

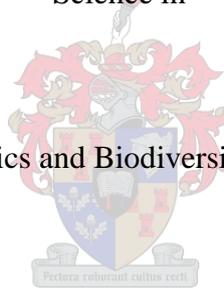
**The Systematic Significance of the Fruit and Seed Morphology and
Anatomy in Selected *Oxalis* L. (Oxalidaceae) species**

By

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DECLARATION

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

Signature..... Date.....

ABSTRACT

At present a proper systematic classification of the southern African members of *Oxalis* L. (Oxalidaceae) does not exist. The most recent and comprehensive revision of the genus based on macro-morphological characters is out-dated (published 60 years ago (Salter, 1944)). The external morphology of the flowers of the southern African *Oxalis* species is reasonably well-studied, but little is known about the anatomy thereof. A pilot study of fruit and seed morphology and anatomy of nine selected southern African *Oxalis* species (Obone, 2003) already revealed some trends to demarcate two main groups. This confirmed the systematic value of some of the characters already proposed by Salter (1944).

The aim of the present study was to assess the potential systematic value of fruit and seed morphology and anatomy of 32 *Oxalis* species. The selection was done such that the included species would represent the main sections proposed by Salter (1944), the pollen types proposed by Dreyer (1996) and the different clades revealed by the phylogenetic tree compiled by Oberlander *et al.* (2004).

Although the species sampling was very low (20% of the southern African taxa), 35 potentially informative characters were identified in fruit and seed morphology and anatomy. These characters may be grouped into three character types, namely autapomorphic characters, randomly distributed characters and systematically informative characters. The first two character types were particularly useful in species-specific characterization. The third group of linked characters could be used to demarcate two major groups of species, those producing endospermous seeds and those producing exendospermous seeds. The three types of characters may prove to be taxonomically informative if more species-inclusive studies are performed.

The cluster analysis strongly supported the demarcation of endospermous and exendospermous groups with 100% bootstrap support. Low bootstrap values were observed for subgroups within each of the major groups. This is probably due to low taxon sampling. Therefore clustering based on fruit and seed morphology should be considered with extreme caution within the two groups. Despite these limitations of sample size, fruit and seed morphological and anatomical characters have proven to be systematically informative at the infra-generic level.

Key words: *Oxalis*, fruit morphology, fruit anatomy, seed morphology, seed anatomy, endospermous seeds, exendospermous seeds.

OPSOMMING

‘n Toepaslike sistematiese klassifikasie sisteem is tot dusver nie beskikbaar vir die suider Afrikaanse lede van *Oxalis* L. (Oxalidaceae) nie. Die mees onlangse en volledige hersiening van die genus, gebaseer op makromorfologiese kenmerke, is verouderd (60 jaar gelede gepubliseer (Salter, 1944)). Die eksterne morfologie van die blomme van suidelike Afrikaanse *Oxalis* spesies is redelik goed bestudeer, maar min is bekend oor die anatomie daarvan. ‘n Loodsstudie van vrug- en saadmorfologie en -anatomie van nege geselekteerde suider Afrikaanse *Oxalis* spesies (Obone, 2003) het alreeds sekere tendense getoon om twee hoofgroepe mee af te baken. Dit het die sistematiese waarde van sommige van die eienskappe wat reeds deur Salter (1944) voorgestel is gestaaf.

Die doel van die huidige studie was om die potensiële sistematiese waarde van vrug- en saadmorfologie en -anatomie van 32 *Oxalis* spesies te ondersoek. Die seleksie is so gedoen dat die ingeslote spesies die hoof seksies voorgestel deur Salter (1944), die stuifmeeltipes voorgestel deur Dreyer (1996), en die verskillende klades in die filogenetiese boom van Oberlander *et al.* (2004), sou verteenwoordig.

Alhoewel die spesies verteenwoordiging baie laag was (20 van die suider Afrikaanse taksa), is 35 filogeneties potensieel belangrike vrug- en saadmorfologiese en –anatomiese kenmerke geïdentifiseer. Hierdie kenmerke kan in drie kenmerk-tipes verdeel word, nl. autapomorfe kenmerke, lukraak verspreide kenmerke en sistematiese insiggewende kenmerke. Die eerste twee tipes kenmerke was veral nuttig vir spesie-spesifieke karakterisering. Die derde groep gekoppelde kenmerke kon gebruik word om twee hoof groepe van spesies, die wat endospermiese sade produseer en die wat eksendospermiese sade produseer, mee af te baken. Die drie tipes kenmerke mag van taksonomiese belang wees in meer spesie-inklusiewe studies.

Fenetiese analise het die afbakening van endospermiese en eksendospermiese groepe sterk ondersteun met 100% bootstrap ondersteuning. Lae bootstrap waardes is waargeneem vir subgroepe binne elk van die hoof groepe. Dit kan moontlik toegeskryf word aan ‘n lae takson verteenwoordiging. Fenetiese groepe, gebaseer op vrug- en saadmorfologie en –anatomie moet dus baie versigtig oorweeg word binne die twee hoofgroepe. Ten spyte van die beperkings van monster grootte, is daar bewys dat vrug- en saadmorfologiese en –anatomiese eienskappe sistematies insiggewend is op die infra-generiese vlak.

Sleutel woorde: *Oxalis*, vrugmorfologie, vruganatomie, saadmorfologie, saadanatomie, endospermiese saad, eksendospermiese saad.

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CONTENTS

ABSTRACT	1
ACKNOWLEDGEMENTS	5
CHAPTER I: INTRODUCTION	8
1.1 Literature review.....	10
1.2 Hypothesis.....	12
1.3 Key questions.....	12
CHAPTER II: MATERIALS AND METHODS	14
2.1 Morphological and anatomical studies.....	14
2.2 SEM and VP studies.....	15
2.3 Statistical analysis using NTSYSpc.....	18
2.4 Terminology.....	19
CHAPTER III: RESULTS	22
3.1 Fruit morphology.....	22
3.1.1 Shape and size.....	22
3.1.2 Ridges on the fruit.....	22
3.1.3 Intra-ocular constrictions between seeds.....	24
3.1.4 Indumentum of the exocarp.....	24
3.1.5 Beak and beak length.....	24
3.1.6 Number of seeds per locule.....	24
3.2 Fruit anatomy.....	24
3.2.1 Fruit lobing and shape of septum between two adjacent locules.....	24
3.2.2 Pericarp thickness.....	26
3.2.3 Shape of exocarp cells.....	26
3.2.4 Secondary metabolite deposits.....	26
3.2.5 Endocarp indumentum.....	26
3.2.6 Channels in the pericarp.....	27
3.2.7 The testa of young seeds.....	27
3.2.8 The inner integument of young seeds.....	27
3.2.9 Endosperm in young seeds.....	31
3.3 Seed morphology.....	31

3.3.1 Shape and size.....	31
3.3.2 Seed colour.....	31
3.3.3 Presence and position of trichomes on the cotyledons of mature seeds	31
3.4 Seed anatomy.....	33
3.4.1 The inner integument of mature seeds.....	33
3.4.2 Endosperm in mature seed.....	33
3.4.3 Cotyledon development	33
3.4.4 Secondary metabolite deposits in the cotyledons.....	35
3.4.5 Number of channels and their position within a cotyledon.....	35
3.5 Inner integument structures (SEM/VP).....	35
3.6 Phenetic analyses.....	35
CHAPTER IV: DISCUSSION	54
4.1 Introduction.....	54
4.2 Variation in the inter-specific fruit and seed morphological and anatomical characters.....	54
4.3 The utility of fruit and seed morphology and anatomy in the identification of major groups	56
4.4 Comparison of the major groups identified by fruit and seed characters to the groupings proposed in previous studies.....	57
4.5 The contribution of fruit and seed characters towards the classification of the southern African members of <i>Oxalis</i>	63
CHAPTER V: CONCLUSION	65
REFERENCES	67
APPENDIX I	73
APPENDIX II	77

CHAPTER I

INTRODUCTION

Oxalis L. belongs to the family Oxalidaceae, which comprises annual or perennial herbs, shrubs and trees. Members of this family occur in Africa, Madagascar, Asia and America (Leistner, 2000). Although complete consensus is still lacking, most systematists regard the family as composing of about seven genera and *ca.* 900 species. Two of these genera *Oxalis* and *Biophytum* DC. occur in South Africa (Leistner, 2000).

Historically the Oxalidaceae was placed in the order Geraniales along with primitive dicotyledonous families, but different authors included different families in the Geraniales. Takhtajan (1980) included the Geraniaceae, Lepidobotryaceae, Hypseocharitaceae and Biebersteiniaceae; Cronquist (1981) included the Geraniaceae, Limnanthaceae, Tropaeolaceae and Balsaminaceae; Dahlgren (1983) included the Geraniaceae, Zygophyllaceae, Erythroxylaceae, Balanitaceae, Humiriaceae, Linaceae and Lepidobotryaceae and Thorne (1983) included the Geraniaceae, Balsaminaceae, Tropaeolaceae and Limnanthaceae

Recent molecular phylogenetic analyses of the angiosperms have improved our understanding of the systematic position of the family. Chase *et al.* (1993) indicated two possible positions for the placement of the Oxalidaceae based on *rbcL* DNA sequence data. An initial search placed the family as a sister clade to the Geraniaceae in the Rosid II clade. This agrees with the proposed classifications of Cronquist (1981) and Takhtajan (1980). A second search showed an unexpected placement of the Oxalidaceae as a sister clade to a clade consisting of members of the Cunoniaceae, Cephalotaceae, Tremandraceae and Eucryphiaceae, but this time within the Rosid I clade (Chase *et al.*, 1993). This contradiction in the two searches is due to the representatives used during the different analyses. In the second search members of *Oxalis* were used as representatives of Oxalidaceae, contrary to the former (also seen as the traditional placement) where the genus *Hypseocharis* Remy was used as a representative for the family. These results were clarified by the DNA analysis of the *rbcL* gene of the families traditionally included within Geraniales by Price & Palmer (1993). They showed a split within Oxalidaceae, where *Hypseocharis* was found closely related to Geraniaceae and the rest of Oxalidaceae were placed as sister to a clade comprising *Eucryphia* Cav. and *Cephalotus* Labill. The Angiosperm phylogeny Group (APG, 1998) proposed a placement of Oxalidaceae within a new order, the Oxalidales within the Eurosids I. Savolainen *et al.* (2001) upheld the establishment of a separate order for the family within their nearly complete

analysis of the phylogeny of the eudicots, based on combined *atpB* and *rbcL* sequence data. Their study confirmed the placement of the family in the Oxalidales within the Eurosids I clade in the Rosids. The position of the Oxalidales was confirmed by APG II (2003).

Controversy prevails about the genera to be included in the Oxalidaceae. Genera traditionally included in the Oxalidaceae are *Biophytum* (70 species), *Oxalis* (ca. 800 species), *Averrhoa* L. (2 species), *Sarcotheca* Blume (11 species), *Dapania* Korth (3 species), *Lepidobotrys* Engl. (1 species) and *Hypseocharis* Remy (9 species) (Boesewinkel 1985). Knuth (1930) included these seven genera along with *Eichleria* Progel (2 species) in the Oxalidaceae. Hutchinson (1959) placed *Averrhoa* in the Averrhoaceae with the Rutales, while *Dapania* and *Sarcotheca* were transferred to the Lepidobotryaceae in the Malpigiales. More recent authors have consented to the inclusion of *Oxalis* (type genus), *Biophytum*, *Dapania*, *Sarcotheca*, *Averrhoa*, *Hypseocharis* and *Lepidobotrys* into the Oxalidaceae (Cronquist, 1981, Boesewinkel, 1985, Rama Devi & Narayana, 1990). However, the results of the Price & Palmer's (1993) molecular study suggested a split in the Oxalidaceae, separating *Hypseocharis* from *Averrhoa* and *Oxalis*, placing it next to Geraniaceae. The erroneous position of *Hypseocharis* in Oxalidaceae was already questioned by Willis (1985), who demonstrated morphological differences between *Hypseocharis* and Oxalidaceae, but also between *Hypseocharis* and Geraniaceae. Boesewinkel's (1988) study on *Hypseocharis* seed coat anatomy confirmed its position within the Geraniaceae and Rama Devi (1991) pointed to a greater resemblance of *Hypseocharis* to the Geraniaceae than to Oxalidaceae with regard to its floral vasculature and the staminal arrangement. The arrangement of these characters in *Hypseocharis* is very similar to that of *Monsonia* L. and *Sarcocaulon* (DC) Sweet, both genera included in the Geraniaceae.

Oxalis is of particular interest because it is the largest and most widespread genus in the family (Judd *et al.*, 1999). It is also one of the two genera of Oxalidaceae that occur in South Africa (Leistner, 2000) and represents the seventh largest genus in the Cape Flora (Goldblatt & Manning, 2000). *Oxalis* has two centres of diversity, one in the area stretching from South America to the southern part of North America, the other in the southwestern Cape region of South Africa (Denton, 1973, Oberlander *et al.*, 2002). Within South Africa, the main diversity centre is located in the Cape Town-Hottentot's Holland area, while two secondary centres are found in the Clanwilliams-Nieuwoudtville and Kamiesberg areas (Oberlander *et al.*, 2002).

In South Africa, *Oxalis* is one of the most prolific genera in terms of number of taxa and sizes of populations. *Oxalis* is represented by *ca.* 211 species (270 taxa), (Salter, 1944, Olivier, 1993) of which a large number is limited as threatened species (57 taxa within the region are listed in the Red Data Book; Hilton-Taylor, 1996). More than 200 *Oxalis* species are found in Namaqualand and the Western Cape, while a few species such as *Oxalis smithiana* Eckl. & Zeyh. reach KwaZulu-Natal and Mpumalanga (Rourke, 1996). In the Western Cape, *Oxalis* species flower from autumn throughout winter into spring and appear almost everywhere. Flowers present a variety of colours, including almost the entire colour spectrum except blue. Species are commonly called sorrel or “surings” because of the acid flavour of the leaves and stems (Rourke, 1996). This also refers to the name of the genus. The name *Oxalis* was derived from the Greek words $\acute{\omicron}\xi\delta\varsigma$ and $\acute{\alpha}\lambda\varsigma$ meaning acid and salt, which is characteristic of the chemical composition of leaves and bulbs of these plants. Humans in the Northern Cape sometimes collect *Oxalis* species for food. In some communities the leaves of *Oxalis pes-caprae* L. are eaten like spinach (Dreyer, 1996). In America tubers of *Oxalis corniculata* L., *Oxalis tuberosa* Mol., and *Oxalis deppei* Loddiges are used as food source (Subrahmanyam, 1995).

1.1-Literature review

The genus *Oxalis* was first described by Linnaeus (1753). At the same time he described eight species. His son described five more species (Linnaeus f., 1781). Jacquin (1795) recognized 78 *Oxalis* species in his revision of the genus, but did not acknowledge heterostyly. Later, Savigny (1797) added three more *Oxalis* species to the list. Sonder (1860), in his revision of the southern African *Oxalis* species, described another 47 new species and recognized 108 species confined to southern Africa. However, many of his names were later reduced to synonymy. Knuth (1930), in a revision of *Oxalis*, extended the work on this genus by including taxa from all over the world. Salter (1944) published the most comprehensive morphological revision of the southern Africa taxa. He described 65 new taxa and acknowledged 208 existing species. He classified these species into 11 sections and 13 subsections. This work was mainly based on morphological data and contained very little or no information on the anatomy, karyology and embryology.

In order to improve Salter’s (1944) morphological classification, several co-workers have added new data from different fields of study. Dreyer (1996) completed a palynological revision of all the southern African *Oxalis* species. Her work emphasised the potential taxonomic value of palynological characters in both the intra- and infrageneric classification

of the genus. As a result, four pollen types (A, B, C and D) and 19 pollen subtypes were identified. Her results agreed with some of Salter's (1944) sections, but questioned the demarcation of others. Oberlander *et al.* (2004) undertook a preliminary DNA based molecular phylogeny of the genus, based on the *trnL-F* sequence data. These results agreed better with the palynological classification of Dreyer (1996) than with the morphological classification of Salter (1944). Their work thus suggested that the morphology and alpha-taxonomy of *Oxalis* needed to be re-assessed.

Salter (1944), in his revision of the genus, did attend to the morphology of the reproductive structures of *Oxalis* and included descriptions of these structures, but he did not consider them in great detail. Broad descriptions of the position and shape (usually ovoid) of the ovary, locule number (5), the number of ovules per locule and the indumentum were given. He regarded the presence or absence of calli on the ovary as taxonomically important within the genus. The type and shape (elongated or not elongated) of the fruit (capsule) was recorded, but the fruit length could not be correlated to the number of seeds contained therein. Therefore the length of the capsule was not used as an informative character. Salter (1944) also described the seed structure and gave an indication of the presence or absence of endosperm. This character allowed the identification of major divisions within the genus, which also agreed to some extent, with his classification based on vegetative structures. All species with endospermous seeds were placed in the first four sections, while all species with exendospermous seeds were placed in the latter five sections. This morphological seed variation is linked to different seedling strategies. Exendospermous seeds are soft and greenish due to the presence of a well-developed and photosynthetic embryo. After ejection by a translucent, membranous and elastic outer integument, the inner integument consists merely of a smooth delicate sheath that breaks off as the embryo is ejected (Salter, 1944; Obone, 2003). These are short-lived seeds that germinate immediately in a moist medium, and have been reported to survive for only three days under adverse conditions (Du Plessis & Duncan, 1989).

Endospermous seeds are generally smaller and the persistent inner testa covering the endosperm is hard and brown, with a more or less rugose surface (Salter, 1944; Obone, 2003). The colour and proportionate size of the embryo to the ripe seed, and the amount of endosperm varies in different species (Salter, 1944). Germination of these types of seeds is slow (Salter, 1944), and seeds are thought to go through a resting period during the following dry summer months, only to germinate during the following winter (Dreyer, pers. obs.).

Anatomical features have played an increasingly important role in the elucidation of phylogenetic relationships in *Oxalis* (Singh 1999). Eiten (1963) and Denton (1973) studied the species of the sections *Corniculatae* and *Ionoxalis*, respectively. They described the ornamentation and banding patterns of the testa and illustrated the seeds of various species.

In summary, Salter (1944) divided the South African *Oxalis* species into 11 sections (nine indigenous and two naturalised) and 13 subsections, based on morphological characters. This classification presented some shortcomings as was indicated by Dreyer (1996) in her palynological revision of the genus and by Oberlander *et al.* (2004) in their preliminary molecular analysis. It thus seems that some of the sections delimited by Salter (1944) are grouped as natural entities, but other sections seem to be artificial compilations of unrelated species. A pilot study of the ovary, fruit and seed morphology and anatomy of some South African members of *Oxalis* (Obone, 2003) provided promising new data. These data were mainly derived from the seed anatomy and included the distinction between endospermous and exendospermous seeds previously proposed by Salter (1944). This preliminary study also showed some congruence with the molecular data. Thus the aims of the present study were to:

1. evaluate the potential systematic value of the fruit and seed morphology and anatomy among South African members of *Oxalis*.
2. compare the fruit and seed morphological and anatomical characters with the results of Salter (1944), Dreyer (1996) and Oberlander *et al.* (2004) in order to test the systematic value of fruit and seed characters.

1.2-Hypothesis

Morphological and anatomical characters of the fruit and seed of *Oxalis* can contribute significantly toward an improved new systematic classification of the southern African members of *Oxalis*.

1.3-Keys questions

- 1-Do the fruit and seed morphology and anatomy of selected *Oxalis* taxa display species-specific character variation?
- 2-Can combinations of these characters be used to identify seed and fruit types in the genus?
- 3-How do these new potentially informative characters compare with the results of:
 - a-Salter's (1944) alpha-taxonomic classification of southern African *Oxalis* ?
 - b-Dreyer's (1996) palynological classification of southern African *Oxalis* ?

c-Oberlander *et al.* (2004) molecular phylogeny of southern African *Oxalis* ?

4-Can these fruit and seed characters contribute toward achieving a new, improved classification of the southern African members of the genus?

CHAPTER II

MATERIALS AND METHODS

Thirty-two species, representing the nine native and one of the two naturalised southern African *Oxalis* sections delimited by Salter (1944), were selected for inclusion in this study. Species selection was done such as to also coincide with the species currently used in the reconstruction of the molecular phylogeny of the southern African members of the genus (*Oberlander pers. com*). Furthermore, the species selection also aimed to represent all of the major pollen types recorded in the genus by Dreyer (1996). Living material was obtained from the J.S. Marais Park, the living collection in the Botanical Garden of the University of Stellenbosch and different natural localities in the Western and Northern Cape Provinces, South Africa (Table 2.1). A voucher specimen for each species was deposited in the Herbarium of the University of Stellenbosch (STEU). For each species numerous fruits and seeds were collected from different plants of the same population to study the morphology and anatomy and to assess the range of variation displayed by the various characters studied.

2.1-Morphological and anatomical studies

Observations of the shape and external structures of the fruits and seeds were done using a stereomicroscope. For anatomical studies fresh material was fixed in Formalin-Alcohol-Acetic acid (5:90:5, FAA) before dehydration and infiltration with wax according to the Ethanol-Butanol technique proposed by Johansen (1940). The wax imbedded material was then sectioned (15 μm thick) using a rotary microtome and the sections were mounted on microscope slides using Haupt's reagent and stained using the Alcian Green-Safranin series (Joel, 1983), Sudan III and Sudan IV (Ruzin, 1999) staining solutions. Slides were permanently mounted using DPX glue for the AGS method and slides stained with Sudan III and IV were temporary mounted in glycerine. All the slides were studied with the help of a Nikon YS 100 light microscope (LM). Sudan III proved to be inefficient in characterizing cuticles within the testa, but Sudan IV proved to be effective. For anatomical studies of herbarium specimens, the dry material first had to be reconstituted before sections and permanent slides could be made. Dry fruits and seeds were softened with Aerosol OT (Ayensu, 1967), but instead of embedding the material in Spurr's resin (Spurr, 1969) as proposed by Peterson *et al.* (1978), material was embedded using the technique of Johansen (1940). Although the softening method of Ayensu (1967) has proven to be successful in many previous histological studies (Ayensu, 1967, Peterson *et al.*, 1978), it was not effective for the reconstituting of flattened, dry fruits and seeds of *Oxalis* specimens. Due to these problems

and the general scarcity of fruits and seeds on herbarium material, the results discussed in the present study were mainly obtained from slides prepared from fresh material.

2.2-SEM and VP studies

The ultra-structure of seeds and inner integuments were studied with the aid of a scanning electron microscope (SEM), using both standard and Variable Pressure procedures (VP) at the EM centre of the Stellenbosch University. For the SEM analyses, dried seeds were mounted onto aluminium stubs using clear nail varnish as glue. The stubs were then sputter-coated with a gold-palladium layer and studied with the aid of a Leo 1430 VP 7 K V SEM. Scanning electron micrographs were taken at fixed magnifications of 150x and 10.000x to facilitate direct comparison between specimens. This technique could not be applied to all the species, and proved problematic, especially for those species with soft cotyledons. The strong vacuum effect led to a total distortion and a dramatic drying of the seed. To study the structure of the seed inner testa of such species, VP methods were applied in addition to the normal SEM procedures. Variable pressure operation is appropriate for the study of damp, alcohol preserved or soft samples that will lose their shape under high vacuum conditions. The VP analyses were also performed on a Leo 1430 VP 7 K V SEM, but at considerably lower vacuum conditions than in normal SEM scans. The material was not sputter-coated with a gold-palladium layer, but kept as natural as possible instead. In general VP micrographs are less clear than normal SEM scans.

Table 2.1: Specimens examined for fruit and seed characters. For each species the taxonomic position, collector and collector's number, University of Stellenbosch project number, locality, and pollen type (according to Dreyer, 1996) are given.

Taxon	Collector & collector's number	Project number	Locality	Pollen type (Dreyer, 1996)
SECTION CORNICULATAE				
<i>O. corniculata</i> L.	Dreyer 602	MO 3	J.S. Marais Park Stellenbosch	C2
SECTION CERNUAE SUBSECTION EU-CERNUAE				
<i>O. pes-caprae</i> L. var. <i>pes-caprae</i>	Gebregziabher 2	MO 6	J.S. Marais Park Stellenbosch	C2
SECTION OPPOSITAE SUBSECTION SUBINTEGRAE				
<i>O. luteola</i> Jacq. var. <i>luteola</i>	Oberlander 26	MO 257	J.S. Marais Park Stellenbosch	C3
<i>O. ambigua</i> Jacq.	Oberlander 140	MO 555	10 km north of Gharies	C3
<i>O. obtusa</i> Jacq. var. <i>obtusa</i>	Obone 1	O 764	J.S. Marais Park Stellenbosch	C3
SUBSECTION BIFURCATAE				
<i>O. heterophylla</i> D.C.	Oberlander 163	MO 608	Houhoek Pass, Grabouw	C4
SECTION STICTOPHYLLAE				
<i>O. purpurea</i> L.	Oberlander 24	MO 255	J.S. Marais Park Stellenbosch	C3
SECTION FOVEOLATAE				
<i>O. furcillata</i> Salter	Dreyer 702	MO 133	Springbok	C2
SECTION CAMPANULATAE				
<i>O. natans</i> L.	Walton 340	MO 607	Elandsberg Private Nature Reserve	C2

SECTION LATIFOLIOLATAE				
<i>O. tenella</i> Jacq.	Dreyer 768	MO 264	Between Citrusdal and Clanwilliam	D1
<i>O. stenoptera</i> Turcz	Oberlander 138	MO 553	Nuwerus	C10
<i>O. aridicola</i> Salter	Dreyer 778	MO 274	Between Pakhuis and Botterkloof Passes	D1
SECTION CRASSULAE				
<i>O. louisae</i> Salter	Dreyer 708	MO 139	Kamieskroon	C10
SECTION ANGUSTSTAE				
SUBSECTION PARDALES				
<i>O. sp.</i>	B.Bayer 7300	MO 511	North West Southkloof	C2
<i>O. grammophylla</i> Salter	Dreyer 670	MO 101	Between Pakhuis and Botterkloof Passes	C2
SUBSECTION SESSILIFOLIATAE				
<i>O. meisneri</i> Sond.	Oberlander 88	MO 468	Tulbagh	C9
<i>O. viscosa</i> E.Mey. ex Sond	Oberlander 136	MO 550	Between Clanwilliam and Klaver	C2
<i>O. hirta</i> L. var. <i>hirta</i>	Dreyer and Oberlander 7	MO 13	J.S. Marais Park Stellenbosch	D1
<i>O. tenuifolia</i> Jacq.	Oberlander 27	MO 258	J.S. Marais Park Stellenbosch	C8
<i>O. cf. urbaniana</i> Schltr.	Bayer 7392	MO 601	Saron	C8
<i>O. multicaulis</i> Eckl. & Zeyh.	Oberlander 158	MO 595	Elandsberg Private Nature Reserve	C8
SUBSECTION XANTHOTRICHAE				
<i>O. pillansiana</i> Salter	Oberlander 135	MO 549	Between Clanwilliam and Klaver	C10

SUBSECTION <i>LINERARES</i>				
<i>O. pusilla</i> Jacq.	Oberlander 36	MO 309	Tienie Versveld Reserve, Darling	C2
<i>O. glabra</i> Thunb.	Oberlander 161	MO 605	J.S. Marais Park Stellenbosch	C8
<i>O. xantha</i> Salter	Oberlander 136	MO 551	Between Pakhuis and Botterkloof Passes	D1
<i>O. ciliaris</i> Jacq. var. <i>ciliaris</i>	Oberlander 28	MO 301	Theronsberg Pass, Ceres	D1
<i>O. oreophila</i> Salter	Dreyer 774	MO 270	Pakhuis Pass	D1
<i>O. versicolor</i> L.	Oberlander 35	MO 308	Tienie Versveld Reserve, Darling	C8
SUBSECTION <i>GLANDULOSAE</i>				
<i>O. ebracteata</i> Savign.	Dreyer 766	MO 262	Piekenier's Kloof Pass	C8
<i>O. droseroides</i> E.Mey. ex Sond.	Oberlander 79	MO 362	Tulbagh	C13
<i>O. clavifolia</i> Sond.	Oberlander 141	MO 556	20 km North of Gharies	C2
NOT ALLOCATED TO A SECTION				
<i>O. monophylla</i> L.	Dreyer and Oberlander 3	MO 9	J.S. Marais Park Stellenbosch	C10

2.3-Statistical analysis using NTSYS-pc

The present study assessed the variation in fruit and seed morphological and anatomical characters, and tried to use this variation to identify possible fruit or seed types among the species studied. Characters analyses were done using the software package **NTSYS-pc**, which provides an effective method for multivariate data analyses (Rohlf, 2004).

The 35 potentially informative characters identified in this study were coded to a binary character matrix. Recoding resulted in 78 binary (present/absent) characters. The data matrix was created in Microsoft Excel and imported into Ntedit to convert it into an NTSYS matrix format. In NTSYS, a SIMQUAL study was performed to compute various association coefficients for qualitative data with unordered states using the Dice coefficient. Clustering was done using SAHN, which performs the sequential, agglomerative, hierarchical, and

nested clustering methods as defined by Sneath & Sokal (1973). UPGMA linkage was used to construct the phenogram.

A heuristic search of 1000 replicates of random taxon-addition, with TBR branch swapping was performed using the PAUP 4.0 beta 10 software (Swofford, 2000) on a Power Mac G5/Dual 2 GHz PC. Internal nodes support was assessed using the bootstrap (Felsenstein, 1985), with 1000 replicates of simple taxon addition and TBR branch swapping.

2.4-Terminology

The terminology of Radford *et al.* (1974) and Stearn (1966) was used to describe fruit and seed morphology and the inner seed coat surfaces. The illustrations of seed surfaces (Figure 2.1) were compiled from Murley (1951) and were used consistently throughout this study to describe the inner integument patterns. An illustration of a cross-section of a developing seed coat of *Averrhoa bilimbi* L. (Oxalidaceae) was obtained from Boesewinkel (1985) and used as reference to interpret the testas of *Oxalis* seeds (Figure 2.2). Figures 2.3 and 2.4 represent schematic drawings of longitudinal sections through young and mature seeds, respectively. These diagrams were used as basic templates for the interpretation of seed age and structure.

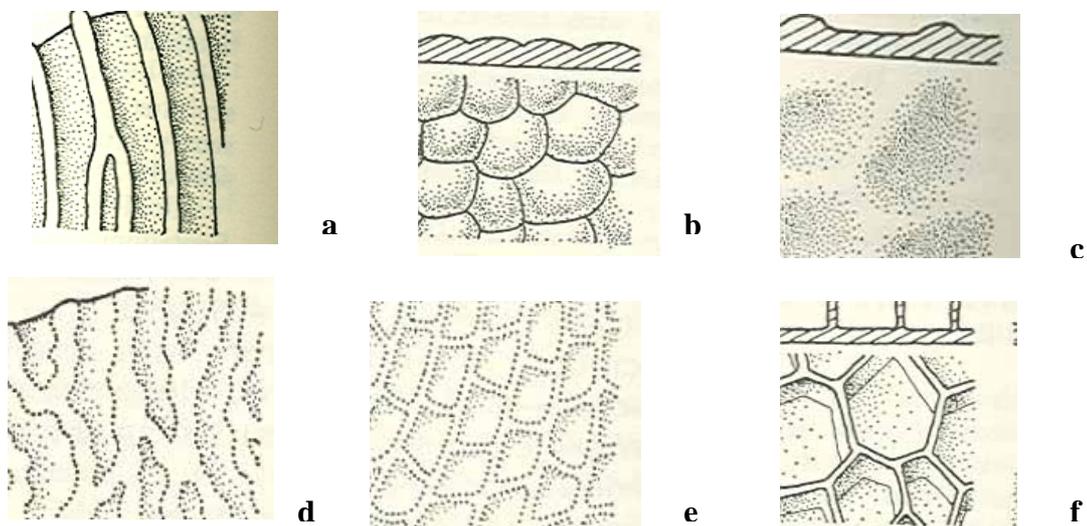


Figure 2.1: Illustrations representing the inner integument patterns as seen with the SEM/VP: a-ribbed; b-colliculate; c-alveolate; d-rugose; e-scalariform and f-reticulate

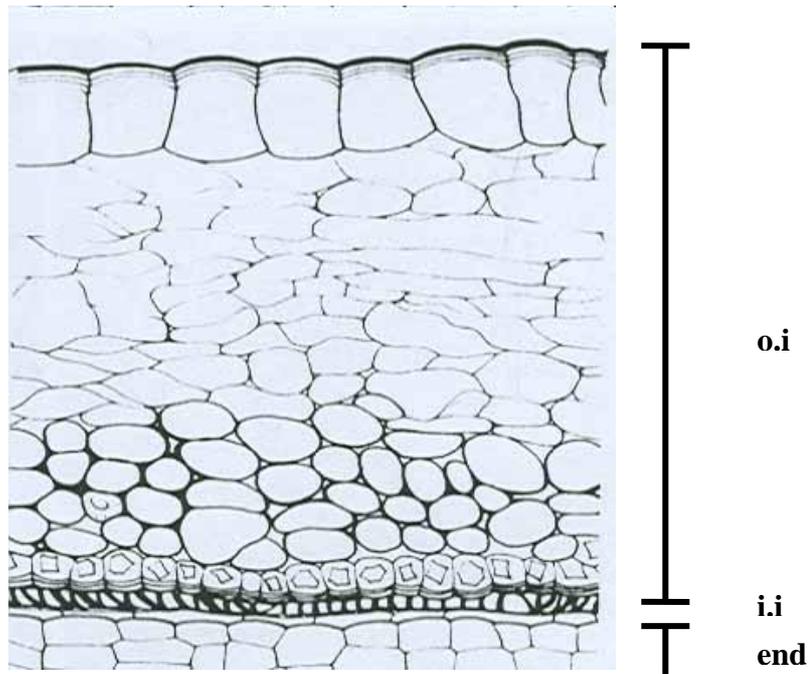


Figure 2.2: Cross section of a developing seed coat of *Averrhoa bilimbi* (Oxalidaceae) after Boesewinkel (1985); **end** = endosperm, **i.i** = inner integument, **o.i** = outer integument. Details of the seed coat are given in section 3.2.7.

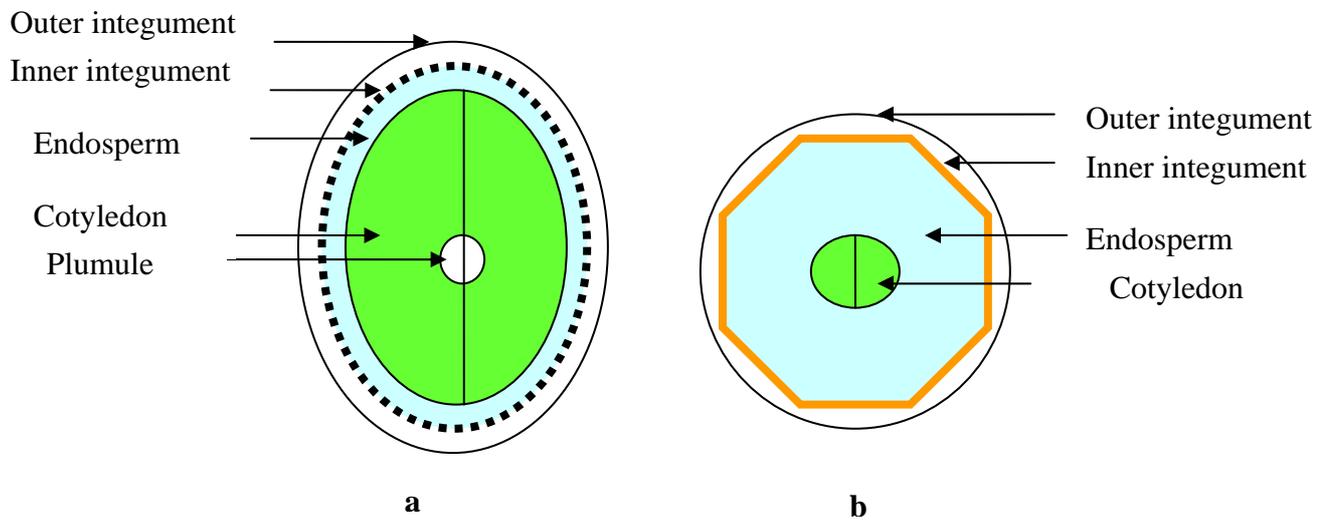


Figure 2.3: Schematic presentations of longitudinal sections through young *Oxalis* seeds; **a**-ex-endospermous seed and **b**-endospermous seed. Details of the seed coat are given in section 3.2.7.

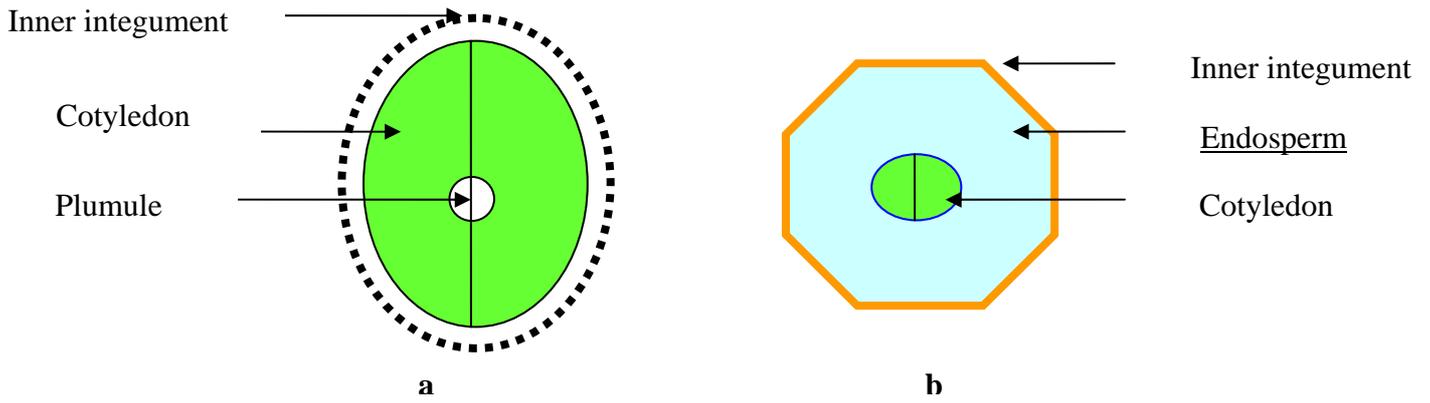


Figure 2.4: Schematic presentations of longitudinal sections through mature *Oxalis* seeds after ejection of the seed by shedding the outer integument; **a**- exendospermous seed and **b**- endospermous seed. Details of the inner seed coat are given in section 3.2.8.

CHAPTER III

RESULTS

A total of 35 fruit and seed characters were identified and compared among the 32 species studied. Some of these characters showed consistent variation between different species, and were thus found to be potentially systematically informative, while other characters were found to be identical in all species studied. All these characters are listed below and the different character states are discussed in some details. Figures of morphological (SM), anatomical (LM) and ultra-structural (SEM/VP) characters are referred to in the text. Tables 3.1 and 3.2, in which all the informative characters are summarized, are supplied at the end of this chapter.

3.1-FRUIT MORPHOLOGY

In all southern African *Oxalis* species fruits are capsules, with five carpels forming five locules and the seeds always display axile placentation. Dried fruits dehisce along the main vein of the carpels, so that the fruits are defined as loculicidal capsules.

3.1.1-Shape and size

Three main fruit shapes were identified, namely oblong, ovoid to broadly ovoid and spheroid to broadly spheroid (Figure 3.1 a-c). Four of the seven endospermous species have oblong fruits, while the other three have ovoid to broadly ovoid fruits. None of the endospermous species has spheroid fruits. Thirteen of the twenty-five exendospermous species have spheroid to broadly spheroid fruits, eight species have ovoid to broadly ovoid fruits and only four exendospermous species have oblong fruits (*O. tenella*, *O. sp. subsection Pardales*, *O. grammophilla* and *O. xantha*). In terms of dimensions, oblong fruits are *ca.* 5 – 15 mm X 2 – 4 mm, ovoid fruits are *ca.* 2.5 – 5 mm X 2.5 – 6 mm and spheroid fruits are *ca.* 1.5 – 6 mm X 1.5 – 6 mm. Fruit sizes and shapes are summarised in Table 3.1.

3.1.2-Ridges on the fruit

Dehiscence zones occur along the main vein of each carpel and in some species prominent ridges also occur along these veins. Prominent ridges occur in three endospermous species (*O. corniculata*, *O. ambigua* and *O. purpurea*), and also in 11 of the 25 exendospermous species (Figure 3.1 b & c).

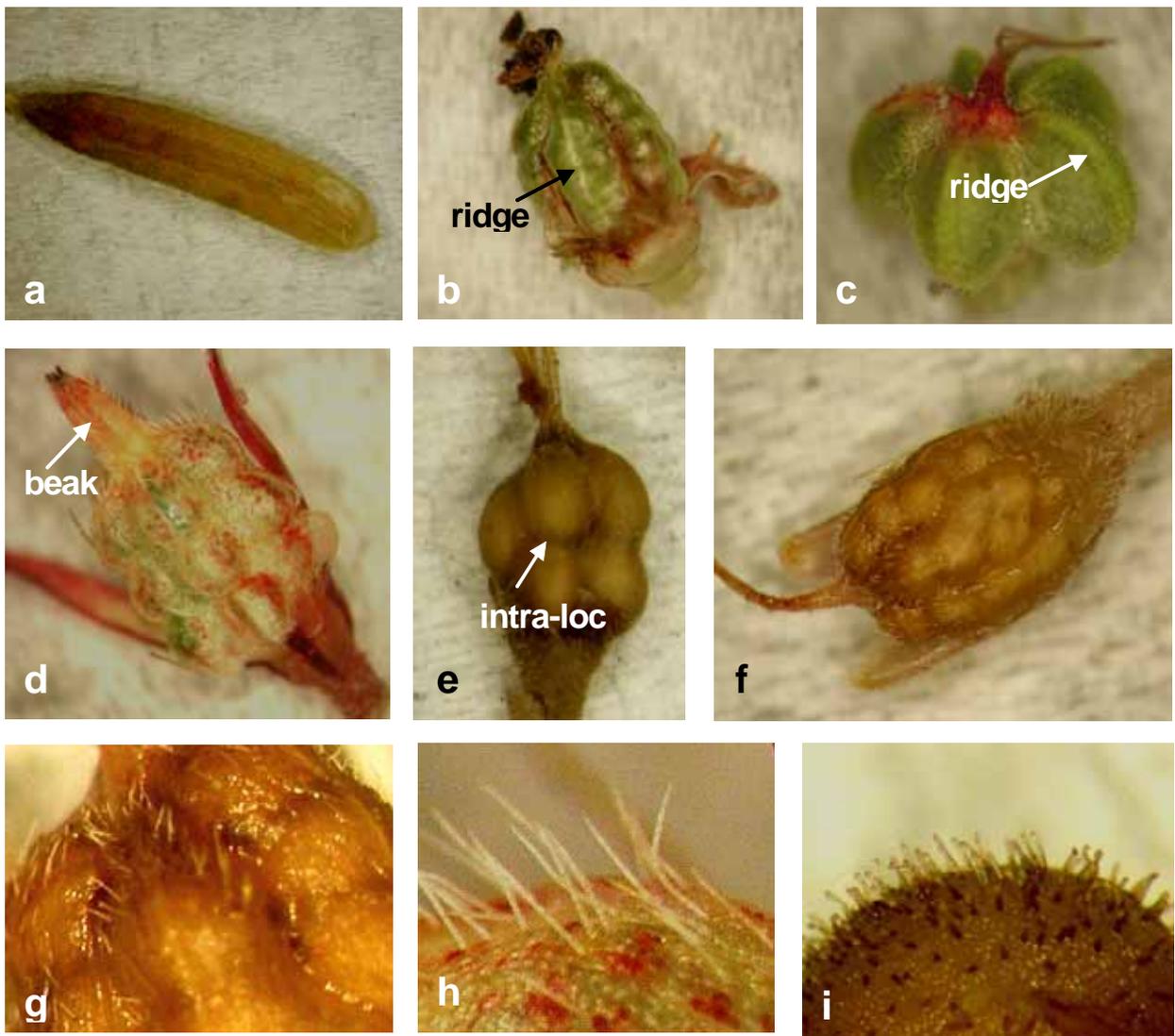


Figure 3.1: **a – c.** Fruit shapes and ridges in *Oxalis*. **a** - *O. corniculata* fruit, oblong with a clear ridge along each locule. **b** - *O. purpurea* fruit, ovoid and with a clear ridge along each locule. **c** - *O. tenuifolia* fruit, broadly spheroid and with a clear ridge along each locule. **d – f.** Intra-locular constrictions: **d** - *O. glabra* fruit, clear constrictions between seeds. **e** - *O. aridicola* fruit, clear constrictions between seeds. **f** - *O. luteola*, intra-locular constrictions poorly defined. **g - i** Type of trichomes found on fruits. **g** - *O. ambigua* fruit with simple hairs. **h** - *O. glabra* fruit with multicellular hairs. **i** - *O. multicaulis* fruit with glandular hairs. **intra-loc**= intra-locular constriction between seeds of the same locule.

3.1.3-Intra-locular constrictions between seeds

This type of constriction was mostly observed in ovoid (and one spheroid (*O. pusilla*)) fruits of exendospermous species (Figure 3.1 d & e). In total, 11 of the 25 species presented intra-locular constriction between seeds of their fruits. This character was consistently absent from endospermous species.

3.1.4-Indumentum of the exocarp

Although the exocarp of all *Oxalis* fruits displayed an indumentum, three different types of trichomes were identified and they were distributed in one of two distinct patterns. Hairs can be scattered all over the fruit (5/7 of the endospermous species) or be restricted only to the upper part of the fruit (14/25 exendospermous species). The three hair types that occur on the fruits include simple, glandular and multicellular hairs. Hairs on a species can be restricted to one type, or different hair types may be mixed (Salter, 1944) (Figure 3.1 g-i).

3.1.5-Beak and beak length (if present)

A beak (fruit extension) was found in two endospermous species (*O. corniculata* and *O. pescaprae*) and in 14 exendospermous species. Beak length ranges between 0.5 and 2 mm, with the longest beaks observed on fruits of *O. corniculata*, *O. xantha* and *O. glabra* (Figure 3.1 d).

3.1.6-Number of seeds per locule

The number of seeds per locule in oblong fruits varies from 5 (*O. heterophylla*) to 10, and is seldom less than 5. Ovoid or broadly ovoid fruits usually have 3 seeds per locule (seldom less), spheroid fruits have 2 seeds per locule (*O. pillansiana*, *O. pusilla* and *O. aridicola* (Figure 3.1 e)) and broadly spheroid fruits always have only 1 seed per locule. In the latter case the fruits have flattened apices (*O. tenuifolia* (Figure 3.1 c), *O. multicaulis* and *O. monophylla*).

3.2-FRUIT ANATOMY

3.2.1-Fruit lobing and shape of septum between two adjacent locules

Three main lobing types were observed among fruits of *Oxalis*, namely deeply lobed, moderately lobed and vaguely lobed. In deeply lobed fruits, locules are almost separate (there is no septum between two adjacent locules); in moderately lobed fruits locules are partially

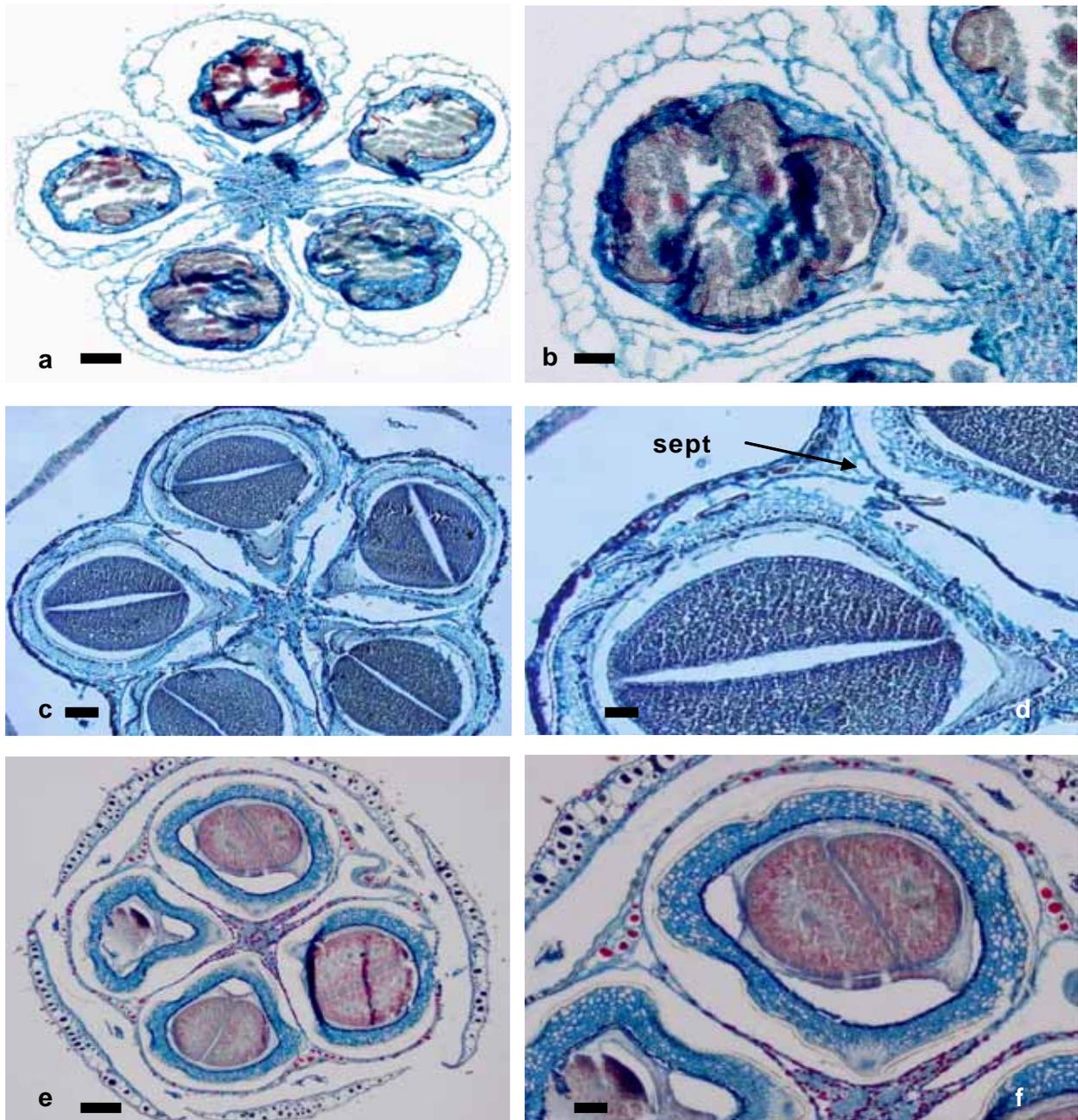


Figure 3.2: **a**, **c** and **e**. Fruit lobing. **a** - *O. obtusa* fruit, deeply lobed. **c** - *O. monophylla* fruit, moderately lobed. **e** - *O. glabra* fruit, vaguely lobed. **b**, **d** and **f**. Septum shape. **b** - Closer view of locules of *O. obtusa* fruit, septum well-separated. **d** - Closer view of locules of *O. monophylla*, septum partially separated. **f** - Closer view of locules of *O. glabra* fruit, septum fused. **sept** = Septum. Scale bar = 0.2 mm in **a**, **c** and **e**. Scale bar = 0.1 mm in **b**, **d** and **f**.

fused and in vaguely lobed fruits locules are fused and separated by a septum (Figure 3.2 d). Fruits of endospermous species are mostly deeply lobed (6 species), with only one that is moderately lobed (*O. pes-caprae*) and these fruits are never vaguely lobed. In contrast, fruits of exendospermous species are mostly moderately lobed (15 species), with some also deeply lobed (4 species) or vaguely lobed (5 species). The fruit lobing of the remaining species (*O. droseroides*) could not be matched with any of these categories, due to insufficient material available.

3.2.2-Pericarp thickness (Figure 3.3)

The pericarp is composed of the exocarp, consisting of one layer of epidermal cells, the mesocarp, consisting of parenchymatic cells and the endocarp, consisting of one layer of epidermal cells. Although this character varies among species, the mesocarp in endospermous species usually consists of more than three cell layers, whereas in the exendospermous species it is mostly between two and three cell layers thick.

3.2.3-Shape of exocarp cells (epidermis)

The epidermal cells of the exocarp in endospermous species are irregular in shape, and they usually have jagged edges (Obone, 2003) (Figure 3.3 a). In contrast, the exocarp cells of exendospermous species are usually round (Figure 3.3 b), oblong or five-sided, and they have more or less smooth edges.

3.2.4-Secondary metabolite deposits within the fruit wall (pericarp) and/or within the fruit centre

Secondary metabolites here refer to all the chemical substances such as phenolic compounds found in any fruit or seed tissue and which stained red with Alcian green stain. These compounds were deposited either only within the fruit centres (Figure 3.3 f) with no secondary compounds in the pericarp (Figure 3.3 a), or both within the pericarp (Figure 3.3 b) and the centre of the fruit as in many exendospermous species.

3.2.5-Endocarp indumentum (if present)

In endospermous species there are hairs on the inner layer of the fruit walls (endocarp). The indumentum ranges from densely hairy with a combination of simple and glandular hairs in two species (*O. luteola* (Figure 3.3 c) and *O. purpurea*) to less densely hairy with simple hairs only in *O. pes-caprae*, *O. ambigua*, *O. obtusa* and *O. heterophilla* and glabrous in *O. corniculata*. No hairs were found on the endocarp of any of the exendospermous species.

3.2.6-Channels in the pericarp (fruit wall)

Channels were found in the pericarp of two species, one endospermous species (*O. ambigua*, Figure 3.3 d) and one exendospermous species (*O. sp. subsection Pardales*).

3.2.7-The testa of young seeds (Figure 3.4)

The testa of both seed types is composed, from the periphery going inwards, of the outer integument, inner integument and a very thin cuticle covering the endosperm (Figure 3.4 e & f). The outer integument comprises an epidermis with a thick cuticle that stains red in Sudan IV and three to four layers of more or less crushed parenchymatic cells (the number varies within species). The innermost cell layer of the outer integument contains undetermined substances (dots, Figure 3.4 e & f) in all species. Comparing the seed coat structure of *Oxalis* to that of *Averrhoa* (Boesewinkel, 1985), similar structures can be recognized like an exotesta with a distinct cuticular layer and the middle layer of crushed cells of the outer integument. The inner integument of endospermous *Oxalis* species is similar to that of *Averrhoa* (Boesewinkel, 1985). This is not surprising, since the seeds of *Averrhoa* are also endospermous. The structure of the inner integument differs between the two seed types of *Oxalis* and will be discussed in the next section.

3.2.8-The inner integument of young seeds

In the inner most layer of the inner integument of the exendospermous seeds, phenolic compounds occur as dark dots (Figure 3.5 f). No special techniques were employed to identify the different phenolic compounds in the seeds, but the AGS (Alcian Green Safranine) staining that was used, stained all phenolic compounds red to almost black. Beneath this dark-dotted inner integument there is a very thin cuticle, staining pinkish with Sudan IV, although it was sometimes difficult to observe (Figure 3.4 c & d). Unlike in exendospermous seeds, the inner integument of endospermous seeds (Figure 3.5 b & d) consists of a reddish layer (or brown when stained with Sudan IV) comprising elongated, thick-walled lignified cells (yellow when stained with Sudan IV). This fibrous cell layer appears more or less wavy in the seven species studied (Figure 3.5 a). The fibrous layer is covered by a thin, pinkish cuticle. The thick-walled lignified cells seem to be similar to the isometric and tangentially flattened cells of the inner pigment-layer of *Averrhoa* described by Boesewinkel (1985).

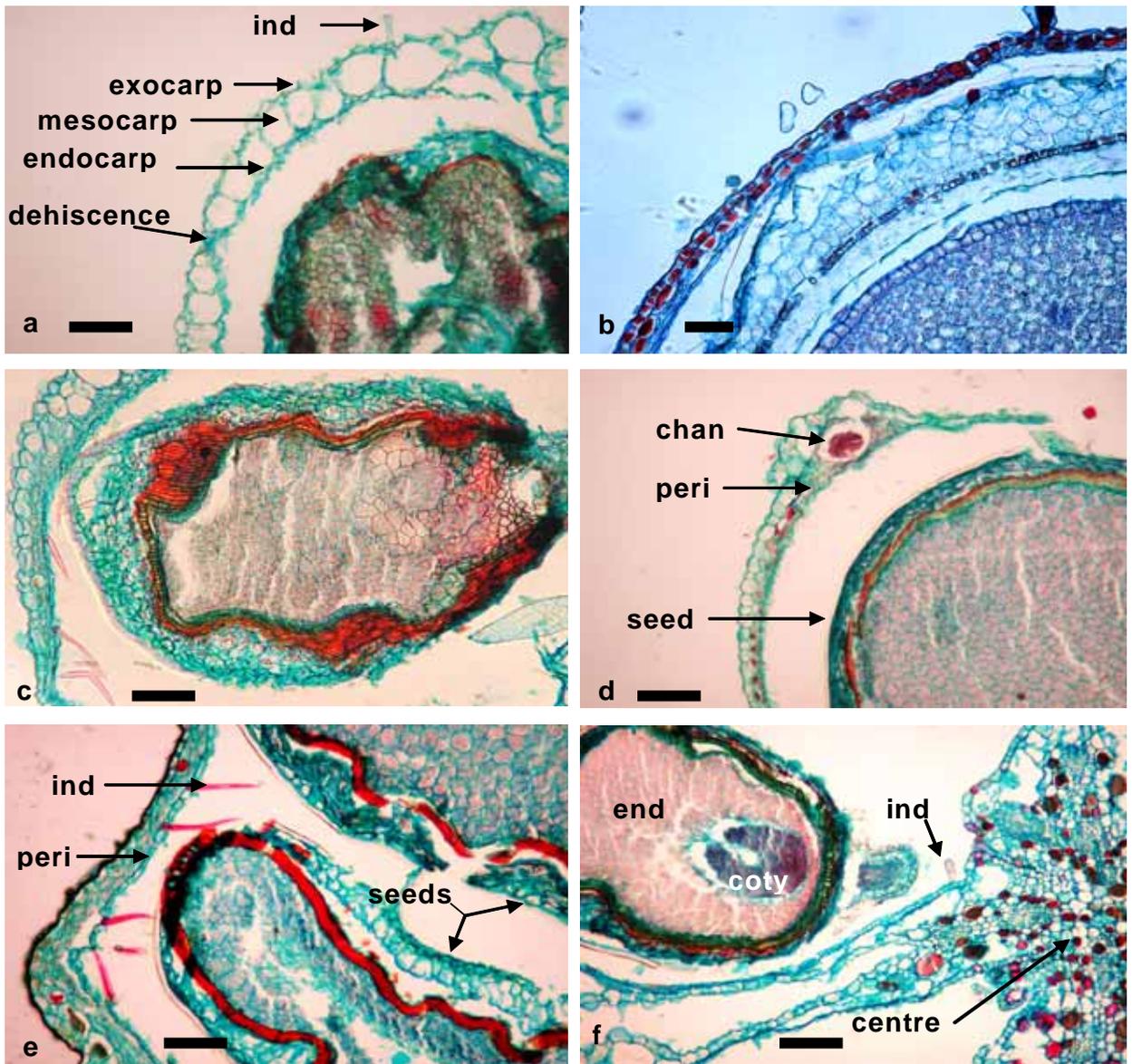


Figure 3.3: **a - e** Pericarps of fruit of *Oxalis* species. **a** - *O. obtusa* fruit, pericarp 3 cell layers thick, exocarp 1 layer of cells with jagged edges and mesocarp 1 layer of large parenchymatic cells and 1 cell layer of the endocarp. No secondary metabolite deposits within the pericarp. **b** - *O. monophylla* fruit, pericarp 3 cell layers thick with round cells and secondary metabolites present within the fruit wall. **c** - *O. luteola* fruit, pericarp 3-4 cell layers thick, hairs present in the endocarp. **d** - *O. ambigua* fruit, pericarp 3-4 cell layers thick, secondary metabolites and channels present. **e** - *O. purpurea*, pericarp 4 cell layers thick, secondary metabolites and hairs present. **f** - *O. luteola* fruit centre, glandular hair and secondary metabolites present. **chan** = channel, **centre** = fruit centre **coty** = cotyledon, **end** = endosperm, **ind** = indumentum and **peri** = pericarp. Scale bar = 0.1 mm.

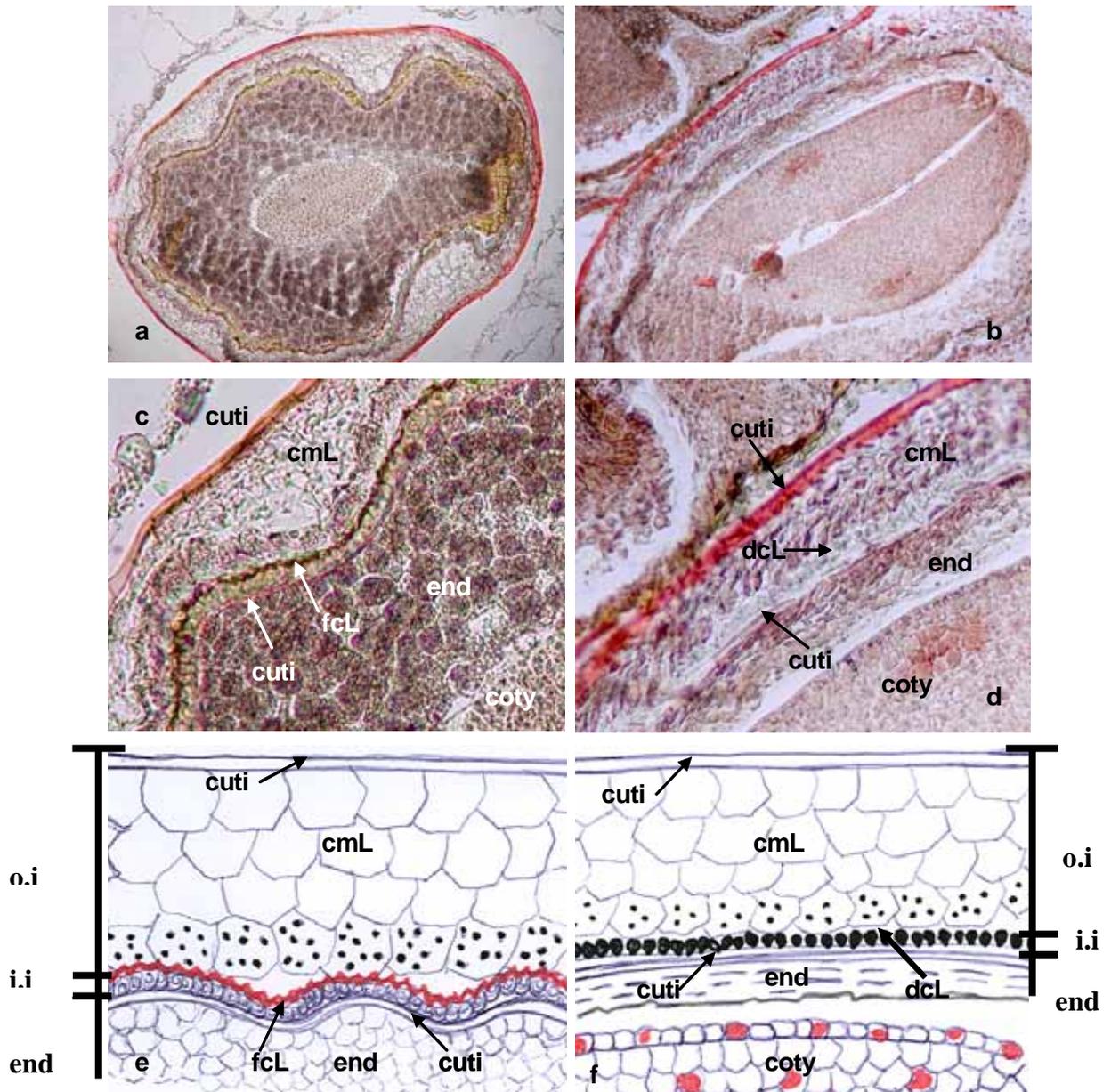


Figure 3.4: a-d. Cuticles within testas of young seeds of *Oxalis*. **a** - *O. heterophylla* young seed, with a cuticle on the epidermis of the outer integument. **b** - *O. grammophylla* young seed, with a cuticle on the epidermis of the outer integument. **c** - A closer view at a seed of *O. heterophylla*, two distinct cuticles, one on the outer integument and the other on the innermost layer of the inner integument. Crushed parenchymatic cells present in the middle layer of the outer integument. Wide cells containing dark substances within the outer integument. Fibrous and wavy cell layer present (stained yellow). **d** - A closer view of *O. grammophylla* seed, two distinct cuticles present, crushed parenchymatic cells present in the middle layer, cells containing dark substances in the outer integument and the dark dotted cell layer in the inner integument. **e** - **f**. Schematic presentations of cross sections through young *Oxalis* seed coats. **e** - Endospermous seeds. **f**-ex-endospermous seeds. **cmL** = crushed cells of the middle layer, **coty** = cotyledon, **cuti** = cuticle, **dcL** = dotted cell layer, **end** = endosperm, **fcl** = fibrous cell layer, **i.i** = inner integument, **o.i** = outer integument. Scale bar = 0.1 mm.

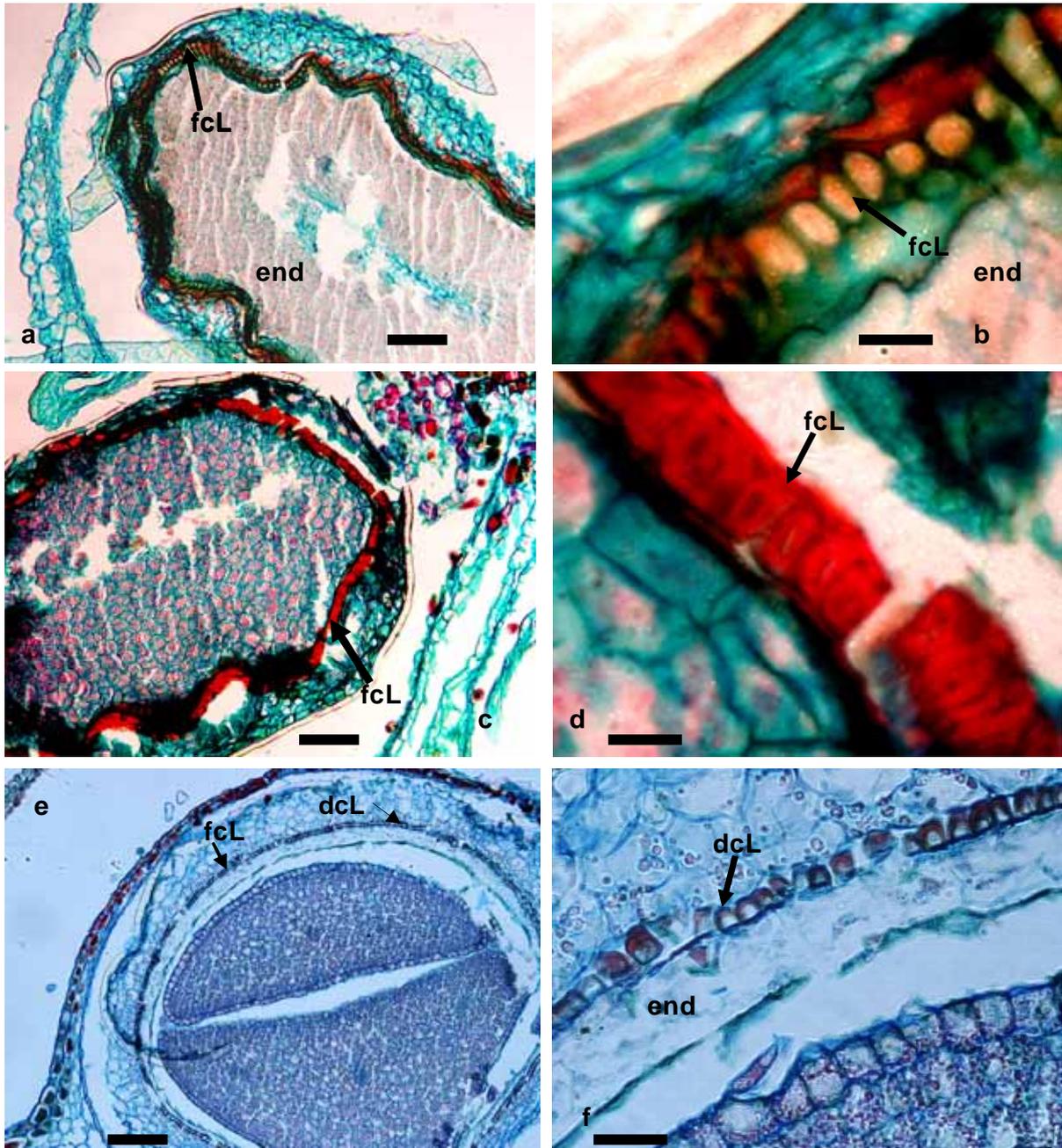


Figure 3.5: a, c and e. Inner integument types in young seeds of *Oxalis*. a - *O. luteola* young seed, orange and wavy inner integument. c - *O. purpurea* young seed, red and wavy inner integument. e - *O. monophylla* young seed, very thin, dotted inner integument. b, d and f. closer view of a, c and e. b - Closer view of *O. luteola* seed, showing thick cell walls of fibrous cells. d - Closer view of *O. purpurea* seed, showing thick and red cell wall of fibrous cells. f - Closer view of *O. monophylla* seed, showing cells with dark substances present in the innermost layer of the outer integument and dark dots composing the inner integument. **dcL** = dark dotted cell layer. **end** = endosperm. **fcL** = fibrous cell layer. Scale bar = 0.1 mm.

3.2.9-Endosperm in young seeds

Endosperm was found in all young seeds (Figure 3.4 e & f), but the endosperm was already starting to disintegrate in all exendospermous seeds examined. In all cases the embryos were considerably more advanced than the embryos of the endospermous seeds examined.

3.3-SEED MORPHOLOGY

The variation in the morphology of angiosperm seeds and the relative constancy of seed structures in narrow taxonomic units permit the use of seed characteristics in taxonomic studies (Esau, 1977). The most important seed morphological characters are shape, size, testa surface, position of the hilum and the presence or absence of specialized structures such as an aril, caruncle or elaiosome. Of the entire range of seed morphological characters assessed in the present study, only shape, size, colour and the presence or absence of hairs on the epidermis of the cotyledons revealed significant variation to be considered potentially systematically informative.

3.3.1-Shape and size

The shape of the seeds varies from elliptic or widely elliptic (endospermous seeds, Figure 3.6 a-c) to obovoid (exendospermous seeds, Figure 3.6 d-h). Elliptic seeds are 0.9 – 1.2 mm long and 0.6 – 1 mm in diameter. Obovoid seeds measure 1 – 4 mm in length and 0.8 – 3 mm in diameter. The sizes and shapes of all seeds are summarised in Table 3.1.

3.3.2-Seed colour

The colour of the seeds studied ranged between orange/brown to green or dark green (Figure 3.6). The seven endospermous species all had orange to brown seeds, while the exendospermous species all had green to dark green seeds.

3.3.3-Presence and position of trichomes on the cotyledons of mature seeds

Trichomes were only found on the cotyledons of mature exendospermous seeds of all the species, except *Oxalis natans*, *Oxalis* cf. *urbaniana* and the three species containing channels within their cotyledons. These hairs may be found scattered all over the cotyledons (Figure 3.6 e & g) or they can be restricted to the margins of the cotyledons (Figure 3.6 f & h).

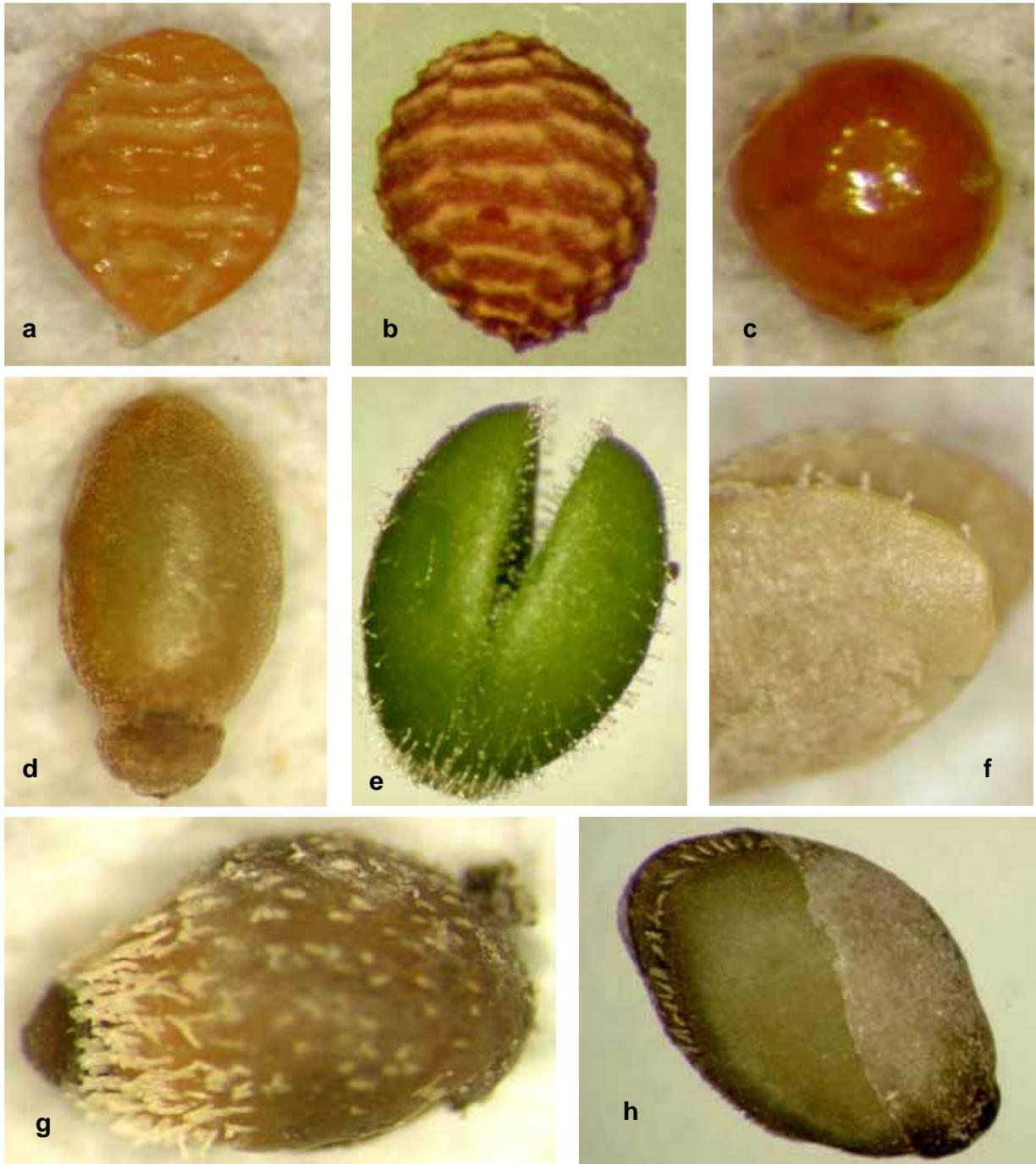


Figure 3.6: **a - c** Mature endospermous seeds. **a** - *O. corniculata* seed, small elliptic and orange seed. **b** - *O. pes-caprae* seed, widely elliptic and brown seed. **c** - *O. ambigua* seed, elliptic and brown seed. **d - h** Mature exendospermous seeds. **d** - *O. xantha* seed, obovoid and photosynthetic seed without trichomes. **e** - *O. glabra* seed, obovoid and photosynthetic seed, pillose. **f** - *O. tenella* seed, obovoid and photosynthetic seed with trichomes on the margins of the cotyledons. **g** - *O. louisae* seed, obovoid and photosynthetic seed, pillose. **h** - *O. hirta* seed, obovoid and photosynthetic seed with trichomes on the margins of the cotyledons.

3.4-SEED ANATOMY

Seed anatomical characters have proven to be very valuable in determining taxonomic relationship (Esau 1977). In the present study five seed anatomical characters were identified as being potentially informative. They are the presence or absence of well-defined cotyledons, the presence or absence and position of channels within the cotyledons, and the presence of either a red phenolic and wavy inner integument or a dark-dotted phenolic inner integument.

3.4.1-The inner integument of mature seeds (Figures 3.7 g, h & e)

At maturity, the seeds of all *Oxalis* species are ejected from the fruit through an explosive dehiscence of the outer integument. The dispersed seeds are thus only covered by the inner integument. The inner integument in both young and mature seeds differ between endospermous and exendospermous species. In endospermous seeds, the thickness of the layer and the extent of waviness are variable between different species. The layer with elongated, thick-walled lignified cells is thinner and much wavier in *O. corniculata*, *O. pes-caprae*, *O. heterophylla* and *O. purpurea* than in the three remaining species, where the layer is thicker and less wavy (sometimes only vaguely wavy). The same dark-dotted phenolic layer that was observed in young seeds was also observed in the older seeds of exendospermous species. This layer is restricted to exendospermous species. The thickness of this layer and the shape of the dotted cells are identical in almost all of the species, with the exceptions of *O. louisae* and *O. monophylla*, in which this cell layer was thicker.

3.4.2-Endosperm in mature seed

Endosperm was observed only in the mature seeds of all of the endospermous species (Figure 3.7 b & c), and no trace of endosperm was found in mature exendospermous seeds.

3.4.3-Cotyledon development

Fully developed embryos with fleshy cotyledons were restricted to all species that produce non-endospermous seeds (Figure 3.7 d & e), whereas poorly developed embryos were found in all endospermous seeds (Figure 3.7 a - c). Almost all exendospermous seeds were composed of two well-developed cotyledons, except in some individuals of *O. hirta*, where an embryo with three or four cotyledons was found (Figure 3.7 f).

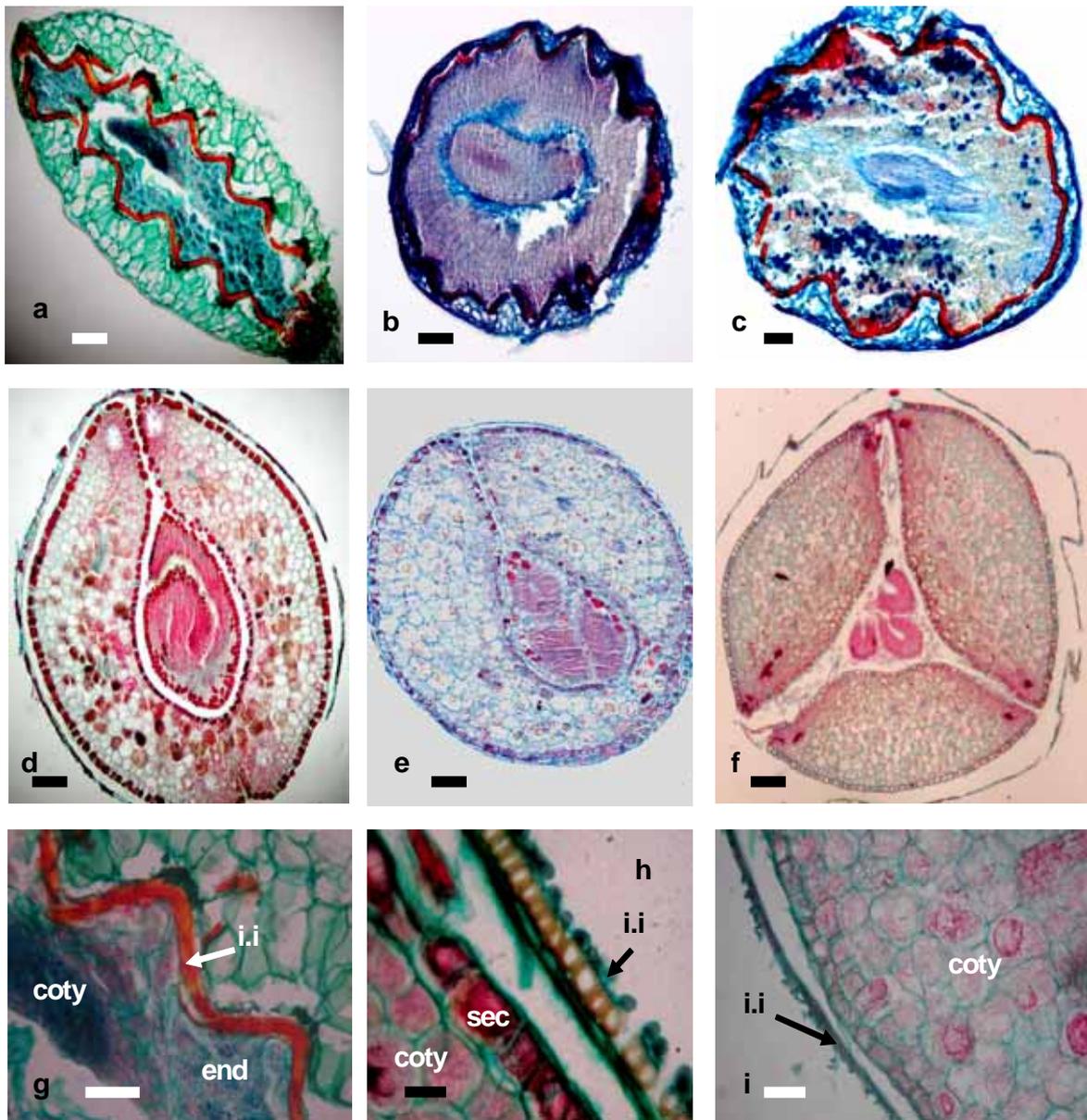


Figure 3.7: **a - c** Cross section through mature endospermous seeds. **a** - *O. corniculata* seed, inner integument obviously wavy. Endosperm present, but cotyledons not well - developed. **b** - *O. pes-caprae* seed, inner integument less wavy, endosperm present. **c** - *O. obtusa* seed, inner integument less wavy and endosperm present. **d - f** mature exendospermous seeds. **d** - *O. xantha* seed, cotyledons well developed, endosperm absent. **e** - *O. glabra* seed, cotyledons well developed endosperm absent. **f** - *O. hirta* seed, three well - developed cotyledons, endosperm absent. **g - i** Types of inner integuments. **g** - *O. corniculata*, fibrous cells and wavy inner layer. **h** - *O. louisae* seed, dotted cell layer. **i** - *O. cf pillansiana* seeds, dark dotted cell layer. **chan** = channels, **coty** = cotyledon, **end** = endosperm, **i.i** = inner integument, **sec** = secondary metabolites. Scale bar = 0.1 mm.

3.4.4-Secondary metabolite deposits in the cotyledons

Secondary metabolites were mainly found deposited in well-developed cotyledons of exendospermous species. Deposits were found in the outer layers of the cotyledons. These deposits could be restricted to the epidermis only (as in *O. aridicola* and *O. glabra*), or it could be deposited in both the epidermis and within the cotyledon (as in *O. furcillata* and *O. xantha*, amongst others) (Table 3.1).

3.4.5-Number of channels and their position within a cotyledon (if present)

Channels were restricted to the cotyledons of the exendospermous seeds of *O. sp. subsection Pardales*, *O. grammophylla* and *O. xantha*. The channels were distributed in two different patterns among these three species: (1) along the outer edges of the cotyledons (*O. grammophylla*) and (2) only at the tip of the cotyledon (*O. sp. subsection Pardales* and *O. xantha* (Figure 3.7 d)).

3.5-INNER INTEGUMENT STRUCTURES (SEM, VP)

The scanning electron microscope is a highly efficient tool for the study of seed structure. In the present study the SEM was used to study the abaxial sculpture of the inner integument of the selected *Oxalis* species (Figure 3.8). The following seven sculptural patterns were identified: ribbed (*O. corniculata*), rugose (*O. pes-caprae*), reticulate (*O. xantha*), alveolate (*O. cf. urbaniana*), scalariform (*O. aridicola*), colliculate (*O. louisae*) and smooth (*O. grammophylla*). Two of these patterns, namely ribbed and rugose, were exclusively found in two endospermous species and colliculate was found in only one exendospermous species (Table 3.1).

3.6-PHENETIC ANALYSES

A cluster analysis was performed for all 32 species using fruit and seed morphological and anatomical characters, including the fine-structure of the inner integument of the seeds in order to demarcate main groups based on these data. The resultant groups are discussed in Chapter 4. At the same time the results are compared to the proposed palynological groupings of Dreyer (1996) and the main lineages of the species-level DNA based phylogenetic reconstruction of *Oxalis* (Oberlander *et al.*, 2004 and Oberlander, in prep). The phenogram depicting the similarities/dissimilarities between the taxa (Figure 3.9) is presented at the end of Chapter 3.

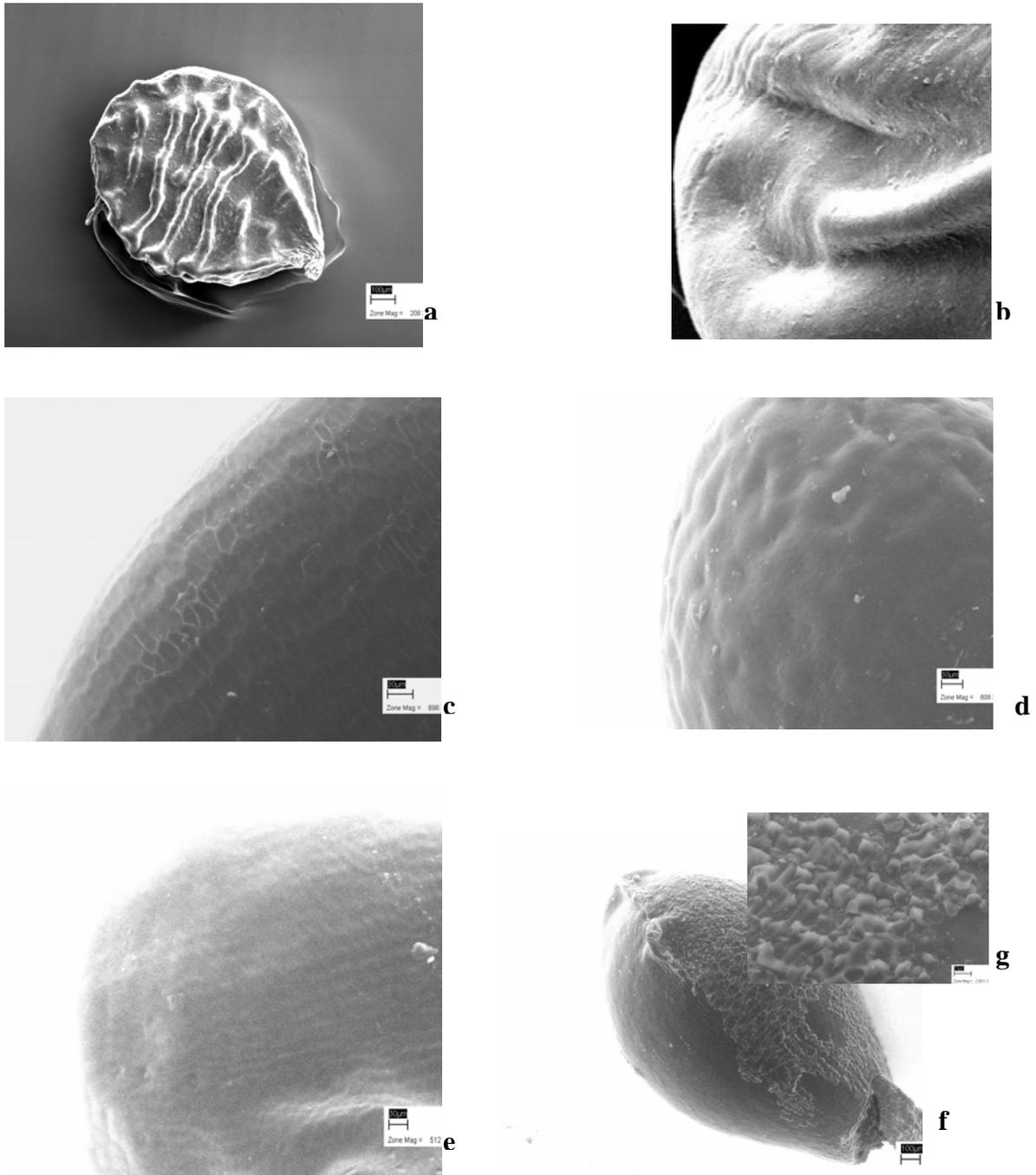


Figure 3.8: **a - g.** Structure of the abaxial side of the inner integument SEM-VP. **a-** *O. corniculata* seed, inner integument ribbed. **b -** *O. pes-caprae* seed, inner integument rugose **c-***O. xantha* seed, inner integument reticulate. **d -** *O. urbaniana* seed, inner integument alveolate. **e -** *O. aridicola* seed, inner integument scalariform. **f –** *O. louisae* seed, inner integument colliculate. **g -** *O. louisae* seed, a closer view of the colliculate pattern.

The phenogram retrieves two major clusters that only connect at the 24% similarity level (76% different (Figure 3.9)). The first cluster (A) includes the first seven species (all producing endospermous seeds) and the second cluster (B) the 25 remaining taxa (all producing exendospermous seeds). Within cluster (B) there is one species, *O. glabra*, that is sister to and 52.1% similar to a group containing two subclusters **B1** and **B2**. The latter two subclusters are about 54.7% similar. These three main clusters (Figure 3.9) are discussed in more detail under separate headings below.

Cluster A (species producing endospermous seeds)

***O. corniculata* - *O. pes-caprae* subgroup**

Within the cluster A, *O. obtusa* and *O. heterophylla* are the most similar (82.8%), and they are sister to *O. corniculata* at a similarity level (SL) of 77.5%. *O. pes-caprae* joins this cluster at SL = 73% (Figure 3.9).

***O. luteola* - *O. purpurea* subgroup**

In the second subcluster within cluster A, *O. ambigua* is found to be 80.7% similar to *O. purpurea*, while these two species collectively show a 79% similarity to *O. luteola* (Figure 3.9).

The two subclusters in the cluster A group together at SL = 64.1%.

Cluster B (species producing exendospermous seeds)

Subcluster B1

***O. furcillata* - *O. grammophylla* subgroup**

Subcluster B1 contains nine species, of which *O. cf. urbaniana* and *O. versicolor* are the most similar (SL = 82.6%) (Figure 3.9). *O. furcillata* is sister to and ca. 72.3% similar to these two species. Another group composed of *O. sp. subsection Pardales* and *O. grammophylla* (ca. 78.1% similar) is sister to and ca. 64.8% similar to the group including *O. cf. urbaniana*, *O. versicolor* and *O. furcillata*.

***O. meisneri* – *O. multicaulis* subgroup**

O. meisneri and *O. pusilla* show a similarity of 68.24% to each other, while *O. viscosa* and *O. multicaulis* are 74.9% similar. These two subgroups (*O. meisneri* - *O. pusilla* and *O. viscosa* - *O. multicaulis*), in turn, show a SL = 63.5%.

The two groups included in subclade B1 are similar at *ca.* 57.4%.

Subcluster B2

***O. natans* - *O. oreophila* subgroup**

Subcluster B2 includes fifteen species and within this subcluster, *O. natans* and *O. monophylla* are the most similar (SL = 81.9%). *O. oreophila* resolves as sister to this group at the 80% similarity level.

***O. hirta* - *O. droseroides* subgroup**

O. ebracteata and *O. droseroides* are 89% similar and form a sister group to *O. tenuifolia* at the 74.9% similarity level. *O. hirta* retrieves as 72.3% similar to the group containing *O. ebracteata*, *O. droseroides* and *O. tenuifolia*.

The first two subgroups in subcluster B2 (*O. natans* - *O. oreophila* and *O. hirta* - *O. droseroides*) group together at the 71.6% similarity level.

***O. tenella* - *O. aridicola* subgroup**

O. tenella and *O. aridicola* are very similar at a SL = 78.1% and form a sister group to the *O. natans* - *O. droseroides* group at the 60.7% similarity level.

***O. xantha* - *O. ciliaris* subgroup**

O. xantha and *O. ciliaris* are 76.84% similar and form a sister group to the large group of 9 species ranging from *O. natans* to *O. aridicola* (SL = 60% similarity) (Figure 3.9).

***O. stenoptera* - *O. clavifolia* subgroup**

O. stenoptera and *O. louisae* are 64.1% similar, while *O. pillansiana* and *O. clavifolia* are 67.6% similar. These two groups join at the 59.4% similarity level.

The large subgroup formed by 11 taxa from *O. natans* to *O. ciliaris* is sister to a smaller subgroup that includes *O. stenoptera*, *O. louisae*, *O. pillansiana* and *O. clavifolia* (SL = 56.7%).

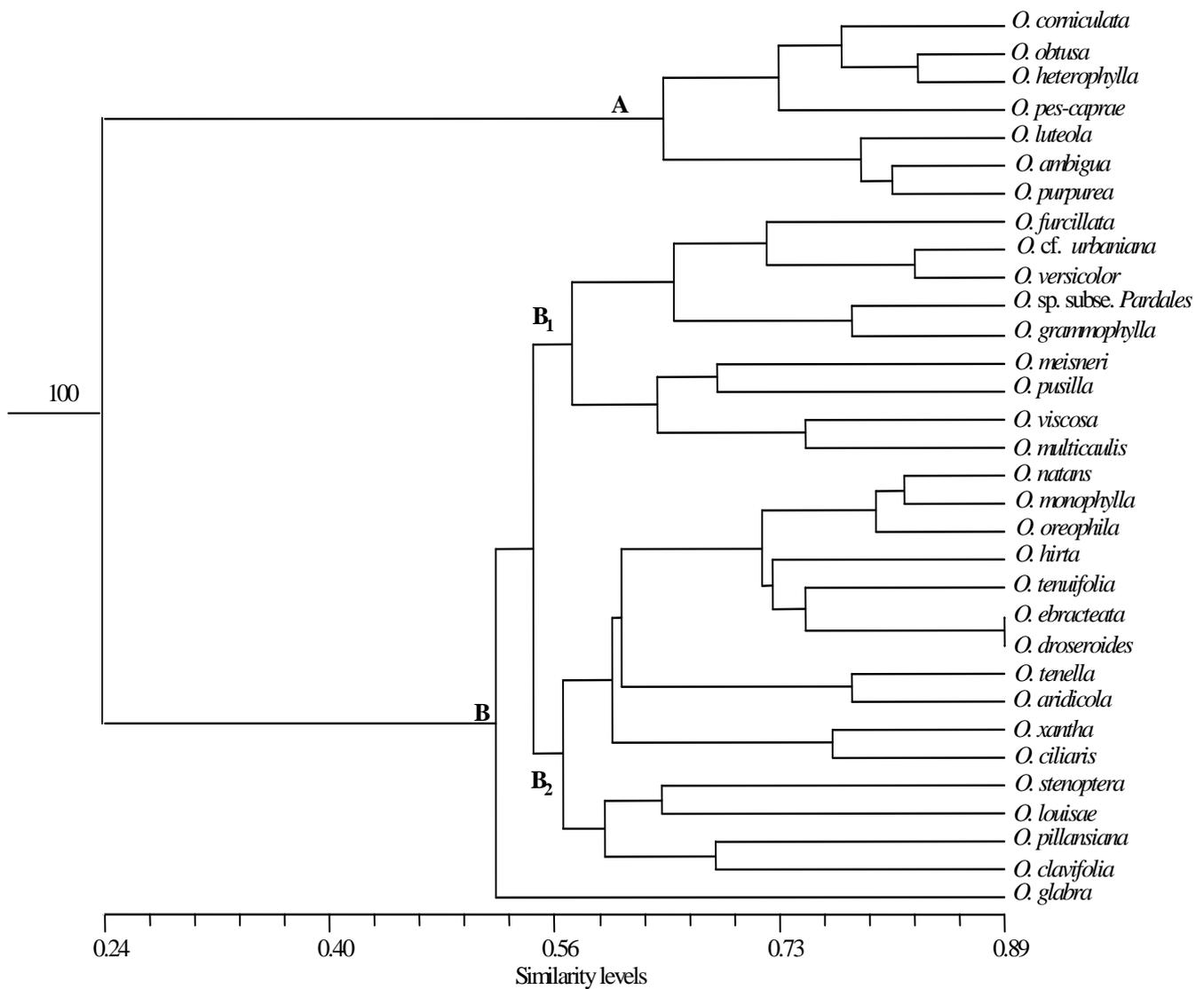


Figure 3.9: UPGMA phenogram of 32 *Oxalis* species, based on 78 fruit and seed characters (cophenetic correlation coefficient $r = 0.92$). The number above the main branch indicates bootstrap support (Felsenstein, 1985).

Table 3.1: A list of morphological and anatomical characters used in the assessment of fruit variation between *Oxalis* selected taxa

FRUIT CHARACTERS	<i>O. corniculata</i>	<i>O. pes-caprae</i>	<i>O. luteola</i>	<i>O. ambigua</i>	<i>O. obtusa</i>
Fruit shape	oblong	oblong	ovoid	ovoid	oblong
Fruit length (mm)	8 - 15	7 - 9	3.5 - 4	2.5 - 5	5 - 12.
Fruit diameter (mm)	2 - 3.5	1.5 - 3	2.5 -3.5	2 - 2.5	2 - 3.5
Fruit ridging (present/absent)	present	absent	absent	present	absent
Intra-locular constriction between seeds (present/absent)	absent	absent	absent	absent	absent
Indumentum position on the exocarp	scattered all over	scattered all over	upper part only	upper part only	scattered all over
Type of hairs on exocarp	multicellular	simple	glandular	simple	simple
Fruit beak (present/absent)	present	present	absent	absent	absent
Beak length (mm)	1 - 2	1.5	0	0	0
Number of seeds per locule	3 - 9	5 - 6	3 - 4	3 - 11	4 - 6
Fruit lobing	deeply lobed	moderately lobed	deeply lobed	deeply lobed	deeply lobed
Type of septum	well divided	partially divided	well divided	well divided	well divided
Pericarp thickness (number of cell layers)	4	3 - 4	4	4 - 5	3
Shape of exocarp cells (epidermis)	jagged edges	jagged edges	jagged edges	round	jagged edges
Secondary metabolites deposits within the pericarp and/or the fruit centre	fruit centre	fruit centre	fruit wall/ fruit centre	fruit wall/ fruit centre	fruit centre
Endocarp indumentum (if present)	absent	simple	multicellular	simple	simple
Channels in the pericarp (present/absent)	absent	absent	absent	present	absent
Testa thickness (number of cell layers)	3 - 4	4 - 5	3 - 4	3 - 4	3 - 4
Red phenolic layer lining the inner testa (present/absent)	present	present	present	present	present
Dark-dotted phenolic layer lining the inner testa (present/absent)	absent	absent	absent	absent	absent
Endosperm in the young seed (intact/disintegrating)	intact	intact	intact	intact	intact

FRUIT CHARACTERS	<i>O. heterophylla</i>	<i>O. purpurea</i>	<i>O. furcillata</i>	<i>O. natans</i>	<i>O. tenella</i>
Fruit shape	oblong	ovoid	ovoid	broadly spheroid	oblong
Fruit length (mm)	5.5 - 10	2 - 4.0	2.5 - 5	3 - 4mm	4.5 - 7
Fruit diameter (mm)	2 - 3.5	3 - 4.0	2.5 - 5	2.5 - 4.5	2 - 4.5
Fruit ridging (present/absent)	absent	present	absent	absent	present
Intra-locular constriction between seeds (present/absent)	absent	absent	present	absent	present
Indumentum position on the exocarp	scattered all over	scattered all over	upper part only	upper part only	scattered all over
Type of hairs on exocarp	glandular	simple	multicellular	glandular	mixed
Fruit beak (present/absent)	absent	absent	present	present	present
Beak length (mm)	0	0	0.5	0.5	1 - 2.5
Number of seeds per locule	6 - 10	10	2	1	2 -3
Fruit lobing	deeply lobed	deeply lobed	moderately lobed	moderately lobed	vaguely lobed
Type of septum	well divided	well divided	partially divided	partially divided	fused
Pericarp thickness (number of cell layers)	3	4	3	3	2
Shape of exocarp cells (epidermis)	oblong	oblong	jagged edges/ oblong	round	oblong
Secondary metabolites deposits within the pericarp and/or the fruit centre	fruit centre	fruit wall/ fruit centre	fruit wall/ fruit centre	fruit wall/ fruit centre	fruit centre
Endocarp indumentum (if present)	simple	multicellular	simple	absent	absent
Channels in the pericarp (present/absent)	absent	absent	absent	absent	absent
Testa thickness (number of cell layers)	3 - 4	3 - 4	3 - 4	4 - 5	3 - 4
Red phenolic layer lining the inner testa (present/absent)	present	present	absent	absent	absent
Dark-dotted phenolic layer lining the inner testa (present/absent)	absent	absent	present	present	present
Endosperm in the young seed (intact/disintegrating)	intact	intact	disintegrating	disintegrating	disintegrating

FRUIT CHARACTERS	<i>O. stenoptera</i>	<i>O. aridicola</i>	O. louisae	<i>O. sp. subsection Pardales</i>	<i>O. grammophylla</i>
Fruit shape	ovoid	broadly spheroid	broadly spheroid	oblong	oblong
Fruit length (mm)	3 - 4mm	3 - 4mm	4 - 5.5	4.5	3.5 - 5
Fruit diameter (mm)	4 - 5mm	3 - 3.5	4 - 6.5	2.5 - 3	2.5 - 4
Fruit ridging (present/absent)	present	absent	present	absent	absent
Intra-ocular constriction between seeds (present/absent)	absent	present	absent	present	present
Indumentum position on the exocarp	scattered all over	scattered all over	scattered all over	upper part only	upper part only
Type of hairs on exocarp	multicellular	glandular	glandular	multicellular	simple
Fruit beak (present/absent)	absent	present	absent	absent	absent
Beak length (mm)	0	1 - 1.5	0	0	0
Number of seeds per locule	1 - 2 seeds	1 - 2 seeds	2 - 3 seeds	2 - 3 seeds	2 - 4 seeds
Fruit lobing	deeply lobed	vaguely lobed	deeply lobed	moderately lobed	moderately lobed
Type of septum	well divided	fused	well divided	partially divided	partially divided
Pericarp thickness (number of cell layers)	3	2	3 - 4	3	3 - 4
Shape of exocarp cells (epidermis)	oblong	round	round	jagged edges/oblong	oblong
Secondary metabolites deposits within the pericarp and/or the fruit centre	fruit centre	fruit centre	fruit centre	fruit centre	fruit centre
Endocarp indumentum (if present)	absent	absent	absent	absent	simple
Channels in the pericarp (present/absent)	absent	absent	absent	present	absent
Testa thickness (number of cell layers)	3 - 4	3 - 4	4 - 5	3 - 4	3 - 4
Red phenolic layer lining the inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining the inner testa (present/absent)	present	present	present	present	present
Endosperm in the young seed (intact/disintegrating)	disintegrating	disintegrating	disintegrating	disintegrating	disintegrating

FRUIT CHARACTERS	<i>O. meisneri</i>	<i>O. viscosa</i>	<i>O. hirta</i>	<i>O. tenuifolia</i>	<i>O. cf. urbaniana</i>
Fruit shape	ovoid	ovoid	broadly spheroid	broadly spheroid	ovoid
Fruit length (mm)	2.5 - 3	2	2.5 - 4	2.5 - 3	3.5 - 4
Fruit diameter (mm)	2 - 2.5	2.5	3 - 4.5	2.5 - 4	2.5 - 3.5
Fruit ridging (present/absent)	absent	absent	present	present	absent
Intra-locular constriction between seeds (present/absent)	absent	absent	absent	absent	absent
Indumentum position on the exocarp	upper part only	upper part only	upper part only	upper part only	upper part only
Type of hairs on exocarp	multicellular	mixed	simple	simple	mixed
Fruit beak (present/absent)	present	absent	present	absent	present
Beak length (mm)	1.5	0	1	0	0.5
Number of seeds per locule	3	2	1	1	3
Fruit lobing	moderately lobed	moderately lobed	vaguely lobed	moderately lobed	moderately lobed
Type of septum	partially divided	partially divided	fused	partially divided	partially divided
Pericarp thickness (number of cell layers)	3	2	3	3	2
Shape of exocarp cells (epidermis)	round	oblong	round	round	pentagonal
Secondary metabolites deposits within the pericarp and/or the fruit centre	central fruit	fruit wall/ fruit centre			
Endocarp indumentum (if present)	absent	absent	absent	absent	absent
Channels in the pericarp (present/absent)	absent	absent	absent	absent	absent
Testa thickness (number of cell layers)	3 - 4	4 - 5	4 - 5	3 - 4	3 - 4
Red phenolic layer lining the inner testa (present/absent)	absent	present	absent	absent	absent
Dark-dotted phenolic layer lining the inner testa (present/absent)	present	absent	present	present	present
Endosperm in the young seed (intact/disintegrating)	disintegrating	disintegrating	disintegrating	disintegrating	disintegrating

FRUIT CHARACTERS	<i>O. multicaulis</i>	<i>O. pillansiana</i>	<i>O. pusilla</i>	<i>O. glabra</i>	<i>O. xantha</i>
Fruit shape	broadly spheroid	spheroid	spheroid	ovoid	oblong
Fruit length (mm)	1.5 - 2	2.5 - 4.5	1.5 - 2.8	4.5 - 6.0	9.5 - 10.5
Fruit diameter (mm)	2 - 3.0	3.0 - 4.0	2.0 - 3.0	3.0 - 4.5	2 - 3.0
Fruit ridging (present/absent)	present	absent	absent	absent	absent
Intra-locular constriction between seeds (present/absent)	absent	present	present	present	present
Indumentum position on the exocarp	scattered all over	upper part only	upper part only	upper part only	scattered all over
Type of hairs on exocarp	glandular	glandular	simple	simple	simple
Fruit beak (present/absent)	absent	present	present	present	present
Beak length (mm)	0	0.5	1.0 - 1.5	0.5	1.0 - 2.0
Number of seeds per locule	1	1 - 2	2	3 - 4	3 - 9
Fruit lobing	moderately lobed	deeply lobed	moderately lobed	vaguely lobed	moderately lobed
Type of septum	partially divided	well divided	partially divided	fused	partially divided
Pericarp thickness (number of cell layers)	2	3	3	3	3
Shape of exocarp cells (epidermis)	round	round	pentagonal	pentagonal	pentagonal
Secondary metabolites deposits within the pericarp and/or the fruit centre	fruit wall/ fruit centre	fruit centre	fruit wall/ fruit centre	fruit wall/ fruit centre	fruit wall/ fruit centre
Endocarp indumentum (if present)	absent	absent	absent	absent	absent
Channels in the pericarp (present/absent)	absent	absent	absent	absent	absent
Testa thickness (number of cell layers)	4 - 5	4 - 5	4 - 5	4 - 5	3 - 4
Red phenolic layer lining the inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining the inner testa (present/absent)	present	present	present	present	present
Endosperm in the young seed (intact/disintegrating)	disintegrating	disintegrating	disintegrating	disintegrating	disintegrating

FRUIT CHARACTERS	<i>O. ciliaris</i>	<i>O. oreophila</i>	<i>O. versicolor</i>	<i>O. ebracteata</i>	<i>O. droseroides</i>
Fruit shape	spheroid	broadly spheroid	ovoid	broadly spheroid	broadly spheroid
Fruit length (mm)	5.5 - 6	2.5 - 4.5	2.5 - 4	2 - 2.5	3
Fruit diameter (mm)	3 - 3.5	2.5 - 4	2 - 3.0	2.5 - 4	4 - 4.5
Fruit ridging (present/absent)	present	absent	absent	present	present
Intra-locular constriction between seeds (present/absent)	absent	absent	present	absent	absent
Indumentum position on the exocarp	scattered all over	scattered all over	upper part only	scattered all over	scattered all over
Type of hairs on exocarp	simple	simple	glandular	simple	simple
Fruit beak (present/absent)	present	present	present	absent	absent
Beak length (mm)	1	0.5	0.5	0	0
Number of seeds per locule	1 - 2	1	2 - 3	1	1
Fruit lobing	moderately lobed	moderately lobed	moderately lobed	vaguely lobed	?
Type of septum	partially divided	partially divided	partially divided	fused	?
Pericarp thickness (number of cell layers)	3	2	3	3 - 4	?
Shape of exocarp cells (epidermis)	oblong/ pentagonal	round	oblong/ pentagonal	round/ pentagonal	?
Secondary metabolites deposits within the pericarp and/or the fruit centre	fruit centre	fruit wall/ fruit centre	fruit wall/ fruit centre	fruit centre	?
Endocarp indumentum (if present)	absent	absent	absent	absent	absent
Channels in the pericarp (present/absent)	absent	absent	absent	absent	?
Testa thickness (number of cell layers)	3 - 4	3 - 4	3 - 4	3 - 4	3 - 4
Red phenolic layer lining the inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining the inner testa (present/absent)	present	present	present	present	present
Endosperm in the young seed (intact/disintegrating)	disintegrating	disintegrating	disintegrating	disintegrating	disintegrating

FRUIT CHARACTERS	<i>O. clavifolia</i>	<i>O. monophylla</i>
Fruit shape	ovoid	broadly spheroid
Fruit length (mm)	2.5 - 3.5	2.8 - 3.5
Fruit diameter (mm)	2 - 2.5	4.0 - 5.0
Fruit ridging (present/absent)	absent	present
Intra-locular constriction between seeds (present/absent)	present	absent
Indumentum position on the exocarp	upper part only	scattered all over
Type of hairs on exocarp	mixed	glandular
Fruit beak (present/absent)	absent	absent
Beak length (mm)	0	0
Number of seeds per locule	3	1
Fruit lobing	deeply lobed	moderately lobed
Type of septum	well divided	partially divided
Pericarp thickness (number of cell layers)	2	3
Shape of exocarp cells (epidermis)	round	round
Secondary metabolites deposits within the pericarp and/or the fruit centre	fruit centre	fruit wall/ fruit centre
Endocarp indumentum (if present)	absent	absent
Channels in the pericarp (present/absent)	absent	absent
Testa thickness (number of cell layers)	3 - 4	4 - 5
Red phenolic layer lining the inner testa (present/absent)	absent	absent
Dark-dotted phenolic layer lining the inner testa (present/absent)	present	present
Endosperm in the young seed (intact/disintegrating)	disintegrating	disintegrating

Table 3.2: A list of morphological, anatomical and SEM/VP characters used in the assessment of seed variation between selected *Oxalis* taxa

SEEDS CHARACTERS	<i>O. corniculata</i>	<i>O. pes-caprae</i>	<i>O. luteola</i>	<i>O. ambigua</i>	<i>O. obtusa</i>
Seed shape	elliptic	widley elliptic	elliptic	elliptic	elliptic
Seed length (mm)	1.0 - 1.1	1.0 - 1.10	0.9 - 1.0	0.7 - 0.8	0.9
Seed diameter (mm)	0.8 - 1	0.9 - 1.0	0.6 - 0.9	0.6	0.6 - 0.8
Seed colour (orange to brown or green)	orange to brown	orange to brown	orange to brown	orange to brown	orange to brown
Type of indumentum on mature seeds (if present)	absent	absent	absent	absent	absent
Position of hairs on the cotyledon	n/a	n/a	n/a	n/a	n/a
Red phenolic layer lining inner testa (present/absent)	present	present	present	present	present
Dark-dotted phenolic layer lining inner testa (present/absent)	absent	absent	absent	absent	absent
Endosperm in mature seed (present/absent)	present	present	present	present	present
Cotyledons development	not well - defined	not well - defined	not well - defined	not well - defined	not well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	absent	absent	absent	absent	absent
Channels in the cotyledon and their position	absent	absent	absent	absent	absent
Number of channels per cotyledon	0	0	0	0	0
Inner integument structure	ribbed	rugose	smooth/reticulate	alveolate	alveolate/reticulate

n/a = not applicable

SEEDS CHARACTERS	<i>O. heterophylla</i>	<i>O. purpurea</i>	<i>O. furcillata</i>	<i>O. natans</i>	<i>O. tenella</i>
Seed shape	elliptic	elliptic	obovoid	obovoid	obovoid
Seed length (mm)	1.1 - 1.2	0.9	1.10 - 1.70	2.9	2 - 2.5
Seed diameter (mm)	0.9	0.9	0.9 - 1	1.9	1.5 - 1.80
Seed colour (orange to brown or green)	orange to brown	orange to brown	greenish	whitish	green
Type of indumentum on mature seeds (if present)	absent	absent	glandular	absent	multicellular
Position of hairs on the cotyledon	n/a	n/a	cotyledon's margins	n/a	cotyledon's margins
Red phenolic layer lining inner testa (present/absent)	present	present	absent	absent	absent
Dark-dotted phenolic layer lining inner testa (present/absent)	absent	absent	present	present	present
Endosperm in mature seed (present/absent)	present	present	absent	absent	absent
Cotyledons development	not well - defined	not well - defined	well - defined	well - defined	well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	absent	absent	outer/within the cotyledon	outer/within the cotyledon	outer cotyledon
Channels in the cotyledon and their position	absent	absent	absent	absent	absent
Number of channels per cotyledon	0	0	0	0	0
Inner integument structure	alveolate	alveolate/reticulate	?	alveolate/scalariform	scalariform

n/a = not applicable

SEEDS CHARACTERS	<i>O. stenoptera</i>	<i>O. aridicola</i>	O. louisae	<i>O. sp. subsection Pardales</i>	<i>O. grammophylla</i>
Seed shape	obovoid	obovoid	obovoid	obovoid	obovoid
Seed length (mm)	2	1.90 - 2.5	1.90 - 2	1.2 - 1.8	1.2 - 1.8
Seed diameter (mm)	1.10 - 1.5	1.90 - 2	1.1 - 1.9	1.0 - 1.10	1.0 - 1.10
Seed colour (orange to brown or green)	green	green	whitish	green to redish	green to redish
Type of indumentum on mature seeds (if present)	multicellular	multicellular	glandular	absent	absent
Position of hairs on the cotyledon	cotyledon's margins	cotyledon's margins	scattered all over	n/a	n/a
Red phenolic layer lining inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining inner testa (present/absent)	present	present	present	present	present
Endosperm in mature seed (present/absent)	absent	absent	absent	absent	absent
Cotyledons development	well - defined	well - defined	well - defined	well - defined	well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	outer/within the cotyledon	outer cotyledon	outer/within the cotyledon	outer/within the cotyledon	outer/within the cotyledon
Channels in the cotyledon and their position	absent	absent	absent	cotyledon tips	cotyledon edges
Number of channels per cotyledon	0	0	0	2	4 - 6 channels
Inner integument structure	alveolate	alveolate/scalariform	colliculate	smooth	smooth

n/a = not applicable

SEEDS CHARACTERS	<i>O. meisneri</i>	<i>O. viscosa</i>	<i>O. hirta</i>	<i>O. tenuifolia</i>	<i>O. cf. urbaniana</i>
Seed shape	obovoid	obovoid	obovoid	obovoid	obovoid
Seed length (mm)	1.5 - 2	1.0 - 2	2.5 - 4	0.9 - 1.5	1.0 - 2.0
Seed diameter (mm)	0.9 - 1.5	0.9 - 1.5	1.5 - 3	0.8 - 1.5	0.9 - 1.2
Seed colour (orange to brown or green)	green	green	green	green	green
Type of indumentum on mature seeds (if present)	multicellular	?	glandular	glandular	absent
Position of hairs on the cotyledon	scattered all over	?	cotyledon's margins	cotyledon's margins	n/a
Red phenolic layer lining inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining inner testa (present/absent)	present	present	present	present	present
Endosperm in mature seed (present/absent)	absent	absent	absent	absent	absent
Cotyledons development	well - defined	well - defined	well - defined	well - defined	well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	absent	outer cotyledon	outer cotyledon	outer cotyledon	outer cotyledon
Channels in the cotyledon and their position	absent	absent	absent	absent	absent
Number of channels per cotyledon	0	0	0	0	0
Inner integument structure	?	?	reticulate	scalariform/scalariform	alveolate/scalariform

n/a = not applicable

SEEDS CHARACTERS	<i>O. multicaulis</i>	<i>O. pillansiana</i>	<i>O. pusilla</i>	<i>O. glabra</i>	<i>O. xantha</i>
Seed shape	obovoid	broadly obovoid	obovoid	obovoid	obovoid
Seed length (mm)	1.5 - 2	1.0 - 2	1.0 - 1.5	0.9 - 1.5	2.0 - 2.5
Seed diameter (mm)	1	1.0 - 2	0.9 - 1.5	0.8 - 0.9	1.1 - 1.80
Seed colour (orange to brown or green)	green	green	green	green	green
Type of indumentum on mature seeds (if present)	absent	multicellular	multicellular	glandular	absent
Position of hairs on the cotyledon	n/a	scattered all over	cotyledon's margins	scattered all over	n/a
Red phenolic layer lining inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining inner testa (present/absent)	present	present	present	present	present
Endosperm in mature seed (present/absent)	absent	absent	absent	absent	absent
Cotyledons development	well - defined	well - defined	well - defined	well - defined	well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	outer cotyledon	outer cotyledon	absent	outer cotyledon	outer/within the cotyledon
Channels in the cotyledon and their position	absent	absent	absent	absent	cotyledon tips
Number of channels per cotyledon	0	0	0	0	2
Inner integument structure	?	scalariform	smooth	smooth	alveolate/reticulate

n/a = not applicable

SEEDS CHARACTERS	<i>O. ciliaris</i>	<i>O. oreophila</i>	<i>O. versicolor</i>	<i>O. ebracteata</i>	<i>O. droseroides</i>
Seed shape	obovoid	obovoid	obovoid	obovoid	obovoid
Seed length (mm)	2.0 -2.5	2.0 - 2.5	1.5 - 2	1.8 - 2.0	2.3 - 2.5
Seed diameter (mm)	1.5 - 2.0	1.5 - 2	1	1.2 - 1.7	1.5 - 1.8
Seed colour (orange to brown or green)	green	green	green	green	green
Type of indumentum on mature seeds (if present)	absent	?	absent	multicellular	multicellular
Position of hairs on the cotyledon	n/a	?	n/a	scattered all over	scattered all over
Red phenolic layer lining inner testa (present/absent)	absent	absent	absent	absent	absent
Dark-dotted phenolic layer lining inner testa (present/absent)	present	present	present	present	present
Endosperm in mature seed (present/absent)	absent	absent	absent	absent	absent
Cotyledons development	well - defined	well - defined	well - defined	well - defined	well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	outer/within the cotyledon	outer/within the cotyledon	outer cotyledon	absent	outer cotyledon
Channels in the cotyledon and their position	absent	absent	absent	absent	absent
Number of channels per cotyledon	0	0	0	0	0
Inner integument structure	alveolate	alveolate/scalariform	alveolate	?	?

n/a = not applicable

SEEDS CHARACTERS	<i>O. clavifolia</i>	<i>O. monophylla</i>
Seed shape	obovoid	obovoid
Seed length (mm)	1.1 - 1.5	2
Seed diameter (mm)	1.0 - 1.1	1.5
Seed colour (orange to brown or green)	green	green
Type of indumentum on mature seeds (if present)	glandular	glandular
Position of hairs on the cotyledon	scattered all over	scattered all over
Red phenolic layer lining inner testa (present/absent)	absent	absent
Dark-dotted phenolic layer lining inner testa (present/absent)	present	present
Endosperm in mature seed (present/absent)	absent	absent
Cotyledons development	well - defined	well - defined
Secondary metabolite deposits in the outer cotyledon and/or within the cotyledon	outer cotyledon	outer cotyledon
Channels in the cotyledon and their position	absent	absent
Number of channels per cotyledon	0	0
Inner integument structure	scalariform	?

CHAPTER IV

DISCUSSION

In an attempt to address the key questions posed in the introduction, information assessed in the previous section will be discussed under the following headings: **4.1** Introduction. **4.2** Variation in the inter-specific fruit and seed morphological and anatomical characters. **4.3** The utility of fruit and seed morphology and anatomy in the identification of major groups. **4.4** Comparison of the major groups identified by fruit and seed characters to the groupings proposed by previous studies: (A) Salter's (1944) morphological classification, (B) Dreyer's (1996) palynological classification and (C) Oberlander *et al.*'s (2004) molecular phylogeny. Section **4.5** general discussion explores the contribution of fruit and seed characters towards the classification of the southern African members of *Oxalis*.

4.1-Introduction

The morphological evaluation of fruits and seeds of 32 South African *Oxalis* species revealed several potentially systematically informative characters (Table 3.1 and 3.2). In his revision of the South African taxa, Salter (1944) already described some of the morphological characters of the capsule and seed considered in the present study. However, since he could not find clear correlation between some of these characters (i.e. number of ovules per locule and the elongated capsule shape), he included only a few fruit and seed characters in his morphological classification of the genus. He based the delimitations of the species mainly on vegetative characters. A pilot study of the fruit and seed anatomy of nine *Oxalis* species (Obone, 2003) revealed several useful characters that were not considered or described by Salter (1944). Some of these seed characters were regarded as potentially informative and served as framework in the planning phase of the present study. Several additional new characters are described in the present study. They include trichomes found on the endocarp and cotyledons, a lignified cell layer in the inner layer of the seed coat, channels within cotyledons and the inner integument structures.

4.2-Variation in the inter-specific fruit and seed morphological and anatomical characters

As outlined in the results, 35 potentially informative characters were identified in the present study. These may be grouped into autapomorphic characters, diversely scattered characters without any clear phylogenetic signal and a third group of characters that can be used to define groups (Appendix II). The three types of characters may prove to be taxonomically

informative in future, when more species are studied. No autapomorphic fruit characters are discussed in great detail here, but it should be noted that the ribbed, rugose and colliculate structures of the inner integument of the testa in mature seeds are diagnostic for *O. corniculata*, *O. pes-caprae* and *O. louisae*, respectively. These differences may become significant in a more species-inclusive analysis.

The fruit characters that occur randomly in species with endospermous and exendospermous seeds (Appendix II), include fruit shape (oblong or ovoid), fruit ridging, trichomes on the outer pericarp, presence or absence of a beak, pericarp thickness and the shape of exocarp cells, secondary metabolites deposited in the fruit walls, the presence or absence of channels in the pericarp and the thickness of the testa in young seeds. Oblong and ovoid fruits occur in both endospermous and exendospermous species (section 3.1.1). In species that produce exendospermous seeds, oblong fruits are observed in only 4/25 species (section 3.1.1) and can thus be considered as diagnostic of *O. sp. subsection Pardales*, *O. grammophylla*, *O. tenella* and *O. xantha*. The remaining 21 exendospermous species have ovoid and spheroid fruits, usually containing one to five seeds per locule. Salter (1944) noticed that species with exendospermous seeds have between one and five (rarely six) ovules or seeds per locule. He thus considered the eight ovules found in *O. fragilis* Salter as an exception. Salter (1944) found no direct relationship between the elongated fruit and the number of seeds per locule in exendospermous species, but he suggested that the elongated fruit shape could be of taxonomic importance. This correlation between fruit elongation and the number of ovules or seeds per locule observed in the present study can be regarded as useful in the delimitation of some exendospermous species.

In the species studied, the type of trichomes and their position on the exocarp vary from glandular hairs scattered all over the fruit in *O. heterophylla* (endospermous) and *O. multicaulis* (exendospermous) to non-glandular (simple) hairs occurring only on the upper part of the fruits in *O. ambigua* (endospermous) and *O. grammophylla* (exendospermous). The remaining fruit characters, such as the fruit ridging, presence or absence of a beak, pericarp thickness, shape of exocarp cells and secondary metabolites deposits in the fruit walls also occur in both endospermous and exendospermous species. The randomly scattered characters do not necessary support all groupings within the phenogram, but with more species included in a study, these characters may prove to be phylogenetically informative (Table 3.1 and 3.2).

The difference between endospermous and exendospermous seeds is considerable, and will be discussed in more detail in the next section (section 4.3). The only seed characters that appear to vary randomly between these two groups of species are the testa thickness in young seeds (number of cell layers) and some inner integument structures (SEM/VP), (Figure 3.8). These characters may prove to be phylogenetically informative within these two groups of species. Other characters that show considerable variation amongst exendospermous species are the presence or absence and position of trichomes on the cotyledons, secondary metabolite deposits in the cotyledons and the presence or absence of channels within the cotyledons. The lack of hairs (trichomes) and the presence of channels in the cotyledons are diagnostic characters for *O. sp. subsection pardales*, *O. grammophylla* and *O. xantha*, all three are exendospermous species.

4.3-The utility of fruit and seed morphology and anatomy in the identification of major groups

Characters used to demarcate major groups included: Fruits shape (spheroid), seed shape, fruit and seed sizes, extent of intra-locular constrictions of the fruit, endocarp indumentum, fruit lobing and the shape of the septum, the number of seeds per locule, the type of lining of the inner integument of the testa, seed colour and the presence or absence of endosperm in mature seed (Appendix II).

The phenetic analysis revealed two distinctly different clusters of species (76% different), (Figure 3.9). All seven species included in cluster A (Figure 3.9) produce endospermous seeds. All members of this cluster have hairs on the endocarp, all have oblong or ovoid (and never spheroid) fruits and they usually contain more than five seeds per locule. Their fruits are either deeply or moderately lobed (never vaguely), with pericarps usually composed of epidermal cells with jagged edges (Obone, 2003) and containing small orange seeds. The inner testa of the seeds of the endospermous species is characterized by a layer of elongated, thick-walled, lignified cells. Endosperm is still present and clearly visible inside the mature seeds.

Two subclusters (Figure 3.9) resolved within this cluster based on fruit shape (oblong or ovoid), mesocarp indumentum (less hairy with non-glandular trichomes in *O. heterophylla*, *O. obtusa* and *O. pes-caprae*, and very hairy with glandular and non-glandular trichomes in *O. luteola* and *O. purpurea*), the shape of the parenchymatic cells forming the mesocarp and secondary metabolite deposits in the fruit walls (present in fruit centres of all the four species

included in the first subcluster and in both pericarp and fruit centres of members of the second subcluster). Bootstrap values of the subclusters were so low that recognition of these subclusters as separated entities is questionable.

Cluster B (Figure 3.9) includes all the species that produce exendospermous seeds. Members of this cluster have fruits of three different shapes, namely oblong, ovoid and spheroid, with the latter two being the most common types. The number of seeds per locule usually ranges between 1 – 4, but in some species one locule may include as many as nine seeds (*e.g. O. xantha*). Ridges and intra-locular constriction of the fruit are particularly obvious in many of the species in this cluster. Mature seeds are bigger than those of species in clade A. The embryos have fully developed photosynthetic cotyledons that are covered by a thin, dark-dotted inner integument. Once the inner integument tears away, the indumentum (trichomes) on the cotyledons of many of these species become visible. The included species also display two of the seven sculpture patterns of the inner integument (scalariform and colliculate) observed in this study. The endosperm already starts to disintegrate in the young seeds of the species in this cluster, so that no trace of endosperm remains in the mature seed

4.4-Comparison of the major groups identified by fruit and seed characters to the groupings proposed by previous studies: (A) Salter's (1944) morphological classification, (B) Dreyer's (1996) palynological classification and (C) Oberlander *et al.*'s (2004) molecular phylogeny.

The number of species for which fruit and seed characters were evaluated in the present study was very limited, which may cause considerable conflict between the datasets. However, these comparisons can be useful, as they may single out individual characters that carry phylogenetic signals despite the limited sample size.

4.4.1-Cluster A

Salter's (1944) morphological classification

Cluster A includes members of the following four sections defined by Salter (1944): *Corniculatae*, *Oppositae* (subsections *Subintegrae* and *Bifurcatae*), *Cernuae* (subsection *Eu-Cernuae*) and *Stictophyllae* (Table 4.1). Most sections are represented by one species only, except for section *Oppositae*, which is represented by the following four species: *O. obtusa*, *O. heterophylla*, *O. luteola* and *O. ambigua*.

Dreyer's (1996) palynological classification

All seven species with endospermous seeds have reticulate pollen (type C) and the following three reticulate subtypes are represented: C2 (micro-reticulate) in *O. corniculata* and *O. pes-caprae*, C3 (finely-reticulate) in *O. obtusa*, *O. luteola*, *O. ambigua* and *O. purpurea* and C4 (reticulate) in *O. heterophylla* (Table 4.1). According to Dreyer (1996) the three pollen subtypes (C2, C3 and C4) are thought to be very closely related.

Oberlander et al. (2004) trnL-F based phylogeny

Five of the taxa included in cluster A are members of Clade II (Oberlander *et al.*, 2004), while *O. corniculata* is sister to all other southern African *Oxalis* species and *O. heterophylla* is included in an unresolved clade of the Oberlander *et al.* (2004) molecular phylogeny (Table 4.1).

Cluster A was strongly retrieved in the phenetic analysis (bootstrap support of 100%), and includes two subclusters: the *O. corniculata* - *O. pes-caprae* subgroup and the *O. luteola* - *O. purpurea* subgroup. The included species of section *Oppositae* namely *O. obtusa* and *O. heterophylla* (subsections *Subintegrae* and *Bifurcatae* respectively) were found to have very similar fruit and seed characters. Fruit and seed characters of these two species are less similar to *O. corniculata* (77.5% SL) and *O. pes-caprae*. Fruit and seed characters of *O. ambigua* and *O. purpurea* are more similar to one another than either of these species are to *O. luteola*.

Salter (1944) placed *O. luteola* (section *Oppositae*) and *O. purpurea* (section *Stictophyllae*) into different sections, and did not regard them to be closely related. But as outlined above, these two species share the same pollen subtypes (C3) and the molecular phylogeny shows that they are all members of the same Clade II. Fruit and seed morphology and anatomy thus confirm the affinity between these two species as proposed by the palynology and the molecular phylogeny.

4.4.2-Cluster B

Salter's (1944) morphological classification

Cluster B includes taxa from five different sections (*Foveolatae*, *Angustatae* (subsections *Sessilifoliatae*, *Lineares*, *Pardales*, *Glandulosae* and *Xanthotrichae*), *Campanulatae*, *Latifoliolatae* and *Crassulae*) and one species (*O. monophylla*) that has not been allocated to any section (Table 4.1). Within cluster B, *O. glabra* (section *Angustatae*, subsection *Lineares*) is sister to a group containing subclusters B1 and B2. Subcluster B1 is mainly composed of species from *Angustatae* subsections *Sessilifoliatae* (*O. cf. urbaniana*, *O. meisneri*, *O. viscosa*

and *O. multicaulis*), *Lineares* (*O. versicolor* and *O. pusilla*) and *Pardales* (*O. sp* and *O. grammophylla*), except for *O. furcillata*, which belongs to section *Foveolatae*. Subcluster **B2** includes species from sections *Campanulatae* (*O. natans*), *Angustatae* (subsections *Sessilifoliatae* (*O. tenuifolia* and *O. hirta*), *Lineares* (*O. oreophila*, *O. xantha* and *O. ciliaris*), *Glandulosae* (*O. ebracteata*, *O. droseroides* and *O. clavifolia*) and *Xanthotrichae* (*O. pillansiana*)), section *Latifoliolatae* (*O. stenoptera* and *O. tenella*), section *Crassulae* (*O. louisae*) and the species not allocated to any section (*O. monophylla*).

Dreyer's (1996) palynological classification

Cluster **B** includes species with two different pollen types, namely reticulate pollen (type C) and supra-areolate (type D) (Table 4.1). *O. glabra*, which is sister to the group containing subclusters **B1** and **B2**, has reticulate pollen of the subtype C8. Subcluster **B1** exclusively includes species with reticulate pollen of the subtypes C2 (micro-reticulate), C8 (finely reticulate) and C9 (reticulate). Subcluster **B2** is palynologically more heterogeneous, and includes both reticulate and supra-areolate pollen types (C and D). The pollen subtypes C2, C8 (finely reticulate), C10, C13 (rugose-reticulate) and D1 (supratectal areolae) are represented. As discussed above, pollen subtypes C2, C3, C4 and C7, C8, C9 are regarded to be very closely related (Dreyer, 1996). Pollen subtype C13, found in *O. droseroides*, is monotypic and it isolates this species as being palynologically unique in the genus. Pollen subtype C10 is closely related to subtypes C11 and C12 (Dreyer, 1996), so that these pollen subtypes bear taxonomic significance as a unit.

Pollen type D as a whole seems to be phylogenetically hugely informative within the genus. D-type pollen was recorded from species in the sections *Latifoliolatae* and *Angustatae* (subsections *Sessilifoliatae* and *Lineares*) (Dreyer, 1996). Dreyer (1996) questioned the co-occurrence of pollen types C and D in the same section, and suggested that this casts considerable doubt on the accuracy of the taxonomic classification.

Oberlander et al. (2004) trnL-F based phylogeny

With regard to the molecular phylogeny, cluster **B** also seems to be heterogeneous and very complex. It includes members from four different clades, namely Clade II, the *O. glabra* Clade, the *O. pardalis* Clade and the *O. hirta* Clade. Within cluster **B** of the present study, members of Clade II and the *O. glabra* Clade are found in both subclusters **B1** and **B2**. In contrast, members of the *O. pardalis* Clade are restricted to subcluster **B1**, while members of

the *O. hirta* Clade are restricted to subcluster **B2** of the present study. The sister species to the group formed by subclusters **B1** and **B2** resolves into the *O. glabra* Clade.

Relationships within cluster **B** seem more complex than in cluster **A**. However, even if levels of similarity between *O. glabra* and the group containing subclusters **B1** and **B2** (52.1% similarity) or between the two latter clades (*ca* 54.7% similarity) are not very high, strongly supported small groups can be distinguished within both clusters **B1** and **B2**.

***O. furcillata* - *O. grammophylla* subgroup**

Within subcluster **B1** *O. cf. urbaniana* and *O. versicolor* are similar in terms of fruit and seed characters, and they also have the same pollen (subtype C8). Both species are also members of the *O. glabra* Clade (Oberlander *et al.*'s, 2004). Therefore, in terms of fruit and seed morphology and anatomy these two species agree with the palynology and molecular data. *O. furcillata* is sister to and *ca.* 72.3% similar to the group containing these two species. Although pollen data of the three species *O. furcillata*, *O. cf. urbaniana* and *O. versicolor* show similarities, the species are included in totally different sections in the morphological classification of Salter (1944). The molecular phylogeny also shows very distant affinities between these three taxa. *O. furcillata* is included in Clade II, while *O. cf. urbaniana* and *O. versicolor* both resolve in the *O. glabra* Clade. Fruit and seed characters thus do not shed much light on the affinities of these three species.

A second group within **B1** is composed of two very similar species (*ca* 78.1%) *O. sp.* subsection *Pardales* and *O. grammophylla*. Both of these species belong to section *Angustatae*, subsections *Pardales*. Salter (1944) considered this section as distinct and natural. The two species share the same pollen (subtype C2) and are both members of the same *O. pardalis* Clade (Oberlander *et al.*, 2004). In this instance the results of the present study are congruent with results of Salter (1944), Dreyer (1996) and Oberlander *et al.* (2004).

***O. meisneri* - *O. multicaulis* subgroup**

Fruit and seed characters reflect a close similarity between *O. meisneri* and *O. pusilla* (68.24% similar). Both species belong to section *Angustatae*, but to different subsections thereof (*Sessilifoliae* and *Lineares*, respectively). They both resolve into the *O. glabra* Clade (Oberlander *et al.*, 2004) and they have pollen of the subtypes C9 (*O. meisneri*) and C2 (*O. pusilla*). *O. viscosa* and *O. multicaulis* are 74.9 % similar. They belong to section *Angustatae*, subsection *Sessilifoliae*, they have related pollen types (C2 and C8

respectively), but resolve into two different clades. Fruit and seed characters thus do not contribute additional information about relationships between these four species, but do support the molecular phylogeny in the case of the first two species.

***O. natans* - *O. oreophila* subgroup**

Within subcluster **B2**, *O. natans* and *O. monophylla* are very similar (81.9% similar), despite the fact that *O. natans* belongs to section *Campanulatae* and *O. monophylla* has not been allocated to any section. The two species have different pollen (subtypes C2 and C10, respectively), and resolve into two different clades (*O. glabra* Clade and Clade II respectively) in the molecular phylogeny. In this case, the small sample size assessed for fruit and seed characters may be misleading. Similarly, fruit and seed information obtained from the present study is not sufficient to reveal the true affinity between *O. oreophila* and the group including *O. natans* and *O. monophylla*, despite the strong similarity between them. *O. oreophila* belongs to a totally different section (*Angustatae*), and has pollen of the type D1. This species also resolves into a totally different clade in the molecular phylogeny (*O. hirta* Clade).

***O. hirta* - *O. droseroides* subgroup**

O. ebracteata and *O. droseroides* in subcluster **B2** are *ca.* 89% similar in terms of fruit and seed characters. Both species belong to section *Angustatae* (subsection *Glandulosae*) and both resolve into the *O. glabra* Clade (Oberlander *et al.*, 2004). Their pollen is, however, very dissimilar, belonging to subtypes C8 and C13, respectively with C13 being a monotypic pollen type and C8 a quite common pollen type in the genus. Therefore, fruit and seed characters agree with both Salter's (1944) classification and the Oberlander *et al.*'s (2004) phylogeny, but are not completely congruent with the palynological classification of Dreyer (1996). The missing data for *O. droseroides* may obscure the significance of fruit and seed characters to some extent.

Relationships between *O. tenuifolia* and the group composed of *O. ebracteata* and *O. droseroides* (*ca.* 74.9% similar) and also between *O. hirta* and the group containing *O. ebracteata*, *O. droseroides* and *O. tenuifolia* (*ca.* 72.3% similar) are the most complex in the **B2** subcluster. Both pollen types D and C occur within these groups, and these groups also include species from three different subsections of *Angustatae* (*Lineares*, *Glandulosae* and *Sessilifoliatae*). In addition these species resolve into two different clades (*O. hirta* Clade and

O. glabra Clade) in the molecular phylogeny. This may probably be due to the limited number of species sampled in the fruit and seed morphological and anatomical analysis.

***O. tenella* - *O. aridicola* subgroup and *O. xantha* - *O. ciliaris* subgroup**

O. tenella and *O. aridicola* are ca. 78.1% similar in terms of fruit and seed characters. They are both members of section *Latifoliolatae*, and share pollen of the same subtype (D1). Both species also resolve into the *O. hirta* Clade in the molecular phylogeny. In this case it seems as though the fruit and seed characters that resolve these two species together as being so similar do have significant phylogenetic importance. Two other species, *O. xantha* and *O. ciliaris*, were also found to be very similar in terms of the characters evaluated in the present study (ca. 76.84% similar). They both belong to the section *Angustatae* subsection *Lineares*, and share pollen of subtypes D1. Both species also resolve together into the *O. hirta* Clade in the molecular phylogeny. So in this case fruit and seed morphology and anatomy again agree with the Salter (1944), Dreyer (1996) and Oberlander *et al.* (2004) classifications, suggesting that the fruit and seed characters that resolve them as being similar are systematically significant. *O. tenella* and *O. aridicola* have many fruit characters in common such as the indumentum scattered all over the exocarp, presence of a fruit beak, a vaguely lobed fruit and a fused fruit septum (Table 3.1). In addition, they share almost all the seed characters except the size of the seed and the inner integument structure (Table 3.2). *O. xantha* and *O. ciliaris* also share many fruit characters such as indumentum scattered all over the exocarp, the presence of simple hairs on the exocarp, the presence of a beak, a moderately lobed fruit and a partially divided fruit septum (Table 3.1). Furthermore, these two species also share almost all seed characters with the exception of channels only found in *O. xantha* (Table 3.2).

***O. stenoptera* - *O. clavifolia* subgroup**

Two groups of species, *O. stenoptera* and *O. louisae* (ca. 64.1% similar) and *O. pillansiana* and *O. clavifolia* (ca. 67.6% similar), resolve with slightly reduced levels of similarity within this subcluster. Species from the first group belong to different sections (*Latifoliolatae* and *Crassulae*, respectively), but they share the same pollen type and subtype (C10), and both resolve to Clade II in the molecular phylogeny. Species in the second group (*O. pillansiana* and *O. clavifolia*) also resolve together in the molecular phylogeny (both Clade II), but they belong to different subsections of section *Angustatae*. The two species have distinctly different reticulate pollen subtypes (C10 in *O. pillansiana* and C2 in *O. clavifolia*). No real systematically informative pattern can thus be deduced from the seed and fruit morphological and anatomical patterns observed for these four species.

4.5-The contribution of fruit and seed characters towards the classification of the southern African members of *Oxalis*

Results of the present study cannot be considered systematically very informative at the infra-sectional levels, for many reasons. Firstly, sample size will obviously have influenced the data. *Oxalis* is represented by *ca.* 211 species (270 taxa) in southern Africa (Salter, 1944, Olivier, 1993), and fruit and seed morphology and anatomy of only 32 (20%) of these species were assessed in the present study.

Secondly not all Salter's (1944) sections and subsections were represented and some of them were limited to single taxon sampled. Members of only nine of his eleven sections and one unclassified species were included in the study. Moreover, among the four endospermous sections that were studied (seven species in total), two sections were represented by a single taxon. Of the five exendospermous sections considered here (including section *Foveolatae*), two were represented by only one species each.

Thirdly, only species with two of the four pollen types (C and D) were represented in the present study. These reduced sampling strategies are probably mainly responsible for the very low bootstrap values observed within subclusters A and B of the phenetic analysis. Due to the under-sampling, this analysis probably presents many "false species groupings", many of which conflict with the results of other datasets.

Despite these limitations, results of the present study clearly highlight a major difference between the endospermous and exendospermous groups, and are thus very significant at the infra-generic level. In addition, these results are very useful for species-specific characterization as was discussed in section 4.2. Comparison to previous studies did also show that fruit and seed characters can, despite the limited sample size, support small taxonomic groupings, where they agree with both palynological and molecular phylogenetic groupings (section 4.4). I believe that further studies on fruit and seed morphology and anatomy of the entire genus will significantly support the phylogenetic groupings that are emerging through the ongoing molecular phylogenetic assessment of the southern African members of *Oxalis*, and are well-worth pursuing further.

Table 4.1: Comparison of results of the present study with Salter's (1944) classification, Dreyer's (1996) palynology and Oberlander *et al* (2004) molecular phylogeny. Clades from the updated and unpublished phylogeny based on *trnL-F* and ITS data (Oberlander *pers. com*) are indicated in bold typeface.

Cluster	Sub-cluster	Taxon	Salter's (1944) sections		Dreyer (1996) Pollen type	Oberlander <i>et al.</i> (2004) <i>trnL-F</i> based phylogeny	
			Sections	Subsections			
A		<i>O. corniculata</i>	<i>Corniculatae</i>	-	C2	Sister to southern African <i>Oxalis</i>	
		<i>O. obtusa</i>	<i>Oppositae</i>	<i>Subintegrae</i>	C3	Clade II	
		<i>O. heterophylla</i>	<i>Oppositae</i>	<i>Bifurcatae</i>	C4	Unresolved	
		<i>O. pes-caprae</i>	<i>Cernuae</i>	<i>Eu-cernuae</i>	C2	Clade I	
		<i>O. luteola</i>	<i>Oppositae</i>	<i>Subintegrae</i>	C3	Clade II	
		<i>O. ambigua</i>	<i>Oppositae</i>	<i>Subintegrae</i>	C3	Clade II	
		<i>O. purpurea</i>	<i>Stictophyllae</i>	-	C3	Clade II	
B	B1	<i>O. furcillata</i>	<i>Foveolatae</i>	-	C2	Clade II	
		<i>O. cf urbaniana</i>	<i>Angustatae</i>	<i>Sessilifoliae</i>	C8	<i>O. glabra</i> Clade	
		<i>O. versicolor</i>	<i>Angustatae</i>	<i>Lineares</i>	C8	<i>O. glabra</i> Clade	
		<i>O. sp.subse. Pardales</i>	<i>Angustatae</i>	<i>Pardales</i>	C2	<i>O. pardales</i> Clade	
		<i>O. grammophylla</i>	<i>Angustatae</i>	<i>Pardales</i>	C2	<i>O. pardales</i> Clade	
		<i>O. meisneri</i>	<i>Angustatae</i>	<i>Sessilifoliae</i>	C9	<i>O. glabra</i> Clade	
		<i>O. pusilla</i>	<i>Angustatae</i>	<i>Lineares</i>	C2	<i>O. glabra</i> Clade	
		<i>O. viscosa</i>	<i>Angustatae</i>	<i>Sessilifoliae</i>	C2	Clade II	
		<i>O. multicaulis</i>	<i>Angustatae</i>	<i>Sessilifoliae</i>	C8	<i>O. glabra</i> Clade	
	B2	<i>O. natans</i>	<i>Campanulatae</i>	-	C2	<i>O. glabra</i> Clade	
		<i>O. monophylla</i>	Not allocated to any section	-	C10	Clade II	
		<i>O. oreophila</i>	<i>Angustatae</i>	<i>Lineares</i>	D1	<i>O. hirta</i> Clade	
		<i>O. tenuifolia</i>	<i>Angustatae</i>	<i>Sessilifoliae</i>	C8	<i>O. glabra</i> Clade	
		<i>O. hirta</i>	<i>Angustatae</i>	<i>Sessilifoliae</i>	D1	<i>O. hirta</i> Clade	
		<i>O. ebracteata</i>	<i>Angustatae</i>	<i>Glandulosae</i>	C8	<i>O. glabra</i> Clade	
		<i>O. droseroides</i>	<i>Angustatae</i>	<i>Glandulosae</i>	C13	<i>O. glabra</i> Clade	
		<i>O. tenella</i>	<i>Latifoliolatae</i>	-	D1	<i>O. hirta</i> Clade	
		<i>O. aridicola</i>	<i>Latifoliolatae</i>	-	D1	<i>O. hirta</i> Clade	
		<i>O. xantha</i>	<i>Angustatae</i>	<i>Lineares</i>	D1	<i>O. hirta</i> Clade	
		<i>O. ciliaris</i>	<i>Angustatae</i>	<i>Lineares</i>	D1	<i>O. hirta</i> Clade	
		<i>O. stenoptera</i>	<i>Latifoliolatae</i>	-	C10	Clade II	
		<i>O. louisae</i>	<i>Crassulae</i>	-	C10	Clade II	
		<i>O. pillansiana</i>	<i>Angustatae</i>	<i>Xanthotrichae</i>	C10	Clade II	
		<i>O. clavifolia</i>	<i>Angustatae</i>	<i>Glandulosae</i>	C2	Clade II	
		-	<i>O. glabra</i>	<i>Angustatae</i>	<i>Lineares</i>	C8	<i>O. glabra</i> Clade

CHAPTER V

CONCLUSIONS

Although fruit and seed morphology and anatomy of a relatively small sample of *Oxalis* species were investigated here, 35 potentially systematic informative characters were identified in this study. Among these, the autapomorphic and randomly distributed characters were particularly useful in species-specific characterization. A third group of linked characters were identified that could be used to demarcate two major groups of species, those with endospermous and those with exendospermous seeds.

The endospermous and exendospermous species groups are distinguishable through characters such as fruit shape (spheroid), seed size, the extent of intra-locular constrictions, the endocarp indumentum, lobing and shape of the septum, number of seeds per locules, type of inner integument lining the testa, seed colour, the type of cotyledons development and the presence or absence of endosperm in the mature seeds. The cluster analysis strongly supported the demarcation of these two species groups, with a 100% bootstrap value supporting these clusters. Only a 24% level of similarity exists between endospermous and exendospermous species.

Low bootstrap values were observed within each of the two major groups, despite some strong similarity levels for some of these clusters. This can mainly be ascribed to the limited sample size, and clustering proposed on the basis of fruit and seed characters should thus be considered cautiously within these two main groups. The low taxon sampling size probably also explains the considerable conflict between many of these clusters and the morphological, palynological and molecular datasets.

Despite these limitations of sample size, fruit and seed morphological and anatomical characters have proven to be systematic informative at the infra-generic level, and lead to the demarcation of clearly distinct endospermous and exendospermous species groups. Comparison with both palynological (Dreyer, 1996) and molecular phylogenetic (Oberlander *et al.*, 2004) data has also allowed the identification of individual characters that appear to carry phylogenetic signals (in *O. tenella* - *O. aridicola* and *O. xantha* - *O. ciliaris* subgroups for example), and are thus worthy of further study. I thus believe that further, more species-inclusive studies of fruit and seed morphology and anatomy will support the phylogenetic

groupings that are emerging through the ongoing molecular phylogenetic assessment of the southern African members of *Oxalis*, and are well-worth pursuing further.

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APPENDIX I: Data matrix of 78 fruit and seed characters used in the phenetic analysis of 32 *Oxalis* species.

TAXON	CHARACTERS																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
<i>O. corniculata</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	1	0	0	1	0
<i>O. pes-capreae</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0
<i>O. luteola</i>	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1
<i>O. ambigua</i>	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1
<i>O. obtusa</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0
<i>O. heterophylla</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0
<i>O. purpurea</i>	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0
<i>O. furcillata</i> var. <i>furcillata</i>	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	0	1
<i>O. natans</i>	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1
<i>O. tenella</i>	1	0	0	0	0	1	0	1	0	0	0	1	0	1	0	1	0	0	0	1	1	0
<i>O. stenoptera</i>	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0
<i>O. aridicola</i>	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	1	0
<i>O. louisae</i>	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0
<i>O. sp. subsection Pardales</i>	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
<i>O. grammophylla</i>	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1
<i>O. meisneri</i>	0	1	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1
<i>O. viscosa</i>	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1
<i>O. hirta</i> var. <i>hirta</i>	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	0	1
<i>O. tenuifolia</i>	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1
<i>O. cf. urbaniana</i>	0	1	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	0	1
<i>O. multicaulis</i>	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0
<i>O. pillansiana</i>	0	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	1
<i>O. pusilla</i>	0	0	1	1	0	0	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	1
<i>O. glabra</i>	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	1
<i>O. xantha</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0
<i>O. ciliaris</i>	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	1	1	0	0	0	1	0
<i>O. oreophia</i>	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	1	0	0	0	1	0
<i>O. versicolor</i>	0	1	0	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	1	0	0	1
<i>O. ebracteata</i>	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0
<i>O. droseroides</i>	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0
<i>O. clavifolia</i>	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1
<i>O. monophylla</i>	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0

TAXON	CHARACTERS																					
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
<i>O. corniculata</i>	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	1
<i>O. pes-capreae</i>	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	1
<i>O. luteola</i>	0	1	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	1	0	1
<i>O. ambigua</i>	1	0	1	0	1	0	0	1	0	0	1	1	1	0	1	0	0	1	0	1	0	1
<i>O. obtusa</i>	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	1
<i>O. heterophylla</i>	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	1
<i>O. purpurea</i>	0	1	1	0	1	0	0	1	0	0	1	1	0	0	1	0	1	0	0	1	0	1
<i>O. furcillata</i> var. <i>furcillata</i>	1	0	0	1	0	1	0	0	1	0	1	1	0	0	1	1	1	0	0	1	0	0
<i>O. natans</i>	0	0	0	0	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0
<i>O. tenella</i>	0	0	1	1	0	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0
<i>O. stenoptera</i>	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0	0
<i>O. aridicola</i>	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	1	0	0
<i>O. louisae</i>	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0
<i>O. sp. subsection Pardales</i>	0	0	0	1	0	1	0	0	1	0	0	1	1	0	1	1	1	0	0	1	0	0
<i>O. grammophylla</i>	1	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0
<i>O. meisneri</i>	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0
<i>O. viscosa</i>	0	0	0	0	0	1	0	0	1	0	1	1	0	1	0	0	1	0	0	0	1	0
<i>O. hirta</i> var. <i>hirta</i>	0	0	1	0	0	0	1	0	0	1	1	1	0	0	1	0	0	1	0	0	1	0
<i>O. tenuifolia</i>	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	1	0	0
<i>O. cf. urbaniana</i>	0	0	0	0	0	1	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	0
<i>O. multicaulis</i>	0	0	1	0	0	1	0	0	1	0	1	1	0	1	0	0	0	1	0	0	1	0
<i>O. pillansiana</i>	0	0	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0
<i>O. pusilla</i>	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	1	0	1	0
<i>O. glabra</i>	0	0	0	1	0	0	1	0	0	1	1	1	0	0	1	0	0	0	1	0	1	0
<i>O. xantha</i>	0	0	0	1	0	1	0	0	1	0	1	1	0	0	1	0	0	0	1	1	0	0
<i>O. ciliaris</i>	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	1	1	0	0
<i>O. oreophia</i>	0	0	0	0	0	1	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
<i>O. versicolor</i>	0	0	0	1	0	1	0	0	1	0	1	1	0	0	1	0	1	0	1	1	0	0
<i>O. ebracteata</i>	0	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	1	0	0
<i>O. droseroides</i>	0	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0
<i>O. clavifolia</i>	0	0	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0
<i>O. monophylla</i>	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0

TAXON	CHARACTERS																					
	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
<i>O. corniculata</i>	0	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0
<i>O. pes-capreae</i>	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0
<i>O. luteola</i>	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0
<i>O. ambigua</i>	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0
<i>O. obtusa</i>	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0
<i>O. heterophylla</i>	0	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0
<i>O. purpurea</i>	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0
<i>O. furcillata</i> var. <i>furcillata</i>	1	0	0	1	0	1	0	1	0	?	?	?	?	?	?	?	0	0	1	0	0	1
<i>O. natans</i>	1	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0
<i>O. tenella</i>	1	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0
<i>O. stenoptera</i>	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0
<i>O. aridicola</i>	1	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0
<i>O. louisae</i>	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1
<i>O. sp. subsection Pardales</i>	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
<i>O. grammophylla</i>	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
<i>O. meisneri</i>	1	0	0	1	0	1	0	0	1	?	?	?	?	?	?	?	0	0	1	0	1	0
<i>O. viscosa</i>	1	0	0	1	0	1	0	0	1	?	?	?	?	?	?	?	0	0	1	?	?	?
<i>O. hirta</i> var. <i>hirta</i>	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1
<i>O. tenuifolia</i>	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1
<i>O. cf. urbaniana</i>	1	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0
<i>O. multicaulis</i>	1	0	0	1	0	1	0	0	1	?	?	?	?	?	?	?	0	0	1	0	0	0
<i>O. pillansiana</i>	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0
<i>O. pusilla</i>	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0
<i>O. glabra</i>	1	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1
<i>O. xantha</i>	1	0	0	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0
<i>O. ciliaris</i>	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0
<i>O. oreophia</i>	1	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	?	?	?
<i>O. versicolor</i>	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0
<i>O. ebracteata</i>	1	0	0	1	0	1	0	0	1	?	?	?	?	?	?	?	0	0	1	0	1	0
<i>O. droseroides</i>	1	0	0	1	0	0	1	0	1	?	?	?	?	?	?	?	0	0	1	0	1	0
<i>O. clavifolia</i>	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1
<i>O. monophylla</i>	1	0	0	1	0	0	1	0	1	?	?	?	?	?	?	?	0	0	1	0	0	1

TAXON	CHARACTERS											
	67	68	69	70	71	72	73	74	75	76	77	78
<i>O. corniculata</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. pes-caprae</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. luteola</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. ambigua</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. obtusa</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. heterophylla</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. purpurea</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>O. furcillata</i> var. <i>furcillata</i>	0	1	1	0	1	0	0	0	0	0	1	1
<i>O. natans</i>	0	0	1	0	1	0	0	0	0	0	1	1
<i>O. tenella</i>	0	1	1	0	1	0	0	0	0	0	1	0
<i>O. stenoptera</i>	0	1	1	0	1	0	0	0	0	0	1	1
<i>O. aridicola</i>	0	1	1	0	1	0	0	0	0	0	1	0
<i>O. louisae</i>	1	0	1	0	1	0	0	0	0	0	1	1
<i>O. sp. subsection Pardales</i>	0	0	1	0	1	0	0	1	1	0	1	1
<i>O. grammophylla</i>	0	0	1	0	1	0	1	1	0	1	1	1
<i>O. meisneri</i>	1	0	1	0	1	0	0	0	0	0	0	0
<i>O. viscosa</i>	?	?	1	0	1	0	0	0	0	0	1	0
<i>O. hirta</i> var. <i>hirta</i>	0	1	1	0	1	0	0	0	0	0	1	0
<i>O. tenuifolia</i>	0	1	1	0	1	0	0	0	0	0	1	0
<i>O. cf. urbaniana</i>	0	0	1	0	1	0	0	0	0	0	1	0
<i>O. multicaulis</i>	0	0	1	0	1	0	0	0	0	0	1	0
<i>O. pillansiana</i>	1	0	1	0	1	0	0	0	0	0	1	0
<i>O. pusilla</i>	0	1	1	0	1	0	0	0	0	0	0	0
<i>O. glabra</i>	1	0	1	0	1	0	0	0	0	0	1	0
<i>O. xantha</i>	0	0	1	0	1	0	0	1	1	0	1	1
<i>O. ciliaris</i>	0	0	1	0	1	0	0	0	0	0	1	1
<i>O. oreophia</i>	0	0	1	0	1	0	0	0	0	0	1	0
<i>O. versicolor</i>	0	0	1	0	1	0	0	0	0	0	1	0
<i>O. ebracteata</i>	1	0	1	0	1	0	0	0	0	0	1	0
<i>O. droseroides</i>	1	0	1	0	1	0	0	0	0	0	1	0
<i>O. clavifolia</i>	1	0	1	0	1	0	0	0	0	0	1	0
<i>O. monophylla</i>	1	0	1	0	1	0	0	0	0	0	1	0

APPENDIX II: Table summarising the three classes of characters assessed in the present study.

Autapomorphic characters	
FRUITS	SEEDS
-	Inner integument structures (rugose, ribbed, colliculate)

Characters randomly distributed between species		
FRUITS	SEEDS	EXENDOSPERMOUS SEEDS (ONLY)
Shape (oblong & ovoid)	Inner integument structures	Position of trichomes on the cotyledon
Fruit ridging (present/absent)	-	Secondary metabolites deposits in the outer cotyledon and/or within the cotyledon
Indumentum position on the exocarp	-	Channels within the cotyledon and their position
Type of hairs on the exocarp	-	Number of channels per cotyledon
Fruit beak (present/absent)	-	-
Beak length (if present)	-	-
Pericarp thickness (number of cell layers)	-	-
Shape of exocarp cells	-	-
Secondary metabolites deposits (within the pericarp and/or within the fruit centre)	-	-
Channels within the pericarp (present/absent)	-	-
Testa thickness of young seeds	-	-

Characters used to define major endospermous and exendospermous groups	
FRUITS	SEEDS
Shape (presence/absence of spheroid fruit)	Shape
Length (mm)	Length (mm)
Diameter (mm)	Diameter (mm)
Intra-ocular constriction between seeds (present/absent)	Seed colour (orange to brown/green)
Number of seeds per locule (1 / 2 / 3 / >3)	Type of indumentum of mature seeds (if present)
Lobing	Fibrous cell layer of the inner integument of mature seeds (present/absent)
Type of septum (well divided/ partially divided/ fused)	Dark dotted phenolic layer lining the inner integument of the testa of mature seeds (present/absent)
Endocarp indumentum (if present)	Endosperm in mature seed (present/absent)
Fibrous cell layer of the inner integument of young seeds (present/absent)	Cotyledons Development (well - defined / not well - defined)
Dark dotted phenolic layer lining the inner integument of the testa of young seeds (present/absent)	-
Endosperm in young seeds (present/absent)	-