

**A MOLECULAR PHYLOGENETIC ASSESSMENT OF *OXALIS* L.
SECTION *ANGUSTATAE* SUBSECTION *LINEARES* USING *trnL-trnF*
SEQUENCE DATA**

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or part been submitted at any university for a degree.

SUMMARY

The phylogenetic relationships and taxonomic status of South African *Oxalis* L. species are highly unresolved, both regionally and with regard to precise placement within the genus as a whole. Studies based exclusively on morphological and palynological characteristics have hitherto proved only partially successful in resolving natural groupings among the indigenous taxa of the region. Recent studies involving a few native taxa have indicated the plastid *trnL-trnF* non-coding DNA region as useful for the purposes of reconstructing phylogenetic relationships within the genus. The present study addressed the taxonomic monophyly and relationships of the highly unresolved section *Angustatae* subsection *Lineares*, using DNA sequence data.

The phylogenetic reconstruction of southern African *Oxalis* species renders five of the subsections of section *Angustatae sensu* Salter (1944) polyphyletic, three of them conclusively so. The members of subsection *Lineares* are split between three clades, two of them with strong bootstrap support. None of these three clades consists exclusively of species of subsection *Lineares*. Likewise four of the seven assemblages of related taxa within subsection *Lineares sensu* Salter (1944) are not retrieved as monophyletic. Pollen data *sensu* Dreyer (1996) supports the clades retrieved in this study, whereas very few morphological characters could be plotted as potential synapomorphies for these clades. The resultant phylogenetic reconstruction thus supports palynological data of this subsection, and indicates the urgent need for a revision of the current morphological classification of Salter (1944).

OPSOMMING

Die filogenetiese verwantskappe en taksonomiese klassifikasie van Suid Afrikaanse *Oxalis* L. spesies is nog baie onduidelik, beide binne die gebied en ten opsigte van die presiese plasing binne die genus as 'n geheel. Vorige werk, uitsluitlik gebaseer op morfologiese en palinologiese kenmerke, was tot dusver nog net gedeeltelik suksesvol in die identifikasie van natuurlike groepe binne die inheemse taksa van die gebied. Onlangse studies op 'n paar inheemse spesies het die nut van die plastied *trnL-trnF* nie-koderende DNA area bevestig vir die rekonstruksie van filogenetiese verwantskappe in die genus. Hierdie studie is gerig op die taksonomiese monofilie en verwantskappe van die onnatuurlike seksie *Angustatae* subseksie *Lineares*, deur gebruik te maak van DNA basis-volgorde data.

Die filogenetiese rekonstruksie van Suid Afrikaanse *Oxalis* spesies dui aan dat vyf van die subseksies van seksie *Angustatae sensu* Salter (1944) polifileties is, met sterk steun daarvoor dat drie van hulle onnatuurlik is. Die lede van subseksie *Lineares* is tussen drie verskillende groepe versprei; twee van dié groepe het sterk ondersteuning. Nie een van die drie groepe bestaan uitsluitlik uit spesies van subseksie *Lineares* nie. So ook is vier van die sewe groepe van verwante spesies binne subseksie *Lineares sensu* Salter (1944) polifileties. Stuifmeel data volgens Dreyer (1996) ondersteun die groepe wat deur die DNA volgordes uitgewys is, terwyl baie min morfologiese kenmerke gebruik kan word as potensiële sinapomorfe/kenmerke. Die filogenetiese rekonstruksie ondersteun dus die palinologiese data van die subseksie, en dui op die dringende behoefte aan 'n hersiening van die huidige morfologiese klassifikasie van Salter (1944).

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INTRODUCTION

Despite being one of the largest genera of the Cape Floristic Region at the southern tip of Africa, and an integral part of the flora of the region, very little is known of the phylogenetic relationships of the genus *Oxalis* L. (Oxalidaceae) in the area. Despite their undoubted value, previous taxonomic treatments of the genus in this region do not provide a wholly natural classification for *Oxalis*. None have been based on phylogenetic reconstruction techniques, nor were these treatments able to utilise the recent advances in using DNA-based data for analysis.

The aims of the present study are twofold. The first aim is to utilise phylogenetic reconstruction methods on DNA sequence data of members of *Oxalis* section *Angustatae* subsection *Lineares*, a large and, with regard to morphological characters, unnatural subsection of the genus in southern Africa. The plastid non-coding *trnL-trnF* region is used to produce a phylogenetic reconstruction in order to test the monophyly of this subsection. A sub-objective integrated within the first is to assess the monophyly of the seven assemblages of putatively related taxa within subsection *Lineares*. These objectives are addressed in chapter two of the present study.

The second main aim of this research is to propose more natural relationships within the southern African members of *Oxalis*. This was done by comparing the phylogenetic reconstruction produced in chapter 2 with both the morphological classification of *Oxalis* in southern Africa (Salter, 1944) and a recent palynological review of all the southern African species (Dreyer, 1996). The findings of these comparisons are presented in chapter 3.

Chapters 2 and 3 are preceded by a literature review on the genus *Oxalis* in southern Africa (Chapter 1). Chapter 1 also reviews the problems in the current classification of *Oxalis*, and sketches a brief overview of the utility of DNA-based data in general, and the *trnL-trnF* region in particular, in resolving taxonomic and phylogenetic problems. The impact of the present study on the current taxonomic classification of *Oxalis* in southern Africa, and future problems that need addressing, are briefly discussed in a concluding chapter.

References:

Salter, T. S., 1944. The genus *Oxalis* in South Africa. *J. S. Afr. Bot. Suppl.* 1: 1-355.

Dreyer, L. L., 1996. A palynological review of *Oxalis* (Oxalidaceae) in southern Africa. Ph.D. thesis, University of Pretoria, Pretoria, South Africa.

NOTE

As regards taxon names: The full names and author citations of all taxa used in the phylogenetic analyses of Chapter 2 are presented in Table 1, page 43. Author citations are otherwise left out of the text for ease of reading. Only species not represented in these analyses are printed in full in the text.

CHAPTER 1

Literature Review

The Oxalidaceae: The family Oxalidaceae is widespread and is represented in most temperate and tropical floras. It is an understudied taxon, with little consensus as to the number of and relationships between its constituent genera. The family as a whole was last extensively monographed by Knuth (1930), who included the genera *Oxalis* L., *Biophytum* D.C., *Eichleria* Progel, *Averrhoa* L., *Dapania* Korth., *Sarcotheca* Blume, *Lepidobotrys* Engl. and *Hypseocharis* Remy, the last of which was considered morphologically intermediate between Geraniaceae and Oxalidaceae. Hutchinson (1959) considered *Lepidobotrys*, *Sarcotheca* and *Dapania* as collectively belonging to a separate family, the Lepidobotryaceae, with *Averrhoa* assigned to the monogeneric Averrhoaceae. Veldkamp (1971), however, assigned *Averrhoa*, *Dapania* and *Sarcotheca* to their own family. Takhtajan (1980) segregated the genera *Hypseocharis* and *Lepidobotrys* into their own families, the Hypseocharitaceae and Lepidobotryaceae, respectively. Thorne (1992), in turn, segregated *Hypseocharis* from the Oxalidaceae, and placed it within the Geraniaceae. Chant (1993) segregated *Averrhoa* into a separate family Averrhoaceae, and considered the Oxalidaceae *sensu stricto* to consist of only *Oxalis*, *Biophytum* and *Eichleria*. This classification was based on that of Cronquist (1981). *Averrhoa*, however, exhibits unequivocal morphological relationships with the Oxalidaceae, and most authors include it in the family. *Hypseocharis*, the problematic intermediate, has at various times been assigned to the Geraniaceae, Oxalidaceae and the monogeneric family, the Hypseocharitaceae.

In addition to these genus-level difficulties, most classical higher-order taxonomies have exhibited disagreement as to the precise ordinal placement of this family. Most taxonomists (Takhtajan, 1980; Cronquist, 1981; Thorne 1992) have considered the family as part of the order Geraniales, particularly affiliated to the Geraniaceae. However, the families included in the Geraniales vary dramatically between the various classification systems of this order, with virtually no overall consensus (Price & Palmer, 1993). Price & Palmer (1993) provide a more detailed discussion of these taxonomic problems.

Recent molecular work has significantly improved our understanding of the systematic position of the family Oxalidaceae, but not the relationships of the genera included within it. Results of Chase *et al.* (1993), based on *rbcL* DNA sequence data, indicated two possible positions for the family. The first agrees with a more traditional placement as sister group to the Geraniaceae, similar to the views of Cronquist (1981) and Takhtajan (1980). This placement was a result of an initial heuristic search, not swapped to completion, and placed Oxalidaceae convincingly in the rosoid II clade. A second search included more taxa and swapped at least some of the produced trees to completion, making this search preferable to the initial one. The position of Oxalidaceae in this second search was an unexpected one, as sister to a clade consisting of members of the Cunoniaceae, Cephalotaceae, Tremandraceae and Eucryphiaceae, within their rosoid I clade. Not one of these families has ever before been considered closely related to either the families Geraniaceae or Oxalidaceae *sensu* Cronquist (1981).

It should be noted that only the tree produced by the second search by Chase *et al.* (1993) included members of the genus *Oxalis* as representatives of the Oxalidaceae. The former, more traditional positioning for the family was resolved using the genus *Hypseocharis* as representative of the family Oxalidaceae. This is questionable, due to mounting evidence against *Hypseocharis* being a member of the Oxalidaceae at all (Cronquist 1981, Takhtajan, 1987). Indeed, several more recent phylogenetic studies of the Geraniaceae have removed *Hypseocharis* from the Oxalidaceae entirely, and placed it within the order Geraniales as basal to the Geraniaceae, to which it bears strong morphological resemblance (Thorne 1992; Price & Palmer, 1993). Price and Palmer (1993) also found a sister relationship between *Oxalis* and *Averrhoa*, with bootstrap support (BS) of 84%. This clade was sister to another consisting of *Eucryphia* and *Cephalotus* (BS 85%).

The Angiosperm Phylogeny Group (APG, 1998) expanded on the work of Chase *et al.* (1993). They proposed that the family Oxalidaceae be placed within a new order, the Oxalidales, within the Eurosoid I clade. This clade corresponds with the Rosoid I clade proposed by Chase *et al.* (1993), weakening support for the more traditional placement of Oxalidaceae within the order Geraniales. The APG-defined order Oxalidales (jackknife support 100%), consisting of the families Cephalotaceae,

Connaraceae, Cunoniaceae, Elaeocarpaceae, Oxalidaceae and Tremandraceae, thus corresponds closely with the groupings found by the second search of Chase *et al.* (1993). The APG-defined order Geraniales shares only the families Ledocarpaceae, Vivianaceae and Geraniaceae with previous morphological classifications (Cronquist, 1981; Dahlgren, 1983; Takhtajan, 1987; Price & Palmer, 1993), and included the genus *Hypseocharis* within the Geraniaceae.

Savolainen *et al.* (2000) upheld the establishment of a separate order for the family Oxalidaceae (BS 88%), within their nearly complete analysis of the phylogeny of the eudicots, based upon combined *atpB* and *rbcL* sequence data. They considered the order Oxalidales, in the eurosid I clade, to consist of the following six families: Brunelliaceae, Cephalotaceae, Connaraceae, Cunoniaceae, Elaeocarpaceae and Oxalidaceae. The family Oxalidaceae is sister to the rest of the order. The families Eucryphiaceae and Tremandraceae are still represented, but were embedded within the families Cunoniaceae and Elaeocarpaceae respectively. Savolainen *et al.* (2000) resurrected the family Brunelliaceae, which had been embedded within the Cunoniaceae by the APG study (1998). *Lepidobotrys* is placed within the sister group to the Oxalidales, namely the Celastrales, where it is the strongly supported sister terminal to the genus *Ruptiliocarpon* (bootstrap support 100%). The remainder of the order Geraniales *sensu* Cronquist (1981) is scattered throughout the higher eudicots (Balsaminaceae within the order Ericales, which is sister to the Euasterid clade; Limnanthaceae and Tropaeolaceae deeply embedded within the Brassicales). The type family Geraniaceae was resolved as part of the clade sister to the entire Eurosids II clade. The results of Savolainen *et al.* (2000) therefore suggested that there is considerable phylogenetic distance between the family Oxalidaceae and members of the highly polyphyletic order Geraniales, to which it traditionally belonged.

The *rbcL* gene is considered of great utility in the reconstruction of the overall angiosperm family tree, given its slow mutation rate and consequent low levels of homoplasy (Palmer *et al.*, 1988). There would thus appear to be strong sequence-based support for the removal of the family Oxalidaceae from the order Geraniales and its replacement within the Eurosids I clade of the APG classification. The advent of DNA-based classification systems based upon evidence from molecular data has thus resulted in the elevation of the Oxalidaceae to a separate, ordinal status. This

family has also been significantly distanced from the Geraniales, an order that has undergone extensive rearrangement.

Despite strong support from DNA sequence data for the phylogenetic placement of the family Oxalidaceae, an in-depth literature review revealed no molecular studies, other than those cited, of this taxon at generic level. None of the cited studies have included more than two genera of the family Oxalidaceae *sensu* Cronquist (1981), and thus could not elucidate patterns of relationships within the family itself, or the precise complement of genera contained therein. Further cladistic analyses at the familial and generic levels, using both morphological and molecular data, are awaited. A phylogenetic reconstruction of the family utilising *rbcL* sequence data is currently underway at the University of Leiden (B. Gravendeel, pers. com.).

At present all that can be said with reasonable certainty is that the family Oxalidaceae includes the genera *Oxalis* and *Averrhoa*, for which cladistic evidence is available to support the relationship (Chase *et al.*, 1993; Price & Palmer, 1993; APG, 1998; Savolainen *et al.*, 2000). *Biophytum*, which bears strong morphological resemblance to *Oxalis*, and is included in the family by all classification systems, should also resolve within the family. The genera *Sarcotheca* and *Dapania* are similarly included by most taxonomists, and are usually associated with *Averrhoa*; consequently the probabilities of inclusion within the Oxalidaceae cannot be overlooked.

The genus Oxalis in southern Africa: *Oxalis* is a cosmopolitan genus consisting of roughly 800 species (Chant, 1993). It exhibits two major centres of diversity, namely in southern Central America and in the South-Western Cape of the Republic of South Africa (Knuth, 1930). The genus is morphologically highly variable, consisting of trees, shrubs, annuals and geophytes. Included members of the genus often, but not always, display trifoliate leaves. The leaves exhibit nastic movements in several American sections, a feature shared with *Biophytum*; little is known of such movements in southern African species. Indigenous South African taxa are all geophytic perennials, utilising bulbous underground organs for resource storage, dormancy and asexual reproduction.

The flowers exhibit very little character variation throughout the genus. They are uniformly actinomorphic, funnel-shaped and pentamerous. *Oxalis* is a member of one of only three confirmed angiosperm families (the Oxalidaceae, the Lythraceae, and the Pontederiaceae), whose flowers exhibit the morphologically highly restrictive tristylous reproductive system (Ornduff, 1974). Salter (1944) affirmed that all southern African taxa are morphologically, if not also functionally, tristylous (although this is not always the case for American taxa). Consequently, most floral characteristics are fixed in a set syndrome (Barrett and Richards, 1992). Thus flower characters, which are traditionally considered to be of great taxonomic value, are of little aid in elucidating the natural relationships within the genus.

The genus is an ubiquitous feature of winter in the Cape Region (Cowling and Hilton-Taylor, 1997). *Oxalis* species are often among the first taxa to flower after the first winter rains. The larger, more colourful taxa usually form huge populations, presumably to attract pollinators effectively. Smaller, more cryptic taxa are also presumably insect-pollinated; however, little data are available for pollination vectors of the genus in South Africa. Salter (1944) provided cursory notes on this subject, whilst Crouch (1989) identified blister beetles, thrips and butterflies as pollinators of *Oxalis obliquifolia* Steud ex A. Rich., a widespread species in southern Africa. Crouch (1989) also identified thrips as amongst the main pollinators of the introduced species *Oxalis corniculata*. Oliver (1993) also noted thrips present on the species *Oxalis oculifera*. The manuscript species *Oxalis fragrans*, which bears white, night flowering, strongly scented flowers, could indicate a developing moth-pollination syndrome (Bayer, 1992).

Oxalis seems to be still actively speciating in South Africa, as species complexes and varieties are rife, showing great variation in leaf size and shape, growth habit, flowering time and period, morphological responses to stress and flower colour. This further complicates attempts at classification. More recent attempts by Bayer (1992) to clarify the taxonomic situation within certain sections have not been accepted by all authors (Dreyer, 1993).

Comprehensive published research on South African *Oxalis* taxa is sparse. Aside from the large-scale nomenclatural typification by Knuth (1930), the only other available study pertaining to all of the South African members of the genus is the

definitive monograph written by Salter in 1944. He attempted a natural classification of the genus in the region based almost exclusively on morphological characteristics. No evolutionary hypotheses were postulated and the classification system was not attained through evolutionary reconstruction methods. Indeed, Salter (1944) admitted that many of his postulated sections and subsections were based on scant and questionable characters, and thus had weak cohesion as natural entities. Despite the disadvantages of age and artificial classification, these two monographs remain the only subgeneric-level treatments of South African *Oxalis* available in the literature. Various other authors have contributed in several fields to a further understanding of the genus; however, few South African species were used and the results can thus seldom be integrated into a full-scale treatment of the genus without making extensive assumptions.

The Salter (1944) monograph attempted to classify all indigenous and naturalised taxa in the region presently covered by the Republic of South Africa and Namibia. He classified all taxa into one of 11 sections, of which the first two, sections *Corniculatae* and *Ionoxalis*, contained a single introduced American weed species each. All indigenous taxa were classified into the remaining nine sections. Very few of these sections were based upon or retained from previous taxonomic studies (Knuth, 1930).

Salter (1944) considered the section *Cernuae*, with umbellate inflorescences and deeply incised leaflets, to contain the most primitive indigenous taxa in the region. He considered the remaining species, with single-flowered inflorescences, to form two broad groupings. The first group, containing most of the members of the sections *Oppositae*, *Sagittatae*, *Foveolatae*, *Stictophyllae*, *Campanulatae*, *Crassulae* and *Latifoliolatae*, are (mostly) stemless plants with (mostly) broad leaflets. The other group, containing the remainder of taxa, consists of (mostly) caulescent plants with (mostly) linear to linear-cuneate leaflets. Most of these latter species are contained in the single huge section *Angustatae*.

Salter (1944) admitted that this classification was artificial. Many “stemless” species are found in section *Angustatae*, whereas a variety of strongly caulescent taxa are found in sections *Oppositae* and *Latifoliolatae*. Several taxa in sections *Oppositae* and *Foveolatae* possess the deeply incised leaflets similar to those of section *Cernuae*. Taxa in section *Oppositae* possess the inflated epidermal cells characteristic of section

Foveolatae. Taxa of section *Latifoliolatae*, an entirely artificial section (Salter, 1944), variously show features found in sections *Oppositae*, *Foveolatae* and *Angustatae*.

Salter (1944) stated that, despite the admitted shortcomings of this classification system, the major divisions he instated involved the lowest number of exceptions. With the limited knowledge at his disposal, Salter (1944) considered this system to be the most optimal classification. Of the two available classification systems for the genus in South Africa, the one by Salter (1944) is the most recent, and appears to be the more natural.

Further work on the genus has concentrated on fields other than morphological classification. Heitz (1927), Yamashita (1935), Warburg (1938), Marks (1956), and Dreyer and Johnson (2000) provided chromosome numbers for a few African and American *Oxalis* species. However, to date, chromosome numbers from only 26 taxa native to South Africa are known. Cytology remains a very promising area of research for the genus (Dreyer and Johnson, 2000), which is likely to elucidate further phylogenetic affinities.

Oberlander *et al.* (2002) proposed preliminary biogeographic patterns for *Oxalis* in South Africa based on data from herbarium labels, outlining centres of diversity and endemism for the genus. *Oxalis* is distributed in a pattern that is common to many other taxa of the Cape Region (Oliver *et al.*, 1983). Several peculiarities of distribution could be explained by the propensity of the genus to exploit two Biomes within the region, a feature shared with very few other taxa. Centres of diversity are found on the Cape Peninsula and adjacent flats, the Cederberg/Knersvlakte region and in northern Namaqualand; these centres could indicate the different environmental pressures that are responsible for speciation in the genus.

Despite this paucity in the literature, the various authors dealing with the genus have cautiously agreed on certain points, amongst them the putative monophyly of the South African taxa. Knuth (1930) assigned all known taxa from the region to six sections (sections 32-37), and considered them closely related. Huynh (1969) agreed with this reasoning, citing the palynological similarities and the universal bulbous nature of South African taxa as possible shared characters. However, this hypothesis remains to be tested.

Section *Angustatae* subsection *Lineares*: Section *Angustatae* is the largest South African section as defined by Salter (1944), and contains 82 species and 112 taxa. These taxa are further grouped into 6 subsections: subsection *Pardales*, with 17 taxa; subsection *Sessilifoliae*, with 23 taxa; subsection *Xanthotrichae*, with 3 taxa; subsection *Lineares*, with 54 taxa; subsection *Glandulosae*, with 8 taxa; and subsection *Multifoliolatae*, with 7 taxa. Subsection *Lineares* is the largest of these subsections, but is morphologically also the most weakly defined.

Salter (1944) defined subsection *Lineares* based on the shared possession of the following characters: narrow, linear, oblong or cuneate leaflets, usually three in number, but rarely five or seven; and a caulescent habit, except in *Oxalis albiuscula* Salter, *O. kamiesbergensis* Salter, and *O. oligophylla*. He also expressly stated: “The species included... (*within this subsection*)... are not necessarily all closely related, but affinities are placed as far as possible in juxtaposition.” An attempt was made to group natural species cohesions within subsection *Lineares*, but the monophyly of the subsection remains doubtful.

There are seven such “groups of related taxa”, hereafter referred to as assemblages. Salter (1944) did not refer to them as such, nor number them. However, for the purposes of this study, each assemblage is described below. For ease of reference, the same taxon numbers as in Salter (1944) are used to refer to species assigned to each assemblage, and certain salient characteristics of each assemblage are also listed. Pictures of some of the species included in the current study are provided in Figures 1-8.

Assemblage one consists of the species *Oxalis primuloides*, *Oxalis linearis*, *Oxalis quinata* Savign. and *Oxalis exserta* (spp. 148-151, Salter, 1944). These four species are characterised by a hypercrateriform, densely pubescent corolla tube. The plants are reasonably robust and woody, and grow in the drier inland areas and plateaux around Springbok and Niewoudtville.

Assemblage two includes two species, *Oxalis gracilis* Jacq. (sp. 152) and *Oxalis helicoides* (sp. 153), which both exhibit a polished, corticate stem that often grows in a spiral. The five species of assemblage three (spp. 154-158) are bound together by the single shared character of a mature fruit capsule exerted well beyond the calyx.

Assemblages four (spp. 159-173) and five (spp. 174-181) both include taxa with a scarcely exerted fruit capsule and a simple caulescent stem, as opposed to assemblages one, two and six, which have branching stems. Assemblages four and five differ mainly in that species in the larger assemblage four possess teeth on the longer filaments of the flower, a character lacking in members of assemblage five.

The single “good” species *Oxalis reclinata* (sp. 182) is found in Assemblage six. This species bears a branching main stem, and sometimes cuneate-obovate leaflets. This is perhaps the most weakly defined assemblage.

Assemblage seven contains the three taxa in subsection *Lineares* that are not caulescent, or very shortly so. These are *Oxalis kamiesbergensis* Salter, *Oxalis albiuscula* Salter and *Oxalis oligophylla*. Although *O. kamiesbergensis* and *O. albiuscula* are similar to one another, *O. oligophylla* possesses strikingly different leaflets and occurs in a contrasting habitat to the other two taxa.

Salter (1944) considered these seven assemblages to be mostly natural taxa. However, if not supported by other, stronger characters, the vague characters used to define many of these assemblages could lead equally well to artificial groupings as to natural ones. For instance, *Oxalis albiuscula*, assemblage seven, can possess a very short stem. Does this invalidate the classification of this species? Similarly, *Oxalis reclinata*, the monotypic “good” representative of assemblage six, is defined solely in the Salter (1944) keys by the possession of an uncommon character, namely the occasional occurrence of cuneate-obovate leaflets. It is thus quite possible for even these supposedly natural assemblages to be polyphyletic.

Palynology: Palynological studies by Dreyer (1996) provide an interesting contrast to the morphological classification of Salter (1944). Both reticulate (Figure 9) and supra-areolate (Figure 10) pollen types were observed throughout subsection *Lineares*, and both are also found in section *Angustatae* subsection *Sessifoliolatae* and section *Latifoliolatae*. Specifically, the distribution of different pollen types in section *Latifoliolatae* corresponds closely to the “groups of related taxa” postulated by Salter (1944) for this unnatural section.

Dreyer (1996) regarded reticulate pollen (Type C, Figure 9) as plesiomorphic amongst South African *Oxalis*, with supra-areolate pollen (Type D, Figure 10) as derived. The artificial distribution of the highly derived Type-D pollen is questionable, and contradicts the putative monophyly of subsection *Lineares* (Dreyer, 1996). Huynh (1969) segregated all taxa possessing D-type pollen into a single palynological grouping, but made no comments as to possible phylogenetic implications.

The distribution of the reticulate and supra-areolate pollen types within subs. *Lineares* corresponds very strongly with the groupings within the subsection as postulated by Salter (1944). The species contained in assemblages one, two and six all share the presence of supra-areolate pollen, whereas the pollen of species in assemblage four is entirely reticulate (Chapter 2, Table 1).

Some of the palynological data (Dreyer, 1996) do conflict with the taxon placement of Salter (1944). *Oxalis levis* and *O. comptonii*, grouped in assemblage five (Salter, 1944), possess reticulate pollen in an otherwise uniform assemblage of species with supra-areolate pollen. Likewise *O. oligophylla*, of assemblage seven (Salter, 1944), exhibits C-type pollen in opposition to the other two taxa, which possess supra-areolate pollen. Such fundamental variation in pollen type for these taxa questions the corresponding morphological groupings proposed by Salter (1944).

Morphological characteristics, as defined by Salter (1944), are of little use in a direct phylogenetic analysis of the subsection. This is due to the extreme morphological plasticity of the plant organs in *Oxalis*, probably resulting from a combination of genetic and epharmonic variation. Palynological evidence does, however, support most of the proposed groupings within the subsection, whilst weakening evidence for subsection *Lineares* as a natural taxon. Since the work of Dreyer (1996), the phylogenetic classification of subsection *Lineares* has not been further clarified. As a result, the approach used in the present study was to use DNA-based evidence in order to assess the phylogenetic affinities of this subsection.

trnL-trnF: Phylogenetic assessments utilising molecular data have proven useful in elucidating ambiguous taxon relationships and rival evolutionary theories at virtually every taxonomic level. Sequence data from the *rbcL* gene region of the plastid

genome have in recent years clarified higher order angiosperm relationships to the satisfaction of most systematists (Chase *et al.*, 1993; Savolainen *et al.*, 2001). Family and genus level studies, utilising a variety of chloroplast and nuclear gene/non-coding regions, have proven exceptionally useful at evaluating classification systems, establishing monophyly, studying character evolution, and directing further research. Molecular phylogenetic methods have also been utilised at species and population level.

To date, molecular studies of *Oxalis* have been limited. The most comprehensive work utilised ITS1 nuclear ribosomal and nuclear chloroplast-expressed glutamine synthetase data (Emshwiller and Doyle, 1998). However, this paper deals exclusively with American taxa. No large-scale molecular study of *Oxalis* has been conducted for South African taxa, and they remain phylogenetically understudied and their relationships thus unclear.

The combined *trnL* intron, *trnL* exon and *trnL-trnF* intergenic spacer region of the plastid genome, hereafter referred to as *trnL-trnF*, has not been extensively used with reference to phylogenetic reconstruction in *Oxalis*. This plastid region has been utilised to elucidate species-level relationships in many other angiosperm taxa (Soltis and Soltis, 1998), and at a wide variety of taxonomic levels. Sheahan and Chase (2000) utilised this region in tandem with the *rbcL* gene to reconstruct relationships amongst the genera of the Zygophyllaceae. Their results exhibited strong agreement with previous morphological phylogenetic trees for the family. A separate analysis of South African representatives of this family, also utilising *trnL-trnF* (Makwarela, 2001), confirmed both the reconstruction of Sheahan & Chase (2000), and a recent morphological reclassification of the genus *Zygophyllum* L. (Van Zyl, 2000). Sweeney and Price (2000) indicated the polyphyly of the genus *Dentaria* L. in the Brassicaceae based on data from the *trnL* intron and the gene *ndhF*. Linder and Eldenas (2000) used both morphological and *trnL-trnF* data in their analysis of the African Restionaceae, indicating reasonably strong support for the monophyly of the taxa within this region. Wojciechowski *et al.* (1999) used nuclear ribosomal ITS and plastid *trnL* intron data to analyse the major Fabaceae genus *Astragalus* L., resolving the position of the proposed segregate genera *Astracantha* Podlech and *Orophaca* (T.

& G.) Britton, and indicating the monophyly of the vast majority of *Astragalus* species.

A recent pilot molecular study performed on plastid *trnL-trnF* sequence data of a limited number of South African *Oxalis* taxa has confirmed the potential value of this region in a phylogenetic assessment of *Oxalis* (Johnson, 2000). Although the study by Johnson (2000) included only sixteen South African taxa, her analysis, utilising *trnL-trnF* and nuclear ITS sequences, retrieved clades comparable to several of the sections postulated by Salter (1944). She did, however, retrieve several major departures from this classification as well (Johnson, 2000). ITS sequence data did not prove informative in cladistic analyses, due to too high levels of sequence variation and consequent saturation. In contrast, *trnL-trnF* sequence data provided reasonable resolution in preliminary trees and therefore lent itself to further analysis within the genus.

As one of the largest genera in the Cape Flora, and one of the most threatened (57 Red Data Book taxa within the region, Hilton-Taylor, 1996), *Oxalis* remains a priority for intensive phylogenetic analysis. It should be noted that the number of Red Data Book taxa could be significantly increased once the extremely variable “group species”, such as *Oxalis obtusa* and *Oxalis purpurea*, are studied intensively, and their variation assessed correctly. Huynh (1969) has indicated significant pollen variation within individual taxa, such as within the “group species” *O. purpurea* and *Oxalis eckloniana*. This could indicate much higher levels of variation than those typically assigned to a species. A recent international incentive focussing on the phylogenetic relationships within selected large genera of the Cape Floristic Region has provided further impetus to this study (the Large Cape Genera collaborative incentive). Reconstructions of a complete species-level phylogenetic tree for *Oxalis*, of which the current study represents a starting point, will be integrated into this larger project.

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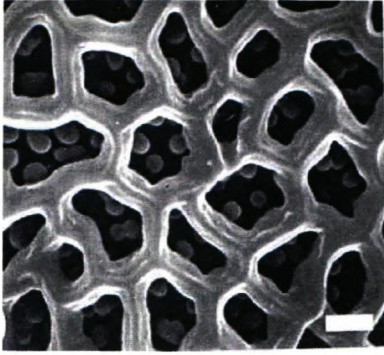


FIGURE 9: Mesocolpial view of a pollen grain of *Oxalis comptonii*. Note the typical reticulate (C-type) nature of the tectum. Scale bar = 1 μ m.

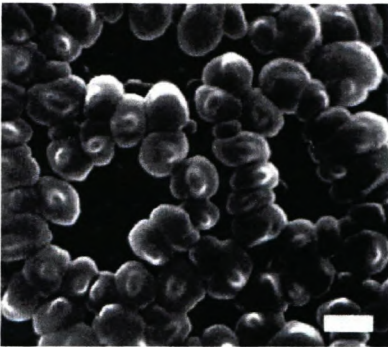


FIGURE 10: Mesocolpial view of a pollen grain of *Oxalis linearis*. Note the prominent supra-tectal structures typical of D-type pollen. Scale bar = 1 μ m.

CHAPTER 2

Evidence to refute the monophyly of *Oxalis* section *Angustatae* subsection *Lineares* based on non-coding *trnL-trnF* plastid sequence data

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Abstract:

Current morphological and palynological studies have only partially resolved the taxonomic classification of *Oxalis* L. in South Africa. Here a molecular phylogenetic reconstruction of relationships within the large section *Angustatae* subsection *Lineares*, using plastid *trnL-trnF* non-coding DNA sequence data, is presented. The results of this study indicate that both section *Angustatae* and three of the subsections contained therein are conclusively polyphyletic. Of the remaining subsections within section *Angustatae*, only subsection *Pardales* was retrieved as being monophyletic. Subsection *Lineares* is shown to be polyphyletic, and the postulated relationships within the subsection are refuted. The members of subsection *Lineares* resolve into one weakly supported and two strongly supported clades in the phylogeny presented here.

Introduction:

The genus *Oxalis* is a large and cosmopolitan taxon with strong representation in the temperate and tropical floras of South and Central America, and in southern Africa (Denton, 1973). The genus exhibits great morphological diversity, including trees, shrubs, herbaceous annuals, and geophytic growth forms. Although the taxonomy of Central American *Oxalis* species is reasonably well established (Denton, 1973;

Lourteig, 1975; Lourteig, 1979), the taxonomy of the southern African representatives still requires intensive research.

Salter (1944) undertook the most recent large-scale taxonomic study of the genus in southern Africa. Despite the undoubted value of this monograph, the study was conducted utilising only macro-morphological data. The author admitted that many of his sectional and subsectional taxa may be artificial, and that the study was only a starting point from which more intensive research and classification could proceed. The taxonomy of southern African *Oxalis* could therefore benefit greatly through the application of modern phylogenetic techniques.

The largest of the sections described by Salter (1944) is section *Angustatae*, which with 82 species contains nearly 40% of the southern African representatives of the genus. The members of section *Angustatae* are defined by the shared possession of a mostly caulescent habit and linear to oblong leaflets that are at least twice as long as broad. However, the taxonomic status of this section has never been phylogenetically tested.

Section *Angustatae* is further divided into six subsections, based on morphological characters such as leaf petiolation, position of the leaves on the stem, number of leaflets per leaf, and the nature and distribution of epidermal hairs on the plant body. Subsection *Lineares* is by far the largest of these subsections.

Subsection *Lineares* consists of 39 species and 54 taxa, arranged into seven assemblages (Chapter 1) of closely related species (Salter, 1944). The relationships both within and among these assemblages are uncertain, and a reassessment of their taxonomic status would benefit from the incorporation of additional characters to those morphological characters used by Salter (1944).

Since the Salter (1944) publication, systematic work on this subsection has been confined to an inclusive palynological study of all South African and Namibian *Oxalis* taxa, including subsection *Lineares* (Dreyer, 1996). Pollen data corroborated several of the assemblages described in subsection *Lineares* (Salter, 1944), whilst questioning the placement of certain taxa in other assemblages. Relationships among these assemblages were also reassessed with regard to the distribution of reticulate and supra-areolate pollen between each assemblage. These two pollen types are radically different, and Dreyer (1996) considered supra-areolate pollen grains to be

more derived than reticulate pollen. Assemblages one, two, five and six consist solely or almost solely of taxa with supra-areolate pollen, a feature shared with groupings of taxa in section *Latifoliolatae* and section *Angustatae* subsection *Sessilifoliatae*. Assemblage four contains taxa with solely reticulate pollen grains, a feature that is considered the relictual state in *Oxalis* (Dreyer, 1996). Assemblages three and seven contained both pollen types, and thus presented additional evidence to regard these groupings as possibly unnatural.

Salter's (1944) classification of subsection *Lineares* as a whole is presumed to be artificial in nature. The recent palynological evidence presented by Dreyer (1996) did not completely agree with the groupings proposed by Salter (1944). It is thus appropriate that additional evidence be sought to elucidate phylogenetic patterns within subsection *Lineares*.

The advent of phylogenetic reconstruction techniques has revolutionised the field of biological systematics and the way relationships are viewed within biological systems. DNA sequencing techniques have similarly affected current classification systems by vastly increasing the number of discrete characters that can be used to reconstruct the evolutionary history of taxa.

As discussed in Chapter 1, plastid gene and non-coding regions have been extensively used in the reconstruction of phylogenies for green plant lineages (Chapter 1). A recent pilot study (Johnson, 2000) of the non-coding plastid *trnL-trnF* region in *Oxalis* indicated the possible utility of this region in resolving the taxonomic problems encountered in the strictly morphological classification of Salter (1944).

The *trnL-trnF* region (typically 900-950 base pairs in length) consists almost exclusively of non-coding intron and spacer sequence, the only exception being the *trnL* 3' exon, which consists of approximately 50 base pairs (Taberlet *et al.*, 1991). Non-coding regions of the plastid genome are exposed to different selective pressures than expressed regions. Although initial assumptions of random, independent mutations and an increased mutation rate have not been entirely merited, non-coding DNA still possesses considerable phylogenetic signal (Kelchner, 2000). Consequently, *trnL-trnF* is an ideal plastid region to use for phylogenetic reconstruction.

The aim of this article is thus to utilise the *trnL-trnF* non-coding plastid sequence region in order to reconstruct a sequence-based phylogeny of *Oxalis* section *Angustatae* subsection *Lineares*. This phylogeny will then be used to evaluate the monophyly of subsection *Lineares* and its segregate assemblages.

Materials and Methods:

Taxonomic and palynological data for all species included in the study are summarised in Table 1. South African species collected over the two-year period of the study were chosen to reflect the classification of *Oxalis* as proposed by Salter (1994). Sequences for other genera within the Oxalidaceae, not indigenous to the South African region, were kindly provided by Dr. Gravendeel (University of Leiden, the Netherlands). These were representatives of the genera *Averrhoa* L., *Sarcotheca* Blume and *Biophytum* D.C., and were used as generic outgroups. Although no molecular evidence has yet supported the relationship between *Oxalis* and *Biophytum*, the latter taxon is considered closely related to the former by virtually all authors, and the morphological resemblance between the two genera is strong (Chapter 1). There is thus justification for including this taxon as well as *Averrhoa bilimbi* Linn., for which *rbcL* evidence supports a close relationship to *Oxalis* (Chapter 1). *Sarcotheca laxa* Knuth has been placed in several conflicting taxonomic positions (Chapter 1) and was included as a more distant putative relative.

Within the genus *Oxalis*, the American taxa are represented by the two species naturalised in southern Africa: *Oxalis corniculata* and *O. latifolia*. Ingroup selection was guided so as to include at least one, and preferably two species from each of the sections and subsections recognised by Salter (1944). Taxa that palynological and/or morphological data had proved recalcitrant to phylogenetic placement were focussed upon. These taxa formed an infrageneric backdrop that could clarify the placement of the subsection *Lineares*, or its component parts, within the genus.

All species in this study were collected during the winter rainfall period (from April to September) of the South-Western Cape in 2001 and 2002. These months correspond to the growth and flowering periods of most native South African *Oxalis* species. Herbarium specimens were prepared for virtually all the collected taxa.

Exceptions were placed in the living *Oxalis* collection at the University of Stellenbosch Botanical Gardens, Stellenbosch. Herbarium specimens were identified both through the use of keys provided by Salter (1944) and by comparison to type specimens in the Compton (NBG) and Bolus (BOL) herbaria, Cape Town. All new herbarium specimens were housed in the Stellenbosch University Herbarium (STEU), Stellenbosch, South Africa. In addition to the living and herbarium collections, samples for DNA sequencing studies (four to five young leaves preserved in silica gel) were also collected for most *Oxalis* taxa encountered in the field.

In total, 24 of the 38 recognised species in subsection *Lineares* were collected over the course of the study. Of the remaining fourteen species, Salter (1944) retained the specific status of two of these with only the greatest reluctance, due to poor herbarium specimens with uncertain or unknown localities. Of the remaining twelve species, five are apparently very localised and the type localities have since been destroyed by agricultural practices and consequently these could not be collected. This stresses the fact that many of these taxa are vulnerable to extinction due to encroaching agricultural development. It is hoped that alternative populations of these species will be located in the future.

DNA was extracted using the 2XCTAB method of Doyle & Doyle (1987). Several species proved difficult to amplify through Polymerase Chain Reactions (PCR) after direct DNA extraction. Further rounds of ethanol precipitation for the extracted DNA of these species resolved this problem, indicating possible interference by secondary metabolites.

Extracted DNA for the *trnL-trnF* region was amplified using the following PCR parameters, (94°C for 1', 65°C for 1', 72°C for 1'30", for 30 cycles, 6' at 72°C, holding temp.: 15°C) utilising the primers 'c' and 'f' (Taberlet *et al.*, 1991). The 100 µl PCR cocktail consisted of 4 µl dNTP's (200 µM), 10 µl Supertherm buffer 455, 10 µl Supertherm MgCl₂ at a 2,5 mmol/L concentration, 1 µl primer C (50 pmol. µl⁻¹), 1 µl primer F (50 pmol. µl⁻¹), 1 µl Taq polymerase containing approx. 1 unit of Taq (produced by the protocol of Pluthero, 1993), 4 µl sample containing 20-50 ng of DNA, and 69 µl of ultra-distilled water.

PCR products were purified using WizardPrep© kits. Purified PCR products were then sequenced using Big Dye™ Terminator RR kits (Applied Biosystems). The same primers utilised for the PCR reactions were used for the dye-terminator reactions, following the protocol: 96°C for 10", 52°C for 30", 60°C for 4', for 35 cycles.

Products of sequencing reactions were analysed on an ABI 377 sequencer, Central Analytical Facility, University of Stellenbosch. Alignment of the complimentary sequences was carried out using the computer alignment program DAPSA (Harley, 1998), with final refining being performed manually.

Phylogenetic analysis was performed using the computer package PAUP 4.0 beta 10 (Swofford, 2000) on an Apple Power PC. All characters were coded as equally weighted, single and discrete. Three phylogenetically informative indels were coded as single, discrete characters with equal weighting. As is standard practice in publication of molecular systematic data, terminal characters at both ends of the sequence region were omitted due to sequence uncertainty. In the current data matrix, this affected the first ten and last eleven characters. A heuristic search of 1000 replicates of random taxon-addition was implemented. TBR branch swapping was performed with the MULPARS and STEEPEST DESCENT options on, and a limit of ten trees saved per replicate to reduce time spent swapping on islands of equally most parsimonious trees. Internal node support was assessed using the bootstrap (Felsenstein, 1985), with 1000 replicates of simple taxon addition and TBR branch swapping.

Results:

Parsimony analysis: The final aligned *trnL-trnF* matrix consisted of 78 taxa and 1080 characters, the length of the region in individual species varying between 900 and 950 base pairs. Maximum parsimony analysis produced 9890 equally parsimonious trees of length 477. Of the 1059 included characters, 314 were variable, with 141 (45%) being potentially parsimony informative. No new groups were recovered by including the three phylogenetically informative indels, nor were bootstrap values significantly altered. One of the equally most parsimonious trees was chosen at random, and is shown in Figure 1. Branch lengths and bootstrap support

values are indicated respectively above and below the branches in the tree. Arrows indicate nodes that collapse in the strict consensus of 9890 trees. Coloured bars indicate clades referred to in the text.

Characteristics of the *trnL-trnF* region for the analysed species are summarised in Table 2. The parsimony analysis yielded a consistency index (CI) of 0,788 and a retention index (RI) of 0,839. This indicates reasonably low levels of homoplasy. Transition versus transversion ratios (ti/tv) were approximately equal (1,1:1,0) for *trnL-trnF* in *Oxalis* (Figure 2). Various authors have found a one-to-one ratio for ti/tv ratios in *trnL-trnF* (Bakker *et al.*, 2000; Reeves *et al.*, 2001); this appears to be a characteristic of the non-coding nature of the *trnL-trnF* region. CI's and RI's for both ti's and tv's were equally high. This, taken in conjunction with the approximately one-to-one ti/tv ratio, indicates equal rates of evolution between ti's and tv's. Concerns regarding possible saturation of ti's due to their increased frequency of change are thus not merited. This justifies the equal weighting scheme used in the general phylogenetic analysis for ti's versus tv's.

***trnL-trnF* tree:** A prominent feature of the phylogenetic tree is the lack of resolution retrieved along the spine of the tree, resulting in a basal polytomy from which all analysed South African taxa, and the American invasive *Oxalis latifolia*, arise (bootstrap support (BS) 99%). *Oxalis corniculata* is sister to this large, unresolved clade, with 100% BS. Although the outgroup taxa have been presented as a monophyletic clade for aesthetic reasons (clade I, Figure 1), in unconstrained analyses *Biophytum* was resolved as sister to *Oxalis*, with moderately strong BS (85%). The two *Biophytum* taxa resolved as sister to one another in all trees, always with 100% BS.

Three major clades arise from the polytomy, as well as the single species *Oxalis commutata*. All three clades have branch lengths of only one step, and receive no bootstrap support. However, various groupings within these clades receive strong support, several of which are completely unexpected in the context of the current taxonomy.

A grouping consisting of section *Cermuae* and the naturalised American taxon *Oxalis latifolia* (clade II) resolves as a cohesive entity in a large percentage of trees,

but is supported by a branch length of one and does not resolve with a bootstrap value above 50%. Included members of section *Cernuae* subsection *Eu-cernuae* do resolve together, but with poor support. *Oxalis latifolia* is the weakly supported sister (58%) to *Oxalis dentata* (section *Cernuae* subsection *Lividae*).

Of the remaining two clades arising directly from the base of the polytomy, the smaller, containing members of a diverse range of sections, is henceforward referred to as clade III (Figure 1). This large group consists of representatives of sections *Cernuae*, *Oppositae*, *Stictophyllae*, *Foveolatae*, *Crassulae*, *Campanulatae*, *Latifoliolatae* and *Angustatae* subsections *Xanthotrichae* and *Sessilifoliatae*. Support for this clade, including *Oxalis caprina*, is less than 50%. However, the clade sister to *O. caprina* receives moderately high bootstrap support (81%). This grouping is henceforth referred to as the “core” clade III.

Despite robust support for the “core” clade III, support for many nodes within this clade is weak and the retrieved groups do not correspond to any morphological or palynological grouping. Many of these groupings are most likely artefacts of sampling bias. The clade containing *Oxalis purpurea*, *Oxalis luteola* and *Oxalis melanosticta* is unexpected, as *O. luteola* has not previously been associated with the other two taxa. There is too little data present in the current study to elucidate more accurate patterns within this group.

The remaining, and largest clade stemming from the base of the polytomy (clade IV), consists of the bulk of section *Angustatae*, as well as representatives of sections *Sagittatae* and *Latifoliolatae*. Despite the few sections contained therein, this group received no bootstrap support and collapsed in the strict consensus tree.

Within this clade, another large grouping (clade IV_B) receives very high bootstrap support (96%). This clade consists of members of sections *Latifoliolatae*, *Angustatae* subs. *Sessilifoliatae* and *Angustatae* subs. *Lineares*. Clade IV_B resolves into two main groupings, with most terminal nodes receiving weak bootstrap support or no resolution. Seven taxa in this clade possess a unique ten base-pair insertion. These taxa are not resolved as monophyletic in this study; instead they form a weakly supported (58%) group that also includes two other taxa lacking this insertion.

A clade consisting entirely of members of section *Angustatae* subsection *Pardales* (clade IV_E) was retrieved with poor BS (63%), whilst two members of section *Sagittatae* (clade IV_C) resolved together strongly. The sister grouping of *Oxalis truncatula* and *Oxalis engleriana* (clade IV_A) received no bootstrap support and collapsed in the strict consensus tree. A small clade (clade IV_D) containing an unexpected but strongly supported grouping of *Oxalis oligophylla*, *Oxalis tomentosa*, and *Oxalis minuta* var. *callosa*, was retrieved with 92% BS. In this instance it is important to note the strongly supported separation of the two recognised varieties of *Oxalis minuta*.

The remainder of clade IV resolves into an unsupported grouping, and contains all other included taxa currently placed in section *Angustatae*. This grouping, henceforward referred to as clade IV_F (Figure 1), did not resolve in the strict consensus tree. It consists of two poorly supported clades, one of which is composed of *Oxalis stenopetala*, *Oxalis phloxidiflora* and *Oxalis tenuipes*, all three of which are members of section *Angustatae* subsection *Lineares*, assemblage 3 (BS 52%, Table 1). The other clade has *Oxalis comptonii* as the unsupported sister to a trichotomy consisting of *Oxalis ebracteata*, a small, strongly supported clade consisting of *Oxalis giftbergensis* and *Oxalis tenuis*, and a large, strongly supported (BS 82%) clade with poor internal resolution. This last clade is henceforth referred to as the “core” clade IV_F. Clade IV_F as a whole was not present in the strict consensus tree, and was not resolved at all in several other identical analyses.

Of the seven assemblages of subsection *Lineares* (Table 1) included in this study, one contains only a single species, and a further two are represented by only a single taxon. Consequently no phylogenetic inferences can be made as regards their current taxonomic placement. The remaining four assemblages, containing the majority of species in subs. *Lineares*, are all polyphyletic.

Members of assemblages one, two and six, as well as some species from assemblages three and five, are included in clade IV_B. Other taxa in clade IV_B are currently placed in section *Angustatae* subsection *Sessilifoliatae* and in section *Latifoliolatae*.

Species found solely in assemblage four, as well as some taxa from assemblages three and five, are gathered into clade IV_F. This clade also contains members of section *Angustatae* subsections *Sessilifoliatae* and *Glandulosae*.

Discussion:

Parsimony analysis yielded a remarkably well-resolved tree with very little homoplasy. Although rate variation does contribute to the robust retrieval of several clades, the *trnL-trnF* region does not possess enough data to provide a large-scale pattern of evolution in *Oxalis*. The transition to transversion ratio of close to parity yields extra evidence to refute downweighting of postulated faster-evolving regions/nucleotides.

Section *Angustatae* as a whole is polyphyletic, with member taxa distributed throughout clades III and IV. Furthermore, only one subsection *sensu* Salter (1944) is retrieved as monophyletic, namely subsection *Pardales*, which is defined by several shared morphological characters. Although it is feasible for the majority of section *Angustatae* to eventually resolve into a single group, taxa from other sections will undoubtedly have to be included in this section in order to satisfy the requirements of monophyly. The present study does not include enough data (both character and taxon sampling) to provide an unambiguous answer to this question at this stage.

Within section *Angustatae*, subsection *Sessilifoliatae* is highly polyphyletic. Indeed, this subsection is split between four, well supported, entirely separate parts of the phylogenetic tree. Two taxa (*Oxalis crocea* and *Oxalis viscosa*) form a moderately supported (BS 79%) clade within clade III. *Oxalis hirta* is placed in clade IV_B. *Oxalis giftbergensis* is the strongly supported (BS 83%) sister to *Oxalis tenuis* (subsection *Glandulosae*) within clade IV_F. The remaining two taxa are found in the “core” IV_F clade. *Oxalis tenuifolia* is the strongly supported sister to *Oxalis stictocheila*, of subsection *Lineares* (BS 86%), whilst *Oxalis urbaniana* is weakly sister (BS 62%) to *Oxalis glabra*, also of subsection *Lineares*.

Two members of subsection *Glandulosae* were included in this study, and both are located in the clade sister to the “core” clade IV_F. *Oxalis ebracteata* and *Oxalis tenuis*

do not, however, resolve as sister taxa. Section *Angustatae* subsection *Multifoliolatae* is also polyphyletic. *Oxalis tomentosa* is included in a strongly supported clade (92%), whilst no bootstrap support underlies the clade in which *Oxalis engleriana* is retrieved. There are not enough data to place *O. engleriana* with any confidence.

Section *Angustatae* subsection *Xanthotrichae* is polyphyletic in this study. The two included taxa belong to different groups of the “core” clade III. Both of these groups are very weakly supported, and the inclusion of more data would clarify the taxonomic situation in subsection *Xanthotrichae*.

Section *Angustatae* subsection *Lineares* is clearly polyphyletic: despite concentrations of taxa in clades IV_B and IV_F, neither of these clades consists solely of species from this subsection. Furthermore, *Oxalis oligophylla* resolves very strongly into clade IV_D, which contains taxa never before associated with members of subsection *Lineares* (Salter, 1944; Knuth, 1930). It is thus apparent that subsection *Lineares* is an unnatural taxon, based on *trnL-trnF* based evidence. Certainly more intensive studies should be focussed on *Oxalis oligophylla*, based on such an unequivocal placement for this species as found in the current study.

As mentioned above, two of the seven assemblages postulated by Salter (1944) are represented by only a single taxon in this study. Another monotypic assemblage is nested within clade IV_B. Any comment on their current taxonomic status, on the basis of *trnL-trnF* sequence data, is thus not justified. The members of the four remaining sections are distributed in such a way that their monophyly can be strongly refuted on grounds of *trnL-trnF* data.

Conclusion:

The phylogenetic analysis of *Oxalis* section *Angustatae* subsection *Lineares* using plastid *trnL-trnF* sequence data provides evidence that *Biophytum* is the sister genus to a monophyletic *Oxalis*. Further evidence is presented to indicate that section *Angustatae* is not a natural taxon. Similarly, subsections *Sessilifoliatae* and *Lineares* are shown to be artificial, based on *trnL-trnF* sequence data. Subsection *Pardales* is weakly resolved as monophyletic. Subsections *Glandulosae* and *Multifoliolatae* are

less conclusively retrieved as polyphyletic. Too little data are available to comment on the monophyletic status of subsection *Xanthotrichae* at this stage, although this taxon does not belong in section *Angustatae*, based on *trnL-trnF* data.

Four of the seven “groups of related taxa”, presented by Salter (1944) as the constituents of subsection *Lineares*, are polyphyletic. The remainder are either monotypic assemblages, or only represented by one taxon in the current study. Further taxa and more informative DNA regions are required to further resolve the putative monophyly and relationships amongst these assemblages.

On the whole, *trnL-trnF* sequence data provided a remarkable degree of resolution for a tree based on a single non-coding region. Nearly 36% of all southern African *Oxalis* species were represented in the current study, and all but eight of these taxa are supported in a clade within *Oxalis* with bootstrap support >50%. Despite overall poor resolution along the spine of the tree, several large clades within *Oxalis* received high bootstrap support levels. Major taxonomic inferences for the southern African members of the genus as a whole are premature at this stage due to biased sampling efforts and too little information derived from the *trnL-trnF* region. However, further studies, involving other gene regions and more inclusive numbers of southern African and American taxa, are planned for the future.

Section *Angustatae* subsection *Lineares* was well sampled in this study. Nearly two thirds of total species diversity for the subsection was included in the analysis, and the degree of sampling and high bootstrap support levels indicate conclusively that, based on *trnL-trnF* data, the subsection is polyphyletic.

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TABLE 1: Genus, section, subsection, assemblage (where appropriate), species name and author citation, project (MO) number and pollen type (Dreyer, 1996) for the species included in this study. Classification and number of species in section/subsection (bold brackets), follows Salter (1944). Question marks (?) indicate species not assessed by Dreyer (1996).

Genus	Section	Subsection	Assemblage	Species	MO number	Pollen type
<i>Sarcotheca</i>	-	-	-	<i>Sarcotheca laxa</i> Knuth	SARLAX	C1-4?
<i>Averrhoa</i>	-	-	-	<i>Averrhoa bilimbi</i> Linn.	AVBIL	C1-5?
<i>Biophytum</i>	-	-	-	<i>Biophytum abyssinicum</i> Steud. ex A. Rich	BIOAB	C1-6?
<i>Biophytum</i>	-	-	-	<i>Biophytum</i> sp.	BIOPHIL	C1-7?
<i>Oxalis</i>	Corniculatae (1)	-	-	<i>Oxalis corniculata</i> L.	MO256	C2
	Ionoxalis (1)	-	-	<i>Oxalis latifolia</i> H.B.K.	MO254	C7
	Cernuae (23)	Eu-cernuae (7)	-	<i>Oxalis pes-caprae</i> L. var. <i>sericea</i> (L. f.) Salter	MO93	C2
			-	<i>Oxalis knuthiana</i> Salter	MO153	C3
		Lividae (3)	-	<i>Oxalis dentata</i> Jacq.	MO49	C3
		Costatae (2)	-	<i>Oxalis purpurascens</i> Salter	MO51	C8
		Stellatae (7)	-	<i>Oxalis caprina</i> L.	MO7	C7
	Oppositae (30)	Subintegrae (22)	-	<i>Oxalis obtusa</i> Jacq. var. <i>obtusata</i>	MO122	C3
			-	<i>Oxalis truncatula</i> Jacq.	MO16	C2
			-	<i>Oxalis luteola</i> Jacq. var. <i>luteola</i>	MO257	C3
		Bifurcatae (8)	-	<i>Oxalis bifida</i> Thunb.	MO19	C2
	Stictophyllae (3)	-	-	<i>Oxalis purpurea</i> L.	MO255	C3
		-	-	<i>Oxalis melanosticta</i> Sond. var. <i>melanosticta</i>	MO33	C2
	Foveolatae (25)	-	-	<i>Oxalis pulchella</i> Jacq. var. <i>pulchella</i>	MO23	C3
		-	-	<i>Oxalis pocockiae</i> L. Bolus	MO37	C2
		-	-	<i>Oxalis punctata</i> L. f.	MO50	C2
		-	-	<i>Oxalis lichenoides</i> Salter	MO281	C2
		-	-	<i>Oxalis furcillata</i> Salter var. <i>caulescens</i> Salter	MO228	C10
	Sagittatae (5)	-	-	<i>Oxalis nidulans</i> E. & Z. var. <i>nidulans</i>	MO212	A
		-	-	<i>Oxalis minuta</i> Thunb. var. <i>minuta</i>	MO163	A
		-	-	<i>Oxalis minuta</i> Thunb. var. <i>callosa</i> Salter	MO230	C7
		-	-	<i>Oxalis eckloniana</i> Presl. var. <i>sonderi</i> Salter	MO39	A
	Campanulatae (5)	-	-	<i>Oxalis dregei</i> Sond.	MO225	C10
	Latifoliolatae (28)	-	-	<i>Oxalis campylorrhiza</i> Salter	MO127	C8
		-	-	<i>Oxalis commutata</i> Sond. var. <i>commutata</i>	MO17	C10
		-	-	<i>Oxalis oculifera</i> E. G. H. Oliver	MO295	C10
		-	-	<i>Oxalis tenella</i> Jacq.	MO70	D1
		-	-	<i>Oxalis aridicola</i> Salter	MO103	D1

TABLE 1 continued:

Genus	Section	Subsection	Assemblage	Species	MO number	Pollen type
	<i>Crassulae</i> (8)	-	-	<i>Oxalis flava</i> L.	MO25	C10
		-	-	<i>Oxalis flaviuscula</i> Salter var. <i>longifolia</i> Salter	MO132	C10
		-	-	<i>Oxalis namaquana</i> Sond.	MO144	C2
		-	-	<i>Oxalis louisae</i> Salter	MO139	C10
		-	-	<i>Oxalis salteri</i> L. Bolus	MO280	C10
	<i>Angustatae</i>	<i>Pardales</i> (11)	-	<i>Oxalis capillacea</i> E. Mey. ex Sond. 1	MO38	C2
			-	<i>Oxalis capillacea</i> E. Mey. ex Sond. 2	MO36	C2
			-	<i>Oxalis leptogramma</i> Salter var. <i>leptogramma</i>	MO28	C2
			-	<i>Oxalis grammophylla</i> Salter	MO101	C2
		<i>Sessilifoliae</i> (15)	-	<i>Oxalis hirta</i> L. var. <i>tubiflora</i> (Jacq.) Salter	MO77	D4
			-	<i>Oxalis crocea</i> Salter	MO124	C7
			-	<i>Oxalis viscosa</i> E. Mey. Ex Sond.	MO73	C2
			-	<i>Oxalis giftbergensis</i> Salter	MO292	C15
			-	<i>Oxalis tenuifolia</i> Jacq.	MO258	C8
			-	<i>Oxalis urbaniana</i> Schltr. var. <i>urbaniana</i>	MO229	C8
		<i>Xanthotrichae</i> (3)	-	<i>Oxalis argillacea</i> Bolus f.	MO282	C10
			-	<i>Oxalis adspersa</i> E. & Z.	MO66	C10
		<i>Lineares</i> (39)	one	<i>Oxalis primuloides</i> R. Knuth	MO142	D1
			one	<i>Oxalis linearis</i> Jacq.	MO110	D3
			one	<i>Oxalis exserta</i> Salter	MO117	D1
			two	<i>Oxalis cf. helicoides</i> Salter var. <i>helicoides</i>	MO119	D1
			three	<i>Oxalis campicola</i> Salter	MO276	D1
			three	<i>Oxalis stenopetala</i> Salter	MO106	C7
			three	<i>Oxalis xantha</i> Salter	MO102	D1
			three	<i>Oxalis tenuipes</i> Salter var. <i>tenuipes</i>	MO296	C12
			three	<i>Oxalis phloxidiflora</i> Schltr.	MO113	C14
			four	<i>Oxalis pallens</i> E. & Z.	MO85	C2
			four	<i>Oxalis glabra</i> Thunb.	MO155	C8
			four	<i>Oxalis pusilla</i> Jacq.	MO182	C2
			four	<i>Oxalis versicolor</i> L. var. <i>versicolor</i>	MO307	C8
			four	<i>Oxalis polyphylla</i> Jacq. var. <i>polyphylla</i>	MO79	C8
			four	<i>Oxalis burtoniae</i> Salter	MO304	C8
			four	<i>Oxalis argyrophylla</i> Salter	MO313	C8
			four	<i>Oxalis stictocheila</i> Salter	MO185	C8

TABLE 1 continued:

Genus	Section	Subsection	Assemblage	Species	MO number	Pollen type
			five	<i>Oxalis ciliaris</i> Jacq. var. <i>ciliaris</i>	MO24	D1
			five	<i>Oxalis burkei</i> Sond.	MO29	D1
			five	<i>Oxalis oreophila</i> Salter	MO97	D1
			five	<i>Oxalis blastorrhiza</i> Salter	MO284	D1
			five	<i>Oxalis comptonii</i> Salter	MO298	C8
			six	<i>Oxalis reclinata</i> Jacq. var. <i>reclinata</i>	MO149	D1
			seven	<i>Oxalis oligophylla</i> Salter	MO293	C8
		<i>Glandulosae</i> (7)	-	<i>Oxalis ebracteata</i> Savign.	MO75	C8
			-	<i>Oxalis tenuis</i> Salter	MO289	C9
		<i>Multifoliolatae</i> (7)	-	<i>Oxalis engleriana</i> Schltr.	MO195	C6
			-	<i>Oxalis tomentosa</i> L. f.	MO62	C8
		Not allocated to section	-	<i>Oxalis monophylla</i> L.	MO9	C10

TABLE 2: Statistics for the *trnL-trnF* plastid region in southern African *Oxalis*

Number of trees	9890
Steps	477
Number of included characters	1059
Number of potentially informative characters	141
Consistency Index (CI)	0,788
Retention Index (RI)	0,839
Transitions (ti's): Number of contributed steps	250
CI	0,836
RI	0,88
Transversions (tv's): Number of contributed steps	227
CI	0,74
RI	0,79
Ti/tv ratio	1,1:1,0

CHAPTER 3

Phylogenetic reconstruction of selected southern African *Oxalis* species based on non-coding plastid *trnL-trnF* sequence data: palynological and morphological comparisons

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Abstract:

The systematics of southern African *Oxalis* is unresolved. This article synthesizes recent advances in the knowledge of the genus in this region, namely a palynological treatment of all recognised taxa, and the first attempt at a DNA sequence-based phylogenetic reconstruction for southern African representatives of the genus. These data are compared to the current morphological classification of *Oxalis* in the region. Palynological and molecular data are largely congruent with one another, and differ substantially from the current morphological treatment in several sections. It is concluded that the combined use of pollen data, molecular data and phylogenetic reconstruction techniques can greatly aid our understanding of the patterns of evolution in the genus, and can clarify the highly homoplasious morphological character states of many southern African *Oxalis* taxa.

Introduction:

The genus *Oxalis* is a common and well-known element of the flora of many temperate climate regions, and is especially well known for its weedy tendencies in disturbed habitats. Despite a near-worldwide distribution, the genus exhibits two major centres of diversity, namely in Central America extending down the Andes mountains into South America, and in southern Africa, more specifically the extreme south-western corner of the continent. Although the classification systems of North

and Central American taxa are reasonably well established (Denton, 1973), southern African taxa require urgent taxonomic revision.

The major work on southern African taxa is a monograph by Salter (1944), in which all species recognised at the time within this region were included. This work collated all data from the region and substantially reduced taxonomic confusion and synonymy in the genus. It attempted a natural classification system for the species in the region, and significantly improved on previous taxonomic classifications of *Oxalis*.

Despite the undoubted nomenclatural and taxonomic value of this monograph, the state of systematic knowledge of the genus *Oxalis* in South Africa is still far from complete. The treatment by Salter (1944) was based exclusively on macro-morphological characters, and many of these characters provide little or ambiguous data as to taxonomic placement. In light of the principle of monophyly, virtually none of the morphological characters underpinning the proposed groupings by Salter (1944) can be regarded as shared and derived for those groupings. Several newly discovered and rediscovered species also challenge the Salter (1944) classification (Oliver, 1995; Kumwenda, 2001). Despite these shortcomings, this treatment remains the authoritative work on *Oxalis* species for southern Africa.

Several recent developments in *Oxalis* research have added to the picture of *Oxalis* evolution in southern Africa. Dreyer (1996) completed an exhaustive study of the palynology of the genus *Oxalis* in southern Africa, studying virtually all currently recognised taxa. Her findings included support for several sections and subsections proposed by Salter (1944). However, many other sections consisted of assemblages of species/taxa with clearly unrelated pollen grain types. Salter (1944) considered many of his own groupings to be unnatural, however, and the palynological evidence further weakened the support for these groupings as natural entities.

A more recent cladistic analysis employing non-coding plastid *trnL-trnF* data, and focussing on the unnatural section *Angustatae* subsection *Lineares* (Salter, 1944; Chapter 2), raised further doubt with respect to the monophyly of many of the Salter (1944) sections. Despite the sampling bias evident in the derived phylogenetic reconstruction, many of the Salter (1944) sections and subsections were retrieved as conclusively polyphyletic on the basis of this DNA sequence data.

Due to the discrepancies between the taxonomic classification of Salter (1944), the palynological findings of Dreyer (1996) and the produced phylogenetic analysis (Chapter 2), a more inclusive, comparative approach was thought necessary. The current study attempted to combine the two more recent additions to our knowledge of *Oxalis* in southern Africa, and to compare their findings with the currently recognised classification of Salter (1944).

Materials and Methods:

As stated above, this paper attempts to provide a comparison between the three data sets currently available for southern African *Oxalis* taxa. Morphological data are derived from Salter (1944). All palynological data and references, unless otherwise indicated, are found in Table 1, from Dreyer (1996). The phylogenetic tree and associated clade names, based on non-coding plastid *trnL-trnF* data, are derived from Chapter 2 of the present study. All data, including author citations for species used in the current study, are summarised in Chapter 2; Table 1, and Chapter 2; Figure 1.

TABLE 1: Description of pollen types identified by Dreyer (1996)

A	Tectum rugulate-reticulate, covered with small sharp supratectal spinules
B	Tectum micro-rugulate with numerous conical, supratectal spines
C1	Tectum micro-reticulate; lumina isodiametrically rounded; mesocolpial intraluminary bacula absent
C2	Tectum micro-reticulate; lumina rounded to oblong to slightly angular; mesocolpial intraluminary bacula absent
C3	Tectum finely reticulate; lumina irregularly angular to rounded; mesocolpial intraluminary bacula absent
C4	Tectum reticulate; lumina large, irregularly angular to slightly rounded; mesocolpial intraluminary bacula absent
C5	Tectum complex, micro-reticulate, consisting of muri on two different levels; lumina isodiametrically rounded to oblong; blunt supratectal spinules present

TABLE 1 conti nued:

C6	Tectum a coarse, open reticulum; lumina large, irregularly angular; supratectal spinules present, intraluminary bacula scattered on the nexine floor of the entire grain
C7	Tectum micro-reticulate; lumina irregularly rounded to oblong; intraluminary bacula scattered on the nexine floor of the entire grain
C8	Tectum finely reticulate; lumina irregularly rounded to angular; intraluminary bacula scattered on the nexine floor of the entire grain
C9	Tectum reticulate; lumina angular to rounded; intraluminary bacula scattered on the nexine floor of the entire grain
C10	Tectum finely reticulate; lumina irregularly angular; intraluminary bacula predominantly clustered along the muri
C11	Tectum reticulate; lumina irregularly angular; intraluminary bacula predominantly clustered along the muri
C12	Tectum coarsely reticulate; lumina irregularly angular; intraluminary bacula clustered along the muri
C13	Tectum rugose reticulate; muri thick, distinctly perforated; lumina irregularly angular; intraluminary bacula clustered along the muri
C14	Tectum coarsely reticulate; muri thin, sometimes perforated; lumina irregularly angular; intraluminary bacula clustered along the muri
C15	Tectum very coarsely reticulate; muri relatively thin, sometimes perforated; lumina large, irregularly angular; intraluminary bacula clustered along the muri
D1	Supratectal areolae solitary, clustered or arranged into an obscure negative reticulum; individual areolae 0.75-2.00 μm in diameter
D2	Supratectal areolae irregular, arranged into a distinct negative reticulum; individual areolae large (1.8 μm in diameter)
D3	Supratectal areolae rounded, arranged into a distinct negative reticulum; individual areolae small (0.89 μm in diameter) but prominently enlarged in apocolpial region
D4	Supratectal areolae irregular, arranged into a coarse ridged pattern; individual areolae large (2.27 X 0.98 μm)

Results and Discussion:

When comparing the current morphological classification to the phylogenetic reconstruction based on *trnL-trnF* data, not a single recognised section (Salter, 1944) is retrieved as being monophyletic. The only two retrieved monophyletic taxa are section *Cernuae* subsection *Eu-cernuae* and section *Angustatae* subsection *Pardales*. Both subsections were, however, grossly undersampled for the phylogenetic analysis. It is also significant that even if every polytomous node were to be resolved in this tree, only two small sections (sections *Sagittatae* and *Stictophyllae*) could possibly be retrieved as monophyletic. Apart from clades where internal topologies are polytomous or weakly supported, most of these radical departures from the current classification appear to be supported by palynological data, and could indicate support for new, more natural infrageneric groupings.

TrnL-trnF data did not resolve the spine of the topology with any bootstrap support. Decent resolution at this level would have allowed far greater clarity in assigning possible synapomorphic morphological characters to major clades. Such resolution would also have allowed robust testing of character evolution proposed by Salter (1944). Strongly supported basal nodes would lead to more conclusive answers, with respect to the monophyly of section *Angustatae* and its subsections, than those retrieved in this analysis. Despite the lack of significant resolution along the spine of the phylogeny, the tree still contains considerable information of interest, and many clades are very strongly resolved.

The results and discussion is structured as follows: Major clades are discussed individually in section A, together with discussions of possible palynological and morphological synapomorphies for these clades. Section B discusses the superimposition of the classification *sensu* Salter (1944) onto the produced phylogenetic tree. Section *Angustatae* subsection *Lineares* is discussed separately in section C, followed by a discussion (Section D) of the utility of currently recognised morphological characters in phylogenetic reconstruction in the genus. A discussion of the molecular and palynological evolution of *Oxalis* in southern Africa, insofar as these patterns can be deduced from the results of the present study, is presented in section E.

Section A: Individual Clades

1) Clade I and *Oxalis corniculata*: higher-order relationships: This study for the first time provides molecular evidence that the genera *Biophytum* and *Oxalis* are closely related (85% BS in unconstrained analyses). This suggests a closer relationship between *Oxalis* and *Biophytum* than between the former taxon and *Averrhoa*, for which such evidence already exists (Price & Palmer, 1993). This is in agreement with the close morphological relationship observed between the two genera, including similar, heterostylous flowers and compound leaves (Chant, 1993). The two included *Biophytum* species consistently grouped together. The bootstrap support for the grouping of *Sarcotheca* with *Averrhoa* (86%) collapsed in the reconstruction represented here; consequently the strong relationship between them could not be represented. Further sampling of genera allied to *Oxalis* will shed greater light on relationships within the family Oxalidaceae.

All included *Oxalis* taxa are retrieved in a monophyletic clade, with 100% bootstrap support. *Oxalis corniculata* (section *Corniculatae*) occupies an expected position as sister to all other *Oxalis* taxa included in the study (BS 99%). This invasive American species bears little resemblance to indigenous South African taxa in its habit and means of vegetative reproduction. The current taxonomic placement of *O. corniculata* (Salter, 1944) is thus corroborated by the position of this species in this study.

2) Clade II: This clade consists of species from section *Cernuae*, and the naturalised American species *Oxalis latifolia*, of section *Ionoxalis*. *Oxalis pes-caprae* and *Oxalis knuthiana* are resolved into a single clade with poor support (69%). This provides tentative support for their current morphological placement in the same subsection (section *Cernuae* subsection *Eu-cernuae*).

Oxalis latifolia (section *Ionoxalis*), the second non-indigenous *Oxalis* included in this study, is placed in a weakly supported (58%), unexpected clade as sister to *Oxalis dentata* (section *Cernuae*, subsection *Lividae*). Although this is not in accordance with current classification, the general morphology of *O. latifolia* is more similar to South African taxa than most other New World *Oxalis* species. In particular it bears close morphological resemblance to members of section *Cernuae*. *Oxalis latifolia* is, in fact, often confused with *Oxalis semiloba* Sond. (section *Cernuae* subsection

Purpuratae) in South African herbaria. However, bootstrap support for this clade is poor (58%). This positioning of *Oxalis latifolia* is by no means conclusive, and awaits further studies.

Oxalis purpurascens is one of only two members of section *Cernuae* subsection *Costatae*, both confined to Namibia. The species resolves to clade II in the current phylogenetic tree, but is unresolved within this clade.

3) Clade III: This clade is of interest due to the unexpected taxonomic diversity of its constituent species. It consists of members of sections *Cernuae*, *Oppositae*, *Stictophyllae*, *Foveolatae*, *Crassulae*, *Campanulatae*, *Latifoliolatae* and *Angustatae* subsections *Xanthotrichae* and *Sessilifoliatae*. There is very little robust internal support for the clade, prohibiting any further major phylogenetic inferences or taxonomic comparisons. *Oxalis caprina* (section *Cernuae* subsection *Stellatae*) resolves as unsupported sister to the rest of the clade, which receives strong bootstrap support (81%, the “core” clade III, Chapter 2). A strongly supported subclade (95%) contains *Oxalis luteola* (section *Oppositae*), *Oxalis purpurea* (Chapter 1, Figure 1) and *Oxalis melanosticta* (both section *Stictophyllae*). Another consists exclusively of *Oxalis crocea* and *Oxalis viscosa* (both section *Angustatae* subsection *Sessilifoliatae*), with a bootstrap value of 79%. None of the other subclades in the “core” clade III has a bootstrap support of more than 61%. Thus there is reasonable evidence based on *trnL-trnF* data to support the “core” clade III, but very little well supported internal structure to provide an idea of relationships.

Oxalis caprina, a notorious weed, is currently placed in section *Cernuae* subsection *Stellatae* (Salter, 1944). Although it shares the umbellate inflorescence and flower colour of members of this subsection, in several analyses for this study (including the final reconstruction) this species was not resolved with other members of section *Cernuae*. *Oxalis caprina* was usually placed as sister to the rest of clade III. This is in contrast to the morphology of this clade, which possesses single-flowered inflorescences, barely incised leaflet apices and a variety of very different flower colours. However, the node linking *O. caprina* to the rest of clade III has a branch length of 1, and collapsed in both the strict consensus and bootstrap consensus tree.

Pollen data further refutes the placement of *O. caprina* in clade III. *Oxalis caprina* possesses pollen of the type C7, a micro-reticulate pollen type that only differs from the very similar pollen type C8 in the size of the reticulum lumina. This pollen type is palynologically more similar to the pollen types exhibited by *O. latifolia* and *O. purpurascens* in clade II, as well as many species in clade IV_F, than to other members of clade III. It must be noted that several taxa embedded in clade III, such as *Oxalis campylorrhiza* and *Oxalis crocea*, also possess pollen of the type C8, or similar. Morphologically, however, these two taxa differ substantially from *Oxalis caprina*. Overall there is thus little support for this node.

Morphologically, the remainder of clade III is very heterogeneous, and includes the majority of acaulescent species included in this study, together with some linear-leaved caulescent taxa and a seasonal aquatic (*Oxalis dregei*). Preliminary attempts to plot morphological data onto the tree indicated that the six species in this study that bear multicellular, non-glandular hairs on the epidermis are spread throughout this clade. Pollen data show a preponderance of pollen type C10 and C2 within this group, with exceptions being close palynological relatives to type C2 (Dreyer, 1996). Considering the weak support for many internal nodes, it is possible that further data will resolve this palynological dichotomy into a terminal C10 clade embedded in an otherwise C2 clade.

Within clade III, the close relationship indicated between the two taxa within section *Stictophyllae* and *Oxalis luteola* (BS 95%), is interesting, in that Salter (1944) did not consider the last taxon to be closely related to this section; however, such a reversal of previous classifications seems to be the norm for this phylogenetic tree. Similarly, although *O. crocea* and *O. viscosa* are closely associated within the Salter (1944) classification, their position within this phylogeny is unequivocal and unexpected: deeply embedded within the “core” clade III (BS 81%) instead of clustered with the other members of *Angustatae* subsection *Sessilifoliatae*.

Oxalis campylorrhiza and *O. crocea* bear pollen of the types C8 and C7 respectively, which are very similar. Although not resolved as sisters in this study, the occurrence of these pollen types in an otherwise predominantly C2/C10 clade is in itself interesting. Pollen types C7 and C8 are not considered particularly closely related to pollen type C10, although a close relationship between pollen types C2 and

C8 is quite likely (Dreyer, 1996). The convincing placement of pollen types C7 and C8 in both clade III (BS 81%) and in various other clades in the phylogeny, suggests that these pollen types have evolved at least twice in the genus, based on the phylogenetic hypothesis presented here.

4) Clade IV_A and *Oxalis bifida*: *Oxalis truncatula* (section *Oppositae* subsection *Subintegrae*) and *Oxalis bifida* (section *Oppositae* subsection *Bifurcatae*) are both members of the artificial section *Oppositae* based on characters that are considered “primitive” for southern African members of the genus (Salter, 1944). Despite this, these species do not resolve close to other members of section *Oppositae*, including putative close relatives, such as *Oxalis obtusa* and *Oxalis luteola*, which are located in clade III. Nor do they resolve close to each other.

Oxalis engleriana belongs to section *Angustatae* subsection *Multifoliolatae*, an admittedly artificial subsection (Salter, 1944), and is a species of the South-Western Cape Renosterveld. This species does not possess any close relatives, other than the highly ambiguous *Oxalis henrici* Bolus f., which was found to be conspecific with *O. engleriana* (Dreyer and van Wyk, 1998). In the present study, *O. engleriana* is placed in a clade as sister to *O. truncatula*. There is no bootstrap support for this placement, and this clade collapses in the strict consensus tree.

5) Clade IV_B: This is one of the most strongly supported clades in the phylogenetic tree, with 96% bootstrap support. The included species are morphologically heterogeneous, and represent members of two different sections (sections *Latifoliolatae* and *Angustatae*; Salter, 1944). Although all species are caulescent and possess linear to linear-cuneate leaves, these two characters are present in many other taxa not included within this clade. There does appear to be a tendency in this group to reduce multicellular and glandular epidermal hairs in favour of simple hairs or none on the plant body, although this does not hold true for the reproductive organs. This character is by no means universal in this clade, and is shared by members of clade IV_F, amongst others. This unexpected clade thus possesses very few currently recognised morphological synapomorphies. However, a strong group of synapomorphies for clade IV_B is the universal occurrence of the unique, derived, supra-areolate pollen type D in, and only in, this clade (Dreyer, 1996). Despite few defining morphological characters, the presence of both a unique, highly derived

pollen type and such strong bootstrap support for the molecular data strongly suggests that this clade is a coherent monophyletic group. Although only approximately half of the taxa with D-type pollen have been included in this study, it seems likely that any other species with D-type pollen added to the matrix will resolve to this clade.

Clade IV_B contains two subclades, one strongly supported (94%) and containing mostly small taxa with apically congested leaves and a wide variety of corolla colours, and the other consisting of larger, more branched taxa with a dull pink to red corolla. This subdivision does not correspond to any previously suggested taxonomic treatment. Ongoing morphological and anatomical studies suggest possible synapomorphies for the bulb in these divisions (Gebregziabher, pers. comm.).

6) Clades IV_C, IV_D and *Oxalis eckloniana*: Clade IV_C consists of two members of section *Sagittatae*, and is retrieved with 94% bootstrap support. *Oxalis eckloniana*, the third member of section *Sagittatae* included in the study, does not resolve to clade IV_C; instead it is placed in an unresolved position within clade IV as a whole. A further postulated member of section *Sagittatae*, *Oxalis minuta* var. *callosa*, is strongly placed in clade IV_D (BS 92%). The remainder of clade IV_D consists of *Oxalis tomentosa* (section *Angustatae* subsection *Multifoliolatae*) and *Oxalis oligophylla* (section *Angustatae* subsection *Lineares*). Section *Sagittatae* is thus polyphyletic based on *trnL-trnF* sequence data, as are section *Angustatae* subsections *Lineares* and *Multifoliolatae*.

The placement of *O. oligophylla* (Chapter 1, Figure 8) with *O. minuta* var. *callosa* and *O. tomentosa* in clade IV_D is unexpected; however, these taxa do share an acaulescent habit and pure white corolla with a yellow tube. The former two taxa also share a glabrous plant body with three linear to linear-oblong leaflets, sepals with conspicuous apical calli, filaments with acute to subacute teeth and a similar habit. None of these characters are synapomorphic for this group, however, and a more detailed morphological and anatomical study is required to assess the possible monophyly of this clade.

7) Clade IV_E: This clade consists of members of section *Angustatae* subsection *Pardales*. Salter (1944) considered this subsection to be a natural entity, based on striate pellucid lines or idioblasts on the leaflets and sepals, and retrorse hairs on the

bulbs of all the included taxa. In the present study the three included members of the subsection, namely *O. grammophylla*, *O. leptogramma* and *O. capillacea* (Chapter 1, Figure 5), were grouped together with poor support (BS 63%). As with section *Sagittatae*, the very low levels of sequence divergence between most of the included taxa are the most probable reason for this poor level of support. Further studies of DNA sequence data from other gene regions could elevate bootstrap support levels for subsection *Pardales*.

Bayer (1992) suggested the reduction into synonymy of all of the species in this subsection under the type species, *Oxalis pardalis*. He based this reduction on the tremendous and inconsistent morphological variation in the characters Salter (1944) utilised to define the individual taxa within the subsection. Until more taxa and characters are included in a molecular study, this suggested taxonomic reshuffling cannot be confirmed or refuted. However, *trnL-trnF* data alone do not contain enough information to accurately determine a satisfactory answer to this problem.

8) Clade IV_F: This large clade consists mostly of species allocated to assemblages three, four and five within subsection *Lineares* in the Salter (1944) revision, with several species included from subsections *Glandulosae* and *Sessilifoliatae*. Bootstrap support for this clade is less than 50%, indicating that at least *Oxalis tenuipes* (Chapter 1, Figure 6), *Oxalis phloxidiflora* and *Oxalis stenopetala* do not possess strong molecular support for placement in this clade. These three species are discussed separately below.

Despite overall poor bootstrap support for clade IV_F, a large subclade within this group does resolve quite strongly (82% BS). This clade, henceforth referred to as the “core” clade IV_F, possesses several interesting taxa and some potential synapomorphies. The “core” clade IV_F will be discussed further below.

O. giftbergensis possesses a unique and highly derived pollen type (type C15, Dreyer, 1996). This pollen type does not bear strong resemblance to other pollen types in clade IV_F, or to the pollen of the sister taxon in this study, *Oxalis tenuis*, which possesses pollen type C9 (Dreyer, 1996). Despite 83% bootstrap support for these two sister species, these two taxa are morphologically extremely interesting in that the former possesses sessile leaves present along the entire length of the stem,

whereas the latter has apically congested, distinctly petiolate leaves. *Oxalis tenuis* also bears remarkable morphological and palynological resemblance to both *O. comptonii* and the more deeply embedded members of clade IV_F, such as *Oxalis pallens*. Moreover, *Oxalis tenuis*, *O. giftbergensis* and *O. comptonii* all occur sympatrically on the same mountain escarpment on the border between the Fynbos and Succulent Karoo biomes (*O. comptonii* and *O. tenuis* are only known from this mountain, the Giftberg). If *O. giftbergensis* and *O. tenuis* are truly sister taxa, the differing nature of their leaf placement and petiolation, regarded as highly significant by Salter (1944), will highlight the impracticality of utilising these characters in a morphological phylogenetic framework for this genus.

The “core” IV_F clade, with 82% bootstrap support, includes mainly taxa from assemblage 5 of subsection *Lineares*. The only exceptions are *Oxalis tenuifolia* and *Oxalis urbaniana*, of subsection *Sessilifoliae*. The taxa in assemblage five, almost exclusively, possess pollen of the type C8. Pollen type C8 occurs in other species in this study, notably in *O. purpurascens*, *O. comptonii*, and in clade IV_D. However, such a concentration of similar pollen in the “core” IV_F clade, a group with reasonably high bootstrap support, appears significant.

Several morphological characters corroborate the relationship between *O. tenuifolia* and *Oxalis stictocheila* (BS 86%), notably the absence or reduction of calli on the leaves and sepals, and the red rim to the corolla. Neither of these characters is, however, restricted to these two species. *O. tenuifolia* bears subsessile, tufted leaflets that appear fasciculate on a long central stem, while the leaves of *O. stictocheila* are apically congested on a much shorter stem. Again if these two taxa are truly sister species, then the taxonomic significance of sessile, cauline leaves versus apically congested, petiolate leaves is brought into question.

Within the “core” IV_F clade, the clade containing *O. tenuifolia* and *O. stictocheila* is embedded in a polytomy that includes very similar species (BS 61%), all displaying to various degrees the coloured rim to one side of the corolla. *Oxalis pallens* and *Oxalis pusilla* (a dwarf species from the Swartland region on the West Coast) possess pollen of the type C2 (Dreyer, 1996). This is interesting in taxa so embedded in a clade possessing the somewhat different pollen type C8. Morphologically, these two taxa

undoubtedly belong to this clade. Further studies could yield more solid evidence as to their precise placement.

Oxalis urbaniana and *Oxalis glabra* (BS 62%) are morphologically very similar, with the chief difference centering on the strikingly ridged bulb of the former taxon. Bayer (1992) synonymised *O. urbaniana* and *Oxalis callimarginata* Weintraub under *Oxalis goniorrhiza* E. & Z., principally because of collections intermediate in morphological characters between these three taxa. Despite searches in the type locality of *O. callimarginata*, and despite repeated searches for the reputedly common *O. goniorrhiza*, only *O. urbaniana* could be located. Consequently no additional comments can be offered in terms of this suggested synonymy. *Oxalis goniorrhiza* and *O. glabra* are morphologically similar, and it can thus be tentatively assumed that *O. urbaniana* and *O. callimarginata* are closely related to these two taxa.

Oxalis burtoniae is a yellow-flowered, multifoliolate species closely associated with granite outcrops along the West Coast of the South-Western Cape. Salter (1944) stated that this species is related to *Oxalis polyphylla*, an extremely common species present in the same area. Salter (1944) thus implied that the former taxon is the result of an edaphically driven speciation event segregating it from the latter. In this study a direct sister-relationship between these two taxa is not supported. Indeed, the species *O. polyphylla* and its myriad varieties and affinities requires a much more in-depth analysis than that presented here.

9) *Oxalis stenopetala*, *Oxalis phloxidiflora* and *Oxalis tenuipes* clade: The placement of these three taxa is of extreme taxonomic interest. All three of these species are placed in assemblage 3 of subsection *Lineares* (Salter, 1944) and receive 52% bootstrap support. The other two members of this assemblage, *O. xantha* and *O. campicola*, are firmly placed in clade IV_B in the present study (96% BS), and possess D-type pollen (Dreyer, 1996). Although *O. stenopetala* and *O. tenuipes* (Chapter 1, Figure 6) are morphologically similar, they are not suggested to be sister taxa in the present study. Indeed, a relationship between them is only weakly supported. In contrast, *O. phloxidiflora*, the strongly supported sister taxon to *O. stenopetala* (BS 88%), is quite distinct in appearance. All three taxa are residents of drier, mountainous inland areas, and their location within the same general area (the

Cederberg and adjacent escarpment) could hint at possible allopatric speciation events leading to the divergence of these taxa.

Palynology does not help to clarify the relationships between *O. phloxidiflora*, *O. stenopetala* and *O. tenuipes*. *Oxalis phloxidiflora* possesses the unique pollen type, C14, which is characterised by a coarsely reticulate tectum and thin, sometimes-perforated muri, and intraluminary bacules clustered along the muri. In this pollen type, the tectum is pluri-columellate under the perforated muri walls. *Oxalis tenuipes* possesses a different, unique pollen type, C12. In these pollen grains the tectum is coarsely reticulate, the lumina irregularly angular and the intraluminary bacules are clustered along the muri, but muri perforations are never present. *Oxalis stenopetala* possesses pollen of the type C7. This pollen type is similar to pollen type C8. From a palynological perspective, the pollen types of these three taxa are not considered closely related (Dreyer, 1996).

Dreyer (1996) did not place the monotypic pollen type C14 particularly close to any other reticulate pollen type, although she considered the evolutionary pathway leading through pollen types C10, C11 and C12 to type C14 to be more likely than through any other pollen type. The monotypic pollen type C12 is considered closely related to types C10 and C11; however, this would place *O. tenuipes* closer to clade III than subsection *Lineares*, which is strongly refuted by both *trnL-trnF* and morphological evidence. This would imply that this pollen type has evolved at least twice in the genus. Moreover, pollen type C7, found in *O. stenopetala*, is not closely related to either pollen type C14 or the C10 pollen type series, but instead shares affinity with pollen type C8.

10) *Oxalis commutata*: Section *Latifoliolatae*, to which *O. commutata* belongs, can be considered to be the “dustbin” section of the genus, with groups of unrelated species possessing no clear affinities elsewhere or to each other being placed here. Like a number of other taxa in the section, *O. commutata* possesses the pollen type C10, and these taxa would thus be grouped closely to one another and to clade III palynologically. However, *O. commutata* does not group here on the basis of *trnL-trnF* data, thus this study does not clarify the phylogenetic placement of this species. Such clarification will have to await a more complete, multi-gene study of the genus as a whole.

Section B: Current classification vs. phylogenetic and palynological data

1) **Section *Cernuae*:** This section is not retrieved as a monophyletic group. Although most of the included taxa in this section resolve to clade II, the unrelated species *Oxalis latifolia* is also weakly retrieved in this clade. Conversely, *Oxalis caprina*, morphologically a definite member of section *Cernuae*, is retrieved without bootstrap support as sister to clade III. Morphologically, the section is bound by the occurrence of deeply incised leaflets and umbellate inflorescences, although exceptions do occur for both characters. Moreover, some species with umbellate inflorescences are currently classified in section *Oppositae*, and some taxa with deeply incised leaflets occur in section *Foveolatae*. The taxonomic situation is thus not clear. Sampling for section *Cernuae* and related sections was poor and an increase in sampling may increase resolution, allowing greater phylogenetic clarity and more definitive statements to be made with regard to the monophyly of this section.

2) **Section *Oppositae*:** This section is considered to be one of the most artificial currently recognised groups in southern African *Oxalis* taxonomy (Dreyer, 1996). This section is morphologically delimited by the occurrence of opposite bracts set at an upper articulation of the peduncle. However, it is uncertain how taxonomically informative this character is, as many other *Oxalis* taxa share subopposite bracts and it is feasible that this character could have evolved more than once. Palynologically, the entire section is very homogeneous (Pollen types C2 and C3 being predominant; Dreyer, 1996); however, this is of little systematic value if this character is regarded as plesiomorphic.

The *trnL-trnF* phylogenetic reconstruction retrieves the four included members of section *Oppositae* in various clades. *Oxalis obtusa* and *Oxalis luteola* are convincingly placed in clade III (BS 81%), whilst *Oxalis truncatula* is the unsupported sister to *Oxalis engleriana* (clade IV_A). *Oxalis bifida* is unresolved within clade IV. Consequently, based on *trnL-trnF* data alone, the section does appear to be polyphyletic. No comment can be made on the monophyly of subsection *Bifurcatae*, as only one species from this subsection was included in the phylogenetic study.

3) Section *Stictophyllae*: This section, with three species, is the smallest defined by Salter (1944). The section is defined by broad leaflets with distinct striolate or black-dotted patterns when dry, and distinctly callose bracts below the middle of the peduncle. The two species included in this study, *Oxalis purpurea* (Chapter 1, Figure 1) and *Oxalis melanosticta*, are placed in a clade with *Oxalis luteola*, a member of section *Oppositae*. This is taxonomically unexpected; however, *O. melanosticta* does bear morphological resemblance to *O. luteola*, particularly in overall habit and flower colour.

4) Section *Sagittatae*: Spreading outer whorls of stamens or pistils in the flower, and sagittate anthers are some of the unique characters defining this section. The section also bears a unique rugulate-reticulate pollen type (designated pollen type A; Dreyer, 1996) and a related, monotypic, micro-rugulate-spinate pollen type (designated pollen type B; Dreyer, 1996). Despite a non-monophyletic section *Sagittatae* resolved in the phylogenetic study, this situation is inconclusive and further sampling is expected to retrieve a monophyletic section *Sagittatae*, excluding *Oxalis minuta* var. *callosa*. A variety of morphological, palynological and *trnL-trnF*-based data provide strong evidence that *Oxalis minuta* var. *callosa* does not belong in this section (Kumwenda, 2001, Chapter 2).

5) Section *Foveolatae*: This section is characterised by leaflets with large epidermal cells that collapse in drying to create a distinct impresso-punctate appearance. On a morphological basis the majority of member taxa appear to belong to this section; however, certain species do not possess this leaflet character. *Oxalis furcillata* in particular, with its smooth, deeply incised, conduplicate leaflets, exhibits little similarity with the rest of the section. Palynological data are highly congruent; little variation from pollen type C2 is observed in the section. However, *Oxalis furcillata* var. *caulescens* possesses pollen of the type C10, in contrast to the type variety of the taxon and the rest of the section. These two pollen types are not considered similar.

The five included members of this section all resolve to clade III. *Oxalis punctata*, *O. lichenoides*, *O. pulchella* and *O. pocockiae* are retrieved in the most deeply embedded subclade of clade III, with members of sections *Oppositae*, *Stictophyllae*, *Crassulae*, *Campanulatae*, *Angustatae* subsection *Xanthotrichae* and the unplaced *O. monophylla*. *Oxalis furcillata* var. *caulescens* is retrieved in a clade near the base of

clade III, as unsupported sister to *Oxalis oculifera*. There is little resolution in this clade, and little further can be said with confidence about these taxa until more data are gathered.

6) Section *Crassulae*: This section contains somewhat succulent species with leaflets that readily fall apart in drying. These species bear large, ovate scales that enclose the base of the petioles, and the petioles are conspicuously articulated to a much widened petiole base. Much of the variation in this section is encountered in the huge group species, *Oxalis flava* (Chapter 1, Figure 2), with many of the remaining taxa being segregates or allies of this large species. Dreyer (1996) questioned the taxonomic placement of two species in this section, namely *Oxalis namaquana* and *Oxalis cathara* Salter, due to markedly different pollen types. Apart from these two species, which bear pollen types C2 and C7 respectively, the rest of section *Crassulae* has pollen of the type C10. The pollen types C7 and C10 are not considered closely related to one another (Dreyer, 1996).

The five members of section *Crassulae* included in the current study are confined to clade III in the phylogenetic study. At present little more can be said due to the low degree of resolution retrieved in clade III, but the members of section *Crassulae* are not retrieved in a monophyletic clade. More data are needed in order to clarify their phylogenetic position.

7) Section *Latifoliolatae*: A section that can be regarded as the “dustbin” section of the Salter (1944) taxonomy, section *Latifoliolatae* includes several groups of clearly related taxa that are not otherwise closely related to each other or to any other of the Salter (1944) sections. The included species are heterogeneous in virtually all aspects, and are grouped here only on the shared characters of broad leaflets and bracts above the middle of the peduncle. Neither of these characters is confined to this section.

Palynologically, each group of related taxa possesses clearly related pollen types. Certain groups contain only pollen of the type C10, others pollen types C2 and C8, and one group the very distinct supra-areolate pollen type D. These groups are thus very dissimilar on a palynological basis, and each group bears more affinity to other sections and groups of taxa than to other members of section *Latifoliolatae*. This strengthens evidence for an unnatural section *Latifoliolatae*.

Based on *trnL-trnF* sequence data, members of section *Latifoliolatae* are distributed throughout the tree. *Oxalis oculifera* (Chapter 1, Figure 3) and *Oxalis campylorrhiza* are strongly retrieved in clade III, whilst *Oxalis tenella* and *Oxalis aridicola*, both species with D-type pollen, resolve to clade IV_B. *Oxalis commutata* is unplaced in this study, resolving along the spine of the topology. This latter species is thus unrelated to the other members of section *Latifoliolatae* on the basis of *trnL-trnF* sequence data. Although the phylogenetic study highlights the putative polyphyly of this section, further character data are needed, and gaps in sampling need to be filled, in order to reclassify section *Latifoliolatae* into more natural segregates.

8) Section *Campanulatae*: This small section consists of seasonal aquatic and marsh plants, characterised by a widely campanulate corolla and the aquatic lifestyle of its constituent taxa. This section is considered natural (Salter, 1944), however, little is known of its relations to the rest of *Oxalis* in the region, and how the aquatic lifestyle evolved from the ancestral terrestrial habit. Palynologically section *Campanulatae* bears affinity to groups in section *Latifoliolatae*, section *Crassulae*, section *Angustatae* subsection *Xanthotrichae* and the unplaced *Oxalis monophylla*.

Only one member of this section was included in the current phylogenetic study, namely *Oxalis dregei*. The *trnL-trnF* tree convincingly places *O. dregei* in clade III; however, no further relations are evident, and there is little resolution within clade III. It is nevertheless noteworthy that most of the taxa with pollen of the type C10 are also included in this clade. Further data are expected to clarify the precise placement of section *Campanulatae*.

9) Section *Angustatae*: This, the largest section *sensu* Salter (1944), is defined by the possession of emarginate, linear to linear-cuneate leaflets that are least twice as long as broad. It is a very heterogeneous section, possessing both caulescent and stemless taxa, and a wide variety of pollen types (Dreyer, 1996; chapter 1).

9.1) Subsection *Xanthotrichae*: This subsection differs from the rest of the section in being completely acaulescent and in possessing multicellular, non-glandular epidermal hairs. Subsection *Xanthotrichae* also differs in that all the taxa in this subsection possess pollen type C10, otherwise not found at all in section *Angustatae*. This pollen type is more similar to that of taxa in sections *Crassulae* and

Campanulatae and parts of section *Latifoliolatae*, than to other pollen types in section *Angustatae*. The phylogenetic study retrieved the included members of subsection *Xanthotrichae* within clade III, and not with the remainder of section *Angustatae*. Consequently, there is strong *trnL-trnF* sequence-based and palynological evidence to suggest that this subsection should not be part of section *Angustatae*.

9.2) Subsection *Pardales*: This subsection is weakly retrieved as monophyletic (63% BS) in the phylogenetic study. This corroborates morphological evidence for this being a natural taxon. This is discussed in more detail in section A6.

9.3) Subsection *Sessilifoliatae*: This subsection is characterised by mostly sessile leaves positioned along the length of the stem, and by most of the peduncles being cauline. Palynologically this is an extremely variable subsection, containing both a wide variety of reticulate C-type pollen and supra-areolate D-type pollen.

The phylogenetic study retrieved subsection *Sessilifoliatae* as conclusively polyphyletic. *Oxalis crocea* and *Oxalis viscosa*, which are morphologically similar, are convincingly placed as sister taxa (BS 79%) in clade III. The sole representative of subsection *Sessilifoliatae* that possesses D-type pollen, *Oxalis hirta* (Chapter 1, Figure 4), is strongly supported as a member of clade IV_B. The species with the monotypic pollen type C15, *Oxalis giftbergensis*, resolves as sister to *Oxalis tenuis*, of subsection *Glandulosae* (BS 83%), in clade IV_F. The remaining two species, *Oxalis tenuifolia* and *Oxalis urbaniana* are retrieved in the “core” clade IV_F, as sisters to taxa associated with subsection *Lineares*. Subsection *Sessilifoliatae* is thus conclusively polyphyletic on both palynological and *trnL-trnF*-based evidence.

9.4) Subsection *Glandulosae*: This subsection is only segregated from subsections *Lineares* and *Sessilifoliatae* by the presence of glandular hairs on the epidermis of the plant body. The member species of this subsection exhibit clear morphological affinities with subsections *Lineares* and *Sessilifoliatae*. Palynologically subsection *Glandulosae* includes species with a preponderance of the pollen type C8, or related types. This indicates possible affinities with parts of subsection *Lineares* and *Sessilifoliatae*.

The two included members of subsection *Glandulosae* are both found in clade IV_F of the *trnL-trnF* tree. *Oxalis tenuis* is retrieved as sister to *Oxalis giftbergensis*, and *Oxalis ebracteata* is unresolved in the clade basal to the “core” clade IV_F. It thus appears that subsection *Glandulosae* is not monophyletic. However, only two of the seven recognised species in the subsection were included in the phylogenetic study. Sampling for this subsection must be increased before further comments can be made on its monophyly or lack thereof.

9.5) Subsection *Multifoliolatae*: The sole character of bearing more than three leaflets per leaf binds this subsection within section *Angustatae*. The species included in this taxon are otherwise dissimilar in morphological terms, with some exhibiting morphological affinities with subsection *Lineares*; several members of subsection *Lineares* possess multifoliolate leaves. From a palynological perspective, this subsection is heterogeneous, including the monotypic pollen type C6, a single species exhibiting pollen type C11 and the remainder bearing pollen of the type C8. These reticulate pollen types are not considered closely related (Dreyer, 1996).

The *trnL-trnF* tree resolves the two included members of subsection *Multifoliolatae* in two separate places. *Oxalis engleriana* is resolved as sister to *Oxalis truncatula*; this receives no morphological or molecular support, and this clade collapses in the consensus tree. *Oxalis tomentosa* is retrieved very strongly in clade IV_D. This clade is completely unexpected from a taxonomic viewpoint, yet palynological similarities and high bootstrap values support clade IV_D. Subsection *Multifoliolatae* thus appears to be polyphyletic.

Section C: Section Angustatae subs. Lineares: taxonomic considerations

The subsection is distributed throughout clades IV_B, IV_D and IV_F in the *trnL-trnF* phylogeny presented here (Chapter 2, Figure 1). It is not retrieved as a monophyletic group. Nothing can be said with respect to the monophyly of *Oxalis* cf. *helicooides* and *O. oligophylla*, which are the sole representatives of assemblages 2 and 7, respectively. However, no other assemblages, as defined by Salter (1944), appear to be natural groups. Consequently, the newly proposed relationships within the entire subsection are dramatically altered from previous morphological classifications.

Clades IV_B and IV_F contain 23 of the 24 included representatives of subsection *Lineares*. Both clades, however, also contain members of other sections or subsections. There is strong bootstrap support upholding clade IV_B and the “core” clade IV_F, which together contain 19 members of subsection *Lineares*. Consequently, most of the taxa included in this subsection are included in two, strongly supported, monophyletic clades; the most notable exception being *O. oligophylla*.

Within subsection *Lineares*, clade IV_F contains members of assemblages three, four and five, and clade IV_B contains all the members of assemblages one, two and six, as well as all taxa with D-type pollen of assemblages three and five. The “core” clade IV_F contains all taxa included in this study that are associated with assemblage four.

The strong molecular support for clades IV_B and the “core” clade IV_F in this study is further corroborated by palynological evidence. If morphological data is found to further support these groupings, there will be considerable pressure to reclassify the subsection *Lineares* into more natural cohesions. Current studies are hinting at possible bulbous (Gebregziabher, pers. comm.) and chromosomal (Dreyer and Johnson, 2000) synapomorphies for both clades IV_B and IV_F. Consequently, preliminary recommendations for taxonomic replacement are merited.

Clade IV_B, with D-type pollen (Dreyer, 1996), is almost certainly a natural taxon. Consequently it is recommended that all D-type taxa from subsection *Lineares* and elsewhere in the genus be removed and brought together into a newly circumscribed, natural taxon. Exact taxonomic placement and status will have to await a complete, phylogenetic revision of the genus *Oxalis*. It is not known whether Salter (1944), who coined the name for the subsection, named subsection *Lineares* after *Oxalis linearis*. Several taxa from this subsection were described before *O. linearis*. It is possible, in light of the naming of other sections and subsections in the genus, that Salter (1944) simply used the name as an epithet describing a salient character of all the member species. If the consistency as regards the naming of the other subsections in section *Angustatae* is to be followed, the latter option seems more probable; however, the nomenclature will have to be consulted before any further name changes are to be implemented.

The “core” clade IV_F also possesses reasonably strong support as a natural taxon. This group will most probably include all the members of assemblage four of the polyphyletic subsection *Lineares*, as well as several obviously related taxa from subsection *Sessilifoliae*. Renaming of this taxon might be necessary to avoid confusion with clade IV_B, which contains *O. linearis*.

Oxalis giftbergensis, *O. tenuis* and *O. ebracteata* could very well be basal members of clade IV_F, as indicated in this study, but there is little evidence to support this. From this study, their position cannot be asserted with any certainty; however, they will most probably be associated with one of the segregates of subsection *Lineares*. Likewise *O. stenopetala*, *O. phloxidiflora* and *O. tenuipes* could also eventually connect to clade IV_F at some basal point, but as the precise placement is not resolved in this study, no comment pertaining to their taxonomy can be offered.

Oxalis oligophylla is quite clearly the most isolated member of subsection *Lineares*, as it bears very little morphological connection to the rest of the subsection. Pollen data suggests a relationship between clade IV_F and the clade containing *O. oligophylla*, however, there is no phylogenetic support for such a connection in this study. Until further evidence can be gathered, it is felt prudent that the placement of *O. oligophylla* within subsection *Lineares* be viewed with extreme caution. Indeed, the entire clade containing *O. oligophylla* merits further intensive research; despite strong bootstrap support, the relationship between *O. oligophylla*, *O. minuta* var. *callosa* and *O. tomentosa* is unexpected from a morphological viewpoint.

As a whole, subsection *Lineares* can be considered as an entirely artificial subsection, whose member taxa are divided into two major clades supported by molecular and palynological evidence, a number of unplaced species whose position will hopefully be resolved at a later stage, and one seemingly unrelated species.

Section D: Morphological considerations

An interesting, recurring feature of large genera in the Cape Flora is the massive species radiation within the restrictive boundaries of a relatively limited number of morphological characters and associated states. Furthermore, these enormous species

radiations are characterised by exhaustive combinations of these characters and character states, which accounts for most of the observed diversity in these genera. This implies considerable degrees of convergence and homoplasy in these taxa. Examples of genera that display this trend include *Aspalathus* L. (Hawkins, pers. comm.) and *Erica* L. *Oxalis* appears to follow this pattern, which makes any attempt at alpha-taxonomy based on these characters extremely difficult. Specifically, the degree of caulescence, the petiolation of the leaflets, the colour of the corolla and the nature of the indumentum macro-morphology exhibit such variation that their use in morphological classifications must be considered questionable.

Major examples of such morphological discrepancies are found in the fundamental classification proposed by Salter (1944). The major division between the umbellate section *Cernuae* and the sections with single-flowered inflorescences is not a fixed one. Several taxa in section *Oppositae* possess inflorescences with two or more flowers. The other character defining section *Cernuae*, the deeply incised leaflets, is also shared with section *Oppositae* subsection *Bifurcatae*, some forms of *O. obtusa*, and two taxa in section *Foveolatae*. Of the remaining taxa, the proposed division between stemless, broad-leaved taxa and caulescent, linear-leaved taxa (Salter, 1944) is violated in almost every recognised section.

Salter (1944) also considered the endospermous nature of the seeds to be taxonomically useful. Member taxa of sections *Cernuae*, *Oppositae*, *Stictophyllae* and *Foveolatae* (except *Oxalis furcillata*) possess endospermous seeds, with the seeds of all remaining indigenous taxa lacking endosperm. Yet this division does not agree with the distribution of any other morphological character.

Other morphological characters, potentially more useful at sectional and subsectional level, also exhibit possible evidence of homoplasy. For example, section *Foveolatae* is defined by the presence of enlarged epidermal cells that collapse on drying, producing an epidermis with a characteristic impresso-punctate appearance. Section *Oppositae* is characterised by the bracts being directly opposite one another, and positioned at an upper articulation of the peduncle. The species *Oxalis obtusa*, of section *Oppositae*, possesses opposite bracts, but clearly also possesses the typical impresso-punctate epidermis of section *Foveolatae*. Similarly, section *Angustatae* subsection *Pardales* is clearly recognised as a natural taxon by the presence of striate

pellucid lines on both the leaflets and the sepals, that turn black in drying. Yet the entirely unrelated section *Stictophyllae* also possesses this character.

This does not mean that organismal-level classification is devoid of taxonomically informative characters. *Oxalis*, however, is so poorly understood in the region that many new, more phylogenetically informative characters are probably still awaiting discovery. A current anatomical bulb study holds great promise in providing “good”, new, systematically informative characters. Other promising fields include karyology, leaf anatomy and studies of seed and fruit characteristics.

Section E: Preliminary evolutionary trends

As mentioned above, morphological characters have probably been subject to considerable homoplasy. Moreover, the phylogenetic tree proposed here exhibits limited resolution, particularly for the spine of the topology. Without well-supported internal resolution in a cladistic reconstruction for South African members of the genus, the phylogenetically informative morphological characters cannot be identified. Patterns of character evolution will only become clear once a robust species-level phylogenetic reconstruction is produced. Despite this, certain trends are already visible, and should be mentioned.

As regards the habit of these plants, the primary change for South African taxa is postulated to be the change from annual/perennial herbs (perhaps even a tree habit) to a geophyte, with a seasonally active lifestyle and prominent underground storage structures. *Averrhoa* L. and *Sarcotheca* Blume are trees, *Biophytum* species are annual or perennial herbs, *O. corniculata* is an annual, and all the other taxa included in this study are bulbous geophytes. The phylogenetic reconstruction proposed here thus supports this theory.

Southern African taxa, however, are not the only members of the genus bearing bulbous underground structures. The American section *Ionoxalis*, to which *O. latifolia* belongs, contains all the *Oxalis* taxa with bulbous structures outside of the southern African region (Denton, 1973). However, these structures are not considered to be homologous to the bulbs of South African taxa. The underground structures of section *Ionoxalis* are fashioned from modified leaf and peduncle bases, with the rest of the

leaf or peduncle serving the usual photosynthetic or reproductive functions. Bulbs in southern African taxa consist of leaves completely modified for storage and asexual reproduction. Denton (1973) describes the fundamental internal differences between the two major bulb types in *Oxalis*. American bulbs are described as scaly, and have a large number of nearly uniform ovate or obovate scales spirally arranged around the stem axis. Southern African bulbs are described as tunicated, with outer scales whose margins often overlap and which enclose a few imbricated inner scales. Denton (1973) further states that these major structural differences in the two bulb types strongly implies that the two bulb types are not homologous, and are derived from unrelated species in the genus *Oxalis*. This hypothesis has never been tested.

Until such time as strong synapomorphic morphological characters are discovered for the molecular systematic groupings of South African taxa, any discussion of their development/evolution would need to be tentative at best. Consequently, further testing of the hypothesis of morphological evolution in South African taxa, as proposed by Salter (1944), will have to await a phylogenetic tree containing well-supported internal resolution.

Palynologically, the situation is somewhat different, in that a hypothesis of the pattern of palynological evolution has been proposed (Dreyer, 1996). Specifically, the various major types and subtypes of pollen in South Africa are considered to have developed from the fundamental tricolpate grains with reticulate tectums common to southern African *Oxalis* (Huynh, 1969; Dreyer, 1996). Other commonly accepted members of the family Oxalidaceae, such as *Sarcotheca*, *Averrhoa*, and *Biophytum*, do exhibit pollen tectums corresponding to the reticulate pollen types C1-C4 described by Dreyer (1996). However, these pollen grains are colpate, not colporate, such as is the case for southern African taxa. This constitutes a significant difference in pollen aperture type. *Oxalis* has apparently undergone a reversal in this respect in that all the indigenous southern African taxa are colpate. This is contrary to general theories of the direction of pollen aperture-type evolution in the angiosperms (Muller, 1979). It should be noted that whilst the majority of southern African *Oxalis* are tricolpate, tetra- and panto-colpate grains are present in the more advanced pollen type A, confined to section *Sagittatae* (Dreyer, 1996). Aberrant grains are also present in various taxa with D-type pollen.

Dreyer (1996) considered pollen types C1-C4 and C7-C9 to be relictual for *Oxalis* in South Africa. Although the reasons for this postulate have mostly been invalidated, the *trnL-trnF* tree upholds this theory. Similar pollen occurs in the closest generic outgroups, as well as in *Oxalis corniculata*; thus there is evidence derived from the *trnL-trnF* tree to support her proposal.

Oxalis corniculata displays pollen of type C2, the most common type found by Dreyer (1996). This is in accordance with both the generic outgroups and with a large percentage of the ingroup. *Oxalis latifolia* possesses the related pollen type C7, and several clades in the analysis exclusively possess pollen of these and closely related types.

Dreyer (1996) further stated that the various major pollen types, namely the rugulate-reticulate pollen type A, the micro-rugulate spinate type B, and the supra-areolate type D, emerged from a central “pool” of reticulate pollen, with pollen types B and D either having evolved directly from pollen type C or via pollen type A. There are too little data in the internal nodes of the *trnL-trnF* tree to support this; however, either evolutionary pathway remains a possibility.

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CONCLUSION:

The findings of this study have conclusively been able to answer its stated objectives. With regard to the first aim of this study, the *trnL-trnF* region possessed enough information to conclude that section *Angustatae* subsection *Lineares* is polyphyletic. The member species of subsection *Lineares* resolve to three clades within the current study, two of them with strong bootstrap support. None of these clades consists entirely of species from subsection *Lineares*. With regard to the assemblages of related taxa in the subsection, none resolve as monophyletic in this study.

The second aim, to compare the produced phylogenetic tree to the current morphological classification and recent palynological data, exhibited new and interesting patterns. On the whole, palynological and *trnL-trnF* sequence-based phylogenetic data were more congruent with each other than with the current classification of *Oxalis* in the southern African region. Certain suites of pollen characters match very well with strongly supported clades in the phylogenetic reconstruction based on *trnL-trnF* data. These shared characters lend strong evidence to support the monophyly of these groups.

On the whole, the *trnL-trnF* tree presented here does not support a monophyletic section *Angustatae* subsection *Lineares*. Instead, several smaller, more natural clades, supported by various palynological characters, are strongly resolved. The systematic significance of several morphological characters in this tremendously variable genus is to be viewed with extreme caution, at least until more data has been gathered with respect to these characters. Only once a well-supported phylogenetic tree with good internal resolution has been reconstructed, will patterns of morphological character evolution become evident. Only then can the state of morphological characters currently used in the classification of the genus be re-evaluated, and systematically useful characters separated from the more homoplasious characters.

Palynological data appears to present a far more accurate picture of evolution and relationships within the genus than macro-morphological criteria, and in conjunction with molecular data can hopefully provide a degree of phylogenetic resolution that has thus far been lacking in studies of South African *Oxalis* taxa. It is to be hoped that the incorporation of both more taxa and more characters (i.e. gene regions) into the

data set, as well as more detailed knowledge of morphological and cytological characters, will provide a better understanding of the phylogenetic relationships in this genus. This issue will be addressed in a forthcoming Ph.D. thesis, which will incorporate and expand upon the current data matrix, in the hope of creating a species-level, multi-gene analysis of *Oxalis* in southern Africa. Hopefully, such knowledge will also prove useful in future studies in elucidating patterns of speciation and extinction in the Cape Floristic Region.