UNIVERSITY OF KWAZULU-NATAL

BIOSYSTEMATIC STUDIES IN SOUTHERN AFRICAN SPECIES OF *STRYCHNOS* L. (LOGANIACEAE)

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BIOSYSTEMATIC STUDIES IN SOUTHERN AFRICAN SPECIES OF *STRYCHNOS* L. (LOGANIACEAE)

by

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Submitted in fulfilment of the academic requirements for the degree of Doctor of Philosophy

in the

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal, Durban

NOVEMBER 2014

PREFACE

The experimental work described in this thesis was carried out in the School of Life Sciences, University of KwaZulu-Natal, Durban from March 2007 to December 2011, under the supervision of Profs Ashley Nicholas, Jenny Lamb and Dr Yogis Naidoo.

The studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION BY SUPERVISORS

We hereby declare that we acted as Supervisors for this PhD student:

Student's Full Name: ADEKUNLE ADEBOWALE

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Thesis Title: BIOSYSTEMATIC STUDIES IN SOUTHERN AFRICAN SPECIES OF *STRYCHNOS* L. (LOGANIACEAE).

Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Science Higher Degrees Office for examination by the University appointed Examiners.

SUPERVISOR: PROFESSOR ASHLEY NICHOLAS

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DECLARATION 1 – PLAGIARISM

I, Adekunle Adebowale, Student Number 205527654

declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

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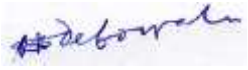
DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1
ADEBOWALE A, NICHOLAS A, LAMB J, NAIDOO Y. 2012. Elliptic Fourier analysis of leaf shape in southern African *Strychnos* section *Densiflorae* (Loganiaceae). *Botanical Journal of the Linnean Society* 170: 542 – 553.

Publication 2
ADEBOWALE A, NAIDOO Y, LAMB J, Nicholas A. 2014. Comparative foliar epidermal micromorphology of Southern African *Strychnos* L. (Loganiaceae): taxonomic, ecological and cytological considerations. *Plant Systematics and Evolution* 300: 127 – 138.

I am the senior author for these two publications. All four authors conceived the research ideas. The first author and Prof Nicholas conducted field works in most cases. I collected data, performed all analyses and interpreted the results under the guidance of the three supervisors. I also wrote the draft manuscripts on which my co-authors commented and critiqued prior to submission. I am also the corresponding author for the publications.



Signed: **A. ADEBOWALE**

ABSTRACT

BIOSYSTEMATIC STUDIES IN SOUTHERN AFRICAN SPECIES OF *STRYCHNOS* L. (LOGANIACEAE)

ADEKUNLE ADEBOWALE Ph.D. thesis, University of KwaZulu-Natal, 2014

Strychnos L. is the largest genus of the pantropical or subtropical family Loganiaceae with about 200 species. Their habits range from trees and shrubs in open areas to lianas in rain forests. The genus is well-known as a source of alkaloids such as strychnine and brucine and other allied compounds, all of which have been used medicinally and in curare formulation for centuries. While taxonomic circumscription of the genus has never been contentious, there is no consensus about infrageneric affiliations, the latest of which recognises 12 sections based on morphological characters. Recent molecular evaluation of the genus on a global scale with the internal transcribed spacer (ITS) marker suggests that many of the currently recognised sections are not monophyletic.

An understanding of regional patterns of evolution, which is relevant for biodiversity conservation, requires an in-depth study of the focus group on a regional scale. Using a multiplicity of approaches from morphological and molecular to biogeographical, this study is an attempt at elucidating diversity patterns at different levels among the southern African species of *Strychnos*.

Various combinations of morphological attributes from branches, leaves, flowers and fruits distinguish seemingly homologous clusters of species, sometimes supported by molecular data. A lack of molecular support for a hypothetical relationship may

indicate case(s) of convergent evolution in these features across the taxa involved. Molecular phylogenies based on the ITS and chloroplast markers confirm the non-monophyletic nature of all but section *Spinosae*. Proposals for sectional re-circumscriptions of the genus are provided.

Patterns of speciation within *Strychnos* suggest a Miocene origin in the rain forests along the South America/Guinea-Congolian axis. Within the southern African subcontinent, the evolution of the genus carries a strong ecological signature along either the forest or savanna biome, with many accompanying morphological adaptations for the respective habitats. The non-synonymy of *S. gerrardii* with *S. madagascariensis* is demonstrated with multiple sources of data, as a case of integrative taxonomy succeeding where single-source data approaches might have failed. Routes to current distribution of the genus in southern Africa are hypothesised to involve a combination of palaeo-climatic oscillations and allopatric speciation, consistent with the process indicated in many other plant groups for the region.

The findings are discussed in the wider context of their implications for taxonomy and biodiversity conservation in the face of climate change, food security and other relevant issues in systematics.

DEDICATION

For the three Ts in my life: Taiwo, ‘Tunmise and ‘Tomide, without whose love and very close technological attention, especially by one of the latter two – in the shape of magically vanishing data via the tea-into-laptop route – this work could have been completed much earlier... but I would not have it any other way!

ACKNOWLEDGEMENTS

I would like to thank my promoter Prof Ashley Nicholas for suggesting this topic and providing me with a stimulating platform to research it. I 'blame' him for every *Strychnos*-related idea popping up in the corners of my head. Thanks for all those philosophical debates that helped sharpen my thinking. I also thank my co-promoters Prof Jenny Lamb and Dr Yogis Naidoo for taking me on an intellectual ride that was both challenging and exciting. Their collective financial support and encouragement ensured the successful completion of the undertaking. Jenny deserves extra mention for being more like an auntie who patiently took me by the hand and guided me to a safe molecular systematics haven. Thanks for all those journal club meetings; they certainly helped.

Other people and institutions contributed to this work in different ways. I am grateful to Ademola Olaniran [Adex] for facilitating my coming to South Africa in the first place, happily hosting me in my first three weeks and helping me to settle down. His home has been a second home to me whenever I am in town. I thank the Glen family (Hugh, René and Melissa and of course … Danica the cat) for adopting me almost as a son. In particular, I thank Dr Hugh Glen, for allowing me to pick that beautiful botanical brain of his. I am grateful to David Styles for several botanical adventures in the KZN area, many of them heart-stopping on cliff edges, all in my bid to hunt down some obscure *Strychnos* specimens, while he chased his rare Crassulas. Mrs Marie Jordaan is gratefully acknowledged for helping me, very early in the work, to recognise some of the taxonomic challenges in South Africa *Strychnos*.

Direct access to collections held by the following herbaria [K, PRE, UDW, PRU, NH, NU, GRA, BM, IFE, and BNRH] is also acknowledged. I thank John and Sandra Burrows for their generosity with information and allowing me to take materials for DNA work from their collections. Ezemvelo KZN Wildlife and eThekwini municipality are acknowledged for permission and access to collect material. I appreciate the effort of the Lowveld Botanic Gardens, Nelspruit for allowing me to sample for DNA in their living collections. I thank all my Conspec lab mates at UKZN for being such fun folks to work with and for always finding creative ways to troubleshoot PCR. The herbarium staff at UDW (Pravin and Edward) have been so helpful assisting me on many collection trips. I thank the Museum staff in the School of AP&ES at University of the Witwatersrand for their support and encouragement. Thanks to Leslie Deysel who skilfully produced the botanical illustrations in Chapter 7. A big thank you to Dr Lena Struwe for making her *in-press* article on Loganiaceae available to me, and also sending me a number of hard-to-come-by publications.

Finally, I would like to express a more-than-deserved gratitude to my immediate family: my wife Taiwo, who has had to temporarily suspend a promising career to support me, and my children, 'Tunmise and 'Tomide for their love and understanding throughout the duration of this programme. They have had to put up with my being absent on several occasions. Thanks a ton guys.

Thanks to my *Strychnos* plants for being such well-behaved, jolly good chaps!

Above all else, I thank God for keeping me in good health and sound mind, to embark on this research and complete it too.

This work was financially supported at various stages by the following institutions: University of KwaZulu-Natal (UKZN) postgraduate tuition bursary, the then Faculty of Science and Agriculture, UKZN for travel and research grants; NRF-Thuthuka bursary awarded through Dr Yogis Naidoo; University of the Witwatersrand SPARC funding for lecturer teaching time buyouts and laptop purchase; School of Animal, Plant and Environmental Sciences, Wits University research grants for new staff.

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CHAPTER 1

INTRODUCTION

Strychnos L. is the largest member of the family Loganiaceae, with a largely circumtropical distribution pattern (Leenhouts, 1962). The Loganiaceae have fascinated botanical taxonomists from very early times due to their heterogenous composition. Depending on treatment, up to 29 genera have been referred to the family, with species numbers estimated to be between 400 and 600 (Bentham, 1856; Takhtajan, 1997; Struwe *et al.* 1994; Leenhouts, 1962; Leeuwenberg and Leenhouts, 1980). The family is essentially tropical or subtropical in its distribution, with a few genera extending into the warm-temperate regions of the Southern Hemisphere. While some genera have wider distribution (e.g. *Strychnos* and *Mitreola* Swainson), a few others are local or regional endemics, such as *Labordia* Gaudich. in Hawaii, *Gardneria* Wallich in China; *Logania* R.Br., *Mitrasacme* Labill. and *Geniostoma* J.R.Forst. & G. Forst. in Australia and New Zealand. The family is not found in Europe, except perhaps in cultivation.

Anatomical evidence from wood structure suggested that many of the genera lumped together in the early conception of the Loganiaceae (e.g. *Geniostoma*, *Buddleja* L. and *Fagraea* Thunb.) belong to more than one distinct family (Moll and Janssonius, 1926; Chalk and Chattaway, 1937; Metcalfe and Chalk, 1950). Following several revision and classification schemes, with the more recent ones incorporating molecular datasets (Backlund *et al.* 2000; Frasier, 2008), a number of genera have been justifiably excluded from the family. These genera are *Anthocleista* Afzel. ex R.Br., *Fagraea* and *Potalia* Aubl. to the Gentianaceae;

Gelsemium Juss. and *Mostuea* Didrichsen to the Gelsemiaceae; *Plocosperma* Bentham to the Plocospermataceae; *Peltanthera* Bentham and *Sanango* Bunting & Duke to the Gesneriaceae; *Polypremum* L. to the Tetrachondraceae; *Androya* H. Perrier, *Buddleja* L., *Emorya* Torr. and *Gomphostigma* Turcz. to the Scrophulariaceae; *Nuxia* Comm. ex Lam. and *Retzia* Thunberg to the Stilbaceae, and finally *Desfontainia* Ruiz & Pav. to the Columelliaceae. Intra-familial groupings currently recognise four tribes. These tribes, Antonieae, Spigelieae, Strychneae and Loganieae, represent a move towards better phylogenetic coherence within the family and a major advance over the previous polyphyletic arrangements of the earlier works, including that of Leeuwenberg and Leenhouts (1980).

The Loganiaceae are a family of trees, shrubs, lianas or herbs. Leaves are nearly always opposite, entire (or finely toothed) and display a prominent midrib. Inflorescences are terminal or axillary cymes, or umbellate as in *Mitrasacme*. Flowers are bisexual with superior ovaries, and are actinomorphic (*Usteria* Willd. has zygomorphic sepals and stamens), with 5- or 4-merous parts (Struwe and Motley, in press). Loganiaceae belong to the order Gentianales (Contortae in the earliest classification schemes) along with the Rubiaceae, Apocynaceae (including Asclepiadaceae), Gentianaceae and Gelsemiaceae (Backlund *et al.* 2000). Recent molecular evidence has elucidated the relationship among members of this order as follows: Loganiaceae is sister to Gelsemiaceae + Apocynaceae; Gentianaceae is in turn sister to the three preceding families, and Rubiaceae is the sister group to all Gentianales (Backlund *et al.* 2000; Frasier, 2008; Struwe and Motley, in press). Thus, Gelsemiaceae occupy an intermediate position between Loganiaceae and Apocynaceae, a position understandable from a

morphological perspective as members of this relatively new family had been traditionally placed with either the Loganiaceae or the Apocynaceae (Endlicher, 1841; Bentham, 1856).

POLLINATION AND BREEDING SYSTEMS IN LOGANIACEAE

The usually small size, bright colour and fragrance of most loganiaceous flowers suggest a high level of entomophily within the family. This has been observed first-hand in *Strychnos*. A strong divergence in floral morphology within *Labordia* Gaudich. has led to speculation of possible bird pollination in some species (Motley and Carr, 1998). Bird and insect pollinations have, however, been documented in *Geniostoma* (Castro and Robertson, 1997). Outcrossing is the common mode of reproduction in Loganiaceae. However, Bruce and Lewis (1960: 33) reported a rare case of cleistogamous reproduction in some populations of *Strychnos henningsii* Gilg from East Africa. While most Loganiaceae possess bisexual flowers, certain members of the tribe Loganieae (*Geniostoma*, *Labordia* and *Logania*) display various degrees of dioecy with some having been described as "*polygamous-dioecious*" (Baillon, 1880). Heterostyly and gynodioecy have been reported in species of *Geniostoma* from Java and New Zealand respectively, and functional dioecy is confirmed for all investigated species of *Labordia* (Struwe and Motley, in press).

CHROMOSOME NUMBERS IN LOGANIACEAE

The base chromosome number in Loganiaceae varies from 8 to 13. The two genera in which polyploidy is common (*Spigelia* L. and *Strychnos*) are also those in which interspecific chromosome number variation has been noted (Struwe and

Motley, in press), suggesting complementary roles for polyploidy and hybridization in the evolution of these genera. Tetraploidy is very common in *Strychnos* although ploidy levels may reach 8- or 10-fold, as documented in *Strychnos angolensis* Gilg and *S. brasiliensis* (Spreng.) Mart. (Gadella, 1963; 1980). Other genera for which chromosome counts exist include *Geniostoma*, *Lobardia*, *Logania*, *Mitreola* and *Usteria* (Miege, 1960; Lewis *et al.* 1962; Keigheri, 1975; Motley and Carr, 1998; Struwe and Motley, in press).

SEED DISPERSAL IN LOGANIACEAE

Seed dispersal in Loganiaceae is mainly by birds and mammals, including humans. A few genera, such as *Antonia* Pohl, *Bonyunia* M.R. Schomb. ex Progel, *Norrisia* Gardner and *Usteria*, have winged, light seeds and are presumed to be wind-dispersed. The colourful and fleshy aspects of seeds in *Labordia* and *Geniostoma* suggest birds as the probable agent of dispersal (Struwe and Motley, in press). *Neuburgia* Blume fruits are buoyant and could potentially be dispersed by water (Leenhouts, 1962). While *Strychnos camptoneura* Gilg et Busse seeds form irregular wings (Struwe and Motley, in press), which offer opportunities for wind dispersal, the seeds of most of the other *Strychnos* species are dispersed by mammals (primates in particular), birds and sometimes ants (Leenhouts, 1962; Pontes, 2005). Seeds of large-fruited *Strychnos* species are usually dispersed by monkeys and other primates, hence the more common name monkey orange, whereas the small-fruited species appear to have a wider range of dispersal agents. In the forest of Kibale National Park, Uganda, red-tailed monkeys (*Cercopithecus ascanius schmidti* Matschie) have been observed to process the seeds of *Strychnos mitis* S. Moore through their guts. This helps remove pulp,

thus reducing fungal pathogen attack and promoting a higher rate of seed germination and survival (Lambert, 2001). Similar symbiotic association between attine ants (Myrmicinae) and *Strychnos ramentifera* Ducke have been documented from the Maraca Island of Brazil (Pontes, 2005). The obligatory fungi-cultivating ants in this case help clean the seeds, preventing fungal infestation and significantly improving seed germination rates.

USES OF LOGANIACEAE

Many genera of Loganiaceae are ethnobotanically valuable, although the genus most well-known for this is *Strychnos*, due to the curarizing and other beneficial properties of its alkaloids (Bisset and Phillipson, 1971). *Neuburgia* and *Norrisia* are used as timber for house flooring in the Malay Peninsula (Leenhouts, 1962), while other members are used for food, in traditional medicine, as remedies for a wide variety of ailments, or as ornamentals (Booker and Cooper, 1961; Zepernick, 1972; Bisset, 1974; Struwe and Motley, in press).

STRYCHNOS AT A GLANCE

The genus *Strychnos* comprises about 200 species, many of which have medicinal properties and a few of which are used for food and carpentry (Bisset, 1974). There are about 75 species in Africa and up to 20 species in a broadly-defined southern Africa, encompassing Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, Swaziland, Zambia and Zimbabwe. Their habits range from trees of various sizes, to shrubs and liana depending on the geography of the species. Taxonomically, the genus is distinct from other members of the

Loganiaceae (Leeuwenberg, 1969), such that generic circumscription has never been controversial. The genus is defined by opposite leaf arrangement, which may sometimes be decussate and is usually inserted on distinct leaf cushions, 3 – 7 nerved vein systems and tetra- or pentamerous floral whorls, with flowers borne on terminal or axillary inflorescence. Ovaries are superior and usually 2-celled, except in *S. spinosa* Lam., where they are 1-celled. Fruits vary in size and are generally globose or nearly so.

At the infrageneric level, there is currently no consensus regarding groupings among *Strychnos* species. This is probably attributable to evolutionary convergence on the one hand, and isolated treatments of the genus on a regional or sub-regional basis on the other (Progel, 1868; Hill, 1917; Sandwith, 1933; Krukoff and Monachino, 1942; Duvigneaud, 1952; Bruce and Lewis, 1960; Leenhouts, 1962; Verdoorn, 1963). Verdoorn's (1963) work was the last, regional treatment of the genus in southern African. The most recent morphology-based attempt at resolving infrageneric groupings from a global perspective was undertaken by Leeuwenberg (1969). Although, his treatment was essentially Afrocentric, it provided the first global groupings of *Strychnos* along sectional lines by integrating all previous works to create a usable, if not entirely satisfactory, framework. His subdivision of *Strychnos* into 12 sections has been adopted by subsequent works as a baseline for testing evolutionary relatedness among *Strychnos* taxa (Ohiri *et al.* 1983; Quetin-Leclercq *et al.* 1990).

A taxonomic treatment that incorporates morphological traits with other sources of data is essential in modern systematic practices. It allows testing for possible

correlation(s) of morphology with other datasets and can therefore help in evaluating the utility or otherwise of various dataset for taxonomic decision making.

MORPHOLOGICAL AND OTHER DIAGNOSTIC FEATURES IN SOUTHERN AFRICAN *STRYCHNOS*

Members of this genus are readily recognisable by the distinctive 3 – 7 veins on their leaves, although separating closely related congeners could be problematic. A number of key diagnostic morphological and other relevant features for southern African *Strychnos* species are summarised in Table 1.1 below.

Table 1.1: Some morphological diagnostic features of southern African *Strychnos*

***Strychnos* species of southern Africa**	**Key Diagnostic features**										
	Colour and texture of tree bark & branches	**Habit & canopy shape**	**Spines present**	**Leaf outline shape**	**Hairy Leaves**	**Leaf epidermal trichome ornamentation**	**No. of floral parts**	**Mean corolla length (mm)**	**Mean sepal length (mm)**	**Fruit size & texture**	**Number of prominent leaf veins**
S. cocculoides Bak.	Rough & corky, young branch reddish	Tree, shrubs; open	Yes	Ovate, orbicular	Yes	Smooth	5	2.5	4	Large, hard	3-7
S. decussata (Pappe) Gilg	Smooth, dark grey/black	Tree,	No	Obovate, ovate-oblong	No	Glabrous	5	5.2	1.4	Small, soft	3
S. gerrardii N.E.Br.	Nearly smooth greyish	Tree; narrow canopy; branches grow vertically	No	Elliptic, oblong	No	Glabrous	4	6.8	3.5	Large, hard	3-5
S. henningsii Gilg	Smooth	Tree, shrubs; spreading rounded canopy	No	Ovate, lanceolate, elliptic	No	Glabrous	5	3.3	1.3	Small, soft	3
S. innocua Del.	Grey brown; nearly smooth, flaking	Tree, shrubs; open, much branched	No	Obovate, elliptic	Yes	Ornamented	4	7.6	2.9	Large, hard	5, prominent on both surfaces
S. madagascariensis Poir.	Pale grey; nearly smooth	Tree, shrubs; open, much branched	No	Obovate, suborbicular or elliptic	Yes, to various degrees	Ornamented	4	7.4	3.1	Large, hard	3-5, not prominent on adaxial surface
S. mitis S. Moore	Grey-brown; smooth	Tree; rounded crown	No	Ovate, elliptic	No	Glabrous	5	3.8	1.8	Small, soft	5
S. potatorum L.f.	Smooth; grey or brown	Tree, shrubs	No	Ovate, elliptic	No	Glabrous	4 or 5	7	2.2	Small, soft	3-5
S. pungens Solered.	Thick rough, fissured; grey or brown	Tree, shrubs	No	Elliptic with sharp apex	No	Glabrous	5	8.5	3	Large, hard	3
S. spinosa Lam.	Rough, shallow fissures, corky; grey or brown	Tree, shrubs; open and spreading	Yes	Orbicular, ovate, obovate	Sometimes	Smooth	5	4.3	5.2	Large, hard	3-7
S. usambarensis Gilg	Smooth; dark brown	Small tree, shrubs, occasionally liana	No	Ovate or elliptic with long acuminate apex	No	Glabrous	4 (5)	2.8	1.5	Small, soft	5

RATIONALE AND AIM OF THE STUDY

Prior to Frasier (2008), there was no adequate molecular framework for testing any hypothesis regarding the evolution of the Loganiaceae in general, and *Strychnos* in particular. Within the southern African subcontinent, a number of studies have treated members of this genus from a purely gross morphological perspective, sometimes with contradictory conclusions regarding the classification and/or placement of some taxa (Hutchinson and Dalziel, 1931; Bruce and Lewis, 1960; Verdoorn, 1963; Leeuwenberg, 1969; 1983). To objectively address such contradictions requires the interrogation of other independent data sources, while at the same time re-examining previously used ones. The current move in systematic circles is the complementary utilisation of multiple sources of independent evidence for biodiversity exploration (Schlick-Steiner *et al.* 2010). Several studies have shown higher success rates for species discrimination when data from multiple sources are combined in the decision-making process; a practice aptly termed "*integrative taxonomy*" (Dayrat, 2005; Padial *et al.* 2010; Riedel *et al.* 2013; Rouhan and Gaudeul, 2014).

Frasier (2008) attempted a worldwide molecular systematic overview of *Strychnos* with about 63% species coverage across the entire geographical range of the genus, using only the nuclear ribosomal internal transcribed spacer (ITS) marker. What is obvious from her work, however, is that many of the species could not be observed directly in the field; an inevitability stemming from the global scale of the work. The global approach does not allow a detailed study of evolutionary dynamics at a regional scale. At regional scales, however, biodiversity conservation is better served by focussed and detailed investigations to address

the unique biotic and abiotic components of a region, and are more likely to garner political support than a generalised global approach (Poiani *et al.* 2000; Younge, 2002). Furthermore, the use of a single molecular marker (ITS in this case) may not reflect a close-enough evolutionary history adequately or with much confidence, especially when using a marker as variable as the ITS.

The overall goal of this study therefore is to evaluate the taxonomy of the genus *Strychnos* within the subcontinent of southern Africa. The specific objectives involve the use of geometric morphometrics, micro-morphology, molecular phylogenetics as well as historical biogeography to test various hypotheses of species boundaries and affiliations in the genus.

GEOGRAPHICAL SCOPE OF THE STUDY

A narrow definition of southern Africa has been adopted here, consistent with Verdoorn (1963). This is to enable for a direct comparison with Verdoorn's work. The taxonomic treatment has therefore excluded such species as *Strychnos angolensis* Gilg, *S. lucens* Baker and *S. xantha* Leeuwenberg, although these taxa are included in some the phylogenetic analyses, to provide a broad base for comparisons.

THESIS LAYOUT

The thesis opens with an introduction (Chapter 1) which gives a summary overview of the systematics of the family Loganiaceae and the genus *Strychnos*. Chapter 2 explores the mathematical quantification of leaf shape by applying the elliptic Fourier method to the regional members of *Strychnos* section *Densiflorae*.

In Chapter 3, leaf epidermal micro-morphological evidence for species delimitation is presented. This is based on SEM and light microscopic studies. The first detailed sets of ITS2 secondary structure models are investigated and presented in Chapter 4 to provide insight into species boundaries and crossability potential among southern African *Strychnos*. Chapter 5 presents the first molecular phylogenetic hypothesis for southern African *Strychnos* that combines both nuclear and chloroplast DNA sequences. This chapter also evaluates the sectional hypothesis of Leeuwenberg (1969) for the African members from an ITS perspective. Chapter 6 attempts a historical biogeographic reconstruction for the observed distribution pattern of southern African *Strychnos* using a combination of DNA sequence data and species distribution datasets. Putting all the previous chapters into perspective, Chapter 7 is an attempt at a new taxonomic classification of southern African *Strychnos*. The chapter gives a thorough history of *Strychnos* and its higher taxonomic ranks within the family Loganiaceae and the order Gentianales. It then proceeds to a taxonomic treatment based on total evidence. The thesis concludes with Chapter 8, which gives a general discussion of the key findings of this study and recommendations for future research.

AUTHOR'S COMMENTS

There are a few repetitions of the major thrusts of this thesis across some of the chapters. For one, it is inevitable because two of the chapters (2 and 3) are already published. The other core chapters (4 – 7) were written as near-publication-ready manuscripts, thus requiring some background information to be provided. The repetitions therefore serve to reinforce certain arguments when independent sources validate a theme.

Due to this publication-readiness approach, I have deliberately used the first person plural “we” or the possessive determiner “our” as appropriate in some of the chapters, consistent with current practices in reporting collaborative work. In addition to Chapters 2 and 3, which are published, these pronouns are used in Chapters 3 – 6. Other chapters (1, 7 and 8) were written in the third person.

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CHAPTER 2

ADEBOWALE A, NICHOLAS A, LAMB J, NAIDOO Y. 2012. Elliptic Fourier analysis of leaf shape in southern African *Strychnos* section *Densiflorae* (Loganiaceae). *Botanical Journal of the Linnean Society* 170: 542 – 553.

The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2012, **170**, 542–553. With 5 figures

Elliptic Fourier analysis of leaf shape in southern African *Strychnos* section *Densiflorae* (Loganiaceae)

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Received 17 January 2012; revised 29 May 2012; accepted for publication 28 August 2012

Leaves can be a useful source of taxonomic information in plants particularly when flowers and fruits are absent during certain periods of the year. In this study, we applied an elliptic Fourier analysis (EFA)-based morphometric technique to assess leaf morphological divergence among four species of southern African *Strychnos* section *Densiflorae*. Using leaf specimen images from field and herbarium collections, we extracted six shape variables [i.e. principal components (PCs)] from the Fourier coefficients and used these variables to describe leaf outline among the species. Our results indicate that the symmetric component of a leaf is the main source of shape differences accounting for 90.25% of total leaf shape variation and captures the more obvious range of observed shapes. PC1 of the symmetric variables describes a wide range of visually observable leaf shape among the species. MANOVA revealed significant interspecific differences except between *S. innocua* and *S. madagascariensis*, which could not be separated by outline analysis. A cross-validated group classification suggests that *S. gerrardii*, with a classification rate of 88.4%, is distinct from *S. madagascariensis*, contrary to some taxonomic treatments. We discuss the value of geometric morphometrics at detecting subtle morphological variations and the evolutionary implications of such variations, which may be undetectable to the human eye. *Botanical Journal of the Linnean Society*, 2012, **170**, 542–553.

ADDITIONAL KEYWORDS: geometric morphometrics – southern African flora.

INTRODUCTION

One of the fundamental goals of systematic biology is the discrimination of taxa based on well-defined diagnostic characters with a view to correctly classifying and identifying future subjects. This is normally achieved by collecting morphological, ecological and/or molecular data. In recent years, the number of molecular studies used for reaching important systematic decisions has risen exponentially, leading to sometimes necessary debates about the relative value of each approach in answering questions with which evolutionary biologists are regularly confronted (Baker, Yu & DeSalle, 1998; Will & Rubinoff, 2004). The appeal of molecular methods has been the strong statistical basis coupled with a perception of considerable reduction in subjectivity. However, from a systematic standpoint, any meaningful interpretation of results from molecular studies is usually placed in a morphological context because such results are used to assign organisms to morphologically predetermined taxonomic categories (MacLeod, Benfield & Culverhouse, 2010). Furthermore, morphology offers a rich, relatively cost-effective and easily accessible source of relevant systematic data (McLellan & Endler, 1998). Morphological variations among organisms often provide insights into ecological and evolutionary forces at work within a group (Wiens & Graham, 2005) and may therefore serve as proxies for a wide range of environmental parameters for which there are no direct means of measuring (Wilf, 1997; MacLeod, 2005). Thus, the molecular revolution,

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rather than threatening it, has reinforced the need for a better understanding of morphological patterns amongst organisms.

A limitation of most morphological methods as practised in the past is the considerable dependence on qualitative description of forms (Dickinson, Parker & Strauss, 1987; Baylac, Villemant & Simbolotti, 2003). Although not entirely without merit, this approach is fraught with subjective and imprecise information especially when dealing with complex biological subjects the shapes of which are not within the sphere of regular human experience. Realization of this shortcoming led directly to the formulation of a quantitative framework for extracting and analysing meaningful morphological information from biological subjects, culminating in the so-called 'morphometric revolution' or geometric morphometrics (GM) (Bookstein, 1991; Corti, 1993; Rohlf & Marcus, 1993; Marcus *et al*., 1996; MacLeod & Forey, 2002; Jensen, 2003; Adams, Rohlf & Slice, 2004).

GM entails the multivariate analysis of Cartesian coordinate data for landmarks and semi-landmarks (Zelditch *et al*., 2004; Slice, 2007). Although the techniques of GM were developed for zoological subjects where type I landmarks (*sensu* Bookstein, 1991) are abundant, they have since been adapted to accommodate other subjects, plants included, where good landmarks are sometimes insufficient or altogether lacking. The outline approach to GM is particularly suited to capturing overall shape in two-dimensional objects such as plant leaves (Ferson, Rohlf & Koehn, 1985; Jensen, Ciofani & Miramontes, 2002; Slice, 2005). Coinciding with the morphometric revolution, the past decade has witnessed an increase in the number of plant biology studies that have employed GM techniques solely or in combination with other methods (for reviews see Hearn, 2009; Lexer *et al*., 2009; Costa *et al*., 2011). Of the outline methods in use, the elliptic Fourier analysis (EFA) approach has been shown to be efficient at group discrimination, even among intraspecific populations, and is being used increasingly in a variety of biological studies (McLellan & Endler, 1998; Hiraoka & Kuramoto, 2004; Milanesi *et al*., 2011).

EFA decomposes a curve into a set of harmonic ellipses each of which is defined by four coefficients or elliptic Fourier descriptors (Kuhl & Giardina, 1982; Ferson *et al*., 1985; Lestrel, 1997). The standardized coefficients can then be used as shape variables in multivariate analysis (Sueur *et al*., 2010). The technique also allows for a reconstruction of shape outline through the inverse Fourier transformation of coefficients (Crampton, 1995; Furuta *et al*., 1995) and is thus useful for visualizing areas of shape changes among subjects of interest.

There is a growing literature on the varied application of the EFA method in the plant sciences. Apart from its application in systematics (Neto *et al*., 2006; Viscosi & Fortini, 2011; Mebatsion, Paliwal & Jayas, 2012), it has found other uses in the fields of agriculture and automatic object identification (Iwata *et al*., 2002; Chaki & Parekh, 2011; Costa *et al*., 2011; Direkoglu & Nixon, 2011). In an instructive study, Iwata *et al*. (2010) combined elliptic Fourier descriptors and quantitative trait loci data in an association mapping to the genetics of rice grains to detect specific markers that could potentially improve yield. A recent study by Terral *et al*. (2010) investigated the possibility of a positive correlation between EFA-derived variables from seed shape and biogeographical patterns. Combining these data with genetics, they unravel the origin and domestication history of ancient European cultivars of grapevine (*Vitis vinifera* L.). Other authors have found the technique useful for uncovering cryptic diversity and for quantifying allometry (Langlade *et al*., 2005; Feng *et al*., 2009; Williams, Hall & Kuklinski, 2012).

Southern African *Strychnos* section *Densiflorae*

Strychnos L. section Densiflorae Duvign. comprises eight species and is endemic to Africa (Leeuwenberg, 1969). Three species (*S. innocua* Del., *S. madagascariensis* Poir. and *S. pungens* Solered.) listed by Leeuwenberg are found in southern Africa. *Strychnos gerrardii* N.E.Br., a fourth taxon found in southern Africa, is closely related to *S. madagascariensis*. Although not recognized by Leeuwenberg, the taxon is included in this study as it is regarded as distinct by many field botanists in SA as evidenced by determinations from herbarium collections, field guides and checklists (Germishuizen *et al*., 2006; van Wyk *et al*., 2011). Verdoorn's (1963) revision treated both *S. gerrardii* and *S. madagascariensis* (synonym *S. dysophylla* Benth.) as subspecies of *S. innocua*. Leeuwenberg, however, reduced *S. gerrardii* to synonymy under *S. madagascariensis* and elevated the latter to specific status. In Leeuwenberg's (1969) treatment, section *Densiflorae* is defined by floral and fruit attributes without much recourse to leaf characteristics (Leeuwenberg, 1969: 31). Where leaves were used as diagnostic characters, the approach was qualitative and open to subjective interpretations. Given that these plants are evergreen, leaves and other vegetative diagnostic features are usually the first characters for identification in the field. Therefore, providing a sound quantitative footing for some of those qualitative descriptions will enhance their systematic value. Moreover, a significant number of herbarium specimens are sterile and the taxonomic

determinations have, for some, been controversial. This study was undertaken to assess the value of leaf shape in the taxonomy of a small well-defined group of *Strychnos* spp.

Vegetatively, the four species are distinguishable by their leaves, bark and canopy structure. *Strychnos gerrardii* has completely glabrous, broadly elliptic–oblong leaves with a narrowing, occasionally subacute apex and cuneate base. Mature trees have secondary branches that grow vertically without spreading. Leaves of *S. madagascariensis* may be glabrous or pubescent and are largely obovate-shaped with a rounded apex and cuneate base. A widely spreading canopy characterizes trees of this taxon. Some non-fruiting leaf specimens may be difficult to assign to one or the other of these two taxa due to possible hybridization between them (Verdoorn, 1963). *Strychnos innocua* is similar to *S. madagascariensis* but with consistently glabrous leaves characterized by prominent reticulate venation on both sides. *Strychnos pungens* can be distinguished from the rest by its tough, elliptical, glabrous leaves with a distinctive sharp apex.

As a contribution to the biology of the southern African *Strychnos* in general, the main aims of this study were to (1) quantify leaf shape in southern African *Strychnos* section *Densiflorae* and (2) estimate interspecific distances and the extent to which leaf shape can discriminate among the four taxa.

MATERIAL AND METHODS

Mature, undamaged leaf specimens were obtained from field or herbarium collections (Table 1; see Appendix 1 for vouchers). For some species where insufficient materials met these criteria, verified images from the JSTOR webpage (http://plants.jstor.org/search? last accessed on 10 April, 2012) were used. All specimens are of southern African origin (Fig. 1; Table 1). Specimens were assigned to species a priori based on a combination of vegetative (tree bark, leaf texture and canopy shape) and reproductive (flowers and/or fruits) features following the diagnoses of Verdoorn (1963) and Leeuwenberg (1969). We excluded leaf shape from our specimen selection criteria to avoid circularity of argument as the investigation hinges on this attribute. Leaves were selected only from specimens with flowers and or fruits, thus reducing our sample size.

For specimens collected by the authors and loose leaves of some herbarium materials, abaxial surface colour images of leaf outlines were captured with a flatbed Canon MP140 scanner at a resolution of 300 dpi. Leaf images from the JSTOR webpage and from other herbarium specimens were first processed with GIMP version 2.6 (Kimball & Mattis, 2006) for similarity in orientation and resolution with the other scanned images. A total of 438 leaf specimens covering the four taxa were sampled, of which 141 met our selection criteria. The images were converted into black-and-white silhouettes and saved as 24-bitmap format with Microsoft Paint tools.

Elliptic Fourier procedure and statistical analysis

We obtained the closed contour of each leaf as chain codes from digital images (Freeman, 1974). The coefficients of the elliptic Fourier (EF) descriptors were rendered invariant to leaf size and rotation but not invariant to starting position of the contour, which was retained at the apex, the most consistent landmark for *Strychnos* leaves. The shape of each leaf was approximated with the first 25 harmonics to generate

Table 1. Sampling details of *Strychnos* species used in the present study

Taxon	Abbreviation	Country	*N*	Leaves sampled
Strychnos gerrardii	Sg	Mozambique	3	26
		South Africa	13	85
		Swaziland	1	5
Strychnos innocua	Si	Botswana	8	46
		Zimbabwe	11	79
Strychnos madagascariensis	Sm	Botswana	5	21
		South Africa	8	58
		Zimbabwe	3	14
Strychnos pungens	Sp	Botswana	4	31
		Namibia	4	27
		South Africa	7	46

N is the number of individuals, which is a combination of field and/or herbarium collections.

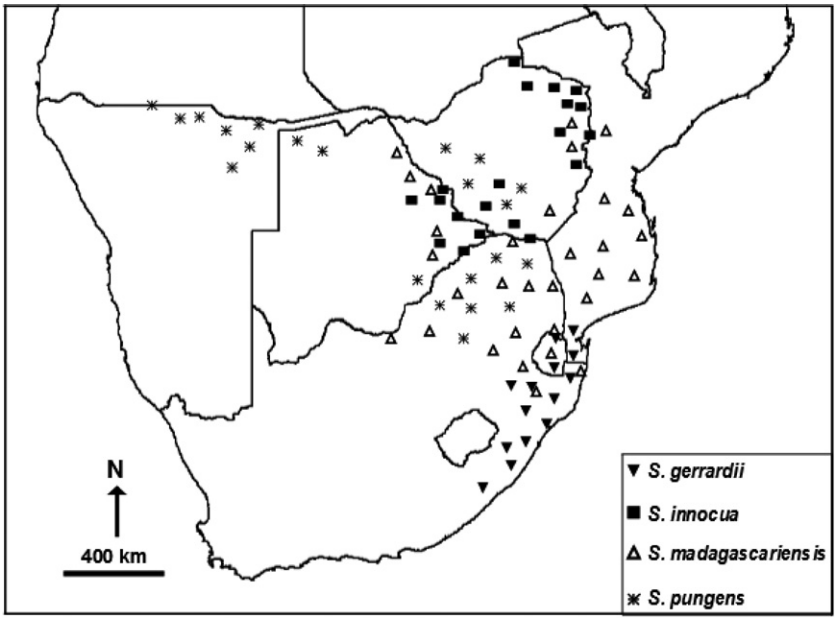

Figure 1. Distribution map of four species of southern Africa *Strychnos*.

$(25 \times 4) - 3$ (i.e. 97) coefficients of the normalized EF descriptors. We arrived at this optimal number of harmonics by visual comparison of reconstructed leaf images at different harmonics with an original silhouette of the same leaf. We performed an exploratory principal component analysis (PCA) on the dataset and then partitioned the EF descriptors into two uncorrelated groups based on whether the coefficients described symmetric or asymmetric aspects of variations (Lexer *et al*., 2009). Using the variance–covariance matrix, a PCA was performed on each set of data separately to reduce dimensionality and summarize the information contained in the coefficients. The partitioning allowed for evaluation of the relative contribution of each dataset to overall leaf shape variation.

Shape variation was summarized by the first six principal components (PCs) otherwise considered as shape variables based on the cumulative percentage of explained variance being $\geq 98\%$. The advantage of PCs is that they are orthogonal to one another and therefore describe independent trends in shape unlike EF coefficients (Andrade *et al*., 2010). To explore the degree of similarity among species, a cluster analysis was performed on mean PC scores using the paired group algorithm. We performed a one-way ANOVA on the entire data set using each of these six PCs as dependent variables and species as predictor variables. Post-hoc analyses were run with Bonferroni's corrected pairwise tests. The contribution of each PC to leaf shape was visualized from the EF coefficients by setting the score for the PC in question to equal the mean ± 2 standard deviations while leaving the scores of the remaining components at the mean. The calculated coefficients were then used for extreme shape reconstructions through the inverse Fourier transformation method (Furuta *et al*., 1995).

The effects of species on shape were further investigated with a single-factor fixed-effect MANOVA. To provide evidence for species discrimination, we performed a canonical variate analysis (CVA) on the six PCs and tested our a priori case assignments by cross-validation to correct for an optimistic error rate. Unequal sample size was corrected for by assignment of prior probabilities proportional to species sample size. EFA, data partitioning and PCA were performed with a suite of programs in the software package SHAPE ver. 1.3 (Iwata & Ukai, 2002) and all statistical analyses were implemented in PAST ver. 2.10 (Hammer, Harper & Ryan, 2001) and Minitab 15.

RESULTS

Leaf outline reconstructions

The first 25 harmonics were sufficient for capturing shape information contained in the outline of the leaves. Main anatomical features of the leaf outline were described by the first 15 harmonics and finer details of the apex and the area around the leaf base were captured by higher order harmonics.

Shape variables and inter-specific variations

Independent shape variables were identified by PCA of Fourier descriptors. Table 2 presents the relative

Table 2. Eigenvalues and contribution of the first six principal components before data partitioning

Component	Eigenvalue	Proportion (%)	Cumulative proportion (%)
1	1.42×10^{-2}	85.25	85.25
2	1.25×10^{-3}	7.49	92.74
3	6.11×10^{-4}	3.68	96.42
4	1.65×10^{-4}	0.99	97.41
5	9.41×10^{-5}	0.57	97.98
6	7.24×10^{-5}	0.44	98.42

contributions of the first six PCs prior to data partitioning. These together accounted for 98.42% of the total variance. Figure 2 shows an ordination plot of the four species in a two-dimensional space defined by PC1 and PC2. The plots of *S. gerrardii* and *S. pungens* are similar along PC1, with mostly negative values, whereas most *S. madagascariensis* and *S. innocua* samples posted positive values along this axis. PC2 separates *S. pungens* from the other three taxa. The trajectories of *S. madagascariensis* and *S. innocua* along both PCs appear similar. The eigenvalues for explained variance show that symmetric variations account for 90.25% of total leaf shape (Table 3).

Table 3. Relative contribution of symmetric and asymmetric components to leaf shape in four *Strychnos* species

Eigenvalues							
	PC1	PC2	PC3	PC4	PC5	PC6	Percentage contribution to overall shape
Symmetric	1.25×10^{-2}	7.78×10^{-4}	2.09×10^{-4}	6.15×10^{-5}	5.58×10^{-5}	2.70×10^{-5}	90.25
Asymmetric	1.22×10^{-3}	1.18×10^{-4}	4.70×10^{-5}	3.63×10^{-5}	3.24×10^{-5}	1.64×10^{-5}	9.75

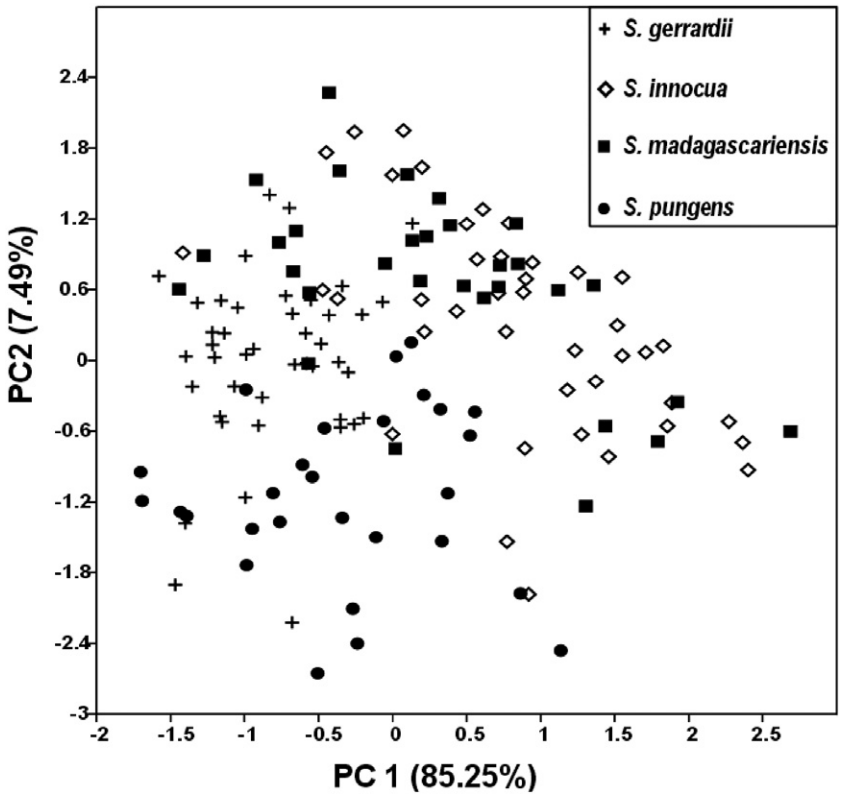

Figure 2. Principal component analysis based on variance–covariance matrix of the unpartitioned dataset from elliptic Fourier coefficients of four southern African *Strychnos* spp. The plot shows PC1 and PC2, which explained 85.25 and 7.49% of the total variation, respectively.

Table 4. Summary of ANOVA results for the first six and first three PCs of the symmetric and asymmetric datasets, respectively

	Symmetric						Asymmetric		
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3
F	37.93*	30.93*	3.92*	13.81*	3.38*	1.97	0.44	0.03	2.82*
P	< 0.0001	< 0.0001	0.01	< 0.0001	0.02	0.121	0.72	0.99	0.04

*Significant.

A one-way ANOVA indicated that symmetric EFA-PCs 1–5 had significant interspecific effects (Table 4). Conversely, only one of the asymmetric EFA-PCs (PC3) had a marginally significant interspecific effect on leaf shape (Table 4). Bonferroni post-hoc tests (Supporting Information Table S1) of multiple comparisons showed that along symmetric PC1 (91.12% of the variance), *S. innocua* and *S. madagascriensis* form an indistinguishable complex; similarly, *S. pungens* and *S. gerrardii* do not differ significantly. However, *S. gerrardii* and *S. madagascariensis*, two taxa treated as synonymous by Leeuwenberg (1969), differed significantly on PCs 1, 2 and 4 (Supporting Information Tables S1, S2, S4). Due to the relatively conserved approach, Bonferroni tests could not resolve the pairwise comparisons for asymmetric PC3. Fisher's least significant difference, however, suggests a significant difference (albeit marginal) between *S. gerrardii* and *S. madagascariensis* (supporting Table S6).

To illustrate the effect of each shape variable, i.e. PC, on overall leaf shape, the scores of the first six PCs were used separately for symmetric and asymmetric variations. For symmetric variation, PC1 captures anisotropy along the major axis of the leaf, which appears to be a function of the length/width ratio. This PC alone explains a wide range of leaf shape encountered among the four *Strychnos* spp. from elliptical to orbicular. PC2 describes the ovate–obovate variation among the samples, whereas PC3 describes fine changes along the outline that reflect how oblong the leaves are. The remaining PCs (4 and 5) expressed fine-scale variations around the apical region that do not readily lend themselves to visual interpretations (Fig. 3), although ANOVA results suggest the difference is due to length/width ratio (supporting Tables S4, S5). Outline reconstructions with asymmetric datasets shows a left–right basal and apical asymmetry on PCs 1 and 2. The other PCs revealed no observable variations across species (Fig. 3).

Because the asymmetric component made little contribution to overall leaf outline variation, multivariate analyses were limited to the symmetric dataset.

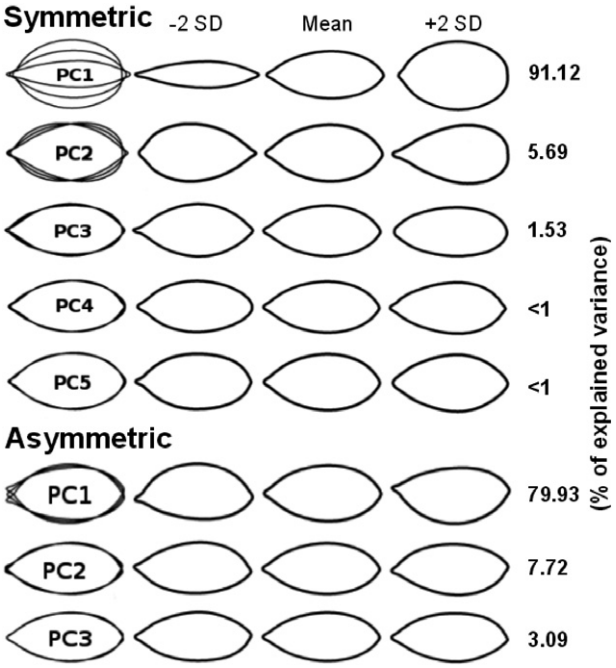

Figure 3. Extreme leaf shape reconstructions using the inverse Fourier function along the first five EFA-PCs from the symmetric data and the first three EFA-PCs from the asymmetric data. The first column shows overlaid drawings of the next three columns along each PC.

As expected, MANOVA based on the six symmetric PC scores revealed a significant effect of species on leaf shape (Wilk's $\lambda = 0.1189$, $F = 23.32$, d.f. = 18 and 373, $P < 0.0001$). With the exception of *S. innocua* and *S. madagascariensis*, all other interspecific groupings showed differences in the multiple comparisons (Table 5). The shape space occupied by each specimen was determined by projecting the scores of each specimen onto the first two CV axes. Canonical variate axis 1 (81.92% of explained variance) distinguishes *S. gerrardii* and *S. pungens* from the *S. innocua*–*S. madagascariensis* complex and CV2 (16.43%) separates *S. pungens* from *S. gerrardii* (Fig. 4). The cross-validation test confirmed the observed discrimination trend with high percentages of correctly

Table 5. Interspecific leaf shape differences from MANOVA of symmetric PC scores: Bonferroni-corrected probabilities of multiple comparisons

	Sg	Si	Sm	Sp
Sg	–			
Si	2.25515E-20	–		
Sm	3.06233E-14	0.433982	–	
Sp	7.49274E-13	6.19172E-23	1.11539E-17	–

Sg, *S. gerrardii*; Si, *S. innocua*; Sm, *S. madagascariensis*; Sp, *S. pungens*.

Table 6. CVA loadings from MANOVA of first six symmetric PCs

Principal component	CV1	CV2
PC1	1.107	–0.421
PC2	**–4.125**	–1.676
PC3	**3.042**	2.065
PC4	–0.125	**–9.731**
PC5	**–4.980**	**4.029**
PC6	–1.710	**–3.437**

PCs are derived from symmetric EF coefficients. CV axes 1 and 2 explain 81.92 and 16.43% of leaf outline variation, respectively. The three PCs with the highest loadings on each axis are indicated by bold type.

classified cases for *S. gerrardii* (88.4%) and *S. pungens* (82.1%) and low percentages for *S. innocua* (65.9%) and *S. madagascariensis* (44.8%). Contrary to expectation, PC5 had the highest loadings on CV1, followed by PCs 2 and 3 (Table 6). The extremely reconstructed outlines along the axes and the CVA scatter plots show that, along the most significant axis, *S. madagascariensis* and *S. innocua* correspond to an obovate–orbicular shape pattern. *Strychnos pungens*, by contrast, corresponds to the elliptical end of the spectrum, and *S. gerrardii* is intermediate. This pattern is supported by field and herbarium observations. Figure 5 presents a clustering relationship based on mean PC scores from the symmetric dataset.

DISCUSSION

Genetic control of leaf shape symmetry and implications of asymmetry

One clear finding from our study is that symmetric variations play a major role in determining leaf shape among these four *Strychnos* spp. Results of ANOVA and MANOVA pairwise comparisons also indicate some degree of heritability for symmetric as opposed to asymmetric variations (Tables 3–5). Our findings of symmetric dominance and apparent heritability are consistent with many other studies in which plant organs (e.g. petals or leaves, fruits or seeds) have been subjected to EFA for the purpose of classification (Yoshioka *et al*., 2004; Torres, Demayo & Siar, 2008; Kawabata, Yokoo & Nii, 2009; Lexer *et al*., 2009). Although genetic control of leaf shape is well established (Kessler & Sinha, 2004; Tsukaya, 2006; Barkoulas *et al*., 2007; Koenig & Sinha, 2007), the inability of our shape variables to discriminate between *S. innocua* and *S. madagascariensis*, two distinct species (Frasier, 2008), would suggest that processes other than pure genetics are at work in determining leaf shape. Other contributors to shape could be epigenetic, environmental and developmental or, more likely, a complex interplay among these processes within the genetic background (Tsukaya, 2005; Klingenberg, 2010). Quantitative traits such as shape are known to be influenced by several genes and are more susceptible to developmental pertubations than their qualitative counterparts (Kessler & Sinha, 2004; Mackay, Stone & Ayroles, 2009).

In contrast to the symmetric variation, only asymmetric PC3 showed any interspecific variation. The extreme PC reconstruction suggests that asymmetry observed among the *Strychnos* spp. is essentially a left–right pattern easily observed at the basal and apical ends of the leaves (see asymmetric PCs 1 and 2 Fig. 3). The phenomenon of fluctuating asymmetry (FA) is applicable in this case (Van Valen, 1962; Yoshioka *et al*., 2004) as *Strychnos* leaves are bilaterally symmetrical under normal circumstances. FA results when individuals from the same population or species are unable to undergo identical development on both sides of the body or body organs that would otherwise exhibit bilateral or radial symmetry (Leary & Allendorf, 1989). It is generally attributed to environmental, developmental or genomic stress (Cowart & Graham, 1999; Roy & Stanton, 1999; Mal, Uveges & Turk, 2002). The level of object asymmetry encountered in this study may be more of a reflection of interspecific variation and not environmental or genomic stress, as studies of FA are usually conducted at smaller scales involving populations of the same species or even leaves on an individual plant. It is therefore difficult to evaluate with certainty the fitness significance of asymmetry in the present study.

Leaf morphospace and interspecific relationships

With the exception of the *S. innocua* and *S. madagascariensis* grouping, symmetric shape variables separated the other taxa including the *S. madagascariensis* and *S. gerrardii* complex. In Leeuwenberg's

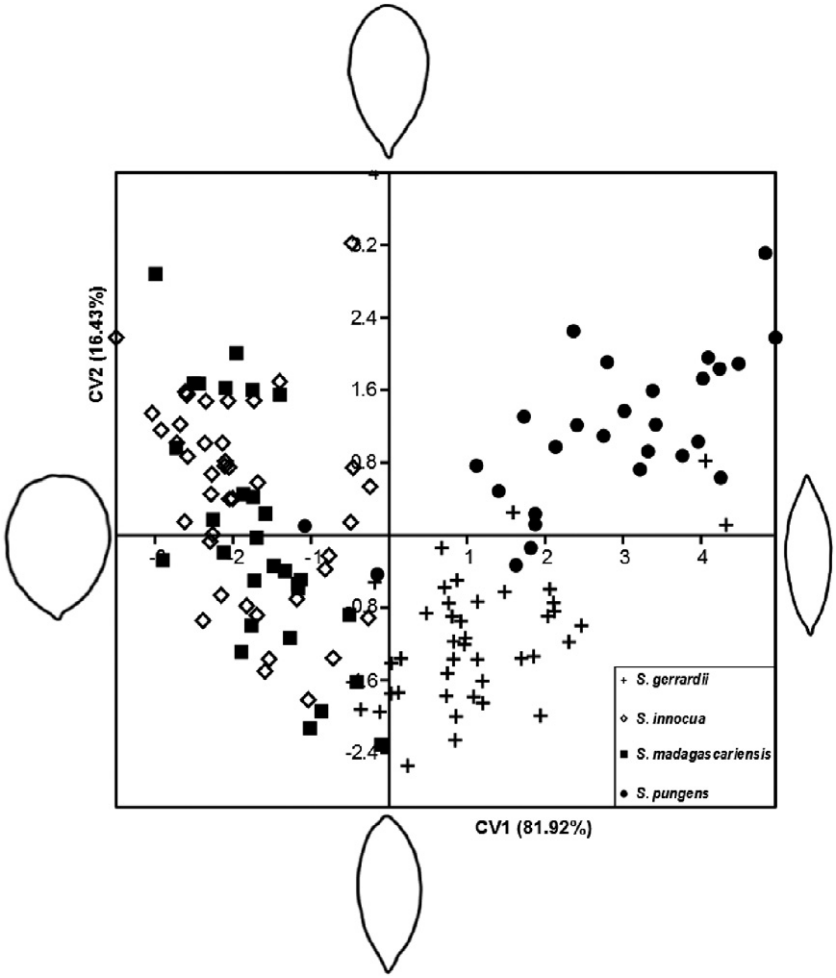

Figure 4. Canonical variate analysis scatter plot of leaf shape from four *Strychnos* spp. Shape variables were extracted from symmetric coefficients of EFA of leaf outline coordinates. Leaf outline reconstructions along each CV axis are shown.

(1969) treatment of the genus, *S. gerrardii* was synonymized to *S. madagascariensis*. In contrast, our analyses of leaf shape data suggest the two taxa can be identified with a high degree of accuracy (Fig. 4), and this is supported by other vegetative differences (Verdoorn, 1963). Most *S. gerrardii* specimens occupy an intermediate position in the morphospace between the two leaf-shape types especially along the first CV axis, and some cases overlap completely with *S. madagascariensis*–*S. innocua* leaf gestalt. This may explain why sterile specimens of *S. gerrardii* are sometimes confused with *S. madagascariensis* in herbarium collections. The potential for hybridization between *S. gerrardii* and *S. madagascariensis* may further confuse the leaf morphological picture in their areas of sympatry, as suggested by Verdoorn (1963). Indeed, some misclassified *S. gerrardii* specimens were sourced from the Tugela valley region of South Africa where putative hybrids between *S. gerrardii* and *S. madagascariensis* have been collected.

The perfect overlap in leaf shape observed between *S. madagascariensis* and *S. innocua* could have both environmental and genetic explanations. First, the species occupy similarly dry open habitats in southern Africa, and thus similarity in leaf shape could reflect evolutionary adaptation to environmental conditions, i.e. evolutionary convergence. Secondly, as shown by multi-gene molecular phylogenetic analysis (Frasier, 2008), *S. pungens* is sister to a clade comprising *S. innocua* and *S. madagascariensis*, possibly implicating some underlying, genetically mediated processes. Similarity of leaf shape may therefore reflect retention in the two species of an attribute in their most recent common ancestor. Verdoorn (1963) hypothesized a close affinity between the two based on morphological evidence. If we thus isolate the group of interest, there is a striking congruence between our cluster analysis result (Fig. 5) and the molecular phylogenetic hypothesis of Frasier (2008), although this may be a chance event associated with

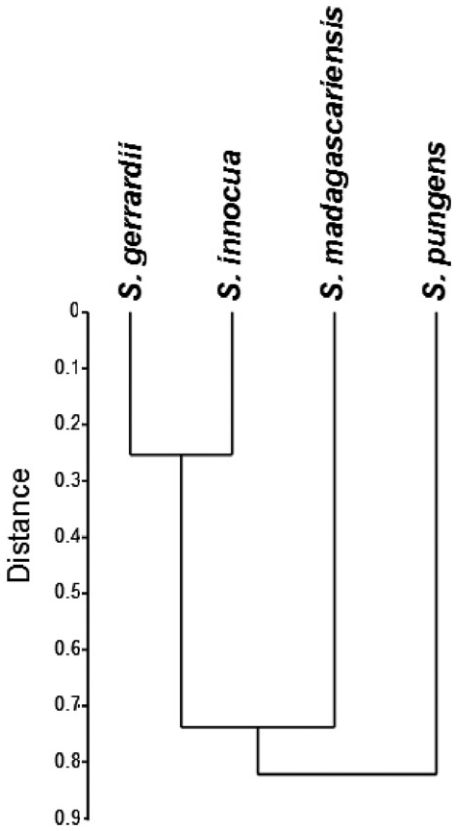

Figure 5. Cluster analysis of leaf outline from four species of *Strychnos* section *Densiflorae*, based on the mean of the first six PC scores of the symmetric dataset.

the small number of species involved. Leaf shape can converge among evidently disparate genetic entities inhabiting similar environments. This, however, is not to disregard the potential phylogenetic signal inherent in some morphometric datasets (MacLeod, 2002).

Strength of geometric morphometrics and limitations of human visual perception

Although there are any numbers of theoretically possible shapes in the morphospace of an organism (Gielis, 2003), there appears to be genetic and developmental canalization into adaptive channels that places constraints on the degree to which any organism can explore the shape space and still be evolutionarily viable. These constraints have functional and hence adaptive implications for shapes in many organisms. By applying the techniques of geometric morphometrics, this study has highlighted the importance of leaf shape in plant systematic studies. Leaf characters, as employed in our study, can prove to be diagnostic at the species level, which may be helpful to end users of the taxonomic enterprise in pragmatic ways. A particular strength of GM methods lies in its ability to detect subtle morphological changes imperceptible to the human eye. For instance, ANOVA uncovered significant interspecific differences in PCs 4 and 5 of the symmetric and PC3 of the asymmetric variables (Table 4). However, looking at the extreme reconstruction (Fig. 3), these differences are barely detectable to the human eye and may be missed altogether. To illustrate the scale to which the human eye can miss important details, symmetric PC5, which had the highest loading on CV1, offered the least visually perceptible variation, from a human viewpoint. It would appear that those quantitative variations most likely to be overlooked are the most important for discriminating closely related taxa. Such subtle variations, if heritable, may have fitness consequences under certain selective pressures. In some insect-pollinated plants, it has been demonstrated that slight variations in petal shape can influence the decision of potential pollinators (Yoshioka *et al*., 2007; Gomez & Perfectti, 2010) or even mate selection in some animal species (Møller & Höglund, 1991). The inability of the approach to discriminate between *S. madagascariensis* and *S. innocua* underlines the limitation of a single approach in solving problems in systematic biology. Nevertheless, geometric morphometrics constitutes an additional tool for analysis of morphological data and may prove valuable as we move in the direction of automated taxonomy.

ACKNOWLEDGEMENTS

This work was supported by the Thuthuka grant of the NRF awarded to Y.N. A.A. thanks Prof. Norman MacLeod of the NHM London for many explanations of geometric morphometric techniques, including free attendance in his taxonomic ordination short course. We also thank two anonymous reviewers for helpful comments and suggestions on an earlier version of the paper.

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APPENDIX 1

S. GERRARDII

Wood JM, 1777 (K); Wood JM, 5624 (PRE); Wood JM, 6163 (PRE); Adebowale A, 1; 2; 3; 8; 11; 12; 17; 23, eight specimens (UDW); Burrows JE, 9052 (BNRH); Moll EJ, 3334 (PRE); Moll EJ, 2409 (PRE); Edwards D, 2601 (NU); Edwards D, 1414; 2823; 2829, three specimens (PRE); Bourquin O, s.n. NH53743 (NH); Nicholas & Ngwenya, 2198 (NH); Ward CJ, 2570; 3198; 4026; 4045; 4052; 4108; 4128, seven specimens (PRE); Tinley KL, 58 (PRE); Burtt-Davy J, 2419 (PRE); Codd LEW & Verdoorn IC, 10176; 10186; 10202; 10204; 10205, five specimens (PRE); Coleman TA, 382 (PRE); Verdoorn IC, 2466 (PRE); Dyer RA, 4347 (PRE); Gerstner, 5063 (PRE); Bayer AJW, 845 (PRE); Huntley BJ, 96 (PRE); Rudatis AGH, 588 (PRE); Boocock JJ, FD5729 (PRE); Stephen JJF, 42 (PRE); Ross JH & Moll EJ, 2297 (PRE).

S. MADAGASCARIENSIS

Rosenfels E, 4 (GRA); Lehmann sn (PRE); Rodin RJ, 4510 (K); Ward CJ, 4079 (NU); Edwards D, 2826 (NU); Fourie SP, M132 (PRE); Glen HF, 2094 (PRE); Glen HF, 2108 (J); Shackleton CM, 730 (J); Raymond 230 (J); Davidson LE, 111 (J); Dyer S, 107 (J); Kerfoot O, K7082 (J); Balkwill K & Balkwill MJ, 9288 (J); Clinning CF, 24 (J); Hemin G, 702 (J); Mogg AOD, 27202; 30127; 31047, s.n. J35583; s.n. J36881 five specimens (J); Adebowale A, 56; 67; 73; three specimens (UDW); van Son TRV, 28860 (PRE); Burtt, 6075 (K); Chase, 4705 (PRE); Barbosa, 2050 (PRE); Schlechter, 11615 (BOL); Wild, 3016 (PRE); Burrows JE, 3900 (BNRH); Burrows JE & Burrows SM, 10718 (BNRH).

S. INNOCUA

Ringoet A, 17786 (BM); Schimper, 1817 (MO); Rowland, s.n. K000426582 (K); Schweinfurth G, 1719 (K); Schweinfurth G, 1432 (K); Schweinfurth G, 1412 (K); Grant, JA s.n., K000426639 (K); Schweinfurth G, 1660 (K); Dalton JT, s.n. K000426583 (K); Barter C, 1160 (P); Antunes vel Dekindt, 1138 (LISC); Goetze W, 1436 (P); No name, s.n. E00193255 (E); Verdick E, s.n. BR0000008949073 (BR); van Wyk P, s.n. PRU074665 (PRU); Congdon s.n. (PRE) s.n. Collection date 21 February 1988; Mogg AOD, 34225 (J); Whellan, 429 (SRGH); Wild, 3016 (PRE); Burtt, 6075 (K); Hodgson, 8/52 (PRE); Greenway PJ, 4851 (K); Burrows JE, 10265 (BNRH); Burrows JE, & Burrows SM, 11104 (BNRH).

S. PUNGENS

Sutton JD, 1018 (PRE); Pegler AM, 1034 (PRE); Rodin RJ, 2663 (K); Laburn RJ, 93 (J); Prosser LN, 1736 (J); Peeters C, Gerioke N & Burelli G, 492 (J); Netshungani EN, 1017 (J); Munday J, 537 (J); Larson TD, 29 (J); Phillips JFV, 2309 (J); Moss CE, 12085 (J); Schroeder CE, s.n. (J) s.n. collection date 28th August 1938; Mogg AOD, 20263; 34007; 36366 three specimens (J); Burtt-Davy J, s.n. PRE14634 (PRE); Story R, 6432 (PRE); De Winter B, 4203 (PRE); Verdoorn IC, 2430 (PRE); Giess JWH, 9514 (PRE); Watt J, 19 (PRE); Strey RG, 3184 (PRE); Theron GK, 1492 (PRE); Repton JE, 1756 (PRE); Miller OB, B965 (PRE); Young RGN, 3001 (PRE); Jacobsen NHG, 2449 (PRE); Snyman JW, 171 (PRE); Schlieben HJE, 9172 (PRE); Gillett JB, 3300 (PRE) Letty CL, 385 (PRE).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Bonferroni post hoc test for symmetric PC1 ANOVA.
Table S2. Bonferroni post hoc test for symmetric PC2 ANOVA.
Table S3. Bonferroni post hoc test for symmetric PC3 ANOVA.
Table S4. Bonferroni post hoc test for symmetric PC4 ANOVA.
Table S5. Bonferroni post hoc test for symmetric PC5 ANOVA.
Table S6. Post hoc test for asymmetric PC3 ANOVA.
Table S7. Group classification summary.

Supplementary information to chapter 2

SI 1: Bonferroni Post hoc test for symmetric PC1 ANOVA

Groups*	Difference	Statistic	P>value	Significant
Sg vs Si	-0.176	10.331	0.000	YES
Sg vs Sm	-0.122	6.714	0.000	YES
Sg vs Sp	-0.023	1.550	0.126	NO
Si vs Sm	0.054	2.217	0.030	NO
Si vs Sp	0.152	6.833	0.000	YES
Sm vs Sp	0.098	4.082	0.000	YES

*** Si = *S. innocua*; Sg = *S. gerrardii*; Sp = *S. pungens*; Sm = *S. madagascariensis***

SI 2 : Bonferroni Post hoc test for symmetric PC2 ANOVA

Groups	Difference	Statistic	P>value	Significant
Sg vs Si	0.010	2.242	0.028	NO
Sg vs Sm	0.020	4.315	0.000	YES
Sg vs Sp	-0.032	6.410	0.000	YES
Si vs Sm	0.009	1.623	0.109	NO
Si vs Sp	-0.042	7.061	0.000	YES
Sm vs Sp	-0.051	8.677	0.000	YES

SI 3: Bonferroni Post hoc test for symmetric PC3 ANOVA

Groups	Difference	Statistic	P>value	Significant
Sg vs Si	-0.000	0.043	0.966	NO
Sg vs Sm	-0.003	0.755	0.453	NO
Sg vs Sp	0.009	2.848	0.006	YES
Si vs Sm	-0.003	0.732	0.467	NO
Si vs Sp	0.009	3.012	0.004	YES
Sm vs Sp	0.012	3.583	0.001	YES

SI 4: Bonferroni Post hoc test for symmetric PC4 ANOVA

Groups	Difference	Statistic	P>value	Significant
Sg vs Si	-0.005	3.373	0.001	YES
Sg vs Sm	-0.007	4.996	0.000	YES
Sg vs Sp	-0.010	8.127	0.000	YES
Si vs Sm	-0.002	0.919	0.362	NO
Si vs Sp	-0.005	2.581	0.012	NO
Sm vs Sp	-0.003	1.798	0.078	NO

SI 5: Bonferroni Post hoc test for symmetric PC5 ANOVA

Groups	Difference	Statistic	P>value	Significant
Sg vs Si	0.005	3.352	0.001	YES
Sg vs Sm	0.003	1.437	0.155	NO
Sg vs Sp	0.002	1.233	0.222	NO
Si vs Sm	-0.002	1.350	0.182	NO
Si vs Sp	-0.003	1.720	0.090	NO
Sm vs Sp	-0.000	0.233	0.817	NO

SI 6: Post hoc test for asymmetric PC3 ANOVA

Group	Mean	Group	Mean	Difference	Fisher LSD	Significant
Sg	0.002	Si	-0.000	0.002	0.003	NO
Sg	0.002	Sm	-0.002	0.005	0.003	YES
Sg	0.002	Sp	-0.001	0.003	0.003	NO
Si	-0.000	Sm	-0.002	0.002	0.003	NO
Si	-0.000	Sp	-0.001	0.001	0.003	NO
Sm	-0.002	Sp	-0.001	0.001	0.004	NO

Note: Bonferroni post hoc did not detect any difference between pairs of species for asymmetric PC3 although ANOVA results suggest some level of difference however marginal. However, Fisher's LSD indicated differences between *S. gerrardii* and *S. madagascariensis*

SI 7: Group classification summary

		Classification				Classification with cross-validation			
					Given Groups				
		Sg	Si	Sm	Sp	Sg	Si	Sm	Sp
Predicted	Sg	39	1	2	3	38	1	4	4
	Si	0	28	9	1	0	27	12	1
Group	Sm	1	12	18	0	2	13	13	0
	Sp	3	0	0	24	3	0	0	23
	Total	43	41	29	28	43	41	29	28
	Correct	39	28	18	24	39	25	13	23
	% Correct	**90.7**	**68.3**	**62.1**	**85.7**	**88.4**	**65.9**	**44.8**	**82.1**

Si = *S. innocua*; Sg = *S. gerrardii*; Sp = *S. pungens*; Sm = *S. madagascariensis*

CHAPTER 3

ADEBOWALE A, NAIDOO Y, LAMB J, Nicholas A. 2014. Comparative foliar epidermal micromorphology of Southern African *Strychnos* L. (Loganiaceae): taxonomic, ecological and cytological considerations. *Plant Systematics and Evolution* 300: 127 – 138.

Plant Syst Evol (2014) 300:127–138
DOI 10.1007/s00606-013-0865-z

ORIGINAL ARTICLE

Comparative foliar epidermal micromorphology of Southern African *Strychnos* L. (Loganiaceae): taxonomic, ecological and cytological considerations

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Received: 4 November 2012 / Accepted: 18 June 2013 / Published online: 3 July 2013

Abstract The micromorphology of the leaf epidermis of 11 species across four sections of southern African *Strychnos* was investigated using light microscopy and scanning electron microscopy. In addition to this, preliminary genome size was assessed with flow cytometry. Qualitative and quantitative results are presented for stomata, trichome and cuticular wax features with an emphasis on the abaxial epidermal surface. A correlated combination of these microscopic features was able to distinguish successfully among the 11 species of *Strychnos* found in the subcontinent. However, micromorphological evidence does not support the current circumscription of the sections. The often-confused *S. gerrardii* and *S. madagascariensis* are distinguishable on leaf micromorphological grounds. Stomata and trichome features show remarkable patterns that largely correlate with the ecological distribution of *Strychnos* species as either forest or savanna inhabitants. The significant variability in stomatal length across species is hypothesized to be indicative of possible existence of variable ploidy levels within the genus in southern African. However, preliminary genome size analyses with flow cytometry appear to be inconclusive.

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Keywords *Strychnos* · Leaf micromorphology · Stomatal index · Flow cytometry · Ploidy level · Stomata size · Trichomes

Introduction

Strychnos L. is the largest and most important genus of the family Loganiaceae, comprising ca. 200 species (Backlund et al. 2000). It is a genus of trees, shrubs and woody climbers distributed across three distinct geographical regions in the continents of Africa, South America and Australasia. Africa has the highest number of species and is regarded as the centre of origin for the genus. A number of the African species are important sources of food and medicine (Frederich et al. 2002; Mwamba 2006). However, the seeds of some of these species are known to be toxic; they contain an alkaloid called strychnine, a poison that has been actively extracted and exploited for its fatal toxins (Ohiri et al. 1983; Philippe et al. 2004).

According to Verdoorn (1963), whose taxonomy was based solely on macro-morphological characters, there are nine species of *Strychnos* in southern Africa (SA). The accumulation of additional collections in different herbaria in SA within the last four decades suggests that species diversity may be higher than earlier reported. The similarity in the macromorphology of some SA species of *Strychnos*, especially leaf morphology, has caused a high degree of misidentification among closely related taxa. Correct identification is important not only for proper documentation of biodiversity but also to avoid accidental poisoning that could result from ingesting the seeds of poisonous species.

The important role of leaf epidermal features in higher plant systematics is well established in the taxonomic

literature (Barthlott 1981; Stace 1984; Zou et al. 2008; Chen et al. 2010; Zhou and Xia 2012). While there has been numerous studies on the poisons and alkaloids of various species of *Strychnos* (Bisset 1972; see review in Philippe et al. 2004), there is virtually no published work, from a taxonomic point of view, on the leaf epidermal features of the genus. Although Bendre (1973) examined trichomes in the family Loganiaceae sensu lato, his work included only a few extra-African *Strychnos* species and did not consider the potential taxonomic value of other epidermal features. This study aims to elucidate the micromorphological features of the leaf epidermis of 11 taxa of *Strychnos* from southern Africa. It covers four of the 12 recognized sections within the genus. This study is part of a larger investigation into the biosystematics of southern African *Strychnos* and is aimed at assessing the taxonomic value of leaf epidermal micromorphology in identifying species of *Strychnos* and in elucidating their possible evolutionary history. The ecological adaptations associated with environmental variation of the epidermal features of each species will also be discussed.

Materials and methods

Specimen voucher information is provided in Table 1. Mature leaf samples were obtained from fresh collections made in the field as well as from herbarium specimens. For SEM, leaf segments approximately 5 mm^2 were sampled from the median portion of each leaf and fixed in 2.5 % phosphate-buffered glutaraldehyde (0.1 M pH 7.2) for 24 h; the leaves were then washed in three different changes of phosphate buffer and postfixed in 1 % aqueous osmium tetroxide for 1 h following Pathan et al. (2008). The leaf samples were again rinsed in water and dehydrated in an ascending ethanol series (25, 50, 75 and 100 %) for 15 min at each concentration. Dehydrated materials were dried in a Hitachi Critical Point dryer HCP-1. Samples were then mounted onto copper stubs and sputter-coated with gold using a Polaron SC500 Sputter Coater for 3 min at 30 mA. Specimens were observed with a Jeol JSM 6100 SEM. In order to examine the consistency of epidermal features, four leaves from separate individuals representing a range of leaf variations were sampled per species and at least two pieces were taken per leaf for examination. Quantitative data were collected from four pieces, each representing individuals sampled per species. An ANOVA test was performed on the stomatal length followed by Scheffe's post hoc test.

As stomatal type and index are sometimes not very clear from SEM images, light microscopy investigation was conducted to access information on these structures. For light microscopy preparations, leaves were cleared according to a schedule modified from Hickey (1973). Median portion of leaves were placed in 5 % sodium hypochlorite ($NaHClO_2$) solution at room temperature for 24 h and then rinsed in distilled water. A few drops of glacial acetic acid were added to neutralize the bleach. The materials were then dehydrated in an ethanol series of increasing strength (25, 50, 75 and 100 %) for 15 min at each concentration, stained in 1 % safranin O for 10 min, mounted in glycerine and observed with an Olympus BH-2 research microscope.

Stomatal and trichome parameters (except stomatal index and type) were taken from SEM images. Stomatal and trichome densities were computed as absolute number per mm^2. Light microscopy images were used to ascertain stomatal type, while stomatal index values were computed using the formula of Salisbury (1927):

$$\text{Stomatal Index} = s/e+s(100)$$

where s is number of stomata per unit area and e is the number of ordinary epidermal cells in the same unit area. The classificatory hypothesis of *Strychnos* taxa used here followed that of Verdoorn (1963), although *S. innocua* Del. (sensu stricto) was not included in her treatment of southern African species. Stomatal terminology was based on that proposed by Dilcher (1974), while the classification

Table 1 Sample table indicating species used in the study, locality and some voucher information

Taxon	Section	Habitat (forest or savanna)	Voucher (Herbarium abbreviations following Holmgren et al. 1990)
S. cocculoides Bak.	Spinosae	Savanna	Story 6343 (PRE)
S. decussata (Pappe) Gilg	Rouhamon	Forest	Adebowale 70 (UDW)
S. gerrardii N.E.Br.	Densiflorae	Coastal forest	Nicholas and Ngwenya 2198 (NH)
S. henningsii Gilg	Breviflorae	Forest	Adebowale 20 (UDW)
S. innocua Del.	Densiflorae	Savanna	Merello, Harder and Nkhoma 999 (PRE)
S. madagascariensis Poir.	Densiflorae	Savanna	Adebowale 73 (UDW)
S. mitis S. Moore	Breviflorae	Wet forest	Adebowale 64 (UDW)
S. potatorum L.f.	Rouhamon	Wet forest	Muller 1802 (WIND)
S. pungens Solered.	Densiflorae	Savanna	Jankowitz 1341 (PRE)
S. spinosa Lam.	Spinosae	Savanna	Schijff and Marais 3686 (PRE)
S. usambarensis Gilg	Rouhamon	Forest	Adebowale 54 (UDW)

of other epidermal characters was based on the work of Wilkinson (1979). Statistical analyses were performed using PAST version 2.05 (Hammer et al. 2001) and GenStat Discovery Edition (2007).

We conducted a preliminary estimate of ploidy levels from DNA content (C value) by flow cytometry (FC) using a few available samples. Approximately 25 mg of healthy leaves of *Strychnos* species were chopped with razor blade and treated in Galbraith's buffer (Galbraith et al. 1983). Fresh leaves of *Solanum lycopersicum* L. (2C = 1.96 pg) were used as internal standard. Samples were filtered through a 50 µm nylon mesh, treated with RNase and incubated at 37 °C for 30 min. Propidium iodide staining protocol of Hanson et al. (2005) was used. Samples were analyzed on a BD FACSAria flow cytometer and data were processed with FACSDiva 6.1.2 software (BD Biosciences, Franklin Lakes, NJ, USA). Samples were run in triplicates with 3,000 nuclei counts per run. DNA content (C value) was calculated according to Dolezel and Bartos (2005), while ploidy level was inferred from mean positions of the G_0/G_1 peak in the DNA histogram of the *Strychnos* specimens relative to the internal standard.

Results

A summary of quantitative and qualitative leaf epidermal micromorphological features are presented in Tables 2 and 3, respectively. Due to the consistency and similarity observed in the qualitative characters on the adaxial surface across the group, the results presented here are for abaxial surfaces except when otherwise indicated.

Trichomes

Four of the species investigated, namely *S. cocculoides* Bak., *S. innocua*, *S. madagascariensis* Poir. and *S. spinosa* Lam. possessed non-glandular hairs on both the abaxial (Figs. 1–4) and adaxial surfaces, while the other species lacked epidermal hairs. There is considerable variation in trichome length across the four taxa, ranging from an average of 110.37 µm in *S. cocculoides* to 238.15 µm in *S. innocua*, as shown in Table 2. Trichome surface ornamentation separates the four pubescent taxa into two groups. Group one comprises *S. spinosa* and *S. cocculoides* both of which have smooth hairs (Figs. 4–5), while the second group comprising *S. innocua* and *S. madagascariensis* have ornamented trichomes (Figs. 6–7). Hairs are usually present on the leaf margins (Fig. 8) and along the main veins where they are usually abundant (Fig. 9). Unique to *S. spinosa* is the presence of hair pockets (domatia) on its abaxial surface even in otherwise glabrous specimens (Fig. 10). Trichomes are significantly more abundant on the abaxial surface than on the adaxial in all the pubescent species observed (Figs. 11–12).

Stomata

All the *Strychnos* species examined in this study are hypostomatic (stomata on abaxial surface only). Stomatal density is species-specific and varies across a wide spectrum from an average of 182 mm^{-2} in *S. mitis* S. Moore to 485 mm^{-2} in *S. cocculoides* (Table 2). Contrary to expectation, stomatal density (SD) and stomatal index (SI) bear no obvious relationship to stomatal length (SL) with respect to sample ecotype. The stomatal type found within

Table 2 Quantitative leaf epidermal characters of southern African *Strychnos*

Species	Stomatal density (mm^{-2}) ± sd	Stomatal index (%)	Stomatal length (µm)	Stomatal breadth (µm)	Trichome length (µm)	Trichome density (ab.) (mm^{-2})	Trichome Density (ad.) (mm^{-2})
Strychnos cocculoides	485.76 ± 46.95	14.84 ± 1.27	17.89 ± 2.34	5.04 ± 1.33	110.37 ± 12.30	35.22 ± 3.02	10.48 ± 1.25
S. decussata	442.80 ± 59.12	16.13 ± 1.46	13.70 ± 1.32	4.65 ± 0.67	–	–	–
S. gerrardii	349.45 ± 49.23	20.68 ± 2.06	16.65 ± 1.67	4.08 ± 1.10	–	–	–
S. henningsii	340.14 ± 57.08	16.25 ± 1.84	8.10 ± 1.08	7.02 ± 0.59	–	–	–
S. innocua	464.85 ± 63.40	15.92 ± 1.04	20.02 ± 1.75	7.50 ± 0.82	238.15 ± 44.63	42.80 ± 3.07	7.40 ± 0.88
S. madagascariensis	470.30 ± 77.34	16.18 ± 1.55	19.14 ± 1.97	5.27 ± 1.06	219.42 ± 21.78	66.85 ± 3.69	11.60 ± 1.54
S. mitis	181.86 ± 41.87	12.51 ± 0.84	13.06 ± 1.84	2.92 ± 0.49	–	–	–
S. potatorum	456.85 ± 91.53	21.15 ± 2.37	15.65 ± 2.70	3.36 ± 1.14	–	–	–
S. pungens	419.57 ± 76.70	17.24 ± 2.83	12.66 ± 1.63	3.82 ± 0.55	–	–	–
S. spinosa	199.50 ± 54.78	16.59 ± 1.44	29.09 ± 2.44	5.46 ± 1.15	188.76 ± 29.82	20.70 ± 2.75	8.52 ± 1.49
S. usambarensis	427.15 ± 52.29	19.68 ± 1.77	5.03 ± 0.74	2.06 ± 0.54	–	–	–

All values based on 25 measurements across four samples per species; *ab.* abaxial, *ad.* adaxial. Values represent arithmetic means

Table 3 Qualitative leaf epidermal characters of *Strychnos* species

Species	Stomatal shape	Stomatal complex elevation	Pattern of anticlinal walls	Wax ornamentation	Trichome
S. cocculoides	Elliptic	Sunken	Weak curved to straight	Coarse warty striation	+
S. decussata	Elliptic	LWE	Straight	Fine striated	−
S. gerrardii	Elliptic	LWE	Straight	Lightly striate around stomata	−
S. henningsii	Circular	LWE	Straight	Smooth	−
S. innocua	Elliptic to angular	Sunken	Straight	Coarse striation	+
S. madagascariensis	Elliptic	LWE	Straight	Wing like striation around stomata	±
S. mitis	Elliptic	LWE	Straight	Smooth	−
S. potatorum	Elliptic	LWE	Straight	Smooth	−
S. pungens	Elliptic	EGC	Weakly curved; straight	Flaky/scaly	−
S. spinosa	Elliptic	LWE	Curved; straight	Fine; around the stomata	±
S. usambarensis	Narrowly elliptic	LWE	Straight	Coarse warty striation	−

LWE level with other epidermal cells, EGC elevated guard cell

+ present, − absent, ± present in some, absent in some

the group is generally anomocytic, in which the epidermal cells bordering the guard cells are indistinguishable from other epidermal cells (Fig. 13). The stomatal complex does not always fit the classical descriptions, however, as there is a tendency towards the weakly actinocytic type in some of the species. The species could be partitioned into two broad groups based on stomatal elevation: those with sunken stomata, *S. cocculoides* and *S. innocua* (Figs. 14–15), and the others without sunken stomata (Figs. 16–24). The guard cells in *S. pungens* Solered. show slight elevation (Fig. 20) while Figs. 25 and 26 highlight the general hypostomatic nature of *Strychnos* species.

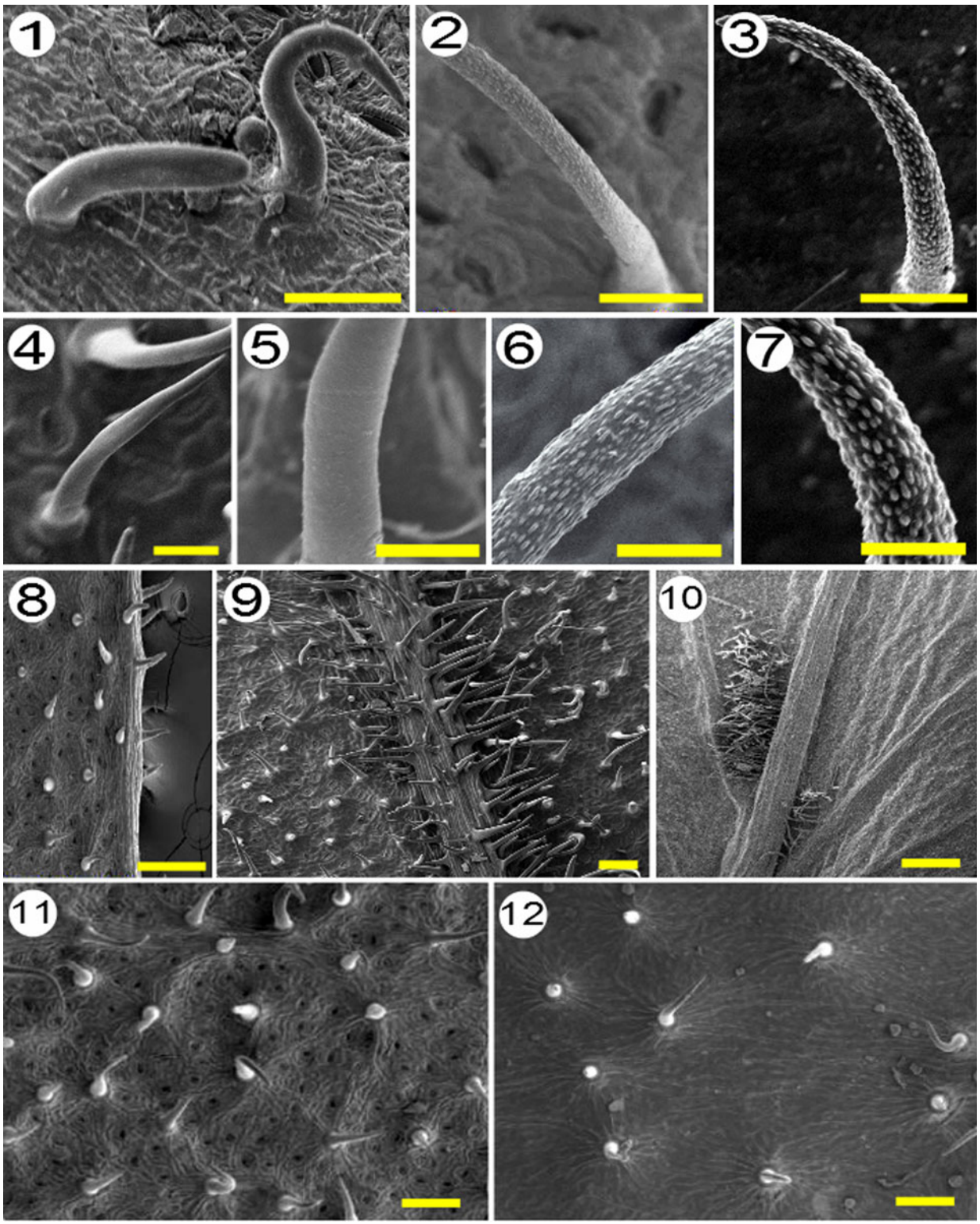
Stomatal shape is elliptic in all species examined with the exception of *S. henningsii* Gilg, which exhibits a decidedly circular outline (Fig. 24). Analyses of variance for SL and density showed significant variation among the species (Table 4 for SL only). Stomatal length, usually regarded as a proxy for ploidy level (Marciniuk et al., 2010), is particularly large in *S. spinosa* and very small in *S. henningsii* and *S. usambarensis* Gilg (Table 2). For the purpose of this study, stomatal length is arbitrarily classified as long (≥21 μm), moderate (11–20 μm) and short (≤10 μm). ANOVA post hoc tests show that there is no ecological signal in stomatal length data (Table 5). Forest species are just as likely to show similarity in their SL with savanna species as they might show with other forest species. Particularly interesting is that *S. spinosa, S. usambarensis* and *S. henningsii* do not have any overlap in SL with any of the other taxa. They rather differ significantly from the other species and from one another (Table 5).

Cuticular ornamentation

SEM observation of cuticular membranes on the leaf epidermis revealed either a smooth surface or some degree of striation. In *S. henningsii, S. mitis*, *S. potatorum* and *S. pungens*, the membrane is smooth or nearly so (Figs. 27–30). *S. decussata* and *S. spinosa* are usually smooth but occasionally display fine striations as shown in Figs. 31 and 32, respectively. Coarse irregular warty striae are characteristic of *S. cocculoides* and *S. innocua* (Figs. 33, 34), while *S. usambarensis* are usually smooth (Fig. 35), occasionally display coarse striations (Fig. 36). *S. gerrardii* N.E. Br. and *S. madagascariensis* have warty striae on their stomata as lateral wings (Figs. 37, 38) (more pronounced in the latter). Generally, the adaxial surfaces are not striated, but when they are, as in *S. madagascariensis*, the striae are usually of the fine warty type (Fig. 39).

Preliminary genome size and ploidy level estimation

Of the 37 specimens of *Strychnos* species subjected to FC analysis, only nine across three species (*S. gerrardii*, *S. henningsii* and *S. pungens*) yielded sufficient nuclei for any meaningful analysis. Estimated 2C DNA content for each of the three species is presented in Table 6. Representative samples of the three species have similar genome size, ranging from 1.88 to 1.92 pg/2C. This is considerably higher than the value of 1.72 pg/4C = 0.86 pg/2C reported for *Strychnos nux-vomica* L. (Table 5; Hanson et al. 2005). In spite of background noise, fluorescence histograms indicate that *S. henningsii* and *S. pungens* samples



Figs. 1–12 SEM of non-glandular trichomes (NGT) found in *Strychnos* species: **1** *S. cocculoides*; **2** *S. innocua*; **3** *S. madagascariensis*; **4** *S. spinosa*; **5** *S. cocculoides* NGT smooth trichome surface; **6** *S. innocua*, trichome with surface ornamentations; **7** *S. madagascariensis*, trichome with surface ornamentations. **8** *S. cocculoides* with hairs on the margin; **9** *S. spinosa* showing dense hairs on the main vein; **10** *S. spinosa*, with domatia on abaxial surface of otherwise glabrous specimen; **11–12** differential trichome density on abaxial and adaxial surfaces of *S. cocculoides*. **11** abaxial surface; **12** adaxial surface. *Scale bar* 50 μm for Fig. 1–4; *scale bar* 100 μm for Fig. 5–9, Fig. 11–12; *scale bar* 400 μm for Fig. 10

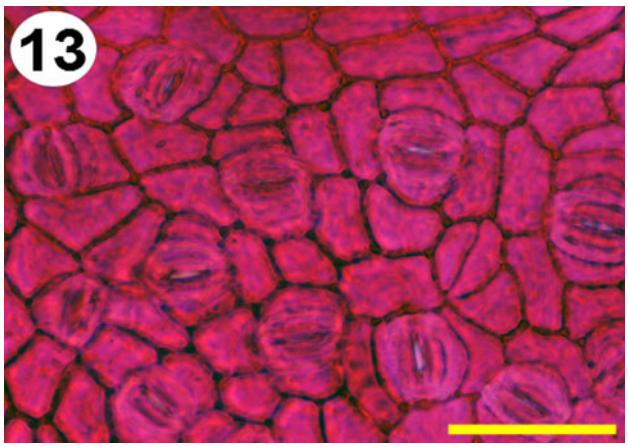


Fig. 13 *S. innocua* LM image displaying anomocytic stomata typical of South African *Strychnos*. *Scale bar* 50 µm

examined are diploid while *S. gerrardii* may be some form of aneuploid from a diploid base number (Fig. 40). We interpreted our FC results with caution owing to high CV values and small sample size.

Discussion

Taxonomic discrimination among southern African *Strychnos*

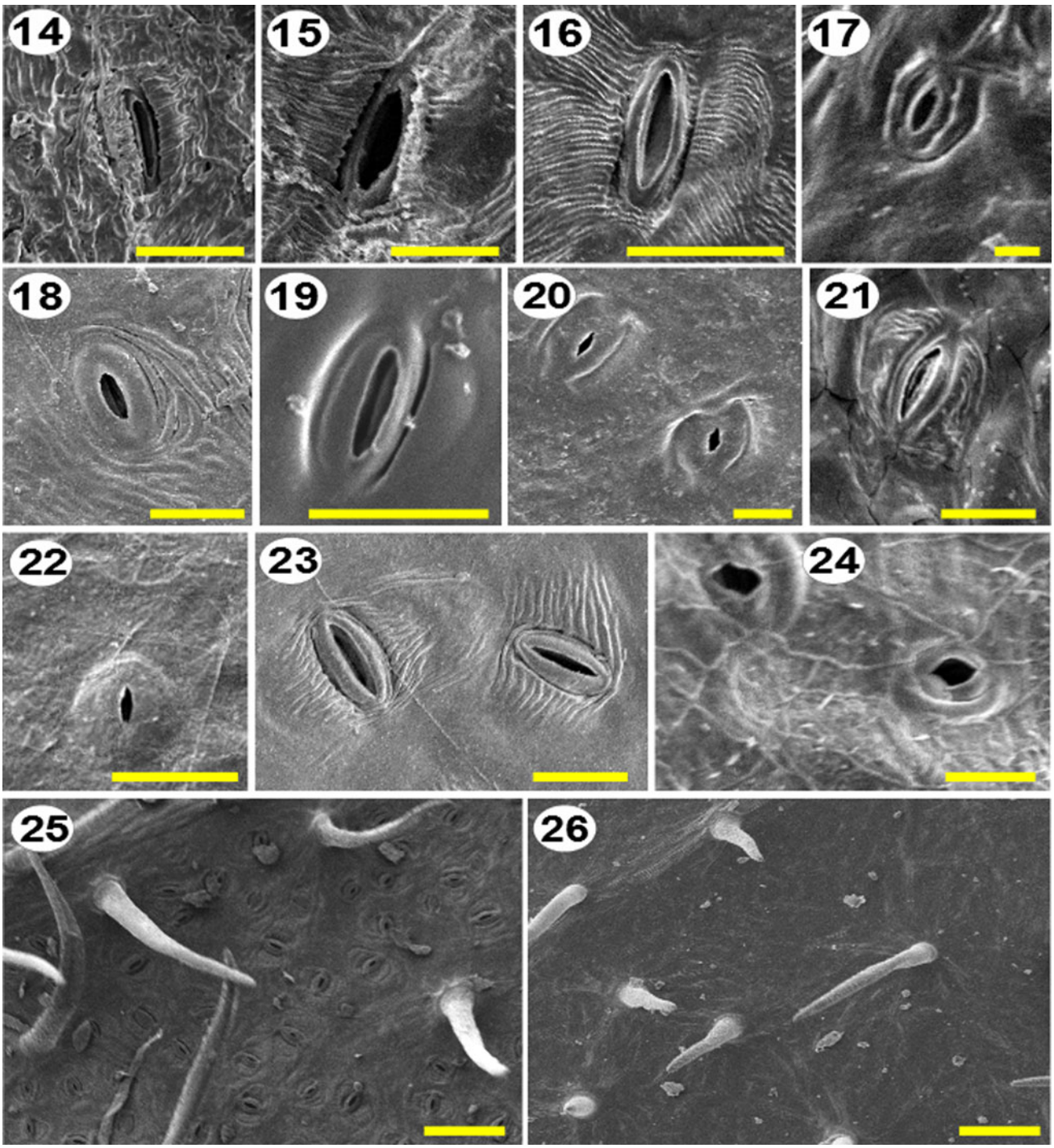
Although leaf epidermal features are sometimes influenced by environmental conditions (Hlwatika and Bhat 2002; Casson and Hetherington 2010), there is ample evidence for their overall genetic control (Cutler and Brandham 1977; Barthlott 1981; Masle et al. 2005). As such, they have been employed for plant species discrimination at various taxonomic ranks (Ghahremaninejad et al. 2012). While no single leaf epidermal feature was sufficient in separating the *Strychnos* species studied here, a combination of correlated features relating to stomata, trichomes and epicuticular wax striations proved effective in discriminating among even closely related taxa. This can be seen within *Strychnos* section Densiflorae Duvign., which comprises four taxa (Table 1). Leeuwenberg (1969) considered *S. gerrardii* as synonymous with *S. madagascariensis* and sunk the former name in favor of the latter. However, the degree of stomatal elevation, stomatal shape and the consistent lack of trichomes in *S. gerrardii* suggest that the two are separate taxa. These differences may reflect the different ecology of *S. gerrardii* (a forest species) and *S. madagascariensis* (a savanna species).

Section *Breviflorae* Prog., comprising *S. mitis* and S. *henningsii,* are forest species and possess smooth epicuticular wax. Their stomatal shape, which is decidedly circular in the latter and narrowly elliptic in the former, however, easily separates these two species (Figs. 19, 24). Members of section *Spinosae* Duvign. (*S. spinosa* and *S. cocculoides*) are separated by their cuticular striations and stomatal size (Tables 2 and 3). Within section *Rouhamon* (Aubl.) Prog., *S. usambarensis* is easily distinguishable from the other two members by its very small stomata and coarse warty cuticular striae. *S. decussata* and *S. potatorum* on the other hand are marginally separated by the convex curvature of epidermal cells, which are smooth, in the latter (Fig. 29) coupled with fine cuticular wax striations in the former.

Leaf micromorphological features do not separate the *Strychnos* species neatly along the sectional categories of Leeuwenberg (1969). This is consistent with the findings of Frasier (2008) which, based on molecular evidence queried the evolutionary basis of such categories in the first place, and recommended a revision along sectional distinctions. While a perfect morphological fit is not necessarily expected among members of the same section, some intra-sectional consistency would strengthen the case for the validity of a natural group. However, the extent to which micromorphological convergence among sectional members is probable remains an open question. From the phylogenetic framework of Frasier (2008), any interpretation of evolutionary affinity in *Strychnos* based on micromorphology must be conducted with considerable degree of circumspection.

Ecological influences on leaf epidermal morphology

Within genetic constraints, environmental factors can exert notable influence on many foliar epidermal features in plants (Klich 2000; Royer 2001; Gielwanowska et al. 2005). Ecophysiological aspects such as light, water availability and CO_2 levels are among the most prominent factors that shape leaf features (Deccetti et al. 2008; Haworth and McElwain 2008). However, it appears that certain features are more susceptible to ecological fluctuations than are others. Royer (2001) and other investigators have found that SI and SD are inversely correlated with CO_2 levels, although SD is much more affected by other environmental stresses than SI. While the two parameters generally decrease with increasing CO_2 levels, the trend is less pronounced in hypostomatous species (Woodward and Kelly 1995), such as *Strychnos*, and even negligible over a small time scale (Haworth et al. 2013). Other factors known to influence SD and SI include but is not limited to altitudinal gradient and atmospheric temperature (Beerling and Chaloner 1993; Haworth and McElwain 2008). On the *average* though, there are significant differences ($P < 0.05$) in total SD and total SI between the forest and savanna species of *Strychnos*. Stomatal frequency is on the *average* higher for the savanna species than forest species. High stomatal frequency is typical of plants in more



Figs. 14–27 SEM of *Strychnos* stomata. **14** and **15**, sunken elliptic stomata. **14** *S. cocculoides*; **15** *S. innocua*; **16** *S. madagascariensis* showing lateral wings of cuticular striae; **17** *S. spinosa*; **18** *S. decussata*; **19** *S. mitis*; **20** *S. pungens* with raised guard cells around stoma; **21** *S. potatorum*; **22** *S. usambarensis*; **23** *S. gerrardii*; **24** *S. henningsii* with circular stomata; **25** *S. madagascariensis* abaxial with stomata and trichomes; **26** *S. madagascariensis* adaxial with trichomes but without stomata. *Scale bar* 20 μm for Fig. 14–24; *scale bar* 100 μm for Fig. 25 and 26

exposed habitats as these are usually areas of high light intensities (Rossatto and Kolb 2010), and could thus play some role in increasing photosynthetic rates for such species (Galmes et al. 2007). Generalizing across taxa is not recommended, however, as different species within the same plant community may show differences in their stomata response to environmental variables (Ferris and Taylor 1994).

Table 4 ANOVA of stomatal length among *Strychnos* species

Source	*df*	SS	MS	F
Between (species)	10	10434.212	1043.421	338.75*
Within species	264	812.929	3.079	
Total	274	11247.142		

* $P < 0.001$

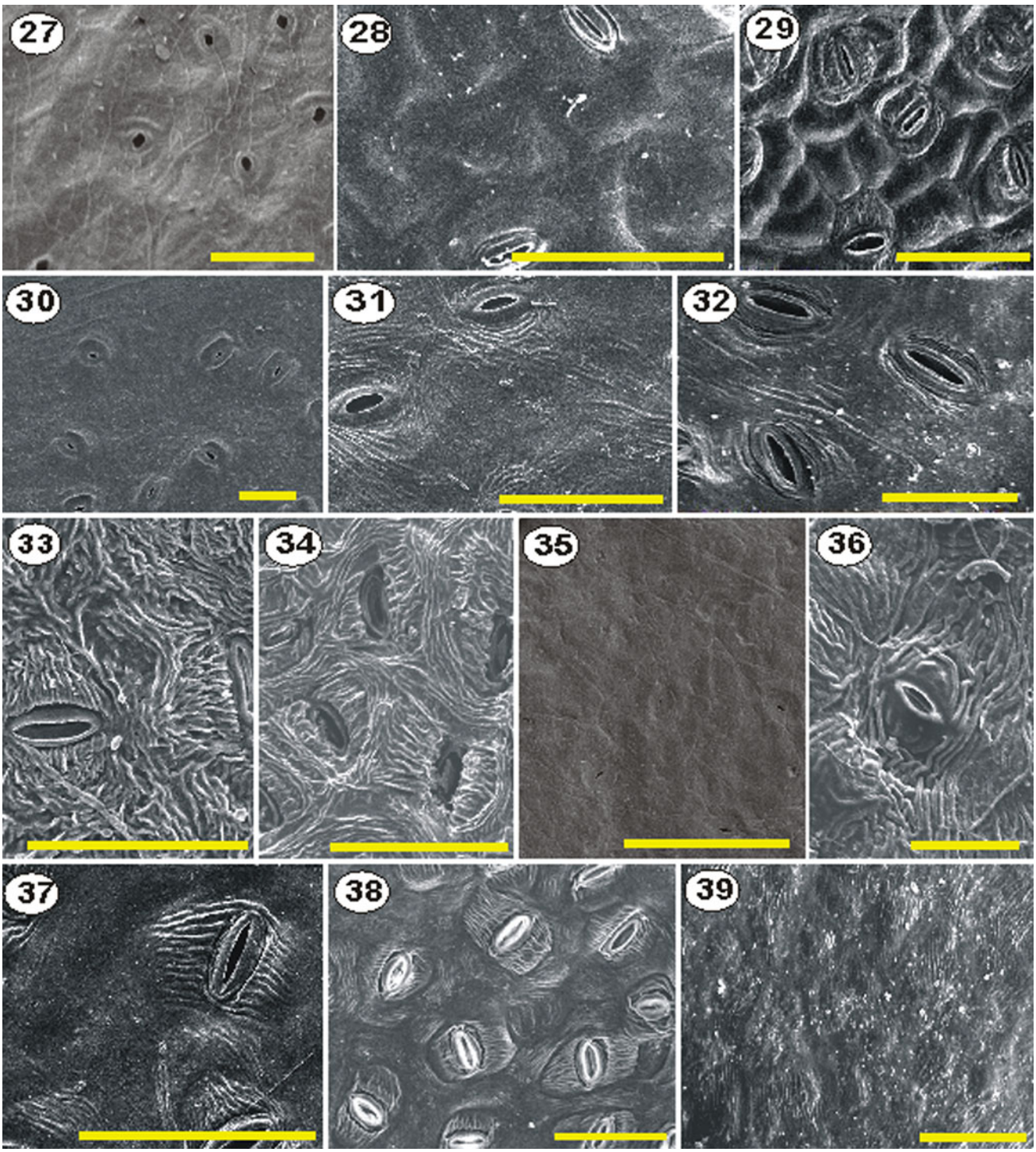
Table 5 Scheffe post hoc test of contrasts among pairs of stomata length means

Group vs Group	Difference	Scheffe statistic	Critical value	Significant?
Coc dec	4.19	8.34	4.321	Yes
Coc ger	1.11	2.22	4.321	No
coc hen	9.78	19.47	4.321	Yes
coc inn	−2.12	4.21	4.321	No
coc mad	−1.34	2.67	4.321	No
coc mit	4.74	9.42	4.321	Yes
coc pot	1.89	3.76	4.321	No
coc pun	5.19	10.32	4.321	Yes
coc spi	−11.36	22.60	4.321	Yes
coc usa	12.90	25.68	4.321	Yes
dec ger	−3.08	6.12	4.321	Yes
dec hen	5.59	11.13	4.321	Yes
dec inn	−6.31	12.55	4.321	Yes
dec mad	−5.53	11.00	4.321	Yes
dec mit	0.55	1.09	4.321	No
dec pot	−2.30	4.58	4.321	Yes
dec pun	1.00	1.98	4.321	No
dec spi	−15.55	30.94	4.321	Yes
dec usa	8.71	17.34	4.321	Yes
ger hen	8.67	17.25	4.321	Yes
ger inn	−3.23	6.43	4.321	Yes
ger mad	−2.45	4.88	4.321	Yes
ger mit	3.62	7.21	4.321	Yes
ger pot	0.78	1.55	4.321	No
ger pun	4.07	8.10	4.321	Yes
ger spi	−12.47	24.82	4.321	Yes
ger usa	11.79	23.47	4.321	Yes
hen inn	−11.90	23.68	4.321	Yes
hen mad	−11.12	22.13	4.321	Yes
hen mit	−5.05	10.04	4.321	Yes
hen pot	−7.89	15.71	4.321	Yes
hen pun	−4.60	9.15	4.321	Yes
hen spi	−21.14	42.07	4.321	Yes
hen usa	3.12	6.21	4.321	Yes
inn mad	0.78	1.54	4.321	No
inn mit	6.85	13.63	4.321	Yes
inn pot	4.01	7.97	4.321	Yes
inn pun	7.30	14.53	4.321	Yes
inn spi	−9.24	18.39	4.321	Yes
inn usa	15.02	29.89	4.321	Yes
mad mit	6.08	12.09	4.321	Yes
mad pot	3.23	6.43	4.321	Yes
mad pun	6.53	12.99	4.321	Yes
mad spi	−10.02	19.93	4.321	Yes
mad usa	14.24	28.35	4.321	Yes
mit pot	−2.85	5.66	4.321	Yes
mit pun	0.45	0.89	4.321	No
mit spi	−16.09	32.03	4.321	Yes
mit usa	8.17	16.26	4.321	Yes
pot pun	3.29	6.56	4.321	Yes
pot spi	−13.25	26.36	4.321	Yes
pot usa	11.01	21.92	4.321	Yes
pun spi	−16.54	32.92	4.321	Yes
pun usa	7.72	15.36	4.321	Yes
spi usa	24.26	48.28	4.321	Yes

coc, *S. cocculoides*; *dec*, *S. decussata*; *ger*, *S. gerrardii*; *hen*, *S. henningsii*; *inn*, *S. innocua*; *mad*, *S. madagascariensis*; *mit*, *S. mitis*; *pot*, *S. potatorum*; *pun*, *S. pungens*; *spi*, *S. spinosa*; *usa*, *S. usambarensis*

Other than the coarse ecological information about the habitat of the sampled species, viz. whether forest or savanna dwelling, we did not carry out measurements on CO_2 levels as it is beyond the scope of our study. Nevertheless, the overarching trend from our data suggests that stomatal frequency is genetically and environmentally determined, and that the tradeoff between the two is species-specific, in accord with the findings of other investigators (Ferris and Taylor 1994; Zhan et al. 2012). It is also well known that trichome density is influenced by environmental changes, with density being positively correlated with levels of water stress (Perez-Estrada et al. 2000; Xiang et al. 2010).

From an ecological perspective, therefore, the 11 species of *Strychnos* studied can be grouped into two broad classes based on their occurrence in either forest or savanna habitats (Table 1). As expected, savanna species exhibited more xeromorphic features than their forest counterparts with some notable exceptions. All four species with non-glandular trichomes are found within the savanna biome. *Strychnos pungens* is the only savanna species lacking hairs, while none of the forest taxa possesses any form of leaf epidermal appendages. The evolution of epidermal trichomes is considered an adaptive strategy for coping with excessive evapotranspiration (Haworth and McElwain 2008), insect/pathogen resistance (Gianfagna et al. 1992; Stenglein et al. 2005) and pollution (Mishra 1982).



Figs. 27–39 SEM of leaf epidermal surfaces showing cuticular variations. All abaxial except Fig. 39. (**27–30**) smooth surface. **27** *S. henningsii*; **28** *S. mitis*; **29** *S. potatorum*; **30** *S. pungens*; **31–32** slight cuticular striations. **31** *S. decussata*; **32** *S. spinosa*; **33–34** coarse irregular warty striae. **33** *S. cocculoides*; **34** *S. innocua*; **35** smooth *S. usambarensis*; **36** coarsely striated *S. usambarensis*; **37** *S. gerrardii*—generally smooth but with lateral wings of striae around stomata; (**38–39**) *S. madagascariensis* **38** showing fine cuticular striation and pronounced lateral wings of striae around stomata; **39** adaxial surface with fine striations. *Scale bar* 20 μm for Figs. 27–39

A higher rate of evapotranspiration is expected in the savanna biome than in forest, which may account for the observed distribution of trichomes among the species. In addition, among the pubescent taxa, trichome density is significantly higher on the adaxial leaf surface than the abaxial. This is consistent with the findings of Liakoura

Table 6 Nuclear DNA content estimation of some *Strychnos* species

Taxon	2C genome size (pg)	% CV	*n**
Strychnos gerrardii	1.92	8.2	5
Strychnos henningsii	1.90	7.6	2
S. pungens	1.88	8.1	2
***S nux-vomica*	0.86	–	–

* Sample size, ** estimated from the 4C value of Hanson et al. (2005)

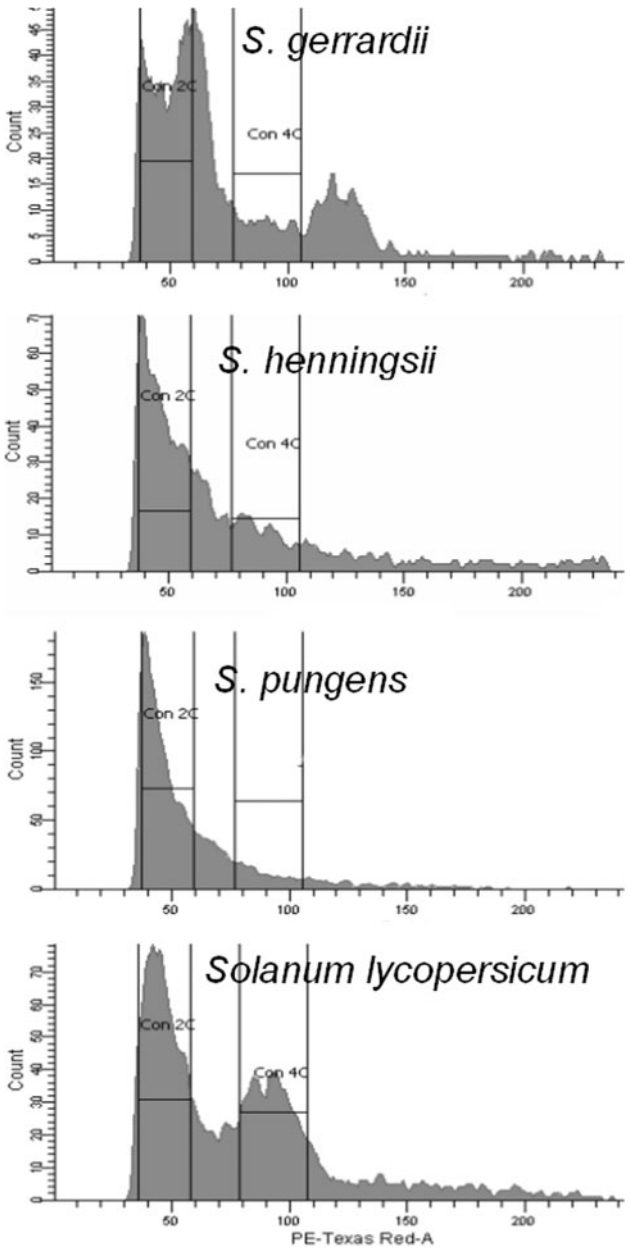


Fig. 40 Histograms of PI fluorescence intensity in the nuclei of *Strychnos* species and internal standard *Solanum lycopersicum*

et al. (1997) and Perez-Estrada et al. (2000) where trichome density was found to be higher in sun-exposed parts than in shaded areas, to deal with excessive radiation.

Two other adaptive leaf features usually associated with plants of arid environments are sunken stomata and thick cuticles. Two of the savanna species possess sunken stomata while the others and their forest-based counterparts either have stomata at the same level as surrounding epidermal cells or occasionally with slightly elevated guard cells. The most obvious explanation for sunken stomata is as an adaptive measure against water loss. This is not consistent with our observations, however, as some savanna species as well as all forest species have similar stomata level. This may indicate that some savanna species have evolved other adaptive strategies for dealing with dry environment and/or that those supposed xeromorphic features might not be *exclusively* associated with dry habitat.

In contrast to the prevailing hypothesis, Haworth and McElwain (2008) questioned the one-dimensional interpretation of supposed xeromorphic features of plants, and documented the occurrence of such features in plants of high water availability environments. A similar trend was observed in the Proteaceae where the incidence of epidermal trichomes and sunken stomata was not biased toward dry climates (Jordan et al. 2008), thus suggesting that they may serve adaptive functions other than reducing water loss.

Cuticular striation does not seem to follow any general ecological pattern in this study. Some of the forest species, such as *S. usambarensis,* have as much warty striations as some savanna species and *S. pungens*, a typical savanna species, does not have any apparent striation.

Is genome size correlated with stomatal size in Strychnos?

Among plants, diploids are known to have smaller stomata than their polyploid relatives (Stebbins 1950; Wilkinson 1979). This positive correlation between stomatal size and genome size in angiosperms has been well-documented (Masterson 1994; Knight and Beaulieu 2008; Hodgson et al. 2010; Inceer and Hayirlioglu-Ayaz 2010; Marciniuk et al. 2010) and applied as a reliable proxy for predicting ploidy level (Aryavand et al. 2003). Given that the majority of angiosperms have polyploidy in their ancestry (Masterson 1994; Soltis et al. 2009), and that polyploidy is common within the family Loganiaceae (Gadella 1980), it is plausible to assume some role for polyploidy in the evolution of *Strychnos*. Although cytotaxonomic data for the genus are rather scanty, currently covering only 32 species, most of the species for which data are available are tetraploids (Frasier 2008) and a base number of $x = 11$ is established for the genus. Of the species in this study, there are published chromosome numbers for *S. cocculoides*, *S. innocua* and *S. spinosa* (Gadella 1980), all of which are $2n = 4x = 44$, further confirming the ubiquity of tetraploidy within the genus.

The consistency of SL as a reliable predictor of ploidy level has been shown to be independent of environmental

conditions (Lomax et al. 2009). Stomatal length data from this study suggest the existence of more than one ploidy level among the *Strychnos* species in the southern African subcontinent. The average difference between short and long stomata as designated here is 22.52 µm (Table 2). This difference is significant ($P < 0.001$) and appear more pronounced than those between tetraploid and hexaploid *Aegilops neglecta* (Poaceae) with much larger absolute SL values and a mere difference of about 9 µm between the ploidy levels (Aryavand et al. 2003). In more comparable families, Chen et al. (2010) reported SL range of 14.9–28.9 µm between diploids and tetraploids *Buddleja* (Buddlejaceae), while Mishra (1997) reported a range of 19.69–29.08 µm in diploid and tetraploid *Coffea* (Rubiaceae). These three genera (*Buddleja*, *Coffea* and *Strychnos*) have close phylogenetic affinity as members of the Euasterid I clade (APG II 2003), thus some commonality in stomatal length and ploidy level pattern may not be out of place. In our present work, we reported SL range of 5.03–29.09 µm among the *Strychnos* species. We thus hypothesize that this wide variation in SL is attributable to variable ploidy levels within the genus.

Conclusion

Contrary to our hypothesis of stomatal length–ploidy level relationship, our flow cytometry results (which we treat with caution) indicate similarity in ploidy levels and genome sizes among the three taxa for which we had data. Thus, a conclusive relationship between ploidy levels, stomatal length and genome size in *Strychnos* will require extensive and definitive flow cytometric analysis across the genus coupled with karyological examination. Owing to the important role of leaf epidermal features not only as a rich source of plant taxonomic information but also as reliable indicator of short- and long-term climate changes, the study of leaf epidermis, even within a systematic framework should incorporate as much ecological information as practicable from the outset.

Acknowledgments The authors thank Sharon Eggers of the Microscopy and Microanalysis Unit of University of KwaZulu-Natal, Westville for guidance on the SEM. AA and YN acknowledge the NRF-Thuthuka scheme for funding part of this research. The authors also thank two anonymous reviewers for their critical but helpful comments on the manuscript.

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CHAPTER 4

ITS2 SEQUENCE AND SECONDARY STRUCTURES FOR CIRCUMSCRIBING SPECIES BOUNDARIES: A CASE STUDY IN SOUTHERN AFRICAN MONKEY ORANGE *STRYCHNOS* L. (LOGANIACEAE)

ABSTRACT

The second internal transcribed spacer (ITS2) continues to play a prominent role in systematic research in spite of legitimate concerns about its phylogenetic utility. We assessed the popularity of the ITS2 marker over a 15-year period (1998 – 2012) via three scholarly search engine queries. Our finding indicates a steady rise in the usage of the marker that spiked during the DNA barcoding revolution of the mid 2000s and has been increasing since. Using sequence and secondary structure attributes, we evaluated the efficiency of the marker to discriminate among southern African *Strychnos* species. Combined phylogenetic analysis of sequence and secondary structure datasets performed better in terms of species resolution than analysis involving primary sequences alone, with 100% and 88.2% taxa discriminations, respectively. Clade I, which corresponds to section *Densiflorae*, is strongly supported and seems to constitute a natural group based on common ancestry. Species boundaries do not always correlate with sexual compatibility as inferred from compensatory base changes (CBCs) patterns. The unusual absence of CBCs within an entire section indicates that their evolution is far more conserved than the speciation process in *Strychnos* and may be a proxy for predicting crossing ability among sectional members. This, along with field observations on hybrid formation

patterns and gene tree paraphyly within the *S. madagascariensis* Poir. complex, suggest that section *Densiflorae* is evolutionarily young. We interpret the observed paraphyly as indicative of a speciation work-in-progress. Lineage sorting is apparently incomplete and divergence may not have been accompanied by character congruence at all levels, thus precluding the definitive achievement of an exclusivity criterion such as reciprocal monophyly. Our results provide additional support for an integrated approach to species delimitation, as speciation is a multi-facetted and dynamic process.

Keywords: ITS2 secondary structure; species delimitation; compensatory base changes; *Strychnos*; DNA barcoding

INTRODUCTION

The ability to identify taxa correctly, especially among closely related taxa, is fundamental to most biosystematic endeavours. Because the criteria used to delimit species are so variable and often debatable, choice of definition can have far reaching implications for conservation and biodiversity assessment as shown by a number of recent studies (Bottin *et al.* 2007; Frankham *et al.* 2012; Herbert *et al.* 2003; Kocot and Santos, 2009; Roca *et al.* 2001; Sanguila *et al.* 2011). The species definition used also influences the type of research questions asked, and thus has enormous implications for biology as a science. While morphology, with their inherent limitations, were arguably the only means previously used to address these questions, the use of molecular markers as complementary or confirmatory tools has

risen exponentially in the last three decades and has enabled the formulation of statistically testable hypotheses about the evolutionary history of life. In recent years, the second internal transcribed spacer region (ITS2) of the nuclear ribosomal DNA has found increased application in taxonomic studies. This is largely due to its high copy number (Alvarez and Wendel, 2003); ease of amplification, high sequence variability (China Plant BOL Group *et al.* 2011) and secondary structure features that apparently correlate with the biological species concept (Coleman, 2009; Mayr, 1982; Ruhl *et al.* 2010). Furthermore, the ITS2 secondary structure has striking homology among eukaryotes (Baldwin *et al.* 1995; Coleman, 2003; Coleman, 2007; Müller *et al.* 2007). This combination of attributes offers opportunity for broad-scale phylogenetic comparison across the eukaryotes, while retaining sufficient nucleotide polymorphisms necessary to separate infrageneric taxa.

Owing to the multi-copy nature of the region and the potential problems of non-homogenization of all ribosomal DNA repeats within a genome (Harpke and Peterson, 2006; Xiao *et al.* 2010), legitimate concerns have been raised about the phylogenetic utility of the ITS region in general (Alvarez and Wendel, 2003; Hughes *et al.* 2002). However, where incomplete concerted evolution of the multiple copies within a lineage is negligible, the region has proved useful for resolving phylogenetic relationships. Indeed, it has been argued that intragenomic variation of the ITS repeats, if present, is limited to a few positions that are never paired in secondary structure (Coleman, 2007) and as such do not compromise the overall usefulness of the marker (Song *et al.* 2012; Wolf *et al.* 2013).

Another characteristic feature of ITS2, which is of taxonomic interest, is the presence of Compensatory Base Changes (CBCs) in the secondary structure. Essentially, a CBC is a pairing position in a proposed double stranded helix where the sequences of two related organisms differ at both positions, yet maintain stable hydrogen bonded pairs (Gutell and Larsen, 1994; Young and Coleman, 2004). Based on a comprehensive analysis of over 1300 related species, Müller *et al.* (2007) posited that the presence of a CBC between two taxa may be regarded as sufficient proof for classifying them as distinct species with about 93% confidence, though it need not be a necessary condition for species differentiation (Wolf *et al.* 2005a). While CBCs on their own are not regarded as causal agents of species formation (Müller *et al.* 2007; Schill *et al.* 2010), they can provide some index for measuring whether sufficient evolutionary time has elapsed for a speciation event to have occurred. Thus, the ITS2 secondary structure can offer some insight into the dynamics of actual or potential gene exchange among related taxa.

The demonstrated independent resolving power of the ITS2 primary sequence and secondary structure in phylogeny reconstruction has resulted in a shift towards the exploration of possibilities for combining the unique information content of each for phylogenies that are more robust. In several groups of organisms where the approach has been applied, the emerging pattern has been that sequence-structure phylogenies are more accurate and better resolved than sequence only phylogenies (Buchheim *et al.* 2011; Keller *et al.* 2010). Often, they offer remarkably more insight

into the evolutionary relationships and processes among the various organisms in question than can be garnered by primary sequence exploration alone (Buchheim *et al.* 2012; Caisová *et al.* 2011; Markert *et al.* 2012).

SOUTHERN AFRICAN *STRYCHNOS* AND DNA BARCODING POTENTIAL OF ITS2

Strychnos L. is a moderately sized genus of trees and liana with about 200 species worldwide (Leeuwenberg, 1969). All the southern African members of the group are important sources of food and local medicines. A number of species like *S. cocculoides* and *S. spinosa* are considered as underutilized and potential crops of the future in arid environments where their hardiness may be advantageous (Mwamba, 2006; Mizrahi *et al.* 2002). In the face of predicted global food shortages and rapid erosion of genetic resources due to anthropogenically induced environmental and climate change, conserving genetic resources for crop improvement among others has never been more important. Fundamental to that effort is the accurate documentation and circumscription of the units of conservation: species. Current taxonomic treatment of southern African *Strychnos* is based on morphological attributes (Leeuwenberg, 1969; Verdoorn, 1963) with unanswered questions regarding species numbers and the unequivocal assignment of some taxa.

Realisation within the DNA barcoding community that there is no foolproof marker for barcoding plants has kept other marker options open. Following a number of studies in several groups, the ITS2 marker appears to be a leading candidate as an alternative or supplementary marker for serious consideration (Chen *et al.* 2010;

Schoch *et al.* 2012). Previous investigations of ITS2 sequence and secondary structure data within a phylogenetic framework have focused on animal, fungal and algal subjects (del Campo *et al.* 2013; Hunter *et al.* 2007; Keller *et al.* 2008; Kocot and Santos, 2009; Ruhl *et al.* 2010; Trizzino *et al.* 2009; Yi *et al.* 2009). Where angiosperms have been involved, the focus has been at higher taxonomic categories, usually at ordinal levels (Biswal *et al.* 2012; Coleman, 2003; Coleman, 2009) whereas comparisons at specific levels have been relatively rare. Any serious consideration of the ITS2 as a marker for barcoding in plants should demonstrate its utility at infrageneric levels, where most pragmatic policy issues surrounding biodiversity conservation usually occur. In this study therefore, we attempt to determine the utility of the ITS2 primary sequence and secondary structure in separating different species of southern African *Strychnos*. We test whether the presence of CBCs in the secondary structure correspond to species boundaries as currently circumscribed. Finally, we examine speciation patterns within the well-defined section *Densiflorae*.

MATERIALS AND METHODS

WEB LITERATURE SEARCH FOR ITS2 RELATED PHRASE

To quantify the trend in the usage of the ITS2 marker and/or secondary structure attributes thereof in scientific research over a 15-year period spanning 1998 – 2012, we queried three scholarly search engines (GoogleScholar, ScienceDirect and SpringerLink) according to the search parameters detailed in Table 4.1. Since the goal here was to discover trends rather than absolute numbers, we scaled down the

number of hits for the GoogleScholar search by a factor of 10 to make for a reasonable comparison with results from the other search engines.

TAXON SAMPLING

Seventeen *Strychnos* species covering eight of the 12 sections within the genus were covered (Table 4.2). Of these, 11 are core SA species as recognized by Verdoorn (1963). The remaining six species were from tropical Africa. *Mitreola petiolata* (J.F. Gmel.) Torr. & A. Gray, *Mitrasacme pygmaea* R. Br. and *Spigelia* spp., all belonging to Loganiaceae, were selected as outgroup taxa (Table 4.3).

DNA EXTRACTION, PCR AMPLIFICATION AND DNA SEQUENCING

Genomic DNA was extracted from silica-dried leaf material or in certain cases herbarium specimens using the QIAGEN DNeasy Plant Mini Kit following the manufacturer's manual with a slight modification; final elutions were carried out using 50µl instead of the recommended 100µl of the elution buffer to obtain higher DNA concentrations. PCR amplification of the ITS2 region was achieved using the universal primer pair "ITS 3": 5'-GCATCGATGAAGAACGCAGC-3' and "ITS4": 5'-TCCTCCGCTTATTGATATGC-3' after White *et al.* (1990). PCR amplifications were performed in 25µl reactions containing 30 – 50ng genomic DNA using the following mix per reaction: 0.8µl PCR-grade water, 2.5µl 10X reaction buffer, 4µl of 25mM $MgCl_2$, 0.5µl of 10mM dNTP, 0.2µl 5 U/µl *Taq* and 4µl of 6 µM primer (forward and reverse). The thermal cycling parameters used were as follows: 2 min at 94^{o}C; 35 cycles of 1 min at 94^{o}C; 2min at 53^{o}C; 2 min at 72^{o}C; final extension of 5 min at 72^{o}C.

PCR products were purified with a Zymoclean^{TM} Gel DNA Recovery Kit. Amplification products were checked on 1.5% agarose gels stained with ethidium bromide. Direct sequencing of PCR products was carried out using the BigDye Terminator Cycle sequencing Kit version3.1 (Applied Biosystems) in a 10µl reaction containing 3µl of the ready reaction mix, 7 pmol of primer and 50 – 100ng of purified PCR product. Forward and reverse sequences were compared and edited with BioEdit (Hall, 1999) to generate consensus sequence for each sample. Sequences were deposited in GenBank (KC609287-KC609321, Table 4.3). We generated 35 *Strychnos* ITS2 sequences in this study, in addition to the 13 sequences downloaded from the GenBank.

PREDICTION OF ITS2 SECONDARY STRUCTURE

The precise boundaries of ITS2 sequences, which are flanked by 5.8S and 28S rRNA genes, were determined for all sequences using the Hidden Markov Models with HMMer 2.3.2 (Eddy, 1998) as implemented for plants on the ITS2 database (Koetschan *et al.* 2010; Koetschan *et al.* 2012; Schultz *et al.* 2006; Selig *et al.* 2008; Wolf *et al.* 2005b). The ITS2 database (ITS2 DB) was queried for homologous secondary structures by selecting a representative sequence of each *Strychnos* taxon. The suggested homologous secondary structure of high quality in most cases was *Mitreola petiolata* (AF054635); this was then used as a template for folding the ITS2 sequences for the other *Strychnos* species via the custom modelling module of the ITS2 DB using default parameters. Sequences that modelled poorly as defined by the parameters were excluded from further secondary structure analysis. As an

additional measure of secondary structure validation, sequences were folded with the stand-alone software RNAstructure version 5.4 (Matthews, 2004; Reuter and Mathews, 2010) using default options. RNAstructure utilizes a minimum free-energy (MFE) algorithm and produces a number of thermodynamically suboptimal structures along with the MFE one. We applied constrained folding by using lowercase nucleotides to force single strandedness in certain part of the structure. With the aid of 4SALE ver. 1.7 (Siebel *et al.* 2006; Siebel *et al.* 2008), sequences and secondary structure information were synchronously aligned and consensus structure was computed and visualized. Secondary structures were redrawn and edited in RNAviz 2.0 (de Rijk *et al.* 2003) and Inkscape v 0.48 (2012). We then conducted pairwise comparison of structures across species for the presence of CBCs and hemi-CBCs (hCBC) in helices II and III within a cladistic concept, taking note of the plesiomorphic status of base pairs producing the mutations (Caisoῦa *et al.* 2011).

SEQUENCE DIVERGENCE AND PHYLOGENETIC ANALYSIS

Primary sequences were aligned with ClustalW (Thompson *et al.* 1994) using the ITS2 secondary structural motif as guide for manual adjustments. Nucleotide substitution model was estimated with jModelTest (Guindon and Gascuel, 2003; Posada, 2008) and MEGA v5.05 (Tamura *et al.* 2011) under the corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC), respectively. The AICc is capable of correcting for relatively small sample sizes. The Kimura 2-parameter model + Gamma distribution (Kimura, 1980) was selected under both criteria as the best substitution model. Intra- and interspecific sequence divergence

were assessed with pairwise calculations under a Kimura 2-parameter model according to the method of Muellner *et al.* (2011). We performed two sets of phylogenetic analyses, one using the primary sequence alignment and the other using the sequence-structure alignment as computed by 4SALE. The aligned primary sequence matrix was subjected to phylogenetic analyses using Maximum Likelihood (ML) and Maximum Parsimony (MP) approaches. Maximum Likelihood analysis was performed with MEGA v5.05, which used a heuristic search algorithm and 1000-fold bootstrap to produce a tree with the highest log likelihood. Maximum Parsimony analysis was performed with the software package PAUP* 4.0b10 (Swofford, 2002). All characters were weighted equally and gaps treated as missing characters. The most parsimonious trees (MPTs) were obtained with heuristic search options of 1000 replicates saving 10 trees per replicate. Branch-swapping was by tree bisection and reconnection (TBR) algorithm with a MulTrees option for keeping multiple equally most parsimonious trees (MPTs). Internal support for the MPT was estimated by using 100 bootstrap pseudoreplicates. A 50% majority-rule consensus tree was constructed from the MPTs. For the sequence-structure phylogenetic analysis, a consensus Neighbour Joining (NJ) tree based on 100 bootstrap replicates was constructed using sequence-secondary alignment structure information simultaneously as implemented in ProfDistS v0.9.9 (Wolf *et al.* 2008) under an ITS2 sequence-structure-specific, general time reversible (GTR) substitution model. Tree output files were read by TreeView (Page, 1996) and illustrated with Inkscape.

RESULTS

ITS2 UTILITY PATTERNS FROM 1998 – 2012

Our two separate search phrases “ITS2” and “ITS2 secondary structure” (Figure 4.1) returned similar trend across the three search engines. Taking the number of hits as a close proxy for studies utilising the ITS2 marker, there has been a consistent and progressive increase in the use of the marker in biological research between the years 1998 and 2012. Relative to the other two engines, GoogleScholar returned a higher number of search hits. The first marked increase in the frequency of ITS2 in scientific literature occurred during the mid 2000s, followed by a significant spike from 2010 onward (Figure 4.1).

PRIMARY SEQUENCE COMPARISONS AMONG *STRYCHNOS*

The mean length of unaligned sequences is 221 nucleotides with the longest found in *S. henningsii* (225) and the shortest in *S. panganensis* (210). As with most angiosperms, ITS2 is GC rich (Hershkovitz and Zimmer, 1996) in *Strychnos*, with an average of 65.9% content across all 48 ingroup sequences, while AT is 34.1% (Supplementary Table 4.1). Intraspecific pairwise genetic distances ranged from 0 – 0.03660 (Mean = 0.00973; SD = 0.01298), while interspecific genetic distances ranged from 0.00375 – 0.22186 (Mean = 0.12733; SD = 0.04612) (Supplementary Tables 4.2 and 4.3). Intraspecific pairwise distance computations excluded *S. panganensis* Gilg and *S. mellodora* S. Moore, as these two species were represented by only one sequence each. Ten species were represented by at least three

sequences, which gave no indication that incomplete concerted evolution of ITS2 repeats could constitute a problem in *Strychnos*. Sequence length and secondary structure appear to be well conserved within the group.

PREDICTED SECONDARY STRUCTURE OF THE ITS2 IN *STRYCHNOS*

ITS2 secondary structures derived from homology models of the ITS2 DB and thermodynamics were similar. In a few cases, structures with the minimum free energy were stereo-chemically improbable and were discarded in favour of energetically suboptimal, but more probable structural, alternatives (as guided by homology modelling). The energetics and helical transfer success from homology modelling are summarised in Table 4.4 Percentage of helical transfer gives an indication of how conserved is the helix in question. The ITS2 secondary structure of *Strychnos* is comparable to those of other eukaryotes (Coleman, 2007). Our predicted structure bears striking similarity to those recently proposed for Loganiaceae (Frasier, 2008) and *Potalia* Aubl. (Molina and Struwe, 2009). A consensus ITS2 secondary structure for the *Strychnos* taxa in this study depicts the typical four helices radiating around a central ring (Figure 4.2). Helix II and helix III are the most conserved, while helices I and IV are much more variable. Helix II is highly conserved across the representatives of the genus, with the exception of *S. angolensis* Gilg and *S. diplotricha* Leeuwenberg. The former has a full CBC just before the terminal loop (Figure 4.3), while the latter has a hemi-CBC at a similar position. This loop is fairly conserved and can be represented as a 5'- UDGC - 3'

motif. In addition to the CBC, the integrity of the proposed secondary structure was further confirmed by the presence in helix II of the hallmark signature in all eukaryotes; the pyrimidine-pyrimidine bulge near the base. Typically, helix III is the longest and though more variable than helix II in *Strychnos*, it has a highly conserved motif on the 5' side. With the exception of *S. diplotricha*, *S. henningsii* and *S. mellodora* a 28-nucleotide sequence 5'-AYGGGCGUCACGACAAGUGGUGGUUGAA- 3' is identical among the other *Strychnos* taxa sampled; it encompasses the 5'-UGGU-3' signature motif and falls within the 30-nucleotide region suggested by Coleman (2003) to be the most conserved ITS2 nucleotide positions among eukaryotes (Figure 4.3).

CBC OR HEMI-CBC-BASED TAXONOMIC GROUPINGS

Based on the presence or absence of CBCs in relevant regions of the four helices, the 17 species studied could be delimited into 14 CBC clades (Coleman, 2000). Of the five taxa in section *Densiflorae* in (Leeuwenberg's, 1969) included in this study, only *S. pungens* has a CBC (in helix IV) relative to the others, in the comparable portion of the helix (Figure 4.4). The remaining four species, *S. gerrardii*, *S. innocua*, *S. lucens* and *S. madagascariensis* constitute a separate CBC clade.

PHYLOGENETIC ANALYSIS AND EVOLUTIONARY TRENDS IN ITS2

The aligned dataset for the primary sequence comprised 234 characters including gaps, which were not coded due to their negligible number. Parsimony tree reconstruction was based on 115 informative characters, a relatively high number

given the alignment length. Phylogenetic relationships inferred by the various methods revealed similar topology although the deeper nodes were either not well-resolved (MP) or supported by weak bootstrap values (ML & NJ). The monophyly of *Strychnos* is well-supported by ML and MP analyses with 94% and 86% bootstrap support (BS), respectively (Figure 4.5). Apart from the poor support for the monophyly of *Strychnos*, the NJ tree computed from sequence-structure data offers comparable node supports to the other trees (Figure 4.6A). It resolved all internal relationships, thereby demonstrating the power of combined ITS2 sequence and secondary structure information in phylogenetic reconstruction. Due to the obvious shortcomings of equating gene trees with species trees, we interpreted the clades here with some caution. There are five principal clades based on the ML tree (Figure 4.5A). Clade I, which corresponds to section *Densiflorae*, is the most consistent with strong BS across all analyses (MP=96; ML=89; NJ=97). It appears to constitute a natural group based on common ancestry. The other clades may represent alternative sectional hypotheses or arbitrary constructs for explanatory purposes. Clade V, for instance, comprises *S. usambarensis* and *S. decussata*, both of which belong to section *Rouhamon* according to Leeuwenberg (1969), as does *S. potatorum* (Clade III). Thus regardless method of analysis, some sectional paraphyly is apparent from the phylogenetic hypotheses presented. A number of other sections are represented by one species each (Table 4.2). It is therefore impossible to assess paraphyly in such groups. Paraphyly is not restricted to sectional grouping, as *S. innocua, S. gerrardii* and *S. madagascariensis* (*S. madagascariensis* complex or SM complex) within section *Densiflorae*, appear to be paraphyletic as well (Figure 4.5).

ITS2 sequences resolved 15 of the 17 *Strychnos* species (88.2% success). A 100% discrimination rate was achieved within the SM complex when secondary structure information was included in tree computation. In most cases, where a species was represented by more than one geographical collection, it was possible to discriminate between specimens from different localities based on intraspecific polymorphisms (Figure 4.6; Table 4.2). This method may therefore be useful for phylogeographic studies. We also noted a case of specimen misidentification: an ITS2 sequence from GenBank labelled as *S. henningsii* (JF937984.1) nested within a strongly supported clade of *S. angolensis* specimens (97% BS; Figure 4.5). Further examination of the secondary structure revealed perfect similarity with *S. angolensis* sequences in addition to the presence of a full CBC in helix II. These traits are restricted to *S. angolensis* (Figures 4.3D; Supplementary Figures 4.1 and 4.2). We thus submit that the specimen from which the sequence was generated is clearly not *S. henningsii* and suggest a confirmation of identity and correction of the GenBank entry.

DISCUSSION

ITS2 SEARCH TREND

Significantly higher number of search hits by GoogleScholar was not surprising as the engine has capability for remotely connecting with several other search engines. The observed pattern of major increase in the usage of the ITS2 marker, especially in the mid 2000s, appears to coincide with the emergence of the DNA barcoding movement following a number of exploratory workshops and landmark publications during the same period (Herbert *et al.* 2003; Schindel and Miller, 2005). The first DNA barcoding

conference held in London in 2005, coupled with the inevitable hunt and testing for other potential DNA barcode regions, may also have played a prominent role. Following the recommendation of *mat*K and *rbc*L markers as land plant barcodes (CBOL, 2009), there has been further interest in barcoding research across most life forms, which may explain more recent ITS2 trends. Unlike the informal, albeit helpful, enthusiasm heatmap of Hollingsworth *et al.* (2011), our result represents a quantitative and repeatable tracking of a marker through scientific literature.

PHYLOGENETIC UTILITY OF ITS2 AND ITS POTENTIAL ROLE AS A PLANT DNA BARCODE MARKER

The increasing popularity of the ITS marker in general and ITS2 in particular for plant phylogenetic reconstruction is at variance with legitimate concerns raised by some authors within the last decade (Alvarez and Wendel, 2003; Vollmer and Palumbi, 2004). This trend in the popularity of ITS2, rather than being an intellectual Allee effect, seems to reflect genuine utility (Feliner and Rosselló, 2007; Schoch *et al.* 2012; Yao *et al.* 2010). The fact that documented cases where these perfectly valid questions could have resulted in wrong phylogenetic inferences are the exception bears out the trend (Draisma *et al.* 2012; Harpke and Peterson, 2006; Ponce-Gordo *et al.* 2011; Xiao *et al.* 2010).

A further contributory factor to the observed pattern is the recognition of the ITS2 region as a potential DNA barcode in plants and fungi (Chen *et al.* 2010; Schoch *et al.* 2012). Indeed, the marker has been practically adopted by the fungal barcoding community. Recent studies have demonstrated the discriminatory power of the

marker across various plant groups from green algae through to angiosperms (Assunção *et al.* 2012; Assunção *et al.* 2013; Chen *et al.* 2010; Gao *et al.* 2010). The *matK* and *rbcL* markers are the currently proposed core barcode regions of choice for land plants (CBOL, 2009). However, the Plant Working Group of the Consortium for the Barcode of Life (CBOL) recognized the various limitations (both technical and in discriminatory ability) of using a plastid only approach and thus recommended the use of supplementary barcodes (Hollingsworth, 2011; Hollingsworth *et al.* 2011). Given the additional evolutionary information a nuclear marker offers, it would appear that the ITS2 marker is playing this "supplementary" role in many plant DNA barcoding undertakings without any formal adoption yet by the plant systematic community. It is our considered view that until the emergence of a better nuclear marker, (and in spite of the whole genome approach to molecular systematics), the ITS region, and by association ITS2, will continue to be prominent in plant systematic research.

SECONDARY-STRUCTURE PHYLOGENY AND PREDICTIVE POWER FOR HYBRID FORMATION

Consistent with the reported improvement in robustness of sequence-structure phylogenies (Keller *et al.* 2010), our sequence-structure phylogeny (SSP) was more robust than sequence only phylogenies (regardless of analysis method) across all clades (Figures 4.5 and 4.6). However, the much deeper nodes of the SSP had less than 50% bootstrap support, possibly reflecting the highly conserved nature of ITS2 secondary structure across the group. Weak support for clades II – V (Figure 4.5A), coupled with the inconsistency in sectional groupings may reflect the limited utility of

one marker to completely resolve phylogenies. Alternatively, it could also imply that current sectional classification of *Strychnos* is not a fair reflection of evolutionary groupings within the genus.

The conserved nature of the ITS2 secondary structure across southern African *Strychnos* as observed in our study, and within the Loganiaceae (Frasier, 2008), and indeed across most eukaryotes (Schultz *et al.* 2005) is consistent with its functional significance for processing of rRNA transcripts (Cote *et al.* 2002; Kos and Tollervey, 2010; Michot *et al.* 1999). If the secondary structure is largely conserved across eukaryotes as is suggested, selective pressure aimed at keeping variation to a minimal within congeneric lineages should be stronger. In groups as taxonomically distant as yeast and humans, Schillewaert *et al.* (2012) demonstrated the activity of a conserved protein, Las1, in pre-rRNA processing at both ends of the ITS2 marker. Given the spatial positioning of the marker, secondary structure folding of ITS2 can bring the 5.8S and the 28S genes into close proximity with each other for functional efficiency during ribogenesis (Keller *et al.* 2009; Schillewaert *et al.* 2012).

The distribution of CBCs in this study is not in perfect accord with recognised species boundaries within the group (Figure 4.5). However, it does not invalidate the established correlation relationship between CBCs and biological species (Coleman, 2000; Muller *et al.* 2007; Wolf *et al.* 2013). Any pair of ITS2 sequences, lacking CBCs, still has a conditional probability of about 23% of belonging to distinct species, although they have a correspondingly higher likelihood (about 77%) of being conspecifics (Muller *et al.* 2007). It is noteworthy that these values were derived

based on the four ITS2 helices. The implication of this approach is that a section like *Densiflorae*, comprises two CBC clades (Coleman, 2000), with *S. pungens* in one, and *S. gerrardii*, *S. innocua*, *S. lucens* and *S. madagascariensis* in the other (Figure 4.4). This latter group of four taxa may potentially constitute a zygotic or Z clade (Coleman, 2000). While we currently lack direct experimental data for elucidating patterns of zygotic compatibility among *Strychnos*, field and herbarium observations of interspecific putative hybrids (Verdoorn, 1963; Leeuwenberg, 1969: 280) suggest that successful hybrids tend to form between members of the same Z clade, which inevitably are also of members of the same CBC clade. A slightly different view suggested by Coleman (2009: 199) hinges potential crossing ability of any two taxa on two factors: overall similarity of their ITS2 sequences and the absence of a CBC or hemi-CBC in "*the longest conserved sequence*", the "*30 nucleotides*" of helix III. From these criteria, the five members of sections *Densiflorae* investigated would constitute a 'biological species', as they could be expected to cross, going by that narrow defintion. Indeed, all recorded cases of putative *Strychnos* hybrids in Africa, have consistently involved members of section *Densiflorae* (Verdoorn, 1963; Leeuwenberg, 1969), such as *S. pungens*, *S. madagascariensis*, *S. lucens* and *S. gerrardii*.

While we recognise that successful hybridization between any two taxa is a multigene phenomenon, and do not attribute crossing ability to a single marker like the ITS2, let alone the helix III, a tiny portion of the ITS2, we note that the correlation between CBC pattern and the biological concept of species Mayr (1982) is strong enough to

suppose that there is some sort of concert in evolution between genes responsible for mating and the ITS (Coleman, 2009).

CONSERVATION IMPLICATIONS OF SPECIES CIRCUMSCRIPTION IN SOUTHERN AFRICAN *STRYCHNOS*

Arguably, the subject of species conceptualization has spawned more debates amongst biologists and philosophers of science than any other topic. De Queiroz (2007; 2011) advanced compelling arguments for unifying seemingly disparate species concepts by identifying a common element. This common property (primary criterion) considers species as "*separately evolving metapopulation lineages*" (De Queiroz, 2007) or a segment thereof. All other properties (secondary properties) upon which differences in species concepts were based are regarded as operational criteria for delimiting species and whose applicability is context specific, depending on the organism in question.

The species debate is pertinent in *Strychnos* where seven African species are on the IUCN Redlist, with status ranging from vulnerable to critically endangered (IUCN, 2012). Within the well-defined section *Densiflorae* in southern Africa for instance, the *S. madagascariensis* or SM complex (comprising *S. gerrardii*, *S. innocua* and *S. madagascariensis*) have been the subjects of various taxonomic revisions (Leeuwenberg, 1969; Verdoorn, 1963), each of which made some tacit underlying assumptions about the nature of species. Although phylogenetic analyses strongly support the monophyly of this section, the exact species boundaries, especially between *S. madagascariensis* and *S. gerrardii,* remain uncertain. Grouping of an

entire section as a single Z-clade (based on helix III, Coleman, 2009) within which successful gene exchange is possible suggests a recent evolutionary origin. It also shows one well-known weakness of the biological species concept: the possibility of successful hybrid formation between disparate taxonomic entities. In certain cases among sexually reproducing organisms, gametic compatibility and taxonomic hierarchies may not necessarily correspond. Successful inter-generic crosses are well known among some plant groups (Inagaki and Tahir, 1992; Kaneko and Bang, 2014) Applying a strict biological species concept to section *Densiflorae* could inadvertently underestimate biodiversity as distinct and generally cohesive members of an entire section could be erroneously reduced to a single species on account of reproductive compatibility amongst them. Under a cladogenetic speciation model (Figure 4.6B), it is conceivable that members of a diverging lineage are sufficiently distinct as taxa based on other features, without having achieved reciprocal monophyly with respect to some gene locus/loci. Indeed, there is ample evidence for such heterogenous rates of character change during speciation (Padial *et al.* 2010) which ensures that lineage divergence will not always be accompanied by character congruence at all levels (Adams *et al.* 2009). This could be the basis of the taxonomic quagmire that resulted in the reduction of *S. gerrardii* (a perfectly valid species, Adebowale *et al.* 2012) to synonymy with *S. madagascariensis* by Leeuwenberg (1969). Paraphyly between *S. madagascariensis* and *S. gerrardii* could therefore be due to incomplete lineage sorting at the ITS2 locus (possibly at many other loci) or hybridization, either of which would implicate the earlier postulated recent divergence hypothesis within the species complex. Adebowale *et al.* (2012) demonstrated that

the two taxa are sufficiently distinct as species by quantitative evaluation of their leaf shape. The emerging picture from the phylogenetic reconstruction in this and related studies (Verdoorn, 1963) is that *S. innocua* is ancestral to *S. madagascariensis* and *S. gerrardii*. These two latter taxa are yet to pass the test of mutual exclusivity.

The concept of two species here is a practical one, as both species differ, not only in overall morphology, but occur in different habitats and thus require different treatment by conservation or environmental managers given the more extensive loss of coastal forest along the KwaZulu-Natal coast line where *S. gerrardii* is naturally found. Increasing anthropogenic degradation of this forest type has caused fragmentation in the distribution of *S. gerrardii*; which is a concern to conservationists. The savanna in which *S. madagascariensis* occurs is much less fragmented and this taxon extends well into tropical Africa and Madagascar, making it less of a conservation concern.

CONCLUSION

ITS2 sequence and structural information is proving increasingly useful for resolving phylogenetic relationships. Although CBC data cannot serve as accurate indicators of species boundaries in *Strychnos*, their absence within a group can be used to predict potential for crossing among group members. While the species debate may continue perhaps for semantic and philosophical reasons, results presented here corroborate other contemporary reasoning that solutions to the species problem will have to be pluralistic and context driven (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). Due to their dynamic nature, and somewhat hypothetical boundaries, a single criterion, rigid

approach to species delimitation is likely to result in difficulties under readily imaginable scenarios. Hence, the need for an integrated approach to species delimitation. If such an approach is properly implemented, within a coherent framework, it can help prevent biodiversity underestimation on the one hand, and taxonomic inflation on the other.

Finally, the increasing dependence of DNA sequence-based biodiversity research on public sequence databases places an enormous responsibility of data accuracy on researchers generating the data. It also requires that curators of such databases (e.g. GenBank) make provisions for correcting and updating erroneous sequence designations when spotted and can be empirically demonstrated. This may constitute one less difficulty to resolve on the way to delimiting species.

ACKNOWLEDGEMENTS

This work was supported by the NRF-Thuthuka grant awarded to the author through Dr Yogis Naidoo. The authors thank Masego Kruger and David Styles for help with collection of *Strychnos* specimens, and the Curator of the National Herbarium Pretoria (PRE) for access to materials. We also thank eThekwini Municipality for access to conservation areas around Durban. The help from John Dyer (Stainbank Nature Reserve), Chris Reim (Pigeon Valley), Feebee Conighe (Hawaan Nature Reserve), and Rob Markham (Shongweni Dam Nature Reserve) is much appreciated.

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Figure 4.5: (A) Maximum likelihood phylogenetic hypothesis based on ITS2 sequence data for Southern African *Strychnos* species. Each coloured taxon or group of taxa in (A) represent CBC clade as inferred from CBC mutations. Clade I is section *Densiflorae* and comprises 2 CBC clades; Clades II – V could be arbitrary constructs for the purpose of explanation. The OTU in red is *S. angolensis* (JF937984.1) misidentified in GenBank entry as *S. henningsii*. (B) 50% Majority-rule consensus tree from 98 most parsimonious trees for the same groups of taxa as in (A). CI (excluding uninformative characters)=0.6103; RI=0.8235.

Figure 4.6: (A) Consensus Neighbour Joining tree based on ITS2 sequence and secondary structure dataset implemented in ProfDistS. (B) Proposed cladogenetic speciation hypothesis within the *S. innocua* - *S. madagascariensis* - *S. gerrardii* complex as inferred from NJ tree (this study), following Adebowale *et al.* (2012) and Verdoorn (1963). Small dashed box represents relatively early stage of divergence where potential for gene exchange is high even if the species can be diagnosed by other means; there is incomplete lineage sorting. Large dashed box represents a future period when reciprocal monophyly has occurred and reproductive isolation may or may not apply. (Readers are referred to the online version to appreciate colour codes and interpretations)

SUPPLEMENTARY INFORMATION

Supplementary Table 4.1: ITS2 nucleotide composition in percentages across all samples

Supplementary Table 4.2: Interspecific genetic distances (p- uncorrected) among 17 taxa of *Strychnos*

Supplementary Table 4.3: Intraspecific genetic distances for 15 taxa of *Strychnos*

Supplementary Figure 4.1: Comparison of ITS2 helices II and III across southern African *Strychnos* species. Key: ang=*S. angolensis*; coc=*S. cocculoides*; dec=*S. decussata*; dip=*S. diplotricha*; ger=*S. gerrardii*; hen=*S. henningsii*; inn=*S. innocua*; luc=*S. lucens*; mad=*S. madagascariensis*; mel=*S. mellodora*; mit=*S. mitis*; pan=*S. panganensis*; pot=*S. potatorum*; pun=*S. pungens*; spi=*S. spinosa*; usa=*S. usambarensis*; xan=*S. xantha*.

Supplementary Figure 4.2: Comparison of ITS2 helices I and IV across southern African *Strychnos* species. Key: ang=*S. angolensis*; coc=*S. cocculoides*; dec=*S. decussata*; dip=*S. diplotricha*; ger=*S. gerrardii*; hen=*S. henningsii*; inn=*S. innocua*; luc=*S. lucens*; mad=*S. madagascariensis*; mel=*S. mellodora*; mit=*S. mitis*; pan=*S. panganensis*; pot=*S. potatorum*; pun=*S. pungens*; spi=*S. spinosa*; usa=*S. usambarensis*; xan=*S. xantha*. The GenBank ITS2 sequences for JF937984.1 and *S. panganensis* are incomplete and so lack helix IV. Note the identical sequences of *S. angolensis* and JF937984.1 (misidentified as *S. henningsii,* Frasier, 2008), even in the highly variable terminal loop.

Table 4.1: Search parameters for ITS2 secondary structures from three scholarly search engines from 1998-2012

	Google Scholar	**ScienceDirect**	**SpringerLink**
Search phrase 1		*"ITS2 secondary structure"*	
Subject filter	Biology, Life Sciences, Medicine, Environmental Sciences, Pharmacology & Veterinary Science	Agricultural & Biological Sciences, Biochemistry, Genetics and Molecular Biology, Immunology & Microbiology	Biomedical and Life Sciences
Specific filters	Anywhere in articles, exclude patents and citations	In full text, journal module, include articles in press	In full text, journal only
Search phrase 2		*"ITS2"*	
Subject filter	Biology, Life Sciences, Medicine, Environmental Sciences, Pharmacology & Veterinary Science	Agricultural & Biological Sciences, Biochemistry, Genetics and Molecular Biology, Immunology & Microbiology	Biomedical and Life Sciences
Specific filters	Anywhere in articles, exclude patents and citations	Journals only, in abstract, title and keywords	Title and abstract only

Table 4.2: Sectional groupings of *Strychnos* species studied (after Leeuwenberg, 1969)

***Strychnos* sections**							
Breviflorae	***Densiflorae***	***Rouhamon***	***Spinosae***	***Brevitubae***	***Dolichantae***	***Lanigerae***	***Penicillatae***
S. henningsii	*S. gerrardii*	*S. decussata*	*S. cocculoides*	*S. mellodora*	*S. xantha*	*S. panganensis*	*S. diplotricha*
S. mitis	*S. innocua*	*S. potatorum*	*S. spinosa*				
S. angolensis	*S. lucens*	*S. usambarensis*	*S. spinosa subsp. lokua*				
	S. madagascariensis						
	S. pungens						

Table 4.3**:** List of taxa used for this study including specimen locality and GenBank accession numbers for ITS 2 sequences of *Strychnos* spp. and outgroups. Taxon in red font is presumed to be misidentified

Species	**ITS2**	**Sample origin**	**Voucher (Herbarium)**
Mitrascame pygmaea	HM622085.1	-	-
Mitreola petiolata	AF054635	-	-
Spigelia hedyotidea	AF177999.1	-	-
Spigelia texana	AF177994.1	-	-
Strychnos angolensis	JF937943.1	-	J. M. and B. Reitsma 1300 (WAG)
Strychnos angolensis (henningsii?)	JF937984.1	-	P. Herman 770 (WAG)
Strychnos angolensis 102	KC609287	Zambia	MG Bingham 9502 (PRE)
Strychnos angolensis 98	KC609288	Zambia	JE Burrows & SM Burrows 10259 (BNRH)
Strychnos cocculoides	JF937961.1	-	CN Nkhoma et al. 122 (WAG)
Strychnos cocculoides 95	KC609289	South Africa	HF Glen NH0134236 (NH)
Strychnos cocculoides C3	KC609290	Namibia	Bartsch, Klaasen & Uiras s.n. WIND80282 (WIND)
Strychnos decussata 10	KC609291	South Africa	A Adebowale 10 (UDW)
Strychnos decussata 18	KC609292	South Africa	A Adebowale 18 (UDW)
Strychnos decussata 60	KC609293	South Africa	Adebowale 60 (UDW)
Strychnos decussata 61	KC609294	South Africa	Adebowale 61 (UDW)
Strychnos decussata 82	KC609295	Mozambique	Burrows JE & Burrows SM 9503 (BNRH)
Strychnos diplotricha	JF937974.1	-	G McPherson 14639 (MO)
Strychnos diplotricha 106	KC609296	Madagascar	Croat 29448 (MO)
Strychnos gerrardii 11	KC609297	South Africa	A Adebowale 11 (UDW)
Strychnos gerrardii 15	KC609298	South Africa	Adebowale 15 (UDW)
Strychnos gerrardii 17	KC609299	South Africa	A Adebowale 17 (UDW)
Strychnos gerrardii 75	KC609300	Mozambique	JE Burrows & SM Burrows 9052 (BNRH)
Strychnos henningsii 20	KC609301	South Africa	A Adebowale 20 (UDW)
Strychnos henningsii 5	KC609302	South Africa	A Adebowale 5 (UDW)
Strychnos henningsii AF	KC609303	Mozambique	S Fourie 1363 (GRA)
Strychnos innocua	JF937987.1	-	A PM de Kruif 541 (WAG)
Strychnos innocua I1	KC609304	Tanzania	Lovette & Kayombo 459 (MO)
Strychnos lucens	JF937991.1	-	H Schmidt et al. 1221 (MO)
Strychnos lucens 99	KC609305	Zimbabwe	Mavi 646 (SRGH)
Strychnos madagascariensis	JF937994.1	-	G McPherson 14555 (MO)
Strychnos madagascariensis 67	KC609306	South Africa	A Adebowale 67 (UDW)
Strychnos madagascariensis 73	KC609307	South Africa	A Adebowale 73 (UDW)
Strychnos mellodora 105	KC609308	Zimbabwe	P van Wyk BSA1865 (PRE)
Strychnos mitis 24	KC609309	South Africa	A Adebowale 24 (UDW)
Strychnos mitis 64	KC609310	South Africa	A Adebowale 64 (UDW)
Strychnos panganensis	JF938019.1	-	P. Antilahimena 85 (MO)
Strychnos potatorum	JF938027.1		EA Banda et al. 3770 (MO)
Strychnos potatorum 77	KC609311	Mozambique	JE Burrows & SM Burrows 10460 (BNRH)
Strychnos potatorum AC	KC609312	Zimbabwe	SP Redfern 23 (GRA)

Strychnos pungens	JF938029.1	-	RRJ van Vuuren 1842 (MO)
Strychnos pungens 49	KC609313	Botswana	Larson 29 (J)
Strychnos pungens 74	KC609314	South Africa	EP Nienaber EN186 (PRE)
Strychnos pungens AN	KC609315	South Africa	PM Burgoyne BPP25 (PRE)
Strychnos spinosa	JF938049.1	-	T Motley
Strychnos spinosa	JF938047.1		L Struwe 1027 (NY)
Strychnos spinosa 48	KC609316	South Africa	B Maguire 8760 (J)
Strychnos spinosa subsp. lokua 84	KC609317	South Africa	W Matthew s.n. (PRU) 091595
Strychnos usambarensis 22	KC609318	South Africa	A Adebowale 22 (UDW)
Strychnos usambarensis 54	KC609319	South Africa	A Adebowale 54 (UDW)
Strychnos xantha	JF938067.1	-	F Malaisse 13311 (WAG)
Strychnos xantha 78	KC609320	Mozambique	JE Burrows & SM Burrows 11326 (BNRH)
Strychnos xantha 90	KC609321	Mozambique	JE Burrows & SM Burrows 10170 (BNRH)

Table 4.4: Helical transfer and Gibbs free energy from homology modelling of *Strychnos* ITS2 secondary structure.

Sn	Taxon	Helical transfer (%) I/II/III/IV	Calculated Free energy (kcal/mol)
1	*S. angolensis* 98	85/100/88/72	-38.02
2	*S. cocculoides* 47	78/100/85/100	-47.0
3	*S. decussata* 10	78/100/91/81	-36.5
4	*S. diplotricha* 106	92/90/91/81	-36.5
5	*S. gerrardii* 11	78/100/94/90	-40.6
6	*S. henningsii* 20	92/100/91/100	-53.5
7	*S. innocua* I1	78/100/94/90	-40.6
8	*S. lucens* 99	78/100/94/90	-39.5
9	*S. madagascariensis* 67	78/100/94/81	-35.4
10	*S. mellodora* 105	85/100/88/90	-43.0
11	*S. mitis* 24	71/100/80/90	-20.52
12	*S. potatorum* 77	92/100/88/90	-43.3
13	*S. pungens* 49	78/100/94/90	-40.6
14	*S. spinosa* 48	71/100/88/90	-35.12
15	*S. spinosa subsp. lokua* 84	71/100/88/90	-37.22
16	*S. usambarensis* 22	78/100/94/72	-33.3
17	*S. xantha* 90	85/100/91/90	-48.2

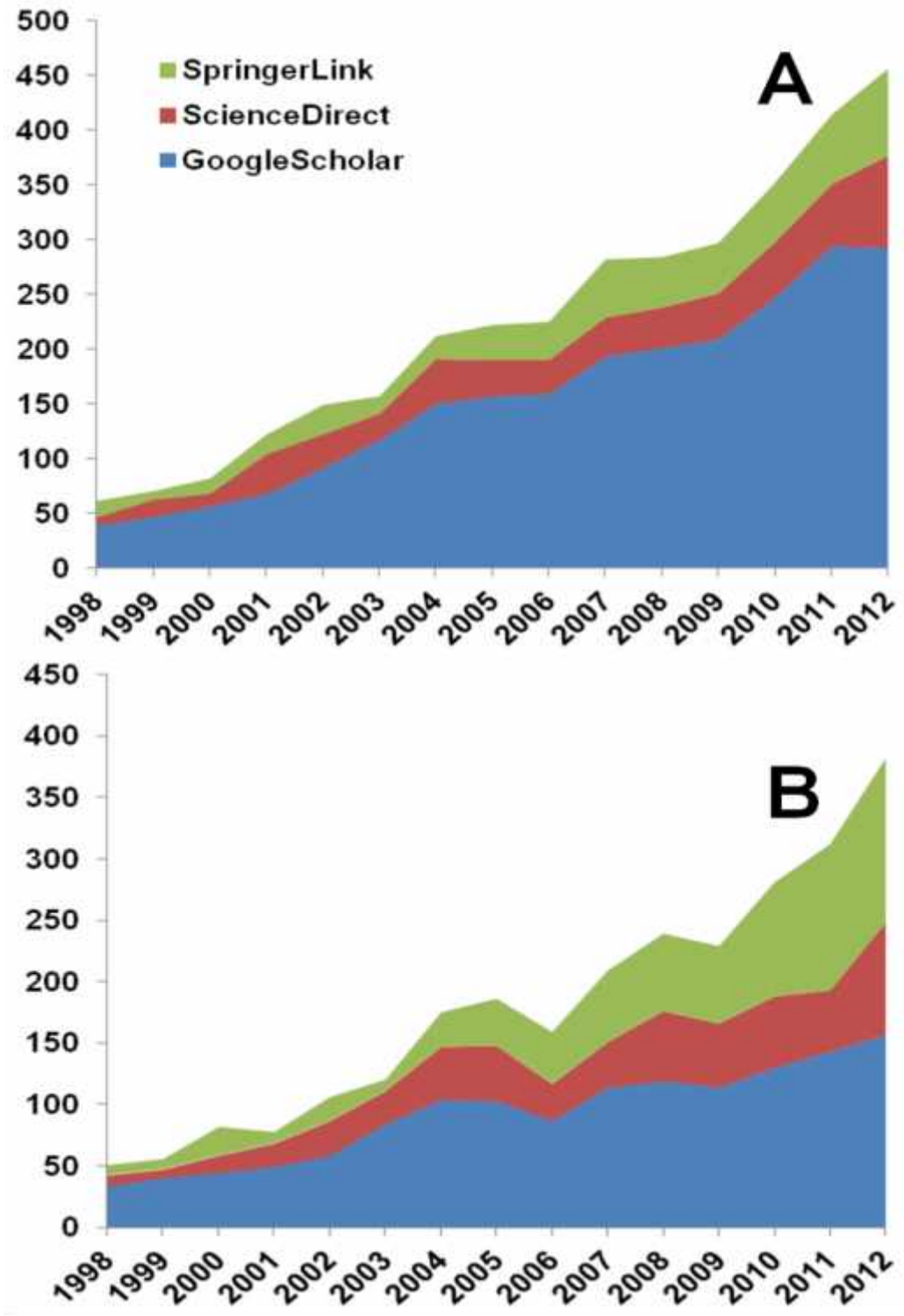
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Figure 4.1

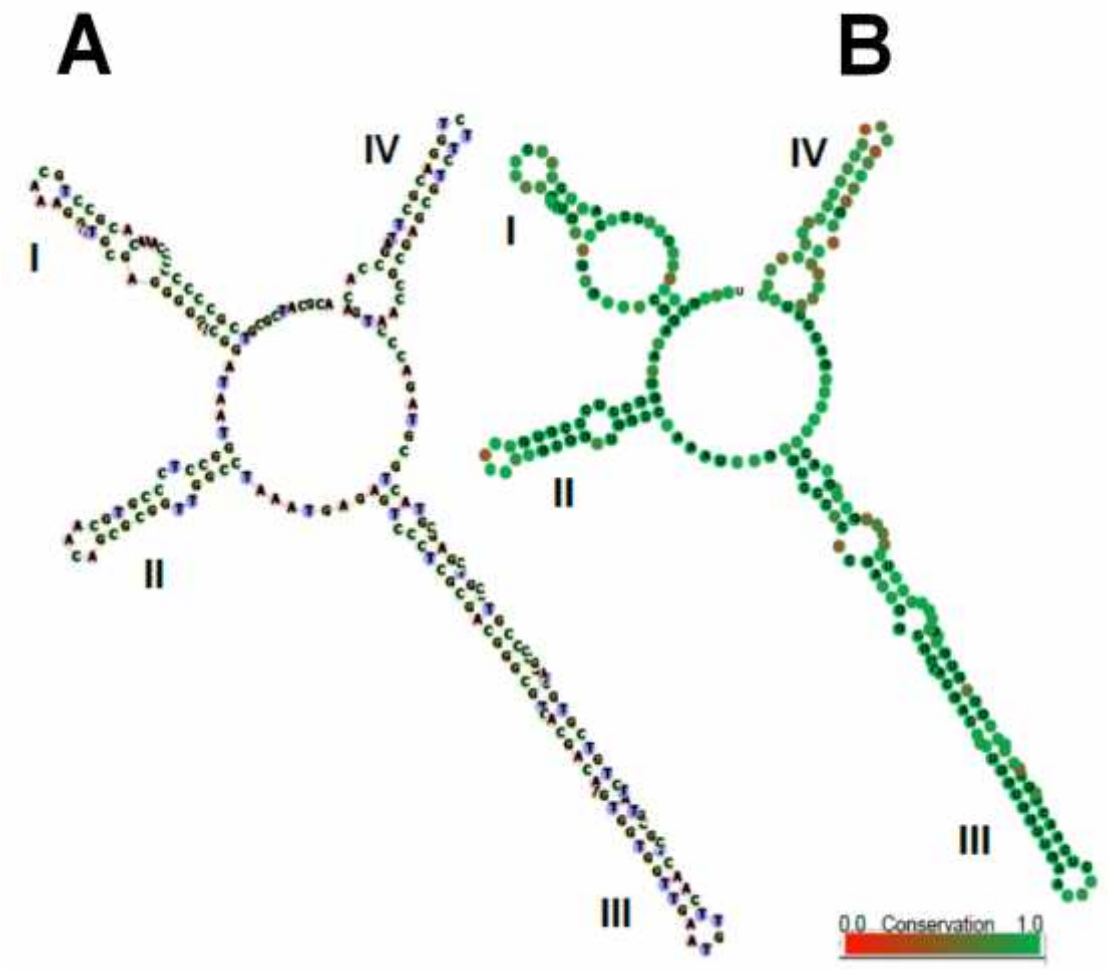
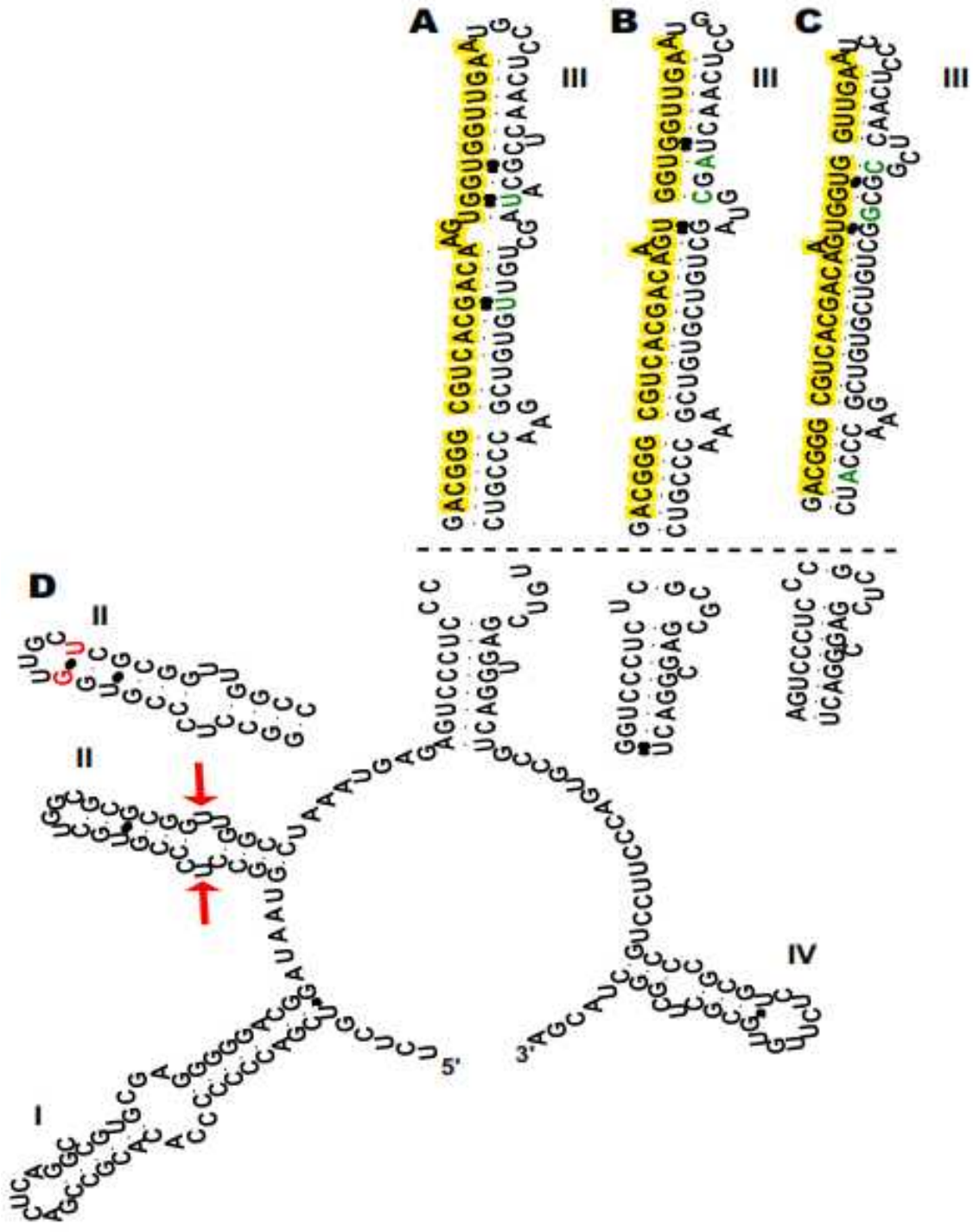


Figure 4.2



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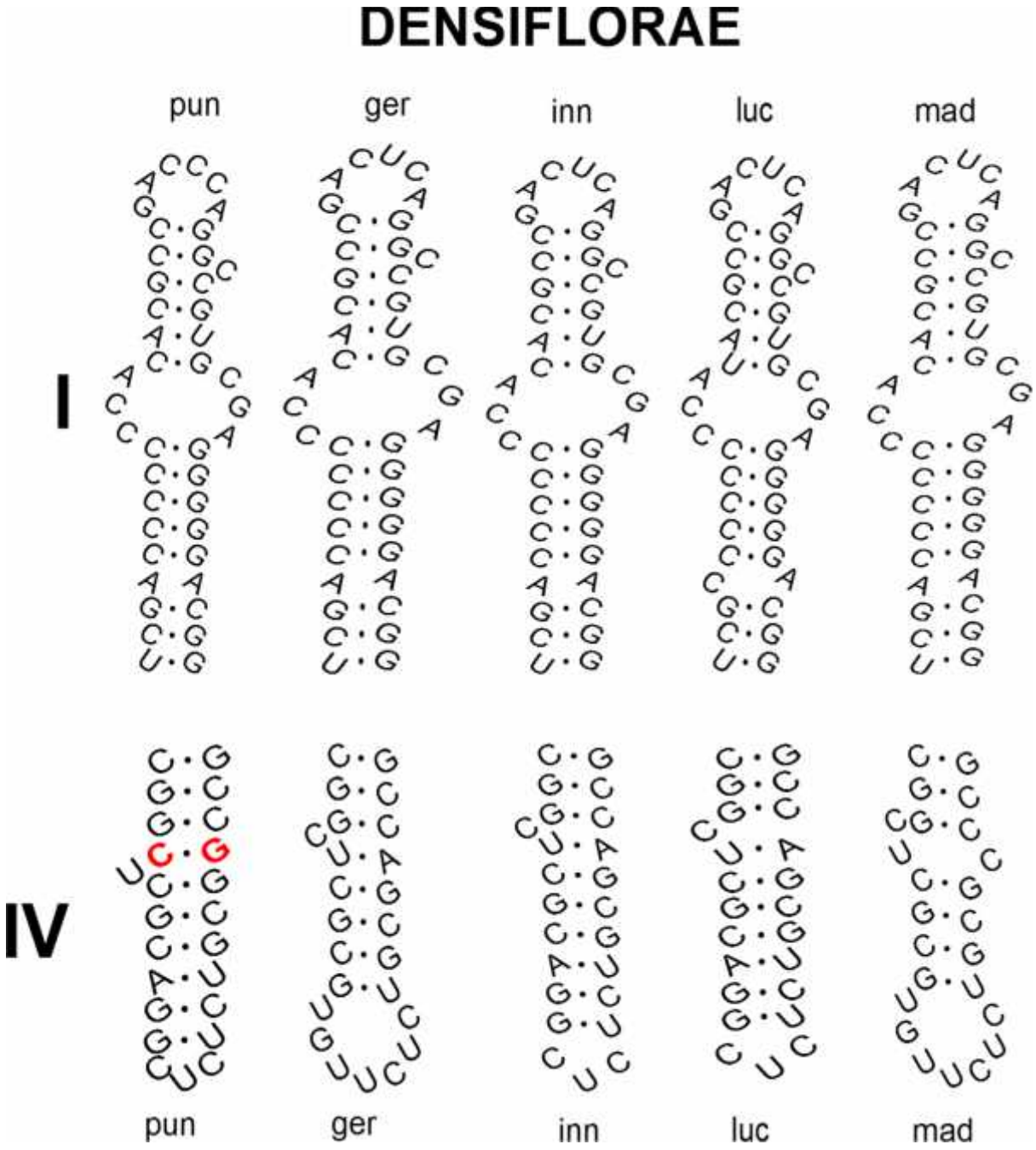
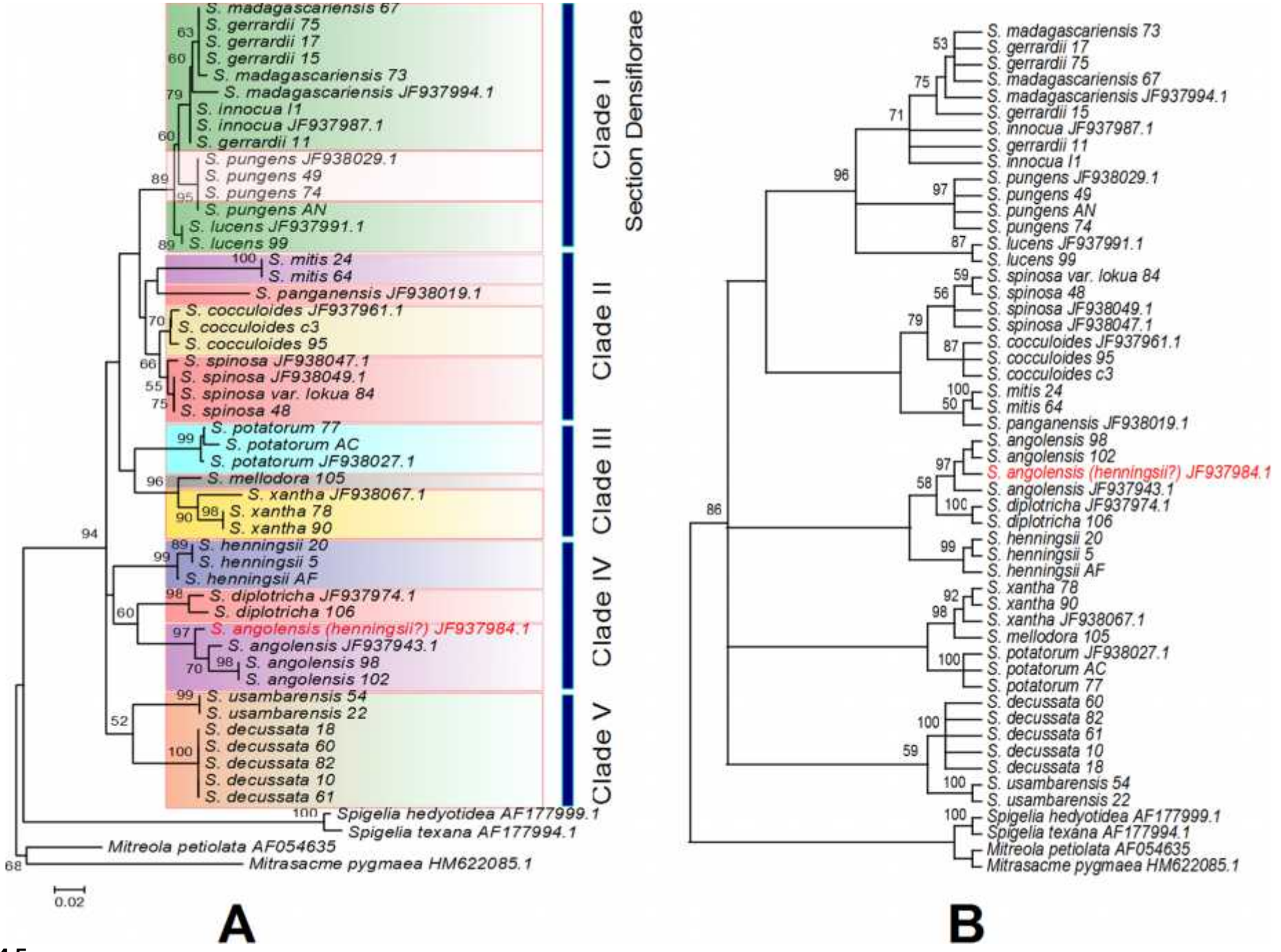


Figure 4.4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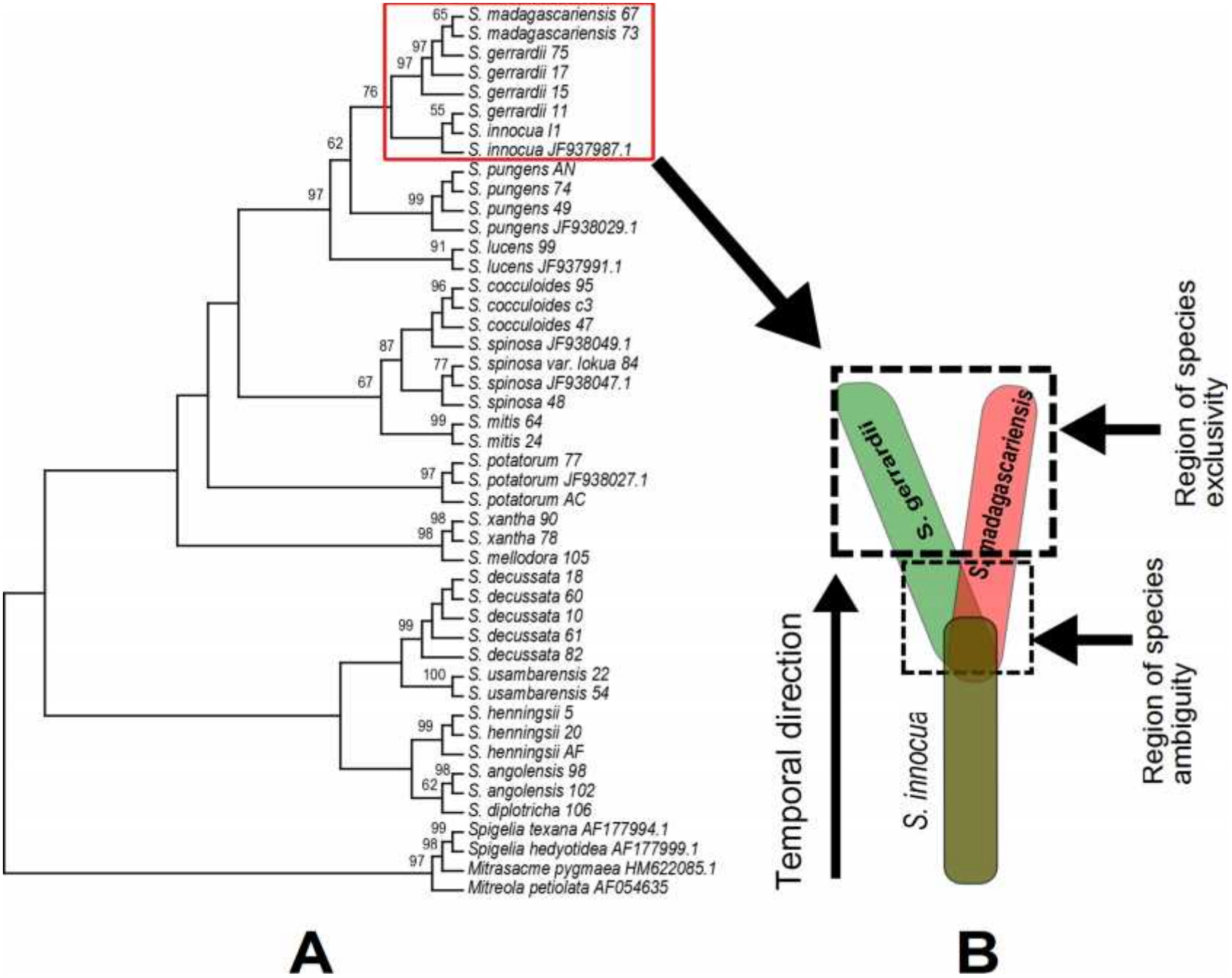
S. innocua I1
S. innocua JF937987.1
S. gerrardii 11
S. pungens JF938029.1
S. pungens 49
S. pungens 74
S. pungens AN
S. lucens JF937991.1
S. lucens 99
S. mitis 24
S. mitis 64
S. panganensis JF938019.1
S. cocculoides JF937961.1
S. cocculoides c3
S. cocculoides 95
S. spinosa JF938047.1
S. spinosa JF938049.1
S. spinosa var. lokua 84
S. spinosa 48
S. potatorum 77
S. potatorum AC
S. potatorum JF938027.1
S. mellodora 105
S. xantha JF938067.1
S. xantha 78
S. xantha 90
S. henningsii 20
S. henningsii 5
S. henningsii AF
S. diplotricha JF937974.1
S. diplotricha 106
S. angolensis (henningsii?) JF937984.1
S. angolensis JF937943.1
S. angolensis 98
S. angolensis 102
S. usambarensis 54
S. usambarensis 22
S. decussata 18
S. decussata 60
S. decussata 82
S. decussata 10
S. decussata 61
Spigelia hedyotidea AF177999.1
Spigelia texana AF177994.1
Mitreola petiolata AF054635
Mitrasacme pygmaea HM622085.1
0.02
Clade I
Clade II
Clade III
Clade IV
Clade V
Section Densiflorae
A
B

Figure 4.5


S. madagascariensis 67
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S. gerrardii 75
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S. gerrardii 11
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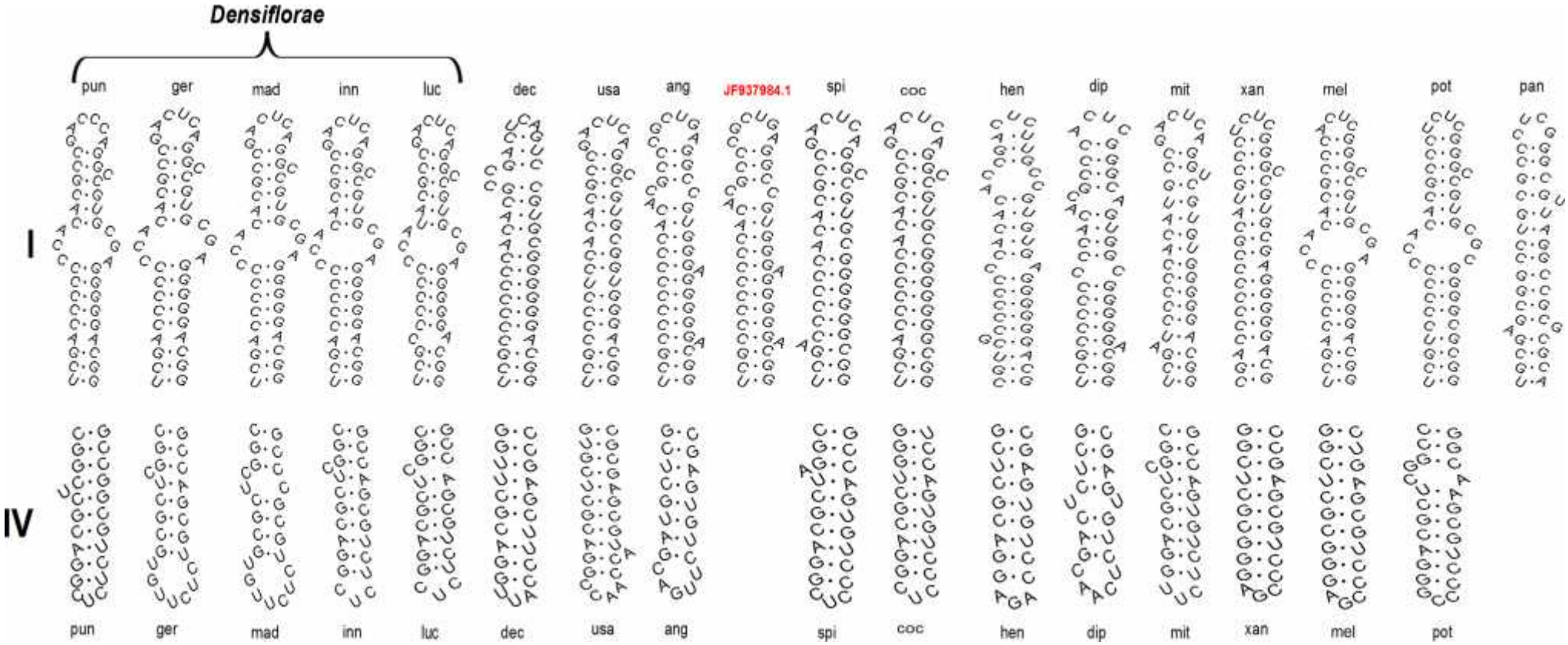
S. innocua JF937987.1
S. pungens AN
S. pungens 74
S. pungens 49
S. pungens JF938029.1
S. lucens 99
S. lucens JF937991.1
S. cocculoides 95
S. cocculoides c3
S. cocculoides 47
S. spinosa JF938049.1
S. spinosa var. lokua 84
S. spinosa JF938047.1
S. spinosa 48
S. mitis 64
S. mitis 24
S. potatorum 77
S. potatorum JF938027.1
S. potatorum AC
S. xantha 90
S. xantha 78
S. mellodora 105
S. decussata 18
S. decussata 60
S. decussata 10
S. decussata 61
S. decussata 82
S. usambarensis 22
S. usambarensis 54
S. henningsii 5
S. henningsii 20
S. henningsii AF
S. angolensis 98
S. angolensis 102
S. diplotricha 106
Spigelia texana AF177994.1
Spigelia hedyotidea AF177999.1
Mitrasacme pygmaea HM622085.1
Mitreola petiolata AF054635
A
S. gerrardii
S. madagascariensis
S. innocua
Temporal direction
Region of species exclusivity
Region of species ambiguity
B

Figure 4.6

Supplementary Figure 4.1



Supplementary Figure 4.2

Supplementary Table 4.1: ITS2 nucleotide composition in percentage across all *Strychnos* samples

	T(U)	C	A	G	Total
S. angolensis	19.2	32.7	16.4	31.8	214.0
*S. angolensis (S. henningsii? JF937984.1)**	17.1	32.9	16.7	33.3	222.0
S. angolensis 102	18.4	32.7	16.6	32.3	223.0
S. angolensis 98	18.4	32.7	16.6	32.3	223.0
S. cocculoides	16.4	33.6	17.3	32.7	220.0
S. cocculoides 95	16.4	34.5	16.4	32.7	220.0
S. cocculoides C3	15.9	34.5	16.4	33.2	220.0
S. decussata 10	18.2	33.6	16.4	31.8	220.0
S. decussata 18	18.2	33.6	16.4	31.8	220.0
S. decussata 60	18.2	33.6	16.4	31.8	220.0
S. decussata 61	18.2	33.6	16.4	31.8	220.0
S. decussata 82	18.2	33.6	16.4	31.8	220.0
S. diplotricha	18.9	33.8	16.2	31.1	222.0
S. diplotricha 106	18.9	33.8	16.7	30.6	222.0
S. gerrardii 11	18.9	34.2	15.8	31.1	222.0
S. gerrardii 15	19.6	33.3	15.5	31.5	219.0
S. gerrardii 17	20.7	32.9	15.3	31.1	222.0
S. gerrardii 75	20.7	32.9	15.3	31.1	222.0
S. henningsii 20	16.5	33.9	16.1	33.5	224.0
S. henningsii 5	16.5	33.9	16.1	33.5	224.0
S. henningsii AF	16.9	33.8	16.0	33.3	225.0
S. innocua	18.9	34.2	15.8	31.1	222.0
S. innocua I1	18.9	34.2	15.8	31.1	222.0
S. lucen 99	18.5	33.8	16.2	31.5	222.0
S. lucens	18.5	33.3	16.7	31.5	222.0
S. madagascariensis	19.8	33.3	15.8	31.1	222.0
S. madagascariensis 67	20.7	32.9	15.3	31.1	222.0
S. madagascariensis 73	20.7	33.3	14.9	31.1	222.0
S. mellodora 105	15.3	36.9	14.4	33.3	222.0
S. mitis 64	21.5	30.6	17.8	30.1	219.0
S. mitis24	21.5	30.6	17.8	30.1	219.0
S. panganensis	19.5	33.3	18.1	29.0	210.0
S. potatorum	19.4	35.1	13.1	32.4	222.0
S. potatorum 77	18.9	35.1	13.1	32.9	222.0
S. potatorum AC	18.0	36.0	12.6	33.3	222.0
S. pungens	17.1	35.1	15.8	32.0	222.0
S. pungens 49	17.1	35.1	15.8	32.0	222.0
S. pungens 74	17.1	35.1	15.8	32.0	222.0
S. pungens AN	17.1	35.1	15.8	32.0	222.0
S. spinosa	17.0	33.5	16.1	33.5	218.0
S. spinosa	16.9	34.2	16.0	32.9	219.0
S. spinosa 48	17.4	33.3	16.4	32.9	219.0
S. spinosa subsp. lokua 84	16.9	33.8	16.4	32.9	219.0
S. usambarensis 22	17.6	32.9	17.6	32.0	222.0
S. usambarensis 54	17.6	32.9	17.6	32.0	222.0
S. xantha	16.7	35.6	14.0	33.8	222.0

S. xantha 90	15.8	35.1	14.4	34.7	222.0
S. xantha78	15.8	35.1	14.4	34.7	222.0
Average	**18.1**	**33.8**	**16.0**	**32.1**	**221.0**

*possibly misidentified sequence from GenBank

Supplementary Table 4.2: Interspecific genetic distances (p- uncorrected) among 17 taxa of *Strychnos*

*	pan	dec	usa	spi	coc	mel	pun	xan	mit	dip	ang	luc	pot	hen	inn	mad
pan																
dec	0.16369															
usa	0.17882	0.09508														
spi	0.09613	0.11308	0.11432													
coc	0.10037	0.10422	0.11255	0.02564												
mel	0.16496	0.11468	0.13535	0.10041	0.11799											
pun	0.11437	0.14905	0.14866	0.07813	0.08062	0.13535										
xan	0.22053	0.16121	0.19280	0.14006	0.14729	0.06347	0.17069									
mit	0.14496	0.18955	0.18024	0.10424	0.10858	0.18955	0.15788	0.22186								
dip	0.18588	0.16452	0.13999	0.13529	0.14641	0.15872	0.15189	0.19891	0.18698							
ang	0.19113	0.16365	0.17290	0.13823	0.14738	0.15558	0.16835	0.18214	0.19181	0.13447						
luc	0.09489	0.12783	0.12758	0.06467	0.06859	0.11468	0.02582	0.15728	0.12159	0.14483	0.14661					
pot	0.16858	0.16196	0.15878	0.08403	0.10992	0.11000	0.11260	0.13785	0.19401	0.16219	0.16838	0.09913				
hen	0.16952	0.11510	0.13113	0.09138	0.10149	0.12209	0.14467	0.16003	0.14524	0.14977	0.12696	0.12358	0.14074			
inn	0.10770	0.13456	0.13435	0.06165	0.06259	0.12120	0.02567	0.15931	0.14268	0.15189	0.15377	0.01519	0.09906	0.13024		
mad	0.12132	0.15136	0.15102	0.07147	0.07241	0.13755	0.03661	0.17736	0.15484	0.16407	0.16589	0.02758	0.11429	0.14194	0.01183	
ger	0.11270	0.13993	0.13965	0.06606	0.06700	0.12644	0.02974	0.16514	0.14831	0.15739	0.15917	0.01913	0.10388	0.13559	0.00375	0.00932

*ang, *S. angolensis*; coc, *S. cocculoides*; dec, *S. decussata*; dip, *S. diplotricha*; ger, *S. gerrardii*; hen, *S. henningsii*; inn, *S. innocua*; luc, *S. lucens*; mad, *S. madagascariensis*; mel, *S. mellodora*; mit, *S. mitis*; pan, *S. panganensis*; pot, *S. potatorum*; pun, *S. pungens*; spi, *S. spinosa*; usa, *S. usambarensis*; xan, *S. xantha*

Supplementary Table 4.3: Intraspecific genetic distances for 15 taxa of *Strychnos* *

**dec	usa	spi	coc	pun	xan	mit	dip	ang	luc	pot	hen	inn	mad	ger
0	0	0.005019	0.006675	0	0.035419	0	0.036629	0.025855	0	0.010093	0.006734	0	0.017035	0.0025

**S. mellodora* and *S. panganensis* not included as they were both represented by one sequence each in the analysis. Mean= 0.00973; SD=0.01298 **ang, *S. angolensis*; coc, *S. cocculoides*; dec, *S. decussata*; dip, *S. diplotricha*; ger, *S. gerrardii*; hen, *S. henningsii*; inn, *S. innocua*; luc, *S. lucens*; mad, *S. madagascariensis*; mit, *S. mitis*; pot, *S. potatorum*; pun, *S. pungens*; spi, *S. spinosa*; usa, *S. usambarensis*; xan, *S. xantha*

CHAPTER 5

MOLECULAR SYSTEMATICS OF SOUTHERN AFRICAN MONKEY ORANGE *STRYCHNOS* L. (LOGANIACEAE) INFERRED FROM NUCLEAR AND PLASTID DNA SEQUENCES

ABSTRACT

Strychnos is the largest genus of the Loganiaceae with about 200 species distributed across Africa (including Madagascar), the Americas, Australia and Asia. Recent molecular phylogenetic effort at elucidating relationships on a global scale provided a useful overview for the genus based on internal transcribed spacer (ITS) data. However, an understanding of evolutionary and ecological patterns at a regional scale is better served by fine scale phylogenetic analysis to resolve species complexes for conservation and allied reasons. In this study, we use plastid (*trn*L-*trn*F, *trn*S-*trn*G) and nuclear ribosomal (ITS) sequence data to infer phylogenetic patterns among members of southern Africa *Strychnos*. We also evaluate sectional validity of previous classifications for African members using the ITS sequence data. Our findings support the monophyly of *Strychnos*, although several of the sections were not monophyletic, thus raising the need for sectional reappraisal of the current classification. *Strychnos xantha* is sister to our expanded representation of southern African taxa, while *S. aculeata* is sister to all African taxa. The uncertain relationships among *S. innocua*, *S. madagascariensis* and *S. gerrardii* were resolved in the phylogenetic analysis of combined datasets. *S. innocua* resolved as sister to the other two species in a well-supported clade. *S. gerrardii* and *S. madagascariensis* are also sister taxa that are not yet reciprocally monophyletic, but possess other features

to distinguish them. The synonymy of *S. gerrardii* with *S. madagascariensis* is therefore rejected. There is clear ecological signal in both the plastid and nuclear datasets, in that both consistently placed forest species at the base of the phylogeny and savanna species in more derived positions. We addressed the adaptive significance of such signals as it relates to fruit and growth forms within the southern African context. In light of our results, some recommendations towards a comprehensive sectional revision of African *Strychnos* are proposed.

INTRODUCTION

Strychnos L. is a diverse genus of lianas, shrubs and trees with most of its species located in the tropics of Africa, South America and Asia (Leeuwenberg, 1969). The genus is renowned for the presence, in several species, of poisonous alkaloids that have been used in curare preparations for hundreds of years. More recently, hitherto pharmacologically-overlooked species have been revealed to be as potent as their popular relatives in the treatment of several ailments (Tchinda *et al.* 2012; Shoko *et al.* 2013).

Despite the large body of studies on the alkaloid chemistry and ethnobotany of *Strychnos*, molecular systematics studies were non-existent before the work of Frasier (2008). Prior to this, taxonomic studies conducted on various continental groups relied predominantly on morphological features (or chemical profiles in a few cases) to reach taxonomic decisions (Krukoff and Monachino, 1942; Krukoff, 1972; Leeuwenberg, 1969; Leeuwenberg and Leenhouts, 1980; Phillippe *et al.* 2004;

Bisset, 1970; Bisset and Phillipson, 1971; Bisset, 1972). These works, excellent in their own rights, were nonetheless fraught with a number of inevitable contradictions arising from evolutionary convergence of morphological forms across various infrageneric groupings in similar geographic areas. The reliability of such classification schemes is thus questionable.

In the most recent treatment of *Strychnos* by Leeuwenberg and Leenhouts (1980), 12 sections were recognized. Eleven of these have representatives in Africa, where about 75 species have been described. At the sub-regional scale, nine species of *Strychnos* have been described in southern Africa (Verdoorn, 1963). Following the various works of Krukoff and Leeuwenberg (cited above), new taxa have been added on the other continents where *Strychnos* occurs (Huft, 1988; McPherson, 2011; Manoel and Guimarães, 2011; Manoel *et al.* 2012), (South America in particular) except in Africa where the last new taxa descriptions were in the work of Leeuwenberg (1969). This is perhaps an indication of the taxonomic neglect the group has suffered in Africa.

The first molecular phylogenetic study in *Strychnos* (Frasier, 2008) treated the genus globally with the aid of the nuclear ribosomal internal transcribed spacer (ITS) marker. Use of the ITS marker in phylogenetics has been shown to resolve evolutionary relationships in several taxonomic groups (Crouch *et al.* 2009; Murillo-A *et al.* 2013; Rybalka *et al.* 2013). Its application in many related families has unraveled complex evolutionary histories (Maurin *et al.* 2007). A multi-locus approach to phylogenetics,

either using several plastid markers or preferably combining plastid and nuclear markers, is recommended in many plant systematics studies, as it offers robust phylogenies, more insight and better explanations of many evolutionary events that may otherwise have gone undetected.

Although Frasier's work gives a good overview of *Strychnos* phylogenetics at a global scale, detailed studies at regional levels are necessary in such an ecologically diverse group, as it offers better opportunity for understanding the evolutionary forces at play within the group, and how these have impacted morphology and phenology. Further, it may be more relevant from a conservation perspective. The *Strychnos madagascariensis* (SM) complex (comprising *S. madagascariensis*, *S. innocua* and *S. gerrardii*) is a case in point. Taxonomic uncertainty surrounding this complex dates to the works of Verdoorn (1963) and Leeuwenberg (1969), where *S. gerrardii* (originally described as a separate species) was effectively reduced to synonymy under *S. madagascariensis* by the latter author. Verdoorn (1963), however, viewed *S. gerrardii* and *S. madagascariensis* as subspecies of *S. innocua*. A slightly different, but related, problem exists within the morphologically heterogeneous *S. spinosa* complex where three subspecies were described by Bruce (1955a), but were later discarded in favour of a single taxon by Leeuwenberg (1969). Resolution of this kind of controversy warrants detailed investigation, which is not the usual goal of a global treatment.

The aims of this study, which uses plastid (*trn*L-*trn*F; *trn*S-*trn*G) and ITS2 markers, were to: (1) examine phylogenetic relationships among southern African *Strychnos* with focus on species complexes and (2) evaluate the scientific and taxonomic confidence in the sectional classification of Leeuwenberg for the African taxa based on the ITS dataset. We also discuss issues of conflicting phylogenetic signals, evolutionary trends in certain traits and species monophyly. To place our finding in context and enable better exploration of these concepts, sampling has been deliberately extended beyond the strict southern African taxa as defined by Verdoorn (1963) by including *S. angolensis*, *S. panganensis* and *S. xantha* from tropical southern Africa. For purposes of discussion therefore, and to avoid confusion with this expanded view, we have labeled Verdoorn's southern African *Strychnos* as the "core southern African *Strychnos*" or core SA *Strychnos* as opposed to our expanded view.

MATERIALS AND METHODS

TAXON SAMPLING

We sampled 29 in-group accessions of *Strychnos* representing 14 species, eleven of which are core southern African (SA) taxa. The following species belong to the core SA taxa: *S. cocculoides* Bak.*; S. decussata* (Pappe) Gilg*; S. gerrardii* N.E.Br.*; S. henningsii* Gilg*; S. innocua* Del.*; S. madagascariensis* Poir.*; S. mitis* S. Moore*; S. potatorum* L.f.*; S. pungens* Solered.*; S. spinosa* Lam. and *S. usambarensis* Gilg. In addition we also include *S. angolensis* Gilg*; S. panganensis* Gilg and *S. xantha*

Leeuw. In order to evaluate the validity of the current sectional limits within African *Strychnos*, we obtained 47 ITS sequences from Frasier (2008) and generated 34 additional sequences in this study. The 81 ITS sequences thus compiled represented 48 species (64% of the African taxa) spread across 10 of the 11 recognised sections proposed by Leeuwenberg (1969) as occurring on the continent (Table 5.1). We generated 29 sequences each, for two plastid markers across the core SA *Strychnos* as well. Five outgroup taxa in Loganiaceae and allied genera were sampled based on Backlund *et al*. (2000).

DNA ISOLATION, PCR AMPLIFICATION AND SEQUENCING

We extracted genomic DNA from field collected, silica-gel-dried leaf material, or herbarium specimens in a few cases using the QIAGEN DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol. Following test amplifications of the nuclear ribosomal internal transcribed spacer (ITS) region and three chloroplast loci (the *trn*L-*trn*F, *trn*S-*trn*G and *trn*D-*trn*T intergenic spacers), we settled on the ITS2 region and the former two plastid markers as we were not able to amplify the *trn*D-*trn*T spacer for most of our samples. Our marker choice is supported by previous systematic studies in the Loganiaceae and allied families such as the Rubiaceae, where the use of similar DNA regions has been successful at infrageneric categories (Andersson and Antonelli, 2005; Cros *et al.* 1998). All PCR amplifications were carried out in 25µl reactions containing: 30-50ng genomic DNA in 9µl; 0.8µl PCR-grade water, 2.5µl 10X reaction buffer, 4µl 25mM $MgCl_2$, 0.5µl 10mM dNTP, 0.2µl 5 U/µl *Taq* and 4µl of 6 µM primer (forward and

reverse). Primer sequences and authorities for each of the loci are provided in Table 5.2.

The ITS2 region readily amplified with this programme: 2 min at 94^{o}C; 35 cycles of 1 min at 94^{o}C; 2min at 53^{o}C; 2 min at 72^{o}C; final extension of 5 min at 72^{o}C. The PCR programme for the amplification of the *trn*L-*trn*F intergenic spacer was: 2 min at 94^{o}C; 30-35 cycles of 30 s at 94^{o}C; 30 s at 52^{o}C; 2 min at 72^{o}C; final extension of 7 min at 72^{o}C. For the *trn*S-*trn*G spacer, we optimised/adapted the second protocol of Shaw *et al*. (2005) as follows: 5 min at 80^{o}C; 35 cycles of 1 min at 95^{o}C; 1 min at 50^{o}C, with a ramp of 0.3^{o}C/s; 5 min at 65^{o}C; final extension of 10 min at 65^{o}C.

PCR products were purified with a Zymoclean^{TM} Gel DNA Recovery Kit (Zymo Research, Orange, CA) following the manufacturer's guide. Amplicons were visualised by electrophoresis in 1.5% agarose gel followed by staining with ethidium bromide. Direct sequencing of PCR products was carried out using the BigDye Terminator Cycle sequencing Kit v 3.1 (Applied Biosystems) in a 10µl reaction containing 3µl of the ready reaction mix, 7 pmol of the corresponding PCR primers and 50 – 100ng of purified PCR products. Chromatograms for forward and reverse sequence reads were assembled and edited with BioEdit (Hall, 1999) to generate consensus sequence for each accession. New sequences from our study were deposited in GenBank (ITS: KC609287 - KC609321 and KM365187; *trn*L-*trn*F: KM365126 - KM365156; *trn*S-*trn*G: KM365157 - KM365186, Table 5.1).

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS

Sequences were pairwise-aligned with the ClustalW algorithm (Thompson *et al.* 1994) as implemented in DAMBE (Xia, 2013) and adjusted by visual inspection where necessary. For ITS2, we used the secondary structure motif as a guide (when possible), while excluding regions of alignment ambiguity prior to analysis. The best fit model of nucleotide substitution was estimated for each locus in jModelTest v 2.1.4 (Darriba *et al.* 2012; Guindon and Gascuel, 2003) under the Akaike Information Criterion (AIC). Pairwise (between markers) incongruence length difference (ILD) tests (Farris *et al.* 1995) were performed in PAUP* v 4.0b10 (Swofford, 2002) prior to phylogenetic analyses. Consequently, phylogeny estimates were based on each individual dataset as well as combined datasets (Table 5.3). For indels, we employed the simple coding algorithm of Simmons and Ochoterena (2000) as implemented in SeqState v 1.4.1 (Müller, 2005).

Using the preferred substitution models suggested by jModelTest, maximum likelihood (ML) analysis with 1,000 bootstrap pseudo-replicates was performed in MEGA 5 (Tamura *et al.* 2011) on individual markers and on the combined dataset to produce majority rule consensus trees. We carried out maximum parsimony on the three-locus concatenated dataset (henceforth referred to as combined dataset) in PAUP*. All characters were weighted equally and unordered. Gaps were treated as missing data in one set of analyses, and coded in another set. The most parsimonious trees (MPTs) were obtained with heuristic search options of 1,000 random addition replicates saving 30 trees per replicate. Branch-swapping was

carried out using the tree-bisection-reconnection (TBR) algorithm with a MulTrees option for keeping multiple equally MPTs. Internal support for clades was evaluated by non-parametric bootstrapping (Felsenstein, 1985) with 1,000 pseudo-replicates. For discussion purposes, bootstrap support (BS) values less than 70% were regarded as weak, 70%-84% as moderate, and 85% and above were considered to be strong. To shed some light on the relationship among the core southern African *Strychnos* taxa, a 12-taxon combined sequence alignment was analysed via the parsimony criterion in PAUP* using the exhaustive search command to find the MPT.

Bayesian Markov chain Monte Carlo (MCMC) inference analyses as implemented in MrBayes v 3.2.1 (Ronquist *et al.* 2012) were performed on a partitioned combined dataset. A six parameter model of nucleotide evolution fitted each of the three loci with gamma distribution applicable only to the ITS2 marker. Consequently, we specified the GTR model for the two plastid markers and the GTR + Γ model for ITS2 with parameters unlinked among partitions. Analyses were run for 5 million generations, sampling every 500 generations. Two independent runs of four chains (one cold, three heated) were performed and the first 1 000 000 generations (20% of the trees) were discarded as burn-in prior to summarizing the posterior. All other settings were retained at their default values. Log files were analyzed in Tracer v 1.5.0 (Rambaut and Drummond, 2009) for convergence assessment and confirmation that effective sample sizes for all parameters were greater than 200. The majority rule consensus tree file produced was edited in FigTree v 1.3.1 (Rambaut, 2006) and Inkscape v 0.48 (www.inkscape.org).

RESULTS

SEQUENCE ATTRIBUTES OF ITS2, *TRN*L-*TRN*F AND *TRN*S-*TRN*G

The length of the second internal transcribed spacer was established using the ITS2 database (Koetschan *et al.* 2012) implementation of the Hidden Markov Models with HMMer 2.3.2 (Eddy, 1998). Across all accessions, complete ITS2 sequence length varied from 210 basepairs (bp) in *S. panganensis* to 225 bp in *S. henningsii*. As the flanking regions – 5.8S and 28S rRNA genes – are incomplete for all the sequences (sequence length across accessions varies from 94 – 128 for the genes), all these regions have been combined with the ITS2 and referred to, for simplicity, as ITS2 in subsequent analysis. Including the flanking regions, sequence length varied between 399 and 404 nucleotide pairs. The ITS2 displayed considerable homoplasy [homoplasy index (HI) excluding uninformative characters is 0.54 for SA *Strychnos* and 0.32 for the African *Strychnos* dataset].

Sequence length for *trn*L-*trn*F ranged from 389 to 407, while that of *trn*S-*trn*G varied from 403 to 512. There is a low level of homoplasy in the plastid markers (HIs excluding uninformative characters are 0.20 and 0.19 for *trn*L-*trn*F and *trn*S-*trn*G datasets, respectively). The larger sequence length variation in the *trn*S-*trn*G intergenic spacer is due to a 104 nucleotide basepair section-defining indel event found in all members of *Densiflorae* sampled (Figure 5.1). Other marker relevant statistics are presented in Table 5.3.

PHYLOGENETIC RELATIONSHIPS AMONG SOUTHERN AFRICAN *STRYCHNOS*

Independent analyses of each of the plastid markers produced trees with several polytomies, a consequence of poor phylogenetic resolution. These are not presented here. The partitioned homogeneity test for the two plastid datasets indicated combinability (P=0.19). They were thus combined and subsequently treated simply as the “plastid dataset”. The combined ITS2 and plastid datasets for SA *Strychnos* showed marginal congruence based on the homogeneity test (P=0.08). Points and possible causes of conflict are noted and discussed. The combined ITS2 and plastid datasets are referred to as the “combined dataset”.

Estimates of phylogeny based on parsimony analysis of the plastid dataset (Figure 5.2a) and maximum likelihood (ML) analysis combined dataset (Figure 5.3) placed an unsupported *S. xantha* basal to the other southern Africa taxa. The monophyly of *Strychnos* has moderate support in the parsimony analysis (BS=84%) and strong support in the ML analysis of the combined dataset. However, there is no resolution regarding the placement of *S. henningsii*, *S. angolensis*, *S. decussata*, *S. potatorum* and *S. usambarensis* (MP tree, Figure 5.2a). In the majority rule consensus tree produced from MP analysis of ITS2 data, there is better resolution, but there is some evidence of topological incongruence, albeit poorly supported, relative to the plastid dataset in the placement of *S. usambarensis* + *S. decussata* clade sister to the other *Strychnos* taxa (Figure 5.2b).

The combined dataset subjected to MP, ML and BI analyses produced similar topology and improved resolution in overall relationships with strong statistical support for the monophyly of *Strychnos* (98/95 BS; 1.00 PP; Figure 5.3). The resolved basal clades had poor support (BS < 50) with *S. xantha* sister to the other SA *Strychnos* species. The SM complex showed paraphyly between *S. gerrardii* and *S. madagascariensis*. In an exhaustive search parsimony analysis of the combined dataset for the core SA species (*sensu* Verdoorn, 1963) however, the complex was resolved. *Strychnos innocua* is sister to the clade comprised of *S. gerrardii* and *S. madagascariensis*. This tree also indicated *S. potatorum* as sister to the core SA taxa (CI excluding uninformative characters=0.67; RI=0.66; tree length=350). However, to better assess evolutionary trends and ascertain basal positions among our expanded SA taxa, we included *S. xantha* in a separate exhaustive search, by replacing *S. gerrardii* (whose clade position is known with certainty to keep the number of taxa to 12, the maximum number of OTUs that PAUP can handle for exhaustive search) with *S. xantha*. The new analysis confirmed *S. xantha* as sister to the other southern African *Strychnos* species (CI excluding uninformative characters=0.64; RI=0.59; tree length=381; tree combining results of both analyses is presented in Figure 5.4). Another notable trend is the consistent placement of forest species at the base of the *Strychnos* phylogeny, reflecting some historical ecological pattern in the molecular dataset of the southern African taxa.

Discounting sections *Dolichanthae* and *Lanigerae*, which were represented by one species each in these analyses, of the other four sections, only *Densiflorae* and

Spinosae appear to be monophyletic for the sampled SA taxa. Sections *Breviflorae* and *Rouhamon* are not monophyletic, having members split in various clades of the tree (Figure 5.3).

SECTIONAL AFFILIATIONS AMONG AFRICAN *STRYCHNOS*

As in the previous analyses, the monophyly of *Strychnos* is maximally supported by Bayesian phylogenetic inference (pp 1.00; Figure 5.5), while the spine of the tree is poorly supported. However, due to wider taxon coverage, there is better insight into sectional affiliations. For ease of interpretation, the phylogeny is grouped into four major clades, none of which is supported. Clade I is mainly an amalgam of the four sections *Densiflorae*, *Lanigerae*, *Spinosae* and the monotypic section *Phaeotrichae*. Excluding *S. henningsii*, which is sister to the clade, this grouping is moderately supported. *S. mitis* is sister to section *Spinosae*, the monophyly of which is invalidated by the placement of *S. staudtii* and *S. phaeotricha*. However, the placement of these two taxa within *Spinosae* is not supported and may therefore not be a true reflection of evolutionary relationships among the taxa in question. We note though, that they are both well-supported within a larger *Spinosae* + *Lanigerae* clade (pp 0.97; Figure 5.5). Section *Lanigerae* has strong support and appears monophyletic based on the sampled taxa. Section *Densiflorae* is paraphyletic due to the placement of *S. staudtii* within the *Spinosae* group. Clade II comprises mainly sections *Rouhamon* and *Brevitubae*, along with *S. malchairii* from section *Breviflorae*. The core *Brevitubae* split from the strongly supported *Rouhamon* to form two smaller clades. Neither of these sections (*Brevitubae* and *Rouhamon*) is monophyletic either.

Clade III comprises a non-monophyletic section *Penicillatae* and the remaining sampled members of *Breviflorae* and *Rouhamon*. Clade IV includes two sections, the monotypic section *Aculeatae* (*S. aculeata*), which is sister to all other African *Strychnos*, and the maximally supported and apparently monophyletic section *Dolichanthae*. With the exceptions of the monotypic sections (*Aculeatae* and *Phaeotrichae*), only two other sections (*Dolichanthae* and *Lanigerae*) appear to be monophyletic, at least based on taxa sampled in this study. Our phylogenetic analyses of the ITS marker for African *Strychnos* do not support the monophyly of the sections: *Breviflorae*, *Brevitubae*, *Densiflorae*, *Penicillatae*, *Rouhamon* or *Spinosae*. Section *Breviflorae* appears to be the most heterogeneous section, as its assigned members are scattered across three of the four major clades.

DISCUSSION

TOPOLOGICAL CONFLICTS AND PHYLOGENETIC UTILITY OF THE VARIOUS MARKERS

Although the combinability of the plastid markers (*trn*L-*trn*F and *trn*S-*trn*G) was supported by statistical test of homogeneity, individual parsimony tree topologies (trees not shown) estimated by each of these markers, displayed poorly supported conflicts, ("soft incongruence" Seelanan *et al.* 1997) such that intralinkage incongruence between the markers may be safely ruled out. The ILD test indicates congruence between ITS2 and plastid markers; yet, tree topologies from the two data sets are different in some respects. The causes of these conflicts, however poorly supported, are still worth exploring.

Phylogenetic incongruence between alternate datasets has generated much debate in molecular systematics and the potential sources and subsequent treatments have been the subject of reviews and various studies (Wendel and Doyle, 1998; Rokas *et al.* 2003; Pelser *et al.* 2010; Davalos *et al.* 2012). Causes include inappropriate model choice, homoplasy, insufficient signals, horizontal gene transfer and incomplete lineage sorting, among several other possibilities (Wendel and Doyle, 1998). The poorly supported basal polytomies in our plastid tree and the lack of robustness at the base of the ITS2 tree for the southern African *Strychnos* makes for a difficult evaluation of the nature of incongruence at play. It would appear though, that the relative paucity of informative sites in the plastid dataset implicates insufficient phylogenetic signal as a contributing factor to the observed incongruence. In addition, the high level of homoplasy in the ITS2 dataset, coupled with the reputation of this marker for sometimes masking divergent events via hybridization or incomplete lineage sorting are plausible alternative explanations for incongruence. These two events may well present similar phylogenetic patterns, which may sometimes be difficult to isolate (Pelser *et al.* 2010). The fact that various analyses of combined datasets, irrespective of method, produced similar topologies and failed to recover robust and congruent basal clades clearly excludes methodological approach as a cause of the observed conflict. A survey of many more plastid markers, perhaps with lower nucleotide substitution rates and combined stronger phylogenetic signal, may hold the key to recovering robust and coherent basal relationships in *Strychnos*.

While the *trn*L-*trn*F tree placed *S. xantha* as sister to all other SA *Strychnos* taxa, the *trn*S-*trn*G suggested *S. mitis* as the sister taxon to the other *Strychnos*. A close examination of each of the plastid datasets revealed that the *trn*L-*trn*F spacer outperformed *trn*S-*trn*G as a source of potentially informative characters (PIC) for phylogeny reconstruction in *Strychnos*, if one considers only variable sites (Table 5.3). Even when constant characters were included, the % PIC was similar for both markers. This is at variance with the findings of a number of studies (Perret *et al.* 2003; Shaw *et al.* 2005; Shaw *et al.* 2007) in which the *trn*S-*trn*G marker consistently outperformed *trn*L-*trn*F in terms of phylogenetic utility. Indeed, in ranking several non-coding plastid markers according to utility across angiosperms, Shaw *et al*. (2005) categorized the *trn*S-*trn*G spacer as tier 1 (considered the most valuable PIC-wise), while *trn*L-*trn*F was in tier 3. Although unexpected, our finding lends further support to the thesis of rate heterogeneity for different markers in different lineages (Sanderson, 1997; Sanderson, 2002). Other studies have also demonstrated that in some plant groups, the *trn*L-*trn*F marker is quite remarkable at resolving phylogenies, performing much better than *mat*K and *rbc*L (Chen *et al.* 2013). Thus conducting a preliminary survey of lineage-specific marker usability prior to undertaking large scale phylogenetic enterprises could be economical in the long run, since utility may not always be generalizable from previous studies.

EVOLUTIONARY TRENDS IN PLANT HABIT, REPRODUCTIVE TRAITS AND ECOLOGY

In *Strychnos*, flowers have evolved to be generally very small (6 – 14 mm in length) and of limited variation in colour (dirty white to pale yellow), such that pollination is

easily effected by small animals e.g. ants and dipterans and presumably wind. This suite of floral traits does not preclude the possibility of pollination by other animal agents, as plants can readily occupy any point on the pollination system continuum (Johnson and Steiner, 2000). There is a fluctuation from 5 to 4-merous floral parts, with the lower number appearing to be stable in the more derived taxa among the southern African members viz. *S. gerrardii* and *S. madagascariensis*. The adaptive significance of this reduction, if any, is not clear in *Strychnos*.

The evolution of the genus in the thick rain forest of Central Africa/South America and its current distribution on the African continent suggests a strong influence of ecology in shaping the distribution of extant taxa. The predominant plant habit within the group is liana, within forests, while a tree/shrub-like habit became more common as the group dispersed into more open habitats on the fringes of forests or savanna woodlands. This is consistent with the phylogenetic hypothesis presented here (Figure 5.4) and in the global assessment by Frasier (2008). The two most basal clades in Frasier's work are all woody climbers found in the forest biome. The tree/shrub-like habit appears to have been a secondarily acquired trait in *Strychnos*, presumably in response to adaptation to a more open landscape, with many African taxa still retaining their ancestral climbing forms. Forest species, with wider distribution ranges, like *S. usambarensis* tend to modify their habit (albeit within genetic boundaries) in response to the surrounding landscape, confirming the influence of ecology on growth forms in *Strychnos*. The evolution of bark features, fruit sizes and fruit hardiness appears to have mirrored the forest-woodland

distribution dichotomy among southern African *Strychnos* (Figure 5.6). Forest-inhabiting species (except *S. gerrardii*) have small soft fruits that are usually 1 – 3 seeded, while their savanna woodland congeners have large, hard, many-seeded fruits (Figure 5.4). The tough bark and hardy fruits play important roles as defensive adaptations against fire outbreak and water shortage, two main features of arid environments (Gashaw *et al.* 2002).

INDELS IN *STRYCHNOS* EVOLUTION

The role of indels in plant evolution has been studied extensively in systematic analyses, and the consensus opinion regarding their utility has made them an integral part of molecular systematics. However, much of this analysis has focused on indels derived from coding regions (Ajawatanawong and Baldauf, 2013; Hilu and Alice, 1999; Garcia-Lor *et al.* 2013). Empirical evidence suggests that non-coding indels are equally structured, with a non-random mode of evolution (Kelchner and Clark, 1997). They contain useful phylogenetic signal, sufficient to define lineages, and offer more robust clade support in monophyletic groupings (Liu *et al.* 2012; Graham *et al.* 2000).

On a finer taxonomic scale, indels can be valuable in phylogenetic analysis as sequence alignments can be prepared with minimal ambiguity and indels can then be meaningfully analysed. The role of indels as firm drivers of evolutionary change in closely related species is well-established (Britten *et al.* 2003; Guo *et al.* 2012). In this study, reliable indels abound in the non-coding chloroplast markers (Table 5.3), a number of which support various taxonomic groupings. Particularly intriguing is the

104-bp deletion event in the *trn*S-*trn*G spacer. Indel events in non-coding regions are usually one or a few nucleotides long (Yamane *et al.* 2006). The presence of this relatively large deletion in all four members of section *Densiflorae*, for which the marker was sampled (Figure 5.1), defines this section. This is particularly pertinent because current phylogenetic placement of *S. staudtii* renders section *Densiflorae* paraphyletic. Therefore sampling *S. staudtii* and the remaining members of the section for *trn*S-*trn*G could once for all resolve the taxonomic composition of this group. Given the phylogenetic position of *Densiflorae*, the loss of a considerable portion of the *trn*S-*trn*G spacer is a recent event in the common ancestor of extant *Densiflorae*. A detailed molecular investigation of this marker and adjacent gene regions across *Strychnos* may provide valuable insight into underlying evolutionary and perhaps ecological processes that led to the observed rapid speciation within this clade.

SPECIES MONOPHYLY AND POSSIBILITIES FOR SPECIMEN MISIDENTIFICATION

A number of non-monophyletic groupings were observed for some species represented by multiple ITS2 sequences generated from different individuals or possibly populations. Two such cases involving *S. angolensis* and *S. henningsii* are addressed here based on strong posterior probability support for the clades in question (≥0.99; Figure 5.5). An ITS sequence sample labeled *S. henningsii* from GenBank (JF937984) nested within a clade of *S. angolensis* specimens with maximum support. Similarly, a sequence with the tag *S. angolensis* (JF937942) nested in a *S. henningsii* clade with strong support. We note that these two GenBank

sequences were generated from herbarium collections in the study of Frasier (2008), who interpreted their paraphyly as cases of possible misdiagnoses or complications arising from the multi-copy nature of the ITS marker. Data on ITS2 secondary structure models in *Strychnos* (Chapter 4) suggested that paraphyly alluded to in these two clades is rather artificial and has more to do with specimen misidentification. Specifically modeling the secondary structure of JF937984 (Frasier's "*S. henningsii* A") showed perfect congruence with *S. angolensis*. Furthermore, ITS2 sequences from multiple specimens of various species of southern African *Strychnos* in this study revealed none of the complications generally associated with the ITS marker for phylogenetic purposes (Alvarez and Wendel, 2003). We note though that one ITS sequence of *S. usambarensis* (JF938064), again from Frasier's study, nested outside three of its conspecifics in southern Africa. However, this does not constitute a problem, as some degree of haplotypes diversity is expected across different populations, especially for a marker like ITS. Besides, all four *S. usambarensis* sequences still nested within a maximally supported clade, quite unlike the *S. angolensis* and *S. henningsii* sequences.

Herbarium specimen misidentification, which we suspect in these two species, is not unusual given the wide range of morphological diversity observed in leaves and inflorescences of *S. henningsii* and *S. angolensis* across their distribution range (Duvigneaud, 1947; Bruce, 1955b; Leeuwenberg, 1969). Sterile specimens of *Strychnos henningsii* may be easily mistaken for *S. decussata* or *S. mitis*, depending on age (Leeuwenberg, 1969). As the developmental age of the sampled herbarium

specimens is not known, this could add a further layer of ambiguity to confuse non-specialists. We thus propose that the specimens tagged "*S. henningsii* A" and "*S. angolensis* A" in Frasier's treatment are *S. angolensis* and *S. henningsii*, respectively. It is recommended that these specimens be reassessed morphologically and with chloroplast DNA markers.

EVALUATION OF SECTIONAL GROUPINGS OF AFRICAN *STRYCHNOS*

Questions surrounding the monophyly of *Strychnos*, addressed by Struwe *et al*. (1994), were recently settled by the work of Frasier (2008) in a global molecular phylogenetic review of the genus. Our results corroborate those of Frasier regarding the monophyly of the group. However, there remains many unresolved infrageneric complexities, mostly at the sectional level. Sections have been merged, split, subsumed and resurrected in the search for better sectional hypotheses. Our results, like that of Frasier (2008), suggest that current sectional delimitations by Leeuwenberg (1969) are artificial, and will thus require modification. The monophyly of sections *Rouhamon*, *Breviflorae*, *Brevitubae* and *Penicillatae* are not supported by our analyses (Figures 5.3 and 5.5). In the southern African *Strychnos* analysis, sections *Densiflorae* and *Spinosae* appear well-supported, although the ITS2 dataset across African taxa only provide unequivocal support for the monophyly of *Dolichanthae* and *Lanigerae* based on sampled taxa, excluding the two monotypic sections.

In Frasier's (2008) assessment, section *Spinosae* is the only monophyletic section recovered. However, her conclusion (which she correctly advised readers to treat with

caution) was based on ITS data alone. Even though we have included some plastid sequences, at least for the southern African taxa, we urge similar caution in the interpretation of our findings, owing to limitations of taxon sampling for the plastid data (only SA species) and other potential pitfalls highlighted in the previous section. Moreover, a comprehensive sectional review should only be attempted when multiple sources of comparative information are available across all (or most) *Strychnos* species. Such an undertaking may require some degree of collaboration to be successful. Nevertheless, we are of the view that certain proposals can still be made towards such anticipated revision. We thus submit some sectional notes based on our observations of African species and available literature.

THE MONOTYPIC SECTIONS: *ACULEATAE* AND *PHAEOTRICHAE*

Of the three monotypic sections (*sensu* Leeuwenberg, 1969) (the third not included being *Scyphostrychnos* represented by *S. camptoneura*, which is a homotypic synonym), only two are included in this study. Their monotypic status is not refuted by our results (Figure 5.5). The sister taxon relationship between *S. aculeata* and the rest of the African taxa in our analysis is consistent with Frasier's (2008) finding. Autapomorphic traits for section *Aculeatae* include the presence of prickles on their stems, the abundance of saponin in fruit pulp and thin medullary rays (Duvigneaud *et al.* 1952; Leeuwenberg and Leenhouts, 1980; Sandberg *et al.* 1969). Section *Phaeotrichae* is nested within section *Spinosae* in a well-supported *Spinosae* + *Lanigerae* and *S. mitis* clade. Its placement in Frasier's (2008; PP=0.55, page 86) work as sister to the *S. jobertiana* group in South America is also poorly supported.

Morphological evidence seems to be in agreement as *S. phaeotricha* is the only known *Strychnos* with pubescent glands on the corolla lobes and filaments (Leeuwenberg, 1969). It is therefore proposed that sections *Aculeatae* and *Phaeotrichae* be tentatively maintained in their current monotypic sectional taxonomic status.

SECTIONS *BREVIFLORAE, ROUHAMON, BREVITUBAE* AND *PENICILLATAE*

Other than the inwardly glabrous short corolla tube, there is wide variation in morphological attributes defining members of section *Breviflorae* as circumscribed by Leeuwenberg (1969). The polyphyletic nature of this group, as revealed by our analyses, is therefore not surprising. Krukoff (1972) subdivided American *Breviflorae* into two subsections based on the nature of the testa: crustaceous or fibrous. However, we refrain from the use of such subsectional classification of the African members, because there is no evidence for the supposed synapomorphy, within the section, of the various character states on which such a classification scheme rests. All but two of the African *Breviflorae* have crustaceous testae, with *S. urceolata* and *S. chromatoxylon* being exceptional in having thick testa. *S. dolichonthrysa* and *S. icaja* both possess thin but woolly seed coats. Given the level of morphological heterogeneity within the group and the extent of polyphyly observed, a wholesale re-circumscription of *Breviflorae* following more comprehensive taxon and molecular marker sampling is recommended.

Sections *Brevitubae*, *Penicillatae* and *Rouhamon* show outright paraphyly. The suites of characters used by Leeuwenberg to classify these sections can be as illuminating as they could be confusing. For instance *S. cuniculina* (section *Brevitubae*) and *S. malchairii* (section *Breviflorae*) both nested within a maximally supported clade of *Rouhamon* (Figure 5.5); a section itself conceived from four sections proposed by Duvigneaud (1952), and for which the only *fairly* consistent character is the presence of a solitary tendril, *when present*. In reworking *Rouhamon*, we therefore suggested the inclusion of *S. culiculina* and *S. malchairii*, as they both possess a number of floral features that define *Rouhamon* and are well-supported by the ITS2 dataset. Similar complexities and contradictions abound in some of the other sections. This necessitates the exploration of other sources of information, including but not limited to cytology, alkaloid chemistry and leaf and floral anatomy.

SECTIONS *DENSIFLORAE* AND *SPINOSAE*

These two sections are endemic to Africa and are characterized by tough, corky or fissured bark and large, hardy, usually edible fruits with many seeds. Their distribution patterns also suggest a common preference for open, dry savanna woodland, with the exception of *S. gerrardii* (section *Densiflorae*), which has an east coastal forest distribution in southern Africa. Members of these two sections are the dominant *Strychnos* species in the southern African region.

Limited taxon coverage in the southern African molecular dataset suggests monophyly for these two apparent sister sections (Figure 5.3). However, the

placement of *S. staudtii* in the ITS2 African dataset invalidates any monophyletic assumptions (Figure 5.5). Section *Spinosae* includes four species, all of which were sampled by Frasier (2008). Her results indicated a strongly supported and monophyletic *Spinosae*. Given the poor support for the clade linking *S. staudtii* to the other members of *Spinosae* in our analysis, and the coherent suite of morphological features uniting the section as circumscribed by Leeuwenberg (1969), we agree with the conclusion of Frasier (2008) as to the monophyly of section *Spinosae*. Results of the combined dataset (our SA taxa) also support monophyly, although only two species were represented in the strict sense.

Anatomically, section *Spinosae* is characterized by well-developed wood parenchyma, thick medullary rays and an absence of interxylary phloem (Duvigneaud *et al.* 1952). These features are relatively constant, and not strongly influenced by geographical location of individual plants. Other morphological features of note include the presence of spines (except in *S. ternata*) on stems and branches, ciliated anthers, short pistils and capitate stigmas (Leeuwenberg, 1969).

Among *Densiflorae, S. staudtii* is the only obvious outlier with respect to a number of floral and fruit attributes (e.g. glabrous gynoecium; two rings of hairs on corolla throat as opposed to one in other species; white fruits as opposed to orange/yellow in other species). Based on our analysis, which is congruent with Frasier (2008), *S. staudtii* should be excluded from *Densiflorae* to restore monophyly to the section. Whatever outcome further sampling from other members of the section might yield (highly

recommended), it appears that retaining *S. staudtii* in *Densiflorae* is not supported by either morphology or molecular data. Future sampling of *S. zenkeri* is crucial, as it is the only other species with small fruit, (2-seeded) and white, just like *S. staudtii* (Leeuwenberg, 1969).

SECTIONS *DOLICHANTHAE* AND *LANIGERAE*

Section *Dolichanthae* is endemic to Africa and as circumscribed by Leeuwenberg (1969) appears to be a natural group based on a consistent set of morphological features. Its monophyly is supported by our analysis of four of its nine species (Figure 5.5). The section is reported to be paraphyletic in Frasier (2008) due to the inclusion of *S. camptoneura* (section *Scyphostrychnos*), a species not included in our analysis. Section *Lanigerae* is well-represented in Africa with 12 species, and better still in Asia with about 20 species. Although paraphyletic in the global analysis, the nine African taxa form a strong monophyletic clade. Synapomorphies for the African member, include paired tendrils, pilose gynoecium, 2-celled ovary and orange-yellow fruit. There is some degree of morphological convergence between the African and some Asiatic members, but whether these similarities have any genetic basis remains to be seen.

CONCLUSION

Currently, there is no consensus on the sectional groupings in *Strychnos*, with some authors ascribing newly described taxa to previously sunken sections (e.g. Manoel *et al.* 2012), while others have suggested the resurrection of some sunken categories

(Frasier, 2008). However, findings from this work and that of Frasier (2008) have provided molecular framework from which future classification hypothesis can benefit. It is strongly recommended that in addition to other data sources, more plastid markers be extensively sampled across the genus, with multiple sampling of some taxa (if and when possible), before conclusive sectional categories are proposed. This would provide a more confident basis for testing hypotheses relating to morphological diversity and ecology of *Strychnos*.

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Table 5.1: Sampled taxa, voucher information and GenBank accession numbers for *trn*L-*trn*F, *trn*S-*trn*G and ITS2 markers. Sectional names are in bold font and follow the scheme of Leeuwenberg (1969). Taxa in red font are presumed misidentifications.

		GenBank accession		
Taxon	Voucher information	*trn*L-*trn*F	*trn*S-*trn*G	ITS
***Aculeatae* Duvign.**				JF937940
S. aculeata Solered.				
***Breviflorae* Prog.**				
S. angolensis Gilg				JF937942
S. angolensis Gilg	JE Burrows & SM Burrows 10259 (BNRH)	KM365151	KM365181	KC609287
S. angolensis/henningsii ?				JF937984
S. henningsii/S. angolensis?				JF937942
S. henningsii Gilg	A Adebowale 20 (UDW)	KM365142	KM365172	KC609301
S. henningsii Gilg	Mauve and Verdoorn 39 (J)	KM365144	KM365174	KC609302
S. henningsii Gilg	P. Van Wyk BSA1880 (PRU)	KM365143	KM365173	KC609303
S. icaja Baill.				JF938044
S. malacoclados C.H. Wright				JF937995
S. malchairii De Wild.				JF937996
S. mitis S. Moore	A Adebowale 24 (UDW)	KM365140	KM365170	KC609309
S. mitis S. Moore	A Adebowale 64 (UDW)	KM365141	KM365171	KC609310
S. afzelii Gilg				JF937941
***Brevitubae* A.W. Hill**				
S. cuniculina Leeuwenberg				JF937967
S. johnsonii Hutch. et M.B. Moss				JF937989
S. millepunctata Leeuwenberg				JF938002

S. samba Duvign.				JF938033
S. cuminodora Leeuwenberg				JF937966
***Densiflorae* Duvign.**				
S. gerrardii N.E.Br.	A Adebowale 11 (UDW)	KM365130	KM365160	KC609297
S. gerrardii N.E.Br.	A Adebowale 15 (UDW)			KC609298
S. gerrardii N.E.Br.	A Adebowale 17 (UDW)	KM365131	KM365161	KC609299
S. gerrardii N.E.Br.	JE Burrows & SM Burrows 9052 (BNRH)			KC609300
S. innocua Del.				JF937987
S. innocua Del.	Lovette & Kayombo 459 (MO)	KM365134	KM365164	KC609304
S. madagascariensis Poir.	A Adebowale 67 (UDW)	KM365132	KM365162	KC609306
S. madagascariensis Poir.	A Adebowale 73 (UDW)	KM365133	KM365163	KC609307
S. pungens Solered.	Larson 29 (J)	KM365135	KM365165	KC609313
S. pungens Solered.	EP Nienaber EN186 (PRE)	KM365136	KM365166	KC609314
S. pungens Solered.	PM Burgoyne BPP25 (PRE)			KC609315
S. staudtii Gilg				JF938052
***Dolichanthae* Duvign.**				
S. asterantha Leeuwenberg				JF937948
S. barteri Solered.				JF938040
S. tricalysioides Hutch. et M.B. Moss				JF938058
S. tricalysioides Hutch. et M.B. Moss				JF938060
S. xantha Leeuwenberg				JF938067
S. xantha Leeuwenberg	JE Burrows & SM Burrows 11326 (BNRH)	KM365138	KM365168	KC609320
S. xantha Leeuwenberg	JE Burrows & SM Burrows 10170 (BNRH)	KM365139	KM365169	KC609321
S. xantha Leeuwenberg	F.J. Breteler 11961 (UZL)	KM365137	KM365167	
S. asterantha Leeuwenberg				JF937947

***Lanigerae* A.W. Hill**				
S. dinklagei Gilg				JF937973
S. fallax Leeuwenberg				JF937979
S. ngouniensis Pellegr.				JF938011
S. panganensis Gilg				JF938019
S. panganensis Gilg		KM365150	KM365180	
S. scheffleri Gilg				JF938034
S. soubrensis Hutch. et Dalz.				JF938039
S. splendens Gilg				JF938050
S. talbotiae S. Moore				JF938054
S. chrysophylla Gilg				JF937960
***Penicillatae* A.W. Hill**				
S. matopensis S. Moore				JF937997
S. mostueoides Leeuwenberg				JF938008
S. mostueoides Leeuwenberg				JF938009
S. pentantha Leeuwenberg				JF938023
S. diplotricha Leeuwenberg				JF937974
***Phaeotrichae* Duvign.**				
S. phaeotricha Gilg				JF938024
***Rouhamon* (Aubl.) Prog.**				
S. campicola Gilg ex Leeuwenberg				JF937957
S. dale De Wild.				JF937968
S. decussata (Pappe) Gilg	A Adebowale 10 (UDW)	KM365152	KM365182	KC609291
S. decussata (Pappe) Gilg	Burrows JE & Burrows SM 9503 (BNRH)	KM365153	KM365183	KC609295

S. elaeocarpa Gilg ex Leeuwenberg				JF937976
S. floribunda Gilg				JF937981
S. potatorum L.f.				JF938027
S. potatorum L.f.	JE Burrows & SM Burrows 10460 (BNRH)	KM365129	KM365159	KC609311
S. potatorum L.f.	SP Redfern 23 (GRA)	KM365128	KM365158	KC609312
S. usambarensis Gilg				JF938064
S. usambarensis Gilg	A Adebowale 22 (UDW)	KM365155	KM365185	KC609318
S. usambarensis Gilg	A Adebowale 54 (UDW)	KM365156	KM365186	KC609319
S. usambarensis Gilg	A Adebowale 6 (UDW)	KM365154	KM365184	
S. boonei De Wild.				JF937953
***Spinosae* Duvign.**				
S. cocculoides Bak.	HF Glen NH0134236 (NH)	KM365149	KM365179	KC609289
S. cocculoides Bak.	Bartsch, Klaasen & Uiras s.n. WIND80282 (WIND)			KC609290
S. congolana Gilg				JF937965
S. spinosa Lam.	B Maguire 8760 (J)	KM365147	KM365177	KC609316
S. spinosa Lam.	A. Abbott 5996 (PRU)	KM365145	KM365175	
S. spinosa Lam.	G.M. Dlamini A2743 (PRE)	KM365146	KM365176	
S. spinosa subsp. *lokua* Bruce	W Matthew s.n. (PRU) 091595	KM365148	KM365178	KC609317
S. ternata Gilg ex Leeuwenberg				JF938055
S. cocculoides Bak.				JF937961
S. spinosa Lam.				JF938049
Outgroup taxa				
Anthocleista grandiflora		KM365126	KM365157	DQ449916
Gardenia				FM204691
Gardneria				JF937930
Neuburgia				JF937935
Spigelia anthelmia		KM365127		KM365187

Table 5.2: Primer pairs and references used for the sequenced molecular markers.

Locus	Primer	Direction	Sequences (5'- 3')	References
ITS2	"ITS 3"	Forward	GCATCGATGAAGAACGCAGC	White *et al.* (1990)
ITS2	"ITS 4"	Reverse	TCCTCCGCTTATTGATATGC	White *et al.* (1990)
*trn*L-F	"e"	Forward	GGTTCAAGTCCCTCTATCCC	Taberlet *et al.* (1991)
*trn*L-F	"f"	Reverse	ATTTGAACTGGTGACACGAG	Taberlet *et al.* (1991)
*trn*S-*trn*G	5'*trn*G2S	Forward	TTTTACCACTAAACTATACCCGC	Shaw *et al.* (2005)
*trn*S-*trn*G	*trn*S^{GCU}	Reverse	AGA TAG GGA TTC GAA CCC TCG GT	Shaw *et al.* (2005)

Table 5.3: Alignment information, model choice and phylogenetic scores for the three regions used in analysis of southern African *Strychnos*.

DNA Marker	Best Fitting Model	Aligned characters including coded indels	Variable /informative characters	Coded indels	No of most parsimonious trees (MPTs)	Tree length for MPTs	CI*/RI/RC
ITS2	TIM3 + Γ + I	421	162/104 (64.2%)	13	13	304	0.68/0.81/0.60
*trn*L-*trn*F	TIM1	429	132/39 (29.5%)	17	7965	152	0.8/0.89/0.82
*trn*S-*trn*G	TPM1uf	549	258/60 (23.3%)	18	1520	301	0.81/0.89/0.85
cpDNA combined	TIM1 + Γ	971	250/72 (28.8%)	31	1269	284	0.8/0.9/0.84
All three combined	Mixed model	1394	414/177 (42.7%)	45	26	598	0.70/0.83/0.68

CI=Consistency Index; RI=Retention Index; RC=Rescaled Consistency Index. *CI computations exclude uninformative characters

FIGURE LEGENDS

Figure 5.1: A 104 basepair indel in the *trn*S-*trn*G intergenic spacer from sampled members of *Strychnos* section *Densiflorae.*

Figure 5.2: Maximum parsimony 50% majority rule trees from analyses of chloroplast (*trn*L-*trn*F; *trn*S-*trn*G) and ITS data for southern African *Strychnos* taxa. (For ITS2, CI excluding uninformative characters = 0.68; RI = 0.81; for plastid dataset, CI excluding uninformative characters = 0.80; RI = 0.90). CI= consistency index, RI = retention index.

Figure 5.3: Maximum likelihood tree from analysis of combined dataset for southern African *Strychnos* (-lnL = 4204.3467). Maximum parsimony and maximum likelihood bootstrap values ≥ 50% are shown above the branches. Bayesian posterior probability values ≥ 0.50 are shown below the branches. Sectional coverage for southern taxa is highlighted in the colour key.

Figure 5.4: Composite maximum parsimony tree from two exhaustive search queries of combined dataset for core southern African *Strychnos* with and without *S. xantha*. Tree estimate is based on analysis of combined *trn*L-*trn*F, *trn*S-*trn*G and ITS markers for a total of 1332 nucleotide basepairs.Colour bars represent evolutionary trends in (a) distribution, (b) fruit size and hardiness, (c) plant growth habit.

Figure 5.5: Bayesian inference consensus tree showing sectional groupings among African *Strychnos* based on analysis of ITS dataset. Posterior probability (PP) values ≥ 0.50 are presented above the relevant branches. ACU = Aculeatae, BRF = Breviflorae, BVT = Brevitubae, DEN = Densiflorae, DOL= Dolichanthae, LAN = Lanigerae, PEN = Penicillatae, PHA = Phaeotrichae, ROU = Rouhamon, SPI = Spinosae.

Figure 5.6: Morphological diversity in southern African *Strychnos*. A= diffuse stem in *S. gerrardii*; B = Erect stem in *S. gerrardii*; C = flaky bark in *S. gerrardii*; D = *S. spinosa* with spread canopy; E = Hard and tough bark of *S. spinosa*; F = smooth bark of *S. decussata*; G = Large hard fruit of *S. madagascariensis*; H = unripe small fruit of *S. usambarensis*; I = ripe small fruit of *S. henningsii*; J = Leaves of *S. gerrardii*. Photo credits: images A, C-I by A. Adebowale; images B and J by A. Nicholas.

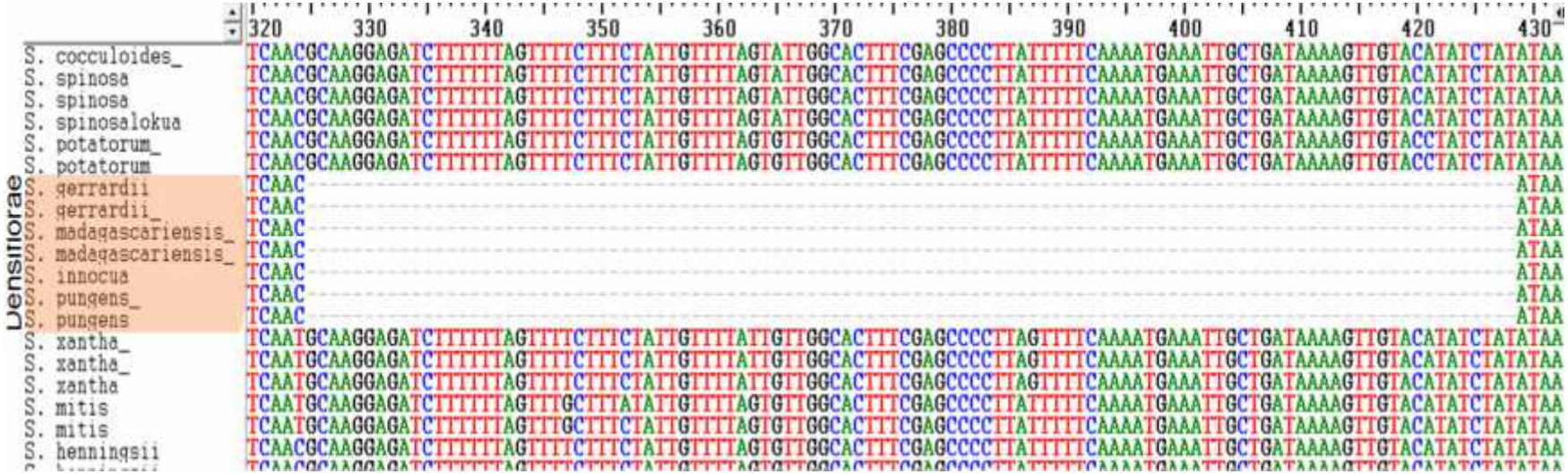
Densiflorae
320
330
340
350
360
370
380
390
400
410
420
430
S. cocculoides_
S. spinosa
S. spinosa
S. spinosalokua
S. potatorum_
S. potatorum
S. gerrardii
S. gerrardii_
S. madagascariensis_
S. madagascariensis_
S. innocua
S. pungens_
S. pungens
S. xantha_
S. xantha_
S. xantha
S. mitis
S. mitis
S. henningsii

Figure 5.1

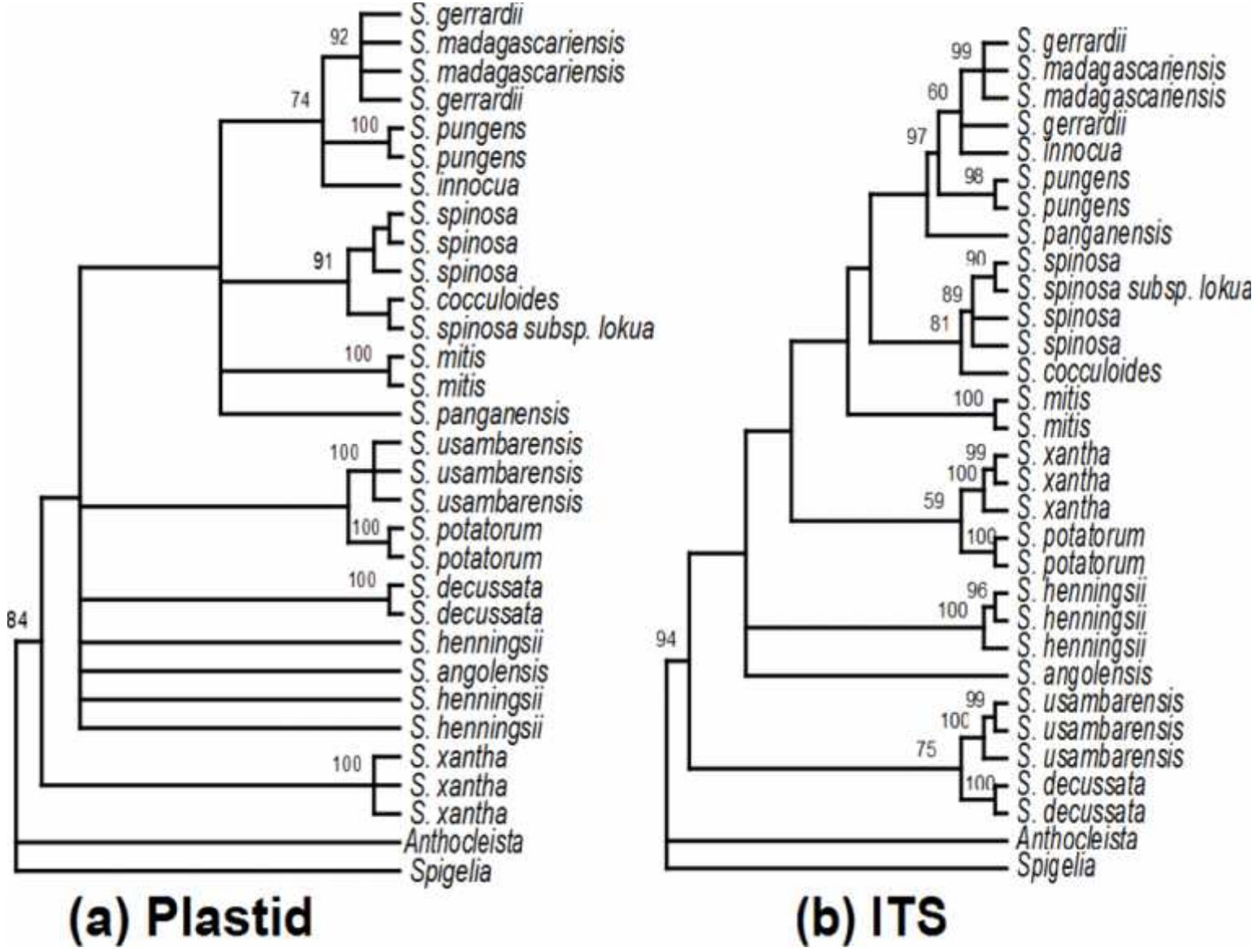
S. gerrardii
S. madagascariensis
S. madagascariensis
S. gerrardii
S. pungens
S. pungens
S. innocua
S. spinosa
S. spinosa
S. spinosa
S. cocculoides
S. spinosa subsp. lokua
S. mitis
S. mitis
S. panganensis
S. usambarensis
S. usambarensis
S. usambarensis
S. potatorum
S. potatorum
S. decussata
S. decussata
S. henningsii
S. angolensis
S. henningsii
S. henningsii
S. xantha
S. xantha
S. xantha
Anthocleista
Spigelia
92
74
100
91
100
100
100
84
100
(a) Plastid
S. gerrardii
S. madagascariensis
S. madagascariensis
S. gerrardii
S. innocua
S. pungens
S. pungens
S. panganensis
S. spinosa
S. spinosa subsp. lokua
S. spinosa
S. spinosa
S. cocculoides
S. mitis
S. mitis
S. xantha
S. xantha
S. xantha
S. potatorum
S. potatorum
S. henningsii
S. henningsii
S. henningsii
S. angolensis
S. usambarensis
S. usambarensis
S. usambarensis
S. decussata
S. decussata
Anthocleista
Spigelia
99
60
97
98
90
89
81
100
99
100
59
100
96
100
94
99
100
75
100
(b) ITS

Figure 5.2

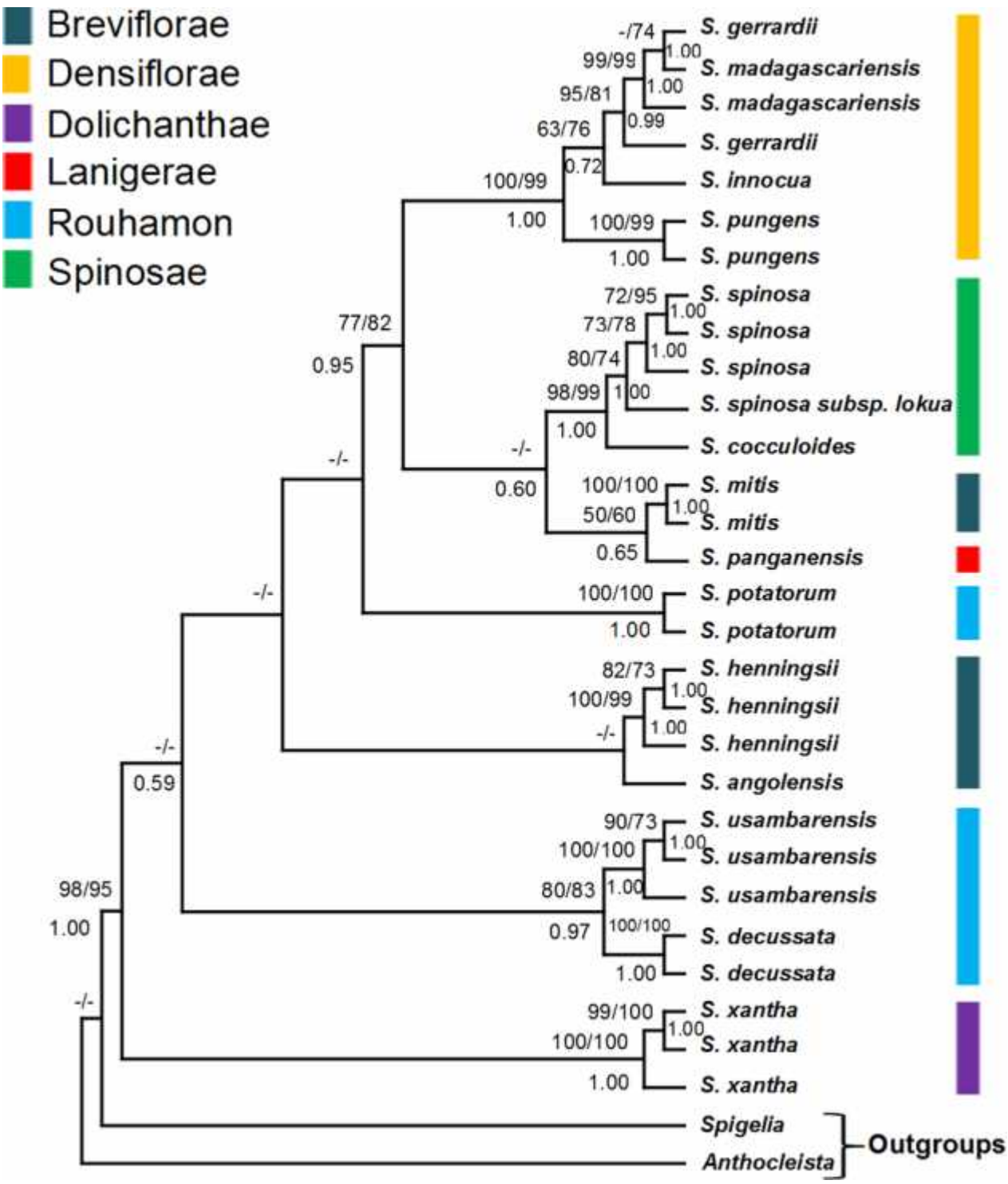
Breviflorae
Densiflorae
Dolichanthae
Lanigerae
Rouhamon
Spinosae
S. gerrardii
S. madagascariensis
S. madagascariensis
S. gerrardii
S. innocua
S. pungens
S. pungens
S. spinosa
S. spinosa
S. spinosa
S. spinosa subsp. lokua
S. cocculoides
S. mitis
S. mitis
S. panganensis
S. potatorum
S. potatorum
S. henningsii
S. henningsii
S. henningsii
S. angolensis
S. usambarensis
S. usambarensis
S. usambarensis
S. decussata
S. decussata
S. xantha
S. xantha
S. xantha
Spigelia
Anthocleista
Outgroups

Figure 5.3

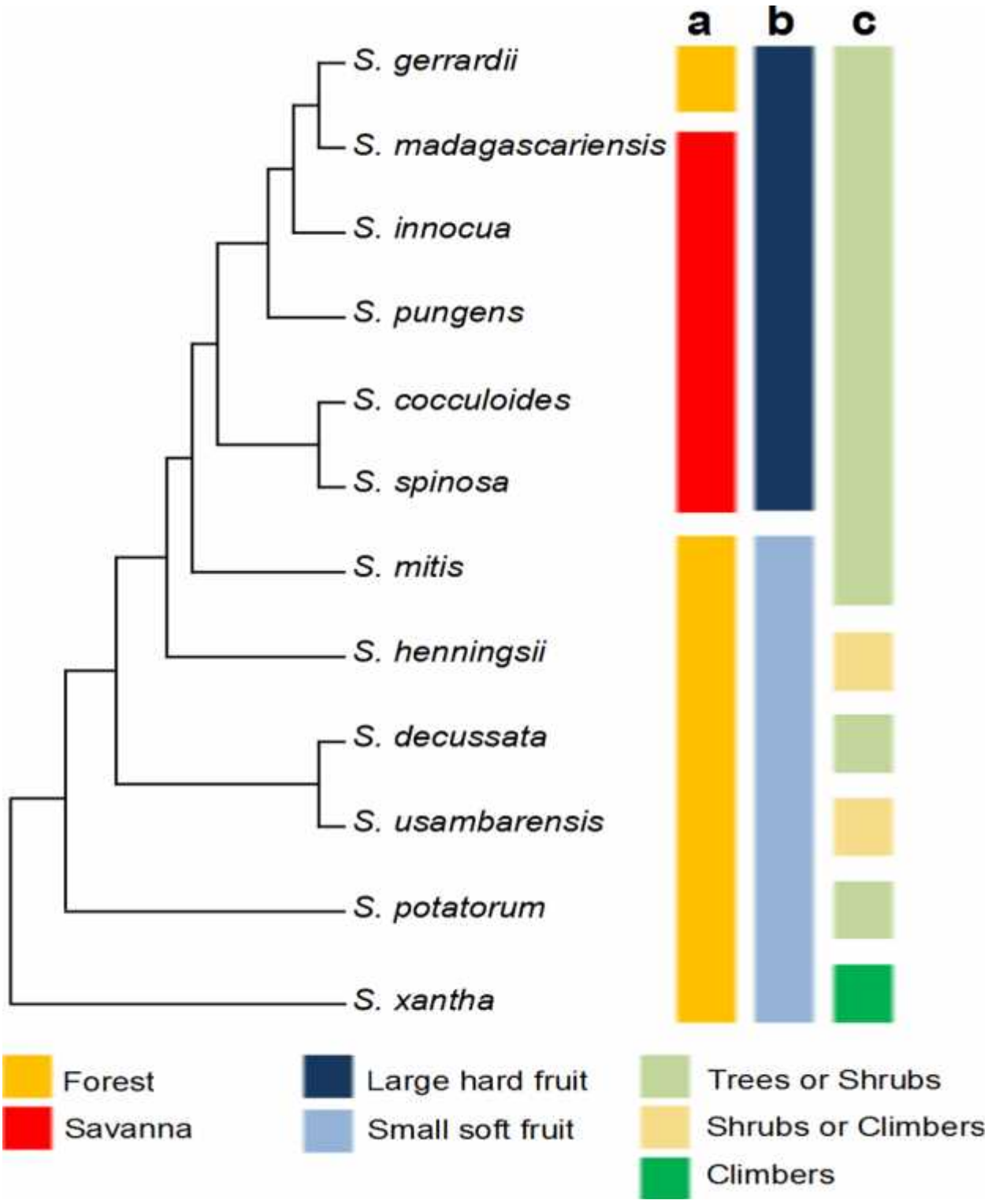
a
b
c
S. gerrardii
S. madagascariensis
S. innocua
S. pungens
S. cocculoides
S. spinosa
S. mitis
S. henningsii
S. decussata
S. usambarensis
S. potatorum
S. xantha
Forest
Savanna
Large hard fruit
Small soft fruit
Trees or Shrubs
Shrubs or Climbers
Climbers

Figure 5.4

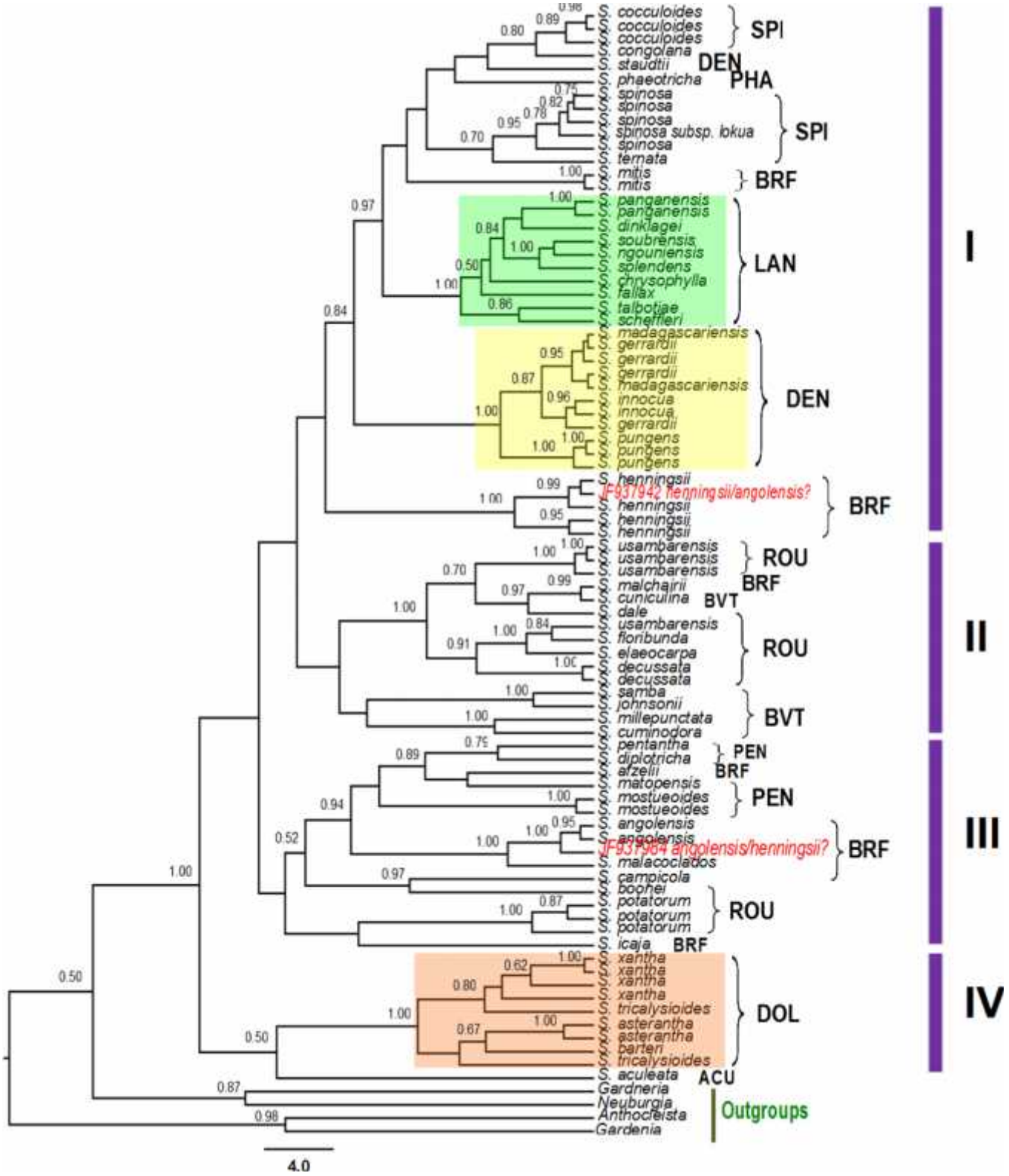
S. cocculoides
S. cocculoides
S. cocculoides
S. congolana
S. staudtii
S. phaeotricha
S. spinosa
S. spinosa
S. spinosa
S. spinosa subsp. lokua
S. spinosa
S. ternata
S. mitis
S. mitis
S. panganensis
S. panganensis
S. dinklagei
S. soubrensis
S. rigouriensis
S. splendens
S. chrysophylla
S. fallax
S. talbotiae
S. scheffleri
S. madagascariensis
S. gerrardii
S. gerrardii
S. gerrardii
S. madagascariensis
S. innocua
S. innocua
S. gerrardii
S. pungens
S. pungens
S. pungens
S. henningsii
JF931942 henningsii/angolensis?
S. henningsii
S. henningsii
S. henningsii
S. usambarensis
S. usambarensis
S. usambarensis
S. malchairii
S. cuniculina
S. dale
S. usambarensis
S. floribunda
S. elaeocarpa
S. decussata
S. decussata
S. samba
S. johnsonii
S. millepunctata
S. cuminodora
S. pentantha
S. diplotricha
S. afzelii
S. matopensis
S. mostueoides
S. mostueoides
S. angolensis
S. angolensis
JF931964 angolensis/henningsii?
S. malacoclados
S. campicola
S. boonei
S. potatorum
S. potatorum
S. potatorum
S. icaja
S. xantha
S. xantha
S. xantha
S. tricalysioides
S. asterantha
S. asterantha
S. barteri
S. tricalysioides
S. aculeata
Gardneria
Neuburgia
Anthocleista
Gardenia
SPI
DEN
PHA
SPI
BRF
LAN
DEN
BRF
ROU
BRF
BVT
ROU
BVT
PEN
BRF
PEN
BRF
ROU
BRF
DOL
ACU
Outgroups
I
II
III
IV
4.0

Figure 5.5

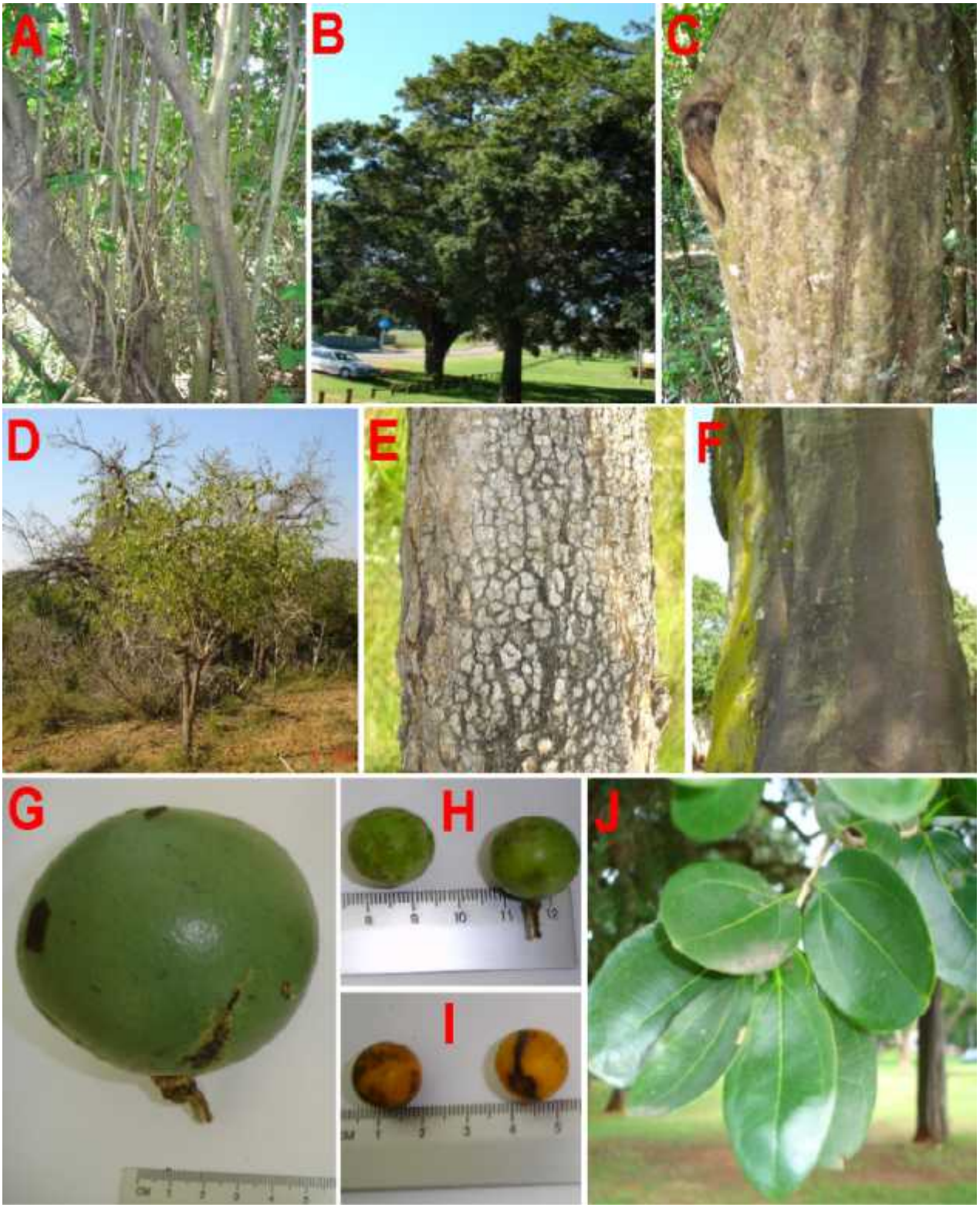

Figure 5.6

CHAPTER 6

DIVERGENCE TIMES ESTIMATES AND HISTORICAL BIOGEOGRAPHY OF SOUTHERN AFRICAN *STRYCHNOS* (LOGANIACEAE)

ABSTRACT

An understanding of earth's geologic past can not only offer insights into the factors influencing the present spatial distribution of organisms, but also proffer plausible explanations for some of the adaptive features accumulated among a given group of closely related taxa over time. Here we investigate divergence times among southern African *Strychnos* using nucleotide sequence data from the internal transcribed spacer of the ribosomal DNA. We also infer ancestral range of 15 southern African *Strychnos* species based on their present day spatial distribution. Bayesian estimates of divergence times implemented in BEAST indicate a middle Miocene origin for SA *Strychnos* at 12.72 myr. This was followed by eight radiations in the late Miocene. The two radiations resulting in the evolution of arid-adaptedness occurred in the late Pliocene-early-Pleistocene epoch. Biogeographic reconstructions with S-DIVA, Bayesian Binary MCMC and DEC suggest that within the context of our analysis, the most recent common ancestors of southern African *Strychnos* occupied the deciduous woodlands of tropical Africa and that dispersal is the major force behind current distribution. The basal species in our reconstructed tree is consistent with the rain forest origin of *Strychnos* along the Guineo-Congolian/South American axis. We argue that global palaeo-climatic oscillations have played a significant role in the evolution of *Strychnos* and specifically that the evolution of arid-adaptation among SA

Strychnos is recent and coincides with periodic and progressive increase in aridity during the Pliocene-Pleistocene epoch. *Strychnos gerrardii* displayed a peculiar distribution pattern by its restriction to the forest, while possessing many arid-adapted traits found in its savanna congeners. This distribution is postulated to be a consequence of vicariant events that happened late in the Pleistocene; events rooted in the repeated cycle of range expansions and contractions of its ancestors that led to its being left in its coastal refugia, while still retaining ancient signature attributes of arid adaptation. Our findings have implications for biodiversity and conservation of *Strychnos* in the face of human-mediated climate change.

Keywords: divergence times estimation; arid adaptation; allopatric speciation; palaeoclimatic oscillations; extinction; relaxed clock; dispersal-vicariance events; BEAST; RASP/S-DIVA

INTRODUCTION

Current distribution of plants and animals, more often than not, is a good reflection of past evolutionary and climatic events. With increasing knowledge about the earth's geologic past comes a better understanding of the processes that have shaped the evolution of life. The ease with which high quality molecular datasets are being generated for reconstructing phylogenies across various lineages has meant that hitherto theoretical conjectures about the history of life may now be tested and the outcome compared with empirical observations. An area that has played a key role in this historical reconstruction effort is the development of powerful computational tools for estimating the ages of diverging lineages within a

phylogenetic context. Although still a relatively young field, it has opened up, among other possibilities, avenues for proposing biogeographic hypotheses and relating the divergence of lineages to climatic changes (Tremetsberger *et al.* 2013).

In estimating divergence times, the application of relaxed clock models (Sanderson, 2002; Drummond and Suchard, 2010), which allows variation in molecular substitution rates across lineages, has been much favoured over the strict clock-like model proposed by Zuckerkandl and Pauling (1962). Relaxed clock models fit most empirical data better (Drummond *et al.* 2006) and appear to be more ubiquitous across most groups of organisms. Some variations of the relaxed model such as Bayesian parametric methods offer opportunities to investigate some of the underlying assumptions of phylogeny reconstructions and evolutionary rate changes over time (Lepage *et al.* 2007). This framework is particularly useful for handling the inevitable uncertainties associated with fossil calibration (Yang and Rannala, 2006) and thus has enabled the combination of information from DNA sequences and available fossils to produce more robust estimates of divergence times among organisms.

Recent developments in historical biogeographic analysis have witnessed integration of dated phylogenies and the distributional range of extant taxa with a view to extrapolating their ancestral range. This approach has paved the way for mapping specific divergence events onto the geological timescale for better evaluation of possible associations between certain geological and cladogenetic events. By relating past events with current distribution patterns, it has also

proved useful in predicting potential impacts of climate change on species distribution under possible future scenarios (Franklin *et al.* 2013), a particularly relevant undertaking for conservation planning.

Of the many analytical methods of historical biogeography in use (for reviews see Crisci *et al.* 2003; Lamm and Redelings, 2009), the dispersal-vicariance approach by Ronquist (1997) implemented in DIVA is by far the most popular. Although some studies (e.g. Donoghue and Moore, 2003; Kodandaramaiah, 2010) have highlighted potential sources of error in the current implementation of DIVA, other authors have developed algorithms to address these issues (Yu *et al.* 2012). One main drawback of DIVA is its sensitivity to the maximum number of ancestral areas required for optimization, which could default to vicariance inference where there is none. Other drawbacks include sensitivity to the exclusion of outgroup taxa, coupled with the inability to distinguish between range expansion and across-barrier dispersal. These limitations have been somewhat addressed in more recent reincarnation of the program. Prominent among them is the dispersal-extinction-cladogenesis (DEC) model of Ree and Smith (2008), implemented in LAGRANGE, which incorporates explicit models of anagenetic as well as cladogenetic changes in geographic range. Yu *et al.* (2010) developed a complementary statistical approach to DIVA called S-DIVA to provide statistical support for ancestral range estimation and more recently Yu *et al.* (2012) have developed an umbrella program RASP (Reconstruct Ancestral State in Phylogenies) to implement LAGRANGE's DEC model, Bayesian Binary MCMC (BBM) and S-DIVA within a user-friendly environment. All of these developments, coupled with sophisticated computational performance in molecular phylogenetics,

have opened up new vistas for formulating and testing competing hypotheses about the spatio-temporal histories of species.

The distribution pattern of southern African *Strychnos* presents unique opportunity to apply biogeographic principles alongside divergence time estimates to investigate historical cause of distribution along two ecological zones such as forest and savanna habitats. General morphological observations suggest that on average, savanna-inhabiting *Strychnos* differ from their forest dwelling congeners by the accumulation of a suite of attributes normally associated with arid adaptation (Leeuwenberg, 1969; Adebowale *et al.* 2014). To complicate the biogeographic landscape, however, *S. gerrardii*, a taxon whose taxonomy has been much debated (Verdoorn, 1963; Leeuwenberg, 1969; Adebowale *et al.* 2012), and that displays all of the attributes of arid-adapted *Strychnos*, has its distribution restricted to the east coastal forests of southern Africa.

The objectives of this study are therefore twofold. One is to provide divergence time estimates for southern African *Strychnos* and the other is to infer the ancestral range of southern African *Strychnos* with a view to unravelling possible causes for the 'discordant' distribution pattern of *S. gerrardii*.

MATERIALS AND METHODS

TAXON SAMPLING

Fifteen taxa representing southern Africa *Strychnos* were sampled. This number is a departure from the nine species recognised by Verdoorn (1963), as we

expanded the definition to include more tropical elements from a wider geographic coverage. We designated *Gardenia hansemannii* K. Schum. FM204691 and *Morinda villosa* Hook. f. AB715225 both in the Rubiaceae, and *Anthocleista grandiflora* L. DQ449916 in the Gentianaceae as outgroups. The first two taxa were included for the purposes of divergence dating, as there are virtually no accepted fossils for Loganiaceae. The *Strychnos* ITS2 dataset used in this study was extracted from those generated from an earlier molecular systematics enquiry into southern African *Strychnos* where voucher information and GenBank accession numbers are already provided (Chapter 5). The ITS2 region has been widely used for species circumscription and is one of the recommended supplementary land plant DNA barcode markers in situations where *mat*K and *rbc*L prove unsuitable (Fazekas *et al.* 2012). In certain cases, the sole use of the marker has proved efficient and economical relative to either the entire ITS region or a combination of plastid markers (Han *et al.* 2013).A preliminary survey of *Strychnos* showed that the ITS region has more potentially informative sites than the most widely used plastid markers (Frasier, 2008; Chapter 5).

PHYLOGENETIC AND DIVERGENCE TIMES ANALYSIS

Phylogenetic relationships and divergences times among the various *Strychnos* taxa were simultaneously estimated by Bayesian inference analysis as implemented in BEAST v2.0.1 (Drummond and Rambaut, 2007) and its associated software. Data were prepared in BEAUti v1.6.2 before analysis, which was then performed under a relaxed clock, GTR base substitution model proposed by jModelTest (Darriba *et al.* 2012) using a calibrated Yule speciation process tree prior. All other settings were retained at default recommendations. The Markov

chain Monte Carlo (MCMC) process was run for 10 million generations with tree and associated parameters sampled every 500th generation to produce a total of 20001 trees. Using TreeAnnotator v 1.6.2, the first 4000 trees were discarded as burn-in and the remaining 16001 trees summarised into a maximum clade credibility tree of mean node heights. The final tree was graphed in FigTree v 1.3.1 (Rambaut, 2006).

FOSSIL CALIBRATION

Since this is a relatively small phylogeny and there are no accepted fossil for any of the ingroup taxa, we elected to impose only one calibration point. Using several calibration points in this case would involve incorporating more outgroup taxa from other families. This may not necessarily improve the accuracy of the dating outcome for the ingroup, but may instead lead to crowding the fossil saturated clade of the resulting phylogeny (Frasier, 2008). Rubiaceae is one of the closest families to the Loganiaceae for which there are a number of reliable fossils. Fossil pollen of *Triporotetradites nachterstedtensis* from the upper Eocene in Germany is related to extant *Gardenia* and is perhaps the oldest accepted *Gardenia* fossil reported to date (Krutzsch, 1970; Muller, 1981). Although leaf impressions of *Strychnos*-like fossils has been reported from the tertiary (Berry, 1938), usage of such macrofossils for calibration is not advisable, because leaf impressions have often been erroneously assigned to a wide variety of taxonomic groups; according to some authors, this renders them unreliable when used as sole source of taxonomic placement (Crane *et al.* 2004). In our view, the same may be said for many pollen fossils. For calibration therefore, we applied the principle of minimum fossil age (Crepet *et al.* 2004; Gandolfo *et al.* 2008) by using the minimum age of

the oldest *Gardenia* pollen fossil. We constrained *Gardenia* and *Morinda* into a monophyletic group, since they both belong to Rubiaceae. We assigned their split a mean age of 41.7 myr with a standard deviation of 4 myr under a normal distribution to give a minimum age of 33.9 myr consistent with fossil evidence and a maximum age of 49.5 myr. Our interpretation of Geologic Time Scale follows Gradstein *et al.* (2012).

SPECIES DISTRIBUTIONS AND BIOGEOGRAPHIC AREAS

There is extensive distribution information available for African *Strychnos* species. We mostly used species distributions published by Leeuwenberg (1969), augmented with more recent georeference information compiled from the Global Biodiversity Information Facility (GBIF) portal (accessed on 10 Jan. 2014) and two herbaria (National Herbarium, Pretoria and the Royal Botanic Gardens Herbarium, Kew). We also added data points created from field observations conducted by the first author. Because of the small number of taxa included, we were able to refine the records by excluding those of uncertain identity and questionable distribution.

Based on the distribution pattern observed, biogeographical regions were defined along the major biomes in sub-Saharan Africa. Consequently four main biogeographic regions were delimited according to the predominant vegetation of the area, which corresponded largely to the current African vegetation map of Mayaux *et al.* (2004). These broadly defined areas are (A) Guineo-Congolian rain forest; (B) Tropical African deciduous woodland; (C) Southern African grassland;

(D) Southern African coastal forest. Terminal taxa were thus coded as occurring in one or more of these four areas.

BIOGEOGRAPHICAL ANALYSIS

The biogeographic history of southern African *Strychnos* was reconstructed by implementing three alternative approaches in RASP v2.1b: Statistical Dispersal-Vicariance (S-DIVA), Bayesian binary MCMC (BBM) and LAGRANGE's Dispersal-Extinction-Cladogenesis (DEC) (Nylander *et al.* 2008; Yu *et al.* 2010; *Yu et al.* 2012; Ree and Smith, 2008). We set the maximum number of ancestral areas to four consistent with our data. However, to minimise bias towards a vicariance default (resulting from having a high number of ancestral areas), a second set of analyses was performed with constraints to limit the number of ancestral areas to two for S-DIVA and one for BBM and DEC. S-DIVA is a parsimony method that identifies events and their associated costs. By averaging the frequencies of ancestral ranges at a node across trees and incorporating information on alternative ancestral ranges, S-DIVA is able to handle uncertainties relating to phylogenies and ancestral area (Yu *et al.* 2010). The other two methods offer greater flexibility in their usage of parametric statistics to specify explicit models for hypothesis testing (Ronquist and Sanmartin, 2011). Specifically, BBM analysis was run with 10 chains for 5 million generations under the F81 + Γ model, with sampling every 500 generations and discarding the first 1000 trees as burn-in. The maximum clade credibility tree derived from BEAST analysis along with the coded distribution data were used for our biogeographic reconstructions in RASP.

RESULTS

PHYLOGENY AND DIVERGENCE TIMES IN SOUTHERN AFRICAN *STRYCHNOS*

The ITS2 alignment from which the phylogeny was reconstructed comprised 402 characters. The BEAST tree was well-resolved with variable degrees of robustness across the clades. The clades corresponding to sections *Densiflorae* and *Spinosae* were strongly supported (pp=1.00 and 0.99, respectively) and the monophyly of *Strychnos* was maximally supported. The basal placement of *S. potatorum*/*S. xantha* clade as sister to the other southern African species was also maximally supported.

Divergence times estimates suggest that the most recent common ancestor (MRCA) of SA *Strychnos* radiated in the mid Miocene with a crown age of 12.72 myr [95% highest posterior density (HPD): 7.14 - 18.05 myr]. Between the late Miocene and early Pliocene, most of the radiation leading to extant taxa within this group had been completed. Two waves of radiation occurred during the late Pliocene - early Pleistocene epochs giving rise to two ecologically important clades, which possessed a unique set of arid-adaptive features in contrast to their preceding forest-dwelling sisters. The first, with a mean age of 3.32 myr (95% HPD: 1.20-5.70 myr), gave rise to *S. cocculoides* and *S. spinosa* in section *Spinosae*, while the other, with an estimated age of 2.46 myr (95% HPD: 0.9-4.0 myr), resulted in four species, all belonging to section *Densiflorae* (Figure 6.1). The most recent split was between *S. gerrardii* and *S. madagascariensis*, which occurred in the late Pleistocene at about 0.17 myr (95% HPD: 0.07-0.82 myr) (Figure 6.1).

HISTORICAL BIOGEOGRAPHY

Ancestral area reconstruction inferred by S-DIVA for various nodes is presented in Figure 6.2. The reconstruction indicates that dispersal has played a predominant role in the present day distribution of SA *Strychnos* species. A minimum of 25 dispersal events and only one vicariance event are postulated to have occurred when the maximum ancestral area was set to four. The number of dispersal and vicariance events remained the same regardless of the number of ancestral areas used for optimizing these analyses. When a smaller number of areas was used (two) however, our analysis recovered an extinction event at node 35 among the outgroup taxa. S-DIVA results suggest that the ancestral area of SA *Strychnos* was the tropical African woodland (Figure 6.2), with a frequency of occurrence of this range being 100% in all but node 21. Nodes 23 and 26 represent SA members of sections *Densiflorae* and *Spinosae*, respectively. From a distribution and abundance points of view, these are the two most successful groups among SA *Strychnos*. According to our analysis, the only vicariance event occurred at node 21, leading to *S. madagascariensis* and *S. gerrardii*. This observation is particularly intriguing, as these taxa have been the subject of a long-standing taxonomic quagmire recently addressed by Adebowale *et al.* (2012), who proposed that they be recognised as distinct species. S-DIVA results presented two competing hypotheses to explain the observed distribution of these two species. The four-ancestral-area assumption favoured a direct vicariance event with no dispersal and postulated a widely distributed ancestor whose range encompassed the deciduous woodlands of tropical Africa through SA grasslands to SA coastal forests prior to the split of the coastal forests from the other two regions. An alternative and equally valid hypothesis under the two-ancestral-area

assumption invokes both dispersal and vicariance as equally probable in explaining the distribution pattern of the two taxa. In this view, the ancestor of *S. gerrardii* and *S. madagascariensis* (node 21) occupied the tropical African woodlands and SA coastal forests prior to range expansion via dispersal to SA grasslands. Subsequently, there was a range contraction and break-up into the present day pattern. Divergence time estimates suggest that this-break up was very recent, occurring late in the Pleistocene.

BBM and DEC analyses presented event histories comparable to S-DIVA, but with a higher frequency of dispersals (30 or 31 as opposed to 26 in S-DIVA) depending on the maximum ancestral area allowed. Both also recovered a vicariance event at node 21, but only DEC detected evidence of extinction within the *Strychnos* lineage for the common ancestor of *S. potatorum* and *S. xantha* at node 20. Whereas S-DIVA indicated a single ancestral range for all but one *Strychnos* clade, DEC and BBM presented a number of possible ancestral ranges with the probability of occurrence at each area graphically depicted at nodes by proportional pie charts (Figure 6.3). DEC estimated fewer combined ancestral areas than BBM and S-DIVA. Furthermore, the MRCA of SA *Strychnos* at node 33 is inferred to have originated in area B (tropical African deciduous woodlands) according to S-DIVA. DEC analysis, however, suggests a higher likelihood of widespread origin encompassing the four geographical areas, while BBM indicates an 85% likelihood of origin in the tropical African deciduous woodlands (B) and 15% in a larger area incorporating B with the southern African coastal forest (BD).

DISCUSSION

UNCERTAINTIES IN DIVERGENCE TIME ESTIMATES

Two major factors stand out as being capable of substantially affecting the outcome of divergence time estimates. The first and apparently more important is the reliability and correct placement of fossil taxa used for calibrating a phylogeny. The second is related to model choice and statistical distribution of *a priori* node calibration. Although using more parameterized substitution models has been proposed as a means to compensate for lack of multiple calibration points (Schenk and Hufford, 2010), specifying a more complex model showed no significant discrepancies in estimated times when we used the more parameterized GTR + Γ + I model (results not shown). This is probably due to the small size, high resolution and relatively robust nature of the phylogeny.

None of the reported fossils for *Strychnos* was considered reliable once we applied the criteria of Muller (1981) and Martínez-Millán (2010), which preferred the use of pollen fossils as opposed to macrofossils due to their abundance, continuous nature in sedimentary deposits and crucially greater reliability for determining the time of first appearance of a plant group (Blackmore, 2007; Thornhill *et al.* 2012). Ironically, there are equally valid arguments in favour of macrofossils that call into question the reluctance of some to use them for calibration. Macrofossils such as leaves, cones and fruits are more complex than pollen and thus offer more features with which to confidently place them on an evolutionary tree (Thornhill *et al.* 2012). Interestingly though, our *Gardenia*-calibrated age estimates of mid-Miocene origin for *Strychnos* accord well with reported cases of *Strychnos* macrofossils. Ettingshausen (1868: 214, cited in

Berry, 1938) reported a *Strychnos* fossil based on a leaf specimen from Bohemia (present day Czech Republic) in the late Miocene, and Berry (1938) reported a Miocene fossil described as *Strychnos patagonica* from leaf impressions found north of Rio Chubut in Patagonia, Argentina. In an earlier work, Berry (1925) provided detailed descriptions of a fossil of *Strychnos mirhojana* from leaf impressions dated to the Miocene. While estimating ages of events for which the evidence is at best inferential will inevitably carry some level of uncertainly, any independent arrival at a similar conclusion from multiple sources lends an added layer of confidence to the outcome.

PLIOCENE-PLEISTOCENE CONDITIONS AND THE EVOLUTION OF ARID-ADAPTED *STRYCHNOS*

A clear trend in our results has been the rapid diversification during the late Pliocene-early Pleistocene of *Strychnos* species adapted to arid environments. Various stages of Miocene-Pliocene-Pleistocene have been characterised as times of cyclically increasing aridity in most parts of the world, including Africa (Bobe and Eck, 2001; Ghinassi *et al.* 2004; Sciscio *et al.* 2013). Although the precise mechanisms responsible for such increase are still the subject of critical enquiry, there is some consensus to suggest that increasing CO_2 levels may have played a major role (Herold *et al.* 2011). Some authors (e.g. Henrot *et al.* 2010) have ascribed an even greater role to changes in sea surface conditions during this period. Climatic changes during this epoch resulted in an increase in global temperature to a level estimated to be about 2 – 3^{o}C higher than present day figures (Haywood and Valdes, 2004). Another important attribute of these palaeo-times, relevant for our context, is the periodic oscillations of conditions between glacial and interglacial cycles.

Oscillations of palaeoclimatic conditions have been potential drivers of speciation in plants and animals, as has been shown by several studies (Rozzi *et al.* 1999; Montgelard and Matthee, 2012; Ikeda *et al.* 2012). The timing of divergent events in arid-adapted *Strychnos* coincide with the cyclical intervals and increased aridity (DeMenocal, 2004), thus suggesting that similar climatic oscillations might have mediated the Pliocene-Pleistocene speciation process in *Strychnos*. Arid-adapted *Strychnos* of southern Africa differ from their forest-adapted congeners essentially by having large hard fruits, tough corky barks (Chapter 5) and a host of other anatomical features that have aided other plant species to cope in similarly dry environments (Gashaw *et al.* 2002; Adebowale *et al.* 2014). It is conceivable from evolutionary dynamics and biology of forest tree species that following abrupt aridification of their habitat, there would have been an initial population decline as a consequence of poor adaptation. Depending on the extent of and tolerance to the arid conditions, such decline may reach a tipping point with a real possibility for local extinctions. Although S-DIVA did not detect extinction within the *Strychnos* lineage, DEC analysis postulated an extinction event leading to *S. potatorum-S. xantha* at node 20 (Figure 6.3A). This may be significant, as the divergence time for this node is in the late Miocene - a time associated with a number of extinctions (Raup and Sepkoski Jr., 1984; Lewis *et al.* 2008). Historical evidence also supports the plausibility of other extinction scenarios during the Pliocene-Pleistocene among other groups during the same period (Chapman *et al.* 1998; Hayward, 2002; Smith and Roy, 2006). This could potentially explain the time gap of about 2 myr between the most derived forest taxa and the earliest diverging arid-adapted clade (Figure 6.1).

ANCESTRAL RANGE AND POTENTIAL MIGRATION ROUTE OF PRESENT DAY SOUTHERN AFRICAN *STRYCHNOS*

A number of biogeographical conclusions may be drawn from the results of our study. The ancestors of southern African *Strychnos* are postulated to have had a range in tropical African deciduous woodlands (B). This area may not be regarded, in strict terms, as a savanna biome. Indeed, African palaeo-vegetation reconstruction suggests that a considerable portion of this area was part of an extensive forest spanning the Guineo-Congolian rainforest (A) and a large part of Tropical African woodland (B) during the Eocene to mid-Miocene (Kissling *et al.* 2012). There was a reduction of this forested area during the Pliocene; a reduction that has continued unabated to the present day (Kissling *et al.* 2012). The implication is that the Guineo-Congolian forest (A) and much of Tropical African woodland (B) were part of a continuous forest during the Eocene-mid Miocene, the latter period of this time frame corresponding with the crown age of *Strychnos*. It is therefore reasonable to assume some earlier migration from the forest of Guinea-Congo to present day African woodland (B) and the grassland of southern Africa (C). In theory, such dispersal could have reached the coastal forests of southern Africa (D). By extension, the original ancestral range of *Strychnos* may include the Guineo-Congolian forests and the tropical woodlands of Africa. This proposition is consistent with the West-Central Africa/South America hypothesis about the origin of *Strychnos* (Frasier, 2008), by which the most basal clades in a global phylogenetic analysis originate from these two regions. Multiple dispersals from this range are corroborated by the preponderance of dispersal as a means of migration from their ancestral ‘home’ to current abode (Table 6.1).

Similar patterns of diversification from forest to arid habitats, during the Miocene, have been reported in related family, the Apocynaceae (Livschultz *et al.* 2011). These authors found climatic evidence suggesting a shift from rainforests to dry forests. The more derived subfamily Secamonoideae, occupies significantly drier habitats relative to its ancestral sister group, the Asclepiadoideae (Livschultz *et al.* 2011). Such a pattern is consistent with our findings, as the more derived southern African *Strychnos* species inhabit drier habitats compared to their ancestral, forest-dwelling congeners.

Strychnos gerrardii represents the only species whose current distribution pattern defied simple and solely dispersal-based explanation. The distribution of this species is postulated by all our biogeographic analyses to have been driven, at least in part, by a vicariance event either with or without accompanying dispersal events. In contrast to the forest-adapted SA *Strychnos* taxa and much in keeping with arid-adapted ones, *S. gerrardii* has large fruit, with very hard and thick pericarps and fairly rough bark (relative to the typical forest species) for reducing water loss and surviving potential wild fires. These features are at variance with its distribution as a coastal forest species and beg the obvious question as to how it acquired such a set of significant but 'non-adaptive' (from a forest point of view) traits. Parallel evolution of such a suite of complex characters is unlikely due to the short time since *S. gerrardii* diverged from its sister taxon *S. madagascariensis*. One parsimonious possibility suggested by our biogeographic results is that the most recent common ancestor (MRCA) of *S. gerrardii* and *S. madagascariensis* occupied a much wider area, encompassing the woodlands and the grasslands (B and C) during the mid-Pleistocene and had already inherited arid-adaptive traits

from its ancestors. However, by the late Pleistocene, habitat fragmentation created isolated populations of this ancestor, some of which migrated eastwards to the coastal forests of southern Africa and evolved to be *S. gerrardii*. The most likely source of habitat fragmentation during the period in question is climatic oscillations; these may have led to the expansion and retraction of forests, thus creating forest refugia of different sizes. However, this hypothesis of direct vicariance does not take into consideration the possibility of gene exchange that may blur evolutionary lines and presumes that such fragmentation occurred only once. While not completely ruling out this hypothesis, an alternative view that incorporates dispersal and vicariance appears more plausible. Palaeo-climatic evidence suggests several episodes of expansion and contraction of biomes (of forests in particular) (Couvreur *et al.* 2008; Smith *et al.* 2012). Given that *S. innocua*, the sister taxon to these two species, occupies both the woodlands and parts of the grasslands [areas B and C; Figure 6.2], it is plausible that the MRCA of *S. gerrardii* and *S. madagascariensis* dispersed into the coastal forests (D) from either the woodlands or grasslands during one of the several expansion/contraction cycles. The geographic isolation leading to a break up of either woodlands-coastal forest (BD) or grasslands-coastal forest (CD) was facilitated during other expansion/contraction cycles and led to *S. gerrardii* being caught up in the coastal forest habitat along the eastern part of southern Africa, while still retaining signature attributes of its recent arid ancestry. Similar distribution trends have been reported for a number of forest-dwelling plants (Couvreur *et al.* 2008; Byrne, 2008) and animals (Smith *et al.* 2012). Among the Annonaceae, Couvreur *et al.* (2008) found empirical evidence of repeated "*connection-isolation events between the East African and Guineo-Congolian*

forests" and predicted a process of evolutionary radiation via multiple vicariance events, similar to our proposal, for many African forest species.

POTENTIAL LIMITATIONS

A limitation of our study stems from spatial partitioning of contiguous and continuous habitat. It is well-known that most habitats present some degree of heterogeneity that renders any sweeping spatial classification, especially at the continental scale used in our study, somewhat arbitrary. Such limitations, though proportional to the biogeographic scale of the study, do not detract from the ability of this type of study to reveal general trends useful for conservation planning and decision making at the relevant scale. Another potential limitation is the sampling distribution of taxa. Since most of the geo-referenced data trace back to herbarium collections, sampling bias may have inadvertently influenced some of our results. It is not unlikely that there are *Strychnos* specimens on the boundaries of our defined geographical areas with potential to alter our interpretations. Such specimens may not have been sampled due to inaccessibility and other logistical reasons, including local population extinctions.

A final potential limitation of our study is the biogeographic analytical methods employed. Our results underscored two inherent tendencies of S-DIVA: i) underestimation of dispersal events, and ii) favouring an unrealistically extensive range for ancestors by incorporating all areas occupied by their daughter taxa (Drovetski, 2003). However, we do note that all three methods of analysis detected the all-important node 21 vicariant event. The use of various analytical approaches is recommended, as it allows alternate hypotheses to be constructed

using the same data. They also have the possibility of detecting other underlying processes outside the capability of any single method.

CONCLUSION AND IMPLICATION

Our results are the first attempts at estimating divergence times in *Strychnos* and indicate that southern African *Strychnos* originated in the mid-Miocene and has a distribution which was strongly influenced by palaeo-ecological events from the late Miocene to the late Pleistocene. This includes oscillating patterns of range expansions and contractions. Arid-adapted *Strychnos* taxa are more derived than their forest relatives and their evolution coincided with increased aridification during the late Pliocene. The general pattern is consistent with the forest origin of *Strychnos* in the Guineo-Congolian/South American axis and a subsequent migration via several dispersals to other parts of Africa. The most recent radiation that gave rise to *S. gerrardii* and *S. madagascariensis* is consistent with an allopatric speciation model closely linked with a combination of dispersal and vicariance events following repeated cycles of forest expansions and contractions. Our findings have implications for conservation planning. Habitat fragmentation, although generally undesirable in a stable system, can create refugia for the maintenance of local gene pools and aid short distance dispersal with the potential for future adaptive radiation under abrupt environmental change. The ability to project such changes and accurately model species responses to them is a continuing community effort that could help mitigate some of the inevitable consequences of climate change.

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Table 6.1: Node ages and biogeographic events for southern African *Strychnos*. Node numbers correspond to those in Figures 6.2 and 6.3.

Nodes	Estimated ages (myr)	95% HPD (myr)	No. of biogeographic events		
			Dispersal	Vicariance	Extinction
19	40.9	33.1-48.7	3	0	0
20	9.20	4.7-14.3	3	0	0 (1)**
21	0.17	0.07-0.82	0	1	0
21*	0.17	0.07-0.82	0	1	0
22	1.56	0.7-2.68	3	0	0
23	2.46	0.9-4.0	1	0	0
24	5.23	2.5-8.1	2	0	0
25	0.63	0.07-1.80	4	0	0
26	3.32	1.20-5.70	1	0	0
27	6.50	3.85-9.72	0	0	0
28	7.70	4.28-11.53	0	0	0
29	6.55	3.0-10.2	4	0	0
30	9.65	6.15-14.3	0	0	0
31	8.19	3.95-12.72	2	0	0
32	10.58	6.55-15.07	0	0	0
33	12.72	7.14-18.05	0	0	0
34	31.26	20.2-43.6	1	0	0
35	42.80	33.7-51.85	2	0	0

*analysis with maximum ancestral area set to 2 as opposed to 4. ** value in bracket is from DEC analysis

Figure legends

Figure 6.1: BEAST Bayesian inference phylogenetic tree showing divergence time estimates among southern African *Strychnos* species. Posterior probability values are shown above branches, divergence times are shown below the branches. Abbreviations: PLI=Pliocene; PLE=Pleistocene; HOL=Holocene.

Figure 6.2: Ancestral area reconstruction from S-DIVA (RASP) mapped onto *Strychnos* phylogeny tree topology derived from BEAST. Branch support values are the same as in Figure 1. Green star on node 21 indicates vicariance event. Pie charts at internal nodes represent marginal probabilities for each alternative ancestral area. Biogeographical regions: A=Guineo-Congolian rain forest; B=Tropical Africa deciduous woodland; C=Southern African grassland; D=Southern African coastal forest. Geographical area partitioning into A, B, C and D based on Mayaux *et al.* (2004) and Couvreur *et al.* (2008).

Figure 6.3: Ancestral range reconstructions by A=Dispersal-extinction-cladogenesis (DEC); and B=Bayesian binary MCMC (BBM) mapped on tree topology derived from BEAST analysis. Reconstruction optimized with a maximum ancestral area of 1. Green star on node 21 indicates vicariance event. Red square on node 20 indicates extinction event. Geographical areas are as in Figure 2. Alternative ancestral ranges at nodes are shown by pie charts. Colour key to possible ranges at different nodes; black with asterisk represent other ancestral ranges; Biogeographical regions: A=Guineo-Congolian rain forest; B=Tropical Africa deciduous woodland; C=Southern African grassland; D=Southern African coastal forest.

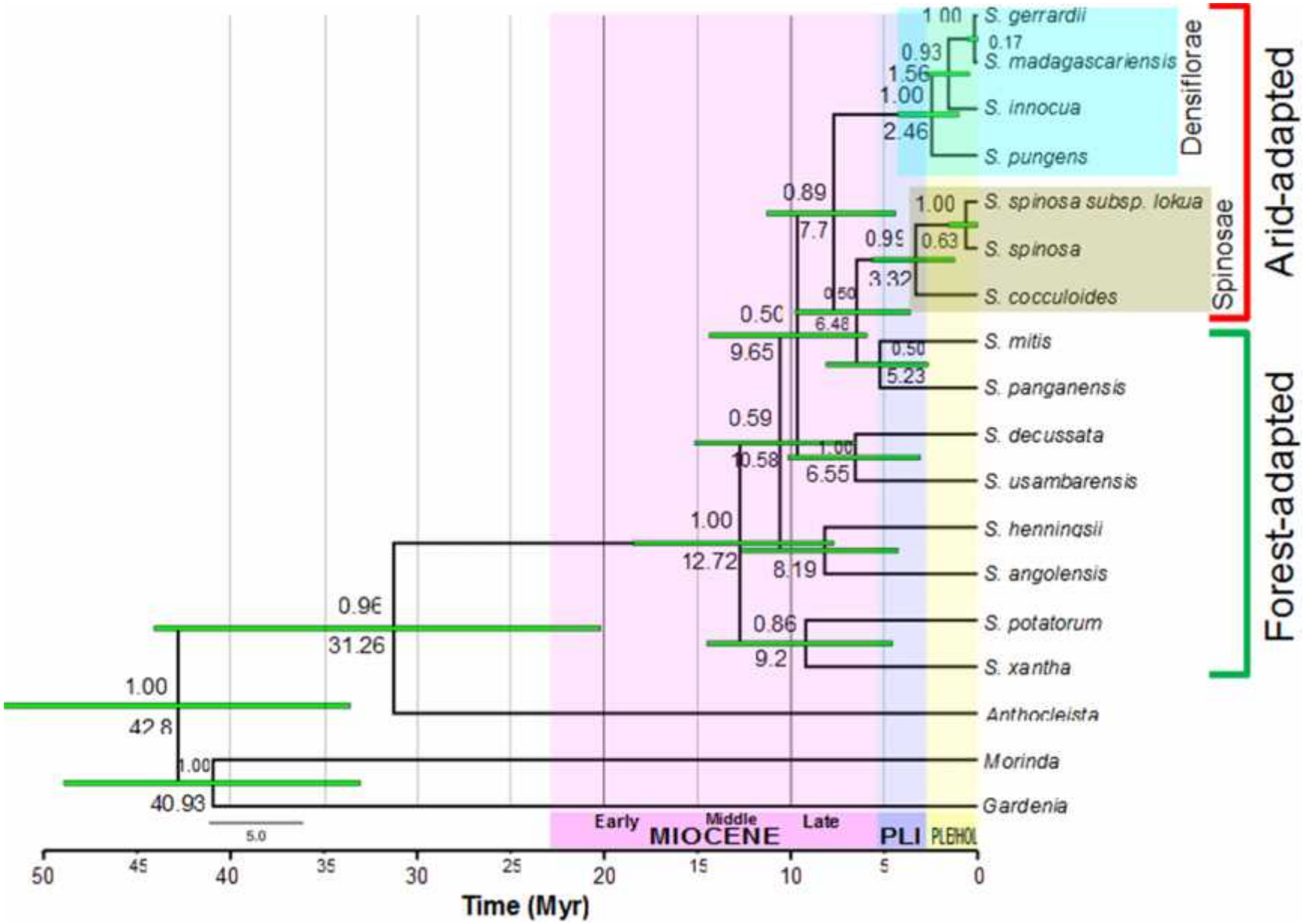
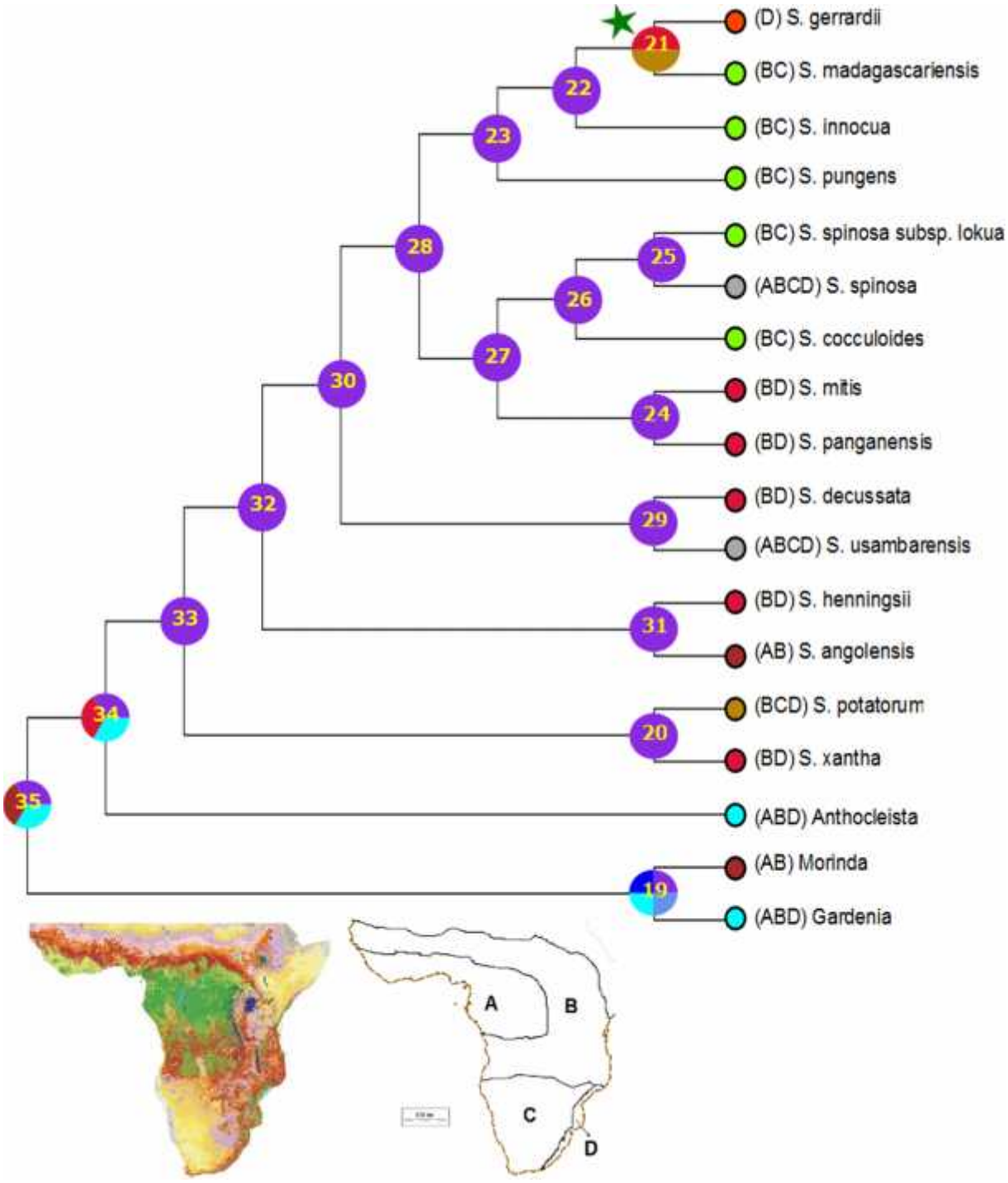

S. gerrardii
S. madagascariensis
S. innocua
S. pungens
S. spinosa subsp. lokua
S. spinosa
S. cocculoides
S. mitis
S. panganensis
S. decussata
S. usambarensis
S. henningsii
S. angolensis
S. potatorum
S. xantha
Anthocleista
Morinda
Gardenia
Densiflorae
Spinosae
Arid-adapted
Forest-adapted
Early
Middle
Late
MIOCENE
PLI
PLEHOL
Time (Myr)

Figure 6.1


(D) S. gerrardii
(BC) S. madagascariensis
(BC) S. innocua
(BC) S. pungens
(BC) S. spinosa subsp. lokua
(ABCD) S. spinosa
(BC) S. cocculoides
(BD) S. mitis
(BD) S. panganensis
(BD) S. decussata
(ABCD) S. usambarensis
(BD) S. henningsii
(AB) S. angolensis
(BCD) S. potatorum
(BD) S. xantha
(ABD) Anthocleista
(AB) Morinda
(ABD) Gardenia
21
22
23
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32
29
33
31
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35
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A
B
C
D

Figure 6.2

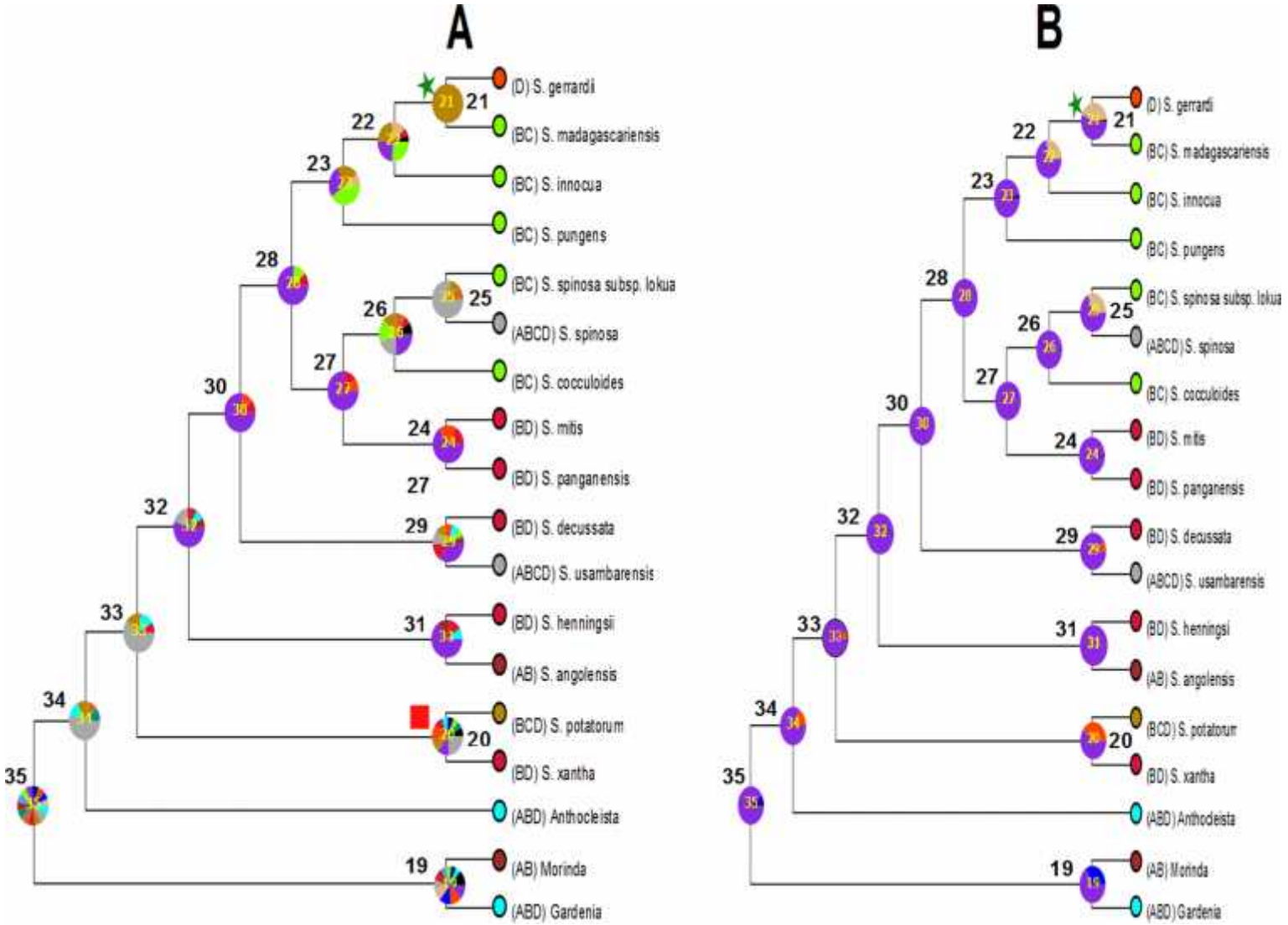
A
(D) S. gerrardi
(BC) S. madagascariensis
(BC) S. innocua
(BC) S. pungens
(BC) S. spinosa subsp. lokua
(ABCD) S. spinosa
(BC) S. cocculoides
(BD) S. mitis
(BD) S. panganensis
(BD) S. decussata
(ABCD) S. usambarensis
(BD) S. henningsii
(AB) S. angolensis
(BCD) S. potatorum
(BD) S. xantha
(ABD) Anthocleista
(AB) Morinda
(ABD) Gardenia
B
(D) S. gerrardi
(BC) S. madagascariensis
(BC) S. innocua
(BC) S. pungens
(BC) S. spinosa subsp. lokua
(ABCD) S. spinosa
(BC) S. cocculoides
(BD) S. mitis
(BD) S. panganensis
(BD) S. decussata
(ABCD) S. usambarensis
(BD) S. henningsi
(AB) S. angolensis
(BCD) S. potatorum
(BD) S. xantha
(ABD) Anthocleista
(AB) Morinda
(ABD) Gardenia

Figure 6.3

CHAPTER 7

A SYSTEMATIC AND TAXONOMIC STUDY OF SOUTHERN AFRICAN *STRYCHNOS* L. (LOGANIACEAE)

ABSTRACT

Strychnos L. is presented here in the context of its history within the Loganiaceae and the Gentianales. A taxonomic treatment of *Strychnos* in southern Africa is given without sectional classification. Eleven species are currently recognised in southern Africa, as *S. gerrardii* has been resurrected to specific status. Diagnostic taxonomic keys, synonyms, descriptions, distribution maps and botanical illustrations are provided for all recognised taxa.

INTRODUCTION

Strychnos is the largest genus of the Loganiaceae and the species are well represented across the tropics, with some reaching farther into the Southern Hemisphere. Infraspecific classifications have been contentious within the genus and recent evidence suggests that most of the currently recognised sections are not monophyletic. The aim of this treatment is to present an overview of the genus with a focus on the southern African members, using multiple sources of information. The taxonomic approach adopted is species level and not sectional level; such sectional classification requires more information than is currently available.

MATERIALS AND METHODS

Field surveys were conducted during different seasons in order to appraise the distribution and phenological patterns of *Strychnos* L. across most of its distribution range, especially in South Africa. National and international herbaria (Appendix 1) were consulted for the examination of type and other representative specimens for collection of additional distribution data and assessment of the extent of morphological diversity within the genus. Excluding live materials from the field, over 1200 herbarium specimens, including more than 30 type specimens of *Strychnos* loaned from 10 institutions, were studied. Where access to particular specimens was logistically prohibitive, a number of virtual herbaria were queried (A, MO, NY, U, US and WAG). Gross morphological and micro-morphological features of leaves were investigated to elucidate patterns of infra-generic groupings (Adebowale *et al.* 2014). Macro-morphology of flowers was also studied. Elliptic Fourier analysis was applied to extract useful leaf shape information from leaf outlines (Adebowale *et al.* 2012). This provided a quantitative basis for an otherwise qualitative attribute, in studying members of *Strychnos* section *Densiflorae*, thus reducing subjectivity inherent in the use of leaf shape attributes.

Phylogeny reconstructions of southern African as well as African species of *Strychnos* were performed using molecular data from DNA sequences of two plastid markers (*trn*L-*trn*F and *trn*S-*trn*G) and the nuclear ribosomal ITS region (Chapter 5). ITS2 secondary-structure molecular modelling was also applied to test species boundaries within the genus (Chapter 4).

TAXONOMIC HISTORY OF *STRYCHNOS* IN CONTEXT OF ITS FAMILY (LOGANIACEAE), ORDER (GENTIANALES) AND HIGHER RANKS

Although there has been little or no controversy regarding the identity of *Strychnos* as a genus, the family Loganiaceae and the order Gentianales, to which it belongs, have had a chequered taxonomic history. A historical overview of the hypothetical conceptions of the order and family proposed by a variety of taxonomic authorities follows here.

HISTORY OF GENTIANALES

Gentianales is a member of the Asteranae, a large informally recognised clade (Chase and Reveal, 2009) that comprises three superorders, the Asterids, Campanulids and Lamiids (APG III, 2009). The Lamiids, as currently circumscribed by APG III, have four crown orders including the Gentianales and the stem family Boraginaceae. The Gentianales were formally described by Lindley (1833) as an assemblage of plants with contorted floral aestivation to which the name Contortae had earlier been applied by Bartling (1830). Such a broad definition was bound to bring together taxa from very different evolutionary histories, as was demonstrated by Schnarf (1931), using only embryological evidence. Over the space of about 160 years (from the 1830s to 1990s), the composition of the order has altered with successive classification schemes. Predictably, this has also affected intra-ordinal concepts of relationships. Bentham and Hooker (1862 – 1883) effectively separated what is now known as Gentianales into two orders, Rubiales and Gentianales, and recognized six families within the latter order namely Oleaceae, Salvadoraceae, Loganiaceae, Gentianaceae, Apocynaceae and Asclepiadaceae. Possibly due to the high taxonomic value accorded to the presence of an inferior ovary and absence of

internal phloem, the Rubiaceae was considered a member of the order Rubiales along with Caprifoliaceae, Adoxaceae, Valerianaceae and Dipsacaceae. Harvey and Hooker (1868) adopted this system without modification in their arrangement of South African plants, thus keeping the families Rubiaceae and Loganiaceae (*sensu lato*) in the two different orders. This exclusion of the Rubiaceae from the Gentianales persisted well into the late 1950s when it was first included into the core Gentianales by Wagenitz (1959). Even then, one highly regarded publication of the 1980s (Cronquist, 1981) maintained this Rubiaceae-exclusion stance.

In Bessey's works (1897; 1915), which also were strongly influenced by the Bentham and Hooker system, the same six families were recognised as belonging to the Gentianales. Engler and Prantl's classification system (Engler and Prantl, 1887 – 1915) for Gentianales and Loganiaceae is not radically different from Bentham and Hooker's in its treatment of the order either. Their series 'contortae' was the taxonomic equivalent of Bentham and Hooker's Gentianales, with minor constitutional arrangements. Authors following Engler and Prantl's system (e.g. Knoblauch, 1892; Solereder, 1892) included essentially the same groups of taxa within Gentianales and Loganiaceae as Bentham and Hooker, with very minor exceptions.

An intriguing aspect to botanical taxonomy during the earlier part of its development as a scientific discipline was the general reluctance to openly question, or at least re-examine 'established' views. Perhaps it was a lack of adequate tools with which to conduct such re-examination from a different perspective, or it was the fear of reprisals (professional suicide being an obvious

example) should one be found in the wrong if leading authorities of the day are directly contradicted. In any event, taxonomists of the 20th century had access to better facilities, new techniques and more sophisticated equipment with which to empirically interrogate their subjects and minimise subjectivity in their judgement and production of classificatory hypotheses. They were more likely to venture their reasoned opinion, regardless of received views, thus ushering in exciting times for the field.

Reflecting the somewhat subjective nature of taxonomic rank assignment, Hutchinson (1969) elevated some assemblages that most authors would regard at the rank of families to ordinal levels. He recognised four orders: Loganiales, Apocynales, Rubiales and Gentianales. The first three orders were referred to the subphylum 'Lignosae' while his Gentianales was classified in the subphylum 'Herbaceae'. He recognised the close affinity among his Apocynales, Loganiales and Rubiales as well as the polyphyletic nature of the Loganiales. However, he had a very narrow concept of the Gentianales, represented by two families, Gentianaceae and Menyanthaceae. His conception of Apocynales as comprising four families Apocynaceae, Periplocaceae, Asclepiadaceae and Plocospermataceae is illuminating, as it reflects phylogenetic relatedness as well as convergence evolution. The Plocospermataceae were later transferred to the Lamiales based on molecular evidence (Oxelman *et al.* 1999).

Cronquist (1981) presented one of the most ambitious single-authored modern classification of the angiosperms based on the synthesis of a multitude of data; another equally worthy and ambitious project was that of Takhtajan (1997 and

2009). Although still influenced by the earlier works of De Candolle (1845) and Bentham and Hooker (1862 – 1883) (e.g. recognition of the order Rubiales as separate from the Gentianales), Cronquist's largely successful effort changed a number of the previous classification paradigms based on cumulative empirical evidence. He recognised six families within the Gentianales viz. Apocynaceae, Asclepiadaceae, Loganiaceae, Saccifoliaceae, Gentianaceae and Retziaceae, and correctly excluded Buddlejaceae, Menyanthaceae, and Oleaceae based on anatomical signatures of internal phloem and endosperm.

In a marked departure from the taxonomic coherence towards which preceding works seemed to be progressing, Goldberg (1986) adopted a very inclusive definition of Gentianales, sometimes referring an entire family on the basis of a single chemical attribute. This created a disparate set of 10 families, including three, which were subsequently transferred to separate orders viz. Columelliaceae to the Rosales, Cuscutaceae syn. Convolvulaceae to the Solanales and Menyanthaceae to the Asterales. Perhaps, in recognition of the need for a comprehensive review of angiosperm classification, Thorne (1992) employed a multidisciplinary approach making use of every source of relevant taxonomic data available to him. He produced a system which overturned his earlier work (Thorne, 1983) and returned some measure of coherence to the Gentianales. He recognised six families namely Apocynaceae, Dialypetalanthaceae, Gentianaceae, Loganiaceae, Rubiaceae and Saccifoliaceae. Nominally, these families mirror current circumscription of the order, as the members of Dialypetalanthaceae are now referred to the Rubiaceae (Fay *et al.* 2000; APG II, 2003), and Saccifoliaceae have been transferred to Gentianaceae (Struwe *et al.*

2002: 48). Following this approach of total evidence, Nicholas and Baijnath (1994) produced a consensus overview of the Gentianales, and arrived at conclusions concurring with those Thorne (1992).

Struwe *et al.* (1994) produced the first analytical cladistic study of the order using an array of morphological, embryological and chemical characters. They proposed seven families, including a newly described family (Geniostomaceae) and elevated *Gelsemium* to family rank (Gelsemiaceae), effectively creating two newly circumscribed families. This arrangement, modified by splitting some accepted families and including the Plocospermataceae from the Lamiales, formed the basis of Takhtajan's (1997) conceptualization of the Gentianales. His most recent work on angiosperm classification (Takhtajan, 2009) continued with this tradition of splitting by recognising 11 families within the order, largely due to splitting of Rubiaceae into a host of smaller families.

TAXONOMIC HISTORY OF LOGANIACEAE

The opening statement in Bentham's (1856: 52 – 53) notes on Loganiaceae captures the essence of the taxonomic challenge posed by this family. "*The group of plants collected under the name of Loganiaceae can scarcely be said to constitute a natural order, but rather one of those artificial assemblages, which, in the present state of our knowledge of plants, we are obliged to interpose between some of the great families, to receive anomalous genera rejected from them*". Thus, the Loganiaceae became the convenient ground for genera that did not fit neatly into related families. It would take almost a century and half after Bentham's poignant observation before a coherent picture of Loganiaceae emerged.

However, the early realisation of the polyphyletic nature of the family helped focus research endeavours towards resolving the problem.

The Loganiaceae (*sensu lato*) were first described by Martius (1827) based on only five genera: *Gaertnera* Lam. (*nom. cons*.), *Pagamea* Aubl., *Usteria, Genistoma* and *Logania*. The first two of these were presumably placed in the Loganiaceae by Martius because the ovaries in these genera are superior. They were subsequently transferred to the Rubiaceae despite this condition. Meisner (1840) included several other genera described by various authors and recognised 10 tribes in four subfamilies. Bentham (1856) dispensed with the subfamily category and reduced the number of tribes to four, incorporating *Norrisia* into the family for the first time. The Bentham and Hooker's (1876) system recognised only three tribes with a more complete enumeration of the family by reinstatement of Gelsemieae as a tribe comprising *Gelsemium* Juss., *Mostuea* Didrichsen and a new genus *Plocosperma* Bentham. Using a combination of morphological and anatomical data, Solereder (1892) distinguished two subfamilies Loganioideae and Buddlejoideae, the first of which he further divided into six tribes. He recognised a total of 25 genera. His classification scheme was highly regarded and it was retained in large part by Leenhouts (1962) and Leewenberg and Leenhouts (1980) in their respective proposals. Following the works of several other authors on various groups within the broad Loganiaceae, Leeuwenberg and Leenhouts (1980) circumscribed the Loganiaceae into 10 tribes and 29 genera. An excellent taxonomic review of the Loganiaceae up to 1980 is presented by Leeuwenberg and Leenhouts (1980). *Retzia* Thunberg (a South African endemic) and *Desfontainia* Ruiz & Pav. (a South American endemic), two

genera regarded as being of doubtful origin in Leenhouts' (1962) system, were nevertheless included in Loganiaceae, maintaining their own monotypic tribes, Retzieae and Desfontainieae, respectively.

In spite of the recognition of the heterogeneous nature of the Loganiaceae, most authors have retained a broad approach to its classification. However, Struwe *et al.* (1994) deviated from previous traditional approaches and proposed a new scheme for Loganiaceae and allied families. In one of the most restrictive groupings ever made for the family Loganiaceae, based on a ring of hairs in the corolla mouth, and apocarpous and semi-inferior ovaries, they recognised only three genera, *Logania, Mitreola* and *Mitrasacme*. Eight genera including *Strychnos* were referred to the Strychnaceae, while *Anthocleista*, *Fagraea* and *Potalia*, formerly of the tribe Potalieae (Leenhouts, 1962), were referred to the Gentianaceae. Based primarily on DNA sequence data, the Strychnaceae are now included in the current circumscription of Loganiaceae (Backlund *et al.* 2000). The transfer of the tribe Potalieae to the Gentianaceae is consistent with an earlier proposal by Bureau (1856), which excluded all genera of uncertain affinity from the Loganiaceae and reconstituted a family of 11 genera. The current position of Potalieae within the Gentianaceae is further supported by the presence of unique combinations of seco-iridoid compounds, specifically sweroside and swertiamarin found only in the Gentianaceae (Jensen, 1992). The family Strychnaceae of Struwe *et al.* (1994) comprises some of the better-recognised loganiaceous genera including *Strychnos*, *Neuburgia, Gardneria, Spigelia, Antonia, Bonyunia, Norrisia* and *Usteria*, although morphological synapomorphies for the group are rather weak, accommodating too many exceptions to be acceptably useful as

diagnostic features (Struwe *et al.* 1994: 193). Takhtajan (1997; 2009) reduced Loganiaceae to a single genus *Logania*, treating all the currently recognised tribes as distinct families in their own right.

GENTIANALES, LOGANIACEAE AND THE RISE OF MOLECULAR SYSTEMATICS

Despite considerable progress of the classifications based on morphology and chemical profiles, there were inherent limitations to the extent of resolution that could be achieved. This was largely due to the paucity of morphological attributes available for use and the subjectivity involved in assigning cardinality to some characters. Subjective decision-making processes are inevitable in morphological cladistics studies. For instance, the decision as to what constitutes a plesiomorphic versus an apomorphic character state is not always clear cut and can lead to character polarization schemes marred by unintended systematic bias. Also, homoplasy (convergent evolution) is now known to be rife in morphological characters. In light of these limitations, recent advances in the application of molecular data (DNA sequences), as a *complementary* tool in taxonomic decision making have been a welcome development for modern systematics. The classifications of Gentianales in general and Loganiaceae in particular, have been illuminated by such advances.

Olmstead *et al.* (1992) were one of the first studies to use DNA sequence data to place members of the order Gentianales and other taxa broadly associated with it phylogenetically. Although taxon sampling was very limited, their phylogenetic overview of the Asteridae (*sensu* Takhtajan, 1980; Cronquist, 1981) using the chloroplast *rbc*L gene, showed the Gentianales to be closely allied to the

Boraginales, Solanales, Scrophulariales and Lamiales in a strongly supported clade. Restriction site mapping of chloroplast DNA by Downie and Palmer (1992) provided possibly the first molecular evidence for the monophyly of a narrow sense Gentianales by the placement of Buddlejaceae deep within an unresolved Lamiales/Scrophulariales clade, as opposed to its previous position within the Gentianales. Such exclusion of misplaced taxa from the order was supported by Bremer *et al.* (1994), whose investigations convincingly demonstrated the validity of excluding *Retzia*, *Desfontainia* and *Buddleja* (syn. *Nicodemia*) from the Gentianales based on *rbc*L sequence data. Further molecular work, with extensive sampling by several authors (Olmstead *et al.* 1993; Olmstead *et al.* 2000; Backlund *et al.* 2000; Jiao and Li, 2007; Frasier, 2008) showed that the order Gentianales, as currently accepted, comprises five families. Intra-ordinal relationships have also been clarified. The Rubiaceae is the sister group to all Gentianales, sequentially followed by Loganiaceae, Gelsemiaceae, Gentianaceae and Apocynaceae (including the Ascelpiadaceae). This arrangement is strongly supported by recent findings of Refulio-Rodriguez and Olmstead (2014) in a taxon-rich phylogeny of Lamiidae using ten gene regions and more than 17,000 basepairs of nucleotide sequences.

There are not many molecular systematic studies devoted to the Loganiaceae as a group. Nevertheless, the work of Downie and Palmer (1992) already provided preliminary molecular evidence for the polyphyletic nature of the Loganiceae (*sensu* Leeuwenberg 1980). A more comprehensive investigation conducted by Backlund *et al.* (2000) proposed detailed relationships among the Gentianales as well as membership composition of the Loganiaceae. The Loganiaceae was

treated as comprising 13 genera along in two well-supported lineages. Although Backlund *et al.* (2000) did not undertake tribal subdivision of the family, they found strong support for the clade corresponding with the tribe Antonieae of Leeuwenberg and Leenhouts (1980). This treatment, however, overlooked three genera in the molecular analysis of Loganiaceae. *Phyllangium* Dunlop and *Schizacme* Dunlop, two new Australian endemic genera split from *Mitrasacme* Labill. Dunlop (1996), and *Norrisia*. Frasier (2008), in what may be regarded as the single most detailed molecular phylogeny of the Gentianales and Loganiaceae to date, used molecular and morphological datasets to elucidate infra-familial groupings of taxa (for the Loganiaceae) and incorporated the three neglected genera. Her results support the monophyly of Loganiaceae as circumscribed by Backlund *et al.* (2000). Although molecular data were lacking for *Phyllangium*, *Schizacme* and *Usteria*, combined analysis of Loganiaceae-level data clarified tribal affinities and indicated monophyly for each of the four currently recognised tribes, namely Antonieae, Spigelieae, Strychneae and Loganieae. Frasier (2008) provided the first phylogenetic support for the monophyly of the tribe Strychneae with morphological synapomorphies such as the presence of fleshy placenta and indehiscent fruit (Struwe and Albert, 1997). Even without molecular information, Frasier rightly suggested the placement of *Phyllangium* and *Schizacme* within the tribe Loganieae, a proposal since validated by recent molecular phylogenetic work (Gibbons *et al.* 2012). Preliminary evidence is also emerging (Gibbons *et al.* 2012) that may result in reducing *Labordia* (at least, some species) to synonymy under *Geniostoma* and confirming the polyphyletic nature of *Mitreola.* Pursuing the latter to its nomenclatural conclusion, Gibbons *et al.* (2013) described a new genus *Adelphacme* KL Gibbons, BJ Conn & MJ Henwood. Evidence has also emerged

that the two sections in the genus *Logania* are not monophyletic; each forms a distinct monophyletic cluster, indicating two genera rather than one (Foster *et al.* 2014a). The previous *Logania* has now been split by Foster *et al.* (2014b) into two genera with the description of another new genus *Orianthera* C.S.P. Foster & B.J.Conn. Thus, the current number of described genera of the Loganiaceae stands at 17, in four tribes as summarized in Table 7.1.

Table 7.1: Genera and Tribes of Loganiaceae

Antonieae	**Loganieae**	**Spigelieae**	**Strychneae**
Antonia Pohl	*Logania* R.Br.		*Gardneria* Wall.
Bonyunia M.R. Schomb. ex Progel	*Geniostoma* J.R. Forst. & G. Forst.	*Spigelia* L.	*Neuburgia* Blume
Norrisia Gardner	*Labordia* Gaudich		*Strychnos* L.
Usteria Willd.	*Mitreola* L.		
	Mitrascame Labill.		
	Schizacme Dunlop		
	Phyllangium Dunlop		
	Adelphacme K.L. Gibbons, B.J. Conn & M.J. Henwood		
	Orianthera C.S.P. Foster & B.J.Conn		

TAXONOMIC HISTORY OF *STRYCHNOS*

Strychnos L. was first formally described by Linneaus (1753) in his Species Plantarum based on *S. nux-vomica* L., the type species, and later by others (De Candolle, 1845; Hill, 1917; Krukoff and Monachino, 1942; Leenhouts, 1962; Leeuwenberg, 1969). The name is derived from the Greek word '*strychnon*' which is a generic name for many poisonous plants regardless of their taxonomic affinity. It means "acrid" or "bitter" in allusion to the bitter-tasting and usually poisonous alkaloid compounds found in members of the group (Quattrocchi, 1999). The name was also applied to the genus by Theophrastus (a student of Plato and Aristotle), although *Strychnos* is also the Greek name for the 'nightshade' genus

Solanum (Don, 1837). There are about 200 species of *Strychnos* worldwide (Leenhouts, 1962).

That *Strychnos* belongs to the Loganiaceae has never been in doubt in past taxonomic assessments, though its earlier infrageneric placement was uncertain. Meisner (1840) placed it in the subfamily Strychnoideae along with genera such as *Antonia, Larbodia* and *Gardneria*. Bentham and Hooker's (1876) system referred it to a large, but patently heterogeneous, tribe Loganieae alongside *Buddleja, Spigelia, Desfontainia* and *Nuxia* amongst many other genera. By the time of Leeuwenberg and Leenhouts' (1980) revision of the Loganiaceae, a settled position for *Strychnos* had been found within the tribe Strychneae along with two of its closest phylogenetic allies, *Gardneria* and *Neuburgia*. This morphologically-derived placement was backed up by molecular data (Frasier, 2008). While the taxonomic placement of *Strychnos* seems secured, infra-generic arrangements are still a work-in-progress.

One of the earliest subgeneric classifications of *Strychnos* was by Progel (1868), later modified by Solereder (1892). They grouped *Strychnos* into three sections (*Longiflorae*, *Intermediae* and *Breviflorae*), which, from the names, indicated the sole dependence on corolla tube length attributes in their demarcation. This circumscription was retained in its entirety by Krukoff and Monachino (1942) in their treatment of American *Strychnos*. Hill (1917), however, using the type and position of hairs on the inner side of the corolla as additional distinguishing feature, split section *Intermediae* into two. Hill's groupings, just nominally different from Solereder's, are sections *Brevitubae*, *Lanigerae*, *Penicillatae* and *Tubiflorae*

(*Longiflorae*). The importance of the African species in any infrageneric classifications was noted by Leenhouts (1962). Lacking adequate information on the African species at the time, he refrained from any sectional categorisation of *Strychnos* in his work. Using morphological (especially anatomical) characters, Duvigneaud (1952) recognised 17 sections and many series within the genus in Africa. Five of these sections were new (*Aculeatae, Spinosae, Phaeotrichae, Densiflorae* and *Dolichanthaea*) and endemic to the continent. They are still retained, thus validating Leenhouts' assertion that "…*any subdivision of the genus as a whole will primarily have to be framed on the African species*" (Leenhouts, 1962). In an extensive systematic revision of African *Strychnos*, Leeuwenberg (1969) distinguished 12 sections, 11 of which are found on the continent, in what he thought to be "*a more or less natural*" arrangement. His treatment subsumed many of Duvigneaud's sections and combined sectional groupings from previous works into a manageable, but nonetheless 'para-polyphyletic', dozen. He also created a new monotypic section *Scyphostrychnos* represented by *S. camptoneura* Gilg et Busse. These 12 sections and their geographical distributions are as follows: *Strychnos* (America and Asia); *Rouhamon* (America and Africa); *Breviflorae* (America and Africa); *Penicillatae* (Africa and Asia); *Aculeatae* (African endemic); *Spinosae* (African endemic); *Brevitubae* (Africa and Asia); *Lanigerae* (Africa and Asia); *Phaeotrichae* (African endemic); *Densiflorae* (African endemic); *Dolichanthae* (African endemic) and *Scyphostrychnos* (African endemic).

Recent molecular systematic reconstruction of the evolutionary relationships among *Strychnos* species suggested that several of the sectional groupings are

artificial assemblages that warrant further investigation before sections are finally demarcated. (Frasier 2008; Chapter 5). The emerging picture is that section *Spinosae* is the only monophyletic infrageneric grouping supported by both morphology and molecules. Other than the monotypic sections (*Aculeatae, Phaeotrichae* and *Scyphostrychnos*), the monophyly of the other sections was not supported; the monophyletic validity of some is only retained within their geographical region (e.g. section *Lanigerae* for African species).

Frasier (2008) and Chapter 5, have provided some molecular framework and made proposals that may be relevant for future clarifications of the infrageneric groupings of *Strychnos*.

Nine species of *Strychnos* were recognised in the southern African region by Verdoorn (1963). While seven of these are readily diagnosable, two species complexes have been taxonomically problematic; *S. innocua* and *S. spinosa*. The taxonomic challenge posed by these two is connected with their wide distributions across the African dry-lands. One outcome of the distribution pattern was the proliferation of infra-specific categories in different geographical areas (Gilg, 1893; Baker, 1895) based on the unsupported assumption that no single species could be so widely distributed. Thus, a long list of synonyms exists for some of these species (Bullock and Bruce, 1938; Leeuwenberg, 1969). Bruce and Lewis (1956), in an attempt to resolve the *S. innocua* complex, distinguished two species (*S. innocua* and *S. dysophylla*), each of which was further reduced to two subspecies. These authors noted the morphological affinity between *S. dysophylla* subsp. *engleri* (Gilg) Bruce and Lewis and the earlier described *S. gerrardii* N.E.Br.

Among the southern African members, Verdoorn (1963) regarded *Strychnos innocua* subsp. *dysophylla* (Benth.) Verdoorn and *S. innocua* subsp. *gerrardii* (N.E.Br.) Verdoorn, as distinct taxa although she still treated them at the sub-specific rank to maintain the complex. Leeuwenberg (1969) reduced all the names previously applied to *S. dysophylla*, *S. gerrardii* and their affiliates including infra-specific ones, to synonyms under *S. madagascariensis* Poiret according to the nomenclatural rule of priority (Mcneill *et al.* 2012 i.e. Melbourne Code). Within the southern African region, however, *Strychnos gerrardii* N.E.Br. is well-recognised by ecologists and field botanists as distinct from *S. madagascariensis*, although their close evolutionary history is equally obvious (Adebowale *et al.* 2012). The non-synonymy of the two taxa has been demonstrated by micro-morphological and DNA sequence data (Adebowale *et al.* 2014; Chapters 4 and 5).

The taxonomy of the *S. spinosa* complex is probably less clear-cut than the *S. innocua* complex. This complex was classified into three subspecies viz. *S. spinosa* subsp. *spinosa*, *S. spinosa* subsp. *volkensii* and *S. spinosa* subsp. *lokua* (Bruce 1955). In revisiting the morphological evidence of Bruce, and taking geographical variation of specimens across Africa into consideration, Leeuwenberg (1969), in agreement with Verdoorn (1963), concluded that the complex cannot be subdivided into infra-specific categories. Molecular evidence (Chapter 5) on the complex is inconclusive owing to limitations in obtaining good materials for DNA work. Therefore, pending further studies, the present work recognises 11 species of *Strychnos* in southern Africa, with a possibility for the discovery of new taxa, especially in the more tropical enclaves of the region.

ETHNOBOTANY AND ALKALOID CHEMISTRY OF *STRYCHNOS*

Since the discovery that the first *Strychnos* species known to science (*S. nux-vomica* L.) has potent toxicological properties, interest in the possible application of other species of *Strychnos* has increased. The indigenous tribes of the Americas and their African counterparts have long used extracts of species of *Strychnos* in hunting as part of their curare formulation for arrow and dart poisons (Bisset and Phillipson, 1971; Quetin-Leclercq *et al.* 1990). They have also been used as ordeal poisons in some communities as a means of deciding innocence or guilt among contending parties (Philippe *et al.* 2004). The bark of *S. toxifera* Schomb. ex Benth. is an important ingredient of calabash curare. The chemical basis of these toxic properties is the unique alkaloid combinations found in virtually all *Strychnos* species, which has made them one of the chemically most investigated groups of plants (Martin and Vanderwal, 2009; Beemelmanns *et al.* 2013). While the usage of the American species tended to be more for poison formulation, the Asian and African members are a valuable source of food and medicine for indigenous people. Extracts from different parts of the plant have been used in both preventive and curative traditional medicine for a variety of ailments. *Strychnos* have been used to treat, among others, snakebites (Chatterjee *et al.* 2004), ulcers and wounds (Bonamin *et al.* 2011), abdominal complaints (Bero *et al.* 2009), tick infestation of farm animals (Madzimure *et al.* 2013) and malaria (Hoet *et al.* 2006). Chewing sticks of some species have been found to improve dental hygiene due to their effectiveness (i.e. effectiveness of the plant extracts) against caries-inducing *Streptococcus* bacteria (Ohiri *et al.* 1983).

Two well-known alkaloids found in *Strychnos* are strychnine and brucine. Only a few species contain Strychnine-like alkaloids, which are usually concentrated in the seeds, bark and roots. A recent report, however, indicated that there might be sufficiently high concentrations in the leaves of certain species to cause poisoning of an adult human (Dasari and Naha, 2011). Over 300 different alkaloids have been isolated from various species of *Strychnos*, with potentially wide ranging biological activities (Frederich *et al.* 2003). The genus thus constitutes a large pool of pharmacological diversity, the value of which may well extend beyond our current understanding.

All large-fruited savanna and some forest species of African *Strychnos* are important sources of food. Indeed, many of these species are underutilized and not well known outside their natural habitat in spite of their high nutritive and economic value (Mwamba, 2006; National Research Council, 2008). *Strychnos potatorum* L.f. is well known in India for its water-cleaning properties. From an anthropocentric perspective, at least seven species of *Strychnos* (*S. cocculoides* Bak, *S. gerrardii* N.E.Br., *S. innocua* Del., *S. lucens* Bak., *S. madagascariensis* Poir., *S. pungens* Solered. and *S. spinosa* Lam.) are an important food sources due to the large fruits that contain copious amounts of delicious pulp. All these species are also somewhat hardy and arid-adapted (except *S. lucens*), which makes them valuable food resources in the face of climate change.

GEOGRAPHICAL DISTRIBUTION AND ECOLOGY OF *STRYCHNOS*

The genus *Strychnos* is pantropical in distribution and is separated into three geographical groups in Africa, America and Australasia (Leeuwenberg, 1969).

Most of the Neotropical *Strychnos* species occur within the 20^{o} latitude on either side of the equator, with Brazil appearing to be a local centre of diversity. On the African continent, the Guineo-Congolian forest area is the centre of species diversity. *Strychnos* species found in this area display striking similarity of habit, growth forms and leaf attributes with their South American relatives, fuelling speculations of a common Gondwanic ancestry, which is not supported by divergence time estimates (Chapter 6). African species occupy a wide variety of habitats ranging from different forest types (coastal, gallery, and woodland) to open woodland areas and dry bushlands. *Strychnos spinosa*, *S. innocua* and *S. usambarensis* are the most widely distributed on the continent. *Strychnos potatorum* is the only species found in Africa and Asia. Its phylogenetic position, relative to other Asian taxa (Frasier, 2008), suggests that it is indeed an African species that must have been transported, possibly along ancient trade routes to Asia (Leeuwenberg, 1969), where it has been much celebrated for its medicinal uses. Bisset *et al.* (1973), however, question this view of *S. potatorum* dispersal by Arab traders. They argued that it is widely distributed across India, is little cultivated, and has a history in Ayurvedic medical compendia going back to about 2000 year or earlier. They thus surmised that *S. potatorum* arrived in Asia, possibly by natural dispersal. There are a number of other plant species (e.g. *Calotropis procera* (Aiton) W.T. Aiton, *Carissa spinarum* L. and *Pergularia daemia* (Forssk.) Chiov.), in the same order Gentianales as *Strychnos*, with similar Afro-Asia distribution patterns.

On a coarse scale, African *Strychnos* can be partitioned into two categories based on their distribution pattern: forest and savanna species. This partitioning is not

strictly adhered to in nature, as several of the 'true savanna' species sometimes penetrate into adjacent forests (e.g. *S. madagascariensis* and *S. innocua*), which may also explain in part, their relatively wider distribution. Some species such as *S. spinosa* tend not to be found in forests, though they may occupy diverse habitats (Leeuwenberg, 1971). Most of the species in West-Central Africa are woody climbers found in thick rain forests (Chapter 5). *Strychnos gerrardii* has a particularly intriguing distribution; it has adaptive characteristics typical of a savanna plant, but occupies forests along the south-eastern coastline of Africa. Biogeographical analysis, coupled with molecular dating of divergence times among the southern African species, suggests that its distribution is a consequence of allopatric speciation, mediated by evolutionarily recent climatic oscillations (Chapter 6). The same mechanism appears to have been responsible for the recent evolution of arid-adaptedness among the southern African species (Chapters 5 and 6).

KARYO-TAXONOMY OF *STRYCHNOS*

Few karyological studies have been undertaken in *Strychnos*. Other than the works of Gadella and a handful of other workers (compiled in Gadella, 1980), we are not aware of any karyological study for the genus. In total, 32 species of *Strychnos* have been examined karyologically, 26 of them from Africa. The basic chromosome number for the genus is x = 11, with reported 2n counts ranging from 24 in *S. spinosa*, *S. nux vomica* L. and *S. minor* (Mohrbutter, 1936) to 110 in *S. brasiliensis* (Spreng.) Mart. (Gadella, 1980). Counts for other species and repeated counts by other authors (cited in Gadella, 1980) indicated that the most

common diploid number encountered in *Strychnos* is 2n = 44, with 2n = 88 reported for *S. angolensis* Gilg and *S. malacoclados* C.H. Wright (Gadella, 1963).

A clear indication from these numbers is that polyploidy has played a prominent role in the evolution of the genus, with the decaploidy in *S. brasiliensis* being the highest level of ploidy reported thus far and tetraploidy the most common. The basal position of *S. brasiliensis*, *S. angolensis* and *S. malacoclados* in the molecular phylogenetic framework for the genus (Frasier, 2008; Chapter 5), immediately suggests that ancestral *Strychnos* had higher 2n ploidy levels and thus chromosome numbers, with a trend towards lower 2n numbers in more derived lineages.

CONSERVATION STATUS OF *STRYCHNOS*

All 11 species of southern African *Strychnos* in this treatment are categorised as of least concern by the South African National Biodiversity Institute (SANBI) [Raimondo *et al.* 2009]. On a global scale, only five *Strychnos* species, out of the seven listed on the IUCN site [http://www.iucnredlist.org/search accessed on 28th July 2014], have a vulnerable or worse conservation status. Three of these five species, which are all forest-dwelling, (*Strychnos millepunctata* Leeuwenberg, *Strychnos staudtii* Gilg and *Strychnos elaeocarpa* Gilg ex Leeuwenberg) are found in Africa. While *Strychnos* species are not seriously threatened, the skewed trend in the direction of forest taxa as the vulnerable ones, calls for some concern not just for forest *Strychnos*, but for other species that occupy similar ecological niches. It is quite probable too that the IUCN report cited above may not reflect the current state of affairs for many of the species. Given the rapid rate of global forest

decline, it is likely that many forest-dwelling species (including *Strychnos*) are more threatened, sometimes to the point of extinction, than can be updated by any agency. This highlights the urgency of documenting as much biodiversity as possible before they are lost.

MORPHOLOGICAL CHARACTERS OF TAXONOMIC VALUE IN SOUTHERN AFRICAN *STRYCHNOS*

No single morphological trait is sufficient to adequately discriminate among the 11 *Strychnos* species in this treatment. However, there is a suite of certain morphological traits, whose presence, absence, size, colour or other configurations, are useful for diagnosing among closely-related members. These include leaf, trichome, fruit, flower, bark, branchlets. These traits are not treated individually in this section, as they have been adequately covered in various parts of this work.

TAXONOMIC TREATMENT

Strychnos L. Sp. Pl. ed. I: 189. (1753); Gen. Pl. ed. 5: 86 (1754); Bentham, J. Linn. Soc. Bot. 1: 75 (1856); Solered., Engler & Prantl, Nat. Pflanzenf. 4 (2): 37 (1892); Hill, Kew Bull. 121 (1917); Krukoff & Monachino, Brittonia 4: 248 (1942); Duvign., Bull. Soc. Roy. Bot. Belg. 85: 9 (1952); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae: 12 (1960); Leenhouts, Fl. Malesiana 1 (6): 343 (1962); Verdoorn, Fl. S. Afr. 26: 136 (1963); Leeuwenberg, Mended. Landb. 69(1): 1-316 (1969); Leeuwenberg, Die Naturlichen Pflanzenfamilien Angiospermae: Ordnung Gentianales Fam. Loganiaceae: 35 (1980).

Type species: *S. nux-vomica* L.

Bremia Harv. In Hooker, Lond. Journ. Bot. I: 25 (1842). Type species: *B. spinosa* (Lam.) Harv. Ex. DC.

Heterotypic synonyms: *Rouhamon* Aubl., Hist. Pl. Guian. I: 93 (1775)

Atherstonea Pappe, Sylva Cap. 2nd ed. 29 (1862). Type species: *A. decussata* Pappe (= *S. decussata* (Pappe) Gilg).

Armed or unarmed trees or shrubs, usually climbers in tropical forests. Lianas with curved tendrils. Branches and branchlets, if armed, with axillary or terminal, straight or recurved spines. *Leaves* opposite or decussate, entire, glabrous to densely pubescent, orbicular to elliptic, 3 – 7 nerved from near the base; stipules absent or reduced to an interpetiolar ridge. *Inflorescence* axillary or terminal, cymose; cymes simple or panicled. *Flowers* 4 – 5 merous. *Calyx* 4 or 5-lobed, nearly completely divided; lobes orbicular to linear lanceolate, as long as corolla to sometimes longer; lobes usually sparsely hairy on the outside, glabrous within, ciliate along the margin. *Corolla* 4 – 5 lobed, salver-shaped, cream or whitish, sometimes pale green; lobes valvate in bud, triangular to oblong, inner face often bearded; tube glabrous or papillose within, sometimes bearded at the throat. *Stamens* 4 – 5, epipetalous, inserted on corolla tube near the throat; filaments short, glabrous; anthers exserted or sub-exserted, narrowly oblong, slightly bifid at the base. *Ovary* 1 – 2 celled, with many ovules; style simple, straight, if hairy usually only at the base; stigma pale white, capitate, faintly bilobed. *Fruit* a berry, globose or nearly so, 10 – 140 mm in diameter, rind leathery or woody, mostly yellow to orange, less often green when mature, sometimes blue-black; pulp juicy, fleshy, often edible. *Seeds* 1 to numerous, embedded in pulp, variously shaped, flattened, subglobose or coffee bean-shaped, with hilum in various positions; testa

sometimes becoming cartilaginous; endosperm copious, hard; embryo small and surrounded by endosperm.

KEY TO SOUTHERN AFRICAN SPECIES OF *STRYCHNOS*

1a. Fruit small, usually up to 30 mm in diameter, 1 – 2 seeded; rind thin and leathery leaves never pubescent ……..……2

1b. Fruit large, up to 140 mm in diameter, many seeded, rind thick and woody; leaves usually pubescent, sometimes glabrous....……..…..6

2a. Leaves coriaceous to subcoriaceous (leather-textured), usually less than 60 mm long ……..……....3

2b. Leaves thin-textured, mostly more than 60 mm, up to 150 mm long ……..……..……5

3a. Leaves decidedly broadest in the lower half, ovate to lanceolate, apex usually elongated, acuminate; branchlets dark brown; flowers 4 merous ..……...…**11. *S. usambarensis***

3b. Leaves broadest at or above the middle, sometimes rhomboid, apex broadly rounded or shortly acuminate; branchlets pale or dark grey; flowers 4 or 5-merous …….....4

4a. Branchlets 4-angled, without lenticels; leaves 3-veined, another pair of vein often present but not distinct, ovate, broadly elliptic or rhombic, leathery, glossy green above; flowers 4 merous; fruit round, 8 – 15 mm in diameter, fleshy, yellow, orange or red when ripe, 1-seeded; seed deeply grooved on one side much like coffee beans ……..**4. *S. henningsii***

4b. Branchlets not 4-angled, with pale brown lenticels; leaves 3 – 5 veined, obovate to broadly elliptic, leathery, glossy, dark green above; flowers 5 merous; fruit round, 8 – 15 mm in diameter, fleshy, usually orange or red, 1 – 2 seeded; seeds compressed and slightly concave ……..……**2. *S. decussata***

5a. Leaves sometimes with domatia in the axils of lateral veins; branchlets often pubescent with a pair of persistent cataphylls near the base; inflorescence pubescent; calyx petaloid, lobes broad; fruit round, 10 – 20 mm in diameter, fleshy, yellow or orange when ripe ……..….**7. *S. mitis***

5b. Leaves without domatia; branchlets glabrous, dichotomously branched with a ring-like scar near the base; inflorescence glabrous; calyx not petaloid, lobes longer than broad; fruit round, 15 – 25 mm in diameter, fleshy, blue-black when ripe ……..….**8. *S. potatorum***

6a. Inflorescence terminal on main branches or on short lateral twigs; sepals long and narrow, often as long as the petals; lateral and terminal branchlets often armed with spines……..…….……..……..…….7

6b. Inflorescence axillary, sometimes terminal; sepals short and broad or shorter than half the length of petals; lateral and terminal branchlets never armed with spines……..…….……..…….8

7a. Bark thickly and persistently corky with longitudinal fissures; branchlets reddish-brown or purplish, with long hairs, armed with pairs of curved axillary spines; fruit dark green with distinct white speckles …..……**1. *S. cocculoides***

7b. Bark rough but not very corky, without fissures; branchlets dull and pale-coloured, sometimes finely pubescent, armed with pairs of straight or curved axillary spines; fruit green with faint or no speckles ……**10. *S. spinosa***

8a. Leaves elliptic, glabrous, apex abruptly narrowed and tipped with a conspicuous straight spine..……..……**9. *S. pungens***

8b. Leaves elliptic to orbicular, pubescent or glabrous, apex rounded, broadly tapering or just tapering, apex never with a spine ……..9

9a. Bark nearly smooth, flaky; main stem upright, up to 15 m high with several upright secondary branches; leaves completely glabrous, elliptic to oblong, apex subacute or narrowly obtuse…**3. *S. gerrardii***

9b. Bark rough; multi-stemmed with spreading canopy, tree up to 8 m high; leaves usually pubescent, at least on abaxial veins, rarely smooth, broadly elliptic, obovate-oblong to suborbicular, apex rounded to broadly tapering……..….10

10a. Leaves borne as clusters towards end of twigs on dwarf stubby spur-branchlets, without prominent leaf veins; fruits 50 – 100 mm in diameter……..……**6. *S. madagascariensis***

10b. Leaves not borne as clusters on end of twigs, with very prominent pale veins on both surfaces; fruit 40 – 75 mm in diameter ……..……..…..**5. *S. innocua***

1. *S. cocculoides* Bak., Bull. Misc. Inform., Kew 1895: 98. (1895) and Fl. Trop. Afr. 4(1): 533. (1903); Hiern, Cat. Welw. Afr. Pl. 3: 704. (1898); Gilg & Busse in Engler, Bot. Jahrb. 36: 110. (1905); Duvign., Lejeunia 13: 114. (1949) and Bull. Soc. Roy. Bot. Belg. 85: 20 (1952); Bruce, Kew Bull. 1955: 38. (1955); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 16 (1960); Verdoorn, Fl. S. Afr. 26: 149. (1963); Leeuwenberg, Mended. Landb. 69(1): 86 (1969). **Figure 7.1.1 page 206.** Type: Angola: Huila District, between Lopollo and Monino, Welwitch 4779 (lectotype, designated by Leeuwenberg (1967) as holotype BM!; isotypes: COI, G, K!, LE, LISU, P!).

S. dekindtiana Gilg, Notizbl. Bot. Gart. Berlin 2: 258. (1899); Baker, Fl. Trop. Afr. loc. cit. p. 534. Type: Angola: Huila District, Dekindt 1032, partly (holotype not seen, destroyed in B; lectotype: P; the LISU sheet of this number is *S. pungens* Solered.).

S. goetzei Gilg in Engler, Bot. Jahrb. 28: 123. (1899) and in op. cit. 32: 179. (1902); Baker in Fl. Trop. Afr. loc. cit. p. 534. Type: Tanzania: Iringa/Ulanga District, Utschungwe (Uzungwa) Mountains, Goetze 643 (holotype not seen, destroyed in B; no isotypes seen).

S. paralleloneura Gilg et Busse, Ioc. cit. p. 112. Type: Angola: Huila District, Keputu Mt., near Otchipongolo, Dekindt 1037 (LISC, lectotype).

S. schumanniana Gilg in Baum, Kuene-Samb. Exped. 330. (1903); Baker in Fl. Trop. Afr. I.c. p. 624. 1904; Prain & Cummins in Fl. Cap. 4(1): 1054. 1909. Type: Angola: Cubango R., near Massaca, Baum 290 (G, lectotype; isotypes: BM!, E!, K!, W).

S. suberifera Gilg et Busse in Engler, Bot. Jahrb. 36: 107. (1905). Type: Tanzania: near Lindi, Mayanga, Busse 2524 (lectotype G; isotypes: BM!, BR!, EA!, HBG, LY). Homotypic synonym: *S. spinosa* var. *suberifera* (Gilg et Busse) Aubrév., Fl. Soud.-Guin. 441. 1950.

S. suberosa T.R. Sim, Forest Fl. and Forest Resources Port. E. Afr. 90 (1909). Type: Mozambique: Magenja da Costa, near Lourenço Marques, Sim 6013; Obermeyer, AA and Verdoorn, IC 3 (holotype PRE!); Democratic Republic of Congo, Justin G., *s.n.* 894899 (paratype BR!).

S. thomsiana Gilg et Busse, Ioc. cit. p. 111. Syntypes: Angola: Huila District, Keputu Mt., Dekindt 9a and 9 b (holotype destroyed in B; no iso-syntypes seen).

Small trees or deciduous shrubs, 1 – 8 m high, with longitudinally ridged, thick, corky branches; young branchlets reddish or blackish purple, densely spreading-pubescent or rarely glabrous; branchlets often armed with pairs of

recurved or straight spines, sometimes branches terminate in a straight spine. *Leaves* shortly petiolate, petiole 1.7 – 9 mm long, oblong-elliptic, broadly ovate to orbicular, usually broadest below the middle, 25 – 65 mm long, 15 – 40 mm wide, usually pubescent on both surfaces, broadly rounded, broadly acuminate, retuse or emarginate at the apex; base rounded, cuneate or subcuneate, rarely subcordate; 3 – 7 nerved at or just above the base; venation prominent and conspicuous on abaxial surface. *Inflorescence* cymose, terminal, sometimes with a subglobulose appearance, congested, usually pubescent, peduncle pubescent, 6 – 22 mm long, pedicels sparsely pubescent. *Flowers* 5-merous, rarely 4 or 6 merous. *Calyx* 5-lobed, pale green, narrowly linear-lanceolate, broad and connate at the base, 3 – 6 mm long, 1 – 1.5 mm wide, usually slightly shorter than the corolla, pubescent. *Corolla* 5-lobed, pale green to greenish yellow, sparsely pubescent or glabrous without, dense fringe of hairs on the throat, lobe lanceolate with acute to acuminate thickened apex, tube 1 – 1.5 x as long as the lobes. *Stamens* inserted at the base of the corolla tube, filament glabrous; anthers oblong or elliptic, 1.2 – 1.8 mm long, 0.8 – 1.2 mm wide, deeply cordate at the base, densely bearded at the base, ciliated all round. *Ovary* globose, subglobose, elongately conical (rounded inverted funnel-like) or broadly ovoid, papillose at least in the upper portion, 2-celled; style very short (0.4 – 1 mm). *Fruit* large, hard, dark green, yellow or orange, minutely speckled, globose, 35 – 100 mm in diameter, many seeded (10 – 100); pulp edible. *Seeds* compressed, plano-convex, up to 25 mm across.

Etymology of specific epithet: From the Greek word ‘*kokkos*’ meaning berry, and ‘*oikes*’ indicating the resemblance of fruits to a berry.

Vernacular names: corky-bark monkey orange (English); kurkbasklapper (Afrikaans); morapa (North Sotho); muhumi (Shona); maguni (Ovambo).

Distribution and habitat: Gabon, Congo-Brazzaville, Democratic Republic of the Congo, Angola, Kenya, Tanzania, Zambia, Zimbabwe, Malawi, Mozambique, Namibia, Botswana and South Africa. It is a dry woodland species. It occurs in sandy soil or rocky terrains and occupies altitudinal range from 400 – 2000 m. Southern African distribution map is depicted in Figure 7.1.2.

Affinity: Closely related to *S. spinosa*, *S. congolana* and *S. ternata* as members of section *Spinosae*. *S. spinosa* is the only species with a 1-celled ovary, the others have 2 cells; *S. cocculoides* shares its highly corky, reddish-brown bark and recurved spines with *S. congolana*, and leaf shape and venation pattern with *S. spinosa*. However, in the wild, these species are not easily confused with one another.

Notes: The specific epithet is rather odd as the fruit, though shaped like a berry, is much bigger than a berry and is the result of the small (possibly immature) nature of the fruit on the type specimen. The stable number of floral parts is five. However, in some specimens, four [*CJ Ward & J Hines 10339* (UDW, NU)] and six [*W Giess 9481* (PRE, WIND)] were encountered, sometimes with varying numbers on flowers of the same inflorescence.

Selected collections: SOUTH AFRICA: Mpumalanga, Loskop Dam Nature Reserve, *Burrows 6323* (BNRH); Waterval, General Hertzog's Farm, near Pretoria, *Pole-Evans s.n.* 13 May 1933 (K, SRGH, PRE); Limpopo Province*, Glen 4080* (NH); Magaliesberg, near Wonderboom, *Schlechter 3630* (BM, BOL, K); Wonderboom Reserve, Pretoria, *Repton 1757* (PRE); Pretoria, *Robertson PRF*

2155 (PRF); Meintjes Kop, Pretoria, *Mogg 16423* (PRE); Rooikop, *Pole-Evans 281*(EA). NAMIBIA: Kavango River valley, *Ward & Hines 10339* (UDW, NU); North east of Grootfontein, *Schoenfelder 222* (PRE); no locality information, *Story 4916* (PRE); Kuring-Kuru, *Giess 9481* (PRE, WIND); Tarikora, Ndiyona Rest camp, *Muller & Giess 451* (PRE, WIND); Main road to Liambezi from Bucalo, *Muller & Irish 3176* (PRE); Okavango Territory, Mbambi Camp between Kuringiki and Katwitwi, *De Winter & Marais 5014* (PRE); near Oshikango, Ovamboland, *Rodin 2664* (BOL, K, MO). ANGOLA: Rocada, Chicusse, *Borges 302* (PRE, LUAI); near Luanda, *Welwitsch 6019* (BM, K, LISU); between Alto dos Cruzes and Quicune, *Welwitsch 4763* (BM, K, LISU), *4767* (BM, LISU). BOTSWANA: Bechuanaland Protectorate, *Miller B/554* (PRE); Ngamiland (?), *Curson 3311* (PRE); ibid. *Miller B/434* (FHO); 300 km NW. of Molepolole, *Story 4916* (K, PRE); Palapye, *de Beer 569* (K, SRGH); Botswana: Tsodilo Hills, *Banks 7* (PRF); North of Kalahari, *Schoenfelder 4* (PRE). MOZAMBIQUE: Mandimba, *Pedro & Pedragâo 3487* (EA); Zambezia, between Alto Ligonha and Alto Molócuè, *Barbosa & de Carvalho 4406* (K, SRGH). ZAMBIA: Chipya, *Cottrell 82* (GRA).

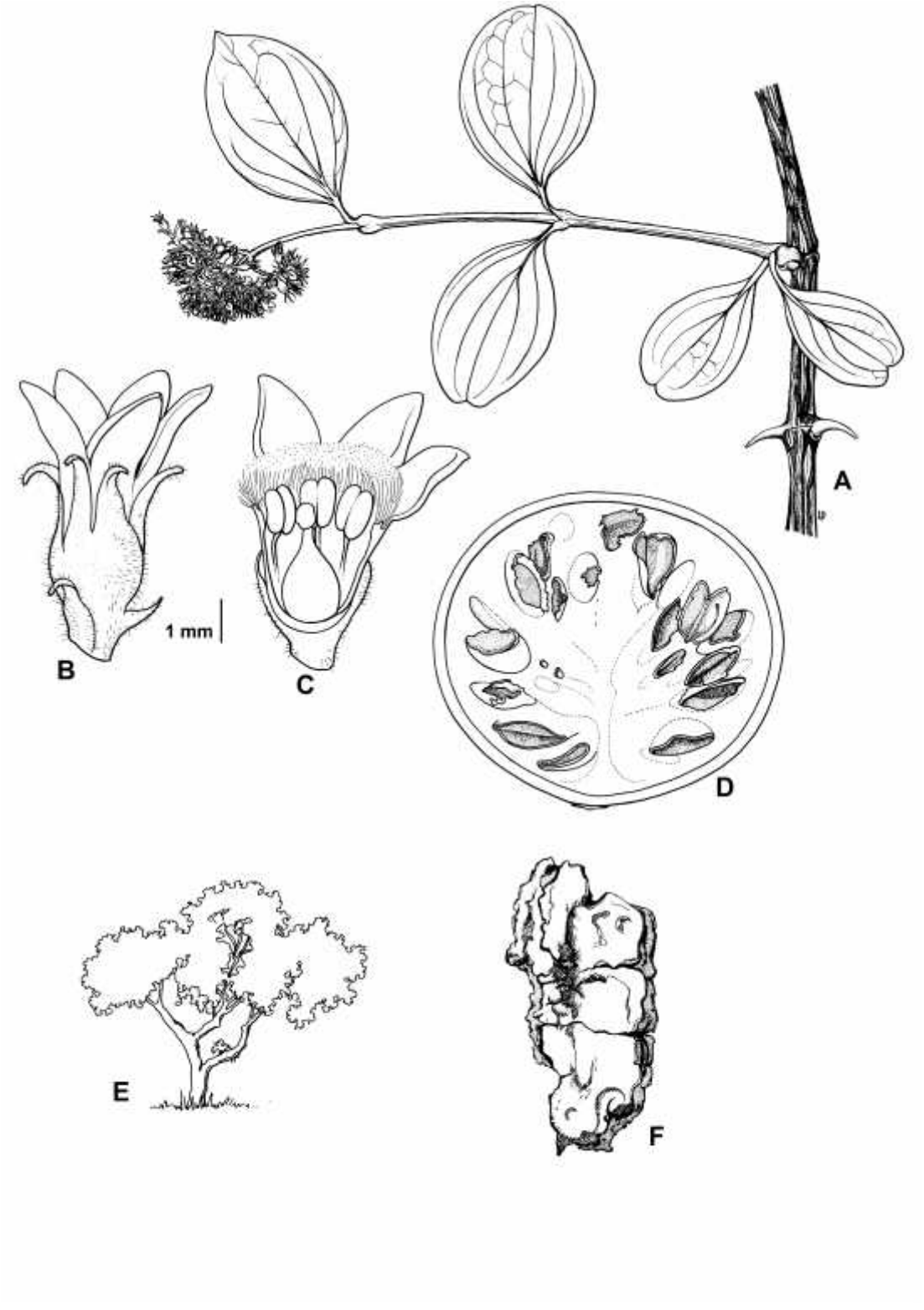

Figure 7.1.1 *S. cocculoides*: A = branchlet, leaf & inflorescence x ½, *Borges 302* (PRE); B & C = flowers *Balkwill, Balkwill & GV Cron 6799* (J); D = cross section of fruit x ½, *de Winter & Marais 5014* (PRE); E = canopy shape drawing, not to scale; F = corky bark x ½, *de Winter & Marais 5014* (PRE). Illustrations by Leslie Deysel.

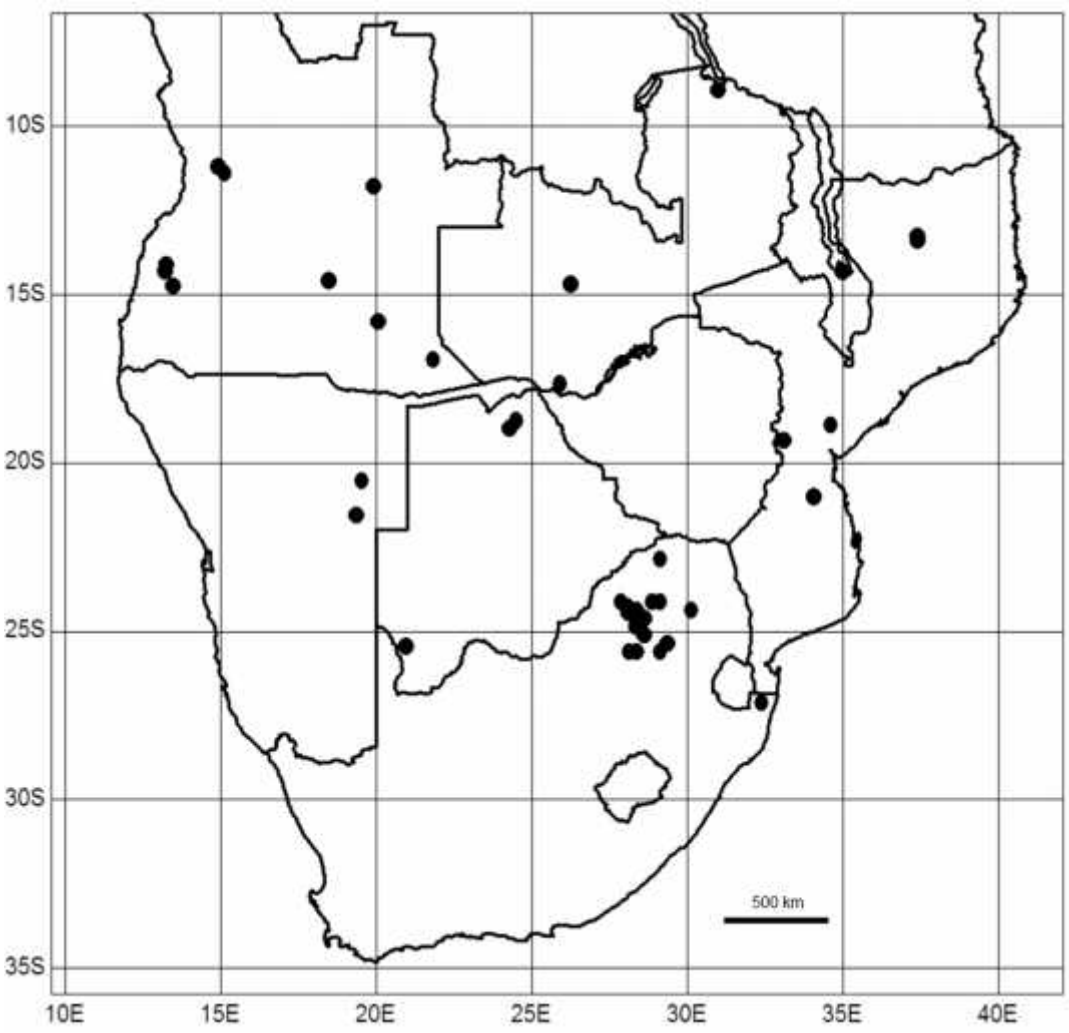

Figure 7.1.2: Distribution map of *Strychnos cocculoides* southern Africa

2. *S. decussata* (Pappe) Gilg in Engler, Bot. Jahrb. 28: 121. (1899); Verdoorn, Bothalia 3: 587-588 (1939) and 7: 11. (1959); Bruce, Kew Bull. 1956: 156. (1956); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 29. (1960); Verdoorn, Fl. S. Afr. 26: 139 (1963). Leeuwenberg, Mended. Landb. 69(1): 100 (1969). **Figure 7.2.1. page 212.**

Type: South Africa: Cape Province, Bathurst, Kowie, Atherstone s.n. (TCD, neotype; iso-neotype: K!).

Basionym: *Atherstonea decussata* Pappe, Silv. Cap. 2nd ed. 29. 1862.

S. atherstonei Harv., Thes. Cap. 2: 41, t. 164. (1863) nom. Illegit.

S. baculum Harv. in syn.; Prain & Cummins in Fl. Cap. 4 (1): 1051 (1909); Marloth, Fl. S. Afr. 3(1): 49, pi. 14C (1932) (as *atherstonii)*; Duvigneaud, Bull. Soc. Roy. Bot. Belg. 85: 30 (1952).

S. boinensis Jumelle et Perrier, Ann. Mus. Col. Marseille Sér. 2. 5: 403 (1907). Type: Madagascar: Boina, Ankaladina Forest, banks of Betsiboka R., Perrier de la Bâthie 1380 (P, holotype; isotype: K!).

Erect tree, 2 – 15 m tall, with smooth pale to dark grey bark. Branchlets with numerous lenticels, terete to sub-quadrate. *Leaves* shortly petiolate, glabrous on both sides, petiole 2.5 – 6 mm long, obovate, ovate-oblong, elliptic or rhombic; lamina subcoriaceous, 25 – 50 mm long, 12 – 25 mm wide; apex retuse or broadly acuminate; base cuneate, subcuneate or rounded; 3-nerved from or just above the base, with a fainter pair of submarginal and marginal nerves; margins slightly

inrolled. *Inflorescence* axillary and sometimes terminal, predominantly cymose, occasionally racemose on an elongated axis; peduncle glabrous or pubescent, 2 – 12 mm long; pedicel sparsely hirsute, 1.8 – 4 mm long. *Flowers* mostly 5-merous, occasionally 4-merous, scented. *Calyx* 5-lobed, connate at the base, lobes ovate, 1 – 1.8 mm long, 0.8 – 1.1 mm wide, shortly ciliate. *Corolla* 5-lobed, creamy white, 4 – 6.5 mm long, about 3 - 5 x as long as the calyx, glabrous outside, dense long hairs in the throat; tube shortly campanulate; lobe oblong, lanceolate or narrowly triangular with an acute to acuminate apex. *Stamens* exserted, arising in the sinuses of the corolla lobes, filament glabrous, short (0.8 – 1.1 mm long), narrowing from a slight broadened base; anthers oblong, 0.8 – 1.2 mm long, 0.7 – 1 mm wide, glabrous, cordate at the base. *Ovary* ovoid to globose, glabrous, 2 - celled; style slender, considerably longer than ovary (1.7 – 3.7 mm); stigma capitate, sometimes bilobed. *Fruit* small, soft, usually orange or red, 8 – 15 mm in diameter, subglobose, 1 – 2 seeded; pulp edible. *Seeds* ellipsoid, slightly longer than broad with a slight depression (hilum) on one side, about 10 mm across.

Etymology of specific epithet: Originally *Antherstonea decussata* Pappe; *Strychnos antherstonei* Harv., named after the English botanist William Guybon Antherstone 1814-1898. Specific epithet decussata is based on the oppositely decussate leaf arrangement.

Vernacular names: Cape-teak (English); Kaapse Kiaat (Afrikaans); umHlamahlala, umKhangele (isiXhosa); umLahlankosi, umPhathwenkosi (isiZulu).

Distribution and habitat: Kenya, Tanzania, Zambia, Zimbabwe, Mozambique, Malawi, Madagascar, Swaziland and South Africa. Common at low altitudes in coastal thicket and woodlands or wet coastal forest, along watercourses or on

termitaria. Altitudinal range: 0 –1500 m. Southern Africa distribution map is supplied in Figure 7.2.2.

Affinity: Leaves may sometimes be confused with the highly variable leaves of *S. henningsii*. However, the leaves of *S. decussata* are generally smaller.

Notes: Leaves of Madagascar specimens tend to have rather acuminate apices in contrast to their mainland African conspecifics.

Selected collections: SOUTH AFRICA: Limpopo, Zwigodini Village, Mutale, *Adebowale 70* (UDW); Burman Bush Nature Reserve, *Adebowale 16* (UDW); Tembe Elephant Park, Western boundary, corridor between Tembe & Ndumo, *Van Wyk 1323* (NH); Louwsburg, *MacDevette 712* (NH); Mtubatuba District, *Ward 4532* (K); Dukuduku-Futululu Forest, *Strey 5476* (K, PRE); ibid., *9429* (NH, NU); Manguzi Forest, *MC Ward 2164* (NH); Kruger National Park, next to Levuvhu River, *Venter 9923* (PRE); Ubombo, False Bay Park, *Ross 2327* (NH); Ubombo, Ingavuma District, Flood Plain of Pongola River, *Furness 629* (NU); Hluhluwe Game Reserve, *CJ Ward 3963* (NU); Berea near Durban, *Medley-Wood 5496* syn. of *S. antherstonei* Harv. (GRA); Eastern Cape, Alexandria forest, *Archibald 6084* (GRA); South Eastern Cape, Port Elizabeth, *Burrows 5666* (GRA); Eastern Cape, coastal grassland and pockets of riverine forest near Nqabe river, *Dold 528* (GRA). MADAGASCAR: Toliara Province, ca. 20 km East of Morombe, *Phillipson 5586* (GRA). MOZAMBIQUE: Macanga, Massamba-Metenge road, de Campos *Andrada 1703* (COI); Chiniziua, Beira region, *Gomes e Sousa 4346* (K). Gaza, 15 km South of Massangena, Save River Valley *Hornbey 2485* (SRGH); between Limpopo and Nuanetsi Rivers, *Smuts 337* (BM, K, PRE); Licuati Forest Camp, Bela Vista area, *Burrows 9503* (BNRH). ZIMBABWE: Melsetter District, *Chase*

4704 (BM, BR, K, MO, PRE, SRGH, WAG); Kariba Gorge, *Goldsmith 34/59* (SRGH); bank Manora River, Urungwe District, *Phipps 927* (K, LISC, SRGH); Unsengesi River, Darwin District, *Whellan 890* (K, SRGH); Manavegadzi, Gwanda District, *Drummond 5747* (SRGH); Chitsa's Kraal, Sabi-Lundi District, *Wild 3397* (BR, K, S, SRGH); Ndanga District, *Armitage 107/55* (SRGH); East of Chipinda Pools, Ndanga District, *McGregor 75/51* (FHO, SRGH); Triangle, Ndanga District, *Mylne 33/51* (FHO, SRGH); Beitbridge District, *Davies 2832* (K, SRGH).

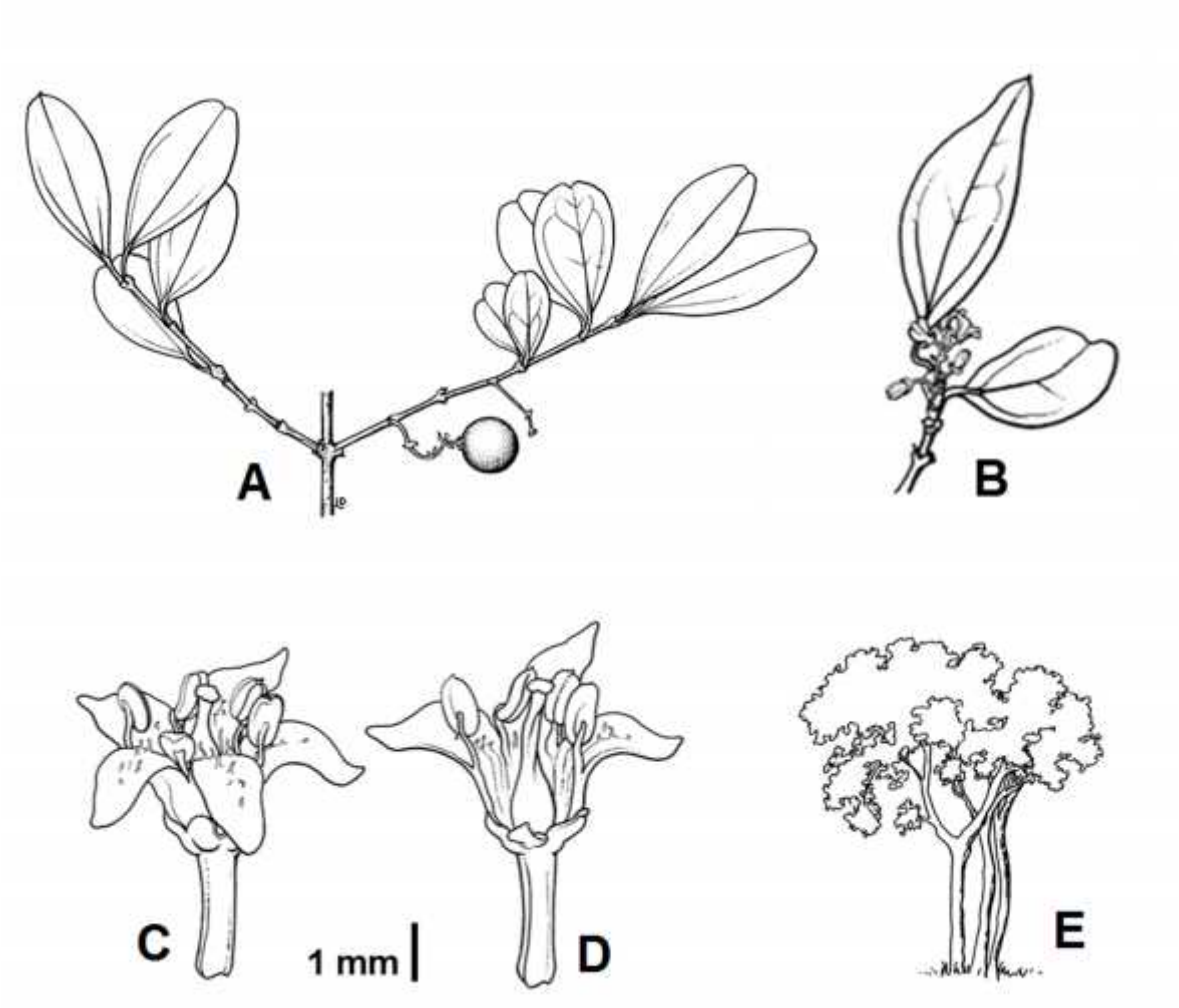

Figure 7.2.1. *S. decussata*: A = branchlet with leaves and a fruit x 1, *Furness 629* (NU); B = leaves and some flowers in bud x 1, *Louwsburg & MacDevette 712* (NH); C & D = flowers, *Louwsburg & MacDevette 712* (NH); E = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

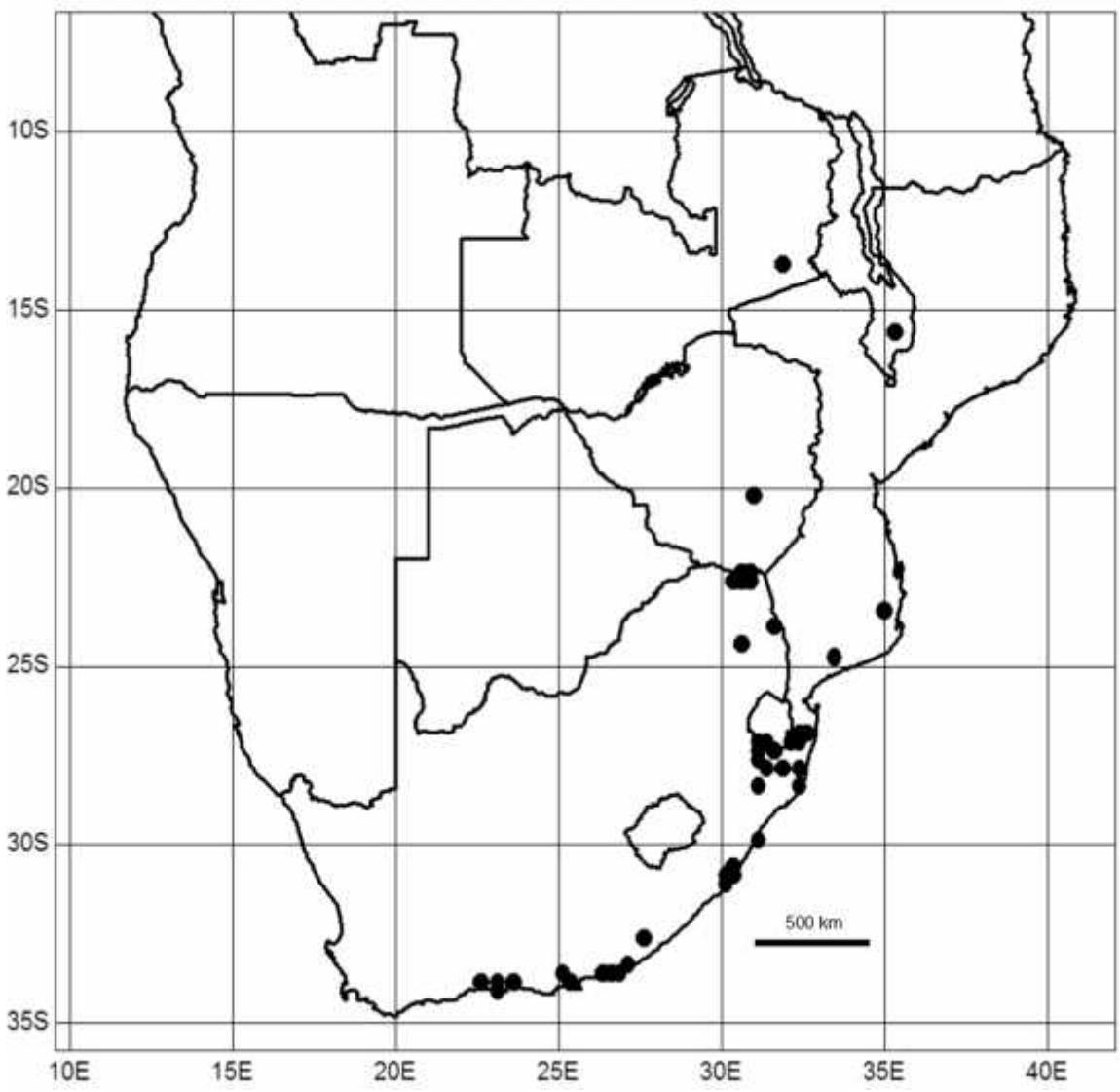

Figure 7.2.2: Distribution map of *Strychnos decussata* in southern Africa

3. *S. gerrardii* N.E.Br.,

N.E.Br., Kew Bull. 1896: 162 (1896); Wood & Evans, Natal Plants 1: 16 (1899); Prain & Cummins, Fl. Cap. 4, 1: 1053 (1909). **Figures 7.3.1 & 7.3.2 page 218 - 219.**

Type: South Africa: Berea. Natal, Wood J.M. 5624; 1421; 1777 (syntypes: NH!, PRE!).

S. innocua subsp. *gerrardii* (N. E. Br.) Verdoorn, Bothalia 7: 12. 1958 and FI. S. Afr. 26: 147 (1963).

S. madagascariensis Poir. (*sensu* Leeuwenberg, 1969) excluding all synonyms except *S. innocua* subsp. *gerrardii* N. E. Br.

Trees or shrubs up to 20 m high with secondary branches growing vertically upwards, not spreading to form a wide canopy; bark greyish, nearly smooth; branchlets slender, glabrous, smooth and lenticellate. *Leaves* shortly petiolate, completely glabrous, petiole 1 – 5 mm long or sometimes subsessile, oblong or elliptic, occasionally loosely obovate, subcoriaceous; apex obtuse, acute, subacute or broadly acuminate; base cuneate, attenuate or convex; lamina 39 – 92 mm long, 10 – 41 mm wide, 3 – 5 nerved from the base. *Inflorescence* axillary cymes, in clusters; peduncle glabrous, rarely pubescent, sparsely if so, 2 – 6 mm long; pedicel glabrous, 2 – 4 mm long. *Flowers* 4-merous. *Calyx* 4-lobed, pale green; sepal lobes elongate-rhomboid, subobovate to broadly ovate, 2.5 – 4 mm long, ciliate along the margin. *Corolla* 4-lobed, greenish yellow, densely pubescent on the throat, 8 – 11.5 mm long, about 3 X as long as the calyx; petal

lobes ovate to linear lanceolate with acute apices. *Stamens* inserted at the mouth of the corolla tube, filament extremely short or sessile, glabrous; anthers oblong, 1.5 – 2 mm long, 0.8 – 1 mm wide, glabrous, deeply cordate. *Ovary* narrowly ovoid or subglobose, 2-celled, dense long hairs on the upper part of ovary and the lower part of short style (2.2 – 3 mm long); stigma capitate. *Fruit* large, hard, globose bluish green to orange yellow, 50 – 80 mm in diameter, many seeded, pulp edible. *Seeds* pale, more or less plano-convex, up to 25 mm across.

Etymology of specific epithet: Named after William Tyrer Gerrard; a botanist and avid plant collector in South Africa.

Vernacular names: Coastal monkey orange, False black monkey orange (English); Kusbosklapper, Basterswartklapper (Afrikaans); Umguluguhla, Mgulugulu (isiZulu);

Distribution and habitat: Kenya, Tanzania, Madagascar, Mozambique, Swaziland and South Africa. It occurs in mainly dry coastal and dune forests, sometimes on margins of wet coastal forests. Altitudinal range 0 – 500 m. Southern African distribution map is depicted in Figure 7.3.3.

Affinity: Within a broad southern African distribution, it is evolutionarily closely allied to *S. innocua*, *S. pungens*, *S. lucens* and *S. madagascariensis*. It is, however, mostly confused with *S. madagascariensis*; a taxon with which it was reduced to synonymy by Leeuwenberg (1969).

Notes: The reduction of *S. gerrardii* to a synonmy of *S. madagascariensis* by Leeuwenberg appears to have been an exercise in taxonomic convenience. To be fair, he recognised the "*very variable*" nature of *S. madagascariensis*, and

provided some details for distinguishing the component members of this complex. Future workers need to look further into the complex. His admission of the tentative nature of his arrangement stated that "…*it is difficult to conceive that all specimens* [of *S. madagascariensis*] *cited above belong to one single species*…" (Leeuwenberg 1969: 171). It is quite clear then that the complex comprises at least two taxa, one of which is *S. gerrardii*. The name *S. gerrardii* N.E.Br. is already well-known to botanists and ecologists in Africa, where the species occurs. The name has been applied by taxonomic practitioners in southern Africa to well-circumscribed groups of specimens that fit the above description, and it is the listed name in checklists. The name is therefore resurrected for conservation and pragmatic reasons.

Selected collections: SOUTH AFRICA: Stella Cemetery, Durban, *Adebowale 1* (UDW); Pigeon Valley Park, Durban, *Adebowale 11* (UDW); Burman Bush Nature Reserve, *Adebowale 17* (UDW); Mabibi Forest, *Edwards 2601* (NU); Manzengwenya area, *Ward 2199* (NH); North of Josini dam, *Ward 2252* (NH); Burman Bush Durban, *Bourquin s.n.* 22 December 1963*, NH 53743* (NH); Durban, Burman Bush Nature Reserve, *Nicholas & Ngwenya 2198* (PRE, possibly NH); Lower Tugela District, 15 miles north of Stanger, *Edwards 2831* (NU); Lower Tugela District, Hlogwene, *Moll 3334* (NU); Durban, *Huntley 96* (PRE); Lala Nek, *Strey 10314* (NU); between Kranskop and Mkandhla, *Bayer 845* (NU, PRE); Maputaland, Sodwana Nature Reserve, *Van Wyk BSA 2447* (PRU); Oribi Gorge Nature Reserve, along Gilbraltar road, *Abbott 8076* (NH); Jameson Drift, Tugela, *Bayer 506* (NU); Umbilo Road, *Rehmann 8148* (Z); Durban, *Burtt-Davy 2419*

(BM). MOZAMBIQUE: between Quissanga and Ingoane, *Barbosa 2050* (LISC, PRE).

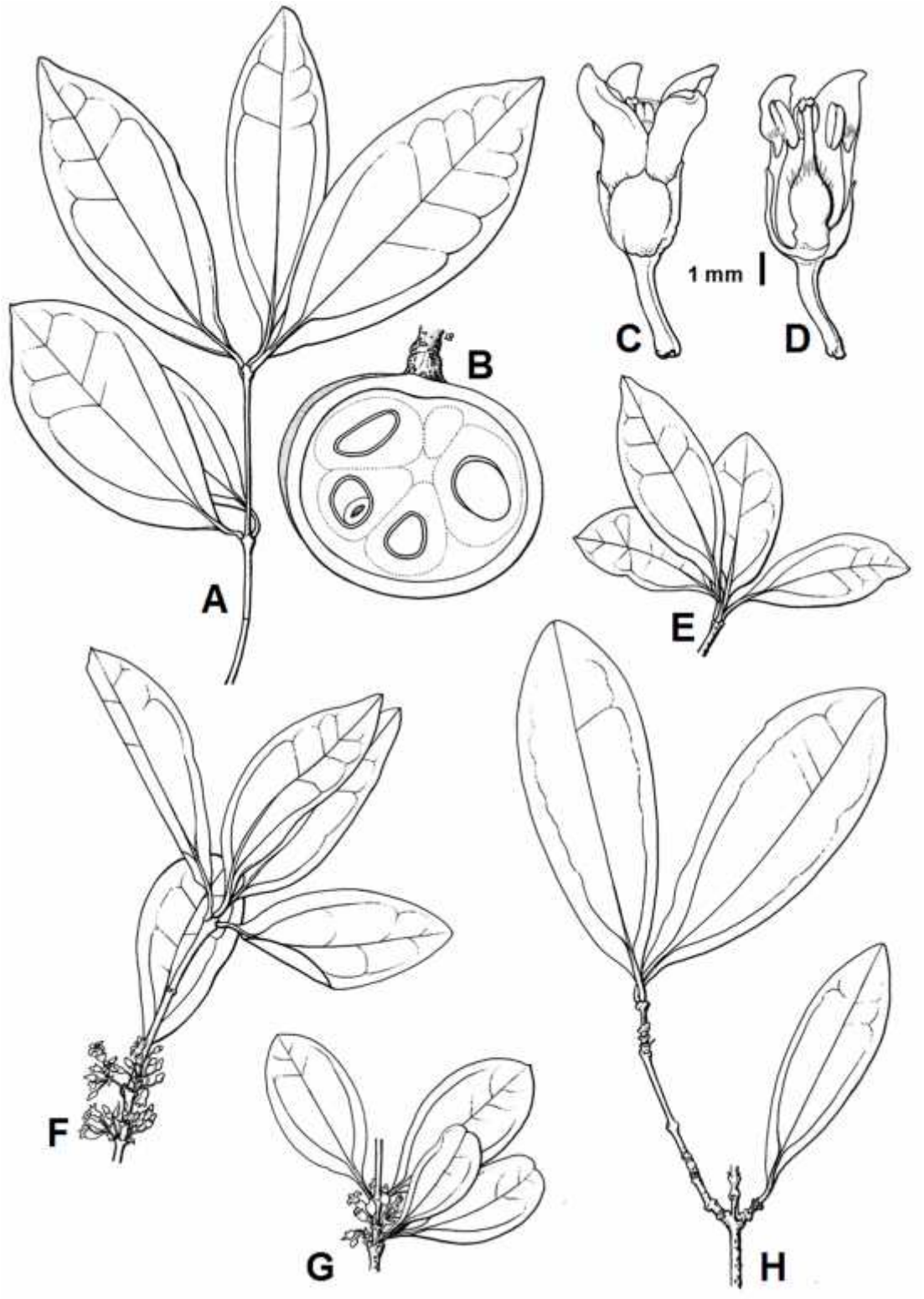

Figure 7.3.1 *S. gerrardii*: A & H = branchlet with leaves x ½, *Edwards 2601 & 1414* (NU); B = L.S. through a fruit x ¾, *Edwards 1414* (NU); C & D = flowers, *Huntley 96* (PRE); E = leaves x ¾, *Bourquin s.n.* NH53743 (NH); F & G = leaves and flowers in bud, x ¾, Wood 5624 (Type; PRE). Illustrations by Leslie Deysel.

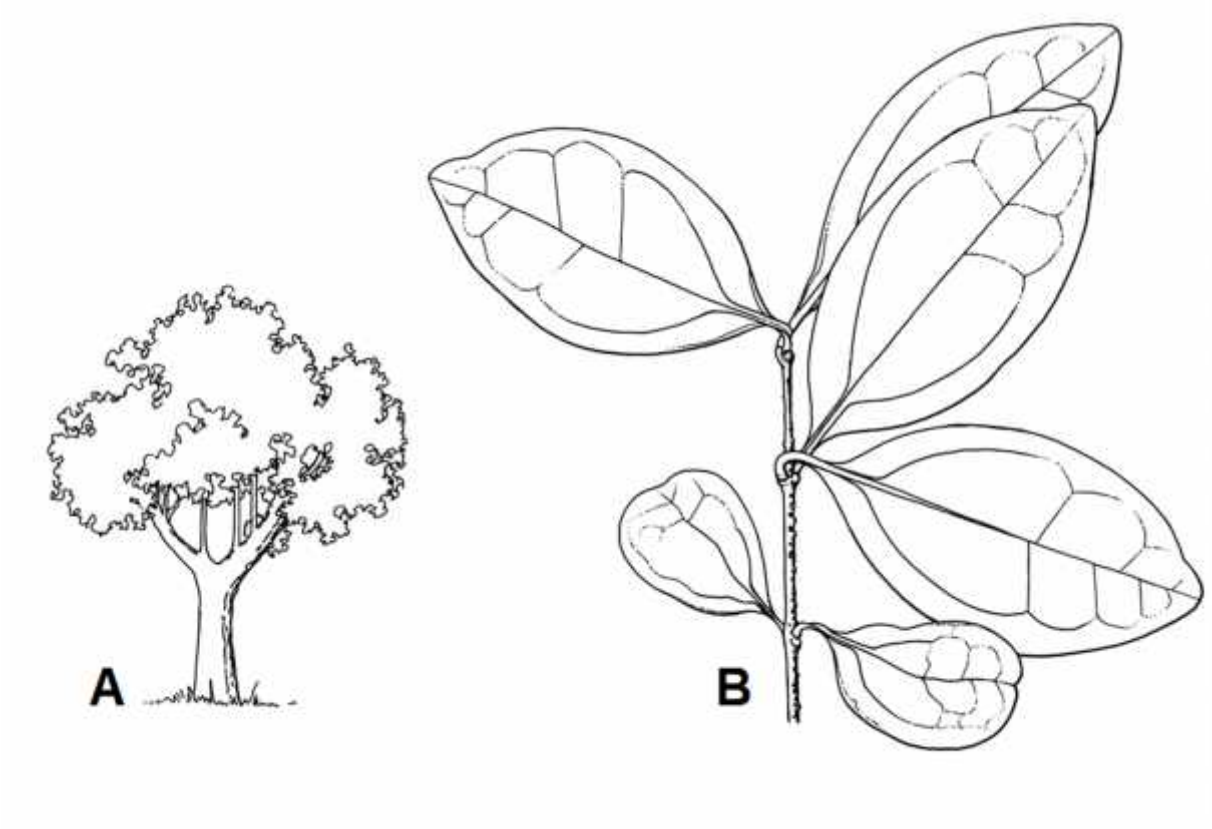

Figure 7.3.2 *S. gerrardii*: A = canopy shape drawing, not to scale; B = branchlet and leaves x ¾, *Nicholas & Ngwenya 2198* (NH). Illustrations by Leslie Deysel.

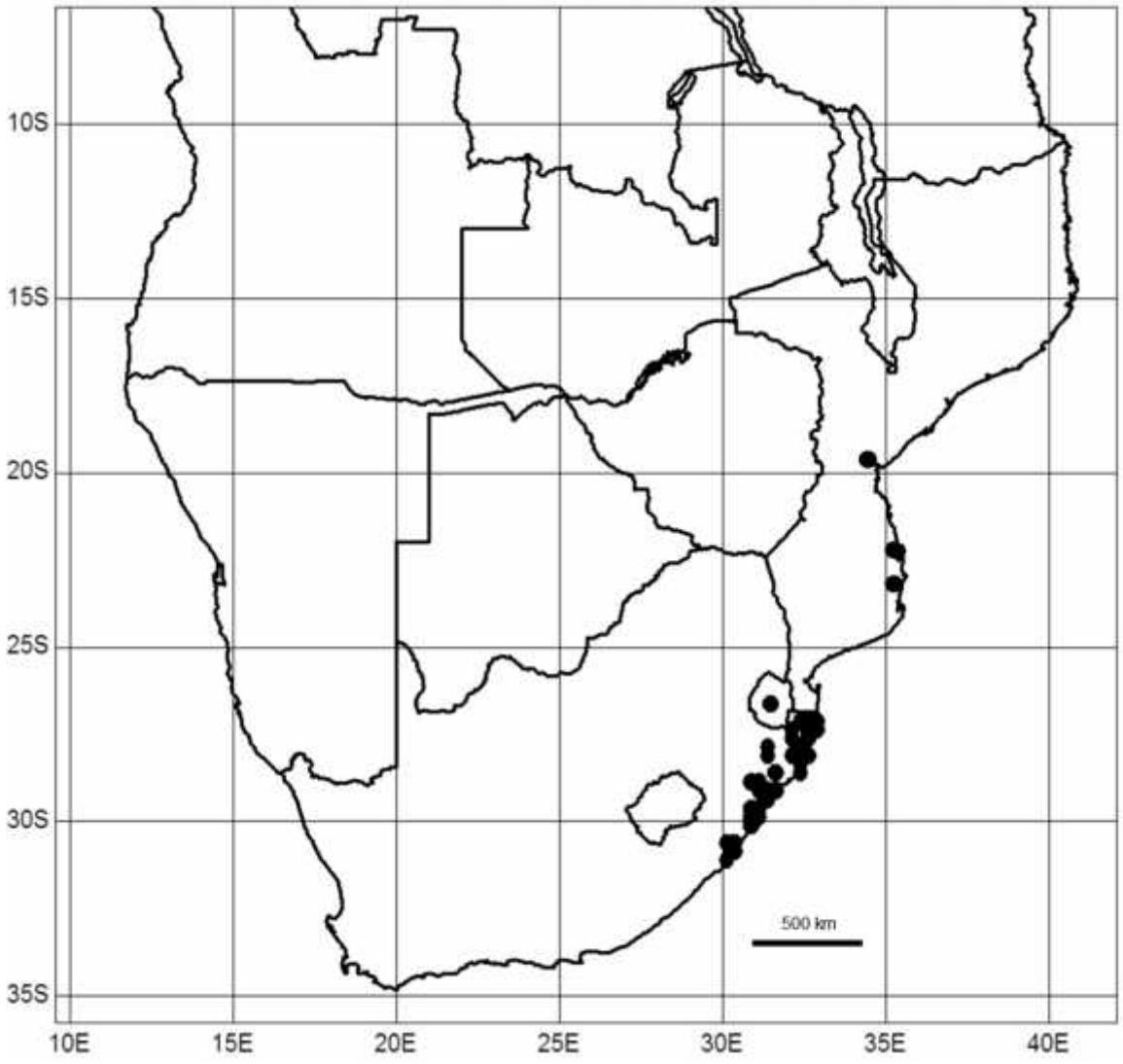

Figure 7.3.3: Distribution map of *Strychnos gerrardii* in sourhern Africa

4. *S. henningsii* Gilg in Engler, Bot. Jahrb. 17: 569. (1893); TR Sim, Forests and Forest Flora Col. Cape Good Hope 273 (1907) (with *S. utilis* Sim as synonym); Prain & Cummins, Fl. Cap. 4(1): 1052 (1909); Marloth, Fl. S. Afr. 3(1): 48. (1932); Verdoom, Bothalia 3: 587-588, f. 1. (1939); EA Bruce, Kew Bull. 1955: 127 (1955); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 32. (1960); Verdoorn, Fl. S. Afr. 26: 140, f. 18. 3. (1963); Leeuwenberg, Mended. Land. 69(1): 126 (1969). **Figure 7.4.1 page 227.** Type: S. Africa: Cape Province, Pondoland, near Umnonono, Bachmann 1745 (E, lectotype).

Heterotypic synonyms: *S. holstii* Gilg in Engler, Abh. Preuss. Akad. Wiss. 36 (1894) and in Engler, Pflanzenw. Ost-Afr. C: 310 (1895); Baker, Fl. Trop. Afr. 4(1): 529 (1903); Duvigneaud, Bull. Séanc. Inst. Roy. Col. Belg. 20: 585 (1949). Types: Tanzania: E. Usambara Mts., Mashewa, Holst 8833a (holotype not seen, destroyed in B, no isotypes seen) & Pare District, S. Pare Mts. Between Chôme and Vudea, Greenway 6562 (K, neotype, designated by E. A. Bruce in 1955, I.c.; iso-neotype: EA).

S. sennensis Bak., Kew Bull. 1895: 97 (1895) and in FI. Trop. Afr. 4(1): 529 (1903). Type: Mozambique: Valley of Zambesi R., opposite Senna, Kirk s.n. (K!, holotype).

S.pauciflora Gilg in Engler, Bot. Jahrb. 28: 121 (1899); Prain & Cummins, Ioc. cit. p. 1053. Type: Mozambique: Lourenço Marques, Schlechter 11682 (holotype destroyed in B; lectotype: BM!, isotype K!).

S. procera Gilg et Busse in Engler, Bot. Jahrb. 36: 97 (1905). Type: Tanzania: Lindi District, Island in Lake Lutamba, Busse 2506 (G, lectotype; isotypes: BM, BR, EA, G, HBG, WAG). Homotypic synonym: *S. holstii* var. *procera* (Gilg et Busse) Duvign., Bull. Inst. Roy. Col. Belg. 20: 587 (1949).

S. albersii Gilg et Busse, Ioc. cit. p. 99. Type: Tanzania: W. Usambara Mts., Kwai, Albers 380 (holotype not seen, destroyed in B; lectotype: EA, photograph in K!, negative 2346).

S. elliottii Gilg et Busse, Ioc. cit. Type: Kenya: near Nairobi, Guy S. Baker in coli. C.F. Elliot 176 (holotype destroyed in B; lectotype: K!).

S. myreioides S. Moore, Journ. Bot. 45: 52 (1907). Type: Uganda: Bonyoro District, Butiaba Piain, Bagshawe 841 (BM!, holotype; isotypes: ENT, US).

S. reticulata Burtt-Davy et Honoré, Kew Bull. 1932: 270 (1932). Type: Kenya: sin. loc, Conservator of Forests 40 (K!, holotype; isotypes: FHO, WAG). Homotypic synonym: *S. holstii* var. *reticulata* (Burtt-Davy et Honoré) Duvign., Ioc. cit. p. 587.

S. barbata Chiov., Fl. Somalia 2: 305 (1932) (as *Strichnos*). Type: Somalia: Oltregiuba, Uama Ido, Senni 262 (FI, holotype).

S. ligustroides Gossw. et Mendonça, Cart. Fitogeogr. Angola 120 (1939), no latin description provided; Duvigneaud, Bull. Soc. Roy. Bot. Belg. 85: 31 (1952). Type:

Angola: Luanda, Musseque de Viana, Gossweiler 10327 (COI, lectotype; isotypes: A, BM!, COI, K!, WAG).

S. holstii var. *reticulata* forma *condensata* Duvign., Bull. Inst. Roy. Col. Belg. 20: 588 (1949). Type: Congo: Léopoldville, Lufu R. Valley, Vivi, opposite Matadi, Duvigneaud 418 (BR, lectotype).

S. holstii vox. reticulata forma *laxiuscula* Duvign., Ioc. cit. Type: Congo: Katanga, Lukafu, near Lubumbashi, Duvigneaud 1248 (BR, lectotype; isotype: WAG).

Tree or shrub, 2 – 10 m high, with spreading rounded crown. Branchlets subquadrangular, pale, ashy or pale brown, swollen at the nodes with persistent petiole-bases; lenticels few and inconspicuous. *Leaves* glossy green, thinly coriaceous, variable in shape and size, ovate, ovate-lanceolate, elliptic, oblong-elliptic or rhombic, subsessile; lamina 20 – 90 mm long, 10 – 60 mm wide, glabrous on both surfaces; apex rounded, acute or acuminate; base rounded, cuneate or rarely subcordate, 3-nerved from the base, often with 1, (rarely 2) submarginal nerves on each side; tertiary nerves reticulate, prominent of both surfaces. *Inflorescence* axillary, sometimes terminal, congested usually compound cymes; peduncle short, 2 – 20 mm long, sparsely pubescent or glabrous. *Flowers* fragant, 5-merous, sometimes cleistogamous, sessile or with very short pedicels. *Calyx* pale green, connate at the base, sepal lobes broadly ovate-orbicular, length and width of similar dimension, about 1 – 1.5 mm, minutely ciliate. *Corolla* yellow or cream-coloured, subrotate, 2.2 – 3 X as long as the calyx, glabrous outside, slightly bearded within at the base of lobes; tube short; petal lobes thick, ovate-

deltoid. *Stamens* inserted at the mouth of corolla tube, just exserted, filament glabrous; anthers elliptic, 0.8 – 1.1 mm long, 0.5 – 0.8 mm wide, cordate at the base, glabrous. *Ovary* globose, glabrous, 2-celled; style short, 0.6 – 1.1 mm long; stigma capitate. *Fruit* ovoid, small, 8 – 15 mm in diameter, dark green turning yellow, orange or red with maturity, usually 1-seeded. *Seeds* ellipsoid, pale brown, glabrous, with an elongated, ridged groove down one side, like coffee-bean, 8 – 12 mm across.

Etymology of specific epithet: Named after the German mycologist, Paul Christoph Henning, (1841-1908); who worked at the Royal Botanic Gardens, Berlin-Dahlem.

Vernacular names: Red bitterberry, walking stick, Natal teak (English); Rooibitterbessie, Koffiehardepeer (Afrikaans); Umqalothi, Umdunye (isiZulu); Umnonono (isiXhosa).

Distribution and habitat: Sudan, Ethiopia, Somalia, Democratic Republic of the Congo, Angola, Uganda, Kenya, Tanzania, Zambia, Malawi, Zimbabwe, Mozambique, Swaziland, Madagascar and South Africa. It is a forest species occuring in bushveld and dry areas along river courses. Altitudinal range 0 – 2000 m. Southern African distribution map is depicted in Figure 7.4.2.

Affinity: It is closely allied to *S. mitis* in terms of the habit, inflorescences and external floral features. Phylogenetically, it is closely allied to *S. angolensis*, as co-members of section *Breviflorae*.

Notes: Leaves of *S. henningsii* are quite variable, which sometimes results in misidentification and confusion with *S. decussata*. In certain localities reproduction by cleistogamy has been observed.

Selected collections:SOUTH AFRICA: KZN, Umhlanga, Hawaan Forest Nature Reserve, *Adebowale 20* (UDW); KZN, Hluhluwe Game Reserve, *Ward 3927* (NU); North east of IsiZululand, Ingwavuma District, *Tinley 892* (NU); KZN, Kosi Bay, Maputaland, *Crouch 972* (NU); KZN, Port Shepstone, Oribi Nature Reserve, Hoope Falls trails, on river bank, *Abbott 7155* (NH); Eastern Cape, Kei road, *Nicholson s.n.* 16 May 1972 (GRA); Eastern Cape, Komgha, *Flanagan 1102* (BM, BOL, FHO, GRA, K); E. Cape, Pirie forest, King Williamstown, *Dold 1752* (GRA); Limpopo, Woodbush Forest *Botha PRF2908* (PRF); Koedoeks Bush, 30 km from Elandshoek Station, *Johnstone PRF1478* (PRF); Kruger National Park, Tialbye, *van der Schijff 4210* (B, K, LISC, MO); Mkuze Game Reserve, Ubombo District, *Ward 3610* (NH); Mkuze Poort, Ubombo District, *Ward 4069* (WAG); Hlabisa, *Gerstner 3849* (B, EA, K, SRGH); near Durban, Tugela River, *Gerrard & M'Ken 1917* (BM, K); Umtumengwana Forest, Wakeni, *Forest Dept. 7255* (K, PRF); Eastern Cape, Kologha, Stutterheim, East London Division, *Hutchins 2884* (K, NBG); Eastern Cape, Willowvale, *Acocks 12275* (PRE); E. Cape, Peri Forest, near King Williamstown, *Galpin 5908* (PRE); ibid., *Elliot 979* (K); East London, *Sim 2172* (BOL, PRE). MOZAMBIQUE: Inhaca island, 23 miles East of Laurenco Marques, *Mogg 27208* (K), *27431, 27696 and 28294,* (J, K); Coastal Forest at Cape Inhaca, near light house, *Mauve & Verdoorn 39* sheets I & II (J); Mocuba District, *Faulkner 55* (K); District of Manica et Sofala, *Muller & Gordon 1328A* (K); Nampula Province, Moma District *Fourie 1363* (GRA). SWAZILAND: Hlatikulu

Forest, *Boocock PRF 5318* (PRF); Murray's Farm, Ubombo Mountains, *Miller 8/49* (BM, FHO). ZIMBABWE: Mangazi River Valley, Meisetter District, *Goldsmith 82/62* (K, LISC, PRE, SRGH); Mtilikwe River, Bangara Falls, Fort Victoria District, *Wild 4374* (K, MO, PRE, SRGH); Sabi River, Ndanga District, *Hall 30* (NBG); Upungure, Chipinga District, *Farrell 199* (PRE, SRGH); Upper Msaswi River, Chipinga District, *Mowbray 22* (K, PRE, SRGH); Sabi-Lundi Rivers, Junction, Ndanga District, *Chase 2259* (BM, PRE, SRGH); ibid., *Wild 3363* (BR, K, PRE, S).

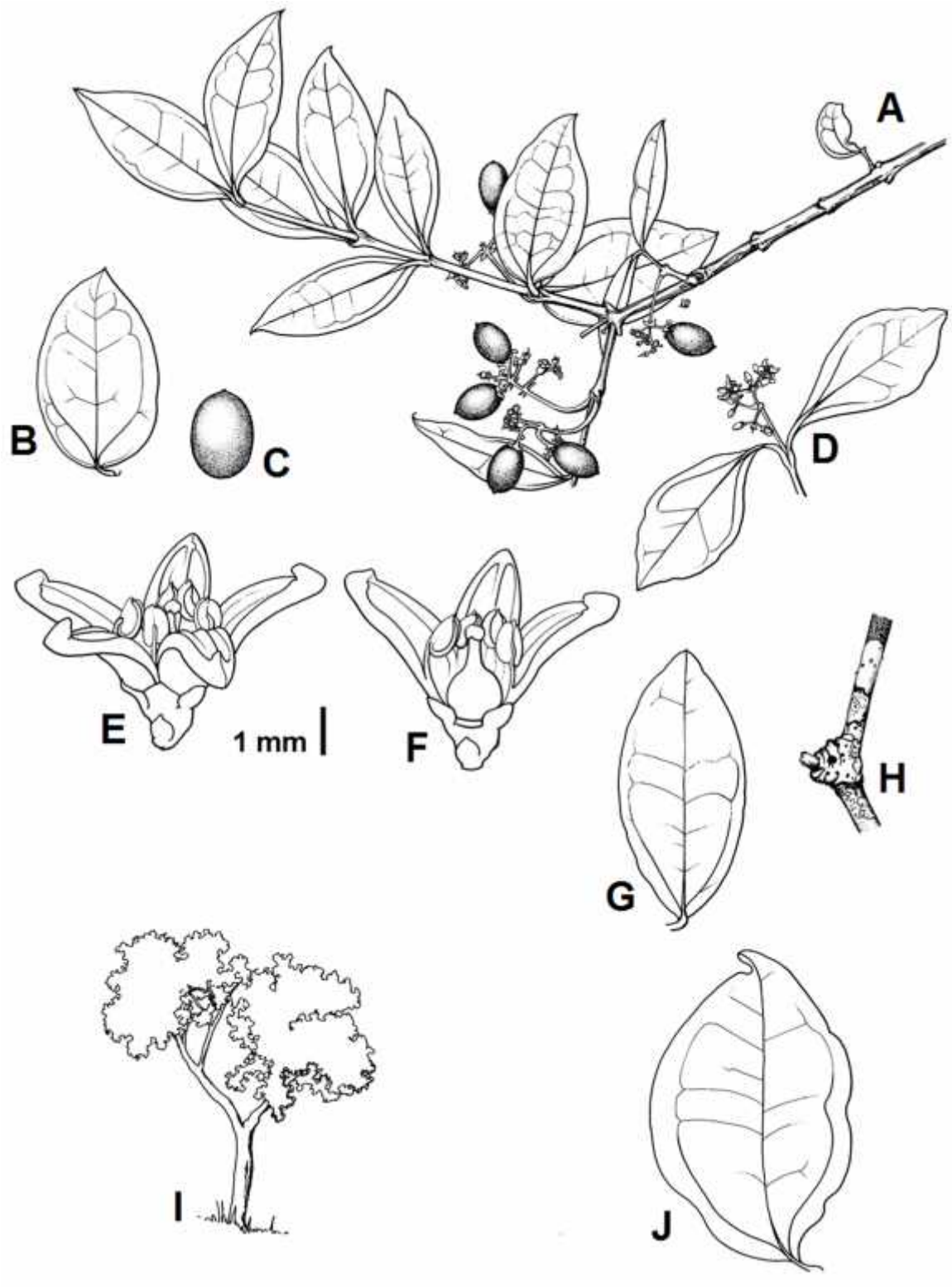

Figure 7.4.1 *S. henningsii*: A = branchlet with leaves & fruits x ½, *Tinley 892* (NU); B = leaf x ½, *Ward 3927* (NU); G = leaf x ¾ *Crouch 972* (NU); J = leaf x ¾, *Strey 8986* (PRE); C = fruit x ½, *Ward 3927* (NU); D = leaves and inflorescence x ½, *Crouch 972* (NU); E & F = flowers, *Abbott 7155* (NH); H = small branchlet with lichen cover x ¾, *Crouch 972* (NU); I = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

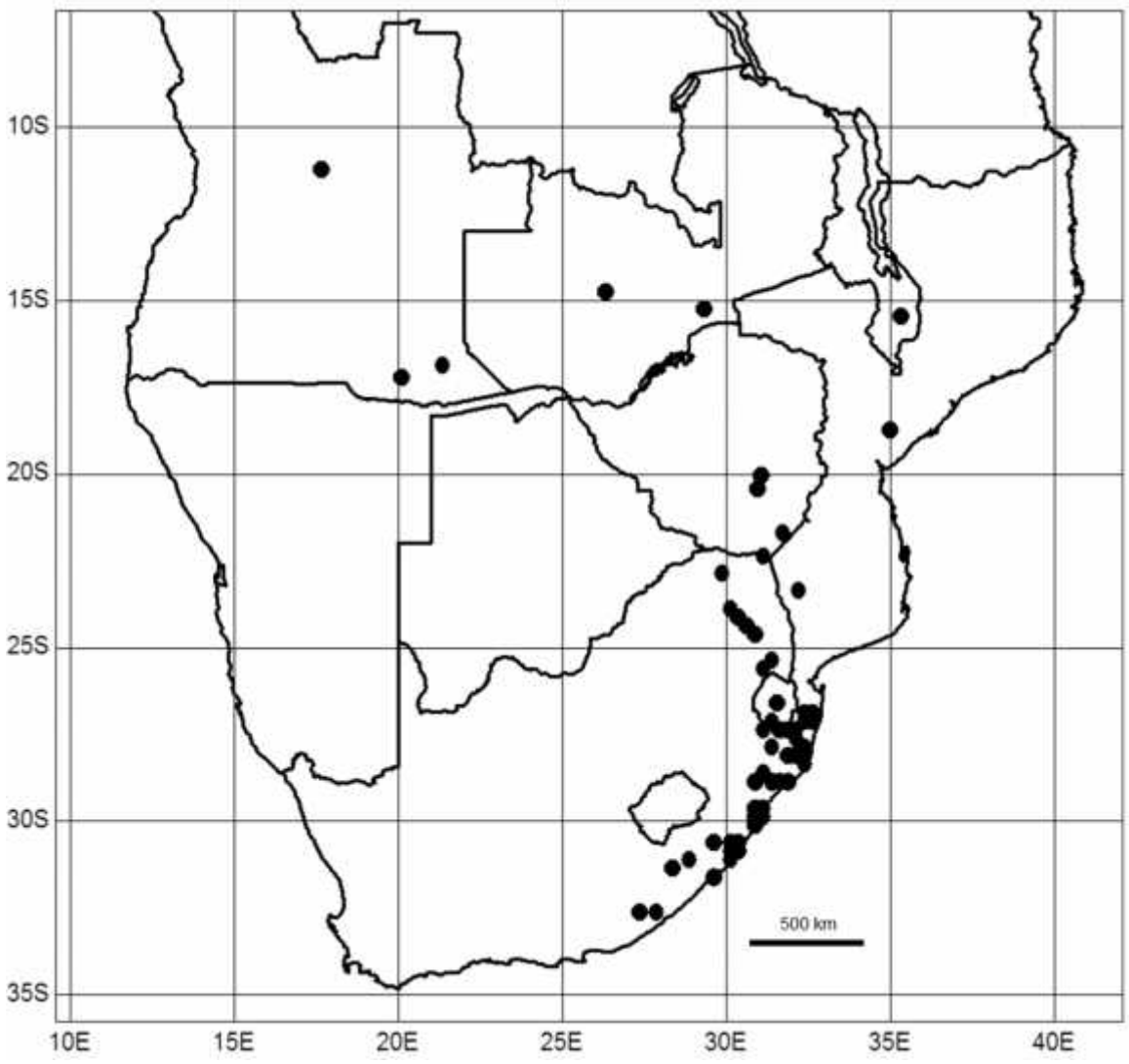

Figure 7.4.2: Distribution map of *Strychnos henningsii* in southern Africa

5. *S. innocua* Del., Cent. Pl. Méroë 53. (1826) = in Calliaud, Voyage à Méroé 4: 343 (1827); Mérat & De Lens, Diet. Mat. Med. 6: 556. (1834); De Candolle, Prod. 9: 17. (1845); Baker, Fl. Trop. Afr. 4(1): 532. (1903); Bullock & Bruce, Kew Bull. 1938: 46 (1938), partly (excluding synonyms *S. unguacha* var. *refusa* Chiov., *S. lokua* A. Rich., and other synonyms moved to *S. madagascariensis)*; Chevalier, Rev. Bot. Appliq. 27: 360 (1947); Aubréville, Fl. Soud.-Guin. 440 (1950), partly (excluding synnonyms *S. burtonii*); Bruce & Lewis, Kew Bull. 1956: 270 (1956) and Fl. Trop. E. Afr. Loganiaceae 25 (1960), partly (as for subsp. *innocua*); Onochie & Leeuwenberg, Fl. W. Trop. Afr. 2nd ed. 2: 496 (1963); Verdoorn, Fl. S. Afr. 26: 144 (1963); Leeuwenberg, Mended. Landb. 69(1): 138 (1969). **Figure 7.5.1 page 235.** Types: Ethiopia: Quamamyl, Calliaud s.n. (not seen); Ethiopia: Tigré, Tacazze R. Valley, Schimper 1817 (P, neotype: isoneotypes: BM, BR, Fl, G, HAL, K, L, LE, M, MO, P, S, UPS, W).

Heterotypic synonyms: *S. unguacha* A. Rich., Voy. Abyss. Bot. Atlas t. 73 (1847) and Tent. Fl. Abyss. 2: 52 (1851); Bentham, Journ. Linn. Soc. 1: 103 (1856); Gilg in Engler, Bot. Jahrb. 17: 562 (1893); Baker, Fl. Trop. Afr. 4(1): 534 (1903).

Homotypic synonyms: *S. unguacha* var. *typica* Gilg, Ioc. cit. p. 563. *S. simiarum* (Höchst.) Gilg ex A. Chev., Rev. Bot. Appliq. 27: 362 (1947).

S. innocua var. *pubescens* Solered. in Engler, Bot. Jahrb. 17: 556 (1893); as var. of subsp. *innocua:* Bruce & Lewis, Ioc. cit. p. 271 and Ioc. cit. p. 26; Onochie & Leeuwenberg, Ioc. cit. p. 41. Type: Nigeria: Nupe, Barter 1160 (holotype not seen, destroyed in B; lectotype: K!; isotype seen: P).

Homotypic synonyms: *S. unguacha* var. *pubescens* (Solered.) Gilg, Ioc. cit. p. 565. *S. triclisioides* Bak., Kew Bull. 1895: 98 (1895) and in Fl. Trop. Afr. 4(1): 533 (1903); Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 22 (1931).

S. *unguacha* var. *dschurica* Gilg, Ioc. cit. p. 565. Type: Sudan: Djurland, near Wau, Schweinfurth 1672 (holotype not seen, destroyed in B; lectotype: K!; isotype: P). Homotypic synonyms: *S. dschurica* (Gilg) Gilg in Engler, Bot. Jahrb. 36: 92 (1905); Chevalier, Et. Fl. Afr. Centr. Fr. 1: 203 (1913) and Expl. Bot. Afr. Occ. Fr. 443 (1920) (as *dshuricd*). *S. penduliflora* Bak. in Fl. Trop. Afr. 4(1): 531 (1903).

S. unguacha var. *grandifolia* Gilg in Engler, Bot. Jahrb. 17: 564 (1893). Type: Sudan: Equatoria Province, Djurland, Seriba, Kurshook Ali, Schweinfurth 1719 (K!, lectotype; isotype: P!). Homotypic synonym: *S. xerophila* Bak., Kew Bull. 1895: 98 (1895) and Fl. Trop. Afr. 4(1): 534 (1903).

S. unguacha var. *microcarpa* Gilg, Ioc. cit. p. 564. Type: Sudan: Bongoland, Seriba Ghattas, near Tondi, Addai, Schweinfurth 1432 (holotype not seen, destroyed in B; lectotype: K).

S. unguacha var. *steudneri* Gilg, Ioc. cit. p. 563. Type: Sudan: Bongoland, Seriba Gir, Schweinfurth 1412 (K, lectotype).

S. fischeri Gilg, Ioc. cit. p. 565; Baker in Fl. Trop. Afr. 4(1): 535 (1903). Type: Tanzania: Shinyanga District, Usule, Fischer 300 (holotype not seen, destroyed in B; no isotype seen).

S. alnifolia Bak., Kew Bull. 1895: 150 (1895) and Ioc. cit. p. 532. Type: Nigeria: Interior, Western Lagos, Rowland anno 1893 (K!, holotype).

S. unguacha var. *polyantha* Gilg in Engler, Bot. Jahrb. 30: 374 (1901). Type: Tanzania: Mbeya District, near Kananda, Goetze 1436 (holotype not seen, destroyed in B; lectotype: G; other isotypes: A, BM!, BR, L, P!).

S. unguacha var. *obovata* De Wild., Ann. Mus. Congo Sér. 4. 1: 98 (1903). Type: Congo: Katanga, Lukafu, Verdick 3 Aug. 1899 (BR, holotype).

S. *huillensis* Gilg et Busse in Engler, Bot. Jahrb. 36: 104 (1905). Type: Angola: Huila, Dekindt 6 a (E, lectotype).

Deciduous shrubs or small, often much-branched, trees, 2 – 12 m high. Bark pale grey or brownish, nearly smooth, powdery, flaking near the base of trunk. Branches, grey-brown, lenticellate or not; branchlets glabrous or pubescent. *Leaves* subsessile or shortly petiolate, glabrous or pubescent; petiole 2 – 7 mm long; lamina coriaceous, matt or dull, glaucous, with pale green reticulate veins on both surfaces, obovate, elliptic, or narrowly obovate, 65 – 135 mm long, 32 – 60 mm wide; apex rounded or subacute; base cuneate or less often rounded; 3 – 7 nerved from or above the base, secondary veins distinct; tertiary nerves reticulate and distinctly prominent on both surfaces. *Inflorescences* axillary cymes, usually several together, very short, few-flowered; peduncle 5 – 12 mm long, pubescent to nearly glabrous; pedicels very short, pubescent. Bracts small, sepal-like, nearly glabrous and sometimes with large basal colleters. *Flowers* 4-merous. *Calyx* pale

green, free, subequal, the inner slightly smaller, ovate, broadly ovate, or suborbicular, 0.8 – 1.5 x as long as wide, 1.7 – 3.5 mm long, 1.5 – 2.5 mm wide, rounded at the apex, ciliate, glabrous or pubescent without, glabrous within. *Corolla* greenish, white or yellowish; 2 – 3.5 x as long as the calyx, glabrous outside, a ring of white lanate hairs in the throat and on the inner base of the lobes; lobes thick, narrowly triangular, acute or subacute. *Stamens* inserted at the mouth of the corolla tube; filaments extremely short or non-existent, glabrous; anthers oblong, about twice as long as wide, 1.2 – 2 mm long, 0.6 – 1 mm wide, glabrous, deeply cordate almost sagittate at the base. *Ovary* narrowly ovoid to oblong often with a disk-like base, pilose in the upper region, otherwise glabrous, 2-celled; style thick, 3 – 4.5 mm long, hairy at the base like the ovary at the apex; stigma capitate. *Fruit* large, hard, globose, orange or yellow, nearly mature bluish-green, 40 – 75 mm in diameter, many seeded (8 – 50 seeds); rind granular, skin slightly shining; pulp orange, edible. *Seeds* flattened or not, obliquely ovate, elliptic, or tetrahedral, 15 – 18 mm across.

Etymology of specific epithet: The specific epithet means harmless, unharmed, lacking poisonous/harmful properties.

Vernacular names: Monkey orange, dull-leaved *Strychnos*, wild orange (English); Umquaqua (Chindau); Mkwakwa, mgulungungulu, mtonga (Swahili);

Distribution and habitat: Guinea, Mali, Côte d'Ivoire, Ghana, Burkina Faso, Togo, Benin Republic, Niger, Nigeria, Cameroon, Central African Republic, Chad, Sudan, Ethiopia, Democratic Republic of the Congo, Angola, Rwanda, Burundi, Uganda, Tanzania, Zambia, Malawi Zimbabwe and Mozambique. It occurs in open

deciduous woodlands and on rocky koppies. Altitudinal range 0 – 1600 m. Southern African distribution map is depicted in Figure 7.5.2.

Affinity: It is closely allied morphologically, geographically and phylogenetically to *S. madagascariensis* from which it can be distinguished by the absence of dwarf lateral shoots and the presence of prominent reticulate venation on both surfaces of the leaves.

Notes: The assignment of distribution for *S. innocua* has been somewhat affected by confusion caused by its close affinity with *S. madagascariensis*. Bullock and Bruce (1938) suggested that the species occurs in South Africa, citing a number of specimens from the Potgietersrus and Zoutpansberg areas in Limpopo Province, and the Tugela area in KwaZulu-Natal, which they classified as belonging to this species. A closer inspection of these specimens and several others collected around the same locality by IC Verdoorn, AJM Leeuwenberg and the current author [e.g. *Galpin 11624* (BOL, K, PRE); *Obermeijer, Schweickerdt & Verdoorn 161* (PRE); *Gerrard 1660* (PRE, K)] indicate that they are *S. madagascriensis* specimens with a few even tending towards *S. gerrardii*. Thus my view is that while the existence of *S. innocua* in South Africa may not be ruled out, especially in the northern parts of Limpopo and Mpumalanga provinces, there are no specimens as yet in our collections to suggest the occurrence of this species in South Africa. Of note as well is the occasional fluctuation in the number of floral parts between 4 and 5, even on the same specimen, as shown by stamens and corolla of *Merello, Harder & Nkhoma 999* (PRE) which are 5-merous, while the calyx is 4-merous in some flowers. This may represent a transitional stage from 5- to 4-merous flowers in *Strychnos*.

Selected collections: TANZANIA: Mbeya, *Congdon s.n. 21 February 1988* (PRE, MO); Kigoma, Gombe National Park, *Gerean, Mbago, Kkajombo & Mpongo 6052* (PRE, MO). ZAMBIA: Mweru Wantipa National Park, *Merello, Harder & Nkhoma 999* (PRE); Ndola woodland, *Cottrell 53* (GRA); Solwezi, Lumwana Mining Company, at round about near construction site, *Burrows 10265* (BNRH). CAMEROON: Sonja boundary of Bénoué Reserve, *Leeuwenberg 7482* (PRE). ZIMBABWE: Central Umvukwe Mountains *Rodin 4430* (PRE); Msengesi Camp, Darwin District, *Whellan 941* (LISC, PRE); Lomagundi, *Jack 1243* (K, SRGH); ibid., *Eyles 5526* (SRGH), *5527* (SRGH); Lomagundi District, *Thornevill 1200* (SRGH); Msengaisi (?), *Jack 4032* (SRGH); Chipoli, Mazoe District, *Moubray SRGH 89321* (BR, K, LISC, S, SRGH); Mrewa District, *Moll 590* (K, WAG). MOZAMBIQUE: Zambezia, Namagoa Estate, Mocuba District, *Faulkner 214* (EA, K, PRE, S, SRGH).

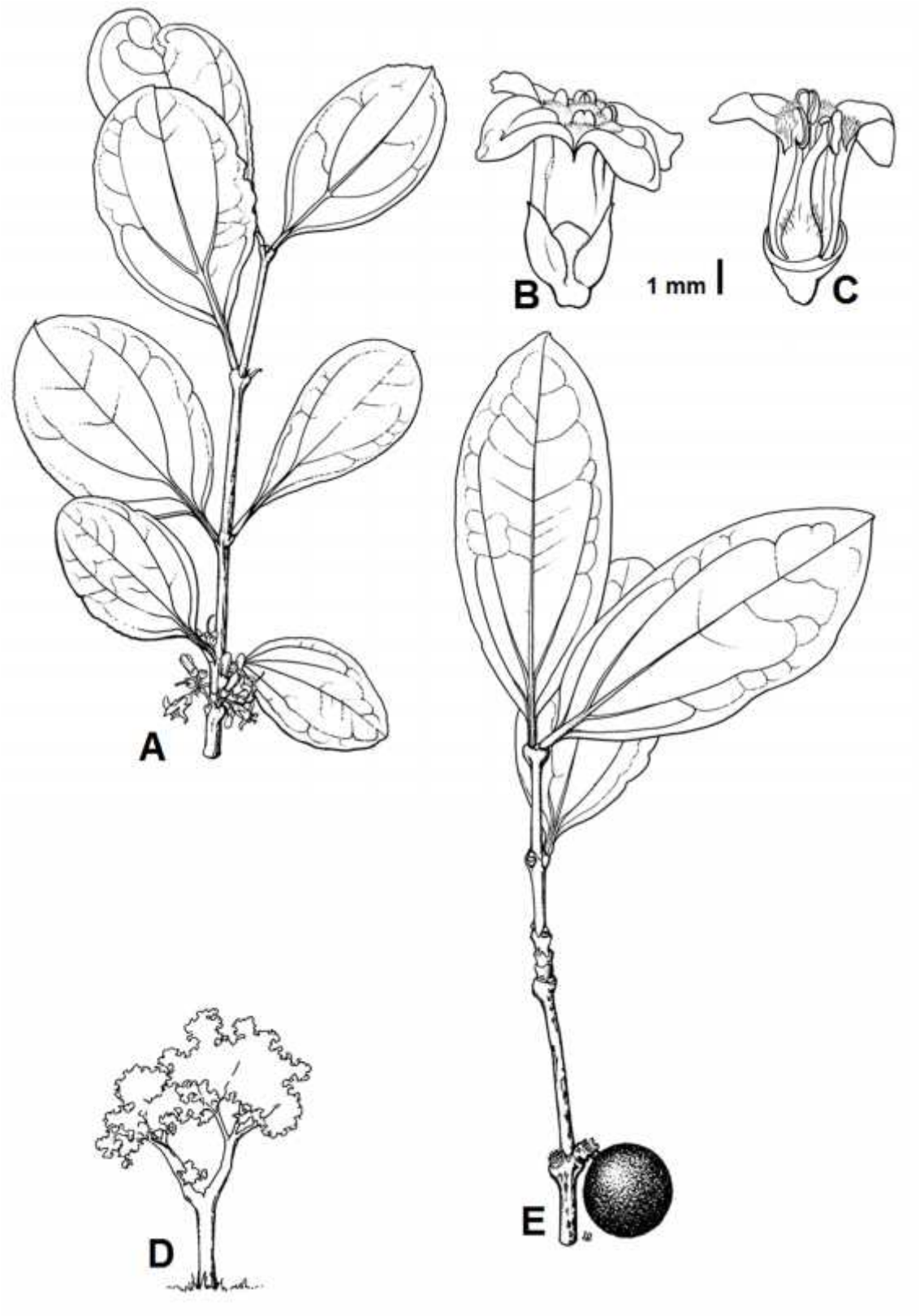

Figure 7.5.1 *S. innocua*: A = branchlet with leaves and flower buds x ¾, *Van Wyk 1531* (PRU); B & C = flowers, *Van Wyk 1531* (PRU); D = canopy shape drawing, not to scale; E = branchlet with leaves and a fruit x ¾, *Congdon* s.n. (PRE). Illustrations by Leslie Deysel.

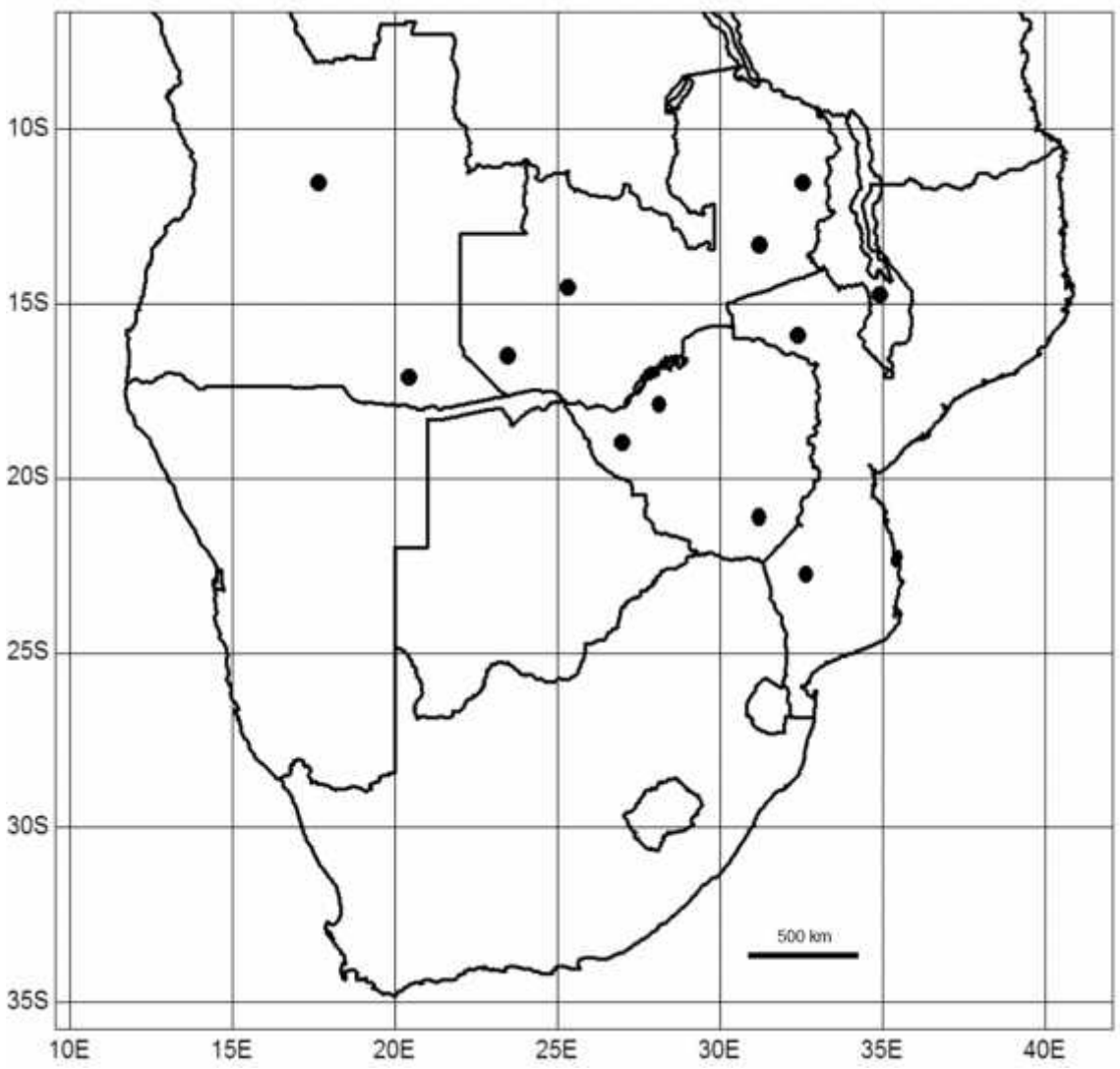

Figure 7.5.2: Distribution map of *Strychnos innocua* in southern Africa

6. *S. madagascariensis* Poir.

Lamarck, Encyc. 8: 696 (1808); Dubuisson, extract of Du Petit-Thouars in Desvaux, Journ. Bot. Paris 1: 250 (1809); Sprengel, Syst. 1: 672 (1825) (cites Du Petit-Thouars); G. Don, Gen. Syst. 4: 65 (1838); Spach, Veg. Phan. 8: 488 (1839) (cites Poiret); De Candolle, Prod. 9: 16 (1845); Jumelle & Perrier, An. Mus. Col. Marseille Sér. 2. 5: 398 (1907); Verdoorn, Fl. S. Afr. 26: 145 (1963); Leeuwenberg, Mended. Landb. 69(1): 160 (1969) excluding the following synonym: *S. innocua* subsp. *gerrardii* (N. E. Brown) Verdoorn. **Figure 7.6.1 page 244.** Type: Madagascar: Foulpointe, *Du Petit-Thouars s.n.* (P, holotype; photographs in PRE and WAG).

Heterotypic synonyms: *S*. *dysophylla* Benth., Journ. Linn. Soc. 1: 103 (1856); Baker, Fl. Trop. Afr. 4(1): 533 (1903); Prain & Cummins, Fl. Cap. 4(1): 1054 (1909); Bruce and Lewis, Ioc. cit. p. 273 and Ioc. cit. p. 27. Type: Mozambique: Delagoa Bay, Forbes 62 (K, holotype; isotype: P).

Homotypic synonyms : *S. randiaeformis* Baill., Bull. Mens. Soc. Linn. Paris 1: 246 (1880).

S. unguacha var. *dysophylla* (Benth.) Gilg in Engler, Bot. Jahrb. 17: 564 (1893); Schinz, Mém. Herb. Boissier 10: 56 (1900) (as *ungaschd*). *S. innocua* subsp. *dysophylla* (Benth.) Verdoorn, Bothalia 7: 12 (1958) and in Fl. S. Afr. 26: 145 (1963), partly (excluding. Repton 1882).

S. vacacoua Baill., Bull. Mens. Soc. Linn. Paris 1: 275 (1880); Jumelle & Perrier, Ioc. cit. Type: Madagascar: Antsingui Mts., near Diégo-Suarez, Bernier 260 (P! lectotype).

S. baroni Bak., Journ. Linn. Soc. 22: 504 (1887). Type: Central Madagascar: sin. loc, Baron 4648 (K!, holotype).

S. dysophylla subsp. *engleri* (Gilg) Bruce & Lewis Kew Bull. 1956: 275 (1956). Type: Portuguese East Africa.

S. engleri Gilg E.J. 17: 568 (1893): Die Pflanzenw. Ost-Afr. C: 310, t. 38 (1895); Fl.Trop. Afr. 4(1): 532 (1903): T.T.C.L.: 275 (1949).

S. wakefieldii Bak., Kew Bull. 1895: 98 (1895); Fl.Trop. Afr. 4(1): 532 (1903): Trees and Shrubs of Kenya Colony: 126 (1936); Check-lists of the Forest Trees and Shrubs of the British Empire, Tangayika Territory: 276 (1949). Type: Kenya, Mombasa (holotype: K!).

S. quaqua Gilg in Engler, Bot. Jahrb. 17: 567 (1893) and 32: 176 (1902) and 36: 101 (1905); Baker, Ioc. cit p. 531. Type: Mozambique: Quilimane, Stuhlmann 1041 (holotype not seen, destroyed in B, photographs seen in FHO and K; lectotype: HBG, photograph in K!, negative 2490).

S. unguacha var. *micrantha* Gilg in Engler, Bot. Jahrb. 17: 563 (1893). Type: Tanzania: Pangani, Stuhlmann 76 (holotype not seen, destroyed in B; no isotype seen); this specimen is also paratype of *S. behrensiana* Gilg et Busse).

S. burtoni Bak., Kew Bull. 1895: 98 (1895) and Fl. Trop. Afr. 4(1): 533 (1903). Type: Mozambique: Manica et Sofala, Chupanga, Kirk 368 (K!, lectotype, designated by Bruce & Lewis). Homotypic synonym: *S. innocua* subsp. *burtonii* (Bak.) Bruce & Lewis, Kew Bull. 1956: 272 (1956) and Fl. Trop. E. Afr. Loganiaceae 26 (1960); Verdoorn, Fl. S. Afr. 26: 145 (1963); Leeuwenberg, Act. Bot. Neerl. 14: 219 (1965).

S. mocquerysi Aug. D.C., Bull. Herb. Boissier Sér. 2. 1: 577 (1901). Type: Madagascar: Maroa, near Antongil Bay, Mocquerys 360 (G, lectotype; isotype: Z).

S. behrensiana Gilg et Busse in Engler, Bot. Jahrb. 32: 175 (1902) and 36: 100 (1905); Baker, Fl. Trop. Afr. 4(1): 531 (1903); De Wildeman, Mem. Inst. Roy. Col. Belg. 8(13): 28 (1946) (as *belviensiana).* Type: Tanzania: Sachsenwald, near Dar-es-Salaam, Busse 15 (LY, lectotype; isotype: G).

S. leiocarpa Gilg et Busse in Engler, Bot. Jahrb. 36: 103 (1905). Type: Tanzania: Lindi District, near Mtange, Busse 2458 (LY, lectotype; isotypes: BR, EA).

S. melonicarpa Gilg et Busse, Ioc. cit. p. 101, figure 2B. Type: Tanzania: Pangani District, near Mnyuzi, Busse 2266 (holotype not seen, destroyed in B; lectotype: LY; other isotypes seen: EA, P: fruit only).

S. pachyphylla Gilg et Busse, Ioc. cit. p. 96; Bruce & Lewis, FI. Trop. E. Afr. Loganiaceae 35 (1960). Type: Tanzania: W. Usambara Mts., Kwai, Eick 332 (holotype destroyed in B; no isotype seen).

S. polyphylla Gilg et Busse, Ioc. cit. p. 104. Type: Tanzania: Kilwa District, Matumbi Mountains, Busse 3058 (BM!, lectotype; isotypes: BR, EA, G, HBG, LY).

S. stenoneura Gilg et Busse, Ioc. cit. p. 103. Type: Tanzania: Lindi District, near Mayanga, Busse 2537 (HBG, lectotype; isotypes: BM!, BR, EA, LY, WAG).

S. innocua subsp. *burtonii* var. *glabra* Bruce et Lewis, Kew Bull. 1956: 273 (1956) and FI. Trop. E. Afr. Loganiaceae 26 (1960). Type: Tanzania: Tanga District, Kwamkembe-Pongwe, Greenway 4851 (EA, holotype; isotypes: FHO, K!).

Shrub or small, much-branched tree, 2 – 12 m high, deciduous, with widely spreading canopy. Bark mostly pale grey or greyish-white, nearly smooth. Branchlets lenticellate, glabrous to pubescent, thick and bearing contracted lateral branchlets with congested prominent leaf scars. *Leaves* membranous to coriaceous, obovate, oblong, suborbicular or oblong elliptic, densely to sparsely pubescent on both surfaces or at least on the abaxial veins, subsessile, petiole usually under 3 mm long, rarely reaching 5 mm or more, petiole glabrous or pubescent; lamina variable in size, 33 – 86 mm long, 16 – 50 mm wide; apex rounded, acute, subacute or retuse; base cuneate, subcuneate, convex or rounded; 3 – 5 nerved from or just above the base, distinct secondary veins

present, faint submarginal vein pair present; tertiary venation reticulate, not or slightly prominent on adaxial surface. *Inflorescences* axillary cyme, usually in clusters, very short and nearly fasciculate, few-flowered. Peduncle, branches and pedicels very short, less than 3 mm long, pubescent or glabrous. *Flowers* 4-merous. *Calyx* pale green, free or nearly so, subequal, the inner slightly smaller, ovate, broadly ovate, suborbicular or smoothly rhomboid, 2.5 – 3.5 mm long, 2 – 3 mm wide, rounded or obtuse at the apex, ciliate. *Corolla* whitish or yellowish, brush-like ring of white lanate hairs in the throat and just at the base of the lobes, 2.5 – 3.3 x as long as the calyx; tube cylindrical or nearly so; lobes thick, narrowly triangular, acute or subacute, spreading. *Stamens* inserted; filaments extremely short, inserted at the mouth of the corolla tube, glabrous; anthers more or less sessile, oblong, 1.2 – 2 mm long, 0.8 – 1 mm wide, glabrous, deeply cordate at the base. *Ovary* elongate-ovoid, or oblong, hirsute at the apex, rest further glabrous, often with a disk-like base, 2-celled; style thick 1.5 – 4 mm long, base hairy like the ovary at the apex; stigma capitate. *Fruit* large, hard, orange or yellow, nearly mature bluish-green, globose, 50 – 100 mm in diameter, many seeded 15 – 75 seeds, skin, slightly shining. Pulp orange, slimy, edible. *Seeds*, flattened or not, elliptic, or tetrahedral, 10 – 25 mm across.

Etymology of specific epithet: first described from Madagascar.

Vernacular names: Black monkey orange (English); Swartklapper, botterklapper (Afrikaans); morapa (Sotho); mogorwagorwana (Tswana); umKwakwa, umGluguza (isiZulu).

Distribution and habitat: Somalia, Kenya, Tanzania, Zambia, Malawi, Zimbabwe, Mozambique, Botswana, Swaziland, Madagascar and South Africa. In open

deciduous woodlands, rocky koppies, coastal bush; altitudinal range 0 – 1800 m. Southern African distribution map is depicted in Figure 7.6.2.

Affinity: closely related to *S. innocua* (in particular) and *S. gerrardii*.

Notes: The conception of *S. madagascariensis* here excludes synonyms applied to *S. gerrardii* in this treatment to create a more recognisable and coherent species. All other synonyms recognised by Leeuwenberg (1969) have been retained.

Selected collections: SOUTH AFRICA: Transvaal, *Fourie M132* (PRE); Tugela valley, Krantzkop, *Dyer 4347* Sheets I & II (PRE); Mpumalanga, Loskop Dam Nature Reserve, *Adebowale 73* (UDW); Shongweni Nature Reserve, *Adebowale 28* (UDW); Tugela, *Gerrard 1660* (PRE, K); Lower Tugela Valley, below Maqumbi, *Edwards 3060* (NU); Nkandla District, half mile North west of Middledrift, *Edwards 2826* (NU); Shongweni Dam, Pinetown, *Edwards 2862* (NU); Kruger National Park, *Marais 910* (PRE); IsiZululand, Lower Umfolozi District, from Empangeni, on Eshowe road, *Ward 4046* (PRE, NU); Mpumalanga, Komatipoort, *Coetzee 1356* (PRE); Middleburg, Loskop Dam Nature Reserve, *Mogg 31047* (PRE); Tembe Elephant Park, *Ward 1050* (NH); Ncemane District, *Ward 16* (NU); Umbombo District, Mkuzi Games Reserve, *Ward 4079* (NU); Maputaland, Lake Amanzimnyama, *Felton & Thornhill 177* (NH, PRU); Mthonjaneni District, Mhlatuzi Valley Nkwenkwe, *Ward 4042* (NU); Hluhluwe Games Reserve, IsiZululand, *Ward 2572* (NU); Umfolozi District, Umfolozi Game Reserve, *Ward 4057* (NU); Mtunzini District, IsiZululand, *Guy & Ward 61* (NU); Limpopo, Waterberg, Mooismersiesfontein Farm, top of Krantzes overlooking Sterkstroom, *Glen 2094*

(PRE). SWAZILAND: Lebombo Mountains, Umbuluzi Gorge, North bank of Mbuluzi River, *Culverwell 1227* (PRE). MOZAMBIQUE: North-East of Chibuto 240 km from Maputo & 65 km from Xai-Xai, *Johnson & Blood 701* (GRA); Mozambique: Zambesia, 220 km E of Quelimane, *Dold 3138* (GRA). ZIMBABWE: Darwin, *Chorley 6051* (SRGH); Chipoli, Mazoe District, *Moubray SRGH 8777 A* (K, LISC, PRE, SRGH); Victoria Falls, *Armitage 46/60* (SRGH); ibid., *Sim 19283* partly (PRE); Gwampa Forest Reserve, Nkai District, *Armitage 28/60* (SRGH); Kariangwe, Sebungwe District, *Lovemore 193* (PRE, SRGH); Umniati River, Hartley District, Gowe, *Whellan 429* (SRGH); Umtali District, *Chase 6256* (K, PRE, SRGH), *6260* (K, LISC, PRE, SRGH); Umtali Commonage, *Chase s.n. PRE 29242* (PRE); Buhera District, *Davies 626* (SRGH); Hot Springs, Melsetter District, *Chase 4705* (BM, COI, LISC, MO, PRE, SRGH).

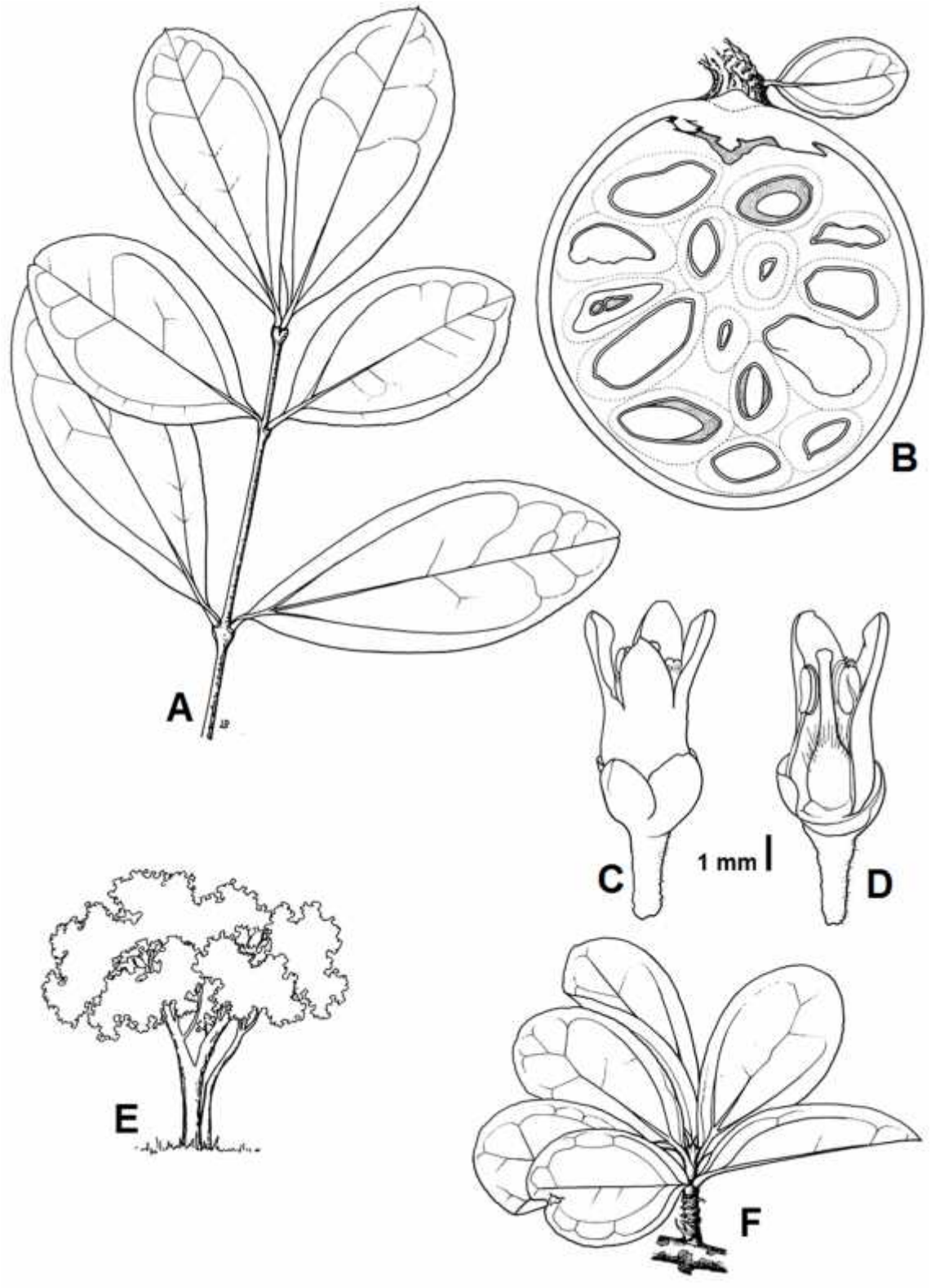

Figure 7.6.1. *S. madagascariensis*: A = branchlet with leaves x ½, *Edwards 2826* (NU); B = cross section of fruit x ¾, *Ward 4079* (NU); C & D = flowers *Mogg 31047* (J); E = canopy shape drawing, not to scale; F = cluster of leaves on a shortened branchlet x ½, *Ward 4079* (NU). Illustrations by Leslie Deysel.

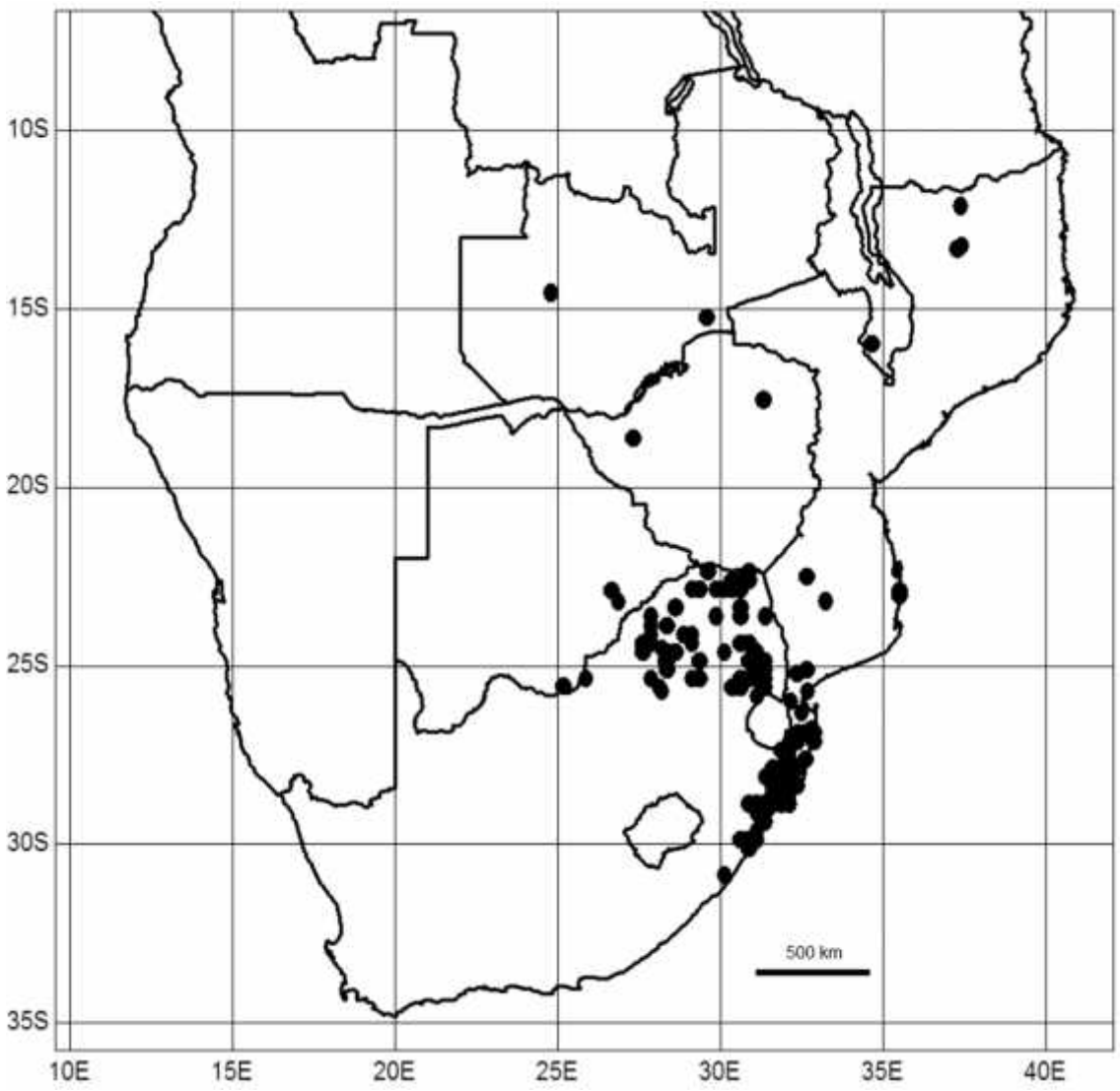

Figure 7.6.2: Distribution map of *Strychnos madagascariensis* in southern Africa

7. *S. mitis* S. Moore, Journ. Linn. Soc. 40: 146 (1911); Duvigneaud, Bull. Soc. Roy. Bot. Belg. 85: 23 (1952) and 86: 106 (1953); Bruce & Lewis, FI. Trop. E. Afr. Loganiaceae 21 (1960); Verdoorn, Fl. S. Afr. 26: 141 (1963); Onochie & Leeuwenberg, Fl. W. Trop. Afr. 2nd ed. 2: 43 (1963), partly (excl. Scott Elliot 5418); Verdoorn, Fl. S. Afr. 26: 141 (1963); Leeuwenberg, Meded. Landb. Wag. 69(1): 190 (1969). **Figure 7.7.1 page 250.** Type: Zimbabwe: Chirinda Forest, Swynnerton 17a (BM!, lectotype; isotypes: K!, Z).

Heterotypic synonym: *S. adolphi-frederici* Gilg in Mildbraed, Wiss. Ergebn. Deutsch. Zentr.-Afr. Exped. 1907-08. 2: 531 (1914). Type: Congo: Orientale, Semliki R. Valley, Mildbraed 1997 (holotype destroyed in B; lectotype: PRE!).

Tall, branched, evergreen tree with rounded crown, 6 – 40 m high (rarely shorter), without spines or tendrils. Bark smooth, grey or grey-brown. Branches and branchlets pale grey, lenticellate, ascending, glabrous or sometimes pubescent. *Leaves* shortly petiolate, 40 – 130 mm long, 17 – 50 mm wide, petiole 3 – 7 mm long, glabrous or with acarodomatia in the axils of abaxial nerves, lamina shining and dark green above, paler beneath, coriaceous (not thick), broadly to narrowly elliptic, oblong, or sometimes ovate or ovate-elliptic; apex acute to acuminate; base cuneate, subcuneate to obtuse; 5 – nerved, one pair of distinct secondary veins from about 10 mm above the base, and a faint submarginal pair from the base. *Inflorescence* both axillary and terminal compound cymes, dense, much shorter than the leaves. Peduncle, branches and bracts beneath pubescent. Peduncle usually short, 3 – 7 mm long; pedicels pubescent. *Flowers* 5 (4) -merous even within a single inflorescence (see note).

Calyx pale green to petaloid, connate at the base, broadly ovate or orbicular, 1.5 – 1.8 mm long, 1.5 – 1.8 mm wide, obtusely acute at the apex, ciliate, pubescent to glabrous outside, glabrous and with colleters at the base inside. *Corolla* cream, yellow, or green, 2 – 2.7 x as long as the calyx, rounded at the apex; tube shortly campanulate, densely bearded at the throat of tube or base of lobes, otherwise glabrous within; lobes thick at the apex, triangular to ovate. *Stamens* just exserted; filaments very short, glabrous, inserted at the base of the corolla tube; anthers oblong, 1 – 1.2 mm long, 0.6 – 0.9 mm wide, deeply cordate at the sparsely bearded base. *Ovary* ovoid-conical, glabrous, 2-celled; style 1 – 1.5 mm long; stigma capitate. *Fruit* small, soft, subglobose, yellow or orange, 10 – 20 mm in diameter, 1 – 2 seeded. *Seeds* pale ochraceous, subellipsoid, usually flattened at one side, not grooved; hilum conspicuous in the middle of the flattened side; up to 12 mm across.

Etymology of specific epithet: mild, gentle, bland, without spines an epithet which could be equally applied to most of the forest species of *Strychnos*.

Vernacular names: Yellow Bitterberry (English); Geelbiterbessie (Afrikaans); munono, umphatsankosi (Swazi); Umnono, Umanono or Umqalothi (isiZulu).

Distribution and habitat: Sudan, Ethiopia, Democratic Republic of the Congo, Angola, Uganda, Kenya, Tanzania, Zimbabwe, Mozambique, Swaziland, South Africa and Comoro Islands. It occurs in upland and lowland rain forests, gallery forests, coastal bush and is also quite common along river courses. Altitudinal range 0 – 2300 m. Southern African distribution map is depicted in Figure 7.7.2.

Affinity: Resembles *S. henningsii* from which it can be distinguished by its generally larger leaves with decidedly more acuminate apex.

Notes: *S. mitis* closely resembles *S. mellodora* in terms of the size of flowers, leaf venation and habit. They, however, differ in that *S. mellodora* has glabrous anthers and are pseudomonomerous with a 1-celled ovary, while *S. mitis* has sparsely bearded anthers and a 2-celled (bicarpellate) ovary. The number of floral parts is usually five. In some specimens of *S. mitis* (e.g. *J.G Williams s.n. EAH 11028*; PRE & EA) however, the number may vary between four and five. One other observation of note is that I encountered only one natural population of *S. mitis* in South Africa during the course of field exploration. Given that most herbarium collection sites were close to a water body, the drying up of such water bodies could spell disaster for *S. mitis*. It appears that healthy populations exist in Kenya, Zimbabwe and Mozambique, where it shares similar habitat with *S. mellodora*.

Selected collections: SOUTH AFRICA: Vernon Crookes Games Reserve, *Adebowale 24;* ibid., *Adebowale 25* (UDW); Umtamvuna Nature Reserve, Fish Eagle trail, *Abbott 3001* (NH); Gwaloweni Forest, Lebombo Mountains, *Edwards 2929* (K, NU), *2934* (K, PRE); ibid., *Tinley 464* (K, NH, PRE, NU); Mpumalanga, 26 km from Nelspruit towards Kaapmuiden, *Jaarsveld 1062* (PRE); Eastern Cape, Mzwane Forest, near Port St. Johns, *Fegen 2956* (FHO, K, PRE, WAG), 5602 (NBG, PRF); Limpopo, Cyprus Kloof, Letaba District, *Renny 197* (PRE); Mtataspruit-Kloof, Letaba District, *Scheepers 1249* (WAG); Ingwavuma, *Gerstner 3771* (NH); Ngome, *Gerstner 5206* (PRE); Hluhluwe Game Res., Hlabisa District, *Codd 9624* (K); ibid., *Ward 2674* (NH); Eshowe, *Kotze 27* (PRF); Eastern Cape Province, Lusikisiki, *Pont 1090* (Z); ibid., Ntsubane Forest, *Fraser s.n. PRF 2349*

(PRE, PRF), *2405* (PRE, PRF); Mlotane Forest, Lusikisiki, *Fegen s.n. PRF 2743* (PRE, PRF), Mzwane Forest, Lusikisiki District, *Fegen s.n. PRF 6993* (PRF); Mzwane Forest, near Port St. Johns, *Fegen 2956* (FHO, K, PRE, WAG), *5530* (PRF), *5602* (NBG, PRF); Ismentone Forest, Port St. Johns, *Forester PRF 1973* (PRF); Manubi Forest, Kentani District, *van der Merwe 7* (PRF). KENYA: Mountain Marsabit Forest, *Williams & Adamson EAH 11028* (EA, K, PRE). UGANDA: Moroto, Lia River, *Wilson 1589* (PRE, EA, K). MOZAMBIQUE: Jardin Vasco da Gama, *Balsinhas 1897* (PRE). SWAZILAND: Ubombo Mountains, 6 km South of Stegi, *July 1953* (K); Hlatikulu Forest, *Boocock 25* (PRE, PRF). ZIMBABWE: Zimbabwe Botanical Gardens Harare, *Van Wyk BSA3193* (PRE, PRU).

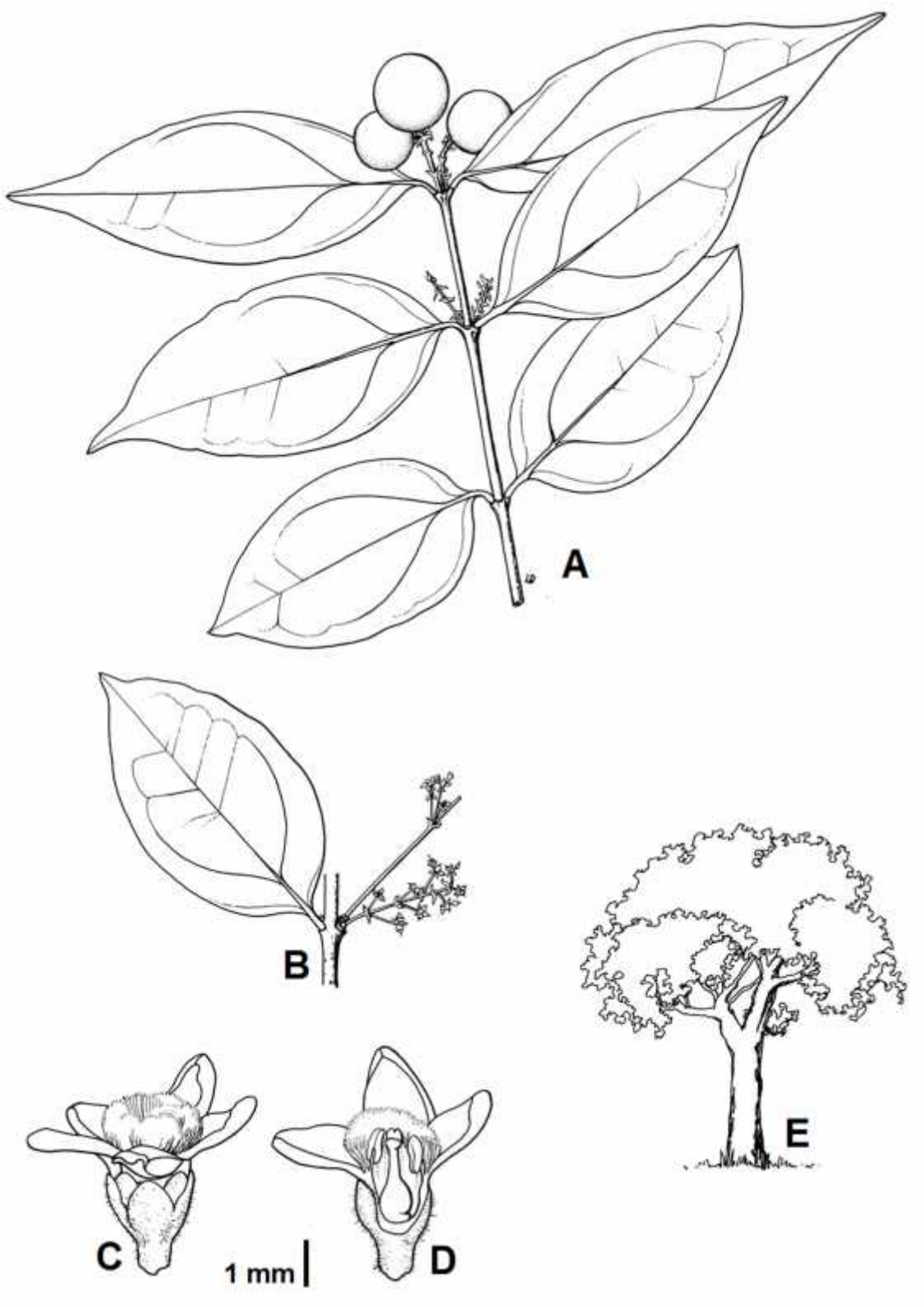

Figure 7.7.1 *S. mitis*: A = branchlet with leaves and fruits x ½, *Balsinhas 1897* (PRE); B = branchlet showing axillary inflorescence x ½; C & D = flowers, *van Jaarsveld 1062* (PRE); E = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

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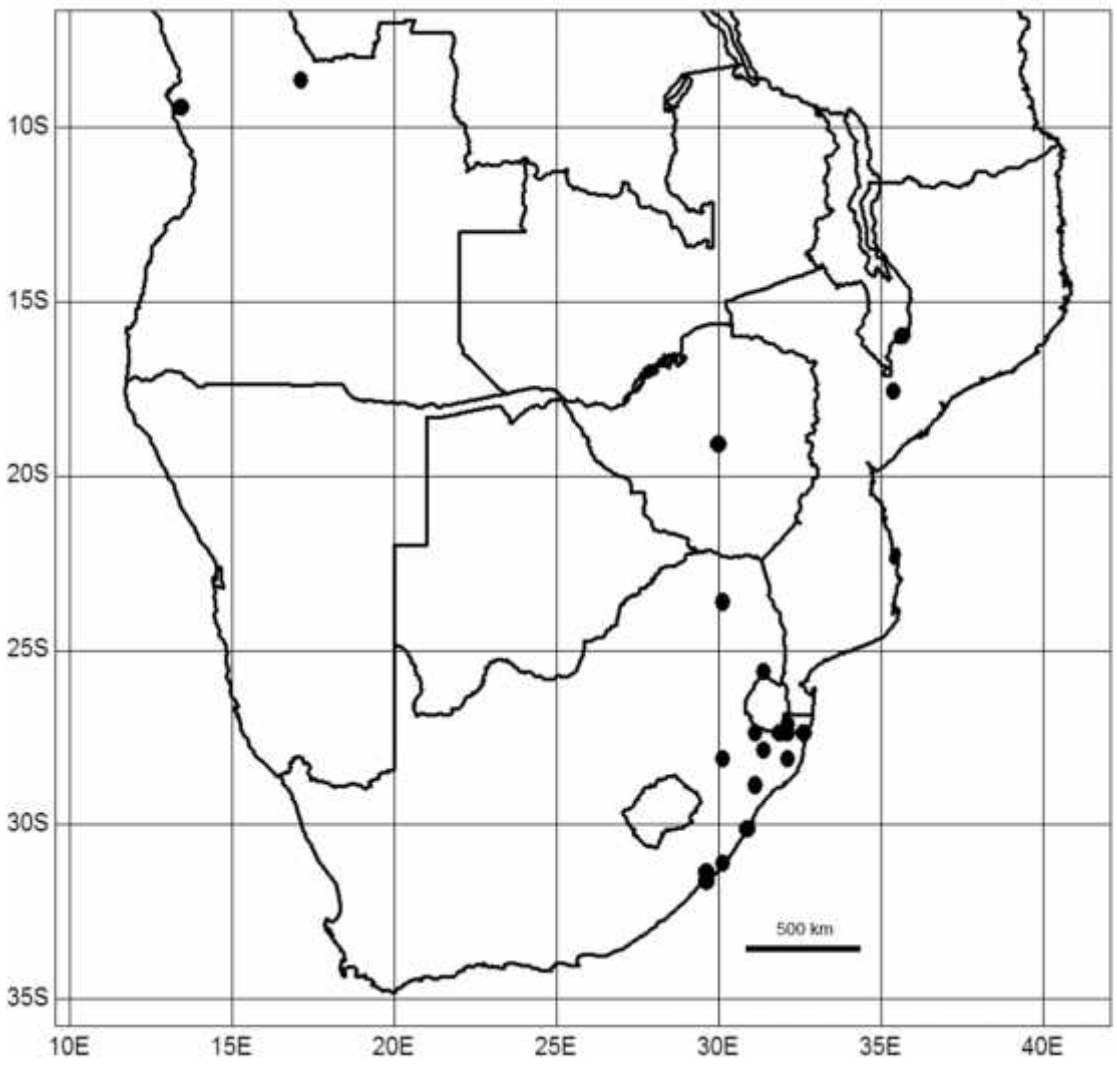

Figure 7.7.2: Distribution map of *Strychnos mitis* in southern Africa

8. *S. potatorum* L. f., Suppl. 148. (1781); Gaertner, Fruct. 2: 477 (1791); Lamarck, Illustr. 2: 38 (1794) (as *potatoria*); Roxburgh, PI. Coast Coromandel 1: 9, pl. 5 (1795); Willdenow, Sp. PI. 1: 1052 (1797); Du Petit-Thouars in Diet. Sc. Nat. 6: 426 (1806) and Not. Hist. Genre Caniram ou *Strychnos* 6 (1806) which has nearly the same text; Dubuisson & Du Petit-Thouars in Desvaux, Journ. Bot. Paris 1: 249 (1809); Poiret in Lamarck, Enc. 8: 696. (1808); Roxburgh, Fl. Ind. 1: 576 (1832); Mérat & De Lens, Diet. Mat. Med. 6: 563 (1834); Bojer, Hort. Maurit. 205 (1837); G. Don, Gen. Syst. 4: 65 (1838); Spach, Veg. Phan. 8: 487 (1839); De Candolle, Prod. 9: 15 (1845); Wight, Illustr. 2: t. 156. (1850); Bentham, Journ. Linn. Soc. 1: 103 (1856); Dalziel & Gibs, Bombay Fl. 156 (1861); Thwaites, Enum. PI. Zeyl. 425 (1864); Brandis, Forest Fl. India 317 (1874); Kurz, Forest Fl. Br. Burma 2: 167 (1877); CB Clarke in JD Hooker, Fl. Br. Ind. 4: 90 (1883); Lanessan, PI. Ut. Col. Fr. Paris 636. (1886); Sagot & Raoul, Man. Prat. Cult. Trop. Paris 297 (1893); Trimen, Fl. Ceylon 3: 176 (1895); Cooke, Fl. Bombay 2: 186 (1904); Brandis, Indian Trees 474 (1906); Bourdillon, Forest Trees Travancore 270 (1908); Dop, Bull. Soc. Bot. Fr. Mém. 19: 18 (1910); AW Hill, Kew Bull. 1917: 154 (1917); JS Gamble, Fl. Madras 868 (1921); HH Haines, Botany Bihar and Orissa 2: 572 (1922) (reprint 1961: 592); Verdoorn, Fl. S. Afr. 26: 143 (1963); Leeuwenberg, Meded. Landb. Wag. 69(1): 218 (1969). **Figure 7.8.1 page 256.** Type: India: Madras, in mountains, Koenig *anno* 1876 (S, lectotype).

Heterotypic synonyms: *S. tetankotta* Retz., Obs. 2: 12 (1781); JF Gmelin, Syst. 2: 387 (1791) (as *telankottd*); Jackson, Index Kew. 2: 1010 (1895) (as *tettankotta*). Type: India, Koenig s.n. in herb. Retzius (LD, holotype). *S. titou-cote* Gaertn., Fruct. 2: 477 (1791). Type not seen.

S. stuhlmannii Gilg in Engler, Bot. Jahrb. 17: 570 (1893); Baker, Fl. Trop. Afr. 4(1): 529 (1903); Duvigneaud, Bull. Soc. Roy. Bot. Belg. 85: 33 (1952) and 86: 108 (1953); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 33 (1960); Verdoorn, Fl. S. Afr. 26: 143 (1963). Type: Mozambique: Tete Province, Zambesi R., opposite Chiramba (cited as Shinamba), Kirk July 1859 (K!, lectotype).

S. heterodoxa Gilg in Engler, Bot. Jahrb. 28: 118 (1899); Baker, Ioc. cit. p. 530. Type: Tanzania: Uhehe, Makinde Steppen, between Iringa and Njombe, Goetze 519 (holotype destroyed in B; lectotype: BM!; isotypes: BR, K!).

Trees or sometimes shrubs, 4 – 18 m high with lenticellate, smooth pale grey or brown bark. Branches dichotomously branched and without spines or tendrils. Branchlets glabrous, with protruding persistent cup-like petiole bases, growing point sometimes modified into a spine-like tip 1 – 3 mm long. *Leaves* shortly petiolate, 60 – 160 mm long, 30 – 80 mm wide, glabrous on both surfaces, petiole 1.5 – 6 mm long; lamina dark green above, paler beneath, thinly coriaceous, membranous when young, elliptic, broadly to narrowly ovate; apex acute, acuminate or sometimes obtuse; base cuneate or rounded; 3 – 5 nerved from or above the base; secondary veins distinct pale green or yellowish. *Inflorescence* simple axillary cymes, usually in the axils of the upper leaves or near the base of branchlets from a leaf-like bract. Peduncle and pedicels slender, glabrous, peduncle 3.5 – 8.5 mm long. *Flowers* (4-) 5-merous, scented. *Calyx* dark green, connate at the base, ovate, or sometimes oblong 1 – 2.5 mm long, 0.8 – 1.5 mm wide with acute apex, not ciliate, glabrous on both sides. *Corolla* white,

cream or yellow, glabrous outside, bearded inside near the middle or near the base of the lobes and often also in the throat; 3.5 – 5 x as long as the calyx, tapering at the apex; lobes oblong, acute, spreading. *Stamens* exserted; filaments glabrous, inserted at the mouth of the corolla tube; anthers oblong, 1.1 – 2 mm long, 0.7 – 1 mm wide, deeply cordate at the base, glabrous. *Ovary* ovoid or conical, glabrous, narrowed into the style, 2-celled; style thick, 2.5 – 4.7 mm long; stigma small, capitate. *Fruit* small, soft, blue-black, globose, 15 – 25 mm in diameter, 1-seeded. Pulp purplish. *Seed* slightly glossy, pale brown, compressed-globose, up to 16 mm across.

Etymology of specific epithet: From the Latin word '*potator oris*' meaning drinker perhaps in allusion to its water clearing properties; resembling a potato, presumably referring to the fruit which looks more like a grape than a potato.

Vernacular names: Black Bitterberry, Grape *Strychnos*, clearing nut (English); Swartbitterbessie (Afrikaans); Nirmali (Hindi); Mulombelombe (Caprivi in Namibia); Mutupa (Venda).

Distribution and habitat: Democratic Republic of the Congo, Burundi, Tanzania, Zambia, Malawi, Zimbabwe, Mozambique, Namibia, Botswana, South Africa, Madagascar, India, Sri Lanka and Myanmar (Burma). In gallery forest, open-woodland, semi-evergreen bushland, often on river banks, on banks of dry riverbeds, or on termite mounds. Altitudinal range 0 – 1600 m. Southern African distribution map is depicted in Figure 7.8.2.

Affinity: Not really like any of the other *Strychnos* species known from southern Africa. Unique in possession of blue-black grape-like fruits and dichotomously branching pattern.

Notes: This is the only *Strychnos* found on two continents; Africa and Asia. It is indigenous to Africa, but is widely cultivated in Asia where the seeds are used to clear water for drinking. The fruits are pounded and used as fish poison in Africa. Could be threatened due to habitat loss, as its preferred habitat is along water courses.

Selected collections: SOUTH AFRICA: Limpopo, Tete Vondo Forest Station, Soutpansberg, *B.B.O. PRF12135* (PRF); Masisi, Louis Trichardt District, *van der Schijff 5245* (PRE); South bank of Pafuri River, *Codd 5397* (PRE); 15 km Northwest of Punda Maria, near Levubu River, *Codd 5381* (BM, MO); Punda Maria, *Codd & de Winter 5525* (K); Limpopo, Near Pafuri, *Nienaber EN219* (PRE). BOTSWANA: Near Kwando river, 18^{o} 06' S; 23^{o} 21'E, *Smith 2467* (PRE, SRGH, MN); Ngamiland, *Curson 109* (PRE). TANZANIA: *Greenway & Kanuri 14217* (PRE). MALAWI: Mulanje District, Litchenya Forest Reserve, *Chapman & White 8427* (PRE). NAMIBIA: Katima Mulilo, on southern bank of Zambezi river, *Müller 1802* (PRE). MOZAMBIQUE: District of Monica & Sofala, Chemba District, Estação Experimental de C.I.C.A., *Lemos & Macuacua 111* (PRE); ibid. *Burrows & Burrows 10460* (BNRH). ZIMBABWE: Lomangundi District, Mangula area, *Jacobsen 4055* (PRE); Gutu District, near Alheit, *Johnson J353B* (NU); South East lowveld, Malilangwe, small reserve on the southern boundary of Gonarezhou National Park, *Redfern 23* (GRA). ZAMBIA: Ndola, *Cottrell 171* (GRA).

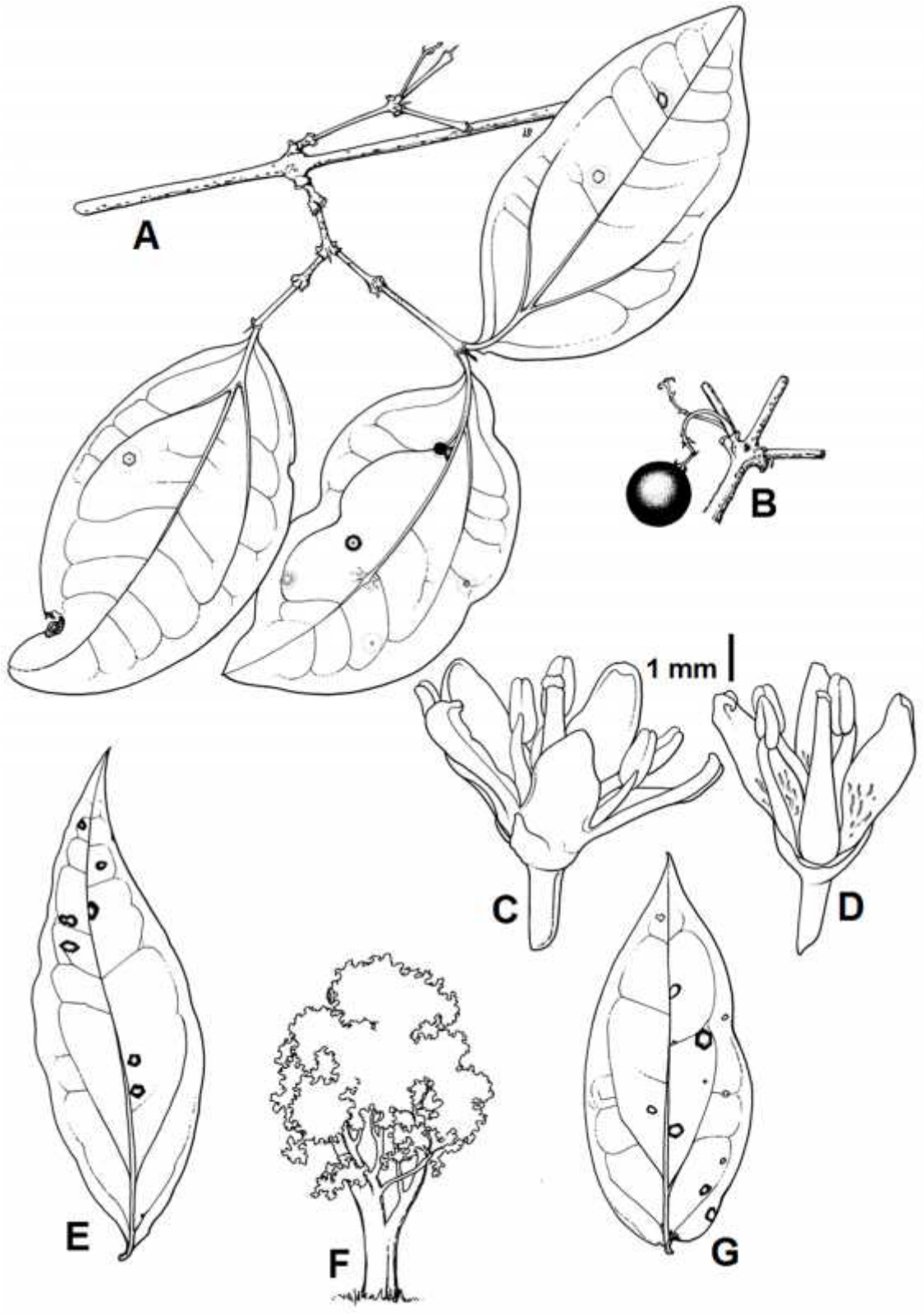

Figure 7.8.1 *S. potatorum*: A = branchlet with leaves x ½, *Muller 1802* (PRE); B = fruit in axil of a branchlet x ½, *Nienaber EN 219* (PRE); C & D = flowers, *Netshungani 956* (J); E & G = leaves x ½, *Nienaber EN 219* (PRE); F = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

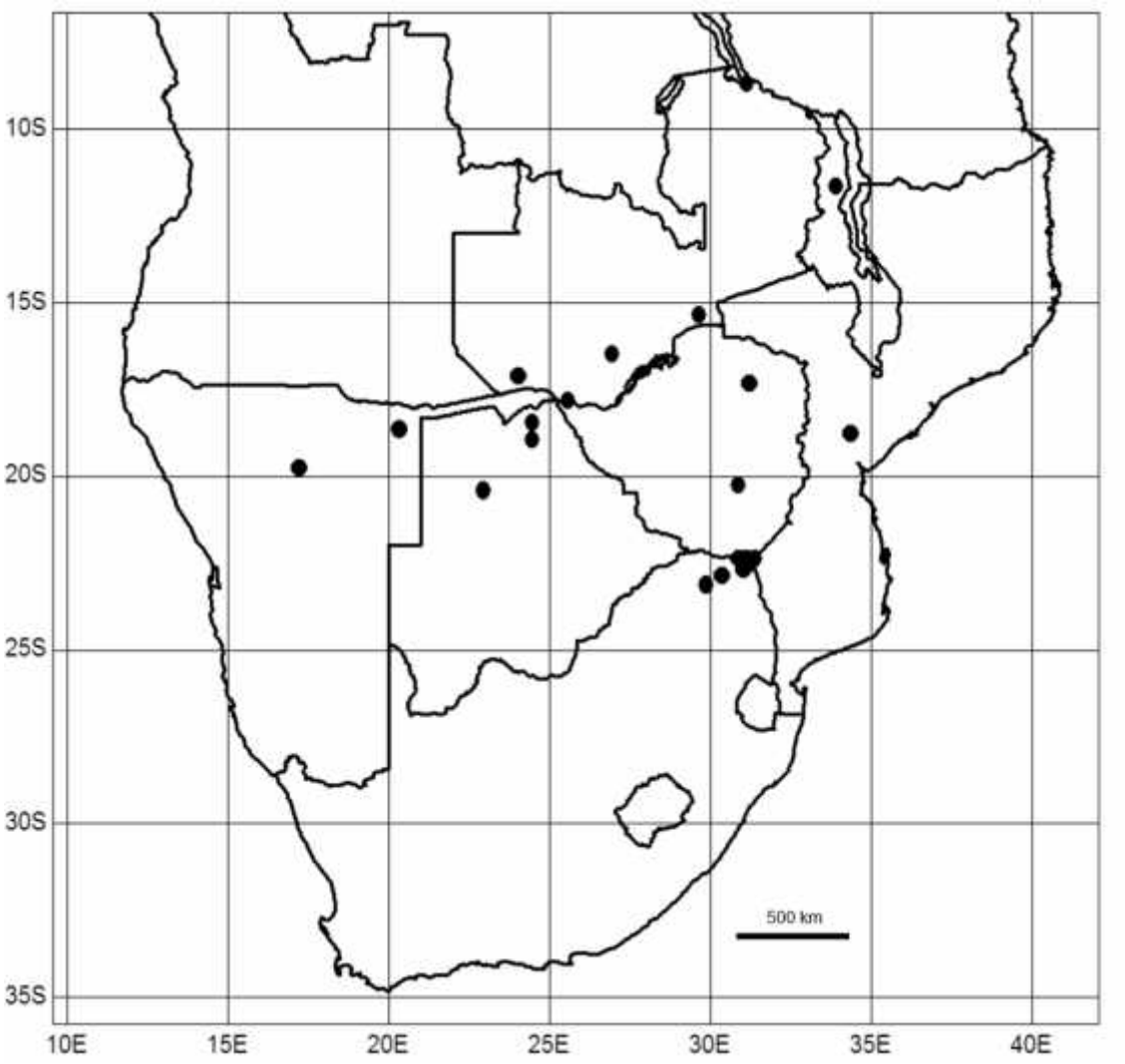

Figure 7.8.2: Distribution map of *Strychnos potatorum* in southern Africa

9. *S. pungens* Solered., Engler & Prantl, Nat. Pflanzenf. 4(2): 40 (1892) and in Engler, Bot. Jahrb. 17: 554 (1893); Hiern, Cat. Welw. Afr. PI. 3: 704 (1898); Gilg, Engler, Bot. Jahrb. 32: 176 (1902); Baker, FI. Trop. Afr. 4(1): 530 (1903); Prain & Cummins, Fl. Cap. 4(1): 1051 (1909); E. A. Bruce, Kew Bull. 1956: 268 (1956); Bruce & Lewis, FI. Trop. E. Afr. Loganiaceae 24 (1960); Verdoorn, Fl. S. Afr. 26: 144 (1963); Leeuwenberg, Meded. Landb. Wag. 69(1): 224 (1969). **Figure 7.9.1 page 262.** Type: Tanzania: Dodoma District, Saranda, Fischer 374 (K!, lectotype; isotypes: BM!, BR, LE, P).

Heterotypic synonyms: *S. occidentalis* Solered., Ioc. cit. and as syn. Ioc. cit. Type not cited; united with above by Solereder. Lectotype: Angola: Huila, Monino (Jan.) Welwitsch 4778 (BM!, lectotype; isotypes: COI, G, K!, LISU, P).

S. henriquesiana Bak., Bol. Soc. Brot. 11: 86 (1893) and FI. Trop. Afr. 4(1): 528 (1903); Gilg & Busse in Engler, Bot. Jahrb. 36: 92 (1905) (with S. *mucronata* Bolus in syn.). Type: Angola: Malanje Province (Aug.) Marques 13 (K!, holotype).

S. sapini De Wild., Comp. d. Kasai 382. 1910. Type: Congo (Kinshasa): Kasai, Bienge, Sapin D 23, Oct. 1907 (BR, holotype; isotypes: BR, K!, LY).

Evergreen tree or shrub, 2 – 8 m high with thick, rough and fissured grey or brown bark, which may be smooth higher up or in younger trees. Branches densely lenticellate; branchlets glabrous or occasionally with few short hairs. *Leaves* subsessile, petiole usually less than 3 mm long, glabrous on both surfaces, 30 – 80 mm long, 10 – 30 mm wide; lamina shining and dark green

above, hardly or not paler and less shining beneath, coriaceous, rigid, elliptic, narrowly elliptic or obovate; apex acute or subacute with the midrib excurrent as a pungent spine 2 – 4 mm long; base cuneate or rounded; 3-nerved, from or above the base, nerves prominent; additional pair of submarginal veins faint. *Inflorescences* axillary cymes, clustered, subsessile, simple or compound. Peduncle, and the very short pedicels pubescent; peduncle 2 – 4 mm long; floral bracts ovate-oblong, about 1.6 mm long. *Flowers* 5-merous. *Calyx* green, sepal lobes nearly free, unequal, imbricate, ovate, broadly ovate or orbicular, 2 – 4 mm long, 2 – 3 mm wide, acute, obtuse or rounded at the apex, ciliate, glabrous on both sides. *Corolla* greenish-cream, 2.4 – 3.2 x as long as the calyx, tapering and obtuse at the apex, glabrous outside, a ring of white lanate hairs in the throat and at the base of the lobes; tube cylindrical or nearly so; lobes thick, narrowly triangular, acute, spreading. *Stamens* just exserted; filaments glabrous, inserted at the mouth of the corolla tube; anthers oblong, 1 – 2 mm long, 0.5 – 1 mm wide, glabrous, deeply cordate at the base. *Ovary* ovoid or oblong, pilose towards the apex, 2-celled, gradually narrowed into the style, often with a disk-like base; style thick, 3 – 5 mm long, hairy at the base like the ovary apex; stigma capitate. *Fruit* large, hard, orange or yellow, nearly mature bluish-green, globose, 50 – 130 mm in diameter, many-seeded with about 20 – 100 seeds; skin granular, slightly shining; pulp yellow, edible. *Seeds* flattened, more or less planoconvex, obliquely ovate, elliptic, up to 30 mm across with thick, very short, erect hairs.

Etymology of specific epithet: pungens from the Latin base *pungō* or *pungēs* refers to the prickling nature of the leaf apex.

Vernacular names: Mudo, Mugati, Mumbumi (isiShona); Spiny-leaved monkey orange (English); Umgwai (Ndebele); Stekelblaarklapper, Botterklapperboom (Afrikaans); Mukubudu (Venda).

Distribution and habitat: Congo-Brazzaville, Democratic Republic of the Congo, Angola, Tanzania, Zambia, Malawi, Zimbabwe, Botswana, Namibia and South Africa. It grows in open woodland, usually in dry areas on rocky slopes, or at the base of stony koppies. Altitudinal range 0 – 2000 m. Southern African distribution map is depicted in Figure 7.9.2.

Affinity: It is phylogenetically allied to the other southern African members of section Densiflorae such as *S. innocua*, *S. madagascariensis* and *S. gerrrardii*. It is, however, distinct from other *Strychnos* taxa as the only one with very stiff, tough leaves with a very sharp and pointy apex.

Notes: Verdoorn reported that it could also be 4-merous, especially the calyx. The fruits are not as palatable as those of *S. spinosa*, *S. madagascariensis* or *S. cocculoides* with which it shares large hard fruits. A decoction of the root is used for treating stomach ache and bronchitis.

Selected collections: SOUTH AFRICA: Rustenberg, *Pegler 1034* (PRE); Pretoria, 600 m NE of Voortrekker Monument, *Court 16* (GRA); NorthWest Province, Rustenburg District *Turner 26* (PRE); Magaliesbergen, *McLean s.n.* BOL5710 (BM, BOL, K, NBG); Pretoria District, *Repton 1882* (PRE); *Verdoorn 2430; 2431* (PRE, WAG); Johannesburg, Gilfillan in herb. *Galpin 6153* (GRA, K); Middelburg District, *Marloth 11756* (A); ibid., *Thode A 1627* (PRE); Aapies Poort,

Rehmann 4161 (BM, K); ibid. *Schlechter 3621* (BOL, K, WAG). BOTSWANA: Ngamiland, *Miller B/420* (PRE); Kanye District, *Yalala 171* (K, WAG). NAMIBIA: Kavango Area 1, Kavango River Valley, *Ward & Hines 10340* (NU, UDW); near Oshikango, Ovamboland, *Rodin 2663* (BOL, K, MO); Okavango Territory, *de Winter 3758* (K, M), 3868 (PRE), 4203 (K, M, PRE). ANGOLA: Buila, Gambos, Chibemba na picada, *De Menezes 621* (PRE, LUAI). ZIMBABWE: near Victoria Falls, *Kirk 1860* (K); Sebungwe District (Oct.) *Davies 1565* (SRGH); Umvuma-Mtao, *Brain 6446* (MO), *6447* (SRGH), *6674* (SRGH).

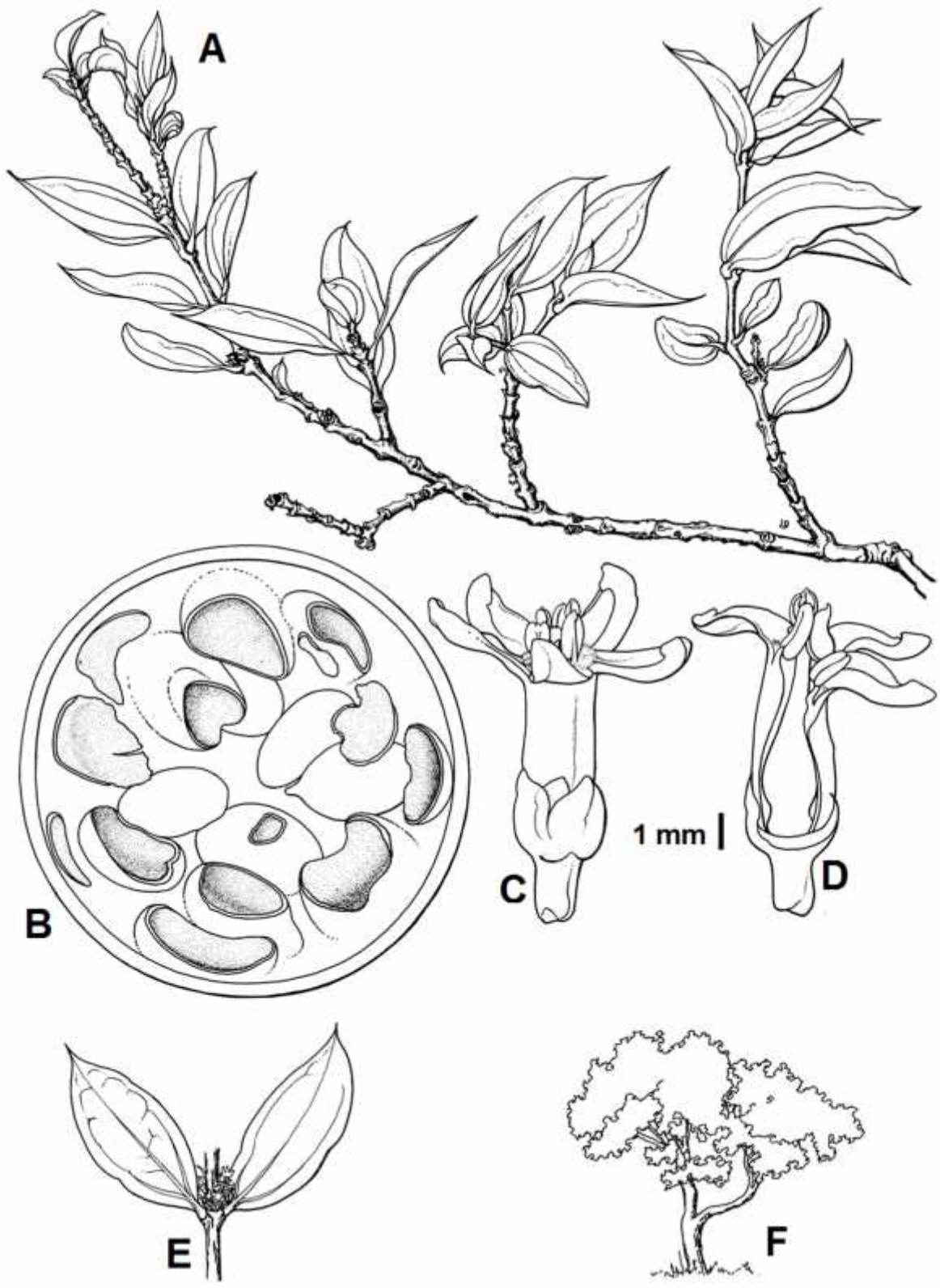

Figure 7.9.1 *S. pungens*: A = branchlet with leaves x ¾, *R.S. Martin 5* (J); B = cross section of fruit showing some seeds x ¾, *Pegler 1034* (PRE); C & D = flowers, *Mogg 20263* (J); E = leaf attachment to stem/branch; axillary inflorescence x ½, *Pegler 1034* (PRE); F = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

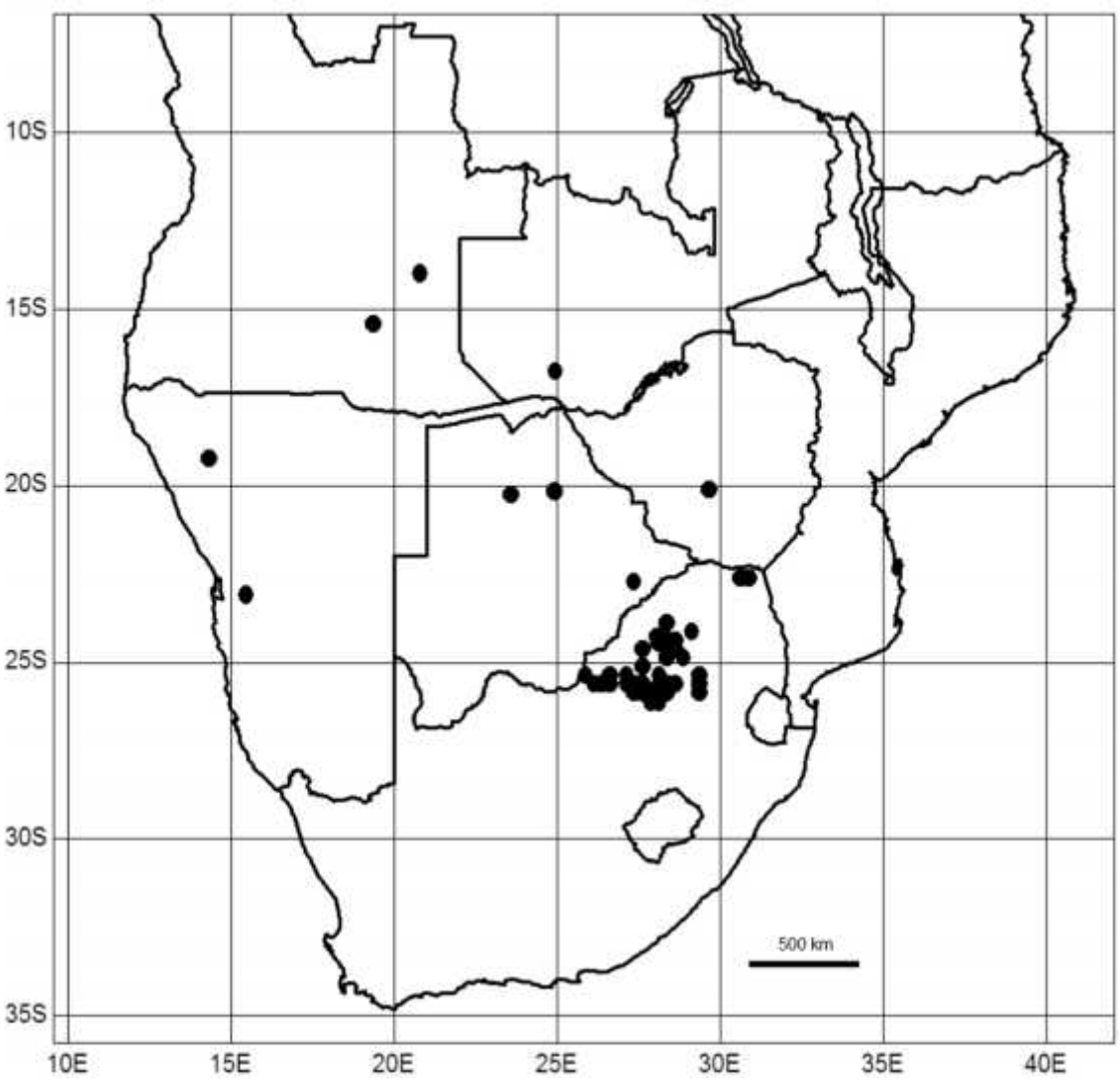

Figure 7.9.2: Distribution map of *Strychnos pungens* in southern Africa

10. *S. spinosa* Lam., Illustr. 2: 38. 1794; Poiret in Lamarck, Ene. 8: 697 (1808); Sprengel, Syst. 1: 672 (1825); Mérat & De Lens, Diet. Mat. Med. 6: 565 (1834); Harvey in Hooker, Lond. Journ. Bot. 1: 25 (1842); Baker, Fl. Trop. Afr. 4(1): 536 (1903); Jumelle & Perrier, Ann. Mus. Col. Marseille Sér. 2. 5: 395 (1907); Prain & Cummins, Fl. Cap. 4(1): 1058 (1909); Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 22, f. 186 (1931); Marloth, Fl. S. Afr. 3(1): 48 (1932); Chevalier, Rev. Bot. Appliq. 27: 355 (1947), partly (excluding synonyms *S. xerophila* and *S. schumanniana*); Duvigneaud, Lejeunia 13: 109 (1949) and Bull. Soc. Roy. Bot. Belg. 85: 20 (1952); Aubréville, Fl. Soud.-Guin. 438 (1950); EA Bruce, Kew Bull. 1955: 40 (1955), (excluding spme synonyms of *S. madagascariensis*); Coates Palgrave, Trees Centr. Afr. 204 – 207 (1957); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 17 (1960), partly (excluding syn. *S. madagascariensis*); Verdoorn, Fl. S. Afr. 26: 147 (1963); Onochie & Leeuwenberg, Fl. W. Trop. Afr. 2nd ed. 2: 41 (1963); Leeuwenberg, Act. Bot. Neerl. 14: 219 (1965); Leeuwenberg, Meded. Landb. Wag. 69(1): 239 (1969). **Figures 7.10.1 and 7.10.2 pages 274 – 275.**

Type: Madagascar: sin. loc., herb. Lamarck s.n. (P, holotype, photograph in K). Homotypic synonyms: *Brehmia spinosa* (Lam.) Harv. ex D.C., Prod. 9: 18 (1845); Bentham, Journ. Linn. Soc. 1: 108 (1856); Baker, Fl. Maurit. 235 (1877), partly (excl. syn. *S. madagascariensis*). *S. vuntac* Boj., Hort. Maurit. 205 (1837), partly (excl. syn. *S. madagascariensis*). *S. vontac* Du Petit-Thouars ex Spach, Veg. Phan. 8: 490 (1839).

Heterotypic synonyms: *S. flacurtii* Desv. ex Dubuisson et Du Petit-Thouars in Desvaux, Journ. Bot. Paris 1: 251 (1808); Mérat & De Lens, Diet. Mat. Med. 6: 565

(1834); Flückiger, Arch. Pharm. 230: 351 (1892) (as *flacourtii*). Type: Madagascar: sin. loc, Du Petit-Thouars s.n. (P, isotype).

S. lokua A. Rich., Tent. Fl. Abyss. 2: 53 (1851); Bentham, Journ. Linn. Soc. 1: 103. (1856); Duvigneaud, Lejeunia 13: 112 (1949) and Bull. Soc. Roy. Bot. Belg. 85: 21 (1952). Type: Ethiopia: Tacazzé R. Valley, near Tchélatchékanné, Quartin Dillon & Petit 412 (P, holotype, and two isotypes; photograph of one sheet in K!). Homotypic synonym: *S. spinosa* subsp. *lokua* (A. Rich.) EA Bruce, Kew Bull. 1955: 42. (1955); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 20. (1960).

S. laxa Solered. in Engler, Bot. Jahrb. 17: 554 (1893). Type: Nigeria: Nupe, Barter 1140 (holotype destroyed in B; lectotype: K!; isotypes: GH, P, W).

S. buettneri Gilg in Engler, Bot. Jahrb. 17: 574 (1893); Baker, Fl. Trop. Afr. 4(1): 535 (1903). Types: Togo: Bismarckburg, Ketschenke Ck., Büttner 370 (syntype destroyed in B) and Jegge Ck., Büttner s.n. (syntype destroyed in B).

S. gracillima Gilg, loc. cit. p. 573; Baker, loc. cit. p. 536. Type: Sudan: Djurland, near Seriba Ghattas, Schweinfurth 1344 (holotype destroyed in B; no isotype seen).

S. schweinfurthii Gilg, loc. cit. p. 568; Baker, loc. cit. p. 525. Type: Congo: Orientale, Monbuttu Land, near Munsa's Dorf, Schweinfurth 3509 (holotype destroyed in B; no isotype seen).

S. tonga Gilg, Ioc. cit. p. 575; in Engler, Pflanzenw. Ost-Afr. C: 311, (1895); Baker, Ioc. cit. 527. Type: Mozambique: Quilimane, Stuhlmann 1039 (HBG, lectotype; photograph in K!, negative 2492).

S. volkensii Gilg, Abh. Kön. Akad. Wiss. Berlin 1894: 25 (1894); in Engler, Pflanzenw. Ost-Afr. C: 311. (1895); Notizbl. Bot. Gart. Berlin 1: 76. (1895); Hiern., Cat. Welw. Afr. PI. 3: 702 (1898); Baker, Ioc. cit. p. 536; Duvigneaud, Lejeunia 13: 111 (1949) and Bull. Soc. Roy. Bot. Belg. 85: 21 (1952). Type: Tanzania: Tanga, Kilimanjaro (Jan.) Volkens 103 (G, lectotype; isotype: BM!). Homotypic synonym: *S. spinosa* subsp. *volkensii* (Gilg) EA Bruce, Kew Bull. 1955: 40 (1955); Bruce & Lewis, FI. Trop. E. Afr. Loganiaceae 19 (1960).

S. miniungansamba Gilg, Notizbl. Bot. Gart. Berlin 1: 77 (1895); Baker, Ioc. cit. p. 536. Type: Angola: Kahungula, Büchner 617 (holotype not seen, destroyed in B; no isotype seen).

S. carvalhoi Gilg in Engler, Bot. Jahrb. 28: 123 (1899); Baker, Ioc. cit. p. 535. Type: Mozambique: between Mussoril and Cabeceira, Carvalho s.n. 1884—'85 (holotype destroyed in B; lectotype: COl).

S. sansibariensis Gilg, Ioc.cit. p. 124; Baker, Ioc. cit. p. 535. Type: Tanzania: Zanzibar, sin. loc, Stuhlmann 1011 (HBG, lectotype, photograph in K!, negative 2371).

S. euryphylla Gilg et Busse in Engler, Bot. Jahrb. 32: 179 (1902); in op. cit. 36: 108 (1905); Baker, loc. cit. p. 526. Type: Tanzania: Kilossa, Usagara, Busse 174 (G, lectotype; isotypes: HBG, LY, P; photograph of HBG sheet in K!, negative 2491).

S. megalocarpa Gilg et Busse in Engler, Bot. Jahrb. 32: 180 (1902); Baker, loc. cit. p. 526. Type: Tanzania: Handeni District, Kwa-Ssulanga (Kwa-Zuranga), Busse 323 (holotype destroyed in B; no isotype seen).

S. omphalocarpa Gilg et Busse, loc. cit. p. 181; Baker, loc. cit. p. 525. Type: Tanzania: Handeni District, Kwa Mdoë, near Handeni, Busse 322 (holotype destroyed in B; lectotype: G).

S gracillima var *paucispinosa* De Wild., Ann. Mus. Congo Sér. 4. 1: 97 (1903) and Bull. Jard. Bot. Brux. 2: 372 (1910). Type: Congo (Kinshasa): Katanga, Lukafu, Verdick 48 (BR, holotype).

S. emarginata Bak. in FI. Trop. Afr. 4(1): 537 (1903); Duvigneaud, Lejeunia 13: 114 (1949) and Bull. Soc. Roy. Bot. Belg. 85: 21 (1952). Type: Sudan: Djurland, Seriba Ghattas, Schweinfurth 1396 (K!, holotype).

S. spinosa var. *pubescens* Bak., loc. cit. Type: Guinea: near Moria, Scott Elliot 4801 (K!, lectotype; isotypes: BM!, GH).

S. gilletii De Wild., Ann. Mus. Congo Sér. 5. 1: 176 (1904); Baker, loc. cit. p. 624. Type: Congo (Kinshasa): Léopoldville Province, Kisantu, Gillet 134 (BR, 2 sheets, lectotype; photograph of one sheet in K!, negative 2207).

S. cardiophylla Gilg et Busse in Engl., Bot. Jahrb. 36: 110 (1905). Type: Tanzania: Kilwa District, Singino Hills, just S. of Kilwa Kavinje, Busse 3011 (holotype not seen, destroyed in B; lectotype: EA, photograph in K!, negative 2283).

S. cuneifolia Gilg et Busse, loc. cit. p. 109. Type: Tanzania: Lindi District, Lake Lutamba, Busse 2519 (holotype in B; lectotype: HBG; isotypes: BR, EA, LY; photograph of EA sheet in K!, negative 2285).

S. harmsii Gilg et Busse, loc. cit. p. 109. Type: Tanzania: Lindi District, Rondo Plateau, Busse 2560 (EA, lectotype; isotypes: EA, LY; photographs of both EA sheets in K!, negatives 2293 and 2294).

S. leiosepala Gilg et Busse, loc. cit. p. 111. Type: Angola: Huila, Dekindt 499 (COI, lectotype; isotypes: COI, LISC, MPU).

S. radiosperma Gilg et Busse, loc. cit. p. 108. Type: Tanzania: Kilwa District, Matumbi Mountains, near Mirungamo, Busse 3061 (holotype destroyed in B; lectotype: HBG; isotypes: BM!, BR, EA, LY; photograph of EA sheet in K!, negative 2286).

S. rhombifolia Gilg et Busse, loc. cit. p. 107. Type: Sudan: Djurland, Seriba Ghattas, Schweinfurth 1407 (holotype destroyed in B; no isotype seen).

S. unguacha var. *refusa* Chiov., Fl. Somala 2: 305 (1932) (as *Strichnos).* Type: Somalia: Oltregiuba, Licchitore, Senni 481 (FI, lectotype).

S. mueghe Chiov., Race. Miss. Consol. Kenya 83 (1935); AW Hill as., Ind. Kew. Suppl. 9: 270 (1938) (as *meughe*). Type: Kenya: Mt. Kenya, 'Saraka Steppe', Balbo 687 (TOM, lectotype).

S. djalonis A. Chev., Expl. Bot. Afr. Occ. Fr. 1: 442. 1920, nomen; Rev. Bot. Appliq. 27: 358 (1947). Type: Guinea: Ditinn Chevalier 12179 bis (P, holotype, photograph in K!, negative 2301; isotype: LY).

Shrub or small tree, 3 – 9 m high, with spreading habit. Bark pale to dark grey or brown, shallowly fissured, irregularly corky. Branches often with recurved or straight spines; branchlets glabrous or shortly pubescent, sometimes terminating in a straight spine. *Leaves* on main axis sometimes ternate; 28 – 128 mm long, 17 – 80 mm wide; shortly petiolate, petiole 2 – 13 mm long, glabrous or pubescent; lamina coriaceous, orbicular, broadly to narrowly elliptic, ovate, or obovate; apex rounded, mucronate, acute, acuminate or occasionally emarginate; base cuneate, subcuneate, rounded or occasionally subcordate; 3 – 7 nerved, distinct, veins often pale on both sides, with domatia in the angles of the main abaxial veins; glabrous or pubescent on both sides. *Inflorescence* terminal, sometimes axillary; peduncle and pedicels sparsely pubescent (never glabrous);

peduncle 4 – 10.5 mm long. Bracts linear or nearly so, upper sepal-like, lower larger, sparsely pubescent outside. *Flowers* 5-merous. *Calyx* pale green, connate at the base, narrowly deltoid to linear, 3.5 – 6 mm long, 0.8 – 1.2 mm wide, apex acuminate, base minutely ciliate or not, outside sparsely pubescent at the base and glabrous at the apex or entirely glabrous, inside glabrous and usually with some hairs at the base. *Corolla* pale green to greenish-white, 0.7 – 2.6 x as long as the calyx, glabrous within except for a dense fringe of hair at the throat; lobes deltoid, subacute, erect or nearly so. *Stamens* inserted at the base of the tube, filaments glabrous, about as long as the anthers; anthers inserted near the base of the corolla, anthers oblong or elliptic, 1 – 1.2 mm long, 0.7 – 1 mm wide, deeply cordate and densely bearded at the base. *Ovary* globose, broadly ovoid, shortly pubescent, pseudomonomerous, 1-celled; stigma subsessile, oblong; style very short, 0.3 – 0.7 mm long. *Fruit* yellow or when nearly mature yellow-green or green, large, hard, resembling an orange, globose, slightly shiny and warty outside, 50 – 130 mm in diameter, many-seeded with about 10 – 100 seeds; pulp yellow, edible. *Seed* pale brown, obliquely ovate or elliptic, flattened, more or less plano-convex, about 10 – 30 mm across.

Etymology of specific epithet: The epithet refers to the abundance of thorns/spines on the branches.

Vernacular names: Groenklapper, Doringklapper (Afrikaans); spiny monkey orange, Natal orange, elephant orange (English); umhahli (isiNdebele); Mutamba (Shona); Morapa (Sotho); umKwakwa (Swahili); Muramba (Venda); iHlala (isiZulu); Nsala (Tswana).

Distribution and habitat: Senegal, Gambia, Mali, Guinea, Sierra Leone, Côte d'Ivoire, Burkina Faso, Ghana, Togo, Benin Republic, Niger, Nigeria, Cameroon, Central African Republic, Chad, Sudan, Ethiopia, Somalia, Democratic Republic of the Congo, Rwanda, Burundi, Angola, Uganda, Kenya, Tanzania, Zambia, Malawi, Zimbabwe, Mozambique, Namibia, Botswana, Swaziland, South Africa, Comoro Islands, Madagascar, Seychelles and Mauritius. It is typically a savanna species occurring in woodlands, bushveld, or sometimes gallery and sand forests. Altitudinal range 0 – 2200 m. Southern African distribution map is depicted in Figure 7.10.3.

Affinity: Closely allied to *S. cocculoides* and treated under that species.

Notes: It is a fairly variable species morphologically, which is to be expected given its extensive distribution range across both mainland Africa and African islands. Bruce (1955) grouped the tropical elements of this species into three subspecies based on leaf shape and size. Such classifications have been shown by Verdoorn (1963) and Leeuwenberg (1969) to be inapplicable, as the tree features are more or less identical across the various forms. Genetic (DNA) evidence by the current author seems to suggest that the level of sequence divergence between what was typified as *S. spinosa* subsp. *lokua* and *S. spinosa* subsp. *spinosa* does not warrant their treatment as separate taxa. It may well be that there are distinct clusters of genetic variants good enough for infra-specific recognition. However, until such rich genetic data are available, the current treatment follows that of Verdoorn and Leeuwenberg in recognising a single taxon without subspecies.

The Sheet I of *Reynolds 2467* (PRE) has *S. spinosa*, sheet II has fruit of *S. madagascariensis* with leaves of *S. spinosa*.

Fruits of *S. spinosa* are eaten when fresh and the pulp is quite tasty when fully ripe. The wood is used in carpentry and the leaves extracts have pesticidal properties.

Selected collections: SOUTH AFRICA: Mkusi Game Reserve, *Goodman 612* (NU); Limpopo, Scheema Farm, near Kampersrus, *Abbott 5996* (PRU); Pongolapoort, *Ward 4121* (NU); Mpumanalnga, Nelspruit region, on road to May Farm, Sheets I & II of *Reynolds 2467* (PRE); Hluhluwe Game Reserve, *Ward 2622* (NH, PRE); KZN, *Ross & Moll, 1815* (PRE); Ndumu Game Reserve, *Matthews s.n.* 2002, PRU 091595 (PRU); ibid., Bella Vista, *Moll 4253* (NH, NU); *Tinley s.n.* February 1964 PRE49945 (PRE); Mpumalanga, Nelspruit District, Kruger National Park, near Pretorius Kop Camp, *Codd 4412* (PRE); Nelspruit District, Numbi Forerst, *Marais & van der Schijff 1254*; *van der Schijff & Marais 3686* (PRE); Mpumalanga, Komatipoort, *Van Wyk & Pienaar 4694* (PRE); Research Station, Nelspruit, *Liebenberg 2858* (PRE); Sibasa, Kruger National Park, Wambia, *van der Schijff & Marais 3683* (PRE); KZN, Maphumulo District, *Moll 2202* (NU); Mtubatuba, Dukuduku State Forest, *Nicholas 1636* (NH); Inanda, near Umzinyathi Falls, *McClean 1097 & Ogilvie 2810* (PRE, NH); Amatikulu Nature Reserve, *Ward 2128* (NH); Dumisa, *Rudatis 1140* (BM, E, K, PRE)**;** Umgeni River valley, PMB, *Hill 3187* (GRA); Sibayi District, IsiZululand, *Derrick 66* (GRA); Eastern Cape, Komgha District, *Flanagan 2375* (GRA); Crocodile Poort, near Barberton, *Galpin 1075* (BOL, GRA, K, PRE, SRGH); Limpopo, Letaba District, *Scheepers 809* (PRE);. NAMIBIA: Okavango Native Territory, *De Winter & Marais 4798* (PRE, K). SWAZILAND: *Kemp 933* (PRE, SDNH); Manzini, *Dlamini A2743* (PRE). ZAMBIA: Ndola, *Cottrell 65* (GRA); Southern Province, Choma, Nansai Farm, *Bruce-Miller 43* (GRA). MOZAMBIQUE: Zambezia, Mocuba, *Dold 3193* (GRA); Kongoni River,

Zambesi Delta, *Kirk s.n.* 1859 (K), 1861 (K). ZIMBABWE: Salisbury, *Brain 10835* (SRGH); *Eyles 4548* (K, SRGH); Melsetter District, *Goodier & Phipps 303* (EA, K, M, PRE, S, SRGH).

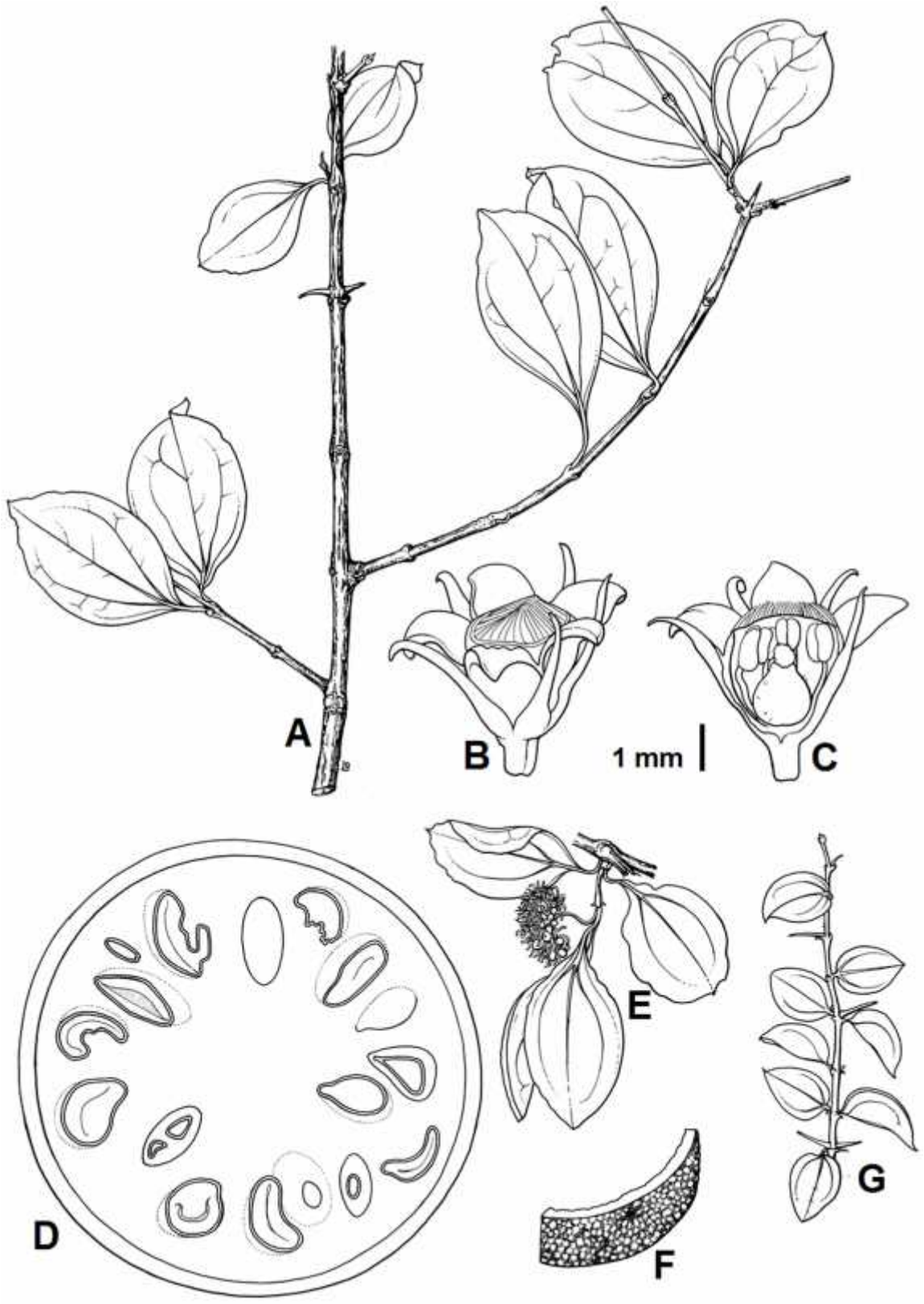

Figure 7.10.1 *S. spinosa*: A = branchlet with leaves and thorns x ¾, *C.J. Ward 2622* (PRE); B & C = flowers, *M.C. Ward 2365* (NH); D = C.S of fruit showing some seeds x ¾, *C.J. Ward 2622* (PRE); E = leaves and inflorescence x ¾, *M.C. Ward 2365* (NH); F = fruit rind showing conspicuous granulations x ¾, possibly subsp. *lokua* Matthews s.n. (PRU); G = juvenile leaves with spines x ¾, Van der Schijff & Marais 3686 (PRE). Illustrations by Leslie Deysel.

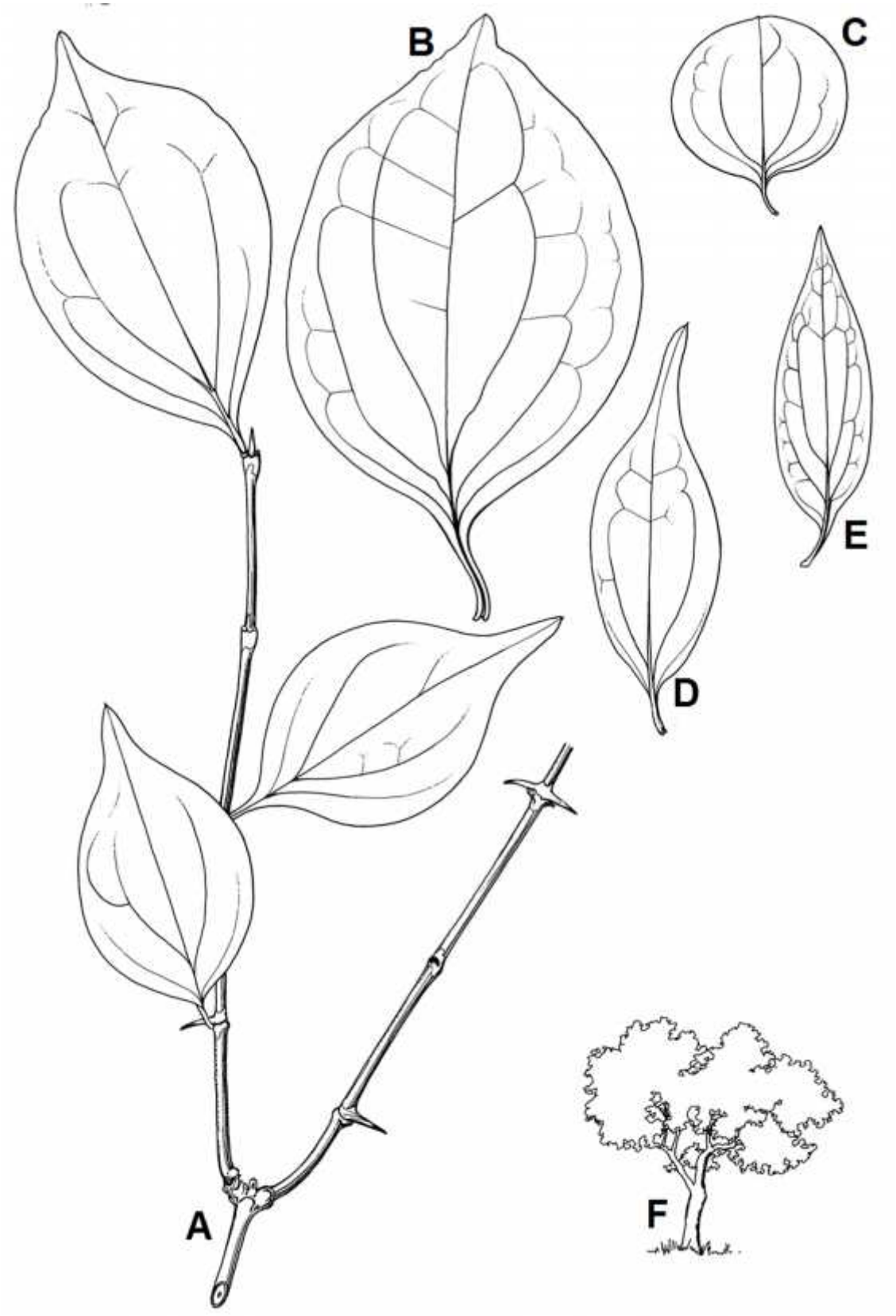

Figure 7.10.2 *S. spinosa*: A = branchlet with leaves and thorns x ¾, *Van Wyk & De V. Pienaar 4568* (PRE); B = leaf x ½, *Van Wyk & De V. Pienaar 4694* (PRE); C & E = leaves x ½, *Scheepers 809* sheets I & II (PRE); D = leaf x ½, Matthews s.n. possibly subsp. *lokua* (PRU); F = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

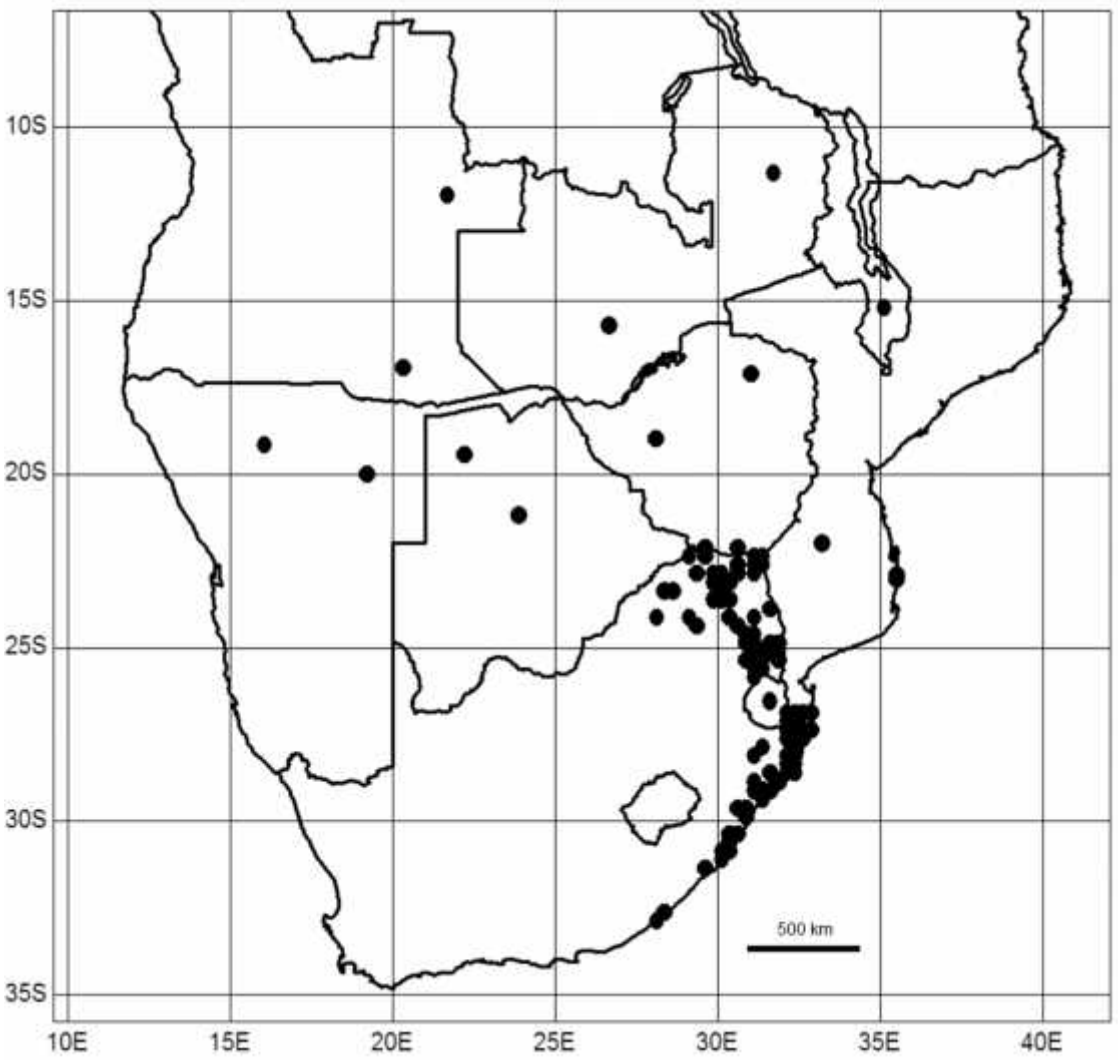

Figure 7.10.3: Distribution map of *Strychnos spinosa* in southern Africa

11. *S. usambarensis* Gilg, Abh. Preuss. Akad. Wiss. 1894: 36 (1894) and Engler, Pflanzenw. Ost-Afr. C: 311 (1895); Baker, Fl. Trop. Afr. 4(1): 526 (1903); EA Bruce, Kew Bull. 1955: 627 (1956); Verdoorn, Fl. Pl. Afr. Plate 1242 (1957); Bruce & Lewis, Fl. Trop. E. Afr. Loganiaceae 34 (1960); Leeuwenberg, Act. Bot. Need. 11: 47 (1962); Verdoorn, Fl. S. Afr. 26: 140 (1963); Onochie & Leeuwenberg, Fl. W. Trop. Afr. 2nd ed. 2: 44 (1963); Leeuwenberg, Meded. Landb. Wag. 69(1): 267 (1969). **Figure 7.11.1 page 281.**

Type: Tanzania: E. Usambara Mts., Mashewa, Holst 3582 (holotype destroyed in B; lectotype: K!; isotypes: HBG, M, W, Z).

Heterotypic synonyms: *S. cerasifera* Gilg in Engler, Pflanzenw. Ost-Afr. C: 311 (1895); Baker, loc. cit. p. 53; Bruce & Lewis, loc. cit. p. 35. Type: Tanzania: coastal region, unlocalized, Stuhlmann 6089 (holotype destroyed in B; no isotype seen).

S. distichophylla Gilg, loc. cit. p. 310; Baker, loc. cit. 525; Bruce & Lewis, loc. cit. p. 35. Type: Tanzania: Biharamulo District, Kimoani (Kimwani) Plateau, Stuhlmann 3397 (holotype not seen, destroyed in B; no isotype seen).

S. micans S. Moore, Journ. Linn. Soc. 40: 146 (1911); Verdoorn, Bothalia 3: 587-588 (1939). Type: Zimbabwe: Chirinda Forest, Swynnerton 125 (BM!, holotype; isotypes: K!, Z).

S. cooperi Hutch. et M. B. Moss in Fl. W. Trop. Afr. 2: 24 (1931) and Kew Bull. 1937: 355 (1937); Duvigneaud, Lejeunia 11: 74 (1947). Type: Liberia: Dukwia R., Cooper 300 (K!, holotype; isotypes: A, BM!, F, FHO, GH, NY, US).

Shrub, liana, or much branched small trees, 3 – 15 m high or more depending on the habit; bark dark brown, thin and smooth. Branches conspicuously lenticellate, dark brown, covered with a pale skin, which later splits and peels off; branchlets glabrous, pale brown. Tendrils occasional, solitary, only present in climbing shrubs or lianas. *Leaves* petiolate, 40 – 80 mm long, 11 – 39 mm wide; petiole 3.5 – 8 mm long, glabrous; lamina dark green above, paler beneath, coriaceous or thinly so, ovate, narrowly ovate, elliptic or narrowly elliptic; apex distinctly acuminate, usually mucronate at the very apex; base cuneate, rounded, or occasionally subcordate, glabrous on both surfaces; usually 3, or occasionally 5-nerved from above the base; tertiary venation reticulate, inconspicuous on the adaxial side. *Inflorescence* axillary cymes, lax or congested, much shorter than the leaves, few-flowered. Peduncle and pedicels glabrous or shortly pubescent; peduncle 2 – 6.5 mm long. Bracts sepalloid, glabrous. *Flowers* predominantly 4- (occaisionally 5) -merous. *Calyx* pale green, connate at the base; lobes ovate, broadly ovate or triangular, 0.7 – 1.5 mm long, 0.6 – 1.2 mm wide, acute or obtuse, minutely ciliate, glabrous on both sides or shortly pubescent outside. *Corolla* white or yellow, 3 – 4 x as long as the calyx, glabrous or minutely pubescent outside, inside with a ring of pilose hairs in the throat; tube short; lobes acute, recurved from below the middle. *Stamens* exserted; filaments glabrous, inserted at the mouth of the corolla tube; anthers suborbicular or oblong, 0.8 – 1.1 mm long, 0.4 – 1 mm wide, glabrous, deeply cordate at the base. *Ovary* narrowly

ovoid, glabrous, rather abruptly narrowed into the style, 2-celled; style 0.8 – 1.8 mm long; stigma capitate or faintly bilobed. *Fruit* small, soft, globose or nearly so, smooth skin, pale green or yellow, glaucous, shortly stipitate within the calyx, 10 – 18 mm in diameter, 1-seeded; pulp orange. *Seed* pale brown, slightly depressed or ellipsoid, shortly and densely pubescent, smooth, with a central hilum at one side, up to 13 mm across.

Etymology of specific epithet: The epithet refers to the Usambara Mountains in North-East Tanzania, from where it was first formally described.

Vernacular names: Stipe-fruited *Strychnos*, Blue bitterberry (English); Bloubitterbessie (Afrikaans);

Distribution and habitat: Guinea, Sierra Leone, Liberia, Côte d'Ivoire, Ghana, Nigeria, Congo-Brazzaville, Democratic Republic of the Congo, Rwanda, Uganda, Kenya, Tanzania, Zambia, Zimbabwe, Mozambique, Swaziland and South Africa. It is a forest species found in upland and lowland rain forests and secondary forests, especially on river banks, in deep valleys, gallery forest, semi-evergreen, and coastal evergreen bushland. Altitudinal range 0 – 2000 m. Southern African distribution map is depicted in Figure 7.11.2.

Affinity: It is quite unique among the southern African *Strychnos* species and will not be confused with any other species. The combination of a distinctive leaf shape with fairly longly acuminate apex and brown branchlets allows easy identification of even sterile specimens.

Notes: The West and Central African specimens of *S. usambarensis* are climbers, while those from southern African and some parts of East Africa are small trees.

Root extracts are used for arrow poison. Other alkaloids of *S. usambarensis* have potential as anti-malarial and cancer treatment.

Selected collections: SOUTH AFRICA: KZN, Hluhluwe Game Reserve, *Guy 118* (NU); Umzinto, Umpambinyoni River Valley, *Moll 3583* (NH); Isipingo beach, Durban, *Ward 3757* (NU); Hell's gate peninsula, Durban, *Nicholas & MacDevette 1267* (NH); Mkomasi river, South coast, *Ward 12644* (NH, NU, UDW); Limpopo, Sibasa District, *Van Warmeloo s.n.* 19 December 1951 (PRE); Lake St Lucia, False Bay Park, *Taylor 693* (NH); False Bay Camp site, near Flat 2, *Adebowale 54* (UDW); Kenneth Stainbank Nature Reserve, Yellow wood Park, Durban, *Adebowale 4* (UDW); Oribi Gorge Nature Reserve, Port-Shepstone, *Nichols 640* (NH)**.** MOZAMBIQUE: Salamanga, *Jasen & Macuacua 7731* (PRE); Manica e Sofala, Inhamitanga *Simäo 1282* (PRE); Lourenço Marques, Maputo, *Hornby 2547* (BM, PRE, SRGH). ZIMBABWE: Southeast of Murahwa's hill, *Chase 8512* (NU); Matobo District, *Guy 1/58* (K, SRGH); ibid. *Plowers 1465* (BR, MO, PRE, SRGH); km 45 of Uvuma-Fort Victoria road *Grout 22/47* (FHO, SRGH); Fort Victoria, Eyles Herb. *Q.V.M. 6601* (SRGH); Umtali District, *Fisher 1525* (SRGH); Vumba, *Ferrar 4099* (PRE); Chirinda Forest, Chipinga District, *Goldsmith 137/62* (BR, K, LISC, WAG). SWAZILAND: Hlatikulu Forest, *Boocock 22* (PRE, PRF). ZAMBIA: Kafwala, *Fanshawe 7003* (K); Kalomo, *Mitchell 15/30* (K).

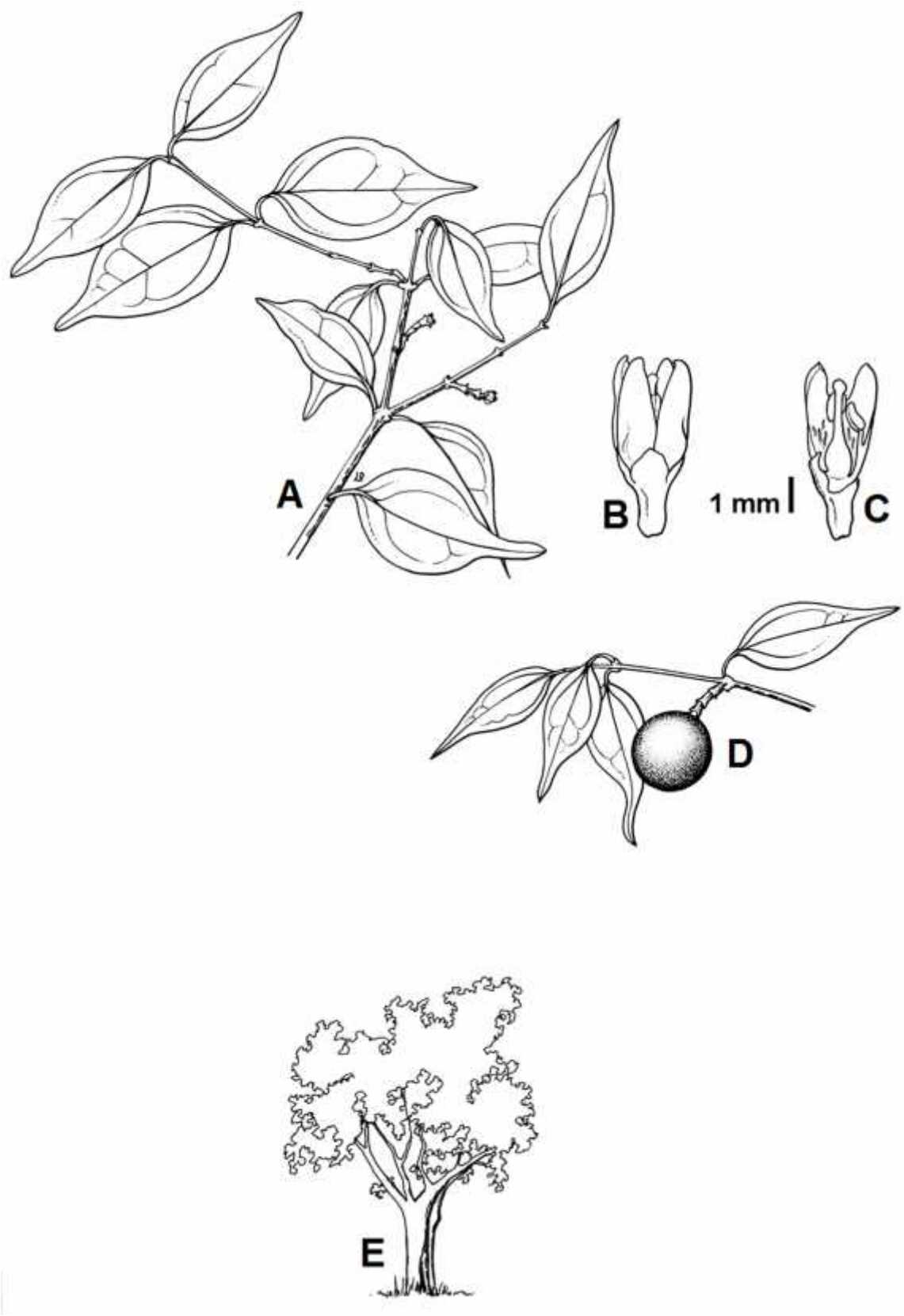

Figure 7.11.1 *S. usambarensis*: A = branchlet with leaves x ¾, *C.J. Ward 12644* (NU); B & C = flowers x ¾, *Taylor 693* (NH); D = branchlet with leaves and a fruit x ¾, *C.J. Ward 12644* (NU); E = canopy shape drawing, not to scale. Illustrations by Leslie Deysel.

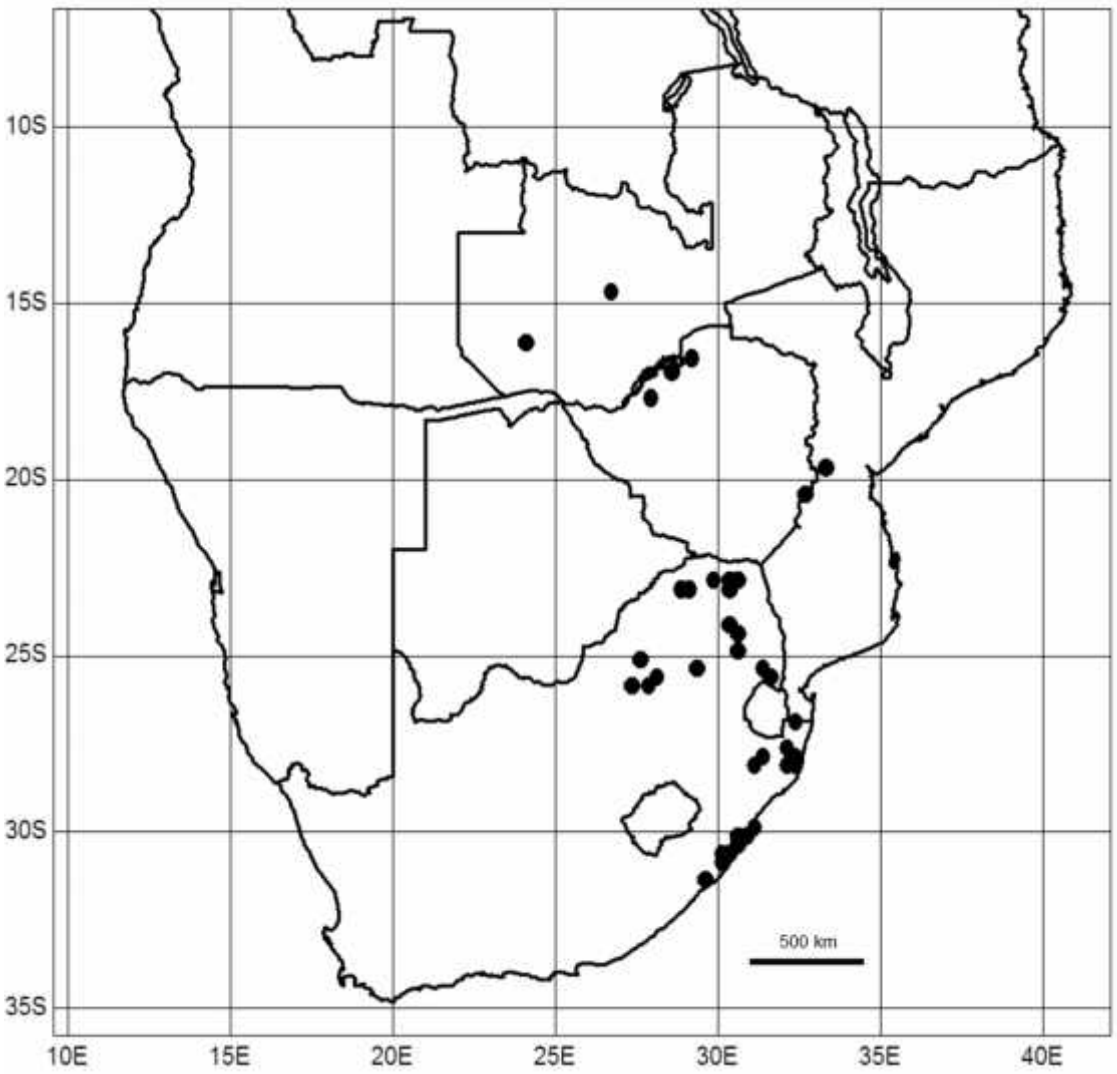

Figure 7.11.2: Distribution map of *Strychnos usambarensis* in southern Africas

SPECIMENS OF UNCERTAIN TAXONOMIC PLACEMENT AND POSSIBLY HYBRID ORIGIN

A number of specimens appear to be morphological intermediates between pairs of well circumscribed species prompting speculations of potential hybrids. Indeed, that hybridization has played a key role in the evolution of Loganiaceae in general, and *Strychnos* in particular, is supported by molecular data (Frasier, 2008). Frasier showed molecular evidence of ancestral hybrid events for some *Strychnos* taxa through their shared polymorphisms. Secondary structure analysis of ITS2 sequences (Chapter 4) predicted clades where hybrids are likely to be found. Incidentally, all of the reported cases of putative hybrid specimens from southern Africa agree with the ITS2 prediction, for which we have data. A significant overlap in distribution range of the putative parents of these hybrid specimens lends added weight to hypotheses about their hybrid origin. It is therefore the considered opinion of this author that the following specimens could be hybrids.

S. pungens* x *S. innocua ZAMBIA: Luanshya *DB Fanshawe 1402* (K! BR, FHO, SRGH, WAG). TANZANIA: Ngulu District, *Peter 34795* (B). ZIMBABWE: Urungwe District *R Goodier 468* (BR, K, LISC, PRE, SRGH); ibid., Chipani, *Whellan 668* (MO, PRE!, SRGH). The specimens have typical *S. pungens* leaf apices and texture, but with the overall ovate leaf shape and branchlet features of *S. innocua.*

S. pungens x ***S. madagascariensis*** SOUTH AFRICA: Nylsvlei Nature Reserve, *K. Balkwill 12092* (K). The leaves have pungent tip and slightly crenate margins combined with a near obovate shape and other vegetative features typical of *S. madagascariensis*. A number of similar specimens have been collected from the northern slope of the Magaliesberg area, according to Verdoorn (1963).

S. innocua* x *S. lucens. DEMOCRATIC REPUBLIC OF THE CONGO: Katanga, near Lubumbashi *Quarré 1927* (A, BR). Leeuwenberg (1969) reported this specimen as an intermediate between *S. innocua* and *S. lucens* based on its branches, leaves, inflorescences and flowers. It has small fruits, which sometimes occur in *S. lucens*.

S. gerrardii* x *S. madagascariensis SOUTH AFRICA: Nkandla district, *D Edwards 1414* (PRE). A specimen considered as *S. innocua* subsp. *dysophylla* (Benth.) Verdoorn and as *S. innocua* subsp. *burtonii* var. *burtonii* (Bak.) Bruce et Lewis. Leeuwenberg, however, designated it as *S. madagascariensis*, although he regarded the subspecific epithets as representing a different gestalt. In the view of the present author, this specimen represents a potential hybrid. Near Tugela River, *LE Codd & IC Verdoorn 10187*, *10203* (PRE); these specimens were designated by Verdoorn as a putative hybrid, an assessment with which I agree, as the specimens combine the features of *S. gerrardii* and *S. madagascariensis*. Marianhill, *Marloth 5669* (PRE). There appears to be abundant potential for hybridization between these two taxa in the Tugela Valley area of South Africa where the species are sympatric.

Finally, it is noteworthy that the five taxa highlighted under hybridization here all belong to the same evolutionarily recent section *Densiflorae*, and helix IIs in their ITS2 secondary structure are identical. Identical structure of helix II has been suggested as a probable molecular fingerprint for biological species (Coleman, 2009). This may aid their ability to freely exchange genes. A lack of hybrid

specimens between any two among these five may therefore be due to geographical isolation rather than genetic incompatibility.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

In chapters 2 – 7 of this thesis, I have employed a variety of approaches to explore the diversity of southern African members of *Strychnos*. Taking a cue from the view of Noss (1990) that consideration of biodiversity should be expanded to various levels to include genes, species, populations, communities, ecosystems and landscapes, I have endeavoured to focus on as many levels as possible, given the constraints of competing resources. This molecule-to-morphology approach, coupled with historical biogeography, has witnessed the use of DNA sequences, mathematical models of shape and molecular markers in conjunction with traditional alpha-taxonomic methods to ask pertinent questions and provide what I hope are some answers concerning the evolution of *Strychnos*. I have presented a number of unique findings, including the presence of a large, section-defining indel in the plastid marker *trnS – trnG* for section *Densiflorae* and unravelled the unusual distribution pattern observed in *S. gerrardii*. Furthermore, the first species-level ITS2 secondary structure models are provided here for southern African *Strychnos*. What follows are summary highlights of key findings from this work and discussions on their implications and potential importance from a broad perspective.

SPECIES CONCEPTS, SPECIES BOUNDARIES AND PHYLOGENETIC AFFILIATIONS IN *STRYCHNOS*

No major taxonomic discourse is considered complete without some reference to the species debate. My goal here is not to recap these seemingly endless debates

as excellent reviews are available elsewhere (Mayden, 1997; Coyne and Orr, 2004; Hausdorf, 2011). Rather it is to explain what *my results* suggest regarding the nature of species using *Strychnos* as a model. It is inevitable that some generalizations will be made. Whether consciously or not, every taxonomist invariably employs some species concepts in their decision-making process. It helps though to be aware of such concepts and their underlying assumptions. In an excellent critique of species concepts, De Queiroz (2007) identified a common thread to all the seemingly incompatible concepts; their property of being "*separately evolving metapopulation lineages… or segments thereof*." Separation of the theoretical concepts of species from the operational criteria for species delimitation as advocated by De Queiroz (2005; 2007; 2011) has been adopted in this work within the context of an integrated taxonomy. Such an operational view permits the recognition of species, provided some measure of evolutionary divergence can be established without necessarily meeting all other criteria, which are considered secondary.

Results of the ITS2 secondary structure models indicate that a number of *Strychnos* species are not reproductively isolated from each other, indicating the absence of genetic incompatibility (Chapter 4). These species are, however, very distinct morphologically and in their plastid DNA. That these taxa can successfully exchange genes is supported by patently hybrid individuals found in areas of geographic overlap among some of these taxa (Leeuwenberg, 1969; Chapter 7). It could well be that the rate of speciation, (as shown by evolution of some complex characters) in certain groups, exceeds the rate at which genes controlling compatibility/crossability evolve. Thus applying the biological species concept

gratuitously here will result in classifying members of section *Densiflorae* as a single species. The conservation implications of such massive lumping are very obvious if every gene-exchanging or potentially gene-exchanging group of taxa are regarded as a single species. In contrast, the dynamic nature of the speciation process immediately suggests a real possibility of taxonomic inflation under this view as any accepted species taxon will be undergoing some degree of divergence in its respective populations, however limited. It is clear that agreeing on an exact point when species status is achieved is a rather arbitrary decision influenced by the potentially subjective view of the taxonomist and the groups of organisms under consideration (Claridge, 2010; Mishler, 2010). It seems indeed that in theory the species debate may never go away; at least not any time soon.

Phylogenetic aggregations among the African taxa reveal several natural clades, which may equate to sections. However, the sectional categories of Leeuwenberg are not supported, with the exception of section *Spinosae* (Frasier, 2008; Chapter 5). Section *Lanigerae* is paraphyletic based on ITS data (Frasier, 2008), although monophyletic for the African elements. Whether plastid datasets will corroborate the overall paraphyletic relationship of the section awaits further investigation. In resolving difficulties such as this, we should be mindful that taxonomic rules were made for the taxonomist and not the other way round, for the ultimate purpose of achieving a functional classification scheme. Indeed, taxonomic hierarchies above the species level (e.g. sections) are considered arbitrary (Claridge, 2010). Extending this view to species as a logical appendage of a higher category, some authors have queried the very reality of species (Mishler, 2010). Nevertheless, it is equally recognised that natural aggregates of organisms will of necessity possess

a number of unifying attributes. Therefore the existence of a continuum among these clusters does not invalidate the reality of either aggregate. If anything, it confirms the dynamic nature of the evolutionary processes producing such clusters in the first place.

A parsimonious approach to dealing with the paraphyly highlighted in section *Lanigerae*, therefore, is to expand it to accommodate the two 'offending' Asian taxa, *S. axillaris* (section *Penicillatae*) and *S. umbellata* (section *Brevitubae*), since they both belong, along with the other *Lanigerae* members, in a maximally supported clade (Frasier, 2008). Similar reasoning will necessitate excluding *S. staudtii* from section *Densiflorae* to restore monophyly and perhaps make it a monotypic section on its own. Most of the other non-monophyletic sections will require wholesale reconstitution following expanded taxon and gene sampling.

MORPHOLOGY, MORPHOMETRICS AND REDUCTION OF SUBJECTIVITY IN TAXONOMIC DECISIONS

The vast array of morphological diversity in *Strychnos*, from the macroscopic to the microscopic, necessitates that such variations should be captured as accurately as possible to harness their taxonomic potential. Regardless of the strength of the molecular methods, which this author readily acknowledges and happily employs, morphological data analysis will continue to play a significant role in modern systematics for the pragmatic reason that morphologies are the first port of call in the field, museums and herbaria. Further, the very nature of systematics mandates that morphological descriptions of taxa be seriously encouraged, however tedious, slow or expert-driven they may be. For one, hanging DNA sequences, a very informative series of …AAACCGGTTGTGT…, of

an endangered animal or plant in any museum will not attract takers. However bringing the actual animal (live or stuffed) or the plant will certainly elicit a reaction from the public, thus creating better awareness.

One of the major arguments against morphological data in systematics is the high level of subjective decision making involved. Many species are described based on qualitative features which, upon close examination by other experts, may reveal aspects completely missed or interpreted differently. Clearly, there is a limit to the achievable resolution of the human eye; a limit imposed by the laws of physics and amplified/varied across individual observers by the laws of genetics. A combination of these limitations, perhaps coloured by the bias of individual taxonomists, is a ripe recipe for subjectivity. The various aspects of what constitutes something as seemingly simple as leaf shape are potential minefields for taxonomic conflicts. A leaf apex described as acute by one observer may be classified as shortly acuminate by another, with each convinced of the correctness of their terminologies. Such an approach, which is still used in alpha taxonomy, has created an array of artificial differences where there are none, despite the best efforts and intentions of practitioners to produce a common glossary of terms. In addition, it has been shown that among supposed experts, achieving self-consistency in taxonomic identification can be quite challenging due to human factors related to fatigue, boredom and prior expectations (Culverhouse *et al.* 2014).

The field of geometric morphometrics (GM) was recently developed to mitigate the subjective tendencies inherent in the taxonomic use of qualitative features and

render them in repeatable mathematical terms. Although the basic principle, as currently practiced, dates to the work of Thompson (1917), the mathematical foundations were laid in the pioneering works of Kendall (1977) and Bookstein (1977; 1986) and later refined in the early 1990s (Rohlf, 1990; Bookstein, 1991; Rohlf and Marcus, 1993). Various applications of GM have allowed for precise quantification of minute shape details, usually imperceptible to the human eye, but nevertheless useful in systematics due to the evolutionary importance of small heritable variations. Adebowale *et al.* (2012), using elliptic Fourier analysis to capture leaf outline in *Strychnos* section *Densiflorae*, successfully distinguished between the closely related *S. gerrardii* and *S. madagascariensis* based on the symmetric component of leaf shape. So powerful are the applications of similar and related techniques for taxonomic ends that some enthusiasts have advocated for an automated decision-making system for taxonomy (Gaston and O'Neill, 2004; Macleod *et al.* 2010). While such automation may not address all our taxonomic needs, there seems to be a genuine case for their consideration, especially in labour-intensive fields such as palynology, where identification is very crucial (Holt and Bennett, 2014) and other areas where there are tens of thousands of archival materials for which routine human identification is the required norm (Gaston and O'Neill, 2004; Culverhouse *et al.* 2014).

PALAEO-CLIMATIC CHANGES AND THE EVOLUTION OF ARID-ADAPTEDNESS IN SOUTHERN AFRICAN *STRYCHNOS*

An interesting discovery in this research was the strong ecological signature found in the molecular datasets, whether nuclear or plastid. Predominantly forest-inhabiting species occupy basal positions and all predominantly savanna-dwelling species are in derived positions in all molecular phylogenetic hypotheses. What is

further intriguing is the remarkable fine scale coincidence between estimated divergence times for the savanna species with the period of increasing aridification across Africa (Chapter 6). Morphological signatures of arid-adaptedness are evident in the corky, rough bark and large, hard fruits of the savanna species. This is even made more remarkable by the absence of such signatures in all forest species, except *S. gerrardii*. The unique distribution of this exemplar taxon has been demonstrated to be a consequence of repeated range expansion and contraction of its arid-adapted ancestor during the Pleistocene. It is quite likely that somewhere in the genome of southern African *Strychnos*, there will be an equally strong genetic imprint of arid-adaptation, since the evolution of such complex traits must have been preceded by intense directional selection pressure.

The mean estimated crown age of southern African *Strychnos* is ca. 13 myr, implying that the genus is slightly older than this, originating within the Miocene. In the context of Loganiaceae phylogeny (Frasier, 2008), this estimate is consistent with recent findings of Foster *et al.* (2014) for the Australian endemic *Logania*, whose origin traces back to the Miocene and where rapid radiation occurred during increased aridification of mainland Australia. Although Foster *et al.* (2014) did not explore possible evidence of arid-adaptedness in *Logania*, it appears that the same biogeographic process is driving evolution in the two genera on different continents. A common feature for both is the rapid diversifications during increased aridity. Increase in aridity has long been associated with habitat fragmentation.

SUGGESTIONS FOR FUTURE RESEARCH

Since a molecular phylogenetic framework is now available for *Strychnos*, an area of study worth pursuing is the establishment of chromosome numbers for the other species for which there are no published counts. Cytotaxonomic data exist for only about 16% of *Strychnos* species, which is very low given the actual and potential value of the group. If such data are combined with flow cytometric analyses, the phenomenon of gigantism in relation to stomatal length as a potential proxy for ploidy level estimation in *Strychnos* could be better evaluated.

The evolution of a number of complex traits in response to aridification in *Strychnos* immediately suggests that there may be other adaptive traits. Current understanding of drought-tolerance mechanisms in plants indicates the possibility of anatomical modifications in the root, stem and leaf tissues consistent with aridity/drought-tolerance levels. The Kranz anatomy, typical of many C4 plants inhabiting arid areas, might be worth investigating in *Strychnos* to assess levels of correlation, if any, between habitat and anatomy. Such findings could prove valuable in an era of climate change. All the arid-adapted taxa are also a potential food sources. Some species, such as *S. cocculoides* and *S. spinosa*, have been actively cultivated for food in arid regions of the world (Jerabek, 1934; Sitrit *et al.* 2003). As a genomic dimension to the enquiry, the genes responsible for drought tolerance could be isolated and characterised.

Following on from the food potential is the need to assess the level of population genetic diversity among several species of *Strychnos*, starting with such widely distributed and highly regarded taxa as *S. cocculoides* and *S. spinosa*, and then

moving on to the other large-fruited species. This could help discover unique haplotypes for each species; a first step in population-level biodiversity conservation. Still on the population genetic front, it would be interesting to understand the extent and nature of divergence between African and Asian populations of *S. potatorum*, as this is the only *Strychnos* species found on both continents. Such studies might elucidate the route by which this species migrated from Africa to Asia and perhaps turn up some anthropologically-relevant details along the way.

For a clearer phylogenetic picture to emerge within the genus there is need to generate extensive chloroplast DNA datasets to complement available nuclear data. Such a wealth of molecular datasets will allow the question of global sectional classification to be better addressed. Section *Densiflorae* should be studied in detail, as it has proved to hold some of the keys towards a better ecological and evolutionary understanding of the genus. The *trnS-trnG* marker should be specifically explored for *Densiflorae* to probe the reliability of the unusually large indel as a sectional molecular signature of diagnostic value.

Due to the capacity of Next Generation Sequencing (NGS) technologies to readily generate large number of sequence data, and the potential of such data at clarifying phylogenetic relationships and better demarcating species boundaries, NGS should be applied to *Strychnos* systematics. A possible starting point would be to produce phylogenies based on plastid genomes representative of all the sections of *Strychnos*.

On a final note, elucidation of the complexity of relationships among any group of biological entities, at whatever hierarchical level, seems to require multiple sources of preferably independent data for better resolution. Such data, in the context of a study like this, appear to derive from a cooperative triumvirate of genes, geography and gestalt.

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APPENDIX 1

A List of herbaria cited in this work, from where specimens were either loaned directly, or where other duplicates not seen by the author reside.

Abbreviation	Full meaning
A	Arnold Arboretum, Harvard University
B	Botanischer Garten und Botanisches Museum, Berlin-Dahlem.
BM	The Natural History Museum, London
BNRH	Buffelskloof Nature Reserve
BOL	University of Cape Town, Bolus Herbarium
BR	National Botanic Garden of Belgium
COI	University of Coimbra Portugal
E	Royal Botanic Garden Edinburgh
EA	National Museums of Kenya, Nairobi Kenya
ENT	Ministry of Natural Resources Entebbe, Uganda
FHO	University of Oxford
FI	Natural History Museum Firenze
G	Conservatoire et Jardin botaniques de la Ville de Genève
GH	Harvard University
GRA	Albany Museum Grahamstown South Africa
HAL	Martin-Luther-Universität, Halle
HBG	Biozentrum Klein-Flottbek, Hamburg
IFE	Obafemi Awolowo University herbarium, Ile-Ife.
J	University of the Witwatersrand, Moss Herbarium, Johannesburg
K	Royal Botanic Gardens, Kew
LE	V. L. Komarov Botanical Institute, Saint Petersburg Russia
LISC	Jardim Botânico Tropical Instituto de Investigação Científica Lisboa Portugal
LISU	Museu Nacional de História Natural e da Ciência, Lisboa Portugal
LUAI	ex-Centro Nacional de Investigação Cientifica (CNIC), Luanda Angola
LY	Université Claude Bernard, Lyon France
M	Botanische Staatssammlung München, Munich Germany
MO	Missouri Botanical Garden, Saint-Louis Missouri USA
MPU	Université Montpellier 2, Monpellier

	France					
NBG	South African National Biodiversity Institute, Cape Town					
NH	South African National Biodiversity Institute, Natal Herbarium Durban					
NU	University of KwaZulu-Natal, Pietermaritzburg campus					
NY	New York Botanical Garden, Bronx					
P	Muséum National d'Histoire Naturelle, Paris					
PRE	South African National Biodiversity Institute (SANBI), National Herbarium, Pretoria					
PRF	South African Forestry Research Institute, Pretoria					
PRU	University of Pretoria, Pretoria					
S	Swedish Museum of Natural History, Stockholm					
SDNH	Swaziland National Herbarium, Manzini					
SRGH	Botanic Garden, Harare Zimbabwe					
TCD	Trinity College, Dublin Ireland					
U	National Herbarium of the Netherlands, Herbarium Utrecht, Leiden					
UDW	University of KwaZulu-Natal, Westville campus, Durban					
UPS	Uppsala University, Uppsala					
US	Smithsonian Institution, Washington, USA					
W	Naturhistorisches Museum Wien, Austria					
WAG	Wageningen University, Wageningen					
WIND	National Botanical Research Institute, Windhoek					

APPENDIX 2

WORDLE ABSTRACTS-BASED WORD CLOUD
From abstracts in this thesis