A synopsis of the genus *Octopoma* N. E. Br. (Ruschioideae) using morphology, 5S NTS and psbA – trnH spacer regions.

A THESIS SUBMITTED TO THE DEPARTMENT OF BOTANY, FACULTY OF SCIENCE, UNIVERSITY OF CAPE TOWN, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN SYSTEMATICS AND BIODIVERSITY SCIENCE.

JUNE 2003

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ACKNOWLEDGEMENTS

Many thanks are directed to my supervisor Associate Professor Terry Hedderson for teaching me that molecular systematics does not require luck, but the proper approach. It was through him that I learnt that one needs not to be fortunate to obtain DNA sequences, but requires the "know how" part of it!!!
I would also like to thank Dr Cornelia Klak for introducing and guiding me throughout a journey in the world of Mesembs. Ms Miranda Waldron is also thanked for helping me with the electron microscope work and Dr Tony Verboom for lending and teaching me to use his wonderful computer equipment.

Tracey Nowell and Nicola Bergh are also thanked for perfecting my skills on operating the delicate laboratory equipment and handling the carcinogenic chemicals.

Jack Henning and Ruvimbo Mapaya will also be remembered for their endless encouragement and company throughout the turbulent study period.

Finally, I would like to thank the Southern African Botanical Diversity Network (SABONET) for funding my studies. SABONET is a GEF project implemented by UNDP's regional centre for Southern Africa, Gaborone, Botswana.

DEDICATION

This project is dedicated to Pamwe Tema for the patience and hardships she went through during my entire study period.

ABSTRACT

The genus *Octopoma* N. Br. is nested within the Ruschioideae in Aizoaceae. It has been thought that eight species make up the genus. The classification was based mainly on the possession of eight locules. This study was, therefore, undertaken to test the monophyly of the genus, and to draw up new taxonomic circumscriptions. Morphology, nuclear (5S NTS) and chloroplast DNA (psbA – trnH) were used to determine phylogenetic relations of the species. Cladistic analyses were based on maximum parsimony (MP) and maximum likelihood (ML) of PAUP*. The morphological data set indicates *Octopoma* to be non-monophyletic by splitting it into two main groups. One of the groups comprises Little Karoo *Octopoma*s, which are *O. octojuge* and *O. quadrisepalum*. The other group comprises Namaqualand *Octopoma*s, which are *O.* c. f. *connatum*, *O. inclusum*, *O. rupigenum*, *O.* sp 9152, *O.* sp. 9132 and *Octopoma subglobosum*. Under, MP, the 5S NTS data is partially resolved and fails to indicate whether *Octopoma* is monophyletic or not. However, under ML analysis, it is resolved and also indicates *Octopoma* to be non-monophyletic. The psbA –trnH data is unresolved under both MP and ML. But when the two DNA data sets are combined under both MP and ML, their results support those of the morphological data set by indicating that *Octopoma* is non – monophyletic. The same groups observed in the morphological data sets are also observed when the two data sets are combined. The total evidence data also indicates *Octopoma* to be non – monophyletic. The same two groups as described above are also observed. It was therefore concluded that *O. octojuge* and *O. quadrisepalum* are the core of *Octopoma*. As such, *O.* c. f. *connatum, O. inclusum, O. rupigenum, O.* sp. *9152, O.* sp. *9132* and *Octopoma subglobosum* are excluded from the genus *Octopoma*. However, it could not be concluded that *O. octojuge* and *O. quadrisepalum* form a bitypic genus since taxonomic positions of *O. tetrasepalum* and *O. abruptum* in the Ruschioideae are still unknown. It was also not possible to determine genera to which these Namaqualand *Octopoma*s should be sunk because overall taxa sampling for the study were inadequate. It was therefore recommended that the study should be improved by including at least 60 *Leipoldtia* and 100 *Ruschia* species because they are closely related to *Octopoma*.

Key words: Octopoma Ruschioideae Aizoaceae Maximum parsimony Maximum likelihood 5S NTS PsbA trnH Monophyletic Namaqualand Little Karoo Morphology Taxonomy.

TABLE OF CONTENTS

CHAPTER 3: MOLECULAR INVESTIGATIONS......................................33

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

'If you plan for a year, plant rice, if you plan for a decade plant trees, but if you plan for a lifetime, educate.' (Van Oudtshoorn, 1992).

CHAPTER 1

GENERAL INTRODUCTION

1.1. Current classification scheme for *Octopoma.*

Octopoma is nested within the Aizoaceae, a family comprising succulent annual to perennial herbs, subshrubs and shrubs (Hartmann, 2001). Although the Aizoaceae, a member of the Centrospermae, has a worldwide distribution (Figure 1. 1), most of its species are found in the winter rainfall region of Southern Africa (Hartmann, 1991; 1993).

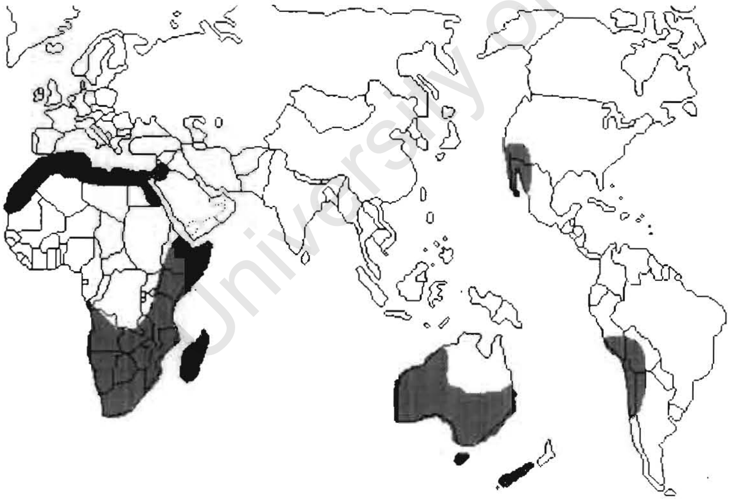


Figure 1. 1. World distribution map for the Aizoaceae.

Putative synapormophies for the Aizoaceae include possession of epidermal bladder cells, perigynous flowers, very long funicles, absence of wood rays in the normal and secondary xylem as well as the possession of expanding tissue that acts on a hydrochastic base (Bittrich & Hartmann, 1988). Five subfamilies (Hartmann, 2001) make up the Aizoaceae; the Aizooideae, Mesembryanthemoideae, Ruschioideae, Sesuviodeae and the Tetragonioideae.

Octopoma is a member of the Ruschioideae (Hartmann, 2001). The Ruschioideae was first divided into five tribes and 22 subtribes (Schwantes, 1971). Then Hartmann (1988, 1990) replaced Schwantes 's (1971) classification by creating 12 groups, based mainly on ruit structure (Hartmann, 1988). However, leaf epidermal cells were also discovered to be important in the delimitation of taxa within the Ruschioideae. Hence, in addition to fruit types and anatomical structures, Hartmann (1991, 1998b) utilised leaf epidermal cells to determine the 12 groups (Table 1. 1.).

Within the Ruschioideae, *Octopoma* is considered part of the Leipoldtiinae, which forms a putative monophyletic group with Lampranthinae and Ruschiinae (Dehn, 1992). The monophyly is supported by sclerotinized fruits and firm, full covering membranes that possess additional closing devices at their distal ends (Dehn, 1992). Locules in the Leipoldtiinae and the Ruschiinae are partially to completely blocked by closing bodies at the exit, and the feature is considered advanced (Dehn, 1992). These features are to some extent common between *Octopoma, Ruschia* and *Leipoldtia* L. Bolus, which implies that the three genera are closely related.

Table 1. 1. Group members of the Ruschioideae (Tribble 1998 & Hartmann 1998).

Group	Species
Aptesia	*Apatesia* N.E.Br., *Carpanthea* N.E.Br.,Conicosia N.E.Br., *Hymenogyne* Haworth., *Caryotophora* Leistner, *Saphesia* N.E.Br., *Skiatophytum* L.Bolus
Cleretum	*Aethephyllum* N.E.Br., *Cleretum* N.E.Br., *Dorotheanthus* Schwantes
Mitrophyllum	*Dicrocaulon* N.E.Br., *Diplosoma* Schwantes, *Jacobsenia* L.Bolus & Schwantes, *Meyerophytum* Schwantes, *Mitrophyllum* Schwantes, *Monilaria* Schwantes (Schwantes), *Oophytum* N.E.Br., *Glottiphyllum* N.E.Br., *Disphyma* N.E.Br.
Delosperma	*Corpuscularia* Schwantes, *Delosperma* ss. N.E.Br., *Drosanthemum* Schwantes, *Malephora* N.E.Br., *Mestoklema* N.E.Br. ex Glen, *Trichodiadema* Schwantes, *Lampranthus* pp., *Oscularia* Schwantes, *Gibbaeum* N.E.Br. (Haworth), *Imitaria* N.E.Br., *Muiria* N.E.Br.
Stomatium	*Frithia* N.E.Br., *Chasmatophyllum* Dinter & Schwantes, *Hammeria* Burgoyne, *Rabiea* N.E.Br., *Rhinephyllum* N.E.Br., *Stomatium* Schwantes, *Mossia* N.E.Br., *Neohenricia* L.Bolus, *Faucaria* Schwantes, *Orthopterum* L.Bolus
Titanopsis	*Aloinopsis* Schwantes, *Deilanthe* N.E.Br., *Ihlenfeldtia* Hartmann, *Nananthus* N.E.Br., *Prepodesma* N.E.Br., *Titanopsis* Schwantes, *Vanheerdea* L.Bolus ex Hartmann, *Didymaotus* N.E.Br., *Tanquana* Hartmann & Liede, Dinteranthus Schwantes, *Lapidaria* N.E.Br. (Dinter & Schwantes), *Lithops* N.E.Br., Schwantesia Dinter
Dracophilus	*Dracophilus* Dinter & Schwantes (Schwantes), *Hartmanthus* S.A.Hammer, *Jensenobotrya* Herre, *Juttadinteria* Schwantes, *Namibia* Schwantes (Schwantes), *Nelia* Schwantes, *Psammophora* Dinter & Schwantes, *Ruschianthus* L.Bolus, *Conophytum* N.E.Br.
Bergeranthus	*Bergeranthus* Schwantes, *Machairophyllum* Schwantes, *Carruanthus* Schwantes (Schwantes), *Hereroa* Dinter & Schwantes (Schwantes), *Rhombophyllum* Schwantes (Schwantes), *Bijlia* N.E.Br., *Cerochlamys* N.E.Br.
Lampranthus	*Antegibbaeum* Schwantes ex C.Weber, *Braunsia* Schwantes, *Carpobrotus* N.E.Br., *Circandra* N.E.Br., *Enarganthe* N.E.Br., *Erepsia* N.E.Br., *Esterhuysenia* L.Bolus, *Lampranthus* ss. N.E.Br., *Namaquanthus* L.Bolus, *Scopelogena* L.Bolus, *Smicrostigma* N.E.Br., *Vlokia* S.A.Hammer, *Wooleya* L.Bolus, *Zeuktophyllum* N.E.Br.
Ruschia	*Acrodon* N.E.Br., *Arenifera* Herre, *Astridia* Dinter, *Ebracteola* Dinter & Schwantes, *Khadia* N.E.Br., *Marlothistella* Schwantes, *Polymita* N.E.Br., *Ruschia* ss. Schwantes, *Stayneria* L.Bolus
Leipoldtia	*Antimima* N.E.Br., *Argyroderma* N.E.Br., *Cephalophyllum* N.E.Br., *Cheiridopsis* N.E.Br., *Cylindrophyllum* Schwantes, Fenestraria N.E.Br., *Hallianthus* Hartmann, *Jordaaniella* Hartmann,, *Leipoldtia* sl., *Octopoma* ss. N.E.Br., *Odontophorus* N.E.Br., *Ottosonderia* L.Bolus, *Pleiospilos* N.E.Br., *Schlechteranthus* Schwantes *Vanzijlia* L.Bolus
Eberlanzia	*Amphibolia* L.Bolus ex Herre, *Eberlanzia* Schwantes, *Ruschianthemum* Friedrich, *Stoeberia* Dinter & Schwantes

Since the description for *Octopoma* was based on *O. octojuge*, which possesses narrow valve wings and big closing bodies, it was assumed that the features were constant for the genus. However, it was later found that not all *Octopoma* species posses valve wings (Bittrich & Hartmann, 1988). *Octopoma* was subsequently characterised. in contrast to *Leipoldtia*, by its possession of contagious expanding keels, absence of valve wings and presence of connate leaves. Unfortunately, the problem was not fully solved because *O. octojuge*, the type specimen, does have valve wings. The problem prompted Hartmann (1998), to reassess *Octopoma*. Hartmann 's (1998) analysis suggested that five of the eight *Octopoma* species; *O. connatum* (L. Bolus) N. E. Br., *O. tetrasepalum* (L. Bolus) H. E .K Hartmann, *O. inclusum* (L. Bolus) N. E. Br. *O. rupigenum* (L. Bolus) L. Bolus and *O. abruptum* (A. Berger) N. E. Br., posses a fruit type similar to that of species in the *Ruschia* (Table 1. 2). The resemblant characters were the possession of closing rodlets and small closing bodies (Hartmann, 1998).

Table 1. 2. Diagnostic characters for the *Ruschia* and *Leipoldtia* groups (Hartmann, 1998).

Ruschia group	**Leipoldtia group**
• Consistent absence of valve wings	• Broad valve wings present
• Small, rod shaped closing bodies	• Large round shaped closing bodies
• Convex covering membranes	• Concave covering membranes
• Closing rodlets present	• Closing rodlets absent
• Fruit tops conical, dome to high dome	• Fruit tops mainly round

The remaining three *Octopoma* species; *O. quadrisepalum* (L. Bolus) H. E. K. Hartmann, *O. octojuge* (L. Bolus) N. E. Br. and *O. subglobosum* (L. Bolus) L. Bolus, possess a fruit capsule similar to that of the *Leipoldtia* species (Table 1. 2). The similarities include the possession of big closing bodies and valve wings. Based on these results, Hartmann (1998; 2001) proposed the splitting of *Octopoma* species into two groups, the *Ruschia* and *Leipoldtia* groups.

Hartmann (1998) and Chesselet et al. (2000) also pointed that *Octopoma* species do not form a natural group and can therefore be split into at least two groups. Based on this Hartmann (1998; 2001) emphasised that *O. octojuge*, *O. quadrisepalum* and *O. subglobosum* are the only *Octopoma*s that belong to the *Leipoldtia* group. The rest of the *Octopoma*s, namely; *O. inclusum, O. connatum, O. abruptum, O. tetrasepalum* and *O. rupigenum*, belong to the *Ruschia* group, a group that is closely related to the *Leipoldtia* group (Hartmann, 1998; 2001). Members of the *Ruschia* group are characterised by round, dome and high dome-shaped fruit tops, consistent absence of valve wings, presence of closing rodlets, rod shaped closing bodies and convex covering membranes (Hartmann, 2001).

However, Hartmann's (1998; 2001) hypothesis for splitting *Octopoma* species into two groups was accompanied by some complications. The first complication is that Hartmann (1998; 2001) recommended *O. subglobosum* to be grouped with *O. octojuge* and *O. quadrisepalum* mainly based on the observation that the trio possess big closing bodies and narrow valve wings (Hartmann, 1998:71; 2001: 99). Contrarily, Hartmann (1998: 71, Figure 45) stated that *O. subglobosum* has **small** closing bodies. Then the question was, which character state for closing body size does *O. subglobosum* really possess? On the other hand valve wings might not provide a substantial basis for classification, because their presence and width can depend on the freshness of the specimen (Brown, 1930).

The second problem is that circumscription of *Octopoma* was also based on single herbarium specimens (Hartmann, 2001). Various authors (e. g. Glen, 1986) therefore postulated the accumulation of errors related to placement of species. The problem was regarded as serious to the extent that numerous studies recommended the transfer of species to other genera. For instance, one species of *Octopoma* was transferred to *Zeuktophyllum* (Hartmann, 1998). Hartmann (2001) and Chesselet et al. (2000) also suggested that species of the genus *Octopoma* do not form a natural group because most species in this genus were placed there only because they possessed eight locules (Hartmann, 1998). According to Hartmann (1998) the species differ so widely in most other features that *Octopoma* can be divided into at least two groups .

1. 2. History of *Octopoma* N. E. Br.

The genus *Octopoma* N. E. Br. was erected by Brown (1930) to accommodate *Octopoma octojuge* (L. Bolus) N. E. Br., the species selected as the generitype. Dr John Muir collected the type specimen in 1926 at Klein Karoo, South Africa. The specimen is housed at the Bolus herbarium and was first described as *Mesembryanthemum octojuge* L Bolus., in 1927. From Bolus's (1927) report it appears the specimen was difficult to place as evidenced by the following statement:

> A distinct species which I have been unable to place satisfactorily in any of the 41 genera given by Mr Brown in the Gardens Chronicle (Nov. 1925). Pending therefore, the creation of a new genus, or the widening of the limits of one of the existing genera, to hold it, I have included it in *Mesembrianthemum*, in the wider sense (Bolus, 1927: 78).

Following creation of the new genus Mesembryanthemum Linn, Phillips (1928) published a book, which was also meant to accommodate diagnostic keys for the genus *Mesembryanthemum* Linn. by Brown (1925). Unfortunately, upon copying the diagnostic keys for *Mesembryanthemum* into Phillips 's (1928) book, structural characters for *M. umbellatum* Linn., the type for *Mesembryanthemum*, and its allied genera were omitted. Therefore, when the genus *Ruschia* Schwantes was erected, Schwantes (1926) sank all *Mesembryanthemum* species described by Brown (1925) into the genus *Ruschia* Schwantes (1926) also used *M. umbellatum*, the type for *Mesembryanthemum*, as the type for the newly erected genus, *Ruschia*. Since all plants under *Mesembryanthemum* were shifted to *Ruschia*, Bolus (1930) also changed *Mesembryanthemum octojuge* L. Bolus to *Ruschia octojuge* (L. Bolus) L. Bolus. The name changing pandemonium displeased Brown (1930) as indicated by the following statement:

> Dr Schwantes has instead of correcting the mistake, founded his genus *Ruschia* upon the identical type structure possessed by *M. umbellatum*. Linn., and has selected another species to be the type of *Mesembryanthemum*. This attempt to replace the genus *Mesembryanthemum*, Linn., as emended by myself, by another generic name of his own not only does not conform with the code of honour held among scientific workers, but is contrary to all rule and precedent, and the science of botany would soon become very chaotic if proposed types of genera that have been examined by authors were allowed to be changed in this way at the will of every writer (Brown, 1930: 14).

After explaining the error, Brown (1930) reallocated *Ruschia* species tampered with by Schwantes (1926) back to the genus *Mesembryanthemum*. As part of the renaming process, Brown (1930) recognised some species that tended to be constantly 8 – locular as distinct. Hence, Brown (1930) formulated the genus *Octopoma* so as to group together all species possessing eight locules. The name *Octopoma* stems from Greek, "*okto* -", for eight, and "- *poma*" for lid or cover (Hartmann, 2001). The genus *Octopoma* is well established and retains its validity up to the present day.

The preceding summary, therefore, suggests that there is considerable taxonomic confusion that neccessiates investigation of the position of *Octopoma* in the Ruschioideae. This investigation was aimed at conducting a more thorough analysis that included both morphological and molecular investigations, since no molecular studies of the genus have ever been performed. Molecular investigations involved the chloroplast (psbA –trnH) and nuclear (5S NTS) spacer regions.

1. 3. Objectives.

1. To investigate whether *Octopoma* species form a monophyletic group.
2. To test Hartmann' s hypothesis that *Octopoma* species could be split into at least two groups.
3. To Identify relevant taxonomic positions within the Ruschioideae for the various *Octopoma* species.

'Biologists must constantly keep in mind that what they see was not designed but rather evolved.' Crick, F. H. C., Nobel Laurate 1962.

CHAPTER 2

MORPHOLOGY AND CLADISTIC ANALYSIS OF *Octopoma*

2. 1. Introduction

Morphology currently provides bases for identification and construction of taxonomic systems in plants (Jones & Luchsinger, 1987). For example, leaf epidermal surfaces have been used in conjunction with fruit morphology to recognise 12 groups in the Ruschioideae (Hartmann, 1988; 1991; 1998). Fruits of the Ruschioideae are sometimes referred to as capsules and the names can be used interchangeably. The shape of the capsule is important and is divided into the fruit-top and fruit-base (Figure 2. 1). In the Ruschioideae, the fruit-base has only two forms, the funnel and the round shaped form, while the fruit-top can be round, dome, high-dome or rectangular shaped (Plates 1-5).

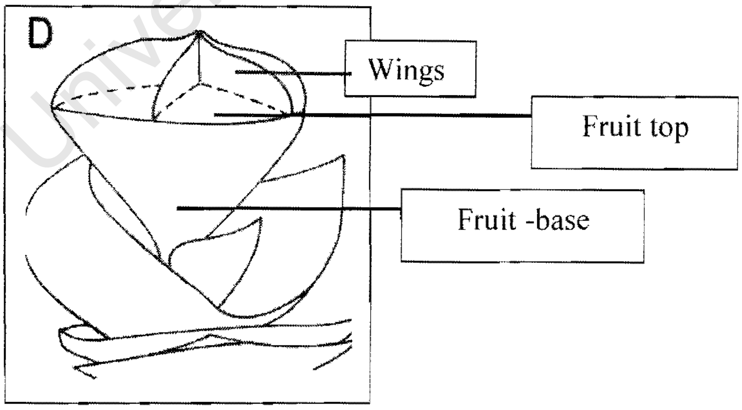


Figure 2.1. Diagram of a Ruschioideae fruit (Hartmann, 1998c)

The fruit-top has wings (Figure 2.1) that divide it into segments (Hartmann, 1988; 1991; 1998). These segments are known as locules and vary from five to 24 in number. Each of these locules has a cover known as a valve (Figure 2. 2). When the capsule is soaked in water the valves open up to reveal internal features of the valve (Figure 2. 2).

These internal features are very important in the taxonomy of the Ruschioideae. The internal features (Hartmann, 1988; 1991; 1998; 1998b; 2001) include valves (V), valve wings (VW), closing bodies (CB), covering membranes (CM), closing rodlets (CR) and (EK) expanding keels (Figure 2. 2). Closing bodies (CB) and closing rodlets (CR) are two different structures. Closing bodies are rod to circular shaped organs that control the release of seeds from the capsule by blocking or opening the locules. These closing bodies are located between the covering membranes and valves. Closing rodlets are membranous extensions of covering membranes and are located beneath the covering membranes. Valve wings are just translucent membranes located along side the valves and might be present or absent depending on the species. Expanding keels are highly sclerotised, rod-like, substances located at the centre of the valve. These expand in length when they get wet and by so doing cause the capsule to open up. Expanding keels originate above the placenta and extend towards the sides of the valve apex (Figure 2. 2).

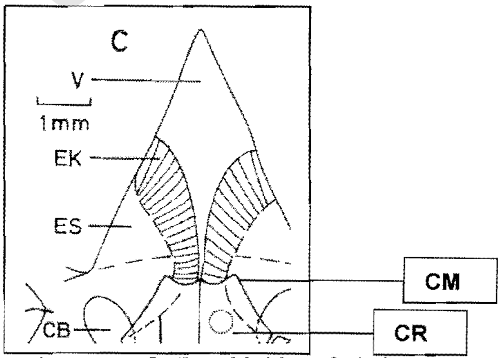


Figure 2. 2. Interior parts of a Ruschioideae fruit (Hartmann 1998c).

These above mentioned and illustrated features have also played a major part in the taxonomy of *Octopoma*s. For instance, it has been assumed that all *Octopoma* species fall within the *Leipoldtia* group (consult Table 2. 1 to see members of this group). Members of the *Leipoldtia* group are recognised by round-topped fruits, broad valve wings, recurved covering membranes, large closing bodies, absence of closing rodlets and more than five locules, a condition known as multilocular (Hartmann, 2001).

Seed characters are also considered important in the taxonomy of the Ruschioideae. For instance, *Octopoma connatum* has D-shaped seeds, while *O. subglobosum* has pear-shaped seeds (Bolus, 1928; 1930; 1963).

2. 1. 1 Assumptions considered when using morphological characters in this study

Homology, 'equivalence of parts', serves as an important tool in comparative studies and forms a basis for most biological generalisations (Jardine, 1969; Guedes & Dupuy, 1976; Blackmore & Barnes, 1987; Patterson, 1988; Coddington, 1994; McKitrik, 1994). In this study, fruit and vegetative features were used to construct a data matrix for primary homology assessment, as recommended by Browner & Schawaroch (1996). However, it was assumed that characters in comparison were for semaphorant plants (same condition). For instance, if closing bodies for *Octopoma subglobosum* were assessed, it was assumed that the documented ones in which the plant under study are compared to, were, for example, of the same maturity stage, unless stated otherwise.

Multistate characters were left unordered in this study because the practice of ordering multistate characters is unnecessary (Hauser & Presch, 1991). Ordered characters are not as informative as perceived and might increase, decrease or have no effect on tree resolution (Hauser & Presch, 1991).

2. 1. 2. Limitations

There tends to be character variability even among members of the same species in the Ruschioideae. The most common ones include variability in the number of locules, fruit top shape and the length of the columella (Hartmann, 2001).

2. 1. 3 Objectives

1. To investigate phylogenetic relations of *Octopoma* species using morphological characters.
2. To provide a morphological contrast to subsequent molecular analysis.

2. 2. Materials and methods

2. 2. 1. Taxon sampling

Eight *Octopoma* species were included in the study, of which *Octopoma* sp. 9152 and *Octopoma* sp. 9123 could not be identified to species level. Additional taxa were used to test the monophyly of *Octopoma*. These additional taxa were selected from the *Leipoldtia* and *Ruschia* groups because it is hypothesised that *Octopoma*s are nested within these groups (Tables 2. 1 & 2. 2). One genus was selected from the *Eberlanzia* group to minimise bias towards particular groups. Outgroups were selected from the *Delosperma* group, a group that is considered distantly related (Tribble and Hartmann, 1998) to the *Leipoldtia* and *Ruschia* groups (Table 2. 1.).

Table 2.1. Members of the Ruschioideae from which specimen sampling was done.

RUSCHIOIDEAE			
Delosperma group	Leipoldtia group	Ruschia group	Eberlanzia group
Corpuscularia Schwantes	*Antimima* N.E.Br.	*Acrodon* N.E.Br. *	*Amphibolia* L.Bolus ex Herre
Delosperma ss. N.E.Br.	*Argyroderma* N.E.Br.	*Arenifera* Herre	*Eberlanzia* Schwantes
Drosanthemum Schwantes*	*Cephalophyllum* N.E.Br	*Astridia* Dinter	*Ruschianthemum* Friedrich
Malephora N.E.Br.	*Cheiridopsis* N.E.Br.	*Ebracteola* Dinter & Schwantes	*Stoeberia* Dinter & Schwantes*
Mestoklema N.E.Br. ex Glen	*Cylindrophyllum** Schwantes	*Khadia* N.E.Br.	
Trichodiadema Schwantes	*Fenestraria* N.E.Br.	*Marlothistella* Schwantes	
Oscularia Schwantes*	*Hallianthus* Hartmann*	*Polymita* N.E.Br.	
Lampranthus pp*	*Jordaaniella* Hartmann*	*Ruschia* ss. Schwantes*	
	Leipoldtia sl.	*Stayneria* L.Bolus	
	Octopoma ss. N.E.Br. *		
	Odontophorus N.E.Br.		
	Ottosonderia L.Bolus		
	Pleiospilos N.E.Br.		
	Schlechteranthus Schwantes*		
	Vanzijlia L.Bolus*		

*Genera used in the study

Table 2.2. Specimens used for the project.

Voucher specimen	Location	Collector and No:	Specimen status	Use
Octopoma octojuge	Bol	Klak 290	Fresh	SEM, morphology and DNA
Octopoma octojuge	Bol	Leipoldt 30854	Herbarium	Morphology
Octopoma octojuge	Bol	Vlok 5. 11	Herbarium	Morphology
Octopoma octojuge	Bol	Anon	Herbarium	Morphology
Octopoma quadrisepalum	Bol	Bruyns 9010	Fresh	SEM, morphology and DNA
Octopoma quadrisepalum	Bol	Bolus 20 719	Herbarium	Morphology
Octopoma quadrisepalum	Bol	Frames 19 080	Herbarium	Morphology
Octopoma inclusum	Bol	Klak 474	Fresh	SEM, morphology and DNA
Octopoma inclusum	Bol	Jaarsveld 5423a	Herbarium	Morphology
Octopoma inclusum	Bol	Van Keerde 274	Herbarium	Morphology
O. inclusum	Bol	Pillans 17 758	Herbarium	Morphology
O. sp.	Bol	Bruyns 9152	Fresh	SEM. morphology and DNA
O. sp.	Bol	Bruyns 9132	Fresh	SEM. morphology and DNA
O. c.f. connatum	Bol	Bruyns 9104	Fresh	SEM. morphology and DNA
O. connatum	Bol	Bolus 8792	Herbarium	Morphology
O. connatum	Bol	Acork 15 100	Herbarium	Morphology
O. connatum	Bol	Klak 632	Herbarium	Morphology
O. connatum	Bol	Pillans 5794	Herbarium	Morphology
O. connatum	Bol	Acorks 14 859	Herbarium	Morphology
O. subglobosum	Bol	Bruyns 9146	Fresh	SEM. morphology and DNA
O. subglobosum	Bol	Pillans 5844	Fresh	SEM. morphology and DNA
O. subglobosum	Bol	Klak 267	Herbarium	Morphology
O. subglobosum	Bol	Salter 4598	Herbarium	Morphology
O. subglobosum	Bol	Bruyns 7610	Herbarium	Morphology
O. subglobosum	Bol	N.B.G. 1770/48	Herbarium	Morphology
O. rupigenum	Bol	Klak 433	Fresh	SEM. morphology and DNA
O. rupigenum	Bol	Williamson 3053	Herbarium	SEM. morphology and DNA
O. rupigenum	Bol	Klak 468	Herbarium	Morphology
O. rupigenum	Bol	Bolus 1504/33	Herbarium	Morphology
O. rupigenum	Bol	Klak 468	Herbarium	Morphology
Leipoldtia frutescens	Bol	Bruyns 9149	Fresh	SEM, morphology and DNA
Leipoldtia c. f. *comapacta*	Bol	Bruyns 9093	Fresh	SEM. morphology and DNA
Leipoldtia klaverensis	Bol	Bruyns 9081	Fresh	SEM. morphology and DNA
Vanzijlia annulata	Bol	Bruyns 9087	Fresh	SEM. morphology and DNA
Vanzijlia annulata	Bol	Acorks 15 023	Herbarium	Morphology
Vanzijlia annulata	Bol	Bolus 15 021	Herbarium	Morphology
Vanzijlia annulata	Bol	Salter ??	Herbarium	Morphology
Vanzijlia annulata	Bol	Arbuthnot 17 391	Herbarium	Morphology
Vanzijlia annulata	Bol	Peers 1394/33	Herbarium	Morphology
Leipoldtia rosea	Bol	Bruyns 9157	Fresh	SEM. morphology and DNA
Ruschia hexamera	Bol	Klak 780	Fresh	SEM. morphology and DNA
Ruschia sandbergensis	Bol	Klak 478	Fresh	SEM. morphology and DNA
Ruschia goodiae	Bol	Bruyns ??	Fresh	SEM. morphology and DNA
Acrodon bellidiflorus	Bol	Klak 179	Fresh	SEM. morphology and DNA
Cylinndrophyllum hallii	Bol	Bruyns 7874	Fresh	SEM. morphology and DNA
Sclecteranthus hallii	Bol	Bruyns 259	Fresh	SEM. morphology and DNA
Hallianthus planus	Bol	Bruyns 7310	Fresh	SEM. morphology and DNA
Jordaaniella spongiosa	Bol	Klak 879	Fresh	SEM. morphology and DNA
Jordaaniella spongiosa	Bol	Pillans 17 752	Herbarium	Morphology
Jordaaniella spongiosa	Bol	Heise 8462	Herbarium	Morphology
Jordaaniella spongiosa	Bol	Van Wyk 147	Herbarium	Morphology
Lampranthus bicolor	Bol	Klak??	Fresh	Ultra structure
Lampranthus bicolour	Bol	Klak??	Fresh	DNA
Oscularia deltoides	Bol	Klak??	Fresh	SEM. morphology and DNA
Oscularia deltoids	Bol	Bartlett 1965/22	Herbarium	Morphology
Oscularia deltoids	Bol	Klak 273	Herbarium	Morphology
Stoeberia carpii	Bol	Klak ??	Fresh	SEM. morphology and DNA
Storberia carpii	Bol	Van Neerde 27 741	Herbarium	Morphology
Stoeberia carpii	Bol	Dinter 8148	Herbarium	Morphology
Stoeberia carpii	Bol	Bruyns 8848	Herbarium	Morphology
Stoeberia carpii	Bol	Bruyns 7301	Fresh	SEM. morphology and DNA

2. 2. 2. Character acquisition methods

- Fruit morphology

Internal and external parts of fruits were observed and drawn under a Leica MS5–Wild 308 700-dissection microscope. Magnifications were varied from 6.3X to 40X, depending on details of interest (Plates 1 –10).

- Leaf samples [Scanning electron microscope (SEM)]

Fresh leaves were cut into samples of about 25.0 mm^2 and transferred into labelled bottles. Adding full strength chloroform to the samples, and allowing them to stand for 25 minutes removed wax layers. Wax layers were removed so that they could not obscure papillae patterns. The de-waxed samples were dehydrated in sequential ethanol treatments of 50%, 70%, 80%, 90% and 99% respectively, with samples being held at each ethanol treatment for at least four hours. Then the samples were dried, mounted and spatter coated using Gold palladium. Observation of the samples was done at an angle of 35 degrees from the electron gun. Working distances between 12.0 and 23.0 mm were used. Magnifications were varied between 64.8X and 563.0X.

- Seeds

For seeds, wax removal, dehydration and critical point drying were not necessary.

The seeds were mounted and coated with gold palladium. Then the samples were observed at an angle of 35° in the vacuum chamber. Working distances of 15.0 – 28.0 mm and magnifications of 40.1X – 133.0X were used.

- Macroscopic features

All other characters were obtained from descriptions in Hartmann's (2001) recent classification scheme of the Aizoaceae.

Character coding

Multistate and reductive coding methods were utilised in this study. Multistate methods were used to avoid the problems that could arise due to linked characters (Pimetel and Riggins, 1987). Reductive coding was used to enable the detection of homoplasy (Wilkinson, 1995).

2. 2. 3. Character list

Fruit characters (See Figures 2. 1. and 2. 2. for illustrations)

1. Expanding keels contagious **(1)**; diverging **(0)**

Contagious expanding keels (Plate 10) that resembled those of *Octopoma* species were observed in *Leipoldtia* c. f. *compacta*, as opposed to those divergent ones as in other *Leipoldtia* species (Plates 8 & 9).

2. Closing bodies large (i. e covering the entire locule) **(0)**; small **(1)** (not covering the entire locule); absent **(2)**

Closing bodies are located at distal ends of the locule and are considered to promote seed dispersal (Lockyer, 1932; Volk, 1960; Ihlenfeldt 1971). Variability in the absence or presence of closing bodies within a species has only been recorded in the genus *Mitrophyllum* (Poppendieck, 1976). *Stoeberia carpii* has no closing bodies (Hartmann, 2001). Hartmann (1998; 2001) reported *O. subglobosum* to have large bodies. However, in this study, small closing bodies (i. e. not covering the entire locule) were observed in *Octopoma subglobosum*.

3. Valves rectangular at the base **(1)**; Ovate at the base **(0)**

4. Columella short **(1)** ; long **(0)**

Shorter columellae are those in which the columella's length ends almost at the base of the capsule, while long columellae are those that extend throughout the longitudinal sectional length of covering membranes (Plate 6). *Leipoldtia klaverensis* and *Octopoma* sp. PVB 9123 were found to have shorter columellae (Plate 7) rather than a long columella observed in other *Octopoma* species (Plate 6).

5. Valve wings consistently absent **(1)**; Valve wings mainly present **(0)**

6. Valve wings rectangular **(1)**; Valve wings tapering towards valve tips **(0)**

7. Stiff and recurved covering membranes at distal margins present **(1)**; Absent **(0)**

8. Multilocules absent **(1)**; Present **(0)**

Species with more than 5 locules are regarded as multilocular (Hartmann, 1998; 2001). Up to 24 - locular plants may be found in the Ruschioideae, e.g. in *Argyrodema delaetii* (Maas, 1928). *Octopoma* species have been documented to have a constant number of 8 locules (Brown, 1930), but in this study, 7 locules were recorded in *Octopoma* sp. PVB 9123.

9. Fruit-top shape dome **(1)**; Round **(2)** ; Square **(3)**; flattened **(4)**

The fruit top shape referred above was as observed from the longitudinal side view.

A rectangular-shaped top was observed in *Leipoldtia* c. f. *compacta* (Plate 5), as opposed to round and dome shaped tops (Plates 1 - 4) that have been reported in *Leipoldtia and Octopoma* species, respectively. Such a fruit top was a new record for *Leipoldtia* species.

10. Fruit-base funnel shaped **(1)**; Bell shaped base **(0)**

11. Fruit rims well pronounced **(1)**; Not well pronounced **(0)**

12. Closing rodlets present **(1)**; Absent **(0)**

Closing rodlets are membranous extensions of covering membranes and are located beneath covering membranes (consult section 2. 1. 0 to see how they differ from closing bodies).

13. Covering membranes undulate **(0)**; Convex **(1)**; Straight **(2)**

14. Fruit hygrogastic **(1)**; Xerochastic **(0)**

15. Expanding keels can open repeatedly **(1)**; Open once and get damaged **(0)**

Inflorescence characters

16. Flowers solitary **(1)**; Compound **(0)**

17. Filamentous staminodes visible during anthesis **(1)**; Not visible **(0)**

Three types of androecia are found in all plants (Ihlenfeldt, 1960). Type 1 are those where staminodes can not be clearly distinguished. Type 2, those in which filamentous & petaloid staminoda can be clearly distinguished from stamens. Type 3 are those without filamentous staminodes. Most species in the Ruschioideae are of the Type 2 androecia.

Leaf characters (Macroscopic)

18. Leaves finger shaped **(4)**; Cymbiform **(3)**; Club shaped **(2)** ; Sickle shaped **(1)** ; Connate, Connate- triquetrous **(0)**

19. Spines on leaves present **(1)**; Absent **(0)**

20. Keels on leaves long **(2)**; Short **(1)**; None **(0)**

21. Leaf colour grey – blackish **(1)**; Greenish **(0)**

Leaf epidermal surface (microscopic) characters

Octopoma inclusum, O. subglobosum, O. connatum, O. rupigenum, Octopoma sp. PVB 9152 and *Leipoldtia rosea*, have rough-surfaced leaves with protruding papillae (Plate 11). *O. quadrisepelum, O. octojuge, Hallianthus planus, Sclecteranthus hallii, Stoeberia* *carpii, Leipoldtia klaverensis* and *Acrodon bellidiflorus* had leaf surfaces that were almost smooth to smooth (Plate 12). *Ruschia sandbergensis* and *Ruschia goodiae* had leaf surfaces that were intermediate between the *Octopoma inclusum* category and the *Octopoma quadrisepelum* category. The leaf surfaces were rough but with no papillae (Plate 13).

22. Leaf texture rough and with leaf papillae **(1)**; Smooth with no papillae **(0)**

23. Stomatal index high **(1)**; Stomatal index low **(0)**

Stomatal index is the total number of stomata per unit area divided by the total number of stomata in a leaf. It is thought to be a better measure than stomatal density because it has less error bias (Lake et al., 2001).

24. Leaves with bladder like cells **(1)**; Without bladder like cells **(0)**

25. Less than 50% of plant side branches connate **(1)**; More than 50% connate **(0)**

26. Free parts of leaves almost globose **(1)**; spreading **(0)**

Seeds

Seed shape appeared to have some taxonomic value. For instance *Octopoma* c. f. *connatum* had D – shaped seeds (Plates 14 C - D), *O. subglobosum* had a pear-shaped seed (Plate 14 A). Unfortunately, not all the investigated fruit fruits had seeds and seed –

shapes for such species could not be obtained from literature either. Hence, seed - shape was excluded from the data matrix.

27. Seeds with low papillae **(1)**; No papillae **(0)**

28. Seeds smaller than closing bodies **(1)**; Equal to or larger than seed **(0)**

The data matrix is presented in (**Appendix 1**.).

2. 3. Cladistic analysis

The morphological data (**Appendix 1**) were analysed using PAUP* version 4.0b10a (Swofford, 1998) under the parsimony criterion. All characters were unordered (Fitch, 1971) and uninformative characters were excluded.

2. 3. 1. Search algorithm

Heuristic searches were conducted using 1000 random addition sequences, the tree bisection reconnection (TBR) branch swapping algorithm, saving multiple trees (MulTrees), and with steepest descent in effect. A jackknife analysis (Davis, 1993) in PAUP* version 4. 0b4a (Swofford, 1998) was performed to investigate the stability of clades and branch support in general. Ten thousand replicates, with 33.67% character deletion and using the 'emulate-Jac' option, were performed via "stepwise addition". Outgroups were selected from the Delosperma group, a group considered to be distantly related to the Leipoldtia and Ruschia groups from which ingroups were sampled. The outgroups, therefore, were *Oscularia deltoides, Lampranthus bicolor* and *Drosanthemum schoelandianum* (Table 1. 1.). The strict consensus criterion was adopted so as to obtain a tree representing components common to all the most parsimonious trees (Anderberg and Tehler, 1990).

Successive weighting

Successive weighting was executed so as to check for information obstruction that could be due to homoplasy (Kitching et al., 1998). The maximum value of the rescaled consistency index (rc = ci x ri), was used (Farris, 1989). A base weight of 10 was assigned to all characters.

2. 4. RESULTS

Unweighted tree

Analysis of the morphological data set for the 25 taxa included 28 characters, of which 27 were parsimony informative. The analysis retained 16 most parsimonious trees ($L = 68$, $CI = 0.529$, $RI = 0.677$, $RC = 0.358$), of which the strict consensus is presented in Figure 2. 3.

For discussion purposes, groups are represented as A, B, C, D, E, F, G and H and these correspond to nodes (A, B, C, D, E, F, G and H) of the tree. The phylogeny recovered from the morphology data set is well resolved except for group A (node A, Figure 2 .3).

The genus *Octopoma* is non-monophyletic and forms two groups (node A and D). Group A comprises *Octopoma* species of Namaqualand, which are *O.* c. f. *connatum*, *O.* sp. 9152, *O. rupigenum*, *O. inclusum*, *O. subglobosum* and *O.* sp. 9152. Phylogenetic relations of these *Octopoma*s (group A) to other members of the Ruschioideae are unresolved (node A). Branch support for the grouping of these *Octopoma* species is also weak (JK<50%).

Group D comprises *Octopoma* species of the Little Karoo, which are *O. octojuge* and *O. quadrisepalum.* The group also has weak support (JK = 53%) and is resolved as sister to group E, which consists of the three subgroups F, G and H. Subgroup F comprises *Ruschia goodiae*, *R. sandbergensis R. hexamera* and *Acrodon bellidiflorus*. Subgroup G comprises *Leipoldtia frutescens, L. rosea and L. klaverensis.* The subgroup is strongly supported (JK = 90) but its position within group E is unresolved (Figure 2. 3). Subgroup H is also unresolved and weakly supported (JK < 50%) within group E.

Weighted tree

The weighted data set stabilised on the second search and produced 16 most parsimonious trees, of which the strict consensus (L = 228, CI= 0. 702, RI = 0. 830, RC = 0. 562) is presented in Figure 2. 4. The weighted tree (Figure 2. 4) is identical to the unweighted tree (Figure 2. 3).

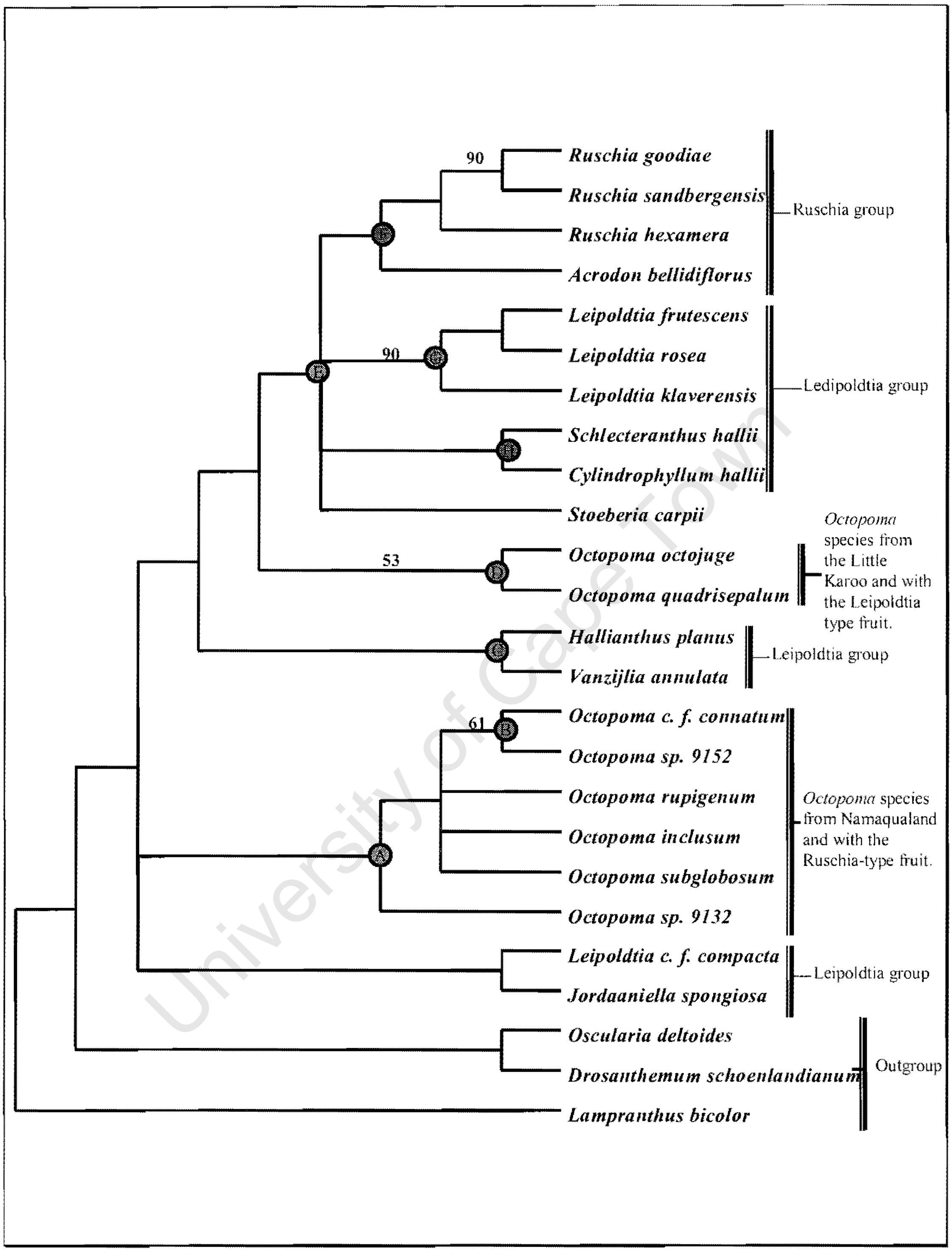


Figure 2. 3. Strict consensus of 16 most parsimonious trees for unweighted morphological data. Tree length = 68. CI = 0.52944, HI = 0.4706, RC = 0.3583, RI = 0. 6768. Numbers on branches indicate Jackknife values. Letters at nodes designate groups discussed in the text.

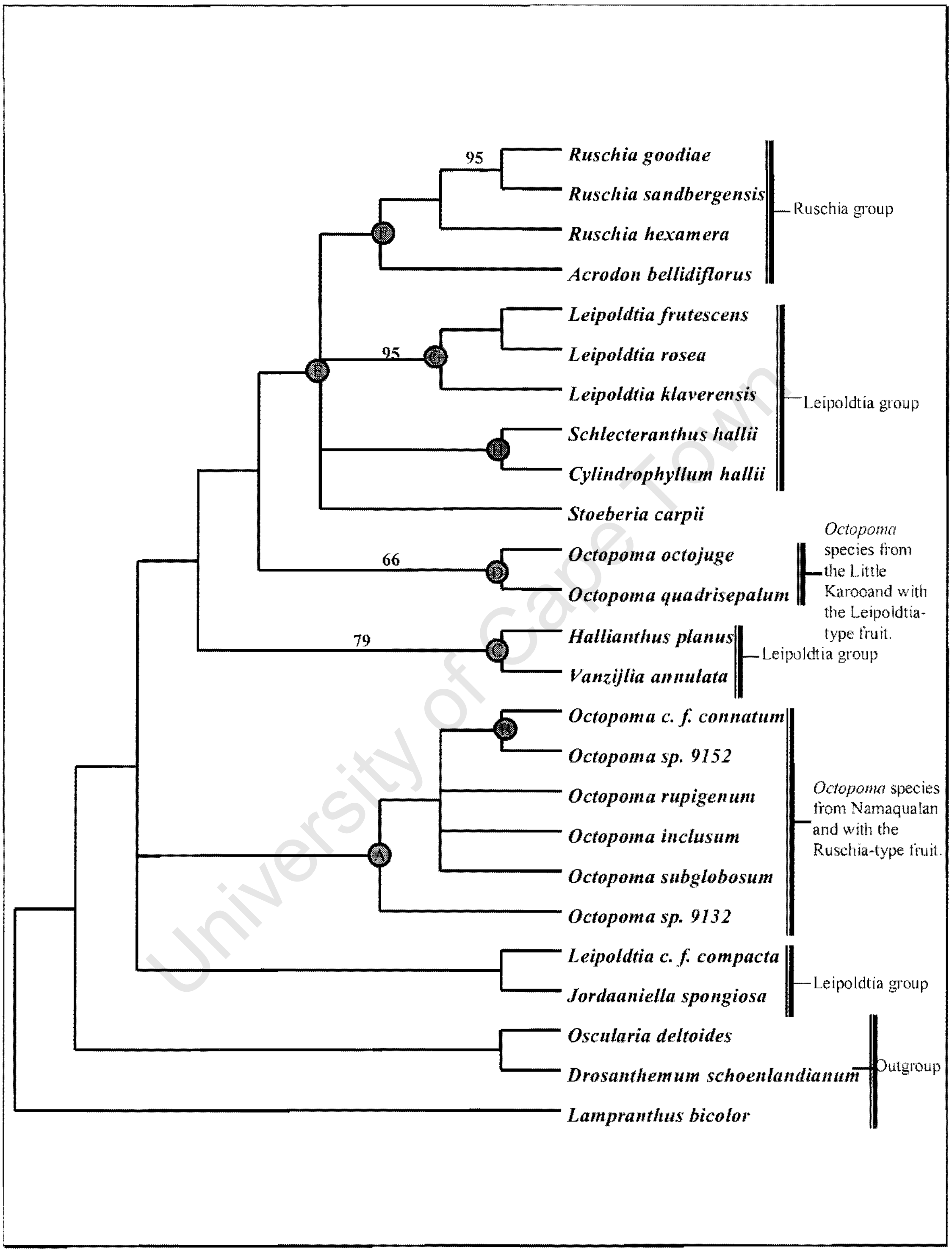


Figure 2. 4. Strict consensus of 16 most parsimonious trees recovered from weighted morphological data. Tree length = 228, CI =0.7018, HI = 0.2982, RI = 0.8296 and RC = 0.5822. Numbers on branches indicate Jackknife values. Letters at nodes designate groups discussed in the text.

2. 5. DISCUSSION

The morphological data indicate that *Octopoma* is non-monophyletic. The results supported Chesselet et al. (2000) who hypothesised that *Octopoma* species do not form a natural group, and Hartmann (1998; 2001) who hypothesised the existence of at least two groups of *Octopoma* species. Morphological analyses of this study are congruent with the results of Chesselet et al. (2000) and Hartmann (1998; 2001) because two groups of *Octopoma* were observed. One group comprised Little Karoo *Octopomas*, which are *O. octojuge* and *O. quadrisepalum*. The other group comprises *Octopomas* of the Namaqualand, which are *O.* c. f. *connatum*, *O.* sp. 9152, *O. rupigenum*, *O. inclusum*, *O. subglobosum* and *O.* sp. 9132.

But even if the study supported Chesselet et al. (2000) and Hartmann's (1998; 2001) hypotheses, it failed to determine phylogenetic relations of Namaqualand *Octopoma*s (node A) to other members of the Ruschioideae. The failure to resolve phylogenetic relations of these Namaqualand *Octopoma*s to other members of the Ruschioideae could have been due an inadequacy in the number of characters, or due to insufficient taxa in the study.

On the other hand, phylogenetic relations of *O. octojuge* and *O. quadrisepalum* (node D) to other members of the Ruschiodeae were resolved. The results supported Tribble and Hartmann (1998) who suggested that the *Ruschia* group is closely related to the *Leipoldtia* group (nodes D and E). However, the branch joining these Little Karoo

*Octopoma*s (*O. octojuge* and *O. quadrisepalum*), as sister taxa to group E members (node E), was weakly supported (JK < 50%). On the other hand the *Leipoldtia* species that lay unresolved within group E (node E) were expected to pair with *O. octojuge* and *O. quadrisepalum* because according to Hartmann (1998) *Leipoldtia* species are more closely related to *Octopoma*s than any other species in the Ruschioideae. But that did not happen probably because they were not enough characters to detect relations between these taxa.

The fact that branch support values and consistency index values increased, when the data set was weighted, suggested the presence of homoplasy in the data set (Kitching et al., 1998). The homoplasius characters were therefore traced and are represented in chapter six.

Another indication from the morphological data is that *O. subglobosum* is not related to *O. octojuge* and *O. quadrisepalum* as Hartmann (1998; 2001) had suggested. Some of the reasons for this contradiction could be that Hartmann's (1998; 2001) suggestion was based on only one character. According to Hartmann (1998; 2001), *O. subglobosum* is supposed to group with *O. octojuge* and *O. quadrisepalum* because the trio share big closing bodies. However, in this study, small rather than big closing bodies were observed for *O. subglobosum*. Besides the size of closing bodies, *O. subglobosum* has closing rodlets while *O. octojuge* and *O. quadrisepalum* do not have this feature. The other difference is that *O. subglobosum* does not have valve wings, while *O. octojuge* and *O. quadrisepalum* do.

2. 6. Conclusions

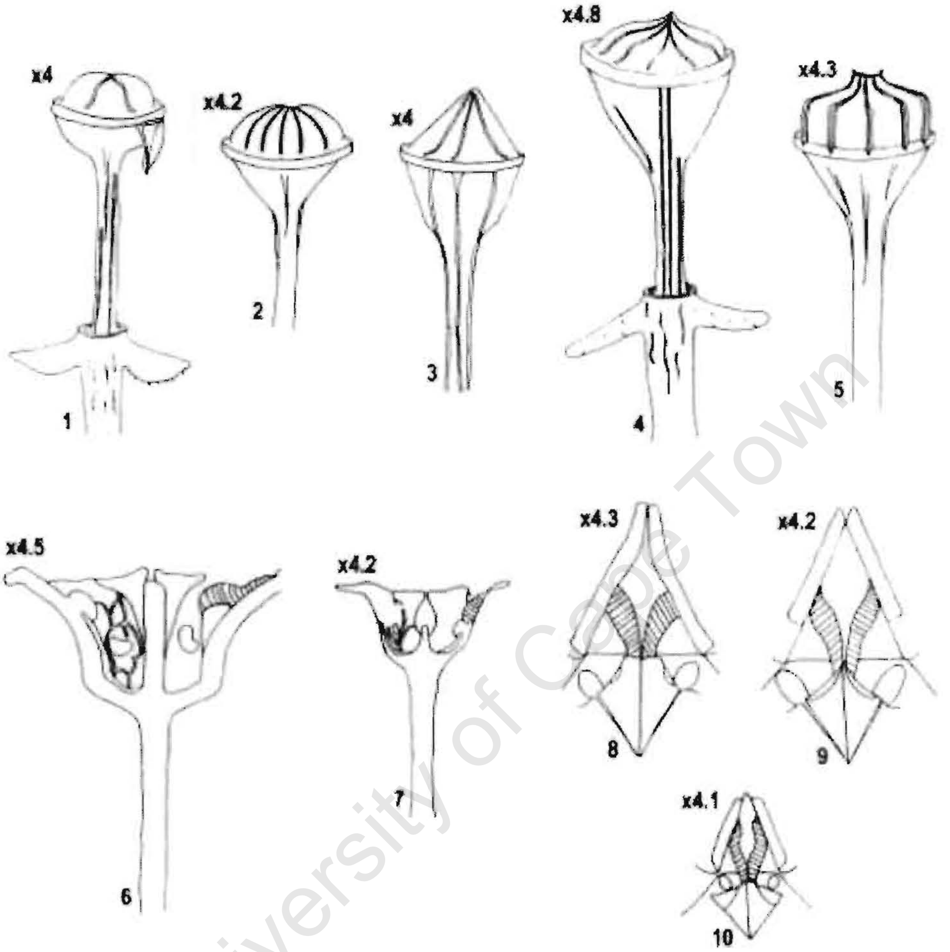
Octopoma species do not form a monophyletic group. At least two groups of *Octopoma* species exist. *Octopoma* species with the *Ruschia*-type fruit belong to a group that is different, but closely related to that of *Octopoma* species that form the *Leipoldtia*-type fruit. Branch support for all the *Octopoma* groupings was low and therefore the morphological data could not be used alone to construct new taxonomic circumscriptions for the genus.

2. 7. Recommendations

Molecular investigations should be done to check if the same conclusions would be arrived at as for the morphological data. Sampling for the study also needs to be broadened to include at least 60 species from *Leipoldtia* and about 100 from *Ruschia*. Type specimens for all the sampled genera also need to be included.

2. 8. Limitations of the study

The number of taxa in the study had to be kept as minimal as possible due to the short study period. But in practice this kind of study requires, in addition to *Octopoma*s, lots of species sampling from genera of *Leipoldtia* and *Ruschia*.



Plates 1 – 10. Fruit shapes: 1).*Octopoma c. f. connatum* (Round), 2). *L. frutescens* (Round), 3). *Octopoma* sp 9123 (High-dome), 4).*Octopoma* c. f. *connatum* (Dome) and 5). *Leipoldtia* c. f. *compacta* (Rectangular). **Columella type:** 6). Long as in *Octopoma* c. f. *connatumm*, 7). Short as in *Octopoma* sp 9123'. The Columella for *Leipoldtia klaverensis* was also as in plate 7. **Internal structure of capsules as observed in**: 8). *Leipoldtia klaverensis* (Diverging), 9). *Leipoldtia frutescens* (Diverging) and 10. *Leipoldtia c. f. compacta* (contagious).

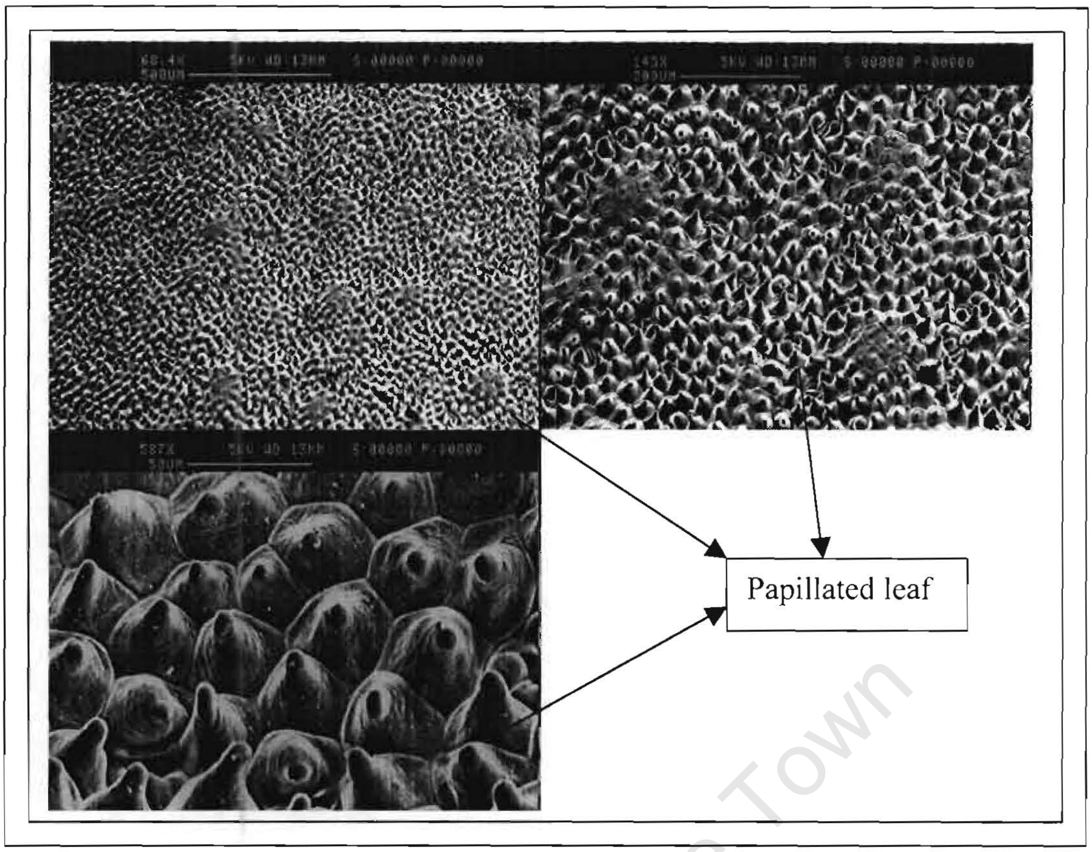


Plate 11. Plate: 11 SEM papillated leaf surface as observed in *Octopoma rupigenum*.

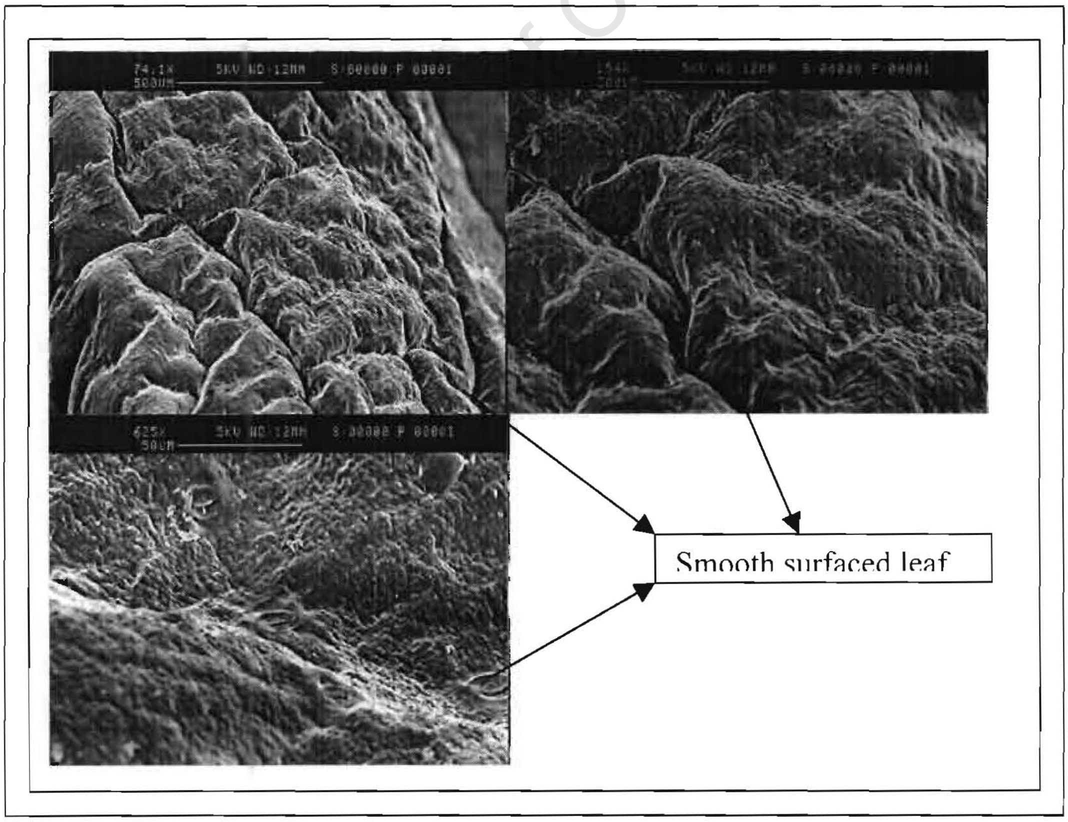


Plate 12: Smooth leaf surface as observed in *Octopoma octojuge*.

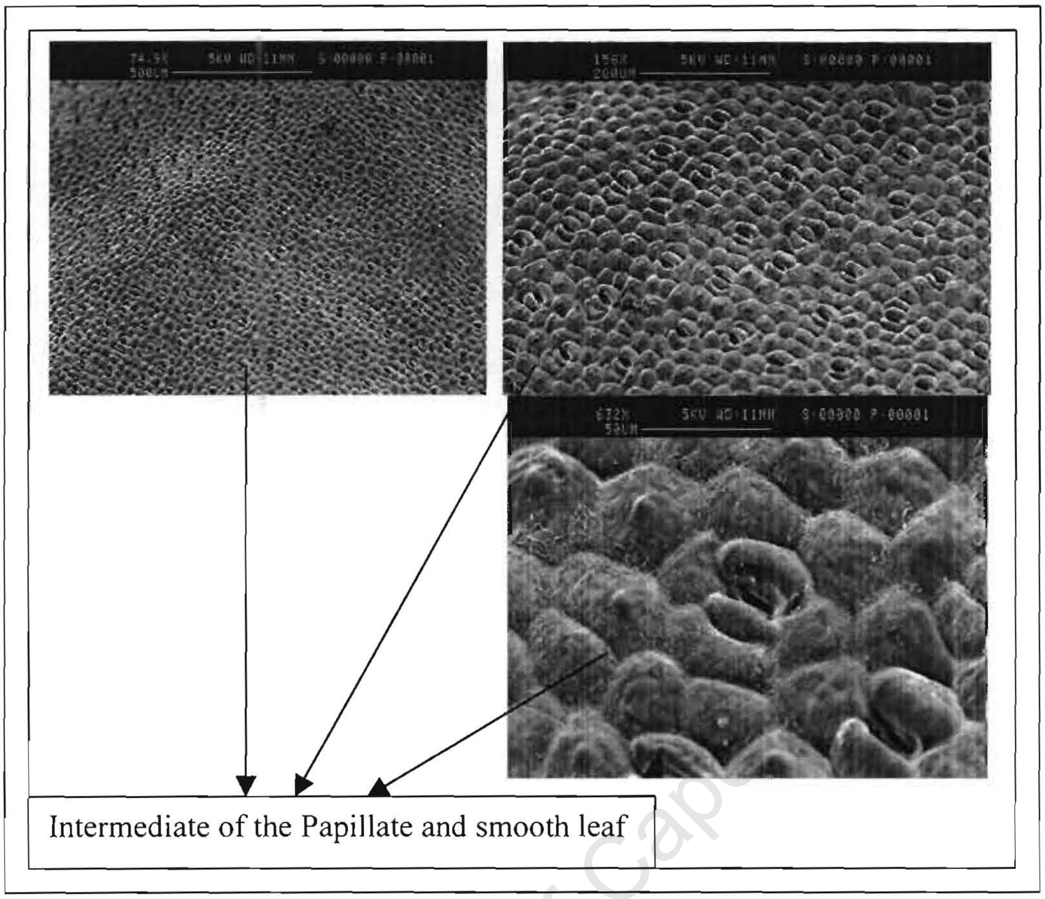


Plate 13: SEM leaf surface for *Ruschia sandbergensis*.

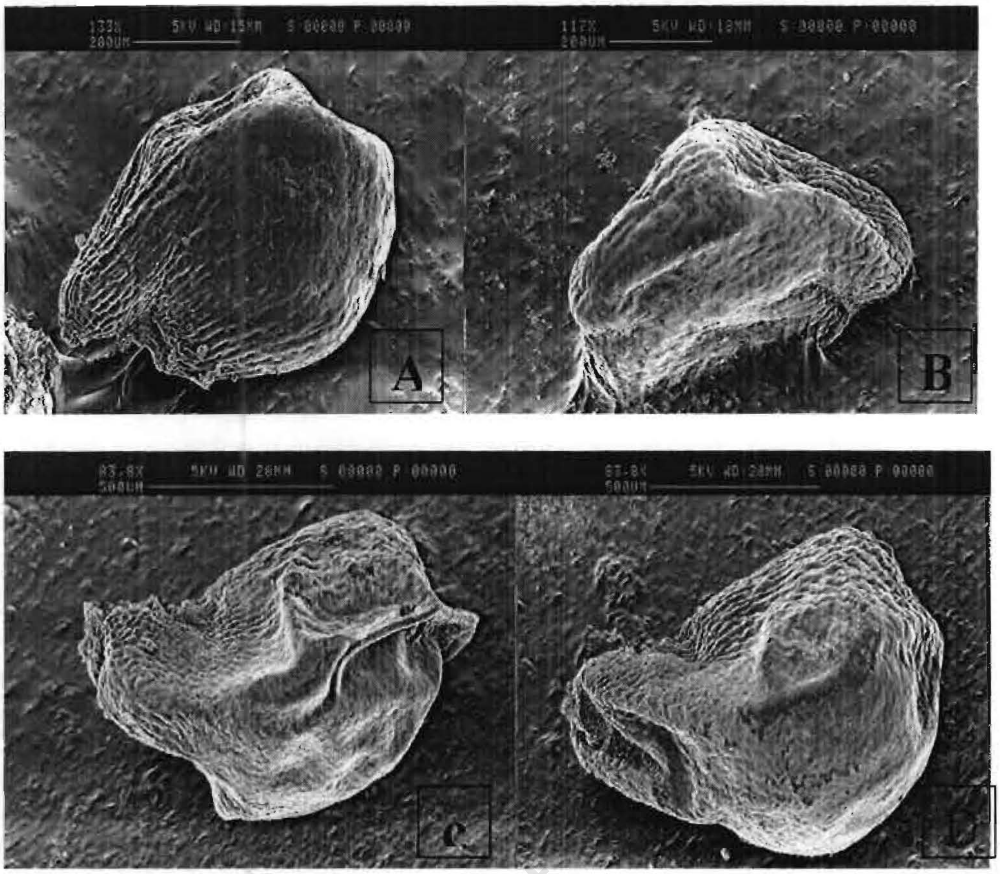


Plate 14: A – D, SEM front view of seed photographs for *Octopoma subglobosum*, *Vanzijlia annulata*, *Octopoma* c. f. *connatum*, *Octopoma* c. f. *connatum*, respectively.

"If you don't succeed at first, change the rules". Anon

CHAPTER 3

Molecular investigations

3. 1. Introduction

While morphological datasets provide indirect genomic information, molecular techniques provide the direct translation of the genetic code (Crawford, 1990). Molecular techniques, especially DNA sequences, are therefore sometimes regarded as the most reliable source of phylogenetic characters (Hillis, 1987). The phylogenetic characters are usually obtained by sequencing the mitochondrial, chloroplast or nuclear regions of living organisms (Griffiths et al., 1993).

Comparing trees based on nuclear and chloroplast markers can be particularly valuable at lower taxonomic levels, providing a window into evolutionary processes that could not be achieved with either genome alone (Soltis & Soltis, 1998: 1). The nuclear and chloroplast markers used in this study were the 5S NTS and the psbA – trnH spacer, respectively. The selection of a DNA region for molecular studies is not easy and the choice is usually based on the extent to which regions were found useful in previous studies. Items to consider when selecting regions include homoplasy levels, mutation rates and the taxonomic level(s) at which the locus is utilisable (Systema, 1990). The 5S NTS and psbA-trnH spacer regions were selected based on qualities that they have exhibited in previous studies (e. g. Sang, 1996; Klak et al., 2003).

3. 1. 1. The 5S Non Transcriber Spacer (5S NTS)

The 5S NTS region is a region between repeats of the 5S subunit nrRNA gene (Long & David, 1980; Hori & Osava, 1987; Sastri et al., 1992; Schneeberger and Cullis, 1992; Crown et al., 1996).

The NTS is divided into three regions; the 3'- downstream, midspacer and the 5'- upstream regions. The 3'- downstream region follows the 3' end of the 5S- gene and is characterised by strings of T's and a TATAT motif downstream. One function of the 3'- downstream region is the termination of transcription (Scoles et al., 1988). On the 5' end of the 5S- gene is the 5'- upstream region. The region is 60 – 90bp long and is the most conserved part of the NTS. Between the 3'-downstream and 5'- upstream regions, is the least conserved region of NTS called the midspacer (Scoles et al., 1988).

Generally NTS sequences are more variable than ITS (internal trancriber spacer) sequences, and may therefore be more informative (Soltis & Soltis, 1998). The 5S NTS data offers the potential to resolve interspecific and intergeneric relationships for the taxa in question. Mutations in the 5S NTS are relatively free from selective constraints and can therefore be readily fixed. That enables the 5S NTS to act as an information reservoir for recent phylogenetic events (Scoles et al., 1988; Baum & Johnson, 1994; Kellogg & Appels, 1996; Crown et al., 1996). Examples of the 5S NTS studies include highly resolved gene trees that were obtained from the phylogenetic studies of *Gossypium*, while the 5S gene failed to resolve species relations in this group (Crown et al., 1996). The region also helped to resolve interspecific relationships within *Cypripedium*, *Phoenix*, *Silene* and *Miscanthus* (Soltis & Soltis, 1998).

Although the 5S NTS genome acts as a good information reservoir for recent phylogenetic events, it has the disadvantage of occurring in multiple copies, which sometimes make DNA sequencing difficult (Kellogg & Appels, 1996). However, reliable phylogenies can still be constructed despite the presence of concerted evolution and these multiple copies (Baker et al., 2000).

3. 1. 2. The psbA – trnH spacer region

The psbA – trnH is a spacer region in the chloroplast genome bordered by the psbA and trnH genes. Psb genes constitute photosystem "b", which is also known as photosystem II (Barber et al., 1987).

The psbA – trnH intergenic spacer region is located in the large single copy (LSC) of the chloroplast genome. As a noncoding region, the psbA – trnH region is under less functional constraints and therefore evolves rapidly. Evidence for rapid evolution of the region was observed in Magnoliaceae, where sequence divergence rates of 1.57%, 0.073% and 0.077% were recorded for the psbA – trnH spacer region, trnK intron and matK coding region, respectively (Sang, 1996). The psbA –trnH spacer region is therefore ideal for use at species and generic levels (Sang, 1996; Heetderks & Evans, 2001).

Base substitutions, insertions and deletions have been identified as valuable characteristics of the psbA – trnH region (Sang, 1996). Aldrich et al. (1988) reported insertions and deletions in the psbA – trnH spacer region to be bordered by AT – rich direct repeats. The reason for the occurrence of direct repeats is associated with slipped strand mispairing during DNA replication or repair (Palmer, 1985a). Direct repeats might also be due to processes of recombination (Bowman & Dyer, 1986; Palmer et al., 1987; Michalowski et al., 1987). However, not all insertions and deletions in the chloroplast genome are bordered by direct repeats (Doebley et al., 1989; Zurawski et al., 1984).

In another study, a 13 base insertion in the psbA – trnH spacer region revealed evidence of hybridisation in the alloploid taxa *Erythronium elegans* and *E. quinaultense* (Allen et al., 2002). Another example is that in which insertions and deletions were used as maternal markers to detect hybridisation in the genus *Cylindropuntia* of Cactaceae (Agrawal & Salywon, 2001). Insertions and deletions were also found to serve as species-specific markers in studying chromosomal evolution in 35 species of cholla cacti (Salywon & Pinkava, 2002).

In a study involving 32 species of Paeoniaceae, the psbA – trnH region registered higher mutation rates than the trnL – trnF spacer region. Hence, it was concluded that the psbA – trnH region is more useful than the trnL – trnF region in revealing phylogenetic relations at intrageneric level (Sang, 1996; 1997).

Another study of the psbA – trnH spacer region identified a 210 base insertion in *Medicago sativa* (Aldrich et al. 1998). The insertion comprised an inverted repeat stem – loop structure 5' to trnH. Portions of these insertions were also found in *Pisum sativum* and *Glycine max*. The interpretation of the results was that the insertion in question was present in the common ancestor of *Pisum sativum*, *Glycine max* and *Medicago sativa* (Aldrich et al., 1998).

Drawbacks of the psbA - trnH region include short sequences, of about 27 base pairs, that are bordered by long inverted repeats. Such sequences can frequently undergo inversions that lead to homoplasious mutations (Sang, 1996; Jansen and Palmer, 1987; Doyle et al., 1992; Rauberson and Jansen, 1992).

3. 1. 3. Previous studies in the Ruschioideae

Classification of *Octopoma* using molecular approaches has never been attempted. Therefore, this study will be the first of its kind. However, 5S NTS, trnL-F and nuclear internal transcribed spacer (ITS) regions have been used to study the phylogenetic position of the *Lampranthus* group in the Ruschioideae (Klak et al., 2003). The study concluded that the 5S NTS region is suitable for studying relationships in the Ruschioideae at generic level. The trnL –trnF and ITS regions were found unsuitable for generic and species studies in the Ruschioideae because of inadequate levels of sequence divergence.

3. 1. 4. Analysis protocol

Two approaches were used to evaluate the data. One of the approaches was maximum parsimony and was employed as described in section 2. 3. 1., chapter 2. The other approach, currently applicable to DNA data, was the maximum likelihood criterion.

Maximum likelihood uses explicit stochastic models to calculate the probability that a tree could give rise to the observed data (Crawford, 1990). Since the likelihood of a tree refers to the probability of the data given the tree and not the other way round (Felsenstein, 1981), likelihood methods allow for the choice of a tree among other trees based on predictions they imply (Lewis, 1998). Then a tree with the highest score is taken as the best estimate of the phylogeny (Felsenstein, 1981).

Substitution rates vary not only from lineage to lineage, but also from site to site. Hence the best way to cater for this site to site heterogeneity is to assume independent substitutions for each site. The disadvantage of that would be the existence of too many parameters in the model, thus calling for tremendous computing efforts. An alternative is to assign sites to a few rate categories. The gamma distribution is used to do this (Lewis, 1998).

The gamma distribution density function can take two qualitatively different shapes. The shapes are dependent on the value of the gamma parameter, which ranges from zero to infinity. Values of the shape parameter that are equal or less than one imply low substitution rates for most sites and quite high substitution rates for very few sites. Inequality in relative rates becomes larger when the shape parameter approaches zero (Lewis, 1998).

When the value of the shape parameter equals ten, it implies that all sites have an equal rate of substitution (normal distribution). In summary, complete homogeneity (equal substitution) of rates occurs when the shape parameter equals infinity, while complete heterogeneity of rates occurs when the shape parameter approaches zero (Lewis, 1998).

3. 1. 5. Objectives

1. To use molecular data to investigate phylogenetic relationships of *Octopoma* species.
2. Evaluate the utility of the psbA-trnH spacer and 5S NTS for resolution of relationships in the Ruschioideae.

3. 2. Materials and methods

3. 2. 1. Taxon sampling

All taxa from morphological analysis were also sequenced, with the exception of *Octopoma* sp. 9132, which failed to yield usable DNA.

3. 2. 2. DNA extraction

The CTAB extraction method of Saghai – Maroof et al. (1984) and modified by Doyle and Doyle (1987) was used to isolate genomic DNA from 24 fresh sampled species. Success of DNA extraction was checked with 1% agarose gel electrophoresis. An intercalating dye, ethidium bromide, was used to aid visualisation of DNA under ultraviolet light (300 nm). Photographing of the gels was done with a UV transilluminator, connected to a cyberteck CSI computer and video copy processor.

The polymerase chain reaction (PCR) was used to amplify the entire 5S NTS region using the forward primer P3 and the reverse primer P4 (Cox et al., 1992; Table 3. 1), and the psbA-trnH spacer region using the forward primer psbAf and reverse primer trnHr (Shinokazi et al., 1986; Table 3.1).

Table 3. 1. Primers used in PCR and sequencing of species in the Ruschioideae.

DNA sequences (spacer regions)	**Primer**	**Sequence 5' – 3'**
5S NTS (Cox et al., 1992)	P3	GAGAGTAGTACATCGATGGG
	P4	GGAGTTCTGACGGGATCCGG
PsbA-trnH (Shinokazi et al., 1986)	PsbAf	GTTATGCATGAACGTAATGCT
	TrnHr	CGCGCATGGATTCACAATA

Amplification was performed on an ABI Gene Amp PCR system 2700. Reaction mixtures were prepared in 35.485 µl volumes as follows: 24.02 µl PCR water, 3.75 µl 10X strength buffer, 3.75 µl Magnesium chloride, 1.12 µl (100ng/ul) of each primer, 1.50 µl dNTP, 0.225 µl (5U/µl) taq and 2 µl template DNA.

Thermocycling reactions were performed under the following conditions; 94 °C (2 min), to ensure denaturation of double – stranded DNA template, 1 cycle denaturing step of 94 °C (1 min), annealing step of X °C for 1 min (X = 55 for 5S NTS and 52 for psbA-trnH spacer) and an extension step of 72 °C (1 min), and another extension step of 72 °C (5 min) to complete the unfinished DNA strands. The reaction was done in 30 cycles. All PCR reactions included a negative control. Success of amplification was evaluated by running products on an agarose gel as described above. The QIAquick PCR purification kit (QIAGEN) was used to purify the PCR products according to the manufacture's instructions.

3. 2. 3. DNA sequencing

DNA templates were sequenced in 10µl, fluorescent dye – labelled reactions. Primers P3, P4, psbAf and trnHr were used in conjuction with the ABI prism dye terminator cycle sequencing ready reaction kit, from PE Biosystems. Twenty-five cycles of 96 °C (30 sec), 50 °C (15 sec), 60 °C (4 min) were used for denaturation, annealing and extension, respectively. Sequence products were resolved on an ABI 3100 automated sequencer according to the manufacture's instructions.

3 .2. 4. Assemblage and alignment of sequences

Sequences were assembled using the SeqMan module of the Lasergene software package (DNASTAR Inc, 1994). The alignment was done easily and manually in MegAlign (DNASTAR Inc, 1994). Aligned sequences were saved in Nexus format for use with PAUP* version 4.0b10 (Swofford, 1998) and MacClade (Maddison and Maddison, 1992). Simple indel coding was adopted since the gaps could be easily aligned (Simmons and Ochoterena, 2000). A number of indels were derived from the alignment (Table 3. 2).

Table 3. 2. Coded indels for the 5S NTS and psbA-trnH regions (See **Appendix 1** for alignment details).

Region	**Insertion/deletion**	**Position**	**Species**
5S NTS	5'ATTGAGG3' 5'-------------3' 5'-----3'	165 -170 193 - 204 210 - 216	*R. goodiae, R sandbergensis* *O. octojuge, O. quadrisepalum, R. hexamera* *O. octojuge, O. quadrisepalum*
PsbA-trn	5'TCCTTTCCTTGTTTT3' 5'TTTCCTTGTTTTTGAATTTAC3'	570 – 584 605 - 624	*O. inclusum, O. subglobosum* *O.* c. f. *connatum, O. sp. 9152*

3. 2. 5. Cladistic analyses

Both separate and combined analyses of the PsbA – trnH spacer and 5S NTS data sets were performed. The data sets were analysed using maximum parsimony and maximum likelihood criteria as implemented in PAUP* 4. 0b10 (Swofford, 1998). Uninformative characters were excluded in the case of maximum parsimony.

3. 2. 5. 1. Maximum parsimony

Heuristic searches with, 1000 random taxon addition replicates, were executed using the tree bisection reconnection (TBR) branch swapping algorithm saving multiple trees (MulTrees), steepest descent. A jackknife analysis (Davis, 1993) in PAUP* version 4. 0b4a (Swofford, 1998) was performed to investigate the stability of clades and branch support in general. Ten thousand replicates, with 33.67% of characters deleted, and using the 'emulate-Jac' option, were performed via "stepwise addition". Outgroups were selected from the *Delosperma* group, a group considered to be distantly related to the *Leipoldtia* and *Ruschia* groups (see above) from which ingroups were sampled. The outgroups, therefore, were *Oscularia deltoides, Lampranthus bicolor* and *Drosanthemum schoelandianum* (Table 1. 1.). The strict consensus criterion was adopted so as to obtain a tree representing components common to all the most parsimonius trees (Anderberg and Tehler, 1990).

3. 2. 5. 2. Maximum likelihood

Rates of variation among sites were assumed to follow a gamma type of distribution with shape parameter alpha (Yang, 1993). The gamma distribution was partitioned such that sites were assigned to four equal rate categories (Yang, 1993) and each category was characterised by its median. Tree scores were evaluated under the relevant substitution model for the data set. Model parameters were estimated on one of the most parsimonous trees and fixed for subsequent analyses. For the 5S NTS and psbA-trnH data sets, settings corresponded to the GTR + G + I model, while combined analysis of the psbA-trnH/5S NTS data, utilised the GTR + G model (Table 3. 2). That was to allow ML analysis to be performed under a model that matches the pattern and rates of nucleotide substitution in the combined data. The proportion of invariable sites (P_{inv}), α and rate substitution matrices were estimated using discrete approximations (Yang, 1994). Branch support was checked using likelihood Jackknife with 100 replicates, as implemented in PAUP* version 4. 0b10 (Swofford, 1998). Heuristic searches consisting of 1000 random addition sequence replicates, with MULPARS, STEEPEST DESCENT options and TBR branch swapping in effect, were performed under the as – is option. The molecular clock was not enforced.

3. 3. RESULTS

3. 3. 1. Maximum parsimony (MP) analysis of the 5S NTS region

The 5S NTS data set had short sequences, the longest being only 184 bp (*Lampranthus bicolor, Drosanthemum deltoides* and *Oscularia schoelandianum*). The shortest sequence was 106 bp (*Schechteranthus hallii*). However, the aligned length over the 24 taxa was 301 characters, of which 129 (43%) were variable and 56 (19%) parsimony informative. Three indels were coded (Table 3. 2). Under the parsimony criterion, four trees were retained (L = 186, CI = 0. 602, RI = 0. 641 and RC = 0. 386), of which the strict consensus is presented in Figure 3. 1.

Octopoma species fall into two main groups. One of these group is a strongly supported (JK =97%) grouping (A) of the two Little Karoo species, *O. octojuge* and *O. quadrisepalum*. The second group (B) is unsupported and it includes Namaqualand species, which are *O.* c. f. *connatum*, *O.* sp. 9152, *O. subglobosum*, *O. rupigenum* and *O. inclusum* as well as *Leipoldtia* c. f *compacta* and *Ruschia hexamera.* Within this latter group, relationships are well resolved, but not supported by the jackknife.

Some portions of the 5S NTS tree (Figure 3.1) resemble those of the morphological tree (Figure 2. 3). For example, group B (Figure 3. 1), which comprises *Octopoma*s of Namaqualand, was also recovered in the morphological tree (group A, Figure 2. 3). The only difference was that in the morphology tree, *Ruschia hexamera* and *Leipoldtia* c. f. *compacta* were placed outside this group. Group A (Figure 3. 1) was also recovered in the morphological tree (group D, Figure 2. 3). Almost all of group C taxa (Figure 3. 1) were also recovered as a group in the morphological tree (node E, Figure 2. 3).

Outgroups (*Lampranthus bicolor, Drosanthemum schoelandianum* and *Oscularia deltoides*) for the morphological tree also resembles those for the 5S NTS tree, except that in the 5S NTS tree, *Oscularia deltoides* is treated as an ingroup taxon (Figure 3. 1)

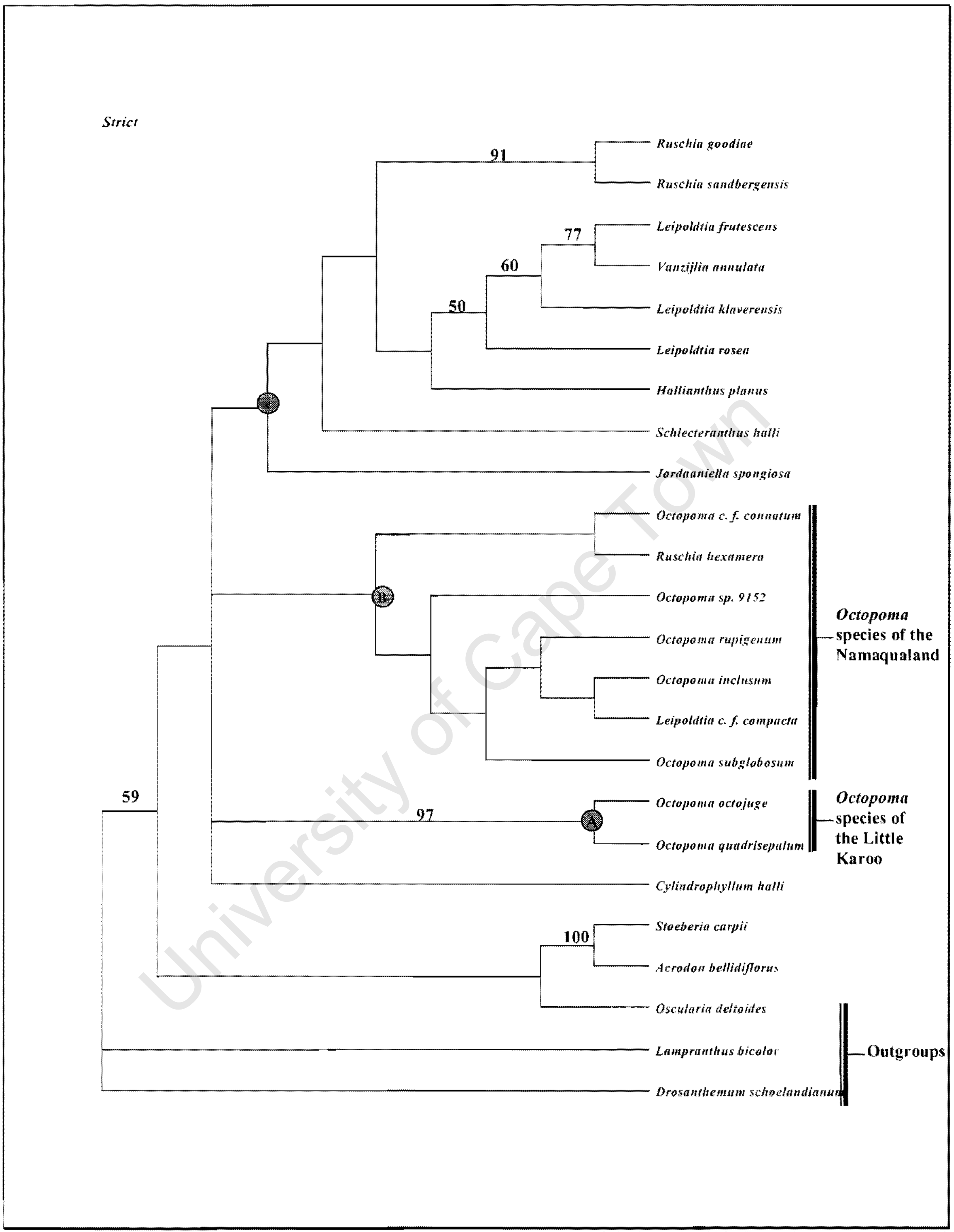


Figure 3. 1. Strict consensus of four most parsimonous trees (L = 186, CI = 0. 602, RI = 0. 641, RC = 0. 386 obtained from analysis of the 5S NTS data set. Numbers above branches designate jackknife values, while letters designate groups.

3. 3. 2. Maximum parsimony analysis of the psbA-trnH spacer region.

The psbA – trnH spacer region also had short sequences, the longest being only 325 bp long (*Leipoldtia klaverensis, L. rosea, L.* c. f. *compacta, L. frustescens*). The shortest sequence was 240bp (*R. sandbergensis*). Three indels were also coded (Table 3. 2). The analysis included 329 characters, of which 29 (9%) were variable and 17 (5%) parsimony informative. Trees retained were 97 400 and their strict consensus (L = 27, CI = 0. 630, RI = 0. 783 and RC = 0. 493) is presented in Figure 3. 2.

The psbA-trnH tree (Figure 3. 2) is mainly unresolved including all of its most parsimonious trees, indicating less variability in the data. Hence, only three groups (A, B, C) were recovered. Group A encompasses all members of the Ruschioideae (node A) but is weakly supported (JK = 65). Group B is strongly supported (JK = 99) and comprises *O.* c . f. *connatum* and *O.* sp. 9152). Group C is moderately supported and comprises *O. inclusum* and *O. subglobosum*. But since the psbA-trnH tree is mainly unresolved, phylogenetic relations of groups B and C to other members of the Ruschioideae are also unresolved.

The psbA –trnH region retains *Drosanthemum schoelandianum* and *Lampranthus bicolor* as outgroup taxa just as with the 5S NTS and morphological (also retained *Oscularia deltoides*) data set.

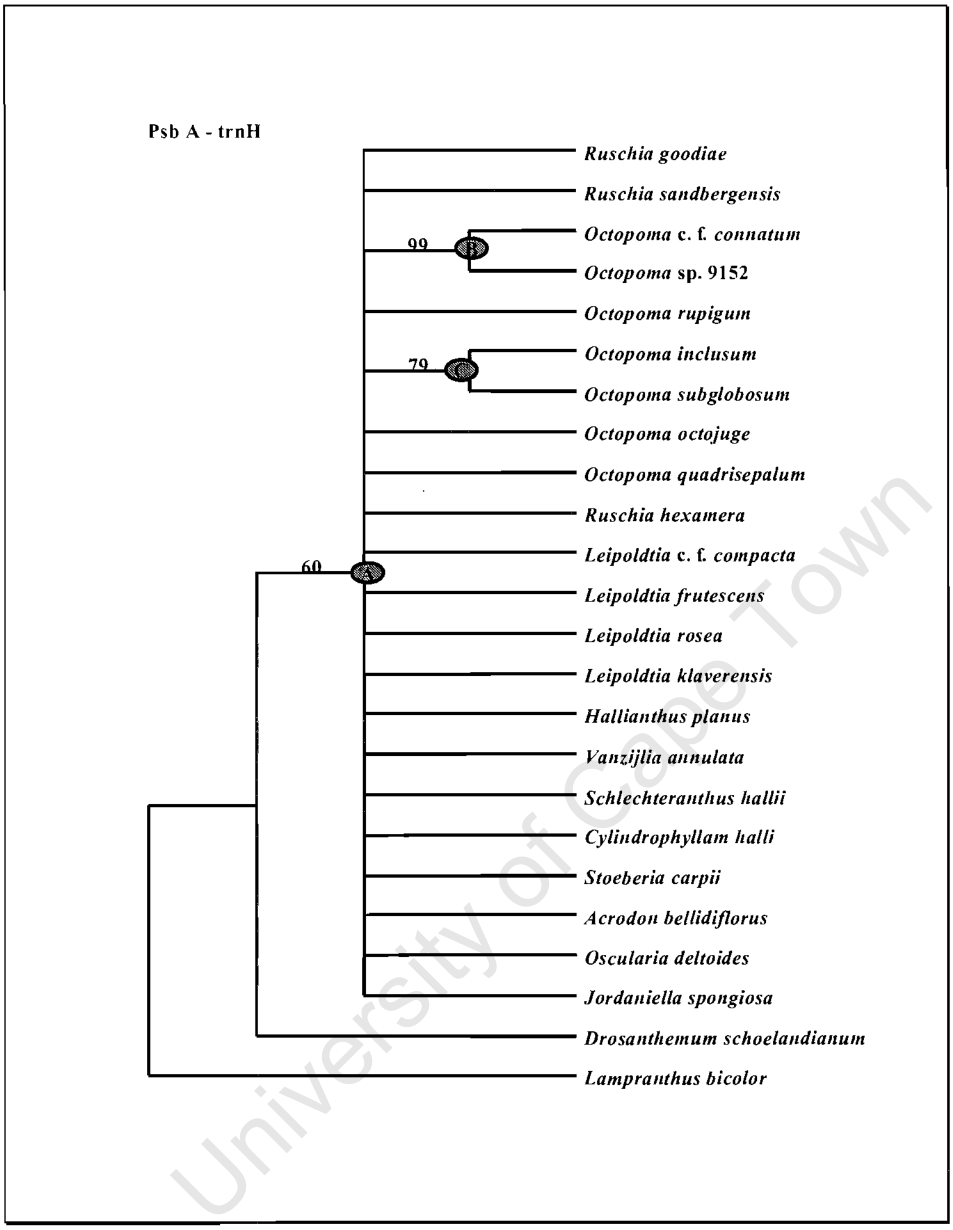


Figure 3. 2. Cladogram for strict consensus of 97 400 most parsimonious trees trees (L = 27, CI = 0. 630, RI = 0. 783, RC = 0. 493) obtained from maximum parsimony analysis of the psbA-trnH data set. Numbers above branches designate jackknife values, while alphabets designate groups.

3. 3. 3. Parsimony analysis of the 5S NTS /psbA –trnH data sets combined.

The analysis involved 24 taxa with 698 characters, of which 168 (24%) were variable and 92 (13 %) parsimony informative. Six most parsimonous trees (L = 238, CI = 0. 567, RI = 0. 602, RC = 0. 342) were recovered, of which the strict consensus is presented in Figure 3. 3. The combined data tree is well resolved and the tree topology is similar to that retained in the 5S NTS data set. Just like the morphology and 5S NTS trees, the combined psbA-trnH/5S NTS tree also does not support the monophyly of *Octopoma*. Two groups of *Octopoma* are present (nodes C and D).

Group D comprises *Octopoma*s of Namaqualand, which are *O. inclusum*, *O. subglobosum*, *O. rupigenum, O.* sp. 9152 and *O.* c. f. *connatum*. Support for the grouping of these *Octopoma*s is low (JK < 50%) and *Leipoldtia* c. f. *compacta* also groups with these Namaqualand *Octopoma*s. Taxa composition of this group D (Figure 3. 2), is also similar to that of group A (Figure 2. 3) in the morphological tree and E in the 5S NTS (Figure 3. 1) tree. However, the 5S NTS tree has an additional taxon, *Ruschia hexamara*, while the morphology tree does not have *Leipoldtia* c. f. *compacta* in its group A.

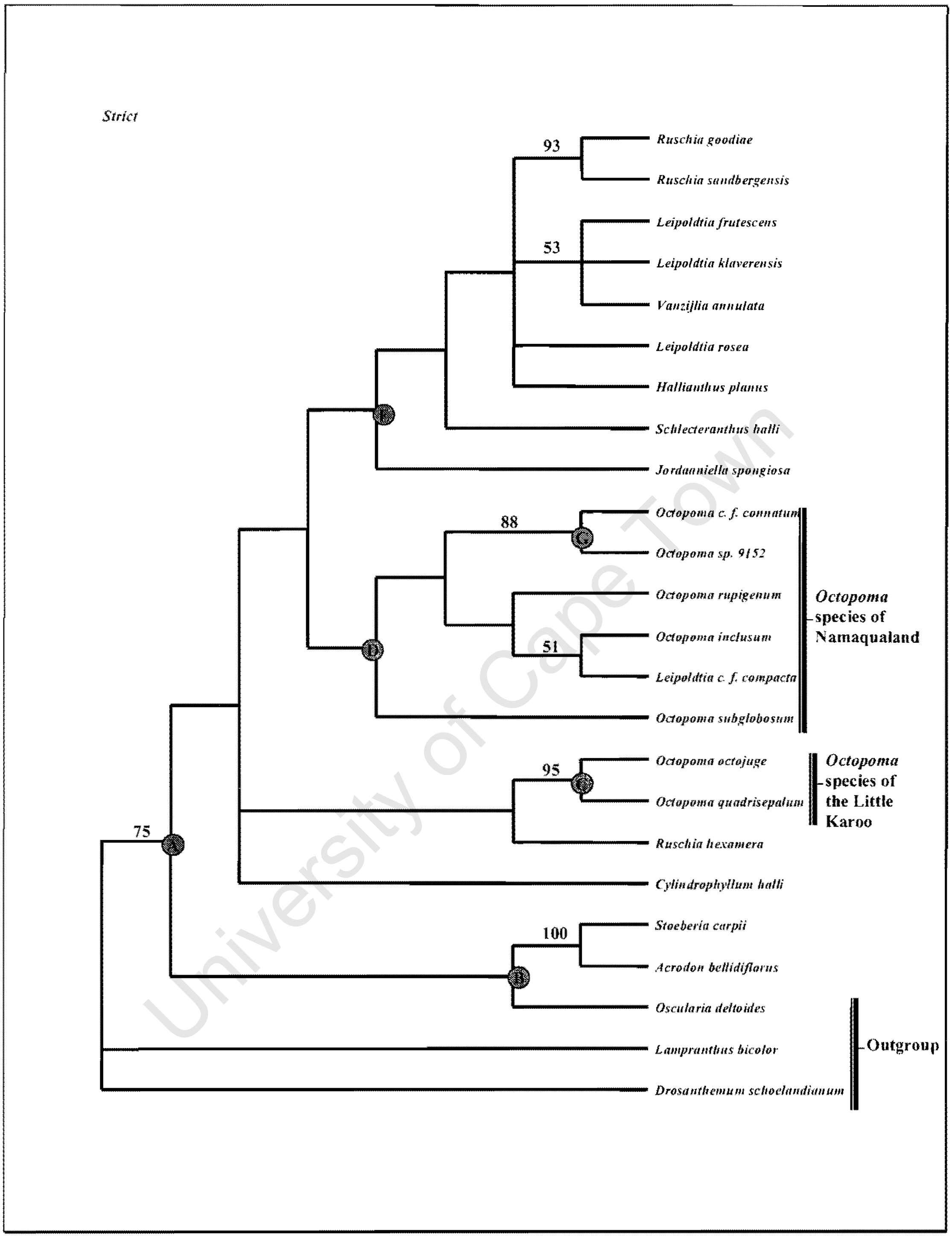


Figure 3. 3. Strict consensus of six most parsimonious trees obtained (L =238, CI = 0. 567, RI = 0. 602, RC = 0. 342) from analysis of the combined 5S NTS and psbA-trnH data sets. Numbers above branches designate jackknife values, while letters designate groups.

3. 3. 4. Maximum likelihood (ML) analysis of 5S NTS, psbA – trnH and, combined 5S NTS/psbA –trnH data sets.

The proportion of invariant sites, substitution model and gamma shape parameters estimates are different for the 5S NTS and psbA –trnH regions (Table 3. 2). Two trees were retained for the 5S NTS data, 16 for the psbA –trnH and two for the combined data sets. Likelihood scores were 5S NTS =- 1330.8, psbA –trnH = -556.4 and combined = -1448. 5 (Table 3. 2). The value of the shape parameter is close to one for the 5S NTS region, close to zero for the combined data set and infinite for the psbA – trnH data set (Table 3. 2). Gamma parameters for individual gene regions imply equal rates of base substitution (normal distribution), while the combined data set has unequal rates of base substitution because the combined gene regions evolved under different models. Both the 5S NTS and psbA – trnH regions are AT-rich with empirical base frequencies of A = 0. 306, C = 0. 129, G = 0. 175, T = 0. 388 and A = 0. 294, C = 0. 134, G = 0. 204, T = 0. 377 respectively.

Table 3. 3. Model parameter estimates for likelihood analysis of the nuclear (5S NTS) and chloroplast (psbA-trnH) data sets.

Data set	**Model**	**Ln Likelihood**	**P_{inv}**[1]	**Gamma Shape**	**Rate substitution matrix**					
					A-C	**A-G**	**A-T**	**C-G**	**C-T**	**G-T**[2]
5S NTS	GTR + G+ I	-1330. 8	0.1	0.9	3.5	2.9	2.4	4.5	2.3	1
PsbA-trn	GTR + G+ I	-556. 5	-	Infinity	3.6	1.3	7.9	2.6	4.5	1
Combined	GTR + G	-1448. 5	0.0	0.2	9.7	4.8	2.2	8.0	7.3	1

1. Proportion of invariable estimates 2. Reference frequency

GTR + G + I = General time reversible with an estimated gamma distribution shape parameter and proportion of invariant estimates (sites).

GTR + G = General time reversible with an estimated gamma distribution.

Most of the major groups retained in maximum parsimony are also recovered in maximum likelihood analysis. However, the groups are better resolved under maximum likelihood. For instance, in maximum parsimony analysis of the 5S NTS data set, phylogenetic relations of Little Karoo *Octopomas* to other members of the Ruschioideae are unresolved. These phylogenetic relations, however, get resolved in maximum likelihood. The group gets resolved as sister taxa to a group that comprises *Ruschia goodieae, Ruschia sandbergensis, Leipoldtia frutescens, Leipoldtia rosea, Leipoldtia klaverensis, Vanzijlia annulata, Hallianthus planus, Schechteranthus hallii, Stoeberia carpii, Acrodon bellidiflorus* and *Jordaaniella spongiosa*. This phylogenetic relationship can also be seen in the morphology tree (Figure 2. 3). Support for this grouping increased from 97% in the maximum parsimony tree, to 99% in maximum likelihood. Maximum likelihood for the 5S NTS data set (Figure 3. 4., node D) just like maximum parsimony (Figure 3. 1., node E), also places *Ruschia hexamera* and *Leipoldtia* c. f. *compacta* in the group that comprises Namaqualand *Octopomas*, which are *O. inclusum*, *O.* c. f. *connatum*, *O. rupigenum*, *O.* sp. 9152 and *O. subglobosum*.

In the combined data set, ML just like MP resolves the group comprising Namaqualand *Octopomas* as sister taxa to the group comprising *R. goodiae, R. sandbergensis, L. frutescens, Vanzijlia annulata, L. rosea, Hallianthus planus, Schechteranthus hallii* and *Jordaaniella spongiosa.* However, the phylogenetic position of the group comprising *O. octojuge* and *O. quadrisepalum* is unresolved in MP (Figure, 3. 3, node C), but gets resolved in ML (Figure 3. 6., node B). The group gets resolved as sister taxa to all Ruschioideae members in the study, except for *Drosanthemum schoelandianum* and *Lampranthus bicolor*.

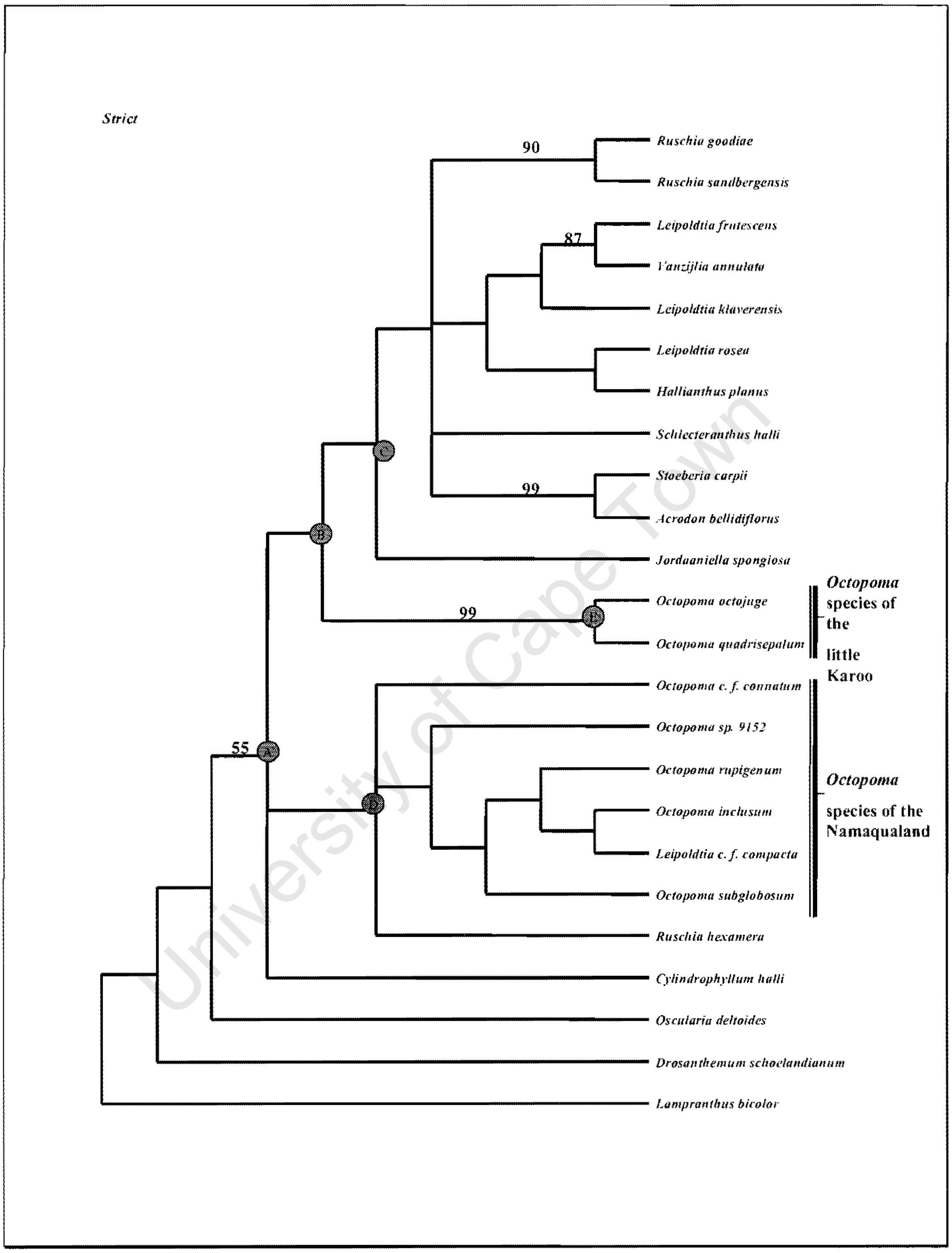


Figure 3. 4. Strict consensus of two trees retained under maximum likelihood analysis of the 5S NTS data set. Numbers above branches designate jackknife values, while letters designate groups.

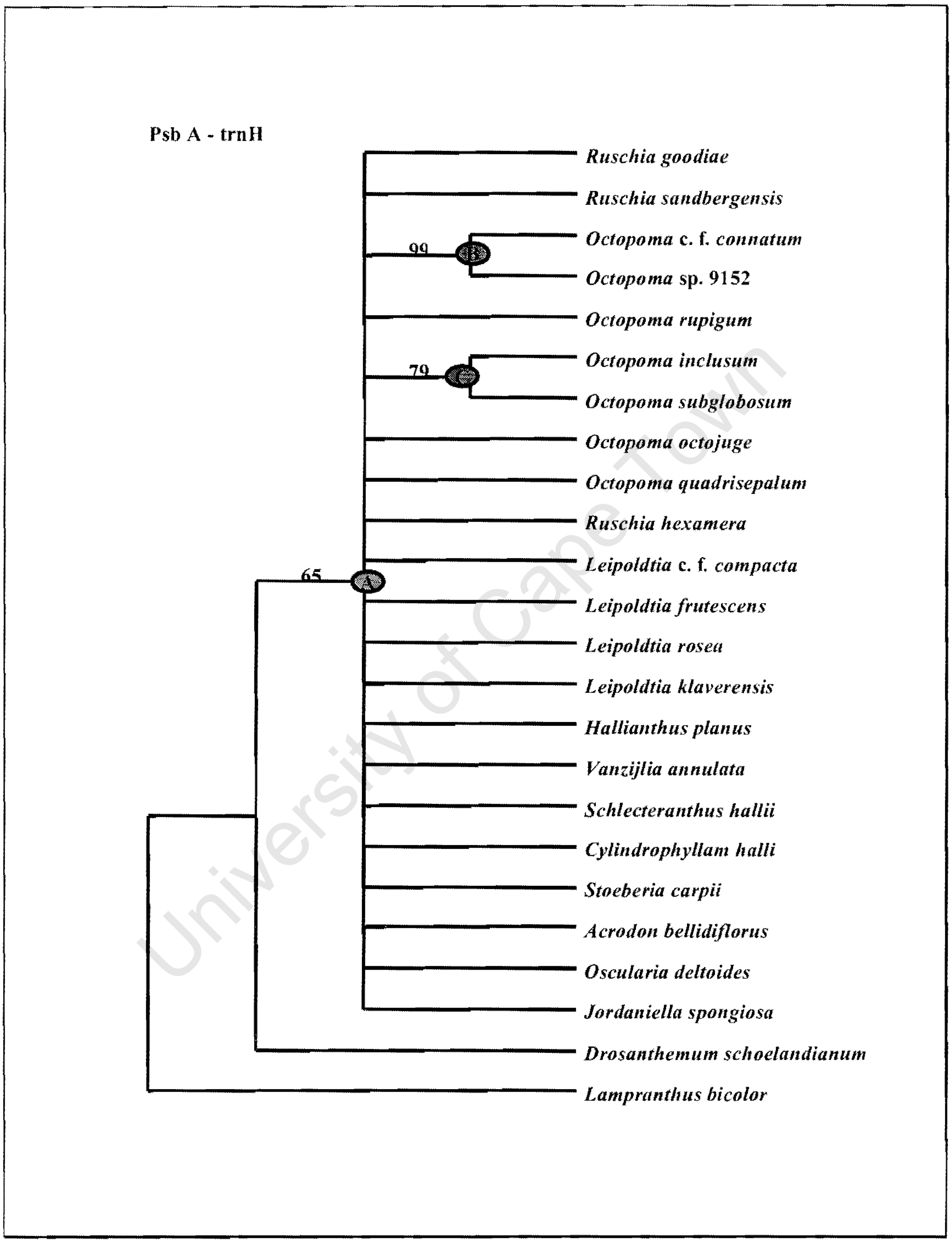


Figure 3. 5. Strict consensus of 16 maximum likelihood trees obtained from analysis of the psbA-trnH data set. Numbers above branches designate jackknife values, while letters designate groups.

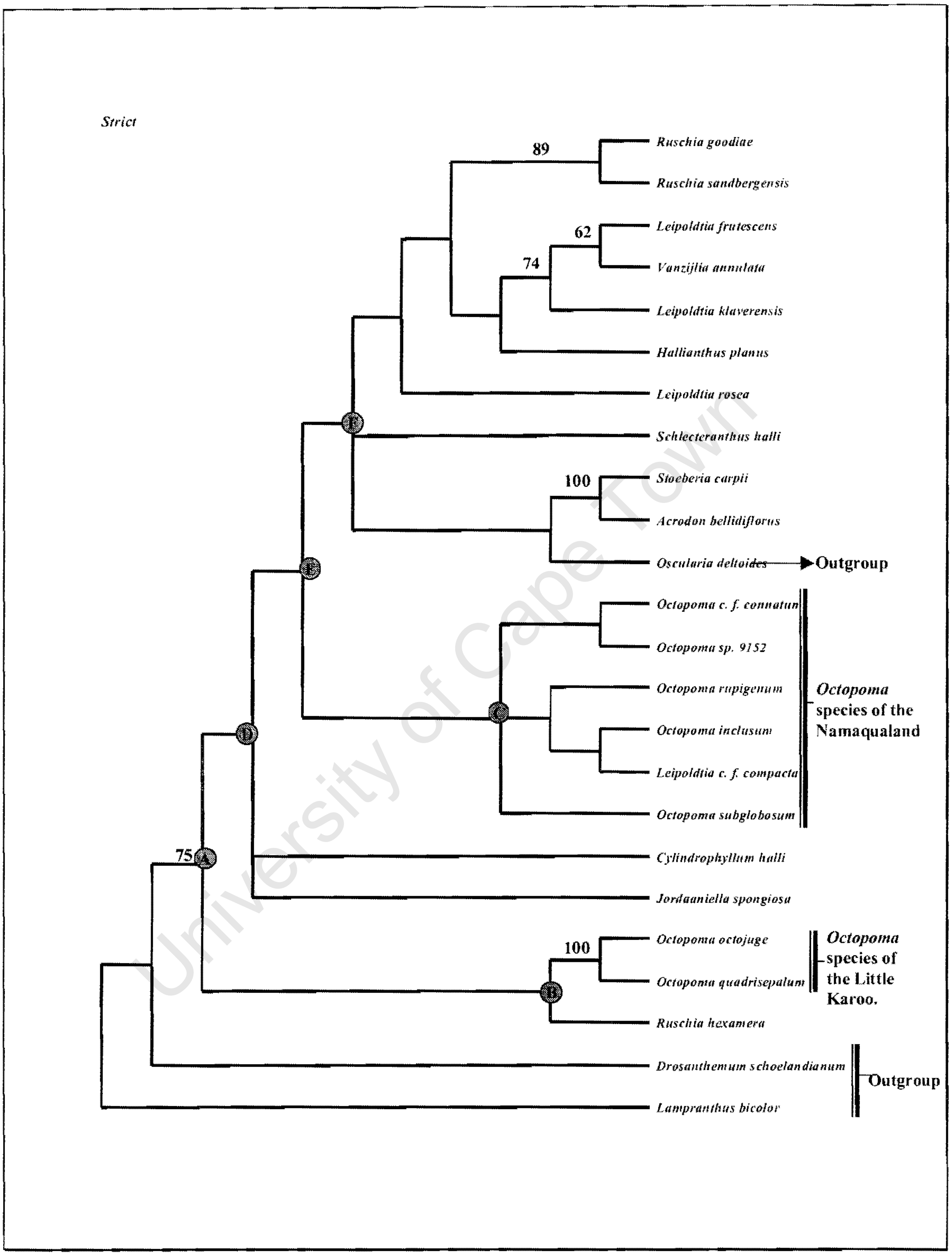


Figure 3. 6. Strict consensus of two maximum likelihood trees obtained from combined analysis of the 5S NTS and psbA-trnH data sets. Numbers above branches designate jackknife values, while letters designate groups.

3. 4. Discussion

3. 4. 1. Comparison of the 5S NTS and psbA –trnH data sets.

Sequences

The psbA – trnH region had longer sequences than the 5S NTS. Both regions were AT-rich. The 5S NTS sequences supported Scoles et al., (1988) who reported the region to be characterised by a string of T's and a TATAT motif downstream. On the other hand, the psbA –trnH sequences supported Palmer (1985a), who reported insertions and deletions in the region to be surrounded by AT-rich direct repeats due to slipped strand mispairing during DNA replication. Both the 5S NTS and psbA-trnH sequences had indels (Table 3. 2), but the psbA-trnH had more non-codable insertions than the 5S NTS.

Utility of the regions

The psbA – trnH region had less sequence variability (9%) than the 5S NTS region (43%). The numbers of informative sites were also less for the psbA – trnH (5%) than for the 5S NTS (19%). The psbA-trnH region was therefore less useful when used alone, and only became useful when combined with the 5S NTS data set. The 5S NTS data also failed to indicate whether *Octopoma* is monophyletic or not. But upon combining the two data sets, it was indicative that *Octopoma* is non – monophyletic, although with low branch support. That therefore supported Soltis & Soltis (1998: 1), who stated that comparing trees based on nuclear and chloroplast markers can be particularly valuable at lower taxonomic levels, providing a window into evolutionary processes that could not be achieved with either genome alone. Also, the combined data sets produced better -resolved trees than when the 5S NTS data set was used alone. For example, relationships among the three main clades found in the NTS data (Figure 3. 1), were better resolved when the 5S NTS and psbA-trnH data sets were combined (Figure 3. 3; Figure 3. 6).

3. 4. 2. Relations of *Octopoma* to other members of the Ruschioideae

According to these molecular studies, *Octopoma* is non- monophyletic and can be divided into two groups. The results support Chesselet et al. (2000) who reported that *Octopoma* that does not form a natural group, and Hartmann (1989, 2001) who hypothesised the existence of at least two groups of *Octopoma* based on morphological data.

However, phylogenetic relations of these groups of *Octopoma* to other members of the Ruschioideae were not resolved. Placement of the Namaqualand *Octopoma*s, for example, was inconsistent between analyses. Phylogenetic relations of these *Octopoma*s to other members of the Ruschioideae were unresolved in the maximum parsimony analysis of the 5S NTS data set. These were resolved in the combined data set but differently, depending on the analysis. In the maximum likelihood tree, these were placed sister to a group, which in addition to other taxa, contained *Stoeberia carpii*, *Acrodon bellidiflorus* and *Oscularia deltoides* (Figure 3. 6, node F).

Therefore, a hypothesis might be formulated from these inconsistent phylogenetic relations. It could be that members of the Ruschioideae are so closely related that their genetic constitution is similar. That was also shown by lack of sequence variability (9%) in the psbA-trnH region.

3. 4. 3. Relations among *Octopoma* species.

Although two groups of *Octopoma*s were identified, it was only the group of Little Karoo *Octopomas* (*O. octojuge* and *O. quadrisepalum*) that was strongly supported (JK> 96%) in the analysis. As in the morphological analysis, the molecular analysis also excluded *O. subglobosum* from the relationship of *O. octojuge* and *O. quadrisepalum*. The result, therefore, contradicted Hartmann' s hypothesis that *O. subglobosum* should be grouped with the Little Karoo species. The group comprising Namaqualand *Octopoma*s, which are *O. inclusum, O. rupigenum, O.* c. f. *connatum, O. sp. 9152* and *O. subglobosum*, was unsupported (JK < 50%) in all the molecular analysis. In some cases, the group also comprised species from other genera such as *Leipoldtia* c. f. *compacta* and *Ruschia hexamera*. Therefore more complete analysis containing lots of species from *Ruschia* and *Leipoldtia* must be done to determine actual taxonomic relations within this group.

3. 5. Conclusions

Octopoma is non-monophyletic and two groups have been identified so far. One group comprises Little Karoo *Octopoma*s, *which* are *O. quadrisepalum* and *O. octojuge*. The other group comprises Namaqualand *Octopoma*s, which are *O. inclusum, O. rupigenum, O.* c. f. *connatum, O.* sp. 9152 and *O. subglobosum*. However, it appears these Namaqualand *Octopoma*s might be split further by detailed studies.

3. 6. Recommendations

This study should be improved by identifying additional, more informative markers and then adding more species from genera of *Leipoldtia* (about 60 species) and *Ruschia* (about 100 species).

The method of taxonomic congruence is not a way to play it safe but involves committing to hypotheses that may not be sanctioned by all the data (Barret, et al, 1991: 487).

CHAPTER 4

TOTAL EVIDENCE

4. 1. Introduction

Advances in systematics have been synonymous with data expansion (Bull et al., 1993). Such data sets include morphology, anatomy, DNA data, allozymes, chemataxonomy and cytological data (Jones and Luchsinger, 1987). However, these data sets do not always provide the same estimates of phylogeny, even for the same taxa (Bull et al., 1993; Levasseur and Lapointe, 2001). To solve the problem, various options have been suggested. These include; (1) taxonomic congruence, (2) intermediate methods (3) global congruence (hybrid methods) and (4) Total evidence (character congruence).

Taxonomic congruence involves separate analysis of data sets and then combining them by means of consensus techniques (Swofford, 1991; Bull et al., 1993; Huelsenbeck et al., 1994; Miyamoto and Fitch, 1995). Intermediate methods involve the use of statistical heterogeneity tests for deciding on whether taxonomic congruence or total evidence should be used (Mickevich and Farris, 1981; Rodrigo et al., 1993; Farris et al., 1995 Huelsenbeck and Bull, 1996). Global congruence evaluates the congruence between total evidence and taxonomic congruence (Levasseur and Lapointe, 2001). It tries to cross – corroborate trees obtained by total evidence and those obtained by taxonomic congruence (Levasseur and Lapointe, 2001).

In this study, total evidence approaches were utilized because; (1) Trees from total evidence approaches are usually more resolved than those from taxonomic congruence approaches (Cracraft and Mindell, 1989; Kluge, 1989). (2) Information pertaining to certain phylogenetic relationships might only be present in some data sets and therefore combing the data sets avails the information to all (Miyamoto and Fitch, 1985; Kluge, 1989; Barret et al., 1991; Donoghue and Sanderson, 1992).

4. 1. 1. Objectives

1. To determine the phylogenetic relations of *Octopoma* species using morphological and molecular data sets.

4. 2. Materials and methods

Molecular and morphological data sets were combined according to normal procedures (Miyamoto, 1985; Kluge, 1989; Barret et al., 1991; Donoghue and Sanderson, 1992). All characters were unordered and of equal weight. All the analysis was performed using heuristic approaches as described in chapter 2, except that here uninformative characters were not excluded.

4. 3. Results

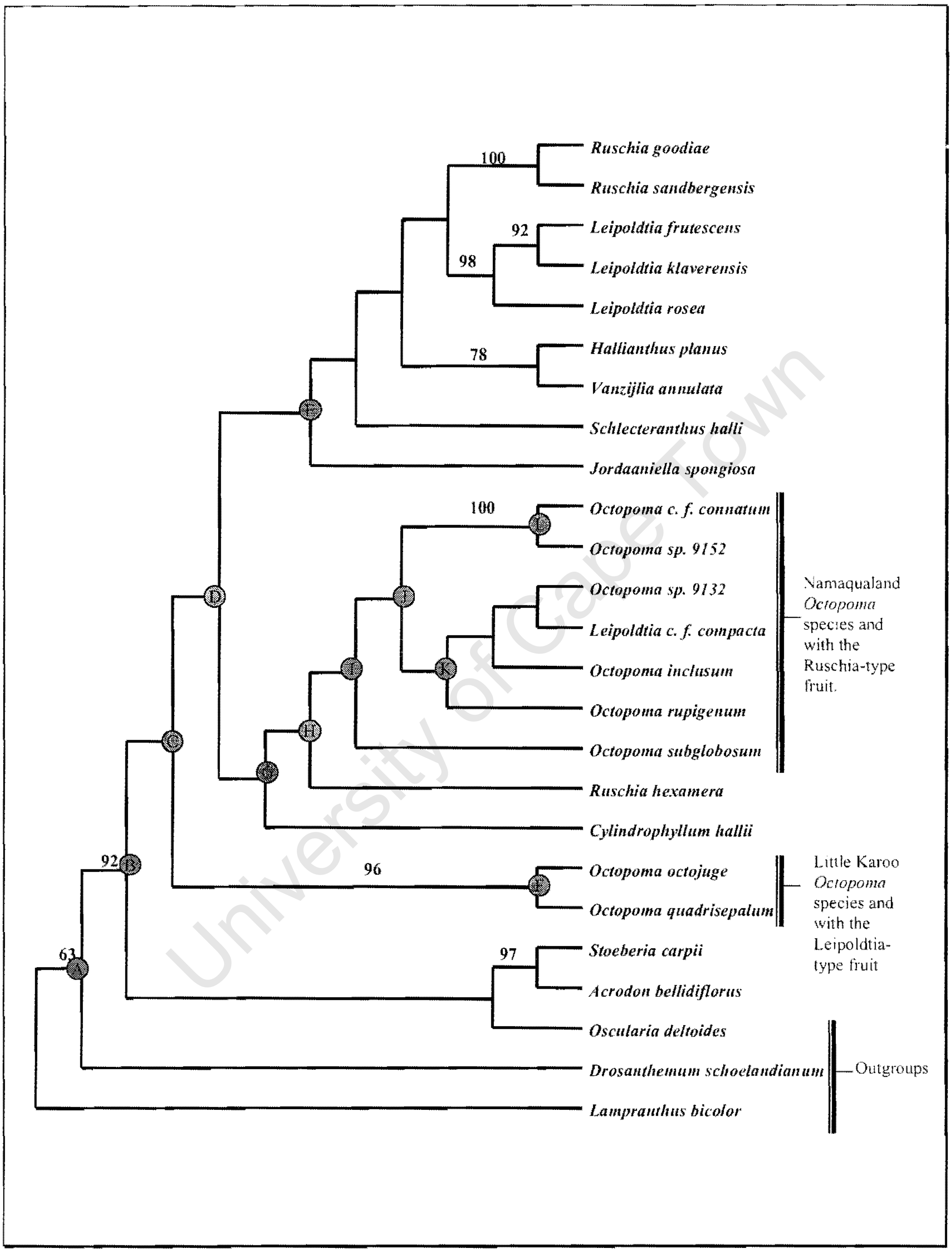


Figure 4.1. Single most parsimonious tree recovered from total evidence. Tree length = 69, CI = 0.6630, RI = 0. 7903, RC = 0. 5335. Numbers on branches indicate Jackknife values and letters designate groups.

The data analysis included 25 taxa and 726 characters. 195 (27%) of these characters were variable and 118 parsimony informative (16%). In this analysis only one most parsimonous tree (L = 69, CI = 0. 6630, RI = 0. 7903, RC = 0. 5335) was obtained (Figure 4.1).

The Ruschioideae (node B) forms a strongly supported group (JK = 92). Within the Ruschioideae, two groups of *Octopoma* can be identified (nodes F and G). One of the groups comprises Namaqualand *Octopoma*s (node G), which are *O.* c. f. *connatum*, *O.* sp. 9152, *O.* sp. 9132, *O. inclusum, O. rupigenum* and *O. subglobosum*. The group is weakly supported (JK < 50) and also contains *Leipoldtia* c. f. *compacta*, *Ruschia hexamera* and *Cylindrophyllum hallii*. This group is also recovered in the morphology, 5S NTS and combined analyses, but with minor differences. In the morphology tree the group lacks *Leipoldtia* c. f. *compacta*, *Ruschia hexamera* and *Cylindrophyllum hallii*. The group lacks only *Cylindrophyllum hallii* in the maximum parsimony and likelihood analysis of the 5S NTS data set. In the combined molecular analysis, both *Ruschia hexamera* and *Cylindrophyllum hallii* are absent from the group.

Phylogenetic relations of these Namaqualand *Octopoma*s to other members of the Ruschioideae are well resolved. These *Octopoma*s are resolved as sister taxa of group F (node F), which comprises *Ruschia goodiae, R. sandbergensis, L. frutescens, L. klaverensis, L. rosea, Hallianthus planus, Vanzijlia annulata, Schlechteranthus hallii* and *Jordaaniella spongiosa.* The same phylogenetic relations of these *Octopoma*s to other members of the Ruschioideae are also observed in the maximum parsimony analyses of the combined molecular data set.

The other group comprises *Octopoma* species of the Little Karoo, which are *O. octojuge* and *O. quadrisepalum* (group F). The group is strongly supported (JK = 96). This group is retained in all the analysis in the study, except for in the psbA –trnH trees, which are otherwise almost unresolved. This group is resolved as sister to the major group D (node D), which comprises Namaqualand *Octopoma*s (group G) and its sister group E, as described in the above paragraph.

4. 4. Discussion

Octopoma seems clearly not to be monophyletic. Chesselet et al. (2000) have noted that *Octopoma* species do not form a natural group, and Hartmann (1998; 2001) hypothesized that the genus constitutes at least two natural groups.

The group comprising *O. quadrisepalum* and *O. octojuge* was strongly supported (JK = 96%) and has some diagnostic morphological characters that distinguish it from other *Octopoma* species. Diagnostic characters for this clade are the absence of closing rodlets and the possession of big closing bodies. The presence of valve wings might be another diagnostic character, but reliance on the character is discouraged because their presence or absence might depend on the freshness of the specimen (Brown, 1930). The concept of *Octopoma* should probably be restricted to this clade since its type is *O. octojuge.*

However, *Octopoma subglobosum* did not group with *O. octojuge* and *O. quadrisepalum* as Hartmann (1998; 2001) suggested. *O. subglobosum* possesses small closing bodies. Hence, claims of grouping *O. subglobosum* with *O. octojuge* and *O. quadrisepalum* based on big closing bodies are not valid. Other characters (e.g. fruit-top shape) also contradict the grouping of *O. subglobosum* with *O. octojuge* and *O. quadrisepalum*.

On the other hand, the group (node I) comprising Namaqualand *Octopoma* species was weakly supported. The group also contains *Leipoldtia* c. f. *compacta*. Because of weak support for this group, it was concluded that these *Octopoma* species might still require further splitting. The results also supported Hartmann (1998; 2001) who mentioned that *Octopoma* species might be split into at least two groups. There is also a need for more sampling of the larger genera *Leipodtia* and *Ruschia* to determine the final circumscription of this clade.

4. 5. Conclusions

Octopoma species do not form a monophyletic group. *Octopoma octojuge* and *Octopoma quadrisepalum* are the 'core' *Octopoma*. The genus *Octopoma* is probably bitypic, but this conclusion should be avoided until all validly identified *Octopoma* species are included in the study. *O. subglobosum* does not belong to the same group as- *Octopoma octojuge* and *Octopoma quadrisepalum*. Further investigation is crucial to determine proper taxonomic placement for *O. subglobosum*, *O. inclusum*, *O.* c. f. *connatum*, *O.* sp. 9152, *O.* sp. 9132 and *O. rupigenum*. These *Octopoma* species might still require further splitting into more groups. If the species are split further, *O. connatum* and *O.* sp. 9152 might still group together because in 98% of the analysis, the two grouped together and with a very high branch support (JK $\geq$ 90%).

4. 6. Recommendations

1. A detailed study comprising all *Octopoma* species and adequate sampling of *Leipoldtia* and *Ruschia* species, together with their type specimens must be done to finalize taxonomic circumscription of the genus *Octopoma*.

2. Diagnostic keys in Hartmann's (1998; 2001) classification of *Octopoma* species must be rectified because Hartmann (1998; 2001) made an error of assigning big rather than small closing bodies to *O. subglobosum.*

The "crisis" in taxonomy is not a figment of the imaginations of taxonomists, but can be objectively documented in the published literature."

Winston and Metzge (1998).

Chapter 5

Taxonomy

5. 1. Introduction

The genus *Octopoma* is not monophyletic. Cladistic analysis of morphology, molecular and total evidence data divided *Octopoma* species into two main groups. According to Judd et al., (1999) a group deserves generic status if; (1) branch support for the clade is strong. (2) There is at least one morphological character to characterize the clade. (3). The group in question is large enough. The first group of *Octopoma* species comprises *O. octojuge* and *O. quadrisepalum*. Hartmann (1998; 2001) grouped *O. subglobosum* with *O. octojuge* and *O. quadrisepalum*. However, Hartmann's (1998; 2001) grouping had no broad morphological evidence. Under Hartmann 's (1998: **71**; 2001: **189**) diagnostic keys, *O. subglobosum* is supposed to group with *O. octojuge* and *O. quadrisepalum* because the trio has big closing bodies and narrow valve wings. But *O. subglobosum* **does not** have big closing bodies. This study showed it, and Hartmann (1998: **73**; 2001: **190**) also reported *O. subglobosum* to have small closing bodies. But the issue of concern is why would Hartmann (1998; 2001) assign two character states for closing bodies in *O. subglobosum*? If it was a mistake, this study recommends rectification of the diagnostic keys by Hartmann (1998: **71**; 2001: **189**). On the other hand, narrow valve wings are not good diagnostic characters because they might be present or absent in the same species depending on specimen freshness (Brown, 1930). At the same time Hartmann (1998; 2001) overlooked the presence or absence of closing rodlets as an important diagnostic character. In this study, the absence of closing rodlets in *O. octojuge* and *O. quadrisepalum* is considered an important and unique character, which excludes *O. subglobosum* from this group because *O. subglobosum* possesses closing rodlets.

Based on the above arguments, the clade for *O. octojuge* and *O. quadrisepalum* is regarded as the core of *Octopoma* because; (1) *O. octojuge* is the type of *Octopoma*. (2) All cladograms in the study, except for psbA – trnH data, indicated a branch support of more than 95% for the grouping of *O. octojuge* and *O. quadrisepalum*. (3) Among *Octopoma* species, *O. octojuge* and *O. quadrisepalum* were the only species that lacked *closing rodlets* and *leaf papillae*. Hence the species deserve generic status and grouping of their own because they meet Judd et al.'s (1999) criteria for awarding generic status to a clade.

The other group was that of *O. subglobosum*, *O. inclusum*, *O.* sp. 9152, *O.* c. f. *connatum*, and *O. rupigenum*. The group had unique characters of having *leaf papillae* and sharing the presence of contagious expanding keels with *Leipoldtia* c. f. *compacta*. But the group cannot be awarded generic status because support for their clade was very weak (JK < 57%) in all analyses. *Leipoldtia* c. f. *compacta*, *Ruschia hexamera* and *Cylindrophyllum hallii* also tended to interfere and mix with this group of *Octopoma* species. Further enquiry in this matter is therefore required for determining the exact placement of these *Octopoma* species. The only thing that can be done for now is to exclude *O.*

subglobosum, *O. inclusum*, *O.* sp. 9152, *O.* sp. 9132, *O. connatum*, and *O. rupigenum* from the genus *Octopoma*. Precise placement of the remaining species would therefore have to be reserved for further investigations that involve lots of *Leipoldtia* and *Ruschia* species, including their types.

On the other hand, formal placement of *O. tetrasepalum* and *O. abruptum* cannot be confirmed because the specimens were not available for the study. Therefore it is not clear as to whether *O. tetrasepalum* and *O. abruptum* will remain with the 'core' *Octopoma*, or will have to be removed from the 'core' *Octopoma* group. For purposes of concluding this study, *O. tetrasepalum* and *O. abruptum* were therefore regarded as species of uncertain placement.

5. 2. Assumptions

5. 2. 1. Species concepts

The recognition of a species in this study followed the phylogenetic species concept (sensu Mishler and Theriot) as defined by Mishler and Theriot (2000). A species is the least inclusive taxon recognized in a formal phylogenetic classification (Mishler and Theriot, 2000: 46). As with all hierarchical levels of taxa in such a classification, organisms are grouped into species because of evidence of monophyly. Taxa are ranked as species rather than at some higher level because they are the smallest monophyletic groups deemed worthy of formal recognition, because of the amount of support for their monophyly and or because of their importance in biological processes operating on the lineage in question (Mishler and Theriot, 2000: 46).

5. 2. 2. Systematic changes

Changes in taxonomic circumscription in this study will be as according to Jeffrey's (1978) definition. A change in circumscription may involve either the union of two or more taxa previously considered distinct or the splitting of what was previously considered to be one taxon into two or more distinct taxa (Jeffrey, 1978: 14).

5. 3. New generic circumscription

5. 3. 1. *Octopoma* N. E. Br.GC 1930: 72; GC 1930: 126; Hartmann Bradleya 1998: 70 – 74. TYPE: *O. octojuge* (L. Bolus) N. E. Br. GC 1930: 72; GC 1930: 126; (BOL!).

Habit: Densely branched shrubs up to 25 cm high and in diameter. **Internode** ochre to dark coloured. **Leaves** mostly connate and dark green. **Inflorescence**, solitary, bracteoles mostly embrace the base of the flower. $K_{4\,(-\,5)}$, inner one a little smaller, diameter 30 –35 mm. Inner petals, shorter than the outer. Filamentous staminodes make a central cone around the stamens, all elements are epapillate or the inner ones might have papillae. Nectary, as a crenulate dark ring. **Fruit:** a capsule and raised above the bracteoles. Top convex with low rims, base funnel shaped, covering membranes somehow elevated in the centre and distally a little turned up. Closing ledge very inconspicuos. Closing body big and flat above. Expanding keels with awns. Valve wings narrow and broadest in the middle. 6 – 8 locules. **Ecology**, in sand, granite, or loam soil with quartzite pebbles. Occurs in areas with less than 200mm of winter rainfall, or in March and November. **Distribution,** Namaqualand, Northern Cape, Western districts of Western Cape, South Africa.

Notes:* *Octopoma was described in contrast to ***Leipoldtia*** L. Bolus. Connate leaves, contiguous expanding keels and absence of valve wings in ***Octopoma*** was believed to distinguish the two genera (Brown, 1930). ***Octopoma octujuge*** was later found to posses narrow valve wings and thereby invalidating absence of valve wings as a diagnostic character for all ***Octopoma*** species. It was later found that some species were actually placed under ***Octopoma*** just because of the possession of eight locules. Hence, it was concluded that at least two more genera could be distinguished for species placed under ***Octopoma*** (Hartmann, 1998; 2001). It was also noticed that there was a group of *Octopoma* with a **Leipoldtia - type** of fruit and another one with a **Ruschia - type** of fruit. (Hartmann, 1998; 2001).

1. *O. octojuge* (L. Bolus) N. E. Br. 1930: 72 ≡ *M. octojuge* L. Bolus ABH 1927: 77 ≡ *Ruschia octojuge* (L. Bolus) L. Bolus NM 1930: 175 **Holotype** Muir 3957 (BOL!).

Habit: Low shrubs up to 8 cm high and 15 cm in diameter. **Leaves:** Free parts trigonous and a little spreading. Keel with tiny teeth. **Inflorescence:** Solitary, K_4, 70 – 85 petals. 30 – 40 filamentous staminodes and 180 – 240 filaments, all white and tousled to some extent.

Fruit: 8 - locules, base funnel shaped, tops raised with moderate rims. Covering membranes undulate, with no closing rodlets. Closing bodies big and reaches undersides of covering membranes. Valve wings, narrow and little bit tapering towards the tip and base. **Ecology:** Found in open patches of the Karoo that receive March and November rain of 100 - 200 mm, with loam soils and some quartzite pebbles. Distribution: Ladismith, Laingsburg, Western Cape, South Africa (Figure 5.1).

Note: *O. octojuge* appears smaller than *O. quadrisepalum*.

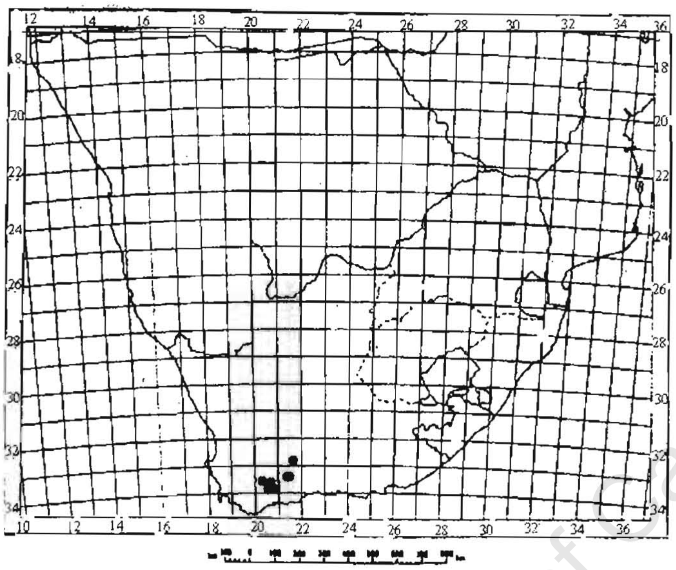


Figure 5. 1. Distribution of *O. octojuge*.

***Herbarium specimens consulted*:**

CAPE:

- **3221** (Carnarvon) : Merweville (-DC), *Leipoldt 30 854* (BOL).
- **3320** (Montagu): Laingsburg (- CB), *Vlok 5. 11* (BOL); Little Karroo, Warmwaterberg Road (- DA), *Ute Schmiedel 11 0024*(BOL); Montagu (- DA), *van Jaarsveld 6151*(NBG); near Ladismith (-DC), *collector anon* (BOL); Montagu (-DC), *Barrydale BH 30853*(BOL); Montagu, warmwaterberg (- DD), *Kerre 12 821*(BOL).

- **3321** (Ladismith): Ladismith (-AD), *Beyth 2496* (BOL); Ladismith (-AD), *Compton* (BOL).

2. *O. quadrisepalum* (L. Bolus) H. E. K. Hartmann Bradleya 1998: 73 ≡ *Ruschia quadrisepala* L. Bolus NM 1930: 175 **Lectotype** Ross Frames 19080 II (BOL!).

Habit: Shrubs, up to 10 cm high and 25 cm in diameter. **Leaves:** Free parts semiglobose, and those in pairs appressed. Keel, smooth. **Inflorescence:** Solitary, with pink petals, K_4.

Fruit: 8 – locules. Fruit base, funnel shaped. Covering membranes undulate, with no closing rodlets. Valve wings narrow and a little bit tapering towards the tip and base. **Ecology:** Associated with shrubs, loam soils with few quartzite pebbles. March and November rains that range between 100 – 200 mm per annum. Distribution: Ladysmith, Laingsburg, Swellendam, Western Cape, South Africa (Figure 5 .2).

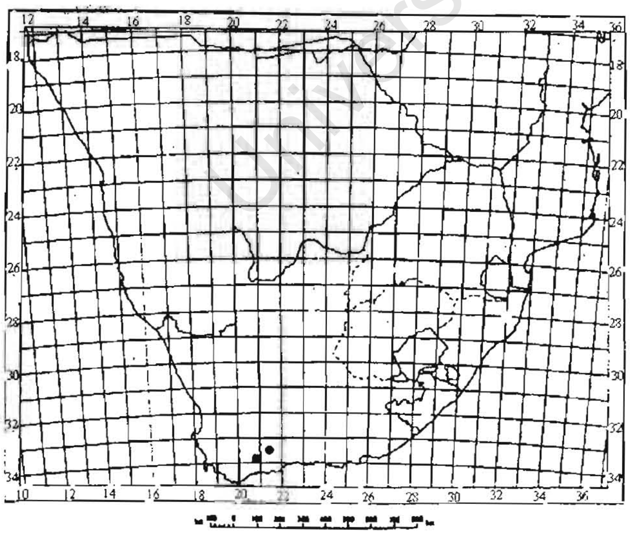


Figure 5. 2. Distribution of *O. quadrisepalum*.

Herbarium specimens consulted

CAPE:

- **3320** (Montagu): Between Lemoenshoek & Springfontein (- DD), *Bolus 20 719* (BOL).
- **3321** (Ladismith): Little Karroo, Ladismith (- AD), *Frames 19 080* (BOL).

5. 3. 2. Species of uncertain placement

1. *O. tetrasepalum* (L. Bolus) H. E. K. Hartmann Bradleya 1998: 74 ≡ *Ruschia tetrasepala* L. Bolus NM 1932: 373 **Holotype** Luckhoff 20203 (BOL).

Habit: Shrubs, up to 13 cm high and with branches in all directions. Stems, basal and up to 30 mm wide. **Leaves:** cymbiform, 3 mm long and 4 mm – 5 mm wide. **Inflorescence:** Solitary. Bracteoles below the middle of the pedicel and bigger than foliage leaves. Petals pink, K_4. No filamentous staminodes. Anthers white, filaments red, with the inner ones being papillate. **Fruit:** 7- locules. Base funnel – shaped. Covering membranes convex and distal. Closing rodlets prominent. Closing bodies shaped into a narrow hook. **Ecology:** Unknown.

Distribution: Vanrhynsdorp, Western Cape, South Africa (Figure 5.3).

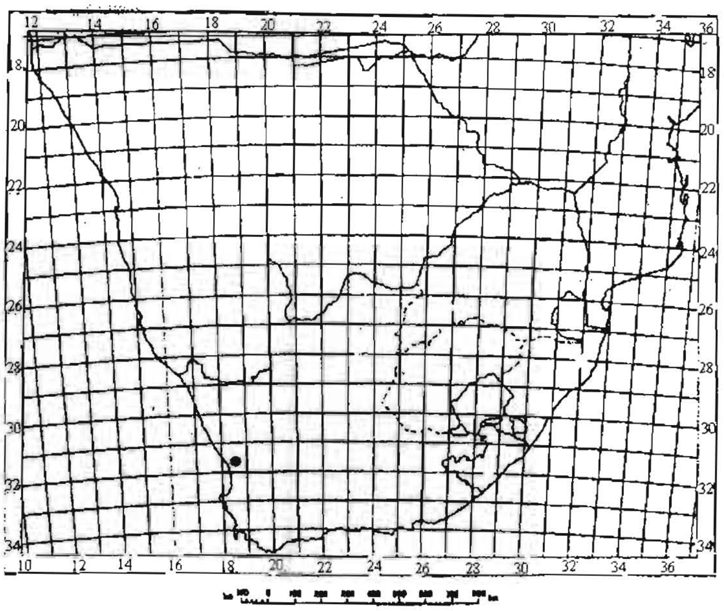


Figure 5. 3. Distribution of *O. tetrasepalum*.

Herbarium specimens consulted:

CAPE:

- **3318** (Vanrhynsdorp): Vanrhynsdorp, between the town and the river (-DA) *Luckhoff 20203* (BOL).

2. *Octopoma abruptum* (A. Berger) N. E. Br. GC 1930: 126 ≡ *M. abruptum* A. Berger 1922: 638 ≡ *Ruschia abrupta* (A. Berger) G. D. Rowley NCSJ 1978: 8 **Holotype** Schlecter 10828 (B, isotype BOL!).

Habit: Shrub up to 25 cm high. **Leaves:** triquetrous, keel minutely denticulate. **Internode:** greyish brown, about 20mm long, rigid. **Inflorescence** - in cymes, bracteolate, rigid. Petals red, K_5. **Fruit:** Funnel shaped base with convex sides, convex covering membranes, closing rodlets prominent, small closing bodies, 20 mm long, expanding keels end in awns, no valve wings, 7 –

8 locules. **Ecology:** hilly areas with less than 125mm of winter rainfall per annum. Distribution: Clanwilliam, Western Cape, South Africa (Figure 5. 4.).

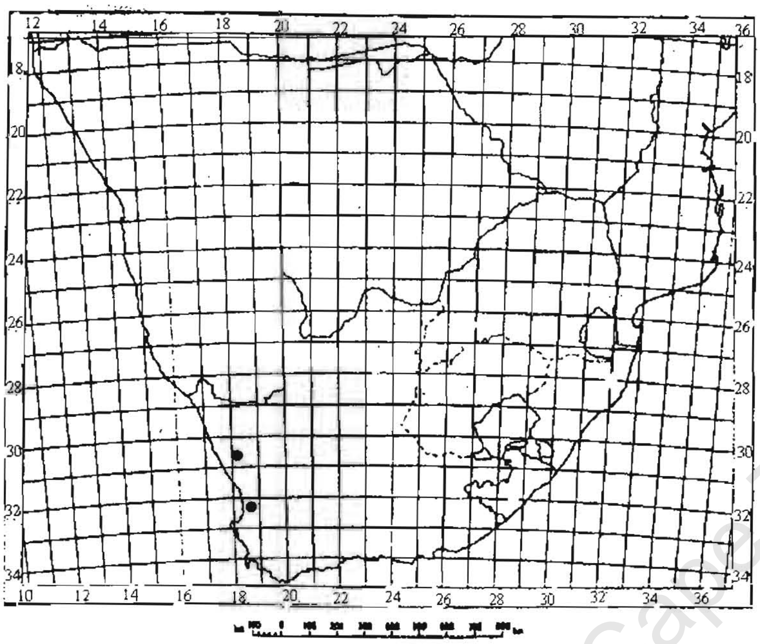


Figure 5. 4. Distribution of *Octopoma abruptum*.

Herbarium specimens consulted:

CAPE:

- **3218:** Between Clan William and Calvinia (- BB), *Schlechter 10 828* (BOL).
- **3018** (Kamiesberg): 6 miles South of Garies (-CA), *Acorks 15 102* (BOL).

5. 3. 3. Species excluded from the genus *Octopoma*

1. *O. connatum* (L. Bolus) L.Bolus 1963: 49 ≡ *Ruschia connata* L. Bolus NM 1928: 139 ≡ *M. connatum* N. E. Br. GC 1930: 32 Holotype Pillans 5794 (BOL!) = *Ruschia conjucta* L. Bolus NM 1931: 32 239 ≡ *O. conjuctum* (L. Bolus) L.Bolus 1966: 128 **Holotype** Mathews 19381 (BOL!).

Habit: Plants form mats. **Leaves**: 13 – 25 mm long and up to 5 mm wide, trigonous to semi – terete, with basal sheaths that separate the two leaves into a pair. Epidermis with a central rounded papillae, wax breaks into thin irregular horizontal plates with age. **Inflorescence**: Long stalks, bracteoles more connate than the foliage leaves and located on the upper half of the stalk. Petals, 40 –50, purple, K_4. Filamentous staminoides, 0 – 32, purple, 120 –205 stamens, purple, sometimes with white bases. **Fruit:** Bell shaped base, sometimes semi- globose, high domed top with low rims, covering membranes convex, with protruding closing rodlets. Small oval closing bodies, with the long axis pointing into the locule, valve wings absent. 8 – locular. **Seed:** Elongate and pear shaped. Testa almost smooth, with low epicuticular rodlets. **Ecology:** Flat gravel located among larger stones, often with quartzite rocks. Winter rainfall less than 125 mm per annum. **Distribution:** Namaqualand, Northern Cape, Vanrhynsdorp, Western Cape, South Africa (Figure 5. 5.).

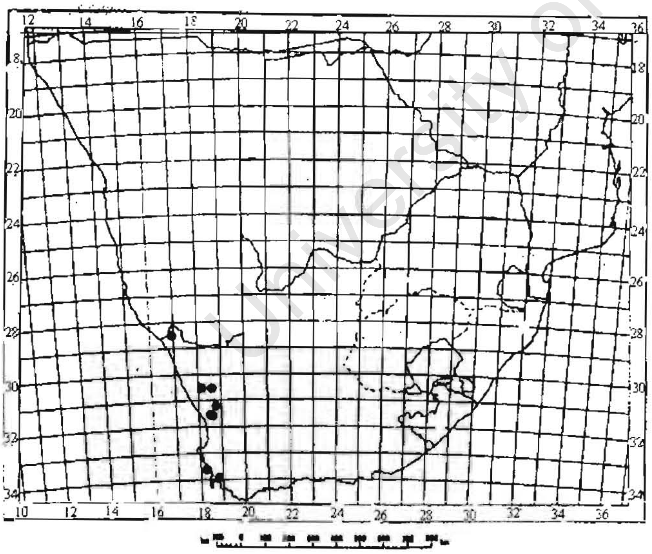


Figure 5. 5. Distribution of *O. connotum*.

Herbarium specimens consulted:

CAPE:

- **3318** (Stellenbosch): Stellenbosch University Gardens (- DD), *Bolus 8792* (BOL).
- **2816:** Richtersveld (- BB), collector not mentioned but writing reported as that of L. Bolus.*Collector no: 9289* (BOL).
- **3018** (Kamiesberg): Kamiesberg (- CA), *Acorks 15 100*, Klipport (-CB), *Bruyns 5372* (BOL).
- **3118** (Vanrhynsdorp): Vanrhynsdorp (- BC), *Klak 632* (BOL), Vanrhynsdorp (- DA), *Matthews 19381* (BOL).
- **3318** (Cape Town): Rosebank (-CD), *Pillans 5794* (BOL).
- **3118** (Vanrhynsdorp): Van Rhynsdorp (- DA), *Acorks 14 859* (BOL).

2. *O. inclusum* (L.Bolus) N.E.Br. GC 1930: 126 ≡ *M. inclusum* L. Bolus ABH 1926: 40 ≡ *Ruschia restuta* G. D. Rowley NCSJ 1978: 8 Lectotype Pillans (BOL!).

Habit: Shrubs, up to 10cm high. **Leaves:** Arranged in four distinct rows, cymbiform, epidermis with low papillae, Wax shaped into plates and platelets. The free parts are 6 – 9 mm long, sheaths, 3 mm long and 4 – 5 mm wide. **Internode**: Invisible. **Inflorescence:** Solitary. Bracteoles almost enclosing the stalk and highly connate. 4 – 5 whorls, petals pinkish – purple, 12 mm long and 1 – 1.5 mm wide. Filamentous staminodes present. Filaments are purple, with white bases, anthers and pollen also white. **Fruit**: 7 – 8 locules, funnel shaped base, top dome shaped with low rims. Covering membranes, convex. Closing rodlets, distinct at the distal end. Closing body, oval, with its long axis pointing into the locule. Valve wings, absent. **Ecology:** Coast plants that prefer winter rain at 100 mm per annum.

Distribution: Namaqualand, Northern Cape, South Africa (Figure 5. 6.).

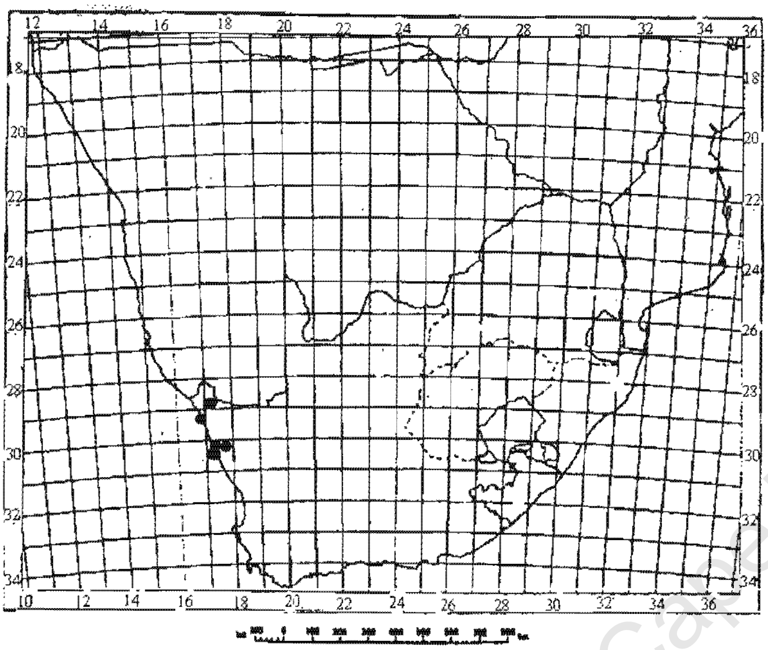


Figure 5. 6. Distribution of *O. inclusum*.

Herbarium specimens consulted:

CAPE:

- **2817** (Richtersveld): Vioolsdrif (- DC), *Williamson 3937* (NBG), Vioolsdrif (DC), *van Jaarsveld 5423a* (NBG).
- **2916** (Port Nolloth): Port Nolloth (- BD), *van Keerde 27 400* (BOL).
- **3017** (Hondeklipbaai): Koingnas (-AB), *Klak 474* (BOL), South of Hondeklipbaai (- AD), *Pillans 17 758* (BOL), South of Hondeklipbaai (- AD), *Pillans 17 911* (BOL), Hondeklipbaai (- BB), *Collector anon* (BOL).

3. *O. rupigenum* (L.Bolus) L. Bolus L Bolus 1967: 306 ≡ *Ruschia rupigena* L. Bolus NM 1935: 520 ≡ *Ruschia rupicola* L. Bolus NM 1933: 415 nomen illeg. Non (Engler) Schwantes ZSK 1926: 187 **Holotype** L. Bolus 1504/33 (BOL!)

Habit: Shrubs up to 20 cm high, with twisted branches. **Leaves**: Triquetrous, 12 mm long and 3 – 4 mm wide, with rough epidermis due to raised dots and fine papillae. **Internode**: 8 – 13 mm long, 4 – 9 mm wide. **Inflorescence:** Solitary. Pedicels 10 – 15 mm long, bracteoles touch the base of the flower. K_5, petals and filamentous staminodes bright pink. Filaments pale to pink. **Fruit:** 8 locules, base funnel – shaped and top dome shaped, covering membranes convex, with a small closing rodlet, closing bodies oval, with the long axis pointing into the locule, valve wings absent. **Ecology**: Associated with rocks. **Distribution**: Clanwilliam, Western Cape, South Africa (Figure 5.7).

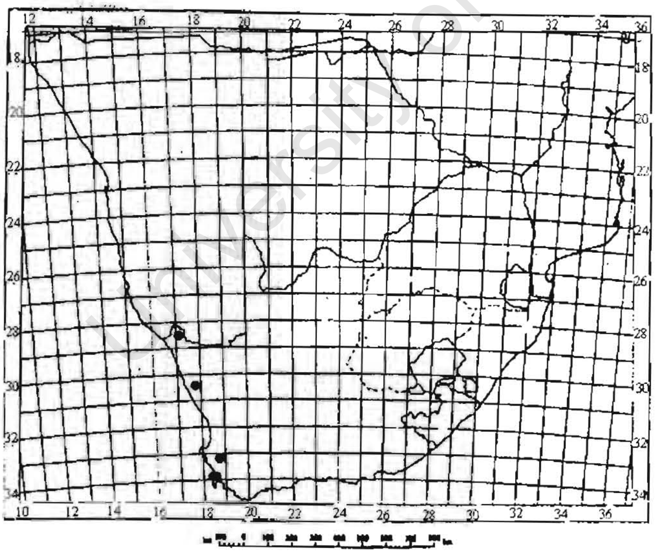


Figure 5.7. Distribution of *O. rupigenum*.

Herbarium specimens consulted.

CAPE:

- **2817** (Vioolsdrif): North of Lekkersing (- CC), *Williamson 3053*(BOL).
- **3017** (Garies): North of Garies near top of mountain (- BD), *Klak 468* (BOL),
- **3318** (Clanwilliam): Clanwilliam (-BB), *Bolus 1504/33* (BOL), Clanwilliam (- CD), *Leipoldt s. n.* (BOL), Pakhuis (- BB) *Klak 433* (NBG), Clanwilliam (- CD), *Esterhuysen 32 204*(BOL).

4. *O. subglobosum* (L. Bolus) L. Bolus L. Bolus 1963: 49 ≡ *Ruschia subglobosa* L .Bolus NM 1928: 140 ≡ *M. reductum* N. E. Br. GC 1930: 33 nomen illeg. Non N. E. Br. GC 1930: 32 **Holotype** Pillans 5844 (BOL!).

Habit: Shrubs up to 20 cm high and 25 cm wide. **Leaves:** A bit connate with bases that are almost separated by an elongate ochre triangle of stem tissue. Free parts spread widely, fatly trigonous, with a marked apical tooth and fine teethed keels. **Inflorescence:** Solitary. K_5, petals purple. Few purple filamentous staminodes surround the central cone of stamens, and filaments have few lateral papillae. Fruit: 7 – 8 locules, base funnel – shaped, top semi – globose, covering membranes prominent closing rodlets. Closing bodies small but reach the undersides of the covering membrane. **Ecology:** Rough sand in pans of Namaqualand granite domes and winter rains less than 100 mm per annum. **Distribution:** Western Namaqualand, Northern Cape, South Africa (Figure 5. 8.).

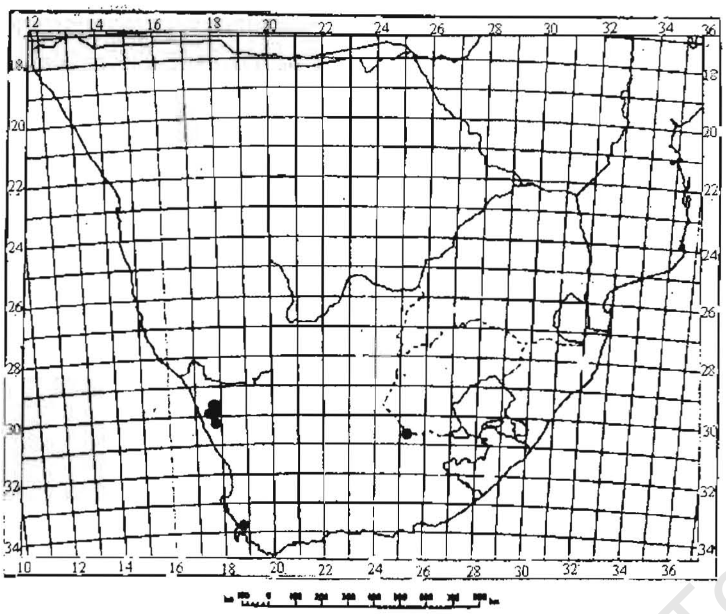


Figure 5. 8. Distribution of *O. subglobusum*.

Herbarium specimens consulted:

- **2917** (Springbok): Hills north of Ookiep (- DB), *Pillans 5844* (BOL); 2 km East of Springbok (- DB), *Klak 267* (BOL); three miles south of Mesklip (-DD), *Salter 4598* (BOL); Mesklip (- DD), *Esterhuysen 7769* (BOL); Little Namaqualand (- DD); *Esterhuysen 7769* (BOL).
- **3017** (Hondeklipbaai): Hondeklipbaai (-BB), *Bruyns 7610* (BOL).
- **3025** (Colesberg): Ratel poort (- CB), *N. B. G. 1770/48* (BOL).

5. *Octopoma* sp. 9152 – This specimen could not be identified to species level. However, its base, covering membranes, closing rodlets and closing bodies resembled those of *Octopoma abruptum*. The specimen was certainly not *O. tetrasepalum* because the specimen's closing bodies were not in the form of narrow hooks (Bolus, 1932; Hartmann, 1998) as they are for *O. tetrasepalum*.

6. *Octopoma* sp. 9132 – This specimen could not be identified to species level as well. Morphological analysis of the specimen excluded it from *Octopoma quadrisepalum* and *Octopoma octojuge*, which, according to this study are the real *Octopoma* species. No molecular data were available for the specimen and therefore, total evidence data in this study, might not have precisely placed this specimen.

"...... this is not the end. It is perhaps, the end of the beginning". Winston Churchill (November 10, 1942).

CHAPTER 6

GENERAL DISCUSSION

6. 1. General comments

This chapter summarises the main conclusions derived from the study. The application of molecular and cladistic analyses, being the first of their kind in the study of *Octopoma*, provided a better understanding of the group and the Ruschioideae in general. It appears the Ruschioideae comprises a number of closely related species. This conclusion was based on the fact that some species in the study tended to occupy inconsistent positions in cladograms (e. g. *Leipoldtia* c. f. *compacta*, *Ruschia hexamera*, and *Cylindriphylum hallii*). That, therefore, tended to obscure identification of phylogenetic relations of *Octopoma* species to other members of the Ruschioideae. The complexity of this *Octopoma* project was probably underestimated because the sampling of taxa for the study appears to have been inadequate. This study ideally would have included extensive taxon sampling from genera close to *Octopoma*, especially *Leipoldtia* and *Ruschia* (Hartmann, 1998; 1998b, 2001). The DNA regions used in the study also did not provide many parsimony informative sites (i. e. 5% for psbA – trnH and 19% for 5S_NTS). But the regions seemed to provide better resolutions when combined. Maybe a third DNA region should have been included. Morphological investigations in this study also supported molecular investigations in the sense that similar groups were recovered in both data sets. The total evidence tree was better resolved (e. g. had more nodes) than any other tree in the study and had higher branch support values. However, trees from these data sets were not necessarily congruent probably because of the presence of homoplasious characters, and lack of parsimony informative sites. Hence, it was found necessary to investigate the evolution of some characters in an attempt to identify homoplasious characters.

6. 2. Phylogenetic relations of *Octopomas* to other members of the Ruschioideae

The main conclusion of the study is that *Octopoma* is non – monophyletic. At least two groups of *Octopoma*s can be identified, a Little Karoo group and a group restricted to Namaqualand. However, taxonomic positions of *O. tetrasepalum* and *O. abruptum* in the Ruschioideae are still uncertain because the species were technically unavailable for the study. On the other hand, phylogenetic relations of Little Karoo *Octopoma*s and Namaqualand *Octopoma*s to other members of the Ruschioideae could not confirmed. For example, although the total evidence tree was fully resolved, nodes indicating phylogenetic relations of *Octopoma*s to other members of the Ruschioideae were poorly supported (JK < 50%). That therefore indicated that these might not be the true phylogenetics relations of these *Octopoma*s to other members of the Ruschioideae. The relations could have been indicated only because members of the Ruschioideae generally resemble one another as has been shown by lack of sequence variability and morphological data (e.g. similar shaped fruit capsules). Therefore true phylogenetic relations of *Octopoma*s to other members of the Ruschioideae can probably be obtained only through increased taxon sampling. The situation is complicated by the fact that genera that are closely related to *Octopoma*, which are *Leipoldtia* and *Ruschia* (Tribble and Hartmann, 1998; Smith et al., 1999; Hartmann, 2001) contain lots of species. For instance, *Leipoldtia* has more than 120 species while *Ruschia* has more than 230 species (Hartmann, 2001). Therefore considering large species abundance in these, it is apparent that there was far much insufficient sampling from these genera. As such this underestimated sampling could have made it difficult to determine the relationships *Octopoma* species to other members of the Ruschioideae.

6. 3. Little Karoo *Octopomas*

The Little Karoo *Octopoma*s, *O. octojuge* and *O. quadrisepalum*, are the 'core' of *Octopoma* because *Octopoma* was described based on *O. octojuge*. The group had strong support (JK > 90) in all the analysis, except in the morphology tree where support was low (JK = 53) and in the psbA – trnH tree that was unresolved. Synapomorphies for these two species include big closing bodies and absence of closing rodlets.

The 5S NTS data set also indicated that the two species share deletions at positions 193 to 204 and 210 to 216. These two species therefore deserve to have generic status because they are strongly supported and have morphological characters that can describe the group (Judd et al., 1999).

6. 4. Namaqualand *Octopomas*

The Namaqualand group included *O. subglobosum*, *O. inclusum*, O. sp. 9152, *O.* c. f. *connatum* and *O. rupigenum*. Morphological synapormophies for the group included the presence of leaf papillae and small closing bodies. These should be excluded from the genus *Octopoma*. However, further investigations need to be performed to determine the generic placement of this group. It seems unlikely that the species will qualify for the status of a genus and clade since the group always contained members from other genera (e. g. *Leipoldtia* c. f. *compacta*, *Ruschia hexamera* and *Cylindrophyllum hallii*) and always had weak support (JK <55). This group might actually need to be subdivided among existing genera once_more species from closely related genera such as *Leipoldtia* and *Ruschia* can be included in this kind of study.

6. 5. Taxonomic position of *Octopoma subglobosum*

The taxonomic position of *O. subglobosum* needs to be explained further because this study contradicted previous studies (e. g. Hartmann, 1998; 2001) in this regard. Both morphological and molecular analysis in this study excluded *Octopoma subglobosum* from Little Karoo *Octopoma*s. This species shares the presence of small closing bodies, absence of valve wings, the presence of closing rodlets and the presence of leaf papillae with Namaqualand *Octopoma*s. The psbA –trnH data set also indicated that *O. subglobosum* shares an insertion (5'TCCTTTCCTTGTTTT3') at position 570 to 584 with *O. inclusum*. In addition to these differences, *Octopoma subglobosum* is a Namaqualand and not a Little Karoo species

6. 6. Character optimization and evolution

Character state optimisation enables the investigator to formulate vivid hypothesis about the history of a character in question (Doyle and Davis, 1998). Characters selected for optimisation in this study were mainly based on their having been used extensively in the classification of the Ruschioideae (e. g. by Bolus, 1927; Lockyer, 1932; Poppendieck, 1976).

6. 6. 1. Evolution of fruit characters

Expanding keels

Expanding keels have been used to contrast *Octopoma* and *Leipoldtia*, with the latter having diverging expanding keels and *Octopoma* the contagious type (Hartmann, 2001). The plesiomorphic state for expanding keels is the contagious type and was observed in the outgroups, as well as in *O. octojuge*, *O. quadrisepalum*, *Stoeberia carpii*, and *Acrodon bellidiflorus* (Figure 6. 1). The presence of contagious keels in the Namaqualand *Octopoma* clade must, therefore, be interpreted as a reversal.

Closing rodlets

Closing rodlets are plesiomorphical present in the study group and their absence is a synapomorphy for *Octopoma octojuge* and *O. quadrisepalum* (Figure 6. 2).

Valve wings

Among *Octopoma* species, the possession of valve wings is unique to *O. octojuge* and *O. quadrisepalum* (Hartmann, 1999; 2001) and it is the plesiomorphic state for the character. *O. octojuge* and *O. quadrisepalum* share this feature with the outgroup taxa *Oscularia deltoides*, *Lampranthus bicolor* and *Drosanthemum Schoenlandianum*. Valve wings evolved independently three times among the sampled Ruschioideae (Figure 6. 3).

Fruit-base shape

Capsules of the Ruschioideae are either funnel or bell shaped at the base (Bolus, 1927; 1931; Brown, 1930). The bell-shaped form evolved independently in the common ancestor of *Octopoma* c. f. *connatum* and *Octopoma* sp. 9152, as well as in *Drosanthemum Schoenlandianum*, *Leipoldtia frutescens*, *L. klaverensis*,and *L. rosea*. The funnel shaped form appears to be the plesiomorphic state and was retained in the rest of the *Octopoma* species. (Figure 6. 4).

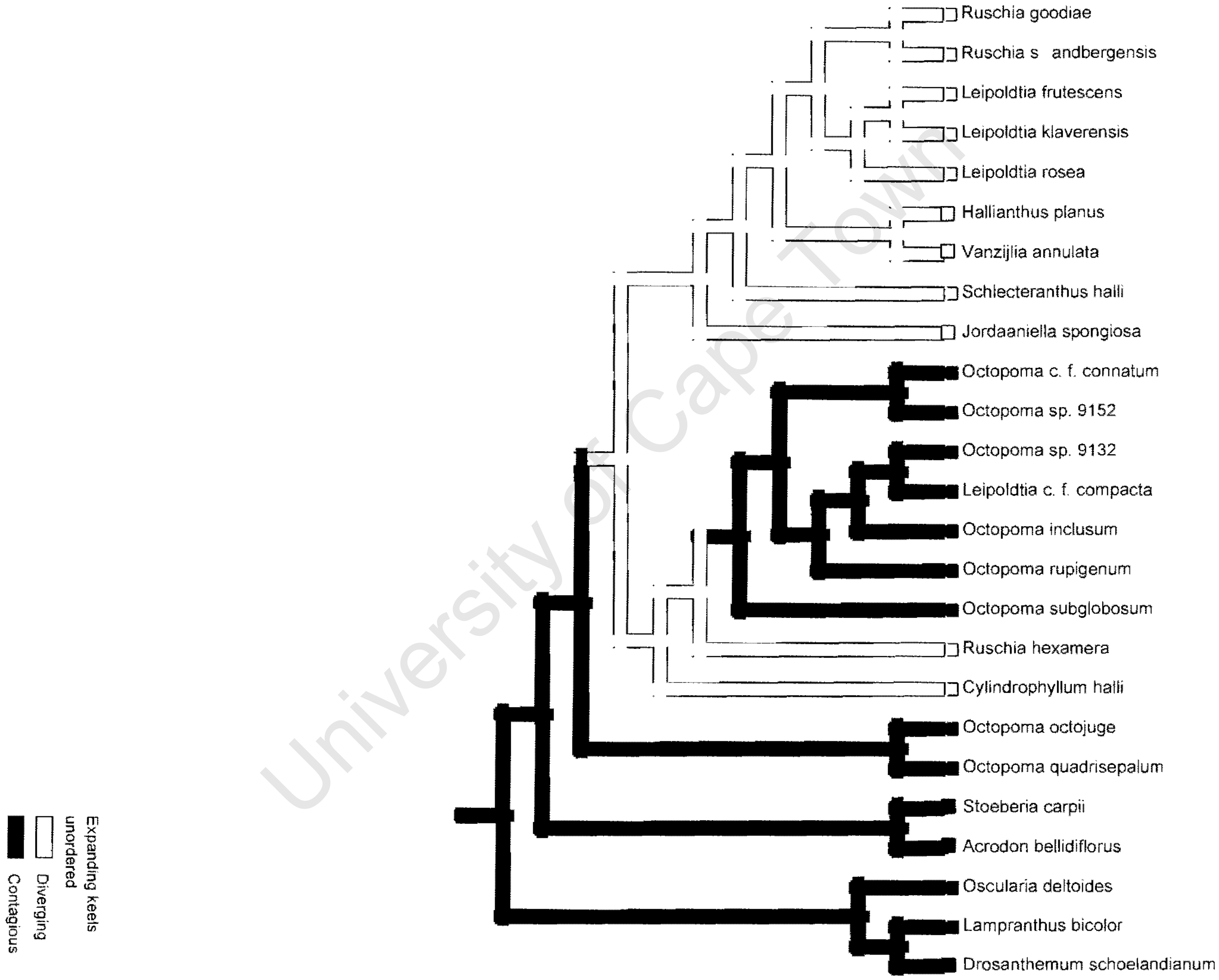


Figure 6. 1. Character optimization for expanding keels.

Figure 6. 2. Character optimization for closing rodlets.

Figure 6. 3. Character optimisation for valve wings.

Figure 6. 4. Character optimisation for fruit-base shape.

6. 6. 2. Evolution of leaf characters

Leaf papillae

Namaqualand *Octopoma* species (*O.* c. f. *connatum*, *O. subglobosum*, *O. inclusum*, *O. rupigenum*, *O.* sp. 9152, *O.* sp. 9132) have leaf papillae on their epidermal cells. *O. octojuge* and *O. quadrisepalum* showed smooth leaves with no papillae. It was also observed that *Octopoma* species possessing leaf papillae were those collected from winter rain regions (i. e. *O.* c. f. *connatum*, *O. subglobosum*, *O. inclusum*, *O. rupigenum*, *O.* sp. 9152, *O.* sp. 9132). On the other hand species without leaf papillae were those collected from summer rain regions (i. e. *O. octojuge* and *O. quadrisepalum*). It was therefore hypothesized that the absence of leaf papillae might be a water conserving mechanism in *Octopoma* species. The absence of leaf papillae might, therefore, be postulated to be an advanced character, which developed to cut down on the rate of transpiration since transpiration rates are normally high in summer because of high temperatures. However, it should be noted that this is just hypothesis and not a proven scientific finding because the fact that the absence of leaf papillae in *Octopoma* work in a functional sense is not an enough reason to presume that it is apomorphic.

The smooth, non-papillate type of leaves was observed to be the plesiomorphic state for this character. The feature then evolved into the rough, papillate type, which is seen *on O.* c. f. *connatum, O.* sp. 9152, *O.* sp 9132, *O. inclusum, O. subglobosum* and *O. rupigenum* (Figure 6. 5). The feature then reversed to the smooth, non-papillate form in *Leipoldtia* c. f. *compacta*. On the other hand, the rough, papillate form evolved independently in *L. rosea*.

High stomatal-index

A high stomatal-index optimises as a synapomorphy for *Ruschia goodiae* and *R. sandbergensis* and it evolved independently at their common ancestor. The feature was absent in all *Octopoma* species and in the outgroup taxa. (Figure 6. 6).

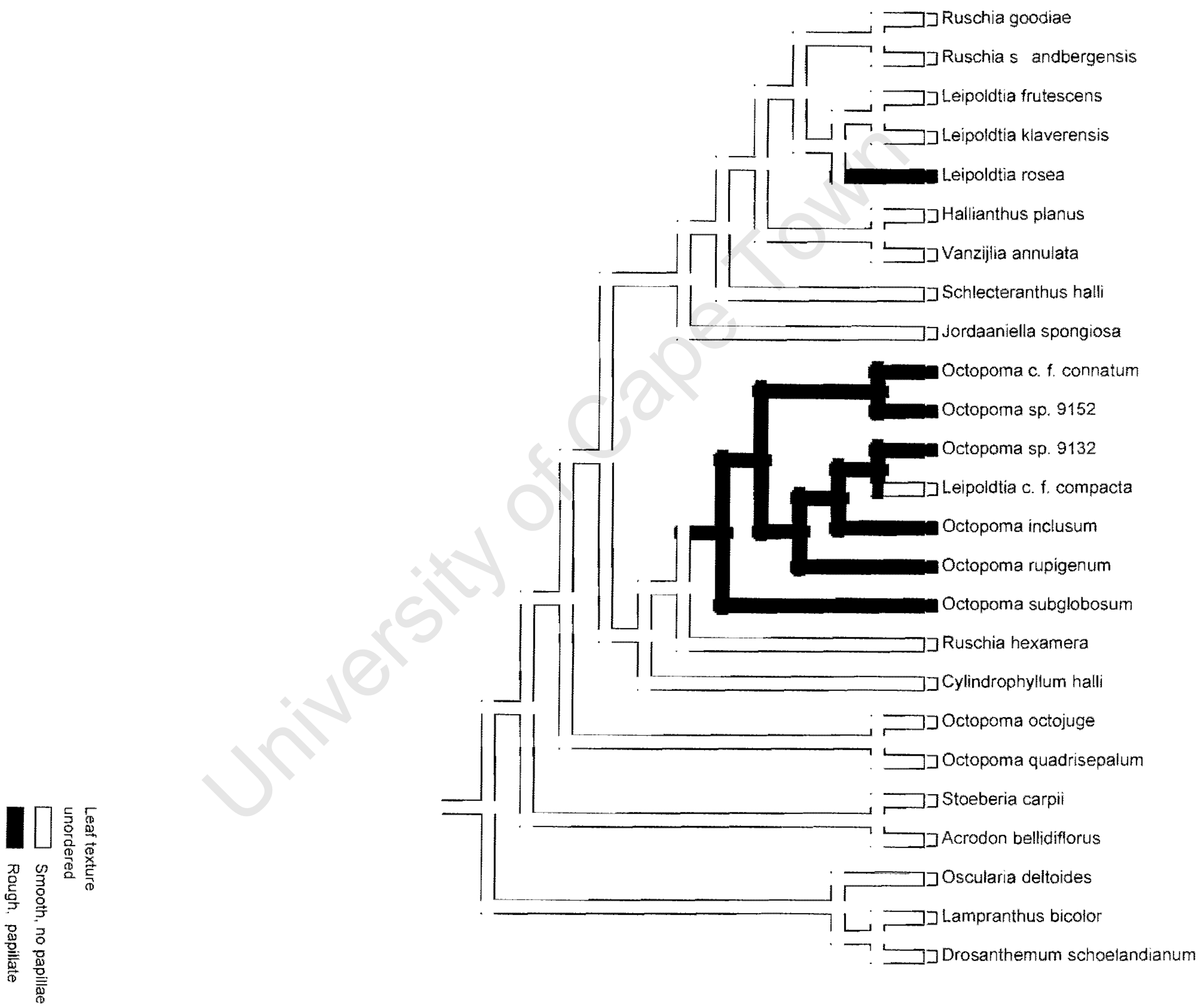


Figure 6. 5. Character optimisation for leaf texture (SEM).

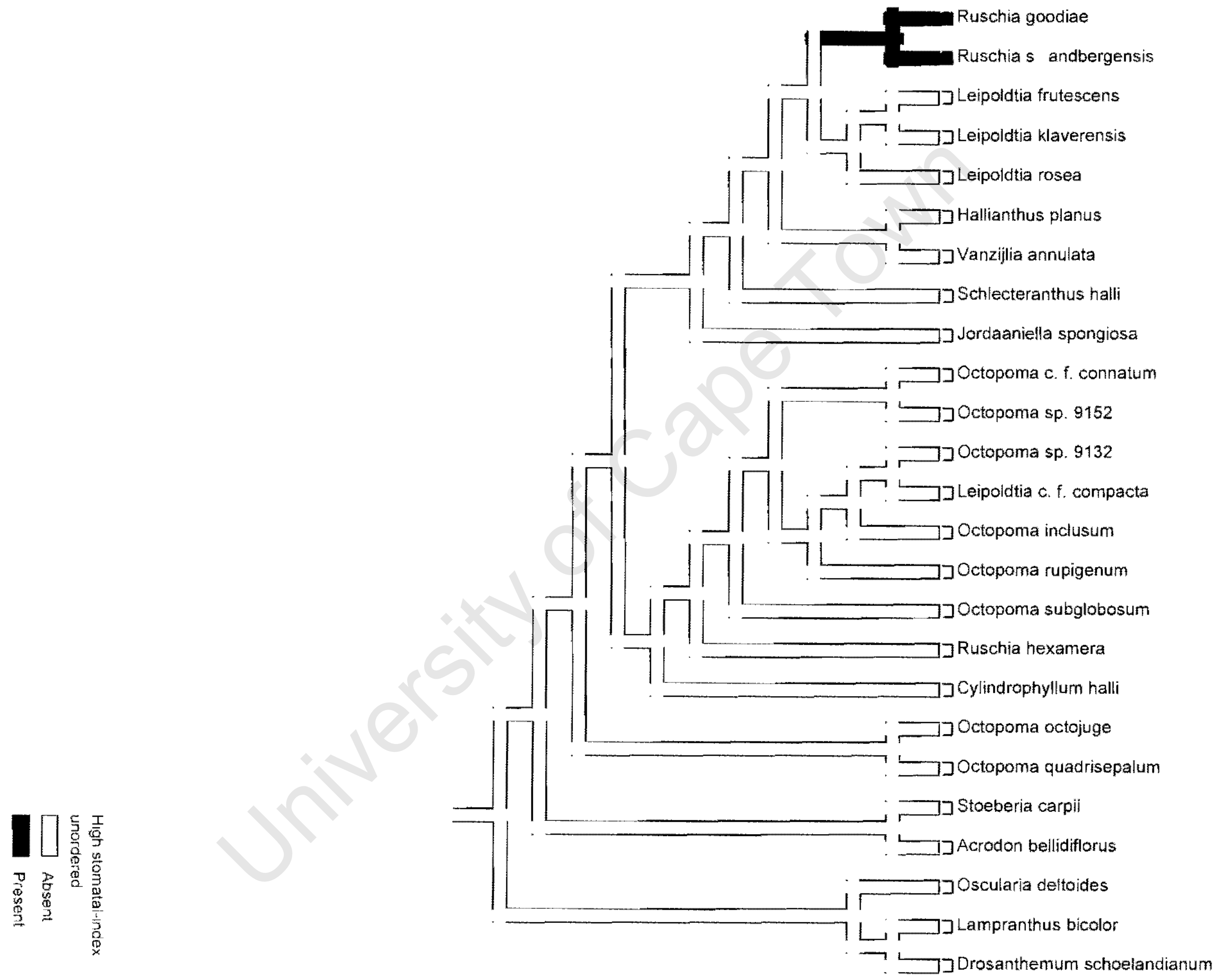


Figure 6. 6. Character optimisation for stomatal index.

6. 7. Conclusions

The morphological, 5S NTS and 5S NTS + psbA-trnH data sets indicated that *Octopoma* is non-monophyletic. Two groups of *Octopoma* exist. One group comprises species of the Little Karoo (*Octopoma octojuge* and *Octopoma quadrisepalum*) and are the core of *Octopoma*. However, it cannot yet be confirmed whether the genus is bitypic because *O. tetrasepalum* and *O. abruptum* were technically missing from the study. The other group comprises *Octopoma* species of Namaqualand, which are *O. inclusum*, *O.* c. f. *connatum*, *O.* sp. 9152, *O.* sp. 9132, *O. rupigenum* and *O. subglobosum*. This group of *Octopoma* species might be split further because it had a low branch support in all the analysis, and included *Leipoldtia* c. f. *compacta* in most cases. The *Octopoma* project is still in its infancy and much work needs to be done. The work should involve all validly identified *Octopoma* species, and lots of *Leipoldtia* and *Ruschia* species, including their type specimens.

6. 7. Recommendations

The study should be continued with the addition of more species from *Leipoldtia* (about 60 species) and *Ruschia* (about 100), including their types. A third DNA marker has to be identified.

6. 8. Limitations

This project was limited by the fact that it was difficult to sample the appropriate species for the study. The inclusion of lots of species from closely related genera would have been suitable, but the time frame was not sufficient for such an exercise.

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APPENDIX 1: DATA MATRIX FOR TAXA IN QUESTION.

CHARACTERS 1 - 28 REPRESENT MORPHOLOGICAL DATA.
CHARACTERS 718-721 ARE 5S NTS INDELS.
CHARACTERS 722-726 ARE PsbA - TrnH INDELS.

SYMBOLS:
P REPRESENTS POLMORPHISM FOR TAXA WITH BOTH CHARACTER STATES 1 AND 2.
? REPRESENTS MISSING DATA
N REPRESENTS A LETTER CHOOSEN ABITRARILY TO SEPERATE 5S NTS FROM THE psbA -trnH DATA.
NUMBERS ABOVE THE DATA SET REPRESENT POSITIONS FOR CHARACTERS.

FOR DEFINITIONS OF DATA SETS SEE THE DATA FILE IN THE COMPACT DISC (CD).

```
[                                       10        20        30        40        50        60]
[                                        .         .         .         .         .         .]

Ruschia_goodiae               0100120141111111000000100100----------------------------CTTT

Ruschia_sandbergensis         01001201??111111000000100100----------------------------CTTT

Octopoma_c._f._connatum       11001200P0011111000001000000----TTGCC--------------------CCC

Octopoma_sp._9132             1011010011011111000001000001????????????????????????????????

Octopoma_sp._9152             1100120010011111000001000000---------------------------CCTTT

Octopoma_rupigenum            1100100011011111000001000000--------C-----------------CCCTTT

Octopoma_inclusum             1100120011011111030001002000------GCC-----------------CCCTTT

Octopoma_subglobosum          1100120011011111000001000000---GTTGCC-----------------CCCTTT

Octopoma_octojuge             1000010021001111000000000000-----------------------------TTT

Octopoma_quadrisepalum        1000010021000111000000000000--GTTGCCC-----------------CCCTTT

Ruschia_hexamera              01001200?101011103000000 2100---------------------------CCTTT

Leipoldtia_c._f._compacta     1100010031011100010100000000-----GCAC-----------------CCCTTT

Leipoldtia_frutescens         0000010020010110011200000000----TGCAA-----------------CCCTTT

Leipoldtia_rosea              0000010020010110011201000000-----TGCC-----------------CCCTTC

Leipoldtia_klaverensis        0001000020010111011200000000--------------------------CCCTTT

Hallianthus_planus            ?110011041010111100000000001---------------------------CCTTT

Vanzijlia_annulata            ?110010011010111100000001000--------------------------CCCTTT

Schlecteranthus_halli         0?000?002101011100001000 0100---------------------------CTTTT

Cylindrophyllum_halli         01000000110??111000010000000------GCC-----------------CCCTTT

Stoeberia_carpii              ?0000010410??101020000002000AAAAAAAA------------------------

Acrodon_bellidiflorus         ?00012004101?110000000000100AAAAAAAA------------------------

Lampranthus_bicolor           12000?01410?1111000000000010CCTCCTGTGAAGTCCTCGTGTTGCACCCCTTT

Oscularia_deltoides           12000?002101?111000000010000ACCTCCTGGGAAGTCCTCGTGTTGCACCCCTTT

Drosanthemum_schoelandianum   ?2000?00400?2110000000010000CCTCCTGGGAAGTCCTCGTGTTGCACCCCTTT

Jordaaniella_spongiosa        ?1000?004101?110040000002000A-----TCAGAACTCC----------------

[                                       70        80        90       100       110       120]
[                                        .         .         .         .         .         .]

Ruschia_goodiae               TT-ACGATTTTTGAAAACTTTTTTTTTTTTTTTTTTTTTT--------------------

Ruschia_sandbergensis         TT-ACGATTTTTGAAAACATTTTTTTTTTTTTTTTTTTTT--------------------

Octopoma_c._f._connatum       TTTTT-ACGATTTTTGTAAATTTTTTATTATTATTTTTTTTTTTTTTTTTAT--------

Octopoma_sp._9132             ????????????????????????????????????????????????????????????

Octopoma_sp._9152             TT-ACGATTTTTGACAAGATTTTTGAAAATTTTATTATTATTTTTTATTTTATTTTTAT

Octopoma_rupigenum            TT-ACGATTTTTGACAAGAATTTTTGTAAATTTTTTATTTTATTTTTTTTTTTTTTTTTT
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Octopoma_inclusum              TT-ACGATTTTTGACAAGA-TTTTTGTAAATTTTTTATTATTATTTTTTATTTTATTTTT
Octopoma_subglobosum           TTGACGAGATTTGAGAAGATTTTTTATTATTATTTTTTATTTTATTTTTTTTTT------
Octopoma_octojuge              TT-ACGATTTTTGACAAAAAAATTTGCCATTATTATTATTATTATTATTATTATTATT
Octopoma_quadrisepalum         TT-ACGATTTTTGACAAAATTTTTGCGATTTTTTTTTTTTTTCGTATTATTAC---TATT
Ruschia_hexamera               TT-ACGATTTTTGACAAAATTTTTGCAA-TCTATCTTTTATTTATTATCTTTTTTATTTT
Leipoldtia_c._f._compacta      TTTACGATTTTTGACAAGATTTTTGAAAA-TTTTTTATTATTATTTTTTATTTTATTTTT
Leipoldtia_frutescens          TTTAAGATTCTTGAAAACTTTTTTTTTTTTTTTTTTTTTTAGC-----------------
Leipoldtia_rosea               TT--CGATTTTTGAAAACTTTTTTTTTTTATA----------------------------
Leipoldtia_klaverensis         TT-ACGATTTTTGAAAACTTTTTTTTTTTAT-----A-----------------------
Hallianthus_planus             TT-ACGATTTTTGAAAACTTTTTTTTTTTTTTTTTTTTTAAA------------------
Vanzijlia_annulata             TT-ACGATTCTTGAAA-CTTTTTTTTTTTTTTTTTTTT----------------------
Schlecteranthus_halli          TTGACGATTTTTGAAAACTTTTTTTTTTTTTTTTTTTTTGT-------------------
Cylindrophyllum_halli          TT-ACGATTTTTGACAAAATTTTTGTAATTTTTTTTTTTTTTTTTTTTTT----------
Stoeberia_carpii               -----------TGCAATTTTTATTTTTTATTTTTT-------------------------
Acrodon_bellidiflorus          -----------TGCAATTTTTATTTTTTATTTTTT-------------------------
Lampranthus_bicolor            TT-ACGATTTCTGACCAATTTTTTGTAATTTTTTTTTT----------------------
Oscularia_deltoides            TT-ACGATTTTTGATTATTTTATTTATTTTTTTTTTTT----------------------
Drosanthemum_schoelandianum    TT-ACGATTTTTGACAA-------------------------------------------
Jordaaniella_spongiosa         -------------ACAAAAAA---GCG---TGCTTGGGTGAGATGTATTT----------

[                                       130       140       150       160       170       180]
[                                         .         .         .         .         .         .]

Ruschia_goodiae                --------AATT-G------------------------AGGTATTGAGG---TAG-ATA
Ruschia_sandbergensis          --------AATT-G------------------------AGGTATTGAGG---TAG-ATA
Octopoma_c._f._connatum        --------AATT-C------------------------GGGT------G----AG-ATA
Octopoma_sp._9132              ????????????????????????????????????????????????????????????
Octopoma_sp._9152              AT------AATTGCC-----------------------GGGTA----------AG-ATA
Octopoma_rupigenum             TTTT----AATT-C------------------------GGGT-----------AG-ATA
Octopoma_inclusum              TTAT----AATT-C------------------------GGGT-----------AG-ATA
Octopoma_subglobosum           --------AATT-C------------------------GGGT-----------AG-ATG
Octopoma_octojuge              TTTT----GGTT-A------------------------AGGT-----------AA-ATA
Octopoma_quadrisepalum         TTTT----GGT--A------------------------AGGT-----------AGAATA
Ruschia_hexamera               TCTTTTCCGTTTATAG----------------------GGGT-----------------
Leipoldtia_c._f._compacta      TTAT----AATT-C------------------------GGGT-----------AG-ATG
Leipoldtia_frutescens          ---T---------G------------------------AGGT-----------GG-ATG
Leipoldtia_rosea               --------AATT-G------------------------AGGT-----------AG-ATA
Leipoldtia_klaverensis         --------AATT-G------------------------AGGT-----------AG-ATG
Hallianthus_planus             --------AATT-G------------------------AGGT-----------AG-ATA
Vanzijlia_annulata             --------AATT-G------------------------AGGT-----------GG-ATG
Schlecteranthus_halli          --------AATT-C----C-------------------AGGT-----------AG-ATA
Cylindrophyllum_halli          --------AATT-GC-----------------------AGGT-----------AG-ATA
Stoeberia_carpii               --------AATT-A------------------------AGGT-----------AA-ATA
Acrodon_bellidiflorus          --------AATT-A------------------------AGGT-----------AA-ATA
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Lampranthus_bicolor             --------AATA---------------------------AGGT-----------AG-ATA
Oscularia_deltoides             --------AAATG--------------------------AGGT-----------AG-GTA
Drosanthemum_schoelandianum     --------AATTWWCCGAGGTTTTWTTTTTTTTTTTTTTAGGTAAAGCCACGGT------
Jordaaniella_spongiosa          --------AATT-C-------------------------AGGT-----------AG-ATA

[                                         190       200       210       220       230       240]
[                                           .         .         .         .         .         .]
Ruschia_goodiae                 ---AACTCCATCC--TGATTT-AATGATTTGCCATTAA-TCGAGAA--------------
Ruschia_sandbergensis           ---AACTCCATCC--TGATTT-AATGATTT-CCATTAA-TCGAGAA--------------
Octopoma_c._f._connatum         ---ACATACA-CC--TGATCTTAAAGATTTTCCGTTAATTCGAGAA--------------
Octopoma_sp._9132               ????????????????????????????????????????????????????????????
Octopoma_sp._9152               ---ACATACA-CC--TGATTT-AATGATTT-CCGTTAA-TCGAGAA--------------
Octopoma_rupigenum              ---AAAT-CA-CC--TGATTT-AATGATTT-CCGTTAA-TCGAGAA--------------
Octopoma_inclusum               ---ACAT-CCACC--TGATTT-AAAGATTT-CCGTTAA-TCGAGAA--------------
Octopoma_subglobosum            AG-ACAT-CA-CC--TGATTT-AATGATTT-CCGTTAA-TCGAGAA--------------
Octopoma_octojuge               ---AAAT-CA-CA-----------TGATTT------AA-TGGAAAACAAAGATCCGAGAA
Octopoma_quadrisepalum          ---AAAT-CC-CA-----------TGATTT------AA-TGGAAAACAAAGATACGAGAA
Ruschia_hexamera                -----------CG-----------AGATTT-CCGTTAA-TCGAAAACAAAGATACGAAAA
Leipoldtia_c._f._compacta       AG-ACAT-CA-CC--CGATTT-AATGATTT-CCGTTAA-TCGAGAA--------------
Leipoldtia_frutescens           A--AAAT-CA-CC--CGATAT-AACGATTT-CCGTTAA-TCGAGAA--------------
Leipoldtia_rosea                ---AAAT-CA-CC--TGATAT-AACGATTT-CCGTTAA-TCGAGAA--------------
Leipoldtia_klaverensis          AGAGAAT-CA-CC--TGATAT-AACGATTT-CCGTTAA-TCGAGAA--------------
Hallianthus_planus              ---AAATGCA-CC--TGATTT-AACGATTTCCCGTTAA-TCGAGAA--------------
Vanzijlia_annulata              -A-AAAT-CA-CC--TGATAT-CATGATTT-CCGTCAA-TCGAGAA--------------
Schlecteranthus_halli           ---AAATGCA-CC--CGATTT-AATGATTT-CCGTTTA-TGGATAA--------------
Cylindrophyllum_halli           ---AAAT-CA-CC--TGATTT-AATGATTT-CCGTTAA-TCGAGAA--------------
Stoeberia_carpii                ---AAAT-CA-CC--TGATTT-AATGATTT-CCGTTAA-TCGAAAACAAAGATACGAGAA
Acrodon_bellidiflorus           ---AAAT-CA-CC--TGATTT-AATGATTT-CCGTTAA-TCGAAAACAAAGATACGAGAA
Lampranthus_bicolor             ---AAAT-CA-CC--AGATGT-AATGATTT---------TCGAAAACAAAGATAAGAGAG
Oscularia_deltoides             ---AAAT----CCACTGATTT-AATGATTT-CCGTTAA-TCGAGAA--------------
Drosanthemum_schoelandianum     ---AATA--AG----TGATTT----------CCGTTAA-TCGAAAACAAAGATACGAGGA
Jordaaniella_spongiosa          ---AACT-CA-CC--TGATCT-AATGATTT-CCGTTAA-T-GAGAA--------------

[                                         250       260       270       280       290       300]
[                                           .         .         .         .         .         .]
Ruschia_goodiae                 -AGGGGTTAAATGAAAA-TT-CGCGC-AATAG-TATA-----------------------
Ruschia_sandbergensis           -AGGGGTTAAATGAAAA-TT-CGCGC-AATA-----------------------------
Octopoma_c._f._connatum         -AGGGGTTAAATGAAAA-TT-CGCSCCAACGG-TATAATG--------------------
Octopoma_sp._9132               ????????????????????????????????????????????????????????????
Octopoma_sp._9152               -AGGGGTTAAATCGAAAATT-CGCGC-AATG--TATAATGGGTGCGATCATACCAGSACT
Octopoma_rupigenum              -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-T--------------------------
Octopoma_inclusum               -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-TATAA----------------------
Octopoma_subglobosum            -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-TATAATGGGTG----------------
Octopoma_octojuge               CAGGGGTTAAATGGAAA-------------------------------------------
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Octopoma_quadrisepalum        -AGGGGTTAAATGGAAAATT-CGCGC-AATGG-TATAATGGG-G----------------
Ruschia_hexamera              -AGGGGTTAAATGAAAA--T-CGCCC-AA-------------------------------
Leipoldtia_c._f._compacta     -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG--ATAATGGG------------------
Leipoldtia_frutescens         -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG--ATAATGGGGG----C-----------
Leipoldtia_rosea              -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG--ATAATT--------------------
Leipoldtia_klaverensis        -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-CATAATGGGG-----------------
Hallianthus_planus            -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-TATAA----------------------
Vanzijlia_annulata            -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-TATAATGGGGG----------------
Schlecteranthus_halli         -----------TGCAAA-----------GA---TAC------------------------
Cylindrophyllum_halli         -AGGGGTTAAATGAAAA-TTCCGCGCCAATGCCTATAATGGG------------------
Stoeberia_carpii              -AGGGGTTAAATGAAAA-TT-C--------------------------------------
Acrodon_bellidiflorus         -AGGGGTTAAATGAAAA-TT-C--------------------------------------
Lampranthus_bicolor           -AGGGGATAAAAGAAAA-TT-CGCGC-AATGG-TATAATGGGTGCGATCATACCAGCACT
Oscularia_deltoides           -AGGGGTTAAATGAAAA-TT-CGCGC-CATGG-TATAATGGGTGCGATCATACCAGCACT
Drosanthemum_schoelandianum   -AGGGGTTAAAAGAAAA-TT-CGCGC-AGAGG-TATAATGGGTGCGATCATACCAGCACT
Jordaaniella_spongiosa        -AGGGGTTAAATGAAAA-TT-CGCGC-AATGG-TATAATGGTGGCGAT------------

[                                      310       320       330       340       350       360]
[                                      .         .         .         .         .         .]
Ruschia_goodiae               --------------------------NNNNNNNNNN---TTGTTATGC-ATGAAACGTAA
Ruschia_sandbergensis         --------------------------NNNNNNNNNN------------------------
Octopoma_c._f._connatum       --------------------------NNNNNNNNNN----TGTTATGC-ATGAA-CGTAA
Octopoma_sp._9132             ????????????????????????????????????????????????????????????
Octopoma_sp._9152             AATGCACCGGATCCCGTCAGAACTCCNNNNNNNNNN------AATGC-------------
Octopoma_rupigenum            --------------------------NNNNNNNNNN---TTGTTATGC-ATGAAACGTAA
Octopoma_inclusum             --------------------------NNNNNNNNNN---TTGTTATGC-ATGAAACGTAA
Octopoma_subglobosum          --------------------------NNNNNNNNNN---TTGTTATGC-ATGAA-CGTAA
Octopoma_octojuge             --------------------------NNNNNNNNNN-----GTTATGC-ATGAA-CGTAA
Octopoma_quadrisepalum        --------------------------NNNNNNNNNN----TGTTATGC-ATGAA-CGTAA
Ruschia_hexamera              --------------------------NNNNNNNNNN----TGTTATGC-ATGAA-CGTAA
Leipoldtia_c._f._compacta     --------------------------NNNNNNNNNN---TTGTTATGC-ATGAAACGTAA
Leipoldtia_frutescens         --------------------------NNNNNNNNNN---TTGTTATGCAAGGAAACG-AA
Leipoldtia_rosea              --------------------------NNNNNNNNNNTTTTTGTTATGC-ATGAA-CGTAA
Leipoldtia_klaverensis        --------------------------NNNNNNNNNN---TTGTTATGC-ATGAAACGTAA
Hallianthus_planus            --------------------------NNNNNNNNNN---TTGTTATGCAATGAAAACGAA
Vanzijlia_annulata            --------------------------NNNNNNNNNN---TTGTTATGC-ATGAAACGTAA
Schlecteranthus_halli         --------------------------NNNNNNNNNN-----GTTATGC-ATGAAACGTAA
Cylindrophyllum_halli         --------------------------NNNNNNNNNN-------------------CGTAA
Stoeberia_carpii              --------------------------NNNNNNNNNN-----GTTATGCCATGAA-C-TAA
Acrodon_bellidiflorus         --------------------------NNNNNNNNNN-------------------CGTAA
Lampranthus_bicolor           AATGC---------------------NNNNNNNNNN---TTGTTATG-CATGAA-CGTAA
Oscularia_deltoides           AATGC---------------------NNNNNNNNNN---TTGTTATGC-ATGAA-CGTAA
Drosanthemum_schoelandianum   AATGC---------------------NNNNNNNNNN---TTGTTATGC-ATGAA-CGTAA
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Jordaaniella_spongiosa          --------------------------NNNNNNNNNN----------------------AA

[                                       370       380       390       400       410       420]
[                                        .         .         .         .         .         .]
Ruschia_goodiae                 TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Ruschia_sandbergensis           -----------------------------C--TATCGAAG-CTCCATCTACAAA------
Octopoma_c._f._connatum         TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Octopoma_sp._9132               ????????????????????????????????????????????????????????????
Octopoma_sp._9152               ---TCACAACTTCCCTCTAGACCTAGCGTGCGTATCGAAGCCTCCATCTACAAA------
Octopoma_rupigenum              TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Octopoma_inclusum               TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Octopoma_subglobosum            TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Octopoma_octojuge               TGCTCACAACTTCCCTCTAGACCTAGCTGT--TATCGAAG-CTCCATCTACAAA------
Octopoma_quadrisepalum          TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Ruschia_hexamera                TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Leipoldtia_c._f._compacta       TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAATAGAAAT
Leipoldtia_frutescens           TGCTCACAACTTCCCTCTAGACTTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Leipoldtia_rosea                TGCTCACAACTTCCCTCTAGACCTAGCCGC--TATCGAAG-CTCCATCTACAAA------
Leipoldtia_klaverensis          TGCTCACAACTTCCCTCTAGACTTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Hallianthus_planus              TGCTCACAACTTCCCTCTAGACTTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Vanzijlia_annulata              TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Schlecteranthus_halli           TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Cylindrophyllum_halli           TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Stoeberia_carpii                TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACTTC------
Acrodon_bellidiflorus           TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Lampranthus_bicolor             TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATTGAAG-CTCCATCTACAAA------
Oscularia_deltoides             TGCTCACAACTTTCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------
Drosanthemum_schoelandianum     TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATTGAAG-CTCCATCTACAAA------
Jordaaniella_spongiosa          TGCTCACAACTTCCCTCTAGACCTAGCTGC--TATCGAAG-CTCCATCTACAAA------

[                                       430       440       450       460       470       480]
[                                        .         .         .         .         .         .]
Ruschia_goodiae                 TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Ruschia_sandbergensis           TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAA-----GGGG
Octopoma_c._f._connatum         TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Octopoma_sp._9132               ????????????????????????????????????????????????????????????
Octopoma_sp._9152               TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Octopoma_rupigenum              TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Octopoma_inclusum               TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Octopoma_subglobosum            TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Octopoma_octojuge               TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Octopoma_quadrisepalum          TGGATAAATTTTCGTTTTTTTGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Ruschia_hexamera                TGGATAAATTTTCGTTTTTTTGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Leipoldtia_c._f._compacta       TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
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Leipoldtia_frutescens            TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Leipoldtia_rosea                 TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAG-----GGG
Leipoldtia_klaverensis           TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Hallianthus_planus               TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Vanzijlia_annulata               TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Schlecteranthus_halli            TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Cylindrophyllum_halli            TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Stoeberia_carpii                 TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Acrodon_bellidiflorus            TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Lampranthus_bicolor              TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Oscularia_deltoides              TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Drosanthemum_schoelandianum      TGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG
Jordaaniella_spongiosa           CGGATAAATTTTCGTTTTT-TGTTTAGTTTAGTGTATACGAGTTATTGAAAGTAAAGGGG

[                                         490       500       510       520       530       540]
[                                          .         .         .         .         .         .]
Ruschia_goodiae                  CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Ruschia_sandbergensis            CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTTTT--
Octopoma_c._f._connatum          CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Octopoma_sp._9132                ????????????????????????????????????????????????????????????
Octopoma_sp._9152                CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Octopoma_rupigenum               CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Octopoma_inclusum                CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTCCTT-
Octopoma_subglobosum             CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTCCTTT
Octopoma_octojuge                CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTTTT--
Octopoma_quadrisepalum           CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Ruschia_hexamera                 CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTTTT--
Leipoldtia_c._f._compacta        CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTT-ATCCCTTGTTTT--
Leipoldtia_frutescens            CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Leipoldtia_rosea                 CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTTTT--
Leipoldtia_klaverensis           CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Hallianthus_planus               CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTTTT--
Vanzijlia_annulata               CAGTACCGATTTCTTGAAAGAA------ATTGGTGATTGCTCCTTTC---CTTGTTTT--
Schlecteranthus_halli            CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTCTTTT--
Cylindrophyllum_halli            CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTA---CTTGTTTT--
Stoeberia_carpii                 CAGTACCGATTTCTTGAAAAAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Acrodon_bellidiflorus            CAGTACCAATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Lampranthus_bicolor              CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Oscularia_deltoides              CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Drosanthemum_schoelandianum      CAGTACCGATTT-TTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--
Jordaaniella_spongiosa           CAGTACCGATTTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTC---CTTGTTTT--

[                                         550       560       570       580       590       600]
[                                          .         .         .         .         .         .]
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Ruschia_goodiae               ----------------------------------------------GAATTTACTTCATA
Ruschia_sandbergensis         ----------------------------------------------GAATTTACTTCATA
Octopoma_c._f._connatum       ----------------------------------------------GAATTTACTTCATG
Octopoma_sp._9132             ????????????????????????????????????????????????????????????
Octopoma_sp._9152             ----------------------------------------------GAATTTACTTCATG
Octopoma_rupigenum            ----------------------------------------------GAATTTACTTCATA
Octopoma_inclusum             -------------------------------TCCTTTCCTTGTTTTGAATTTACTTCA--
Octopoma_subglobosum          ATTCTTGAAAGAACAAGAAATTGGTGATTGCTCCTTTCCTTGTTTTGAATTTACTTCATA
Octopoma_octojuge             ----------------------------------------------GAATTTACTTCATA
Octopoma_quadrisepalum        ----------------------------------------------GAATTTACTTCATA
Ruschia_hexamera              ----------------------------------------------GAATTTACTTCATA
Leipoldtia_c._f._compacta     ----------------------------------------------GAATTTCCTTCATA
Leipoldtia_frutescens         ----------------------------------------------GAATTTACTTCATA
Leipoldtia_rosea              ----------------------------------------------GAATTTACTTCATA
Leipoldtia_klaverensis        ----------------------------------------------GAATTTACTTCATA
Hallianthus_planus            ----------------------------------------------GAATTTACTTCATA
Vanzijlia_annulata            ----------------------------------------------GAATTTACTTCATA
Schlecteranthus_halli         ----------------------------------------------GAATTTACTTCATA
Cylindrophyllum_halli         ----------------------------------------------GAATTTACTTCATA
Stoeberia_carpii              ----------------------------------------------GAATTTACTTCATA
Acrodon_bellidiflorus         ----------------------------------------------GAATTTACTTCATA
Lampranthus_bicolor           ----------------------------------------------GAATTTACTTCATA
Oscularia_deltoides           ----------------------------------------------GAATTTACTTCATA
Drosanthemum_schoelandianum   ----------------------------------------------GAATTTACTTCATA
Jordaaniella_spongiosa        ----------------------------------------------GAATTTACTTCATA

[                                      610       620       630       640       650       660]
[                                        .         .         .         .         .         .]
Ruschia_goodiae               TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Ruschia_sandbergensis         TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Octopoma_c._f._connatum       CTCCTTTCCTTGTTTTGAATTTACAATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Octopoma_sp._9132             ????????????????????????????????????????????????????????????
Octopoma_sp._9152             CTCCTTTCCTTGTTTTGAATTTACAATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Octopoma_rupigenum            TATT--------------------AATATGAATAGTGCATTTGTAACTAAATAGGAAAGG
Octopoma_inclusum             -ATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Octopoma_subglobosum          TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Octopoma_octojuge             TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Octopoma_quadrisepalum        TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Ruschia_hexamera              TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Leipoldtia_c._f._compacta     TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Leipoldtia_frutescens         TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Leipoldtia_rosea              TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Leipoldtia_klaverensis        TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
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Hallianthus_planus           TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Vanzijlia_annulata           TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Schlecteranthus_halli        TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Cylindrophyllum_halli        TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGA----
Stoeberia_carpii             TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Acrodon_bellidiflorus        TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Lampranthus_bicolor          TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Oscularia_deltoides          TATT--------------------AATATGAATAGTGCATTTGTAAGT------------
Drosanthemum_schoelandianum  TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG
Jordaaniella_spongiosa       TATT--------------------AATATGAATAGTGCATTTGTAAGTAAATAGGAAAGG

[                                      670       680       690       700       710       720]
[                                        .         .         .         .         .         .]

Ruschia_goodiae              GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAT----------------010
Ruschia_sandbergensis        GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGATCCCCACCATGCGCGA-010
Octopoma_c._f._connatum      GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCC-ACCATGCGCG--101
Octopoma_sp._9132            ????????????????????????????????????????????????????????????
Octopoma_sp._9152            GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGA-----------------100
Octopoma_rupigenum           GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGATCCC-ACCATGCGCGA-101
Octopoma_inclusum            GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAT----------------101
Octopoma_subglobosum         GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCACC-ATGCGCGAA000
Octopoma_octojuge            GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCACC-ATGCGCGA-000
Octopoma_quadrisepalum       GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCACCCATGCCCGA-100
Ruschia_hexamera             GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGT-------------------001
Leipoldtia_c._f._compacta    GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGATCCCCACCATGCGCGA-000
Leipoldtia_frutescens        GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGATCCCCACCATGCGCGA-000
Leipoldtia_rosea             GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCCACCATGCGCGA-000
Leipoldtia_klaverensis       GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGATCCCCACCATGCGCGA-000
Hallianthus_planus           GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGT-------------------000
Vanzijlia_annulata           GGCGGATGTAGCCAAATGGATCAAGGCAGTGGATTTGTGATCCCACC-ATGCGCGAA000
Schlecteranthus_halli        GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCACC-ATGCGCGA-000
Cylindrophyllum_halli        ------T------AAG-G------GGC---GG-------------------------000
Stoeberia_carpii             GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGA-----------------000
Acrodon_bellidiflorus        GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGA-----------------000
Lampranthus_bicolor          GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCACC-ATGCGCGA-000
Oscularia_deltoides          ---------------------------------------------------------000
Drosanthemum_schoelandianum  GGCGGATGTAGCCAAGTGGATCAAGGCAGTGGATTTGTGAATCCACC-ATGCGCGA-000
Jordaaniella_spongiosa       GGCGGATGTAGCCAAGTGGATCAAGGCAGTGRATTTGTGA-----------------000

[                                   ]
[                                   ]

Ruschia_goodiae              0N0000
Ruschia_sandbergensis        0N0000
Octopoma_c._f._connatum      0N0011
Octopoma_sp._9132            ??????
Octopoma_sp._9152            0N0011
Octopoma_rupigenum           0N0000
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Octopoma_inclusum	0N1100
Octopoma_subglobosum	1N1100
Octopoma_octojuge	1N0000
Octopoma_quadrisepalum	0N0000
Ruschia_hexamera	0N0000
Leipoldtia_c._f._compacta	0N0000
Leipoldtia_frutescens	0N0000
Leipoldtia_rosea	0N0000
Leipoldtia_klaverensis	0N0000
Hallianthus_planus	0N0000
Vanzijlia_annulata	0N0000
Schlecteranthus_halli	0N0000
Cylindrophyllum_halli	0N0000
Stoeberia_carpii	0N0000
Acrodon_bellidiflorus	0N0000
Lampranthus_bicolor	0N0000
Oscularia_deltoides	0N0000
Drosanthemum_schoelandianum	0N0000
Jordaaniella_spongiosa	0N0000