

TAXONOMIC VARIATION, SPECIES LIMITS AND PHYLOGENETIC RELATIONSHIPS IN OLINIACEAE

Submitted by

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Date: March 2012

DECLARATION

I, Ramagwai Joseph Sebola Student number 8901772H declare that:

- i) This thesis, except where otherwise indicated, represents my own research work on the taxonomic variation, species limits and phylogenetic relationships in Oliniaceae in the School of Animal Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg;
- ii) This thesis does not contain data, figures or writing, unless specifically acknowledged, copied from other researchers;
- iii) This thesis has not been submitted for any degrees or examination at any other University.

Signed at Johannesburg on the day of , **2012**.

Signature

ABSTRACT

A new classification of the Oliniaceae is presented. The monogeneric Oliniaceae is one of the smallest, but lesser known myrtalean families that has not been monographed since Cufodontis' (1960) revision, despite advances in analytical tools. In this thesis, a combination of morphometric phenetic (principal component analysis, principal coordinate analysis, and cluster analysis) and phylogenetic (cladistics) methods of analyses were used to critically evaluate the morphological variation; to determine the best taxonomic characters (quantitative and qualitative); and, based on these characters, to circumscribe species within *Olinia*; to propose and test hypotheses of phylogenetic relationships; and to provide a new classification of the Oliniaceae. Data were gathered from herbarium specimens and from populations in the field in order to gain an understanding of intra- and inter-specific and population level variation.

In this study, the basic assumptions often made by most plant systematists with regard to characters thought to be useful in the taxonomy of plant taxa, and the methods of character analysis often employed are tested. It is established that there are noteworthy exceptions to some of the commonly held indefensible views of *a priori* taxonomic knowledge of specific groups, especially in the Oliniaceae; instead the most objective means of assessing the relative merits of different data sets should be sought. It is also recognised in this study that one of biggest impediments to understanding species-level variation and diversity is not so much the lack of data and the sources (herbarium specimens or population level data), but rather the flawed methodology used to analyse data.

The classification presented in this study reflects remarkable morphological variation within *Olinia*, which is the basis for identification of species. The major delimiting characters between species were found to

be the shapes of petals and leaves, presence or absence of indumentum and the degree of hairiness on floral parts, and the form of inflorescence units (i.e. being either compact or spreading). Insect infestations and their effect on the floral biology and morphology (size and length) in *Olinia* are discussed in the context of species circumscriptions. Different types of floral galls are associated with particular species groups, whereby flowers of some species twist and exhibit characteristic tubercles along elongated ridges of the hypanthium when infected by insects, completely different from flowers that expand and swell when infected.

The polymorphic or ochlo-species *O. rochetiana* complex was found to exhibit great morphological variation that does not correlate with geography, and hence not easily delimited using traditional methods. A scientific, systematic procedure and technique for analysing morphological variation in suspected ochlo-species is presented, and it involves numerical phenetic or morphometric methods of analysis through separate, yet sequential stages. The technique involves calibration of the character set, sub-sampling of the taxonomic operational units (OTU's), and a stepwise approach to analysing unresolved clusters or groups. These procedures are simple to perform and reduce the biases often involved in the delimitation of clusters in phenetic analyses. The standard taxon is identified and used to calibrate the character list in Cluster Analysis in order to inform the taxonomic decisions on where to delimit taxa on phenograms by using the level of phenetic dissimilarity at which members of the standard taxon join each other before they join other clusters as the criterion for delimitation of taxa. In order to avoid the calibration of the data set being influenced by a single concept of a standard taxon, more than one standard taxon can be included in the analyses. The consistency of retrieving similar clusters in Cluster Analysis can be verified by analysing representative sub-samples of the data matrix. This provides the confidence with which to accept or reject the delineated clusters. Therefore, the consistent retrieval of the same groups in the different analyses of the sub-samples using different OTU's and numbers of OTU's

of groups suggests that the groups are reliable based on the set of characters used, and that the groups obtained do not depend on the total number of OTU's or individual OTU's used in the analyses, but rather on the interpretation of variation among the studied taxa represented by the OTU's. The stepwise approach becomes useful when there is difficulty in the interpretation of phenetic similarities of clusters in ordination analysis. Accordingly, distinct clusters or groups of unquestionable distinctness in the ordination space are removed from the analysis, thereby increasing availability of ordination space to allow the remaining groups to spread beyond their original positions. During stepwise analysis, different suites of characters that correlated with other ordination axes often become dominant or active in separating the remaining clusters or groups of OTU's. This technique is recommended as a standard procedure in phenetic analyses since it improves the confidence that can be assigned to resultant clusters or groups, and also allows for finer resolution and clearer visualisation of phenetic similarities of unresolved clusters or groups.

The morphometric analysis of variation has established that *O. huillensis*, hitherto reported to occur only in Angola, is widespread in southern Africa and exhibits geographic segregates characterised as *O. huillensis* subsp. *huillensis* (largely the Angolan material with papery leaves), *O. huillensis* subsp. *burttdavii* (for plants with very short petioles; leaves that are broadly elliptic to obovate and leathery; inflorescences that are compact with shorter internodes), and *O. huillensis* subsp. *discolor* (for plants with slender branches; longer petioles; thin, glossy leaves; inflorescences with longer internodes; and thin walls of floral tubes/hypanthia). The persistence of bracts and bracteoles through and after anthesis or their deciduousness before or at anthesis, including the reduction or not of axes of the inflorescence are critical distinguishing features among some sympatric species (*O. capensis* and *O. ventosa*, and between *O. micrantha* and *O. emarginata*) which are often confused. Two species (*O. radiata* and *O. micrantha* Decne.) are protected under the South African National

Forests Act (Act No. 84 of 1998). The cladistic analyses corroborate and support the monophyly of *Olinia* as earlier demonstrated (Conti *et al.* 1996; Schönenberger & Conti 2003). Two major clades are recognisable at sectional levels: Section *Olinia* and Section *Rochetiana* with the latter representing all tropical and subtropical taxa (*O. rochetiana sensu stricto*, *O. ruandensis*, *O. usambarensis*, *O. huillensis* subsp. *huillensis*, *O. huillensis* subsp. *burttdavii*, and *O. huillensis* subsp. *discolor*) whereas the former section, which includes the type species *O. ventosa* (L.) Cufod. for the genus *Olinia* and bears the autonym, represents all the temperate taxa (*O. emarginata*, *O. radiata*, *O. capensis*, *O. micrantha*, *O. ventosa*, and *O. vanguerioides*). The recognition of these two sections leaves *Olinia* phylogenetically natural (i.e. monophyletic), and from a taxonomic point of view the two sections or species groups have unique morphological synapomorphies.

Accepting that plant taxa should be reproductively isolated and phenotypically distinct (Rieseberg *et al.* 2006), the discrete non-overlapping phenotypic clusters obtained in the phenetic analyses were considered to represent taxonomic entities. Therefore, the phenetic and ecological concepts of species are applied at the specific and subspecific levels, respectively. Variation in the polymorphic *O. rochetiana* species complex is best explained using the ochlo-species concept (White 1962). Types (holotypes, isotypes, and iconotypes) were designated and cited for all species and their synonyms. A full taxonomic account of Oliniaceae worldwide is provided with an identification key, descriptions and distribution maps for all taxa recognised.

DEDICATION

To Tlou Solomon and Esther Mapula Sebola, my deceased parents who taught me respect for humankind and instilled a sense of responsibility to others.

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Chapter 1.

General Introduction to the Study

1.1 Background

a) Systematics and taxonomic history

Phylogenetic studies within the Myrtales have retained the Oliniaceae as a distinct family (Conti *et al.* 1996 & 1997; Schönenberger & Conti 2003) within the “OPRA” clade (i.e. Oliniaceae-Penaeaceae-Rhynchocalycaceae-Alzateaceae) or the “CAROP” clade when Crypteroniaceae is included (Clausing & Renner 2001). Knowledge of the phylogenetic relationships between members of the Oliniaceae is desirable, but this has been rendered impossible by lack of detailed information on the taxonomic limits of species and their phytogeographic affinities. Oliniaceae, the ‘hard pear’ family, includes a single genus *Olinia* Thunb. and is characterised by a shrubby to large arborescent habit with quadrangular young branches; and flowers arranged in paniculate inflorescences with branches ending in either three or nine-flowered cymes (Dahlgren & Thorne 1984; Weberling 1988). *Olinia* is endemic to the African continent and the majority of species (*O. rochetiana*, *O. vanguerioides*, *O. huillensis*, *O. usambarensis*, *O. ruandensis*, *O. radiata*, *O. emarginata* and *O. micrantha*) occur in tropical, subtropical and temperate areas, whereas only two species occur in the winter-rainfall areas of south- western Cape (*O. capensis* and *O. ventosa*). The seemingly aberrant record of *O. ventosa* on the island of St. Helena is regarded as a relatively recent introduction from the Western Cape (Hutchinson 1926 & 1973). The habitats for most species are consistently mountain forests to forest margins with occasional occurrence in rocky outcrops. The earliest revisions of the Oliniaceae were by Klotzsch (1836) and Sonder (1862) who dealt mainly with the southern African species, Hiern (1871) for tropical species, and Decaisne (1877) whose treatment of *Olinia* was worldwide. Since then, there have been several regional revisions of the Oliniaceae,

dealing with species occurring in particular floral regions of the African continent (Hofmeyr & Phillips 1922; Burtt Davy 1926; Cufodontis 1960; Fernandes & Fernandes 1962; Verdcourt 1975 & 1978; Verdcourt & Fernandes 1986).

The delimitation of taxa, which includes their description, naming, ranking within a hierarchical arrangement (Sneath & Sokal 1973), the testing of hypotheses of relationships and generation of classifications are the primary goals of this study, which is focussed on clarifying the taxonomic limits of species within the Oliniaceae. The complex variation within *Olinia rochetiana sensu lato* presents special taxonomic problems that merit assessment of variation from a wide range of sources in order to interpret accurately and objectively the pattern of variation. This species complex exhibits high levels of overlapping morphological variation, and is widely distributed in tropical and tropical East Africa, particularly along the high mountain ranges of East Africa (Elgon, Ruwenzori, Kilimanjaro, Aberdare, Kenya, Meru). Included in the *O. rochetiana* complex are *O. huillensis*, *O. ruandensis*, *O. discolor*, *O. volkensii*, *O. usambarensis* and *O. macrophyla*, all of which Verdcourt (1975, 1978) treated as conspecific. Species with such complicated variation patterns have been described by White (1962) as ‘ochlo-species’ to distinguish them from clearly identifiable species that show clear discrete variation. Such species are polymorphic, geographically widespread, have variation patterns not correlated with geography and therefore cannot be subdivided satisfactorily based on macro-morphological and ecological features alone (Cronk 1998; Henderson 2006).

Some general concepts and methodology for taxonomic/systematic analyses are reviewed in order to provide the rationale and appropriate philosophical context to avoid unintended confusion on their application in this study. These include taxa, species, phenetics, cladistics, classifications, and nomenclature. In taxonomy and systematic biology, the term taxon (or taxa in plural) can be any group of organisms ranging from form, variety or subspecies through species, genera and families to

kingdoms. For more detailed discussion and arguments on these and related concepts in botanical studies, reference is made to Mayr (1987), Templeton (1989), Nixon and Wheeler (1990), Wheeler and Platnick (2000), APG I (1998), APG II (2003), Stevens (2004), Van Door *et al.* (2004), Soltis *et al.* (2007).

The terms taxonomy and systematics are used here synonymously in the sense of Stace (1989) to refer to the study and description of variation, the investigation of the causes and consequences of this variation, and the analysis of the data obtained to produce a system of classification. Classifications are constructed for various purposes, and in plant systematics (as is the case in this study) these can be for phylogenetic/phyletic classifications (concerned with reflecting the evolutionary pathways and relationships among taxa), or phenetic classifications, which reflect the overall present-day similarity and/or, dissimilarity of organisms under study. In phenetic numerical taxonomy, the term operational taxonomic units (OTU's) as proposed by Sokal and Sneath (1963) are often applied to the units of study or terminal taxa, while in a phylogenetic or a cladistic sense the alternate term, evolutionary unit (EU), is used.

Phenotypic variation in some flowering plant groups does not assort readily into discrete categories (Mishler & Donoghue 1982; Rieseberg & Willis 2007). Therefore, the search for a satisfactory concept of species still remains a central focus for botanists (Graybeal 1995; Maze *et al.* 2005; Monsch 2005; Rieseberg *et al.* 2006), but is often complicated by: i) the lack of a single, all encompassing definition; ii) the use of the term 'species' in several different senses (White 1962; Sneath 1976; Van Valen 1976; Wiley 1978 & 1980; Paterson 1985; Raven 1986; Nixon & Wheeler 1990; Baum 1992; Kornet 1993; Kornet & McAllister 1993; Mayden 1997; Cronk 1998; Van Door *et al.* 2004; Rieseberg & Willis 2007); iii) the need to simultaneously reconcile recent advances in evolutionary theory, with recent advances in systematic theory; iv) with empirical requirements of

objectivity and testability (Baum 1998) and v) with constraints imposed by the formal Linnaean nomenclatural system (Brandon & Mishler 1987).

The biological species concept requires that entities or species are treated as groups of natural populations capable of interbreeding, and are reproductively isolated from other such groups of populations (Raven 1986). The emphasis is on genetic isolation rather than on morphological limits, which is unattractive for plant taxonomists because in plants the morphological and genetic limits do not always coincide (Stace 1989). The general problems associated with using herbarium specimens as the sole source of data are well known (Sneath 1976; Henderson 2006). Amongst much else this may include missing data as a result of poorly collected, pressed and mounted specimens. In this case, much of the biological information becomes incomplete due to distorted or incomplete organs, and the tendency of collectors to not record all vital information. In this project, effort will be made to supplement herbarium specimens of some taxa with field based data on the population structure, floral biology and ecological characteristics. Thus, for the majority of taxa in this study a reliance on herbarium specimens as the main source of data would prohibit a thorough assessment of the applicability of the biological and the composite concepts of species. The data to be analysed should allow for an assessment of the applicability of the phenetic concept of species, which considers the species level as that at which distinct phenetic clusters can be observed (Sneath 1976). The ecological concept of species on the other hand relates to a lineage or closely related set of lineages, which occupies an adaptive zone minimally different from that of any other lineage in its range (Van Valen 1976). Although the recognition of infra-specific taxa of several degrees of rank such as subspecies, variety, sub-variety and form is discouraged as causing confusion in classifications (Stebbins 1950), there are, however, situations where such categorisations are justified and required (White 1993; Cronk 1998).

The term 'ochlo-species' is of no nomenclatural significance, but one which is convenient for discussions and it means an irregular crowd or mob

(White 1962), and thus needs a wide range of data sets to understand the pattern of variation. Verdcourt's (1975 & 1978) and Verdcourt and Fernandes' (1986) concept of *O. rochetiana* is broad, and thus presents challenges to understanding species limits in Oliniaceae. According to Rieseberg and Willis (2007) plant speciation is characterised by the evolution of barriers to genetic exchange between previously interbreeding populations. Accordingly, the pre-pollination, or more generally pre-zygotic, barriers such as eco-geographic isolation and temporal isolation will impede mating or fertilisation between species, thus contributing more to total reproductive isolation than do post-zygotic barriers such as hybrid sterility. The totality of information to be considered for analysis in this project should allow for the testing and determination of acceptance or otherwise of the phenetic concept of species, which recognises species as distinct clusters in a phenetic space and are diagnosable from other such clusters by unique features (Sneath 1976). The ecological concept of species is considered for interpretation and characterization of morphological variation at the population level (within and between population variations).

Studies that focus on delimitation of taxa and testing of hypotheses of relationships employ two primary methods of data analysis (phenetic and cladistic analyses). These methods are commonly applied in plant systematic/taxonomic studies at either the ordinal and familial levels (Young & Watson 1970; Conti *et al.* 1996; Schönenberger & Conti 2003), generic level (West 1987; Schrire & Lewis 1996), specific level (Mogea 1999; Leht 2005; Bortiri *et al.* 2006; Otieno *et al.* 2006; Mizianty 2006; Tyler 2006; Vander Kloet & Dickinson 2005; Xiang *et al.* 2006) or infra-specific levels (Boyd 2002; Henderson 2002) to produce classifications that reflect phylogeny. A range of analytical tools such as cluster analysis, principal component analysis, principal coordinate analysis, and detrended correspondence analysis are available for data manipulation and graphic representation of variation in plant systematics (Henderson 2006), and below is a review of selected methods and techniques deemed relevant and appropriate for data analyses in this study.

b) Methodology in systematic studies

Phenetics and cladistics are approaches and methods used with increasing frequency to organise biological information and to construct classifications instead of relying upon intuitive methods of data assessment and manipulation. In these methods, the entities under study and the characters and character states under consideration are stated explicitly and are therefore available to other workers for re-interpretation or any other manipulation. This improves the level of objectivity and reduces the subjective element within the resultant classifications (Sneath & Sokal 1973; Stace 1989).

Phenetics

Phenetics, which is often synonymised with numerical taxonomy, aims to be objective, explicit and repeatable both in the evaluation of taxonomic relationships and in the erection of taxa (Sneath & Sokal 1973; Stevens 2000). This approach and method emphasises repeatability and objectivity as compared to the circularity, imprecision, speculative nature, and somewhat unscientific approaches to systematics that characterised traditional or alpha taxonomy (Davis & Heywood 1963; Stace 1989; Stuessy 1990). However, this method lacks the ability to distinguish between homology (true similarity of parts due to common evolutionary origin or ancestry) and homoplasy (superficial similarity of parts that have different origins). Thus, the similarity observed does not reflect true relationships (Sneath 1976; Siebert 1992). Analogous structures could have a similar function, but different origins, through convergent evolutionary development (Patterson 1982; Stevens 1984; Patterson 1988; Wagner 1989).

The application of numerical phenetics to develop and test hypotheses regarding species limits assumes that: 1) the larger the number of

characters on which the classification is based the better the classification will be; 2) all characters should be given equal *a priori* importance; and 3) classifications are based on overall phenetic similarity, and phylogenetic inferences can be made from the taxonomic structure revealed by the classification (Sneath 1976). According to Stuessy (1990) phenetic classification involves: the selection of taxa or individuals for study, usually called operational taxonomic units (OTU's); selection of characters (ideally more than 100 characters); description and measurements of character states; comparison of character states to a) determine a measure of overall similarity or phenetic relationship between pairs of OTU's, and b) determine the taxonomic structure i.e. detection of possible groups and subgroups among all OTU's; and ranking all OTU's into categories of taxonomic hierarchy. These procedures will be followed in this study, and an attempt will be made to test some of the underlying assumptions.

Two numerical phenetic techniques dominate as the main approaches for representing the taxonomic structure in a data set, namely clustering and ordination methods of analysis. Ordination techniques are concerned with approximating the OTU's of a dissimilarity matrix by the distances (usually Euclidean) generated by a set of points plotted in a few dimensions (Gower 1988). An ordination analysis aims to represent phenetic relationships of objects (e.g. populations or individuals) by the scattering of points in reduced dimensional space (Baum 1986; Chandler & Crisp 1998), and can also identify multiple overlapping patterns (Faith & Norris 1989). Commonly used ordination techniques include principal components analysis; principal coordinate analysis and multidimensional scaling, and these are explored in this study for analysis of data and to portray the OTU's in the first three dimensions.

Cluster analysis is one of the many methodologies that falls into the realm of phenetics, and is often utilised in devising phenetic classifications. The objective of a clustering technique is to group the operational taxonomic

units (OTU's) into clusters so that the elements within a cluster are more similar to each other than they are to OTU's outside the cluster (Sneath & Sokal 1973). Common clustering methods used in taxonomic investigations include the Unweighted Pair Group method of Arithmetic Averages (UPGMA), single linkage or complete linkage (Crisci *et al.* 1979; Hill 1980; Duncan & Baum 1981; Balfour & Linder 1990; Crompton *et al.* 1990; Small & Brookes 1990; Small & Fawzy 1991; Van den Borre & Watson 1994; Vincent & Wilson 1997; Chandler & Crisp 1998; Hodalova & Marhold 1998; Bartish *et al.* 1999; Small *et al.* 1999; Marcussen & Borgen 2000). Cluster analysis will be used in this project at the specific and infraspecific ranks in order to test the species integrity and to present graphical representation and statistical information about the variation of characters within species, and at the population level.

The 'calibration' of the data set, 'verification' of taxonomic groups and performing 'stepwise analyses' are techniques intended to address the methodological problems inherent in the application of phenetic methods (mainly ordination and cluster analysis). Amongst much else these may include whether the groups obtained are dependent on the variation represented by particular specimens or on variation between taxa to which the specimens belong, or on the lack of resolution on ordination axes. . These techniques will be formally presented in Chapter 2.

Phylogenetics

Although Lamarck (1810) initiated the idea of 'genealogical classification', it was not until fifty years later (Darwin 1859) and immediately afterwards (Haeckel 1866 & 1868; Lankester 1877) that the concept of phylogenetic classification became popularized as the central goal of systematics. Hennig's (1965 & 1966) work advanced and increased the rigour of phylogenetic analysis and formalised cladistic methodology which was readily accepted and applied in zoological studies, but reluctantly applied in botanical studies (Bremner & Wanntorp 1978). Phylogenetic

systematics is generally considered synonymous with cladistics although this must not be confused with phyletic or evolutionary classificatory approach, which also bases classifications on phylogeny (Stuessy 1990). The progressive change within an evolutionary line over time is referred to as phyletic evolution (Stuessy 1990). Cladistics as a method of systematic biology (Patterson 1980) is used to reconstruct genealogies of organisms and construct phylogenetic classifications (Scotland 1992). Cladistic analysis is based on two premises: 1) that evolution occurs at or below specific level, since no cladogram would result if evolution occurred above the specific level (Ashlock 1974); and 2) that each group has a unique ancestry, which precludes reticulate evolution. In plants, this poses a problem considering that speciation occurs often as a consequence of hybridization (Linder 1988). The aim of cladistics is to group taxa into monophyletic groups by synapomorphies (Humphries & Funk 1984), wherein a monophyletic group would contain all and only descendants of a common ancestor as opposed to a paraphyletic group which would contain some but not all descendants of a common ancestor. A polyphyletic group would contain diverse elements derived from different ancestors. The basic steps (adapted from Linder 1988; Mishler 2005; Stuessy & König 2008; Jensen 2009) in cladistic analysis would include 1) selecting the operational taxonomic units or evolutionary units; 2) selecting characters of evolutionary interest; 3) describing and measuring character states; 4) determining or erecting hypotheses of homology of characters and character states; 5) constructing transformation series and determining polarity of these series to root the character state networks; 6) constructing a basic data matrix; 7) selecting algorithms and generating cladograms; and 8) using the cladograms to assess the hypotheses on polarity and homology about the characters or characters states, and repeating the process until the cladogram with the most congruent distribution of character states is obtained; and 9) constructing a classification based on the cladogram.

However, cladistic methodology is not without critics, and the criticisms centre around instabilities created on classifications and nomenclature,

and the lack of ‘comprehensiveness’ and ‘completeness’ of resultant classifications due to exclusion of paraphyletic groups and emphasis on monophyletic groups (Nooteboom 1988; Brummitt 1996a; Brummitt 1997; Sosef 1997; Cavalier-Smith 1998). This is contrary to the basic purpose of phylogenetic classification (i.e. creation of a comprehensive and all inclusive phylogenetic classification of all taxa, both extant and extinct). In transformed cladistics, a number of overly restrictive premises originally formulated by Hennig have been discarded, namely that speciation is only dichotomous; that parental species disappear following speciation, forming two daughter species; that each species is defined by a unique autapomorphy; and that sister groups should receive equal ranking (Linder 1988; Van Welzen 1997; de Queiroz & Gauthier 1992). Cladistic analysis, as a tool for phylogenetic reconstruction, will be used in this project with species of Oliniaceae as terminal taxa to assess their phylogenetic relationships.

Compared to numerical phenetic methods of analysis (Sokal & Sneath 1963), the application of cladistic methods in botanical studies only gained prominence in the last three decades as the most robust methods to analyse relationships at ordinal and familial levels (Conti *et al.* 1996; Fan & Xiang 2003), generic level (Xiang *et al.* 1993; Schrire & Lewis 1996; Xiang *et al.* 1998; Funk *et al.* 2007), and species level (Xiang *et al.* 2006). The phenetic and cladistic methods as outlined here are considered appropriate, operationally rigorous and robust enough to analyse various sets of data in this study to provide an improved classification of Oliniaceae.

Classifications and Nomenclature

In the strict Hennegian sense, classifications should reflect and recognise only monophyletic groups of taxa (i.e. a group that contains all and only the descendants of the most recent common ancestor). The approach for converting cladograms into classifications is meant to minimise novelty, but maximise information content (Funk 1985; Linder 1988) in which

there is formal recognition of those clades with the highest confidence ratings and maximally stable classifications (Linder 1991). Several indices or measures are available to determine the robustness of clades (i.e. how well the clades are supported by the character set), and these include the clade stability index (Davis 1993), the character jackknife (Penny & Hendy 1986; Farris *et al.* 1996), the data set removal index (Gatesy *et al.* 1999) and the character bootstrap (Felsenstein 1985). Therefore, classifications have become hypotheses that are testable through cladistic methods which are objective and repeatable (Linder 1988; Stuessy 1990).

The basic tenet of nomenclature is the creation of stability (i.e. giving priority to older and more familiar names) in classifications (Brummitt 1996b; de Queiroz 1997). However, when more knowledge and understanding of the group accumulates the necessary changes should be effected to improve the classification and its predictive value (de Queiroz & Donoghue 1988; Schrire & Lewis 1996). Where possible, type specimens (holotypes, isotypes, syntypes, etc.) of all names previously recognised in *Olinia* will be studied and included in the morphometric phenetic analyses in order to resolve the nomenclature of taxa recognised in this study. All taxa recognised in this study will be described and named in accordance with the principles of the International Code of Botanical Nomenclature (ICBN, Staffleu *et al.* 1972; Greuter *et al.* 2000; McNeill *et al.* 2006).

1.2 Research Problem

The delimitation and recognition of species and infraspecific taxa within *Olinia* has been the source of taxonomic uncertainty and confusion (Rao & Dahlgren 1969; Tobe & Raven 1984; Schönenberger & Conti 2003); and this is largely due to inadequate sampling of some species, some questionable distributional records and intergrading morphological similarities. Extensive intraspecific morphological variation in most species has led to the recognition and description of numerous subspecies (Sim 1907; Cufodontis 1960). The revision of tropical species by Hiern (1871) led to the recognition

of only one species, *O. cymosa* Thunb. In the *Forests and forest flora of the colony of the Cape of Good Hope*, Sim (1907) also recognised only *O. cymosa* Thunb., but with three varieties namely var. *latifolia* Sond., var. *intermedia* Sim and var. *acuminata* (Klotzsch) Sond. on the basis of leaf shapes and sizes. With access to fresh material of *Olinia* in South Africa and collaboration with the Director of Kew and the Curator of the Botanical Department of the British Museum, the account of Hofmeyr and Phillips (1922) limited the concept of *O. cymosa* Thunb. to include var. *latifolia* and var. *intermedia*, but elevated var. *acuminata* to specific level, *O. acuminata* Klotzsch, and recognised a new species *O. radiata* Hofmeyr & Phill. However, Burt Davy (1926) recognised two species for the *Flora of Transvaal*: *O. emarginata* Burt Davy and *O. usambarensis* Gilg. He described *O. emarginata* and typified it on Galpin 1275, a specimen collected from Barberton, Mpumalanga.

The comparative study of the tropical and southern African material of *Olinia* by Cufodontis (1960) led to the recognition and typification of *O. ventosa* (L.) Cufod. as a species endemic to the Western Cape and distinct from the Angolan material of *Olinia* (Welwitsch 991) on the basis of petal and sepal shapes and sizes. Relying on the shape and size of leaves and inflorescences, Fernandes and Fernandes (1962) recognised a distinct species, *O. huillensis*, for the Angolan material as clearly separate from the South African *O. ventosa* (L.) Cufod., and other tropical taxa (*O. aequipetala*, *O. usambarensis* and *O. discolor*). However, Verdcourt's (1975 & 1978) work for tropical East Africa led to the recognition of only one variable species, *O. rochetiana* A. Juss., and subsuming of about nine species into synonymy pending further investigation. This scenario was maintained for the Flora of Mozambique (Verdcourt & Fernandes 1986), except that *O. vanguerioides* Bak. was also recognised. Since then, species delimitation in tropical and east tropical Africa remained problematic and was never comprehensively investigated. The work of Verdcourt (1975 & 1978) represents a 'lumping' approach (Mayr & Ashlock 1991; White 1993) in that he believed in broad taxa, and avoided emphasis on finer divisions of variation. A survey of the material available indicates that the morphological variation within *O. rochetiana*

sensu Verdcourt, particularly of floral features, can be delimited into more than one taxon. *Olinia vanguerioides* Bak. seems restricted to the mountain ranges along south-eastern Zimbabwe. It is surprising and interesting to note that the geographic range of this species remains restricted to and has never been expanded beyond Zimbabwe despite active botanical exploration in the southern African region since Verdcourt's (1975 & 1978) treatment of the Oliniaceae.

The application of general inflorescence terminology of Johnson and Briggs (1984) and Weberling (1988) has proved to be limiting for a full description of the structural variation among species groups in *Olinia*. The interpretation of floral whorls in *Olinia* has generated divergent views on the relative positions of the sepals and petals (Rao & Dahlgren 1969; Cronquist 1981; Dahlgren & Van Wyk 1988; Schönenberger & Conti 2003; Von Balthazar & Schönenberger 2006). Without exception, the main inflorescence axis in *Olinia* ends with a terminal flower, the secondary inflorescence axes branch off the main inflorescence axis and lead to inflorescence units, each comprising a triad of flowers which can either be reduced making the triad compact and tight, or elongated. Therefore, the lack of consensus among workers on the interpretation of floral whorls in *Olinia*, the poorly-delimited species, taxonomic inconsistencies and instability in the application of taxonomic names, as well as unresolved species relationships all point to the need for an overall revision of the Oliniaceae, with circumscription of species using empirical phenetic techniques, and determination of phylogenetic relationships among all taxa recognised within the Oliniaceae.

1.3 Research Aims and Objectives

Previous studies of the Oliniaceae, except Decaisne (1877) and Cufodontis (1960), have all been limited to regional and geographical areas, have not documented the taxonomic variation throughout the geographic range of taxa, and have not portrayed the evolutionary trends and relationships.

That the interpretation (definition and circumscription) of the floral structures is not yet agreed by different workers (Rao & Dahlgren 1969; Weberling 1988; Schönenberger & Conti 2003; Von Balthazar & Schönenberger 2006) presents an even bigger challenge for the taxonomy of the Oliniaceae. The limited information and understanding of species limits and phylogenetic relationships in Oliniaceae warrants an investigation, and it is the primary goal of this project to add new knowledge to the understanding of the taxonomic variation, species limits and phylogenetic relationships in Oliniaceae.

Therefore, the overall aim of this study is to provide a monograph of the Oliniaceae (with full taxonomic descriptions, nomenclature with full synonymy, keys for the identification of taxa, distribution maps, lists of cited specimens); and to provide a phylogeny and classification of Oliniaceae. The specific objectives are to:

1. Assess the species limits and integrity within the Oliniaceae by studying variation in macro- and micro-morphological characters for all taxa in Oliniaceae throughout their entire geographic distribution.
2. Evaluate and determine the systematic value of morphological variation within the *O. rochetiana* complex through the application of numerical phenetic methods of data analysis.
3. Investigate the relevant species concept(s) that would be appropriate to explain the variation in the *O. rochetiana* complex.
4. Investigate the phylogenetic relationships between members of the Oliniaceae based on morphological data, compare these to the results based on a molecular data set, and propose a classification based on these relationships.
5. To provide a monograph (new worldwide classification) of Oliniaceae, with full taxonomic descriptions, nomenclature with full synonymy,

keys for the identification of taxa, distribution maps and lists of cited specimens.

1.4 Approach to the study

This study was conducted in three major phases: the first phase focussed on categorisation and interpretation of morphological variation and delimitation of taxa; the second phase focussed on cladistic analysis as a means to assess and infer phylogenetic relationships among taxa in Oliniaceae; and the third phase was concerned with the preparation of full taxonomic descriptions, nomenclature with full synonymy, identification keys and distribution maps for all taxa recognised in Oliniaceae. Initially, a survey of macro-morphological features of specimens of Oliniaceae housed at the CE Moss Herbarium (J) and those at the National Herbarium, Pretoria (PRE) was conducted. For all recognised taxa the type specimens, and where possible cited specimens, were studied in order to gain an understanding of the concept of each taxon. These specimens were supplemented by loaned specimens from other herbaria worldwide that were known to hold extensive collections of materials from the African continent, notably B, BM, BOL, ETH, K and SAM. More than five hundred specimens were studied; and the relatively large sample of herbarium specimens was intended to cover the known range of taxonomic variation, and to aid in the descriptions of taxa recognised in this study.

During study visits to BM, BOL, K, NBG and PRE there was access to, and examination of a large number of *Olinia* specimens covering a wide range of geographic distribution in tropical and tropical East Africa. This led to field work being confined to South Africa (Limpopo Province, Mpumalanga, Gauteng, North West Province and Western Cape) to study inter-specific and intra-specific variation in *Olinia* populations, and to record physiognomic features and ecological conditions. Both light and scanning electron microscopy were used to investigate variation in micro-morphological features on vegetative and floral parts. Anatomical features

of the leaf were studied at the Jodrell Laboratory (Herbarium, Kew Botanical Gardens) and assessed for their taxonomic value.

The variation was studied, recorded, summarised and presented through appropriate statistical indices for phenetic and cladistic analyses as documented in the various chapters to address the stated objectives of this project.

1.5 Outline of Dissertation

This thesis is presented as a compilation of papers, published or ready for submission for publication in peer refereed scientific journals. Each paper constitutes a chapter, and covers one of the key objectives outlined. Chapter 1 introduces the scope and context of this study, taxonomic problems in the Oliniaceae, and outlines the study aims and objectives.

Chapter 2 focuses on refining methodology and techniques for numerical phenetic analysis with the aim of addressing some of the manifold inherent problems of numerical phenetics such as the choice of inappropriate analytical tools (principal coordinate analysis, principal component analysis, multi-dimensional scaling, and cluster analysis) for various sets of data. The rationale and justification for using specific techniques are explained. These techniques are then applied in an investigation, analysis and interpretation of morphological variation within the *Olinia rochetiana* complex. This chapter addresses objective 1 fully, and partially addresses objectives 2 and 3.

Chapters 3 and 4 focus on problematic taxa of Oliniaceae occurring in southern Africa, and provide a basis for recognising species using numerical phenetic methods of data analysis. Both chapters 3 and 4 address objective 1. The analysis and interpretation of morphological variation among taxa occurring in tropical east Africa is presented in Chapter 5, which addresses objectives 2 and 3.

Chapter 6 focuses on the phylogenetic relationships among members of the Oliniaceae, and addresses objective 4 in full.

A synthesis in the form of a monograph of the Oliniaceae is presented in Chapter 7. In this chapter, a new comprehensive classification of the Oliniaceae throughout its known range of geographic distribution is outlined. A multi-purpose key is provided that can be used (both in the field and in the herbarium/laboratory) for identification of taxa within *Olinia*. This chapter addresses objective 5. Chapter 8 presents general discussion and conclusions of the study; and areas for further investigations are also elucidated.

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