A systematic study of *Boerhavia* L. and *Commicarpus* Standl. (Nyctaginaceae) in southern Africa

M. Struwig
(B.Sc; M. Env. Sc.)

Thesis submitted in fulfillment of the requirements for the degree Philosophiae Doctor in Environmental Sciences at the Potchefstroom campus of the North-West University

Supervisor: Prof. S.J. Siebert

Co-supervisor: Dr. A. Jordaan

Assistant supervisor: Prof. S. Barnard

November 2011

ACKNOWLEDGEMENTS

First and foremost I would like to thank my Heavenly Father for the opportunity and for the courage and strength to complete this study to the best of the abilities that He gave me.

Very special thanks to Prof. S.J. Siebert for his endless patience, guidance and encouragement.

I would like to thank the following persons and institutions:

- Dr. A. Jordaan and Prof. S. Barnard for their guidance and assistance with the morphological, anatomical, palynological and molecular work
- Mr L. Meyer and Ms E. Klaassen (WIND) for their assistance with fieldwork in Namibia (2009 & 2010)
- Prof. A.E. van Wyk for teaching me the methodology of acetolizing pollen
- The curators of the following herbaria for access to their Nyctaginaceae collection: BLFU, BOL, GRA, J, KMG, KSAN, NH, NMB, NU, PRE, PRU, PUC, UCBG, UNIN, WIND and ZULU
- Dr. L.R. Tiedt and Ms W. Pretorius at the Laboratory of Electron Microscopy of the North-West University for technical assistance and guidance with the SEM, TEM and light microscopic work
- Ms M.J. du Toit for assistance with the maps
- Prof. L. du Preez for the use of the African Amphibian Conservation Research Group's microscope
- DNA Sequencer of the Central Analytical Facilities, Stellenbosch University for the DNA sequencing laboratory work
- Dr. C.M.S. Mienie and DNAbiotec (Pty) Ltd. who taught me valuable skills needed for molecular laboratory work
- Dr. Jane Wright for presenting an excellent phylogenetic course
- Friends and family for their support and encouragement

ABSTRACT

The Nyctaginaceae Juss. is a small flowering plant family of about 30 genera and 400 species mainly found in the tropical and subtropical regions of the New World. In Africa, six genera and 49 species occur, and in southern Africa, five genera and 19 species. *Boerhavia* L. and *Commicarpus* Standl. are the most species rich genera in southern Africa, with seven and eight species respectively. These species have not previously been studied taxonomically nor phylogenetically, and the objective of this study was to provide a systematic and phylogenetic treatment of these groups for southern Africa.

Plant material was collected from the diversity centres of *Boerhavia* and *Commicarpus* in southern Africa. Leaves, flowers and anthocarps stored in ethanol were measured and examined with a stereomicroscope and surface studies conducted with a scanning electron microscope (SEM). Material fixed in paraformaldehyde was embedded, sectioned, stained and examined with a light microscope. Pollen samples were acetolized before examination with a SEM. Sequencing analyses were done with a DNA Sequencer and neighbor-joining and maximum likelihood trees drawn. Distribution and habitat information were obtained from voucher specimens from various herbaria.

The pollen grains are uniform in shape and sculpture and provide no diagnostic characters with which to distinguish between species. They do, however, provide broad characters to distinguish between the genera.

Morphological, anatomical and molecular investigations, however, provided a series of characters to distinctly discern between *Boerhavia* and *Commicarpus*. Morphologically, *Boerhavia* and *Commicarpus* differ in growth form, inflorescence type, shape and indumentum of the upper and lower part of the flower, and shape and indumentum of the anthocarp. Anatomically, *Boerhavia* can be distinguished from *Commicarpus* by the Kranz anatomy around the minor veins of the leaves (which is absent in *Commicarpus*) and the sclerenchyma bundles which are present within the rib and inter-rib areas of the anthocarp (sclerenchyma bundles are

only present in the rib area in *Commicarpus*). Molecular analyses group *Boerhavia* and *Commicarpus* as distinct clades with high bootstrap support. The differentiation is such in southern Africa, that *Mirabilis* and *Acleisanthes*, two non-African genera, are even placed intermediately between the two.

The different species of *Boerhavia* and *Commicarpus* can be distinguished by the lower, coriaceous part of the flower and the anthocarp, as the arrangement of the glands, ribs and trichomes on these structures is species specific. The anatomy of the stems, leaves and anthocarps of the different species is uniform and uninformative and cannot be used to distinguish between the species. Molecular analyses support the distinction of the different species as defined by the morphology, and group the morphologically similar *C. fruticosus* and *C. squarrosus* in close relation. The aliens, *Boerhavia cordobensis*, *B. diffusa* var. *diffusa* and *B. erecta*, group together and the indigenous *B. coccinea* var. *coccinea*, *B. deserticola*, *B. hereroensis* and *B. repens* subsp. *repens* group together. The widely distributed *C. plumbagineus* and *C. helenae* var. *helenae* are closely related to each other, as are *C. pentandrus* and *C. decipiens* which are limited in theitr distribution to the African.

This systematic study has shown that *Boerhavia* and *Commicarpus* are two distinct genera in southern Africa with well-defined species. This provides a workable classification system for southern Africa. This classification requires to be further refined by combining the morphology, anatomy, palynology and phylogenetics of the southern African *Boerhavia* and *Commicarpus* species into a single phylogeny. The phylogenetic investigations are, however, incomplete as the molecular analyses still need refinement and incorporation of more genes and taxa.

Keywords: *Boerhavia*, *Commicarpus*, Nyctaginaceae, phylogeny, southern Africa, systematics

OPSOMMING

Die Nyctaginaceae Juss. is ‘n klein blomplantfamilie met ongeveer 30 genera en 400 spesies wat hoofsaaklik in die tropiese en subtropiese dele van die Nuwe Wêreld voorkom. Ses genera en 49 spesies is in Afrika teenwoordig waarvan vyf genera en 19 spesies in Suider-Afrika voorkom. *Boerhavia* L. en *Commicarpus* Standl. is die mees spesieryke genera in Suider-Afrika, elk met onderskeidelik sewe en agt spesies. Hierdie spesies is nog nie vantevore taksonomies of filogeneties ondersoek nie, en die doel met hierdie studie was om ‘n sistematies-filogenetiese ondersoek van dié twee genusgroepe in Suider-Afrika te doen.

Plantmateriaal is in die diversiteitsentrums van *Boerhavia* en *Commicarpus* in Suider-Afrika versamel. Materiaal van blare, blomme en antokarpe wat in etanol gestoor is, is gemeet en met ‘n stereomikroskoop ondersoek. Oppervlakstudies is met ‘n skandeerelektronmikroskoop (SEM) gedoen. Materiaal gefikseer in paraformaldehied, is ingebed, gesny, gekleur en met ‘n ligmikroskoop ondersoek. Stuifmeel is geasetoliseer voordat dit met die SEM ondersoek is. Basispaarvolgorde-analises is met ‘n DNS-volgorde-analiseerder gedoen en verwantskapsluitingsbome (*neighbor-joining*) en maksimum waarskynlikheidsbome (*maximum likelihood*) is geteken. Verspreidings- en habitatinligting is verkry vanaf bewyseksemplare van verskeie herbaria.

Stuifmeelkorrels van hierdie genera is eenders in vorm en skulptuur en alhoewel die twee genera in breë van mekaar onderskei kan word, is daar nie diagnostiese kenmerke waarmee op spesievlak onderskei kan word nie.

Morfologiese, anatomiese and molekulêre ondersoeke het ‘n reeks van kenmerke opgelewer waarmee *Boerhavia* en *Commicarpus* onderskei kan word. *Boerhavia* en *Commicarpus* verskil morfologies in groeivorm, in die tipe bloeiwyse, in die vorm en skulptuur van die boonste en onderste dele van die blom en die vorm en skulptuur van die antokarp. Anatomies kan *Boerhavia* van *Commicarpus* onderskei word op grond van die teenwoordigheid van Kransanatomie om die syare van die blare (wat afwesig is by *Commicarpus*) en die sklerenchiembondels wat in die rib-

en tussenrib-areas van die antokarp teenwoordig is (sklerenchiembondels is slegs teenwoordig in die rib-areas van *Commicarpus*). Molekulêre analises groepeer *Boerhavia* en *Commicarpus* in aparte klades wat elk deur hoë statistiese ondersteuning (*bootstrap support*) ondersteun word. Die indeling is sodanig dat *Mirabilis* en *Acleisanthes*, twee nie-Afrika genera, in 'n tussenposisie tussen die twee bestudeerde genera groepeer.

Die verskillende *Boerhavia* en *Commicarpus* spesies kan van mekaar onderskei word op grond van die rangskikking van die kliere, ribbe en trigome op die onderste, leeragtige deel van die blom en die antokarp wat spesiespesifiek is. Die anatomie van die stingels, blare en antokarpe van die verskillende spesies is eenvormig en verskaf nie inligting waarmee die spesies van mekaar onderskei kan word nie. Molekulêre analises ondersteun die indeling van die verskillende spesies soos morfologies bepaal en groepeer die morfologies eenvormige *C. fruticosus* en *C. squarrosus* in nou verwantskap. Die uitheemse *Boerhavia cordobensis*, *B. diffusa* var. *diffusa* en *B. erecta* groepeer saam en die inheemse *B. coccinea* var. *coccinea*, *B. deserticola*, *B. hereroensis* en *B. repens* subsp. *repens* groepeer saam. Die wydverspreide *C. plumbagineus* and *C. helenae* var. *helenae* is nou verwant, asook *C. pentandrus* en *C. decipiens*, wat in hul verspreiding tot Afrika beperk is.

Die sistematiese studie toon dat *Boerhavia* en *Commicarpus* twee afsonderlike genera in suider Afrika met goed gedefinieerde spesies is. Dit verskaf 'n werkbare klassifikasiesisteem vir suider Afrika. Die klassifikasiesisteem kan egter verfyn word deur die morfologie, anatomie, palinologie en filogenie van die suider Afrikaanse *Boerhavia*- en *Commicarpus*-spesies in 'n enkele filogenie te kombineer. Die filogenetiese ondersoek is egter onvolledig omdat die molekulêre analises verder verfyn moet word en meer gene en taksa bygevoeg moet word.

Keywords: *Boerhavia*, *Commicarpus*, Nyctaginaceae, filogenie, suider Afrika, sistematiek

LIST OF ABBREVIATIONS

A	Adenine
B	Botanischer Garten und Botanisches Museum Berlin-Dahlem, Germary
BISH	Herbarium Pacificum, Bishop Museum, Hawaii, USA
BLFU	Geo-Potts Herbarium, University of the Free State, Bloemfontein, South Africa
BM	Herbarium, The Natural History Museum, London, United Kingdom
BM-SL	Sloane Herbarium, The Natural History Museum, London, United Kingdom
BOL	Bolus Herbarium, University of Cape Town, Rondebosch, South Africa
Bp	Base pair
DNA	Deoxyribonucleic Acid
E	Endemic
ESEM	Environmental Scanning Electron Microscope
F	Herbarium, Field Museum of Natural History, Chicago, United States of America
G	Guanine
GH	Herbarium, Harvard University, Cambridge, United States of America
GRA	Selmar Schonland Herbarium, Albany Museum, Grahamstown, South Afirca
I_{cong}	Congruency Index
ITS	Internal Transcribed Spacer
J	Charles E. Moss Herbarium, University of the Witwatersrand, Johannesburg, South Africa
K	Herbarium, Royal Botanic Gardens, Kew, United Kingdom
KMG	Herbarium, McGregor Museum, Kimberley, South Africa
KNP	Skukuza Herbarium, South African National Parks, Skukuza, South Africa
KSAN	Kimberley South African National Parks Herbarium, South African National Parks, Kimberley, South Africa
L	Nationaal Herbarium Nederland, Leiden University branch, Leiden, Nederland
LINN	Herbarium, Linnean Society of London, London, United Kingdom
M	Herbarium, Botanische Staatssammlung München, München, Germany
MA	Herbario, Real Jardín Botánico, Madrid, Spain
MEGA	Molecular Evolutionary Genetics Analysis
MO	Herbarium, Missouri Botanical Garden, Saint Louis, USA
NCBI	National Center for Biotechnology Information
*ndh*F	subunit F of NADP dehydrogenase
NH	KwaZulu-Natal Herbarium, South African National Biodiversity Institute, Durban, South Africa
NMB	Herbarium, National Museum, Bloemfontein, South Africa
NP	National Park
NR	Nature reserve
NSW	National Herbarium of New South Wales, Royal Botanic Gardens, Sydney, Australia
NU	Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg, South Africa
PCR	Polymerase Chain Reaction
PRE	National Herbarium, South African National Biodiversity Institute, Pretoria, South Africa

PRU	H.G.W.J. Schweickerdt Herbarium, University of Pretoria, Pretoria, South Africa
PUC	A.P. Goossens Herbarium, North-West University, Potchefstroom, South Africa
rDNA	Ribosomal DNA
RNA	Ribonucleic Acid
SAM	Herbarium, South African Museum, Cape Town, South Africa
SEM	Scanning Electron Microscope
T	Thymine
TEM	Transmission Electron Microscope
UCBG	Herbarium, University of Botswana, Gaborone, Botswana
UNIN	Larry Leach Herbarium, University of Limpopo, Sovenga, South Africa
WIND	National Herbarium of Namibia, National Botanical Research Institute, Windhoek, Namibia
Z	Herbarium, University of Zürich, Switzerland
ZULU	Herbarium, University of Zululand, Kwa-Dlangezwa, South Africa

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES