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Tracing History

*Phylogenetic, Taxonomic, and Biogeographic Research in
the Colchicum Family*

BY

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Abstract

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This thesis concerns the history and the intrafamilial delimitations of the plant family Colchicaceae. A phylogeny of 73 taxa representing all genera of Colchicaceae, except the monotypic *Kuntheria*, is presented. The molecular analysis based on three plastid regions—the *rps16* intron, the *atpB-rbcL* intergenic spacer, and the *trnL-F* region—reveal the intrafamilial classification to be in need of revision. The two tribes Iphigenieae and Uvularieae are demonstrated to be paraphyletic. The well-known genus *Colchicum* is shown to be nested within *Androcymbium*, *Onixotis* constitutes a grade between *Neodregea* and *Wurmbea*, and *Gloriosa* is intermixed with species of *Littonia*.

Two new tribes are described, Burchardieae and Tripladenieae, and the two tribes Colchiceae and Uvularieae are emended, leaving four tribes in the family. At generic level new combinations are made in *Wurmbea* and *Gloriosa* in order to render them monophyletic. The genus *Androcymbium* is paraphyletic in relation to *Colchicum* and the latter genus is therefore expanded.

An investigation of the distribution of colchicine within the expanded Colchicaceae is conducted to evaluate the potential of colchicine as a synapomorphy of the re-circumscribed family. The results demonstrate presence of colchicine in all genera previously not examined in Colchicaceae and in the genus *Burchardia*, earlier reported to lack colchicine. Hence, demonstrating colchicine to be a synapomorphy for the family.

An attempt to date the phylogeny of the order Liliales together with a dispersal-vicariance (DIVA) analysis indicates that the split between Colchicaceae and Alstromeriaceae+Luzuriagaceae represents a vicariance event following the disintegration of the Australian-Antarctican-South American link, ~34 million years ago. Further, the DIVA analysis indicates that Colchicaceae originated in Australia, first reached Asia and North America, and later Africa, from where they expanded to Europe and also dispersed back to Australia.

Keywords: biogeography, Colchicaceae, colchicine, dating, phylogeny, taxonomy

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Papers included in the thesis

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I **VINNERSTEN, A. & K. BREMER.** 2001. Age and biogeography of major clades in Liliales. *Am. J. Bot.* 88 (9): 1695–1703.
- II **VINNERSTEN, A. & G. REEVES.** 2003. Phylogenetic relationships within Colchicaceae. *Am. J. Bot.* 90 (10): 1455–1462.
- III **VINNERSTEN, A. & J. MANNING.** Submitted. A new classification of Colchicaceae. *Taxon*.
- IV **MANNING, J. & A. VINNERSTEN.** Submitted. The genus *Colchicum* L. redefined to include *Androcymbium* Willd. based on molecular evidence. *Taxon*.
- V **VINNERSTEN, A. & K. BREMER.** Manuscript. Historical biogeography of Colchicaceae inferred from dispersal-vicariance analysis.
- VI **VINNERSTEN, A. & S. LARSSON.** Manuscript. Colchicine distribution in Colchicaceae investigated by mass spectrometry.
- VII **VINNERSTEN, A., T.D. MACFARLANE & A.L. CASE.** Manuscript. A note on *Iphigenia novae-zelandiae* (Colchicaceae).

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All papers included, except **Paper IV**, are written by the first author with comments and suggestions given by the co-authors. The papers were also planned in cooperation with the co-authors. The first author is responsible for all analyses and the major part of the laboratory work, except in **Paper VI**, where S.L. is responsible for the mass spectrometry analysis. **Paper IV** is jointly written by the authors whereas the first author is responsible for the nomenclatural combinations.

Important note. Paper **III**, **IV** and **VII** of this thesis are manuscripts that contain the descriptions of new tribes and new combinations of several species. Two papers have been submitted to a journal, and in order to make clear that the names of these new tribes and combinations are not validly published in this thesis the Latin diagnoses in the tribal descriptions, and the references to the basionyms of the new combinations, necessary according to the International Code of Botanical Nomenclature, are omitted.

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FEM TIDLÖSA ÅR (Swedish summary)



De flesta har en föreställning om hur en liljeväxt ser ut och slår du upp familjen Liliaceae i en äldre flora så känner du säkert igen arter som pärlhyacint, gräslök och liljekonvalj. Öppnar du däremot en modern flora så finns ingen av dessa arter kvar i familjen. De enda svenska eller naturaliserade representanterna som idag räknas till Liliaceae är brandlilja, krollilja, tigerlilja samt arter av vårlök, tulpan och släktet *Fritillaria* som inkluderar Upplands landskapsblomma kungsängsliljan (Krok och Almquist, 2001). Att på det här sättet förändra och omdefiniera gamla välkända begrepp kan uppfattas som en godtycklig modernisering men så förhåller det sig inte, utan de nya avgränsningarna är resultatet av många års systematisk forskning.

Ända sedan Darwin publicerade sina idéer om evolution (1859) har systematiker strävat efter att beskriva och klassificera världen utifrån ett släktskapsperspektiv, ett så kallat naturligt system. Det innebär att en växts namn inte bara berättar vilken art det är, utan också om dess historia och vilka dess närmaste släktingar är. Splittrandet av den gamla familjen Liliaceae är alltså inte godtyckligt utan beror helt enkelt på att vår kunskap om växternas släktskap har ökat och förändrats. Idag vet vi att vårlök och liljekonvalj inte är ”syskon” i en och samma familj utan snarare avlägsna ”bryllingar” med flera par ”kusiner” däremellan.

I min egen forskning har jag ”släktoforskat” på en liten växtfamilj som heter Colchicaceae. Det är en nära släkting till familjen Liliaceae och de två utgör tillsammans med fem andra familjer ordningen Liliales. ”Min” familj har inga naturliga svenska representanter men kan däremot ses odlad i många svenska trädgårdar. Vanligast är *Colchicum autumnale* (se bild ovan), eller tidlösa som det svenska namnet är. Det är en cirka decimeterhög krokuslik växt som utan blad blommar på senhösten (jmf det engelska namnet naked ladies). För att se vilda colchicacéer måste du bege dig till Medelhavsregionen, Sydafrika eller Australien. Några få arter finns också i Nordamerika. Störst variation uppvisar familjen i Kapregionen i Sydafrika. Det var dit jag åkte för att närmare studera växterna, samla in material och extrahera DNA.

I min forskning har jag framförallt tittat på hur de olika släktena inom familjen står i relation till varandra. I min andra artikel (**II**) visar jag att släktskapsförhållandena på flera punkter inte ser ut som man hittills antagit. För att förmedla den informationen, och få klassificeringen att spegla

släktskapen inom familjen, har jag beskrivit två nya tribusar och slagit ihop några av de gamla släktena (tribus är en nivå, en mellanrang, mellan släkte och familj). Dessutom har flera arter behövt byta släkte med påföljden att ca 70 arter fått nya namn (artikel **III**, **IV** och **VI**).

När man klassificerar och namnger grupper av växter, så som släkten och familjer, strävar man alltså efter att namnge grupper som har en gemensam förfader och där ingen avkomma är utelämnad (en så kallad monofyletisk grupp). Om det ska vara någon mening med att namnge en grupp räcker det inte med att den är monofyletisk utan den måste också vara igenkänningsbar, helst med minst en unik karaktär (en synapomorfi) som förenar alla de inkluderade arterna.

Under drygt 60 år uppfattades Colchicaceae som en väl avgränsad familj med flera unika karaktärer men på 1990-talet framkom, genom flera oberoende DNA-studier, att det fanns flera släkten som borde inkluderas i familjen men som saknade de definierande karaktärerna.

En av de mer välkända karaktärerna för arterna inom ”gamla” Colchicaceae är den kemiska substansen kolchicin. Det är en mycket giftig alkaloid som påverkar celldelningen och som använts både inom växtförädling, för att skapa polyploider (växter med dubbel eller flerdubbel kromosomuppsättning), samt vid framställning av preparatsnitt för kromosomräkning (då substansen ”fryser” celldelningen). Medicinskt har kolchicin länge använts för att behandla gikt och på senare år har även cancerforskningen intresserat sig för substansen.

Kolchicin bildas genom en ganska avancerad biosyntetisk process och det är därför troligt att förmågan att producera kolchicin enbart har uppstått en gång och sedan gått i arv (Kite et al., 2000). Tidigare har två av de nya släktena i Colchicaceae, *Burchardia* och *Uvularia*, undersökts och man kunde då inte påvisa något kolchicin (Potěšilová, 1987; Kite et al., 2000). För att ta reda på hur utbredningen av kolchicin ser ut i dagens Colchicaceae, och var i släktträdet förmågan att producera kolchicin uppstod, har jag undersökt representanter från nio släkten. Några representanter från gamla familjen som tidigare inte blivit undersökta och därtill de nya släktena samt ett släkte från familjen som är närmast släkt med Colchicaceae (artikel **VI**). Mina studier visar att alla nuvarande släkten inom familjen innehåller kolchicin och att förekomsten av kolchicin alltså fortfarande är en unik karaktär för Colchicaceae (ett släkte är fortfarande ej undersökt då inget material fanns att tillgå).

Efter att ha kartlagt släktskapsförhållandet inom familjen har jag ägnat mig åt att försöka ta reda på var familjen ursprungligen kommer ifrån, ungefär hur gammal den är och hur den spridit sig i världen. Artikel **V** innehåller en biogeografisk analys av familjen där jag, med hjälp av dagens utbredningsmönster och kännedom om släktträdet, presenterar en hypotes om spridningsvägar och ursprungliga utbredningsområden. Tillsammans med den datering som gjorts i artikel **I** av hela ordningen Liliales framträder

ett mönster som visar att förfadern till Colchicaceae förmodligen fanns i Australien på den tiden då Australien satt ihop med Sydamerika via Antarktis. När landförbindelsen bröts, under eocene 55-34 miljoner år sedan, kom en del av den ursprungliga populationen att isoleras i Sydamerika och så småningom utvecklas till systerfamiljerna till Colchicaceae, medan populationen i Australien utvecklades till Colchicaceae. Familjen har sedan spridit sig från Australien till Nordamerika, troligen via östra Asien och den tempererade landförbindelse som fanns för ca 34 miljoner år sedan. Colchicaceae har dessutom spridit sig från Australien, över Indiska oceanen, till Sydafrika och vidare norrut. Kolonisationen av Sydafrika innebar många nya levnadsvilkor vilket återspeglar sig i den stora variation som vi idag ser hos de afrikanska arterna av Colchicaceae.



Figure 1 Colchicum asteroides (formerly *Androcymbium asteroides*). A species restricted to the northern Cape in South Africa (illustration by John Manning).

INTRODUCTION



Figure 2 *Wurmbea punctata* (formerly *Onixotis punctata*). The species is endemic to the Cape region, South Africa (illustration by A. Vinnersten).

The flora of the southwestern part of South Africa is unlike any other flora in the world. In fact it is so different that it is often regarded as a separate floristic kingdom, one of six, in the world (Good, 1974; Takhtajan, 1986; Goldblatt and Manning, 2000). The region, known as the Cape, is regarded as one of the richest in terms of botanical diversity. Even though it is a temperate region, it is comparable to some of the world's tropics, which comprise the most species-rich habitats on earth. Almost 69% of the vascular plants in the Cape are endemic to the area, i.e. the species is unique to the region. And botanists have been fascinated by the flora ever since the first Europeans came here in the 16th century.

The area has a Mediterranean climate with dry summers and winter rains, which sustain a unique heath vegetation known as fynbos, where shrubs with needle-like sclerophyllous leaves predominate (fynbos is Afrikaans and refers to fine-leaved vegetation). The floristic composition of the vegetation is unusual, demonstrated by the dominance of plant families as Ericaceae, Proteaceae, and Restionaceae.

One of the unique features distinguishing the Cape flora from the surrounding area, is the large amount of geophytes (seasonal herbaceous perennials with a bulb, corm or rhizome). A remarkably large number of these are petaloid monocotyledons, predominantly Iridaceae (Goldblatt and Manning, 2000). This results in a brushy landscape that literally explodes in colour after the winter rain and the frequently recurrent fires, which are an important part of the Cape ecology.

One of the families of petaloid monocots regarded to have a centre of diversification in the Cape, is the Colchicaceae (Nordenstam, 1998). It is a rather small family comprising mainly arid and semi-arid species. Most members of the family have an underground corm, which enables the plant to be dormant during the dry periods and subsequently flower, following the rainy season. In my research I have concentrated on the relationship between the genera within Colchicaceae (paper II). The result of the increased knowledge of the phylogeny in this family, is a revision of the generic

delimitations (paper **III**, **IV** and **VII**) and the proposal of a new tribal classification (paper **III**).

In 1998 the circumscription of Colchicaceae was expanded (Nordenstam, 1998) and it has since then been largely accepted. The larger Colchicaceae is inevitably a more heterogeneous family and no single diagnostic character uniting the family was found. Paper **VI** explores the potential of the alkaloid colchicine as a synapomorphy by examining the distribution of the substance in the family.

Although most of the species in Colchicaceae are seasonal perennials in temperate dry habitats in Africa and Eurasia, a few are confined to the rain forests and wet sclerophyll forests in Australia. This scattered distribution and different preferences in habitat raise several interesting questions. In papers **I** and **V** we try to unravel the biogeographic history of this family. In order to find out when and where it originated and how the species became established in their current areas.



Figure 3 Fynbos vegetation with *Leucospermum*, Proteaceae, in the foreground, Fernkloof Nature reserve, South Africa. Photo: Per Kornhall.

COLCHICACEAE –the old perspective



Colchicaceae is a horticulturally well known family with common garden plants such as the glory lily, bellworts and naked ladies (or autumn crocus, not to be confused with the Swedish “höstkrokus” which is an actual *Crocus* and belongs to the Iridaceae). The species most commonly grown in flowerbeds is *Colchicum autumnale* (figure to the left; from Engler, 1888).

Morphology, distribution and habitat

Colchicaceae is a family of mainly perennial geophytes but vines and herbs are also included. Until 1998 the family comprised 13 genera, a small group of morphologically homogeneous plants, all having a special corm (Fig. 3), which in some species has been described as a bulb and in others as a rhizome. Despite the different appearances, all the corms are vertically orientated and externally covered with brown or black tunics, formed annually by the basal part of the sheath of the lower leaf. The corm has two buds. One lower, which is the renewal bud that later develops into the new corm, and one upper, which is a reserve bud (Buxbaum, 1936). Besides the corm, all representatives are characterised by parallel-veined sheathing leaves, dry capsules and the alkaloid colchicine (Nordenstam, 1982; Dahlgren et al., 1985).

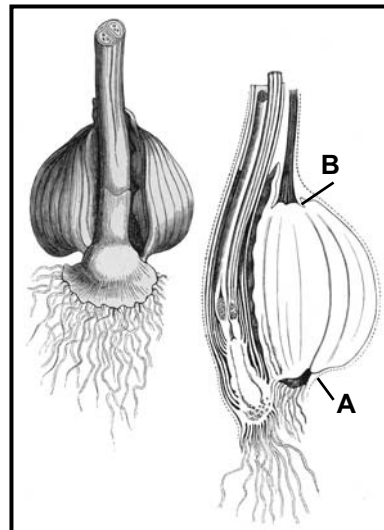


Figure 4 The underground corm of *Colchicum autumnale* **A**: position of the renewal bud, **B**: position of the reserve bud (from Engler, 1888).

Towards the end of the last century, the circumscription of the family was expanded to an inevitably more heterogeneous family of 19 genera (Nordenstam, 1998), including species with rhizomes, non-sheathing leaves, reticulate venation and berries (Fig. 4).

The majority of the species are seasonal perennials distributed in temperate to arid habitats in Africa to Eurasia. Additional species are found in the tropical and subtropical regions of the Old World. One genus, comprising five species, is distributed in North America (*Uvularia*) whereas a few species are confined to the rain forests and wet sclerophyll forests in Australia. The distribution of *Wurmbea* has puzzled several botanists (Buxbaum, 1936; Raven and Axelrod, 1974; Nordenstam, 1982), being evenly represented in Africa and Australia but nowhere else. The phylogeny and estimated age (paper I) indicate that the genus must have reached Australia by long-distance dispersal (see paper V for further discussion). The two closely related genera *Iphigenia* and *Camptorrhiza* show similar distributions with a disjunct pattern including southern Africa and India. However, the former genus is also represented in Madagascar and Socotra.

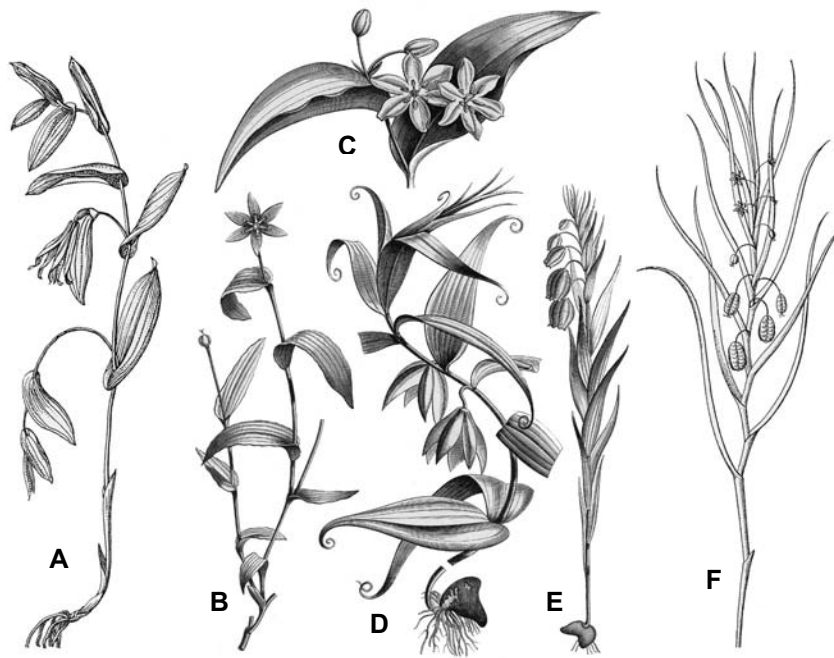


Figure 5 Representatives of six different genera from the family Colchicaceae. **A** *Uvularia* **B** *Schelhammera* **C** *Tripladenia* **D** *Gloriosa* (formerly *Littonia*) **E** *Sandersonia* **F** *Iphigenia*. **A-C** are rhizomateous genera whereas **D-F** have a corm (modified from Engler, 1888 and Krause, 1930).

Phytochemistry

Members of the narrowly circumscribed Colchicaceae are well known to contain the alkaloid colchicine (Fig. 5). Colchicine is a substance widely used in the past as a remedy against gout, a disease caused by deposits of uric acid in the joints. Despite the long use of colchicine as a drug, it is highly toxic due to its anti-mitotic properties demonstrated by the severe damage to livestock caused in Africa (Watt and Breyer-Brandwijk, 1962). The same properties are utilised to produce polyploids in plant breeding since it does inhibit cell division but not chromosome division. In present times, there is a renewed interest for the use of colchicine as a possible anti-tumour drug. Since the alkaloid itself is too poisonous for humans, a derivate of the substance is used to treat e.g. leukemia (Evans et al., 1981; Finnie and van Staden, 1991; Samuelsson, 1999).

Colchicine is today commercially extracted from *Colchicum* and *Gloriosa* but is known to occur in varying amounts in many of the genera within Colchicaceae (Šantavý, 1967; Potěšilová et al., 1969, 1987; Kite et al., 2000). In paper VI we examined the distribution of colchicine in the family to evaluate the possibility of using colchicine as a synapomorphy for the re-circumscribed family.

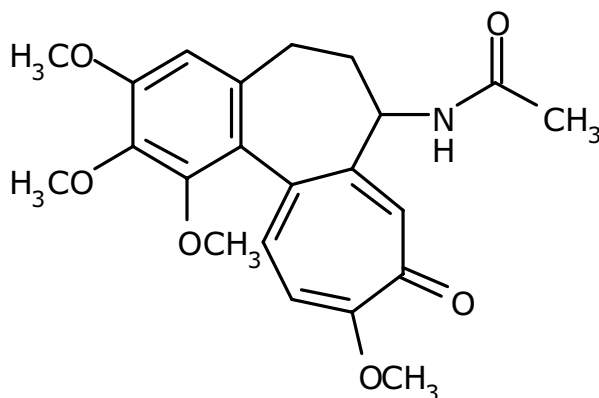


Figure 6 The chemical structure of colchicine. The molecule consists of an aromatic ring (above left), a 7-membered coal ring (above right) and the characteristic tropolone ring (below right) (Samuelsson, 1999).

Historical outline

De Candolle was the first to use the family name Colchicaceae in 1805. His circumscription of the family comprised the type genus *Colchicum*, described 50 years earlier by Linnaeus (1753), and five additional genera. Today three of these genera are placed in different families (Liliaceae, Tofieldiaceae and Melanthiaceae; Kubitzki, 1998; APG, 2003), whereas the other three are all included in the genus *Colchicum*. Most subsequent authors did not follow De Candolle but treated the taxa as part of Liliaceae sensu lato.

More than 100 years later Buxbaum (1925, 1936, 1937) conducted several thorough morphological investigations of the Lilioideae, resulting in the description of a new subfamily, the Wurmbeoideae, to which he referred 13 genera, viz. *Neodregea*, *Dipidax* (= *Onixotis*), *Wurmbea*, *Anguillaria*, *Androcymbium*, *Colchicum* (incl. *Bulbocodium* and *Merendera*), *Baeometra*, *Iphigenia*, *Iphigeniopsis* (= *Camptorrhiza*), *Ornithoglossum*, *Gloriosa*, *Littonia*, and *Sandersonia*. These genera form a homogenous group, initially distinguished by the possession of a special corm (see above). The later detection of the alkaloid colchicine, provided a good chemical character for the group. Later classifications routinely recognised this group of genera as the family *Colchicaceae* (Dahlgren, 1985; Nordenstam, 1986).

During the last decade of the 20th century, the use of nucleotide sequence data in phylogenetic reconstructions demonstrated some aberrant genera to belong within the family, viz. *Burchardia*, *Disporum*, *Petermannia*, *Tripladenia*, and *Uvularia* (Chase et al., 1993, 1995; Rudall et al., 1997). The family was thus expanded (Nordenstam, 1998) to include 19 genera and 225 spp. (Table 1).

Table 1: The previous informal classification of Colchicaceae, incorporating Nordenstam's (1982, 1998), and Dahlgren et al.'s (1985) classifications.

	Wurmbeoideae		Uvularioideae
	<i>Burchardia</i> ¹	<u>Uvulariaceae</u> ²	<i>Disporum</i>
<u>Anguillariceae</u>	<i>Neodregea</i>		<i>Uvularia</i>
	<i>Onixotis</i>		<i>Schelhammera</i>
	<i>Wurmbea</i>		<i>Tripladenia</i>
<u>Bacometreae</u>	<i>Baeometra</i>		<i>Kuntheria</i>
<u>Colchiceae</u>	<i>Androcymbium</i>		
	<i>Colchicum</i>		
<u>Iphigenieae</u>	<i>Camptorrhiza</i>		
	<i>Gloriosa</i>		
	<i>Hexacyrtis</i>		
	<i>Iphigenia</i>		
	<i>Littonia</i>		
	<i>Sandersonia</i>		
	<i>Ornithoglossum</i>		

¹Nordenstam (1998) placed the genus in the family and indicated it to be placed with the wurmbaeoid genera based on molecular evidence (Chase et al., 1993, 1995). However, he did not place it in any tribe.

² Previously all the genera in Uvulariaceae have been placed in Uvulariaceae (Dahlgren et al., 1985) or Convallariaceae (Brummitt, 1992). When Dahlgren et al.'s classification was published, *Kuntheria* was not yet described but included in *Schelhammera* as *S. pedunculata*.

Based on molecular evidence, the monotypic Australian genus *Petermannia* is often regarded as a member of the family (Rudall et al., 1997). Subsequent molecular studies have all used the same *rbcL*-sequence placing the genus as a sister taxon to *Tripladenia* (APG, 1998; Rudall, 2000; paper I). However, morphology does not support such a close relationship of the two taxa, leaving the position of *Petermannia* ambiguous (J. Conran, University of Adelaide, personal communication). This questioned position of *Petermannia* is due to misidentified material. The source of DNA used for the original "Petermannia" *rbcL* sequence published in 1997 is a *Tripladenia* (M. Chase, Royal Botanic Gardens, Kew and J.I. Davis, Cornell University, personal communication). A recent two-gene analysis by J.I. Davis (in prep.) shows that a positively identified specimen of *Petermannia cirrosa* does not belong within Colchicaceae, instead it forms a sister taxon to a clade of Colchicaceae, Alstroemeriaceae, and Luzuriagaceae. The genus is therefore not included in my work on Colchicaceae.

COLCHICACEAE –the new perspective



Figure 7 *Gloriosa superba* or the glory lily is a very variable species distributed in the tropics of the Old World. The species delimitations in the genus are difficult and the genus has often been regarded to be monotypic (from Engler, 1888).

Phylogeny

In order to sort out the intrafamilial relationships of the expanded Colchicaceae, 73 taxa representing roughly a third of all species were sampled. When collecting material to a phylogenetic study, the aim is to get samples representing all the groups affected by the study and to make sure that as much of the variation as possible is sampled. In reality, however, it is more often the access of available material that is determining and restricting the taxa included. In paper **II**, all to me available material was included representing all 19 genera except the monotypic genus *Kuntheria* from Australia.

The phylogeny of the expanded Colchicaceae (Nordenstam, 1998) was reconstructed in paper **II**. Since the morphology of Colchicaceae has been already rather well studied it is a strict molecular investigation (Buxbaum, 1925, 1936; Sterling, 1973a, b, c, 1974a, b, 1975, 1977; Macfarlane, 1980; Nordenstam, 1982, 1986).

Three different plastid regions were used in order to produce the phylogeny—the *rps16* intron, the *atpB-rbcL* intergenic spacer, and the *trnL-F* region (Fig. 8). All these regions are evolving rather fast since they all comprise, at least partly, non-coding DNA. The regions have proven to be suitable to infer phylogenies at generic and lower levels (Taberlet et al., 1991; Manen et al., 1994; Oxelman, 1997).

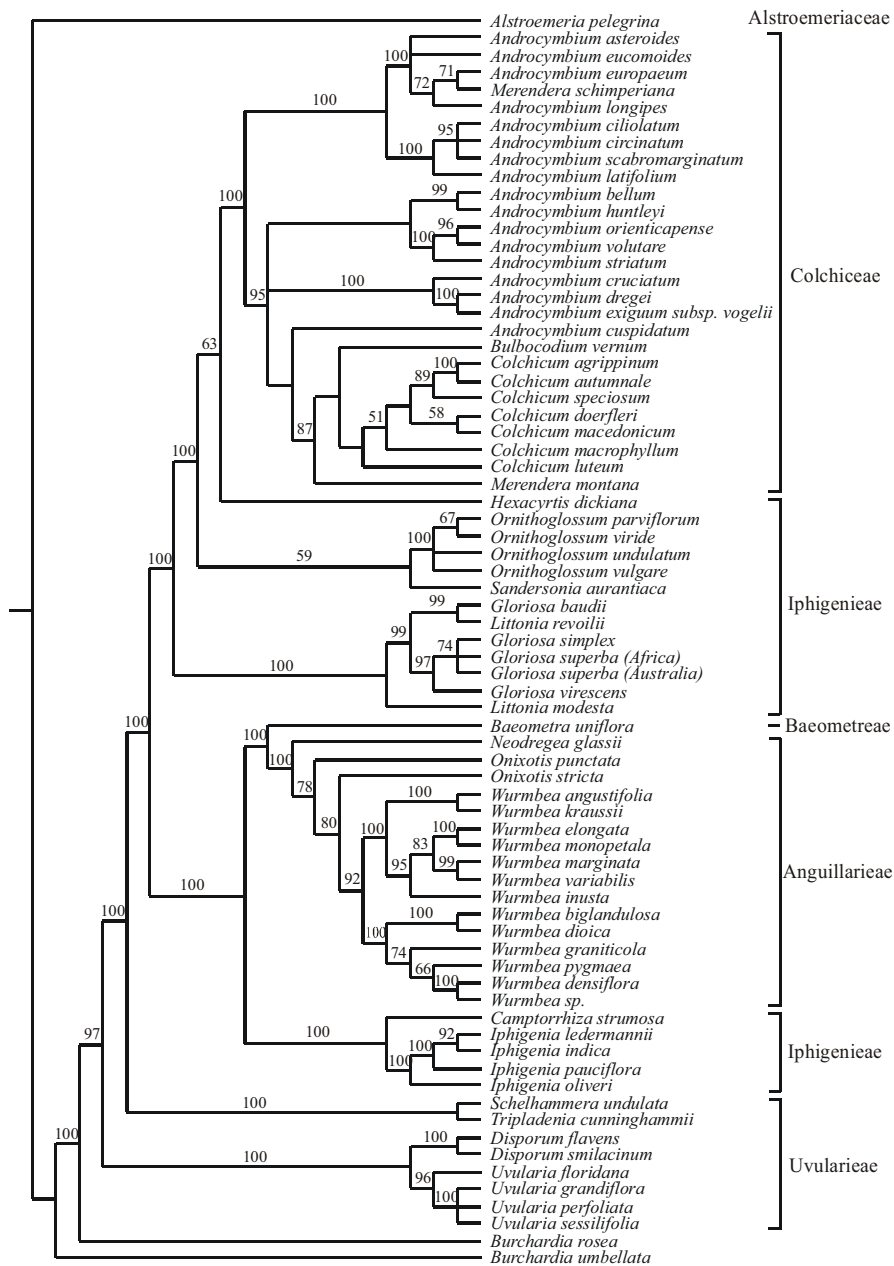


Figure 8 The strict consensus tree from the analysis in paper II. The cladogram is based on a combined analysis of sequences from the *rps16* intron, the *atpB-rbcL* spacer and the *trnL-F* region. Jackknife support values are shown above the branches. The brackets show the old tribal delimitation of the family. In the figure some old generic and species names have been kept to facilitate the recognition of their placement in the phylogenetic tree (*Bulbocodium* and *Merendera* at generic level and *Gloriosa baudii*, *G. simplex* and *G. virescens* at species level).

The compiled matrix, comprising 3830 DNA characters, was analysed both separately and combined. The combined analysis yielded 324 most parsimonious trees, where most of the topology variation is due to the limited number of parsimony-informative characters within the genus *Androcymbium*. The strict consensus tree is shown in Fig. 7 (for more detailed description of methods see paper II).

The phylogeny demonstrates two of the five tribes to be paraphyletic along with the genera *Burchardia* and *Onixotis*. Species of *Gloriosa* and *Littonia* are intermixed and the genus *Colchicum* is demonstrated to be nested within *Androcymbium* rendering the latter paraphyletic.

A synapomorphy

Paper II confirms that Colchicaceae sensu Nordenstam (1998) is a well-supported monophyletic group, however in need of taxonomical revision (paper III, IV and VII). The extended family seems to lack an easily identified morphological synapomorphy. Colchicine, formerly considered to be a unique character for the family (see above), is reported to be lacking in some of the genera now included in the family and some are still not examined. In paper VI we investigated the distribution of colchicine within the expanded family to evaluate the potential of colchicine as a synapomorphy of the family.

A total of nine samples representing the recently included genera *Disporum*, *Schelhammera*, and *Uvularia* together with the two previously unstudied genera from Colchicaceae (Kite et al., 2000), *Hexacyrtis* and *Neodregea*, were investigated. Furthermore, one representative of the sister group, *Alstroemeria* (Alstroemeriaceae) and one genus known to contain colchicine, *Baeometra*, were included in the study. Previous reports on presence of colchicine in *Tripladenia* by Potěšilová et al. (1969) (as *Kreysigia* according to Kite et al., 2000) are based on material from *Kreysigia multiflora* Rchb. Since *Kreysigia* has a confused nomenclature, with synonyms both as *Schelhammera* and *Tripladenia*, it is unclear what material was actually used in the study and material from *Tripladenia* was therefore also included.

By submitting plant extracts of the samples to nanospray mass spectrometry a cleavage pattern, much like a fingerprint, is obtained. This pattern for all samples was compared to the known pattern of pure colchicine (paper VI).

The results clearly demonstrate the presence of colchicine in all the examined genera from the Colchicaceae sensu lato. No trace of the alkaloid was detected in the sister group (*Alstroemeria*), hence demonstrating colchicine to be a good synapomorphy for the family.

DATING



Figure 9 *Wurmbea capensis*, one of the first species recognised in this genus. *Wurmbea* is a disjunct genus with ca. 40 species evenly distributed between Southern Africa and Australia (from Thunberg, 1799).

Our understanding of the evolution of flowering plants is constantly increasing by the access of better resolved phylogenies with good support (Källersjö et al., 1998; Soltis et al., 1998; Soltis, Soltis, and Chase, 1999; APG II, 2003). With the better understanding of the relationship among flowering plants the question of dating phylogenies comes into focus. Being able to put an approximate date to all the splits in the tree of life would not only satisfy our natural curiosity for history but also convey important information regarding clades of the same age, which is needed for comparison in evolutionary biology and historical biogeography.

Geological history

The configuration of the landmasses has changed during the earth history due to the movement of the continental plates. The present outline of the continents began to form in the Early–Mid Jurassic (~180 million years ago, mya) when the supercontinent Pangaea started to break up into the southern continent Gondwana and the northern counterpart Laurasia. The landmasses of Laurasia, comprising North America, Europe, and Asia, have then been joined together in various constellations over time (Sanmartin, 2001) whereas Gondwana has followed a gradual disintegration.

The initial break-up of Gondwana is estimated to ~165-150 mya (Scotese et al., 1988; Hallam, 1994). The disintegration then followed several steps starting with the separation of a western part, comprising Africa and South America, from an eastern part, comprising Australia, Antarctica, Madagascar, and India, ~162 mya (McLoughlin, 2001). In the Early Cretaceous the South Atlantic Ocean started to open between South America and Africa resulting in the final isolation of Africa estimated to 80-85 mya (Scotese et al., 1988; Goldblatt, 1993; Hallam, 1994). The opening of the South Atlantic Ocean also initiated a rotation of the continents and South America and Antarctica were subsequently brought together, enabling floral exchange

between South America and Australia via a habitable Antarctica. The Antarctic link offered a significant dispersal route of southern temperate biota in the Late Cretaceous-Early Tertiary (Woodburne and Case, 1996; Sanmartin and Ronquist, in prep.). The connection across Antarctica followed a gradual disintegration during the Paleogene 65-34 mya (Hallam, 1994; Sanmartin and Ronquist, in prep.) ending with the establishment of the Antarctic circumpolar current and the onset of the first Antarctic glaciation.

How to date

In paper I the order Liliales is dated using two different approaches, the mean branch length method, mbl (Gustavsson and Bremer, 1997) and non-parametric rate smoothing, nprs (Sanderson, 1997). The first method is based on the assumption that mutations occur randomly in the DNA and that they appear with approximately the same change rate (often referred to as the molecular clock). The idea behind the non-parametric rate smoothing is to accept local differences of rate in the tree. By assuming that changes in substitution rates are inherited from an ancestral lineage to its immediate descendants the method smoothes the rate differences over the tree.

To be able to translate the calculated substitution rates into dates both methods need to be calibrated with at least one reference point, viz. a dated fossil or some other known date that is possible to connect to a speciation event. With the reference point and the substitution rates, an approximated minimum age for all the nodes in the given tree can be estimated.

The current distribution of the order Liliales, with mainly Southern Hemisphere species distributed on several continents, indicates that the different families of the order might have originated before the final isolation of the landmasses once forming the ancient continent Gondwana (Africa, South America, Australia, Antarctica, Madagascar, India). Raven and Axelrod (1974) discussed the biogeography of Liliiflorae and concluded that the ancestor of the orders would have been old enough to have existed while South America and Australia were connected via Antarctica. Later attempts to date all flowering plants indicate the ancestral lineage of Liliales to date back to the Early Cretaceous ~100 mya when dispersal between the landmasses of Africa, South America and Antarctica were still possible (Bremer, 2000; Wikström et al., 2001).

In paper I we compare the two different dating methods and address the questions of historical biogeography of Liliales. A phylogeny of 40 genera and all seven families of the Liliales based on *rbcL* sequences is dated, extending the results from Bremer's (2000) and Wikström et al.'s (2001) dating of orders and larger groups of flowering plants.

The result of dating

The two different dating methods used gave very similar results. The mbl method yielded somewhat younger estimates in more than half of the nodes. Generally the differences lie within the confidence intervals calculated and in no case do the differences in dating between mbl and nprs affect the biogeographical conclusions drawn in the paper.

Four clades were found to date back to the Cretaceous around 65 mya (Fig. 9) when the South American, Antarctic, and Australian parts of Late Cretaceous Gondwana were still interconnected, supporting the dating of Bremer (2000) and Wikström et al. (2001). The disintegration of Gondwana is likely to have brought about four vicariance events within the Liliales. The isolation of South America from Australia due to the termination of the Antarctic link during the late Eocene ~34 mya (Hallam, 1994) is likely to have caused a vicariance between *Lapageria*+*Philesia*, and *Ripogonum* in Smilacaceae and the split between Alstroemeriaceae and Luzuriagaceae. The basal split in Melanthiaceae and one within Liliales possibly represent a North America–Eurasia vicariance event. Other possible intercontinental vicariance events were not easily aligned with the separation of the continents, partly due to the distribution of Colchicaceae, which seemed more convoluted than in the other families. This turned out to be due to insufficient sampling of the family (see below and **V**).

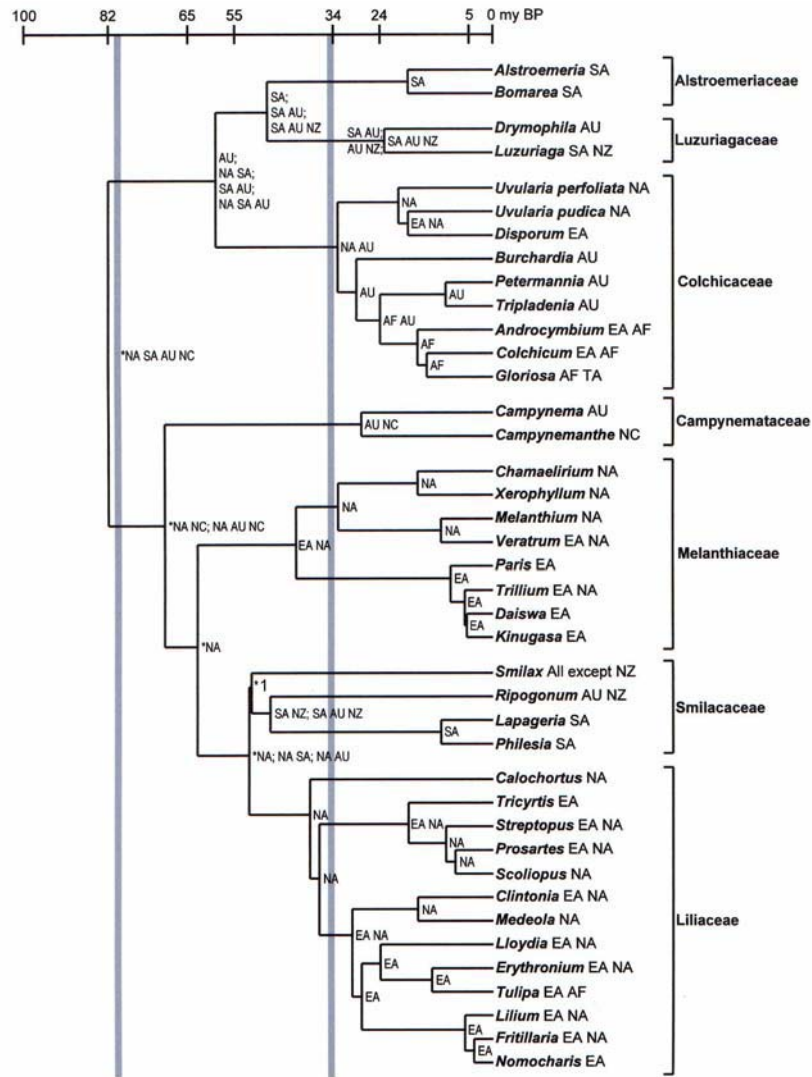


Figure 10 The phylogeny of Liliales with nodes arranged according to the age estimates in paper I. Grey bars show final break-up of Africa and South America, 80 mya, and the final break-up of the South American-Antarctic-Australian connection, 34 mya. Ancestral distributions are according to the DIVA optimisation in paper I. EA=Eurasia, NA=North America, SA=South America, AF=Africa, TA=tropical Asia, AU=Australia, New Guinea, and Tasmania, NC=New Caledonia, and NZ=New Zealand. *The number of areas allowed at each node is restricted to four. ¹Numerous alternative optimisations, see Fig. 3 in paper I for all alternatives.

Biogeography of Colchicaceae

The distribution of Colchicaceae with largely Southern Hemisphere representatives points to a possible Gondwana origin. However, the family is also represented in Europe, Asia and North America but notably there are no South American species. In paper I the dispersal-vicariance (DIVA) analysis found the basal split within the family to represent a North American–Australian vicariance. The estimated date of the split (~34 mya) implies that the ancestral lineage leading to Colchicaceae existed throughout Eocene when South America and Australia were connected via Antarctica. This led to the hypothesis that the family originally might have been American and Australian, later having become extinct in South America. This hypothesis rested on the topology of the tree, which showed the East Asian *Disporum* nested inside the North American *Uvularia* and the *Disporum*+*Uvularia* clade sister to the rest of the family. Hence the DIVA analysis postulated an Australian and North American ancestral distribution for the Colchicaceae.

The sampling of Colchicaceae in paper I was however too poor to explain satisfyingly the peculiar distribution pattern of the family. The establishment of a robust phylogeny comprising all genera, but the monotypic Australian genus *Kuntheria*, in paper II rendered the possibility to conduct a thorough biogeographical analysis of the family (paper V).

The extensive sampling in paper II shows a different topology (Fig. 7) with the Australian *Burchardia* as sister to the rest of the family and *Disporum* and *Uvularia* as sister genera. The DIVA analysis in paper V does not postulate the combination of Australia and North America at the basal node of Colchicaceae but instead it postulates Colchicaceae to be originally Australian and the split between Colchicaceae and the sister group Alstroemeriaceae+Luzuriagaceae to involve a vicariance event between Australia and South America (Fig. 10).

Further, the DIVA analysis shows the Australian tribes Burchardieae and Tripladenieae to be relicts from the ancestral distribution. The tribe Uvularieae seems to represent an East Asian–North American expansion where the family reached North America (*Uvularia*) via East Asia (*Disporum*) probably via the first Beringian bridge (~34 mya), a well known dispersal route for warm temperate groups in the Mid-Tertiary (Sanmartin, 2001). The tribe Colchiceae is an originally African group that later dispersed and became established also in Eurasia and Australasia (paper V).

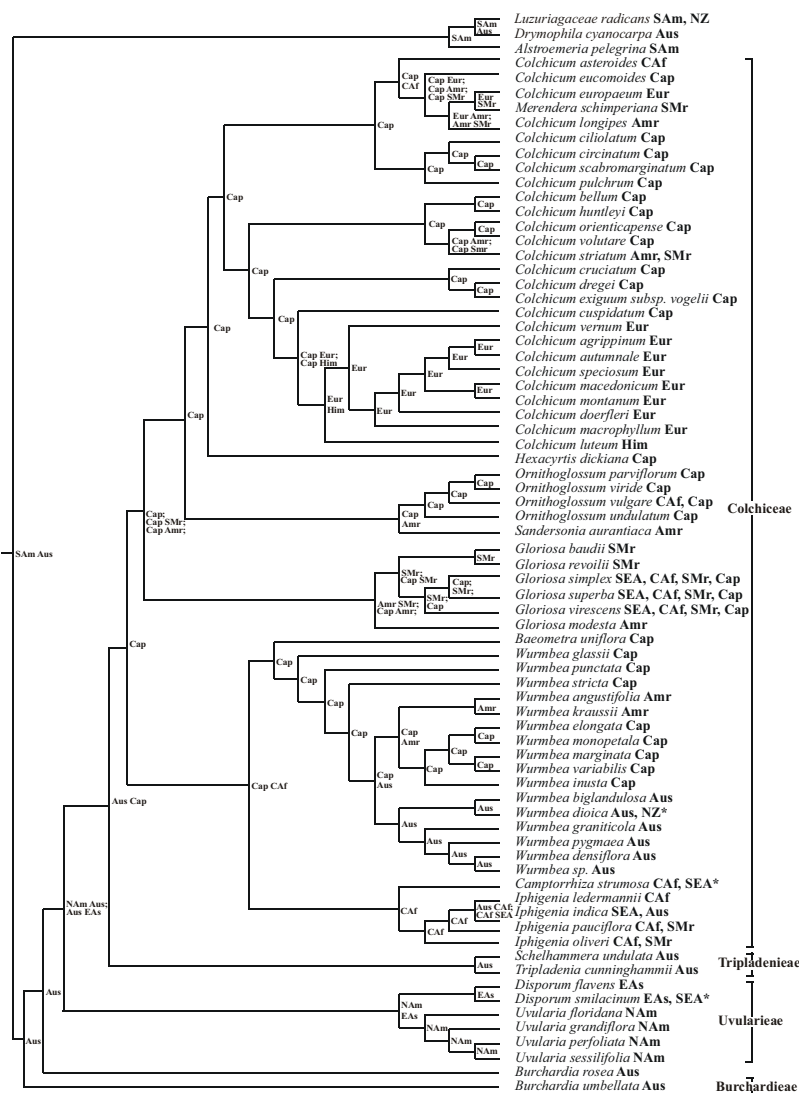


Figure 11 One of the most parsimonious trees from the analysis in paper II with distributions optimised for each node by dispersal-vicariance analysis (DIVA; Ronquist, 1996, 1997). Alternative optimisations are separated by semicolons, **Eur** = Europe to Caucasus, **Him** = the mountainous area in Himalaya, Pakistan, and Afghanistan, **SEA** = South East Asia, **EAs** = Temperate East Asia, **NAm** = North America, **SAM** = South America, **Aus** = Australia, **Cap** = Cape region, **CAF** = Tropical and subtropical Africa, **Amr** = the Afromontane archipelago-like region, and **SMr** = the Somali–Masai region.

*The species has a more restricted distribution but since the phylogeny lacks representatives of the more peripheral distributions within the genus they are added here so that the total distribution of the genus is represented.

TAXONOMY



Figure 12 *Wurmbea glassii*, formerly the sole representative of the genus *Neodregea*. The species is restricted to the arid parts of the Cape region and Namibia (from Wright, 1913).

Modern taxonomists strive to create a natural classification, i.e. a classification reflecting the evolutionary history of taxa. In order to contribute to such a classification we need a clear view of the relationships, or phylogeny, of the taxa we want to classify. The phylogeny provides the settings for the circumscription and nomenclature of a taxon, regulated under the International Code of Botanical Nomenclature, ICBN.

In the classification, currently used in the botanical society (see Cantino and Queiroz, 2000, for a different view), taxa sharing a common ancestor are grouped into larger and larger hierarchical entities (e.g. species into genera, genera into families and families into orders etc.). There are, however, no rules as to what to include in a taxon (i.e. how many species should constitute a genus, or how many genera a family should include). The assembling of taxa into ranks is subjective and a source of many taxonomical debates.

In recent years a few attempts to formalise criteria to choose between alternative circumscriptions of a taxon, under the ICBN, have been published. The criterion of monophyly is the primary one, followed by stability of the taxon (support for the group), stability of the nomenclature, maximising the phylogenetic information, and ease of recognition of the group (APG, 1998; Backlund and Bremer, 1998). The relative importance of the four subordinate principles varies with each particular case (see paper III for a more detailed discussion).

A new classification of Colchicaceae

The phylogenetic analysis in paper II clearly demonstrates Colchicaceae sensu Nordenstam (1998) to be in need of a taxonomical revision. Two of the five tribes and the three genera *Androcymbium*, *Burchardia*, and *Onixotis* are paraphyletic. Further, species of *Gloriosa* and *Littonia* are intermixed (Fig. 12). Paper III concerns the tribal delimitations and the circumscription of the smaller genera, whereas paper IV deals with the two larger genera in the family, *Androcymbium* and *Colchicum*.

Generic circumscriptions

The close relationship between the genera *Colchicum* and *Androcymbium* was first suggested by Buxbaum (1925) and they have since then been invariably allied in the tribe *Colchiceae* (Buxbaum, 1936; Nordenstam, 1982, 1998; Dahlgren, 1985). Both genera are acaulescent or short-stemmed and have androecial nectaries situated at the base of the filaments, unlike the perigonal nectaries that characterise other genera in the family. They are also unique in the family in having 2(-4)-porate pollen.

The molecular phylogeny (Fig. 12) shows two well-supported clades within *Androcymbium*, one comprising the European and some of the South African species of *Androcymbium* (100% jackknife support) and the other comprising *Colchicum* nested within the rest of the South African species of *Androcymbium* (95% jackknife support). Paper IV discusses the various alternatives to achieve a monophyletic delimitation of the genera and unfortunately none of the alternatives keeping the name *Androcymbium* renders a stable, morphologically recognisable taxon.

The two species of *Onixotis* do not form a monophyletic group but a grade between the monotypic *Neodregea* and *Wurmbea*. In view of the molecular data and the morphological variation in *Wurmbea* (Macfarlane, 1980: tables 1 and 2; Nordenstam, 1986) we advocate a merge of the three genera *Onixotis*, *Neodregea*, and *Wurmbea* into a morphologically well-circumscribed genus that is characterised by an ebracteate inflorescence of sessile flowers.

A merge of the two genera *Gloriosa* and *Littonia* has earlier been suggested (Nordal and Bingham, 1998) but the authors chose to await more data before doing the formal combinations. The molecular evidence in paper II supports Nordal and Bingham's results and the two genera are merged.

Furthermore, the two species representing the genus *Burchardia* in the analysis do not form a monophyletic group. The genus currently comprises five species (Macfarlane, 1987), three of which are similar in having basal tepal nectaries (*Burchardia bairdiae*, *B. multiflora* and the type species *B. umbellata*), whereas the remaining two species *B. monantha* and *B. rosea* lack nectaries. Further investigations are needed to resolve the circumscription of the genus and any taxonomical changes are presently premature.

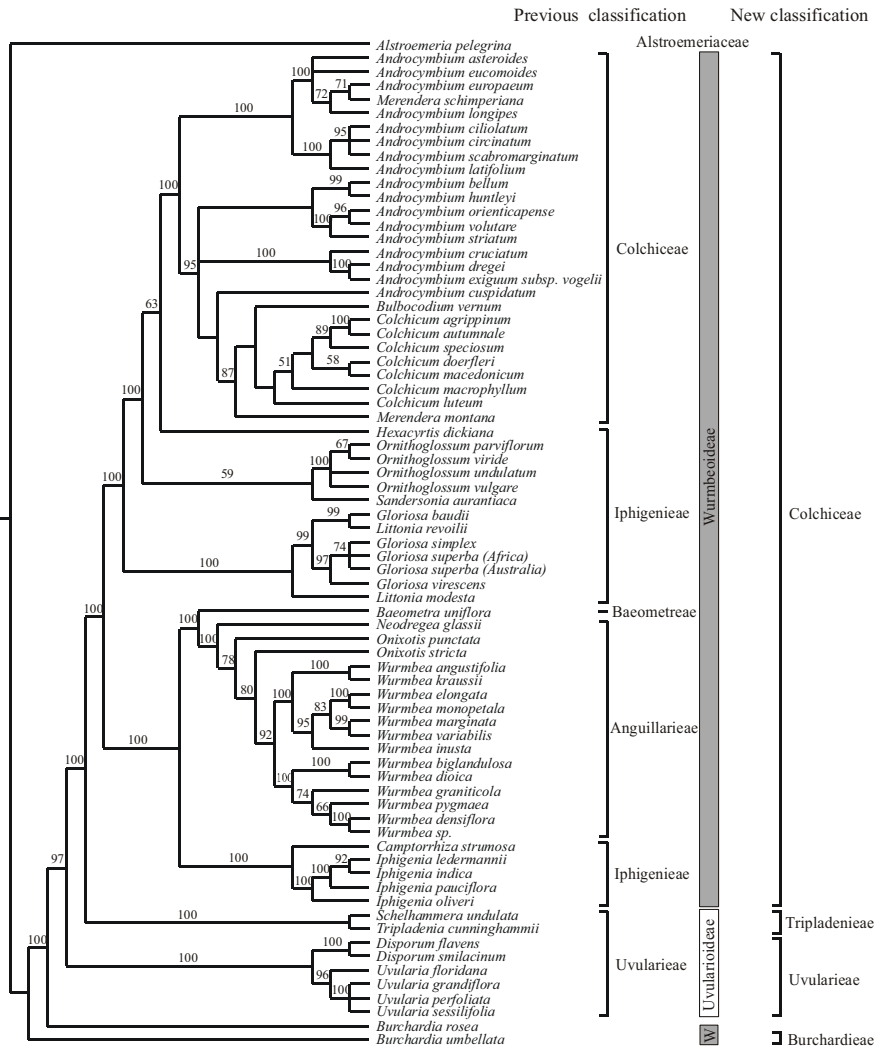


Figure 13 The strict consensus tree from the molecular analysis in paper II with jackknife support values above the branches and the new classification from paper III and IV in the margin.

Tribal circumscriptions

The previous classification of Colchicaceae (Table 1) comprised five tribes of which three are monogeneric as a result of the new generic delimitations, Anguillarieae, Colchiceae, and Baecometreae. The remaining two, Iphigenieae and Uvularieae are paraphyletic (Fig. 12).

The phylogeny presents several different options rendering a monophyletic tribal classification and after evaluating the alternatives (paper III) we chose to include all the genera of the former Colchicaceae (Nordenstam, 1982) in the tribe Colchiceae. To comply with the monophyly criterion we have refined Uvularieae to include only the genera *Uvularia* and *Disporum*, which necessitates the description of a new tribe to accommodate the genera *Tripladenia*, *Schelhammera*, and *Kuntheria*. The latter genus is placed here based on morphological characters, e.g. the branching habit, a cataphyllous rhizome, and erect flowers often in umbel-like cymes.

The monophyly criterion also imposes the erection of a new monogeneric tribe for the genus *Burchardia*. Because the generic delimitation in this genus is not yet settled the tribe will here be taken to include only the type species, *B. umbellata*, awaiting an investigation of which species actually belong in the genus *Burchardia*.

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