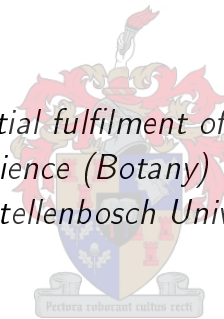


The phylogenetic and potential functional  
significance of leaf anatomical and  
physiological traits of southern African  
*Oxalis*

by

Michelle Jooste

*Thesis presented in partial fulfilment of the requirements for the  
degree of Master of Science (Botany) in the Faculty of Science  
at Stellenbosch University*



Department of Botany and Zoology,  
University of Stellenbosch,  
Private Bag X1, Matieland 7602, South Africa.

Supervisors:

Prof. L.L. Dreyer Dr. K.C. Oberlander

March 2016

# Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: ..... March 2016 .....

Copyright ©2016 Stellenbosch University  
All rights reserved.

# Contents

Declaration	i
Contents	ii
List of Figures	v
List of Tables	x
Abstract	xi
Opsomming	xii
Acknowledgments	xiii
<b>1 Chapter 1 - Introduction</b>	<b>1</b>
1.1 The Greater Cape Floristic Region	1
1.2 <i>Oxalis</i> in southern Africa	1
1.3 <i>Oxalis</i> leaf anatomical traits	3
1.4 References	5
<b>2 Chapter 2 - The phylogenetic significance of leaf anatomical traits of southern African <i>Oxalis</i></b>	<b>7</b>
2.1 Introduction	7
2.2 Materials and Methods	8
2.2.1 Morphology	11
2.2.2 Stomata	12
2.2.3 Epidermal, mesophyll and vascular tissue measurements	13
2.3 Results: Leaflet morphology	14
2.4 Results: Epidermal traits	16
2.4.1 Epidermal pavement cells	17
2.4.2 Trichomes	24
2.4.2.1 Non-glandular hairs	25
2.4.2.2 Glandular hairs	25
2.4.2.3 Leaflet ciliation	29
2.4.3 Stomata	31
2.5 Results: Mesophyll	34
2.5.1 Mesophyll arrangement	35
2.5.2 Mesophyll dimensions	37
2.5.3 Crystals	41
2.5.4 Cavities in mesophyll tissue	41
2.6 Results: Vascular tissue	43

2.7	Summary of Oberlander <i>et al.</i> (2011) clades with unique combinations of traits (Figure 2.36)	49
2.8	Discussion: Leaf morphology	53
2.9	Discussion: Epidermal tissue traits	53
2.9.1	Epidermal pavement cells	53
2.9.1.1	Irregular type pavement cells	53
2.9.1.2	Papillose type pavement cells	54
2.9.1.3	Semi-swollen type pavement cells	54
2.9.1.4	Swollen type pavement cells	55
2.9.2	Trichomes	55
2.9.3	Stomata	57
2.9.3.1	Stomatal position	57
2.9.3.2	Stomata in crevice	58
2.9.3.3	Stomatal position and evolution	58
2.9.3.4	Stomatal length, density and ploidy level	58
2.9.3.5	Stomatal complex	58
2.10	Discussion: Mesophyll tissue traits	59
2.10.1	Mesophyll arrangement type	59
2.10.2	Mesophyll thickness	59
2.10.3	Cavities within the mesophyll	60
2.10.4	Crystals	60
2.11	Discussion: Vascular tissue traits	61
2.12	Conclusion	63
2.13	References	64
<b>3</b>	<b>Chapter 3 - The mode of photosynthesis and the potential functional significance of leaf anatomical and physiological traits of southern African <i>Oxalis</i></b>	<b>71</b>
3.1	Introduction	71
3.2	Materials and methods	73
3.2.1	Carbon isotope analysis	73
3.2.2	Leaflet conductance data	73
3.2.3	Leaflet anatomy	74
3.2.3.1	Stomatal length and density	74
3.2.3.2	Stomatal index (SI)	74
3.2.4	Stomata and ploidy level	74
3.2.5	Specific Leaf Area (SLA)	75
3.2.6	Statistical analysis	75
3.3	Results: Photosynthetic pathway	76
3.3.1	Isotope analysis	76
3.3.2	Stomatal conductance	77
3.3.3	Leaflet anatomical and physiological traits	78
3.4	Results: Stomatal conductance data relative to leaf physiological traits	82
3.4.1	Stomatal conductance relative to stomatal size and densities	82
3.4.2	Stomatal dimensions	87
3.4.3	Leaflet physiology (SLA and mesophyll thickness)	94
3.5	Discussion	98
3.6	Conclusion	102
3.7	References	103
<b>4</b>	<b>Chapter 4 - Conclusion</b>	<b>108</b>

4.1	Chapter 1 conclusions . . . . .	108
4.2	Chapter 2 conclusions . . . . .	108
4.3	References . . . . .	110
4.4	List of addenda . . . . .	111
<b>References</b>		<b>112</b>

# List of Figures

2.1	A randomly sampled nuclear ITS phylogenetic tree from BEAST, used to sample and study southern African <i>Oxalis</i> taxa, including the <i>Oxalis</i> clade numbers and names as described by Oberlander <i>et al.</i> (2011).	10
2.2	Line drawing depicting the three main regions of <i>Oxalis</i> leaves: the semi-amplexicaul basal region, the petiole and the lamina (may be divided into one to multiple leaflets). Scale bar represents 5 mm and the drawing is based on a dry leaf of <i>O. depressa</i> (MO506).	12
2.3	Light microscope photographs of wax-embedded and stained leaflet material illustrating the dimensions of leaflet tissues that were measured for bifacial and isobilateral mesophyll arrangement types observed in <i>Oxalis</i> taxa. (a) <i>O. pulchella</i> (MO559), (b) <i>O. ebracteata</i> (MO262).	13
2.4	Photographs of pressed leaf material showing variability in number of leaflets per leaf in southern African <i>Oxalis</i> taxa. Species are listed from left to right, top to bottom within each image and each scale bar represents 1 cm. (a) <i>O. dregei</i> (MO398), <i>O. dregei</i> (MO398), <i>O. monophylla</i> (MO584), <i>O. monophylla</i> (MO584), <i>O. nortieri</i> (MO503), <i>O. monophylla</i> (MO1476), (b) <i>O. purpurea</i> (MO410), <i>O. fenestrata</i> (MO1527), <i>O. livida</i> (MO731), <i>O. sonderiana</i> (MO839), <i>O. attaquana</i> (MO45), <i>O. punctata</i> (MO343), <i>O. convexula</i> (MO368), <i>O. imbricata</i> (MO427), <i>O. depressa</i> (MO471), <i>O. dines</i> (MO823), <i>O. gracilis</i> (MO554), <i>O. glabra</i> (MO306), <i>O. oreophila</i> (MO505), <i>O. meisneri</i> (MO468), <i>O. confertifolia</i> (MO497), <i>O. deserticola</i> (MO1141), (c) <i>O. filifoliolata</i> (MO1490), <i>O. flaviuscula</i> (MO929), (d) <i>O. zeyheri</i> (MO590), <i>O. engleriana</i> (MO485), (e) <i>O. cathara</i> (MO582), <i>O. cathara</i> (MO582).	14
2.5	Phylogenetic distribution of southern African <i>Oxalis</i> taxa with less than three, three or more than three leaflets per leaf, using an ITS-based tree.	15
2.6	Phylogenetic distribution of flat and conduplicate leaflet types observed in southern African <i>Oxalis</i> taxa, using an ITS-based tree.	16
2.7	Light microscope photographs of epidermal peels depicting irregular, papillose, semi-swollen and swollen epidermal pavement cell types observed in southern African <i>Oxalis</i> taxa. (a) " <i>O. magnifolia</i> " (MO1524), (b) <i>O. uliginosa</i> (MO394), (c) <i>O. fenestrata</i> (MO1527), (d) <i>O. inconspicua</i> (MO569), (e) <i>O. monophylla</i> (MO584), (f) <i>O. goniorrhiza</i> (MO370), (g) <i>O. lateriflora</i> (MO887), (h) <i>O. livida</i> (MO361), (i) <i>O. pes-caprae</i> (MO849), (j) <i>O. pulchella</i> (MO559), (k) <i>O. foveolata</i> (MO1466), (l) <i>O. bullulata</i> (MO123).	18
2.8	Line drawings of transverse sectioned leaflet material depicting the four different epidermal pavement cell types observed in southern African <i>Oxalis</i> species. Scale bars represent 100 $\mu\text{m}$ . (a) Irregular type, (b) Papillose type, (c) Semi-swollen type, (d) Swollen type.	19
2.9	A boxplot indicating the AD and/or AB epidermal cell heights ( $\mu\text{m}$ ) of irregular, papillose and semi-swollen and swollen epidermal cell types in southern African <i>Oxalis</i> taxa. The symbols a and b were significantly different at $p < 0.001$ , and b and c were significantly different at $p < 0.01$ . A black line represents the median, a box represents the first and third quartiles, the whiskers represent the 95% confidence intervals and the circles represent outliers.	20

2.10	The phylogenetic distribution of adaxial epidermal cell types in southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	21
2.11	The phylogenetic distribution of adaxial epidermal cell types in southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	22
2.12	Light microscope photographs of epidermal peels indicating vein-associated elongated AB epidermal cells for taxa with irregular and swollen AB epidermal cell types. (a) <i>O. extensa</i> (J1216), (b) <i>O. fenestrata</i> (MO1527), (c) <i>O. ericifolia</i> (MO1143), (d) <i>O. pocockiae</i> (MO562), (e) <i>O. bullulata</i> (MO123), (f) <i>O. obtusa</i> (MO556). . . . .	23
2.13	Light microscope photographs of hand-sections of fresh leaflet material depicting the protruding and non-protruding mid-rib types observed in <i>Oxalis</i> taxa. The main vein vascular tissue is indicated with an arrow in each image. (a) <i>O. adspersa</i> (MO1223), (b) <i>O. obtusa</i> (MO566), (c) <i>O. convexula</i> (MO368). . . . .	23
2.14	The phylogenetic distribution of elongated and non-elongated vein-associated AB epidermal cell types found in southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	24
2.15	The phylogenetic distribution of trichome location (AD or AB surfaces) on leaflets of southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	26
2.16	Line drawings of trichome types identified on AD and AB leaflet surfaces in the studied <i>Oxalis</i> taxa. . . . .	27
2.17	The phylogenetic distribution of trichome types (glandular hairs and non-glandular hairs) observed in southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	28
2.18	The phylogenetic distribution of the presence or absence of clavate multi-cellular hairs located on AD and AB leaflet surfaces of southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	29
2.19	The phylogenetic distribution of glandular hair and non-glandular hair ciliation types observed on the leaflets of southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	30
2.20	Line drawings of the four stomatal complex types identified in the studied <i>Oxalis</i> taxa. Scalebar = 100 $\mu\text{m}$ . (a) <i>O. extensa</i> (J1216), (b) <i>O. convexula</i> (MO368), (c) <i>O. pulchella</i> (MO863), (d) <i>O. salteri</i> (MO1137). . . . .	31
2.21	Line drawings of transverse leaf sections and light microscope photographs of epidermal peels that show two groups of <i>Oxalis</i> taxa with exceptional stomatal arrangements. a) Group 1 includes epistomatic taxa with additional vein-associated stomata on the AB surface ( <i>O. cathara</i> MO582) and b) Group 2 includes hypostomatic taxa with additional vein-associated stomata on the AD surface ( <i>O. compressa</i> MO519). . . . .	32
2.22	The phylogenetic distribution of southern African <i>Oxalis</i> taxa with epistomatic, hypostomatic and amphistomatic leaflets on an ITS-based tree. . . . .	33
2.23	The phylogenetic distribution of southern African <i>Oxalis</i> taxa with epistomatic, hypostomatic and amphistomatic leaflets (with and without additional stomata in AB (Group 1) and AD (Group 2) leaflet crevices) on an ITS-based tree. . . . .	34
2.24	Light microscope photographs of wax-embedded and stained leaflet material depicting bifacial, isobilateral Type 1 and isobilateral Type 2 mesophyll arrangement observed in <i>Oxalis</i> taxa. (a) <i>O. commutata</i> (MO1224), (b) <i>O. foveolata</i> (MO1466), (c) <i>O. purpurea</i> (MO410), (d) <i>O. falcatula</i> (MO476), (e) <i>O. nortieri</i> (MO503), (f) <i>O. multicaulis</i> (MO883), (g) <i>O. oreophila</i> (MO270), (h) <i>O. tenella</i> (MO264), (i) <i>O. gracilis</i> (MO554). Scalebars represent 100 $\mu\text{m}$ . . . . .	36
2.25	The phylogenetic distribution of mesophyll arrangement in southern African <i>Oxalis</i> taxa, using an ITS-based phylogenetic tree. . . . .	37
2.26	The phylogenetic distribution of the section heights (including AD epidermal, palisade parenchyma, spongy parenchyma and AB epidermal layer heights) of southern African <i>Oxalis</i> taxa, using an ITS-based phylogenetic tree. . . . .	39

2.27	Boxplots of the relative dimensions (percentage values) of epidermal tissue and mesophyll tissue measured in bifacial, isobilateral Type 1 and isobilateral Type 2 <i>Oxalis</i> leaflets. . . . .	40
2.28	Light microscope photographs of crystals observed in wax-embedded and stained leaflet mesophyll of studied <i>Oxalis</i> taxa. (a) <i>O. zeyheri</i> (MO590), (b) <i>O. flaviuscula</i> (MO929), (c) <i>O. convexula</i> (MO368), (d) <i>O. tenella</i> (MO264), (e) <i>O. callosa</i> (MO532), (f) <i>O. convexula</i> (MO368), (g) <i>O. foveolata</i> (MO1466), (h) <i>O. depressa</i> (MO464), (i) <i>O. depressa</i> (MO464). Scalebars represent 50 $\mu\text{m}$ . . . . .	41
2.29	Light microscope photographs of wax-embedded and stained leaflet material depicting cavities with and without epithelial inner linings observed in the mesophyll of the studied <i>Oxalis</i> taxa. (a) <i>O. namaquana</i> (MO809), (b) <i>O. pulchella</i> (MO559), (c) <i>O. obtusa</i> (MO567), (d) <i>O. camelopardalis</i> (MO469), (e) <i>O. multicaulis</i> (MO883), (f) <i>O. purpurea</i> (MO410). Scalebars represent 100 $\mu\text{m}$ . . . . .	42
2.30	The phylogenetic distribution of cavities (with and without epithelial cell linings) observed in the mesophyll tissue of southern African <i>Oxalis</i> taxa, using an ITS-based tree. . . . .	43
2.31	The phylogenetic distribution of southern African <i>Oxalis</i> taxa with pinnate and palmate venation, using an ITS-based tree. . . . .	44
2.32	Light microscope photographs of wax-embedded and stained leaflet material and hand-sections of fresh leaflet material depicting embedded and protruding mid-ribs observed in <i>Oxalis</i> taxa. (a) <i>O. luederitzii</i> (J1210), (b) <i>O. eckloniana</i> (MO521), (c) <i>O. engleriana</i> (MO485), (d) <i>O. adspersa</i> (MO1223), (e) <i>O. hirsuta</i> (MO1586), (f) <i>O. nortieri</i> (MO503), (g) <i>O. depressa</i> (MO464), (h) <i>O. bowiei</i> (MO502), (i) <i>O. compressa</i> (MO519). Scalebars represent 100 $\mu\text{m}$ . . . . .	46
2.33	Light microscope photographs of wax-embedded and stained leaflet material depicting vascular tissue with and without a sclerenchymous sheath observed in <i>Oxalis</i> taxa. (a) <i>O. brasiliensis</i> (MO391), (b) <i>O. bowiei</i> (MO502), (c) <i>O. namaquana</i> (MO809), (d) <i>O. oreophila</i> (MO270), (e) <i>O. ciliaris</i> (MO329), (f) <i>O. odorata</i> (MO1549). Scalebars represent 100 $\mu\text{m}$ . . . . .	47
2.34	The phylogenetic distribution of southern African <i>Oxalis</i> taxa with and without a protruding mid-rib (vascular tissue), using an ITS-based tree. . . . .	48
2.35	The phylogenetic distribution of southern African <i>Oxalis</i> taxa with and without a sheath around vascular tissue, using an ITS-based tree. . . . .	49
2.36	The phylogenetic tree of southern African <i>Oxalis</i> taxa with illustrations of the typical leaf types observed in each clade with exceptions as discussed in the text. Three epidermal cell types are depicted, namely irregular, semi-swollen ( <i>lonoxalis</i> clade, Clade 2 and Clade 3) and swollen (sub-clade of Clade 7) epidermal cell types. Conduplicate leaflets are indicated by a leaf lamina at an acute angle to the mid-rib. All leaflet cross-sections are orientated with respect to the <i>lonoxalis</i> cross section. Taxa from Clade 8 had flat and conduplicate leaflets with bifacial or isobilateral Type 1 mesophyll arrangements. AD - adaxial leaflet surface, AB - abaxial leaflet surface. . . . .	52
3.1	Histogram indicating the distribution of $\delta^{13}\text{C}$ isotope data of southern African <i>Oxalis</i> species collected from the common garden collection and field localities. . . . .	76
3.2	Boxplot indicating the range of $\delta^{13}\text{C}$ values of leaves collected in the common garden collection and from the field. A black line represents the median, a box represents the first and third quartiles, the whiskers represent the 95% confidence intervals and the circles represent outliers . . . . .	77
3.3	Boxplot indicating the stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ) of southern African <i>Oxalis</i> species measured on both the adaxial and abaxial surfaces of plants growing in the common garden collection and the field. . . . .	78



- 3.4 Light microscope photographs of wax-embedded leaflet material depicting southern African *Oxalis* species with relatively thick epidermal cells (AD or AB epidermal cells). (a) *O. attaquana* (MO45), (b) *O. dentata* (MO474), (c) *O. falcata* (MO476), (d) *O. eckloniana* (MO521), (e) *O. lateriflora* (MO887), (f) *O. commutata* (MO1224). Scalebars represent 100  $\mu\text{m}$ . . . . . 79
- 3.5 Light microscope photographs of wax-embedded leaflet material depicting vascular tissue surrounded by a sheath observed in southern African *Oxalis* taxa. (a) *O. oreophila* (MO270), (b) *O. xantha* (MO273), (c) *O. ciliaris* (MO329), (d) *O. confertifolia* (MO358), (e) *O. callosa* (MO532), (f) *O. odorata* (MO1549). Scalebars represent 100  $\mu\text{m}$ . . . . . 79
- 3.6 Photographs of three *Oxalis* species with semi-succulent to succulent leaves (a) *O. pulchella* (MO559), (b) *O. foveolata* (MO1466), (c) *O. convexula* (MO368). Edited light microscope photographs of epidermal peels of the AD leaflet surface of three *Oxalis* species (stomata are highlighted with red circles, where each circle represents a single stoma) to illustrate the low stomatal densities (d) *O. pulchella* (MO559), (e) *O. foveolata* (MO1466), (f) *O. convexula* (MO368). Light microscope photographs of wax-embedded and transverse-sectioned leaflet material depicting relatively dense mesophyll arrangement with cavities within the mesophyll (g) *O. pulchella* (MO559), (h) *O. foveolata* (MO1466), (i) *O. convexula* (MO368). Scalebars represent 100  $\mu\text{m}$ . . . . . 81
- 3.7 Photographs of three *Oxalis* species with herbaceous leaves (a) *O. salteri* (MO1137), (b) *O. hygrophila* (MO230), (c) *O. bowiei* (MO502). Edited light microscope photographs of epidermal peels of the leaflet surfaces of three *Oxalis* species (stomata are highlighted with red circles, where each circle represents a single stoma) to illustrate the stomatal densities (d) AD surface of *O. salteri* (MO1137), (e) AD surface of *O. hygrophila* (MO230), (f) AB surface of *O. bowiei* (MO502). Light microscope photographs of wax-embedded and transverse-sectioned leaflet material depicting the common mesophyll arrangement observed among the majority of studied species with bifacial mesophyll arrangement type (g) *O. salteri* (MO1137), (h) *O. hygrophila* (MO230), (i) *O. bowiei* (MO502). Scalebars represent 100  $\mu\text{m}$ . . . . . 82
- 3.8 Boxplot indicating the total (pooled) stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ) of adaxial and abaxial leaflet surfaces (including additional vein-associated stomata) for southern African *Oxalis* species with epistomatic, hypostomatic and amphistomatic leaflets. The symbols a and b are significantly different at  $p < 0.05$ , and b and c are significantly different at  $p < 0.001$ . . . . . 83
- 3.9 Scatterplot of stomatal central axis lengths ( $\mu\text{m}$ ) in comparison to stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ). . . . . 84
- 3.10 Boxplot indicating stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ) of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomata. The symbols a and b were significantly different at  $p < 0.001$  ( $F = 20.06$ ,  $df = 504$  and 3). . . . . 85
- 3.11 Boxplot indicating stomatal central axis lengths of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomata. The symbols a and b were significantly different at  $p < 0.01$  ( $F = 13.36$ ,  $df = 167$  and 3). . . . . 86
- 3.12 Boxplot indicating log transformed stomatal densities of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomatal positions on leaflets. The symbols a and b were significantly different at  $p < 0.01$  ( $F = 32.69$ ,  $df = 171$  and 3). . . . . 87
- 3.13 Light microscope photographs of three examples of the smallest and largest stomata observed within southern African *Oxalis* species. (a) AD surface of epistomatic *O. salteri* (MO1137), (b) AD surface of epistomatic "*O. magnifolia*" (MO1524), (c) AD surface of epistomatic *O. purpurea* (MO344), (d) AB surface of hypostomatic *O. fenestrata* (MO1527), (e) AD surface of amphistomatic *O. nortieri* (MO503), (f) AD surface of epistomatic *O. furcillata* (MO564). . . . . 88

3.14	Histograms and scatterplot indicating stomatal lengths and densities of all stomata (AD and AB) for all studied <i>Oxalis</i> taxa. Multiple $R^2 = 0.262$ , Adjusted $R^2 = 0.256$ , $F = 47.55$ , $df = 1, 134$ , $p < 0.001$ . . . . .	89
3.15	Scatter-plot indicating the relationship between stomatal length and ploidy levels in the <i>Oxalis</i> taxa studied. Multiple $R^2 = 0.071$ , Adjusted $R^2 = 0.056$ , $F = 4.67$ , $df = 1, 61$ , $p < 0.05$ . . . . .	90
3.16	Boxplot indicating the Stomatal Index of epistomatic, hypostomatic and amphistomatic leaves from southern African <i>Oxalis</i> species sampled from the garden collection and various field localities. The symbols a and b were significantly different at $p < 0.01$ , b and c were significantly different at $p < 0.05$ . . . . .	91
3.17	Light microscope photographs of <i>Oxalis</i> taxa with epistomatic leaflets with additional vein-associated stomata on the AB surface, and hypostomatic leaflets with additional vein-associated stomata on the AD surface. (a) AD surface of epistomatic <i>O. cathara</i> (MO582), (b) AD surface of epistomatic <i>O. flava</i> (field), (c) AD surface of epistomatic <i>O. psammophila</i> (field), (d) AB surface of <i>O. cathara</i> (MO582), (e) AB surface <i>O. flava</i> (field), (f) AB surface <i>O. psammophila</i> (field), (g) AD surface of <i>O. compressa</i> (MO519), (h) AD surface of <i>O. tetraphylla</i> (MO392), (i) AD surface of <i>O. pes-caprae</i> (field), (j) AB surface of <i>O. compressa</i> (MO519), (k) AB surface of <i>O. tetraphylla</i> (MO392), (l) AB surface of <i>O. pes-caprae</i> (field). . . . .	92
3.18	Boxplot indicating stomatal central axis lengths of <i>Oxalis</i> taxa with epistomatic, hypostomatic and amphistomatic stomatal positions on leaflets. The stomatal lengths of the additional vein-associated stomata that occur on the AB surface of epistomatic leaflets and on the AD surface of hypostomatic leaflets were included in this boxplot. The symbols a, b and c were significantly different at $p < 0.001$ . . . . .	93
3.19	Boxplot indicating the stomatal conductance ( $\text{mmol/m}^{-2}\text{s}^{-1}$ ) of southern African <i>Oxalis</i> species measured on both the adaxial (AD) and abaxial (AB) surfaces of epistomatic (Epi), hypostomatic (Hypo) and amphistomatic (Amphi) leaflets, and leaflets with additional vein-associated stomata located in the crevice of leaflets (Epi-ABcrev and Hypo-ADcrev). The symbols a, b and c were significantly different at $p < 0.001$ . . . . .	94
3.20	Boxplot indicating the log surface area ( $\text{cm}^2$ ) of southern African <i>Oxalis</i> leaves sampled from the living collection and the field. The symbols a and b were significantly different at $p < 0.001$ . . . . .	95
3.21	Boxplot indicating the total dry weight (g) of southern African <i>Oxalis</i> leaves sampled from the living collection and the field. . . . .	95
3.22	Boxplot indicating the log specific leaf area ( $\text{cm}^2/\text{g}$ ) (SLA) of southern African <i>Oxalis</i> leaves sampled from the living collection and the field. The symbols a and b were significantly different at $p < 0.01$ . . . . .	96
3.23	Boxplot indicating log mesophyll thickness ( $\mu\text{m}$ ) (palisade and spongy parenchyma tissue) of southern African <i>Oxalis</i> with epistomatic, hypostomatic and amphistomatic leaflets. The symbols a and b were significantly different at $p < 0.05$ . . . . .	97
3.24	Boxplot indicating log leaflet section thickness (total adaxial and abaxial epidermal cell height and the mesophyll thickness) ( $\mu\text{m}$ ) of southern African <i>Oxalis</i> with epistomatic, hypostomatic and amphistomatic leaflets. The symbols a and b were significantly different at $p < 0.05$ . . . . .	97

# List of Tables

2.1	The <i>Oxalis</i> clade numbers and names as described by Oberlander <i>et al.</i> (2011). Clades correspond to those mentioned in the text and figures throughout this study . . . . .	11
-----	---	----

## Abstract

The southern African *Oxalis* radiation is extremely morphologically variable and few characters are known as synapomorphies supporting DNA-based clades, hindering species identification, taxonomic revision and understanding of functional trait evolution in this clade. Sixty-eight leaflet anatomical traits of 109 southern African *Oxalis* species were assessed in search of phylogenetically significant characters that delineate clades. This study showed that the combination of six leaflet anatomical traits (stomatal position, adaxial epidermal cell types, abaxial epidermal cell types, mesophyll type, sheath around vascular tissue and degree of leaflet conduplication) clearly support various clades defined by previous DNA-based phylogenetic work. Despite the phylogenetic patterns detected in the aforementioned traits, other leaflet anatomical traits were highly variable and showed no phylogenetic pattern: these traits could possibly hold functional significance. The information gathered in this study will aid in the taxonomic revision of this speciose member of the Greater Cape Floristic Region (GCFR) and provide a basis for future hypotheses regarding its radiation.

Limited previous work on *Oxalis* has shown evidence for CAM photosynthesis in some South American species and preliminary anatomical work on southern African *Oxalis* hinted at Kranz anatomy, typically associated with  $C_4$  photosynthesis. We assessed stable carbon-isotope data, stomatal conductance and anatomical traits for 67 southern African *Oxalis* species to test whether any indigenous species show alternative modes of photosynthesis. All assessed taxa followed an exclusively  $C_3$  photosynthetic pathway. The measured stomatal conductance data of southern African *Oxalis* species showed that many species make minimal effort to prevent water loss, even though *Oxalis* was previously regarded as a water-conservative genus (Kluge and Ting (1978); Proches *et al.* (2006a); Biocyclopedia: *Oxalis* (2012)).

Additionally we observed that the majority of southern African *Oxalis* species have epistomatic leaflets, which is regarded as the rarest stomatal position among all angiosperms. Interestingly, epistomatic leaflets had significantly smaller stomata (which enables fast response to changes in environmental conditions) and significantly higher conductance rates (which enables carbon fixation to take place at a faster rate). We propose that this strategy would be favoured under the strongly seasonal environment in which the majority of southern Africa *Oxalis* grows (winter rainfall Mediterranean climate), as this enables these plants to take advantage of their growing conditions to ensure high productivity, rapid growth and successful carbon storage into their below-ground organs.

We propose that the strategy of small adaxially-located stomata with high conductance rates might be an overlooked key innovation driving diversification into one of the largest geophytic plant lineages within the GCFR.

## Opsomming

Die suider-Afrikaanse *Oxalis* radiasie is morfologies geweldig variërend, en min kenmerke is bekend as sinapomorfies wat DNA-gebaseerde klades ondersteun, wat spesie-identifikasie, taksonomiese hersiening en 'n begrip van funksionele kenmerk evolusie in hierdie klade bemoeilik. Agt-en-sestig blaartjie anatomiese kenmerke van 109 suider Afrikaanse *Oxalis* spesies is ondersoek op soek na filogeneties beduidende kenmerke wat klades afbaken. Die studie het aangedui dat die kombinasie van ses blaartjie-anatomiese kenmerke (stomata posisie, adaksiale epidermale sel-tipes, abaksiale epidermale sel-tipes, mesofil sel-tipe, skede rondom vaatweefsel en graad van blaartjie toevouing) verskeie klades wat deur vroeëre DNA-gebaseerde filogenetiese werk gedefinieer is, ondersteun. Ten spyte van die filogenetiese patrone waargeneem in hierdie kenmerke, was ander blaartjie anatomiese kenmerke hoogs variërend en het min filogenetiese patrone gewys: hierdie kenmerke mag funksioneel betekenisvol wees. Die inligting wat in hierdie studie vergader is sal die taksonomiese hersiening van hierdie spesie-ryke lid van die Groter Kaapse Floristiese Streek (GKFS) steun en 'n basis daarstel vir toekomstige hipoteses ten opsigte van sy radiasie.

Beperkte vroeëre werk op *Oxalis* het bewys gelewer vir CAM fotosintese in sommige Suid-Amerikaanse spesies en voorlopige anatomiese werk op suider-Afrikaanse *Oxalis* het Kranz anatomie, wat tipies met  $C_4$  fotosintese geassosieer word, gesuggereer. Ons het stabiele stikstof-isotoop data, stomata geleiding en anatomiese kenmerke van 67 suider-Afrikaanse *Oxalis* spesies ondersoek om te toets of enige inheemse spesies van alternatiewe tipes fotosintese gebruik maak. Alle taksa wat ondersoek is maak uitsluitlik van  $C_3$  fotosintese gebruik. Die stomata geleidingsdata wat gemeet is was hoër as wat verwag is. Hierdie inligting het die wyse waarna hierdie genus gekyk word omgekeer, siende dat *Oxalis* vroeër as water-konserwatief beskou is, maar hierdie data dui aan dat baie *Oxalis* spesies minimale pogings aanwend om water te bespaar.

Addisioneel het ons waargeneem dat die meerderheid suider-Afrikaanse spesies epistomatiese blaartjies het, wat beskou word as die skaarste stomata posisie binne die angiosperme. Interessant genoeg, het epistomatiese blaartjies beduidend kleiner stomata (wat vinnige reaksie op veranderinge in omgewingskondisies moontlik maak) en beduidend hoër geleidingstempos (wat dit moontlik maak vir stikstof fiksering om teen 'n vinniger tempo plaas te vind). Ons stel voor dat hierdie strategie verkies sou word onder die sterk seisoenale omgewing waarin die meeste suider-Afrikaanse *Oxalis* spesies groei (winter reënval Mediterreense klimaat), aangesien dit hierdie plante in staat stel om voordeel te trek uit hulle groei-omstandighede en hoë produktiwiteit, vinnige groeitempo en suksesvolle stikstofstoring in hulle ondergrondse organe verseker.

Ons stel voor dat die strategie van klein, adaksiaal-geplaasde stomata met hoë geleidingstempos 'n onopgemerkte sleutel innovasie mag wees wat die diversifikasie tot een van die grootste geofietise plant-ontwikkelingslyne in die GKFS mag dryf.

## Acknowledgments

I would like to express my sincerest gratitude to my supervisors, Prof. L.L. Dreyer and Dr K.C. Oberlander for their continuous support, guidance, training and encouragement throughout this study.

I would like to thank Prof. G.F. Midgley for his invaluable assistance with advice and guidance on plant physiological data, and who generously offered to pay for additional isotope analysis that was not originally included in our research plan and budget.

I thank The United Nations Educational, Scientific and Cultural Organization (UNESCO) for the Man And Biosphere (MAB) Young Scientist Research Award of 2014, The South African Association for Women Graduates (SAAWG) for the Bertha Stoneman Award and the Cape 300 Foundation, all of which provided financial support for this study.

I thank Prof. J. Suda (Charles University, Prague) who kindly provided the genome size data of the Stellenbosch University Botanical Gardens' Living *Oxalis* collection.

I thank A. Soltoff who helped with the counting of stomata and epidermal cells to calculate Stomatal Index (SI) values for Chapter 2.

I would like to thank the Stellenbosch University and Department of Botany and Zoology for the use of their facilities and equipment.

I would like to thank my parents, sister, B. Marais and X. von Stein for their help, love and support in every way possible.

# Chapter 1

## Introduction

### 1.1 The Greater Cape Floristic Region

The unique biogeographic region located at the southwestern tip of the African continent, namely the Greater Cape Floristic Region (GCFR), is globally renowned for its extremely species-rich and diverse flora (Born *et al.*, 2006). The GCFR essentially encompasses the entire winter-rainfall area of the Cape. Climates across the GCFR are variable, with a Mediterranean climate of strongly seasonal winter-rainfall in the southwest of the region, extensive summer precipitation and aseasonal rainfall in the eastern half of this region and semi-arid conditions to the west and north-west of the region (Linder, 2003). The landscape typically consists of a mosaic of coarse-grained, nutrient poor sandstone soils on mountain slopes and richer shale soils of nutrient-intermediate status in valleys, with smaller patches of limestone and granite adding to the geological diversity (Linder, 2003). The variation in rainfall between localities, together with the variety of different soils, creates an unusually high number of local habitats and ecological niches available to plant life, reflected in the different vegetation types and very high species richness and diversity in this area of the GCFR (Manning and Goldblatt, 2012).

Over 17% of all species present in the GCFR are represented by geophytes with bulbs, corms, tubers or rhizomes. Both geophytic and annual growth forms have been recognized as adaptive strategies for seasonal climates (Raunkiaer (1934); Burns (1946); Svoskin (1960); Rees (1966)). Geophytes utilize underground storage organs to escape harsher seasons unfavourable for growth and occur in regions with reliable climatic seasonality (Manning and Goldblatt, 2012). The well-defined and reliable seasonal differences between summer and winter climates in the Cape (Schulze *et al.* (1978); Campbell (1983)) are considered to be the reason why geophytic growth forms are favoured in this region. The genus *Oxalis* (Oxalidaceae) is the sixth largest geophytic lineage and the largest geophytic genus in the GCFR (Manning and Goldblatt, 2012).

### 1.2 *Oxalis* in southern Africa

The taxonomic placement of the Oxalidaceae has been controversial, as previous classification was based mainly on morphology and growth form traits (Cronquist (1981); Takhtajan (1997); Thorne (2000)). The family used to be included in the Geraniales close to the Geraniaceae, but molecular systematic studies by Chase *et al.* (1993) and Price and Palmer (1993) proposed entirely new affinities for the Oxalidaceae. The Angiosperm Phylogeny Group (APG III, 2009) retrieves the family in the Oxalidales, along with families such as the Connaraceae, Cephalotaceae, Cunoniaceae, Elaeocarpaceae, Brunelliaceae and Huaceae. Oxalidaceae includes five genera, of which *Oxalis* L. is by far the largest with more than 500 species. This family is morphologically variable and includes

trees, shrubs, lianas, annuals and geophytes, which occur mainly in tropical and subtropical regions (Chant (1978); Takhtajan (1997); Leistner (2000); Judd *et al.* (2008)). Two genera, *Oxalis* and *Biophytum* D.C., occur in South Africa (Leistner, 2000). Two centres of diversity for *Oxalis* are known, one in South America and the other in southern Africa (Denton (1973); Oberlander *et al.* (2002)). Approximately 220 *Oxalis* species and about 270 taxa (Salter (1944); Oliver (1993)) have been recorded from southern Africa and more than 90% of these species are known to be endemic to South Africa (Manning and Goldblatt, 2012). Within southern Africa, *Oxalis* is by far the most diverse and has the highest number of endemic species within the GCFR (Oberlander *et al.*, 2002). Three centres of *Oxalis* biodiversity have been identified, namely the Cape Peninsula/Hottentots Holland region, the Clanwilliam/Niewoudtville region and the Kamiesberg region (Oberlander *et al.*, 2002). All members of the southern African *Oxalis* lineage are geophytic (Gebregziabher, 2004); in contrast to only 31% of the species from the South American diversity centre (Lourteig, 2000).

*Oxalis* is the sixth largest geophytic lineage and the largest geophytic genus in the GCFR (Manning and Goldblatt, 2012). It is one of several geophytic taxa that are extensively shared between the Cape Core Region (CCR) and the Extra Cape Region (ECR) in southern Africa (Oberlander *et al.*, 2002) and displays substantial species richness within both regions (Manning and Goldblatt (2012); Snijmann (2013)). *Oxalis* lineages from the ECR were found to be much younger than lineages from the CCR (Verboom *et al.*, 2009). Due to the evolution of geophytism in *Oxalis* and its distribution in both the CCR and ECR, this genus offers an outstanding opportunity to study various hypotheses regarding the evolution and diversification of the Cape Flora (Oberlander *et al.*, 2011). Unfortunately the size and complexity of southern African *Oxalis* has resulted in the southern African members being understudied, and until recently their systematic relationships have been poorly understood.

Summing up the major contributions of Jacquin (1795), Linnaeus (1753), Savigny (1797) and Sonder (1806), the most recent major work to feature South African *Oxalis* in a global context within the Oxalidaceae was by Knuth (1930). Subsequent to that, the three most recent and comprehensive studies on southern African *Oxalis* were by Salter (1944), Dreyer (1996) and Oberlander *et al.* (2011). After 20 years of extensive fieldwork and a detailed study of living and herbarium material, Salter published the most comprehensive taxonomic revision of the southern African *Oxalis* in 1944. He described 65 new taxa and acknowledged 208 existing species in this study, which greatly contributed to the knowledge base of southern African *Oxalis* (Dreyer, 1996). However, only characters visible to the naked eye or through light microscopy, for example plant-, leaf- and bulb morphology, were considered, a limiting factor acknowledged by the author himself (Salter, 1944). Very few anatomical characters were included, which further limited the ability to discover phylogenetic affinities. The work has also been criticised in the literature for not covering the full geographic range of species and for the limited herbarium and living material on which some new taxa were based (Bayer, 1992). However, Salter (1944) did provide a solid taxonomic foundation and a stable set of species names on which further insight into the evolutionary radiation of southern African *Oxalis* could be founded.

The next major study of southern African *Oxalis* was completed by Dreyer (1996), who presented a detailed review of the palynology of southern African members of the genus. She identified four major pollen types based on the tectum structure, namely micro-reticulate, micro-rugulate spinate, reticulate and supra-areolate pollen types. Supra-areolate pollen is unique within the angiosperms, but based on Salter's (1944) classification it occurred in two different sections and multiple subsections of the southern African *Oxalis* genus. Pollen characters are highly conservative in comparison to floral or vegetative characters (Judd *et al.*, 2008). The chances of at least two independent origins for such a rare pollen type therefore seemed very unlikely (Dreyer, 1996). Despite the well-defined and easily-recognisable pollen type groupings there was poor congruence between the morphological



classification proposed by Salter (1944) and the palynological classification of Dreyer (1996).

The most recent major study on southern African *Oxalis* species, a reconstruction of a large scale DNA sequence-based phylogeny, was published by Oberlander *et al.* (2011). Three molecular markers and three different inference methods were used to determine the phylogenetic relationships between three-quarters of all indigenous southern African *Oxalis* species (Oberlander *et al.*, 2011). All *Oxalis* from southern Africa formed a clade, and despite substantial incongruence between different molecular markers, it resolved the previously unclear basal relationships between the major southern African lineages. The phylogeny proposed by Oberlander *et al.* (2011) was incongruent with the morphological taxonomy of Salter (1944), as all recognised sections proved to be non-monophyletic. The proposed phylogeny corresponded very well with previous phylogenetic studies (Oberlander *et al.*, 2004), and with the palynological classification by Dreyer (1996) and provides a framework for future systematic work on this genus. The incongruence between the molecular phylogeny and the morphological classification has highlighted the need for more independent datasets that include morphological characters as well as data from other disciplines such as anatomy, karyology, reproductive biology and ecology.

To our knowledge, possible associations between biogeographical attributes of species that belong to the southern African *Oxalis* clades have not been formally tested. Coarse-scale biogeographic reconstructions have shown that the southern African radiation has a South American origin, and that southern crown African *Oxalis* most likely originated in the GCFR, where the vast majority of species are located (Oberlander *et al.*, 2011). Within the GCFR, there appears to be little phylogenetic pattern in distribution ranges. The sum of species distribution ranges within the major clades in this study tends to cover much or all of the GCFR, although again this has not been subject to formal testing (Dreyer and Oberlander *pers. comm.*). Future studies could possibly prioritise the compilation of such datasets, such that anatomical or ecological datasets could be compared to biogeographical datasets to assess patterns within the phylogenetic context of this genus.

### 1.3 *Oxalis* leaf anatomical traits

The majority of *Oxalis* species (South African and global) have three leaflets per leaf (Salter (1944); Denton (1973); Lourteig (2000)). Three to 13 leaflets per leaf have been recorded in the sect. *lonoxalis* (Denton, 1973) and one to 29 leaflets per leaf have been recorded in southern African *Oxalis* (Salter, 1944). This trait has been included as a taxonomically useful morphological trait in the current classification of *lonoxalis* and the southern African *Oxalis* genus. Various comparative leaf anatomical studies include epidermal pavement cell types (Benitez and Ferrarotto (2009); Moon *et al.* (2009); Soh and Parnell (2011)): sinuous epidermal cell types (?; Storey (2006)), angular types (Wood, 1874), and papillose types (Haberlandt (1914); Wiggins and Porter (1971)) have been reported in South American and European *Oxalis* species. Bladder epidermal cells have been observed on the AB leaflet surface of South American *O. carnosus* (Steudle, 1983) and swollen epidermal cells have been described in southern African *Oxalis* (Salter, 1944). Taxonomic work on *Oxalis* section *lonoxalis* reported that pubescence, trichome types, densities and lengths were taxonomically significant traits, but considerable variation of trichome traits on the plant in general, and leaves specifically, for species in the *lonoxalis* clade have been recorded (Denton, 1973). Hypostomatic leaflets have been documented in North American (Denton, 1973) and South American *Oxalis* (dos Reis and Alvim (2013); Steudle (1983)). A single known record of amphistomatic leaflets in *Oxalis* is known in *O. latifolia* (dos Reis and Alvim (2013), Fig.3), but stomatal distribution seems to be different between AD and AB surfaces and stomata are sparse on the AD surface and confined to above the midrib vascular tissue. Anomocytic and paracytic stomatal complex types have been

reported in Oxalidaceae (Metcalf (1979); Freire *et al.* (2005); dos Reis and Alvim (2013)).

To our knowledge, mesophyll arrangement types have only been recorded in three south American *Oxalis* species (citeDosReis2013; Toma *et al.* (2007)), which are the typical angiospermous bifacial condition. *Oxalis* are well-known for their high concentrations of calcium oxalate present in leaf tissue (Jones and Luchsinger (1987); Dellagrecia *et al.* (2015); Šircelj *et al.* (2010); Abhilash *et al.* (2011)). Trends of calcium oxalate deposits in some *Ionoxalis* species have been described, but these traits were not as taxonomically useful as previously assumed (Denton, 1973). Pinnate venation types were recorded in Oxalidaceae in general Jones and Luchsinger (1987) and sect. *Ionoxalis* (Denton, 1973). The trait of a protruding vascular mid-rib has been recorded in three South American species (dos Reis and Alvim, 2013) and the presence of sheaths have been described around the periphery of the vascular bundles in *O. corniculata* (Toma *et al.*, 2007). Despite the above-mentioned leaflet anatomical traits, it is obvious that there is scope for much more in-depth study of *Oxalis* leaflets and there are doubtless many traits that have not yet been assessed or reported in the available literature.

Angiosperms show a great wealth of external morphological characters of potential taxonomic value (Carlquist (1961); Radford *et al.* (1974); Metcalfe (1979); Stuessy (1990)) and it has been proposed that the leaf is “perhaps anatomically the most varied organ of angiosperms” (Carlquist, 1961). Carlquist (1961) also claimed that most systematic studies of angiosperms would benefit from the use of comparative anatomy due to its demonstrated utility. The first main aim of this study was thus to compile a leaflet anatomical dataset of as many southern African *Oxalis* species as possible, and assess variation of these traits in a phylogenetic context.

The second main aim of this study was to explore the mode(s) of photosynthesis among southern African *Oxalis* species in a leaf anatomical and physiological context. This represents the first well-sampled and extensive physiological research on southern African *Oxalis* (almost 50% of the southern African genus). This main aim is explored in more detail in Chapter 3.

The main findings of this thesis are discussed in Chapter 4.

## 1.4 References

- Bayer, M. (1992). Salter's revision of South African *Oxalis* (Oxalidaceae) and some new combinations. *Herbertia*, vol. 48, pp. 58–69.
- Born, J., Linder, H.P. and Desmet, P. (2006). The Greater Cape Floristic Region. *Journal of Biogeography*, pp. 1–16.
- Burns, W. (1946). Corm and bulb formation with special reference to the Graminae. *Transactions and Proceedings of the Botanical Society of Edinburgh*, vol. 34, pp. 316–347.
- Campbell, B.M. (1983). Montane plant environments in the fynbos biome. *Bothalia*, vol. 14, pp. 283–298.
- Carlquist, S. (1961). *Comparative plant anatomy: A guide to taxonomic and evolutionary applications of anatomical data in angiosperms*. Rinehart Winston, New York.
- Chant, S.R. (1978). *Oxalidaceae*, in: Heywood, V. H. (Ed.), *Flowering Plants of the world*. Oxford University press, Oxford.
- Chase, M.W., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mshler, D., Duvall, M.R., Price, R.A., Hills, H.G., Qru, Y.L., Kron, K.A. and et al. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcl*. *Annals of the Missouri Botanical Garden*, vol. 80, pp. 528–580.
- Cronquist, A. (1981). *An integrated system of classification of flowering plants*. Columbia University Press, New York.
- Denton, M.D. (1973). *A monograph of Oxalis Section Ionoxalis (Oxalidaceae) in North America*.
- Dreyer, L.L. (1996). A palynological review of *Oxalis* (Oxalidaceae) in southern Africa.
- Gebregziabher, A.K. (2004). Systematic significance of bulb morphology of the southern African members of *Oxalis* L. (Oxalidaceae).
- Jacquin, N. (1795). *Icones planarium rariorum, Vol. 3*. Wappler, Vienna.
- Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J. (2008). *Plant systematics: a phylogenetic approach, 3rd edition*. Sinauer Associates, INC., Sunderland, Massachusetts, USA.
- Knuth, R. (1930). *Oxalidaceae*, in Engler, A. (Ed.), *Das Pflanzenreich 95 IV. Leipzig*.
- Leistner, O.A. (2000). Seed plants of southern Africa: families and genera, Strelitzia 10.
- Linder, H.P. (2003). The radiation of the Cape flora, southern Africa. *Biological Review*, vol. 78, pp. 597–638.
- Linnaeus, C. (1753). *Species Plantarum, 1st edition*. Impensis Laurentii Salvii, Stockholm.
- Lourteig, A. (2000). *Oxalis* L. subgenera *Monoxalis* (Small) Lourteig, *Oxalis* Trifidus Lourteig. *Bradea*, vol. 7, pp. 201–629.
- Manning, J. and Goldblatt, P. (2012). *Plants of the Greater Cape Floristic Region 1: the Core Cape Flora*. Strelitzia 29, South African National Biodiversity Institute, Pretoria, South Africa.

- Metcalfe, C.R. (1979). *Anatomy of the dicotyledons, 2nd Ed. Vol. 1: systematic anatomy of leaf and stem, with a brief history of the subject*. Oxford Science Publications, Clarendon, Oxford.
- Oberlander, K.C., Dreyer, L.L. and Bellstedt, D.U. (2011). Molecular phylogenetics and origins of southern African *Oxalis*. *Taxon*, vol. 60, no. 6, pp. 1667–1677.
- Oberlander, K.C., Dreyer, L.L., Bellstedt, D.U. and Reeves, G. (2004). Congruence of trnL-F and palynological data sets in the southern African *Oxalis* L section Angustatae subsection Lineares. *Taxon*, vol. 53, pp. 977–985.
- Oberlander, K.C., Dreyer, L.L. and Esler, K.J. (2002). Biogeography of *Oxalis* (Oxalidaceae) in South Africa: a preliminary study. *Bothalia*, vol. 32, pp. 97–100.
- Oliver, E.G.H. (1993). Oxalidaceae: a new species of *Oxalis* from the Western Cape. *Bothalia*, vol. 23, pp. 72–74.
- Price, R. and Palmer, J. (1993). Phylogenetic relationships of the Geraniaceae and Geraniales from rbcL sequence comparisons. *Annals of the Missouri Botanical Garden*, vol. 80, pp. 661–671.
- Radford, A.E., Dickison, W.C., Massey, J.R. and Bell, C.R. (1974). *Vascular Plant Systematics*. Harper and Row Publishers.
- Raunkiaer, C. (1934). *The Life Forms of Plants and Statistical Plant Geography*. Clarendon Press, Oxford.
- Rees, A.R. (1966). The physiology of ornamental bulbous plants. *Botanical Review*, vol. 32, pp. 1–23.
- Salter, T.M. (1944). The genus *Oxalis* in South Africa: a taxonomic revision. *Journal of South African Botany*, vol. 1, pp. 1–355.
- Savigny, M.J.C.L. (1797). *Encyclopedie methodique Botanique, Vol 4*. Agasse, Paris.
- Schulze, E.D., Ellis, R., Schulze, W. and Trimborn, P. (1978). Diversity, metabolic types and delta<sup>13</sup>C carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia*, vol. 106, pp. 352–369.
- Snijmann, D. (2013). Plants of the Greater Cape Floristic Region: The Extra Cape flora, *Strelitzia* 30.
- Sonder, O.W. (1806). *Oxalidaceae*, in: *Harvey, W.H., Sonder, O.W. (Eds.), Flora Capensis, Vol. 1*. Robertson, Cape Town.
- Stuessy, T.F. (1990). *Plant taxonomy*. Columbia University Press, New York.
- Svoskin, I.P. (1960). Specific biological characteristics of bulbous geophytes as related to their past and present ecology. *Botanicheskii Zhurnal*, vol. 45, pp. 1073–1078.
- Takhtajan, A. (1997). Floristic regions of the world.
- Thorne, R. (2000). The Classification and Geography of the Flowering Plants: Dicotyledons of the classic Angiospermae. *The Botanical Review*, vol. 66, pp. 441–647.
- Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F. and et al. (2009). Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, vol. 51, pp. 44–53.

## Chapter 2

# The phylogenetic significance of leaf anatomical traits of southern African *Oxalis*

### 2.1 Introduction

Within southern Africa, *Oxalis* has by far the highest number of species and the greatest morphological diversity within the Greater Cape Floristic Region (GCFR) (Oberlander *et al.*, 2002). Approximately 220 *Oxalis* species and about 270 taxa (Salter (1944); ?) have been recorded from southern Africa and more than 90% of these species are known to be endemic to South Africa (Manning and Goldblatt, 2012). Three centres of *Oxalis* biodiversity have been identified, namely the Cape Peninsula/Hottentots Holland region, the Clanwilliam/Niewoudtville region and the Kamiesberg region (Oberlander *et al.*, 2002). All members of the southern African *Oxalis* lineage are geophytic (Gebregziabher, 2004); in contrast to only 31% of the species from the South American diversity centre (Lourteig, 2000). The genus *Oxalis* offers an outstanding opportunity to study various hypotheses regarding the evolution and diversification of the Cape Flora (Oberlander *et al.*, 2011).

The taxonomic placement of the family Oxalidaceae has been controversial, as previous classification was based mainly on morphology and growth form traits (Cronquist (1981); Takhtajan (1997); Thorne (2000)). The southern African *Oxalis* radiation is extremely morphologically variable and few characters are known as potential synapomorphies supporting clades (Oberlander *et al.*, 2011), hindering species identification, taxonomic revision and understanding of functional trait evolution in this clade. Due to the size and complexity of the southern African *Oxalis* genus, this has resulted in the southern African members being understudied, and until recently their systematic relationships have been poorly understood (Oberlander *et al.*, 2011). Salter (1944) divided the southern African *Oxalis* taxa into 11 sections and 13 subsections based on morphological characters. Dreyer (1996) and Oberlander *et al.* (2011) highlighted some of the shortcomings of this classification and suggested that none of the described sections were grouped as natural entities, as none of the sections were found to be monophyletic. The incongruence between the molecular phylogeny and the morphological classification thus emphasized the need for the re-evaluation of morphological characters in *Oxalis* through the use of other independent datasets.

*Oxalis* leaves are tremendously variable in number and shape of leaflets, leaf and leaflet size, degree of leaflet conduplication, petiole length and shape, nature of the epidermis and indumentum attributes (Salter, 1944). Preliminary investigations on selected characters of southern African *Oxalis* highlighted the potential systematic significance of various vegetative and reproductive characters.

These characters included the morphology and anatomy of bulbs, fruits, seeds and seedlings (De Villiers (2001), Gebregziabher (2004), Obone (2005)). Anatomy is a classical source of data used in plant taxonomy, as homologies of morphological character states can easily be identified through anatomical traits (Stuessy, 1990). These traits are useful for identification purposes and for the determination of phylogenetic relationships between taxa (Metcalfe (1979), Judd *et al.* (2008)). However, recent research into anatomical characters of *Oxalis* has been lacking, apart from a study by Singh (1999) who showed that anatomical traits are important in the clarification of phylogenetic relationships among South American *Oxalis* species.

It is thus reasonable to expect that the genus *Oxalis* may display a wealth of systematically significant leaf anatomical traits that may further clarify relationships between the southern African taxa, but to date this has not been assessed. Our main objective was to search for potential leaf anatomical synapomorphies among phylogenetically representative taxa to support the monophyly of southern African *Oxalis* clades (Oberlander *et al.*, 2011).

## 2.2 Materials and Methods

Plant material was collected from the *Oxalis* living collection in the Stellenbosch University Botanical Gardens and supplemented with plant material collected in the field. Plants in the living collection have been exposed to the same watering and day-night light regime over the past number of years and the effects of environmentally induced anatomical variation in plants should therefore be minimised. The phylogenetic tree of Oberlander *et al.* (2011) was used to guide taxon selection, to ensure even sampling across all of the main clades. Our sampling included 149 accessions (109 species) of native taxa and three non-native out-group accessions (three species) (Figure 2.1 and Appendix 1). All accessions are living plants from the Botanical Garden's collection, and each accession comprises of up to five individual plants. Garden accession numbers are reported as MO-numbers given in parenthesis after species names throughout this chapter. A minimum of five mature leaves were sampled per accession, and in taxa with more than one leaflet, central leaflets were studied. Leaflets were sampled from different plants, but for some taxa from the living collection there was limited material (between one and three plants per accession). Fresh leaflet material was fixed in Formalin-Acetic-Acid (FAA), dehydrated in an alcohol series and gradually infiltrated with and embedded in paraffin wax (Johansen, 1944). A rotary microtome (Leitz, Germany) was used to cut transverse sections that ranged in thickness between 10 and 12  $\mu\text{m}$ . Sections were stained using the Safranin-Alcian-blue (Bailey, 2001) differential staining methods and DPX glue was used to preserve these sections as permanent slides.

Leaf anatomical traits were studied using a Nikon ECLIPSE E400 light microscope and photographed using a Leica MC 170 HD camera and LAS CORE software (Leica, Switzerland). Digital images and permanent slides were used as reference material for comparative anatomical studies between the selected species. Additional hand sections were made from fresh leaf material to verify selected traits. A total of 68 leaf anatomical traits representing dermal, ground and vascular tissue were recorded (Appendix 1), of which 22 were qualitative (discrete) and 46 quantitative (continuous). To our knowledge, many of the discrete and continuous characters described in our study have not previously been assessed or described for *Oxalis* species. Continuous traits (*e.g.* mesophyll thickness) were measured to scale on images imported to ImageJ (Abràmoff *et al.*, 2004). The data presented in the study by dos Reis and Alvim (2013) on three South American *Oxalis* species were included in our analysis as discrete data for outgroup species. Trees derived from the nuclear Internal Transcribed Spacer region were used in this study, as ITS is more variable than plastid data and preliminary genome-level studies indicate that ITS-derived trees are more consistent with the species tree than

plastid-derived phylogenies (K.C. Oberlander, unpublished data). Trees were reconstructed using an expanded ITS data set from Oberlander *et al.* (2011), including sequences from a number of newly-collected taxa and extensive outgroup sampling of the entire genus *Oxalis* and family Oxalidaceae. Trees were generated in BEAST v.1.7.5 (Drummond *et al.*, 2012) using parameter settings for priors and Markov Chain Monte Carlo parameters as in Oberlander *et al.* (2009), with a normal prior of 56 million years (+/- 3.5 million years) on crown Oxalidaceae. The taxa corresponding to Clade 7 (Figure 2.1) were forced to monophyly, as many taxa in this clade have large deletions in ITS1 that negatively affect the resolution of this clade. However, both morphological characters (Salter, 1944) and preliminary genome-level data convincingly support these taxa as a monophyletic unit (K.C. Oberlander, *pers comm.*). Also, log likelihood and parameter values for unconstrained BEAST analyses were identical to clade-constrained analyses. Convergence of parameter values and trees on the same posterior was checked in Tracer v.1.5. A burnin of the first 25% of trees was removed, on a total of two independent runs of  $1 \times 10^7$  generations. All discrete and continuous traits were separately plotted on a sample of phylogenetic trees chosen at random from the posterior distribution. The tip MO numbers on the tree were replaced by our accessions of the same species (Addendum I). We present the results in this study on a single tree (Figure 2.1), although the patterns we observed were qualitatively similar on different trees. All analyses were performed in R (R-Core-Team, 2014) using the *ape* (Paradis *et al.*, 2004) and *geiger* (Harmon *et al.*, 2008) packages, as well as custom scripts to remove tree tips without data. For ease of reference to the study of Oberlander *et al.* (2011), we followed the same clade names and numbers in this study (Figure 2.1), except for the poorly characterised Clade 9, which is not retrieved in ITS data sets with greater taxon sampling and is not discussed further.

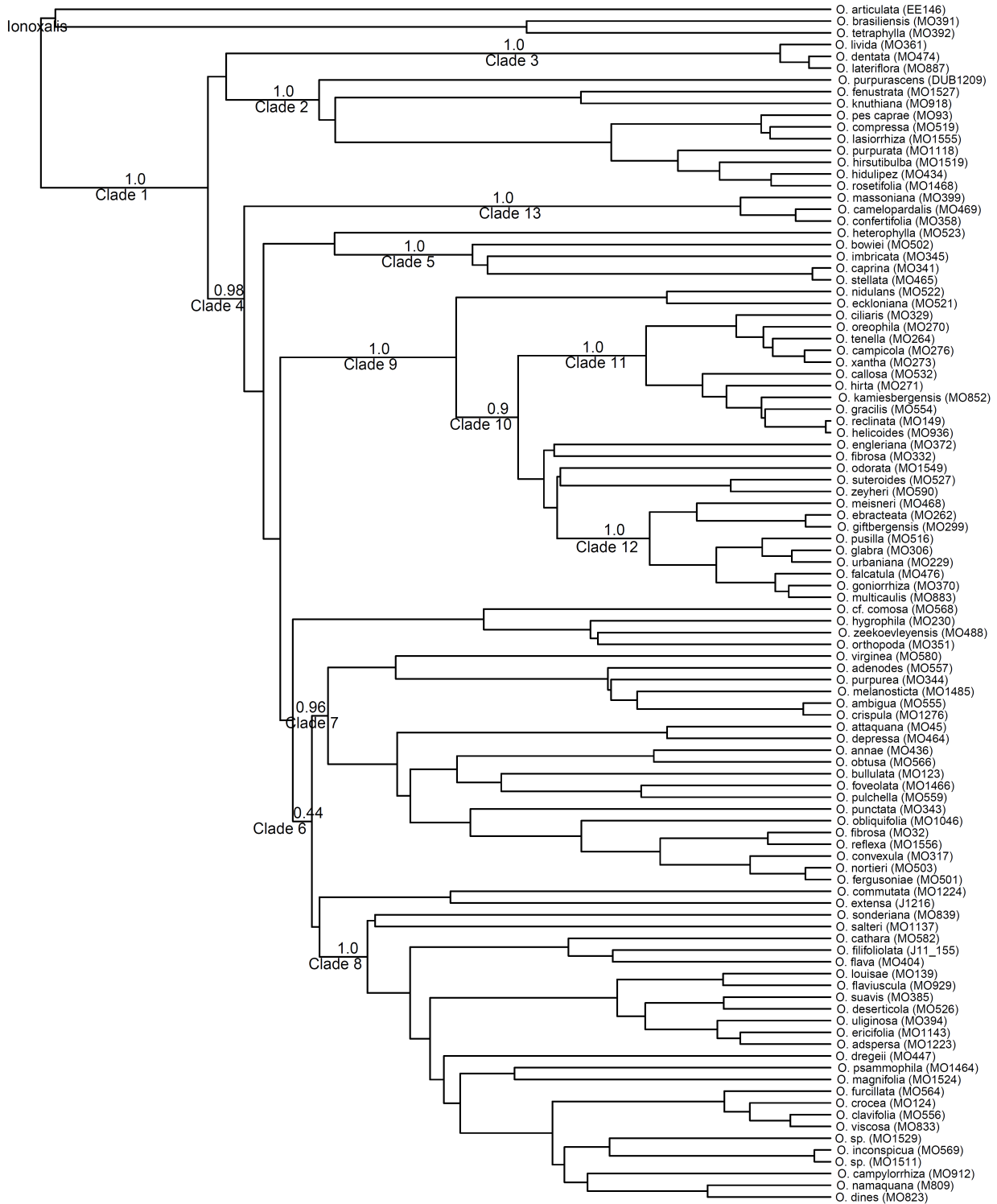


Figure 2.1: A randomly sampled nuclear ITS phylogenetic tree from BEAST, used to sample and study southern African *Oxalis* taxa, including the *Oxalis* clade numbers and names as described by Oberlander *et al.* (2011).



Table 2.1: The *Oxalis* clade numbers and names as described by Oberlander *et al.* (2011). Clades correspond to those mentioned in the text and figures throughout this study

Clade	Description
Clade 1	South African <i>Oxalis</i>
Clade 2	<i>O. pes-caprae</i> and relatives
Clade 3	Sect. <i>Cernuae</i> subsect. <i>Lividae</i>
Clade 4	Core South African <i>Oxalis</i>
Clade 5	<i>O. stellata</i> and relatives
Clade 6	Clade 6
Clade 7	<i>O. purpurea</i> and relatives
Clade 8	<i>O. flava</i> and relatives
Clade 9	Clade 9
Clade 10	Clade 10
Clade 11	<i>O. hirta</i> and relatives
Clade 12	<i>O. glabra</i> and relatives
Clade 13	Sect. <i>Angustatae</i> subsect. <i>Pardales</i>

### 2.2.1 Morphology

The plant architecture and internal structure of leaves in the genus *Oxalis* conforms to the vascular plant architecture as described by Esau (1953) and Mauseth (1988). *Oxalis* leaves can be divided into three main regions (Figure 2.2): a semi-amplexicaul basal region, a petiole and a lamina, which is divided into one to multiple leaflets, usually three (Salter (1944); Matthews and Endress (2002)). Articulations separate the three main regions of the leaf, which allow nastic movement of the petiole and leaflets (Salter (1944); Robb (1963); Levy and Moore (1993); Pedersen *et al.* (2006)). The leaf base often extends into several stipule-like structures that protrude beyond the basal articulation of the leaves of some *Oxalis* species (Salter (1944); Levy and Moore (1993)). The leaf base is variously succulent depending on the species, and the basal articulation between the leaf base and the terete part of the petiole often serves as an abscission zone (Salter (1944); Lourteig (2000)).

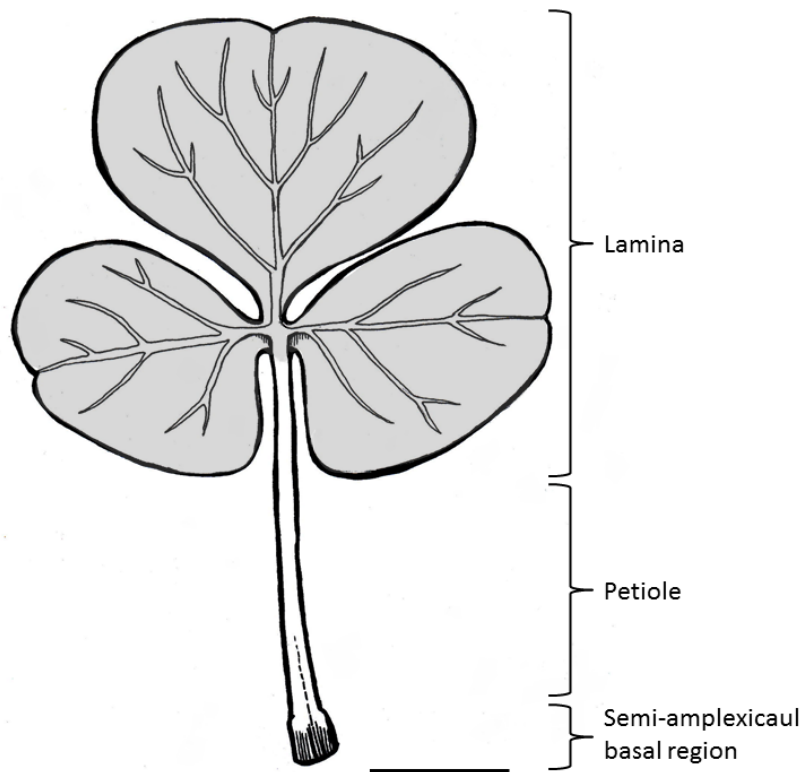


Figure 2.2: Line drawing depicting the three main regions of *Oxalis* leaves: the semi-amplexicaul basal region, the petiole and the lamina (may be divided into one to multiple leaflets). Scale bar represents 5 mm and the drawing is based on a dry leaf of *O. depressa* (MO506).

The leaves of all studied taxa were collected, pressed and oven-dried at 50°C (Appendix 3). The number of leaflets per leaf for all studied species were documented. Many *Oxalis* taxa have conduplicate leaflets (adaxial (AD) leaflet halves folded along the central vein to face each other, with the fold facing the stem and the abaxial (AB) sides of the leaflets facing outwards). We coded leaflets as conduplicate or flat and sought to measure the degree of conduplication using the angles of the leaflets relative to the central vein in ImageJ and photographs of hand-sections of fresh leaflet material. Flat leaflets had angles ranging from 111.5° to 180.0°, while conduplicate leaflets had angles ranging from 40.6° to 139.7°. This overlap between the angles of the flat and conduplicate leaflets is due primarily to locally increased folding around the central vein. The angle of the fold was measured only closely around the central vein. Some leaflets had slightly conduplicate leaflets in the area around the central vein, but the rest of the leaflet was flat; these leaves were grouped as conduplicate leaflets.

### 2.2.2 Stomata

Epidermal impressions were made to study the adaxial (AD) and abaxial (AB) leaflet surfaces of mature leaves collected from the living collection and the field by applying clear nail varnish to fresh leaflet material. The nail varnish layers were peeled off with clear cellotape and stuck onto microscope slides for photography. Ten measurements of stomatal central axis length, stomatal density, epidermal cell density and epidermal cell area were taken in random fields of view for each studied leaflet, and the data from five leaflets were used as average values to represent taxa. Stomatal size as a function of leaflet location (AD or AB) and stomatal density as a function of leaflet location

(AD or AB) were separately determined using a factorial ANOVA, with Tukey post-hoc tests in R (R-Core-Team, 2014). Stomatal central axis length (long axis of stomata) was measured instead of stomatal area, because stomata are dynamic structures that can open and close and this movement influences the short axis length (Willmer and Fricker, 1996), while the long axis length remains constant. Stomatal density and epidermal cell density was estimated by counting the number of cells per field of view at a fixed magnification of 40X times. Cell counts were then converted to cells per mm<sup>2</sup>.

### 2.2.3 Epidermal, mesophyll and vascular tissue measurements

Measurements of mesophyll layer dimensions were made from photographs of permanent slides in ImageJ (Abràmoff *et al.*, 2004). Ten measurements of AD epidermal height, palisade layer height, spongy layer height and AB epidermal height were made for each studied leaflet section (Figure 2.3), and average measurements of five leaflet sections were used as representative average values. These measurements were used to assess the relative layer heights of each tissue type. We must note that the angle at which the leaflets were cut with the microtome and the area of the leaflet that was sectioned could have contributed to variation in our data, although we standardized and strived to always cut perpendicularly into the middle-portion of leaflets. Comparisons of wax-embedded material with fresh material showed that on average, fixed mesophyll layer height was 32.4% smaller than fresh mesophyll layer height and that epidermal layer height was 19.0 % smaller than epidermal layer height. Due to this difference in size, only wax-embedded samples were used for measurements of internal mesophyll traits. The mesophyll dimensions we describe in this study therefore do not reflect the dimensions of fresh living material, but can still be used comparatively, as all leaflet material was processed and preserved following the same protocol. A custom script was used to plot mesophyll dimensions (including AD and AB epidermal cell heights to represent the entire leaflet section height) to corresponding tips on the phylogeny in R (R-Core-Team, 2014) and edited in Paint. We did not gather any continuous vascular tissue data (for example veins per leaf section or dimensions of vascular tissue) from our sections, as these traits were too dependant on the area of the leaf blade sectioned.

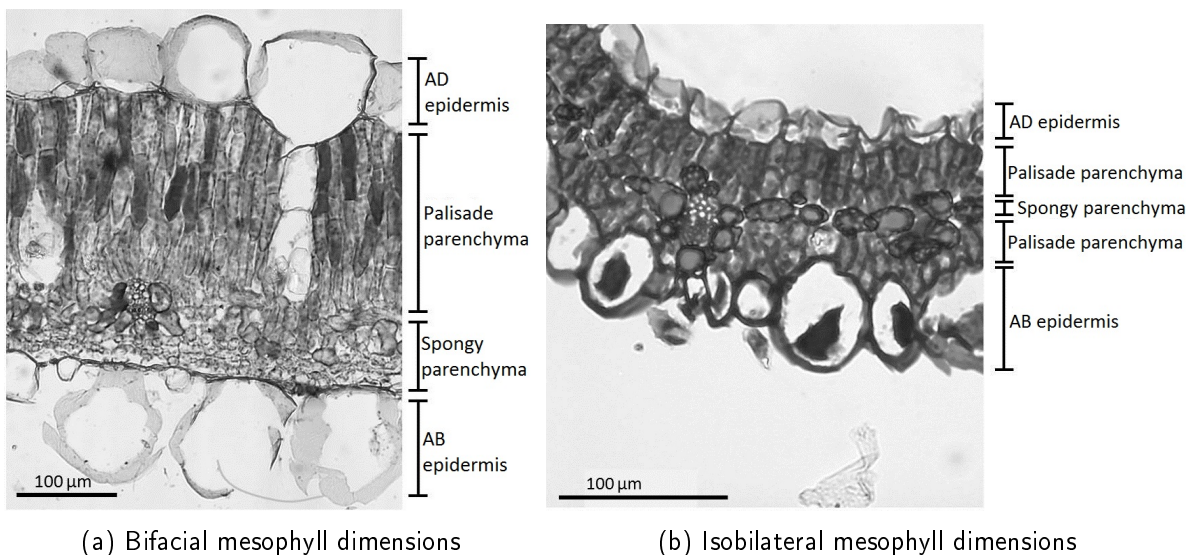


Figure 2.3: Light microscope photographs of wax-embedded and stained leaflet material illustrating the dimensions of leaflet tissues that were measured for bifacial and isobilateral mesophyll arrangement types observed in *Oxalis* taxa. (a) *O. pulchella* (MO559), (b) *O. ebracteata* (MO262).

## 2.3 Results: Leaflet morphology

*Oxalis* taxa have variable numbers of leaflets per leaf, ranging from one, to three (89.2% of studied taxa) up to 29 (Salter, 1944), but with a maximum of eleven leaflets observed in this study (Figure 2.4 and Addendum III). Taxa with three leaflets were by far the most commonly observed, and were distributed throughout the *Oxalis* phylogeny (Figure 2.5). Taxa with more than three leaflets per leaf were found within four clades namely: *lonoxalis* (*O. tetraphylla* MO392), Clade 12 (*O. zeyheri* MO590), Clade 10 (*O. engleriana* MO372) and Clade 8 (*O. ericifolia* MO1143, *O. flaviuscula* MO929, *O. flava* MO404, *O. filifoliolata* J11-155, *O. cathara* MO582). Taxa with single leaflets were located in two clades namely: Clade 7 (*O. nortieri* MO503) and Clade 8 (*O. dregei* MO447, *O. salteri* MO1137) (Figure 2.5).

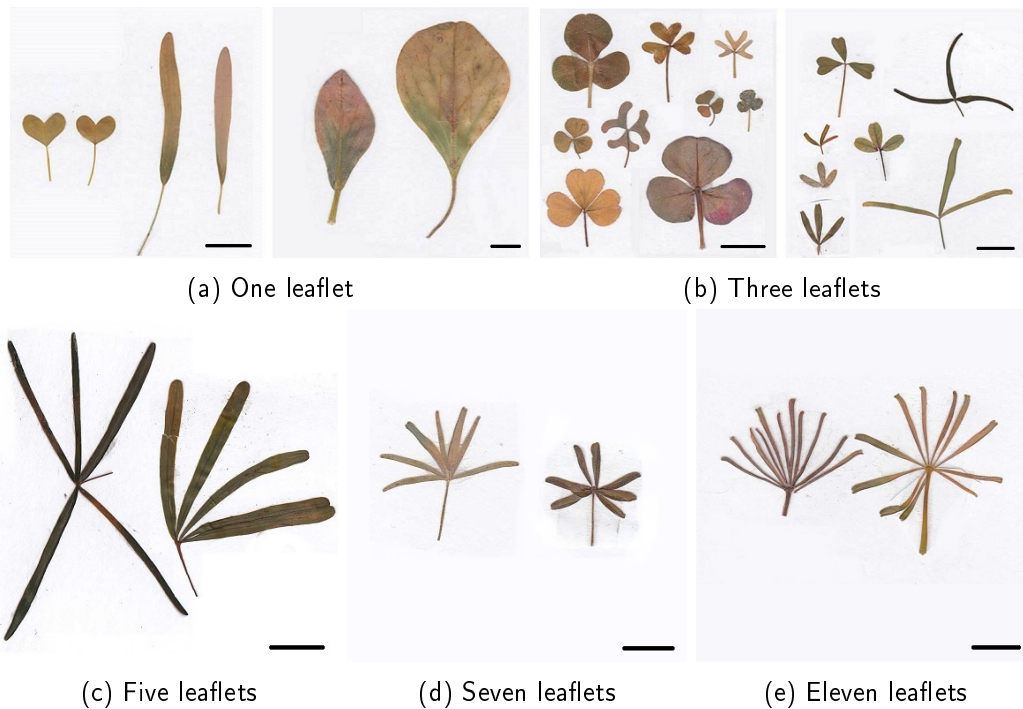


Figure 2.4: Photographs of pressed leaf material showing variability in number of leaflets per leaf in southern African *Oxalis* taxa. Species are listed from left to right, top to bottom within each image and each scale bar represents 1 cm. (a) *O. dregei* (MO398), *O. dregei* (MO398), *O. monophylla* (MO584), *O. monophylla* (MO584), *O. nortieri* (MO503), *O. monophylla* (MO1476), (b) *O. purpurea* (MO410), *O. fenestrata* (MO1527), *O. livida* (MO731), *O. sonderiana* (MO839), *O. attenuata* (MO45), *O. punctata* (MO343), *O. convexula* (MO368), *O. imbricata* (MO427), *O. depressa* (MO471), *O. dines* (MO823), *O. gracilis* (MO554), *O. glabra* (MO306), *O. oreophila* (MO505), *O. meisneri* (MO468), *O. confertifolia* (MO497), *O. deserticola* (MO1141), (c) *O. filifoliolata* (MO1490), *O. flaviuscula* (MO929), (d) *O. zeyheri* (MO590), *O. engleriana* (MO485), (e) *O. cathara* (MO582), *O. cathara* (MO582).

Flat and conduplicate leaflets were observed to be equally common throughout the studied taxa (50.0% each), but did show a phylogenetic pattern. Flat leaflets were observed in all members of the *lonoxalis* clade, Clade 2, Clade 7 and Clade 5 (Figure 2.6) and members from Clades 3, 11, 12 and 13 had conduplicate leaflets. Clade 8 had a random mixture of both flat and conduplicate leaflets.

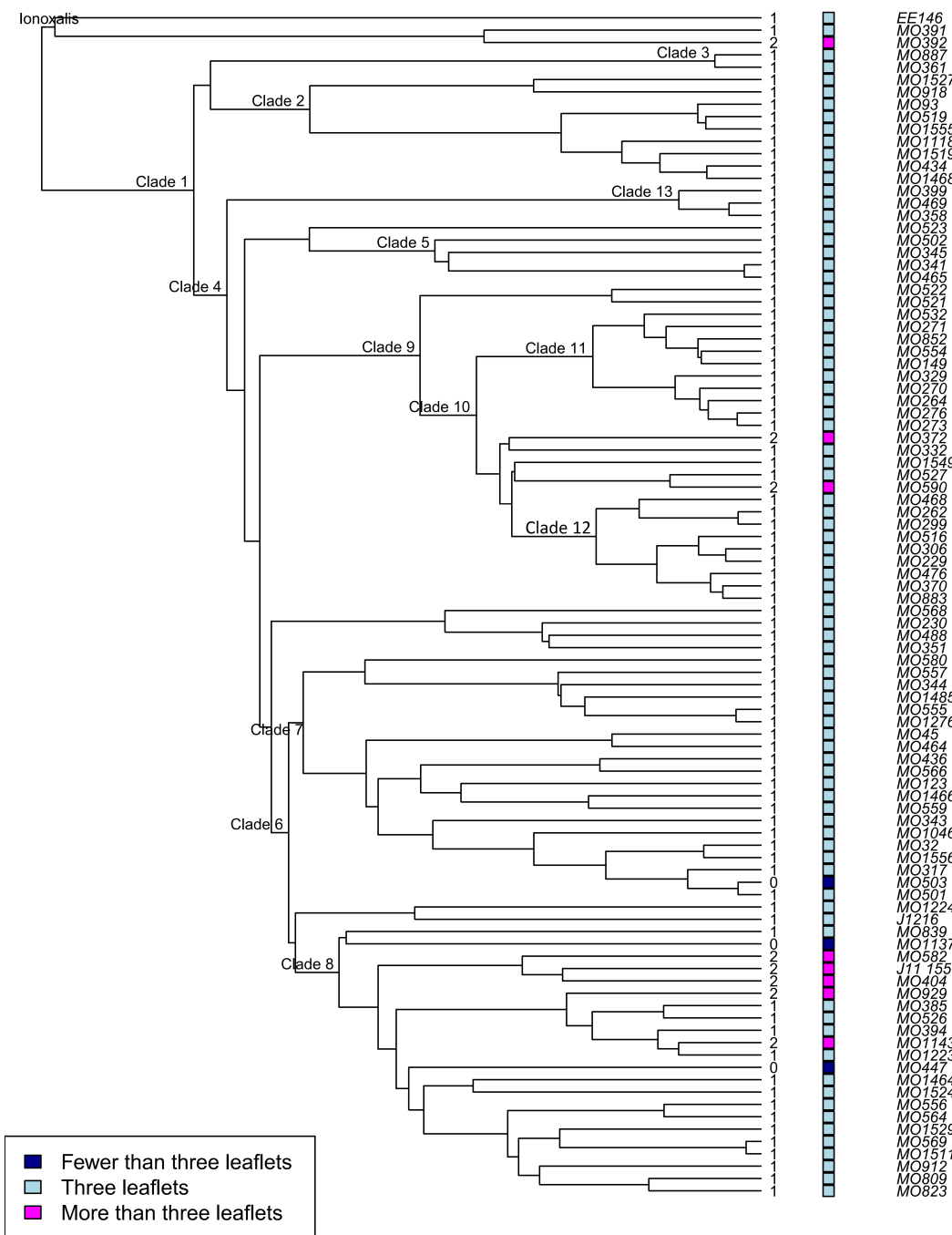


Figure 2.5: Phylogenetic distribution of southern African *Oxalis* taxa with less than three, three or more than three leaflets per leaf, using an ITS-based tree.

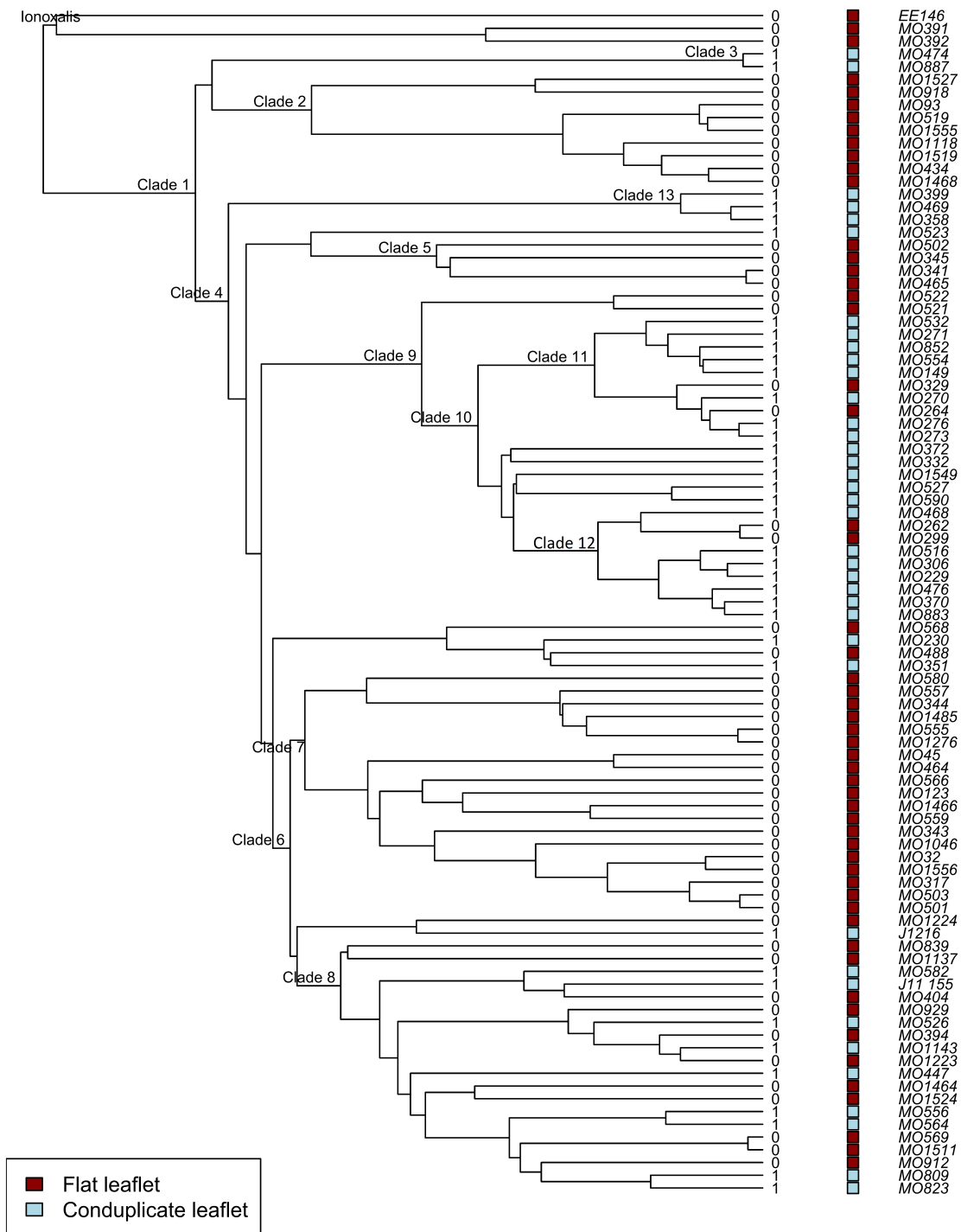


Figure 2.6: Phylogenetic distribution of flat and conduplicate leaflet types observed in southern African *Oxalis* taxa, using an ITS-based tree.

## 2.4 Results: Epidermal traits

Leaflet epidermal morphology and anatomy revealed significant variations in traits such as epidermal cell types, trichome types, ciliation of leaflets and attributes of the stomatal complex.

## 2.4.1 Epidermal pavement cells

Leaflet epidermal pavement cells displayed variable anticlinal and periclinal cell wall shapes. Four epidermal cell types were commonly observed and identified based on the anticlinal wall shape (as seen from above) and the periclinal wall shape (as seen from the side) of each cell. Epidermal pavement cells were classified as irregular, papillose, semi-swollen or swollen epidermal cell types (Figure 2.7).

Irregular epidermal cell types had anticlinal cell walls that varied in shape in a continuum from angular to sinuous shapes (Figures 2.7a, 2.7b and 2.7c). The periclinal walls were always parallel to the base of the epidermal cells (the point where the epidermal pavement cells and the guard cells of the stomata join), meaning that each irregular epidermal cell was uniform in depth (Figure 2.8a). All irregular cells present on a leaflet surface appeared to be relatively similar in shape and size. Papillose cells always had angular anticlinal cell walls with a small, but definite conical protrusion in the centre of the periclinal wall of each cell (Figures 2.7d, 2.7e, 2.7f and 2.8b). All papillose cells present on a leaflet appeared to be uniform in shape and size. The semi-swollen epidermal cell type was defined by a periclinal protrusion tapering regularly towards a point in the centre of each cell, causing each epidermal cell to appear conical in shape (Figure 2.8c). The anticlinal walls of semi-swollen epidermal cells were angular to sinuous in shape (Figures 2.7g, 2.7h and 2.7i). The conical-shaped cells were regularly interspersed by smaller and flatter epidermal cells and stomata. The swollen epidermal cell type was defined by large, rounded cells that appeared to be swollen beyond the average horizontal and vertical perimeters of a 'typical' epidermal cell. When seen from above, the anticlinal cell walls varied from a rounded angular shape to a sphere (Figures 2.7j, 2.7k and 2.7l). When seen from the side, the periclinal wall was completely domed and the base of the epidermal pavement cells was sunken into the mesophyll tissue, below the point where the epidermal pavement cells and the guard cells of the stomata join (Figure 2.8d). The swollen cells were irregularly interspersed by small epidermal cells and stomata.

Only irregular, papillose and swollen epidermal cell types occurred on the adaxial (AD) leaflet surfaces of the studied taxa. Only irregular, semi-swollen and swollen epidermal cell types were observed on the abaxial (AB) leaflet surfaces. Papillose epidermal cells were found only on the AD leaflet surfaces and semi-swollen epidermal cells were found only on the AB leaflet surfaces. Papillose and semi-swollen epidermal cell types occurred in combination with irregular epidermal cell types on the opposite leaflet surfaces. Another important observation was that all taxa with papillose epidermal cells on the AD surface were epistomatic and all taxa with semi-swollen epidermal cells were hypostomatic. The irregular epidermal cells were the most common cell type found on both the AD (65.4%) and AB (85.2%) leaflet surfaces. Irregular cell type on one leaflet surface were seen in combination with papillose and semi-swollen epidermal cell types on the other leaflet surface. Swollen cells always occurred on the AD and AB surfaces of the same leaflet. The heights of the AD and AB epidermal cells were extremely variable in all epidermal cell types and ranged over an order of magnitude (Figure 2.9). The average epidermal cell heights, for the combined data of all epidermal cell types, showed that the AB epidermal cells were significantly thicker than AD epidermal cells ( $t = -4.524$ ,  $df = 133.94$ ,  $p < 0.001$ ). The AB cells of irregular cell types were significantly higher than AD cells ( $t = -4.135$ ,  $df = 92.18$ ,  $p < 0.001$ ), but the AD and AD cells of swollen epidermal cell types were similar in height ( $t = -48.60$ ,  $df = 15.91$ ,  $p > 0.05$ ).

Swollen epidermal cells were observed only in Clade 7 (14 out of 21 taxa) rendering this trait synapomorphic for a sub-clade of Clade 7 (Figure 2.10). Semi-swollen AB epidermal cell types were observed in the *Ionoxalis* clade, Clade 2 and Clade 3 (Figure 2.11). Taxa with papillose type AD cells and taxa with irregular AD and AB epidermal cells were scattered throughout the phylogeny (Figures 2.10 and 2.11). The average AD and AB epidermal cell heights were variable and did not

show any phylogenetic pattern (Addendum II). Taxa from Clades 3 and 7 had relatively large AD epidermal cell heights and taxa from Clades 5, 8, 11 and 12 all had relatively smaller AD epidermal cell heights.

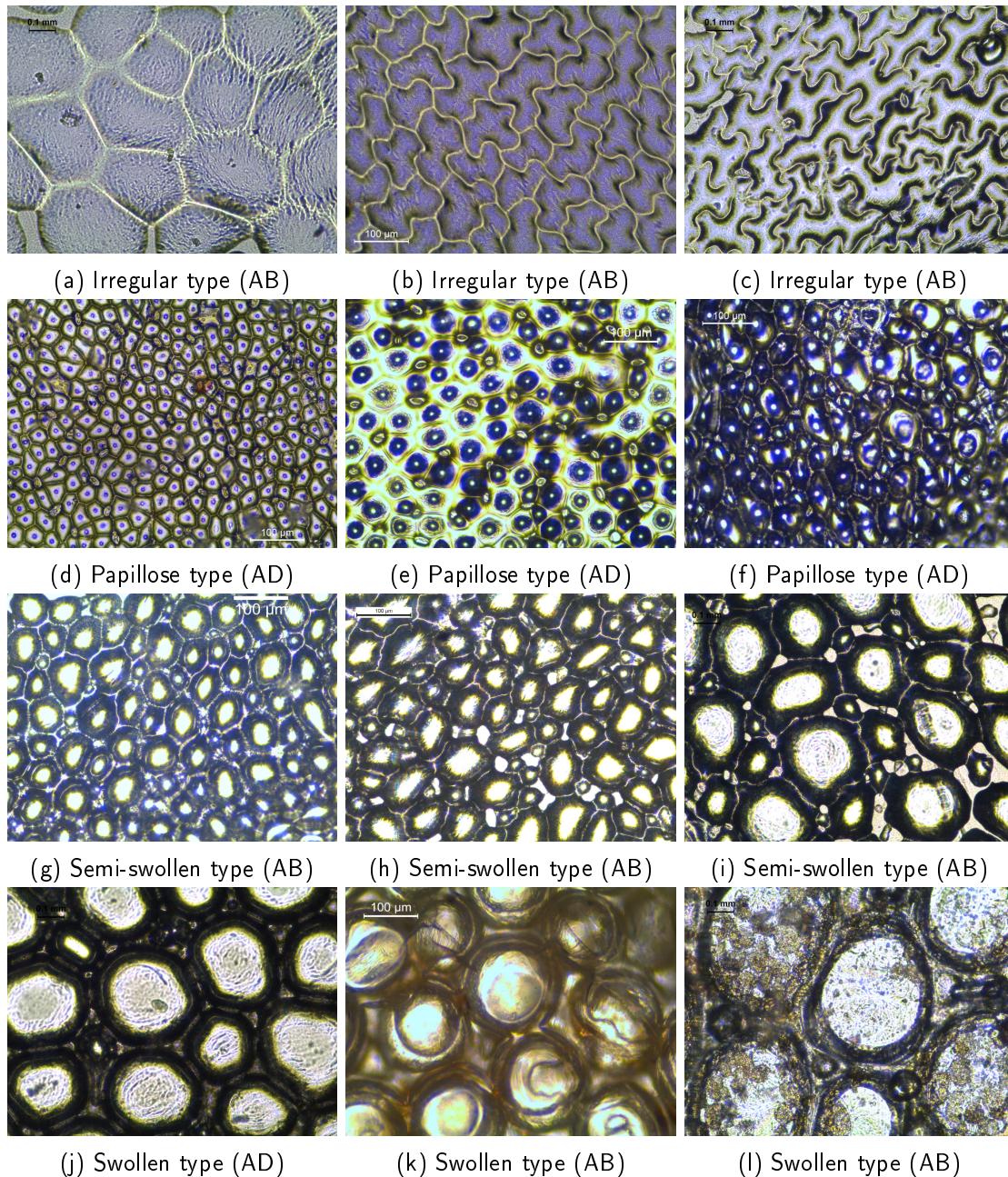


Figure 2.7: Light microscope photographs of epidermal peels depicting irregular, papillose, semi-swollen and swollen epidermal pavement cell types observed in southern African *Oxalis* taxa. (a) "*O. magnifolia*" (MO1524), (b) *O. uliginosa* (MO394), (c) *O. fenestrata* (MO1527), (d) *O. inconspicua* (MO569), (e) *O. monophylla* (MO584), (f) *O. goniorrhiza* (MO370), (g) *O. lateriflora* (MO887), (h) *O. livida* (MO361), (i) *O. pes-caprae* (MO849), (j) *O. pulchella* (MO559), (k) *O. foveolata* (MO1466), (l) *O. bullulata* (MO123).



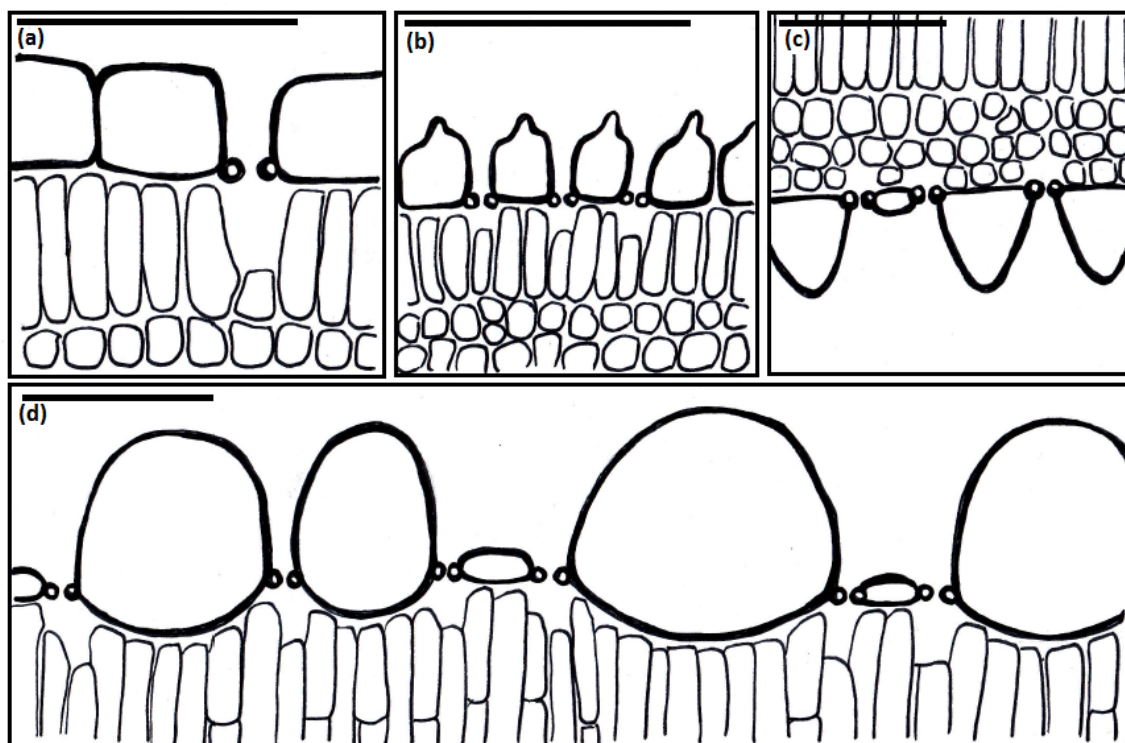


Figure 2.8: Line drawings of transverse sectioned leaflet material depicting the four different epidermal pavement cell types observed in southern African *Oxalis* species. Scale bars represent 100  $\mu\text{m}$ . (a) Irregular type, (b) Papillose type, (c) Semi-swollen type, (d) Swollen type.

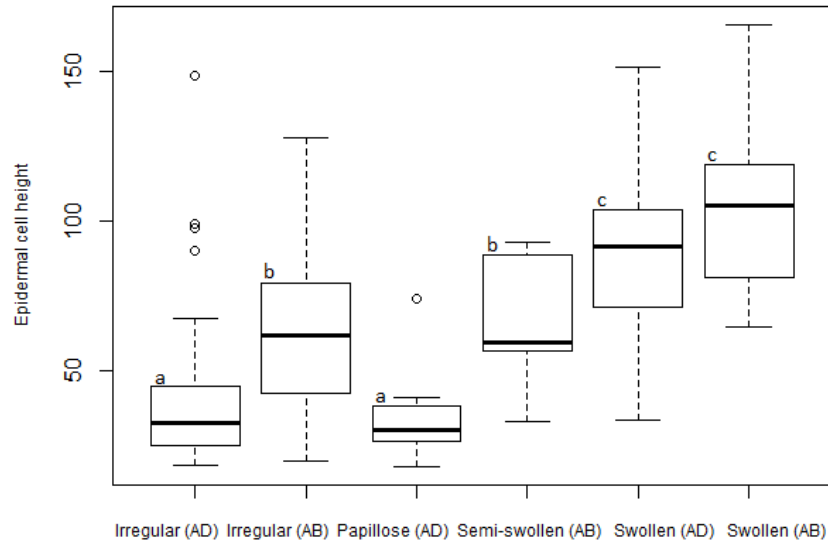


Figure 2.9: A boxplot indicating the AD and/or AB epidermal cell heights ( $\mu\text{m}$ ) of irregular, papillose and semi-swollen and swollen epidermal cell types in southern African *Oxalis* taxa. The symbols a and b were significantly different at  $p < 0.001$ , and b and c were significantly different at  $p < 0.01$ . A black line represents the median, a box represents the first and third quartiles, the whiskers represent the 95% confidence intervals and the circles represent outliers.

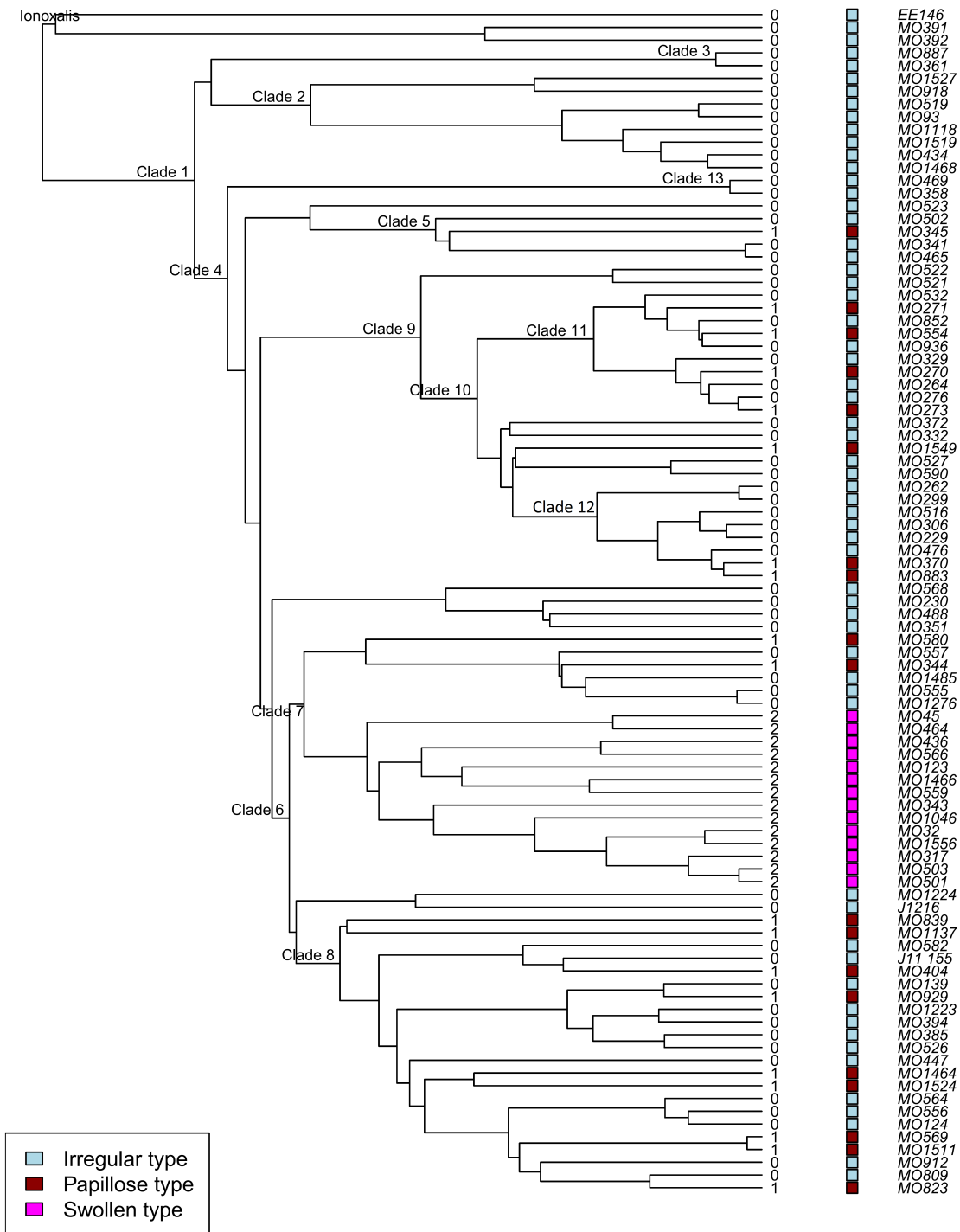


Figure 2.10: The phylogenetic distribution of adaxial epidermal cell types in southern African *Oxalis* taxa, using an ITS-based tree.

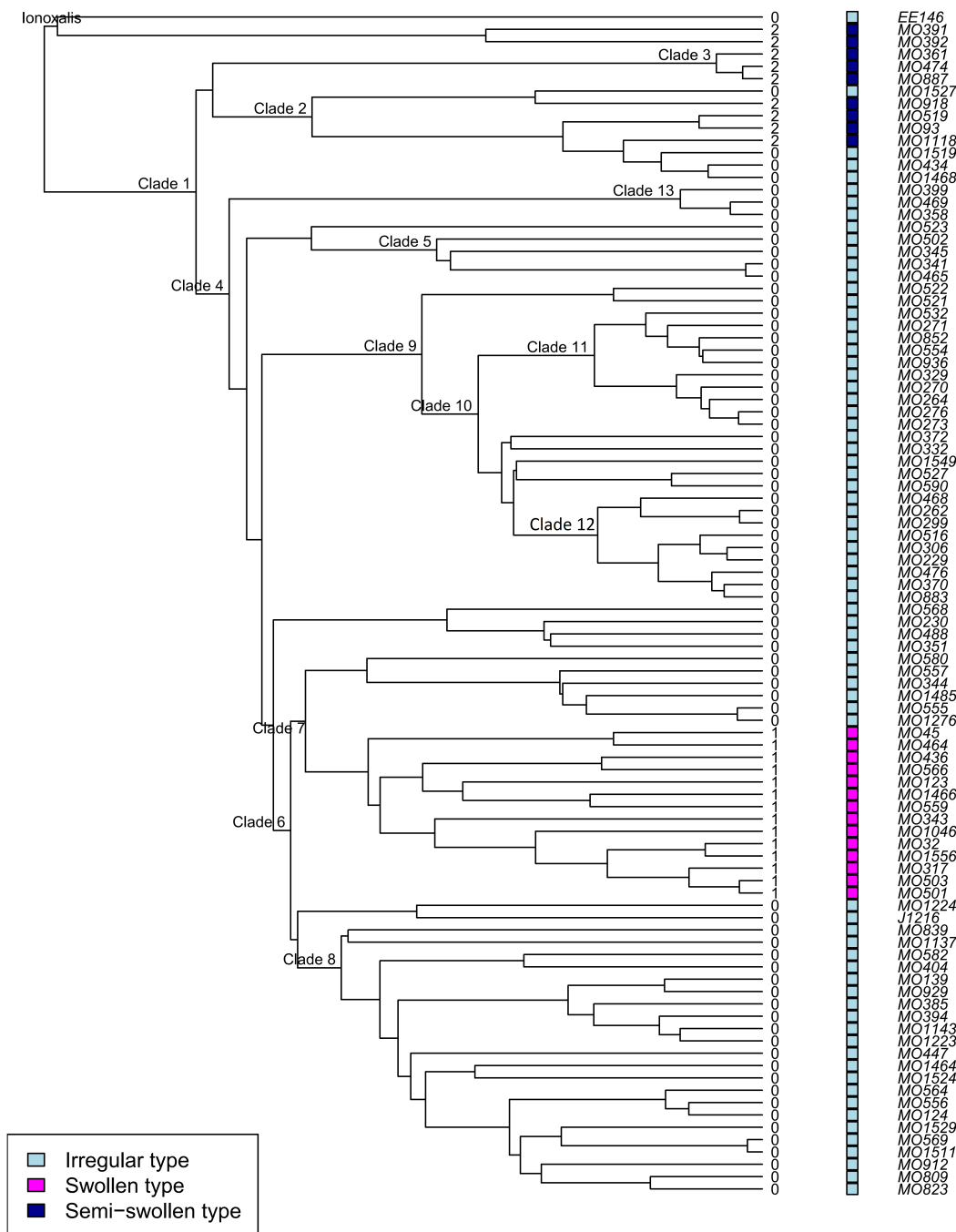


Figure 2.11: The phylogenetic distribution of adaxial epidermal cell types in southern African *Oxalis* taxa, using an ITS-based tree.

More than 80% of the studied taxa displayed an interesting phenomenon of having elongated AB epidermal cells above the central vein of each leaflet, in parallel with the central vein (Figure 2.12). Elongated epidermal cells were rectangular in shape, ranging from long and thin cells (average dimensions: 161.15  $\mu\text{m}$  by 15.24  $\mu\text{m}$ ) to short and wide (average dimensions: 94.57  $\mu\text{m}$  by 32.33  $\mu\text{m}$ ). Elongated epidermal cells were found in taxa with irregular (92.2%) and swollen AB epidermal cell types. Elongated epidermal cells were found in both flat and conduplicate leaflets, co-occurred with protruding and non-protruding veins (Figure 2.13) at almost equal frequency (37.0% vs. 40.9%), and co-occurred with all three epidermal cell types. Therefore there was no detectable association

between elongated AB epidermal cells and protruding veins, epidermal cell type or leaf conduplication to explain this observed phenomenon.

As the majority of studied taxa did have elongated AB vein-associated epidermal cells, taxa without elongated AB epidermal cells were of greater interest (in a phylogenetic sense). Taxa without AB elongated epidermal cells occurred in Clades 2, 11, 12 and the *lonoxalis* clade, and 12 out of 20 (60.0%) studied taxa from Clade 7 did not have this trait (Figure 2.14).

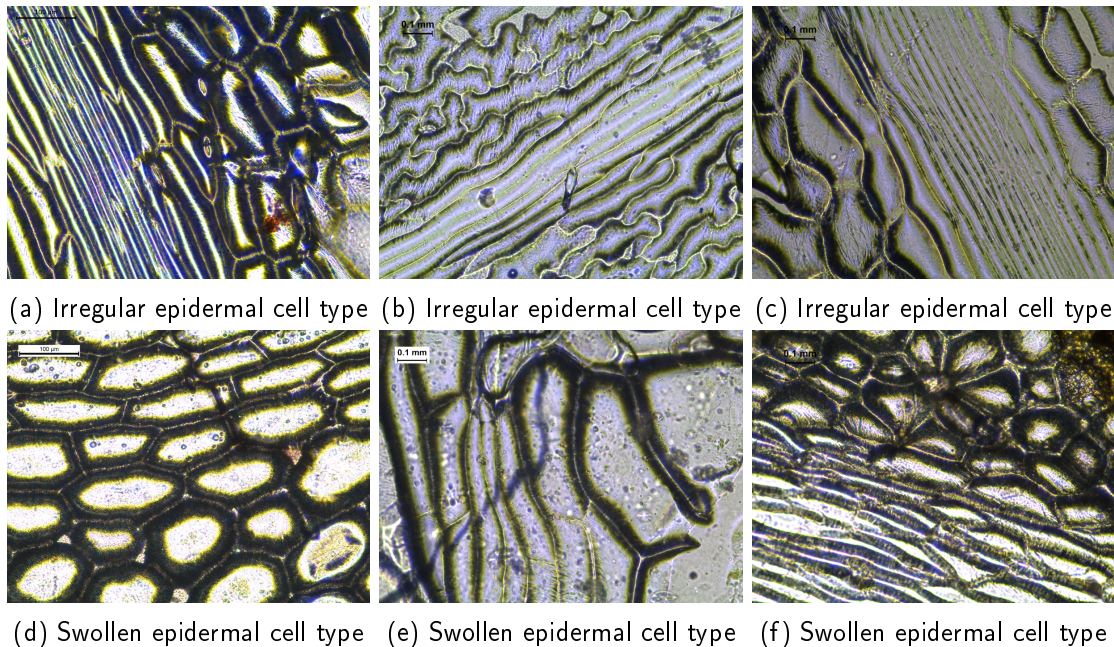


Figure 2.12: Light microscope photographs of epidermal peels indicating vein-associated elongated AB epidermal cells for taxa with irregular and swollen AB epidermal cell types. (a) *O. extensa* (J1216), (b) *O. fenestrata* (MO1527), (c) *O. ericifolia* (MO1143), (d) *O. pocockiae* (MO562), (e) *O. bullulata* (MO123), (f) *O. obtusa* (MO556).

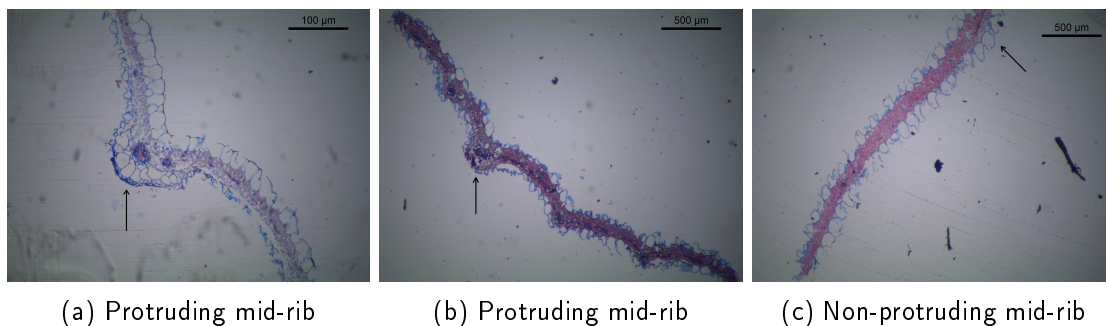


Figure 2.13: Light microscope photographs of hand-sections of fresh leaflet material depicting the protruding and non-protruding mid-rib types observed in *Oxalis* taxa. The main vein vascular tissue is indicated with an arrow in each image. (a) *O. adspersa* (MO1223), (b) *O. obtusa* (MO566), (c) *O. convexula* (MO368).

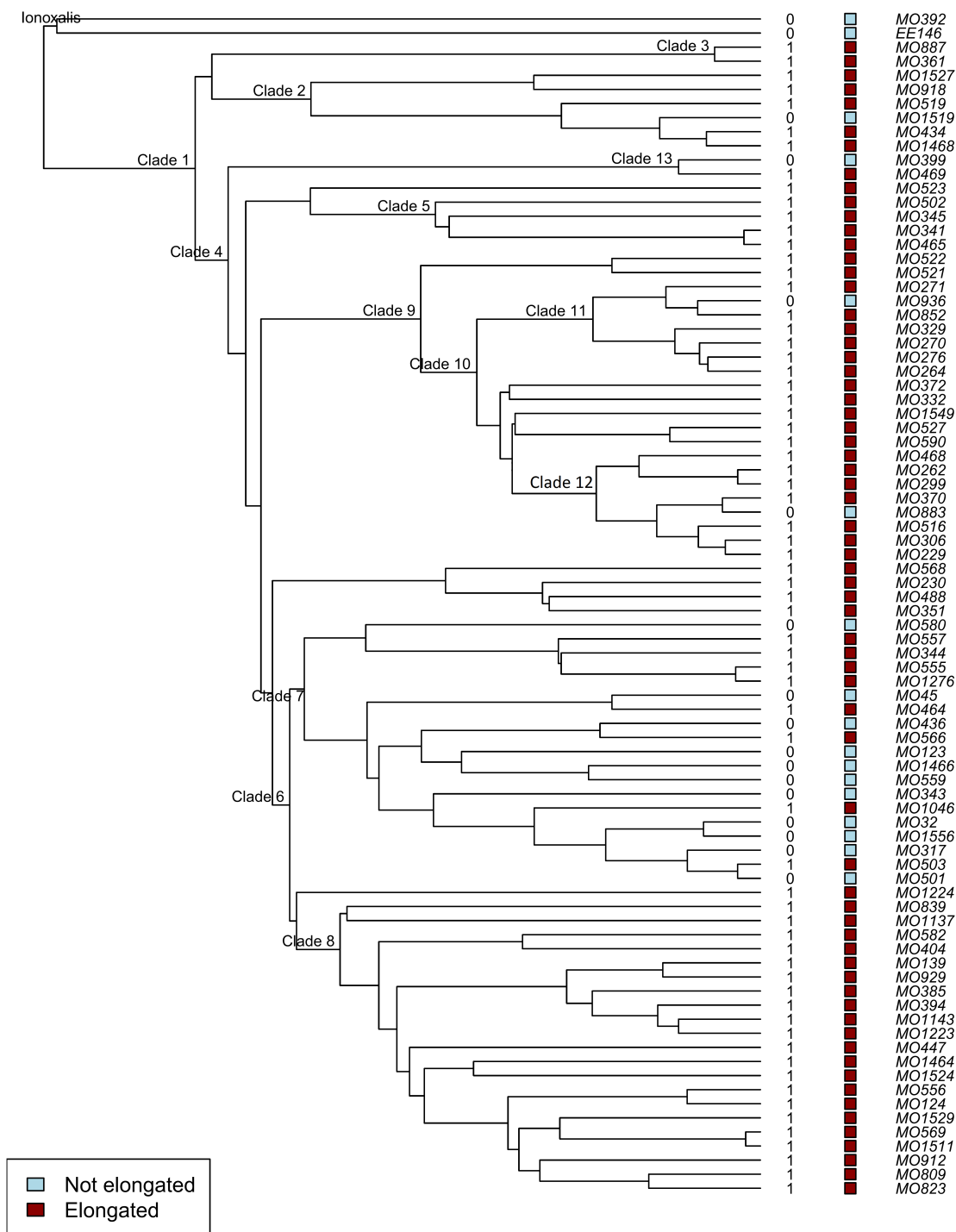


Figure 2.14: The phylogenetic distribution of elongated and non-elongated vein-associated AB epidermal cell types found in southern African *Oxalis* taxa, using an ITS-based tree.

## 2.4.2 Trichomes

Two main types of trichomes were observed among *Oxalis* taxa, namely glandular- or non-glandular hairs, depending on the presence or absence of swollen secretory cells at the tip of the trichome. Two subtypes of glandular and five subtypes of non-glandular hairs were recognised (Figure 2.15). Non-glandular hairs were more common (78.7% across taxa) than glandular hairs. The leaflet epidermal

surfaces of only 29 out of 110 studied *Oxalis* species (26.4%) were glabrous *i.e.* completely free of trichomes, while the other taxa had at least one type of trichome present. Trichomes were most commonly observed on the AB leaflet surface (54.5% across taxa). Taxa with trichomes on both surfaces were less common (18.2%) and only one species (*O. foveolata* MO1466) had trichomes solely present on the AD leaflet surface (Figure 2.15). Considerable variation of trichome traits was observed between individuals from the same species, when specimens from the botanical gardens and the field were compared. Intra-specific variation included presence and absence of trichomes, glandular hair type, non-glandular hair type and the distribution of trichomes on AD, AB or both leaflet surfaces (Addendum I).

### 2.4.2.1 Non-glandular hairs

Five types of non-glandular hairs were observed: short (<150  $\mu\text{m}$ ) uni-cellular hairs (Figure 2.16a), inflated uni-cellular hairs (Figure 2.16b), long (>150  $\mu\text{m}$ ) uni-cellular hairs (Figure 2.16c), long multi-cellular hairs (Figure 2.16d) and clavate multi-cellular hairs (Figure 2.16e). Clavate hairs were differentiated from glandular hair types by the shape of the terminal cell, which was irregularly teardrop-shaped instead of globular as in glandular hairs. Long hairs and clavate hairs were the most common non-glandular hair type among studied taxa (41.3% each). Different combinations of non-glandular hair types were observed. The most common non-glandular hair association was between long hairs and clavate hairs (66.7%). Other combinations all included clavate hairs in association with either short hairs (21.2%), inflated hairs (6.1%) or multi-cellular (3.0%) hairs. Taxa with short-unicellular hairs occurred in Clades 2, 7, 11 and 12 (Addendum II), taxa with inflated single cellular hairs were observed in Clades 2, 3, 7, 8 and 12 (Addendum II), taxa with long single-cellular hairs were observed in all *Oxalis* clades and taxa with long multicellular hairs were observed in one taxon from Clade 7 and three taxa from Clade 8. The type of non-glandular epidermal hair did not appear to show any phylogenetic pattern (Figure 2.17), except that clavate multicellular hairs were absent from the leaflets of all but two species (*O. campylorrhiza* (MO912) and *O. namaquana* (MO809)) in Clade 8 (Figure 2.18). With four exceptions (*O. livida* (MO361) from Clade 3, *O. helicoides* (MO936) from Clade 11, *O. pusilla* (MO516) from Clade 12, *O. tetraphylla* (MO392) from *lonoxalis*), glabrous leaflets were confined to members from Clade 6.

### 2.4.2.2 Glandular hairs

Two types of glandular hairs were observed. Short-stalked glandular hairs (Figure 2.16f) consisted of a round, single-celled head attached to a short, single-celled stalk, whereas long-stalked glandular hairs (Figure 2.16g) had stalks comprised of two or more elongated cells. Long-stalked glandular hairs were observed in 60.0% of studied taxa and were more common than short-stalked glandular hairs. Glandular hairs were observed on the AD leaf surface (6.5%), both the AD and AB surfaces (6.5%), but most commonly on the AB surface of studied taxa (85.7%). The majority of the glandular hairs that were found on the AB surface were seen only directly below the central vascular bundle, following the total length of the central vein of each leaflet. The majority of taxa from Clade 8 had glandular hairs present on their leaflets, but the distribution of glandular-hair types on the phylogeny did not typify individual clades (Figure 2.17).

*Oxalis* trichome traits were extremely variable within and between species and none of these above mentioned trichome traits were synapomorphic to any *Oxalis* clades (Figures 2.15 and 2.17). Within a few clades, the majority of taxa shared the same traits, for example eight out of ten members of Clade 11 all had trichomes located on their AD leaflet surfaces; exceptions were *O. campicola* (MO276) with AB non-glandular hairs and *O. helicoides* (MO936) with no trichomes on their leaflets (Figure 2.15) and in Clade 8, 14 out of 24 taxa all had AD located glandular hairs (Figure 2.15 and

2.17). All taxa from Clade 12 had AD located trichomes (Figure 2.15). The majority of taxa from Clades 2 and 9 had AD located non-glandular hairs (Figure 2.15 and 2.17).

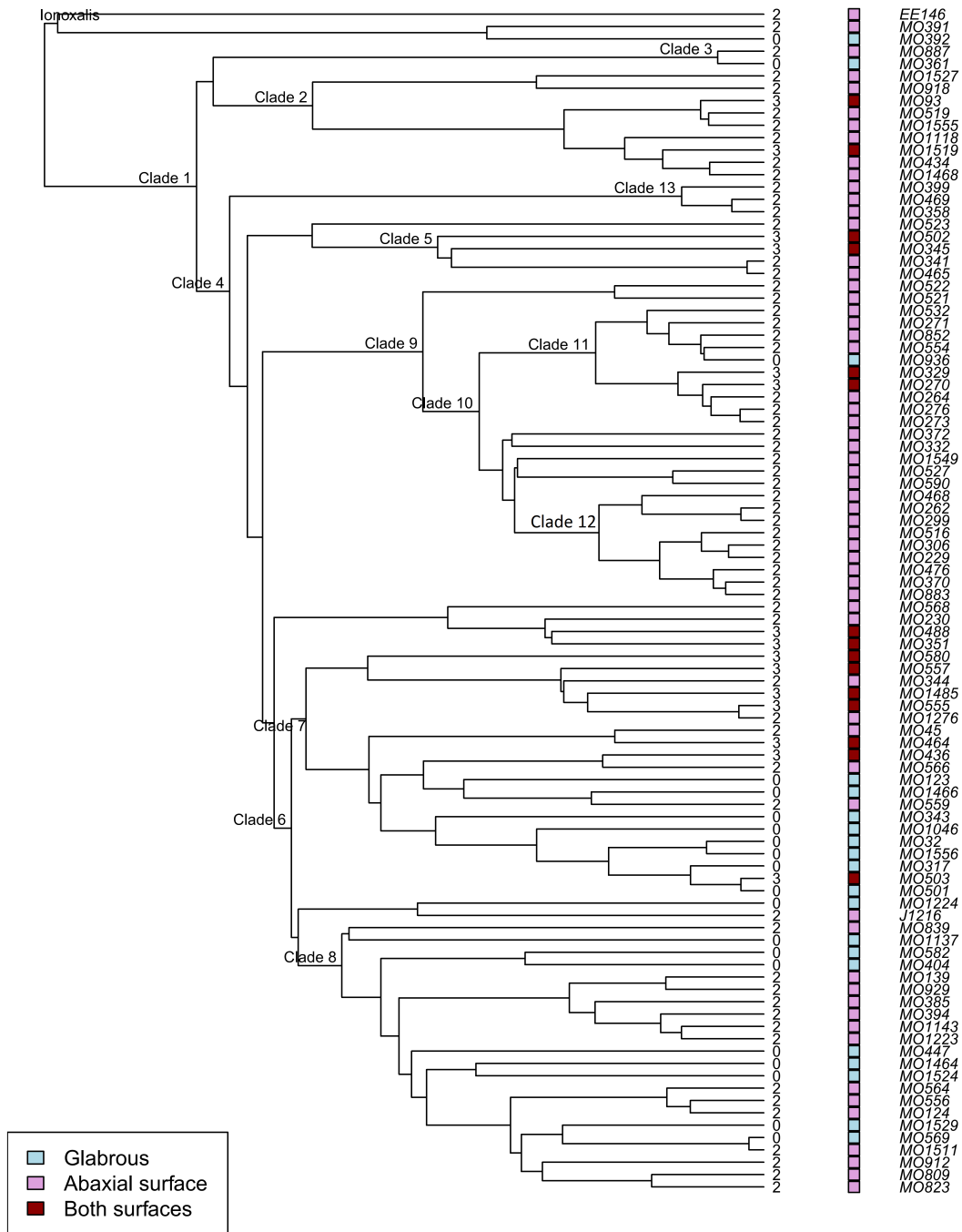


Figure 2.15: The phylogenetic distribution of trichome location (AD or AB surfaces) on leaflets of southern African *Oxalis* taxa, using an ITS-based tree.



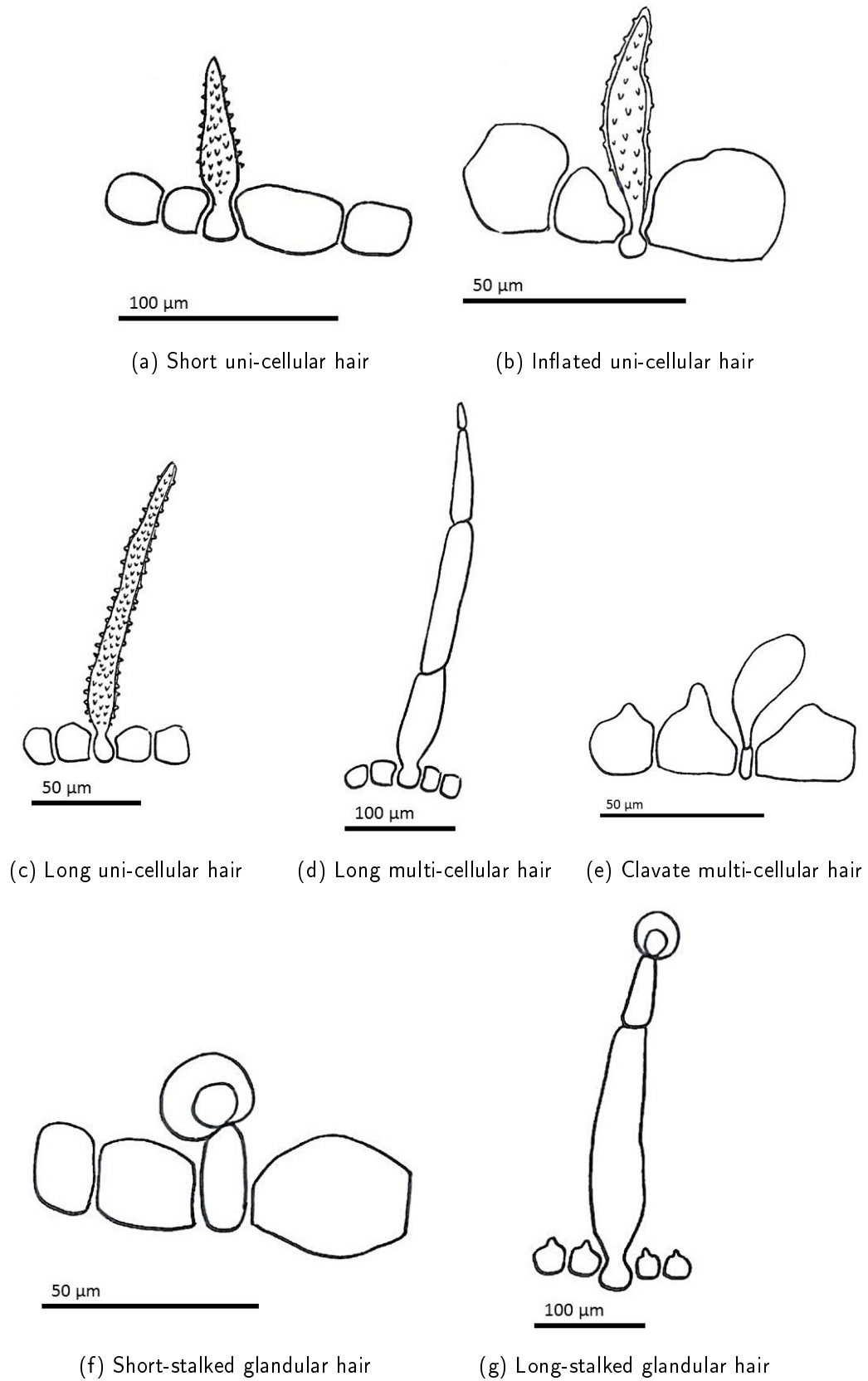


Figure 2.16: Line drawings of trichome types identified on AD and AB leaflet surfaces in the studied *Oxalis* taxa.

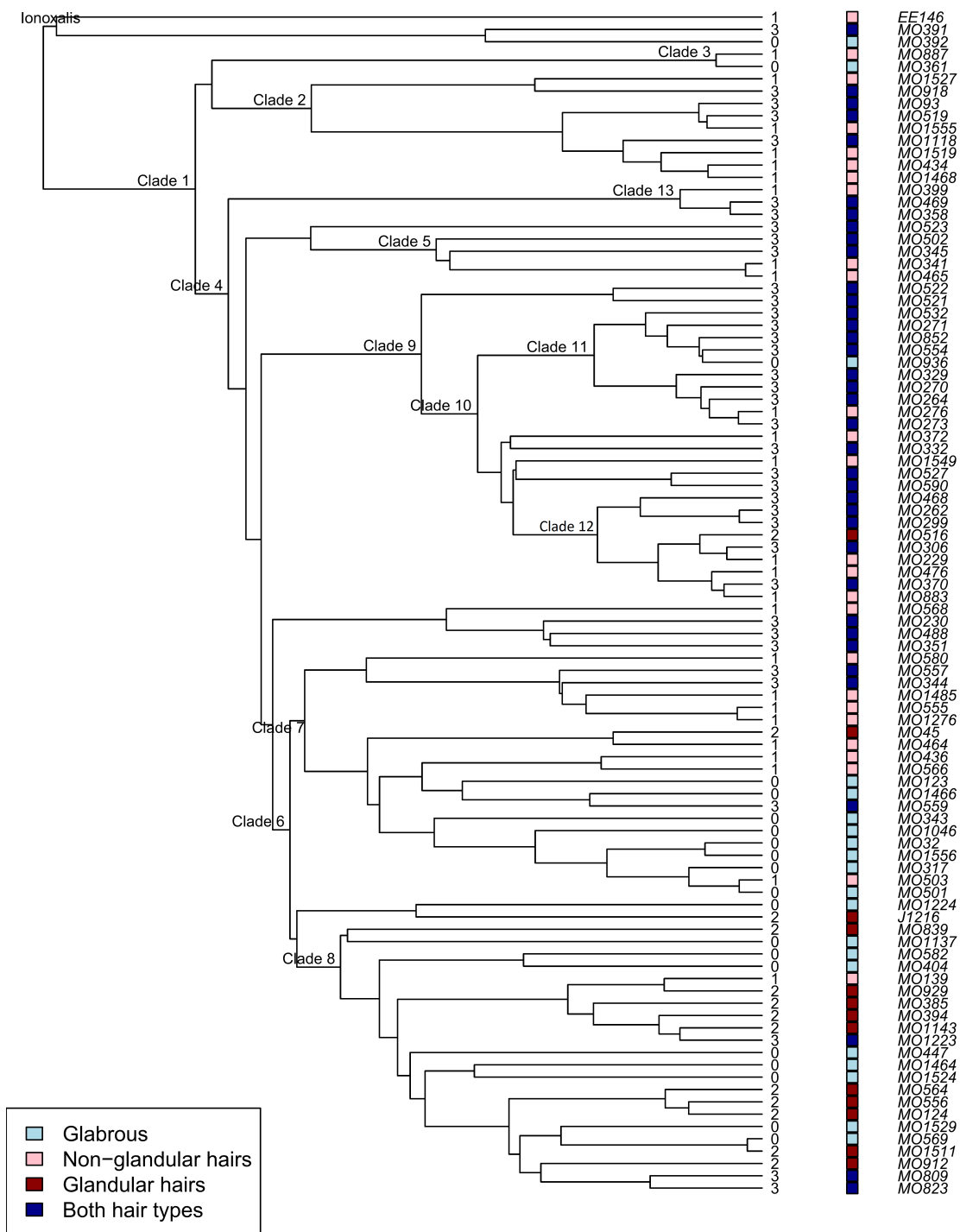


Figure 2.17: The phylogenetic distribution of trichome types (glandular hairs and non-glandular hairs) observed in southern African *Oxalis* taxa, using an ITS-based tree.

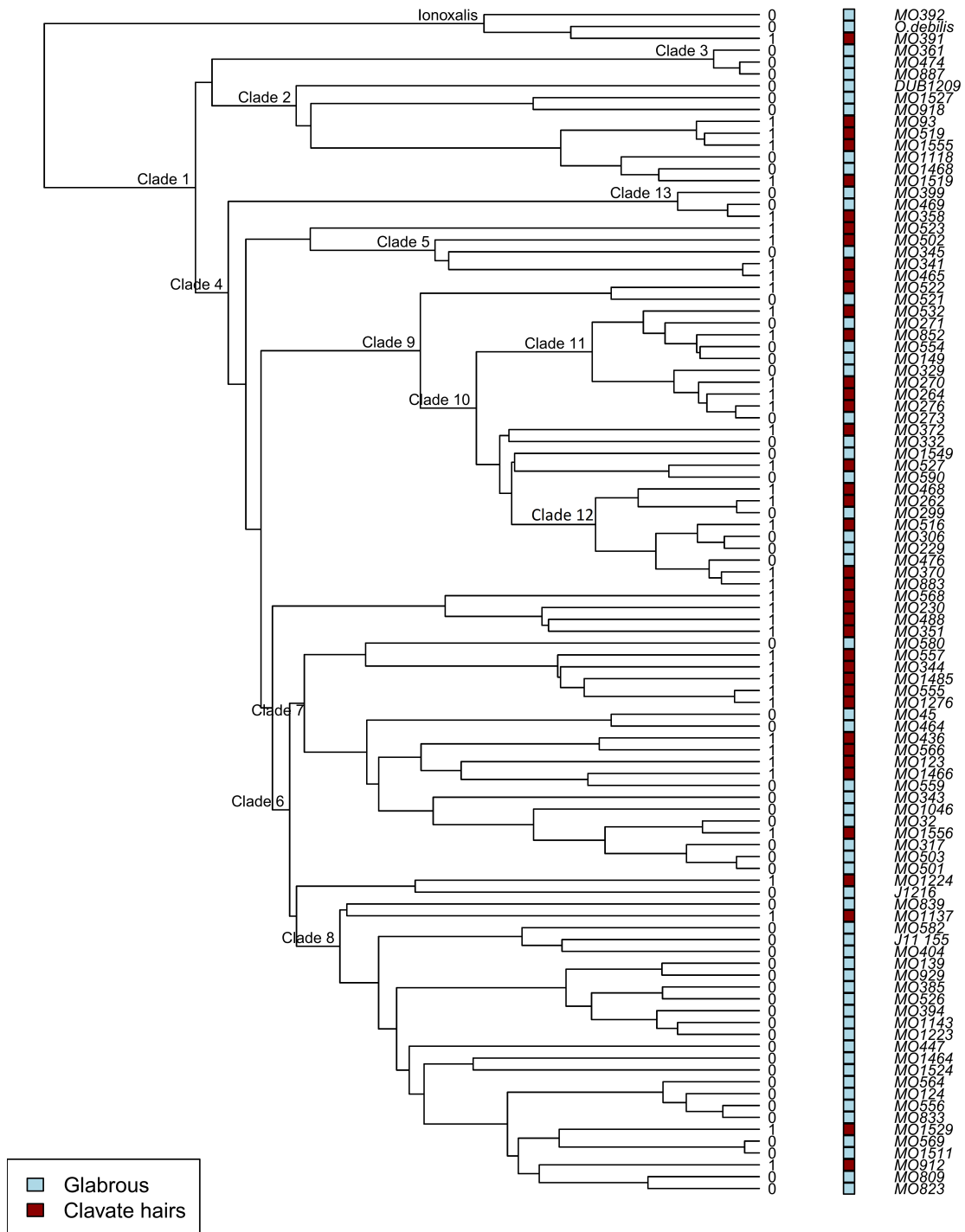


Figure 2.18: The phylogenetic distribution of the presence or absence of clavate multi-cellular hairs located on AD and AB leaflet surfaces of southern African *Oxalis* taxa, using an ITS-based tree.

### 2.4.2.3 Leaflet ciliation

The margins of leaflets were observed to be glabrous, simple hair-ciliate (non-glandular hairs) or glandular hair-ciliate. These ciliation types were equally common, as a third of the studied taxa displayed each of the described ciliation-types. The ciliation types did not show any phylogenetic

pattern, but small clusters of taxa (three to five closely related species) within a clade often shared ciliation types throughout the *Oxalis* phylogeny (Figure 2.19).



Figure 2.19: The phylogenetic distribution of glandular hair and non-glandular hair ciliation types observed on the leaflets of southern African *Oxalis* taxa, using an ITS-based tree.

### 2.4.3 Stomata

Stomatal traits could not be observed for five studied taxa due to extremely dense trichome cover (mostly simple hairs) or swollen epidermal cells that obscured the surrounding stomata (*O. confertifolia* (MO358), *O. dentata* (MO474), *O. hirsuta* (MO586), *O. lasiorrhiza* (MO1555) and *O. pulchella* (MO369)). The transverse leaf sections were used to gather data for these samples e.g. stomatal position could be detected from the sections, but not stomatal lengths or densities. Three known types of stomatal complexes were observed in *Oxalis* (definitions adapted from Radford *et al.* (1974)), namely anomocytic (stoma surrounded by cells that are indistinguishable in size and shape from other epidermal cells (Figure 2.20a); anisocytic (stoma surrounded by three cells, with one cell much smaller than the other two (Figure 2.20b) and actinocytic (stoma completely surrounded by a circle of five or more radially elongated cells (Figure 2.20d). A fourth arrangement type, similar to the anisocytic type, but comprising of three large cells and one small one, was also very commonly encountered, and will be referred to as the 4-celled anisocytic stomatal type (Figure 2.20c). An anomocytic arrangement was the most common type of stomatal complex as it occurred in 96 out of 101 studied taxa (95.0%), followed by 4-celled anisocytic, anisocytic and then actinocytic types. Interestingly, all but one taxon (*O. deserticola* (MO526)) had more than one type of stomatal complex per leaflet surface and ten combinations of stomatal complexes were observed (Addendum I). The combination of anomocytic, 4-celled anisocytic and actinocytic types (15.8%) and anomocytic and 4-celled anisocytic types (11.9%) were the most common of the ten combinations of co-occurring stomatal types.

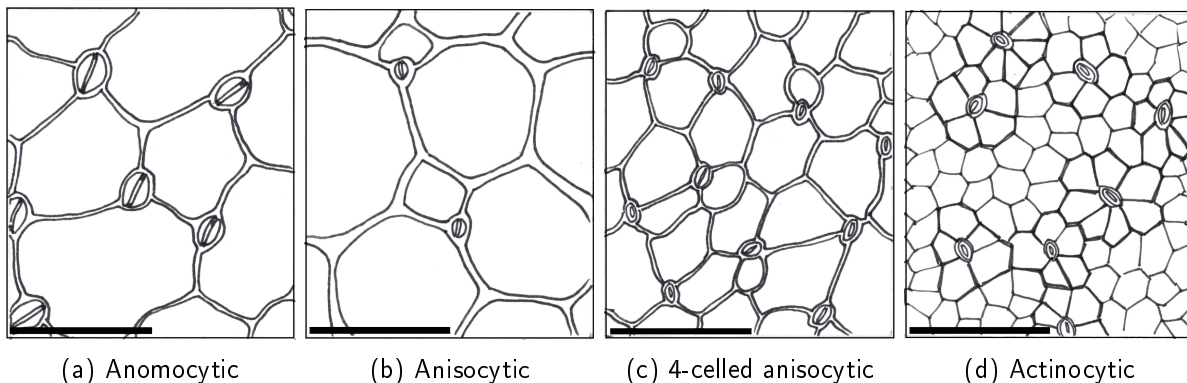


Figure 2.20: Line drawings of the four stomatal complex types identified in the studied *Oxalis* taxa. Scalebar = 100  $\mu\text{m}$ . (a) *O. extensa* (J1216), (b) *O. convexula* (MO368), (c) *O. pulchella* (MO863), (d) *O. salteri* (MO1137).

Epistomatic, hypostomatic and amphistomatic leaflets were observed, but epistomatic taxa were observed in 70 out of 110 taxa (63.6%) and were more common than hypostomatic (14.5%) and amphistomatic (21.8%) taxa. Interestingly, 32 taxa appeared to have the majority of their stomata on one leaflet surface (*i.e.* epistomatic or hypostomatic), but with a few additional stomata on the opposite leaflet surface above the central vein. Thirteen of these taxa (here defined as Group 1) were predominately epistomatic and have conduplicate leaflets, with a few additional vein-associated stomata on the AB leaf surface (Figure 2.21a and Addendum I). Seven taxa (here defined as Group 2) were predominately hypostomatic and had flat leaflets, but with additional stomata on the AD leaflet surface above the central vein (Figure 2.21b and Addendum I). We observed that taxa from Group 1 all had isobilateral mesophyll arrangement, whereas taxa in Group 2 all had bifacial mesophyll arrangement (Figure 2.21). Despite the constancy in these shared traits to the extent that two groups could be recognized, these taxa are phylogenetically not closely related (Figure 2.22). The

observed stomatal-mesophyll correlation may thus not reflect relatedness, but rather some functional significance (data not shown).

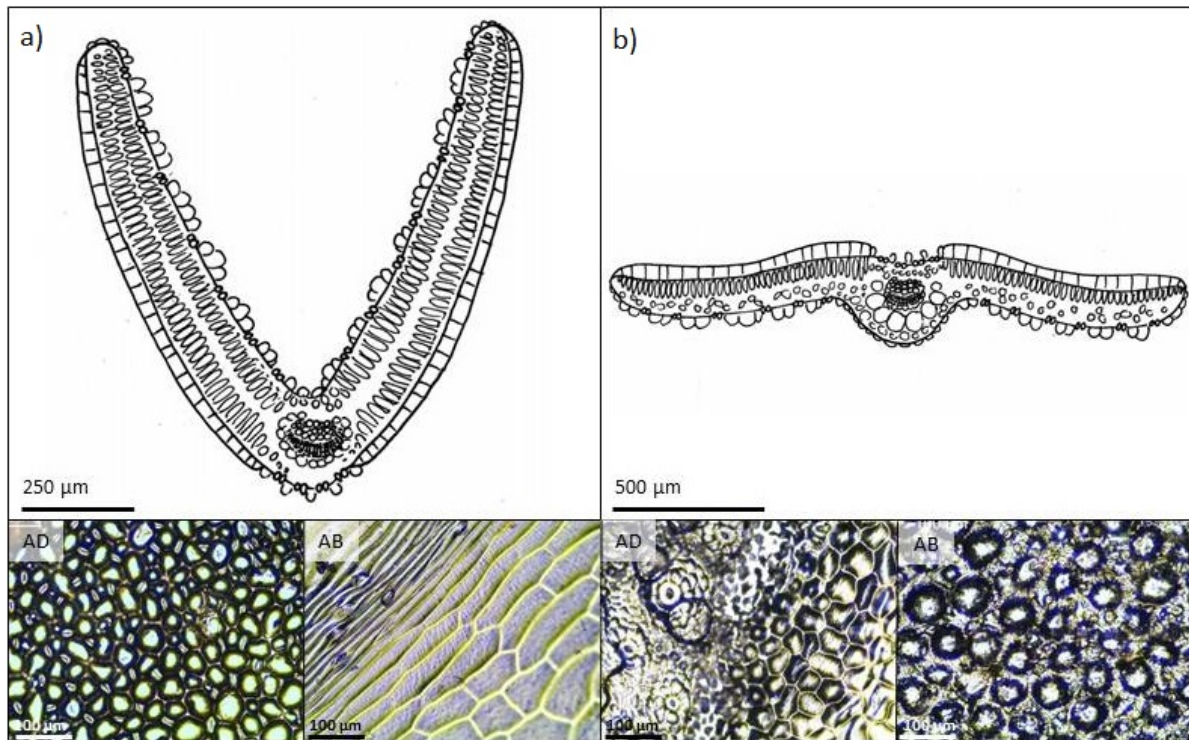


Figure 2.21: Line drawings of transverse leaf sections and light microscope photographs of epidermal peels that show two groups of *Oxalis* taxa with exceptional stomatal arrangements. a) Group 1 includes epistomatic taxa with additional vein-associated stomata on the AB surface (*O. cathara* MO582) and b) Group 2 includes hypostomatic taxa with additional vein-associated stomata on the AD surface (*O. compressa* MO519).

All observed amphistomatic taxa had bifacial mesophyll arrangement and flat leaflets. Epistomatic taxa had both bifacial and isobilateral mesophyll arrangement in both flat and conduplicate leaflets. Hypostomatic taxa had bifacial mesophyll arrangement and leaflets were always flat. Stomata of all taxa appeared to be very slightly sunken below the area where epidermal pavement cells and the first layer of mesophyll cells meet, regardless of the size or shape of the epidermal and mesophyll cells.

No obvious phylogenetic signal in continuous stomatal traits, such as large increases or decreases of stomatal length or density confined to specific clades, were visible in any of the *Oxalis* clades described by Oberlander *et al.* (2011). Stomatal position did, however, show a strong phylogenetic pattern (Figure 2.22). All taxa from the *Ionoxalis* clade, Clade 2, Clade 3 and Clade 5 (except *O. imbricata* (MO345)) had hypostomatic leaflets. The majority of southern African *Oxalis* taxa had AD located stomata as seen in Clades 8 and 9. The well-supported Clade 7 was the only clade that had amphistomatic leaflets (14 taxa). We should note that all taxa from the hypostomatic Clade 2 (except *O. hirsutibulba* (MO1519)) had additional stomata above the mid-rib of the AD leaflet surfaces and that additional stomata on the AB leaflet surfaces of taxa from the epistomatic Clade 8 were commonly observed (Figure 2.23). However, these stomatal traits did not typify any *Oxalis* clades. Neither stomatal central axis lengths nor stomatal densities showed any phylogenetic pattern for AD or AB located stomata (Addendum II).

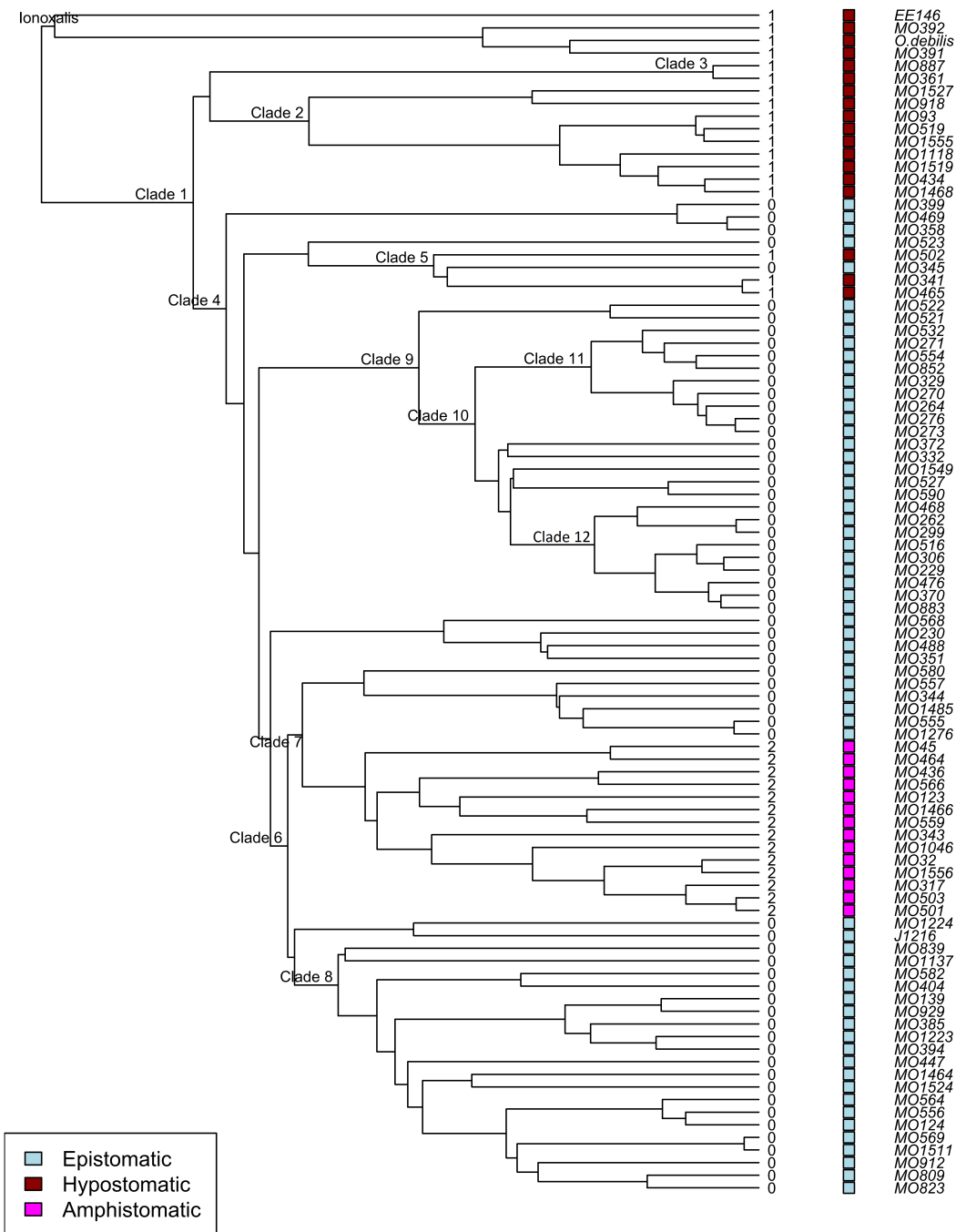


Figure 2.22: The phylogenetic distribution of southern African *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic leaflets on an ITS-based tree.

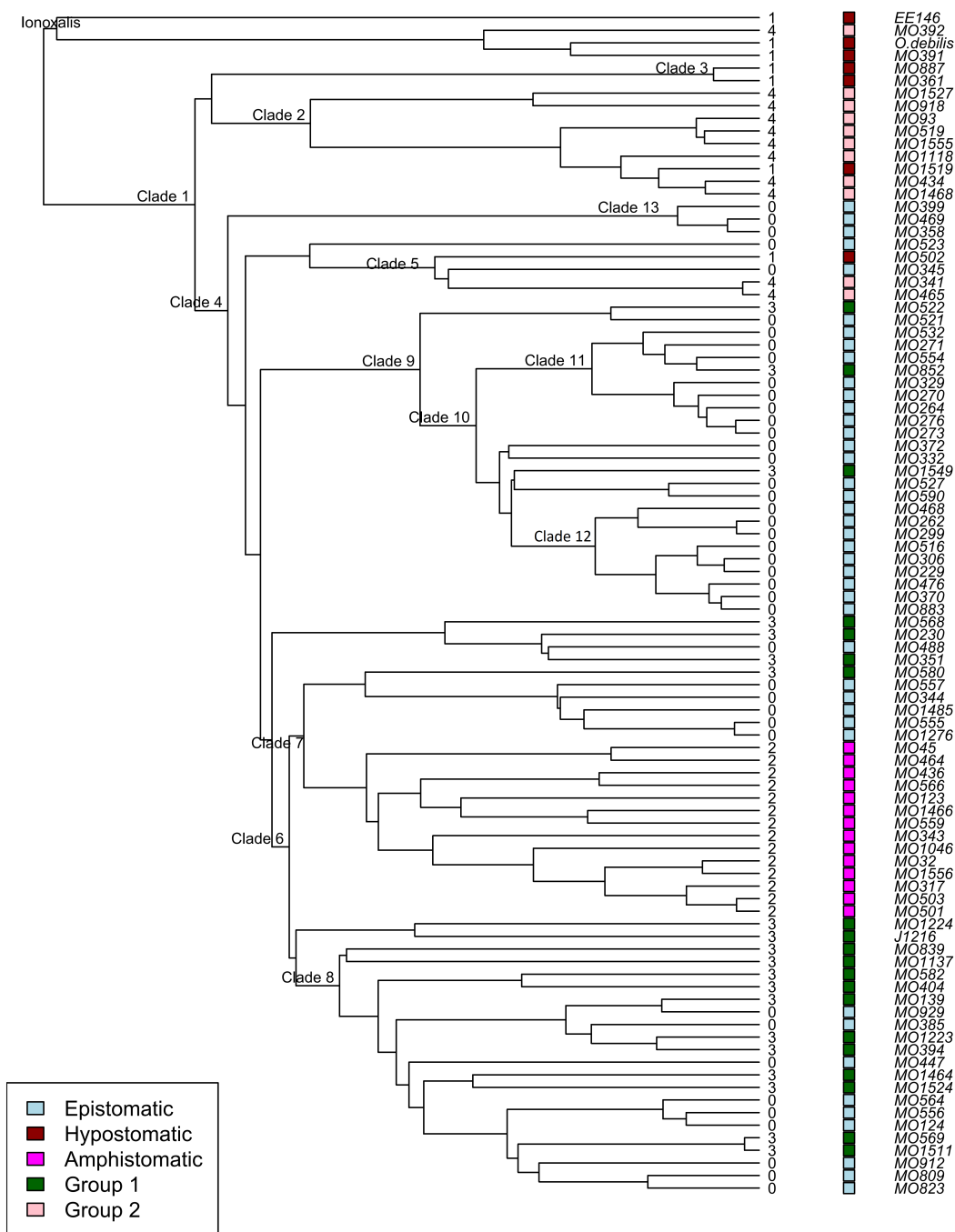


Figure 2.23: The phylogenetic distribution of southern African *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic leaflets (with and without additional stomata in AB (Group 1) and AD (Group 2) leaflet crevices) on an ITS-based tree.

## 2.5 Results: Mesophyll

Leaflet mesophyll anatomy revealed significant variations in traits such as mesophyll type, number of cell layers per mesophyll type, crystal types and distribution and cavities within the mesophyll. The described internal anatomy of *Oxalis* was based only on taxa sampled from the living garden collection, so we cannot comment on any variation of internal structures of taxa from the living



collection and the field.

### 2.5.1 Mesophyll arrangement

Two tissue types were observed in the mesophyll, namely palisade parenchyma and spongy parenchyma. The mesophyll of bifacial taxa were differentiated into one- to five-seriate palisade parenchyma and two- to six-seriate spongy parenchyma. The palisade parenchyma in bifacial arrangements usually comprised slightly over half (51.2%, average = 59.4  $\mu\text{m}$ , range = 24.9  $\mu\text{m}$  - 178.9  $\mu\text{m}$ ) of total mesophyll thickness (spongy parenchyma average = 55.7  $\mu\text{m}$ , range = 19.8  $\mu\text{m}$  - 88.4  $\mu\text{m}$ ). Palisade cells were cylindrical in shape, while the spongy parenchyma cells were round or ovoid. The mesophyll of isobilateral Type 1 taxa were differentiated into an AD and AB layer of one- to three-seriate palisade parenchyma each, and an one- to three-seriate spongy parenchyma located between the palisade layers. Palisade parenchyma comprised almost two thirds (63.2%, average = 72.3  $\mu\text{m}$ , range = 35.6  $\mu\text{m}$  - 186.0  $\mu\text{m}$ ) of total mesophyll thickness (spongy parenchyma average = 25.4  $\mu\text{m}$ , range = 9.5  $\mu\text{m}$  - 61.0  $\mu\text{m}$ ) in leaves of this type. The mesophyll of isobilateral Type 2 taxa was not differentiated into a palisade and spongy layer, but seemed to match only spongy parenchyma (cells round to ovoid). The average mesophyll heights ranged from 58.9  $\mu\text{m}$  to 158.0  $\mu\text{m}$ .

The number of palisade layers was relatively consistent (plus or minus 1 cell layer) between different samples of the same species, but varied between taxa with bifacial and isobilateral mesophyll types. The number of spongy parenchyma layers was variable within and between species, but no detectable phylogenetic pattern was observed (Addendum II).

Mesophyll arrangement types were consistent between multiple samples from the same *Oxalis* species. Bifacial arrangement (*i.e.* the arrangement of AD palisade parenchyma and AB spongy parenchyma; Figure 2.24a, 2.24b and 2.24c) was observed in 55.2% of studied taxa and was therefore more common than an isobilateral arrangement. Two different types of isobilateral arrangements were observed in the remaining taxa: isobilateral Type 1 was defined as mesophyll arrangement with a single layer of round-celled spongy parenchyma located between upper and lower palisade layers (Figure 2.24d, 2.24e and 2.24f), and isobilateral Type 2 was defined as mesophyll arrangement where only uniform oval-shaped palisade parenchyma cells occur in the mesophyll tissue (Figure 2.24g, 2.24h and 2.24i) and spongy parenchyma was completely absent. Isobilateral Type 1 was observed in 26 out of 39 isobilateral taxa (66.7%) and was therefore more common than isobilateral Type 2.

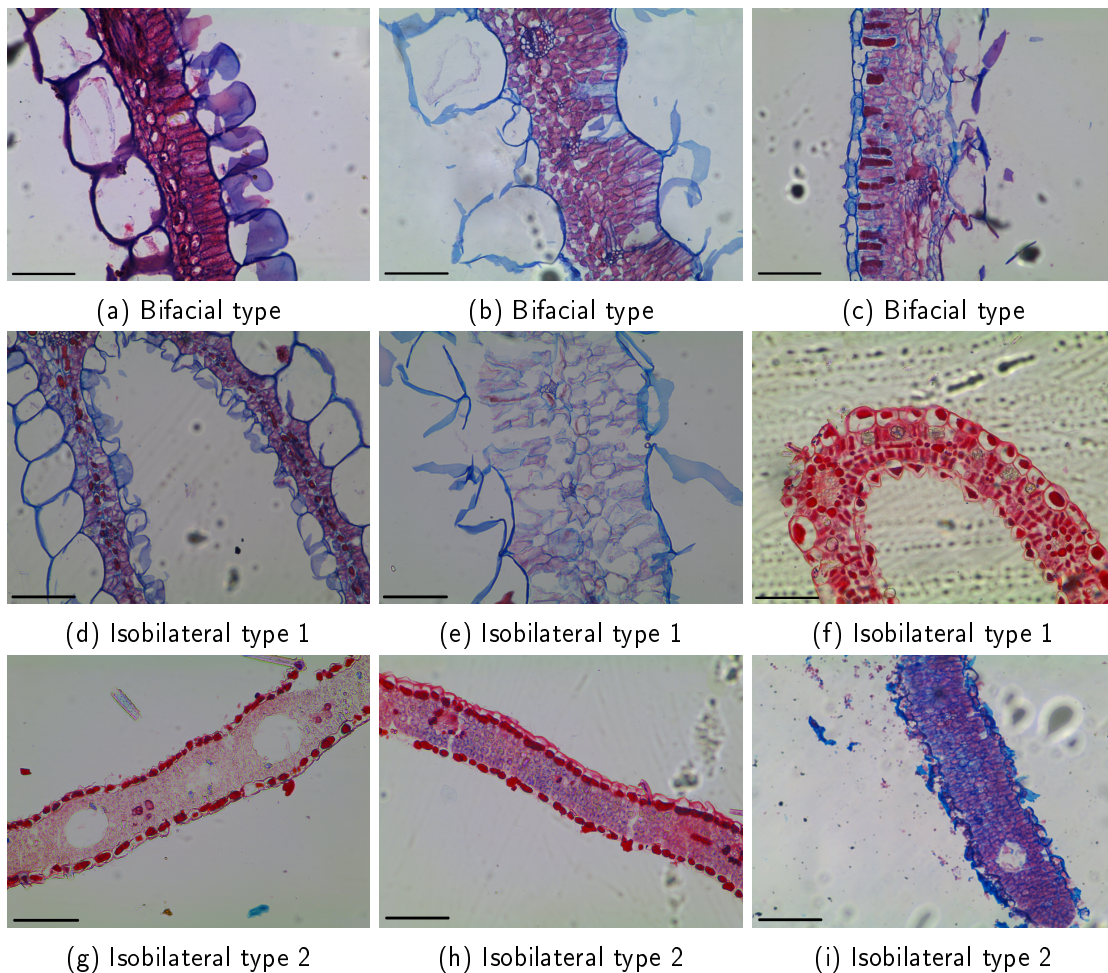


Figure 2.24: Light microscope photographs of wax-embedded and stained leaflet material depicting bifacial, isobilateral Type 1 and isobilateral Type 2 mesophyll arrangement observed in *Oxalis* taxa. (a) *O. commutata* (MO1224), (b) *O. foveolata* (MO1466), (c) *O. purpurea* (MO410), (d) *O. falcatula* (MO476), (e) *O. nortieri* (MO503), (f) *O. multicaulis* (MO883), (g) *O. oreophila* (MO270), (h) *O. tenella* (MO264), (i) *O. gracilis* (MO554). Scalebars represent 100  $\mu\text{m}$ .

Bifacial mesophyll type was the most common mesophyll arrangement type and appears to be ancestral to southern African *Oxalis* (Figure 2.25). All sampled taxa of the *Ionoxalis* clade, Clade 2, Clade 3, Clade 5 and Clade 7 (except *O. nortieri* (MO503) and *O. dilatata* (MO524)) had bifacial mesophyll arrangement types. Taxa from Clade 12 had isobilateral Type 1 arrangement and taxa from Clade 8 had both bifacial and isobilateral Type 1 arrangements. Taxa from Clade 11 and Clade 13 had isobilateral Type 2 mesophyll arrangement (Figure 2.25). Mesophyll type was therefore a trait with a relatively clear phylogenetic pattern.

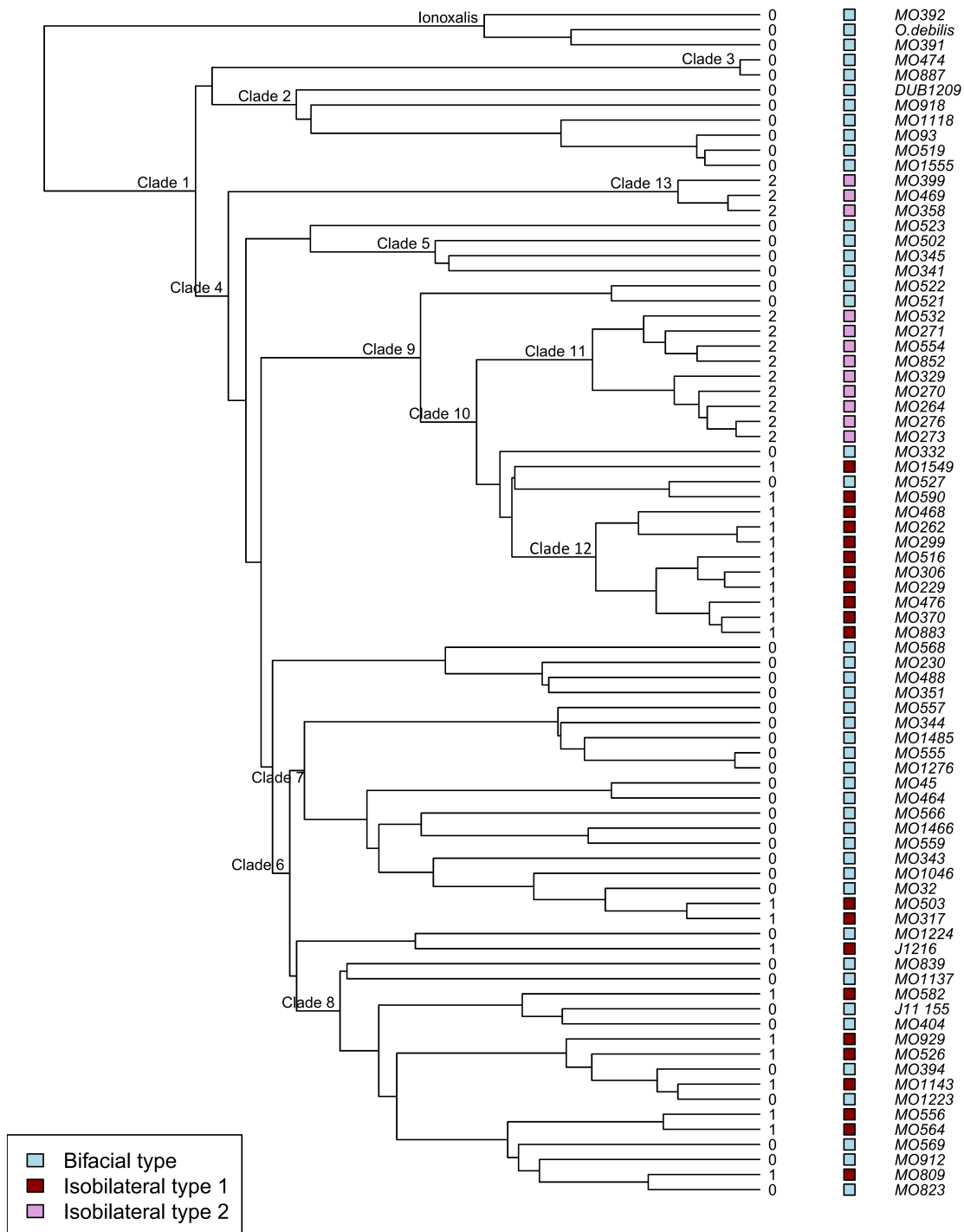


Figure 2.25: The phylogenetic distribution of mesophyll arrangement in southern African *Oxalis* taxa, using an ITS-based phylogenetic tree.

## 2.5.2 Mesophyll dimensions

The average mesophyll heights of bifacial and isobilateral taxa varied from 57.0  $\mu\text{m}$  to 264.0  $\mu\text{m}$  in taxa with bifacial mesophyll, 47.8  $\mu\text{m}$  to 247.0  $\mu\text{m}$  in isobilateral Type 1 taxa and 58.9  $\mu\text{m}$  to 158.0  $\mu\text{m}$  in isobilateral Type 2 taxa. Proportional mesophyll thickness relative to AD and AB epidermal heights also illustrated clear differences. The mesophyll of bifacial leaflets consisted of 21.9% AD

epidermal tissue, 24.2% palisade tissue, 23.8% spongy tissue and 30.2% AB epidermal tissue (Figure 2.27a). On average, isobilateral Type 1 leaflets consisted of 16.3% AD epidermal tissue, 43.4% mesophyll tissue and 36.3% AB epidermal tissue (Figure 2.27b). On average, isobilateral Type 2 leaflets consisted of 18.3% AD epidermal tissue, 61.3% mesophyll tissue and 22.4% AB epidermal tissue (Figure 2.27c). This data can be useful for future identification purposes to discriminate between isobilateral Type 1 and 2 mesophyll. Overall, it is evident that the AD and especially the AB epidermal layers (though only one cell layer thick) massively contribute to the total leaflet thickness of taxa with both bifacial and isobilateral leaflet types.

The average leaflet section heights (including AD epidermal heights, palisade layer heights, spongy layer heights and AB epidermal heights) were variable, but overall, the mesophyll and epidermal cell dimensions of closely related taxa corresponded to the *Oxalis* clades (Figure 2.26) indicating that there is some observable phylogenetic signal in this trait. Within Clades 12, 7 and 3 closely related taxa showed very clear similarity of their mesophyll and epidermal tissue dimensions. From these data we can also see that in the taxa with relatively thicker leaflet sections (within Clade 7), the increased thickness does not rely on the massively swollen epidermal cells found in taxa from this clade, because all the main tissue types (AD and AB epidermal tissue and mesophyll tissue) have proportionally increased thickness.

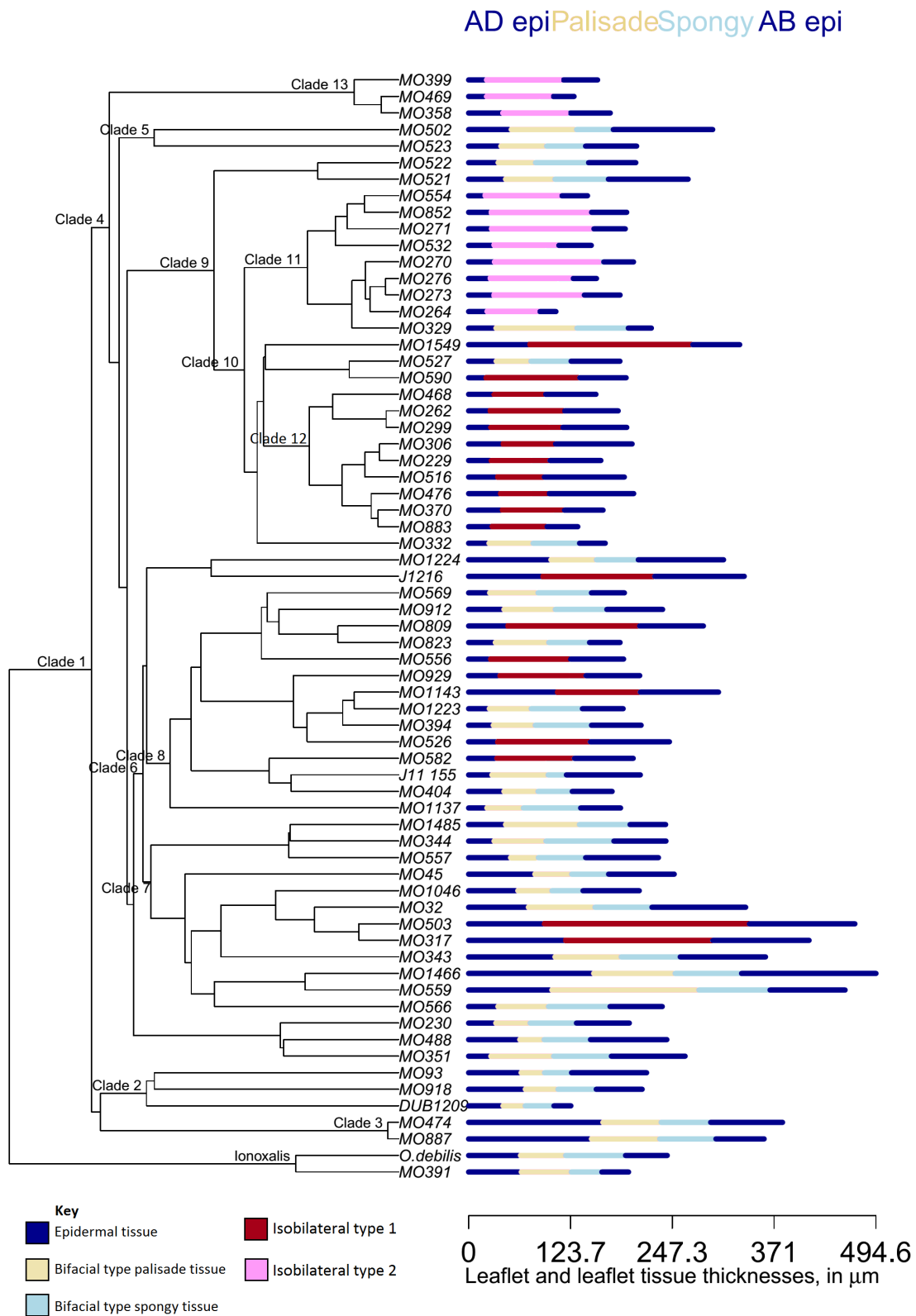
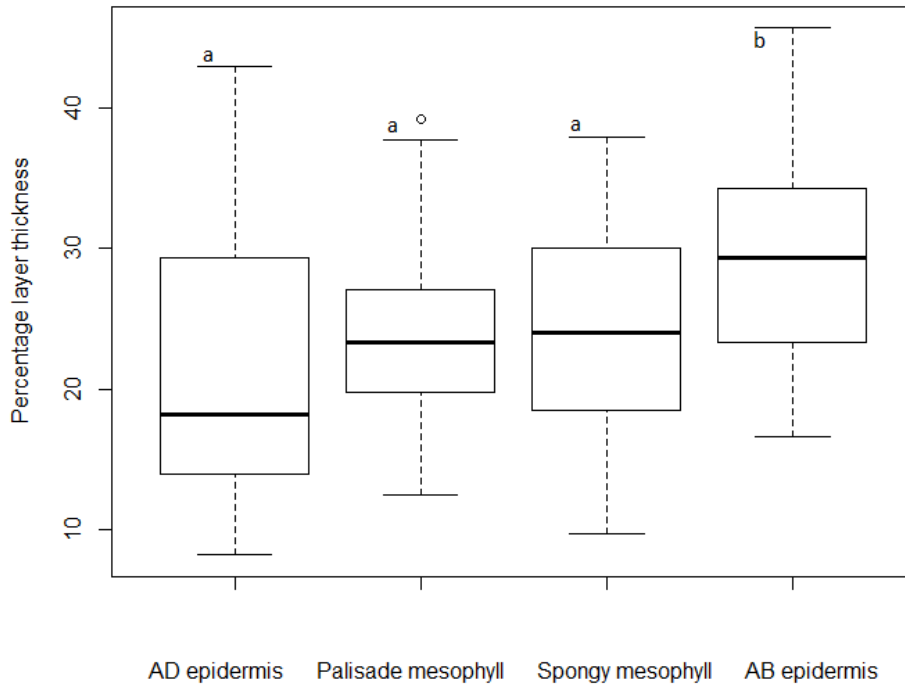
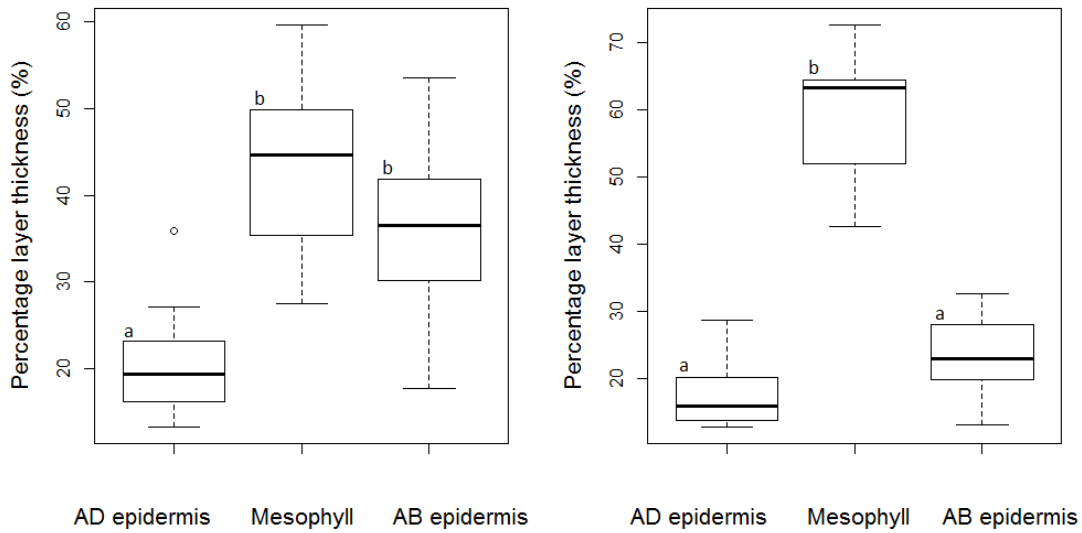


Figure 2.26: The phylogenetic distribution of the section heights (including AD epidermal, palisade parenchyma, spongy parenchyma and AB epidermal layer heights) of southern African *Oxalis* taxa, using an ITS-based phylogenetic tree.



(a) Bifacial type, where the symbols a and b were significantly different at  $p < 0.05$



(b) Isobilateral Type 1, where the symbols a and b were significantly different at  $p < 0.001$

(c) Isobilateral Type 2, where the symbols a and b were significantly different at  $p < 0.001$

Figure 2.27: Boxplots of the relative dimensions (percentage values) of epidermal tissue and mesophyll tissue measured in bifacial, isobilateral Type 1 and isobilateral Type 2 *Oxalis* leaflets.

### 2.5.3 Crystals

Crystals were observed in 90% of studied taxa and four different types of crystals were identified namely: druse crystals (Figures 2.28a, 2.28b and 2.28c), round crystals (Figure 2.28d and 2.28e), prismatic crystals (Figure 2.28g and 2.28h) and crystal sand (Figures 2.28f and 2.28i). Crystals occurred as free individual crystals or in clusters of more than two. Crystals were observed in either the palisade layers, spongy layers or both palisade and spongy layers of *Oxalis* taxa with bifacial and isobilateral mesophyll types. These traits were extremely variable, as multiple crystal types co-occurred within some *Oxalis* species, and no phylogenetic pattern based on any crystal traits could be observed.

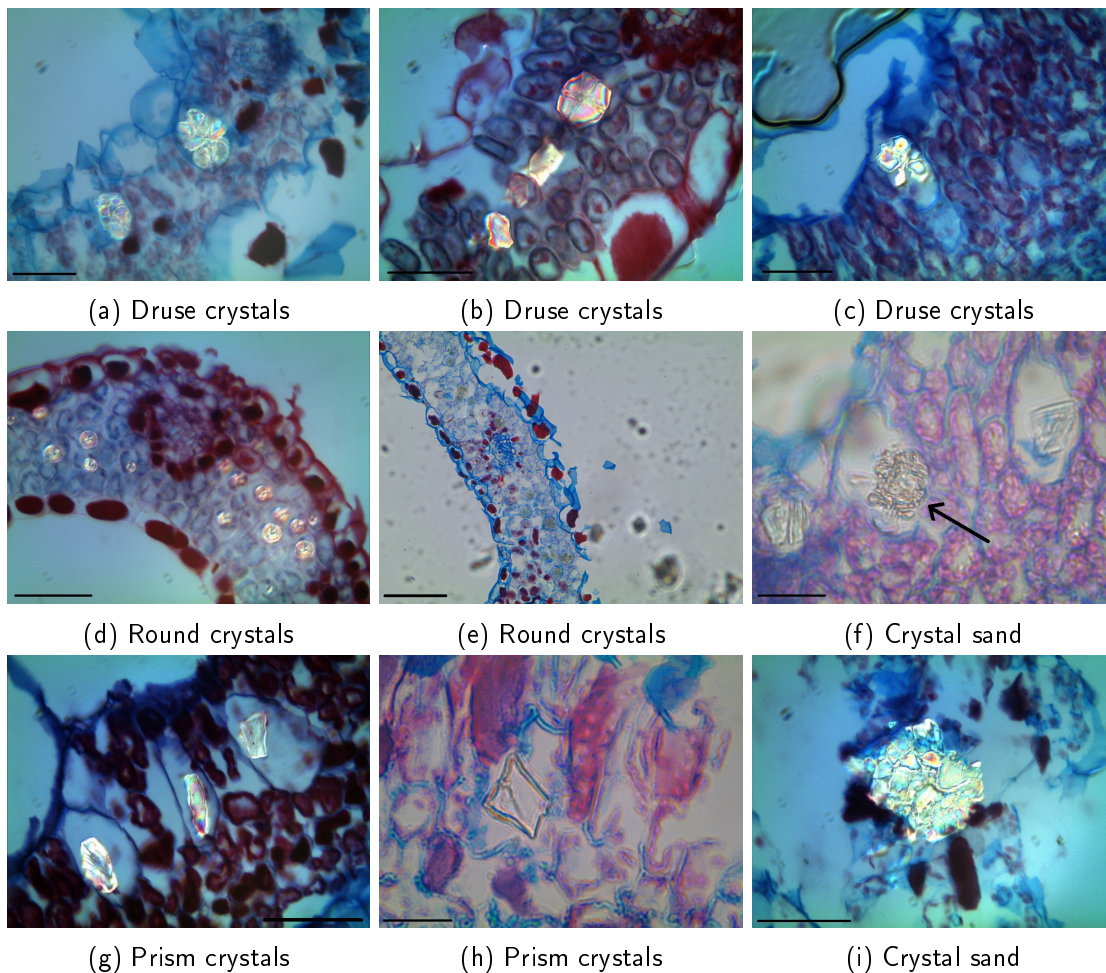


Figure 2.28: Light microscope photographs of crystals observed in wax-embedded and stained leaflet mesophyll of studied *Oxalis* taxa. (a) *O. zeyheri* (MO590), (b) *O. flaviuscula* (MO929), (c) *O. convexula* (MO368), (d) *O. tenella* (MO264), (e) *O. callosa* (MO532), (f) *O. convexula* (MO368), (g) *O. foveolata* (MO1466), (h) *O. depressa* (MO464), (i) *O. depressa* (MO464). Scalebars represent 50 μm.

### 2.5.4 Cavities in mesophyll tissue

Hollow cavities were observed within the mesophyll of 36 out of 81 studied *Oxalis* taxa (44.4%). In 50% of these taxa the cavities had no inner lining (Figure 2.29a, 2.29b and 2.29c), while the other 50% of taxa had cavities with an inner epithelial lining (one cell layer in thickness) (Figure 2.29d,

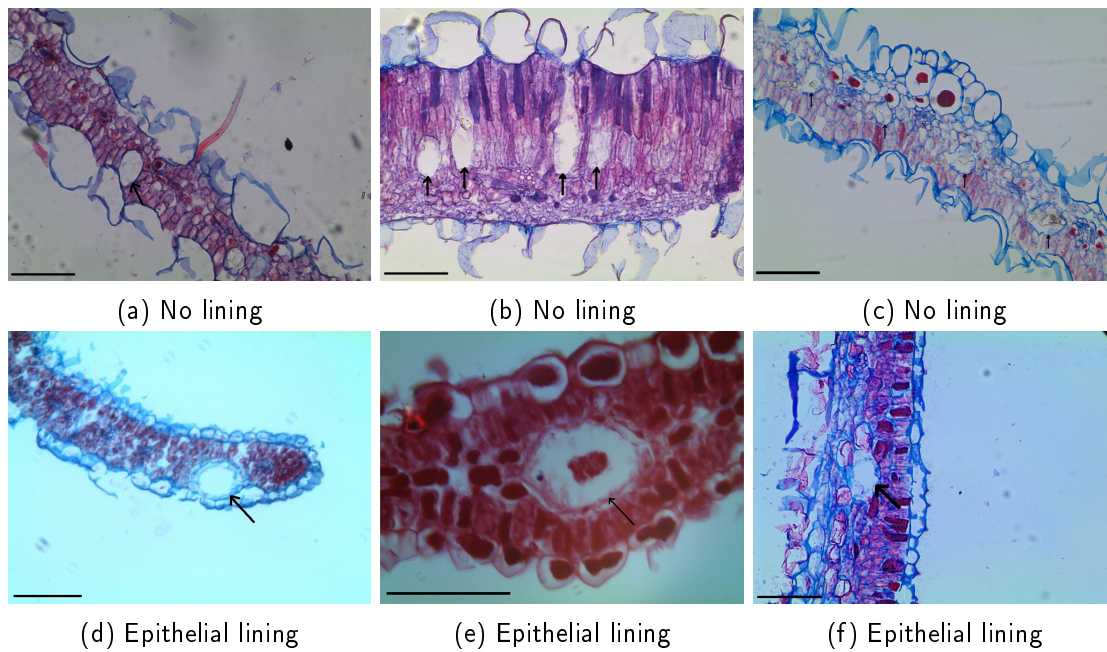


Figure 2.29: Light microscope photographs of wax-embedded and stained leaflet material depicting cavities with and without epithelial inner linings observed in the mesophyll of the studied *Oxalis* taxa. (a) *O. namaquana* (MO809), (b) *O. pulchella* (MO559), (c) *O. obtusa* (MO567), (d) *O. camelopardalis* (MO469), (e) *O. multicaulis* (MO883), (f) *O. purpurea* (MO410). Scalebars represent 100  $\mu\text{m}$ .

2.29e and 2.29f). Cavities were located in either the palisade layer, spongy layer or spanned the full depth of both layers. Cavities (with and without epithelial linings) were either empty or contained various types of crystals. Although this is an interesting phenomenon with possible taxonomic and functional value, we are cautious to describe types and numbers of crystals seen in these cavities in too much detail, as crystals could have moved from their original position throughout the sectioning and staining process. The presence and location of observed cavities in the leaflet mesophyll could have been dependent on the area of leaflet that was sectioned, so many more taxa could potentially have cavities that we could not observe. Taxa without any cavities in their mesophyll were scattered throughout the *Oxalis* phylogeny. Similarly taxa with cavities (with or without epithelial lining) were present in all *Oxalis* clades (Figure 2.30).



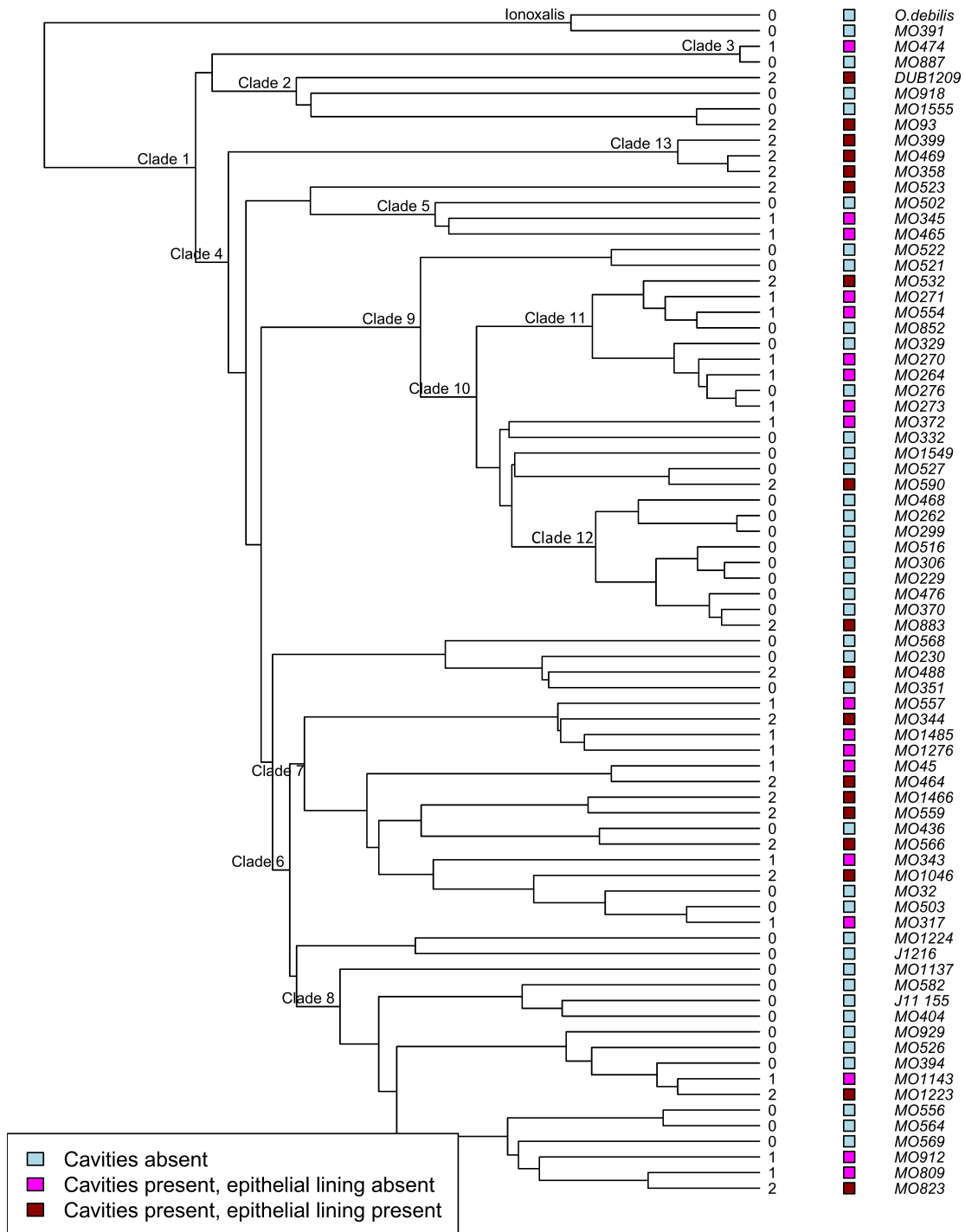


Figure 2.30: The phylogenetic distribution of cavities (with and without epithelial cell linings) observed in the mesophyll tissue of southern African *Oxalis* taxa, using an ITS-based tree.

## 2.6 Results: Vascular tissue

Two types of venation were identified within studied leaflets, namely pinnate (typical central vein and secondary veins branching from the central vein, usually opposite each other) and palmate venation (veins branch from one single point, close to the leaflet articulation point). The number of veins

could not accurately be quantified, due to the preservation of leaflet material. A pinnate venation type was observed in 78 out of 90 studied taxa (86.7%) and was therefore the most common venation type (Figure 2.31). Taxa with palmate venation occurred only in Clade 7 (6 taxa) and Clade 8 (2 taxa).

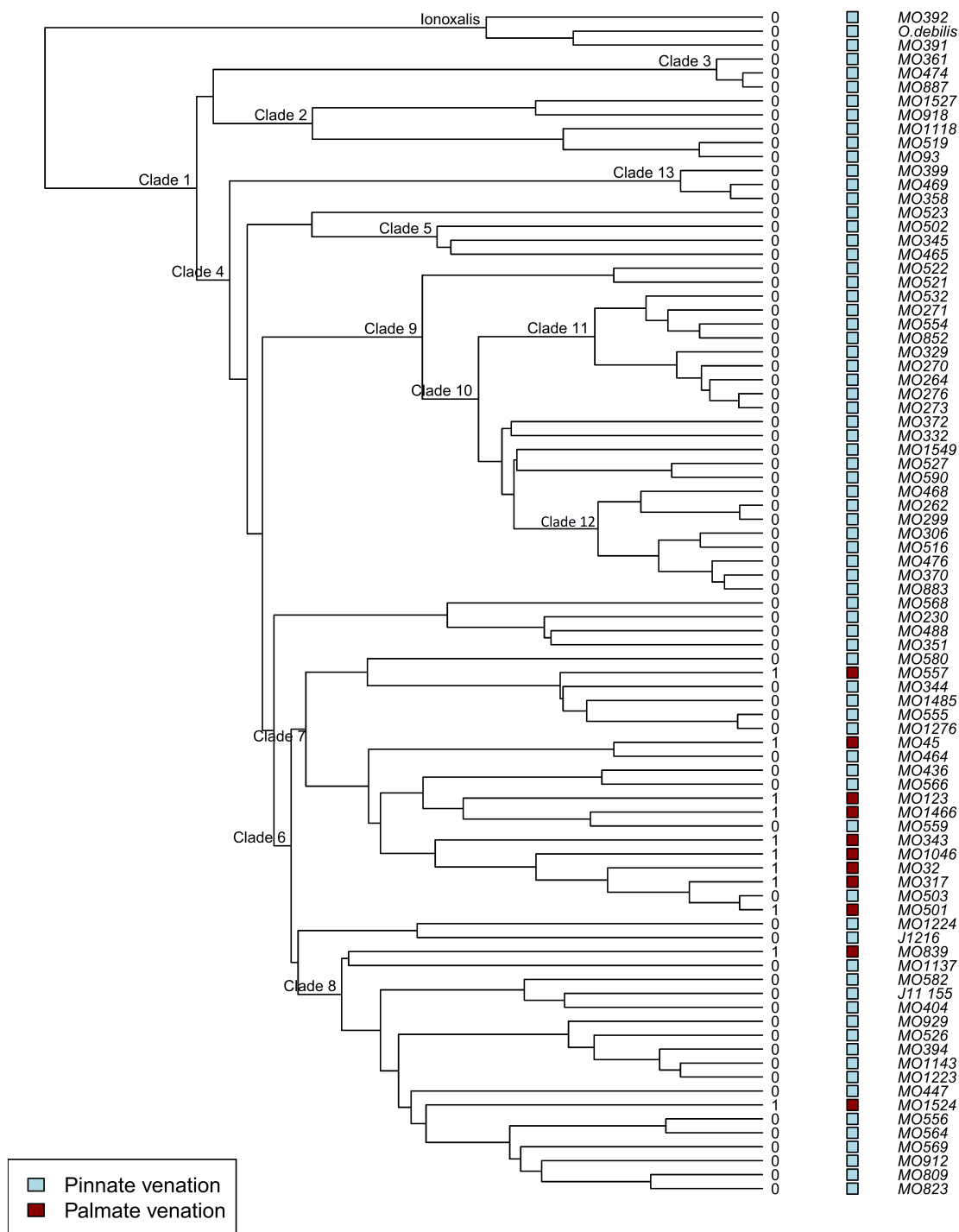


Figure 2.31: The phylogenetic distribution of southern African *Oxalis* taxa with pinnate and palmate venation, using an ITS-based tree.

As in standard angiosperm leaf vascular tissue, the midrib vascular trace of all studied taxa contained xylem that faced the AD surface and phloem that faced the AB surface (Esau, 1953). The vascular bundles were located at the junction of palisade and spongy mesophyll in all taxa with bifacial mesophyll arrangement, and in the middle region of the mesophyll of all isobilateral taxa. The vascular tissue and mid-rib region of 38 out of 89 studied taxa (42.4%) projected outwards on the AB surface (Figures 2.32d, 2.32e and 2.32f), while the remainder of taxa had vascular tissue embedded in the mesophyll (Figures 2.32a, 2.32b and 2.32c). One to three layers of collenchymatous cells occurred between the vascular tissue and the AB epidermis in taxa with a protruding midrib, and palisade parenchyma cells occurred between the vascular tissue and the AD epidermis. These collenchyma cells were massively enlarged relative to the average mesophyll cells and were usually accompanied by AB epidermal cells that were smaller (in width and height) than the surrounding AB epidermal cells (Figure 2.33b). Only 50% of these seemingly smaller cells corresponded to the elongated AB epidermal cells associated with the mid-rib. The vascular tissue of taxa with an embedded midrib was surrounded by palisade and spongy parenchyma cells. The central and lateral vascular bundles of 48 out of 84 studied taxa (57.1%) were surrounded by a single layered sheath that was different in size and shape to the surrounding parenchyma cells in the mesophyll (Figure 2.33). These cells were circular or ovate in shape, and the cell walls and contents stained differently to the surrounding parenchyma cells.

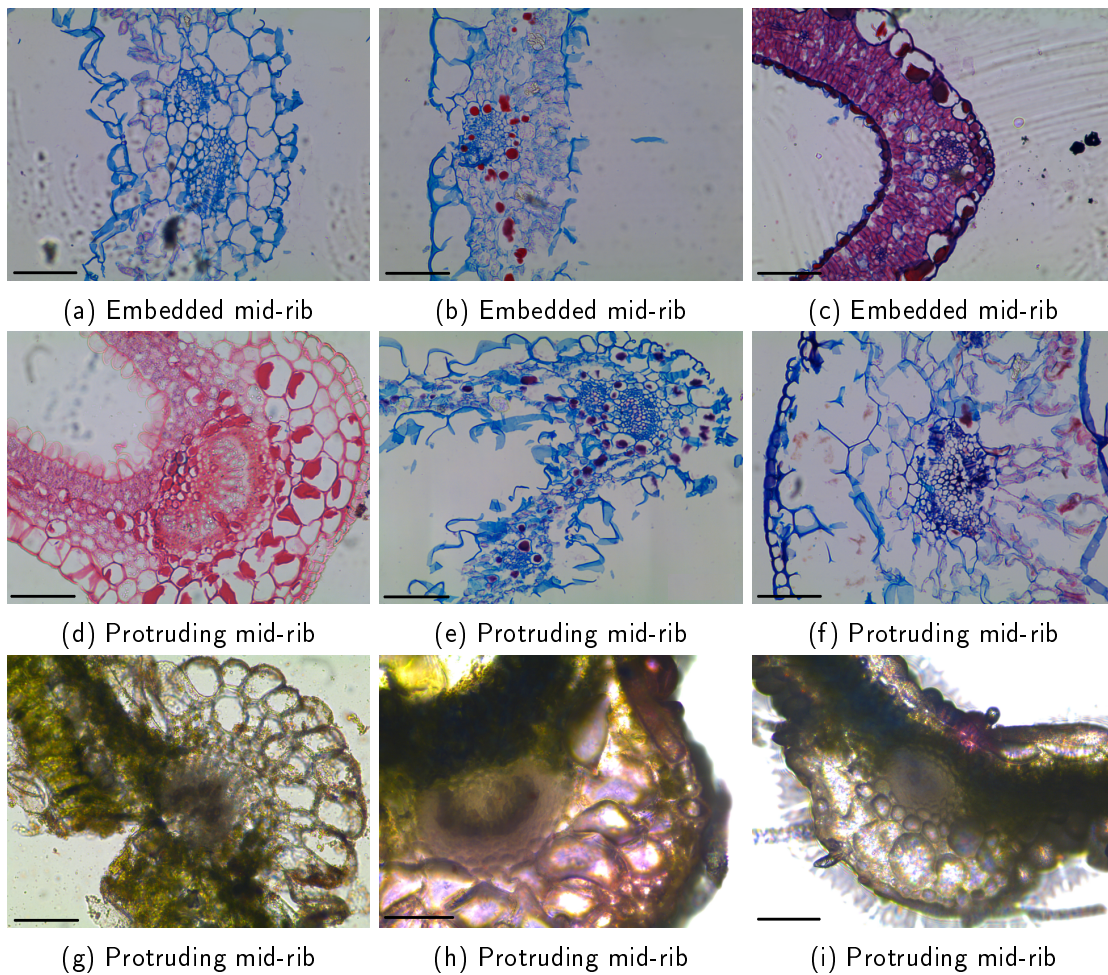


Figure 2.32: Light microscope photographs of wax-embedded and stained leaflet material and hand-sections of fresh leaflet material depicting embedded and protruding mid-ribs observed in *Oxalis* taxa. (a) *O. luederitzii* (J1210), (b) *O. eckloniana* (MO521), (c) *O. engleriana* (MO485), (d) *O. adspersa* (MO1223), (e) *O. hirsuta* (MO1586), (f) *O. nortieri* (MO503), (g) *O. depressa* (MO464), (h) *O. bowiei* (MO502), (i) *O. compressa* (MO519). Scalebars represent 100  $\mu\text{m}$ .

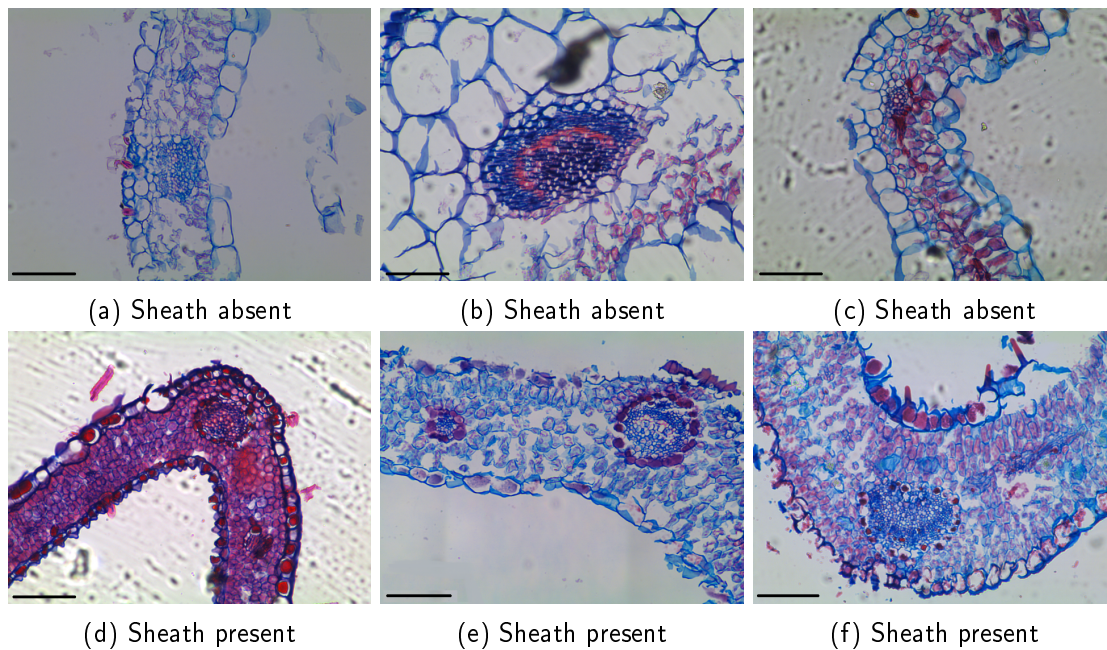


Figure 2.33: Light microscope photographs of wax-embedded and stained leaflet material depicting vascular tissue with and without a sclerenchymous sheath observed in *Oxalis* taxa. (a) *O. brasiliensis* (MO391), (b) *O. bowiei* (MO502), (c) *O. namaquana* (MO809), (d) *O. oreophila* (MO270), (e) *O. ciliaris* (MO329), (f) *O. odorata* (MO1549). Scalebars represent 100  $\mu\text{m}$ .

The presence or absence of a protruding mid-rib was constant throughout taxa of the same species, but this trait was not unique to any *Oxalis* clades (Figure 2.34). The taxa from Clades 11 and 12 (except for *O. ciliaris* (MO329), *O. oreophila* (MO270), *O. glabra* (MO306) and *O. ebracteata* (MO262)) all shared the trait of having a non-protruding mid-rib. The presence or absence of a sheath around vascular tissue was constant throughout taxa of the same species. Taxa with a sheath around vascular tissue were distributed throughout the phylogeny, but a clear pattern emerged as all taxa from Clade 10 (except *O. pusilla* (MO516)) had the sheath around their vascular tissue (Figure 2.35).

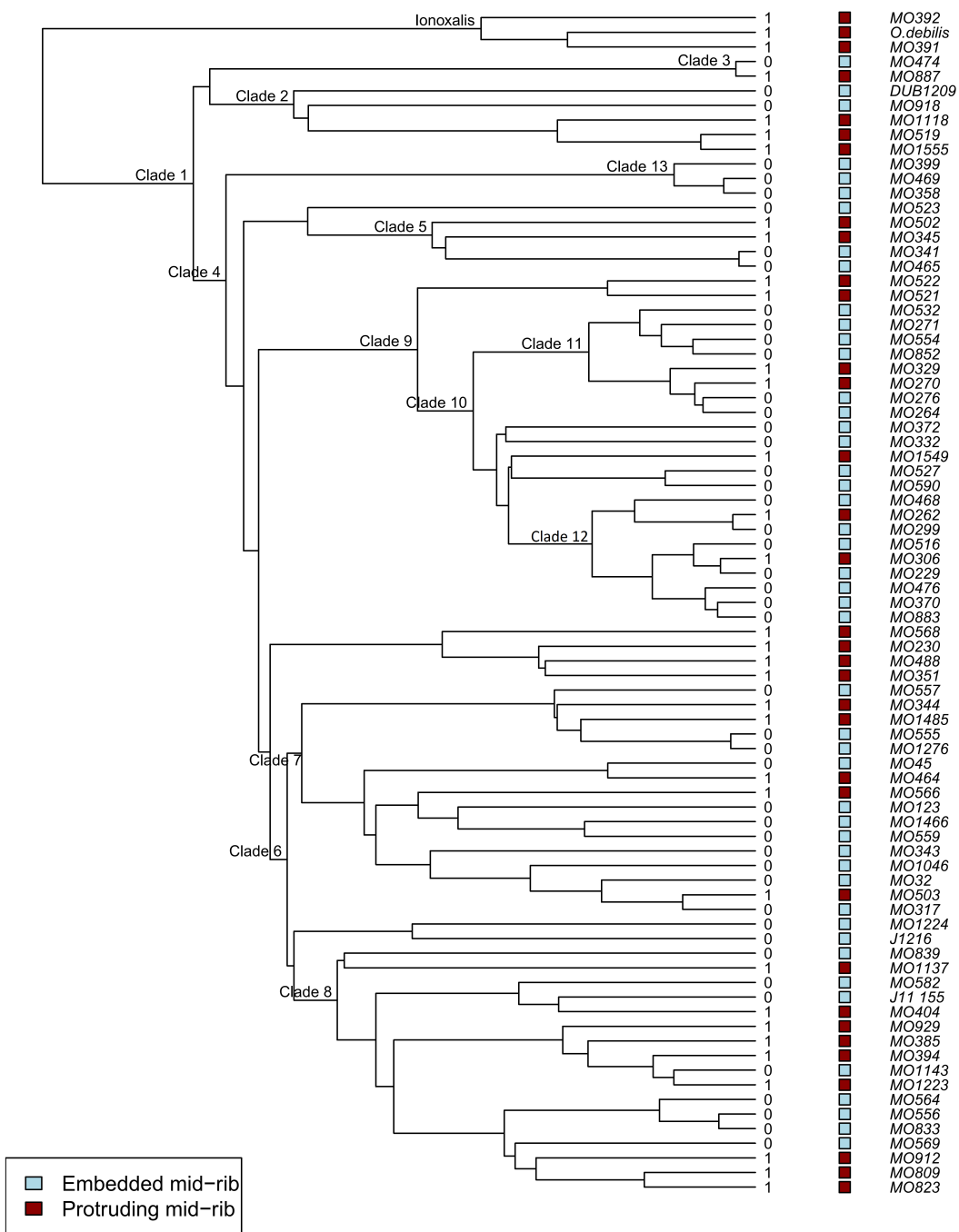


Figure 2.34: The phylogenetic distribution of southern African *Oxalis* taxa with and without a protruding mid-rib (vascular tissue), using an ITS-based tree.

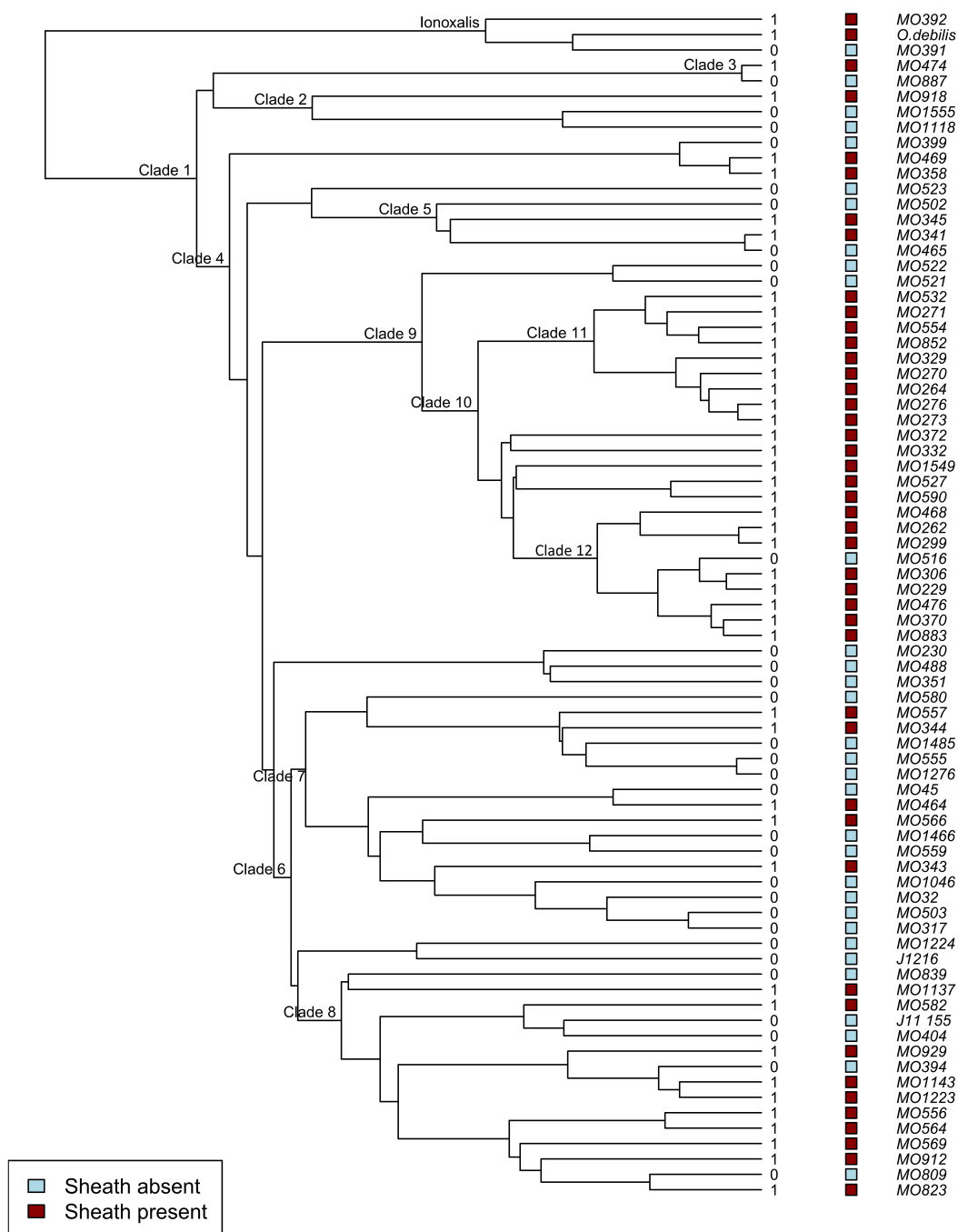


Figure 2.35: The phylogenetic distribution of southern African *Oxalis* taxa with and without a sheath around vascular tissue, using an ITS-based tree.

## 2.7 Summary of Oberlander *et al.* (2011) clades with unique combinations of traits (Figure 2.36)

***lonoxalis* clade** All taxa from the *lonoxalis* clade were characterised by the combination of flat leaflets, AB located stomata (hypostomatic), bifacial mesophyll arrangement, vascular tissue without a sheath and semi-swollen epidermal cell type on the AB leaflet surface.

**Clade 1 - South African *Oxalis*** No phylogenetically significant traits were observed that delineate this clade. Only three closely related outgroups were sampled in our study, but the possibility of detecting synapomorphies for this clade remains until further outgroup samples are studied and included in analyses.

**Clade 2 - *O. pes-caprae* and relatives** All taxa from Clade 2 had flat leaflets. Stomata were always located on the AB leaflet surfaces (hypostomatic) and additional stomata were located on the AD leaflet surfaces above the mid-rib. Semi-swollen epidermal cells were located on the AB leaflet surfaces of most taxa from this clade. Mesophyll arrangement of all taxa was bifacial and vascular tissue was without a sheath.

**Clade 3 - Sect. *Cernuae* subsect. *Lividae*** Taxa from Clade 3 had conduplicate leaflets. The AD epidermal cells were exceptionally thick. Semi-swollen epidermal cells were located on the AB leaflet surfaces in combination with AB located stomata (hypostomatic). Mesophyll arrangement was always bifacial and vascular tissue was without a sheath.

**Clade 4 - Core South African *Oxalis*** AD located stomata (epistomatic leaflets) is a diagnostic trait for the majority of this species-rich southern African *Oxalis* clade (except for Clade 5 with hypostomatic leaflets).

**Clade 5 - *O. stellata* and relatives** All taxa from Clade 5 had flat leaflets with bifacial mesophyll arrangement. Leaflets were hypostomatic (except for one epistomatic taxon). All taxa from this clade had cavities with epithelial linings in their mesophyll and vascular tissue was without a sheath.

**Clade 6** Palmate venation types were observed only in taxa from this clade. All taxa had cavities (with or without epithelial lining) present and vascular tissue was without a sheath.

**Clade 7a - *O. purpurea* and relatives - amphistomatic type** Taxa had flat leaflets with bifacial mesophyll arrangement and vascular tissue was without a sheath. Swollen epidermal cells and stomata on both the AD and AB leaflet surfaces (amphistomatic leaflets) were traits unique and universal to this clade. Elongated AB epidermal cells were absent in many taxa, while palmate venation was common.

**Clade 7b - *O. purpurea* and relatives: *purpurea* type** Taxa from this clade had flat leaflets with bifacial mesophyll arrangement. All leaflets had AD located stomata and vascular tissue was without a sheath.

**Clade 8 - *O. flava* and relatives** Leaflets were epistomatic and additional stomata located on the AB main vein surface were very common in this clade. These additional stomata were located in-between elongated AB epidermal cells. Leaflets were either flat or conduplicate with bifacial or isobilateral Type 1 mesophyll arrangements. The majority of taxa from this clade had glabrous leaflets. If taxa were not glabrous, glandular hairs were commonly observed (this is one of only two clades with glandular hairs). Bifacial or isobilateral mesophyll types were observed and vascular tissue was without a sheath.

**Clade 10** Leaflets from this clade had isobilateral (Type 1 or Type 2) mesophyll arrangements (except for 2 taxa with bifacial mesophyll arrangement). A sheath was present around the vascular tissue in all but one taxon from this clade. Stomata were AD located (epistomatic) and all leaflets were conduplicate.



**Clade 11 - *O. hirta* and relatives** All taxa from Clade 11 had conduplicate leaflets. Stomata were always located on the AD leaflet surfaces (epistomatic) and mesophyll arrangement was always isobilateral Type 2. Sheaths around the vascular tissue were observed in all taxa from this clade. If trichomes were present, they were AD located (80% of taxa).

**Clade 12 - *O. glabra* and relatives** All taxa from Clade 12 had conduplicate leaflets with isobilateral Type 1 mesophyll arrangements. All leaflets were epistomatic and had sheaths around the vascular tissue. Glandular hairs were commonly observed (this is one of only two clades with glandular hairs).

**Clade 13 - Sect. *Angustatae* subsect. *Pardales*** All taxa from Clade 13 had conduplicate leaflets with isobilateral Type 2 mesophyll arrangements. All leaflets were epistomatic, had cavities with epithelial linings in their mesophyll and vascular tissue was without a sheath.

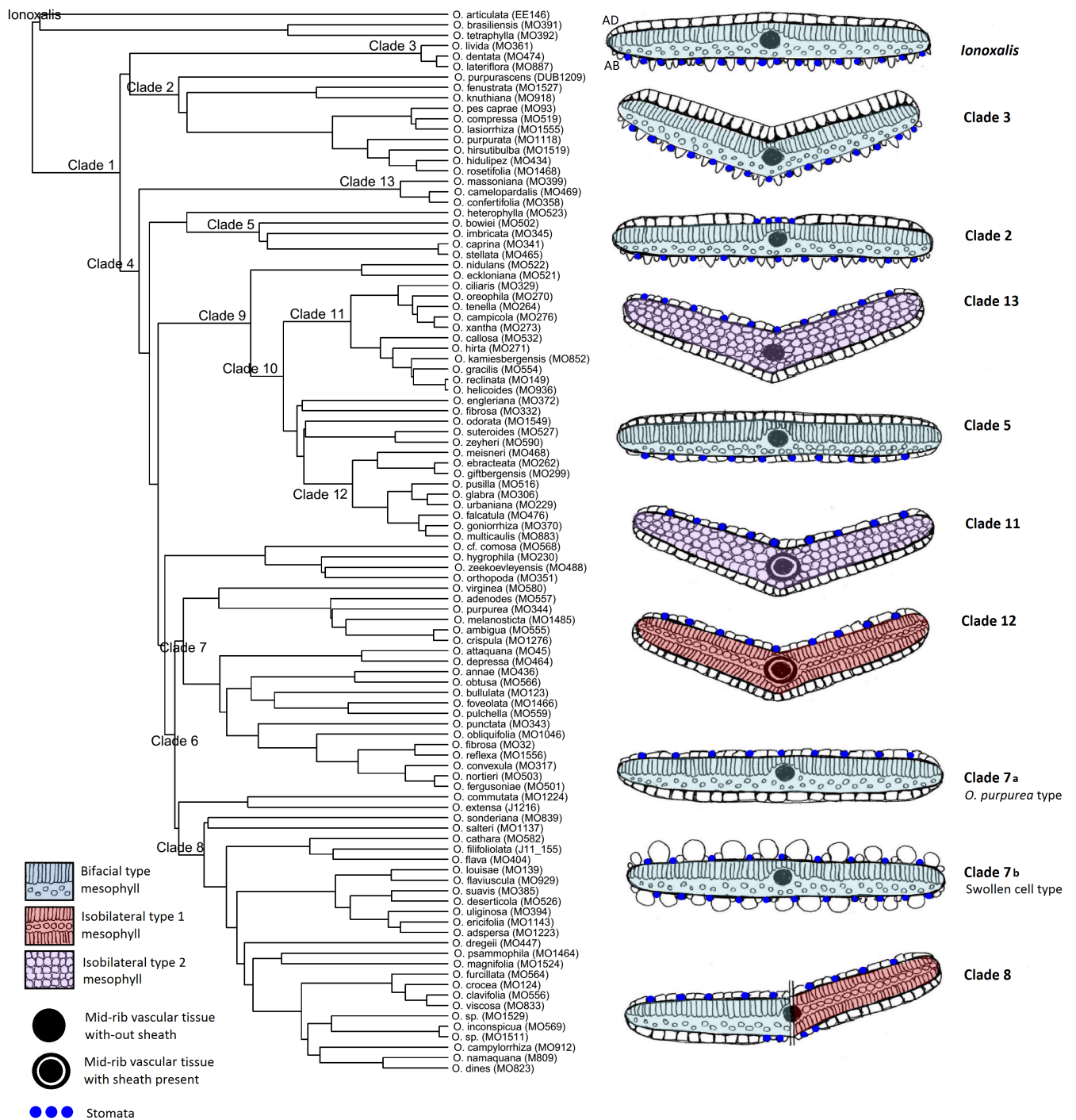


Figure 2.36: The phylogenetic tree of southern African *Oxalis* taxa with illustrations of the typical leaf types observed in each clade with exceptions as discussed in the text. Three epidermal cell types are depicted, namely irregular, semi-swollen (*lonoxalis* clade, Clade 2 and Clade 3) and swollen (sub-clade of Clade 7) epidermal cell types. Conduplicate leaflets are indicated by a leaf lamina at an acute angle to the mid-rib. All leaflet cross-sections are orientated with respect to the *lonoxalis* cross section. Taxa from Clade 8 had flat and conduplicate leaflets with bifacial or isobilateral Type 1 mesophyll arrangements. AD - adaxial leaflet surface, AB - abaxial leaflet surface.

## 2.8 Discussion: Leaf morphology

Despite the relative subjectiveness of flat vs. conduplicate leaflets, this trait showed rather strong phylogenetic pattern and quite clearly defined seven *Oxalis* clades and one cluster of species. To our knowledge this trait has not been described as a taxonomically significant trait in other *Oxalis* species. Leaflet movements induced by light intensity, precipitation and wind speed (Robb, 1963) are phenomena, most commonly linked to diurnal rhythms (where leaflets fold at night) in *Oxalis* (Salter (1944); Denton (1973)). Leaflet movements could cause leaflets to fold over to look like temporary conduplication, but all studied taxa were sampled during the day.

The vast majority of *Oxalis* species (South African and global) have three leaflets per leaf (Salter (1944); Denton (1973)), therefore fewer than three or more than three leaflets per leaf are an exception to the norm. A range from three to 13 leaflets per leaf have been recorded in the sect. *Ionoxalis* (Denton, 1973). These data were used as a taxonomically relevant trait to delineate species, but the study by Denton (1973) did not assess the phylogenetic relationships among species. Perhaps surprisingly, the number of leaflets per leaf did not seem to be phylogenetically informative in terms of defining specific southern African clades. The distribution of this trait clearly contradicted Salter's [1944] morphological classification of *Oxalis* species. He grouped almost all taxa with more than three leaflets in *Angustatae* subsection *Multifoliolatae*, but these species were not considered as being closely related species. Our results highlight Salter's (1944) suspicion of this grouping as being artificial, as taxa with more than three leaflets or less than three leaflets were found in clades throughout the *Oxalis* phylogeny. Taxa with less than three leaflets were found in Clade 7 and Clade 8, and taxa with more than three leaflets were found in Clade 8 and Clade 10. Within Clade 8 the taxa with more than three leaflets appear to be closely related (*O. flaviuscula* (MO929) and *O. ericifolia* (MO1143) are sister taxa and *O. cathara* (MO582), *O. filifoliolata* (J11-155) and *O. flava* (MO404) are sister taxa), but overall taxa with more or less than three leaflets are distantly related. This suggests that leaflet gain or loss has happened multiple times across southern African *Oxalis* clades.

## 2.9 Discussion: Epidermal tissue traits

### 2.9.1 Epidermal pavement cells

Pavement cells are typically unspecialised cell types as their function is to protect the underlying tissue layers (Mauseth, 1988) and to ensure that the more specialised cells are spaced evenly (Korn (1976); Glover (2000)). The pavement cells of eudicot leaves are typically shaped like interlocking puzzle-pieces (sinuous or angular anticlinal cell walls) to provide the leaf with additional mechanical strength (Glover, 2000). Due to the relatively unspecialised morphology of pavement cells, they have traditionally been regarded as insignificant "in the patterning of the epidermis," as their development is regarded as the default process. The AD and AB cells of leaves typically have different features to optimise their function under given environmental conditions (Nakata and Okada, 2013). On the whole, we found that pavement cell traits of southern African *Oxalis* were of considerable taxonomic and phylogenetic significance.

#### 2.9.1.1 Irregular type pavement cells

Various comparative leaf anatomical studies include epidermal pavement cell types (Benitez and Ferrarotto (2009); Moon *et al.* (2009); Soh and Parnell (2011)) and refer to cells with straight or sinuous anticlinal walls as two separate cell types. Sinuous type (*O. violacea* L. and *O. acetosella* L. (Storey, 2006) and angular types *O. europaea* Jord. (Wood, 1874) epidermal cells have been

reported in *Oxalis* species. In southern African *Oxalis* we observed sinuous and angular anticlinal cell walls. However, tremendous variability of these traits was observed as anticlinal walls ranged from straight to sinuous, with a continuum of all possible states in-between. Extreme examples of the intra-species variability of this trait were observed in three taxa (*O. campylorrhiza* (MO912), *O. extensa* (J1216) and *O. fenestrata* (MO1527)) that displayed straight anticlinal walls in the Botanical Gardens' living collection and sinuous walls in the field, and two taxa (*O. campicola* (MO276) and *O. knuthiana* (MO918)) that displayed sinuous anticlinal walls in the Botanical Garden's living collection and straight walls in the field. Due to the variability of anticlinal cell wall shape, we collectively referred to taxa with straight or sinuous anticlinal walls as irregular epidermal cell types. Irregular epidermal cell types occurred in all *Oxalis* clades and this trait was not phylogenetically significant. The variability of this trait might be better explained as a response to environmental factors. The pavement cells of plants growing in shade reportedly have more sinuous anticlinal walls than plants exposed to full sun, which might very well explain the variation we observed (Watson (1942); Esau (1965); Kürschner (1997)). Sinuous epidermal cells provide better mechanical resistance to cell collapse during changes in leaf turgor when cells expand or collapse (Krauss, 1949).

### 2.9.1.2 Papillose type pavement cells

Papillose epidermal cells are known on the AD leaf surface of a number of plant species, such as *O. rubra* A. St.-Hil. (Haberlandt, 1914) and *O. cornellii* Anderson (Wiggins and Porter, 1971). The presence of these papillose cells in southern African *Oxalis* was not phylogenetically significant, but could have functional significance. The shape of papillose epidermal cells has been described to be similar to a biconvex or planoconvex micro-lens that can focus and concentrate light to underlying cell layers (Haberlandt, 1914), thus optimizing light absorption ((Brodersen and Vogelmann, 2007); (Glover, 2007); (Lee, 2007)). A detailed study by Gkikas *et al.* (2015) on the papillose epidermal cells observed on the petals of *O. pes-caprae* showed that papillose epidermal cells helped to increase the amount of light absorbed. They speculated that the absorbed light diffused and scattered within the mesophyll, increasing the amount of usable light in the tissue (which aided in heating-up the mesophyll and caused the flowers to open). If the aforementioned were true for flower petals, the same principles could possibly hold for leaves, providing plants with papillose cells with a photosynthetic advantage.

Although not examined in detail by us as part of this study, epicuticular wax deposits are known from the papillose leaflet surfaces of at least some *Oxalis* species (L.L. Dreyer *pers. comm.*). It has been proposed that a wax-covering on the papillae of AD epidermal cells could significantly decrease the infra-red absorption of a leaf as wax causes light to reflect. Reduced light absorption reduces the heat load of a leaf, which limits the transpiration rate and water loss (Benson and Borrill, 1969). The epicuticular wax observed in southern African *Oxalis* is similar to the wax described by Neinhuis (1997) and Ensikat *et al.* (2011). That study described the occurrence of papillose epidermal cells with epicuticular wax as having a water-repellent function. In *Oxalis* this could be a function to consider, as the stomata are located on the same surface as these papillose epidermal cells.

### 2.9.1.3 Semi-swollen type pavement cells

This epidermal cell type was confined to sect. *Ionoxalis* and Clade 2 and Clade 3, but not all taxa from these clades had this epidermal cell type. The AB epidermal cells of sect. *Ionoxalis* are described as having large epidermal cells relative to the width of the leaflets (Denton, 1973), and agrees with Metcalfe and Chalk's (1950) description of "arched" cells (convex) that resemble palisade cells. We feel confident that our observed semi-swollen AB epidermal cell type is the same as that described by Denton (1973) and Metcalfe and Chalk (1950). We should note that the

semi-swollen epidermal cell type could possibly be confused with the swollen epidermal cell type when looking only at epidermal peels, but when assessing the side view of these cells it is clear that the semi-swollen epidermal cells have a conical-shaped profile, while the swollen epidermal cells have a spherical-shaped profile. The presence of semi-swollen AB epidermal cells in the outgroup (*Ionoxalis*) and two of the deepest diverging clades in southern African *Oxalis* (Clade 2 and Clade 3) leads us to speculate that this was one of the ancestral states of the southern African *Oxalis* genus, and that this trait was lost on the branch leading to Clade 4.

#### 2.9.1.4 Swollen type pavement cells

Swollen epidermal cells found on the AD and AB surfaces of leaflets were unique to a subclade of Clade 7, therefore this trait was regarded as being phylogenetically informative. Swollen epidermal cells have been described in southern African *Oxalis* (Salter, 1944) and in the South American *O. carnososa* Molina (Steudle, 1983). These swollen cells were described by Salter (1944) as being “comparatively large” and the majority of swollen-celled *Oxalis* species were grouped into Salter’s (1944) section *Foveolatae*. This trait was used as one of the defining characteristics of this section. Salter’s (1944) grouping of species was almost identical to our Clade 7, except that he included *O. furcillata*, which does not display the swollen epidermal cell type and is included in Clade 8. Salter (1944) excluded *O. obtusa* from sect. *Foveolatae*, but both the ITS tree and the results of our study indicate that *O. obtusa* does belong in Clade 7. Oberlander *et al.* (2009) suggested that this trait could be a synapomorphy for Clade 7, but our results show that this character state does not typify the entire clade, and would be more correctly considered a synapomorphy for the larger daughter clade within Clade 7. *O. oreithala*, *O. algoensis* (EZ.), *O. fourcadei* (S.), *O. lawsonii* (B.) and *O. senecta* (S.) are other, currently unsampled species in Salter’s (1944) section *Foveolatae*, but we predict that these species will also be included in Clade 7 based on the presence of swollen epidermal cells.

Bladder cells that are superficially similar to our swollen epidermal cell type have been observed on the AB leaflet surface of the distantly related South American *O. carnososa* (Steudle, 1983). Although Steudle (1983) also considered the large AD epidermal cells of *O. carnososa* to be bladder cells, these are clearly not comparable to our swollen type, as they are not swollen out of the epidermal surface and are not interspersed with smaller epidermal cells and stomata. The bladder cells of Steudle (1983) are somewhat deeper ( $163.5 \pm 16.8 \mu\text{m}$  vs  $109.0 \pm 42.1 \mu\text{m}$ ) and thinner ( $126.6 \pm 20.5 \mu\text{m}$ ) as measured using Steudle (1983) images imported into ImageJ, ( $155.4 \pm 57.9 \mu\text{m}$ ) than our swollen epidermal type, but otherwise clearly conform to our definition. It is thus possible that our swollen epidermal cell type serves similar functions as the bladder cells in *O. carnososa*. This character state has evolved more than one in *Oxalis*, however *O. carnososa* and the southern African *Oxalis* clade are distantly related.

These cells aid in water storage (Hill and Hill (1976); Luttge (1976)) and the elimination of metabolically produced salts (Luttge *et al.* (1978)). The physiological study of Steudle (1983) set out to determine the function of the bladder cells of a South American *Oxalis* species (*O. carnososa*) by studying the water relations of these cells. They found that the bladder cells contain large amounts of oxalic acid. This study ruled out the function of water storage in this *Oxalis* species, and speculated that the bladder cells help to get rid of the metabolically produced acid. In southern African *Oxalis* it is possible that these bladder cells fulfil a similar function. Epidermal bladder cells reportedly release their contents (supposedly excess salts namely  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$  (Luttge *et al.* (1987); Adams *et al.* (1998); Agarie *et al.* (2007)) or metabolic compounds including calcium oxalate crystals (Adams *et al.* (1992); Jou *et al.* (2004); Agarie *et al.* (2007))) only when the bladders rupture. The bladder cells of southern African *Oxalis* are ruptured only when predated on by herbivores, so these cells

and their contents could fulfil an anti-herbivorous function (Salter, 1944). Other possible functions of bladder cells include the role of a secondary epidermis to reduce water loss and prevent excessive UV damage (Vogelman *et al.*, 1996).

## 2.9.2 Trichomes

The type, presence, absence and location of trichomes are important diagnostic characters in plant identification and taxonomy (Davis and Heywood, 1963). Previous systematic studies on angiosperm families have included trichome characteristics and successfully delineated clades based on these traits, for example Mentheae (Moon *et al.*, 2009) and *Ballota* (Osman, 2012). The taxonomic work on *Oxalis* section *lonoxalis* reported that pubescence hair types, densities and lengths were taxonomically significant traits. Despite this information from the literature, neither the glandular nor the non-glandular hairs observed in South African *Oxalis* taxa were phylogenetically significant (only two very weak traits of the presence of inflated hairs in members from Clade 7 and Clade 8, and the absence of clavate hairs in Clade 8). The lack of a phylogenetically significant pattern is not too surprising as immense variation in trichome traits (trichome types and position) was observed within and between species.

Substantial trichome variability in non-southern African *Oxalis* species have been reported in other studies (mostly from species descriptions) for example: *O. stricta* L. is reportedly 'sometimes pubescent' (Bryson, 2009), *O. cornellii* has glandular and non-glandular trichomes (Wiggins and Porter, 1971), *O. corniculata* L. has non-glandular trichomes (long single cellular hairs) (Freire *et al.*, 2005) on leaves that are 'pubescent to nearly glabrous' and *O. megalorrhiza* L. (synonym: *O. carnososa* (Molina)) is glabrous. The leaves of *O. simplicifolia* are glabrous (Lorence and Wagner, 2011) and glandular and non-glandular trichomes were described in *O. debilis* Knuth. and *O. corniculata* (dos Reis and Alvim, 2013). These examples show that trichome traits are variable within and between other *Oxalis* species, which mirror our findings.

Several factors might influence our trichome observations beyond phylogenetic relatedness. Trichome presence, type and density can be influenced by local environmental conditions, the age and condition of the plant or by local selective pressures over longer time scales (Hull and Morton, 1975). Trichomes can be shed with age (Salter, 1944). This phenomenon could possibly explain the presence or absence of trichomes we observed between multiple samples from the same species. It is possible that trichome traits were phylogenetically more conservative and phylogenetically informative than our study has shown for leaflets alone. Future phylogenetic studies should assess all trichomes present on the plants. Leaf surfaces have been described as being the "multifunctional interfaces between a plant and environment" (Eller, 1979), so the different types of trichomes we observed could possibly fulfil specific functions depending on the plant's environment. Trichomes are classically regarded as a plant's defence mechanism against insects (eating the leaves) (Levin, 1973). Glandular-haired species were described as being "sticky" to trap insects, and the species *O. droseroides* was described as being "densely clothed" with sticky glandular hairs (Salter, 1944). Glandular hairs often fulfil a secretory function to repel insects or to get rid of excess substances. In southern African *Oxalis* glandular hairs were commonly observed along the margins of leaflets, and below the mid-rib. These glandular hairs could possibly be excreting substances, or preventing insects from coming into contact with the vulnerable leaflet areas. Denton (1973) reported that the glandular heads of the glandular hairs found in sect. *lonoxalis* often contained oily materials. In our study we observed contents within the glandular heads, but did not test composition of contents, so cannot speculate further on the function of these cells. Short and long unicellular non-glandular hairs were commonly observed in southern African *Oxalis* species, and these hair types could possibly assist plants with protection against radiation. Simple hairs supposedly help regulate the radiation

that could indirectly also play a role in the temperature regulation of a leaf (Eller (1979); Eller (1985)).

Variation of trichome traits on the plant in general, and leaves specifically, for species in the *lonoxalis* clade has been recorded (Denton, 1973). Pubescence was suggested to be uniform for geographically restricted species, which is correlated to similar climatic conditions and reduced sexual reproduction (seed sterility) (Denton, 1973). *O. corymbosa* DC. was used as an example of a species that is seed-sterile in North America and has only single-cellular hairs (Denton, 1973). She speculated that this consistent hair type was the result of prolific vegetative propagation. The majority of southern African *Oxalis* species are self-sterile and can be prolifically clonal, so this could influence the trichome types observed.

### 2.9.3 Stomata

Both the environment and evolutionary history of plants play important roles in determining and shaping plant physiology, especially in water-stressed environments (Willson *et al.*, 2008). Stomatal traits determine water-use efficiencies, gas exchange and photosynthetic rates, so it is possible to expect correlated evolution of stomatal traits and leaf anatomical traits in similar environments. However, not much research has focussed on these traits within an evolutionary context (Beerling and Kelly (1996); Brodribb *et al.* (2009); Dunbar-Co *et al.* (2009); Brodribb (2011); Haworth *et al.* (2011)).

#### 2.9.3.1 Stomatal position

The position of stomata on leaflets was phylogenetically significant across southern African *Oxalis* clades. Taxa with hypostomatic leaflets were commonly observed in the non-South African *lonoxalis* clade. A recent comparative study on three South American *Oxalis* species also report all studied species to be hypostomatic (*O. latifolia* H.B.K., *O. debilis*, *O. corniculata*) (dos Reis and Alvim, 2013). *O. latifolia* and *O. debilis* are included in sect. *lonoxalis*, so this data adds to our confidence of stomatal placement in this section. Denton (1973) also found that North American species from *lonoxalis* mostly had hypostomatic leaflets. AB located stomata have been described for *O. carnosa* (Steudle, 1983). Members of *lonoxalis* (*O. corniculata* and *O. carnosa*) are phylogenetically distantly related, so this suggests that AB located stomata are widespread and ancestral in the *Oxalis* genus. We thus propose that AB located stomata are the ancestral state for the southern African *Oxalis* genus. AB located stomata still characterise the species-poor Clade 2 and Clade 3, in contrast to Clade 4, which is characterised almost universally by stomata on the adaxial (AD) leaflet surface.

The major exception to AD stomata in the speciose Clade 4 is in Clade 5, the *Stellata* Clade of Oberlander *et al.* (2011), which apart from a single species (*O. imbricata* (MO345)) is hypostomatic. This implies a fairly complex history of character change for stomatal position in the lineage leading towards Clade 5. However, it must be noted that the *Stellata* Clade was one of the most unexpected clades retrieved by Oberlander *et al.* (2011), comprising a mix of morphologically dissimilar species. Recent phylogenetic results with massively increased sampling from hundreds of low-copy nuclear loci cause this clade to disintegrate, with *O. imbricata* being deeply embedded in Clade 4 and the remainder of the *Stellata* Clade resolving as sister to Clade 3 (R. Schmickl, unpublished data). Both positions are supported by morphological characters (K.C. Oberlander, *pers. comm.*). These new positions imply a much simpler scenario for the evolution of stomatal position deep in the southern African *Oxalis* lineage, with a single, un-reversed change from an ancestral hypostomatic southern African taxon to epistomaty in Clade 4. It is possible that epistomaty could serve as a key innovation for the massive Clade 4, which contains the vast majority of southern African *Oxalis* diversity.

Taxa with amphistomatic leaflets were observed only in Clade 7 and therefore this character was regarded as unique to this clade. Amphistomatic leaves frequently occur in xeric habitats (Parkhurst, 1978) and amphistomaty is regarded as an adaptation to enable the maximum conductance of a leaf (Parkhurst (1978); Gutschick (1984)). It is then possible to assume that taxa with amphistomatic leaflets evolved under conditions where high conductance rates were favoured, and are still maintained, and therefore set current taxa at an advantage (Muir, 2015).

An interesting association between the stomata and epidermal pavement cell types of amphistomatic taxa from Clade 7 was detected, namely all taxa with amphistomatic leaflets had swollen epidermal cell types (bladder cells) on both the AD and AB leaflet surfaces. It is possible that the evolution of these two characters is correlated. A similar association was reported in the Aizoaceae (Bohley *et al.*, 2015). Further research is needed to test this potential association.

### 2.9.3.2 Stomata in crevice

The presence of additional stomata above the mid-rib on either the AD and AB leaflet surfaces was not a trait with a strong enough phylogenetic pattern to typify clades, but frequently occurred in two clades (additional stomata on AD surface of Clade 2 and additional stomata on the AB surface of Clade 8). This trait was not observed in any of the out-group taxa studied, nor mentioned in the literature on southern African or global *Oxalis* species. This phenomenon has been recorded in other angiosperms, for example *Solanum* L. (Benitez and Ferrarotto, 2009).

The spacing of stomata is theoretically not random (Glover, 2000). We agree with this suggestion and reason that these additional stomata above the midrib have functional significance. Leaflet conductance data show that these additional vein-associated stomata (very often less than 30 stomata in total) transpire, or emit enough water vapour, for conductance readings to be detected (Chapter 2).

### 2.9.3.3 Stomatal position and evolution

The presence of additional stomata above the central vein of leaflets is widely scattered throughout the southern African *Oxalis* phylogeny, and is restricted to a small number of species. This suggests that this trait can easily evolve in *Oxalis* species, but it does not persist for a long time. However, it does suggest a viable mechanism for the change from hypostomatic leaflets (*Ionoxalis*, Clades 2 and 3) to epistomatic leaflets (Clade 4), through a viable short-lived intermediate state of having amphistomatic leaflets. This raises the interesting question as to why the majority of species did not maintain this trait of having amphistomatic leaflets?

### 2.9.3.4 Stomatal length, density and ploidy level

The average stomatal central axis lengths and the average stomatal densities of AD and AB located stomata were extremely variable and therefore did not show any phylogenetically significant pattern. The variability of these traits is most probably influenced by other factors, such as ploidy level and environmental conditions. To our knowledge, no previous studies have assessed the stomatal lengths and densities of *Oxalis* species, so we do not have other datasets with which to compare our work. We found a significant negative relationship between stomatal size and stomatal density, a well-known relationship that has been described in many angiosperms (Sack *et al.* (2003); Beaulieu *et al.* (2008); Zhang *et al.* (2012); Jooste (2015) Chapter 3).



### 2.9.3.5 Stomatal complex

The stomatal complex types observed in *Oxalis* did not show any phylogenetic pattern as the anomocytic type occurs in almost all taxa. Anisocytic, 4-celled anisocytic and actinocytic types did not show any phylogenetic pattern, and neither did any combinations of stomatal complex types. We therefore interpret stomatal complex types as being extremely variable. Studies on non-southern African *Oxalis* species reported anomocytic stomatal complex types in *O. corniculata* (Freire *et al.*, 2005), *O. debilis* and *O. latifolia* (dos Reis and Alvim, 2013). Anomocytic and paracytic stomatal complex types have been reported in sect. *Ionoxalis* (Denton, 1973) and Oxalidaceae in general (Metcalf (1979); Freire *et al.* (2005)), but we are unaware of studies showing anisocytic, 4-celled anisocytic and actinocytic stomatal complexes in any other *Oxalis* species.

Studies on stomatal complex types of selected angiosperms have reported that an anomocytic stomatal complex was the most common stomatal type, and that it co-occurred with a variety of other stomatal types (Cantino (1990); Moon *et al.* (2009)). Multiple stomatal types within a leaf are a common occurrence (Branova, 1992), and these traits are suggested to be taxonomically significant. Stomatal complex type, together with stomatal proportions are taxonomically informative (Den Hartog and Baas (1978); Wilkinson (1979); Branova (1992)). Despite the general consensus that stomatal complex type can be taxonomically informative, we could not detect any clear pattern based on these traits among southern African *Oxalis* species. It is possible that there is genuinely no pattern or that we could not detect patterns due to our interpretation and classification system. Previous studies have noted that interpretation of stomatal types for taxonomic use could be problematic (Soh and Parnell, 2011), especially distinguishing anomocytic and anisocytic stomatal types (Metcalf and Chalk (1950); Patel and Inamdar (1971); van Welzen and Baas (1984)). In southern African *Oxalis* multiple combinations of different stomatal complexes were observed, so we concluded that these traits were too variable to detect phylogenetic patterns. The work of Patel and Inamdar (1971) showed that multiple stomatal types could develop during the life history of a species, and this might account for some of the observed variation among *Oxalis* species.

## 2.10 Discussion: Mesophyll tissue traits

### 2.10.1 Mesophyll arrangement type

Mesophyll types have been included in taxonomic and phylogenetic studies such as in the Amaryllidaceae (Traub (1968); Fritsch (1988)), Salsoleae (Botschantzev (1969*b*); Botschantzev (1969*a*); Pyankov *et al.* (2001)) and Adoxaceae (Chatelet *et al.*, 2013). In south American *Oxalis* only bifacial mesophyll arrangement types have been recorded (dos Reis and Alvim (2013), Toma *et al.* (2007)) and this data agrees with the two out-group taxa used in our study. The mesophyll arrangement types observed in southern African *Oxalis* appeared to be phylogenetically significant traits, especially isobilateral Type 1 and isobilateral Type 2 mesophyll arrangements.

The distribution of southern African taxa with isobilateral Type 2 arrangement is regarded as a phylogenetically significant trait for defining Clades 11 and 13. To our knowledge there is no available morphological (Salter, 1944), palynological (Dreyer, 1996) or genetic (Oberlander *et al.*, 2011) evidence to support a close relationship between these two clades. This trait therefore appeared to have evolved twice among southern African *Oxalis* species. Isobilateral mesophyll arrangement (Type 1 and Type 2) is commonly observed in southern African *Oxalis* species, and it is possible that these traits evolved independently, multiple times. Elongated shape of palisade cells increases the surface area to volume ratio, which is beneficial as CO<sub>2</sub> can reach chloroplasts more easily (Turrell, 1936). Reportedly the structure of palisade cells is regarded as optimal for photosynthesis (Chatelet

*et al.*, 2013), so given this information we can speculate that leaves with palisade in the AD and AB layers (isobilateral) are at an even greater advantage, as they could be photosynthetically more efficient, even more so when leaflets are held erect as have been observed in many of these species.

We should mention that the mesophyll arrangement type (isobilateral Type 2) with uniformly rounded palisade cells, could actually be immature cells of isobilateral Type 1, observed due to heteroblasty development that is common among *Oxalis* species (Goebel, 1898). The morphology of heteroblasty in sect *Ionoxalis* has been described, but no anatomical studies have yet been done to assess the internal differences between young and old leaves (Denton, 1973). For our study we strived to select only mature leaves, but there is always a possibility that we accidentally selected younger leaves. However, it seems unlikely that such an argument could apply to two entire clades (Clades 11 and 13). We therefore feel confident in our allocation of this trait, and suggest that mesophyll arrangement type is a good phylogenetically informative trait that can delimit clades within southern African *Oxalis* genus.

## 2.10.2 Mesophyll thickness

The number of palisade or spongy layers, the palisade and spongy layer heights and the percentage palisade to spongy mesophyll data were extremely variable. These traits showed no clear phylogenetic pattern, but reflected (and confirmed) the described mesophyll arrangement types. For example, taxa with isobilateral mesophyll arrangements would have more palisade tissue and less spongy tissue reflected in the number of layers and layer heights. In many leaflets the dividing line between palisade or spongy tissue types was unclear and the angle of sectioning (if not perpendicular) could possibly have obscured cells or a layer of cells. The number of mesophyll cells per layer type could therefore possibly vary with one cell layer (due to misidentification), which would have an effect on the variation observed in our data.

The total mesophyll section heights and total leaflet section heights showed no clear pattern, as both these traits displayed tremendous variability in southern African *Oxalis* and the lack of a clear pattern could be influenced by a few factors. The areas of leaflets sectioned could have varied (even though we standardised to the middle region of a leaflet); within a transverse section of a leaflet we measured the mesophyll height in various positions between the mid-rib and the leaflet margin and calculated average representative values, so a better protocol, standardising to another region of the leaflet, might have yielded less variable results. Also, our sampling possibly included too few replicates to represent a species, as mesophyll thickness could be a trait that is influenced by environmental conditions. There is also no clear association between mesophyll arrangement type and mesophyll thickness.

Plants can respond to and regulate the influence of environmental factors such as light intensity, rainfall and UV-radiation (Manetas *et al.* (1997); Cunningham *et al.* (1999); Burrows (2001); Kerstiens (2006); Cutler *et al.* (2008)), by altering their internal leaf structure, which includes the amount and distribution of palisade and spongy mesophyll tissue (Parkhurst and Mott (1990); Parkhurst (1994); Terashima and Hikosaka (1995); Vogelmann *et al.* (1996)). Leaf structural traits play an important role in the way in which plants adapt to water stress (Ashton and Berlyn (1992); Gratani and Bombelii (1999)) and plants with thicker leaves are more common in arid areas (Cunningham *et al.* (1999); Gratani and Bombelii (1999); Wang *et al.* (2011)). This could be another factor that caused variability in our data, even though all wax-embedded samples were collected only from the living collection (where plants were exposed to the same environmental conditions). The possibility still remains that these plants have not yet properly acclimatised to the garden conditions, but we cannot really make any conclusions without sampling from different field localities too.

### 2.10.3 Cavities within the mesophyll

The presence and absence and the type of cavity (with or without epithelial lining) found in the mesophyll of southern African *Oxalis* taxa did not show any strong phylogenetic pattern (cavities were present in all taxa from Clade 7, except *O. nortieri* (MO503)). To our knowledge, no other studies have reported cavities in the mesophyll of other *Oxalis* species. Variation of this trait could be dependant on leaflet age, as cavities could possibly be absent from young leaves. Even though the sectioning of leaflets was done following our protocol, it is possible that areas of leaflets with cavities were not sectioned, and were therefore not observed. The presence of the epithelial lining was always very clear, so we feel confident in our identification of this trait.

The anatomical study of Retamales *et al.* (2014) showed that the secretory cavities observed in the leaves of Myrtaceae produce lipophilic compounds, according to their histochemical stains. The images they provided in that study look similar to those observed in southern African *Oxalis* species, but it is not possible to draw any further conclusions. In our study no histochemical tests were done, but this is something to consider for future studies. We can, however, note that the majority in cavities of southern African *Oxalis* leaflets seemed to contain crystals.

### 2.10.4 Crystals

The crystals observed in southern African *Oxalis* were extremely variable in terms of their location in the mesophyll layers, whether they were loose or in clusters, free or inside cavities (with or without epithelial lining) and different crystal types that co-occurred in one leaflet. It is not surprising that we did not detect any phylogenetic pattern based on crystal traits. *Oxalis* are well-known for their high concentrations of calcium oxalate present in leaf tissue (Jones and Luchsinger (1987); Šircelj *et al.* (2010); Abhilash *et al.* (2011)). Trends of calcium oxalate deposits in some *Ionoxalis* species have been described, but these traits were not as taxonomically useful as previously assumed (Denton, 1973). Many taxonomic studies on angiosperms have reported that crystal type and their distribution were taxonomically significant traits (Metcalf and Chalk (1950); Metcalfe (1983); Cervantes-Martinez *et al.* (2005); Lersten and Horner (2005); Horner *et al.* (2009); Lersten and Horner (2011)).

In our study a possible explanation for all the above-mentioned variation could be due to the sectioning and staining method used to prepare leaflet material. Crystals are rigid in comparison to the epidermal and mesophyll cells, so it is possible that these crystals were resistant to the microtome blade, and therefore moved from their original position or they could have been damaged or disrupted when cut. Another possibility is that the crystals shifted (different position in the mesophyll, or moved out of the cavities) during the staining procedure. An interesting study on the dietary/nutritional value of an European *Oxalis* species (*O. acetosella*) found that old leaves have higher oxalate concentration (and possibly more crystals) than young leaves (Šircelj *et al.*, 2010). From this information we can speculate that crystal traits could vary between old and young leaves, and could be another explanation for the variability observed (even though we standardised to sample only mature leaves).

Crystal formation is usually associated with membranes or chambers found within a cell vacuole (Franceschi and Horner, 1980). The crystals observed in the majority of southern African *Oxalis* were located inside cavities, with or without epithelial linings. The majority of these cavities were larger than the surrounding mesophyll cells, and were duct-like. We suspect that they are not merely single modified cells, but ducts. There were some taxa that appeared to have 'free' crystals that were located in between the mesophyll cells (and usually no cavities were observed), but these are probably better explained by crystal movement during sectioning and staining.

Calcium oxalate deposits, in the form of cubical crystals (with a tendency to cluster), were documented in sect. *lonoxalis* (Denton, 1973). Reportedly, these deposits were often associated with tannins, which turn the deposits orange, red-orange, red or black in colour. The presence of these deposits in leaves (and the vegetative tissues in general) is reportedly the reason why so few insects or other animals attack the leaves of *Oxalis* (Denton, 1973). Deposits were observed in the tips of sepals, bracts, bulb scales and leaves of *lonoxalis* species (Denton, 1973). Similar deposits were seen in southern African *Oxalis* species from Clade 7 and Clade 13 and were used to define section *Stictophyllae* (which corresponds to Clade 7) and sub-section *Pardales* (which corresponds to Clade 13) (Salter, 1944). In all likelihood these markings correspond to the channels with epithelial linings and the compounds that turn black when dried (Salter, 1944). Clade 7 and Clade 13 are completely unrelated, therefore the presence of this trait could potentially be of phylogenetic significance.

## 2.11 Discussion: Vascular tissue traits

The venation types and vascular tissue traits observed in southern African *Oxalis* did not show strong phylogenetic significance. This is surprising, because leaf venation patterns are diverse and are expected to show strong phylogenetic pattern (Uhl and Mosbrugger (1999); Broadribb and Feild (2010); Walls (2011)).

Palmate and pinnate venation types were identified among southern African taxa. Palmate venation was observed only in a few taxa from Clade 7 and Clade 8. Pinnate venation types were recorded in Oxalidaceae in general (Jones and Luchsinger, 1987) and sect. *lonoxalis* (Denton, 1973), but to our knowledge palmate venation has not previously been recorded in other *Oxalis* species. Leaflet venation types (pinnate or palmate) were not always clear, especially in the lobed, heart-shaped leaflets where the mid-rib is short relative to the two lobes of the leaflet. It is therefore possible that this trait has been simplified as a discrete character, when it possibly should have been treated as a continuous trait (where pinnate and palmate venation were the extremes in a continuum). Our observations of leaf venation type were based on pressed and dried leaflet material, but we feel that this approach was not entirely sufficient, as the dried mesophyll could have obscured the underlying venation pattern. Future studies on *Oxalis* leaflet venation should follow an established protocol to clear, stain and mount leaflets, for example the protocol described by Vasco *et al.* (2014).

The trait of embedded or protruding vascular mid-rib tissue did not show any phylogenetic pattern, as the trait of a protruding mid-rib was commonly observed in taxa from all *Oxalis* clades. This phenomenon has also been recorded in three South American species (dos Reis and Alvim, 2013). Due to the random phylogenetic distribution and common occurrence of this trait, we speculate that it could have more functional significance. Taxa with a protruding mid-rib had large amounts of collenchymous tissue located beneath the vascular bundle, possibly to support and protect the leaf lamina and protect the vascular tissue.

*Oxalis* taxa with sheaths around their vascular bundles were distributed throughout the phylogeny, but all members from Clades 11 and 12 had these sheaths, which we regarded as one of the diagnostic traits of these two clades. Similar sheaths were described around the periphery of the vascular bundles in *O. corniculata* (Toma *et al.*, 2007). The wax embedded leaflet material showed that the arrangement of xylem and phloem inside the vascular tissue was more or less uniformly arranged in all studied taxa, therefore the amount of phylogenetically significant data gathered from transverse sections of vascular tissue was limited.

## 2.12 Conclusion

The southern African *Oxalis* radiation is extremely morphologically variable and few characters are known as potential synapomorphies supporting clades, hindering species identification, taxonomic revision and understanding of functional trait evolution in this southern African clade (Salter (1944); L.L. Dreyer, *pers comm.*). Sixty-eight leaflet anatomical traits of 109 southern African *Oxalis* species were assessed in search of phylogenetically significant characters that delineate clades. This study showed that the combination of six leaflet anatomical traits (stomatal position, adaxial epidermal cell types, abaxial epidermal cell types, mesophyll type, sheath around vascular tissue and degree of leaflet conduplication) clearly support various clades defined by previous DNA-based phylogenetic work. Despite the phylogenetic patterns detected in the aforementioned traits, other leaflet anatomical traits were highly variable and showed no phylogenetic pattern. These traits could possibly hold functional significance and this is explored in more detail in Jooste (2015) (Chapter 3). The information gathered in this study will aid in the taxonomic revision of this speciose member of the Greater Cape Floristic Region and provide a basis for future hypotheses regarding its radiation.

## 2.13 References

- Abhilash, P.A., Nilasha, Pratapan, A., Suresh, V.N., Lizocherian, O., Sunitha, T.K. and Raghu, K.G. (2011). Cardio protective effects of aqueous extract of *Oxalis corniculata* in experimental myocardial infarction. *Experimental and Toxicological Pathway*, vol. 63, pp. 535–540.
- Abràmoff, M.D., Magalhães, P.J. and Ram, S.J. (2004). Image processing with ImageJ. *Biophotonics International*, vol. 11, no. 7, pp. 36–41.
- Adams, P., Nelson, D.E., Yamada, S., Chmara, W., Jensen, R.G., Bohnert, H.J. and Griffiths, H. (1998). Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytologist*, vol. 138, pp. 171–190.
- Adams, P., Thomas, J.C., Vernon, D.M., Bohnert, H.J. and Jensen, R.G. (1992). Distinct cellular and organismic responses to salt stress. *Plant and Cell Physiology*, vol. 33, pp. 1215–1223.
- Agarie, S., Shimoda, T., Shimizu, Y., Baumann, K. and Sunagawa, H. (2007). Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladdercell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. vol. 58, no. 8, pp. 1957–1967.
- Ashton, P.M.S. and Berlyn, G.P. (1992). Leaf adaptations of some *Shorea* species to sun and shade. *New Phytologist*, vol. 121, pp. 587–596.
- Bailey, C. (2001). Botanical sections. *Balsam Post*, vol. 53, pp. 13–17.
- Beaulieu, J.M., Leitch, I.J., Patel, S., Pendharkar, A., Knight, C.A. and Beaulieu, J.M. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist*, vol. 179, pp. 975–986.
- Berling, D.J. and Kelly, C.K. (1996). Evolutionary comparative analyses of the relationship between leaf structure and function. *New Phytologist*, vol. 134, pp. 35–51.
- Benitez, C. and Ferrarotto, M. (2009). Morfología de la epidermis foliar en dos grupos de *Solanum* sección Geminata (Solanaceae). *Caldasia*, vol. 31, no. 1, pp. 31–40.
- Benson, M. and Borrill, M. (1969). The significance of clinical variation in *Dactylis marina* Borril. *New Phytologist*, vol. 68, pp. 1159–1173.
- Bohley, K., Joos, O., Hartmann, H., Sage, R., Liede-schumann, S. and Kadereit, G. (2015). Phylogeny of *Sesuvioideae* (Aizoaceae) Biogeography, leaf anatomy and the evolution of C<sub>4</sub> photosynthesis. *Perspectives in Plant Ecology, Evolution and Systematics*, vol. 17, no. 2, pp. 116–130.
- Botschantzev, V.P. (1969a). Review of species of *Coccosalsola* Fenzl section of genus *Salsola* L. (in Russian). *News Systematics Higher Plants*, vol. 13, pp. 74–102.
- Botschantzev, V.P. (1969b). The genus *Salsola* L. (composition, history of development and distribution). Summary of report on published papers presented instead of doctor degree thesis. Nauka, Leningrad (in Russian).

- Broadribb, T.J. and Feild, T.S. (2010). Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology letters*, vol. 13, pp. 175–183.
- Brodersen, C.R. and Vogelmann, T.C. (2007). Do epidermal lens cells facilitate the absorptance of diffuse light? *American Journal of Botany*, vol. 94, pp. 1061–1066.
- Brodribb, T.J. (2011). Passive origins of stomatal control in vascular plants. *Science*, vol. 331, pp. 582–585.
- Brodribb, T.J., McAdam, S.A.M., Jordan, G.J. and Feild, T.S. (2009). Evolution of stomatal responsiveness to CO<sub>2</sub> and optimization of water-use efficiency among land plants. *New Phytologist*, vol. 183, pp. 839–847.
- Bryson, C. (2009). *Weeds of the South*. University of Georgia.
- Burrows, G.E. (2001). Comparative anatomy of the photosynthetic organs of 39 xeromorphic species from sub-humid New South Wales, Australia. *International Journal of Plant Sciences*, vol. 162, pp. 411–430.
- Cantino, P.D. (1990). The phylogenetic significance of stomata and trichomes in the Labiatae and Verbinaceae. *Journal of the Arnold Arboretum*, vol. 71, pp. 323–370.
- Cervantes-Martinez, T., Horner, T.H., Palmer, R.G., Hymowitz, T. and Brown, A.H.D. (2005). Calcium oxalate crystal macropatterns in leaves of species from groups *Glycine* and *Shuteria* (Glycininae; Phaseoleae; Papilionoideae; Fabaceae). *Canadian Journal of Botany*, vol. 83, pp. 1410–1421.
- Chatelet, D.S., Clement, W.L., Sack, L., Donoghue, M.J. and Edwards, E.J. (2013). The evolution of photosynthetic anatomy in *Viburnum* (Adoxaceae). *International Journal of Plant Science*, vol. 174, no. February, pp. 1277–1291.
- Cronquist, A. (1981). *An integrated system of classification of flowering plants*. Columbia University Press, New York.
- Cunningham, S.A., Summerhayes, B. and Westoby, M. (1999). Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrients. *Ecological Monographs*, vol. 69, pp. 569–588.
- Cutler, D.F., Botha, C.E.J. and Stevenson, D.W. (2008). *Plant anatomy, an applied approach*.
- Davis, P.H. and Heywood, V.H. (1963). *Principles of Angiosperm Taxonomy*. Van Nostrand, Princeton, New Jersey.
- De Villiers, M.J. (2001). A systematic revision of *Oxalis* L. section *Crassulae* T.M. Salter (Oxalidaceae).
- Den Hartog, R.M. and Baas, P. (1978). Epidermal characters of the Celastraceae sensu lato. *Acta Botanica Neerlandica*, vol. 27, pp. 355–388.
- Denton, M.D. (1973). *A monograph of Oxalis Section Ionoxalis (Oxalidaceae) in North America*.
- dos Reis, A.E. and Alvim, M.N. (2013). Anatomia foliar comparada de três espécies do gênero *Oxalis*. *Belo Horizonte, MG*, vol. 3, no. 6, pp. 59–72.
- Dreyer, L.L. (1996). A palynological review of *Oxalis* (Oxalidaceae) in southern Africa.
- Drummond, A.J., Suchard, M.A., Xie, D. and Rambaut, A. (2012). Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, vol. 29, no. 8, pp. 1969–1973.
- Dunbar-Co, S., Sporck, M.J. and Sack, L. (2009). Leaf trait diversification and design in seven rare taxa of the Hawaiian Plantago radiation. *International Journal of Plant Sciences*, vol. 170, pp. 61–75.

- Eller, B.M. (1979). Die strahlungsokologische Bedeutung von Epidermisauflagen. *Flora*, vol. 168, pp. 146–192.
- Eller, B.M. (1985). Epidermis und spektrale Eigenschaften pflanzlicher Oberflachen. *Deutschen Botanischen Gesellschaft*, vol. 98, pp. 465–475.
- Ensikat, H.J., Ditsche-Kuru, P., Neinhuis, C. and Barthlott, W. (2011). Superhydrophobicity in perfection: the outstanding properties of the lotus leaf. *Bellstein Journal of Nanotechnology*, vol. 2, pp. 152–161.
- Esau, K. (1953). *Plant Anatomy*. Wiley, New York.
- Esau, K. (1965). *Plant Anatomy*. John Wiley and Sons Inc., New York, London, Sydney.
- Franceschi, V.R. and Horner, H.T. (1980). Calcium Oxalate Crystals in Plants. *Botanical Review*, vol. 46, pp. 361–427.
- Freire, S.E., Arambarri, A.N.A.M., Bayón, N.D., Sancho, G., Urtubey, E., Monti, C., Novoa, M.C. and Colares, M.N. (2005). Epidermal characteristics of toxic plants for cattle from the Salado river basin (Buenos Aires, Argentina). *Bol. Soc. Argent. Bot.*, vol. 40, no. 3-4, pp. 241–281.
- Fritsch, R.M. (1988). Anatomical investigation of the leaf blade of *Allium* L. (Alliaceae). I. Species having one row of vascular bundles. *Flora*, vol. 181, pp. 83–100.
- Gebregziabher, A.K. (2004). Systematic significance of bulb morphology of the southern African members of *Oxalis* L. (Oxalidaceae).
- Gkikas, D., Argiropoulos, A. and Rhizopoulou, S. (2015). Epidermal focusing of light and modelling of reflectance in floral-petals with conically shaped epidermal cells. *Flora - Morphology, Distribution, Functional Ecology of Plants*, vol. 212, pp. 38–45.
- Glover, B. (2007). *Understanding Flowers and Flowering*. Oxford University Press, Oxford.
- Glover, B.J. (2000). Differentiation in plant epidermal cells. *Journal of experimental botany*, vol. 51, no. 344, pp. 497–505.
- Goebel, K. (1898). *Organographie der Pflanzen*. pp. 1–158.
- Gratani, L. and Bombelli, A. (1999). Leaf anatomy, inclination and gas exchange relationships in evergreen sclerophyllous and drought semideciduous shrub species. *Plant Physiology*, vol. 37, pp. 573–585.
- Gutschick, V.P. (1984). Photosynthesis model for C<sub>3</sub> leaves incorporating CO<sub>2</sub> transport, propagation of radiation, and biochemistry 2. ecological and agricultural utility. *Photosynthetica*, vol. 18, pp. 569–595.
- Haberlandt, G. (1914). *Physiological Plant Anatomy*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. and Challenger, W. (2008). GEIGER: investigating evolutionary radiations. *Bioinformatics*, vol. 24, pp. 129–131.
- Haworth, M., Elliot-Kingston, C. and McElwain, J.C. (2011). Stomatal control as a driver of plant evolution. *Journal of Experimental Botany*, vol. 62, pp. 2419–2423.
- Hill, A.E. and Hill, B.S. (1976). *Elimination processes by glands: mineral ions*. In: *Encyclopedia of plant physiology, N. S., vol. 2: Transport in plants II, pt. B: Tissues and organs*. Springer.
- Horner, H.T., Wanke, S. and Samain, M.S. (2009). Evolution and systematic value of leaf crystal macropatterns: A phylogenetic approach in the genus *Peperomia* (Piperaceae). *International Journal of Plant Sciences*, vol. 170, pp. 343–354.



- Hull, H.M. and Morton, H. L., W.J.R. (1975). Environmental influences on cuticle development and resultant foliar penetration. *The Botanical Review*, vol. 41, pp. 421–452.
- Johansen, D.A. (1944). *Plant Microtechnique*. McGraw-Hill, New York.
- Jones, S.B. and Luchsinger, A.E. (1987). *Plant Systematics (2nd ed.)*. McGraw-Hill Book Company.
- Jooste, M. (2015). The phylogenetic and functional significance of leaf anatomical and physiological traits of southern African *Oxalis*.
- Jou, T., Chou, P.H., He, M., Hung, Y. and Yen, H.E. (2004). Tissue-specific expression and functional complementation of a yeast potassium uptake mutant by a salt-induced ice plant gene mcSKD1. *Molecular Biology*, vol. 54, pp. 881–893.
- Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J. (2008). *Plant systematics: a phylogenetic approach, 3rd edition*. Sinauer Associates, INC., Sunderland, Massachusetts, USA.
- Kerstiens, G. (2006). Water transport in plant cuticles: An update. *Journal of Experimental Botany*, vol. 57, pp. 2493–2499.
- Korn, R.W. (1976). Concerning the Sinuous Shape of Leaf Epidermal Cells. *New Phytologist*, vol. 77, no. 1, pp. 153–161.
- Krauss, B.H. (1949). Anatomy of the vegetative organs of the pineapple, *Ananas comosus* (L.) Merr II. The leaf. *Botanical Gazette - Chicago*, vol. 110, pp. 333–404.
- Kürschner, W.M. (1997). The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/*Q. pseudocastanea* Goeppert) - Implications for their use as biosensors of palaeoatmospheric CO<sub>2</sub> levels. *Review of Palaeobotany and Palynology*, vol. 96, no. 1-2, pp. 1–30.
- Lee, D. (2007). *Nature's Palette, the Science of Plant Colour*. The University of Chicago Press, Chicago.
- Lersten, N.R. and Horner, H.T. (2005). Development of the calcium oxalate crystal macropattern in pomegranate (*Punica granatum*, Punicaceae). *American Journal of Botany*, vol. 92, pp. 1935–1941.
- Lersten, N.R. and Horner, H.T. (2011). Unique calcium oxalate "duplex" and "concentration" idioblasts in leaves of tribe Naucleaeae (Rubiaceae). *American Journal of Botany*, vol. 98, no. 1, pp. 1–11.
- Levin, D.A. (1973). The role of trichomes in plant defence. *The Quarterly Review of Biology*, vol. 48, pp. 3–15.
- Levy, F. and Moore, D. (1993). Population variations of leaflets sleep movements in *Oxalis grandis* (Oxalidaceae). *American Journal of Botany*, vol. 80, pp. 1482–1493.
- Lorence, D.H. and Wagner, W.L. (2011). *Oxalis simplicifolia*. *PhytoKeys*, pp. 53–60.
- Lourteig, A. (2000). *Oxalis* L. subgenera *Monoxalis* (Small) Lourteig, *Oxalis* Trifidus Lourteig. *Bradea*, vol. 7, pp. 201–629.
- Luttge, U. (1976). *Salt glands*. In: *Ion transport in plant cells and tissues*. North-Holland Publishing Co.
- Luttge, U., Fischer, E. and Steudle, E. (1978). Consideration of photosynthetic efficiency at low light as a major determinant of crop photosynthetic performance. *Plant, Cell and Environment*, vol. 1, pp. 121–129.
- Luttge, U., Fischer, E. and Steudle, E. (1987). Membrane potentials and salt distribution in epidermal bladders and photosynthetic tissue of *Mesembryanthemum crystallinum* L. *Plant, Cell and Environment*, vol. 1, pp. 121–129.

- Manetas, Y., Petropoulou, Y., Stamatakis, K., Nikolopoulos, D., Levizou, E. and et al. (1997). Beneficial effects of enhanced UV-B radiation under field conditions: Improvement of needle water relations and survival capacity of *Pinus pinea* L. seedlings during the dry Mediterranean summer. *Ecological Monograph*, vol. 128, pp. 101–108.
- Manning, J. and Goldblatt, P. (2012). *Plants of the Greater Cape Floristic Region 1: the Core Cape Flora*. Strelitzia 29, South African National Biodiversity Institute, Pretoria, South Africa.
- Matthews, M.L. and Endress, P.K. (2002). Comparative floral structure and systematics in Oxalidales (Oxalidaceae, Connaraceae, Brunelliaceae, Cephalotaceae, Cunoniaceae, Elaeocarpaceae, Tremandraceae). *Botanical Journal of the Linnean Society*, vol. 140, pp. 321–381.
- Mauseth, M.L. (1988). *Plant Anatomy*. Benjamin-Cummings, Menlo Park, California, USA.
- Metcalfe, C.R. (1979). *Anatomy of the dicotyledons, 2nd Ed. Vol. 1: systematic anatomy of leaf and stem, with a brief history of the subject*. Oxford Science Publications, Clarendon, Oxford.
- Metcalfe, C.R. (1983). *Secreted mineral substances - Crystals*. In C. R. Metcalfe and L. Chalk [eds.] *Anatomy of the Dicotyledons*, vol. II 2nd edition.
- Metcalfe, C.R. and Chalk, L. (1950). *Anatomy of the dicotyledons*, vol. 2 volumes.
- Moon, H.K., Hong, S.P., Smets, E. and Huysmans, S. (2009). Phylogenetic significance of leaf micromorphology and anatomy in the tribe Mentheae (Nepetoideae: Lamiaceae). *Botanical Journal of the Linnean Society*, vol. 160, no. 2, pp. 211–231.
- Muir, C.D. (2015). *Selection constrains phenotypic evolution in a functionally important plant trait*. Ph.D. thesis, University of British Columbia.
- Nakata, M. and Okada, K. (2013). The Leaf Adaxial-Abaxial Boundary and Lamina Growth. *Plants*, vol. 2, pp. 174–202.
- Neinhuis, C. (1997). Characterization and Distribution of Water-repellent, Self-cleaning Plant Surfaces. *Annals of Botany*, vol. 79, pp. 667–677.
- Oberlander, K.C., Dreyer, L.L. and Bellstedt, D.U. (2011). Molecular phylogenetics and origins of southern African *Oxalis*. *Taxon*, vol. 60, no. 6, pp. 1667–1677.
- Oberlander, K.C., Dreyer, L.L. and Esler, K.J. (2002). Biogeography of *Oxalis* (Oxalidaceae) in South Africa: a preliminary study. *Bothalia*, vol. 32, pp. 97–100.
- Oberlander, K.C., Emshwiller, E., Bellstedt, D.U. and Dreyer, L.L. (2009). A model of bulb evolution in the eudicot genus *Oxalis* (Oxalidaceae). *Molecular Phylogenetics and Evolution*, vol. 51, no. 1, pp. 54–63.
- Obone, C. (2005). The systematic significance of the fruit and seed morphology and anatomy in selected *Oxalis* L. (Oxalidaceae) species.
- Osman, A.K. (2012). Trichome micromorphology of Egyptian *Ballota* (Lamiaceae) with emphasis on its systematic implication. *Pakistan Journal of Botany*, vol. 44, no. 1, pp. 33–46.
- Paradis, E., Claude, J. and Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, vol. 20, pp. 289–290.
- Parkhurst, D.F. (1978). The adaptive significance of stomatal occurrence on one or both surfaces of leaves. *Journal of Ecology*, vol. 66, pp. 367–383.
- Parkhurst, D.F. (1994). Diffusion of CO<sub>2</sub> and other gases inside leaves. *New Phytologist*, vol. 126, pp. 449–479.

- Parkhurst, D.F. and Mott, K.A. (1990). Intercellular diffusion limits to CO<sub>2</sub> uptake in leaves: studies in air and helox. *Plant Physiology*, vol. 94, pp. 1024–1032.
- Patel, R.C. and Inamdar, J.A. (1971). Structure and ontogeny of stomata in some Polemoniales. *Annals of Botany*, vol. 35, pp. 389–409.
- Pedersen, M., Johnsson, A., Maehle, J. and Dalokken, R. (2006). Short period leaf movements in *Oxalis regnелиi*. *Physiologia Plantarum*, vol. 89, pp. 277–284.
- Pyankov, V.I., Artyusheva, E.G., Edwards, G.E., Black Jr., C.C. and Soltis, P.S. (2001). Phylogenetic analysis of tribes Salsoleae (Chenopodiaceae) based on ribosomal ITS sequences: implications for the evolution of photosynthesis types. *American Journal of Botany*, vol. 88, no. 7, pp. 1189–1198.
- R-Core-Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Radford, A.E., Dickison, W.C., Massey, J.R. and Bell, C.R. (1974). *Vascular Plant Systematics*. Harper and Row Publishers.
- Retamales, H.A., Scherson, R. and Scharaschkin, T. (2014). Foliar micromorphology and anatomy of *Ugni molinae* Turcz. (Myrtaceae), with particular reference to schizogenous secretory cavities. *Revista Chilena de Historia Natural*, vol. 87, no. 27, pp. 1–7.
- Robb, S. (1963). *Oxalis latifolia* Kunth. *New Phytologist*, vol. 62, pp. 75–79.
- Sack, L., Grubb, P.J. and Maranon, T. (2003). The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecology*, vol. 168, pp. 139–163.
- Salter, T.M. (1944). The genus *Oxalis* in South Africa: a taxonomic revision. *Journal of South African Botany*, vol. 1, pp. 1–355.
- Singh, G. (1999). *Plant Systematics – an integrated approach*.
- Soh, W.K. and Parnell, J. (2011). Comparative leaf anatomy and phylogeny of *Syzygium* Gaertn. *Plant Systematics and Evolution*, vol. 297, no. 2, pp. 1–32.
- Stuedle, E. (1983). Planta of *Oxalis carnosus* Molina. *Planta*, vol. 159, no. 1, pp. 38–45.
- Storey, M. (2006). *Biolimages: The Virtual Field-Guide (UK)*.
- Stuessy, T.F. (1990). *Plant taxonomy*. Columbia University Press, New York.
- Takhtajan, A. (1997). *Floristic regions of the world*.
- Terashima, I. and Hikosaka, K. (1995). Comparative ecophysiology of leaf and canopy photosynthesis. *Plant, Cell and Environment*, vol. 18, pp. 1111–1128.
- Thorne, R. (2000). The Classification and Geography of the Flowering Plants: Dicotyledons of the classic Angiospermae. *The Botanical Review*, vol. 66, pp. 441–647.
- Toma, C., Gostin, I. and Ivanescu, L. (2007). Histo-anatomical details of the *Oxalis corniculata* L. *Biologie vegetala*, pp. 5–10.
- Traub, H.P. (1968). The subgenera, sections and subsections of *Allium* L. *Plant Life*, vol. 24, pp. 147–163.
- Turrell, F.M. (1936). The area of the internal exposed surface of dicotyledon leaves. *American Journal of Botany*, vol. 23, pp. 255–264.

- Uhl, D. and Mosbrugger, V. (1999). Leaf venation density as a climate and environmental proxy: a critical review and new data. *Palaeogeography, Palaeoclimatology, Palaeoecology*, vol. 149, pp. 15–26.
- van Welzen, P.C. and Baas, P. (1984). A leaf anatomical contribution to the classification of the Linaceae complex. *Blumea*, vol. 29, pp. 453–479.
- Vasco, A., Thadeo, M., Conover, M. and Daly, D.C. (2014). Preparation of Samples for Leaf Architecture Studies, A Method for Mounting Cleared Leaves. *Application in Plant Sciences*, vol. 2, no. 9, pp. 1–4.
- Vogelman, T.C., Nishio, J.N. and Smith, W.K. (1996). Leaves and light capture: Light propagation and gradients of carbon fixation within leaves. *Trends in Plant Science*, vol. 1, no. 2, pp. 65–70.
- Šircelj, H., Mikulic-Petkovsek, M. and Batic, F. (2010). Antioxidants in spring leaves of *Oxalis acetosella* L. *Food Chemistry*, vol. 123, pp. 351–357.
- Walls, R.L. (2011). Angiosperm leaf vein patterns are linked to leaf functions in a global-scale data set. *American Journal of Botany*, vol. 98, pp. 244–253.
- Wang, R., Huang, W., Chen, L., Ma, L., Guo, C. and et al. (2011). Anatomical and physiological plasticity in *Leymus chinensis* (Poaceae) along large-scale longitudinal gradient in Northeast China. *PloS ONE*, vol. 6.
- Watson, R.W. (1942). The effect of cuticular hardening on the form of epidermal cells. *New Phytologist*, vol. 41, no. 4, pp. 223–229.
- Wiggins, I.L. and Porter, D.M. (1971). *Flora of the Galapagos Islands*. Stanford University Press, Stanford California.
- Wilkinson, H.P. (1979). *The plant surface (mainly leaf)*. In: Metcalfe CR, Chalk L (eds) *Anatomy of the dicotyledons, 2nd edn*. Clarendon Press, Oxford.
- Willmer, C. and Fricker, M. (1996). *Stomata, 2nd edn*. Chapman and Hall.
- Willson, C.J., Manos, P.S. and Jackson, R.B. (2008). Hydraulic traits are influenced by phylogenetic history in the drought-resistant and invasive genus *Juniperus* (Cupressaceae). *American Journal of Botany*, vol. 95, pp. 299–314.
- Wood, A. (1874). *Class-Book Of Botany USA and Canada*. A.S Barnes and Co.
- Zhang, S.-b., Guan, Z.-j., Sun, M., Zhang, J.-j., Cao, K.-f. and Hu, H. (2012). Evolutionary association of stomatal traits with leaf vein density in Paphiopedilum, Orchidaceae. *PloS ONE*, vol. 7, no. 6, pp. 1–10.

## Chapter 3

# The mode of photosynthesis and the potential functional significance of leaf anatomical and physiological traits of southern African *Oxalis*

### 3.1 Introduction

*Oxalis* (Oxalidaceae) is the sixth largest geophytic lineage and the largest geophytic genus in the Greater Cape Floristic Region (GCFR) (Manning and Goldblatt, 2012). Geophytes complete their life cycles within a relatively short period of time, which is generally determined by the availability of soil moisture and temperature (Van Rooyen, 1999). Geophytes such as *Oxalis* avoid the arid summers of the Mediterranean climate in the GCFR, as their growing season co-occurs with the winter rainfall period. It has been shown that geophytes have a performance advantage in semi-arid regions, as a substantial portion of their reserves (most importantly, carbon) are carried from one season to the next (Raunkiaer (1907); Pate and Dixon (1981); Ruiters *et al.* (1993a)). Geophytes are characterised by the important life strategy of the allocation and re-translocation of reserves and nutrients between the storage organ and the above-ground plant body (Pate and Dixon (1981); Witkowski (1989); Ruiters *et al.* (1993a); Ruiter (1995)). Geophytes therefore have sufficient reserves to sustain their survival and reproduction between the short periods of their growing season. As these reserves are dependent on the amount of carbon that the plant has fixed and stored during the previous growing seasons, it has been suggested that geophytes could be expected to have 'special' gas exchange characteristics (*i.e.* high photosynthetic CO<sub>2</sub> uptake) (Rossa and von Willert, 1999).

In plants there are three main pathways of carbon fixation, namely C<sub>3</sub>, C<sub>4</sub> and Crassulacean Acid Metabolism (CAM) (Osmond, 1982). These three pathways are defined by various characteristics, including leaf anatomy, the enzymes used in carbon fixation, stomatal behaviour such as timing of opening and growing-season temperatures in which the plants occur (Osmond (1982); Moson (1989); Ehleringer and Monson (1993); Ehleringer *et al.* (1997)). The C<sub>3</sub> mode of photosynthesis is the ancestral and most common photosynthetic pathway for angiosperms (Osmond (1982); Moson (1989); Ehleringer and Monson (1993)). All leaf photosynthetic activity in C<sub>3</sub> plants takes place in the mesophyll tissue (Ehleringer and Monson, 1993) and optimal rates of photosynthesis are achieved between 20°C and 30°C (Larcher, 1995). Under warm (ambient air temperatures warmer than 25°C) and dry conditions photorespiration becomes more prevalent, but the metabolic products of this pathway cannot be used by the Calvin Cycle of the C<sub>3</sub> photosynthetic pathway and therefore represent a loss of carbon and energy to the plant. It is therefore evident that C<sub>3</sub> plants are photo-

synthetically less effective under warm and dry environmental conditions.

C<sub>4</sub> photosynthesis evolved as one of the alternative methods of carbon fixation for plants growing in warmer temperature conditions (25°C to 40°C), high light intensities (light saturation), low CO<sub>2</sub> concentrations, low water availability and low nitrogen levels (Schulze *et al.*, 1996). The leaf structures of C<sub>4</sub> plants are adapted to optimise water- and light-use efficiency (Rao and Rajendrudu, 1989), for example C<sub>4</sub> plants have a much thicker epidermis than C<sub>3</sub> plants, which possibly acts as water storage tissue or a barrier between the chloroplasts in the mesophyll and the unforgiving environmental conditions (Muhaidat *et al.*, 2007). The mesophyll and bundle sheath tissues of C<sub>4</sub> plants are arranged in a radial manner around the vascular tissue, referred to as Kranz anatomy (Brown (1975); Nelson and Langdale (1992)). The bundle sheath cells of C<sub>4</sub> plants do have chloroplasts, unlike C<sub>3</sub> plants (Botha, 1992), which enables both the bundle sheath tissue and the mesophyll cells of C<sub>4</sub> plants to photosynthesise. C<sub>4</sub> plants have higher optimum temperatures (30°C to 40°C) than C<sub>3</sub> plants and broader temperature ranges at which maximum rates (>90%) of gas exchange can be maintained (Berry and Bjorkman (1980); Downton *et al.* (1984)). These specialized anatomical and biochemical properties of the C<sub>4</sub> metabolic pathway enable optimal water-use and light-use strategies for plants in warm and dry conditions. The different photosynthetic pathways of C<sub>3</sub> and C<sub>4</sub> plants discriminate between the <sup>12</sup>C and <sup>13</sup>C isotopes of carbon, meaning that the ratios of these two isotopes can be used to assign plants to various photosynthetic groups (O'Leary, 1988).

CAM plants are usually succulent and also have higher optimal temperatures than C<sub>3</sub> plants (30°C to 40°C) (Larcher, 1995) as well as broader temperature ranges at which optimum rates of gas exchange can be maintained (Berry and Bjorkman (1980); Downton *et al.* (1984)), making this pathway advantageous in hot and dry conditions (25°C to 40°C). CAM plants have the unique adaptation that gaseous exchange and therefore temporary carbon fixation and storage take place during the night when stomata are open. This strategy restricts water loss, due to lower nocturnal transpiration rates within the extreme environments that some of these plants inhabit (Ehleringer and Monson, 1993). Generally CAM plants have a thick cuticle (Gibson (1982); Ogburn and Edwards (2009)), low stomatal density (Gibson, 1986) and succulent leaves filled with undifferentiated mesophyll cells with enlarged vacuoles (Guralnick and Ting, 1987). This causes CAM plants to exhibit increased cell sizes as well as increased mesophyll thickness relative to C<sub>3</sub> and C<sub>4</sub> species (Gibson (1982); Smith and Winter (1996); Winter and Smith (1996); Nelson *et al.* (2005)). Due to large cell sizes, cells are tightly packed within leaves, resulting in less intercellular air space (Smith and Heuer (1981); Maxwell *et al.* (1997)) as well as shorter length of mesophyll surface exposure to intercellular air space per unit area (Nelson *et al.*, 2005) than in comparable C<sub>3</sub> leaves.

There is limited mention of the mode of photosynthesis associated with geophytes, but Larcher (2003) suggested that CAM, reversible and/or intermediate modes of photosynthesis have evolved in geophytes present in semi-arid or arid habitats. Very little is known about the preferred photosynthetic pathways of *Oxalis* species. Putative evidence of CAM photosynthesis in South American *O. megalorrhiza* Jacq. (synonym: *O. carnososa* Molina) was recorded by Kluge and Ting (1978). Both Proches *et al.* (2006a) and Biocyclopedia: *Oxalis* (2012) made passing reference to the possibility that non-C<sub>3</sub> modes of photosynthesis may occur in South American and South African *Oxalis* species, but to date these suggestions have not been verified. De Villiers (2001) detected structures that resemble non-C<sub>3</sub>-photosynthetic anatomies among members of southern African *Oxalis* section *Crassulae*; for example bundle sheath and mesophyll tissue were suspected to be arranged in Kranz anatomy that is typically associated with the C<sub>4</sub> photosynthetic pathway. The anatomical data collected by Jooste (2015) (Chapter 2) identified similar structures that superficially resemble the bundle sheaths, as well as densely packed mesophyll tissue in some species, which resemble the mesophyll arrangement that is often associated with plants that have a CAM photosynthetic

pathway.

All southern African members of *Oxalis* are geophytes, and many species have succulent to semi-succulent above-ground plant parts (Salter (1944); Proches *et al.* (2006b)). Over 17% of all species present in the GCFR are geophytes with bulbs, corms, tubers or rhizomes (Manning and Goldblatt, 2012). Both geophytic and annual growth forms have been recognized as adaptive strategies for seasonal climates (Raunkiaer (1934); Burns (1946); Svoskin (1960); Rees (1966)). Geophytes utilize underground storage organs to escape harsher seasons unfavourable for growth and occur in regions with reliable climatic seasonality (Manning and Goldblatt, 2012). Climates across the GCFR are variable, with a Mediterranean climate of strongly seasonal winter-rainfall in the southwest of the region, extensive summer precipitation and aseasonal rainfall in the eastern part of this region and semi-arid conditions to the west and northwest of the region (Manning and Goldblatt, 2012). The well-defined and reliable seasonal differences between summer and winter climates in the Cape region (Schulze *et al.* (1978); Campbell (1983)) are considered to be a reason why geophytic growth forms are favoured here (Manning and Goldblatt, 2012) raising the real possibility that this group utilizes the CAM photosynthetic pathway.

The aims of this paper were therefore to determine whether carbon isotope analysis and stomatal conductance, together with the various leaf anatomical traits identified by Jooste (2015) (Chapter 2), suggest the presence of any mode(s) of non- $C_3$  photosynthesis among southern African *Oxalis* species. If species are  $C_3$  then we would expect to find their leaves showing  $\delta^{13}C$  isotope values between  $-22\text{‰}$  and  $-37\text{‰}$  and active day-time transpiration (detectable stomatal conductance). If species are  $C_4$ , then we expect that they would have  $\delta^{13}C$  isotope values between  $-7\text{‰}$  and  $-15\text{‰}$ , display Kranz anatomy with bundle sheath cells that contain chloroplasts and would have a high relative epidermal thickness. If species are CAM then we expect to detect low to zero stomatal conductance during the day. Potential anatomical characters of CAM plants would include succulent leaves, low stomatal densities, the absence of chloroplasts in bundle sheath cells and undifferentiated mesophyll cells. If no alternative modes of photosynthesis were detected, we expect that leaf anatomical and physiological traits (e.g. leaf thickness, specific leaf area, stomatal position), together with the stomatal conductance data, could give us insight into the water-use and life-history strategies of this genus, in order to better understand how these plants successfully utilize their growing conditions in such a strongly seasonal environment.

## 3.2 Materials and methods

### 3.2.1 Carbon isotope analysis

Leaf material of the majority of taxa studied in (Jooste (2015); Chapter 2) were collected from the *Oxalis* living collection in the Stellenbosch University Botanical Gardens and various field localities (Addendum I). Leaf samples placed in separate brown paper bags for all species, oven dried at  $50^\circ\text{C}$  for 72 hours and ground into a homogeneous powder with a pestle and mortar. The pestle and mortar were rinsed with distilled water before each new sample was ground. The stable carbon and nitrogen isotope ratios were analysed at the Archaeometry Laboratory in the Department of Archaeology, University of Cape Town. Between 0.6 and 0.7 mg of each powdered sample was weighed and combusted in an automated analyser (Flash 2000) coupled to a continuous-flow isotope ratio mass spectrometer (Delta V Plus). All results were reported in units per mil (‰) with  $\delta^{13}C$  relative to Vienna Pee Dee Belemnite (VPDB). The  $\delta^{13}C$  values were used to distinguish the potential  $C_3$  plants from the potential  $C_4$  plants.

$C_3$  plants typically have  $\delta^{13}C$  values that range between  $-22\text{‰}$  and  $-37\text{‰}$ , while  $C_4$  plants typically

have  $\delta^{13}\text{C}$  values that range between  $-7\text{‰}$  and  $-15\text{‰}$  (Vogel (1978); Vogel (1980); Farquhar *et al.* (1982); Ehleringer and Osmond (1994); O'Leary (1988)). However, due to the overlap between the  $\delta^{13}\text{C}$  values of CAM plants with  $\text{C}_3$  and  $\text{C}_4$  plants, these values could not be used to identify CAM plants.

### 3.2.2 Leaflet conductance data

The stomatal conductance (measured in  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and leaflet temperature ( $^{\circ}\text{C}$ ) were measured for both the adaxial (AD) and abaxial (AB) leaflet surfaces of fully sun-exposed leaves. Stomatal conductance was measured during the peak growing season of *Oxalis* (late-June to early-August) both in the common garden collection and in the field. Three measurements of both the AD and AB surface of each leaflet were taken, and three leaflets per plant were measured. A calibrated Decagon SC-1 porometer (Decagon Devices, Pulman, Washington, USA) was used to take the readings. Measurements were only taken between 10 am and 4 pm to increase the probability that plants were transpiring near their full potential under the given environmental conditions.

### 3.2.3 Leaflet anatomy

The anatomical characters with potential functional and physiological significance were collected from the epidermal peels and sectioned and stained leaflet material of Jooste (2015) (Chapter 2). These traits included epidermal cell and cuticle thickness, stomatal densities and stomatal index (SI), presence of Kranz anatomy and contents of bundle sheath cells and mesophyll densities, arrangement types and dimensions. These data were supplemented by additional epidermal peels for species that were studied in the field (species that were not sampled in the living collection in Chapter 2).

#### 3.2.3.1 Stomatal length and density

Several stomatal physiological traits were studied as stomata play a crucial role in leaf conductance and photosynthesis. Epidermal impressions were made to study the AD and AB leaflet surfaces of mature leaves by applying clear nail varnish to fresh leaflet material. The nail varnish layers were peeled off with clear cello tape and stuck onto microscope slides for photography. Ten measurements of stomatal central axis length, stomatal density, epidermal cell density and epidermal cell area were taken in random fields of view under 40x magnification for each studied leaflet, and the data from five leaflets were used as average values to represent taxa. Stomatal size as a function of leaflet location (AD or AB) and stomatal density as a function of leaflet location (AD or AB) were separately determined using a factorial ANOVA, with Tukey post-hoc tests in R. Stomatal central axis length (long axis of stomata) was measured instead of stomatal area, because stomata are dynamic structures that can open and close and this movement influences their short axis length (Willmer and Fricker, 1996), while the long axis length remains constant. Stomatal and epidermal cell density was estimated by counting the number of cells per field of view at a fixed magnification of 40x times. Cell counts were then converted to cells per  $\text{mm}^2$ .

#### 3.2.3.2 Stomatal index (SI)

The stomatal index (SI) was calculated to normalize the effect of epidermal cell size on stomatal density counts (Salisbury, 1927) as the SI indicates the ratio of stomatal cells to all epidermal cells. Stomatal index (SI) was defined as:

$$\text{SI}(\%) = \frac{\text{stomatal density}}{\text{stomatal density} + \text{epidermal cell density}} \times 100 \quad (3.2.1)$$



Stomatal length and log transformed stomatal density data were plotted and a linear model was fitted to the data using the statistical package (Stats) in R (R-Core-Team, 2014).

### 3.2.4 Stomata and ploidy level

There is a well-known positive relationship between ploidy level and stomatal guard cell length among angiosperms (Beaulieu *et al.* (2008); J. G. Hodgson *et al.* (2010)). The genome size data, interpreted as ploidy level data, of the taxa from the Stellenbosch Botanical Gardens' Living collection were kindly provided by Jan Suda (Charles University, Prague). Both the stomatal length data and the ploidy level data were collected from the same individual plants (exact same accessions from the living collection). Ploidy level and stomatal length data were plotted and a linear model was fit to the data under the expectation that increases in ploidy level would lead to increased stomatal length, using the statistical package (Stats) in R (R-Core-Team, 2014).

### 3.2.5 Specific Leaf Area (SLA)

The surface areas of fresh leaflets were determined with a Li300 area meter. Leaves with more than one leaflet were usually taken apart, to ensure that the leaflets did not overlap when the leaf was measured. Conduplicate leaflets were divided along the central vein to ensure that leaflet surface area was not lost due to conduplication. Three leaves were sampled per accession. After area measurements all leaflet material was oven dried (50°C for 72 hours) so that the corresponding dry leaf weight (g) could be measured. These data were used to calculate the specific leaf area for each leaf, defined as the total fresh leaflet area per leaf, divided by the total dry leaf mass per leaf. The average value (of the three leaves) was used to represent an accession or species.

### 3.2.6 Statistical analysis

All statistical analyses were performed in R (R-Core-Team, 2014). Data were tested for normality (Shapiro-Wilk test). If the data were normally distributed parametric tests were used, and if the data were not normally distributed, non-parametric tests were used. Welch's two sample T-tests and ANOVA's followed by TukeyHSD multiple comparisons of means were used to compare various datasets and physiological traits.

### 3.3 Results: Photosynthetic pathway

#### 3.3.1 Isotope analysis

Eighty species from the garden collection and 25 species from the field were included in the stable carbon isotope analyses in order to test for the presence of photosynthetic pathways alternative to  $C_3$ . The  $\delta^{13}C$  values observed in southern African *Oxalis* ranged from -25.34 ‰ to -32.38 ‰, with a average of -29.70 ‰ (Figure 3.1). Given that the typical  $\delta^{13}C$  values for  $C_4$  plants range between -8 ‰ and -20 ‰ and  $C_3$  plants range between -22 ‰ and -37 ‰ (Vogel (1978); Vogel (1980); Farquhar *et al.* (1982); Ehleringer and Osmond (1994); O'Leary (1988)), it was clear that all southern African *Oxalis* species tested had a non- $C_4$  mode of photosynthesis.

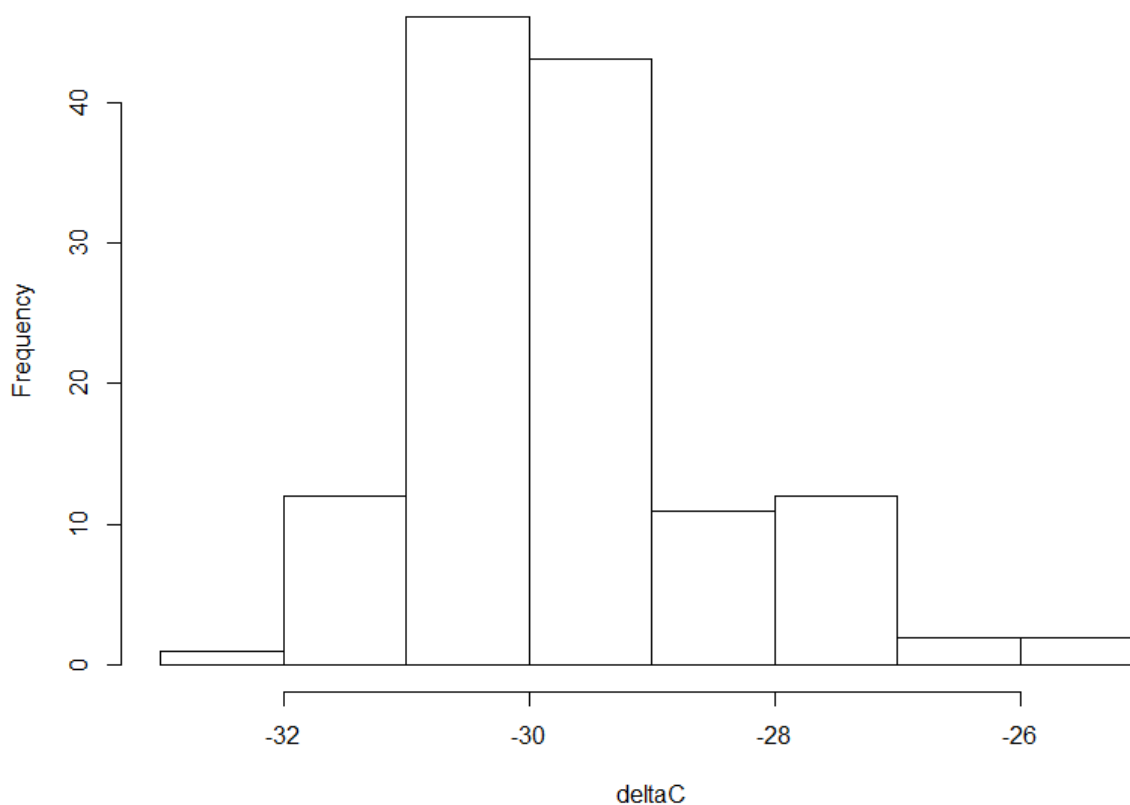


Figure 3.1: Histogram indicating the distribution of  $\delta^{13}C$  isotope data of southern African *Oxalis* species collected from the common garden collection and field localities.

The comparison of  $\delta^{13}C$  values from leaves collected in the common garden collection and the field showed that field collected samples (field average = -28.15 ‰) had statistically significantly higher (more positive)  $\delta^{13}C$  values than species from the common garden (garden average = -30.07 ‰) ( $t = -6.56$ ,  $df = 28.345$ ,  $p < 0.001$ ) (Figure 3.2). The overall  $\delta^{13}C$  values of *Oxalis* species had similar and relatively large ranges in both the garden (4.5 ‰) and the field (5.2 ‰). We observed relatively large within-species variation, for example *O. magnifolia*, with two garden collected samples with  $\delta^{13}C$  isotope values of -28.54 ‰ and -30.11 ‰ and six field collected samples with  $\delta^{13}C$  isotope

values that ranged from -25.34 ‰ to -28.57 ‰. Despite the different ranges of  $\delta^{13}\text{C}$  values in the garden and field collected samples, all samples fell within the  $\text{C}_3$ -photosynthetic range.

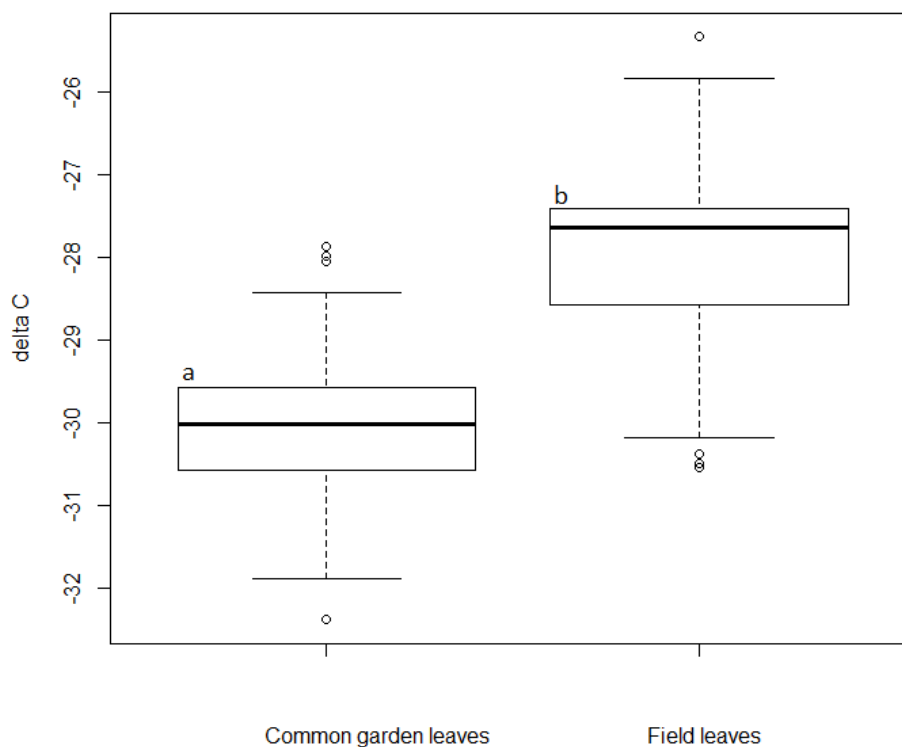


Figure 3.2: Boxplot indicating the range of  $\delta^{13}\text{C}$  values of leaves collected in the common garden collection and from the field. A black line represents the median, a box represents the first and third quartiles, the whiskers represent the 95% confidence intervals and the circles represent outliers

### 3.3.2 Stomatal conductance

The stomatal conductance of 93 species from the Stellenbosch University Botanical Gardens collection and 40 species from various field localities were measured in order to differentiate between  $\text{C}_3$  and CAM plants. The conductance data of 23 of these species were collected from both the common garden and field localities. The stomatal conductance measurements of all *Oxalis* species showed that these plants were transpiring and photosynthetically active during the day. These data showed that species had a non-CAM mode of photosynthesis. The  $\delta^{13}\text{C}$  and stomatal conductance data conclusively showed that all studied species exclusively used the  $\text{C}_3$  photosynthetic pathway.

The overall stomatal conductance of *Oxalis* species ranged from  $3.5 \text{ mmol/m}^{-2}\text{s}^{-1}$  (AD surface of epistomatic leaflet) to  $443.1 \text{ mmol/m}^{-2}\text{s}^{-1}$  (AB surface of amphistomatic leaflet) with an overall average conductance of  $72.2 \text{ mmol/m}^{-2}\text{s}^{-1}$ . The statistical comparison between the conductance data measured from plants growing in the common garden collection and the field showed no significant difference for the AD surface ( $p > 0.05$ ) or the AB surface ( $p > 0.05$ ) ( $F = 7.808$ ,  $df = 127$  and  $3$ ) (Figure 3.3). For this specific comparison the stomatal conductance of both epistomatic leaflets and the AD surface of amphistomatic leaflets were grouped as plants with AD conductance readings,

and both hypostomatic leaflets and the AB surface of amphistomatic leaflets were grouped as plants with AB conductance readings. There were thus no significant differences between the stomatal conductance of garden or field samples, and stomatal conductance data measured from both localities were therefore pooled for a few of the following analyses (clearly indicated if applicable). The leaflet temperature was measured with each stomatal conductance reading and we observed that leaflet temperature ranged between 18.2 °C and 31.9 °C, with an average temperature of 24.1 °C.

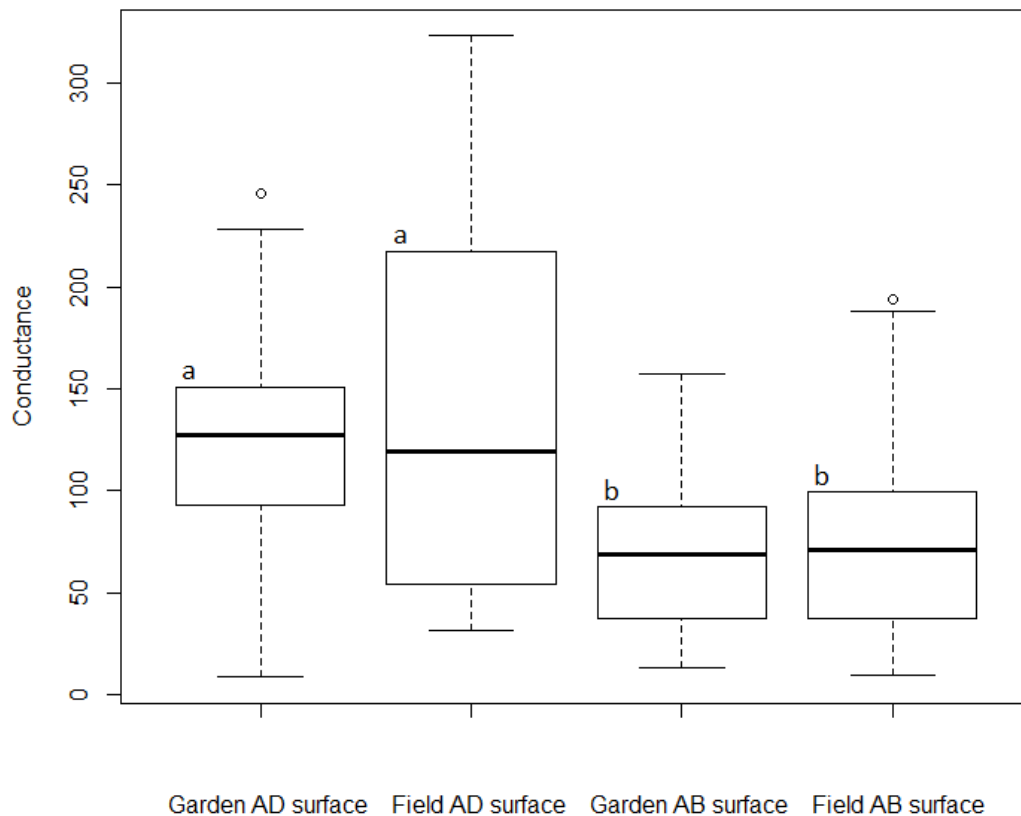


Figure 3.3: Boxplot indicating the stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ) of southern African *Oxalis* species measured on both the adaxial and abaxial surfaces of plants growing in the common garden collection and the field.

### 3.3.3 Leaflet anatomical and physiological traits

A few traits that seemed to resemble the diagnostic traits of alternative modes of photosynthesis were observed in *Oxalis* species (Jooste (2015); Chapter 2). Plants that follow the  $\text{C}_4$  photosynthetic pathway reportedly have a thick epidermis, and a number of southern African *Oxalis* species were observed with epidermal cell layers that appear to be thick relative to the mesophyll tissue layer height (Figure 3.4). Kranz anatomy, with chloroplasts within the bundle sheath cells, is another diagnostic trait of  $\text{C}_4$  plants. Many southern African *Oxalis* species had diagnostic cells that were radially arranged around the vascular tissue, and these cells were collectively referred to as a sheath (Figure 3.6). These cells did, however, not appear to contain any chloroplasts.

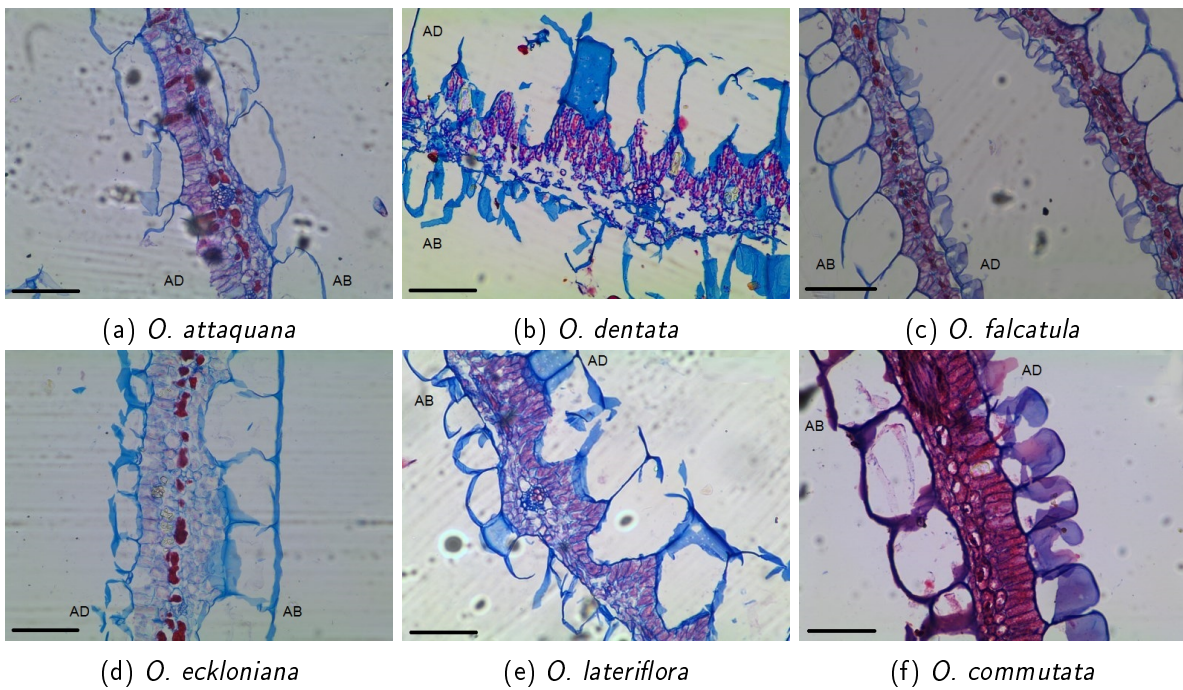


Figure 3.4: Light microscope photographs of wax-embedded leaflet material depicting southern African *Oxalis* species with relatively thick epidermal cells (AD or AB epidermal cells). (a) *O. attenuata* (MO45), (b) *O. dentata* (MO474), (c) *O. falcata* (MO476), (d) *O. eckloniana* (MO521), (e) *O. lateriflora* (MO887), (f) *O. commutata* (MO1224). Scalebars represent 100  $\mu\text{m}$ .

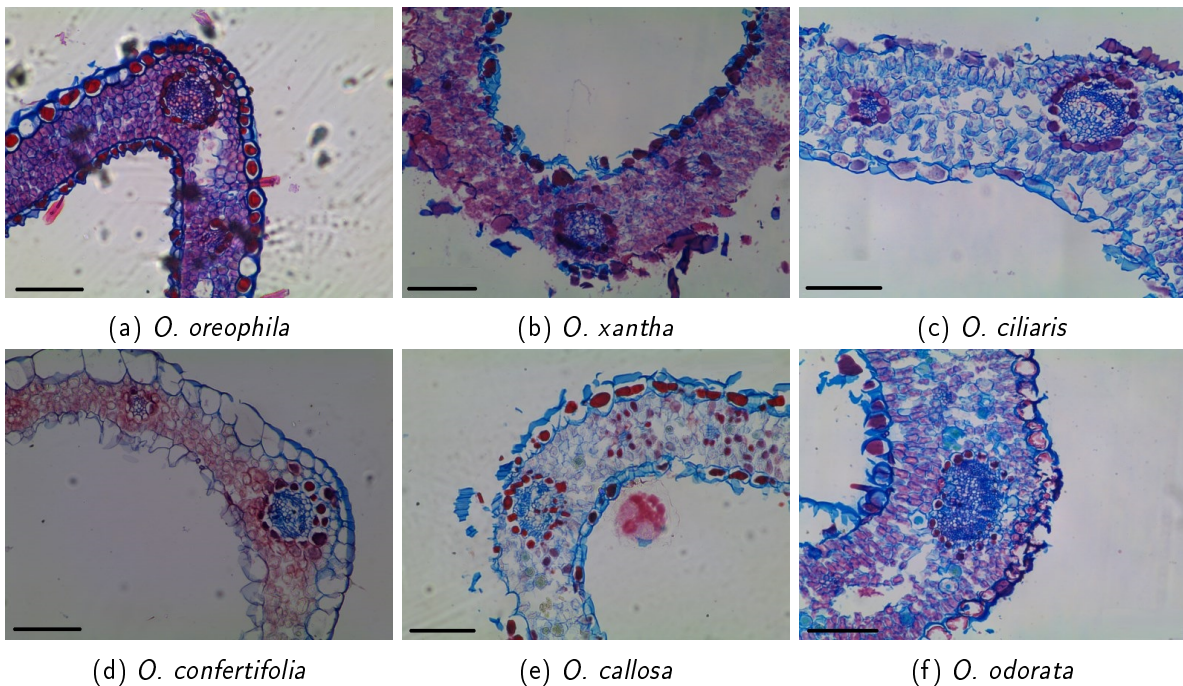


Figure 3.5: Light microscope photographs of wax-embedded leaflet material depicting vascular tissue surrounded by a sheath observed in southern African *Oxalis* taxa. (a) *O. oreophila* (MO270), (b) *O. xantha* (MO273), (c) *O. ciliaris* (MO329), (d) *O. confertifolia* (MO358), (e) *O. callosa* (MO532), (f) *O. odorata* (MO1549). Scalebars represent 100  $\mu\text{m}$ .

CAM plants usually have succulent leaves, thick cuticles and low stomatal densities (Gibson (1982); Gibson (1986); Ogburn and Edwards (2009)). The leaves of CAM plants are typically filled with undifferentiated mesophyll cells (isobilateral mesophyll arrangement type) with enlarged vacuoles and increased cell sizes, increased mesophyll thickness and less intercellular air space relative to  $C_3$  and  $C_4$  species. Among southern African *Oxalis* there are a few species that display many of these anatomical traits typically associated with CAM photosynthesis, even though we have confirmed that they all follow a  $C_3$  photosynthetic pathway. Interestingly, all species that display these characters are closely related and belong to the sub-clade with amphistomatic leaflets with swollen epidermal cell types from the well supported Clade 7 (Jooste (2015); Chapter 2). These species appeared to have succulent leaves (Figures 3.6a, 3.6b and 3.6c) and all species from this sub-clade had relatively lower AD and AB stomatal densities (sub-clade average stomatal density: AD surface = 60.3 stomata/mm<sup>2</sup>, AB surface = 48.1 stomata/mm<sup>2</sup>) than the majority of southern African *Oxalis* species (southern African average stomata density: AB = 113.8 stomata/mm<sup>2</sup>, AB surface = 67.9 stomata/mm<sup>2</sup>) (AD surface:  $t = 6.450$ ,  $df = 80.77$ ,  $p < 0.001$ ) (AB surface:  $t = 2.59$ ,  $df = 37.64$ ,  $p < 0.05$ ) (Figures 3.6d, 3.6e and 3.6f). The SI values for amphistomatic leaflets were significantly higher, meaning that even after taking epidermal cell size and density into account (as these species all had the swollen epidermal cell type), these species still have relatively lower stomatal densities ( $F = 30.23$ ,  $df = 122$  and  $3$ ,  $p < 0.05$ ) than leaflets with other stomatal arrangements (epistomatic or hypostomatic). Not all species from this sub-clade had relatively thick mesophyll tissue, but the species with the thickest mesophyll tissue all belong to this sub-clade. Species from this sub-clade had relatively dense mesophyll arrangement in comparison to the other studied species, but these species did not display the trait of having enlarged vacuoles (but rather extra-cellular cavities) within their mesophyll and the mesophyll arrangement type of the majority of these species were clearly bifacial (Figure 3.6g, 3.6h and 3.6i). These traits are shown in Figure 3.6, with stereotypical  $C_3$  anatomical traits in the studied species shown as comparison in Figure 3.7. The lack of typical  $C_4$  or CAM leaflet anatomical traits is consistent with the  $\delta^{13}C$  isotope and stomatal conductance data of all studied species, and therefore strengthens our conclusion that all southern African *Oxalis* are  $C_3$  plants.

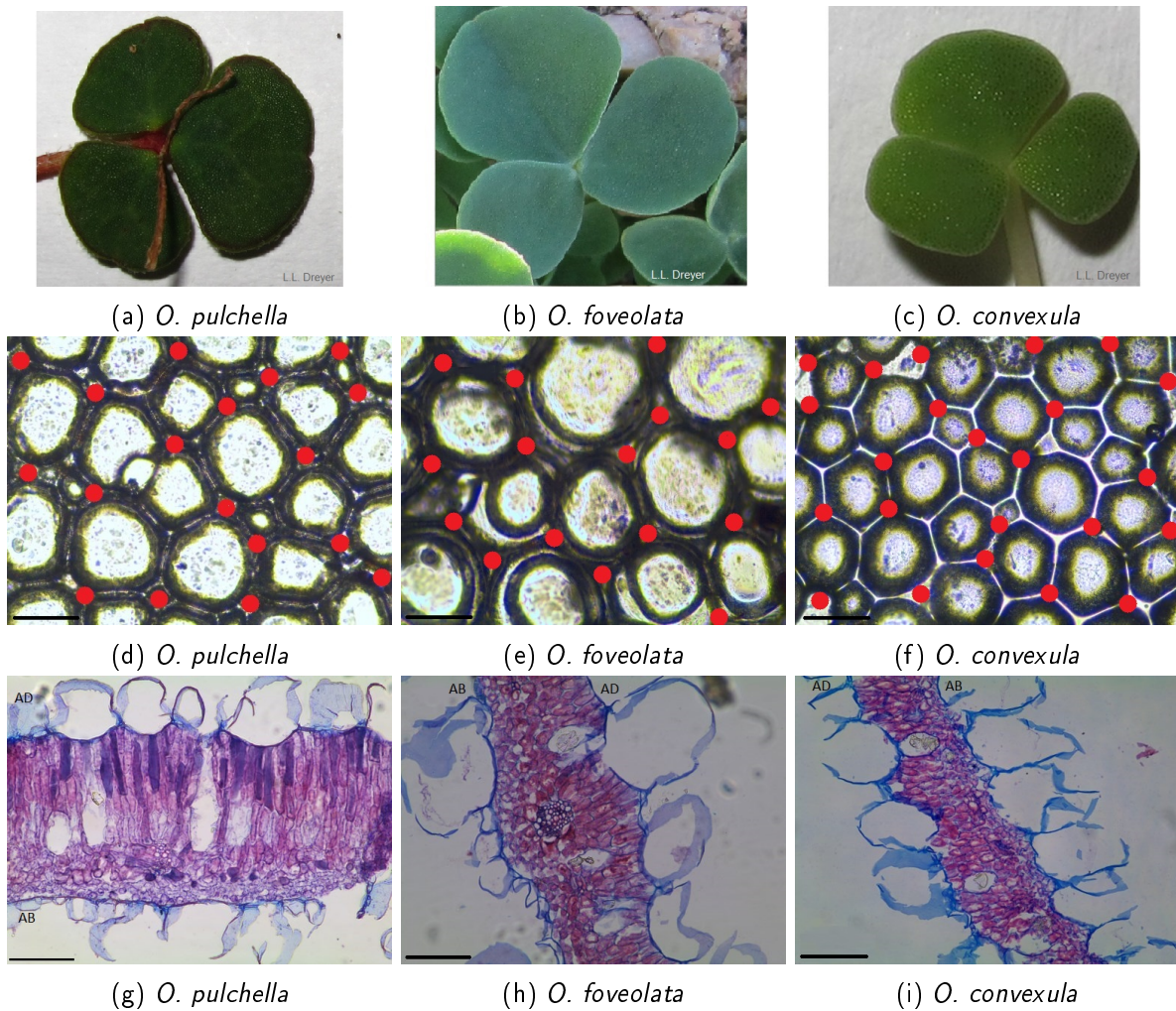


Figure 3.6: Photographs of three *Oxalis* species with semi-succulent to succulent leaves (a) *O. pulchella* (MO559), (b) *O. foveolata* (MO1466), (c) *O. convexula* (MO368). Edited light microscope photographs of epidermal peels of the AD leaflet surface of three *Oxalis* species (stomata are highlighted with red circles, where each circle represents a single stoma) to illustrate the low stomatal densities (d) *O. pulchella* (MO559), (e) *O. foveolata* (MO1466), (f) *O. convexula* (MO368). Light microscope photographs of wax-embedded and transverse-sectioned leaflet material depicting relatively dense mesophyll arrangement with cavities within the mesophyll (g) *O. pulchella* (MO559), (h) *O. foveolata* (MO1466), (i) *O. convexula* (MO368). Scalebars represent 100  $\mu\text{m}$ .

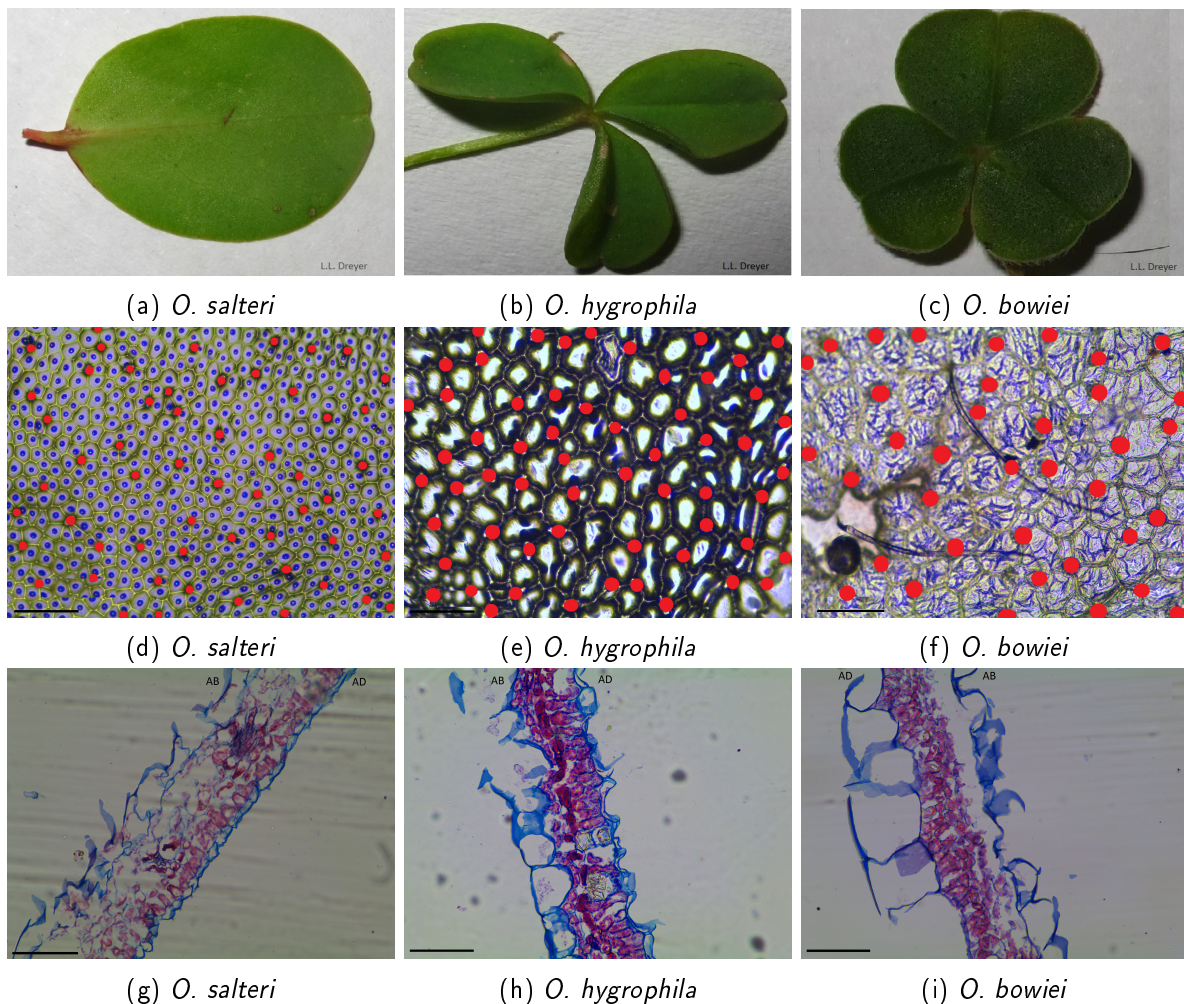


Figure 3.7: Photographs of three *Oxalis* species with herbaceous leaves (a) *O. salteri* (MO1137), (b) *O. hygrophila* (MO230), (c) *O. bowiei* (MO502). Edited light microscope photographs of epidermal peels of the leaflet surfaces of three *Oxalis* species (stomata are highlighted with red circles, where each circle represents a single stoma) to illustrate the stomatal densities (d) AD surface of *O. salteri* (MO1137), (e) AD surface of *O. hygrophila* (MO230), (f) AB surface of *O. bowiei* (MO502). Light microscope photographs of wax-embedded and transverse-sectioned leaflet material depicting the common mesophyll arrangement observed among the majority of studied species with bifacial mesophyll arrangement type (g) *O. salteri* (MO1137), (h) *O. hygrophila* (MO230), (i) *O. bowiei* (MO502). Scalebars represent 100  $\mu\text{m}$ .

### 3.4 Results: Stomatal conductance data relative to leaf physiological traits

#### 3.4.1 Stomatal conductance relative to stomatal size and densities

The stomatal conductance of many *Oxalis* species was similar to geophytes from the Namaqualand region (?), but were reportedly much higher than would be expected for typical southern African  $C_3$  (annual) species. These results warranted further investigation into some of the leaf physiological traits that could possibly enable these plants to have such relatively high conductance rates. We therefore investigated stomatal conductance data relative to stomatal position (epistomatic, hypostomatic and amphistomatic arrangements). The conductance of the AD and AB surface (in-



cluding additional vein-associated stomata) for each leaflet type were pooled to represent the total conductance per leaflet. These data showed that the total conductance of hypostomatic leaflets was significantly lower than that of epistomatic leaflets ( $p < 0.05$ ), and that the total conductance of amphistomatic leaflets was significantly higher ( $p < 0.001$ ) than the other two leaflet types ( $F = 20.29$ ,  $df = 411$  and  $2$ ) (Figure 3.8).

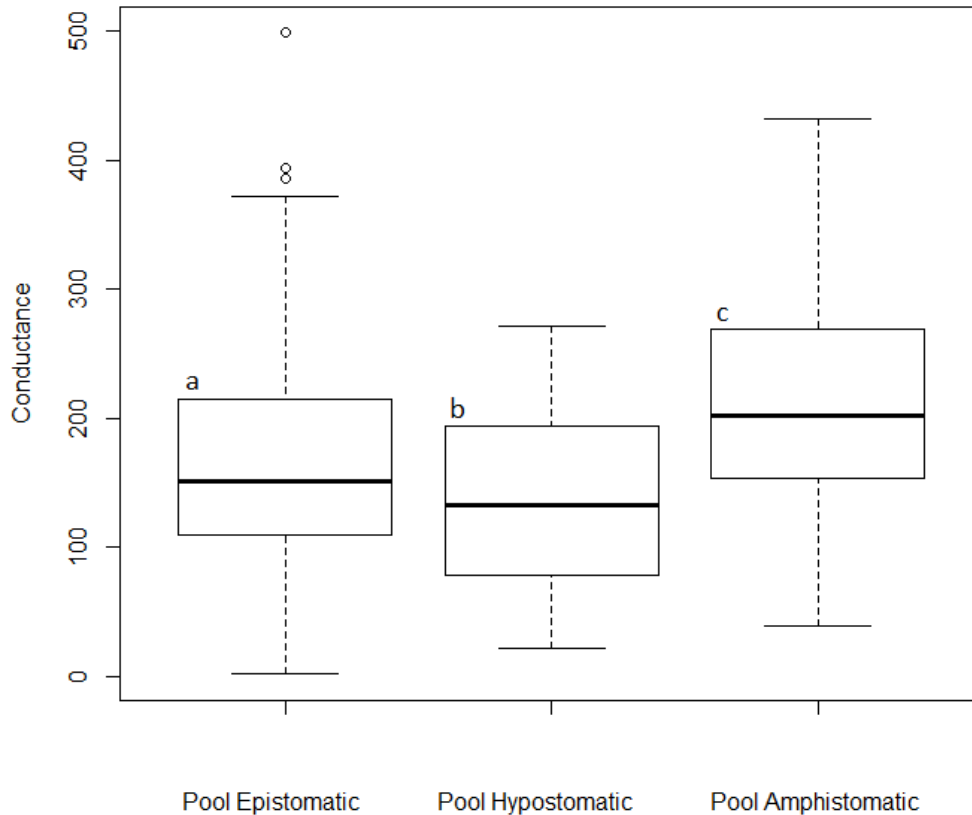


Figure 3.8: Boxplot indicating the total (pooled) stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ) of adaxial and abaxial leaflet surfaces (including additional vein-associated stomata) for southern African *Oxalis* species with epistomatic, hypostomatic and amphistomatic leaflets. The symbols a and b are significantly different at  $p < 0.05$ , and b and c are significantly different at  $p < 0.001$ .

The stomatal conductance and stomatal central axis length were correlated, but this analysis showed only a weakly supported negative relationship (Multiple  $R^2 = 0.073$ , Adjusted R-squared =  $0.061$ , F-statistic =  $5.832$  on  $df = 1$  and  $74$  and  $p$ -value =  $0.0182$ ) (Figure 3.9).

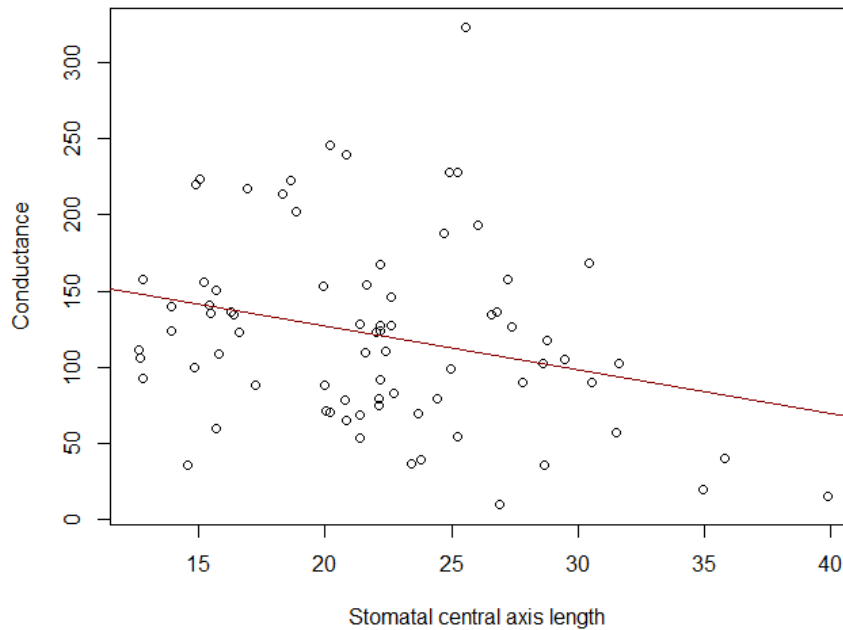


Figure 3.9: Scatterplot of stomatal central axis lengths ( $\mu\text{m}$ ) in comparison to stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ).

When studying stomatal conductance of the different leaflet types (AD surface of epistomatic leaflets, AB surface of hypostomatic leaflets, AD and AB surface of amphistomatic leaflets) relative to stomatal length and densities (Figures 3.10, 3.11 and 3.12) we observed a possible relationship between these traits. A multiple regression was performed where three variables were considered (namely stomatal length, stomatal density and stomatal position) to possibly have an effect on stomatal conductance. This analysis showed that there was no association between stomatal conductance and stomatal length ( $p > 0.05$ ) or stomatal density ( $p > 0.05$ ). Stomatal position was the only considered variable that had a significant effect on stomatal conductance (Multiple  $R^2 = 0.219$ , F-statistic = 7,297,  $df = 52$  and 2,  $p < 0.001$ ).

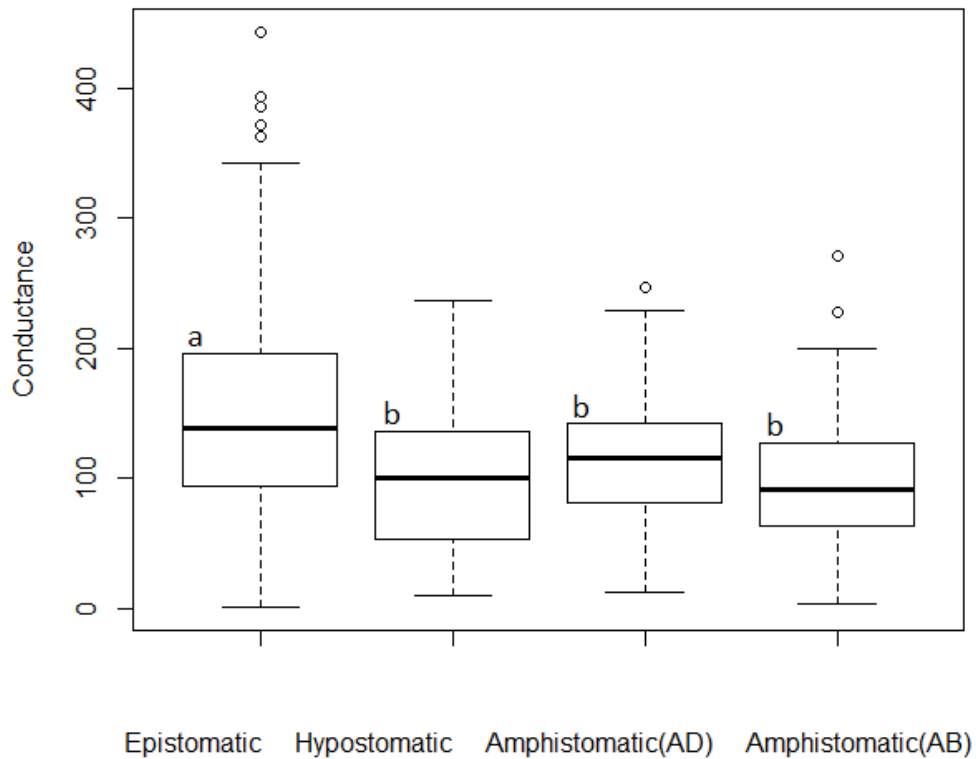


Figure 3.10: Boxplot indicating stomatal conductance ( $\text{mmol/m}^{-2}\text{s}^{-1}$ ) of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomata. The symbols a and b were significantly different at  $p < 0.001$  ( $F = 20.06$ ,  $df = 504$  and  $3$ ).

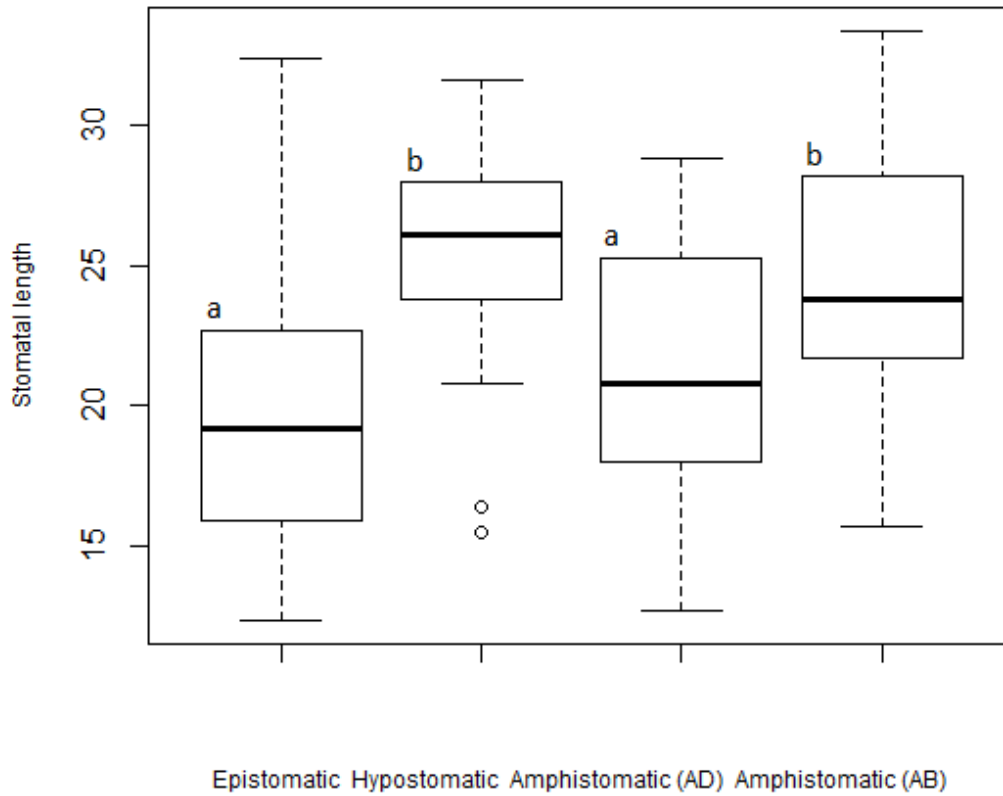


Figure 3.11: Boxplot indicating stomatal central axis lengths of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomata. The symbols a and b were significantly different at  $p < 0.01$  ( $F = 13.36$ ,  $df = 167$  and  $3$ ).

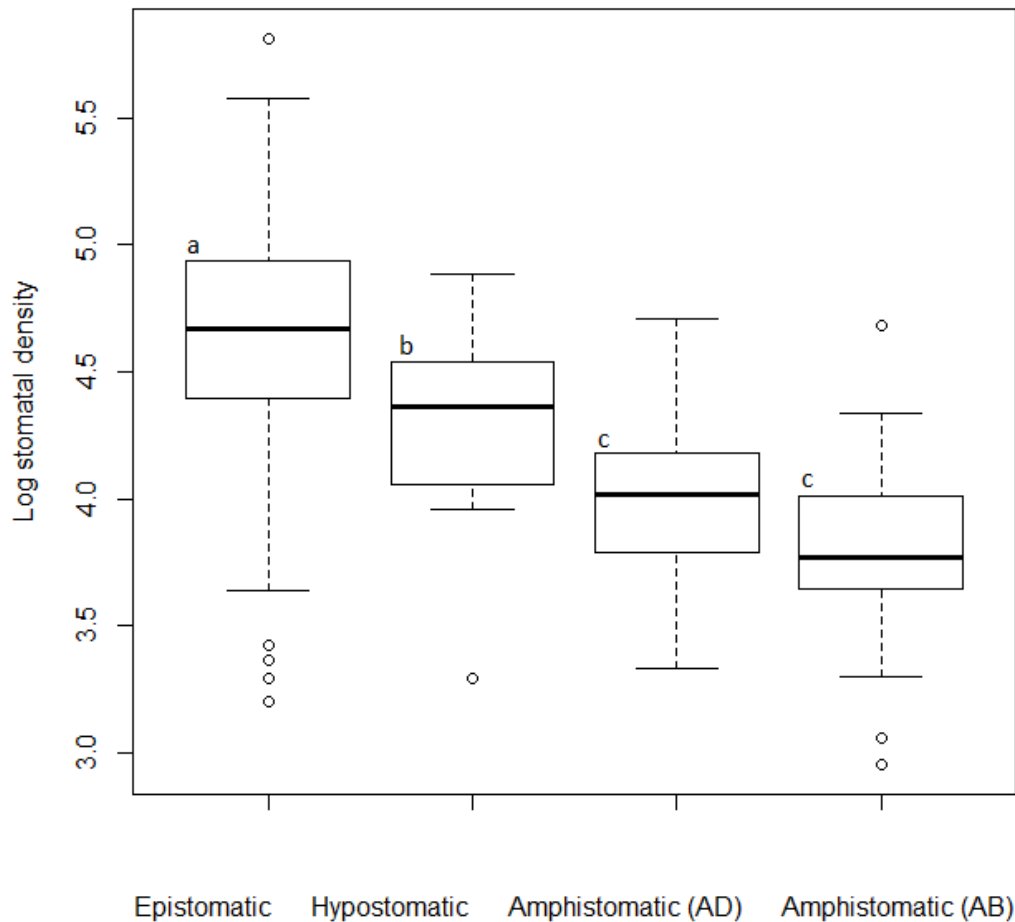


Figure 3.12: Boxplot indicating log transformed stomatal densities of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomatal positions on leaflets. The symbols a and b were significantly different at  $p < 0.01$  ( $F = 32.69$ ,  $df = 171$  and  $3$ ).

### 3.4.2 Stomatal dimensions

Stomatal size and densities varied considerably as average stomatal lengths of studied taxa ranged from  $12.39 \mu\text{m}$  (AD surface of *O. sonderiana* (garden)) to  $33.34 \mu\text{m}$  (AB surface of *O. pocockiae* (field)) (Figures 3.13 and 3.14a) and stomatal densities ranged from 13 stomata/ $\text{mm}^2$  (AB surface of *O. bullulata* (garden)) to 334.8 stomata/ $\text{mm}^2$  (AD surface of *O. sonderiana* (field)) (Figure 3.14b). The stomatal length measurements from taxa growing in the garden living collection were similar compared to taxa collected in the field for AD stomata (AD garden =  $19.27 \mu\text{m}$  (sd = 5.85), AD field =  $20.36 \mu\text{m}$  (sd = 3.88),  $t = 0.87$ ,  $df = 49.36$ ,  $p > 0.05$ ) and AB stomata (AB garden =  $25.12 \mu\text{m}$  (sd = 7.04), AB field =  $25.52 \mu\text{m}$  (sd = 4.03),  $t = 0.18$ ,  $df = 15.34$ ,  $p > 0.05$ ). Stomatal length data from taxa collected in the field were therefore included in our original garden dataset.

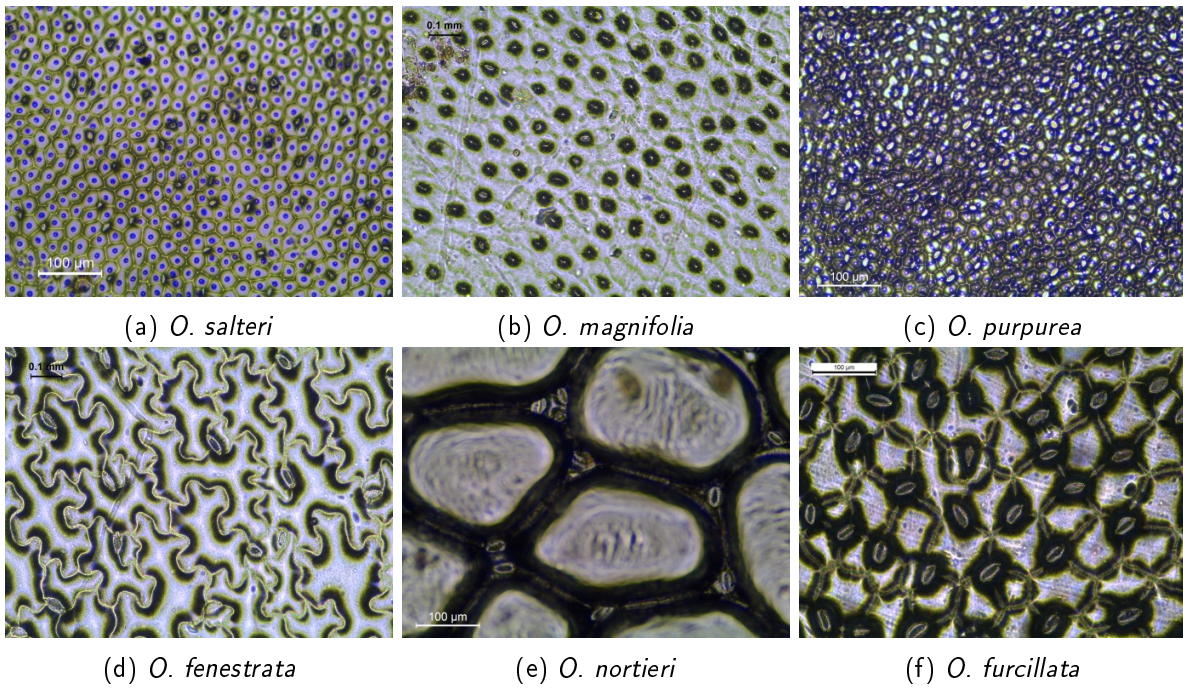
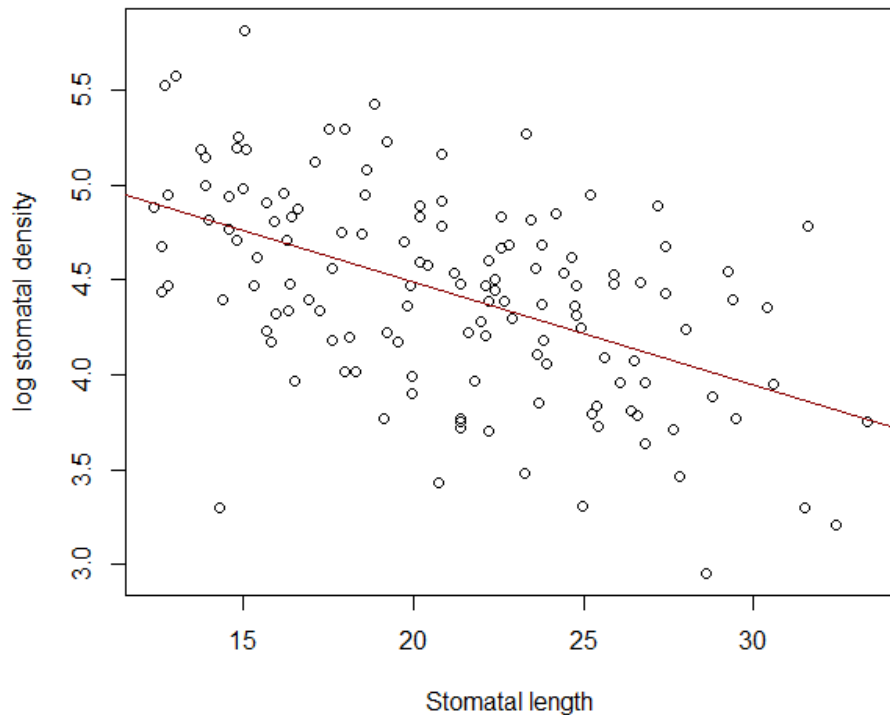
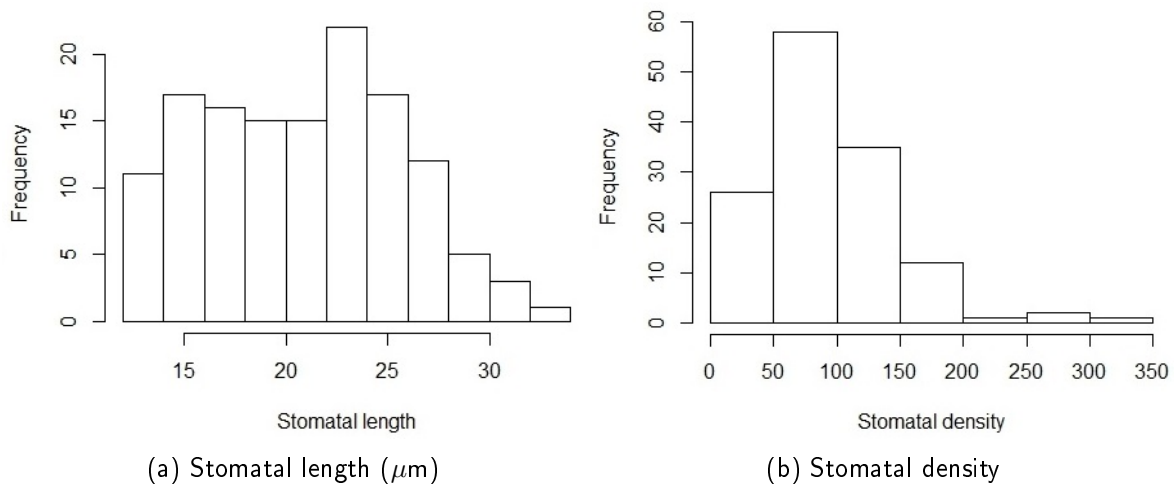


Figure 3.13: Light microscope photographs of three examples of the smallest and largest stomata observed within southern African *Oxalis* species. (a) AD surface of epistomatic *O. salteri* (MO1137), (b) AD surface of epistomatic "*O. magnifolia*" (MO1524), (c) AD surface of epistomatic *O. purpurea* (MO344), (d) AB surface of hypostomatic *O. fenestrata* (MO1527), (e) AD surface of amphistomatic *O. nortieri* (MO503), (f) AD surface of epistomatic *O. furcillata* (MO564).



(c) Stomatal length vs. log stomatal density

Figure 3.14: Histograms and scatterplot indicating stomatal lengths and densities of all stomata (AD and AB) for all studied *Oxalis* taxa. Multiple  $R^2 = 0.262$ , Adjusted  $R^2 = 0.256$ ,  $F = 47.55$ ,  $df = 1, 134$ ,  $p < 0.001$ .

The correlation of stomatal length and log stomatal density showed a strongly supported negative relationship (Multiple  $R^2 = 0.262$ , Adjusted  $R^2 = 0.256$ ,  $F = 47.55$ ,  $df = 1$  and  $134$ ,  $p < 0.001$ ) (Figure 3.14c).

Ploidy level of studied taxa ranged from 2X to 12X, and a few species had variable ploidy levels for example *O. purpurea* and *O. stellata* had 2X and 4X ploidy levels, whereas *O. flava* had 2X, 4X, 6X, 10X and 12X ploidy levels and *O. obtusa* had 2X, 3X, 4X, 5X, 6X and 8X ploidy levels.

The correlation of log stomatal size with ploidy levels showed a weak, positive linear relationship (Multiple  $R^2 = 0.071$ , Adjusted  $R^2 = 0.056$ ,  $F = 4.67$ ,  $df = 1, 61$ ,  $p < 0.05$ ) (Figure 3.15).

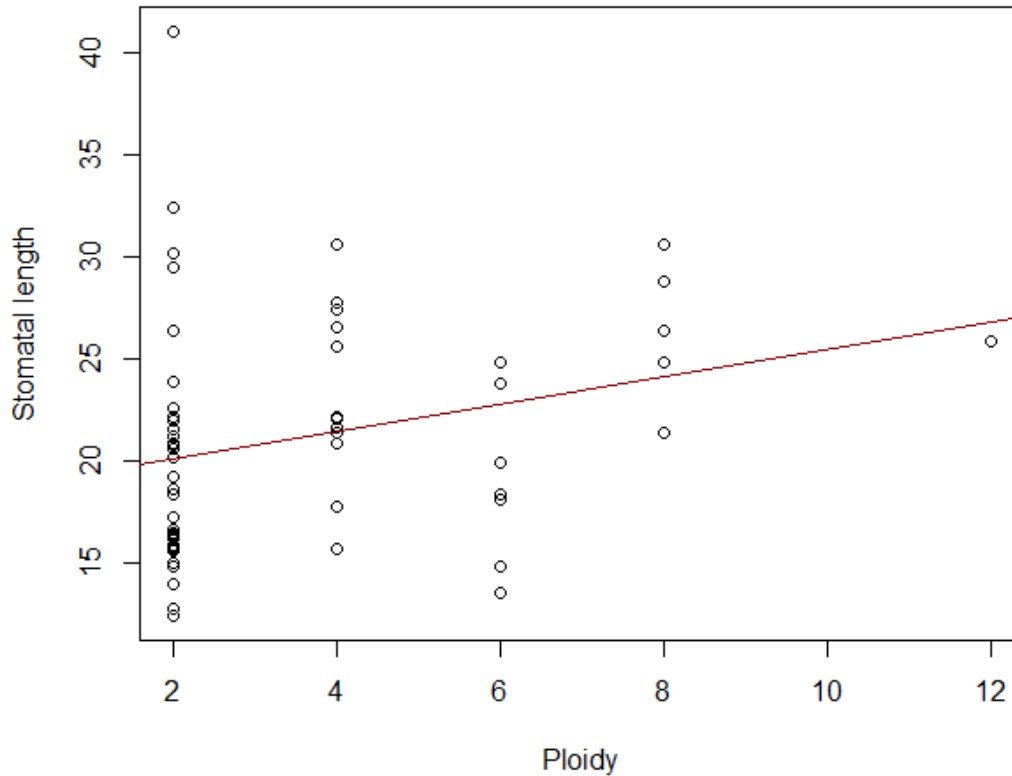


Figure 3.15: Scatter-plot indicating the relationship between stomatal length and ploidy levels in the *Oxalis* taxa studied. Multiple  $R^2 = 0.071$ , Adjusted  $R^2 = 0.056$ ,  $F = 4.67$ ,  $df = 1, 61$ ,  $p < 0.05$ .

The stomatal index of *Oxalis* species ranged from 7.87 (AB surface of *O. kamiesbergensis*) to 66.67 (AD surface of *O. punctata*). The SI of AB located stomata (average = 39.07) were significantly higher than the SI of AD located stomata (average = 31.17) ( $t = -2.98$ ,  $df = 68.25$ ,  $p < 0.01$ ) and no significant difference was detected between the SI of garden and field collected species ( $t = 1.225$ ,  $df = 65.67$ ,  $p > 0.05$ ). For these comparisons the data from epistomatic and the AD surface of amphistomatic species were pooled to represent the AD SI, and the data of hypostomatic and the AB surface of amphistomatic taxa were pooled to represent AB SI. The SI of the AD and AB surface of amphistomatic taxa were similar (AD average = 48.15, AB average = 43.79,  $p > 0.05$ ), but the SI of hypostomatic taxa (average = 37.15) were significantly lower than the SI of amphistomatic taxa ( $p < 0.05$ ), and the SI of epistomatic taxa (average = 26.06) were significantly lower than those of hypostomatic taxa ( $p < 0.01$ ) (Figure 3.16).



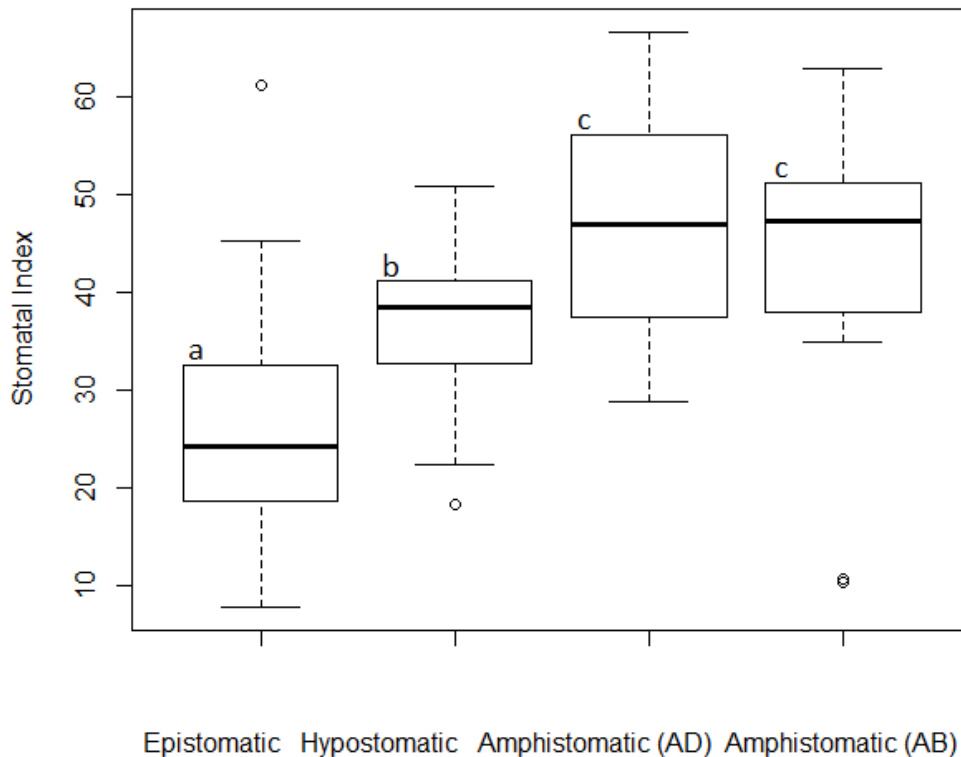


Figure 3.16: Boxplot indicating the Stomatal Index of epistomatic, hypostomatic and amphistomatic leaves from southern African *Oxalis* species sampled from the garden collection and various field localities. The symbols a and b were significantly different at  $p < 0.01$ , b and c were significantly different at  $p < 0.05$ .

Interestingly, 32 taxa appeared to have the majority of their stomata on one leaflet surface (*i.e.* epistomatic or hypostomatic), but with a few additional stomata on the opposite leaflet surface above the central vein. The additional stomata on the AD surface of hypostomatic taxa (Group 2) were of a similar size to the AB stomata (AD =  $25.8 \mu\text{m}$  (sd =  $5.88 \mu\text{m}$ ), AB =  $25.5 \mu\text{m}$  (sd =  $4.65 \mu\text{m}$ )), but the additional stomata on the AB surface of epistomatic taxa (average =  $35.62 \mu\text{m}$  (sd =  $5.77 \mu\text{m}$ )) were significantly larger than the stomata on the AD surface (Group 1) (average =  $19.65 \mu\text{m}$  (sd =  $4.61 \mu\text{m}$ )) ( $t = -9.243$ ,  $df = 12.70$ ,  $p < 0.001$ ) (Figure 3.17).

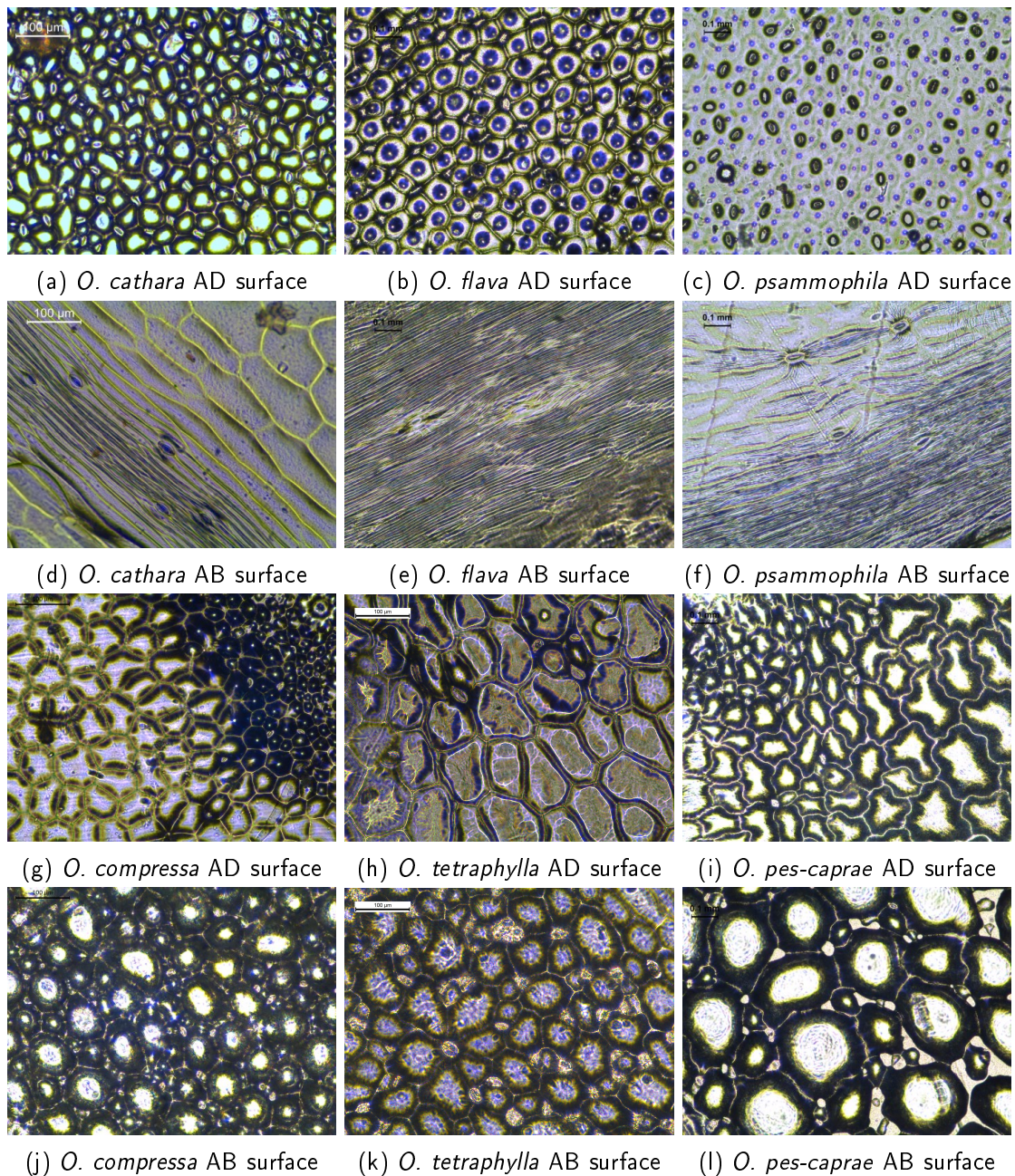


Figure 3.17: Light microscope photographs of *Oxalis* taxa with epistomatic leaflets with additional vein-associated stomata on the AB surface, and hypostomatic leaflets with additional vein-associated stomata on the AD surface. (a) AD surface of epistomatic *O. cathara* (MO582), (b) AD surface of epistomatic *O. flava* (field), (c) AD surface of epistomatic *O. psammophila* (field), (d) AB surface of *O. cathara* (MO582), (e) AB surface *O. flava* (field), (f) AB surface *O. psammophila* (field), (g) AD surface of *O. compressa* (MO519), (h) AD surface of *O. tetraphylla* (MO392), (i) AD surface of *O. pes-caprae* (field), (j) AB surface of *O. compressa* (MO519), (k) AB surface of *O. tetraphylla* (MO392), (l) AB surface of *O. pes-caprae* (field).

The statistical comparison of stomatal sizes based on the position of stomata on a leaflet showed that AD located stomata (epistomatic and AD surface of amphistomatic leaflets) were significantly smaller than AB located stomata (hypostomatic and AB surface of amphistomatic leaflets) ( $F = 29.56$ ,  $df = 186$  and  $5$ ,  $p < 0.001$ ) (Figure 3.18). The additional vein-associated stomata on the AB surface (average length =  $35.62 \mu\text{m}$ ) of epistomatic leaflets were significantly larger ( $p < 0.001$ )

than the stomata from the AD surface (average length = 19.65  $\mu\text{m}$ ). The additional vein-associated stomata on the AD surface (average length = 25.87  $\mu\text{m}$ ) of hypostomatic leaflets were similar in size ( $p > 0.05$ ) to the stomata from the AB surface (average length = 25.21  $\mu\text{m}$ ) ( $F = 29.56$ ,  $df = 186$  and 5) (Figure 3.18). The average stomatal densities ranged from 13 to 335 stomata per  $\text{mm}^2$ .

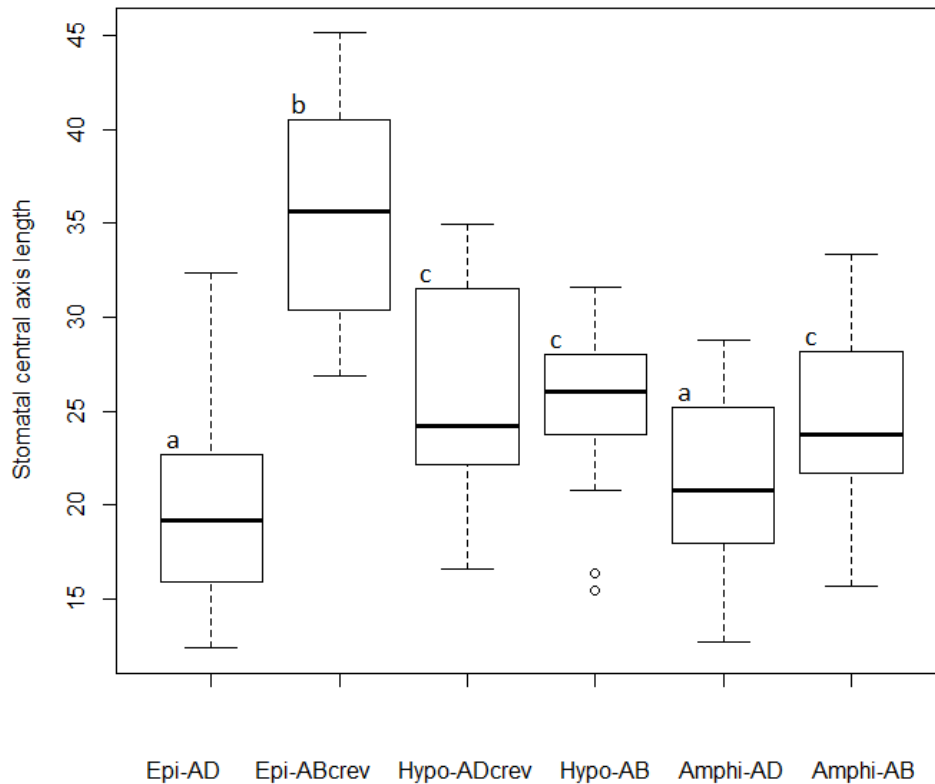


Figure 3.18: Boxplot indicating stomatal central axis lengths of *Oxalis* taxa with epistomatic, hypostomatic and amphistomatic stomatal positions on leaflets. The stomatal lengths of the additional vein-associated stomata that occur on the AB surface of epistomatic leaflets and on the AD surface of hypostomatic leaflets were included in this boxplot. The symbols a, b and c were significantly different at  $p < 0.001$ .

The stomatal conductance of the AD surface (average = 149.12  $\text{mmol/m}^{-2}\text{s}^{-1}$ ) of epistomatic leaflets were significantly higher ( $p < 0.001$ ) than the conductance of the additional AB located vein-associated stomata (average = 44.74  $\text{mmol/m}^{-2}\text{s}^{-1}$ ) found on some epistomatic leaflets (Figure 3.19). This was as expected, because the additional AB located vein-associated stomata occur in very low densities (in some taxa only 30 stomata on the AB surface of a leaflet). The stomatal conductance of the AB surface (average = 103.5  $\text{mmol/m}^{-2}\text{s}^{-1}$ ) of hypostomatic leaflets were significantly higher ( $p < 0.001$ ) than the conductance of the additional AD located vein-associated stomata (average = 53.34  $\text{mmol/m}^{-2}\text{s}^{-1}$ ) ( $F = 45.42$ ,  $df = 622$  and 5). Despite the significantly lower conductance rates of the additional vein associated stomata (of epistomatic and hypostomatic leaflets), these stomata had conductance rates that contribute to the overall conductance of each leaflet.

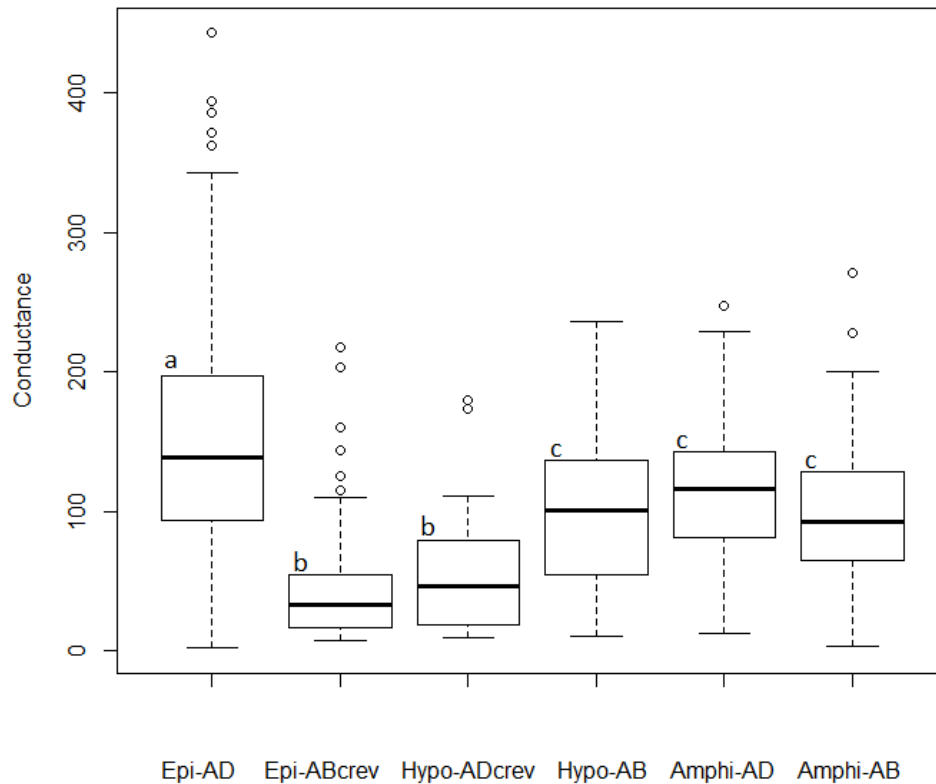


Figure 3.19: Boxplot indicating the stomatal conductance ( $\text{mmol}/\text{m}^{-2}\text{s}^{-1}$ ) of southern African *Oxalis* species measured on both the adaxial (AD) and abaxial (AB) surfaces of epistomatic (Epi), hypostomatic (Hypo) and amphistomatic (Amphi) leaflets, and leaflets with additional vein-associated stomata located in the crevice of leaflets (Epi-ABcrev and Hypo-ADcrev). The symbols a, b and c were significantly different at  $p < 0.001$ .

### 3.4.3 Leaflet physiology (SLA and mesophyll thickness)

The total surface areas of all leaflets per leaf were significantly higher for leaves collected in the field (average =  $7.08 \text{ cm}^2$ ) than from the garden (average =  $1.31 \text{ cm}^2$ ) ( $t = -6.17$ ,  $df = 26.22$ ,  $p < 0.001$ ) (Figure 3.20). It should be noted that many more species were sampled from the garden collection and many of the garden samples were small-leaved species that were not collected from the field, causing these data to be biased. The total dry weight per leaf was similar for leaves collected in the field (average =  $0.047 \text{ g}$ ) and from the garden (average =  $0.048 \text{ g}$ ) ( $t = 0.04$ ,  $df = 25.48$ ,  $p > 0.05$ ) (Figure 3.21).

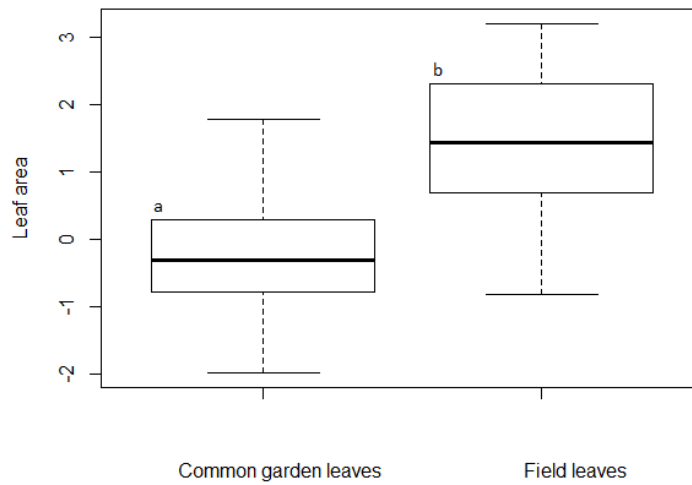


Figure 3.20: Boxplot indicating the log surface area ( $\text{cm}^2$ ) of southern African *Oxalis* leaves sampled from the living collection and the field. The symbols a and b were significantly different at  $p < 0.001$ .

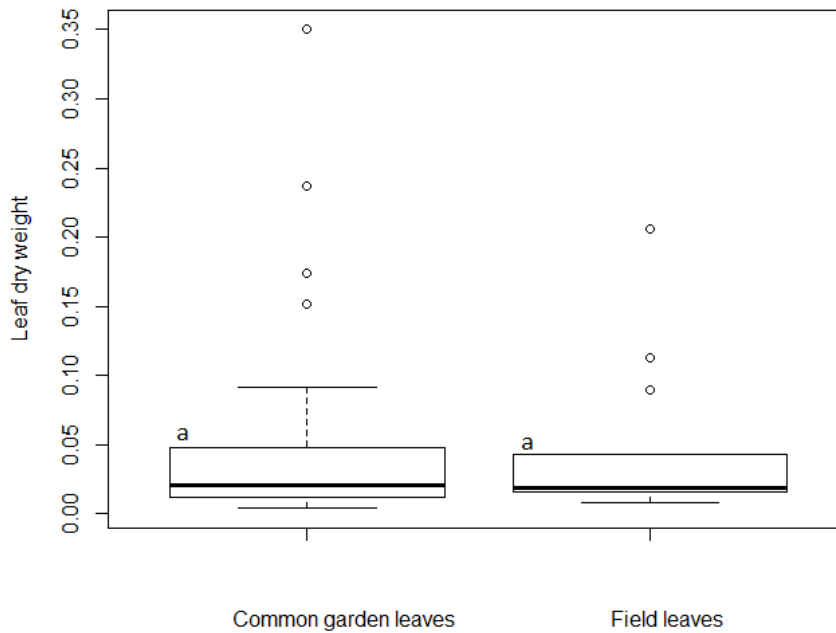


Figure 3.21: Boxplot indicating the total dry weight (g) of southern African *Oxalis* leaves sampled from the living collection and the field.

The SLA of species collected in the garden and the field varied considerably as the SLA ranged from  $29.78 \text{ cm}^2/\text{g}$  (garden sample) to  $649.46 \text{ cm}^2/\text{g}$  (field sample). The SLA of species from the field (average =  $301.25 \text{ cm}^2/\text{g}$ ) were significantly higher than garden species (average =  $159.20 \text{ cm}^2/\text{g}$ )

( $t = -3.28$ ,  $df = 21.67$ ,  $p < 0.01$ ) (Figure 3.22). In this study we had one species (*O. magnifolia*) sampled from the garden and the field with multiple samples from different field localities, and this example illustrated that the SLA within a species can be variable. The SLA of the garden sample of *O. magnifolia* had an average of  $164.22 \text{ cm}^2/\text{g}$  and the SLA of samples from the field varied from  $161.08 \text{ cm}^2/\text{g}$  to  $427.91 \text{ cm}^2/\text{g}$ .

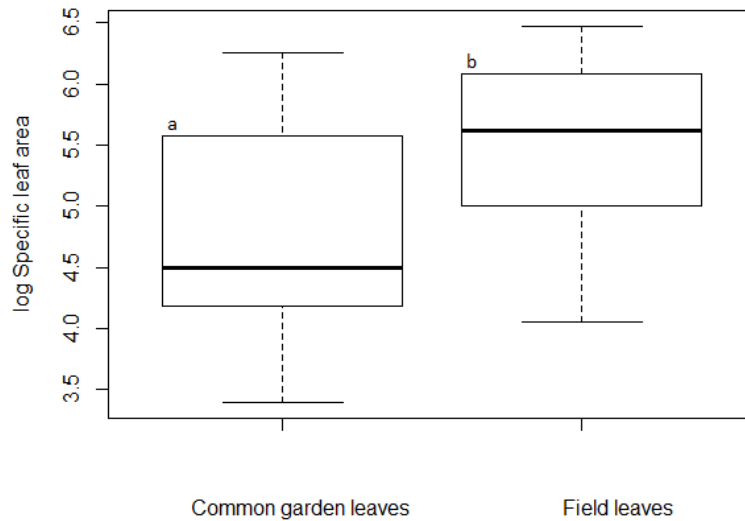


Figure 3.22: Boxplot indicating the log specific leaf area ( $\text{cm}^2/\text{g}$ ) (SLA) of southern African *Oxalis* leaves sampled from the living collection and the field. The symbols a and b were significantly different at  $p < 0.01$ .

The log mesophyll thickness ( $F = 7.446$ ,  $df = 67$  and  $2$ ,  $p < 0.05$ ) (Figure 3.23) and log total leaflet section heights ( $F = 8.430$ ,  $df = 68$  and  $2$ ,  $p < 0.05$ ) (Figure 3.24) of amphistomatic leaflets were significantly higher than epistomatic or hypostomatic leaflets.

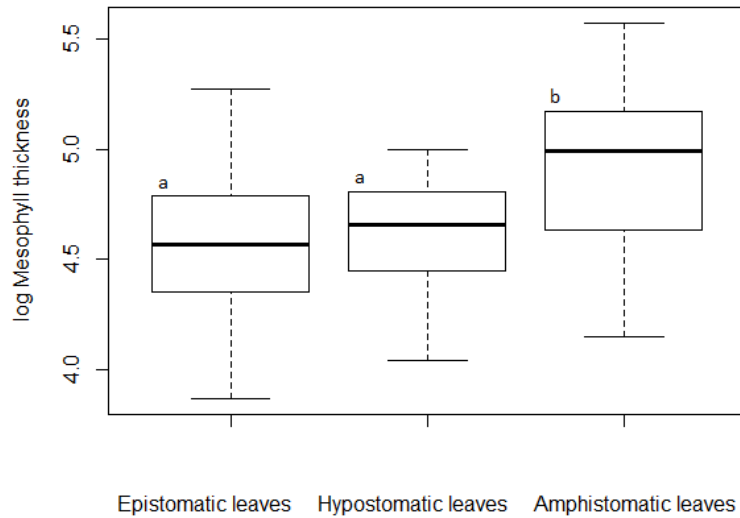


Figure 3.23: Boxplot indicating log mesophyll thickness ( $\mu\text{m}$ ) (palisade and spongy parenchyma tissue) of southern African *Oxalis* with epistomatic, hypostomatic and amphistomatic leaflets. The symbols a and b were significantly different at  $p < 0.05$ .

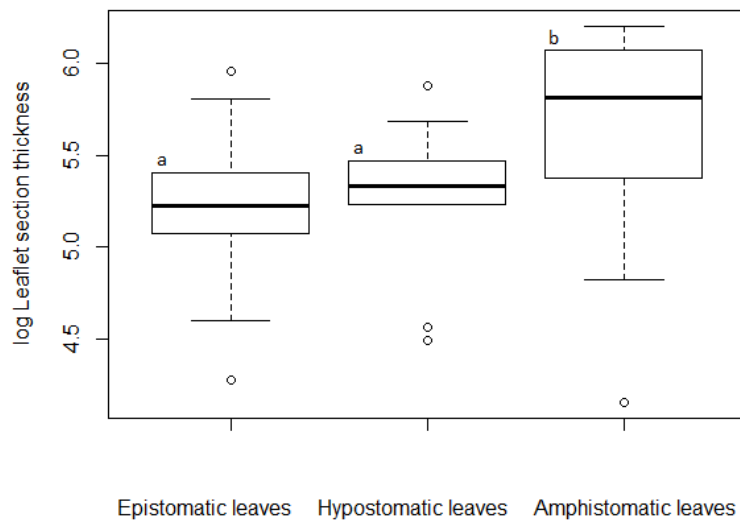


Figure 3.24: Boxplot indicating log leaflet section thickness (total adaxial and abaxial epidermal cell height and the mesophyll thickness) ( $\mu\text{m}$ ) of southern African *Oxalis* with epistomatic, hypostomatic and amphistomatic leaflets. The symbols a and b were significantly different at  $p < 0.05$ .

### 3.5 Discussion

The  $\delta^{13}\text{C}$  isotope data together with the stomatal conductance data gathered in this study conclusively show that all sampled southern African *Oxalis* species follow a  $\text{C}_3$  photosynthetic pathway. Based on the gathered stomatal conductance, leaflet physiological and leaflet anatomical data, we propose a new understanding of the physiology of *Oxalis* in relation to the unique growing conditions and resulting life history of this genus in the winter rainfall region of the Greater Cape Floristic Region (GCFR) of southern Africa.

*Oxalis* was previously regarded as a water-conservative genus (Kluge and Ting (1978); Proches *et al.* (2006a); Biocyclopedia: *Oxalis* (2012)), to the extent that the possibility of a CAM photosynthetic pathway was proposed for some species. The stomatal conductance measurements of southern African *Oxalis* species showed the opposite; that many species make minimal effort to prevent water loss. To our knowledge only one other study has recorded the stomatal conductance of an *Oxalis* species, namely a study on geophytes in Namaqualand by Rossa and von Willert (1999). They measured various photosynthetic traits of geophytes from the Namaqualand region, including *O. flava*, and reported conductance measurements that ranged between  $280 \text{ mmol/m}^{-2}\text{s}^{-1}$  and  $360 \text{ mmol/m}^{-2}\text{s}^{-1}$  for this species. Our own measurements for this species ranged between  $137 \text{ mmol/m}^{-2}\text{s}^{-1}$  and  $228 \text{ mmol/m}^{-2}\text{s}^{-1}$ . The stomatal conductance range of all studied *Oxalis* species ranged from  $31.4 \text{ mmol/m}^{-2}\text{s}^{-1}$  to  $443.1 \text{ mmol/m}^{-2}\text{s}^{-1}$  and different geophytic species from the Namaqualand region reportedly have stomatal conductance rates that range between  $60 \text{ mmol/m}^{-2}\text{s}^{-1}$  and  $600 \text{ mmol/m}^{-2}\text{s}^{-1}$  (Rossa and von Willert, 1999). Both datasets show a wide spread in conductance rates, and comparing our own *Oxalis* dataset to the geophytic dataset, it appears that the stomatal conductance of *Oxalis* is fairly standard to the other measured GCFR geophytes. Importantly, Rossa and von Willert (1999) stated that the studied geophytes (and we suggest southern African *Oxalis* too) tend to have high conductance rates, comparable to those of typical annual plants.

Geophytes from the Namaqualand region inhabit semi-arid areas with predictable summer drought, but their growth and reproductive stages coincide with the strongly seasonal winter rainfall of the Cape, which creates an environment with abundant water availability and moderate temperatures (Ruiters *et al.* (1993b); Ruiters *et al.* (1993c)). Given the active winter growing season of *Oxalis* species, and our interesting observation that the majority of southern African *Oxalis* species have the rare trait of epistomatic leaflets, we propose that there could be an association between the leaflet anatomical and physiological traits of these species.

All non-south African *Oxalis* species are reportedly hypostomatic, as well as the members of two southern African clades (19.6% of studied species). Hypostomatic leaflets had relatively large stomata, relatively low stomatal densities and the lowest stomatal conductance of the three stomatal position strategies pursued by SA *Oxalis*. Epistomatic leaflets are regarded as the rarest leaflet type among angiosperms (Muir, 2015), but given our current sampling the majority of studied southern African species (64.7%) displayed this phenomenon. Clade 4 represents 90% of the southern African *Oxalis* diversity, so given your wide sampling across Clade 4 it is reasonable to assume that all the other species in Clade 4 have AD stomata too. Our study showed that southern African *Oxalis* species with epistomatic leaflets had significantly smaller stomatal lengths and significantly higher stomatal densities than hypostomatic or amphistomatic leaflet types. Epistomatic leaflets had conductance rates higher than that of hypostomatic leaflets and lower than amphistomatic leaflets. Amphistomatic leaflets were identified in all members (14 species) from a sub-clade within Clade 7 of the *Oxalis* phylogeny (Jooste (2015); Chapter 2). Leaf physiological traits associated with this leaflet type included: significantly lower stomatal densities and significantly higher stomatal index



values on both leaflet surfaces, significantly higher total leaflet conductance (pooled AD and AB leaflet data) and many of the thickest leaflets and thickest mesophyll recorded belonged to members of this clade. The stomatal lengths located on the AD surfaces of these species were significantly smaller than the AB surface, and similar in size to respective stomata in epistomatic and hypostomatic species, respectively.

There are various costs and benefits associated with different stomatal positions on leaflets. Hypostomatic leaflets are regarded as the most common leaflet type on a global scale (Muir, 2015) as more than 90% of all plant species are hypostomatic (Salisbury (1927); Leick (1927); Wood (1934); Fitter and Peat (1994)). The prevalence of hypostomatic leaflets among angiosperms is suggested to reflect a cost associated with AD located stomata (Muir, 2015), for example, AD located stomata might increase the leaves' susceptibility to foliar pathogens (Gutschick, 1984), excessive water loss due to evapotranspiration (Foster and Smith, 1986) and potential water-blockage of stomata (Smith and McClean, 1989). It is suggested that there are only a few rare situations when epistomatic leaflets are favoured, for example in plants with unusual epidermal or mesophyll anatomy or in aquatic plants (Muir, 2015). Cape *Oxalis* invaded aquatic habitats at least twice (Oberlander *et al.*, 2014), and all three aquatic *Oxalis* lineages are epistomatic. These southern African examples agree with Muir (2015), indicating that AD located stomata are a necessary pre-adaptation for an aquatic plant where leaves float. Amphistomatic plants have the remarkable ability to separately regulate stomatal opening on the AD and AB surfaces, meaning that when amphistomatic plants are water stressed, they can close the adaxially-located stomata and function as hypostomatic plants by keeping the abaxially-located stomata open (Spinner (1936); Smith and Heuer (1981); Pospisilova and Solarova (1982); Reich (1984)).

The size and abundance of stomata are important traits that determine whole-plant function, because they influence the maximum photosynthetic capacity of leaves by determining the amount of CO<sub>2</sub> that gets absorbed (Broadribb *et al.* (2007); Franks and Beerling (2009)). Smaller stomata on angiosperm leaves enable plants to have a more rapid response to temperature changes in their environment (Hetherington and Woodward (2003); Franks and Farquhar (2007); Drake *et al.* (2013)). An excellent example of how sensitive geophytes are to temperature changes in the Namaqualand region was documented for *Romulea amoena* (Schltr.), while recording continuous gas exchange (Rossa and von Willert, 1999). They captured gas exchange data for this species under berg-wind conditions, which is phenomenon that takes place during winter in the Namaqualand region, causing a rapid increase in temperature (von Willert *et al.*, 1992). Air temperature reportedly rose from 15°C to 25.5°C within a day and consequently the maximum CO<sub>2</sub> exchange dropped from 2.5 to 0.8 μmol m<sup>-2</sup> s<sup>-1</sup> and clearly showed the sensitivity of *R. amoena* to increased temperatures (Rossa and von Willert, 1999). Smaller stomata can be packed more densely on a leaf surface, relative to larger stomata, which means that leaves with smaller stomata would have a higher capacity to absorb CO<sub>2</sub> under favourable conditions (Broadribb *et al.* (2007); Franks and Beerling (2009)), but also to rapidly reduce conductance when conditions become unfavourable (Drake *et al.*, 2013).

Southern African geophytes from the Namaqualand region tend to have high conductance rates (Rossa and von Willert, 1999) and we have suggested that the conductance rates of studied *Oxalis* species are comparable. Plants with high stomatal conductance would have high transpiration rates, and we propose that these high transpiration rates could act as a mechanism of evaporative cooling for these plants. The leaflet temperatures of all studied species ranged between 18.2°C and 31.9°C, which also clearly coincides with the theoretical temperature range of C<sub>3</sub> plants (20°C to 30°C) (Larcher, 1995). The reported optimum temperature for photosynthetic CO<sub>2</sub> uptake of *O. flava* (with epistomatic leaflet type) is 17.9°C (Rossa and von Willert, 1999), and this optimal temperature value was described as being "remarkably low". It was suggested that the geophytes

from the Namaqualand region have low temperature optima in order to prevent photorespiration, which is a wasteful process induced at high temperatures in order to prevent water loss in  $C_3$  plants (Farquhar and Sharkey (1982); Sharkey (1988); Farquhar *et al.* (1989)). It has been proposed that when water supply is not limited, leaf cooling is promoted through increased transpiration (Lu *et al.* (1994); Radin *et al.* (1994)). We therefore propose that the high conductance rates could act as an evaporative cooling mechanism, which helps to maintain a relatively cool leaflet temperature (close to the temperature optima), even when ambient temperatures are much higher during the day. We suspect that these high conductance rates were made possible by the reportedly high soil moisture content during the winter months within the natural habitat of the *Oxalis* species, which enables maximum photosynthesis and growth when environmental conditionals are optimal. Plants with higher stomatal conductance rates can have improved water-use efficiencies and higher carbon fixation rates (Cowan, 1977), which would be favoured by natural selection (Drake *et al.*, 2013).

Given these various anatomical and physiological traits observed in southern African *Oxalis* and the importance of these traits to a plant, we expected to detect associations between stomatal conductance, stomatal length, stomatal density and stomatal position. The outcome of the multiple regression analysis showed that stomatal length and stomatal density had no effect on the stomatal conductance, and that stomatal position (epistomatic, hypostomatic and amphistomatic) was the only variable that significantly affected the stomatal conductance in studied southern Africa *Oxalis* species, although this variable only explained approximately 20% of the variation in our data, indicating that there are potentially other variables that should be considered in future analyses. Only 54 species were included in this analysis (as there were only this many samples with a complete dataset for all variables tested). It is therefore possible that the studied variables do have an effect on stomatal conductance, but that the relationships could not be detected due to the small sample size. It should also be noted that stomatal length and stomatal density are inversely correlated - this would imply that it is possible for the stomatal conductance to increase with one variable, whilst the other variable decreases. This would effectively cancel the effect of these variables all together. Finally, it appears that stomatal position affects stomatal length and stomatal density, so the apparent trend between these variables and conductance is actually being driven by the underlying variable, namely stomatal position.

Two hypotheses have been suggested to try and explain the relationships between plant growth form and stomatal position of herbaceous plants (Muir, 2015), which might be applicable to the studied southern African *Oxalis* species. The first hypothesis suggests that due to the relatively short life spans of herbaceous leaves (Wright *et al.*, 2005), these plants need to have high photosynthetic rates to 'pay' their construction costs over a relatively short period of time (Reich *et al.* (2003); Muir (2015)). The second hypothesis suggests that due to the relatively fast life histories of herbaceous plants, faster growth rates (enabled by higher photosynthetic rates in leaves (Field and Mooney, 1986)) are strongly selected for traits (Muir, 2015). The application of the first hypothesis to southern African *Oxalis* would be invalid as the leaf life spans of epistomatic, hypostomatic and amphistomatic species are the same - one season. The second hypothesis would also possibly be invalid as all southern African *Oxalis* are perennials with relatively short life spans within a growing season. To date, only one data-point has been recorded to give an indication of life span of southern African *Oxalis* species, which indicated that *O. polyphylla* can reach up to 40 years of age Salter (1944). Although this second hypothesis might be true for southern African *Oxalis* species, it remains entirely speculative until further assessed.

It has been shown that plants with amphistomatic leaflets have an increased photosynthetic rate (Parkhurst, 1978). This is because the stomatal distribution of amphistomatic leaflets reportedly optimizes the photosynthetic rate of leaves (Parkhurst (1978); Gutschick (1984)). Several anatomical

and ecological factors have been proposed to favour amphistomatic leaflets. These include high leaf thickness, greater light intensity, lower precipitation, higher altitude and herbaceous growth form. Theoretically, plants with equal stomatal densities on both the AD and AB leaflet surfaces would be able to maximize their photosynthetic rate by minimizing the distance between stomata and the chloroplasts within the mesophyll tissue (Parkhurst (1978); Gutschick (1984); Mott *et al.* (1984); Parkhurst and Mott (1990)) and amphistomatic plants should be able to maintain more efficient temperature control and fix carbon at a faster rate due to higher conductance rates (as previously discussed). Based on these theories, the majority of all plants, as well as all southern African *Oxalis* species, should display this phenomenon. However, among southern African *Oxalis* only 15.2% of all studied species displayed this phenomenon. Amphistomatic leaflets would be regarded as a very successful strategy, but it is apparent that there are also associated costs.

Epistomatic leaflets would be regarded as the second most effective strategy as epistomatic leaflets have the second highest conductance rates, and would have an adaptive advantage over hypostomatic leaflets, which have the lowest stomatal conductance rates. It has been shown that stomatal position is an ecologically relevant plant functional trait that could be valuable for understanding physiological and ecological evolution of plants Muir (2015). Considering these phenomena in an evolutionary perspective, a shift away from hypostomatic leaflets would be a favourable adaptation that would be more advantageous than disadvantageous if the increase in photosynthetic rate outweighs the increased risks, such as increased pathogenic infection, when stomata are AD-located.. Jooste (2015) (Chapter 2) proposed that hypostomatic leaflets were the ancestral state of the southern African *Oxalis* genus. It seems likely that the shift from hypostomatic to epistomatic leaflets took place via an intermediate state with amphistomatic leaflets, as an intermediate state without any stomata would be strongly selected against. The presence of the additional vein-associated stomata observed in many southern African *Oxalis* species is suggested. This trait appears to be fairly common and widely scattered across the tree, which could indicate that it is fairly easy to evolve. For example, the shift from hypostomatic leaflets to amphistomatic leaflets proceeded via hypostomatic leaflets with additional vein-associated stomata on the AD leaflet surface.

It appears that the epistomatic leaflet strategy with conductance rates relatively higher than that of hypostomatic leaflets, but less extreme than amphistomatic leaflets were widely favoured among many southern African *Oxalis* species. However, we should note that amphistomatic leaflets theoretically (and based on our stomatal conductance data) appear to be the most successful strategy, so we have to question why amphistomatic leaflets lost the AB stomata and were driven away from the 'best solution' to become epistomatic? It is possible that selected species (the members from the sub-clade of Clade 7) were anatomically capable of maintaining amphistomatic leaflets, and that these species were at a selective advantage causing this trait to evolve secondarily among the members from this clade (and that it was once again mediated by the presence of the additional vein-associated stomata on the AB surface of epistomatic leaflets). In this study we have only measured a potential benefit of changing stomatal position (*i.e.* stomatal conductance), but we did not measure any associated costs, for example stomatal infection, and the associated costs of stomatal position in *Oxalis* are factors that should be measured and considered in future studies.

We suggest that the shift from hypostomatic to epistomatic leaflets, due to the potential selective advantage of higher conductance rates associated with stomatal position, is a successful strategy for the majority of the *Oxalis* species studied and a possible key innovation for life in the Cape. The success of this strategy can be attributed only to the fact that the growing season of *Oxalis* coincides with the winter rainfall of the GCRF. We suggest that this strategy was so successful, that it has enabled the radiation of *Oxalis* into one of the largest geophytic plant lineages in the GCRF.

### 3.6 Conclusion

Previous limited work on *Oxalis* had shown evidence for CAM photosynthesis in some South American species and preliminary anatomical work on southern African *Oxalis* hinted at Kranz anatomy, typically associated with  $C_4$  photosynthesis. We assessed stable carbon-isotope data, stomatal conductance, leaflet physiological and anatomical traits of 67 southern African *Oxalis* species to test whether any indigenous species show alternative modes of photosynthesis. All assessed taxa followed an exclusively  $C_3$  photosynthetic pathway. The measured stomatal conductance data were higher than expected for *Oxalis* (Kluge and Ting (1978); Proches *et al.* (2006a); Biocyclopedia: *Oxalis* (2012)) and matched the conclusions of limited previous work on geophytes from the Namaqualand region (Rossa and von Willert, 1999). We concluded that the stomatal conductance data indicates that southern African *Oxalis* make a minimal effort to prevent water-loss.

Additionally we observed that the vast majority of southern African *Oxalis* species have epistomatic leaflets, which is regarded as the rarest stomatal position among all angiosperms (Muir, 2015). We suggest that the shift from the ancestral state of hypostomatic leaflets to epistomatic leaflets is a successful strategy for the majority of the *Oxalis* from the GCFR, due to the potential selective advantage of higher conductance rates associated with an AD stomatal position than AB position. We propose that this strategy would be favoured under the strongly seasonal environment in which the majority of southern African *Oxalis* species grow (GCFR - winter rainfall), as this enables these plants to take advantage of their growing conditions to possibly ensure high productivity, rapid growth and successful carbon storage into their below-ground bulbs. We propose that the strategy of adaxially-located stomata with relatively high conductance rates might be an overlooked key innovation driving diversification in one of the largest geophytic plant lineages within the GCFR.

### 3.7 References

- Beaulieu, J.M., Leitch, I.J., Patel, S., Pendharkar, A., Knight, C.A. and Beaulieu, J.M. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist*, vol. 179, pp. 975–986.
- Berry, J. and Bjorkman, O. (1980). Photosynthetic response and adaptation to temperature in higher-plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 31, pp. 491–543.
- Biocyclopedia: *Oxalis* (2012). Accessed: 2014-05-30.
- Botha, C.E.J. (1992). Planmodesmatal distribution, structure and frequency in relation to assimilation in C<sub>3</sub> and C<sub>4</sub> grasses in southern Africa. *Planta*, vol. 187, pp. 348–358.
- Broadribb, T.J., Field, T.S. and Jordan, G.S. (2007). Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology*, vol. 144, pp. 1890–1898.
- Brown, W.V. (1975). Variations in anatomy, associations, and origins of Kranz tissue. *American Journal of Botany*, vol. 62, pp. 395–402.
- Burns, W. (1946). Corm and bulb formation with special reference to the Graminae. *Transactions and Proceedings of the Botanical Society of Edinburgh*, vol. 34, pp. 316–347.
- Campbell, B.M. (1983). Montane plant environments in the fynbos biome. *Bothalia*, vol. 14, pp. 283–298.
- Cowan, I.R. (1977). Stomatal behaviour and environment. *Advances in Botanical Research*, vol. 4, pp. 117–228.
- De Villiers, M.J. (2001). A systematic revision of *Oxalis* L. section *Crassulae* T.M. Salter (Oxalidaceae).
- Downton, W.J.S., Berry, J.A. and Seeman, J.R. (1984). Tolerance of photosynthesis to high temperature in desert plants. *Plant Physiology*, vol. 74, pp. 786–790.
- Drake, P.L., Froend, R.H. and Franks, P.J. (2013). Smaller, faster stomata : scaling of stomatal size, rate of response, and stomatal conductance. *Journal of Experimental Botany*, vol. 64, no. 2, pp. 495–505.
- Ehleringer, J.R., Cerling, T.E. and Helliker, B.R. (1997). C<sub>4</sub> photosynthesis, atmospheric CO<sub>2</sub>, and climate. *Oecologia*, vol. 112, pp. 285–299.
- Ehleringer, J.R. and Monson, R.K. (1993). Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology, Evolution and Systematics*, vol. 24, pp. 411–439.
- Ehleringer, J.R. and Osmond, C.B. (1994). *Stable isotopes*, in: *Pearcy, R.W., Ehleringer, J.R., Mooney, H.A., Rundel, P.W. (Eds.), Plant Physiological Ecology*. Chapman and Hall, London.
- Farquhar, G.D., O'Leary, M.H. and Berry, J.A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Austrian Journal of Plant Physiology*, vol. 9, pp. 121–137.
- Farquhar, G.D. and Sharkey, T.D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, vol. 33, pp. 317–345.

- Farquhar, G.D., von Caemmerer, S. and Berry, J.A. (1989). A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta*, vol. 149, pp. 78–90.
- Field, C. and Mooney, H.A. (1986). The photosynthesis-nitrogen relationship in wild plants. In: Givnish T.J., editor, *On the Economy of Plant Form and Function*. pp. 25–56.
- Fitter, A. and Peat, H. (1994). The ecological flora database. *Journal of Ecology*, vol. 82, pp. 415–425.
- Foster, J. and Smith, W. (1986). Influence of stomatal distribution on transpiration in low-wind environments. *Plant, Cell and Environment*, vol. 9, pp. 751–759.
- Franks, P.J. and Beerling, D.J. (2009). Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. *Plant, Cell and Environment*, vol. 32, pp. 1737–1748.
- Franks, P.J. and Farquhar, G.D. (2007). The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. *Plant Physiology*, vol. 125, pp. 935–942.
- Gibson, A.C. (1982). *The anatomy of succulence*, in: Ting, I.P., Gibbs, M. (Eds.), *Crassulacean acid metabolism*. American Society of Plant Physiologists, Rockvill.
- Gibson, A.C. (1986). *The cactus primer*. Harvard University Press, Cambridge, MA, USA.
- Guralnick, L.J. and Ting, I.P. (1987). Physiological changes in *Portulacaria afra* (L.) Jacq. during a summer drought and rewatering. *Plant Physiology*, vol. 85, pp. 481–486.
- Gutschick, V.P. (1984). Photosynthesis model for C<sub>3</sub> leaves incorporating CO<sub>2</sub> transport, propagation of radiation, and biochemistry 2. ecological and agricultural utility. *Photosynthetica*, vol. 18, pp. 569–595.
- Hetherington, A.M. and Woodward, F.I. (2003). The role of stomata in sensing and driving environmental change. *Nature*, vol. 424, pp. 901–908.
- J. G. Hodgson, J. G. ans Sharafi, M., Jalili, A., Diaz, I., Montserrat-Marti, G., Palmer, C., Cerabolini, B., Pierce, S., Hamzehee, B., Asri, Y. and et al. (2010). Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of Botany*, vol. 105, pp. 573–584.
- Jooste, M. (2015). The phylogenetic and functional significance of leaf anatomical and physiological traits of southern African *Oxalis*.
- Kluge, M. and Ting, I. (1978). *Crassulacean Acid Metabolism: analysis of an ecological adaptation*. Springer-Verlag, Heidelberg, Germany.
- Larcher, W. (1995). *Physiological Plant Ecology, 3rd edition*. Springer, Berlin.
- Larcher, W. (2003). *Physiological Plant Ecology*. Springer, Heidelberg, Berlin.
- Leick, E. (1927). Untersuchungen über den Einflub des Lichtes auf die offnungsweite unterseitiger und oberseitiger Stomata desselben Blattes. *Jahrbucher für Wissenschaftliche Botanik*, vol. 67, pp. 771–848.
- Lu, Z., Radin, J.W., Turcotte, E.L., Percy, R. and Zeiger, E. (1994). High yields in advanced lines of pima cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. *Physiologia Plantarum*, vol. 92, pp. 266–272.
- Manning, J. and Goldblatt, P. (2012). *Plants of the Greater Cape Floristic Region 1: the Core Cape Flora*. Strelitzia 29, South African National Biodiversity Institute, Pretoria, South Africa.
- Maxwell, K., von Caemmerer, S. and Evans, J.R. (1997). Is a low internal conductance to CO<sub>2</sub> diffusion a consequence of succulence in plants with crassulacean acid metabolism? *Australian Journal of Plant Physiology*, vol. 24, pp. 777–786.

- Moson, R.K. (1989). On the evolutionary pathways resulting in  $C_4$  photosynthesis and Crassulacean acid metabolism. *Advances in Ecological Research*, vol. 19, pp. 57–110.
- Mott, K.A., Gibson, A.C. and O'Leary, J.W. (1984). The adaptive significance of amphistomatic leaves. *Plant, Cell and Environment*, vol. 5, pp. 455–460.
- Muhaidat, R., Sage, R.F. and Dengler, N.G. (2007). Diversity of kranz anatomy and biochemistry in  $C_4$  eudicots. *American Journal of Botany*, vol. 3, pp. 362–381.
- Muir, C.D. (2015). *Selection constrains phenotypic evolution in a functionally important plant trait*. Ph.D. thesis, University of British Columbia.
- Nelson, A.E., Tammy, L.S. and Sage, R.F. (2005). Functional leaf anatomy of plants with crassulacean acid metabolism. *Functional Plant Biology*, vol. 32, pp. 409–419.
- Nelson, T. and Langdale, J.A. (1992). Developmental genetics of  $C_4$  photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 43, pp. 25–47.
- Oberlander, K.C., Dreyer, L.L. and Bellstedt, D.U. (2011). Molecular phylogenetics and origins of southern African *Oxalis*. *Taxon*, vol. 60, no. 6, pp. 1667–1677.
- Oberlander, K.C., Roets, F. and Dreyer, L.L. (2014). Pre-Pleistocene origin of an endangered habitat: links between vernal pools and aquatic *Oxalis* in the Greater Cape Floristic Region of South Africa. *Journal of Biogeography*, vol. 42, pp. 1572–1582.
- Ogburn, R.M. and Edwards, E.J. (2009). Anatomical variation in Cactaceae and relatives: trait liability and evolutionary innovation. *American Journal of Botany*, vol. 96, pp. 391–408.
- O'Leary, M.H. (1988). Carbon isotopes in photosynthesis. *Bioscience*, vol. 38, pp. 328–336.
- Osmond, C.B. (1982). Crassulacean Acid Metabolism: a curiosity in context. *Annual Review of Plant Physiology*, vol. 29, pp. 379–414.
- Parkhurst, D.F. (1978). The adaptive significance of stomatal occurrence on one or both surfaces of leaves. *Journal of Ecology*, vol. 66, pp. 367–383.
- Parkhurst, D.F. and Mott, K.A. (1990). Intercellular diffusion limits to  $CO_2$  uptake in leaves: studies in air and helox. *Plant Physiology*, vol. 94, pp. 1024–1032.
- Pate, J.S. and Dixon, K.W. (1981). Plants with fleshy underground storage organs - A Western Australian Survey. Pp. 181-215. In Pate, J. S. and McComb, A. J. (eds), *The Biology of Australian plants*.
- Pospisilova, J. and Solarova, J. (1982). Environmental and biological control of diffusive conductances of adaxial and abaxial leaf epidermes. *Photosynthetica*, vol. 18, pp. 445–453.
- Proches, S., Cowling, R.M. and Du Preez, D.R. (2006a). Patterns of geophyte diversity and storage organ size in the winter-rainfall region of southern Africa. *Diversity and Distribution*, vol. 11, pp. 101–109.
- Proches, S., Cowling, R.M. and Du Preez, D.R. (2006b). Patterns of geophyte diversity and storage organ size in the winter-rainfall region of southern Africa. *Diversity and Distribution*, vol. 11, pp. 101–109.
- R-Core-Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Radin, J.W., Lu, Z., Percy, R.G. and Zeiger, E. (1994). Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proceedings of the National Academy of Sciences, USA*, vol. 91, pp. 7217–7221.
- Rao, A.P. and Rajendrudu, R. (1989). Net photosynthetic rate in relation to leaf anatomical characteristics of  $C_3$ ,  $C_3$ - $C_4$  and  $C_4$  dicotyledons. *Proceedings of the Indian Academy of Sciences*, vol. 99, pp. 529–537.

- Raunkiaer, C. (1907). *Platerigets Livsformer og deres Betydning for Geografien*. Nordisk Verl., Copenhagen.
- Raunkiaer, C. (1934). *The Life Forms of Plants and Statistical Plant Geography*. Clarendon Press, Oxford.
- Rees, A.R. (1966). The physiology of ornamental bulbous plants. *Botanical Review*, vol. 32, pp. 1–23.
- Reich, P. (1984). Relationships between leaf age, irradiance, leaf conductance, CO<sub>2</sub> exchange, and water-use efficiency in hybrid poplar. *Photosynthetica*, vol. 18, pp. 445–453.
- Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J., Oleksyn, J. and et al. (2003). The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences*, vol. 164.
- Rossa, B. and von Willert, D.J. (1999). Physiological characteristics of geophytes in semi-arid Namaqualand, South Africa. *Plant Ecology*, vol. 142, pp. 121–132.
- Ruiter, C. (1995). Biomass and resource allocation patterns within the bulb of the perennial geophyte *Haemanthus pubescens* L. subsp. *pubescens* (Amaryllidaceae) in a periodic arid environment of lowland fynbos, South Africa. *South African Journal of Arid Environments*, vol. 31, pp. 311–323.
- Ruiters, C., McKenzie, B., Aalbers, J. and Raitt, L.M. (1993a). Seasonal allocation of biomass and resources in the geophytic species *Haemanthus pubescens* subspecies *pubescens* in lowland coastal fynbos, South Africa. *South African Journal of Botany*, vol. 59, pp. 251–258.
- Ruiters, C., McKenzie, B., Aalbers, J. and Raitt, L.M. (1993b). Seasonal allocation of biomass and resources in the geophytic species *Haemanthus pubescens* subspecies *pubescens* in lowland coastal fynbos, South Africa. *South African Journal of Botany*, vol. 59, pp. 251–258.
- Ruiters, C., McKenzie, B. and Raitt, L.M. (1993c). Life-history studies of the perennial geophyte *Haemanthus pubescens* L. subspecies *pubescens* (Amaryllidaceae) in lowland coastal fynbos, South Africa. *South African International Journal of Plant Sciences*, vol. 154, pp. 441–449.
- Salisbury, E.J. (1927). On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical Transactions of the Royal Society B*, vol. 216, pp. 1–65.
- Salter, T.M. (1944). The genus *Oxalis* in South Africa: a taxonomic revision. *Journal of South African Botany*, vol. 1, pp. 1–355.
- Schulze, E.D., Ellis, R., Schulze, W. and Trimborn, P. (1978). Diversity, metabolic types and delta<sup>13</sup>C carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia*, vol. 106, pp. 352–369.
- Schulze, E.D., Ellis, R., Schulze, W. and Trimborn, P. (1996). Diversity, metabolic types and delta<sup>13</sup>C carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia*, vol. 106, pp. 352–369.
- Sharkey, T.D. (1988). Estimating the rate of photorespiration in leaves. *Physiologia Plantarum*, vol. 73, pp. 147–152.
- Smith, J.A.C. and Heuer, S. (1981). Determination of the volume of intercellular spaces in leaves and some values for CAM plants. *Annals of Botany*, vol. 48, pp. 915–917.
- Smith, J.A.C. and Winter, K. (1996). *Taxonomic distribution of crassulacean acid metabolism, in: Winter, K., Smith, J.A.C. (Eds.), Crassulacean acid metabolism: biochemistry, ecophysiology and evolution*. Springer-Verlag, Berlin.
- Smith, W.K. and McClean, T.M. (1989). Adaptive relationship between leaf water repellency, stomatal distribution, and gas exchange. *American Journal of Botany*, vol. 76, pp. 465–469.



- Spinner, H. (1936). Stomates et altitude. *Berichte der Schweizerischen Botanischen Gesellschaft*, vol. 46, pp. 12–27.
- Svoskin, I.P. (1960). Specific biological characteristics of bulbous geophytes as related to their past and present ecology. *Botanicheskii Zhurnal*, vol. 45, pp. 1073–1078.
- Van Rooyen, M.W. (1999). *Functional aspects of short-lived plants*. In: W.R. Dean and S.J. Milton (Eds). *The Karoo: Ecological patterns and processes*. Cambridge University Press, Cambridge.
- Vogel, J.C. (1978). Isotopic assessment of the dietary habits of ungulates. *South African Journal of Science*, vol. 74, pp. 298–301.
- Vogel, J.C. (1980). Fractionation of the carbon isotopes during photosynthesis. *Sitzungsberichte der Heidelberger Akademie der Wissenschaften*, vol. 3, pp. 111–135.
- von Willert, D.J., Eller, B.M., Werger, M.J.A., Brinckmann, E. and Ihlenfeldt, H.-D. (1992). *Life strategies of succulents in deserts*. Cambridge University Press, Cambridge.
- Willmer, C. and Fricker, M. (1996). *Stomata, 2nd edn*. Chapman and Hall.
- Winter, K. and Smith, J.A.K. (1996). *Crassulacean acid metabolism: Biochemistry, Ecophysiology and Evolution*. Springer-Verlag, Berlin.
- Witkowski, E.T.F. (1989). Response to nutrient additions by the plant growth forms of sand-plain lowland fynbos, South Africa. *Vegetatio*, vol. 79, pp. 89–97.
- Wood, J.G. (1934). The physiology of xerophytism in Australian plants: the stomatal frequencies, transpiration and osmotic pressures of sclerophyll and tomentose-succulent leaved plants. *Journal of Ecology*, vol. 22, pp. 69–87.
- Wright, I.J., Reich, P.B., Cornelissen, J.H., Falster, D.S., Garnier, E. and et al. (2005). Assessing the generality of global leaf trait relationships. *New Phytologist*, vol. 166, pp. 485–469.

# Chapter 4

## Conclusion

### 4.1 Chapter 1 conclusions

The southern African *Oxalis* radiation is extremely morphologically variable and to date few characters are known as potential synapomorphies supporting southern African clades (Oberlander *et al.*, 2011). To date this lack of knowledge has hindered species identification, taxonomic revision and the understanding of functional trait evolution in this clade (Salter (1944); L.L. Dreyer, *pers comm.*). The objectives of Chapter 2 of our study were to compile a leaflet anatomical dataset of as many southern African *Oxalis* species as possible, to assess variation of these traits in a phylogenetic context and to determine potential leaf anatomical synapomorphies among phylogenetically representative taxa that support the monophyly of southern African *Oxalis* sub-clades (Oberlander *et al.*, 2011).

The findings presented in Chapter 2 showed that a combination of six leaflet anatomical traits (stomatal position, adaxial epidermal cell types, abaxial epidermal cell types, mesophyll type, sheath around vascular tissue and degree of leaflet conduplication) clearly support various southern Africa *Oxalis* clades defined by previous DNA-based phylogenetic work (Oberlander *et al.*, 2011). Despite the phylogenetic patterns detected in the aforementioned traits, other leaflet anatomical traits were highly variable and showed no phylogenetic pattern. We suggested that these traits could possibly hold functional significance and this was explored in more detail in Jooste (2015) (Chapter 3). The extensive leaflet anatomical dataset compiled through this study will aid in the taxonomic revision of this species-rich genus inhabiting of the Greater Cape Floristic Region (GCFR) and provide a basis for future hypotheses regarding its radiation.

### 4.2 Chapter 2 conclusions

*Oxalis* (Oxalidaceae) is the sixth largest geophytic lineage and the largest geophytic genus in the GCFR (Manning and Goldblatt, 2012). Geophytes have sufficient reserves to sustain their survival and reproduction between the short periods of their growing season. As these reserves are dependent on the amount of carbon that the plant has fixed and stored during the previous growing seasons, it has been suggested that geophytes could be expected to have 'special' gas exchange characteristics (i.e., high photosynthetic CO<sub>2</sub> uptake) (Rossa and von Willert, 1999). Larcher (2003) suggested that CAM, reversible and/or intermediate modes of photosynthesis have evolved in geophytes present in semi-arid or arid habitats, similar to the GCFR. To date little is known about the preferred photosynthetic pathways of *Oxalis* species. The aims of Chapter 3 were to explore the mode(s) of photosynthesis by means of carbon isotope analysis and stomatal conductance, together with the various leaf anatomical traits identified by Jooste (2015) (Chapter 2), to determine if any mode(s)

of non- $C_3$  photosynthesis are present among southern African *Oxalis* species.

The outcomes of Chapter 3 showed that all assessed southern Africa *Oxalis* species followed an exclusively  $C_3$  photosynthetic pathway. The measured stomatal conductance data were higher than expected for *Oxalis* (Kluge and Ting (1978); Proches *et al.* (2006a); Biocyclopedia: *Oxalis* (2012)) and matched the conclusions of limited previous work on geophytes from the Namaqualand region (Rossa and von Willert, 1999). We concluded that the stomatal conductance data indicates that southern African *Oxalis* make a minimal effort to prevent water-loss during their growing season.

Additionally we observed that the vast majority of southern African *Oxalis* species have epistomatic leaflets, which is regarded as the rarest stomatal position among all angiosperms (Muir, 2015). We suggested that the shift from the probable ancestral state of hypostomatic leaflets to epistomatic leaflets was a successful strategy for the majority of the *Oxalis* from the GCFR, due to the potential selective advantage of higher conductance rates associated with an AD stomatal position than AB position. We proposed that this strategy would be favoured under the strongly seasonal environment in which the majority of southern African *Oxalis* species grow (GCFR - winter rainfall), as this enables these plants to take advantage of their growing conditions to possibly ensure high productivity, rapid growth and successful carbon storage into their below-ground bulbs. We proposed that the strategy of adaxially-located stomata with relatively high conductance rates might have been an overlooked key innovation driving diversification in one of the largest geophytic plant lineages within the GCFR.

## 4.3 References

- Biocyclopedia: *Oxalis* (2012). Accessed: 2014-05-30.
- Jooste, M. (2015). The phylogenetic and functional significance of leaf anatomical and physiological traits of southern African *Oxalis*.
- Kluge, M. and Ting, I. (1978). *Crassulacean Acid Metabolism: analysis of an ecological adaptation*. Springer-Verlag, Heidelberg, Germany.
- Larcher, W. (2003). *Physiological Plant Ecology*. Springer, Heidelberg, Berlin.
- Manning, J. and Goldblatt, P. (2012). *Plants of the Greater Cape Floristic Region 1: the Core Cape Flora*. Strelitzia 29, South African National Biodiversity Institute, Pretoria, South Africa.
- Muir, C.D. (2015). *Selection constrains phenotypic evolution in a functionally important plant trait*. Ph.D. thesis, University of British Columbia.
- Oberlander, K.C., Dreyer, L.L. and Bellstedt, D.U. (2011). Molecular phylogenetics and origins of southern African *Oxalis*. *Taxon*, vol. 60, no. 6, pp. 1667–1677.
- Proches, S., Cowling, R.M. and Du Preez, D.R. (2006). Patterns of geophyte diversity and storage organ size in the winter-rainfall region of southern Africa. *Diversity and Distribution*, vol. 11, pp. 101–109.
- Rossa, B. and von Willert, D.J. (1999). Physiological characteristics of geophytes in semi-arid Namaqualand, South Africa. *Plant Ecology*, vol. 142, pp. 121–132.
- Salter, T.M. (1944). The genus *Oxalis* in South Africa: a taxonomic revision. *Journal of South African Botany*, vol. 1, pp. 1–355.

## 4.4 List of addenda

### Addendum I

All raw anatomical and physiological data from Chapter 2 and Chapter 3 from this study. Arranged according to species name, but MO-accession numbers are included to highlight multiple accessions for the same species from the Stellenbosch University Botanical Gardens' living collection.

### Addendum II

All additional phylogenetic plots based on discrete and continuous data that were not included as figures in Chapter 2 of this study.

### Addendum III

A collection of photographs of pressed, dried and labelled leaves for the majority of studied *Oxalis* leaves collected from the living collection.