

Chapter 6.

Phylogenetic relationships within *Olinia* (Oliniaceae) inferred from cladistic analysis of morphological data

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ABSTRACT

Phylogenetic relationships within *Olinia* are examined using morphological data. Parsimony cladistic analysis using *Crypteronia paniculata*, *Dactylocladus stenostachys*, *Axinandra coriacea*, *Rhynchochalyx lawsonioides*, *Peddiea africana*, *Penaea mucronata*, and *Alzatea verticillata* as putative outgroups revealed that *Olinia* taxa formed a strongly supported monophyletic clade with two poorly supported subclades. The results also indicate *Alzatea* to be sister to *Olinia*. A preliminary classification of the Oliniaceae is proposed in which the two subclades within *Olinia* are recognized at sectional rank: Sect. *Olinia* for the subclade comprising the southern African species, and Sect. *Rochetiana* for the subclade comprising the tropical east African species. The two subclades within *Olinia* are congruent with particular geographical regions. Optimization of morphological characters on consensus trees indicates synapomorphic character states that support the *Olinia* clade, and the subclade comprising the southern African species (*O. emarginata*, *O. radiata*, *O. capensis*, *O. micrantha*, *O. ventosa*, and *O. vanguerioides*). The subclade comprising tropical east African species (*O. rochetiana sensu stricto*, *O. ruandensis*, *O. usambarensis*, *O. huillensis* subsp. *huillensis*, *O. huillensis* subsp. *burttdavii*, and *O. huillensis* subsp. *discolor*) is not supported by any synapomorphic character states.

Keywords: clade, cladistics, morphology, Myrtales, *Olinia*, Oliniaceae, outgroup, phylogeny.

INTRODUCTION

Phylogenetic relationships of the monogeneric Oliniaceae have been a point of uncertainty for some time, and this is evident by the incongruity of the different positions allotted to the family by various researchers. Sonder (1862) considered *Olinia* to be allied to the Melastomataceae, but did not provide evidence. Since then, several pre-cladistics non-molecular studies (Engler 1894, 1921 & 1964; Engler & Gilg 1924) have suggested relationships with the Thymeleaceae (non-core Myrtales), while post-cladistics molecular studies (Conti *et al.* 1996; Schönenberger & Conti 2003; Stevens 2003; Judd & Olmstead 2004) have placed the Oliniaceae within the core Myrtales. The Oliniaceae and Thymeleaceae are superficially similar, but differ in the inferior ovary (Oliniaceae) with 3-5 locules, each locule with three ovules; the presence of 4-5 spatulate petals which are pubescent at the base and alternate with as many incurved scales; and in the stamens with thickened apical connectives (Dahlgren & Thorne 1984). The Thymeleaceae have anatomical characters in common with Oliniaceae, including intraxylary phloem (Mújica & Cutler 1974). A relationship with the Rubiaceae is also feasible on the basis of anatomical features, which include vestured pits in the vessels and sclerenchymatous idioblasts in the mesophyll (Metcalf & Chalk 1950). However, that the Rubiaceae lack intraxylary phloem, which occurs in Oliniaceae weakens the possible relationship between the two families. Both the anomocytic and paracytic types of stomata are found in the Oliniaceae although the anomocytic stomata often have surrounding cells that extend below the guard cells. In this condition the anomocytic appearance becomes concealed, and leads to an erroneous interpretation of lack of anomocytic type of stomata (Mújica & Cutler 1974). In the Rubiaceae, the stomata are of the paracytic type. The trichomes in Oliniaceae are unicellular, simple and rather thick-walled (Rao & Dahlgren 1969).

On the basis of stem and wood anatomy Solereder (1908) regarded the genus *Olinia* as belonging to the family Lythraceae. Hutchinson (1926) in his first edition of the "*Families of Flowering Plants*" included the Oliniaceae in the order Lythrales, which he considered to comprise mainly herbs (with some minor exceptions) whilst he regarded the order Myrtales as chiefly woody. However, in his subsequent editions (Hutchinson 1959 & 1973) he associated Oliniaceae with the order Cunoniales. In *Flora capensis*, Sonder (1862) accorded *Olinia* an Order "Olinieae"; and it was later transferred to the order "Lythrarieae" by Hiern (1871) in Flora of tropical Africa. This classification was maintained by Bentham and Hooker (1876) in *Genera Plantarum*, and Hofmeyr & Phillips (1922). These classifications were based largely on morphological data. Other workers, including Melchior (1964), Sao (1975), Thorne (1968, 1976 & 1981), Cronquist (1968 & 1981), Takhtajan (1980) and Dahlgren (1975), have maintained the Oliniaceae as a separate family in the order Myrtales based largely on morphological, anatomical and embryological features. These authors, however, differed in what they proposed to be the possible relatives of Oliniaceae within the "core families" of the Myrtales (Table 1). Dahlgren and Thorne (1984) regarded the Oliniaceae to be closely allied to the Combretaceae on the basis of chromosome number ($X=12$), epigynous condition, frequent occurrence of small petal scales; and also their geographical distributions, being centred in Africa.

Molecular studies within the Myrtales (Conti *et al.* 1996; Schönenberger & Conti 2003) place the Oliniaceae within the "OPRA" clade including Penaeaceae, Alzateaceae and Rhynchocalycaceae, or the "CAROP" clade when Crypteroniaceae is added (Clausing & Renner 2001). The Penaeaceae and Oliniaceae share similar floral features such as the obhaplostemonous condition (i.e. stamens arranged in one whorl or row, and are opposite the petals), and in the case of Oliniaceae and Penaeaceae the organs of the outermost whorl are interpreted as teeth of unspecified nature or epicalyx, the middle whorl as calyx, and the innermost whorl as corolla (von Balthazar &

Schönenberger 2006; Schönenberger & Conti 2003); rudimentary stipules (Rao & Dahlgren 1969); pollen features such as the very thick foot layer and tectum, and a thin columella with an infratectal granular layer extending over the subsidiary colpi (Patel *et al.* 1984); and both have African distributions. However, pollen of Oliniaceae differs from that of Penaeaceae with its unique aperture system, with the asymmetric colpi and the half subsidiary colpi (Erdtman 1952). An ephemeral endothecium, regarded as the specialised feature of the Oliniaceae, has also been reported in Penaeaceae (Tobe & Raven 1984) and the two genera *Axinandra* (Crypteroniaceae) and *Rhynchocalyx* (Rhynchocalycaceae).

The homology of the perianth organs in *Olinia* presents polarised views and arguments on the interpretation and definition of the floral parts (Schönenberger & Conti 2003). One view originally held by Gilg (1894) and subsequently adopted by Dahlgren and van Wyk (1988), regards the petal-like lobes as sepals inserted in the mouth of an elongated receptacle, and the interpolated scale-like structures as petals. The opposing view (Hutchinson 1926; Phillips 1926; Verdcourt 1975) interprets the "scales" as petals, which makes the flowers similar to those of Penaeaceae where the stamens are alternisepalous. In Rhynchocalycaceae the small petals alternate with the sepal lobes and are positioned as hooded structures above the stamens.

Phylogenetic studies in the family

To date there have been no comprehensive phylogenetic studies to trace evolutionary lineages within Oliniaceae, although Johnson and Briggs (1984), Van Vliet and Baas (1984), Conti *et al.* (1996 & 1997), and recently Schönenberger and Conti (2003) have attempted to place the family in a phylogenetic context within the order Myrtales.

Table 1. Historical treatments of relationships of the family Oliniaceae

Date	Author(s)	Taxa related to Oliniaceae	Characters used to establish relationships
1897 & 1904	Engler & Engler <i>et al.</i>	Thymelaeaceae	Anatomical features (i.e. intraxylary phloem).
1908	Hiern	Lythraceae (<i>Olinia</i> related to <i>Sonneratia</i> & <i>Strephonema</i>)	Calyx-tube covering the ovary, numerous stamens, and fruits drupaceous.
1950	Metcalf & Chalk	Rubiaceae	Anatomical features (i.e. vestured pits in the vessels, and sclerenchymatous idioblasts).
1964	Melchior	Penaeaceae	Floral features.
1984	Dahlgren & Thorne	Penaeaceae	Floral features and African distribution.
	Dahlgren & Thorne	Combretaceae	Chromosome number (X=12); epigynous condition and African distribution.
	Johnson & Briggs	Penaeaceae	Morphology.
1996	Conti <i>et al.</i>	<i>Penaea</i> , <i>Rhynchocalyx</i> and <i>Alzatea</i>	DNA (<i>rbcL</i> sequence data).
1997	Conti <i>et al.</i>	<i>Penaea</i> , <i>Rhynchocalyx</i> and <i>Alzatea</i>	DNA (<i>rbcL</i> sequence) data and phenotypic (morphological, anatomical, palynological and embryological) data.
2003	Schönenberger & Conti	Rhynchocalycaceae and Penaeaceae	DNA (<i>rps16</i> intron, <i>rpl16</i> intron, <i>trnS-G</i> intergenic spacer, <i>atpB-rbcL</i> intergenic spacer, <i>psbA-trnH</i> intergenic spacer, <i>matK</i> exon (part)).

These authors, except the latter, did not set out to provide an evolutionary hypothesis of Oliniaceae, but to elucidate patterns of evolutionary lineages within the Myrtales. The hypotheses of evolution proposed by Johnson and Briggs (1984), and Van Vliet and Baas (1984) are based on a wide range of anatomical features. Most anatomical characters are, however, unsuitable for such a cladistic approach because of the high probability of lines of parallel specialisation in individual families (Van Vliet & Baas 1984).

The inclusion of *Olinia* in different families by Sonder (1862), Bentham and Hooker (1876) and Solereder (1908) has raised concern on its status, that is, whether it should retain family status; and if not, with which family should *Olinia* be combined or included? Phylogenetic studies within the Myrtales have retained Oliniaceae as a distinct family (Conti *et al.* 1997; Schönenberger & Conti 2003) within the OPRA clade that includes Penaeaceae, Rhynchocalycaceae, and Alzateaceae or Crypteroniaceae (i.e. the CAROP clade of Clausing & Renner 2001). These authors (Conti *et al.* 1997; Schönenberger & Conti 2003; Clausing & Renner 2001) have, however, pointed to the difficulties in identifying terminal taxa for cladistic analyses due to the confusing taxonomy and the blurred species limits within the Oliniaceae. The *rbcL* data (Conti *et al.* 1996) indicate that *Olinia* is sister to *Penaea*, and that both are more closely related to *Rhynchocalyx* than they are to *Alzatea*. Schönenberger and Conti (2003) made reference to eight species in *Olinia*, but sampled only five (*O. emarginata*, *O. capensis*, *O. ventosa*, *O. radiata* and *O. vangueroides*) without specifying the other three species they recognise. Consequently, in this paper an attempt was made to sample all known taxa in *Olinia* worldwide and present hypotheses of intra-familial (Oliniaceae) phylogenetic relationships.

The aim of this study was therefore to provide insights and understanding of the phylogenetic relationships between species of *Olinia* using morphological data. In particular, the following questions were addressed: 1) does the morphological data support the monophyly of *Olinia* as proposed from

molecular studies? 2) which species represent the oldest lineage(s) in the putative phylogeny of Oliniaceae based on morphological data? 3) which family/families in the Myrtales (including all possible outgroups) is/are most closely related to Oliniaceae based on the morphological data set? 4) how do various outgroups affect the topology and putative phylogeny within the Oliniaceae? 5) what are the trends in morphological character state evolution within Oliniaceae? and 6) what morphological character states support various clades within the Oliniaceae?

MATERIALS AND METHODS

Taxon sampling

Previous phylogenetic studies (Conti *et al.* 1996; Clausen & Renner 2001; Schönenberger & Conti 2003; Rutschmann *et al.* 2004), did not sample all taxa in *Olinia* and thus could not adequately test the monophyly of *Olinia*. In order to address this limitation, all taxa in *Olinia* (including infra-specific taxa) recognized as a result of morphometric phenetic analyses (Sebola & Balkwill 1999, 2006 & 2009) were considered as part of the ingroup for phylogenetic analyses (Table 2). These taxa were circumscribed according to their possession of unique combination of character states, and represent the currently known range of taxonomic variation in *Olinia* worldwide. Therefore, the inclusion of infra-specific taxa as terminals in this study is justified by the requirement to test for the monophyly of the ingroup, *Olinia*.

Outgroup analysis is regarded as one of the most reliable methods for polarisation of character states (Watrous & Wheeler 1981; Humphries & Funk 1984; Stevens 1980 & 1984) and is widely used in phylogenetic studies. Although it is not necessary to include more than one outgroup in a cladistic analysis (Nixon & Carpenter 1993; Leht 2005), sampling of outgroups was based on published records (Conti *et al.* 1996, 1997 & 2002; Schönenberger & Conti 2003; Rutschmann *et al.* 2004) to include *Alzatea verticillata* Ruiz & Pav., *Axinandra coriacea* Baill., *Crypteronia paniculata* Bl., *Dactylocladus stenostachys* Oliv., *Peddiea africana* Harv., *Penaea mucronata* L. and

Rhynchocalyx lawsonioides Oliv. for rooting the trees. *P. africana*, a non-Myrtales and a member of the Thymeleaceae (Thymeleales *sensu* Cronquist (1981), and Dahlgren and Thorne (1984), was selected as an outgroup in order to assess which family/ies of the OPRA clade *sensu* Conti *et al.* (1996, 1997) and Schönenberger and Conti (2003) are most closely related to the Oliniaceae based on morphological data. Given that Alzateaceae and Rhynchocalycaceae are monotypic and were included in the molecular phylogenetic studies that included *Olinia* (Conti *et al.* 1996 & 1997; Schönenberger & Conti 2003), effort was made in this study to sample the same species of *Penaea*, *Dactylocladus* and *Axinandra* that were used in the molecular phylogenetic studies.

Selection and coding of characters

The definition of characters, delimitation of character states, and the formulation and assessment of primary homology (Appendix 1) were done in line with Hawkins *et al.* (1997) for the construction of a cladistic data matrix. Inapplicable data were coded following the procedure of Maddison (1993). The character states were discrete, and assumed to be under unique genetic control (Stevens 1991; Gift & Stevens 1997). The data matrix (Appendix 2) comprises 91 morphological characters and 19 taxa. All characters in the data matrix were treated as unordered and of equal weight (Meacham 1984; Sanderson & Donoghue 1989; Baum & Estabrook 1996) under non-additive parsimony (Fitch 1971).

Cladistic methods of analysis

In order to test for the monophyly of *Olinia*, and to test the effect of various outgroups on tree topology and putative phylogeny of the Oliniaceae, analyses were performed on 1) the data set containing all known taxa in *Olinia*, including all putative outgroups, and 2) on data sets each with a different outgroup. Although it is not necessary to have more than one outgroup to root the trees (Nixon & Carpenter 1993; Wheeler *et al.* 1993; Wiegmann *et al.* 1993; Struwe *et al.* 1994), the advantage is that this approach brings significant test of the monophyly of the ingroup (Barriel & Tassy 1998). Heuristic parsimony analyses were performed on the data set using Winclada

ver. 1.00.08 (Nixon 2002). Parsimony uninformative characters (i.e. those that lack information to resolve relationships among the taxa of interest (Siebert 1992)) were excluded from the analyses.

A tree bisection-reconnection branch swapping (TBR + TBR) on Wagner trees was generated from 1000 random taxon additions, with the program set to hold 1000 most parsimonious trees (MPTs) in memory for each search. A strict consensus tree was generated from the most parsimonious trees. Given the conservative nature of strict consensus trees which may contain components not found in any of the original most parsimonious trees, and may lead to little resolution of trees (Siebert 1992), the Nelsen consensus tree was generated to show areas of conflict and agreement compared to the most parsimonious trees. The slow optimisation option was applied on characters. Branches with low or no support were collapsed. The relative internal support for each clade in the consensus tree was calculated using Bootstrap analysis (Felsenstein 1985) with a maximum of 1000 replications. Branches with a bootstrap value of <50% were considered poorly supported and values were not shown on the strict consensus trees, 50 – 59% as weakly supported, 60 – 79% as moderately to well supported, and $\geq 80\%$ as well supported.

RESULTS

The strict consensus tree (Figure 1,a) from the analysis in which all the putative outgroups were included and *P. africana* was the principal outgroup indicates that *Olinia* is strongly supported (100%) as monophyletic, but with poorly supported subclades (<50% bootstrap support) separating the southern African species (*O. capensis*, *O. ventosa*, *O. emarginata*, *O. micrantha*, *O. radiata*, and *O. vanguerioides*) from the tropical east African species (*O. huillensis* subsp. *huillensis*, *O. huillensis* subsp. *burttdavii*, *O. huillensis* subsp. *discolor*, *O. rochetiana*, *O. ruandensis*, and *O. usambarensis*). *A. verticillata* branches off closest to the *Olinia* clade, and this suggests that *Alzatea* is sister to *Olinia*, contrary to the findings that either *Penaea* is sister to *Olinia*

(Johnson & Briggs 1984; Conti *et al.* 1996; Clausing & Renner 2001; Schönenberger & Conti 2003) or *Rhynchochalyx* is sister to *Olinia* (Morley & Dick 2003). Within the southern African species group, there is moderate to good support (74%) for the sister relationship between *O. emarginata* and *O. micrantha* whilst there is poor (<50%) clade support for the sister relationship between *O. radiata* and *O. vanguerioides*, and weak clade support (50%) for sister relationship between *O. capensis* and *O. ventosa*. The close relationship between the *O. huillensis* subspecies is highly supported (90%), with the sister relationship between subsp. *burttdavii* and subsp. *discolor* also receiving good support (83%).

In the Nelsen consensus tree (Figure 1,b) *Olinia* still remains monophyletic with a very strong bootstrap support (100%) but with a large polytomy involving all the southern African species (*O. capensis*, *O. ventosa*, *O. radiata*, *O. vanguerioides*, the well supported clade for sister species *O. emarginata* and *O. micrantha*), and the poorly supported clade for all tropical east African species. Clade support for sister relationship between *Axinandra coriacea* and *Dactylocladus stenostachys* is strong (80%), and this correlates with both species belonging to the same family, Crypteroniaceae. All the clades with poor bootstrap support (<50%) collapse in the Nelsen consensus tree (Figure 1,b), thus indicating that these nodes represent areas of conflict with the most parsimonious trees. The search for shared derived characters (synapomorphies), including both plesiomorphic and apomorphic character states that diagnose species relationships was performed using the slow character optimisation option within WinClada (Nixon 2002). The interpretation was made following De Pinna's (1991) concept of homology and synapomorphy (shared, derived character states) in which homology means equivalence of parts, and the notion that two or more structures can only be considered homologous if at some level they represent a single synapomorphy for a group. This is equivalent to the recognition of homology as similarity due to descent from a common ancestor (Smith 1990).

Table 2. Taxa included in the cladistic analysis

Ingroup

Olinia Thunb.

- O. capensis* (Jacq.) Klotzsch
- O. emarginata* Burtt Davy
- O. huillensis* Welw. Ex A&R Fern. subsp. *huillensis*
- O. huillensis* subsp. *burttdavii* Sebola
- O. huillensis* subsp. *discolor* (Mildbr.) Sebola
- O. micrantha* Decne.
- O. radiata* Hofm. & Phill.
- O. rochetiana* A. Juss.
- O. ruandensis* Gilg
- O. usambarensis* Gilg ex Engler
- O. vanguerioides* Baker f.
- O. ventosa* (L.) Cufod.

Outgroups

- Alzatea verticillata* Ruiz & Pav.
- Axinandra coriacea* Baill.
- Crypteronia paniculata* Bl.
- Dactylocladus sternostachys* Oliv.
- Peddiea africana* Harv.
- Penaea mucronata* L.
- Rhynchochalyx lawsonioides* Oliv.

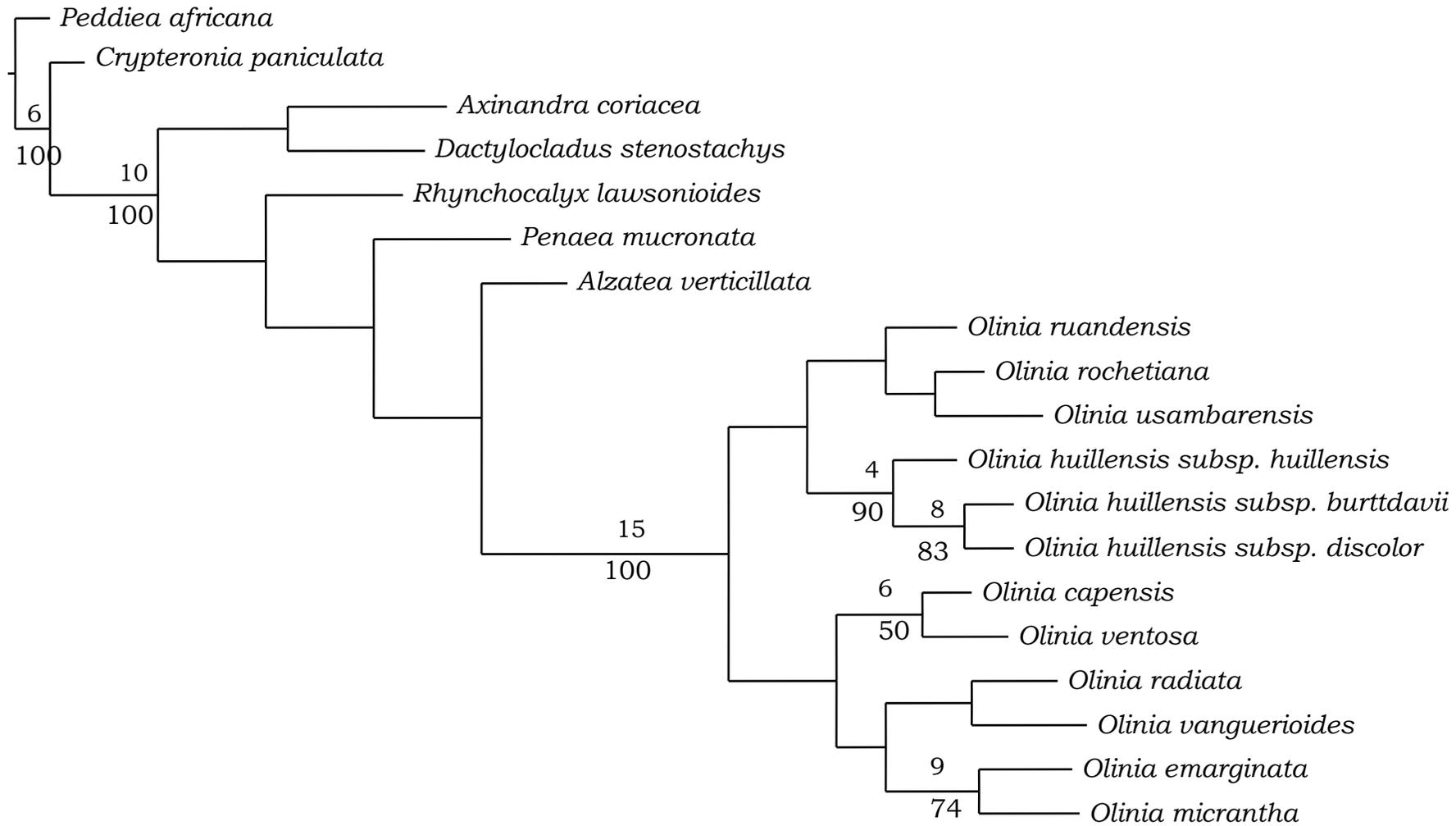


Figure 1(a). Strict consensus tree of four MPT's generated from parsimony analysis of morphological data with *Peddiea africana* as the outgroup (L = 171, CI = 61, RI = 61). Minimum branch length shown above the branches and bootstrap values are indicated below branches for branches with bootstrap values $\geq 50\%$.

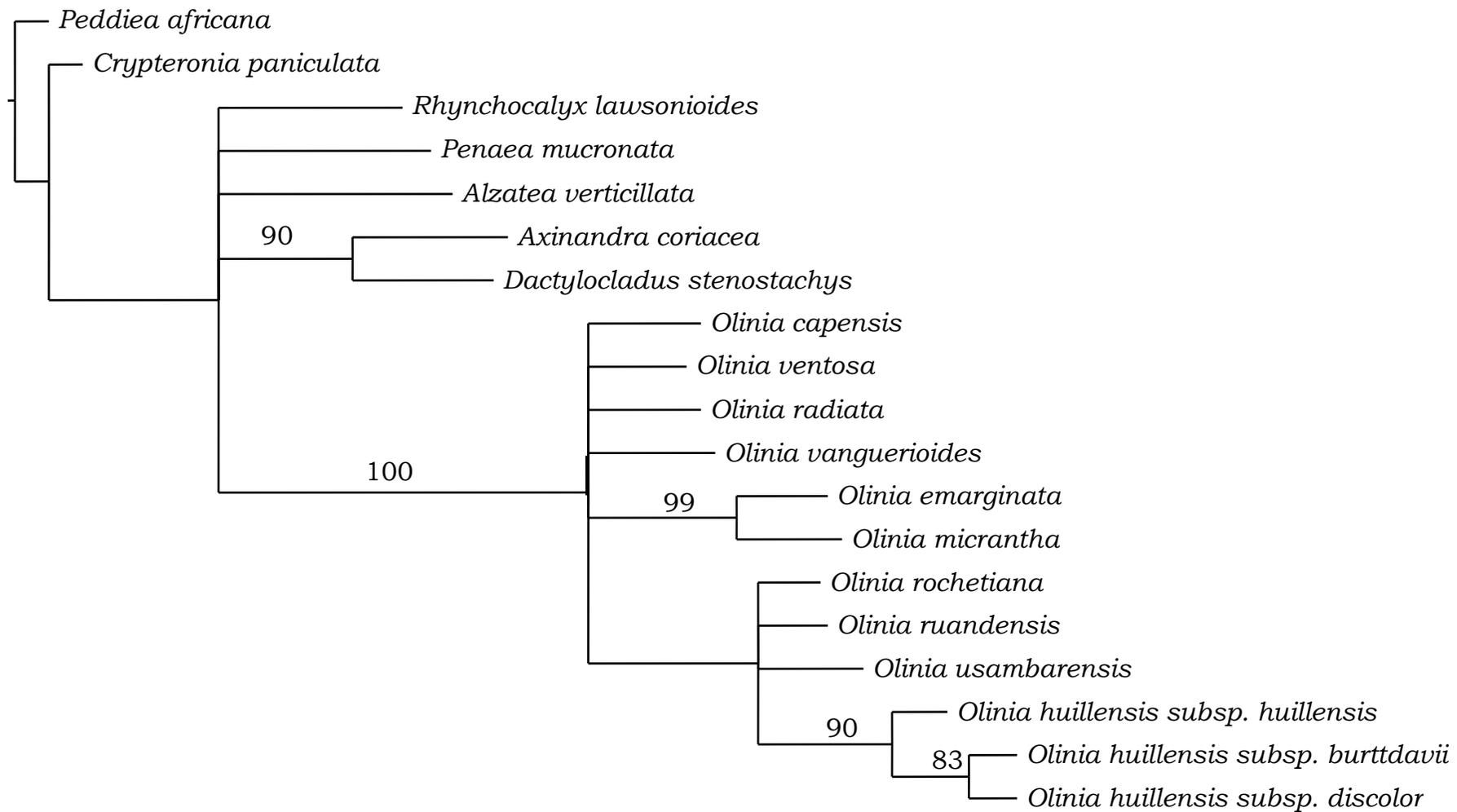


Figure 1(b). Nelsen consensus tree of four MPT's generated from parsimony analysis of morphological data with *Peddiea africana* as the outgroup (L = 329, CI = 36, RI = 49). Bootstrap values are indicated above branches for branches with bootstrap values $\geq 50\%$.

The optimization of characters on the strict consensus tree (Figure 1,c) indicates a significant number of characters with synapomorphic states supporting the *Olinia* clade. These include cymose inflorescences; bract and pedicel surfaces that are markedly pubescent; pedicels that end with blunt teeth or “calyculi”; petals that are oblong to spatulate; hypanthia or floral tubes that are adnate to, and extend above the ovary; an oval to subglobose indehiscent fruit that has a circular hypanthium scar at the tip; stamens that are alternisepalous and inserted at the inner rim/mouth of the floral tube.

Some characters indicate convergence as a result of independent multiple origins among the lineages within *Olinia*. These include the internodes of flowering branches that are shorter than the inflorescence axis having arisen independently in the clade comprising tropical east African species, and in the clade consisting mainly of southern African temperate species, particularly the subclade comprising *O. capensis* and *O. ventosa*. Clade B comprising southern African species (Figure 1,c) is supported by the following synapomorphic character states (i.e. between 10–12 secondary veins counted on both sides of midrib, secondary veins branching at an angle of 30°–45°, the angle of leaf base measured on adaxial surface $\geq 25^\circ$, a markedly pubescent inflorescence unit, a hypanthium rim that ends with bifid blunt teeth, a thickened and globular stigma, 4 or 5 seeds per locule), and this strengthens the need to formally recognise this clade as taxonomically distinct from clade A comprising the tropical east African species of *Olinia*. However, the lack of synapomorphic characters to support some clades within the tropical east African species group is indicative of homoplasious character states in the data set, and this suggests the existence of problems with homology in this study.

The tropical east African clade is not supported by any synapomorphies. Instead, there are homoplasious character states involving leaf blade that is either ovate or obovate, the adaxial and abaxial leaf surfaces that are concolorous, a cymose inflorescence, inflorescence axis and units that are sparsely pubescent; while the southern African clade is supported by synapomorphies involving secondary vein pairs of between 10 – 12, branching angle of secondary veins being 30° – 45°, angle of leaf apex that is 15° – 25°, and a hypanthium rim that ends with minute bifid blunt teeth. The South

African species are particularly defined by flowers arranged in axillary 9-flowered cymes, pedicel ending that is smooth or entire and without minute blunt teeth or “calyculi”, and three or fewer seeds per locule.

Within the tropical east African clade, sister species relationships for *O. ruandensis* and the species pair of *O. rochetiana* and *O. usambarensis* is supported by the leaf lamina with about 10 – 12 secondary vein pairs, leaves that are sub-sessile or with very short (0.5 – 2.0 mm long) petioles, sparsely pubescent petiole and peduncle surfaces, the bracts that persist after anthesis, and flowers without galls, the style surface that is markedly pubescent, and the fruit that is sub-globose. However, only one character, the hypanthium outer surface that is sparsely pubescent, supports the sister relationships between *O. rochetiana* and *O. usambarensis*. The clade for the subspecies of *O. huillensis* is supported by a shrubby life form with less than 3 m high, internodes of flowering branches being equal to or longer than the inflorescence axis, about 6 – 9 secondary vein pairs, leaf margins being revolute or slightly in-rolled, leaves with very short petioles (< 0.5 mm long) or almost sessile, peduncle and the base of petal adaxial surface sparsely pubescent; and the sister relationship between *O. huillensis* subsp. *burttdavii* and *O. huillensis* subsp. *discolor* is defined by inflorescence units or paracladia that are open and loosely arranged, ebracteate flowers, petals that are spatulate or linear to narrowly elliptic, a sparsely pubescent base of adaxial petal surface, and the width of the circular hypanthium scar being narrower than 3.0 mm.

The sister relationship between *O. emarginata* and *O. micrantha* is supported by leaf apices that are notched or emarginate, the number of secondary vein pairs being fewer than twelve, the branching angle of secondary veins being between 10° – 29°, the angle of leaf apex measured on adaxial surface being 5° – 14°, leaves that are sub-sessile or with a petiole 0.5 – 2.0 mm long, the peduncle surfaces that are sparsely pubescent, the bases of adaxial petal surfaces that are sparsely pubescent, the style surface that is sparsely pubescent, the fruit (drupe) that is oblong, smooth, not fleshy, and/or without prominent ribs, and less than three seeds per locule.

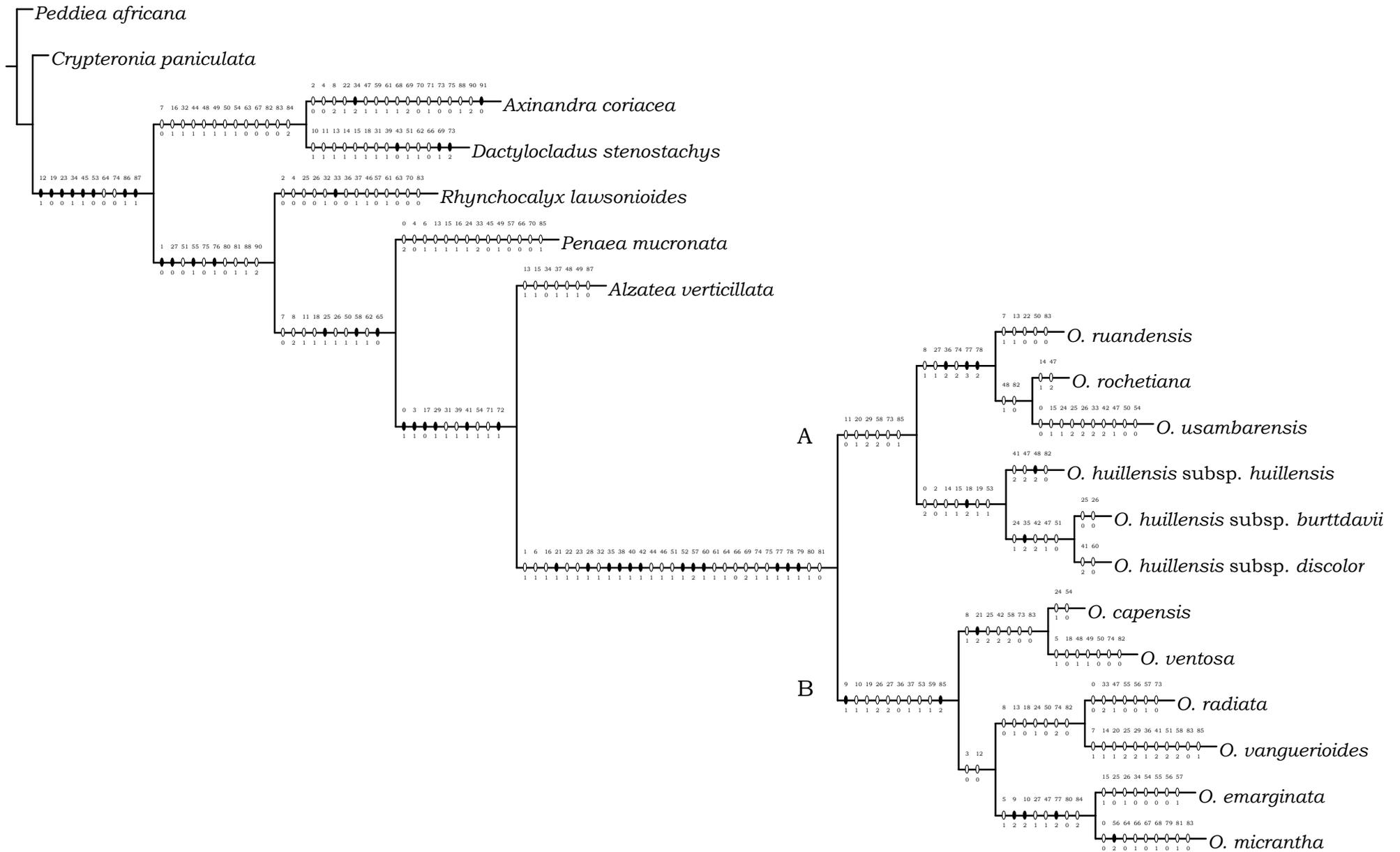


Figure 1(c). Strict consensus tree of four MPTs generated from parsimony analysis of morphological data with *Peddiea africana* as the outgroup (L = 171, CI = 61, RI = 61). Symbols: filled circles (●) = synapomorphic and autapomorphic character states, empty circles (○) = homoplasious character states (font reduced to allow visualization of all characters on each node).

The sister relationship between *O. capensis* and *O. ventosa* emerges in all the analyses, and is supported by the leaf blade that is ovate to obovate, the number of secondary vein pairs being between 10 and 12, the adaxial and abaxial leaf surfaces that are concolorous, inflorescence units ending in cymes of nine compact flowers, the apices of petals being truncate to rounded, and glabrous fruit surface. The axillary stipule is synapomorphic for the subclade comprising *O. huillensis* subspecies. The subclade comprising *O. rochetiana sensu stricto*, *O. ruandensis* and *O. usambarensis* is supported by pedicels that end with minute blunt teeth or “calyculi”, and an oblong fruit (drupe). The sister relationship between *O. rochetiana* and *O. usambarensis* is not supported by any synapomorphy.

In order to assess the effect of various outgroups on the topology and phylogenetic relationships within the Oliniaceae, separate parsimony analyses were conducted on the data matrices in which only one of the outgroups was included without the others, and these analyses yielded in each case strict consensus trees of different lengths, topology, CI and RI indices (Table 3). The use of either *C. paniculata*, *P. africana*, or *R. lawsonioides*, yielded the strict consensus trees (Figures 2, 3 & 4) with shortest length, similar tree topology, very good support for the monophyly of *Olinia*, and better internal clade support for species relationships as in Figure 1(a) compared to when either *A. verticillata*, *P. mucronata*, *A. coriacea* or *D. stenostachys* was used to root the trees. Two major clades similar to those in Figure 1(a) emerge within *Olinia* (Figure 2), with the clade for tropical east African species receiving good support (79%) whilst support for the clade representing the South African species is still poor (<50%). Clade support is very strong for sister relationships amongst the *O. huillensis* subspecies, and amongst the South African species at 95% and 91%, respectively.

Although the use of *P. africana* yielded the consensus tree with the shortest length, the rooting of the trees with *C. paniculata* yielded almost a similar consensus tree but with one step longer and one more clade with > 50% bootstrap support. Similarly, rooting the trees with *R. lawsonioides* led to a consensus tree with a higher number of clades having >50% bootstrap support, but with a polytomy that involves four taxa. The number of taxa in

polytomies increases when either *A. verticillata* or *P. mucronata* is used to root the trees, and even more taxa in polytomies are encountered when either *A. coriacea* or *D. stenostachys* is used to root the trees (results not shown), thus showing poor resolution of species relationships.

The *Olinia* clade was split into two subclades when either *C. paniculata*, *P. africana*, *R. lawsonioides* or *A. verticillata* was used as the only outgroup in the parsimony analysis (Figures 2, 3, 4 & 5), and the two subclades are similar to clades A & B in Figures 1,c and 2. It is the use of *P. africana* as the only outgroup in the analysis that yielded a consensus tree (Figure 3) with the shortest length (L= 176) that also exhibits similar topology and subclades to the consensus tree (Figure 1, a) obtained when *P. africana* was the principal outgroup and analysed with the other outgroups. In this analysis (Figure 3), the clade for southern African species is still weakly-supported (50%), while the support for the clade comprising tropical east African species is poor (<50%). The pattern of species relationships among the tropical species is similar to that observed for previous analyses (Figure 1,a), with strong support (83%) for sister relationship between *O. huillensis* subsp. *burttdavii* and *O. huillensis* subsp. *discolor*. The use of *P. mucronata* as the only outgroup in the parsimony analysis led to general lack of resolution of species relationships within *Olinia* (Figure 6). Although the southern African species form their own polytomy, these are however nested within a large polytomy involving the tropical species of *Olinia*. The clade for *Olinia* splits into three subclades (Figure 6). The subclade for southern African species receives good support (86%) compared to the moderate support (70% and 73%) when either *R. lawsonioides* or *A. verticillata* is used to root the trees (Figures 4 & 5). The sister species relationship between *O. capensis* and *O. ventosa* is weak (56%) and this species pair forms a polytomy with the rest of the southern African species. A similar topology is obtained when *A. verticillata* is used to root the trees (Figure 5). The clade for tropical east African species splits into two subclades, the poorly supported (>50%) *O. ruandensis*, *O. rochetiana* and *O. usambarensis* species group and the moderately supported (68%) *O. huillensis* subspecies group.

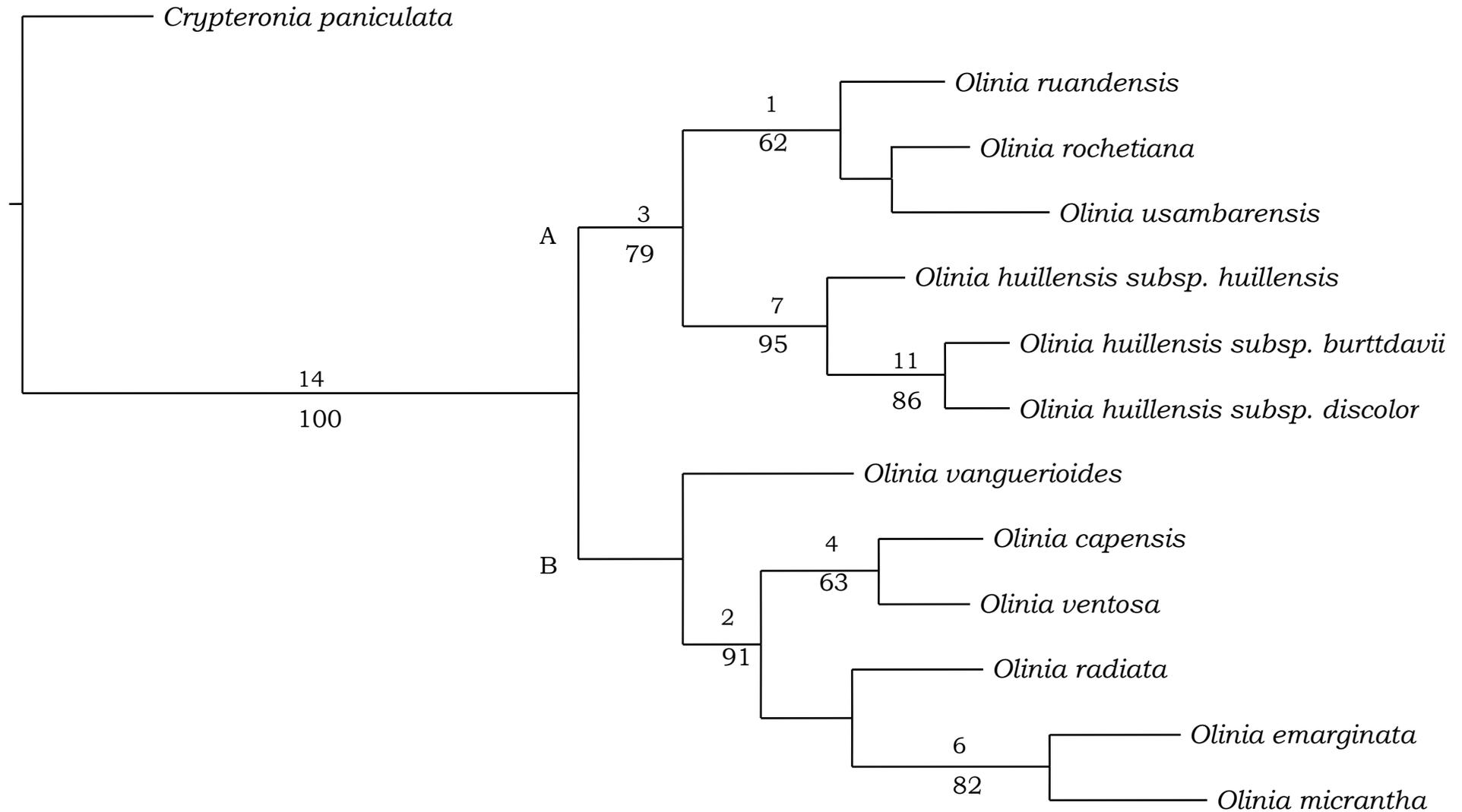


Figure 2. Strict consensus tree of four MPT's generated from parsimony analysis of morphological data with *C. paniculata* as the only outgroup. Tree length = 177, CI = 46, RI = 66. Minimum branch length shown above the branches and bootstrap values are indicated below branches for branches with bootstrap values $\geq 50\%$. A and B represents clades for the tropical east African species and the southern African species, respectively.

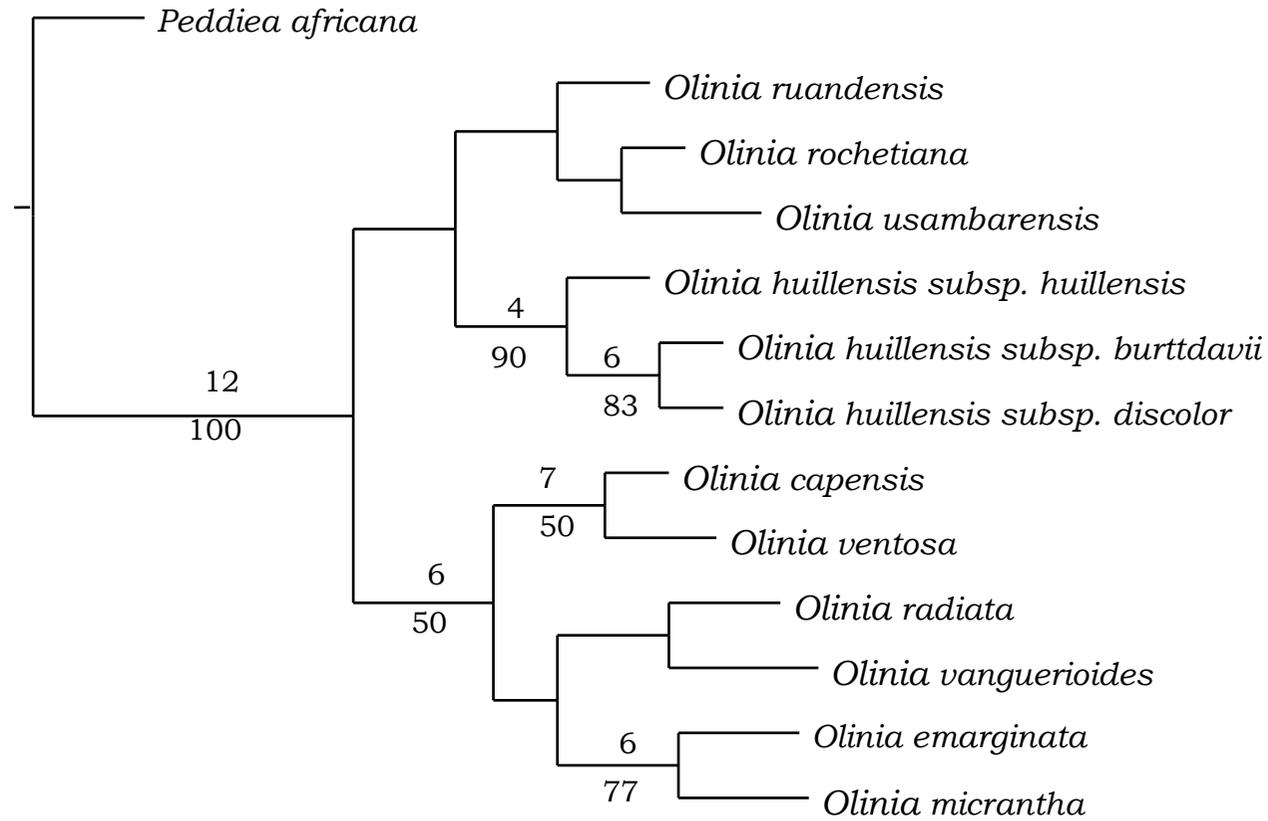


Figure 3. Strict consensus tree of four MPT's generated from parsimony analysis of morphological data with *P. africana* as the only outgroup. Tree length = 176, CI = 48, RI = 65. Minimum branch length shown above the branches and bootstrap values are indicated below branches for branches with bootstrap values $\geq 50\%$.

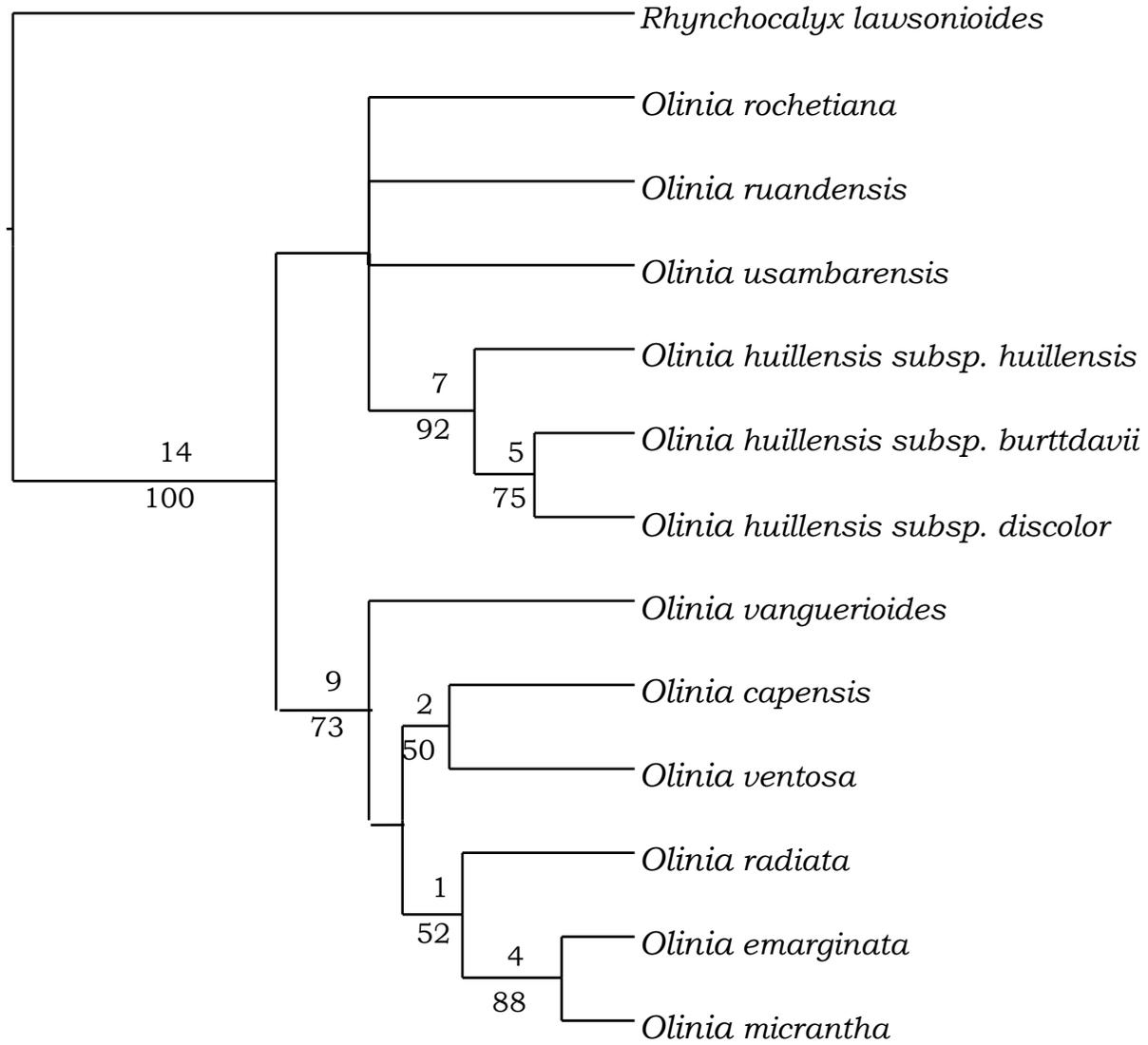


Figure 4. Strict consensus trees of 103 MPT's generated from parsimony analysis of morphological data in which *R. lawsonioides* was the only outgroup. Tree length = 180, CI = 57, RI = 56. Minimum branch length shown above the branches and bootstrap values are indicated below branches for branches with bootstrap values $\geq 50\%$.

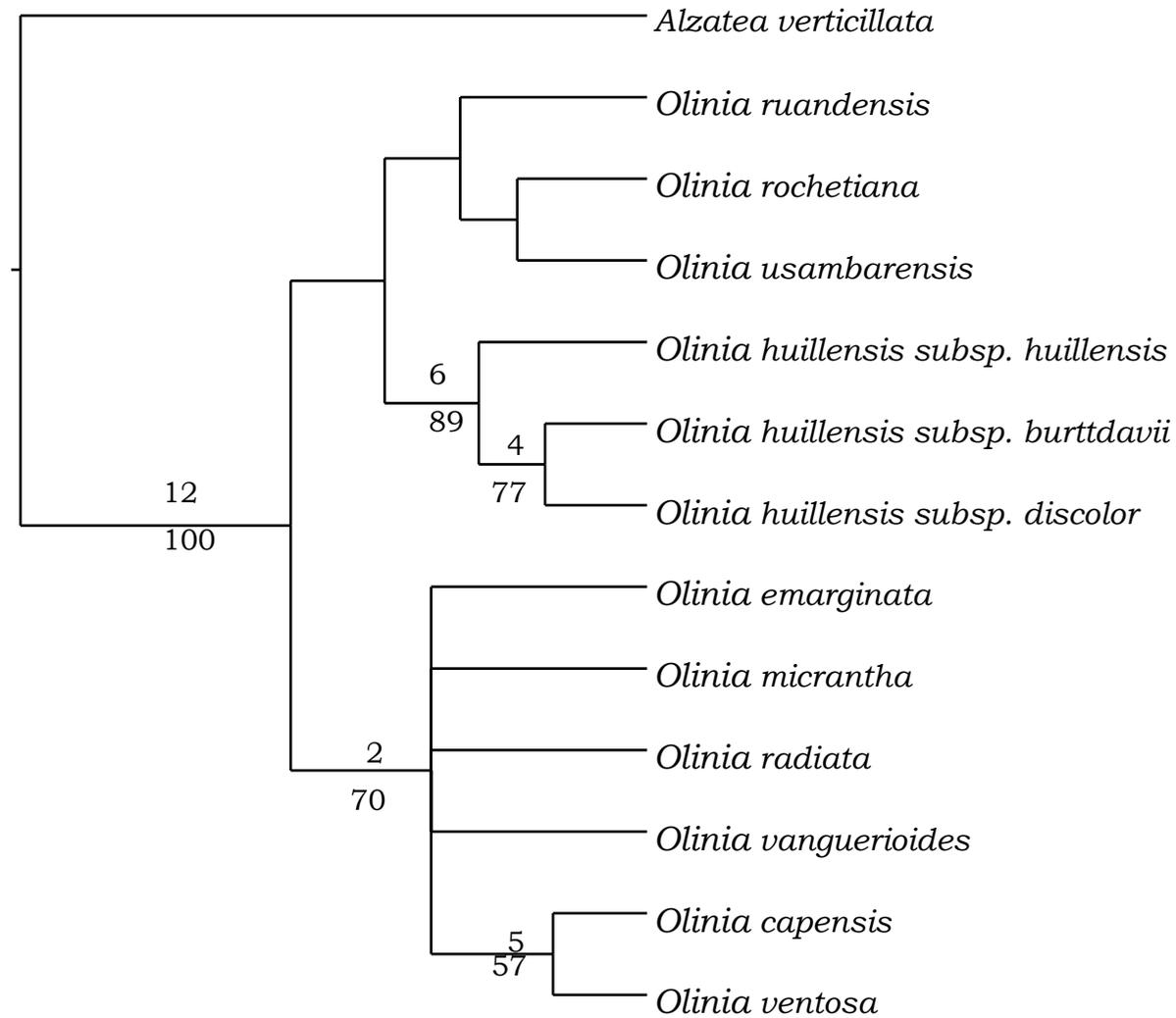


Figure 5. Strict consensus trees of 103 MPT's generated from parsimony analysis of morphological data in which *A. verticillata* was the only outgroup. Tree length = 179, CI = 50, RI = 52 Minimum branch length shown above the branches and bootstrap values are indicated below branches for branches with bootstrap values $\geq 50\%$.

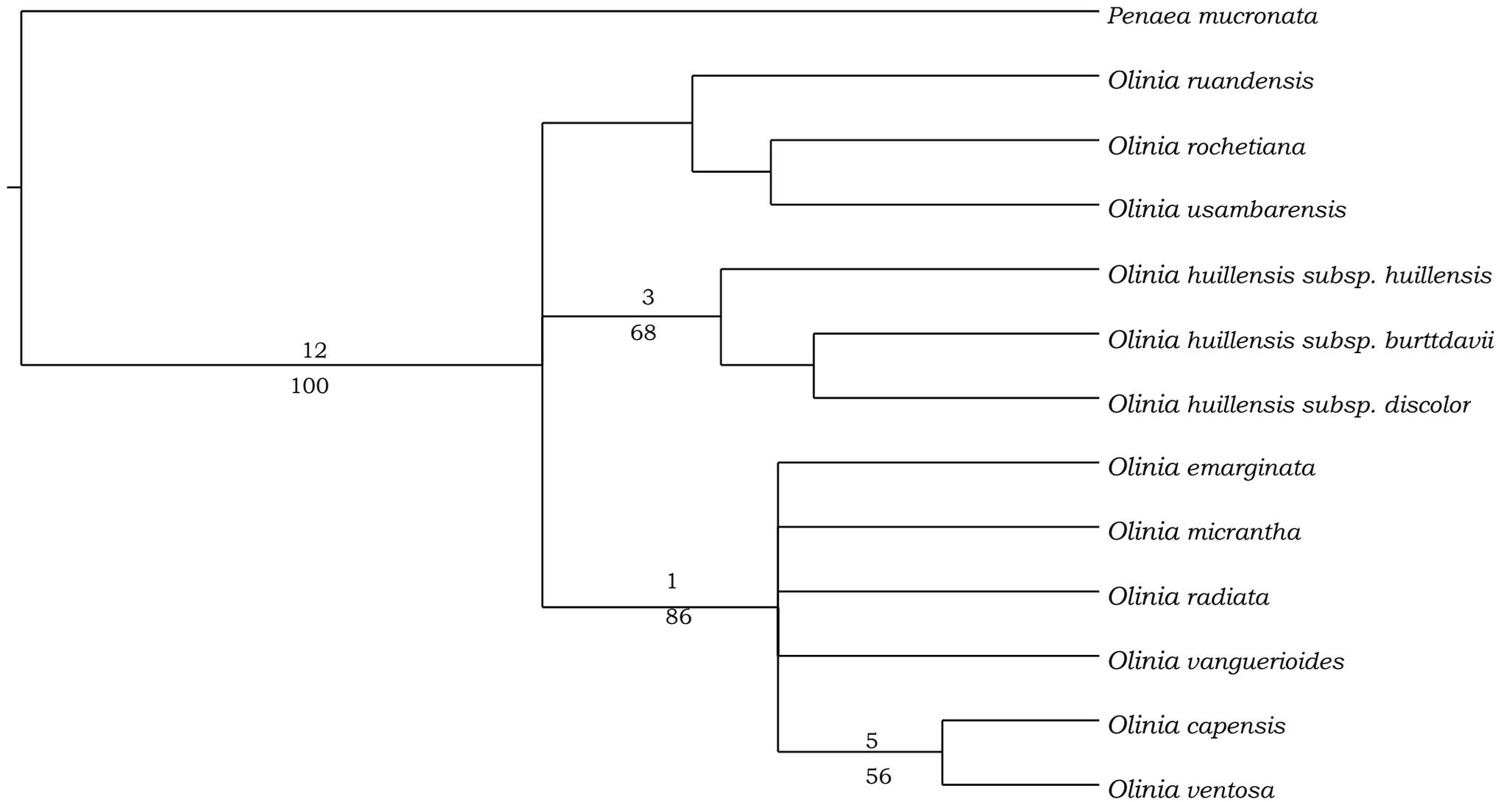


Figure 6. Strict consensus tree of 103 MPT's generated from parsimony analysis of morphological data in which *P. mucronata* was the only outgroup. Tree length = 192, CI = 52, RI = 46. Minimum branch length shown above the branches and bootstrap values are indicated below branches for branches with bootstrap values $\geq 50\%$.

Table 4. Summary of cladistic analyses conducted to determine phylogenetic relationships within *Olinia*. “CI”= consistency index, “RI” = retention index, “L” = tree length, “MPTs” = most parsimonious trees, “SCT” = strict consensus tree, BS = bootstrap support.

Data matrix description	Relevant Figure in text	MPTs				SCT					
		No. of trees	L	CI	RI	No. of shortest trees	L	CI	RI	No. of clades ≥50% BS	No. of taxa in polytomy
Data matrix including all putative outgroups with <i>P. africana</i> in principal position.	Figure 1(a)	1005	205	52	63	8	177	61	61	4	0
Data matrix including only <i>C. paniculata</i> as an outgroup.	Figure 2	107	226	48	56	4	177	46	66	6	0
Data matrix including only <i>P. africana</i> as an outgroup.	Figure 3	107	271	52	63	4	176	48	65	5	0
Data matrix including only <i>R. lawsonioides</i> as an outgroup.	Figure 4	103	175	58	59	5	180	57	56	6	4
Data matrix including only <i>A. verticillata</i> as an outgroup.	Figure 5	103	163	55	59	5	179	50	52	4	5
Data matrix including only <i>P. mucronata</i> as an outgroup.	Figure 6	103	171	57	58	5	192	52	46	3	5
Data matrix including only <i>A. coriacea</i> as an outgroup.	Not shown	107	221	41	52	4	205	40	55	2	8
Data matrix including only <i>D. stenostachys</i> as an outgroup.	Not shown	107	329	40	48	4	280	36	54	2	9

DISCUSSION

Relationships and taxonomic implications

The results point to some noteworthy taxonomic possibilities and patterns of species relationship within *Olinia*. In all the analyses *Olinia* is monophyletic, and is in accord with the criteria that the level of monophyly ultimately recognized as a genus should be from the node most strongly supported by diagnosing characters (Schrire & Lewis 1996). Twelve synapomorphic character states diagnose the *Olinia* clade (Figure 1,c), and these character states are important in differentiating *Olinia* from its closest relatives. Contrary to Barriel and Tassy's (1998) assertion that when multiple outgroups are used the first listed outgroup (i.e. the outgroup in principal position) is merely the first in the order and it is not more 'out' than the others, it appears that in this study the topology on the cladogram is influenced largely by which of the outgroups is designated as principal outgroup. *P. mucronata* and *R. lawsonioides* show closer sister relationship to each other than each is to *A. verticillata*, which appears to be a sister species to *Olinia*. This is contrary to previous studies (Johnson & Briggs 1984; Conti *et al.* 1997; Clausing & Renner 2001; Schönenberger & Conti 2003) which supported sister relationships between Oliniaceae and Penaeaceae and between Alzateaceae and Rhynchocalycaceae although with low to moderate bootstrap support (Schönenberger & Conti 2003).

Within *Olinia* there are two distinct lineages the species composition of which correlate with their geographical distributions. The results indicate that irrespective of which of the putative outgroups is placed in the principal position in the analysis, the tropical east African species of *Olinia* form a clade separable from that for southern African taxa. Therefore, these results allow for preliminary classification of the Oliniaceae to be made (Table 4), pending phylogenetic analysis involving a combined data set of both morphology and molecular data sets. Although these clades will be formally treated in the monograph of the Oliniaceae (Chapter 5), a comment on the

probable, and/or appropriate taxonomic ranks for these clades is worthwhile and desirable. The assignment of rank for these clades should be guided, at least by consideration of two factors, namely the clade support and the branch length in the phylogenetic analysis (Schrire & Lewis 1996) including other relevant taxonomic factors. Carpenter (1993) and Swofford & Olsen (1990) recommend that division of taxa, and rank, should be guided by estimates of branch length which is the measure of distance between taxa and nodes, generated from inferring a phylogenetic hypothesis. In this study the low level of support (i.e. $\leq 50\%$ for the clade consisting of the tropical east African species) and the moderate support (i.e. 50%, 70%, 73% and 86% for the clade comprising southern African species) in some analyses suggest that these clades should be recognised at a rank below genus level, possibly at the sectional rank in accordance with explicit methods to recognise genera (Carpenter 1993; Swofford & Olsen 1990; Schrire & Lewis 1996). Recognition of these clades at the sectional level is strengthened by consideration of the length of branches for these clades, which are considerably shorter compared to the length of the branch which separates the *Olinia* clade from the outgroups. The minimum branch lengths are equivalent of the Bremer decay indices, and are useful indicators of branch support and tree stability (Bremer 1994) in phylogenetic studies. The characters supporting the two major clades (A&B in Figure 2) are traditionally used in species delimitation of taxa within *Olinia* (Verdcourt 1975 & 1978; Verdcourt & Fernandes 1986; Sebola & Balkwill 1999, 2006 & 2009), and are therefore of high significance in the taxonomy of *Olinia* to warrant recognition of the two clades at sectional rank (Table 4).

The clade comprising mainly the tropical east African species should accordingly be assigned the epithet Sect. *Rochetiana* since it includes the earliest specific name (*O. rochetiana* A. Juss.) from the region named in honour of Rochet d'Hericourt. The other clade which comprises mainly the temperate and winter-rainfall species occurring in South and southern Africa, should be assigned the autonym *Olinia* (i.e. Sect. *Olinia*) by virtue of including the type species of *Olinia*, namely *O. ventosa* (L.) Cufod.

All the species of *Olinia* sampled in the molecular study of Schönerberger and Conti (2003) constitute one subclade for the southern African species in this morphological study (clade B of Figure 2). The sister relationship between *O. capensis* and *O. ventosa*, the only species of *Olinia* receiving winter rainfall in the south-western Cape of South Africa, is weakly supported (63%) in this study while in the molecular study the same clade forms a trichotomy that includes *O. radiata* with very strong support (97%). It is interesting to note that *O. vanguerioides*, which occurs on quartzite outcrops in the Copper Belt Region of Zimbabwe, is poorly associated with *O. radiata* (<50% bootstrap support) when *Peddiea africana* is used to root the trees (Figures 1,a; 3), and forms part of a polytomy with the predominantly South African taxa *O. emarginata*, *O. micrantha* and *O. radiata* when *A. verticillata* and *P. mucronata* are used as outgroups (Figures 5 & 6).

Table 4. Preliminary classification of the Oliniaceae based on cladistic analysis of morphological data.

Family:	Oliniaceae Arnott ex Sonder
Genus:	<i>Olinia</i> Thunb.
Section A:	<i>Olinia</i> comprising <i>O. capensis</i> (Jacq.) Klotzsch, <i>O. emarginata</i> Burt Davy, <i>O. micrantha</i> Decne., <i>O. radiata</i> Hofmeyr & Phill., and <i>O. ventosa</i> (L.) Cufod.
Section B:	<i>Rochetiana</i> (A. Juss.) Sebola comprising <i>O. huillensis</i> subsp. <i>huillensis</i> Welw. ex A&R Fernandes, <i>O. huillensis</i> subsp. <i>burttdavii</i> Sebola, <i>O. huillensis</i> subsp. <i>discolor</i> (Mildbr.) Sebola, <i>O. rochetiana sensu stricto</i> A. Juss., <i>O. ruandensis</i> Gilg, <i>O. usambarensis</i> Gilg ex Engl., and <i>O. vanguerioides</i> Baker f.

The sister relationship between *O. emarginata* and *O. vanguerioides* has good support (81%) in Schönenberger and Conti's (2003) molecular study, contrary to the lack of support for this relationship in this morphological study. Morphological synapomorphies have not been identified between *O. emarginata* and *O. vanguerioides*, and therefore their sister relationship is considered dubious. *O. emarginata* grows into a large tree, occurs on rocky outcrops and along streams in temperate regions of South Africa, and has characteristic small to medium elliptic leaves. *O. vanguerioides*, on the other hand has a shrubby habit, occurs in rocky outcrops and along mountain ravines and streams in Zimbabwe. The leaves are large, elliptic with a prominent midrib and conspicuous net venation.

The clade suggesting a sister relationship between *O. emarginata* and *O. micrantha* is well to strongly supported (Figures 1,a; 2; 3; & 4), and this reflects the close morphological similarities between the two species (Sebola & Balkwill 1999). Although both species have similar leaf shapes (narrow to broadly elliptic) and similar ranges of leaf dimensions (20 – 40 × 10 – 18 mm), major differences between the two species are expressed in floral features. The hypanthium in *O. emarginata* is mostly glabrous and 3.5 – 7 mm long whereas *O. micrantha* exhibits a pubescent hypanthium that is up to 3.0 mm long. *O. micrantha* retains its bracts after anthesis while *O. emarginata* sheds bracts at or before anthesis.

All the taxa in clade A of Figure 2 are members of the *O. rochetiana* species complex of which the taxonomy and species limits were circumscribed using multivariate numerical analyses (Sebola & Balkwill 2009). The phylogenetic relationships within this clade are tested for the first time, and the relationships largely reflect the pattern of clustering and categorisation of the morphological variation as in Figure 4 of Chapter 5. Two subclades are recognisable within clade A: the weakly-supported *O. ruandensis* clade (62% clade support) comprising *O. ruandensis*, *O. rochetiana sensu stricto* and *O. usambarensis*, and the well supported *O. huillensis* clade (95% clade support). The high support for the sister relationships within the *O. huillensis*

clade can be correlated with the close similarities (i.e. 0.08 euclidean distance units that separate *O. huillensis* subsp. *huillensis* from its geographic segregates *O. huillensis* subsp. *burttdavii* and *O. huillensis* subsp. *discolor* (Figure 4, Chapter 5)). The poor support (<50%) for the sister relationship between *O. usambarensis* and *O. rochetiana* in all the analyses highlights the confused taxonomy of these species (Cufodontis 1960; Verdcourt 1975 & 1978; Verdcourt & Fernandes 1986).

There is clear correspondence between species groups established in this study and those proposed by Mújica and Cutler (1974) which were based on leaf anatomical characters (i.e. type of vascular strands, shape and frequency of terminal sclereids). As in this study, the Mújica and Cutler's (1974) groups correlate with geographical distributions of species in that one group comprises those species that occur in southern Africa, while the other group comprises species occurring largely in east tropical Africa. These groups of species are distinguished anatomically by the number of girders in the leaf lamina and distribution of stomata on the leaf adaxial surface; while morphologically the differences are obvious with respect to the leaf adaxial surface that is either smooth or with marked ridges, variation in petiole length, cuneate or decurrent leaf bases, and lateral veins that either loop once or twice before the margins on adaxial surfaces, which is an indication of the degree or extent of development of the hypodermis. Therefore, the results of this morphological study support the same phylogenetic hypothesis of relationships within *Olinia* proposed in the anatomical (Mújica & Cutler 1974) and molecular (Schönenberger & Conti 2003) studies, wherein *Olinia* is well supported as monophyletic, and the existence of two species groups with unique diagnostic characters that correlate with geographical distribution. The species pairs *O. ventosa* and *O. capensis*, and *O. emarginata* and *O. micrantha* more often represent the oldest lineages in the analyses in which various outgroups are used to root the trees. Reduction in the number of parts in floral whorls has occurred once in *O. usambarensis* within Sect. *Rochetiana*, whereas reduction of hypanthium unit length occurred twice in *Olinia* (i.e. *O. capensis* and *O. micrantha*).

Character specialization and evolution

All species of *Olinia* share the presence of concave or hooded 'scales'. The phylogenetic significance of the 'scales' in *Olinia* has not been fully studied, but such structures may afford protection for reproductive organs against possible damage by lepidopteran insects. Among the southern African species of *Olinia* some species (*O. emarginata* and *O. micrantha*) exhibit xeromorphic features as means to adapt to the temperate and drier conditions. These features include reduction in the size of most vegetative and reproductive structures, as well as leathery leaves. Although the number of parts in floral whorls remains constant (5-merous) for most species, except for *O. usambarensis* which exhibits a 4-merous floral condition compared to a 5-merous condition in all other species of *Olinia*, it is the reduction in the size of flowers, leaves and fruits that are characteristic of some species groups associated with drier conditions. Although the 4- and 5-merous conditions are both very common within the Myrtales, the 5-merous condition is considered to be ancestral (Dahlgren & Thorne 1984) despite the 4-mery having been proven to have arisen very early and subsequently became dominant in several evolutionary lines (Cronquist 1981; Eyde 1975). With regard to floral merosity within *Olinia*, differentiation seems to have taken place through reduction from the 5-merous condition typical of all taxa, except *O. usambarensis* which has 4-merous condition. The 5-merous flower condition is shared with *Alzatea*, and *Axinandra*. *Rhynchochalyx* has 6-merous flowers whilst *Penaea mucronata* has a 4-merous flowers.

Within the Myrtales there is general tendency for reduction as well as multiplication of stamens. According to Dahlgren and Thorne (1984) the occurrence of numerous stamens (polystemony) within some Myrtales constitutes a derived condition that has evolved from a diplo- or haplostemonous state, especially in large flowers. In this study, numerous stamens are found particularly in *Axinandra coriacea*, and treated as primitive. Diplostemony (condition in which stamens are arranged in two

whorls, the outer whorl/stamens being opposite the sepals and inner whorl/stamens opposite the petals) is considered basic and widespread in the Myrtales (Dahlgren & Thorne 1984). However, *Olinia* shows an obhaplostemonous condition in that stamens are arranged in only one whorl, and are alternisepalous and opposite the petals. This condition is also observed in *Penaea mucronata*. In *Olinia* the fruit is an indehiscent drupe compared to a woody capsule in Rhynchocalycaceae and Crypteroniaceae (*Axinandra*, *Crypteronia* and *Dactylocladus*). *Olinia* shares alternisepalous stamens with *P. mucronata* and Crypteroniaceae. The leaf tip, or more precisely the tip of the midvein, ends in a glandular swelling or mucro in all species of *Olinia*, *Rhynchocalyx*, and *P. mucronata*. The mucro is absent in *Alzatea*, *Axinandra*, *Crypteronia* and *Dactylocladus*. Stamen arrangement is therefore of no significance in the assessment of phylogenetic relationships within the Oliniaceae. On the other hand, floral merosity, shape and size of floral parts, venation pattern, and fruit dimensions appear to be important characters that show synapomorphic states for some species groups within *Olinia*.

O. radiata and *O. micrantha* have the smallest flowers, but the largest fruits in *Olinia*. Therefore, the morphological features which show adaptation for xeric environments are found among the species within Sect. *Olinia*, distinct from those species in Sect. *Rochetiana* which exhibit large and broad leaves associated with tropical and rain forest conditions. Leaves are leathery and coriaceous with in-rolled or wavy margins among the species occurring in the drier temperate regions (*O. emarginata*, *O. huillensis*, and *O. vanguerioides*), whilst species occurring in tropical moist environments, mostly those in Sect. *Rochetiana*, exhibit broad but thin and papery leaves.

Except for *O. capensis* and *O. ventosa* (the species occurring in the winter rainfall area of the southwestern Cape) and *O. usambarensis* which occurs in the moist tropical areas, the loss of bracts and bracteoles has occurred in all other species of *Olinia*. The distribution and occurrence of indumentum on the floral and vegetative features appears to be influenced by the climatic

conditions in which the species occur. This is particularly evident in the indumentum found on vegetative features compared to that on floral structures which tends to have much more constant distributions and occurrence.

CONCLUSIONS

This study intended to explore the phylogenetic relationships within the Oliniaceae, as well as to determine which of the three outgroups yielded tree topology with the shortest length and highest internal clade support compared to the others when placed in the principal position. The inclusion of all putative outgroups (*A. coriacea*, *C. paniculata*, *D. stenostachys*, *R. lawsonioides*, *P. mucronata*, *A. verticillata*, and *P. africana*) in the analysis resulted in the strict consensus trees that show best internal clade support compared to when either *P. mucronata* or *A. verticillata* was placed in the principal position. The exclusive use of *P. africana* as the only outgroup in the analysis yielded a consensus tree with the shortest length and highest internal clade support, followed by the use of *C. paniculata*, *R. lawsonioides*, *A. verticillata* and then *P. mucronata*. Among the outgroups, *A. verticillata* is placed closest to *Olinia*, thus it can be concluded that *A. verticillata* is the sister species to *Olinia*.

The shortest trees with the highest consistency (CI) and retention (RI) indices showed similar topology, although the trees differ in the levels of internal clade support. In all the strict consensus trees (Figures 1,a; 2 – 6), *Olinia* is well supported, and is monophyletic with two major subclades segregating species groups on the basis of geographical distribution. It is therefore proposed to recognise the two subclades of *Olinia* in two sections: Sect. *Olinia*, an autonym for the subclade that bears the type species for the genus *O. ventosa* (L.) Cufod., and comprises all the temperate species occurring largely in South and southern Africa; and Sect. *Rochetiana* based on Rochet d'Héricourt' specimen no. 18 collected during his voyages to Abyssinia (now Ethiopia) from 1842 to 1844, which is the holotype for *O. Rochetianana* A.

Juss. Also, included in this section are all the east tropical African taxa of *Olinia*. It has become evident in this study that the trees with shortest length do not necessarily represent a better estimation of relationships, but could reflect the existence of polytomies among species. Instead, a combination of tree length, clade support and branch length should be used in determining a better estimation of phylogenetic relationships.

It is evident in this study that morphological data alone are insufficient to fully resolve the species relationships in *Olinia*, more specifically the relationships among the South African taxa that form a polytomy. The floral features, in particular the perianth organs, provided more parsimony-informative variation compared to vegetative features. The low internal support for some of the clades is indicative of the relatively high homoplasy level in the data set. This also reflects the difficulty of species delimitation within *Olinia* wherein most species are delimited largely on a combination of quantitative autapomorphies or unique character states. However, most of these quantitative characters show overlapping ranges between some species of *Olinia*. The importance of overlapping characters as phylogenetically informative has been demonstrated (Thiele 1993; Rae 1998; Swiderski *et al.* 1998). Further phylogenetic studies in the Oliniaceae using an expanded data set of combined morphological and molecular characters will be desirable in order to improve clade support as bootstrap values are strongly influenced by the number of characters (Felsenstein 1985; Sokal & Shao 1985; Sanderson 1989; Bremer *et al.* 1999). In summary, this cladistic analysis demonstrates that *Olinia* has two major species groups, which are recognised here at sectional rank. The two sections have unique morphological synapomorphies that correlate well with anatomical features (Mújica & Cutler 1974). Sister relationships with good support are established between *O. ventosa* and *O. capensis*, between *O. emarginata* and *O. micrantha*, and between *O. huillensis* subsp. *burttdavii* and *O. huillensis* subsp. *discolor* while there is poor support for sister relationship between *O. Rochetianana* *sesu stricto* and *O. usambarensis*.

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APPENDICES

Appendix 1. Characters and character states used in the cladistic analysis.

Habit.

1. Life form: large tree, more than 10 m high = 0; medium or slender tree, 3 - 10 m high = 1; shrub, less than 3 m high = 2.
2. Terminal and young branches (including inflorescence axes) in transverse section: round = 0; quadrangular = 1.
3. Length of internodes of flowering branches: equal to or longer than inflorescence axis = 0; shorter than inflorescence axis = 1.

Leaf.

4. Blade basic shape: elliptic to broadly elliptic = 0; ovate or obovate = 1.
5. Base: decurrent = 0; cuneate = 1.
6. Apex: acuminate to acute = 0; notched or emarginate = 1.
7. Leaf tip: without areola/mucro = 0; with areola/mucro or glandular swelling = 1.
8. Blade texture: rigid or stiff to leathery = 0; papery = 1.
9. Number of secondary veins counted on both sides of midrib (lateral vein pairs): >12 = 0; 10 - 12 = 1; 6 - 9 = 2.
10. Branching angle of secondary veins: > 45° = 0; 30° - 45° = 1; 10° - 29° = 2.
11. Angle of leaf apex measured on adaxial surface: ≥ 25° = 0; 15° - 24° = 1; 5° - 14° = 2.
12. Angle of leaf base measured on adaxial surface: ≥ 25° = 0; 15° - 24° = 1; 5° - 14° = 2.
13. Coloration of leaf surfaces: discolorous with distinct paleness = 0; concolorous = 1.
14. Visibility of secondary veins: visible on adaxial surface = 0; invisible on adaxial surface = 1.
15. Leaf margin: flat = 0; revolute or slightly in-rolled = 1.
16. Leaf attachment: distinctly petiolate = 0; sub-sessile or sessile = 1.
17. Stipule condition: well developed and conspicuous = 0; rudimentary or absent = 1.

18. Stipule position: intrapetiolar or axillary = 0; interpetiolar = 1.

Petiole.

19. Petiole length: > 2.0 mm long = 0; sub-sessile or 0.5 – 2.0 mm long = 1; sessile or < 0.5 mm long = 2.

20. Petiole surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.

Inflorescence.

21. Inflorescence type: paniculate or thyrsoid = 0; cymose = 1.

22. Inflorescence units ending in: multi-floral cymes or panicles = 0; cymes of 3-flowers = 1; cymes of 9 compact flowers = 2.

23. Inflorescence axis length: longer than the branch internodes = 0; shorter or equal to branch internodes = 1.

24. Number of units or paracladia per inflorescence axis: > 6 = 0; ≤ 6 = 1.

25. Inflorescence architecture/organisation: loose or sparse = 0; dense and compact = 1.

Indumentum.

26. Inflorescence axis indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.

27. Inflorescence unit indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.

28. Peduncle surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.

29. Indumentum type: multicellular = 0; unicellular = 1.

Flower.

30. Floral arrangement: in multi-floral panicles = 0; in axillary 9-flowered cymes = 1; in trichotomous or 3-flowered cymes = 2.

31. Flower symmetry: regular or actinomorphic = 0; irregular or zygomorphic = 1.

32. Ovary position: perigynous = 0; epigynous = 1.

33. Perianth aestivation: valvate = 0; imbricate = 1.

34. Number of parts per whorl: 6-merous = 0; 5-merous = 1; 4-merous = 2.

35. Flower bracteates = 0; ebracteate = 1.

36. Pedicel ending: smooth or entire = 0; with minute blunt teeth or

- 'calyculi' = 1.
37. Floral tube/ hypanthium: absent = 0; present = 1.
 38. Hypanthium / floral tube: sepaloid = 0; petaloid = 1.
 39. Floral tube: short, saucer-shaped at or below ovary = 0; extend above ovary = 1.
 40. Hypanthium/ floral tube free = 0; adnate or semi-adnate to ovary = 1.
 41. Petals cucullate or hood-like and folded = 0; open and flattened = 1.
 42. Petal shape: lobate and unguiculate or clawed = 0; oblong to spatulate = 1; linear to narrowly elliptic = 2.
 43. Petal apex: acuminate to apiculate = 0; shortly mucronate = 1; truncate to rounded = 2.
 44. Petal margin: wavy to slightly serrate = 0; entire = 1.
 45. Sepal condition: large and showy = 0; minute and scale-like = 1.
 46. Petal condition: minute and scale-like = 0; large and showy = 1.
 47. Petal insertion: below hypanthium rim = 0; on the hypanthium rim = 1.
 48. Hypanthium outer surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
 49. Hypanthium inner surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
 50. Petal abaxial surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
 51. Base of petal adaxial surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
 52. Pedicel surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
 53. Floral galls: absent = 0; present = 1.
 54. Bract persistence after anthesis: not caducous = 0; bracts caducous = 1.
 55. Bract texture: leathery = 0; papery = 1.
 56. Bract shape: triangular = 0; narrow, linear to oblong = 1; broadly rounded to spatulate = 2.
 57. Bract adaxial surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.

58. Bract abaxial surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
59. Hypanthium rim ends: without bifid blunt teeth = 0; with bifid blunt teeth = 1.

Stamen.

60. Stamen position: alternipetalous = 0; alternisepalous = 1.
61. Stamen insertion: midway or bottom of floral tube = 0; at inner rim/mouth of floral tube = 1.
62. Filament length: longer than the anthers = 0; shorter or equal to the length of anthers = 1.
63. Level of filament attachment on dorsifixed anthers: basal or median = 0; apical = 1.
64. Anthers enclosed or covered by: petal lobes = 0; sepal or scales = 1.
65. Connective: enlarged and thickened = 0; thin and narrow = 1.
66. Anthers basifixed = 0; dorsifixed = 1.
67. Connective position: at the front of thecae = 0; at the back of thecae = 1.
68. Anther dehiscence: introrse = 0; extrorse or latrorse = 1.

Carpel.

69. Stigma shape: flat or punctate to capitate = 0; thickened and globular = 1.
70. Gynoecium: superior = 0; semi-inferior/superior = 1; inferior = 2.
71. Style length: longer than 3.0 mm = 0; shorter than 3.0 mm = 1.
72. Style position: protruding through hypanthium / floral tube or mature bud = 0; included in the floral tube or mature bud = 1.
73. Style remnant: persistent and conspicuous at tip of fruit = 0; inconspicuous at fruit tip = 1.
74. Style surface indumentum: glabrous = 0; sparsely pubescent = 1; markedly pubescent = 2.
75. Hypogynous disk: present = 0; absent = 1.
76. Locule number: > 5 = 0; 2 – 5 = 1.

Fruit.

77. Fruit type: woody capsule = 0; drupe = 1.

78. Fruit shape: spherical = 0; ovate or obovate = 1; oblong = 2; subglobose to broadly elliptic = 3.
 79. Fruit dehiscence: dehiscent = 0; indehiscent = 1.
 80. Tip of mature fruit: without a conspicuous circular rim or hypanthium scar = 0; with a circular hypanthium scar = 1.
 81. Fruit fleshiness: fleshy = 0; not fleshy = 1.
 82. Width of circular hypanthium scar: wider than 3.0 mm = 0; narrower than 3.0 mm = 1.
 83. Pericarp: fissured and/or with prominent ribs = 0; smooth and/or without prominent ribs = 1.
 84. Indumentum on fruit surface: glabrous = 0; pubescent = 1.
 85. Number of seeds per locule: ≥ 6 = 0; 4 – 5 = 1; ≤ 3 = 2.
 86. Plant: dioecious = 0; monoecious = 1.
 87. Petals absent or rudimentary = 0; present = 1.
 88. Sepals absent or rudimentary = 0; present = 1.
 89. Flower unisexual = 0; bisexual = 1.
 90. Flower bract: absent (ebracteate) = 0; single bract per flower = 1; 2 or more bracts per flower = 2.
 91. Stamen number: twice number of sepals/petals = 0; as many as sepals/petals = 1.
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Appendix 2. Datamatrix used in the cladistic analysis of *Olinia*

Taxa / Characters	00000	00001	11111	11112	22222	22223	33333	33334	44444	44445	55555	55556	66666	66667	77777	77778	88888	88889	99	
	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890
<i>Peddiea africana</i>	01101	00100	00000	00101	00010	22200	00010	01000	00010	00000	02010	01100	00011	11100	10011	10100	1n110	00010	1	
<i>Crypteronia paniculata</i>	01101	00100	00000	00101	00010	22200	00010	01000	00010	00000	02010	01100	00011	11100	10011	10100	1n110	00010	1	
<i>Axinandra coriacea</i>	01000	00020	00100	01100	00100	22200	00112	01000	00011	10111	12001	01101	01000	11012	01000	00300	1n002	01111	2	
<i>Dactylocladus stenostachys</i>	01101	00000	11111	11110	00000	22200	01111	01001	00001	10011	11001	01100	00100	10001	10020	10300	1n002	01101	1	
<i>Rhynchocalyx lawsonioides</i>	00000	01100	00100	00100	00000	00000	00101	00100	00010	11000	00000	11000	01000	11100	00010	01000	01100	01111	2	
<i>Penaea mucronata</i>	20100	01020	01110	10110	00001	11000	00021	01000	00010	00001	10000	11010	00110	00100	00010	01000	01110	11111	2	
<i>Alzatea verticillata</i>	10111	00020	01110	11010	00000	11001	01010	01101	01010	10011	10001	11110	00110	01100	11110	01000	01110	01011	2	
<i>Olinia emarginata</i>	11101	11022	21000	11011	01110	01111	01110	10111	11111	11100	11110	00111	11111	00102	11111	11111	00112	21111	2	
<i>O. micrantha</i>	01101	11022	21000	01011	01110	12111	01111	10111	11111	11100	11111	12211	11110	01012	11111	11110	01102	21111	2	
<i>O. capensis</i>	11111	01011	11100	01011	02111	22211	01111	10111	11211	11000	11110	11221	11111	00102	11101	11111	10100	21111	2	
<i>O. ventosa</i>	11111	11011	11100	01001	02110	22211	01111	10111	11211	11011	01111	11221	11111	00102	11100	11111	10000	21111	2	
<i>O. radiata</i>	01101	01001	11010	01001	01111	12211	01121	10111	11111	11100	01111	00111	11111	00102	11102	11111	10010	21111	2	
<i>O. vanguerioides</i>	11101	01101	11011	01001	11111	22212	01111	11111	12111	11000	02111	11221	11111	00102	11112	11111	10000	11111	2	
<i>O. huillensis</i> subsp. <i>huillensis</i>	21011	01020	00101	11021	11110	11012	01111	11011	12111	11220	11111	11220	11111	00102	11101	11111	10010	11111	2	
<i>O. huillensis</i> subsp. <i>burttdavii</i>	21011	01020	00101	11021	11111	00012	01111	21011	11211	11100	10111	11220	11111	00102	11101	11111	10110	11111	2	
<i>O. huillensis</i> subsp. <i>discolor</i>	21011	01020	00101	11021	11111	11012	01111	21011	12211	11100	10111	11220	01111	00102	11101	11111	10110	11111	2	
<i>O. rochetiana</i>	11111	01010	00101	01010	11110	11112	01111	12011	11111	11210	11101	11220	11111	00102	11102	11121	10010	11111	2	
<i>O. ruandensis</i>	11111	01110	00110	01010	11010	11112	01111	12011	11111	11000	01101	11220	11111	00102	11102	11121	10100	11111	2	
<i>O. usambarensis</i>	01111	01010	00100	11010	11111	22112	01121	12011	11211	11110	01100	11220	11111	00102	11102	11121	10010	11111	2	