 200 species inhabiting a wide range of habitats particularly in the winter rainfall areas of the western and southern Cape.

Many species are morphologically extremely plastic, which makes species identification and description of new species challenging based solely on morphological criteria. Here we report the discovery of a new species, *Oxalis saltusbelli* Dreyer & Roets, from the Cape Floristic Region of South Africa.

Placement of this taxon within the genus was based on DNA sequence-based analyses of the Internal Transcribed Spacer (ITS) region and morphological comparisons with related species. The new species is currently only known from the Oorlogskloof Nature Reserve. It has sticky bulb tunics, multifoliate leaves, with variable leaflet shapes ranging from oblong to linear, depending on age, concave petioles and lilac flowers. This study brings the number of *Oxalis* species in South Africa to 201 and highlights the urgent need for molecular and morphological re-evaluation of all taxa in this morphologically and ecologically diverse plant lineage.

Key words: Oxalidaceae, *Oxalis*, new species, phylogeny, South Africa, taxonomy

## Introduction

*Oxalis* is represented by 200 species in southern Africa (Salter, 1944, Ornduff, 1973, Oliver, 1993, Williamson, 1999, Kumwenda et al., 2004, Manning & Goldblatt, 2008), of which 120 occur within the Cape Floristic Region (CFR) (Goldblatt & Manning, 2000). Within southern Africa, species in this morphologically and ecologically diverse genus are characterized by having true bulbs that bear seasonal leaves and flowers at the end of a subterranean stem. Most CFR species flower during the wetter and cooler winter months. Within the CFR, *Oxalis* species occur in both Fynbos and Succulent Karoo vegetation, where they occupy habitats ranging from extremely xeric to aquatic (in seasonal pools) across the CFR. As in other heterostylous taxa, flower structure is conservative (Barrett, 1992). Many species also display flower color-polymorphism, making it nearly impossible to distinguish species using floral morphology alone.

The southern African members of the genus were last revised by Salter (1944), with more recent work confined to descriptions of individual species (Salter, 1944, Ornduff, 1973, Oliver, 1993, Williamson, 1999, Kumwenda et al., 2004, Manning & Goldblatt, 2008) and some minor taxonomic changes (Bayer, 1991). It is generally recognized that the current morphological classification is artificial. This can largely be ascribed to considerable vegetative plasticity in the genus. In addition to many morphologically narrowly-defined species, the current taxonomy also includes numerous species complexes that display considerable morphological variation (Salter, 1944). This is, for example, illustrated by the eleven species formally included in section *Angustatae* subsection *Pardales* by Salter (1944). These were recently synonymized under *Oxalis pardalis* Sond. s. l. (Bayer, 1993), despite considerable variation in morphology across this complex. Other well-known, variable and problematic *Oxalis* species complexes include *O. flava* L., *O. purpurea* L., *O. obtusa* Jacq. and *O. hirta* L. The extreme morphological plasticity in *Oxalis* renders the identification and demarcation of new species among southern African members difficult. It is thus often problematic to discern whether a morphologically unique form does, in fact, represent a new taxon, or whether it is merely a morphological variant of an existing taxon.

In order to revise the systematics and taxonomy of *Oxalis*, we are in the process of completing a DNA-based phylogenetic reconstruction of southern African species. Preliminary results of this study (Oberlander et al., 2004) have enabled us to re-evaluate the significance of several morphological traits used in the taxonomic revision of Salter (1944). These results have also proposed unexpected new relationships between species previously thought to be only distantly related (Salter, 1944). One such example is the *O. tomentosa* alliance, a well-supported lineage (bootstrap support of 92%; Oberlander et al., 2004) including the species *O. oligophylla* Salter (section *Angustatae* subsection *Lineares*; Salter, 1944), *O. hygrophila* Dreyer (section *Latifoliolatae*; Kumwenda et al. 2004) and *O. tomentosa* L. (section *Angustatae* subsection *Multifoliolatae*; Salter 1944). Recent research has added *O. palmifrons* Salter (section *Angustatae* subsection *Multifoliolatae*; Salter 1944) to this alliance. The current taxonomic spread of this alliance is reflected in its very diverse morphology, rendering it hard to identify shared morphological traits between these species. It is within this context that a non-flowering specimen collected from the Oorlogskloof Nature Reserve near Nieuwoudtville, Northern Cape, during July 2007 raised considerable interest. This locality was revisited during June 2008, and flowering and fruiting individuals were obtained.

In this study we evaluate the position of this apparent new taxon based on both DNA and morphological evidence. We also demonstrate how the new species displays a unique combination of characters expressed in other species in the *O. tomentosa* alliance.

## **Materials and Methods**

### *Morphological assessment*

The morphology of the newly collected specimens was studied and compared to fresh material of *O. hygrophila*, *O. oligophylla*, *O. palmifrons*, and *O. tomentosa* obtained from the field as well as the *Oxalis* living collection housed in the Stellenbosch University Botanical Garden. This was supplemented by herbarium material from Stellenbosch University (STEU, Stellenbosch, South Africa), Compton (NBG, SANBI, South Africa) and Bolus (BOL, Cape Town) herbaria (Table 1). Both

morphological and ecological traits of these species were included in comparative studies. Pollen grains were studied with the aid of a Nikon YS100 light microscope.

#### *DNA sequencing and molecular analysis*

Phylogenetic reconstruction was based on DNA sequence data obtained from the nuclear Internal Transcribed Spacer region (ITS). DNA extraction and sequencing were performed at the DNA sequencing facility of Stellenbosch University. Following standard cell lysis procedures, DNA extraction used the NucleoSpin (R) 96 Plant genomic DNA extraction kit (Macherey-Nagel). The protocol was performed on a Genesis 200RMP liquid handler (Tecan) using the conditions recommended by the kit manufacturers. PCR, sequencing, contig creation and alignment of the sequence data followed Oberlander et al. (2008). Due to the uncertain placement of the *O. tomentosa* alliance within the context of the southern African lineage, a variety of taxa were included as outgroups (Table 1). All generated sequences were submitted to the NCBI's GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>) (Table 1). Parsimony analyses were conducted in PAUP\* v4.0b10 (Swofford, 2003), using branch and bound searches to find all most parsimonious trees, saving all trees. Support levels for nodes were assessed using nonparametric bootstrap (10 000 replicates) using branch and bound searches. Bayesian Inference was conducted on the same data set using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) under the model of sequence evolution as chosen in MrModeltest v2.2 (Nylander, 2004). Five million generations in two separate analyses were run in order to ensure adequate sampling of the posterior distribution.

#### *Geographical distribution and habitat preferences*

Species geographic distributions were obtained from our own collections, Salter (1944), the National Herbarium Pretoria (PRE) Computerised Information Service database (PRECIS, <http://www.sanbi.org/information/databases.htm>) and the listed herbaria (Table 1).

## **Results**

#### *Morphological assessment*

Morphological data suggested that the newly collected Oorlogskloof specimens are related to the *O. tomentosa* alliance. Morphological characters shared by species in this lineage include their acaulescent habit, sticky blackish-brown bulb tunics, and mostly white flowers. With the exception of *O. tomentosa*, all species are sparse flowerers.

Numerous morphological characters collectively differentiate the newly collected taxon from other species in the *O. tomentosa* alliance (Table 2). Unique diagnostic characters of this taxon include adaxially concave petioles, the presence of short petiolules on all leaflets and its lilac colored flowers.

#### *DNA sequencing and molecular analysis*

The final alignment featured sixteen taxa and 785 characters, with gaps coded as missing data. Of these characters, 46 (6 %) were parsimony-informative. Parsimony branch and bound searches yielded four trees of length 171. The topology of the *O. tomentosa* alliance was identical in all trees. No tree resolved the strongly-supported trichotomy of the newly collected taxon, *O. tomentosa* and *O. palmifrons* (Fig. 1).

Bayesian Inference was conducted under the GTR +  $\Gamma$  model of sequence evolution, as selected by MrModeltest. Both independent runs were deemed to have converged on a stable posterior distribution, and the first 1000 sampled generations were discarded as burnin.

Both parsimony and Bayesian Inference show identical topologies for the *O. tomentosa* alliance. The newly collected taxon is located in a trichotomy with *O. palmifrons* and *O. tomentosa*. The other two member species of the alliance are strongly supported as sister to these three taxa (Fig. 1).

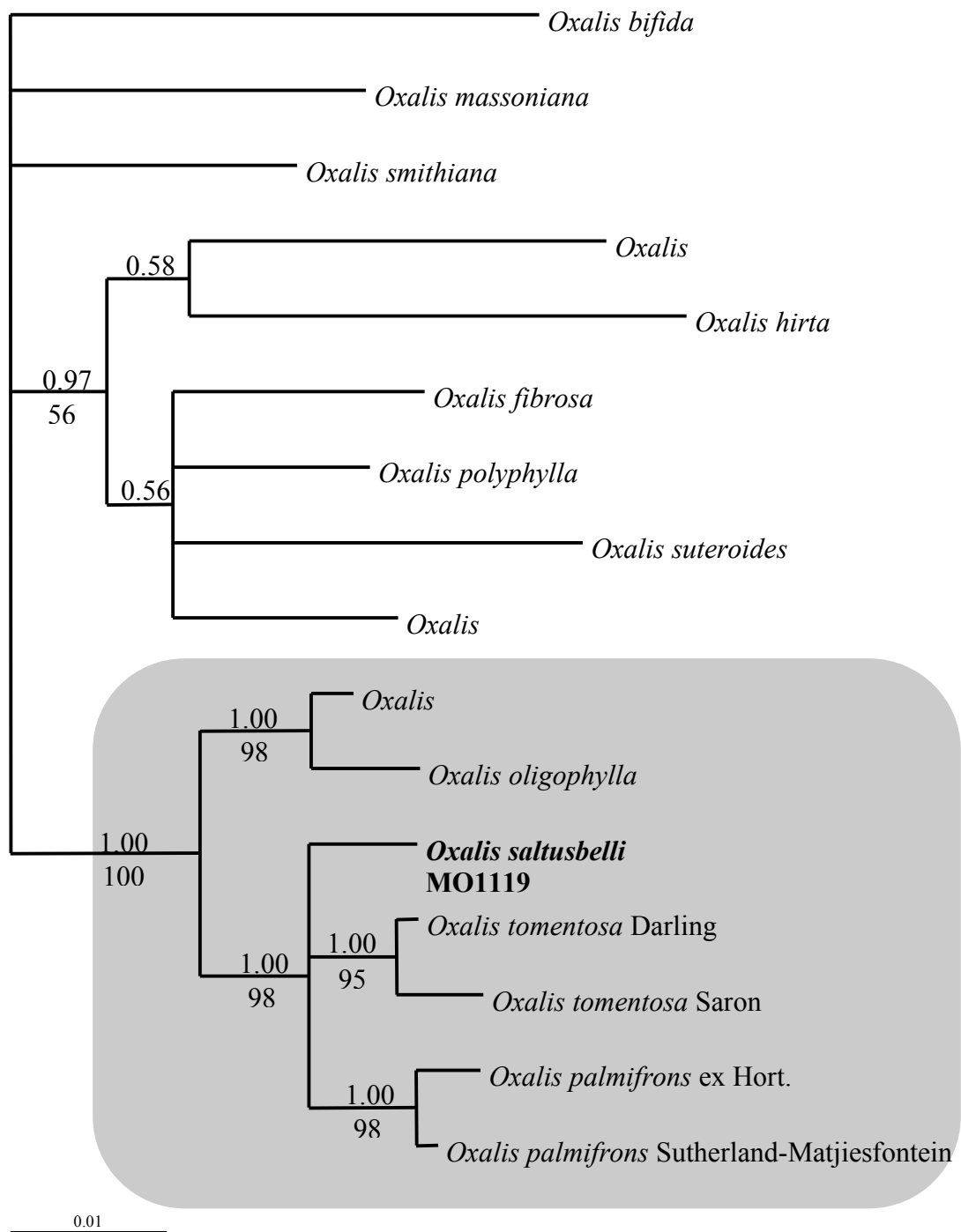


Fig. 1. Bayesian majority-rule consensus tree of the *O. tomentosa* alliance and outgroups. Branch lengths are average number of changes per site. Numbers above branches are Bayesian posterior probability values; numbers below are parsimony bootstrap. The *O. tomentosa* alliance is indicated by grey shading. The new species is bolded.

### *Geographical distribution and habitat preferences*

Biogeographical data of species within the *O. tomentosa* alliance are depicted in Fig. 2. Both *O. tomentosa* and *O. palmifrons* are restricted to shale substrates, with the former species distributed in the low-lying areas of the extreme South-western Cape, while the latter is confined to the Succulent Karoo from Matjiesfontein to the Sutherland district. Both species grow in exposed, sunny positions at low altitudes. *Oxalis hygrophila*, *O. oligophylla* and the newly collected taxon are narrowly endemic sandstone specialists respectively restricted to the Pakhuis Pass, Giftberg and the Bokkeveld Plateau. They all occur at higher elevations of between 400 to 500 m above sea level. *Oxalis hygrophila* prefers seasonally moist seepage bands in full sun, while both *O. oligophylla* and *O. saltusbelli* are closely associated with rocky habitats, where they often occur in shady crevices below rocks.

### **Taxonomy**

Morphological data indicate that the newly collected taxon represents a distinct species, and molecular data confirms a close affinity with *O. palmifrons* and *O. tomentosa* in the *O. tomentosa* alliance. It is thus newly described as follows:

***Oxalis saltusbelli*** Dreyer & Roets, sp. nov. Figs. 3 and 4

Bulbi tunicis viscidis atrobrunneis foliolis valde heterophyllis petiolis adaxiale concavis floribus pallide lilacinis distinguitur.

TYPE. —3119 (Calvinia): Northern Cape Province, Distr., Oorlogskloof Nature Reserve, along Rock Pigeon hiking trail, ca. 675 m, (-CA), 13-06-2008, Dreyer 837 (STEU, holotype; BOL, NBG isotypes).

Geophyte, 10–250 mm tall, aggregated into clumps. *Bulb* obovate to globose, with pointed to bent, narrow apex, 10–60 mm long, tunics blackish-brown, sticky, indumentum densely glandular hairy when young, glabrescent with age. *Rhizome* vertical, up to 200 mm long, with masses of adventitious roots extending from upper part, upper rhizome nodes with prominent straw-coloured, amplexicaul, broadly triangular scales, abaxially covered in long, soft hairs; sheath present, light brown, glabrescent, glandular hairy along entire length, densely villous at apex. *Above-ground stem* absent. *Leaves* 3–15 per plant, smaller, robust, semi-succulent and

reddish green in sun plants; larger, more slender, herbaceous and deep green in shade plants; *petioles* adaxially concave to almost U-shaped, 50–160 mm, sparsely covered with long, soft hairs over its entire length; *leaflets* (6) 8–11 (13), very shortly (0.3–0.5 mm) petiolulate, of unequal length, radially arranged around swollen pulvinus, linear and conduplicate in sun plants, oblong to linear and non-conduplicate in shade plants, abaxially purple, elliptical to oblong in young plants, 10–90 X 0.5–3 mm, apex shallowly emarginate, base attenuate, adaxially glabrous, abaxially sparsely covered with long, soft, hairs when young, glabrescent with scattered hairs restricted to leaflet margin and mid vein in mature leaves, with two rounded to oblong, reddish-orange apical calli abaxially. *Peduncle* 1-flowered, 75–100 mm long, erect, reddish-green, evenly covered with long soft hairs, bracts 2 on upper part of peduncle, alternate, linear to filiform, up to 0.6 mm long. *Sepals* 5.0 X 1.0–1.5 mm, lanceolate-elliptical, apex acute, abaxially covered with long, soft hairs, green becoming reddish, especially along margins. *Corolla* 18.0–20.0 mm long, light lilac with funnel-shaped yellow tube, tube 8.5–9 mm long, petals obovate to broadly spatulate, abaxially with a few long soft hairs on outer margins, ecallose. *Stamens* in 3 series, 2 series per plant, the shortest level 1.5–2 mm, the middle level 2.5–3.0 mm and the longest level 6.2–6.0 mm long, all basally joined for 1.0–1.5 mm; anthers oblong, yellow; filaments white, glandular-hairy over entire length; filament teeth greenish-white, 0.5–2 mm long, apically rounded to obtuse and curved outward. Ovary 1.2–1.5 mm long, ovoid, densely pilose, 5-locules each 1-ovuled; styles 5, separate, in three series with one series per plant, shortest level 1.5 mm long, middle level 3.0 mm long, longest level 6.0 mm long, erect, densely villous; stigmas green, capitate. *Fruit* a globular 5-locular capsule. *Seeds* without endosperm. Pollen tricolpate, triangularly rounded in equatorial view, spherical in polar view, tectum finely reticulate.

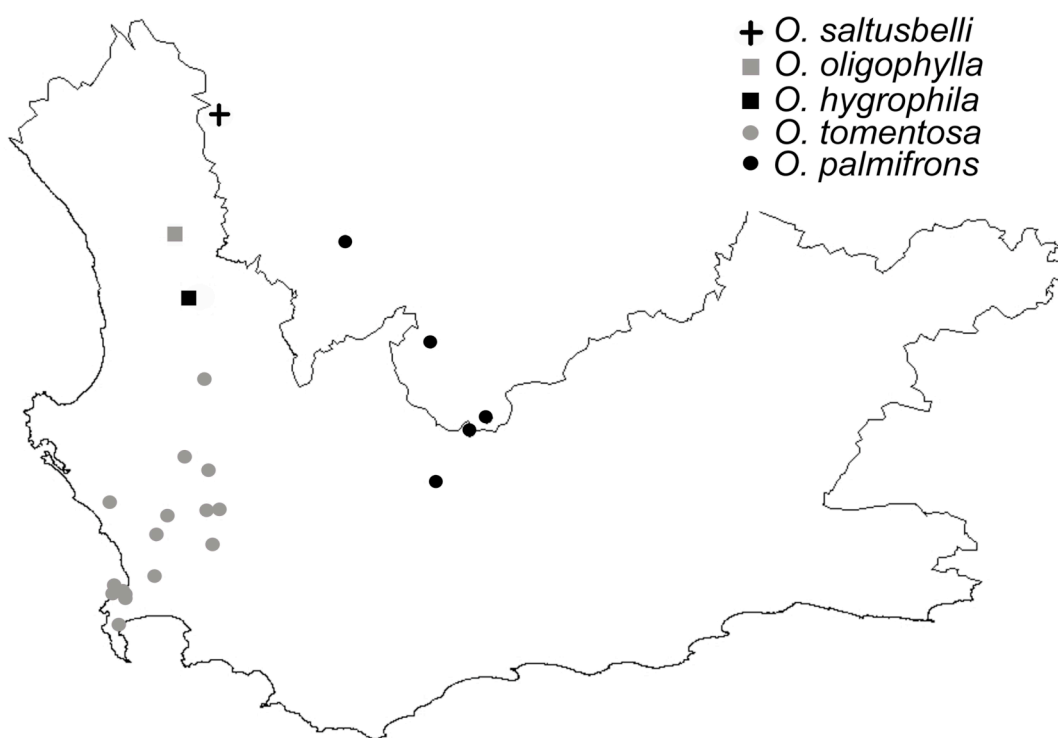


Fig. 2. Geographical distribution of species in the *Oxalis tomentosa* alliance.

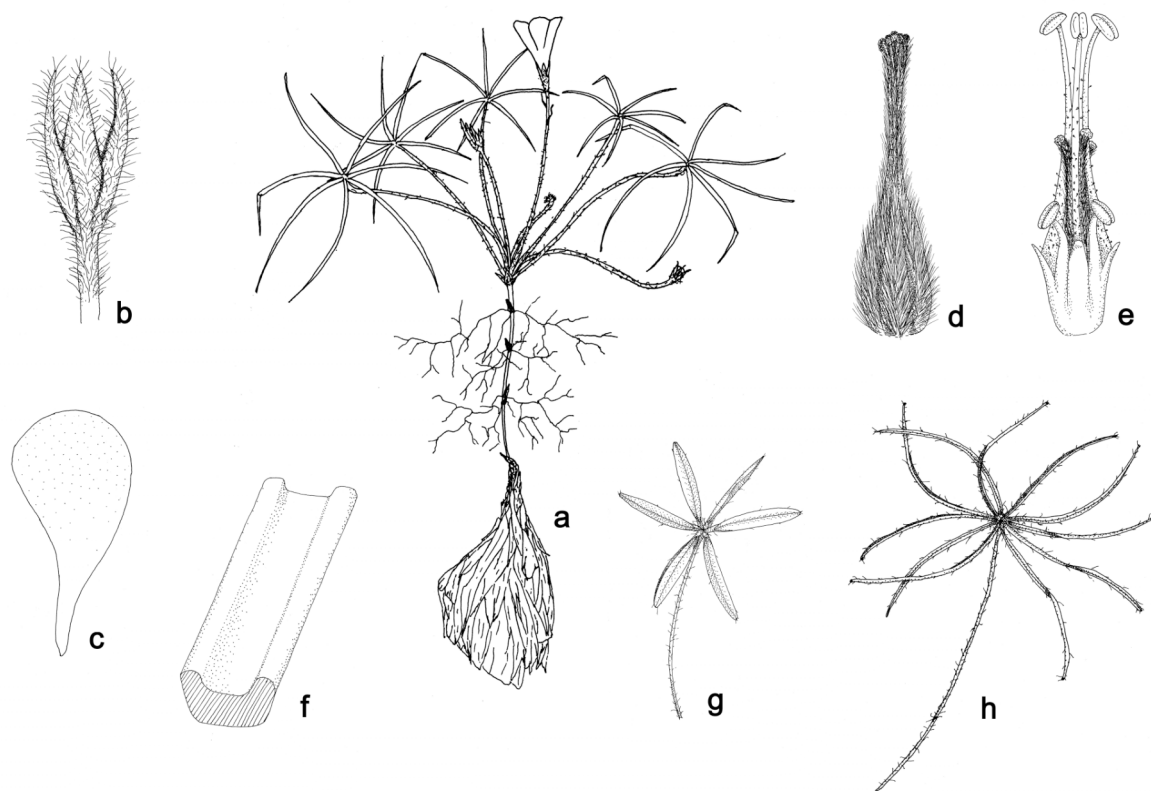


Fig 3. Line diagram of *O. saltusbelli*. a. Mature plant. b. calyx. c. petal. d. gynoecium. e. androecium and gynoecium. f. cross-section of petiole. g. immature leaf. h. mature leaf.

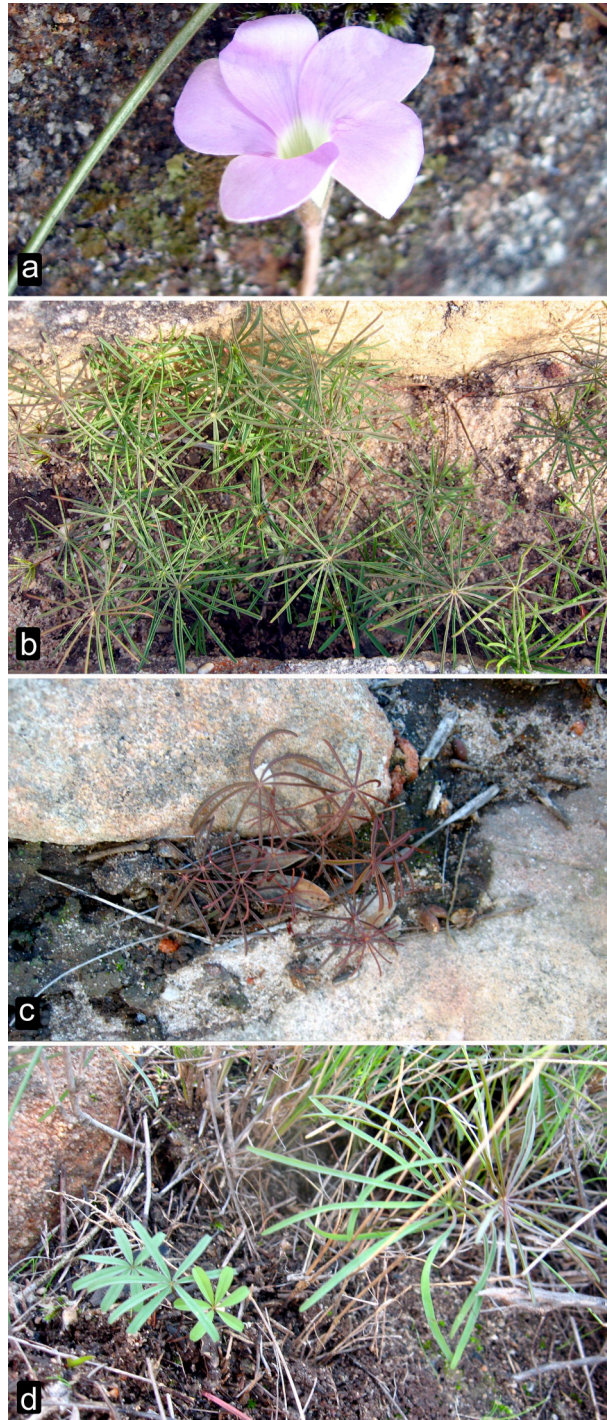


Fig. 4. *Oxalis saltusbelli*. a. flower. b. typical habit. c. mature full sun form. d. juvenile form (left) and mature shade form (right).

## Distribution

*O. saltusbelli* is confined to the Oorlogskloof on the Bokkeveld Escarpment, where it grows along the Rock Pigeon hiking trail and surrounding outcrops (figs. 2, 4b-d). It is found in coarse sand in both exposed, sunny habitats and in moist, shady crevices between sandstone rocks. Plants are found in scattered clusters. Plants flower in June, but flowering is not prolific, with only a few flowers per plant and flowering plants per population.

## Notes

The specific epithet refers to the restricted distribution of this species to the Oorlogskloof National Park, Nieuwoudtville plateau, Northern Cape Province. Superficially this species (especially the sun forms) bears a very strong resemblance to the sympatric species *O. flava* in general appearance. Closer examination reveals obvious differences between these two species, and they remain distinct in sympatry. *Oxalis saltusbelli* lacks the distinct basal petiolar articulations and papery bracts at the leaf bases that characterize *O. flava*. The sticky, blackish-brown tunics of *O. saltusbelli* are also very different to the papery, pale brown bulb tunics produced by *O. flava*.

## Discussion

Both the morphological attributes (Table 2) and molecular phylogenetic placement of *O. saltusbelli* confirm a close affinity of this taxon with the *O. tomentosa* alliance, particularly with *O. tomentosa* and *O. palmifrons*. It differs from *O. tomentosa* in having a less hairy indumentum and a grooved petiole. It differs from *O. palmifrons* in having fewer leaves and a more terete petiole. The new species differs from both in the lilac flowers and more linear leaflets, as well as distribution range and substrate preference. *Oxalis saltusbelli* is morphologically most similar to *O. tomentosa* and *O. palmifrons* in terms of leaflet number and arrangement, rhizome length and bulb shape and size. Like all members of this lineage, *O. saltusbelli* produces very distinct heterophyllous juvenile forms which bear a strong morphological similarity to mature plants of *O. tomentosa*. The habitat and substrate of the new species are comparable to both *O. oligophylla* and *O. hygrophylla*. Unique, diagnostic characters of *O.*

*saltusbelli* include the adaxially concave to almost U-shaped petiole and the lilac flowers.

As the current infrageneric classification is artificial, we refrain from placing this taxon in any currently-recognised section. Future classification systems should nevertheless reflect this species close relationship with members of the *O. tomentosa* alliance.

### **Acknowledgements**

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Table 1. Specimens used in morphological and molecular comparisons.

| Species               | Locality   | Herbarium<br>(Reference nr.) | Collector                 | GenBank<br>accession<br>Number |
|-----------------------|--|------------------------------|---------------------------|--------------------------------|
| <i>O. hygrophila</i>  | Groot Kliphuis, Pakhuis Pass<br>near Clanwilliam | STEU (MO230)                 | Dreyer &<br>Kumwenda 1    | <u><b>EU437024</b></u>         |
| ”                     | Groot Kliphuis, Pakhuis Pass<br>near Clanwilliam | BOL                          | Leipoldt s.n.             | -                              |
| ”                     | Groot Kliphuis, Pakhuis Pass<br>near Clanwilliam | BOL                          | Bolus s.n. BH<br>9400     | -                              |
| ”                     | Western slope of Gifberg                         | BOL                          | Salter 7266               | -                              |
| <i>O. oligophylla</i> | Gifberg Pass                                     | STEU                         | Zietsman &<br>Siqueira 42 | -                              |
| ”                     | Gifberg Pass                                     | STEU (MO293)                 | Dreyer s.n.               | <u><b>EU437023</b></u>         |
| <i>O. palmifrons</i>  | Tweedside  | NBG                          | Salter 6053               | -                              |
| ”                     | Wolwerivier, Ceres                               | BOL                          | Salter 6063               | -                              |
| ”                     | South of Middelpos                               | STEU                         | Suda 113                  | -                              |
| ”                     | Ex. Hort.  | STEU (MO403)                 | Holmes s.n.               | <u><b>EU437021</b></u>         |
| ”                     | between Sutherland and<br>Matjiesfontein         | STEU (MO824)                 | Oberlander s.n.           | <u><b>FJ211166</b></u>         |
| <i>O. saltusbelli</i> | Oorlogskloof Nature Reserve,<br>Nieuwoudtville   | STEU (MO1000)                | Dreyer & Roets<br>25      | -                              |
| ”                     | Oorlogskloof Nature Reserve,<br>Nieuwoudtville   | STEU (MO1119)                | Dreyer 837                | <u><b>FJ211167</b></u>         |
| <i>O. tomentosa</i>   | Road to Saron, 500 m off R44                     | STEU (MO363)                 | Oberlander 80             | <u><b>FJ211168</b></u>         |
| ”                     | Darling  | STEU (MO62)                  | Te Roller 10              | <u><b>EU437022</b></u>         |
| ”                     | Cape Peninsula                                   | BOL                          | Salter 508                | -                              |

|                      |  |              |                 |                        |
|----------------------|--|--------------|-----------------|------------------------|
| ”                    | Malmesbury                                     | BOL          | Salter 2164     | -                      |
| ”                    | Elandsberg Nature Reserve                      | STEU         | Dreyer, s.n.    | -                      |
| <i>O. bifida</i>     | Jonkershoek                                    | STEU (MO19)  | Dreyer 608      | <b><u>EU437017</u></b> |
| <i>O. massoniana</i> | Ex Hort.                                       | STEU (MO399) | Van Wijk 2968   | <b><u>EU437015</u></b> |
| <i>O. smithiana</i>  | Ex Hort.                                       | STEU (MO322) | Bellstedt 699   | <b><u>EU436964</u></b> |
| <i>O. eckloniana</i> | Theronsberg Pass, Ceres                        | STEU (MO39)  | Dreyer 628      | <b><u>EU437025</u></b> |
| <i>O. hirta</i>      | Piekenierskloof Pass, Citrusdal                | STEU (MO77)  | Dreyer 646      | <b><u>EU436971</u></b> |
| <i>O. fibrosa</i>    | Ladysmith                                      | STEU (MO332) | Oberlander 51   | <b><u>EU436958</u></b> |
| <i>O. polyphylla</i> | Silvermine, Cape Peninsula                     | STEU (MO47)  | Ciliers s.n.    | <b><u>EU437010</u></b> |
| <i>O. engleriana</i> | Between Caledon and<br>Villiersdorp            | STEU (MO195) | Oberlander 10   | <b><u>EU436961</u></b> |
| <i>O. suteroides</i> | Between van Rhyn’s dorp and<br>van Rhyn’s Pass | STEU (MO527) | Oberlander s.n. | <b><u>EU436963</u></b> |

Table 2. Comparison of the morphological characters of species within the *O. tomentosa* alliance.

| Character                      | <i>O. hygrophila</i>   | <i>O. oligophylla</i>   | <i>O. palmifrons</i>                    | <i>O. saltusbelli</i>   | <i>O. tomentosa</i>            |
|--------------------------------|------------------------|---|---|---|--------------------------------|
| Bulb shape                     | ovate with acute apex  | oval to oval-oblong, often deformed                                       | ovoid with attenuate-acicular apex      | obovate to globose, with pointed apex   | oblong-ovoid with pointed apex |
| Bulb length                    | 9–11 mm                | 15–30 mm  | up to 40 mm                             | 10–60 mm  | 15–20 mm                       |
| Bulb tunics colour and texture | light brown, thin      | black, sticky, hard   | dark brown, rather hard                 | blackish-brown, sticky, rather hard   | greyish brown                  |
| Rhizome                        | not enclosed in sheath | not enclosed in sheath, with adventitious roots extending from upper part | not enclosed in sheath                  | enclosed in tunic sheath, with masses of adventitious roots extending from upper part | enclosed in tunic sheath       |
| Leaf number                    | 10–12                  | 1–2(3)  | very numerous                           | 10–12 (20)  | 5–15                           |
| Leaf indumentum                | glabrous               | adaxially glabrous, abaxially sparsely glandular pilose                   | adaxially glabrous, abaxially pubescent | sparsely covered with long, soft hairs  | pubescent on both surfaces     |
| Petiole                        | 23–56 mm               | 60–120 mm   | up to 20 mm                             | 50–160 mm   | up to 50 mm                    |

|                       |   |                                       |                      |  |                                    |
|-----------------------|---|---------------------------------------|----------------------|--|------------------------------------|
| length                |   |                                       |                      |  |                                    |
| Petiole cross-section | round   | round                                 | compressed           | adaxially concave                                  | round to flattened                 |
| Leaflet number        | 3   | 3                                     | 20–29                | (6) 8–11 (13)                                      | 10–20                              |
| Leaflet arrangement   | arranged at 90° angles to one another   | arranged at 90° angles to one another | palmate              | peltately arranged around swollen pulvinus         | peltately spreading                |
| Leaflet shape         | narrowly elliptic to elliptic, conduplicate   | narrow linear, conduplicate           | oblong, conduplicate | linear, oblong to elliptical, open to conduplicate | oblong-cuneate, often conduplicate |
| Leaflet calli         | 2, apical, round to oblong, sometime with 1–6 less conspicuous calli along upper margin | absent                                | absent               | 2, apical, round to oblong                         | absent                             |
| Petiolule             | sometimes present in medial leaflet   | absent                                | absent               | present, 0.3–0.5 mm                                | absent                             |
| Peduncle length       | 25–60 mm  | 120–160 mm                            | 5–15 mm              | 75–100 mm  | 50–100 mm                          |

|                    |         |        |        |            |        |
|--------------------|---------|--------|--------|------------|--------|
| Flower<br>colour   | white   | white  | white  | pale lilac | white  |
| Calli on<br>petals | present | absent | absent | absent     | absent |

Chapter 6: An unusual new species of *Oxalis* (Oxalidaceae) from the Knersvlakte,  
South Africa

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**Abstract**

*Oxalis ericifolia* differs from all other southern African *Oxalis* in having massive papillate protrusions on the adaxial leaflet epidermis, broadly swollen peduncular articulations, a wine-red androecium and an extremely short petal claw, less than one fifth the length of the total petal. Other unusual features include the red colour of the claw and the substrate-induced flattening of the bulbs. It was discovered in a unique locality from which two other endemic *Oxalis* species have been described. A phylogenetic analysis of the nuclear ITS region places the new species in the informally recognized *Oxalis flava* clade.

Key words: new species, Oxalidaceae, *Oxalis ericifolia*, palynology, phylogeny,  
South Africa, taxonomy

## **Introduction**

The taxonomy of southern African *Oxalis* is complicated by the large number of species present in the region (*ca.* 200), and by the presence of many species complexes, which are inconsistently subdivided into varieties and forms (Salter, 1944). In addition, many morphological characters are environmentally plastic, resulting in a single species displaying considerable morphological variation between different microhabitats. These problems complicate the recognition of potentially new taxa. Given the current dearth of knowledge on ecological or reproductive characters of the genus, new taxa are most confidently recognized on the basis of an assemblage of unique morphological characters.

A new taxon collected in June 2008 presented a suite of morphological characters unobserved anywhere else in southern African *Oxalis*. At first glance the filiform petioles and translucent white adaxial leaflet colour were so dissimilar to any known described species that non-flowering individuals were not immediately recognizable as a member of the genus. Further investigation showed that Salter (1944) had indeed collected this species (Salter 2498), but did not describe it due to a lack of flowering material and the sporadic seasonal above-ground appearance of the plants. Collection of flowering material confirmed that this species is new, and consequently we describe it here as *Oxalis ericifolia* Oberlander & Dreyer.

## **Materials and Methods**

### *Morphological assessment*

The morphology of the newly collected specimens was studied and compared to all described species (Salter, 1944; Ornduff, 1973; Oliver, 1993; Williamson, 1999;

Kumwenda et al., 2004) as well as with all species in the *Oxalis* living collection housed in the Stellenbosch University Botanical Garden. In addition material of the newly-collected specimens was compared to all known and unidentified *Oxalis* species housed in the Stellenbosch University (STEU), Compton (NBG) and Bolus (BOL) herbaria.

### *Palynology*

Fresh pollen grains were mounted in water for immediate study with the aid of a Nikon YS100 light microscope (LM). Pollen samples were also prepared for Scanning Electron Microscope (SEM) analyses by mounting them onto aluminium stubs using double-sided carbon tape. They were sputter-coated with gold-palladium and studied using a Leo 1430 VP7 scanning electron microscope (SEM).

### *DNA sequencing and molecular analysis*

To determine potential systematic relationships, we performed phylogenetic inference of DNA sequence data obtained from the nuclear Internal Transcribed Spacer region (ITS). DNA extraction and sequencing were performed at the DNA sequencing facility of Stellenbosch University. Following standard cell lysis procedures, DNA extraction used the NucleoSpin (R) 96 Plant genomic DNA extraction kit (Macherey-Nagel). The protocol was performed on a Genesis 200RMP liquid handler (Tecan) using the conditions recommended by the kit manufacturers. PCR, sequencing, contig creation and alignment of the sequence data followed Oberlander et al. (unpublished). Preliminary ITS data suggested a close relationship to the *Oxalis flava* clade (Oberlander et al., unpublished). The ITS sequences of all species within this clade were included in an analysis to more precisely place this new taxon (Table 1). All

generated sequences were submitted to the NCBI's GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>) (Table 1). Parsimony analyses were conducted in PAUP\* v4.0b10 (Swofford, 2003), using heuristic searches to find all most parsimonious trees. Starting trees were generated using random taxon addition, and 1 000 TBR branch-swapping replicates, saving ten trees per replicate, were implemented. Support levels for nodes were assessed using nonparametric bootstrap (10 000 replicates) using heuristic searches. Bayesian Inference was conducted on the same data set using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) under the model of sequence evolution as chosen in MrModeltest v2.2 (Nylander, 2004). Five million generations in two separate analyses were run in order to ensure adequate sampling of the posterior distribution.

## Results

### *Morphological assessment*

The new taxon displays many characters not otherwise found in southern African *Oxalis* (Fig. 1). Most distinctively, the petal claw of the flower is much shorter than in any other regional *Oxalis*, never more than one fifth the length of the petal and often much shorter. Although there are other southern African *Oxalis* species with campanulate flowers, this is the only taxon to achieve this by contracting the claw, instead of re-orienting the entire petal. The claw is also wine-red, a character shared by other *Oxalis* such as *O. callosa* Knuth and *O. oculifera* E.G.H. Oliver. The peduncles of this new taxon are also unique among southern African *Oxalis* species. The basal articulation of the peduncle is so massively swollen that it appears almost gall-like in comparison to the rest of the peduncle. Unlike any other known species, the three leaflets of this species display very large papillae on the adaxial leaflet

epidermis. The leaflet margins are rolled upward along the entire length of the leaflet, giving the leaves a distinct linear-ericoid appearance. This makes the plants inconspicuous when not in flower. The shiny white adaxial papillae are so large and dense that they are visible with the naked eye between the rolled leaflet margins. Although many known *Oxalis* species produce conduplicate leaves, no other species has such distinctly involute margins. The new taxon also has wine-red filaments, which is another unusual character for the genus.

#### *DNA sequencing and molecular analysis*

Phylogenetic sequence analysis of the new taxon is somewhat equivocal (Fig. 2). Although both parsimony and Bayesian inference agree on the close relationship of this taxon with the *O. flava* clade, parsimony does not support any affinity with the included taxa. Bayesian inference strongly supports a clade containing the new taxon, *O. adspersa* Eckl. & Zeyh., *O. aurea* Schltr. and *O. uliginosa* Schltr.. Although Bayesian methods do tend to overinflate support measures (Cummings et al., 2003), it is known that model-based approaches utilize DNA sequence information more efficiently, and thus provide a better estimate of phylogenetic placement. Moreover, the new taxon is not particularly similar to any of these three species.

#### *Palynology*

##### LM

Pollen grains are tricolpate, isopolar and triangularly rounded in polar view and spherical to oblate in equatorial view. The apocolpium is reduced, and colpus ends are obtusely rounded.



Fig. 1. *Oxalis ericifolia*. (A) Typical clustered habit. (B) side view of immature flower. (C) view of open flower showing wine-red eye at base of corolla. (D) fruit. (E) leaflet articulation. (F) close-up of the adaxial leaf surface showing papillae. (G) swollen basal articulation on peduncle.



SEM (Fig. 3)

Pollen grains are semitectate, with a coarsely reticulate, homobrochate tectum.

Lumina are irregularly angular, abruptly diminishing in size towards the colpi resulting in a distinct colpus margin with rounded lumina. The average lumina diameter is 2.9  $\mu\text{m}$ . Muri are stratified, and have an average thickness of 0.8  $\mu\text{m}$ . The nexine floor is beset with blunt intraluminary bacula, predominantly clustered along the muri. The colpus membrane is coarsely granular.

The morphology of pollen grains of this species corresponds to pollen grains of *O. tenuipes* var. *tenuipes* (Pollen type C12, Dreyer 1996). This pollen type is very similar to pollen types C10 and C11, in that all three types have open reticulate tectums with intra-luminary bacules clustered along the muri. The only consistent difference between these three pollen types is the average lumina diameter, which is larger in pollen type C12 than in the other two types (Dreyer, 1996). Dreyer (1996) concluded that species with reticulate pollen of these three types are probably closely related. Most members of the *O. flava* clade have reticulate pollen of the type C10 or C11.

### **Taxonomy**

Morphological data clearly confirm this newly collected taxon to be a distinct new species of *Oxalis*. It is thus newly described as follows:

***Oxalis ericifolia*** Oberlander & Dreyer, sp. nov. (Fig. 1 & 4)

Geophytum acaule, bulbis complanatis, petiolis tenuibus filiformibus ad filo metallico similibus, foliolis conduplicatis papillas adaxiales egregie magnas habentibus,

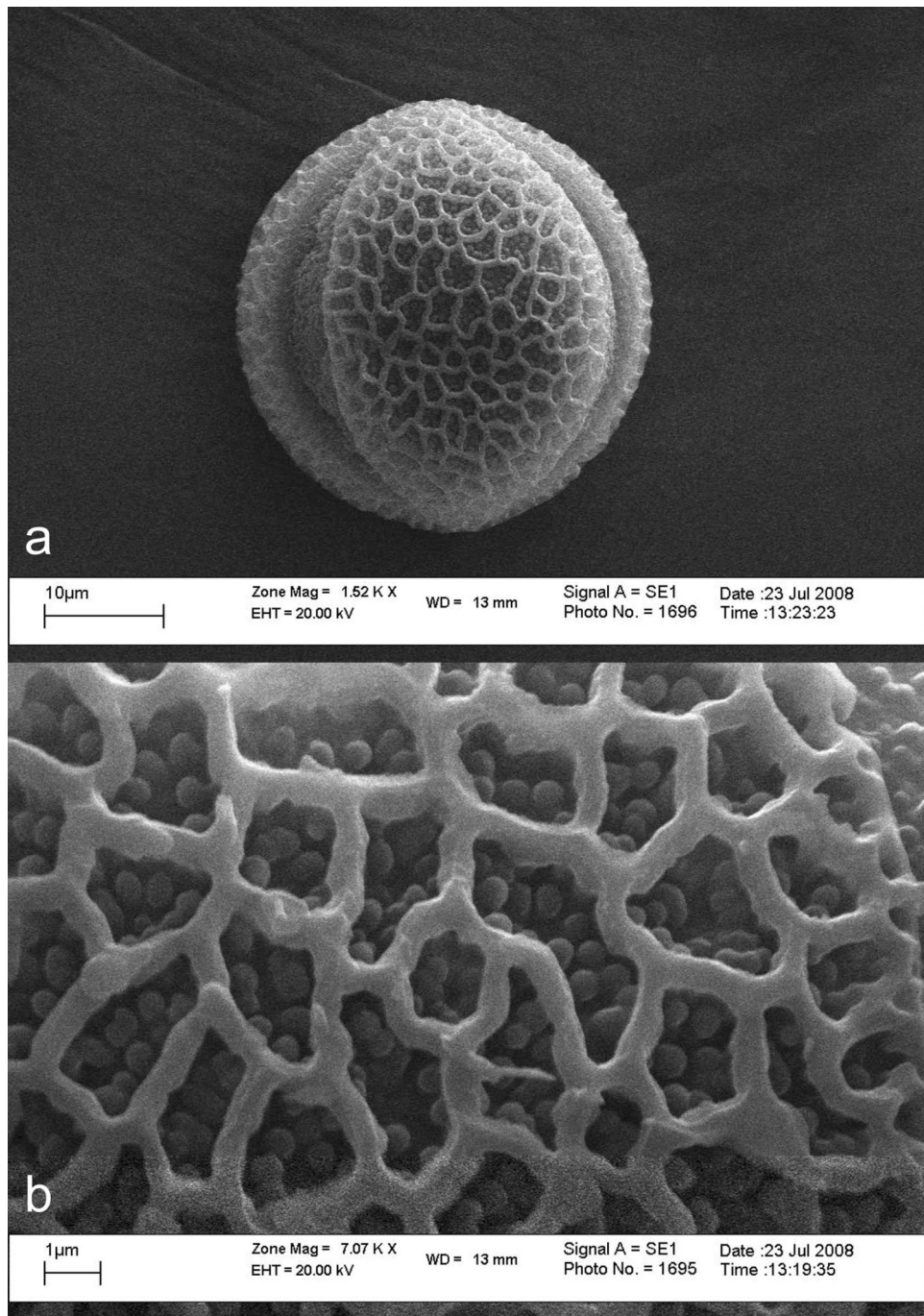


Fig. 3 SEM micrograph of *O. ericifolia* pollen. (A) Whole grain in equatorial view. (B) Mesocolpial portion of the tectum.

pedunculis sub flore crassis et articulis basalibus grandibus, floribus albis centro vinoso-rubris, campanulatis sine tubo floralis.

TYPE. —3119 (Calvinia): Western Cape Province, broken shale outcrop 40 km north east of Vanrhynsdorp on road to Nieuwoudtville, 20-06-2008, Oberlander 500 (STEU, holo.; BOL, NBG iso.).

Slender, stemless geophyte, 40–70 mm tall, clump-forming, variously covered with hyaline pluricellular hairs, glabrescent with age. *Bulbs* aggregated into complex system of old rhizomes, sheaths, adventitious roots and developing bulbs; with new years growth penetrating through remains of previous years; individual bulbs lanceolate to obovate, flattened due to growth in between layers of substrate, 10–20 mm long, tunics papery, dark brown to black, glabrous. *Rhizome* 45–160 mm long or longer, white to orange, succulent, often very thick, densely glandular pubescent towards apex, glabrescent towards bulb, densely enclosed in fibrous mass of old tunics and bulb remains, leaf bases on upper rhizome imbricated by 3–5 scales, scales sparsely ciliate. *Above-ground stem* absent. *Leaves* apically congested at tip of rhizome, 3–17 per plant, erect, all of similar height; petiole 15–30 mm long, filiform, up to 0.4 mm in diameter, rigid, wiry, red, leaves densely glandular hairy when young, becoming less so with age; leaflets 3, palmate, erect, rigid, sessile to very shortly petiolulate, involute, shortly glandular-pubescent when young, glabrescent with age, 15–28 x 2.0–2.5 mm, apex rounded to slightly emarginate, adaxial epidermis beset with cylindrical, translucent papillae. *Peduncle* 1-flowered, 35–55 mm long, slightly thicker than petioles, swollen apically, basal articulation massively swollen, almost gall-like in appearance, glabrescent with age, shiny. *Sepals* narrowly

triangular to lanceolate, 2.0–3.0 x 1.0–1.5 mm, acute, wine red, glabrescent with glandular hairs along margins; bracts 2, filiform, alternate, variable in position but mostly in middle or upper part, sometimes appressed to calyx, wine red, glabrous. *Corolla* campanulate, white with base of petals wine red, floral tube absent; petals oblanceolate, minutely clawed, 12–16 mm long, apex truncate or rounded, occasionally subacute, ecallose. *Stamens* in 3 series, 2 series per plant, shortest 2.0–3.0 mm long, middle 4.0–5.0 mm long and longest 6.0–7.0 mm long, basally adnate; anthers oblong, yellow; filaments wine red, covered with red glandular hairs; filament teeth oblong to spatulate, adnate to longer filaments, obtuse, glabrous; pollen bright yellow. *Ovary* narrowly ovoid, 1.2–1.4 mm long, glabrous, 5-locular with 2–3 ovules per locule; styles 5, free, sparsely glandular hairy in distal part, in 3 series with 1 series per plant, shortest 1.5 mm long, middle 4.0 mm long, longest 7.0 mm long; stigmas yellow-green, cup-shaped, fimbriate. *Fruit* a 5-locular capsule, globular, not longer than sepals, light green. *Seeds* without endosperm. Pollen tricolporate, triangularly rounded in equatorial view, spherical in polar view, tectum coarsely reticulate with intra-luminary bacules clustered along the muri.

### **Distribution and Ecology**

*O. ericifolia* was discovered in a single locality, growing in association with *O. deserticola* Salter and *O. melanograptia* Salter. It is currently only known from this extremely rocky band on the southwestern face of a broken shale outcrop 40 km north east of Vanrhynsdorp. Despite an apparently restricted distribution range, the plants were found to be locally abundant. Plants were scattered in loose clumps across the

broken shale bands, where they grew in full sun. Individuals of all three morph types were present, and numerous individuals bore fertile capsules. *O. ericifolia* flowers in June.

### **Diagnosis and Relationships**

The specific epithet refers to the distinctly ericoid leaflets. Unlike *Erica* leaves, however, the leaflets of *O. ericifolia* are rolled adaxially, and almost completely enclose an unusually papillate adaxial epidermis. Other diagnostic characters of this species include the filiform petioles, the swollen articulation on the peduncle, the wine-red filaments and petal-bases, and the very short claw.

In terms of relationships, several characters suggest that this species belongs in an informal group of species termed the *O. flava* clade (Oberlander et al., submitted). ITS data strongly support a membership in this clade. The pollen types of *O. ericifolia* and other members of the *O. flava* clade are very similar. Another potential character that supports this relationship is the presence of uniseriate pluricellular hairs, which is very common amongst members of the *O. flava* clade. Salter (1944) suggests an affinity with *O. pulvinata* Salter, which differs in being entirely glabrous, in having multifoliolate leaves, and in the well-developed claw of the petal.

It would seem surprising that such an unusual species as *O. ericifolia* has not been described in the period between initial discovery and the present, especially given that it is sympatric with *O. deserticola* (described by Salter and Compton; 1935) and *O. melanograptia* (described by Salter and Compton; 1936), two rare species of conservation importance. However, as Salter (1944) mentions, the species appears sporadically, and can go an entire season without appearing above-ground. All three

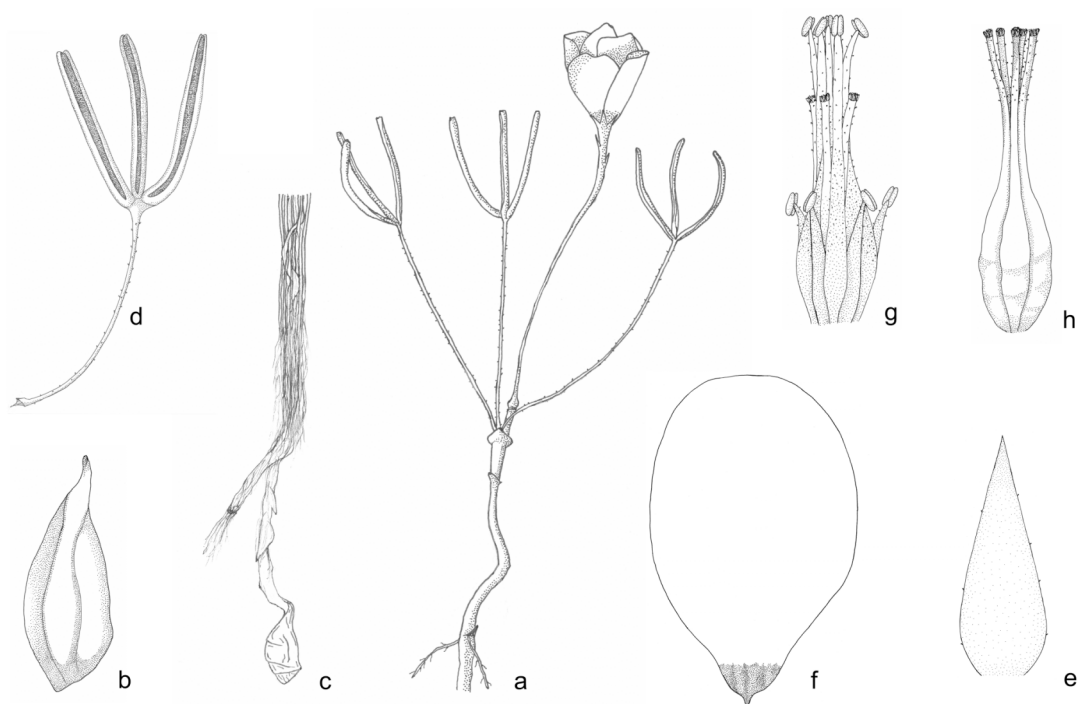


Fig 4. Line diagram of *O. ericifolia*. (A) above-ground plant and rhizome. (B) bulb. (C) young bulb and underground habit. (D) leaf. (E) sepal. (F) petal. (G) androecium and gynoecium of the mid morph. (H) gynoecium.

species are currently only known from this outcrop. Another feasible explanation is that *O. ericifolia* flowers slightly earlier than these two species, and may well have finished flowering by the time the other species are in full bloom. When not in flower the plants are inconspicuous, and hard to recognize as a member of the genus *Oxalis* without close observation.

The discovery of *O. ericifolia* brings the number of recognized southern African *Oxalis* species to 201. Due to the artificial nature of the most recent morphological classification (Salter, 1944), this species is not infra-generically placed within one of the nine currently recognized sections. Molecular phylogenetic results also did not conclusively reveal close affinities, but strongly support inclusion of this species within the *O. flava* clade. This is supported by morphological characters such as the pollen type and the presence of uniseriate, multicellular epidermal hairs. Both of these characters are almost exclusively shared by members of the *O. flava* clade.

### **Acknowledgements**

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Table 1. List of all *Oxalis* taxa included in phylogenetic analyses.

| Species  | Reference number | Genbank Accession Number |
|--|------------------|--------------------------|
| <i>Oxalis adspersa</i> E. & Z.                                       | MO66             | <a href="#">EU436936</a> |
| <i>Oxalis argillacea</i> Bolus f.                                    | MO282            | <a href="#">EU436940</a> |
| <i>Oxalis aurea</i> Schltr   | MO267            | <a href="#">EU436935</a> |
| <i>Oxalis campylorrhiza</i> Salter                                   | MO127            | <a href="#">EU436947</a> |
| <i>Oxalis cathara</i> Salter   | MO582            | <a href="#">EU436931</a> |
| <i>Oxalis</i> cf. <i>flava</i> L.                                    | MO1101           | <a href="#">FJ211169</a> |
| <i>Oxalis</i> cf. <i>canaliculata</i> manuscript species M. B. Bayer | MO583            | <a href="#">EU436946</a> |
| <i>Oxalis clavifolia</i> Sond.                                       | MO556            | <a href="#">EU436951</a> |
| <i>Oxalis comosa</i> E. Mey. ex Sond.                                | MO238            | <a href="#">EU436954</a> |
| <i>Oxalis crocea</i> Salter  | MO124            | <a href="#">EU436950</a> |
| <i>Oxalis deserticola</i> Salter                                     | MO526            | <a href="#">EU436939</a> |
| <i>Oxalis dines</i> Ornduff  | MO653            | <a href="#">EU436943</a> |
| <i>Oxalis disticha</i> Jacq.   | MO596            | <a href="#">EU436942</a> |
| <i>Oxalis dregei</i> Sond.   | MO796            | <a href="#">EU436930</a> |
| <i>Oxalis ericifolia</i> Oberlander & Dreyer                         | MO1143           | <a href="#">FJ211170</a> |
| <i>Oxalis fabaefolia</i> Jacq.                                       | MO152            | <a href="#">EU436925</a> |
| <i>Oxalis flava</i> L.   | MO25             | <a href="#">EU436924</a> |
| <i>Oxalis flava</i> L.   | MO183            | <a href="#">FJ211173</a> |
| <i>Oxalis flava</i> L.   | MO414            | <a href="#">FJ211174</a> |
| <i>Oxalis flava</i> L.   | MO576            | <a href="#">FJ211172</a> |
| <i>Oxalis flava</i> L.   | MO753            | <a href="#">FJ211175</a> |
| <i>Oxalis flaviuscula</i> Salter                                     | MO132            | <a href="#">EU436929</a> |
| <i>Oxalis furcillata</i> Salter                                      | MO228            | <a href="#">EU436952</a> |
| <i>Oxalis inconspicua</i> Salter                                     | MO138            | <a href="#">EU436944</a> |
| <i>Oxalis louisae</i> Salter   | MO139            | <a href="#">EU436928</a> |
| <i>Oxalis monophylla</i> L.  | MO7              | <a href="#">EU436927</a> |
| <i>Oxalis monophylla</i> L.  | MO60             | <a href="#">FJ211177</a> |
| <i>Oxalis monophylla</i> L.  | MO795            | <a href="#">FJ211176</a> |
| <i>Oxalis namaquana</i> Sond.  | MO144            | <a href="#">EU436941</a> |
| <i>Oxalis obtusa</i> Jacq.   | MO194            | <a href="#">EU436922</a> |
| <i>Oxalis pillansiana</i> Salter and Exell                           | MO549            | <a href="#">EU436938</a> |
| <i>Oxalis purpurea</i> L.  | MO300            | <a href="#">FJ211171</a> |
| <i>Oxalis salteri</i> L. Bolus                                       | MO280            | <a href="#">EU436932</a> |
| <i>Oxalis sonderiana</i> (O. Kuntze) Salter                          | MO140            | <a href="#">FJ211178</a> |
| <i>Oxalis</i> sp. affin. <i>campylorrhiza</i>                        | MO120            | <a href="#">EU436948</a> |
| <i>Oxalis stenoptera</i> Turcz.                                      | MO553            | <a href="#">EU436934</a> |
| <i>Oxalis suavis</i> R. Knuth  | MO385            | <a href="#">EU436937</a> |
| <i>Oxalis uliginosa</i> Schltr                                       | MO394            | <a href="#">EU436933</a> |
| <i>Oxalis viscosa</i> E. Mey. ex Sond.                               | MO73             | <a href="#">EU436949</a> |

Chapter 7: Reassessment of the taxonomic status of *Oxalis fabaefolia* (Oxalidaceae) and the description of a unique form of *O. flava* from the Northern Cape Province of South Africa

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## Abstract

Southern African *Oxalis* taxonomy is complicated by tremendous morphological variation. The widely distributed *Oxalis flava*, for example, currently contains eight morphologically distinct forms. The remaining members of section *Crassulae* display distinctive enough morphological characters to retain specific status, despite resemblance to forms of the broadly defined *O. flava*. Recent collection of a taxon with strong morphological affinities to species in section *Crassulae* generated much interest. In this study we assess the placement of this new taxon to members of section *Crassulae* based on analyses of DNA sequence data of the Internal Transcribed Spacer (ITS) region and morphological comparisons. Results show that most members of section *Crassulae* are distantly related to *O. flava*. However, our morphological and molecular data strongly suggest that the newly collected taxon represents yet another form of *O. flava*. In addition, these data show *O. fabaefolia* to be nested within *O. flava*, suggesting that it should be synonymized under this broadly defined species. Both the new taxon and *O. fabaefolia* display unique morphological characters, allowing them to be considered separate infra-specific taxa of *O. flava*. Thus the taxa *Oxalis flava* var. *unifoliolata* and *O. flava* var. *fabaefolia* are proposed here.

Key words: Oxalidaceae, *Oxalis flava*, *Oxalis fabaefolia*, phylogeny, taxonomy

## 1. Introduction

Southern African members of the large genus *Oxalis* L. include many species complexes that show considerable epharmonic variation across their distribution ranges (Salter, 1944). The *Oxalis flava* L. complex (section *Crassulae*) represents one of the most variable examples. It is widespread in the western coastal belt and inland regions of the Cape Floristic Region (CFR), where it naturally occurs on both shale and sandstone soils. The extreme variation displayed by this species is reflected in its taxonomic history and the number of described forms. *O. flava* was first described by Linnaeus (1753) in *Species Plantarum*. Jacquin (1794) recognized *O. flava* as a valid species, and newly described putatively related species *O. flabellifolia* Jacq., *O. lupinifolia* Jacq. and *O. pectinata* Jacq. He did not recognize the presence of tristyls in *Oxalis*, resulting in his description of the three floral morphs normally found within a single species as separate taxa. Salter (1944) reduced all three of these species to synonyms of *O. flava*, and recognized them as forms based mainly on petiole margin, indumentum, and leaflet and sepal shape. He suggested that numerous characters (including various bulb and contractile root characters) may aid the demarcation of taxonomic groups in this species, although he failed to recognize any additional characters to support such a classification. He conceded that even the forms recognized in his taxonomic treatment displayed some character overlap, rendering the subdivision of this complex group-species almost impossible based on morphological characters alone. The species is currently described as including eight morphologically distinct forms.

Jacquin (1794) also described another seemingly related group of species, *O. fabaefolia* Jacq., *O. crispa* Jacq., *O. asinina* Jacq., *O. lanceaefolia* Jacq. and *O. leporina* Jacq., and distinguished between them based on corolla colour, leaf shape and stilar morph type. Salter (1944) synonymised all of the species in this group under *O. fabaefolia*, placing *O. asinina*, *O. lanceaefolia* and *O. leporina* under *O. fabaefolia* Form B. Salter (1944) maintained *O. fabaefolia* and *O. flava* as separate species based on the presence of winged petioles in the former. He did, however, concede that forms of *O. flava* are scarcely distinguishable from *O. fabaefolia* Form B.

Compared to *O. flava* and *O. fabaefolia*, other species included in section *Crassulae* (*O. pulvinata* Salter, *O. namaquana* Sond., *O. cathara* Salter, *O. salteri* L. Bolus, *O. flaviuscula* Salter and *O. louisae* Salter) are morphologically well-defined. However, like most taxonomic sections currently recognized in southern African *Oxalis*, the monophyly of the section is in question. DNA-based phylogenetic reconstructions of the genus (Oberlander et al. 2004; Oberlander et al., 2008a) showed that members of this section do not form a well-supported monophyletic unit.

Although section *Crassulae* is currently rather loosely defined, several characters are shared by the included species. Members of the section are mostly stemless, somewhat succulent plants with large scales enclosing their petiole bases. The petioles are distinctly basally articulated, and are mostly conspicuously widened below the basal articulation (Salter, 1944). Within section *Crassulae*, unifoliolate leaves are found in three species: *O. fabaefolia*, *O. flava* form G and *O. salteri*. Of these, *O. salteri* is the only species in which mature plants consistently develop only one leaflet per leaf. In both of the other species, the formation of unifoliolate leaves is always the exception, present only in a few (mostly younger) individuals in any given population (Salter 1944). The discovery of a large population of individuals with morphological similarities to members of section *Crassulae*, but with consistently unifoliolate leaves, was thus of considerable interest. This population was discovered at Gannabos between Nieuwoudtville and Loeriesfontein in the Northern Cape Province, South Africa. Superficially this population strongly resembled *O. salteri*, but closer examination revealed numerous morphological characters shared with *O. flava* and *O. fabaefolia*. In addition, *O. salteri* is only known from west of the Nieuwoudtville escarpment.

In this study we evaluate the taxonomic placement of plants from this newly-collected population within *Oxalis* section *Crassulae* using comparative morphology and DNA sequence data. Based on morphological similarities between plants from this population, *O. fabaefolia*, *O. flava* and *O. salteri*, we included material of these three species in all analyses. For the molecular study, multiple accessions of *O. flava* were included to represent the morphologically diverse forms of this species.

## 2. Materials and Methods

### 2.1. Morphological assessment

The morphology of the newly collected specimens was studied and compared with fresh material of *O. flava*, *O. fabaefolia* and *O. salteri* obtained from the field and the *Oxalis* living collection in the Stellenbosch University Botanical Garden. This was supplemented by herbarium material from the Stellenbosch University (STEU), Compton (NBG) and Bolus (BOL) herbaria (Table 1).

### 2.2. DNA sequencing and molecular analysis

Phylogenetic reconstructions were based on DNA sequence data obtained from the nuclear Internal Transcribed Spacer region (ITS). DNA was extracted and sequenced at the DNA sequencing facility of Stellenbosch University. Total genomic DNA was extracted using a NucleoSpin (R) 96 Plant genomic DNA extraction kit (Macherey-Nagel) on a Genesis 200RMP liquid handler (Tecan). PCR, sequencing, contig creation and alignment of the sequence data followed Oberlander et al. (2008a). In order to place the new taxon correctly in the context of southern African *Oxalis*, sequences of seven of the eight species of section *Crassulae* were included, along with other potential close relatives such as *O. monophylla* L. f. (Oberlander et al., 2004; Oberlander et al., 2008a, Oberlander et al., 2008b). All generated sequences were submitted to the NCBI's GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>) (Table 1). Parsimony analyses were conducted in PAUP\* v4.0b10 (Swofford, 2003), using heuristic searches (5 000 replicates). Support levels at nodes were assessed using nonparametric bootstrap (10 000 replicates). Bayesian Inference was conducted using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) under the model of sequence evolution as chosen in MrModeltest v2.2 (Nylander, 2004). Two separate analyses of five million generations each were run.

## 3. Results

### 3.1. Morphological assessment

Morphological characters included in comparisons between *O. flava*, *O. fabaefolia*, *O. salteri* and the newly collected taxon are presented in Table 2. Morphologically the newly collected taxon is closely related to species in *Oxalis* section *Crassulae* based

on the semi-succulent habit and the large papery scales at the nodes. It can be distinguished from all three other taxa by the presence of multicellular glandular hairs on the rhizome, filaments and styles. Also unique to the newly collected taxon is the presence of multicellular hairs on the leaf margins. Except for the winged petioles of *O. fabaefolia*, no constant characters separate *O. fabaefolia* and *O. flava*.

### 3.2. DNA sequencing and molecular analysis

The final alignment included 39 taxa and 803 characters, with 106 (13.2 %) parsimony-informative characters. Gaps were coded as missing data. Parsimony analysis yielded 3202 trees of 371 steps in length. Bayesian Inference was conducted using the GTR + I +  $\Gamma$  model as selected by MrModeltest. The first 1000 sampled generations were discarded as burnin and remaining trees from both runs were combined into a 50 % majority-rule consensus tree (Fig. 1).

Members of *Oxalis* section *Crassulae* did not resolve in a single monophyletic clade. The species *O. namaquana*, *O. cathara*, *O. salteri*, *O. flaviuscula* and *O. louisae* are not sister to *O. flava*, and are consequently not discussed further. The specific placement of these species will be dealt with in a more appropriate article (Oberlander et al., in prep.). All included accessions of *O. flava*, *O. fabaefolia* and the newly-collected taxon resolved in a strongly supported clade (Fig. 1). *Oxalis monophylla* is sister to this clade with strong support. Both the newly collected taxon and *O. fabaefolia* resolved deeply embedded within *O. flava*.

## 4. Discussion

Morphologically the newly-collected taxon shows considerable character overlap with *O. flava*, but is distinct from other species (*O. pulvinata*, *O. namaquana*, *O. cathara*, *O. salteri*, *O. flaviuscula*, and *O. louisae*) currently included within *Oxalis* section *Crassulae*. The only constant characters that regularly distinguish this taxon from all other forms of *O. flava* are the presence of multicellular glandular hairs and the consistent formation of mature unifoliate leaves. Similarly, the only distinction between *O. flava* and *O. fabaefolia* is the presence of winged petioles in the latter species.

Phylogenetically the ITS sequence of the newly-collected taxon resolved deeply imbedded in the *O. flava* complex, with strong support. It forms a well-supported sister relationship with *O. flava* collected from the De Doorns (MO 25) area, more than 200 km further south. This population represents the typical form (form A, Salter, 1994) of *O. flava*. Similarly, the ITS sequence of *O. fabaefolia* is strongly supported as being sister to an accession of *O. flava* (MO 753) collected near Nieuwoudtville. This population of *O. flava* (MO 753) is morphologically very dissimilar to *O. fabaefolia* in being slender and delicate with linear, almost graminoid leaflets oriented vertically. Both taxa are from the same general geographic area (Nieuwoudtville / Vanrhynsdorp), but occupy very different habitats.

*Oxalis flava* represents one of the morphologically most variable species complexes among South African *Oxalis*. Leaflet numbers vary from 2 to 12, leaflet shapes vary from linear to obovate and levels of above-ground stem exsertion vary from absent in most forms to well-developed in populations near Klawer (Dreyer, pers. obs.). This species displays considerable flower colour polymorphism, with more than one colour often occurring sympatrically in the same population. These characters stay constant between forms when these are removed from the field and cultivated under nursery conditions, suggesting a genetic basis for these characters.

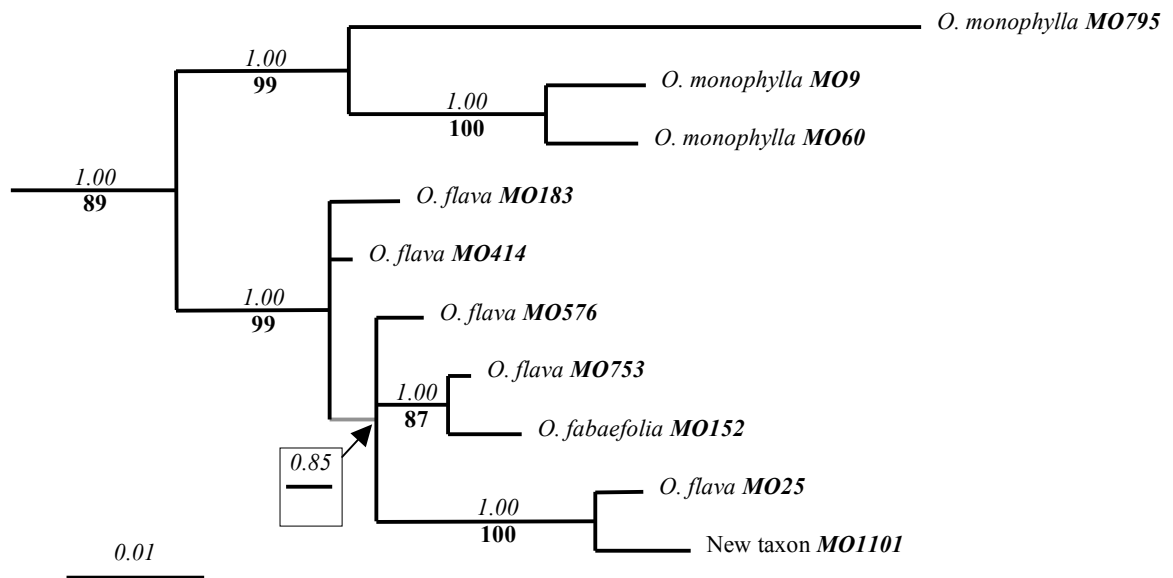


Fig.1. Topology showing majority-rule consensus tree of *O. monophylla* and the *O. flava* species complex. Numbers above the branches indicate Bayesian Posterior Probability, numbers below the branches refer to Parsimony Bootstrap values. Branches that collapse in the parsimony strict consensus tree are indicated in grey. The scale bar indicates average number of changes per site.

The intra-specific demarcation of *O. flava* is thus extremely complicated, and it may be subdivided once focused studies on this complex have been conducted. Preliminary cytological results (Suda, pers. com.) also revealed extensive variation in this species, with ploidy levels of  $2n = 2, 4, 6, 8, 10$  and  $12$  reported. A focused, large-scale, intra-specific phylogeographical and cytological study of *O. flava* is needed to tease apart phylogenetically separate lineages in this complex. In the interests of consistency, we propose that both the newly collected taxon and *O. fabaefolia* be synonymised as conspecific with *O. flava*. In recognition of their distinctive morphological characters and geographical distribution, however, we propose that both taxa be recognized as varieties of *O. flava*.

## 5. Taxonomy

**Oxalis flava** L., Sp. Plant.: 433 (1753); Thunb. Diss.: 23, n. 24 (1781); Jacq. Oxal.: 116, t. 73, t. 78 f. 2 (1794); Edward's Bot. Reg. II: t. 117 (1816); DC. Prodr. I: 702, n. 149 (1824); Eckl. and Zeyh. Enum. I: 95, n. 747 (1836); Salter: 215 (1944).

ICONOTYPE Burm. Afr.: 68, t. 27. f. 4 (1738).

*O. flava* L. var. *thunbergiana* Sond. in Harvey and Sonder Fl. cap. I: 347 (1859-1860).

*O. bulbosa angustis digitatis foliis* Burm. Afr.: 68, t. 27, f. 4 (1738).

*O. bulbosa, simplicicaule* Burm. Afr.: 81, t. 30, f. 1 (1738).

*Acetosella flava* (L.) O. Kuntze in Rev. Gen. I: 91 (1891).

*O. pectinata* Jacq. Oxal.: 118, t. 75 (1794); Eckl. and Zeyh. Enum I: 95, n. 748 (1836); Knuth in Engler's Pflanzenreich Oxal.: 383 (1930) ICONOTYPE: Jacq. Oxal.: t. 75 & 78, f. 2 (1794).  
*O. flava* L. var. *pectinata* (Jacq.) Sond. in Sonder and Harvey Fl. cap. I: 347 (1859-1860).

*O. lupinifolia* Jacq. Oxal.: 115, t. 72 (1794); DC. Prodr. I: 702, n. 148 (1824); Eckl. and Zeyh. Enum. I: 95, n. 745 (1836); Sonder in Sonder and Harvey Fl. cap. I: 348 (1859-1860); Knuth in Engler's Pflanzenreich Oxal.: 382 (1930). ICONOTYPE: Jacq. Oxal.: t. 72 (1794).

*Acetosella lupinifolia* (Jacq.) O. Kuntze in Rev. Gen. I: 91 (1891).

*O. flabellifolia* Jacq. Oxal.: 117, t. 74 (1794); DC. Prodr. I: 702, t. 151 (1824); Ecl. and Zeyh. Enum. I: 95, n. 746 (1836); Sonder in Sonder and Harvey Fl. cap. I: 347 (1859-1860); Knuth in Engler's Pflanzenreich Oxal.: 383 (1930). ICONOTYPE: Jacq. Oxal.: t. 74 (1794).

*O. isosepala* Tod. Nuovi Gen. e Spec.: 36 (1858).

*Acetosella flabellifolia* (Jacq.) O. Kuntze, Rev. Gen. I: 91 (1891).

*O. flava* L. Form A T. M. Salter in Salter: 216 (1944).

*O. flava* L. Form B T. M. Salter in Salter: 216 (1944).

*O. flava* L. Form C T. M. Salter in Salter: 216 (1944).

*O. flava* L. Form D T. M. Salter in Salter: 216 (1944).

*O. flava* L. Form E T. M. Salter in Salter: 217 (1944).

*O. flava* L. Form F T. M. Salter in Salter: 217 (1944).

*O. flava* L. Form G T. M. Salter in Salter: 217 (1944).

*O. flava* L. Form H T. M. Salter in Salter: 217 (1944).

Stemless geophyte, 10.0–190.0 mm tall when in flower. Bulb ovate, widely ovate, widely elliptical or circular, base round, tapering to acute apex, dark brown, reddish brown or light brown; tunics glabrous, hard or papery and smooth. Rhizome 30.0–160.0 mm, tunics thin and soft to thick and hard, mostly glabrous, seldom scantily covered with glandular hair towards apex and glabrescent below; scales mostly present, positioned at regular intervals along rhizome, attachment sheathing. Above-ground stem absent or well-exserted, up to 200.0 mm long, many-branched with short or elongated internodes. Leaves rosulate, 2–30 per plant, petiolate, petioles (5.0) 20.0–30.0 (130) mm long, semi-succulent, round in cross-section, with distinct upper and lower articulations, sometimes narrowly (0.5 mm) to broadly (10.0–12.0 mm) winged, base widened; leaflets 1–12, sessile to shortly petiolulate, erect to palmately spreading, 8.5–63.0 X 11.0–38.0 mm, linear, oblong, elliptical, cuneate, cuneate-obovate or obovate, occasionally beset with small reddish dots, abaxially and adaxially glabrous, occasionally shortly petiolulate, sometimes with multicellular glandular hairs restricted to the margin, base attenuate, obtuse or cuneate, margin entire, more or less cartilaginous, occasionally undulate, apex obtuse to minutely emarginate. Peduncle 1-flowered, 4.0–170.0 mm long, green, glabrous to sparsely covered with long multicellular glandular hairs, base articulated and occasionally widened; bracts 2, alternate, above middle of peduncle to just below sepals, filiform, narrowly oblong or narrowly elliptical, glabrous or occasionally with multicellular glandular hairs, 2.0–5.0 mm long, apex round, occasionally callose. Sepals 4.0–11.0 X 1.0–4.0 mm, shape variable, margin entire, apex round, obtuse, acute to acuminate, occasionally callose, abaxially and adaxially glabrous, sometimes with multicellular

glandular or simple hairs along margins. Petals 14.0–37.0 X 6–19 mm, yellow, white or pale rose mauve with red borders, obovate to spatulate, glabrous, margin entire, apex round or truncate, a few calli occasionally present at the tip, tube broad, yellow, funnel-shaped. Stamens in 3 series, 2 series per plant, mostly with lower level 2.0–4.0 mm long, middle series 4.0–5.0 mm long, longest series 5.0–7.0 mm long; filaments with sparse glandular hairs along entire length, blunt teeth separate to adnate to longer filaments, sometimes apically outwardly deflexed; anthers dorsifixed, oblong, extrorse; pollen yellow, tricolporate, tectum reticulate. Ovary ovoid, 5-lobed, 2.5 mm long, 5-loculed with 3 ovules per locule, translucent, glabrous; styles 5, separate, erect, covered with simple or glandular hairs in the upper half; stigma yellow, fimbriate. Fruit a 5-locular capsule, globular to subglobular, shorter than sepals. Seed exendospermous.

### **Diagnostic characters**

Geophyte with fibrous bulb, mostly semi-succulent. Number of leaflets ranging from 1–12. Petiole round, sometimes winged. Leaflet margins smooth to undulate. Flowers large, funnel-shaped with broad tube, petals white, light pink or yellow.

### **6. Key to the varieties**

Plant entirely glabrous, leaflets 2–5, petiole wings 10.0–12.0 mm wide ... (a) var.

*fabaeolia*

Plant covered with multicellular glandular hairs on most plant parts, leaflets 1, petiole wings up to 0.5 mm wide ..... (b)

var. *unifoliolata*

**(a) *Oxalis flava* L. var. *fabaefolia* (Jacq.) Dreyer & Oberlander, comb. nov. Jacq.**  
*Oxal.* t. 27 (1794). DC. Prodr. I: 697, n. 90 (1824); Sonder in Harvey and Sonder's Fl.  
cap. I: 319 (1859-1860); Knuth in Engler's Pflanzenreich *Oxal.*: 377 (1930); Salter:  
211 (1944). ICONOTYPE: Jacq. *Oxal.* t. 27 (1794).

*O. crispa* Jacq. *Oxal.*: 58, t. 23 (1794); DC. Prodr. I: 697, n. 86 (1824); Eckl. and  
Zeyh. Enum. I: 95, n. 748 (1836); Sonder in Harvey and Sonder's Fl. cap. I: 319  
(1859-1860). ICONOTYPE: Jacq. *Oxal.*: t. 23 (1794).

*O. fabaefolia* Jacq. var. *crispa* (Jacq.) Sond. In Sonder and Harvey Fl. cap. I: 319  
(1859-1860).

*Acetosella crispa* (Jacq.) R. Kuntze in Rev. Gen. I: 90 (1891)

*O. asinina* Jacq. *Oxal.*: 59, t. 24 (1794); DC. Prodr. I: 697, n. 88 (1824); Sonder in  
Harvey and Sonder's Fl. cap. I: 318 (1859-1860); Knuth in Engler's Pflanzenreich  
*Oxal.*: 326 (1930). ICONOTYPE: Jacq. *Oxal.*: t. 24 (1794).

*Acetosella asinina* (Jacq.) O. Kuntze in Rev. Gen. I: 91 (1891).

*O. lanceaefolia* Jacq. *Oxal.*: 61, t. 26 (1794); DC. Prodr. I: 697, n. 89 (1824).

ICONOTYPE: Jacq. *Oxal.*: t. 26 (1794).

*O. leporina* Jacq. *Oxal.*: 60, t. 25 (1794); Salter in Salter: 212 (1944). ICONOTYPE:  
Jacq. *Oxal.*: t. 26 (1794).

### **Diagnostic characters**

Stemless, succulent geophyte. Petioles with prominent wings, 10–12 mm broad. Leaflets 2–5, broadly elliptical to obovate, margins sometimes undulate. Flowers yellow, pale mauve or white.

### **Geographic distribution**

*O. flava* var. *fabaefolia* occurs in a restricted area from Vanrhynsdorp in the west to the foot of the Nieuwoudtville escarpment in the east, but is locally abundant within this region. It prefers clay substrates and is always associated with Succulent Karoo vegetation. The species prefers direct sunlight.

### **Selected specimens studied**

3118 (Vanrhynsdorp): Vanrhynsdorp, at top of Gifberg (–BC), Bayer 1335 (NBG); 500 m from N7, on Mauwerskop road (–BC), Dreyer 642 (STEU); Gifberg summit, (–BC), Dreyer, Roets & Zietsman 22 (STEU); Klawer, 6 km S of Trawal along N7 (–BC), Helme 1300 (NBG); 6 mile S of Vanrhynsdorp (–BC), Salter 5311 (NBG); Vanrhynsdorp(–BC), Suda 82 (STEU); Vanrhynsdorp (–BC), Suda 89 (STEU).

**(b) *Oxalis flava* var. *unifoliolata* Dreyer & Oberlander, var. nov.** Figs 2 and 3.

Geophytum acaule, foliis unifoliolatis, petiolis anguste alatis articulo superno prominenti et in marginibus pilis serrato-denticulatis, floribus flavis vel albis.

TYPE. —3119 (Calvinia): Northern Cape Province, Gannabos, between Nieuwoudtville and Loeriesfontein, (–BC), 12-06-2008, Dreyer 829 (STEU, holotype; BOL, NBG isotypes).

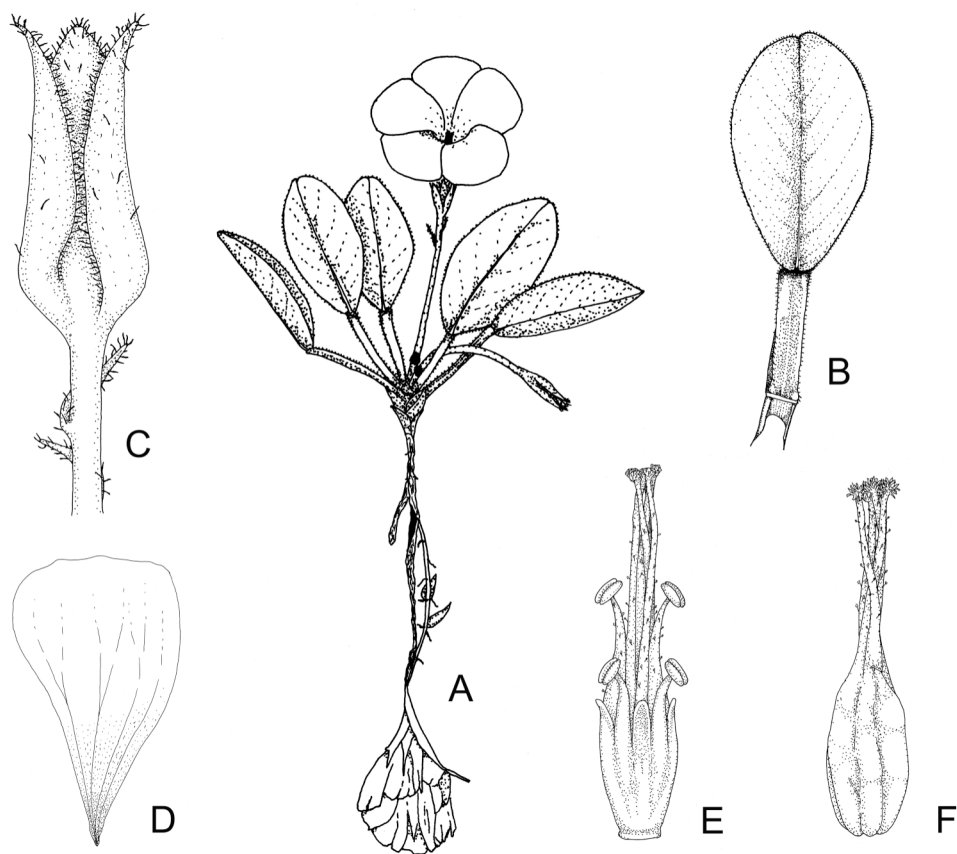


Fig. 2. Line diagram of *O. flava* var. *unifoliolata*. A. Mature plant. B. leaf. C. calyx. D. petal. E. androecium and gynoecium. F. gynoecium.

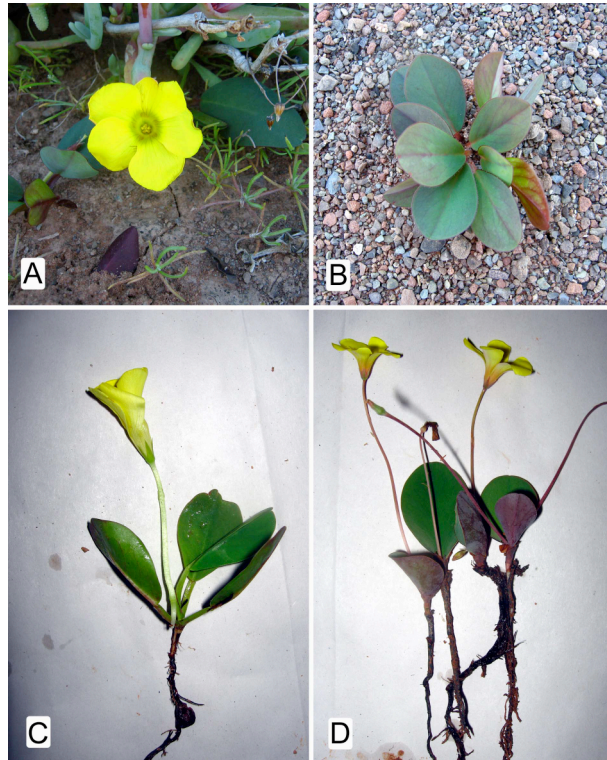


Fig. 3. *Oxalis flava* var. *unifoliolata*. A. flower. B. typical habit. C. side view. D. *Oxalis salteri* side.

Stemless geophyte, 35.0–50 mm tall, aggregated into intertwined clonal clumps. Bulb regularly ovate with acute apex, reddish to chocolate brown, smooth; tunics glabrous, soft. Rhizome 30.0–160.0 mm, scantily covered with thin tunics, scantily covered with multicellular glandular hair towards apex, glabrescent below. Above-ground stem absent. Leaves 2–8 per plant, consistently unifoliate; petioles (6.0) 20.0–30.0 (120) mm, semi-succulent, red with narrow wings (*ca.* 0.5 mm broad), with distinct upper and lower articulations; leaflet 1; 15.0–40.0 X 11.0–28.0 mm, erect and orientated at different angles, enhancing tufted appearance of plant, obovate to elliptical, apex obtuse to minutely emarginated, base obtuse to cuneate, abaxially and adaxially glabrous, but with long multicellular glandular hairs restricted to the margin, adaxially apple green with narrow red margin, abaxially green to red, entire leaflet beset with small reddish dots; petiolule 0.2–1.0 mm long. Peduncle 1-flowered, 20.0–40.0 mm long, green, sparsely covered with long multicellular glandular hairs; bracts 2, alternate, above middle of peduncle, filiform, covered with multicellular glandular hairs, 3.0 mm long. Sepals 8.0–10.0 X 2.0–2.5 mm, lanceolate, acute to acuminate, abaxially and adaxially glabrous, with long multicellular glandular hairs along margins. Corolla 20.0–30.0 mm long, yellow or white with broad, yellow, funnel-shaped tube; petals spatulate with long, narrow claw, glabrous, ecallous. Stamens in 3 series, 2 series per plant, lower level 2.0 mm long, middle series 4.0 mm long, longest series 7.0 mm long; filaments with sparse glandular hair along entire length, blunt teeth separate to adnate to longer filaments, and apically outwardly deflexed; anthers oblong; pollen yellow, tricolporate, tectum reticulate. Ovary ovoid, 5-lobed, 2.5 mm long, 5-loculed with 3 ovules per locule, translucent, glabrous; styles 5, separate, erect, scantily covered with glandular hairs; stigma yellow, fimbriate. Fruit a 5-locular capsule, globular, shorter than sepals. Seed exendospermous.

### **Diagnostic characters**

Stemless semi-succulent geophyte, often clumped. Most plant parts covered with multicellular glandular hairs. Leaves unifoliolate. Petioles exceeding 20.0 mm in length. Flowers yellow or white.

### **Geographic distribution**

Three populations of *Oxalis flava* var. *unifoliolata* were found between Nieuwoudtville and Loeriesfontein. One population occurs about 15 km from Nieuwoudtville along this route, with another large population just beyond the Knersvlakte turnoff on this same road. The third large population was found on the flats at Gannabos, growing below the quiver tree forest at this locality. All three populations comprised of both yellow and white flowered individuals. This taxon is restricted to flat plains on rich clay substrates, where they grow in direct sunlight. Plants are mostly clustered into clonal clumps comprised of many individuals. Natural seed set was found to be abundant.

### **Material examined**

*O. flava* var. *unifoliolata*

NORTHERN CAPE.—3119 (Calvinia): Along Nieuwoudtville to Loeriefontein road, ca. 15 km before turn-off to Gannabos (-AD), Dreyer 845 (STEU); At turn-off to Knersvlakte, on Nieuwoudtville to Loeriesfontein road (-AD), Dreyer 848 (STEU)

### **Notes**

*Oxalis flava* var. *unifoliolata* superficially resembles *O. salteri* in that both species have unifoliolate leaves. Unlike *O. salteri*, which very rarely forms clonal clumps, *O.*

*flava* var. *unifoliolata* individuals are almost exclusively clustered into clonal clumps.

*Oxalis flava* var. *unifoliolata* flowers in June and most plants were already in full fruit by the end of June.

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Table 1

Specimens examined, GenBank accession numbers and locality data for the *Oxalis* species analyzed in this study.

| Species              | Locality  | Herbarium<br>(Ref. nr.) | Collector                         | GenBank<br>accession<br>Number |
|----------------------|---|-------------------------|-----------------------------------|--------------------------------|
| <i>O. fabaefolia</i> | Klawer, 6 km S of Trawal along N7                         | NBG                     | Helme 1300                        | -                              |
| "                    | Vanrhynsdorp, at top of Gifberg                           | NBG                     | Bayer 1335                        | -                              |
| "                    | 6 mile S of Vanrhynsdorp                                  | NBG                     | Salter 5311                       | -                              |
| "                    | 1 km south of Vanrhynsdorp                                | STEU<br>(MO 152)        | Dreyer 721                        | <b><u>EU436925</u></b>         |
| "                    | Gifberg summit  | STEU<br>(MO 997)        | Dreyer,<br>Roets &<br>Zietsman 22 | -                              |
| "                    | 500 m from N7, on Mauwerskop road                         | STEU<br>(MO<br>1133)    | Dreyer 642                        | -                              |
| "                    | Vanrhynsdorp  | STEU                    | Suda 82                           | -                              |
| "                    | Vanrhynsdorp  | STEU                    | Suda 89                           | -                              |
| <i>O. flava</i>      | Roggeveld Escarpment at Ouberg Pass                       | NBG                     | Oliver 9631a                      | -                              |
| "                    | W of Soutriver, 3.5 km S of Sishen-Saldana bridge over N7 | NBG                     | Paterson-<br>Jones 793            | -                              |
| "                    | Between Hopefield and Paternoster                         | NBG                     | Leipoldt<br>3494                  | -                              |
| "                    | Cederberg, Fonteinskloof                                  | NBG                     | Viviers 1308                      | -                              |
| "                    | Pietermeintjies, Laingsburg                               | NBG                     | Compton<br>18034                  | -                              |
| "                    | Geelkuil farm, Malmesbury                                 | NBG                     | Thompson<br>11                    | -                              |
| "                    | Riversdale  | NBG                     | Salter 2293                       | -                              |
| "                    | Karham, Hondeklipbaai                                     | NBG                     | Bayer 2234                        | -                              |
| "                    | Calvinia, Nieuwoudtville Wildflower Reserve               | NBG                     | Perry &<br>Snijman<br>2034        | -                              |
| "                    | Strand, Harmony Reserve, Simonstown                       | NBG                     | Runnalls 999                      | -                              |
| "                    | Worcester, Farm Onse Rus, Brandwach,                      | NBG                     | Walters<br>1955                   | -                              |
| "                    | 11 km SW of Touwsrivier,                                  | NBG                     | Thompson                          | -                              |

|   |  |     |                    |   |
|---|--|-----|--------------------|---|
|   | Worcester  |     | 3001               |   |
| ” | Geelkrans on Katsterdrif just N of Citrusdal                   | NBG | Thompson 1181      | - |
| ” | Southern Kamiesberg, S of Gladdekop                            | NBG | Oliver 9541        | - |
| ” | 3 km SW of Oorlogskloof road, near Bloedsuigersfontein         | NBG | Bayer 2143         | - |
| ” | Near Dassiesklip Farm  | NBG | Bohnen 9015        | - |
| ” | Karoopoort-Ceres intersection, Hottentotskloof                 | NBG | Bayer s.n.         | - |
| ” | Calvinia, 4 km N of Doornbosch                                 | NBG | Bayer 1900         | - |
| ” | Calvinia, Nieuwoudtville Groot Tuin                            | NBG | Pretorius 705      | - |
| ” | Top of Gifberg   | NBG | Van Jaarsveld 4370 | - |
| ” | Nieuwoudtville. Oorlogskloof Nature Reserve                    | NBG | Pretorius 706      | - |
| ” | Botterkloof, 1 mile N of top of pass                           | NBG | Thompson 1163      | - |
| ” | Warm Baths, Clanwilliam  | NBG | Edwards 227        | - |
| ” | Worcester, Mimoswa opposite side of gravel rd to Moordkuilsdam | NBG | Forrester 57       | - |
| ” | Karoskop, Ceres Karoo  | NBG | Bayer 3516         | - |
| ” | Jagersfontein Memorial, Montagu                                | NBG | Bayer 3987         | - |
| ” | Wuppertal, 1 km W Bidouw Farm                                  | NBG | Bayer 4796         | - |
| ” | Fairy Glen, 6 km N of Worcester                                | NBG | Bayer 614          | - |
| ” | Brandvlei Pass, Die Nekkie                                     | NBG | Bayer 671          | - |
| ” | NW Soutpan, Calvinia   | NBG | Bayer 2737         | - |
| ” | 87 km Worcester to Karoopoort, NW Touwsrivier                  | NBG | Bayer 627          | - |
| ” | SE shore of Moordkuil Dam, Worcester                           | NBG | Forrester 52       | - |
| ” | Flats NW of Prince Alfred Hamlet, Worcester                    | NBG | Oliver, 5906       | - |
| ” | Faure, W flats close to national rd, Simonstown                | NBG | Oliver 5899        | - |
| ” | Keiserfontein SW of Nieuwoudtville, Calvinia                   | NBG | Oliver 3390        | - |
| ” | Nieuwoudtville reserve,  | NBG | Perry &            | - |

|   |  |                      |  |                        |
|---|--|----------------------|--|------------------------|
|   | Calvinia   |                      | Snijman<br>2102                        |                        |
| ” | Nieuwoudtville Wild flower<br>reserve                  | NBG                  | Perry &<br>Snijman<br>2066             | -                      |
| ” | Garies, Darter’s Grave                                 | NBG                  | Bayer 2211                             | -                      |
| ” | Montagu, Rietrivier                                    | NBG                  | Bayer 4737                             | -                      |
| ” | De Doorns, Worcester                                   | STEU<br>(MO 25)      | Dreyer 614                             | <b><u>EU436924</u></b> |
| ” | Darling, on Yzerfontein road                           | STEU<br>(MO 183)     | Dreyer 747                             | <b><u>FJ211173</u></b> |
| ” | Nieuwoudtville area, Bokkeveld<br>plateau              | STEU<br>(MO 414)     | Mucina<br>160903/11                    | <b><u>FJ211174</u></b> |
| ” | Ex Hort.   | STEU<br>(MO 576)     | M. B. Bayer<br>7317                    | <b><u>FJ211172</u></b> |
| ” | R316 beyond Pakhuis Pass, on<br>route to Calvinia      | STEU<br>(MO 716)     | Siqueira &<br>Zietsman 22              | -                      |
| ” | Gifberg Pass   | STEU<br>(MO 725)     | Siqueira &<br>Zietsman 29              | -                      |
| ” | 5 km west of Nieuwoudtville                            | STEU<br>(MO 753)     | Mucina<br>030605/11                    | <b><u>FJ211175</u></b> |
| ” | Along N7, 10 km past<br>Clanwilliam on route to Garies | STEU<br>(MO 838)     | Dreyer &<br>Dreyer 6                   | -                      |
| ” | Between Citrusdal and<br>Clanwilliam, along N7         | STEU<br>(MO 901)     | Dreyer &<br>Roets 2                    | -                      |
| ” | 10 km past Ceres, on route to<br>Touwsrivier           | STEU<br>(MO<br>1027) | Dreyer,<br>Roets, Krige<br>& Curran 2  | -                      |
| ” | Summit of Theronsberg Pass                             | STEU<br>(MO<br>1036) | Dreyer,<br>Roets, Krige<br>& Curran 11 | -                      |
| ” | Summit of Theronsberg Pass                             | STEU<br>(MO<br>1037) | Dreyer,<br>Roets, Krige<br>& Curran 12 | -                      |
| ” | Elandsbaai   | STEU<br>(MO<br>1076) | Curran 8                               | -                      |
| ” | Between Jonaskop and<br>Villiersdorp                   | STEU<br>(MO<br>1081) | Dreyer 616                             | -                      |
| ” | 5 km before Clanwilliam, along<br>N7                   | STEU<br>(MO<br>1084) | De Jager 2                             | -                      |
| ” | Clanwilliam above Patryskraal                          | STEU<br>(MO<br>1090) | Mucina<br>010608/1                     | -                      |

|                   |  |                      |                           |                        |
|-------------------|--|----------------------|---------------------------|------------------------|
| "                 | Nieuwoudtville Waterfall   | STEU<br>(MO<br>1096) | Dreyer 624                | -                      |
| "                 | Nieuwoudtville, Farm<br>Willemsrivier, at entrance to<br>farm gate                             | STEU<br>(MO<br>1106) | Dreyer 635                | -                      |
| "                 | Farms leading to entrance of<br>Oorlogskloof   | STEU<br>(MO<br>1111) | De Jager 13               | -                      |
| "                 | Oorlogskloof   | STEU<br>(MO<br>1112) | De Jager 18               | -                      |
| "                 | Nieuwoudtville, 1 km before<br>waterfall   | STEU<br>(MO<br>1113) | De Jager 14               | -                      |
| "                 | N7, just before Doornfontein<br>road   | STEU<br>(MO<br>1129) | De Jager 29               | -                      |
| "                 | Darling  | STEU                 | Suda 34                   | -                      |
| "                 | Yzerfontein  | STEU                 | Suda 64                   | -                      |
| "                 | Clanwilliam  | STEU                 | Suda 75                   | -                      |
| "                 | Calvinia   | STEU                 | Suda 95                   | -                      |
| "                 | Nieuwoudtville   | STEU                 | Suda 75                   | -                      |
| "                 | Vanrhynsdorp   | STEU                 | Suda 160                  | -                      |
| <i>O. salteri</i> | Road leading towards Gifberg   | STEU<br>(MO 755)     | Siqueira &<br>Zietsman 45 | -                      |
| "                 | Suttridge Farm, 21 km on road<br>to Nieuwoudtville   | STEU<br>(MO<br>1087) | Mucina<br>310508/2        | -                      |
| "                 | On road between<br>Nieuwoudtville and<br>Vanrhynsdorp  | STEU<br>(MO<br>1137) | Dreyer 646                | -                      |
| New taxon         | Along Nieuwoudtville to<br>Loeriefontein road, <i>ca.</i> 15 km<br>before turn-off to Gannabos | STEU                 | Dreyer 845                | -                      |
| "                 | At turn-off to Knersvlakte, on<br>Nieuwoudtville to<br>Loeriesfontein road                     | STEU                 | Dreyer 848                | -                      |
| "                 | Gannabos, between<br>Nieuwoudtville and<br>Loeriesfontein,                                     | STEU<br>(MO<br>1101) | Dreyer 829                | <b><u>FJ211169</u></b> |

Table 2

Morphological characters evaluated in comparisons between various *Oxalis* species  
from section *Crassulae*

| Character                               | <i>O. flava</i>   | <i>O. fabaefolia</i>   | <i>O. salteri</i>   | New taxon  |
|---|---|--|---|--|
| Plant indumentum                        | Usually entirely glabrous (Form B with short pluricellular hairs on petioles and sometimes peduncles) | Entirely glabrous  | Entirely glabrous   | Rhizome, filaments and styles with glandular hair; leaflet margins, peduncles, bracts and sepal margins with soft multicellular hair |
| Bulb shape                              | Oval to ovoid   | Ovoid to ovoid-conical   | Globose-conical, often deformed                                   | Oval to ovoid  |
| Petiole base (below basal articulation) | Dilated   | Dilated and scale-like   | Not dilated   | Not dilated  |
| Petiole wings                           | Mostly absent, with narrow cartilaginous margin in Form F   | Foliaceous wings present   | Absent  | Narrow wings present   |
| Leaflet number                          | 2-12 (Form G: juveniles 1)  | 2-5  | 1   | 1  |
| Petiole length                          | 20.0–60.0 mm  | 15.0–25.0 mm   | 3.0–5.0 mm  | 20.0–30.0 mm   |
| Leaflet shape                           | Linear, oblong, cuneate, cuneate-obovate or obovate   | Suborbicular, elliptical, obovate or oblanceolate                      | Broadly elliptical or ovate                                       | Obovate to elliptical  |
| Petal length                            | 14.0–30.0 mm  | 20.0–30.0 mm   | 18.0–23.0 mm  | 20.0–30.0 mm   |
| Petal orientation                       | Flower broadly funnel-shaped, petals curved back at 30–60° beyond tube                                | Flower broadly funnel-shaped, petals curved back at 30–60° beyond tube | Flowers not funnel-shaped, petals curved back at >60° beyond tube | Flower broadly funnel-shaped, petals curved back at 30–60° beyond tube   |

Chapter 8: Taxonomic position of *Oxalis purpurata* Jacq. (Oxalidaceae)

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The most recent major taxonomic work on southern African *Oxalis* L. (Salter, 1944) features a number of poorly-known taxa known from only one or two localities. A significant fraction of these species are described, and only known from, extremely poor data. Some, such as *Oxalis incerta* R. Knuth and *O. linoides* R. Knuth, are described from incomplete herbarium specimens that often lack bulbs or even flowers. Other taxa lack locality data, or appear to be merely aberrant forms of good species. Although these species were upheld by Salter (1944), he retained these taxa only in the interests of completeness, and expressed his gravest doubt about the validity of several.

Recent palynological (Dreyer, 1996) and DNA-based work (Oberlander et al. 2004; Oberlander et al. in prep.) on the genus has clarified the status of a number of these taxa. In these cases, a more definite answer to the uncertain status of these poorly-known species has been achieved. For example, Dreyer and van Wyk (1996) synonymized *Oxalis henrici* Bolus f. under the more widespread *Oxalis engleriana* Schltr on the basis of a unique pollen type shared by both species. In other instances, new species have been described that have clarified relationships between morphologically dissimilar taxa, such as *Oxalis hygrophila* Dreyer (Kumwenda et al., 2004) and *Oxalis saltusbelli* Dreyer and Roets (Dreyer et al., submitted). Also, a phylogenetic scheme based on DNA data has been erected for the entire southern African clade of *Oxalis* (Oberlander et al., in prep.). Despite over eight years of intensive collection, a number of poorly-known taxa were not collected during this time period, and could not be validated and/or systematically placed with any certainty on the basis of DNA data. This was not a problem for species that share putative macro-morphological or palynological synapomorphies which would allow

at least a tentative placement. For some poorly-known taxa, however, the paucity of even this knowledge has meant that little has changed as regards their highly dubious taxonomic status.

One of these poorly known taxa is *Oxalis purpurata* Jacq. To our knowledge, this species has only been found twice. Apart from the original description by Jacquin (1798), Salter (1940) found a population of this species growing amongst rocks in van Rhyn's Pass, now on the border between the Northern and Western Cape, South Africa. He placed the species in section *Cernuae* subsection *Purpuratae* based on the acaulescent habit, the lack of an apical beak to the bulb, the smooth nature of the bulb tunics, and the basal peduncles. This position was upheld in his later monograph on South African *Oxalis* (Salter, 1944). The other two members of this subsection, *Oxalis bowiei* Lindl. and *Oxalis semiloba* Sond., are both summer-rainfall species associated with the coastal regions of the Eastern Cape and Kwazulu-Natal. The resulting biogeographic link between these summer-rainfall species and *O. purpurata* seems unlikely given the current distributions of these three species. In addition, *O. purpurata* differs from both of these taxa in not producing a contractile root and by bearing teeth on the filament bases, characters considered taxonomically important by Salter (1944).

Despite several years of visiting the site, *O. purpurata* has not been rediscovered at van Rhyn's Pass. A recent study of unidentified *Oxalis* in the Compton Herbarium (NBG, Cape Town, South Africa) produced a specimen of *O. purpurata* from the Oorlogskloof Nature Reserve, about 10km south of the Pass (W. A. J. Pretorius 707, NBG 181394). This prompted more extensive recent searches for this species in the Nature Reserve, and in June 2008 a flowering population was discovered in a similar habitat to that described by Salter (1940). Herbarium specimens and living material (MO1118, Dreyer 836) were collected, and a leaf sample was taken for later DNA extraction and sequencing.

Due to the recent date of rediscovery, this species could not be included in large-scale phylogenetic analyses of the genus (Oberlander et al., submitted). However, smaller-scale analyses of the nuclear Internal Transcribed Spacer region (Genbank accession number

FJ426283), following the protocols and procedures of Dreyer et al. (2008), yielded a confident placement within a small clade of species with umbellate inflorescences (Parsimony bootstrap support: 99 %; Bayesian posterior probability: 1.00). This clade corresponds to the *O. pes-caprae* clade of Oberlander et al. (in prep.). Apart from well-known weedy species such as *O. pes-caprae* L., this clade includes taxa from Namibia (e.g. *O. purpurascens* Salter and its allies), Namaqualand (e.g. *O. haedulipes* Salter and its allies) and the western Cape Floristic Region (e.g. *O. compressa* L. f.). The only other sampled member of subsection *Purpuratae*, *Oxalis bowiei*, is not closely related to the *O. pes-caprae* clade, but instead is closely related to other summer rainfall taxa such as *O. tragopoda* Salter and *O. stellata* E. & Z. The extremely close morphological resemblance between *O. bowiei* and *O. semiloba*, and provisional DNA data (K. Oberlander, pers comm.), also makes the latter species very unlikely to be a relative of *O. purpurata*.

The presence of umbellate inflorescences and the almost succulent nature of *O. purpurata* are in agreement with many other members of the *O. pes-caprae* clade. It does differ from all other members of the clade in bearing pale lilac (instead of yellow or white) flowers, and no contractile root. However, neither of these character states are shared by the summer-rainfall taxa. Most other morphological (i.e. teeth present on the filaments, seven or more ovules per carpel, glabrous corolla) and palynological (finely reticulate tectum; Dreyer, 1996) features are congruent with a placement in the *O. pes-caprae* clade. Given the congruent DNA and morphological data, we consider *O. purpurata* to be unrelated to the summer-rainfall species of subsection *Purpuratae*. Instead, a position within the *O. pes-caprae* clade is strongly supported, and we thus place it within this clade as a close relative of *O. pes-caprae*, *O. compressa*, *O. copiosa* Bolus f. and *O. haedulipes*.

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## Chapter 9: Conclusion

In this study I set out to provide a DNA-based phylogenetic hypothesis of relationships with which to explore the evolutionary patterns and processes within southern African *Oxalis*. This was done to redress the lack of knowledge of one of the largest genera in the CFR, the largest geophytic genus in the region, and one that offers great potential insights into the mode of evolution in one of the world's most distinctive biodiversity hotspots. It is a genus that contains some of the world's most noxious weeds. Paradoxically, and unfortunately, it is also a genus in which approximately 25% of recognized southern African species are listed as threatened, even though virtually nothing is known about the ecology or even the distribution range of the majority of these taxa.

As set out in Chapter 2 and Chapter 3 of this study, a new phylogenetic backbone and preliminary classification are now available on which to base further research. The three major divisions of a clearly monophyletic southern African lineage are now well-supported, and offer insights into biogeography, ecology, key innovations and character trait evolution. The phylogeny, and the new classification, agrees with previous phylogenetic work, as well as the current palynological classification of the genus. Many potential macro-morphological characters that can be explored for further support of monophyly of informal groups are proposed. These emphasize the need for more anatomical studies and greater focus on both the bulb and system of vegetative reproduction.

An aspect of this work has allowed greater insight into processes operating beyond the genus *Oxalis*. Explorations of divergence time estimates and duration of occupancy of the Cape have shown why, in addition to a growing body of evidence for other Cape clades, climate-induced speciation theories cannot fully explain the tremendous biodiversity of the region. The hypothesis of a Cape origin for the southern African clade is strengthened, as the few summer-rainfall *Oxalis* lineages are deeply embedded within otherwise CFR clades.

The bulbs of *Oxalis* are one of their major and defining characters, and arguably have played a massive role in their evolution, distribution and potential as weeds. As Chapter 4 attempts to explain, the exaptation of normal photosynthetic petiole bases for storage and protection of underground structures has significant explanatory power in illuminating many questions of *Oxalis* morphology, ecology and radiation. The preliminary understanding of global *Oxalis* relationships offered here allows the first glimpse into explaining the evolution of these structures.

A large amount of basic taxonomic work remains to be completed for this genus, but results presented here will significantly guide such studies. In Chapters 5 to 8 my co-authors and I describe two new species, one mentioned but not formally described by Salter (1944) over sixty years ago, the other completely new to science. Some attempt is made at addressing the species boundaries and nomenclature of the extremely complex group species *O. flava*. Finally, we discuss the taxonomic position of the rare and little-known *O. purpurata*, and place it in a more natural position within the current phylogenetic understanding of the genus. With results of this thesis as a backbone, many more such studies can and will now be undertaken.

There are admitted shortcomings to this work. Although large amounts of data have been applied to the problem of increasing resolution, many clades remain large polytomies. The topology linking the three main southern African subclades is uncertain, and the closest relatives of the southern African clade are still not confidently known. There is evidence of potential hybridization in the incongruent topologies of plastid- and nuclear-derived trees. However, these should not be seen as pitfalls, but rather as challenging new questions for future systematists to address who wish to work on this fascinating group of plants.