An assessment of the subgeneric classification of *Zygophyllum* (Zygophyllaceae) in southern Africa: evidence from noncoding *trn*L–*trn*F chloroplast DNA sequences

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by

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DECLARATION

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

Signature: Date

SUMMARY

Sequence data from the intron and the spacer of the *trn*L–F chloroplast DNA region were used to study the phylogenetic relationships of the genus *Zygophyllum* L. (Zygophylloideae: Zygophyllaceae) in the southern African region. The chloroplast DNA was extracted from both herbarium and silica-gel dried material. Closely related genera, i.e. *Augea* Thunb., *Fagonia* L. and *Tetraena* Maxim. within the subfamily Zygophylloideae and more distantly related genera *Seetzenia* R.Br. ex Decne and *Tribulus* L. were used as outgroups. Sequences revealed length variation mainly due to the presence of indels (insertions and deletions). Phylogenetic analysis using parsimony revealed two distinct lineages for southern African members of *Zygophyllum*, corresponding to the proposed subgeneric classification (Van Huysteen 1937; Van Zyl 2000). There is a strong monophyly support for the sections within the subgenus *Agrophyllum* (Neck.) Endl. However, the transference of the monotypic section *Grandifolia* Engl. from subgenus *Zygophyllum* to *Agrophyllum* is not confirmed, because material of *Z. stapffii* Schinz. was not available. Despite the morphological evidence for the subdivision of the subgenus *Zygophyllum*, the molecular data did not confirm the monophyly for its sections. This could be the result of biased sampling, since all the species used in the analyses, except *Z. cordifolium* L.f. and *Z. morgsana* L., belong to section *Capensia* Engl. The *trn*L region data support the transfer of the monotypic section *Morgsana* Huysst. from subgenus *Agrophyllum* to subgenus *Zygophyllum*. The molecular data also seem to have implications for the biogeography of *Zygophyllum*. The southern African *Agrophyllum* representatives are related to East African and Middle East *Zygophyllum* species, whereas the southern African subgenus *Zygophyllum* members are closely related to Australian *Zygophyllum* species.

Keywords: *Zygophyllum*; *Agrophyllum*; sections; chloroplast DNA; *trn*L–*trn*F region; primers; PCR; parsimony

OPSOMMING

Die volgorde-data van die *trn*L–F chloroplas-DNA gebied is gebruik om die filogenetiese verwantskappe van die genus *Zygophyllum* L. (Zygophylloideae: Zygophyllaceae) in suider Afrika te bestudeer. Die chloroplas-DNA is geëkstraheer van beide herbaria en silica-gel gedroogde materiaal. Naverwante genera binne die subfamilie Zygophylloideae bv. *Augea* Thunb., *Fagonia* L. en *Tetraena* Maxim., sowel as verder verwante genera, soos *Seetzenia* R.Br. ex Decne en *Tribulus* L., was as buite-groepe gebruik. Die lengte-variasie in die volgorde-data kan toegeskryf word aan indels (*insertions* and *deletions*). Filogenetiese analise deur die gebruik van parsimonie het twee duidelike ontwikkelingslyne vir suider-Afrikaanse *Zygophyllum* taksa aangedui. Dit stem goed ooreen met die voorgestelde subgeneriese klassifikasiesisteem vir die genus (Van Huysteen1937; Van Zyl 2000). Daar is 'n sterk ondersteuning vir monofilie van die seksies binne die subgenus *Agrophyllum* (Neck.) Endl. Die oorplasing van die monotipiese seksie *Grandifolia* Engl. vanaf subgenus *Zygophyllum* na subgenus *Agrophyllum* is nie bevestig nie, want materiaal van *Z. stapffii* Schinz. was nie beskikbaar nie. Ten spyte van morfologiese bewyse vir die subdivisie van die subgenus *Zygophyllum* het die molekulêre data nie die monofilie van die seksies bevestig nie. Dit is moontlik as gevolg van eensydige data-insameling, aangesien al die spesies wat in die analise gebruik word (behalwe *Z. cordifolium* L.f. en *Z. morgsana* L.) aan die seksie *Capensia* Engl. behoort. Die *trn*L-gebied data ondersteun die oordra van die monotipiese seksie *Morgsana* Huysst. van die subgenus *Agrophyllum* na die subgenus *Zygophyllum*. Die molekulêre data bied ode inligting oor die biogeografie van *Zygophyllum*. Die suider-Afrikaanse *Agrophyllum* taksa is verwant aan Oos-Afrika en Midde-Oosterse *Zygophyllum* spesies, terwyl lede van die Suid-Afrikaanse subgenus *Zygophyllum* nouverwant is aan *Zygophyllum* spesies in Australië.

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CHAPTER 4

CHAPTER 1

1. INTRODUCTION

1.1 Background

The most recent revision of genus *Zygophyllum* L. in the southern African region was done by Dr. Van Zyl (2000) as part of her PhD. study at the University Stellenbosch. In her work she described several new species and rearranged Van Huysteen's (1937) sections. Her study was mainly based on macromorphological characters and distribution patterns of the different species. With the aid of distribution maps drawn from herbarium specimens, she did extensive field work in order to study the species in their own natural environments over a period of eleven years.

The present study came as a follow up on Van Zyl's work and was undertaken in collaboration with her. The project forms part of my Coursework Masters degree in Systematics and Biodiversity Science Programme at the University of Stellenbosch (Botany Department). Since molecular techniques have became available at the University of Stellenbosch, it was my task to attempt to confirm the Van Zyl (2000) classification using these techniques. This was done with the expertise of the two departments: Biochemistry (Prof. D. U. Bellstedt), Botany (Dr. E. M. Marais and Dr. L. L. Dreyer). This study represents one of the first molecular investigations of the southern African *Zygophyllum* species. The findings of this study are presented as a mini thesis in the form of a publishable paper. In the structure of this mini thesis, the rules for *American Journal of Botany* were followed. The introduction is presented in this chapter. The experimental work is presented in chapter 2, the results or findings in chapter 3 and the discussion in chapter 4. A comprehensive reference list is given after the discussion, and relevant appendices at the end of the thesis.

1.2 Genus description and distribution

The genus *Zygophyllum* L. belongs to the subfamily Zygophylloideae, of the family Zygophyllaceae (Engler 1899, 1931). In the most recent phylogenetic classification of the eudicots (Savolainen *et al.* 2000), the Zygophyllaceae is placed together with the monotypic family Krameriaceae in an order of its own, the Zygophyllales. Engler (1896, 1931) divided the family of about 25 genera into seven subfamilies: Tetradiclidoideae, Augeoideae, Zygophylloideae, Peganoideae, Chitonioideae, Nitrariodeae and Balanitoideae. His Zygophylloideae, including *Zygophyllum*, is the largest subfamily (17 of the 25 genera) and represents the typical taxon within the family (El Hadidi 1975).

Zygophyllum has a worldwide distribution, of which the south-west African region is regarded an important centre of the genus *Zygophyllum* (El Hadidi 1978, Van Zyl & Marais 1999; Figure 1.1). In this region (Angola, Botswana, Namibia and South Africa) most *Zygophyllum* species are found in arid to semi-arid and saline areas along the western and central part of the subcontinent, although some of the species do occur in less harsh conditions along the south coast. According to Van Zyl (2000), the highest number of species is found in the Nama Karoo (as defined by Rebelo 1996) and Succulent Karoo (as defined Cowling and Hilton-Taylor 1997). Only two of the southern African species (*Z. simplex* L. and *Z. decumbens* Del. var. *decumbens*) have a very wide distribution, also occurring in north-African and Asian regions.

Launert (1963), Dyer (1975), El Hadidi (1985) and Retief (2000) described plants in this genus as shrublets or shrubs, rarely herbaceous annuals; leaves opposite, simple or bifoliate, sessile or petiolate, leaflets often fleshy and variable in shape, stipulate; flowers solitary or rarely cymous; sepals 3–5; disc fleshy, angled or lobed; petals 4 or 5, sometimes clawed, imbricate, white, yellow or orange; stamens twice as many as petals, inserted at the base of the disc; filaments terete, with an entire, bifid or 2-partite appendage; ovary usually sessile on the disc, lobed, angled or globose, 3–5-locular, ovules few to many in each locule; style terete; stigma usually simple; fruit a lobed, angled or winged capsule or schizocarp; seeds with or without endosperm, usually mucous producing; embryo usually straight.

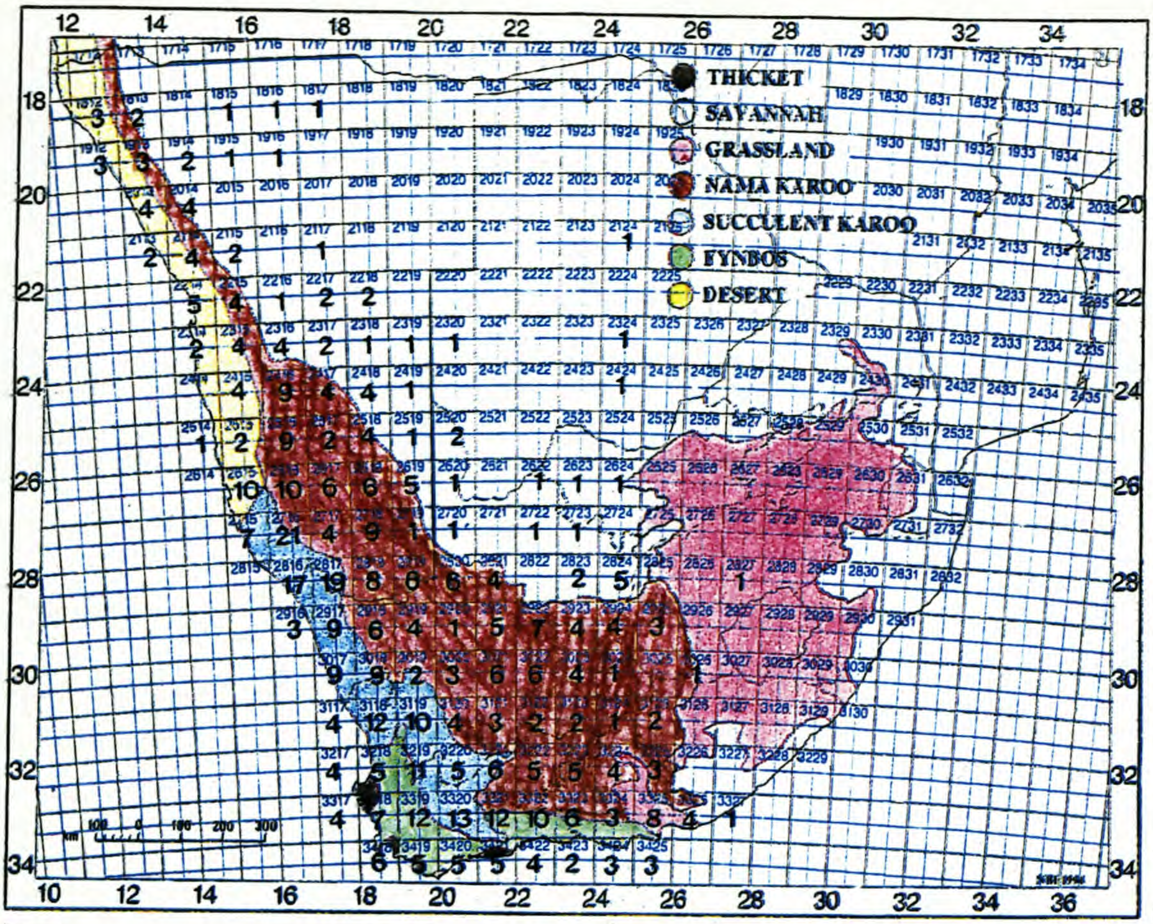

Figure 1.1 Species diversity of the genus *Zygophyllum* in the different biomes in the southern Africa (Van Zyl 2000).

1.3 Taxonomic overview

The genus *Zygophyllum* was first described by Linnaeus (1753). At present an uncertainty still exists about the number of species within *Zygophyllum*, but according to Index Kewensis about 130 species are recognized worldwide. Endlicher (1841) divided the genus into two subgenera with regard to the floral and fruit morphology: subgenus *Fabago* Adans. with capsule splitting in a loculicidal manner and subgenus *Agrophyllum* (Neck) Endl. based on the genus *Agophyllum* (err. Typogr.) described by Necker in 1790. The fruits of subgenus *Agrophyllum* are septicidal schizocarps splitting into indehiscent mericarps. Sonder (1860) described 25 South African species, grouping them together according to leaf characters, ignoring the prominent fruit characters by which the genus is currently divided into two subgenera. Engler (1931), in a worldwide study of *Zygophyllum*, recognised 17 sections, also ignoring the most competent subdivision according to fruit dehiscence. Van Huyssteen (1937) also did a world wide study of *Zygophyllum* with special reference to southern African species. She acknowledged Endlicher's (1841) subgenera but changed the name *Fabago* to subgenus *Zygophyllotypus*. She also rearranged Engler's (1931) sections and added several new sections to the list. Schreiber (1963) dealt with 18 species from Namibia. She typified the names of the species, provided distribution maps and arranged the species alphabetically. Van Zyl (2000) corrected the subgenus name *Zygophyllotypus* to *Zygophyllum*.

In a recent revision of the southern African species of *Zygophyllum*, Van Zyl (2000) described 16 new species to bring the total number of described *Zygophyllum* species in southern Africa to 54. Like previous authors, her study was mainly based on macromorphological characters and distribution patterns of the different species. Many striking differences on vegetative and reproductive morphology confirmed the subdivision of the genus into two subgenera: *Zygophyllum* and *Agrophyllum* (Neck.) Endl. Morphological characters also support the subdivision of the genus into 9 sections. The characters on which the subgeneric split of *Zygophyllum* was based are listed in Table 1.1. Van Zyl (2000) transferred the monotypic section *Morgsana* Huysst. (previously included in subgenus *Agrophyllum* by Van Huyssteen (1937)) to subgenus *Zygophyllum* to which it shows a greater affinity with regard to floral and fruit morphology. Similarly, she transferred the monotypic section *Grandifolia* Engl. (previously included in subgenus *Zygophyllum* by Van Huyssteen (1937)) to subgenus *Agrophyllum*. The following classification of southern African species of *Zygophyllum* was proposed by Van Zyl (2000):

A. Subgenus *Agrophyllum*:

1. Section *Annua* Engl. (3 species)
2. Section *Prismatica* Van Zyl (3 species)
3. Section *Bipartita* Huysst. (9 species)
4. Section *Alata* Huysst.
    Subsection *Alata* (3 species)
5. Section *Cinerea* Huysst. (2 species)
6. Section *Grandifolia* Engl. (1 species)

B. Subgenus *Zygophyllum*:

7. Section *Paradoxa* Huysst. (3 species)
8. Section *Capensia* Engl. (29 species)
9. Section *Morgsana* Huysst. (1 species)

Table 1.1. Summary of the morphological characters on which the subgeneric split of *Zygophyllum* was based (Van Zyl 2000).

Character	**Subgenus *Zygophyllum***	**Subgenus *Agrophyllum***
1. Indumentum	hairs elongate, sometimes short and sparse	trichomes two-armed, T-shaped, appressed
2. Flowers orientation	partially zygomorphic	actinomorphic
3. Sepals	not articulate	articulate
4. Petal colour	majority of species have petals in different shades of yellow	petals white
5. Petal markings	red, brown, khaki or deep yellow; variable in shapes: V-, W-, U-, to M-shaped	markings absent
6. Petal shape	usually constant	varies from spathulate, obovate to oblancoelate
7. Petal orientation	usually patent or reflexed during anthesis	usually extended and stiffy wide-open
8. Filaments length	shorter than or similar in length to style	longer than style, sometimes also than petals
9. Nectar disc	always densely papillate	smooth
10. Fruit dehiscence	loculicidal (capsules)	septicidal (schizocarps)
11. Fruit ridges	present in the majority of species	absent; five-winged fruits
12. Nature of mucilage when seeds are immersed in water	structured, containing long, spiral inclusions of a uniform width	structured, short, spiral inclusions, wine glass-shaped in side view

1.4 Main objectives of the study

The aim of this study was to determine whether molecular data support the morphological classification proposed by Van Zyl (2000) for the southern African members of *Zygophyllum*. Molecular data, especially DNA sequences, have recently received a great deal of attention as a potential source of phylogenetically informative characters that are less ambiguous than non-molecular characters (Chase *et al.* 1993). Judd *et al.* (1999) claim that molecular data are more likely to reflect the true phylogeny than morphological data, because they reflect gene-level changes which are thought to be less subjected to convergence and parallelism than morphological traits. As a result, molecular data are now widely used for generating phylogenetic hypotheses. Both the nuclear and chloroplast genes have been used to reconstruct the phylogenetic relationships of land plants (e.g. Soltis *et al.* 1998; Cox and Hedderson 1999; McDade and Moody 1999; Meerow *et al.* 1999; Molvray *et al.* 1999).

Recent morphological, anatomical and molecular studies have benefited our understanding of the phylogenetic relationships within the Zygophyllaceae. Anatomical studies alone suggested that the monotypic species *Augea capensis* Thunb. is closely related to the type genus *Zygophyllum* (Sheahan and Cutler, 1993). Sheahan and Chase (1996) used the combination of morphological, anatomical and *rbc*L DNA sequence data to divide the family into five subfamilies. Sheahan and Chase (2000) sequenced both the *rbc*L and *trn*LF genes of some southern African members of *Zygophyllum*. In their analyses, the southern African *Zygophyllum* taxa formed two distinct lineages: one corresponding to subgenus *Zygophyllum* and the other to subgenus *Agrophyllum*. The number of species included in the analyses were, however, limited. Thus, the relationships between the southern African *Zygophyllum* taxa were not very conclusive.

In the present study, the more variable noncoding *trn*L–*trn*F chloroplast DNA sequences were used to address phylogenetic questions in *Zygophyllum*. Although the main focus was on southern African species, a few species from other geographical areas were included in the analysis. Species from closely related genera, i.e. *Augea* Thunb., *Fagonia* L. and *Tetraena* Maxim. within the subfamily Zygophylloideae and more distantly related genera *Seetzenia* R. Br. ex Decne and *Tribulus* L. were also used as outgroups.

Key questions include:

(1) What is the molecular level of support for the subgenera *Zygophyllum* and *Agrophyllum*? Do molecular data justify the split of *Zygophyllum* into two separate genera?

(2) Do the molecular data support the sections included within the two subgenera?

(3) Is there molecular support for transferring the monotypic sections *Morgsana* (previously placed in the subgenus *Agrophyllum*) to subgenus *Zygophyllum* and *Grandifolia* (previously placed in the subgenus *Zygophyllum*) to subgenus *Agrophyllum*?

(4) Do molecular data support the split of *Zygophyllum* into four subgenera or into two separate genera? Both the monotypic sections *Morgsana* and *Grandifolia* have unique ways of fruit dehiscence not typical for *Zygophyllum* nor *Agrophyllum*, and might be better treated as subgenera on their own or even separate genera all together.

(5) To indicate geographic affinities of southern African *Zygophyllum* taxa.

1.5 The importance of the study

(1) In the past, different taxonomists had different ideas on the placement of the family Zygophyllaceae. Takhtajan (1969) and Dahlgren (1980) treated it as related to the family Geraniaceae (order Geraniales). Hutchinson (1973) placed it with families like Balanitaceae and Malpighiaceae in the order Malpighiales. Heywood (1978) and Cronquist (1981, 1988) treated Zygophyllaceae as belonging to the Sapindales on the basis of the compound or cleft leaves, well-developed nectary disk, syncarpous ovary with a limited numbers of ovules and producing characteristic triterpenoid bitter substances. Takhtajan (1980, 1983, 1986) and Thorne (1992) treated Zygophyllaceae as belonging to the orders Rutales and Linales, respectively. In Chase *et al.* (1993), members of the order Sapindales were resolved in both the rosid I and rosid II clades, with Zygophyllaceae and its sister family Kramariaceae included within rosid I. The combination of morphological characters, *rbc*L, *atp*B and 18 S nuclear ribosomal DNA placed the two sister families in the new order Zygophyllales (Angiosperm Phylogeny Group 1998). However, the relationship between this order and the other rosids is still unknown. As these unresolved relationships could have resulted from

under-sampling, the results of the present study combined with other current studies on the Zygophyllaceae, should help to resolve this phylogenetic problem.

(2) *Zygophyllum* is one of the larger genera within southern Africa. Along with many genera within the families Mesembryanthemaceae, Chenopodiaceae and Aizoaceae, it often dominates in very harsh environments (Compton 1929; Russel 1987). A good classification of the genus will be useful in other disciplines (e.g. ecology and ecophysiology) as it is important to know the correct genera and species you are working with, regardless the nature of the research.

(3) The genus *Zygophyllum* includes many species that are rare and endangered. We have to know what they are in order to conserve them. If we do not engage ourselves in the systematics of this group, some lineages may become extinct before being discovered.

(4) A better understanding of *Zygophyllum* systematics will also help stock farmers. A greater number of the species in the subgenus *Zygophyllum* are palatable to grazers than in the subgenus *Agrophyllum* (e.g. *Z. lichtensteinianum*, Shearing and Van Heerden 1994). A good classification of the genus will help in advising the farmers which taxa have good pasture potential.

CHAPTER 2

2. MATERIALS AND METHODS

2.1 Plant materials

The *trn*L intron and *trn*L-F spacer sequences of 18 taxa included in the analyses were accessed from Genbank (Table 2.1). Voucher specimens for 22 southern African species from which DNA was extracted as part of this study had previously been deposited at the Stellenbosch University herbarium (STEU). Taxa were chosen to represent both the subgenera and the sections recognised by Van Zyl (2000). These are listed in Table 2.2. Two sequences (*Z. foetidum* Schrad. & Wendtl. and *Z. morgsana* L.) were requested from Bjorn-Axel-Beier (Uppsala, Sweden) who is also involved in molecular studies of the subfamily Zygophylloideae.

2.2 DNA extraction

DNA was extracted from about one year old silica-dried material; when this was not available herbarium material was used (Table 2.2). Total genomic DNA was extracted using a modification of the CTAB method (Doyle and Doyle 1987). A spatula tip of polyvinylpyrrolidine (PVP) was added during extraction of herbarium material. For herbarium material, further purification was achieved using isopropanol precipitation. This procedure appeared to be necessary for DNA prepared from degraded herbarium material (Fay *et al.* 1998; Sheahan and Chase 2000). To preserve herbarium collections, only those specimens with enough leaves have been sampled.

Since PCR reactions are expensive, it was of great importance to determine the quantity and purity of the DNA isolated from the herbarium material. This was assessed from absorbance readings at 260 nm (A_{260}) and 280 nm (A_{280}), using a Beckmann DU 650 spectrophotometer and mini cuvette. Since an $A_{260} = 1 = 50$ μg/ml double-stranded DNA (Maniatis *et al.* 1989), a 1/50 dilution was used for spectrophotometric determinations, using TE buffer diluted with water (1/50 dilution) as a blank. The A_{260}/A_{280} values between 1.8 and 2.0 were considered to yield a sufficiently good PCR product. Smaller and larger ratio values were attributed to DNA degradation and contamination (proteins and phenolic compounds), respectively. The undiluted DNA concentration (μg/μl) was calculated from the A_{260} value.

Table 2.1. Species for which *trn*LF sequences were obtained from Genbank, together with sources of plant materials, sequence lengths, and EMBL database accession numbers. Locality of the *Zygophyllum* species is indicated in brackets, with the species also occurring in southern Africa indicated with an asterix.

Species	Accessions	Sequence lengths	EMBL accession number
Augea capensis Thunb	Chase 718 K	453	AJ387945
Fagonia cretica L.	Chase 3432 K	751	AJ387942
Seetzenia lanata (Willd.) Bullock	Herman 3964 K	831	AJ387956
Tetraena mongolica Maxim.	Sheahan 1994 K	722	AJ387959
Tribulus macropterus Boiss.	Collenette 3/93 K	810	AJ387961
Zygophyllum album L. (Middle East)	Thulin et al. 7977 UPS	728	AJ387963
Z. billardierei DC. (Australia)	S.R.417 Adelaide Botanic Garden	708	AJ387964
Z. coccineum L. (Middle East)	Ryding 1347 K	688	AJ387965
Z. cylindrifolium Schinz.*(southern African)	Craven 3800 WIND	628	AJ387966
Z. decumbens Del.*(south & north Africa)	Thulin *et al.* 7981 UPS (East African??)	741	AJ387967
Z. fabago L. (world wide)	Chase 516 K (unknown locality)	762	AJ387968
Z. fruticulosum DC.(Australian)	Chase 2203 K	729	AJ387969
Z. glaucum F. Muell.(Australian)	Chase 2204 K	669	AJ387970
Z. hildebrandtii Engl. (East African)	Thulin et al. 9012 UPS	709	AJ387971
Z. hirticaule Van Zyl * (southern African)	Van Zyl 3894 NBG holo.; PRE, S, WIND	704	AJ387972
Z. robecchii Engl. (East African)	Thulin et al. 8428 UPS	704	AJ387973
Z. simplex L.* (world wide)	Chase 806 K (unknown locality)	721	AJ387974
Z. xanthoxylum Engl. (worldwide)	Chase 1700 K (unknown locality)	704	AJ387975

Table 2.2. Species for which DNA was extracted at the University of Stellenbosch, together with sources of plant materials. Silica gel dried and herbarium materials are indicated with an **S** and **H** after the collector's number, respectively.

Species	Voucher	Subgenus (Van Zyl 2000)	Section (Van Zyl 2000)
1. *Z. cordifolium* L.f.	Van Zyl 4601 **S**	*Zygophyllum*	*Paradoxa*
2. *Z. cuneifolium* Eckl. & Zeyh.	Van Zyl 4600 **S**	*Zygophyllum*	*Capensia*
3. *Z. decumbens* Del. var. *decumbens*	Van Zyl 4588 **S**	*Agrophyllum*	*Bipartita*
4. *Z. fulvum* L.	Van Zyl 4605 **S**	*Zygophyllum*	*Capensia*
5. *Z. fusiforme* Van Zyl	Van Zyl 4066 **H**	*Zygophyllum*	*Paradoxa*
6. *Z. giessii* Merxm. & A. Schreib.	Van Zyl 4353 **H**	*Agrophyllum*	*Cinerea*
7. *Z. leucocladum* Diels in Schultze	Van Zyl 4479 **H**	*Zygophyllum*	*Capensia*
8. *Z. lichtensteinianum* Cham.	Van Zyl 4594 **S**	*Zygophyllum*	*Capensia*
9. *Z. longicapsulare* Schinz.	Van Zyl 3885 **H**	*Agrophyllum*	*Cinerea*
10. *Z. microcarpum* Cham.	Van Zyl 4591 **S**	*Agrophyllum*	*Alata* subsection *Alata*
11. *Z. morgsana* L.	Van Zyl 4285 **H**	*Zygophyllum*	*Morgsana*
12. *Z. prismatocarpum* Sond.	Van Zyl 4474 **H**	*Agrophyllum*	*Prismatica*
13. *Z. pterocaule* Van Zyl	Van Zyl 4136 **H**	*Agrophyllum*	*Prismatica*
14. *Z. retrofractum* Thunb.	Van Zyl 4597 **S**	*Agrophyllum*	*Bipartita*
15. *Z. rigidum* Schinz.	Van Zyl 4590 **S**	*Agrophyllum*	*Alata* subsection *Alata*
16. *Z. rogersii* Compt.	Van Zyl 3984 **H**	*Zygophyllum*	*Capensia*
17. *Z. schreiberanum* Merxm. & Giess	Van Zyl 4502 **H**	*Zygophyllum*	*Capensia*
18. *Z. spitskopense* Van Zyl	Van Zyl 4606 **S**	*Zygophyllum*	*Capensia*
19. *Z. spongiosum* Van Zyl	Van Zyl 3785 **H**	*Agrophyllum*	*Annua*
20. *Z. stapffii* Schinz.	Van Zyl 3786 **H**	*Agrophyllum*	*Grandifolia*
21. *Z. tenue* Glover	Van Zyl 4593 **S**	*Agrophyllum*	*Bipartita*
22. *Z. teretifolium* Schltr.	Van Zyl 4598 **S**	*Zygophyllum*	*Capensia*

2.3 PCR and agarose gel electrophoresis

The *trn*L-F region was amplified from the purified genomic DNA using the polymerase chain reaction (PCR; Mullis and Faloona 1987). The final concentrations of reagents (in 100 μl reaction volumes) were: 1 × PCR buffer, 1 unit of thermostable DNA polymerase (Taq), 2.5 mM $MgCl_2$, 103.2 nmol primer c and 95.7 nmol primer f, 200 μM dNTP's and the total genomic DNA (chloroplast DNA) used as template (4 μl). Primers used were "c" and "f" (Taberlet *et al*., 1991), which amplify a fragment comprising the *trn*L intron, the 3' *trn*L exon, and intergenic spacer between this exon and the *trn*F gene of the chloroplast genome. The primers were obtained from the University of Cape Town Oligonucleotide Synthesis Unit. For PCR purposes, 50 pmol/μl dilutions were made of each primer. These diluted primer solutions were stable at –20 °C (freezer) for up to four weeks.

The PCR's were carried out in a Hybaid PCR Express Minicycler. The thermal profile consisted of an initial denaturation step (94 °C for 3 minutes), followed by 35 cycles of 94 °C (45 seconds), 54 °C (45 seconds), 72 °C (1 minute) each, and a final elongation step of 6 minutes at 72 °C. The PCR products were identified by electrophoresis of 10 μl of each reaction product in a 2% agarose gel in 1 × TAE (Tris-acetate) buffer. Ethidium bromide (0.5 μg/ml) was added in the gel for visualization of the electrophoresis results under ultra-violet (UV) illumination (Sharp *et al.* 1973).

2.4 Purification of PCR product

The PCR reaction products were purified from an agarose gel for sequencing according to the following procedure. The remaining 90 μl of the final reaction was run in 1 × TAE buffer on a 0.5% agarose gel containing ethidium bromide for the same purpose as described above. The band was cut out under UV illumination and purified from the agarose using Promega Wizard PCR minicolumns (Promega Corp., Maddison, Wisconsin) in accordance with the manufacturer's protocols.

2.5 PCR sequencing

Partial sequencing of the purified product was carried out using the Taq Cycle Sequencing Kit and the same primers as for the PCR. In some cases Taberlet *et al.* (1991) internal primers "e" and Bellstedt *et al.* (2000) "c2" and reverse c2 were also used. Sets of these nested primers are derived from the highly conserved chloroplast *trn*L and *trn*F genes (Table 2.3; Figure 2.1). Sequencing was achieved using the automated ABI sequencer of the Analytical Facility, University of Stellenbosch.

Table 2.3. Sequences of the three primers used for sequencing of these non-coding regions of cpDNA. The B and A in the code refer to each strand of DNA.

Name	Code	Sequence 5'–3'
c	B49317	CGAAATCGGTAGACGCTACG
c2	B…..	GGATAGGTGCAGACTCAAT
c2 reverse	A…..	ATTGAGTCTGCACCTATCC
d	A49855	GGGGATAGAGGGACTTGAAC
e	B49873	GGTTCAAGTCCCTCTATCCC
f	A50272	ATTTGAACTGGTGACACGAG

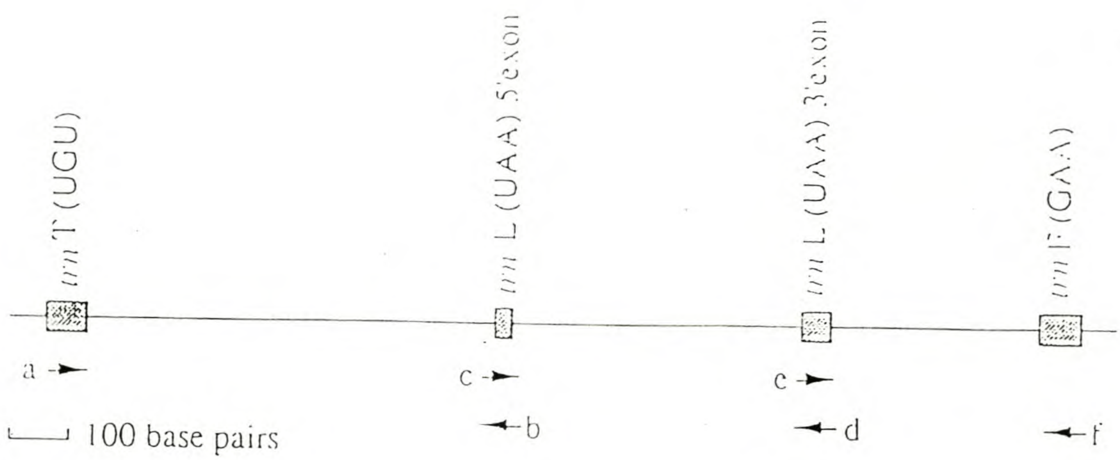

Figure 2.1 Positions and directions of universal primers used to amplify three non-coding regions of cpDNA. Developed from Taberlet *et al.* (1991).

2.6 Choice of outgroups

Augea capensis, *Fagonia cretica*, *Seetzenia lanata*, *Tetraena mongolica* and *Tribulus macropterus* were used as outgroups, based on the analyses of Sheahan and Chase (1996, 2000). In their studies, *Augea*, *Fagonia* and *Tetraena* are nested within *Zygophyllum* to form a large Zygophylloideae clade. However, *Seetzenia* and *Tribulus* are resolved outside the clade with the *Zygophyllum* taxa.

2.7 Sequence alignment

For each taxon and sequenced DNA region, forward (5'–3') and reverse (3'–5') sequences were assembled and checked for inaccurate base calling using the DAPSA (Harley 2000*) computer program. The sequences were initially aligned using the automatic alignment option within DAPSA (Harley 2000*). The *trn*L–F region is known to contain sequence repeats (micro-satellites), poly-A and -T regions (Vendramin *et al.* 1996), which complicate alignment. The data sets were truncated in both the intron and spacer regions to eliminate autoapomorphic sequence ends. This was done on regions of ambiguous alignment and incomplete data (i.e. at the beginning, between the poly A-rich region and the c2 primer binding site, and the end of sequences) were excluded from analyses.

2.8 Data analysis

The gene was analysed in two separate data matrices, one containing 32 taxa (*trn*L intron) and one containing 29 taxa (*trn*L intron and *trn*LF spacer combined). Phylogenetic analyses for each data set were performed using PAUP *: Phylogenetic Analysis Using Parsimony (version 4.0b4a) on an Apple Macintosh PowerPC.

All phylogenetic analyses were conducted using rigorous heuristic searches. All characters were equally weighted and character states were treated as unordered. Indels (insertions and deletions) were coded as a fifth character and assigned a weight of 1 using the method of Giribet and Wheeler (1999). Branches with maximum branch length of zero were collapsed, and all most parsimonious trees were saved. Support for individual nodes was evaluated using the parsimony bootstrap (Felsenstein 1985) and jackknife (Farris *et al.* 1996). These were investigated using 100 replicates of random taxon selections, tree-bisectioning-reconnection (TBR) branch-swapping options and MAXTREE = 200. One tree was held at each step during stepwise addition. The actual deletion percentage for jackknife replicates was 33.3. Both the bootstrap and jackknife 50% majority-rule consensus trees were saved. To search for more resolution, the analyses were repeated for data weighted by the Rescaled Consistency and Retention Index (Farris 1989).

CHAPTER 3

3. RESULTS

3.1 DNA amplification

Of the 22 samples, only 13 allowed DNA amplification. The bands of the PCR products for 8 of these 13 species are shown in Figure 3.1. Species which were found to contain amplifiable chloroplast DNA are *Z. cordifolium*, *Z. cuneifolium*, *Z. decumbens* var. *decumbens*, *Z. fulvum*, *Z. leucocladum*, *Z. lichtensteinianum*, *Z. microcarpum*, *Z. retrofractum*, *Z. rigidum*, *Z. schreiberanum*, *Z. spitskopense*, *Z. tenue* and *Z. teretifolium*. Eleven of these were silica-gel dried materials (Table 2.2). *Z. leucocladum* and *Z. schreiberanum* were the only herbarium samples that allowed DNA amplification. However, the amplification of these two was unsuccessful under standard DNA isolation conditions. A spatula tip of PVP had to be added during the isolation. A small fragment of DNA was amplified in *Z. schreiberanum* (Figure 3.1, lane 4). The amplification of *Z. leucocladum* yielded a good PCR product for sequencing. The A_{260}/A_{280} ratio values for all the remaining herbarium samples were less than 1.8 (unpublished). The lower ratio values might indicate that the extracts contain none or very few templates.

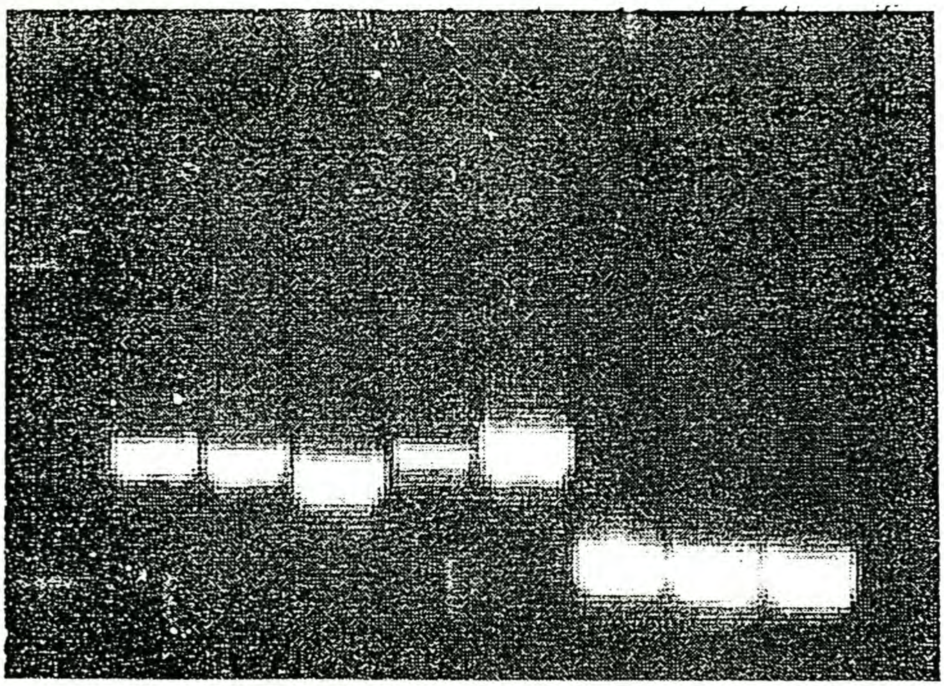

Figure 3.1. Agarose gel electrophoresis of PCR products for 8 species

3.2 *trn*LF sequence characteristics

There is a considerable length variation in the *trn*L intron region between *Seetzenia* and *Tribulus*, and the other taxa (Figures 3.2 & 3.3; Appendices I & II). This length variation resulted from a large number of deletions shared by *Zygophyllum*, *Fagonia*, *Augea* and *Tetraena*. *Seetzenia* and *Tribulus* possess an identical major insertion (bp 261–367). There are also smaller autapomorphic deletions and insertions shared by ingroup taxa.

Species of the subgenus *Zygophyllum* and three Australian species, *Z. fruticulosum*, *Z. billardierei* and *Z. glaucum*, share three minor deletion patterns (bp 73–78; bp 99–103; bp 385–391), respectively. The first deletion pattern is also shared with the widespread species *Z. xanthoxylum* and *Z. fabago*. Another smaller deletion (bp 251) is shared with two of the three Australian species, *Z. billardierei* and *Z. fruticulosum*. *Z. morgsana* possesses an autapomorphic deletion (bp 151–155). All the taxa, except *Z. spitskopense*, possess six base pairs deletion (bp 428–433). The poly A-rich insertion pattern is also followed by the subgenus *Zygophyllum* and the three Australian species (bp 368–378).

Species of sections *Bipartita* and *Annua* share two deletion patterns: bp 172–176 and bp 566, respectively. The first pattern is also found in *Augea capensis*, *Z. album*, *Z. coccineum* and *Z. fabago*. *Z. microcarpum* and *Z. rigidum* (both section *Alata*) are the only *Agrophyllum* species without these deletion patterns. The *trn*LF spacer region shares much less length variation, although with the exception of *Z. decumbens*, species of section *Bipartita* exhibit a distinct deletion pattern (bp 826–919, Figure 3.3). The southern African species of subgenus *Zygophyllum* possess autapomorphic deletions and insertions patterns in the spacer region (bp 883–889; bp 991–997). These insertion patterns are also shared with the three Australian species *Z. fruticulosum*, *Z. billardierei* and *Z. glaucum*.

The two sequences of *Z. decumbens* specimens from different geographical areas were compared. *Z. decumbens* from the northern hemisphere and *Z. decumbens* var. *decumbens* from the southern African region show sequence homology. However, the two specimens differed only with regard to two base changes at bp 109 and 111 in which *Z. decumbens* possesses an A in both positions which are replaced by a T and a G in *Z. decumbens* var. *decumbens*, respectively (Figure 3.2 or 3.3).

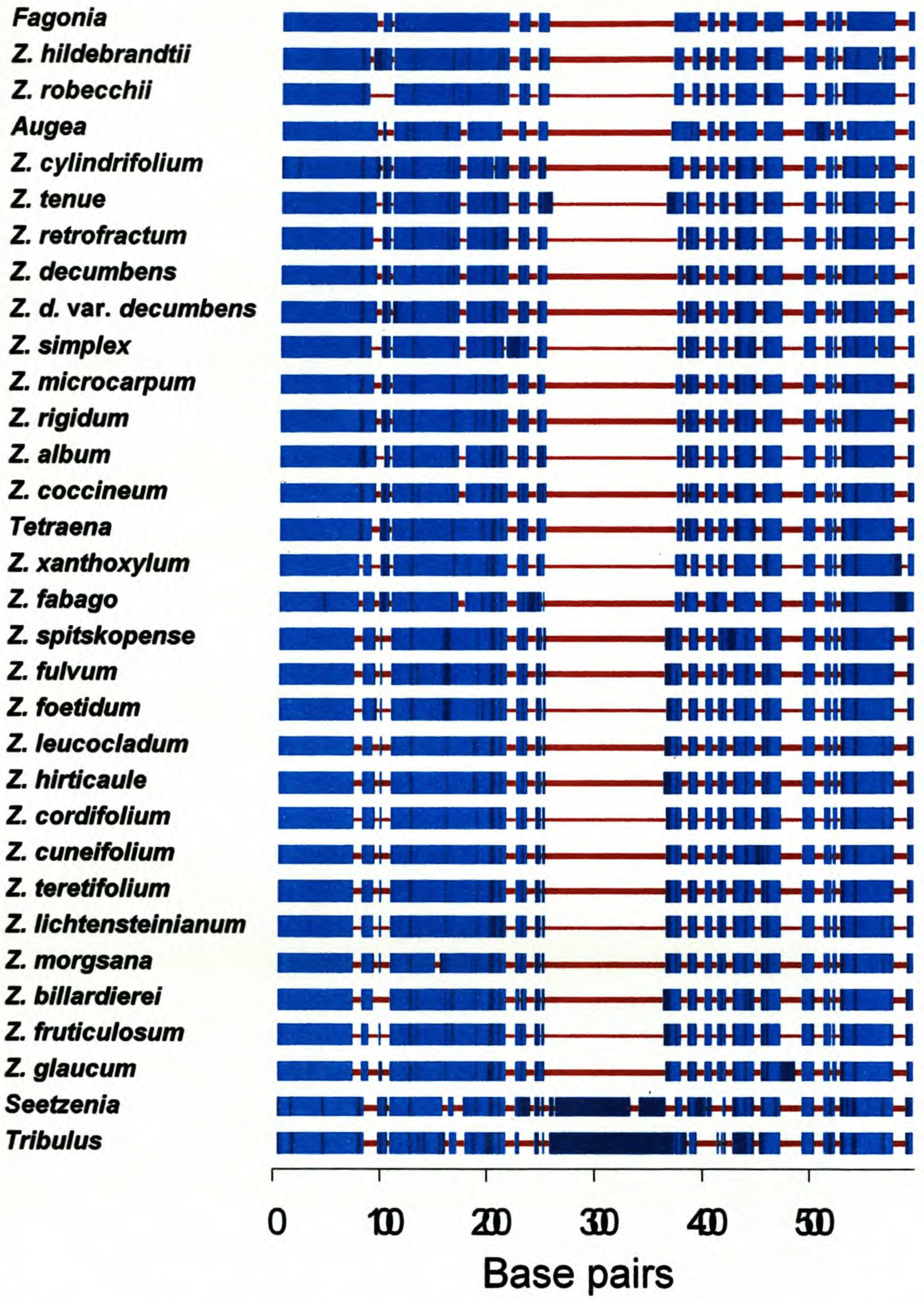

Figure 3.2 General sequence characteristics of the *trn*L intron summarised as sequence cartoons. Homologous sequences are indicated as turquoise bars, mutations (substitutions and insertions) as blue bars and gaps are indicated as red lines. The length of the aligned data set is indicated in base pairs on the x-axis.

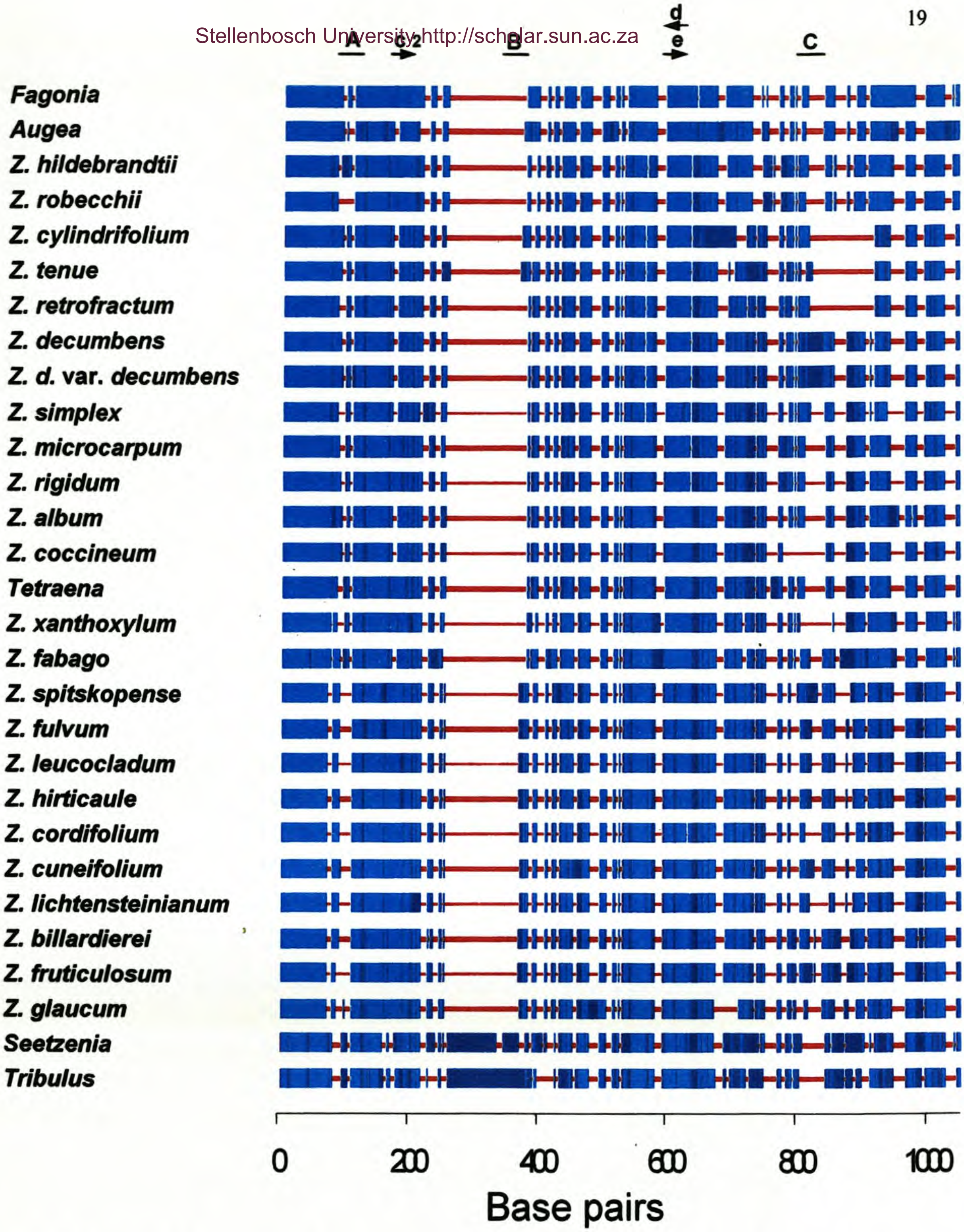

Figure 3.3 General sequence characteristics of the *trn*L intron and the *trn*LF spacer summarised as sequence cartoons. The internal primers binding sites are also indicated. Fifty-six base pairs between the poly A-rich region (A) and the primer c2 were removed from the aligned data set. Homologous sequences are indicated as turquoise bars, mutations (substitutions and insertions) as blue bars and gaps are indicated as red lines. The length of the aligned data set is indicated in base pairs on the x-axis.

3.3 Phylogenetic analysis

Table 3.1. Summary of parsimony analyses. Incl. = number of included characters, Infor = number of informative characters, MPTs = most parsimonious trees, Length = tree length, CI = Consistency Index, RI = Retention Index, RCI = Rescaled Consistency Index and HI = Homoplasy Index.

Data set	Incl.	Infor.	MPTs	Length	CI	RI	RCI	HI
*trn*L intron	604	76	6	215	0.6279	0.8261	0.5187	0.3721
*trn*LF	1053	176	6	498	0.6667	0.8081	0.5387	0.3333

3.3.1 *trn*L intron data set

Alignment of the *trn*L intron data resulted in a total of 604 character sites, of which 449 were invariable (excluding gaps), 155 were variable characters, 76 were phylogenetically informative, 79 were autapomorphic (excluding indels) and 28 were informative indels. Maximum parsimony analysis of the *trn*L intron resulted in 6 most parsimonious trees with a length of 215 steps, a Consistency Index of 0.6279, a Retention Index of 0.8261, a Rescaled Consistency Index of 0.5187 and a Homoplasy Index of 0.3721. The strict consensus tree is shown in Figure 3.4. The bootstrap and Jackknife support (> 50%) are indicated above and below the branches, respectively.

Southern African *Zygophyllum* members display two lineages: one corresponding to subgenus *Agrophyllum* and the other to subgenus *Zygophyllum*. Species of *Agrophyllum* are resolved in a very well supported major clade (bootstrap 93%; jackknife 98%) consisting of *Z. album*, *Z. coccineum* and *Tetraena* (Figure 3.4). Section *Bipartita* and *Annua* together form an unresolved relationship with section *Alata* clade; the clade containing *Z. album* and *Z. coccineum*; and another clade containing *Tetraena*. Species of the section *Bipartita* are resolved as a sister clade to the section *Annua* (represented by *Z. simplex* only). The relationship between these two sections is very well supported (bootstrap of 97%; jackknife 98%). Species in the section *Bipartita* form a well supported clade (bootstrap 71%; jackknife

83%). *Z. retrofractum* and its very well supported (bootstrap 99%; jackknife 96%) monophyletic sister clade consisting of *Z. cylindrifolium* and *Z. tenue* are also very well supported (bootstrap 92%; jackknife 89%). The relationships between *Z. rigidum* and *Z. microcarpum* (both of section *Alata* subsection *Alata*) are well supported (bootstrap 88%; jackknife 83%). However, the relationship between this section (clade) and the other sections of the subgenus *Agrophyllum* remains unresolved.

The subgenus *Zygophyllum* is resolved in a lineage containing the three Australian species *Z. billardierei*, *Z. fruticulosum* and *Z. glaucum*. This major clade is very well supported (bootstrap 98%; jackknife 100%). With the exceptions of *Z. cordifolium* (section *Paradoxa*) and *Z. morgsana* (section *Morgsana*), all southern African species in this clade belong to the section *Capensia*. *Z. foetidum* and its well supported (bootstrap 84%; jackknife 90%) monophyletic sister clade consisting of *Z. spitskopense* and *Z. fulvum* are also strongly supported (bootstrap 90%; jackknife 94%). Other remaining clades in the subgenus remain unresolved. However, *Z. leucocladum* and *Z. hirticaule* show a fairly to weakly supported relationships (bootstrap 59%; jackknife 63%). *Z. cuneifolium* and *Z. teretifolium* also show a weakly supported relationships (bootstrap 50%; jackknife 57%).

3.3.2 *trn*LF data set

Alignment of the *trn*LF data resulted in a total of 1053 character sites, of which 658 were invariable (excluding gaps), 395 were variable characters, 176 sites were phylogenetically informative, 219 were autapomorphic (excluding indels) and 77 were informative indels. Maximum parsimony analysis of the *trn*LF resulted in 6 most parsimonious trees with a length of 498 steps, Consistency Index of 0.6667, Retention Index of 0.8081, Rescaled Consistency Index of 0.5387 and Homoplasy Index of 0.3333; the strict consensus of which is shown in Figure 3.5. The bootstrap and jackknife support (> 50%) are indicated above and below the branches, respectively.

The southern African *Zygophyllum* species show two lineages: one corresponding to the subgenus *Agrophyllum* and the other to the subgenus *Zygophyllum*. The *Agrophyllum* species are resolved in the clade consisting of *Z. album*, *Z. coccineum* and *Tetraena* sp. (Figure 3.5). This major clade is very well supported (bootstrap 100%; jackknife 100%). Species of the section *Bipartita* are resolved as a sister to section *Annua* within this major clade. This relationship is also very well supported (bootstrap 99%; jackknife 100%). Species of the section *Bipartita* form a very well supported clade (bootstrap 100%; jackknife 100%). Within this clade, *Z. decumbens* spp. form a sister relationships with a moderately supported (bootstrap 61%; jackknife 64%) clade containing *Z. cylindrifolium*, *Z. tenue* and *Z. retrofractum*. *Z. retrofractum* form a sister relationships with the well supported (bootstrap 85%; jackknife 95%) monophyletic clade containing *Z. cylindrifolium* and *Z. tenue*. Species in the section *Alata* (*Z. rigidum* and *Z. microcarpum*) form a very well supported monophyletic clade (bootstrap 99%; jackknife 99%), sister to another very strongly supported (bootstrap 98%; jackknife 99%) monophyletic clade containing *Z. album* and *Z. coccineum*. However, their relationship is not well supported (bootstrap 58%; jackknife 69%). These two monophyletic clades form a well supported sister relationship with *Tetraena* (bootstrap 77%; jackknife 88%).

The subgenus *Zygophyllum* is resolved in a very well supported clade (bootstrap 100%; jackknife 100%) containing three Australian species *Z. billardierei*, *Z. fruticulosum* and *Z. glaucum*. The southern African species of the subgenus *Zygophyllum* form a poorly supported clade (less than 50%), sister to the Australian species which form a well supported clade (bootstrap 74%; jackknife 85%). The relationships between the southern African species in the subgenus remain unresolved. However, *Z. spitskopense* and *Z. fulvum* show a very well supported relationship (bootstrap 100%; jackknife 99%). The relationship between *Z. leucocladum* and *Z. hirticaule* is weakly supported (bootstrap 54%; jackknife 62%). This relationship is also revealed by the *trn*L intron tree. The relationships between *Z. cordifolium*, *Z. lichtensteinianum*, *Z. teretifolium* and other species in the subgenus remain unresolved. However, the relationship between *Z. teretifolium* and *Z. cuneifolium* was expected since it was already revealed by the *trn*L intron data set. *Z. morgsana*, *Z. teretifolium* and *Z. foetidum* were excluded from this analysis, since the *trn*LF spacer regions were not available for the three species. *Z. morgsana* and *Z. foetidum* sequences requested from Bjorn-Axel-Beier were sent without the spacer region. In *Z. teretifolium*, the sequence obtained for the spacer region was not good enough to be included in the analysis.

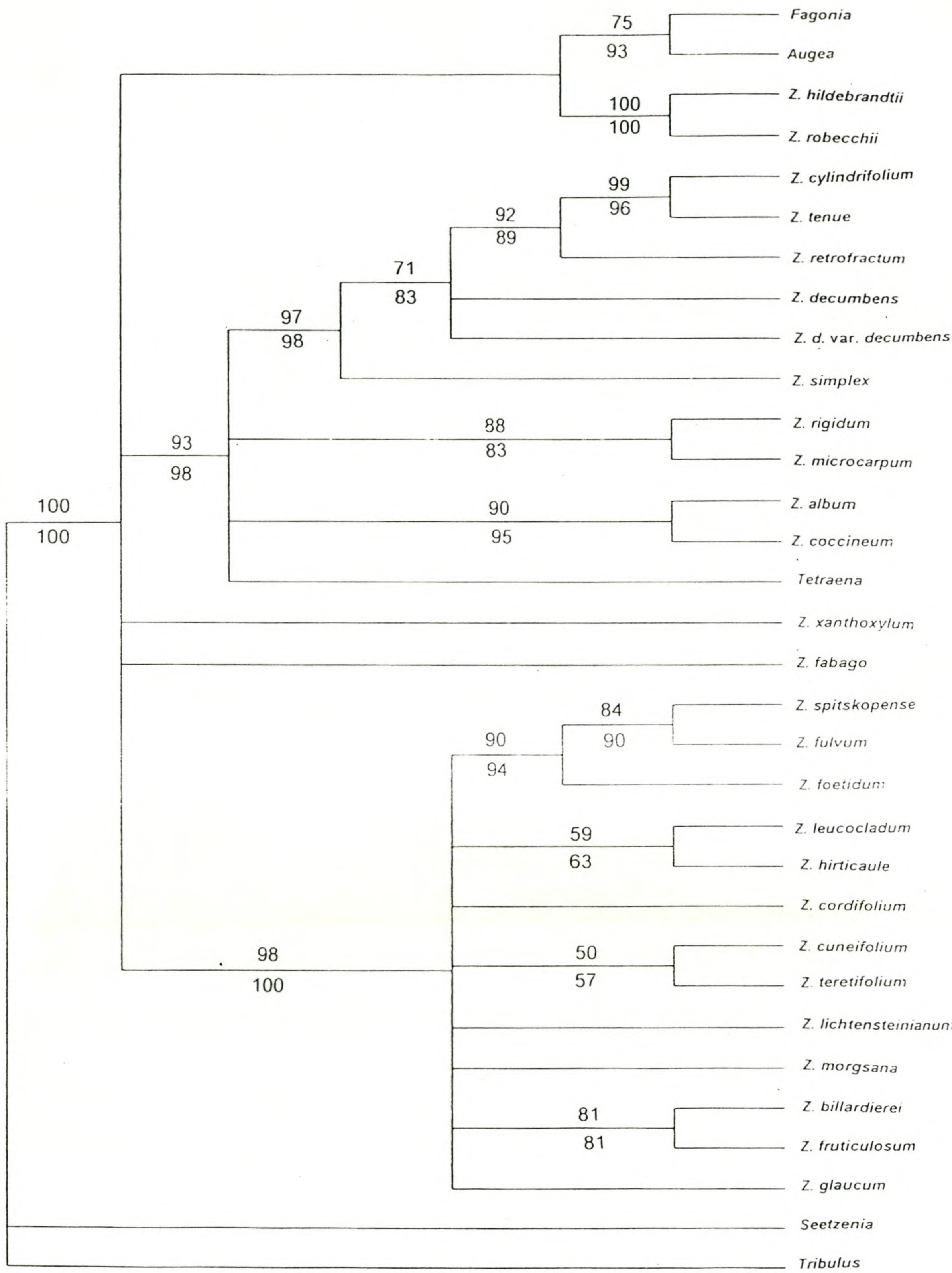

Figure 3.4 The strict consensus of the 6 MPTs found during equally weighting parsimony analysis of the *trn*L intron sequences. Numbers above and below branches are bootstrap and jackknife support values, respectively. If the support was less than 50% it was not indicated.

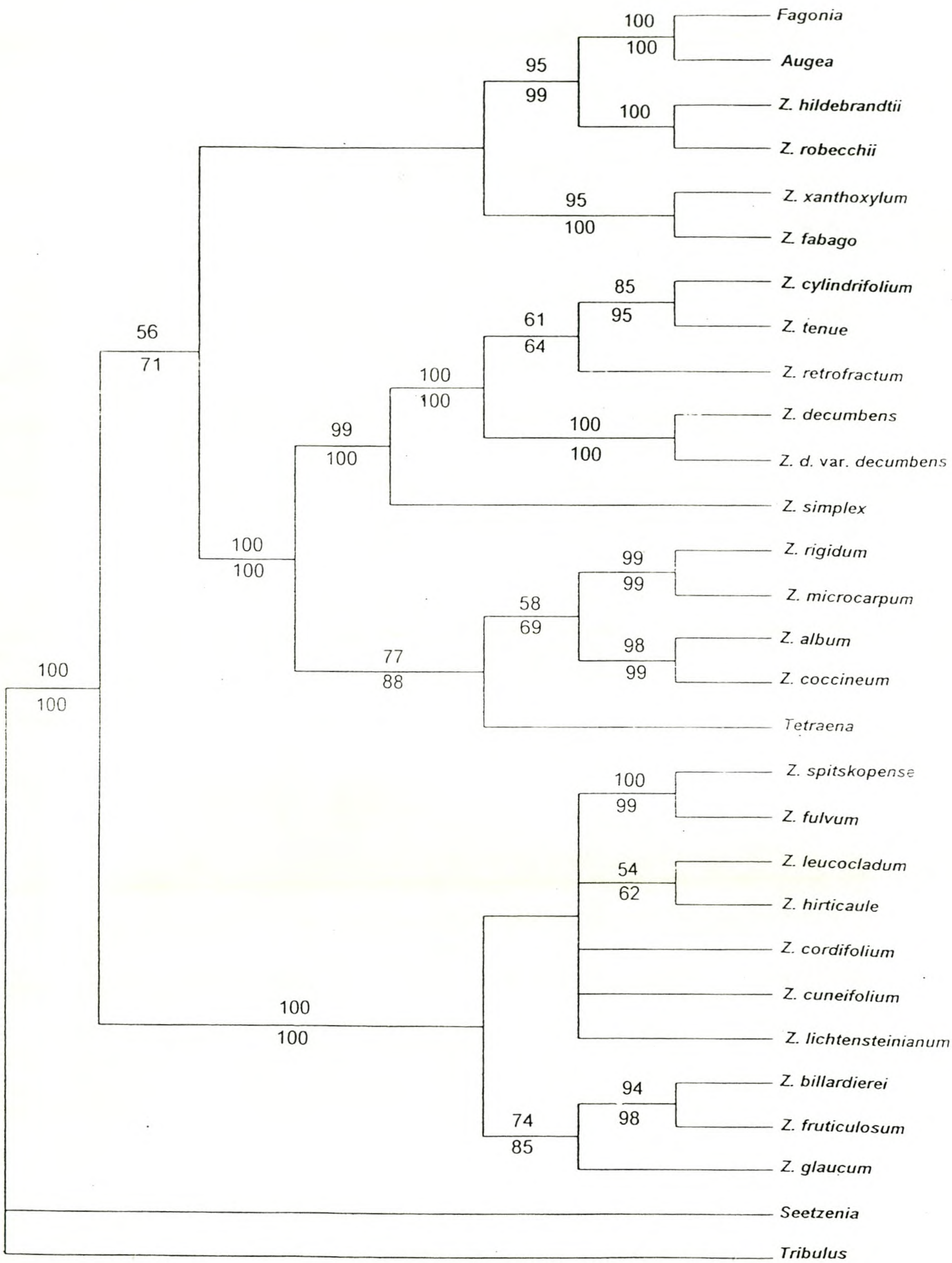

Figure 3.5 The strict consensus of the 6 MPTs found during equally weighting parsimony analysis of the *trn*LF sequences. Numbers above and below branches are bootstrap and jackknife support values, respectively. If the support was less than 50% it was not indicated.

4. DISCUSSION

4.1 DNA amplification

Out of 11 herbarium DNA extracts, only two were found to contain amplifiable chloroplast DNA after the addition of PVP. The use of insoluble PVP helps to remove the PCR inhibiting activities, like phenolic compounds (Loomis & Bataille 1966). The amplification of both the *trn*L intron and spacer was unsuccessful in *Z. schreiberanum*. All the sequences generated using the primer sets were limited and could not form a full *trn*LF stretch. Possibly the DNA of this species is degraded, since herbarium material was used. It is also possible that mutations have occurred in the chloroplast tRNA genes of this species, whereby the primers could no longer recognise their relevant regions (Bellstedt *et al.* 2000).

When collecting, an initial rapid desiccation of specimens is important in limiting the senescence processes (Savolainen *et. al.* 1995). This guarantees the success of silica-gel dried tissues for DNA analysis (Chase & Hillis 1991). The breaking of cellular compartments during the drying stage may produce endogenous hydrolytic damage, which mostly happen in herbarium specimens. Plants such as grasses are easy to dry, but others like *Zygophyllum* species with succulent leaves take longer to dry, thus sometimes giving time for cellular disruption. There seems to be no apparent correlation between the age of a herbarium sample and the success of DNA amplification, as herbarium specimens both less and more than 20 years old have been successfully used in the past (Chase and Sheahan 1996; Savolainen *et. al.* 1995). In this study, most of the one year old herbarium specimens gave negative results. Thus, the conservation of amplifiable DNA in herbarium specimens seems to depend on several factors that are taxon-specific, such as species chemistry. Herbarium specimens will remain an invaluable source of material for molecular studies, since the collection of living plant samples is often difficult. However, different protocols of DNA amplification need to be followed to remove the PCR inhibiting activities and increase the PCR efficiency. These include prolonging of precipitation time in isopropanol to about three weeks at –20 °C. This has been found to be more effective for samples with degraded DNA than the conventional procedure (Fay *et. al.* 1998). In the present study, the extracts were only precipitated overnight.

4.2 Suitability of sequencing primers

The *trn*LF sequences of *Zygophyllum* species have three polyA-rich regions referred to as regions A, B and C (See 79–95 bp; 368–391 bp & 805–842 bp; Figures 3.2 & 3.3 or Appendix I & II). These regions make it difficult to sequence the full *trn*LF of most species using the two external primers by Taberlet *et al.* (1991), i.e. c and f only. Only *Z. retrofractum* could be sequenced in this way (Table 4.1). For most species additional internal primers c2, c2 reverse, d and e were used to generate the full *trn*LF sequences. Some of these primers were unsuccessful in generating sequences (Table 4.1).

The c2 primer presented a successful alternative to the c primer (which consistently gave problems after the polyA-rich region) on sequencing the full *trn*L intron of a number of species. The e and f primers were successful in generating the *trn*LF spacer of most species. Sequencing of the *trn*L intron with the d and c2 reverse primers was unsuccessful in all the species. Primer c2 reverse was designed as part of this study in order to sequence the problematic region between the poly A-rich region (A) and the c2 primer binding site. Since sequencing with the c2 reverse primer failed, this region of about 56 base pairs between the poly A-rich region and the c2 primer binding site was removed from the aligned sequence data sets and excluded from the analyses.

4.3 Phylogenetic relationships within *Zygophyllum*

The two main clades, which are supported in both the *trn*L intron and the *trn*LF trees, correspond to the subgenera proposed by Van Zyl (2000). However, the relationships between the sections in subgenus *Zygophyllum* remain unresolved in both the *trn*L intron and *trn*LF trees. This could have resulted from biased sampling, since all the species used in the analyses, except for *Z. cordifolium* and *Z. morgsana*, belong to section *Capensia* (Van Zyl 2000).

Table 4.1. Sequencing reactions performed with primers as indicated in order to generate full length *trn*L–F sequences.

Species	Primer					
	c	**c2**	**c2 reverse**	**d**	**e**	**f**
Z. cordifolium	successful	successful	unsuccessful	unsuccessful	successful	successful
Z. cuneifolium	successful	successful	unsuccessful	–	successful	successful
Z. d. var. *decumbens*	successful	successful	–	unsuccessful	–	successful
Z. fulvum	successful	successful	unsuccessful	–	–	successful
Z. leucocladum	successful	successful	unsuccessful	unsuccessful	–	successful
Z. lichtensteinianum	successful	successful	unsuccessful	unsuccessful	successful	successful
Z. microcarpum	successful	successful	unsuccessful	–	–	successful
Z. retrofractum	successful	–	–	–	–	successful
Z. rigidum	successful	successful	unsuccessful	unsuccessful	–	successful
Z. schreiberanum	limited	limited	unsuccessful	–	limited	limited
Z. spitskopense	successful	successful	–	unsuccessful	–	successful
Z. tenue	successful	successful	–	unsuccessful	limited	successful
Z. teretifolium	successful	–	unsuccessful	unsuccessful	–	successful

The CI is higher for *trn*LF than for *trn*L intron, and the RI is slightly higher for *trn*L intron than for *trn*LF, indicating that in spite of its greater homoplasy the *trn*L intron is still doing well. However, the *trn*LF data set analysis gave a tree with a better resolution than that given by the *trn*L intron data set. Thus, most of the relationships in the present study were inferred from the *trn*LF tree (Figure 4.1). A summary of morphological characters supporting the individual nodes for southern African members of *Zygophyllum* are listed in Table 4.2.

Subgenus *Agrophyllum*. This subgenus was represented by three sections in the analyses: *Bipartita*, *Annua* and *Alata* subsection *Alata*. Both the *trn*L intron and *trn*LF sequence analyses confirm the sections proposed by Van Zyl (2000). Species from each section form their own well supported clade: *Z. cylindrifolium*, *Z. tenue*, *Z. retrofractum* and *Z. decumbens* form a clade representing section *Bipartita*. Species in this clade have opposite, bifoliate, petiolate leaves; membranous stipules with lacerate margins, two on the ventral and two on dorsal side of the stems (El Hadidi 1980). With the exception of *Z. decumbens* which possesses flowers arranged in a cyme, all other species in this clade have solitary and axillary flowers (or sometimes two together). *Z. tenue* and *Z. retrofractum* show a morphological resemblance in that they are both shrubs with repeatedly arched stems. *Z. tenue* resembles *Z. cylindrifolium* with respect to the fruit morphology, but differs with regard to leaf morphology. Their fruits are obovoid in shape.

Section *Bipartita* is resolved as a sister group to the reinstated section *Annua* (Van Zyl 2000) represented by *Z. simplex*. Section *Annua* was previously treated as section *Bipartita* (Van Huysteen 1937). The relationship between sections *Bipartita* and *Annua* is supported by bipartite staminal scales; young stems with a prominent ventral groove; opposite leaves; smooth nectar discs, prominently 10-lobed, with lobes arranged in 5 pairs, each pair orientated outwards and upwards. *Z. simplex* and two closely related species (*Z. inflatum* Van Zyl and *Z.* . *spongiosum* Van Zyl) were placed in a section of their own (*Annua*) based on habit character. Species in section *Bipartita* are shrubby perennials, while *Z. simplex* and the two newly described species are annual or biennial, erect or prostrate herbs. *Z. simplex* is also reported to be the only *Zygophyllum* species with the C_4 photosynthetic pathway (Crookston and Moss 1972; Sheahan and Cutler 1993). Molecular, physiological and anatomical studies for the two new species in section *Annua* are needed to investigate their relationship with *Z. simplex*.

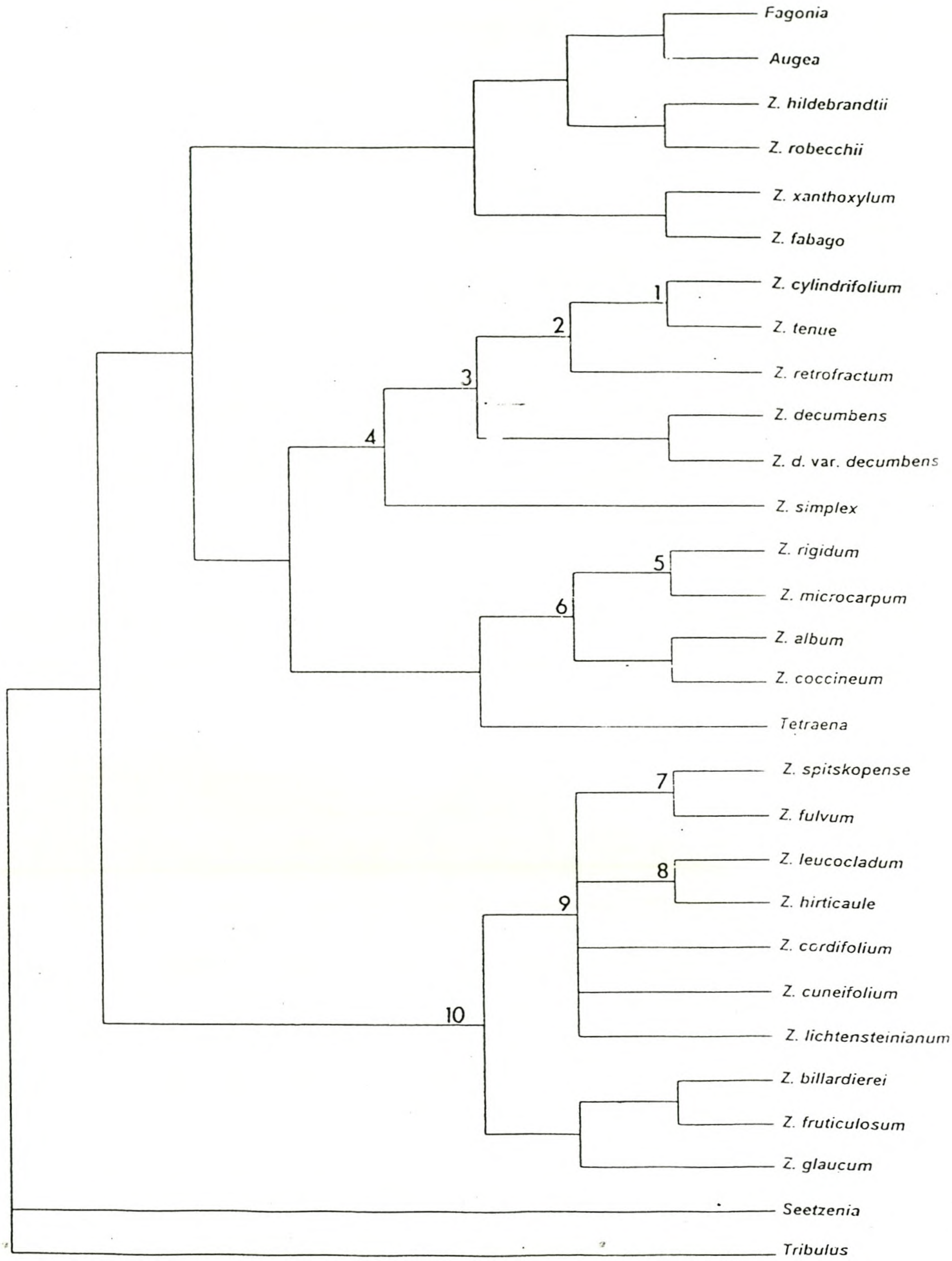

Figure 4.1 The strict consensus of the 6 MPTs found during equally weighting parsimony analysis of the *trn*LF sequences. The node numbers (as explained in Table 4.2) for southern African *Zygophyllum* clades are indicated.

Table 4.2. A summary of morphological characters supporting the individual nodes of the southern African members of *Zygophyllum* (Figure 4.1).

Node	Morphological characters
1	Fruits obovoid in shape
2	Flowers solitary and axillary (or two altogether)
3	Shrubby perennials; opposite, bifoliate, petiolate leaves; membranous stipules with lacerate margins, two on the ventral and two on the dorsal side of the stems; seeds pyriform, testa grainy, becoming transparent when wet, producing structured mucilage with short, spiral inclusions that seem to unravel at the apex
4	Bipartite staminal scales; young stem with weakly to prominent grooves; opposite leaves; smooth nectar discs, prominently 10-lobed, with lobes arranged in 5 pairs, each pair orientated outwards and upwards. Seeds pyriform, when immature attached with a long funicle
5	Young parts densely or sparsely white hairy, with two-armed trichomes, becoming glabrescent with age; stipules rarely fused, two ventral and two dorsal; flowers solitary, axillary; staminal scales simple; nectar disc smooth, 10-angled, with 10 small lobes orientated downwards; mature fruits large, usually with undulate wings; seeds compressed pyriform
6	Solitary flowers; petals white, pink, orange to yellow, spathulate, base with a long claw
7	Sessile, opposite, bifoliate leaves, with triangular rachis apices; stipules triangular, two on ventral and one on dorsal side of the stem
8	Opposite, bifoliate leaves; staminal scales and filament to nectar ratios similar
9	Flowers 1–3 together, axillary; nectar disc papillate; staminal scales simple; mature fruits loculidal capsule, 5-angled or 5-winged
10	Flowers large and yellow

Section *Alata* subsection *Alata* was represented by *Z. rigidum* and *Z. microcarpum*. These two species appear together in a clade which is resolved as a sister to the Middle Eastern clade composed of *Z. album* and *Z. coccineum*. The relationship between the two clades was expected, since *Z. album* and *Z. coccineum* also belong to the subgenus *Agrophyllum* (Van Huyssteen 1937). The two clades unexpectedly form a sister clade to the monotypic genus *Tetraena* (Sheahan and Chase 1996; 2000). Characters that separated *Tetraena* from *Zygophyllum* need to be revised since molecular data always seem to embed it within *Zygophyllum*.

Subgenus *Zygophyllum*. Southern African species of this subgenus appear to be closely related to the three Australian species *Z. fruticulosum*, *Z. billardierei* and *Z. glaucum*. The position of *Z. fruticulosum* embedded within this clade is unexpected, since Van Huyssteen (1937) assigned it to the subgenus *Agrophyllum*. Most of the southern African species in this clade belong to the section *Capensia* (Van Zyl 2000). However, *Z. cordifolium* which belongs to the section *Paradoxa* Van Zyl (2000) forms an unresolved relationships with the clades composed of Australian species; *Z. spitskopense* and *Z. fulvum*; *Z. hirticaule* and *Z. leucocladum*; *Z. cuneifolium*; and *Z. lichtensteinianum*.

In the *trn*L intron tree, *Z. teretifolium* appears as a close relative to *Z. cuneifolium*. The relationship between these two species is shown by swollen nodes, similar filament to scale ratio and large seeds in both species. They only differ with regard to leaf morphology, *Z. teretifolium* with terete, succulent leaflets and *Z. cuneifolium* with cuneate leaflets. With regard to the leaf morphology, *Z. lichtensteinianum* is very similar to *Z. foetidum*. Both have petiolate, bifoliate leaves with asymmetrical obovate leaflets. However, in the *trn*L tree, *Z. foetidum* appears to be related to the clade composed of *Z. spitskopense* and *Z. fulvum*. All three species have either cream to yellow or yellow flowers with red markings. The *Z. spitskopense*-*Z. fulvum* clade is supported by sessile, opposite, bifoliate leaves, with triangular rachis apices; triangular stipules, two on the ventral side and one on the dorsal side of the stem. With regard to floral morphology, *Z. fulvum* shows a close affinity to *Z. cuneifolium* and *Z. hirticaule*, because of the similarity in their staminal scales. The relationship between *Z. hirticaule* and *Z. leucocladum*, as shown by both trees, is also supported by similar staminal scales and filament to scale ratios. However, the two species differ in that the leaves of *Z. hirticaule* are sessile, while those of *Z. leucocladum* are subsessile to sessile. *Z. morgsana*, which is assigned to section *Morgsana* by Van Zyl (2000) also form the same unresolved sister relationship as *Z. cordifolium*. Adding more taxa from this subgenus (mostly from the section *Paradoxa*) might provide more information about the relationships within this clade.

Our results indicate that the *trn*LF region is useful in addressing questions on the reconstruction of the phylogenetic relationships in the genus *Zygophyllum*. Subgenus *Agrophyllum* is more distantly related to subgenus *Zygophyllum* than it is to *Fagonia*, *Augea* and *Tetraena*. There is also a strong support for monophyly of the three sections within the subgenus *Agrophyllum*. However, the transfer of the monotypic section *Grandifolia* from subgenus *Zygophyllum* to *Agrophyllum* is not confirmed. In order to do this it is essential to sample *Z. stapffii*. Despite the morphological evidence for the sections within subgenus *Zygophyllum*, our molecular data do not support the monophyly of all the included sections. The lack of resolution of *Z. cordifolium*, *Z. morgsana*, *Z. lichtesteinianum* in the overall subgenus *Zygophyllum* clade may indicate that these species represent more isolated elements within the subgenus *Zygophyllum* lineage. The addition of more taxa, including members of the unsampled sections, may shed more light on the sectional relationships within subgenus *Zygophyllum*. More evidence may also be gathered from other genes like *rbc*L and the ITS region. Combining independent characters often increases the resolution of the ingroup and the bootstrap support of the internal nodes of the phylogenetic trees (Olmstead & Sweere, 1994; Soltis *et al.* 1998). The *trn*L intron proved informative regarding the transfer of the monotypic section *Morgsana* from subgenus *Agrophyllum* to subgenus *Zygophyllum*. Although unresolved, the *trn*L intron data embed *Z. morgsana* within the subgenus *Zygophyllum* clade. Thus, one can cautiously conclude that morphological characters on which the subgeneric split of the genus was based by Van Zyl (2000) reflect the true phylogeny of the genus.

The molecular data seem to have implications for the biogeography of *Zygophyllum*. The southern African subgenus *Agrophyllum* species are closely related to the northern and eastern African and Middle Eastern species. *Z. rigidum* and *Z. microcarpum* (section *Alata* subsection *Alata*) together form a sister relationship with two Middle Eastern species *Z. album* and *Z. coccineum*. Morphologically, *Z. album* and *Z. coccineum* share the flower size and colour with the southern African members of the subgenus *Agrophyllum*. They all have small and white petals (Zohary 1972). Nothing concerning the fruit dehiscence was mentioned in the literature.

The southern African representatives of subgenus *Zygophyllum* are more closely related to the Australian species *Z. billardierei*, *Z. fruticulosum* and *Z. glaucum*. This southern hemisphere distribution may be explained by vicariance events following the break-up of Gondwana (Raven and Axelrod 1974). The southern African representatives of the subgenus *Zygophyllum* also share flower size and colour with the two Australian species *Z. billardierei* and *Z. glaucum*. They all have large and yellow flowers (Eichler 1981, 1986). The fruit dehiscence of both the species is also loculicidal (Bentham & Mueller 1863). However, the fruit of the other Australian species, *Z. fruticulosum* opens septicidally into indehiscent capsules (Bentham & Mueller 1863). The flowers resemble the ones for the subgenus *Zygophyllum* in that they are also large and yellow (often drying white). Since the mechanism for releasing and dispersing seeds in *Z. fruticulosum* differs from the loculicidal opening of the fruit in subgenus *Zygophyllum*, there is a conflict between molecular and morphological evidence. Morphological characters used to split the genus into two subgenera need to be standardised in order to confirm the relationship between the Australian *Z. fruticulosum* and members of the subgenus *Zygophyllum* as shown by molecular data (Sheahan and Chase 1996, 2000).

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APPENDICES

Appendix I. Aligned *trn*L sequences of 32 taxa, showing only altered sites in sequences *Z. hildebrandtii* to *Tribulus* (using *Fagonia* as a reference).

						60
Fagonia	ATACTAAGTG	ATCACTTTCA	AATTCAGAGA	AACCCTGGAA	TTAGAAATGG	GCAATCCTGA
Z.hildebrandtii	··········	··········	··········	··········	··········	··········
Z.robecchii	··········	··········	··········	··········	··········	··········
Augea	··········	··········	··········	··········	··········	··········
Z.cylindrifolium	N·········	······N···	··········	··········	··········	··········
Z.simplex	··········	··········	··········	··········	··········	··········
Z.decumbens	··········	··········	··········	··········	··········	··········
Z.d.var.decumbens	··········	··········	··········	··········	··········	··········
Z.retrofractum	··········	··········	··········	··········	··········	··········
Z.rigidum	··········	··········	··········	··········	··········	··········
Z.microcarpum	··········	··········	··········	··········	··········	··········
Z.tenue	··········	··········	··········	··········	··········	··········
Z.album	··········	··········	··········	··········	··········	··········
Z.coccineum	··········	··········	··········	··········	··········	··········
Tetraena	··········	··········	··········	··········	··········	··········
Z.xanthoxylum	··········	··········	··········	··········	··········	··········
Z.fabago	··········	··········	··········	··········	···C······	··········
Z.spitskopense	··········	··········	··········	··········	··········	··········
Z.leucocladum	··········	··········	··········	··········	··········	··········
Z.hirticaule	··········	··········	··········	··········	··········	··········
Z.fulvum	··········	··········	··········	··········	··········	··········
Z.cordifolium	··········	··········	··········	··········	··········	··········
Z.cuneifolium	··········	··········	··········	··········	··········	··········
Z.lichtensteinianum	··········	··········	··········	··········	··········	··········
Z.teretifolium	··········	··········	··········	··········	··········	··········
Z.foetidum	··········	··········	··········	··········	··········	··········
Z.morgsana	··········	··········	··········	··········	··········	··········
Z.billardierei	··········	··········	··········	··········	··········	··········
Z.fruticulosum	··········	··········	··········	··········	··········	··········
Z.glaucum	··········	··········	··········	··········	··········	··········
Seetzenia	C·········	··A·······	··········	··········	···A······	··········
Tribulus	C·········	··A·T·····	··········	··········	···A······	··········

						120
Fagonia	GCCAAATCCT	GTTTTCCTAA	AAAAAAAAAA	-----GAAGT	AAATC-GGAT	AGGTGCAGAG
Z.hildebrandtii	··········	·····A·G··	···-----C·	AAAAA···TC	·····-····	··········
Z.robecchii	··········	·····A·G··	···-------	----------	---·-·····	··········
Augea	··········	··········	··········	AA----··T-	------····	·········A
Z.cylindrifolium	··········	······TG··	··········	AAAA-···T·	C····-····	··········
Z.simplex	··········	·····ATG··	···G·-----	------··T·	C····-····	··········
Z.decumbens	··········	······TG··	··········	A-----··T·	C····-····	··········
Z.d.var.decumbens	··········	······TG··	··········	AA----··T·	C····-··T·	G·········
Z.retrofractum	··········	······TG··	·······---	------··T·	C····-····	··········
Z.rigidum	··········	······TG··	··········	A-----··T·	C····-····	··········
Z.microcarpum	··········	······TG··	·········-	------··T·	C····-····	··········
Z.tenue	··········	······TG··	··········	------··T·	C····-····	··········
Z.album	··········	······TG··	G·········	AA--------	C····-····	··········
Z.coccineum	··········	······TG··	··········	AA----··T·	C····-····	··········
Tetraena	··········	······TG··	·······---	-----T··T·	C····-····	··········
Z.xanthoxylum	··········	·······--·	·······---	------··T·	C··T---···	··········
Z.fabago	··········	·······--·	··········	-----T··T·	C····-····	··········
Z.spitskopense	··········	·-------··	··········	A----···--	---·-·····	··········
Z.leucocladum	··········	·-------··	·········-	------··--	---·-·····	··········
Z.hirticaule	··········	·-------··	··········	A-----··--	---·-·····	··········
Z.fulvum	··········	·-------··	··········	A-----··--	---·-·····	··········
Z.cordifolium	·-------··	··········	A-----··--	---·-·····	··········	
Z.cuneifolium	··········	·-------··	··········	AA----··--	---·-·····	··········
Z.lichtensteinianum	··········	·-------··	··········	------··--	---·-·····	··········
Z.teretifolium	··········	·-------··	··········	------··--	---·-·····	··········
Z.foetidum	··········	·-------··	··········	AAA---··--	---·-·····	··········
Z.morgsana	··········	·-------··	··········	A-----··--	---·-·····	··········
Z.billardierei	··········	·-------··	··········	-----·----	-------···	··········
Z.fruticulosum	··········	·-------··	·······---	------··--	---·---···	··········
Z.glaucum	··········	·-------··	·······---	------··T-	--·-----··	··········
Seetzenia	··········	·····A·G··	·---------	-----C··C·	··GAG-····	··········
Tribulus	··········	······TG··	·---------	-----C··A·	···GG-····	··········

						180
Fagonia	ACTCAACGGA	AGCTGTTCTA	AAAAATGGAG	TTGACTGCTG	CATTACGTTA	GTCAAGTCAA
Z.hildebrandtii	······T···	··········	··········	··········	···C······	········G·
Z.robecchii	······T···	··········	··········	··········	···CG·····	········G·
Augea	······T···	··········	··········	······ATG·	··········	-------···
Z.cylindrifolium	······T···	··········	··········	·········A	·····T····	-------···
Z.simplex	······T···	··········	··········	··········	·····T····	-------···
Z.decumbens	······T···	··········	··········	·········A	·····T····	-------···
Z.d.var.decumbens	······T···	··········	··········	·········A	·····T····	-------···
Z.retrofractum	······T···	··········	··········	·········A	·····T····	-------···
Z.rigidum	······T···	··········	··········	··········	·····T····	··········
Z.microcarpum	······T···	··········	··········	··········	·····T····	··········
Z.tenue	······T···	··········	··········	·········A	·····T····	-------···
Z.album	······T···	··········	··········	··········	·····T····	-------···
Z.coccineum	······T···	··T·······	··········	··········	·····T····	-------···
Tetraena	······T···	··········	··········	··········	·····T····	··········
Z.xanthoxylum	······T···	··········	··········	··········	······A···	··········
Z.fabago	······T···	··········	··········	··········	·····A····	-------···
Z.spitskopense	······T···	··········	··········	······AACA	T·A·······	··········
Z.leucocladum	······T···	··········	··········	··········	T·········	··········
Z.hirticaule	······T···	··········	··········	··········	T·········	··········
Z.fulvum	······T···	·C········	··········	······AACA	T·A·······	··········
Z.cordifolium	······T···	··········	··········	··········	T·········	··········
Z.cuneifolium	······T···	··········	··········	··········	T·········	··········
Z.lichtensteinianum	······T···	··········	··········	··········	T·········	··········
Z.teretifolium	······T···	··········	··········	··········	T·········	··········
Z.foetidum	······T···	··········	··········	······AAC·	T·A·······	··········
Z.morgsana	······T···	··········	··········	-----K····	T·········	··········
Z.billardierei	······T···	··T·······	··········	··········	T····A····	··········
Z.fruticulosum	······T···	··T·······	··········	··········	T····A····	··········
Z.glaucum	······T···	··T·······	··········	··········	T·········	··········
Seetzenia	······T···	··········	··········	·········-	--·······-	-------·A·
Tribulus	······T···	····A····C	··········	·····A··CT	---·······	-------···

						240
Fagonia	GGAATCCTTG	CATCGAAACT	TTTTCAAAGA	TAAAGGAT--	-----AACCT	TTTTTTC---
Z.hildebrandtii	··········	··········	·······G··	·G······--	-----·····	··C····---
Z.robecchii	··········	··········	·······G··	·G······--	-----·····	··C····---
Augea	··········	··········	·······G··	----------	-----·····	··C-------
Z.cylindrifolium	·····A····	····A·····	··C-A·····	·G··A···--	-----··A··	··C····---
Z.simplex	·····T····	····A·····	··CAA·····	·N··-···GA	AGAAT··A··	··C····---
Z.decumbens	·····A····	····A·····	··CAA·····	·G······--	-----··A··	··C····---
Z.d.var.decumbens	·····A····	····A·····	··CAA·····	·G······--	-----··A··	··C····---
Z.retrofractum	·····A····	····A·····	··CAA·····	·G··A···--	-----··A··	··C····---
Z.rigidum	······T···	····A·····	··CAA·····	·G······--	-----··A··	··C····---
Z.microcarpum	······T···	····A·····	··CAA·····	·G······--	-----··A··	··C····---
Z.tenue	·····A····	····A·····	··CAA·····	·G··A···--	-----··A··	··C····---
Z.album	··········	····A·····	··CAA·····	CG······--	-----··A··	··C····---
Z.coccineum	··········	····A·····	··CAA·····	CG······--	-----··A··	··C····---
Tetraena	··········	···AA·····	··CAA·····	·G······--	-----··A··	··C····---
Z.xanthoxylum	·T········	········T·	··CAA·····	········--	-----·····	··C····---
Z.fabago	··········	····A·····	··CAA·····	·G······--	-----·····	··C····TAC
Z.spitskopense	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.leucocladum	······T··A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.hirticaule	······T··A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.fulvum	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.cordifolium	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.cuneifolium	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.lichtensteinianum	·········A	··········	·AAAA··T··	·GG·T···--	-----·····	··C····---
Z.teretifolium	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.foetidum	·········A	····A·····	·AAAA·····	·G······--	-----··C··	··C····---
Z.morgsana	·····T···A	··········	·AAAA·····	·G······--	-----··N··	··C····---
Z.billardierei	·········A	··········	·AAAA·····	·G······--	-----·····	--C····---
Z.fruticulosum	·········A	··········	·AACA·····	·G······--	-----·····	··C····---
Z.glaucum	·········A	··········	GAAAA·····	·G······--	-----·····	··C····---
Seetzenia	·A···A···C	G·········	·CAGA·····	·G······--	-----····C	·A·A·A·TAT
Tribulus	······T··C	········T·	GAAGA·····	·G······--	-----····C	----------

```
                                                                                    300
Fagonia               ----TATCAA ACTCTC---- ---------- ---------- ---------- ----------
Z.hildebrandtii       -----··A·· ······---- ---------- ---------- ---------- ----------
Z.robecchii           -----··A·· ······---- ---------- ---------- ---------- ----------
Augea                 -----····· ······---- ---------- ---------- ---------- ----------
Z.cylindrifolium      -----·GA·· ·T·------- ---------- ---------- ---------- ----------
Z.simplex             -----·GA·· ···------- ---------- ---------- ---------- ----------
Z.decumbens           -----·GA·· ···------- ---------- ---------- ---------- ----------
Z.d.var.decumbens     -----·GA·· ···------- ---------- ---------- ---------- ----------
Z.retrofractum        -----·GA·· ···------- ---------- ---------- ---------- ----------
Z.rigidum             -----··A·· ···------- ---------- ---------- ---------- ----------
Z.microcarpum         -----··A·· ···------- ---------- ---------- ---------- ----------
Z.tenue               -----·GA·· ·T·A·AAACT ---------- ---------- ---------- ----------
Z.album               -----··A·· ·A·------- ---------- ---------- ---------- ----------
Z.coccineum           -----··A·· ·A·------- ---------- ---------- ---------- ----------
Tetraena              -----··A·· ···------- ---------- ---------- ---------- ----------
Z.xanthoxylum         -----··A·· ···------- ---------- ---------- ---------- ----------
Z.fabago              TTTC···A·· -·-------- ---------- ---------- ---------- ----------
Z.spitskopense        -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.leucocladum         -----·AA·· -··T------ ---------- ---------- ---------- ----------
Z.hirticaule          -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.fulvum              -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.cordifolium         -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.cuneifolium         -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.lichtensteinianum   -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.teretifolium        -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.foetidum            -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.morgsana            -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.billardierei        -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.fruticulosum        -----··A·· -··T------ ---------- ---------- ---------- ----------
Z.glaucum             -----··A·· ···T------ ---------- ---------- ---------- ----------
Seetzenia             A----··AT· -··T------ ATAC-TACTT AAATATTATA AAAAATAATA TTTAAATAAT
Tribulus              -----··AT- ---·C----- ATACGTACTT AAATAATATT GAAAATATTA TTGAAATAAT
```

```
                                                                                    360
Fagonia               ---------- ---------- ---------- ---------- ---------- ----------
Z.hildebrandtii       ---------- ---------- ---------- ---------- ---------- ----------
Z.robecchii           ---------- ---------- ---------- ---------- ---------- ----------
Augea                 ---------- ---------- ---------- ---------- ---------- ----------
Z.cylindrifolium      ---------- ---------- ---------- ---------- ---------- ----------
Z.simplex             ---------- ---------- ---------- ---------- ---------- ----------
Z.decumbens           ---------- ---------- ---------- ---------- ---------- ----------
Z.d.var.decumbens     ---------- ---------- ---------- ---------- ---------- ----------
Z.retrofractum        ---------- ---------- ---------- ---------- ---------- ----------
Z.rigidum             ---------- ---------- ---------- ---------- ---------- ----------
Z.microcarpum         ---------- ---------- ---------- ---------- ---------- ----------
Z.tenue               ---------- ---------- ---------- ---------- ---------- ----------
Z.album               ---------- ---------- ---------- ---------- ---------- ----------
Z.coccineum           ---------- ---------- ---------- ---------- ---------- ----------
Tetraena              ---------- ---------- ---------- ---------- ---------- ----------
Z.xanthoxylum         ---------- ---------- ---------- ---------- ---------- ----------
Z.fabago              ---------- ---------- ---------- ---------- ---------- ----------
Z.spitskopense        ---------- ---------- ---------- ---------- ---------- ----------
Z.leucocladum         ---------- ---------- ---------- ---------- ---------- ----------
Z.hirticaule          ---------- ---------- ---------- ---------- ---------- ----------
Z.fulvum              ---------- ---------- ---------- ---------- ---------- ----------
Z.cordifolium         ---------- ---------- ---------- ---------- ---------- ----------
Z.cuneifolium         ---------- ---------- ---------- ---------- ---------- ----------
Z.lichtensteinianum   ---------- ---------- ---------- ---------- ---------- ----------
Z.teretifolium        ---------- ---------- ---------- ---------- ---------- ----------
Z.foetidum            ---------- ---------- ---------- ---------- ---------- ----------
Z.morgsana            ---------- ---------- ---------- ---------- ---------- ----------
Z.billardierei        ---------- ---------- ---------- ---------- ---------- ----------
Z.fruticulosum        ---------- ---------- ---------- ---------- ---------- ----------
Z.glaucum             ---------- ---------- ---------- ---------- ---------- ----------
Seetzenia             ATATCAAATG ATTAATGATA ACTCAAATAT ATTTTT---- -----ATATA ATAAAATATT
Tribulus              ATCTCAATTG ATTCATAATG ACTCAAATCT ATTTTTTAGA TTTTCAAATT TATAAATATT
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                                                                                420
Fagonia               ---------- ----AAAATA AAAAAGATAA AGACTTTTG- ------GAAT CAA-----TT
Z.hildebrandtii       ---------- ----······ ··-------- -·······T- ------C··· T·-------·
Z.robecchii           ---------- ----······ ··-------- -·······T- ------C··· T·-------·
Augea                 ---------- ---A······ ·····T···· ·T······T- -------··· ··------·C
Z.cylindrifolium      ---------- -ATA······ ··-------- -T······T- ------C··· ··-------·
Z.simplex             ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.decumbens           ---------- --------·· ·····-·A·· CT······T- ------C··· ··-------·
Z.d.var.decumbens     ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.retrofractum        ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.rigidum             ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.microcarpum         ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.tenue               ---------A GAAA·TT··· ·····-·A·· ········T- ------C··· ··-------·
Z.album               ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.coccineum           ---------- --------·· ·····-·A·· -T······T- ------C··· ··-------·
Tetraena              ---------- --------·· ·····-·A·· ·T······T- ------C··· ··-------·
Z.xanthoxylum         ---------- -------··· ·····A·A-- -······AT- -------··· ··-------·
Z.fabago              ---------- -------··· ·····-·A·· ········T- -------··· ···ATCAA··
Z.spitskopense        ---------A AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.leucocladum         ---------A AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.hirticaule          -------AAA AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.fulvum              ---------A AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.cordifolium         ---------- AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.cuneifolium         ---------- AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.lichtensteinianum   ---------- AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.teretifolium        ---------- AAAA·····C ··-------- -TG·····T- -------··· ··-------·
Z.foetidum            ---------- AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.morgsana            ---------- AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.billardierei        -------AAA AAAA······ ··-------- -T······T- -------··· ··-------·
Z.fruticulosum        --------AA AAAA·····C ··-------- -T······T- -------··· ··-------·
Z.glaucum             ---------- AAAA······ ··-------- -T······T- -------··· ··-------·
Seetzenia             TATATGAAAA --------·G ·····A---- -T······TT TTTTTT··-· T·--------
Tribulus              TCTATGAAAA TATTT·T··G ····T··A·· -·······T- ---------- ---------·
```

```
                                                                                480
Fagonia               GGAAGTT--- ---TAAGAAA GAATCAAATA TGA-----TT TTATCAAATC ATTTACTC--
Z.hildebrandtii       ·······--- ---G······ ·········· ···-----·· ·A··A····· ········--
Z.robecchii           ·······--- ---G······ ·········· ···-----·· ·A··A····· ········--
Augea                 ·······--- ---G······ ·········· ···-----·· ·········· ········--
Z.cylindrifolium      ·A·····--- ---G··TC·· ·········C ·A·-----·· ·G········ ········--
Z.simplex             ·······--- ---G··TC·· ·······C·· ·A·-----·· ·G········ ········--
Z.decumbens           ·······--- ---G··TC·· ·········· ·A·-----·· ·G········ ········--
Z.d.var.decumbens     ·······--- ---G··TC·· ·········· ·A·-----·· ·G········ ········--
Z.retrofractum        ·A·····--- ---G··TC·· ·········· ·A·-----·· ·G········ ········--
Z.rigidum             ·······--- ---G··T··· ··G······· ·A·-----·· ·G········ ········--
Z.microcarpum         ·······--- ---G··T··· ··G······· ·A·-----·· ·G········ ········--
Z.tenue               ·AG····--- ---G··TC·· ·········C ·A·-----·· ·G········ ········--
Z.album               ·······--- ---G··T··· ·········· ·A·-----·· ·G········ ········--
Z.coccineum           ·······--- ---G··T··· ·········· ·A·-----·· ·G········ ········--
Tetraena              ·······--- ---G··T··· ·········· ·A·-----·· ·G········ ········--
Z.xanthoxylum         C······--- ---G······ ·········· ···-----·· ·G··A····· ········--
Z.fabago              ·······--- ---G······ ·········· ···-----·· ·G········ ········--
Z.spitskopense        ·AG····AGA GTTG······ ····T····· ···-----·· ·G··C····· ········--
Z.leucocladum         ·AG····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.hirticaule          ·AG····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.fulvum              ·AG····--- ---G······ ····TC···· ···-----·· ·G··C····· ········--
Z.cordifolium         ·AG····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.cuneifolium         ·AG····--- ---G······ ····TC···· ···TATGA·· ·G··C····· ········--
Z.lichtensteinianum   ·AG····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.teretifolium        ·AG····--- ---G······ ····TC···· ···-----·· ·G··C····· ········--
Z.foetidum            ·AG····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.morgsana            ·AG····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.billardierei        ··G····--- ---G······ ····T···AT ···-----·· ·G··C····· ········--
Z.fruticulosum        ··G····--- ---G······ ····T····· ···-----·· ·G··C····· ········--
Z.glaucum             ··G····--- ---G······ ····T····· ···-----·· ·G··C····· ········GC
Seetzenia             ---····--- ---G··T··· ····A····· ·T·-----·· ·G········ ···C····--
Tribulus              -····-C--- ---G·GTTG· AGGGGT···· AA·-----·C ·G········ ········--
```

```
                                                                                    540
Fagonia              ---------- --------CA AAGTCTGATA TA-------T CTTTTTC-AA AAAAA----G
Z.hildebrandtii      ---------- --------·· ·········· ·--------· A······-·· ··-----TA·
Z.robecchii          ---------- --------·· ·········· ·--------· A······-·· ··-----TA·
Augea                ---------- --------·· ······T··· ··TCTTTTA· ···----··· ·····AA--·
Z.cylindrifolium     ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.simplex            ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.decumbens          ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.d.var.decumbens    ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.retrofractum       ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.rigidum            ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.microcarpum        ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.tenue              ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.album              ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.coccineum          ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Tetraena             ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.xanthoxylum        ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.fabago             ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.spitskopense       ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.leucocladum        ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.hirticaule         ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.fulvum             ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.cordifolium        ---------- --------·· ·········· ·--------· ···G···-·· ··-----TG·
Z.cuneifolium        ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.lichtensteinianum  ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.teretifolium       ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.foetidum           ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.morgsana           ---------- --------·· ·········· ·--------· ·······-·· ··-----TG·
Z.billardierei       ---------- --------·· ·········C ·--------· ·······-·· ··-----TG·
Z.fruticulosum       ---------- --------·· ·········C ·--------· ·······-·· ··-----TG·
Z.glaucum            CAATCATTTA CTC-----·· ·········· ·--------· ·······-·· ··-----TG·
Seetzenia            ---------- --------·· ·········· G--------· ·····AC··· ·------TT·
Tribulus             ---------- --------·· ·········· G--------· ····AAC··· ·------TT·
```

```
                                                                                    600
Fagonia              ATTAGTCAGG CGAGAATAAA GATAGAGTCC CATTCTATAT GTCAATA--- ---------T
Z.hildebrandtii      ·········A ·········· ·········· -······G·· ····G··--- ---------·
Z.robecchii          ·········A ·········· ·········· ·········· ····G··--- ---------·
Augea                ·········A ·········· ·········· ·········· ·······--- ---------·
Z.cylindrifolium     ·········A T········· ·········- ·········· ····G··--- ---------·
Z.simplex            ·········A T········· ·········- ·········· ····G··--- ---------·
Z.decumbens          ·········A T········· ·········- ·········· ····G··--- ---------·
Z.d.var.decumbens    ·········A T········· ·········- ·········· ····G··--- ---------·
Z.retrofractum       ·········A T········· ·········- ·········· ····G··--- ---------·
Z.rigidum            ·········A T········· ·········· ·········· ····G··--- ---------·
Z.microcarpum        ·········A T········· ·········· ·········· ····G··--- ---------·
Z.tenue              ·········A T········· ·········- ·········· ····G··--- ---------·
Z.album              ·········A T········· ·········· ·········· ····G··--- ---------·
Z.coccineum          ·········A T········· ·········· ······C··· ····G··--- ---------·
Tetraena             ·········A T········· ·········· ·········· ····G··--- ---------·
Z.xanthoxylum        ·········A T········· ·········· ·········· ····G··TTT ACAA-----·
Z.fabago             ·········A T········· ·········· ·········· ····G··TTG ACAACAATA·
Z.spitskopense       ·········A T········· ·········· ·········· ····G··--- ---------·
Z.leucocladum        ·········A T········· ·········· ······T··· ····G··--- ---------·
Z.hirticaule         ·········A T········· ·········· ······T··· ····G··--- ---------·
Z.fulvum             ·········A T········· ·········· ·········· ····G··--- ---------·
Z.cordifolium        ·········A T········· ·········· ······T··· ····G··--- ---------·
Z.cuneifolium        ·········A T········· ·········· ······T··· ····G··--- ---------·
Z.lichtensteinianum  ·········A T········· ·········· ······T··· ····G··--- ---------·
Z.teretifolium       ·········A T········· ·········· ·········· ····G··--- ---------·
Z.foetidum           ·········A T········· ·········· ·········· ····G··--- ---------·
Z.morgsana           ·········A T········· ·········· ······T··· ····G··--- ---------·
Z.billardierei       ·········A T········· ·········· ·········· ····G··--- ---------G
Z.fruticulosum       ·········A T········· ·········· ·········· ····G··--- ---------G
Z.glaucum            ·········A T········· ·········· ·········· ····G··--- ---------·
Seetzenia            ··G····G·A T········· ·········· ·······C·· ·······--- ---------C
Tribulus             ·······G·A T········· ·········· ·······C·· ·······--- ---------C
```

Appendix II. Aligned *trn*LF sequences of 29 taxa, showing only altered sites in sequences *Z. hildebrandtii* to *Tribulus* (using *Fagonia* as a reference).

						60
Fagonia	ATACTAAGTG	ATCACTTTCA	AATTCAGAGA	AACCCTGGAA	TTAGAAATGG	GCAATCCTGA
Z.hildebrandtii	··········	··········	··········	··········	··········	··········
Z.robecchii	··········	··········	··········	··········	··········	··········
Augea	··········	··········	··········	··········	··········	··········
Z.cylindrifolium	N·········	······N···	··········	··········	··········	··········
Z.simplex	··········	··········	··········	··········	··········	··········
Z.decumbens	··········	··········	··········	··········	··········	··········
Z.d.var.decumbens	··········	··········	··········	··········	··········	··········
Z.retrofractum	··········	··········	··········	··········	··········	··········
Z.rigidum	··········	··········	··········	··········	··········	··········
Z.microcarpum	··········	··········	··········	··········	··········	··········
Z.tenue	··········	··········	··········	··········	··········	··········
Z.album	··········	··········	··········	··········	··········	··········
Z.coccineum	··········	··········	··········	··········	··········	··········
Tetraena	··········	··········	··········	··········	··········	··········
Z.xanthoxylum	··········	··········	··········	··········	··········	··········
Z.fabago	··········	··········	··········	··········	···C······	··········
Z.spitskopense	··········	··········	··········	··········	··········	··········
Z.leucocladum	··········	··········	··········	··········	··········	··········
Z.hirticaule	··········	··········	··········	··········	··········	··········
Z.fulvum	··········	··········	··········	··········	··········	··········
Z.cordifolium	··········	··········	··········	··········	··········	··········
Z.cuneifolium	··········	··········	··········	··········	··········	··········
Z.lichtensteinianum	··········	··········	··········	··········	··········	··········
Z.billardierei	··········	··········	··········	··········	··········	··········
Z.fruticulosum	··········	··········	··········	··········	··········	··········
Z.glaucum	··········	··········	··········	··········	··········	··········
Seetzenia	C·········	··A·······	··········	··········	···A······	··········
Tribulus	C·········	··A·T·····	··········	··········	···A······	··········

						120
Fagonia	GCCAAATCCT	GTTTTCCTAA	AAAAAAAAAA	-----GAAGT	AAATC-GGAT	AGGTGCAGAG
Z.hildebrandtii	··········	·····A·G··	····----C·	AAAAA···TC	·····-····	··········
Z.robecchii	··········	·····A·G··	···-------	----------	---··-····	··········
Augea	··········	··········	··········	AA----··T-	-------···	·········A
Z.cylindrifolium	··········	······TG··	··········	AAAA-···T·	C····-····	··········
Z.simplex	··········	·····ATG··	···G·-----	------··T·	C····-····	··········
Z.decumbens	··········	······TG··	··········	A-----··T·	C····-····	··········
Z.d.var.decumbens	··········	······TG··	··········	AA----··T·	C····-··T·	G·········
Z.retrofractum	··········	······TG··	·······---	------··T·	C····-····	··········
Z.rigidum	··········	······TG··	··········	A-----··T·	C····-····	··········
Z.microcarpum	··········	······TG··	·········-	------··T·	C····-····	··········
Z.tenue	··········	······TG··	··········	------··T·	C····-····	··········
Z.album	··········	······TG··	G·········	AA--------	C····-····	··········
Z.coccineum	··········	······TG··	··········	AA----··T·	C····-····	··········
Tetraena	··········	······TG··	·······---	-----T··T·	C····-····	··········
Z.xanthoxylum	··········	······---·	·······---	------··T·	C··T----··	··········
Z.fabago	··········	······---·	··········	-----T··T·	C····-····	··········
Z.spitskopense	··········	·--------·	··········	A----··---	---··-····	··········
Z.leucocladum	··········	·--------·	·········-	-----··---	---··-····	··········
Z.hirticaule	··········	·--------·	··········	A----··---	---··-····	··········
Z.fulvum	··········	·--------·	··········	A----··---	---··-····	··········
Z.cordifolium	··········	·--------·	··········	A----··---	---··-····	··········
Z.cuneifolium	··········	·--------·	··········	AA---··---	---··-····	··········
Z.lichtensteinianum	··········	·--------·	··········	-----··---	---··-····	··········
Z.billardierei	··········	·--------·	··········	-----·----	-------···	··········
Z.fruticulosum	··········	·--------·	······----	-----··---	---··-····	··········
Z.glaucum	··········	·--------·	······----	------··T-	--·----···	··········
Seetzenia	··········	·····A·G··	·---------	-----C···C	··GAG-····	··········
Tribulus	··········	······TG··	·---------	-----C··A·	···GG-····	··········

						180
Fagonia	ACTCAACGGA	AGCTGTTCTA	AAAAATGGAG	TTGACTGCTG	CATTACGTTA	GTCAAGTCAA
Z.hildebrandtii	······T···	··········	··········	··········	···C······	········G·
Z.robecchii	······T···	··········	··········	··········	···CG·····	········G·
Augea	······T···	··········	··········	······ATG·	··········	·------···
Z.cylindrifolium	······T···	··········	··········	·········A	·····T····	·------···
Z.simplex	······T···	··········	··········	··········	·····T····	·------···
Z.decumbens	······T···	··········	··········	·········A	·····T····	·------···
Z.d.var.decumbens	······T···	··········	··········	·········A	·····T····	·------···
Z.retrofractum	······T···	··········	··········	·········A	·····T····	·------···
Z.rigidum	······T···	··········	··········	··········	·····T····	··········
Z.microcarpum	······T···	··········	··········	··········	·····T····	··········
Z.tenue	······T···	··········	··········	·········A	·····T····	·------···
Z.album	······T···	··········	··········	··········	·····T····	·------···
Z.coccineum	······T···	··T·······	··········	··········	·····T····	·------···
Tetraena	······T···	··········	··········	··········	·····T····	··········
Z.xanthoxylum	······T···	··········	··········	··········	······A···	··········
Z.fabago	······T···	··········	··········	··········	·····A····	·------···
Z.spitskopense	······T···	··········	··········	······AACA	T·A·······	··········
Z.leucocladum	······T···	··········	··········	··········	T·········	··········
Z.hirticaule	······T···	··········	··········	··········	T·········	··········
Z.fulvum	······T···	·C········	··········	······AACA	T·A·······	··········
Z.cordifolium	······T···	··········	··········	··········	T·········	··········
Z.cuneifolium	······T···	··········	··········	··········	T·········	··········
Z.lichtensteinianum	······T···	··········	··········	··········	T·········	··········
Z.billardierei	······T···	··T·······	··········	··········	T····A····	··········
Z.fruticulosum	······T···	··T·······	··········	··········	T····A····	··········
Z.glaucum	······T···	··T·······	··········	··········	T·········	··········
Seetzenia	······T···	··········	··········	·········-	--·······-	------·A··
Tribulus	······T···	····A···C·	··········	····A···CT	---·······	·------···

						240
Fagonia	GGAATCCTTG	CATCGAAACT	TTTTCAAAGA	TAAAGGAT--	-----AACCT	TTTTTTC---
Z.hildebrandtii	··········	··········	·······G··	·G······--	-----·····	··C····---
Z.robecchii	··········	··········	·······G··	·G······--	-----·····	··C····---
Augea	··········	··········	·······G··	----------	-----·····	··C-------
Z.cylindrifolium	·····A····	····A·····	··C-A·····	·G··A···--	------·A··	··C····---
Z.simplex	·····T····	····A·····	··CAA·····	·N·-····GA	AGAAT··A··	··C····---
Z.decumbens	·····A····	····A·····	··CAA·····	·G······--	------·A··	··C····---
Z.d.var.decumbens	·····A····	····A·····	··CAA·····	·G······--	------·A··	··C····---
Z.retrofractum	·····A····	····A·····	··CAA·····	·G··A···--	------·A··	··C····---
Z.rigidum	······T···	····A·····	··CAA·····	·G······--	------·A··	··C····---
Z.microcarpum	······T···	····A·····	··CAA·····	·G······--	------·A··	··C····---
Z.tenue	·····A····	····A·····	··CAA·····	·G··A···--	------·A··	··C····---
Z.album	··········	····A·····	··CAA·····	CG······--	------·A··	··C····---
Z.coccineum	··········	····A·····	··CAA·····	CG······--	------·A··	··C····---
Tetraena	··········	···AA·····	··CAA·····	·G······--	------·A··	··C····---
Z.xanthoxylum	·T········	········T·	··CAA·····	········--	-----·····	··C····---
Z.fabago	··········	····A·····	··CAA·····	·G······--	-----·····	··C····TAC
Z.spitskopense	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.leucocladum	······T··A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.hirticaule	······T··A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.fulvum	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.cordifolium	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.cuneifolium	·········A	··········	·AAAA·····	·G······--	-----·····	··C····---
Z.lichtensteinianum	·········A	··········	·AAAA··T··	·GG·T···--	-----·····	··C····---
Z.billardierei	·········A	··········	·AAAA·····	·G······--	-----·····	--C····---
Z.fruticulosum	·········A	··········	·AACA·····	·G······--	-----·····	··C····---
Z.glaucum	·········A	··········	GAAAA·····	·G······--	-----·····	··C····---
Seetzenia	·A···A···C	G·········	·CAGA·····	·G······--	-----····C	·A·A·A·TAT
Tribulus	······T··C	········T·	GAAGA·····	·G······--	-----····C	----------

						300
Fagonia	----TATCAA	ACTCTC----	----------	----------	----------	----------
Z.hildebrandtii	-----··A··	······----	----------	----------	----------	----------
Z.robecchii	-----··A··	······----	----------	----------	----------	----------
Augea	-----·····	······----	----------	----------	----------	----------
Z.cylindrifolium	-----·GA··	·T·-------	----------	----------	----------	----------
Z.simplex	-----·GA··	···-------	----------	----------	----------	----------
Z.decumbens	-----·GA··	···-------	----------	----------	----------	----------
Z.d.var.decumbens	-----·GA··	···-------	----------	----------	----------	----------
Z.retrofractum	-----·GA··	···-------	----------	----------	----------	----------
Z.rigidum	-----··A··	···-------	----------	----------	----------	----------
Z.microcarpum	-----··A··	··--------	----------	----------	----------	----------
Z.tenue	-----·GA··	·T·A·AAACT	----------	----------	----------	----------
Z.album	-----··A··	·A··------	----------	----------	----------	----------
Z.coccineum	-----··A··	·A··------	----------	----------	----------	----------
Tetraena	-----··A··	···-------	----------	----------	----------	----------
Z.xanthoxylum	-----··A··	··--------	----------	----------	----------	----------
Z.fabago	TTTC···A··	-··-------	----------	----------	----------	----------
Z.spitskopense	-----··A··	--·T------	----------	----------	----------	----------
Z.leucocladum	-----·AA··	--·T------	----------	----------	----------	----------
Z.hirticaule	-----··A··	--·T------	----------	----------	----------	----------
Z.fulvum	-----··A··	--·T------	----------	----------	----------	----------
Z.cordifolium	-----··A··	--·T------	----------	----------	----------	----------
Z.cuneifolium	-----··A··	--·T------	----------	----------	----------	----------
Z.lichtensteinianum	-----··A··	--·T------	----------	----------	----------	----------
Z.billardierei	-----··A··	--·T------	----------	----------	----------	----------
Z.fruticulosum	-----··A··	--·T------	----------	----------	----------	----------
Z.glaucum	-----··A··	···T------	----------	----------	----------	----------
Seetzenia	A----··AT·	--·T------	ATAC-TACTT	AAATATTATA	AAAAATAATA	TTTAAATAAT
Tribulus	-----··AT-	---·C-----	ATACGTACTT	AAATAATATT	GAAAATATTA	TTGAAATAAT

						360
Fagonia	----------	----------	----------	----------	----------	----------
Z.hildebrandtii	----------	----------	----------	----------	----------	----------
Z.robecchii	----------	----------	----------	----------	----------	----------
Augea	----------	----------	----------	----------	----------	----------
Z.cylindrifolium	----------	----------	----------	----------	----------	----------
Z.simplex	----------	----------	----------	----------	----------	----------
Z.decumbens	----------	----------	----------	----------	----------	----------
Z.d.var.decumbens	----------	----------	----------	----------	----------	----------
Z.retrofractum	----------	----------	----------	----------	----------	----------
Z.rigidum	----------	----------	----------	----------	----------	----------
Z.microcarpum	----------	----------	----------	----------	----------	----------
Z.tenue	----------	----------	----------	----------	----------	----------
Z.album	----------	----------	----------	----------	----------	----------
Z.coccineum	----------	----------	----------	----------	----------	----------
Tetraena	----------	----------	----------	----------	----------	----------
Z.xanthoxylum	----------	----------	----------	----------	----------	----------
Z.fabago	----------	----------	----------	----------	----------	----------
Z.spitskopense	----------	----------	----------	----------	----------	----------
Z.leucocladum	----------	----------	----------	----------	----------	----------
Z.hirticaule	----------	----------	----------	----------	----------	----------
Z.fulvum	----------	----------	----------	----------	----------	----------
Z.cordifolium	----------	----------	----------	----------	----------	----------
Z.cuneifolium	----------	----------	----------	----------	----------	----------
Z.lichtensteinianum	----------	----------	----------	----------	----------	----------
Z.billardierei	----------	----------	----------	----------	----------	----------
Z.fruticulosum	----------	----------	----------	----------	----------	----------
Z.glaucum	----------	----------	----------	----------	----------	----------
Seetzenia	ATATCAAATG	ATTAATGATA	ACTCAAATAT	ATTTTT----	-----ATATA	ATAAAATATT
Tribulus	ATCTCAATTG	ATTCATAATG	ACTCAAATCT	ATTTTTTAGA	TTTTCAAATT	TATAAATATT

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                                                                                   420
Fagonia                  ---------- ----AAAATA AAAAAGATAA AGACTTTTG- ------GAAT CAA-----TT
Z.hildebrandtii          ---------- ----······ ···------- -·······T- ------C··· T·-------·
Z.robecchii              ---------- ----······ ···------- -·······T- ------C··· T·-------·
Augea                    ---------- ---A······ ·····T···· ·T······T- ------···· ·-------·C
Z.cylindrifolium         ---------- -ATA······ ···------- -T······T- ------C··· ··-------·
Z.simplex                ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.decumbens              ---------- ---------· ·····-·A·· CT······T- ------C··· ··-------·
Z.d.var.decumbens        ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.retrofractum           ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.rigidum                ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.microcarpum            ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.tenue                  ---------A GAAA·TT··· ·····-·A·· ········T- ------C··· ··-------·
Z.album                  ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.coccineum              ---------- ---------· ·····-·A·· -T······T- ------C··· ··-------·
Tetraena                 ---------- ---------· ·····-·A·· ·T······T- ------C··· ··-------·
Z.xanthoxylum            ---------- --------·· ·····A·A-- -······AT- -------··· ··-------·
Z.fabago                 ---------- --------·· ·····-·A·· ········T- -------··· ···ATCAA··
Z.spitskopense           ---------A AAAA·····C ···------- -T······T- -------··· ··-------·
Z.leucocladum            ---------A AAAA·····C ···------- -T······T- -------··· ··-------·
Z.hirticaule             -------AAA AAAA·····C ···------- -T······T- -------··· ··-------·
Z.fulvum                 ---------A AAAA·····C ···------- -T······T- -------··· ··-------·
Z.cordifolium            ---------- AAAA·····C ···------- -T······T- -------··· ··-------·
Z.cuneifolium            ---------- AAAA·····C ···------- -T······T- -------··· ··-------·
Z.lichtensteinianum      ---------- AAAA·····C ···------- -T······T- -------··· ··-------·
Z.billardierei           -------AAA AAAA······ ···------- -T······T- -------··· ··-------·
Z.fruticulosum           --------AA AAAA·····C ···------- -T······T- -------··· ··-------·
Z.glaucum                ---------- AAAA······ ···------- -T······T- -------··· ··-------·
Seetzenia                TATATGAAAA --------·G ·····A---- -T······TT TTTTTT··-· T·--------
Tribulus                 TCTATGAAAA TATTT·T··G ····T··A·· -·······T- ---------- ---------·
```

```
                                                                                   480
Fagonia                  GGAAGTT--- ---TAAGAAA GAATCAAATA TGA-----TT TTATCAAATC ATTTACTC--
Z.hildebrandtii          ·······--- ---G······ ·········· ·-------·· ·A··A····· ········--
Z.robecchii              ·······--- ---G······ ·········· ·-------·· ·A··A····· ········--
Augea                    ·······--- ---G······ ·········· ·-------·· ·········· ········--
Z.cylindrifolium         ·A·····--- ---G··TC·· ·········C ·A------·· ·G········ ········--
Z.simplex                ·······--- ---G··TC·· ·······C·· ·A------·· ·G········ ········--
Z.decumbens              ·······--- ---G··TC·· ·········· ·A------·· ·G········ ········--
Z.d.var.decumbens        ·······--- ---G··TC·· ·········· ·A------·· ·G········ ········--
Z.retrofractum           ·A·····--- ---G··TC·· ·········· ·A------·· ·G········ ········--
Z.rigidum                ·······--- ---G··T··· ··G······· ·A------·· ·G········ ········--
Z.microcarpum            ·······--- ---G··T··· ··G······· ·A------·· ·G········ ········--
Z.tenue                  ·AG····--- ---G··TC·· ·········C ·A------·· ·G········ ········--
Z.album                  ·······--- ---G··T··· ·········· ·A------·· ·G········ ········--
Z.coccineum              ·······--- ---G··T··· ·········· ·A------·· ·G········ ········--
Tetraena                 ·······--- ---G··T··· ·········· ·A------·· ·G········ ········--
Z.xanthoxylum            C······--- ---G······ ·········· ·-------·· ·G··A····· ········--
Z.fabago                 ·······--- ---G······ ·········· ·-------·· ·G········ ········--
Z.spitskopense           ·AG····AGA GTTG······ ····T····· ·-------·· ·G···C···· ········--
Z.leucocladum            ·AG····--- ---G······ ····T····· ·-------·· ·G···C···· ········--
Z.hirticaule             ·AG····--- ---G······ ····T····· ·-------·· ·G···C···· ········--
Z.fulvum                 ·AG····--- ---G······ ····TC···· ·-------·· ·G···C···· ········--
Z.cordifolium            ·AG····--- ---G······ ····T····· ·-------·· ·G···C···· ········--
Z.cuneifolium            ·AG····--- ---G······ ····TC···· ···TATGA·· ·G···C···· ········--
Z.lichtensteinianum      ·AG····--- ---G······ ····T····· ·-------·· ·G···C···· ········--
Z.billardierei           ··G····--- ---G······ ····T···AT ·-------·· ·G···C···· ········--
Z.fruticulosum           ··G····--- ---G······ ····T····· ·-------·· ·G···C···· ········--
Z.glaucum                ··G····--- ---G······ ····T····· ·-------·· ·G···C···· ········GC
Seetzenia                ---····--- ---G··T··· ····A····· ·T------·· ·G········ ···C····--
Tribulus                 -···-·C--- ---G·GTTG· AGGGGT···· AA------·C ·G········ ········--
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						540
Fagonia	----------	---CAAAGTC	TGATATA---	----TCTTTT	TC-AAAAAAA	----GATTAG
Z.hildebrandtii	----------	---·······	·······---	-----A····	··-····---	--TA······
Z.robecchii	----------	---·······	·······---	-----A····	··-····---	--TA······
Augea	----------	---·······	·T·····TCT	TTTA······	---·······	AA--······
Z.cylindrifolium	----------	---·······	·······---	----······	··-····---	--TG······
Z.simplex	----------	---·······	·······---	----······	··-····---	--TG······
Z.decumbens	----------	---·······	·······---	----······	··-····---	--TG······
Z.d.var.decumbens	----------	---·······	·······---	----······	··-····---	--TG······
Z.retrofractum	----------	---·······	·······---	----······	··-····---	--TG······
Z.rigidum	----------	---·······	·······---	----······	··-····---	--TG······
Z.microcarpum	----------	---·······	·······---	----······	··-····---	--TG······
Z.tenue	----------	---·······	·······---	----······	··-····---	--TG······
Z.album	----------	---·······	·······---	----······	··-····---	--TG······
Z.coccineum	----------	---·······	·······---	----······	··-····---	--TG······
Tetraena	----------	---·······	·······---	----······	··-····---	--TG······
Z.xanthoxylum	----------	---·······	·······---	----······	··-····---	--TG······
Z.fabago	----------	---·······	·······---	----······	··-····---	--TG······
Z.spitskopense	----------	---·······	·······---	----······	·--····---	--TG······
Z.leucocladum	----------	---·······	·······---	----······	·--····---	--TG······
Z.hirticaule	----------	---·······	·······---	----······	·--····---	--TG······
Z.fulvum	----------	---·······	·······---	----······	·--····---	--TG······
Z.cordifolium	----------	---·······	·······---	-------·G·	·--····---	--TG······
Z.cuneifolium	----------	---·······	·······---	----······	··-····---	--TG······
Z.lichtensteinianum	----------	---·······	·······---	----······	·--····---	--TG······
Z.billardierei	----------	---·······	····C··---	----······	·--····---	--TG······
Z.fruticulosum	----------	---·······	····C··---	----······	··-····---	--TG······
Z.glaucum	CAATCATTTA	CTC·······	·······---	----······	·--····---	--TG······
Seetzenia	----------	---·······	·····G·---	----······	·AC···----	--TT···G··
Tribulus	----------	---·······	·····G·---	----······	AAC···----	--TT······

						600
Fagonia	TCAGGCGAGA	ATAAAGATAG	AGTCCCATTC	TATATGTCAA	TA--------	----TTGACA
Z.hildebrandtii	····A·····	··········	·······-··	··G······G	·---------	----······
Z.robecchii	····A·····	··········	··········	·········G	·---------	----······
Augea	····A·····	··········	··········	··········	·---------	----······
Z.cylindrifolium	····AT····	··········	····-·····	·········G	·---------	----······
Z.simplex	····AT····	··········	····-·····	·········G	·---------	----······
Z.decumbens	····AT····	··········	····-·····	·········G	·---------	----······
Z.d.var.decumbens	····AT····	··········	····-·····	·········G	·---------	----······
Z.retrofractum	····AT····	··········	····-·····	·········G	·---------	----······
Z.rigidum	····AT····	··········	··········	·········G	·---------	----·C····
Z.microcarpum	····AT····	··········	··········	·········G	·---------	----·C····
Z.tenue	····AT····	··········	····-·····	·········G	·---------	----······
Z.album	····AT····	··········	··········	·········G	·---------	----·C····
Z.coccineum	····AT····	··········	··········	·C·······G	·---------	----·CA···
Tetraena	····AT····	··········	··········	·········G	·---------	----······
Z.xanthoxylum	····AT····	··········	··········	·········G	··TTTACAA-	----······
Z.fabago	····AT····	··········	··········	·········G	··TTGACAAC	AATA······
Z.spitskopense	····AT····	··········	··········	·········G	·---------	----·C····
Z.leucocladum	····AT····	··········	··········	·T·······G	·---------	----·C····
Z.hirticaule	····AT····	··········	··········	·T·······G	·---------	----·C····
Z.fulvum	····AT····	··········	··········	·········G	·---------	----·C····
Z.cordifolium	····AT····	··········	··········	·T·······G	·---------	----·C····
Z.cuneifolium	····AT····	··········	··········	·T·······G	·---------	----·C····
Z.lichtensteinianum	····AT····	··········	··········	·T·······G	·---------	----·C····
Z.billardierei	····AT····	··········	··········	·········G	·---------	----GC····
Z.fruticulosum	····AT····	··········	··········	·········G	·---------	----GC····
Z.glaucum	····AT····	··········	··········	·········G	·---------	----·C····
Seetzenia	··G·AT····	··········	··········	··C·······	·---------	----CC·G··
Tribulus	··G·AT····	··········	··········	··C·······	·---------	----CC····

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                                                                             660
Fagonia               ACAATGAAAT TTATAGTGAG AGGAAAATCC GTCGATTTTA TCAA-TGTGG AGGGTTNAAG
Z.hildebrandtii       .......... .......... .......... ......-... .A..A.CGT. ......C...
Z.robecchii           .......... .......... .......... ......-... .A..A.CGT. ......C...
Augea                 .......... .......... .......... .....A.... ....-.CG.. ......C...
Z.cylindrifolium      .......... .......... .......... ......-... NA..-.CGTA ....NN.NNN
Z.simplex             .......... .......... .....C.... ......-... .A..-.CGT. ......C...
Z.decumbens           .......... .......... .......... ......-... .A..-.CGT. ......C...
Z.d.var.decumbens     .......... .......... .......... ......-... .A..-.CGT. ......C...
Z.retrofractum        .......... .......... .......... ......-... .A..-.CGT. ......C...
Z.rigidum             .......... .......... .......... ......-... .A..A.CGT. ......C...
Z.microcarpum         .......... .......... .......... ......-... .A..A.CGT. ......C...
Z.tenue               .......... .......... .......... ......-... GA..-.CGT. ......C...
Z.album               .......... .......... .......... .......... .A..-.CGT. ......C...
Z.coccineum           .......... .......... .......... .......... .A..-.CGT. ......C...
Tetraena              .......... .......... .......... .......... .A..-.CGT. ......C...
Z.xanthoxylum         .......... .......... .......... .......... .A..-.CGT. ......C...
Z.fabago              .......... .......... .......... .......... .A..-.CGT. ......C...
Z.spitskopense        .......... ...A...... .......... .......... .A..-.TGT. ......C...
Z.leucocladum         .......... ...A...... .......... .......... .A..-.TGT. ......C...
Z.hirticaule          .......... ...A...... .......... .......... .A..-.TGT. ......C...
Z.fulvum              .......... ...A...... .......... .......... GA..-.TGT. ......C...
Z.cordifolium         .......... ...A...... .......... -......... .A..-.TGT. ......C...
Z.cuneifolium         .......... ...A...... .......... .......... .A..-.TGT. ......C...
Z.lichtensteinianum   .......... ...A...... .......... .......... .A..-.TGT. ......C...
Z.billardierei        .T........ ...A...... .......... .......... .A..-.TGT. ......C...
Z.fruticulosum        .......... ...A...... .......... .......... .A..-.TGTN ......C...
Z.glaucum             .......... ...A...... .......... .......... .A..-.TGT. ......C...
Seetzenia             .......... .......A.. .......... ..T......T .A..-.CGT. ......C...
Tribulus              .......... .......A.. .......... .......... .A..-.TGT. ......C...
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                                                                             720
Fagonia               TCCCTTTATC CCCAAAA--- -------AAG TCCTGGTTGA ATCCCGNAAT GATCTATCTT
Z.hildebrandtii       .....C.... .......--- --------.. ...C...... .....TT... ..........
Z.robecchii           .....C.... .......--- --------.. ...C...... .....TT... ..........
Augea                 .......... .......TTA TCCCAAA... .......G.. ......T... ......G...
Z.cylindrifolium      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNN------ ----------
Z.simplex             .....C.... .......--- --------.. ...C....C. .....TT... ..GA......
Z.decumbens           .....CC... .......--- --------.. ...C....C. .....TG... ...A......
Z.d.var.decumbens     .....C.... .......--- --------.. ...C....C. .....TT... ...A......
Z.retrofractum        .....C.... .......--- ---------. ...C....C. .....TT... ...A......
Z.rigidum             .....C.... .......--- --------.. ...C....C. .....TT... ..........
Z.microcarpum         .....C.... .......--- --------.. ...C....C. .....TT... ..........
Z.tenue               .....C.... .......--- ---------. ...C.A---- -....TTC.. ...A......
Z.album               .....C.... .......--- --------.. ........C. .....TT... .......T..
Z.coccineum           .....C.... .......--- --------.. ........C. .....TT... .......T..
Tetraena              .....C.... .......--- --------.. ...C....C. .....TT... ...A......
Z.xanthoxylum         .....C.... .......--- --------.. ...C...... .....TT... .......---
Z.fabago              .....C.... .......--- --------.. ...C...... .....TT... .......---
Z.spitskopense        .....C.... .......--- --------.. .....A.... .....TT... ..........
Z.leucocladum         .....C.... .......--- --------.. .......... .....TT... ..........
Z.hirticaule          .....C.... .......--- --------.. .......... .....TT... ..........
Z.fulvum              .....C.... .......--- --------.. .....A.... ....ATT... ..........
Z.cordifolium         .....C.... .......--- --------.. .......... .....TT... ..........
Z.cuneifolium         .....C.... .......--- --------.. .......... .....TT... ..........
Z.lichtensteinianum   .....C.... .......--- --------.. .......... .....TT... ..........
Z.billardierei        .....C.... .......--- --------.. .......... ....TTT... ..........
Z.fruticulosum        .....C.... .......--- --------.. .......... ....TTT... ..........
Z.glaucum             .....C.... ...N.----- ---------- ---------- ---------. ..........
Seetzenia             .....C.... .......--- --------.. A.TC.AG.T. C....-T... T..T......
Tribulus              .....C.... .......--- --------G. C.---...T. C....-T... T..T......
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						780
Fagonia	CCCTCTCGTT	C-------AA	-A-AAA--AA	AAA----TTC	-A--------	TTCTGTTTC-
Z.hildebrandtii	T·········	T-------··	----··TT··	···AGAA···	-·AAA-----	··--·A··TG
Z.robecchii	T·········	T-------··	----··TT··	N··AGAA···	-·AAA-----	··--·A···G
Augea	··········	---------·	-·-···TT··	···AAA-···	-·--------	·········-
Z.cylindrifolium	A···A·····	TTTTT--G··	T·G···TTTC	···----···	G---------	··A·····T-
Z.simplex	A··GA····N	TTTTT--G··	T·G···TTC·	···----···	G---------	··A·····T-
Z.decumbens	G···A·····	TTTTT--G··	T·G···TTTC	···----···	G---------	··A·····T-
Z.d.var.decumbens	G···A·····	TTTTT--G··	T·G···TTTC	···----···	G---------	··A·····T-
Z.retrofractum	A·-·A·····	TTTTT--G··	T·G···TTC·	···----···	C---------	··A·····T-
Z.rigidum	A···A··A··	TTTT---G··	T·T···TTC·	···----···	G---------	··A·····AT
Z.microcarpum	A···A··A··	TTTT---G··	T·T···TTC·	···----···	G---------	··A·····AT
Z.tenue	A···A··A··	TTTTTTTG··	T·G···TTTC	···----···	G---------	··A·····--
Z.album	A···A··A··	TTTT---G··	T·T···TT··	···----···	G---------	··A·····A-
Z.coccineum	A···A··A··	TTTT---G··	T·T···TTC·	···----···	G---------	··A·····A-
Tetraena	A···A··A··	TTTT---G··	T·T···TTC·	···-------	G·AAAATTCG	··A·····A-
Z.xanthoxylum	-T········	TTTT---G··	T·G···TTC·	···----···	G---------	··A·····G-
Z.fabago	-·········	TTTG---G··	T·G···TTC·	···----···	G---------	··A······-
Z.spitskopense	A·········	TGTT---G··	T·G···TTC·	···----···	G---------	··A······-
Z.leucocladum	A·········	TGTT---G··	T·G···TTC·	···----···	G---------	··A······-
Z.hirticaule	A·········	TGTT---G··	T·G···TTC·	···----···	G---------	··A······-
Z.fulvum	A·········	TGTT---G··	T·G···TTC·	···----···	G---------	··A······-
Z.cordifolium	A·········	TGTT---G··	T·G···TTCG	···----···	G---------	··A······-
Z.cuneifolium	A·········	TGTT---G··	T·G···TTC·	···----···	G---------	··A······-
Z.lichtensteinianum	A·········	TGTT---G··	T·G···TTC·	···----···	G---------	··A······-
Z.billardierei	A·····A···	TGTTT----·	T·G···TT··	···----···	G---------	··A······-
Z.fruticulosum	A·····A···	TGTTT----·	T·G···TTC·	···----···	G---------	··A······-
Z.glaucum	A······A·N	TCTT---G··	T·G···TTC·	N··----···	G---------	··A······-
Seetzenia	AT··TAACA·	·TCTTTCGTT	AGC·G-TT··	···----···	G---------	··A·····A-
Tribulus	AG·----C·C	TCTTTTCGTT	ATT·GTTC··	···----···	G---------	··A·C····-

						840
Fagonia	-----TTATT	TATTC-TAC-	GCT-AAAAAA	AAAAAAAA--	----------	----------
Z.hildebrandtii	GTTTT·····	G·····-··-	···T······	········--	----------	----------
Z.robecchii	G-TTT·····	G·····-··-	···T······	···-------	----------	----------
Augea	-----·····	G·····-··-	··········	···-------	----------	----------
Z.cylindrifolium	----T·····	G·····-··-	···T······	········AA	A---------	----------
Z.simplex	-----·····	G·····-··-	···T······	········AA	AAA-------	----------
Z.decumbens	----T·····	G·····-··-	···T·····G	···T····AG	CTTAAAAATC	AATAAAAAAA
Z.d.var.decumbens	----T·····	G·····-··-	···T·····G	···T····AG	CTTAAAAATC	AATAAAAAAA
Z.retrofractum	--TTT·····	G·····-··-	···T······	········AA	----------	----------
Z.rigidum	TTTTA·····	G·····-··-	···T······	···-------	----------	----------
Z.microcarpum	TTTTA·····	G·····-··-	···T······	···-------	----------	----------
Z.tenue	---TT····C	G·····-··-	···T··----	--·TT···AA	AAAAA-----	----------
Z.album	-----·····	G·····-··-	···T······	···-------	----------	----------
Z.coccineum	-----·----	----------	----------	----------	----------	----------
Tetraena	-----·····	G····TAC--	···T······	···-------	----------	----------
Z.xanthoxylum	-----····G	G········-	··AT···---	----------	----------	----------
Z.fabago	-----····G	G········-	·T-·······	········AA	AC--------	----------
Z.spitskopense	-----·····	G·--------	--·A······	···T····AA	AAAAATTGAT	GCTC------
Z.leucocladum	-----·····	G·--------	-----·····	···T····AA	AAAAAC----	----------
Z.hirticaule	-----·····	G·--------	-----·····	···T····AA	AAAAC-----	----------
Z.fulvum	-----·····	G·--------	-----·····	···T·C··AA	AAAAAC----	----------
Z.cordifolium	-----·····	G·--------	-----·····	···C·-----	----------	----------
Z.cuneifolium	-----·····	G·--------	·----·····	···T···TAA	AAAAAAAAC-	----------
Z.lichtensteinianum	-----·····	G·--------	--·-······	···T····AA	AAA-------	----------
Z.billardierei	-----·····	G····T··TT	·A-·······	···T····AA	AAA-------	----------
Z.fruticulosum	-----·····	G····T··TT	·A-·······	···TT···AA	AAAAAAAA--	----------
Z.glaucum	-----·····	G··NN-·TNN	T---------	---·T·N·NA	----------	----------
Seetzenia	-----·C···	C········-	T··TTCCGGG	----------	----------	----------
Tribulus	-----·C···	C········T	CT·T·G·---	----------	----------	----------

						900
Fagonia	--GATCTGAG	CATCCATTTT	----------	------TTTT	TTT-GA-GG-	-ATATATGAT
Z.hildebrandtii	--········	···AA-····	TT--------	-------··C	CA--------	G·········
Z.robecchii	--········	···AA-····	T---------	-------··C	CA------·-	G·········
Augea	-A········	··········	----------	--------··	··A-----·-	-·········
Z.cylindrifolium	----------	----------	----------	----------	----------	----------
Z.simplex	--········	A···A·····	----------	-------··C	·A·T·TG··T	G······T··
Z.decumbens	-A········	A··AA·····	----------	-------··C	·A·T·TG··T	G······T··
Z.d.var.decumbens	-A···A····	A··AA·····	----------	-------··C	·A·T·TG··T	G······T··
Z.retrofractum	----------	----------	----------	----------	----------	----------
Z.rigidum	----······	A··AA·····	----------	-------··C	·A·T·TG··T	G·········
Z.microcarpum	----······	A··AA·····	----------	-------··C	·A·T·TG··T	G·········
Z.tenue	----------	----------	----------	----------	----------	----------
Z.album	----······	A··AA·····	----------	-------··C	·A·T·TG··T	G········C
Z.coccineum	----······	A··AA·····	----------	-------··C	·A·T·TG··T	G·········
Tetraena	----······	A··AA·····	----------	-------··C	·A·T·TG··T	G·········
Z.xanthoxylum	----------	-------···	----------	-------··C	·A·T·TG··T	G·········
Z.fabago	-A········	···AA·····	TT-------T	TTCTAT···C	·A·T·TG··T	G·········
Z.spitskopense	-A········	····A·····	TTT-------	----------	------TTAT	A·········
Z.leucocladum	-A········	···AA·····	T---------	-------··C	---------T	A·········
Z.hirticaule	-A········	···AA·····	TT--------	-------··C	---------T	A·········
Z.fulvum	-A········	····A·····	T---------	-------··G	·A-------T	A·········
Z.cordifolium	-A········	···AA·····	TTT-------	-------··C	·A--------	-·········
Z.cuneifolium	-A········	···AA·····	TT--------	-------··C	·A--------	-·········
Z.lichtensteinianum	----------	--·AA·····	TTT-------	-------··C	---------T	A·········
Z.billardierei	-A········	···AA·····	TTTTTTATAA	----------	------TTAT	A·····G···
Z.fruticulosum	-A········	···AA·····	TTTTTTATAA	---------·	A·AAT·TTAT	A·····G···
Z.glaucum	-A···N····	···NN··N·N	TN--------	-----T···C	·A--------	-·····G···
Seetzenia	-----·C···	··GAA-····	T--TATTATA	AAAAG---·A	··GT··TATA	TGA··GAT··
Tribulus	-----·C···	··GAA·····	TTTTCTTATC	AAAAG---·C	··GT··TAT-	----·GAT··

						960
Fagonia	ACACATA---	--AAAAAGAA	CATCCTTTCG	CGTAGAATTC	CTGTTTCTCC	CCCCCCCTTT
Z.hildebrandtii	·······---	--C·······	········AA	··········	·······C·T	TTT-------
Z.robecchii	·······---	--C·······	········AA	··········	·······C·T	TTT-------
Augea	·······---	--·····T··	·········C	··········	·C······TT	TTTTTTT··C
Z.cylindrifolium	----------	---------·	·····C··AA	····N····G	T·A···A---	----------
Z.simplex	·······---	--······-·	········AA	··········	··--------	----------
Z.decumbens	·······---	--······-·	········AA	·········G	T·A···----	----------
Z.d.var.decumbens	·······---	--······-·	········AA	·········G	T·A···----	----------
Z.retrofractum	----------	---------·	·····C··AA	·········G	T·A···----	----------
Z.rigidum	·······---	--········	········AA	··········	··T···A---	----------
Z.microcarpum	·······---	--········	········AA	··········	··T···A---	----------
Z.tenue	----------	---------·	·····C··AA	·········G	T·A···----	----------
Z.album	·······---	--········	········AA	··········	··TAACG·AG	AATT··T···
Z.coccineum	·······---	--········	········AA	··········	··T···A---	----------
Tetraena	·······---	--········	········AA	··········	··T···A---	----------
Z.xanthoxylum	·······---	--C·······	········AA	··········	·······CTT	TTTTTA----
Z.fabago	G······AAA	AAC·······	········AA	··········	·······C·T	TTATTA----
Z.spitskopense	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.leucocladum	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.hirticaule	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.fulvum	·······---	--T·······	········AA	··C·······	··C····C·T	T---------
Z.cordifolium	·······---	--T···C···	·······GAA	··C·······	··C····C·T	TT--------
Z.cuneifolium	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.lichtensteinianum	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.billardierei	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.fruticulosum	·······---	--T·······	········AA	··C·······	··C····C·T	TT--------
Z.glaucum	·······---	--T·······	···T····AA	··C·······	··C····C·T	----------
Seetzenia	··GT···---	---······-	·G··T···A·	AAGGT····A	·······AAA	AAT-------
Tribulus	··G--·----	---·······	····T···A·	·AA··G····	·CA···TAAA	T---------

						1020
Fagonia	TTTTTTTTTT	TTTATGATTC	ACATTTT---	----------	-GAATTGACA	TAGGACTACG
Z.hildebrandtii	---------·	··········	·······---	----------	-T········	········A·
Z.robecchii	---------·	··········	·······---	----------	-T········	········A·
Augea	---------·	··········	·······---	----------	-·········	········A·
Z.cylindrifolium	---------·	···C·T····	·······T--	----------	-C········	C······CA·
Z.simplex	---------·	··A·······	·······T--	----------	-T········	C······CA·
Z.decumbens	---------·	···C·T····	·······T--	----------	-·········	C······CA·
Z.d.var.decumbens	---------·	···C·T····	·······T--	----------	-·········	C······CA·
Z.retrofractum	---------·	···C·T····	·······T--	----------	-·········	C······CA·
Z.rigidum	---------·	··········	·······T--	----------	-T········	·······CA·
Z.microcarpum	---------·	··········	·······T--	----------	-T········	·······CA·
Z.tenue	---------·	···C·T····	·······T--	----------	-C········	C······CA·
Z.album	·A-------·	·········-	·······T--	----------	-T········	·······CA·
Z.coccineum	---------·	·········A	·······T--	----------	-T········	·······CA·
Tetraena	---------·	··········	·······T--	----------	-T········	·······CA·
Z.xanthoxylum	---------·	··········	·······T--	----------	-·········	········A·
Z.fabago	---------·	··········	·······T--	----------	-·········	········A·
Z.spitskopense	---------·	··········	·······T--	ATTTTTT---	-T········	········A·
Z.leucocladum	---------·	··········	·······T--	CTTTTTT---	-T········	········A·
Z.hirticaule	---------·	··········	·······T--	ATTTTTT---	-T········	········A·
Z.fulvum	---------·	··········	·······T--	ATTTTTT---	-T········	········A·
Z.cordifolium	---------·	··········	·······T--	ATTTTTT---	-T········	········A·
Z.cuneifolium	---------·	··········	·······T--	CTTTTTT---	-T········	········A·
Z.lichtensteinianum	---------·	··········	·······T--	ATTTTTT---	-T········	········A·
Z.billardierei	---------·	··········	······GA--	-TTTTTT---	-T········	········A·
Z.fruticulosum	---------·	··········	·······G--	ATTTTTG---	-T········	········A·
Z.glaucum	---------·	··A·······	·······T--	ATTTTTT---	-T········	········A·
Seetzenia	---------·	·GA·······	·······TGT	CTTTTTTTTT	T·········	···A····A·
Tribulus	---------·	·AA·······	·A·····TGT	CTTTTT----	-T········	···A····A·

				1053
Fagonia	TCATCTAGTA	AAA-------	-TAAAA-TGA	TGA
Z.hildebrandtii	··········	··--------	-·····A···	···
Z.robecchii	··········	··--------	-·····A···	···
Augea	··········	···TAATAAA	A·····A·T·	···
Z.cylindrifolium	··········	··--------	--------··	A··
Z.simplex	··········	··--------	--------··	A··
Z.decumbens	··········	··--------	--------··	A··
Z.d.var.decumbens	··········	··--------	--------··	A··
Z.retrofractum	··········	··--------	--------··	A··
Z.rigidum	··········	··--------	--------··	A··
Z.microcarpum	··········	··--------	--------··	A··
Z.tenue	··········	··--------	--------··	A··
Z.album	··········	··--------	--------··	A··
Z.coccineum	G·········	··--------	--------··	A··
Tetraena	··········	··--------	--------··	A··
Z.xanthoxylum	··········	···TG-----	-·····-···	A··
Z.fabago	··········	···TG-----	--····-···	A··
Z.spitskopense	··········	··--------	--------··	A··
Z.leucocladum	··········	··--------	--------··	A··
Z.hirticaule	··········	··--------	--------··	A··
Z.fulvum	··········	··--------	--------··	A··
Z.cordifolium	··········	··--------	--------··	A··
Z.cuneifolium	··········	··--------	--------··	A··
Z.lichtensteinianum	··········	··--------	--------··	A··
Z.billardierei	··········	··--------	--------··	A··
Z.fruticulosum	··········	··--------	--------··	A··
Z.glaucum	··········	··--------	--------··	A··
Seetzenia	·A···CG···	··--------	-·····-···	G··
Tribulus	··C··G····	··--------	--------··	G··