MOLECULAR SYSTEMATICS OF *IRIDACEAE*: A COMBINED ANALYSIS OF FOUR PLASTID DNA SEQUENCE MATRICES

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Abstract - *Iridaceae* are one of the largest families of *Lilianae* and probably also among the best studied families of monocotyledons. To further evaluate generic, tribal and subfamilial relationships, we have produced four plastid DNA data sets for 57 genera of *Iridaceae* plus outgroups: *rps*4, *rbc*L (both protein coding genes), and the *trn*L intron snd the *trn*L-F inter-gene spacer. All four matrices produce highly congruent, although not identical trees, and we thus analysed them in a combined analysis, which produced a highly resolved and well supported topology. In each of the individual trees, some genera or groups of genera are misplaced relative to Goldblatt's and Rudall's morphological cladistic studies, but the combined analysis produced a pattern much more similar to these previous ideas of relationships. In the combined tree, all subfamilies were resolved as monophyletic clades, except *Nivenioideae*, which formed a grade in which *Ixioideae* were embedded. The achlorophyllous *Geosiris* (sometimes referred to *Geosiridaceae* or *Burmanniaceae*) fell within the nivenioid grade. Most of the tribes are monophyletic, except for *Ixieae*, *Watsonieae* and *Sisyrinchieae*, but the topology within *Ixioideae* is not strongly supported due to extremely low levels of sequence divergence. *Isophysis* is sister to the rest of the family, and *Diplarrhena* falls in a well supported position as sister to *Irideae*/*Sisyrinchieae*/*Tigridieae*/*Mariceae*; *Bobartia* of *Sisyrinchieae* is supported as a member of *Irideae*.

Key words - *Iridaceae*, systematics, DNA.

Introduction

The petaloid monocot family *Iridaceae* comprises some 1800 species in ca. 65 genera (Goldblatt, 1990, 1991), representing one of the largest families of the superorder *Lilianae* (*sensu* Dahlgren *et al.*, 1985). Members of *Iridaceae* are typically characterised by isobilateral equitant leaves, styloid crystals and flowers with only three stamens. Although worldwide in distribution, the family is centred in Africa where there are some 1000 species, most of which are restricted to southern Africa.

Rigorous and multidisciplinary studies by many authors have, to date, failed to produce a consensus sub-familial classification. The first phylogenetic classification of *Iridaceae*, using cladistic techniques (Goldblatt, 1990), formed the basis of the most recent classification of the family. This analysis used 52 characters from phytochemistry, cytology, pollen structure, anatomy and morphology to identify four major clades. Given subfamily status (Goldblatt, 1991), these were designated *Isophysidoideae*, *Nivenioideae*, *Iridoideae* and *Ixioideae*. In turn, *Iridoideae* comprised tribes *Mariceae, Tigrideae, Iridineae* and *Sisyrinchieae*, and subfamily *Ixioideae* comprised tribes *Pillansieae, Watsonieae* and *Ixieae*.

In a subsequent cladistic analysis of *Iridaceae*, Rudall (1994) used 33 characters which included more anatomical characters than were used by Goldblatt (1990). This analysis recognised the four subfamilies and seven tribes *sensu* Goldblatt. However, the relationships among the subfamilies found in the two separate analyses are not identical. The principal areas of conflict concern the relationship of *Ixioideae* to the rest of the family and the placement of *Isophysis*. In Goldblatt's scheme, *Ixioideae* were the most derived clade whereas Rudall's analysis placed them sister to the remainder of the family. Also, Goldblatt (1990) defined *Isophysis* as the sister taxon to the rest of the family, whereas Rudall used a different outgroup and placed *Isophysis* sister to *Nivenioideae*. In Rudall's (1994) analysis, *Isophysis* together with *Nivenioideae* then formed the most derived clade.

The most recent phylogenetic representation of *Iridaceae* is that of Souza-Chies *et al*. (1997), using molecular data derived from the region coding for protein four of the plastid small ribosomal subunit (*rps*4). This tree, inferred by the interpretation of a relatively small number of molecular characters (approximately 600 base pairs), placed *Isophysis* as the sister taxon to the rest of the family. Subfamily *Ixioideae* formed a well supported clade, although there was little resolution within it, and subfamily *Nivenioideae* did not form a monophyletic group but rather a paraphyletic grade with *Ixioideae* as its terminal clade. In this analysis the monophyly of subfamily *Iridoideae* was not supported, but there was no evidence to refute its monophyletic status. Therefore *rps*4 alone provided insufficient evidence to evaluate the monophyly of this subfamily.

Few non-molecular characters remain to be studied that could resolve the conflicts among the phylogenetic interpretations of *Iridaceae*. This study includes molecular characters from three additional plastid regions as a source of phylogenetic information and combines these data with the supplemented *rps*4 data of Souza-Chies *et al*. (1997) into a single matrix. The three plastid DNA regions sequenced are the *trn*L (UAA) intron, the *trn*L-*trn*F (GAA) intergene spacer (collectively known as the *trn*L-F region) and the gene for the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase (*rbc*L). The aim of this analysis is to enhance the current understanding of *Iridaceae* phylogeny and to elucidate some presently unresolved key questions, among which the following are the most pertinent:

(i) The relationships among the four subfamilies (Goldblatt, 1991) which includes the proper placement of *Isophysis*, a Tasmanian endemic lacking one synapomorphy often assumed for *Iridaceae*, the inferior ovary. Earlier treatments had assigned *Isophysis* to its own family (Bentham and Hooker, 1883).

(ii) The familial and tribal position of the Madagascan achlorophyllous saprophyte, *Geosiris*, which in the past has been referred to *Burmanniaceae*, assigned to its own family *Geosiridaceae* (Jonker, 1939) or placed in subfamily *Nivenioideae* (Goldblatt *et al*., 1987; Goldblatt, 1990).

(iii) Correct delimitation of *Iridoideae*, including the proper status of tribe *Sisyrinchieae* within this subfamily. Of particular interest is the placement of the African genus *Bobartia* in *Sisyrinchieae* (Goldblatt & Rudall, 1992) as all other members of this tribe are American or Australasian.

(iv) Generic and tribal relationships in *Ixioideae*.

MATERIAL AND METHODS

Plant material and herbarium vouchers used in this analysis are listed in Table 1. Total genomic DNA was extracted from 1.0g fresh leaf or flower tissue or 0.15-0.2 g silica-dried tissue using the 2XCTAB method described by Doyle and Doyle (1987). Herbarium material of *Klattia flava* was extracted using a modified 2XCTAB method (Fay *et al*., 1997) with propan-2-ol instead of ethanol for precipitation of the DNA and a two week precipitation period at 20°C. All DNA extracts were purified by cesium-chloride ethidium-bromide equilibrium density gradients (1.55g/ml). Purified, total DNAs were dialysed in 1X TE buffer and stored at 80°C.

Three plastid regions, *rbc*L, *trn*L intron and *trn*L-F intergene spacer were amplified for the 57 species of *Iridaceae* and six outgroup taxa listed in Table 1. Those genera not represented in the *rps*4 analysis of Souza-Chies *et al*. (1997) were amplified to achieve conformity between the four data sets.

Twenty to fifty nanograms of total genomic DNA were used as a template for Taq-mediated amplification. Amplification of the *rbc*L gene was carried out using a forward primer that matched the first 20 base pairs of the exon and a reverse primer beginning at either position 1360 or 1368 on the complementary strand. Amplification using these primers produced a 1388 or 1391 base pair fragment of the *rbc*L exon. In some cases amplification of the complete gene was not possible due to degradation of the genomic DNA. In these cases the gene was amplified in two parts using internal primers 636F and 724R (this reverse primer does not work for dicotyledons). For *rbc*L four sequencing reactions per taxon were required with primers 1F, 636F, 724R and 1360R/1368R (Muasya *et al*., 1998). In most cases greater than 80% overlap was achieved.

Primers «c» and «f» (Taberlet *et al*., 1991) were used to amplify the intron and intergene spacer region between the *trn*L 3' and *trn*F exons. The amplified fragment varied in length from approximately 650 to 900 base pairs and resulted in an aligned matrix of 1250 base pairs. For cases in which complete amplification of the «c» to «f» region failed, internal primers «d» and «e» (Taberlet *et al*., 1991) were used to amplify the gene in two non-overlapping segments. Only two sequencing reactions, with primers «c» and «f», were required in cases for which complete amplification of the *trn*L-F region was successful. Greater than 80% overlap was achieved in most cases. All *trn*L-F sequences were easily aligned by eye. Four discrete gaps were also coded as 0/1 characters, otherwise gaps were coded as missing.

A fragment including the *rps4* gene, an intergene spacer and the *ser*-tRNA gene was amplified using primers *rps5'* and *t*RNAS (Souza-Chies *et al*., 1997). The resulting amplified fragment was approximately 800 base pairs in length. Only the 600bp *rps*4 exon was used in this analysis. As for *trn*L-F, all *rps*4 sequences were aligned by eye.

TABLE 1

Goldblatt 1990	Accession/Voucher	Literature Citation		
Iridaceae				
		*rbc*L	*trn*L	*rps*4
Subfamily Isophysidoideae				
Isophysis tasmanica (Hook.)T.Moore	J.Bruhl (I-2)	Chase *et al.* (1993)	this paper	Souza-Chies *et al.* (1997)
Subfamily Nivenioideae				
Nivenia corymbosa Baker	Goldblatt (I-14), MO		this paper	Souza-Chies *et al.* (1997)
Aristea coerulea (Thunb.) Vahl.	Piketbon 9362 (I-15), MO		this paper	-
Aristea platycaulis Bak.	MNHN (S 69-1198)	-	-	Souza-Chies *et al.* (1997)
Witsenia maura (L.) Thunb.	Orchand 35 (I-12)	this paper	this paper	this paper
Klattia flava G.J.Lewis.	Goldblatt B656 (I-240), MO	this paper	this paper	this paper
Patersonia glabrata R. Br	UNSW 21494 (I-18)	this paper	this paper	-
Patersonia fragilis Aschers & Graebn.	MNHN (S 92-20)	-	-	Souza-Chies *et al.* (1997)
Geosiris aphylla Baill.	Prance 30781 (MWC 1458), K	Rudall *et al.* (1997)	this paper	this paper
Subfamily Iridoideae				
Tribe Sisyrinchieae				
Bobartia gladiata (L.f)Ker Gawl.	Goldblatt 9490 (I-16), MO	this paper	this paper	Souza-Chies *et al.* (1997)
Libertia ixioides Spreng.	M.W. Chase (I-218), K	this paper	this paper	-
Libertia formosa Grah.	Geneve (Chile)	-	-	Souza-Chies *et al.* (1997)
Diplarrhena latifolia Benth.	M.W. Chase (I-220), K	this paper	this paper	Souza-Chies *et al.* (1997)
Orthrosanthus chimboracensis Bak	M.W. Chase (I-231), K	this paper	this paper	this paper
Orthrosanthus polystachyus Benth	Goldblatt 9297, MO	Duvall *et al.* 1993	this paper	this paper
Sisyrinchium micranthum Cav.	Henrich (I-208), MO	this paper	this paper	-
Sisyrinchium sp. Benth. &Hook.	Campinas (Herbarium)	-	-	Souza-Chies *et al.* (1997)
Olsynium filifolium (Gaudich.) Goldbl	M.W.Chase (I-243), K	this paper	this paper	this paper
Solenomelus pedunculatus Hochr.	M.W. Chase (I-222), K	this paper	this paper	Souza-Chies *et al.* (1997)

Tribe Iridieae				
Subtribe Homeriinae				
Moraea namequemontas Goldbl.	Folken & Vonter 627 (I-44), PRE	this paper	this paper	-
Moraea spathulata Klatt.	MNHN (EB 90-1494)	-	-	Souza-Chies *et al.*,(1997)
Homeria cookii L.Bolus	Goldblatt & Manning 9672B (I-183), MO	this paper	this paper	this paper
Galaxia sp.	Goldblatt & Nanni 10254 (I-228), MO	this paper	this paper	this paper
Gynandriris sisyrincium Parl.	M.W. Chase (I-107), K	this paper	this paper	this paper
Subtribe Iridinae				
Dietes robinsoniana Klatt	Pickard 3377 (I-8), MO	this paper	this paper	-
Dietes robinsoniana Klatt	MNHN (S 88-82)	-	-	Souza-Chies *et al.*,(1997)
Iris unguicularis Poir.	M.W. Chase (I-100), K	this paper	this paper	Souza-Chies *et al.*,(1997)
Belamcanda chinensis Adans.	MNHN (JA 84-1389)	this paper	this paper	Souza-Chies *et al.*,(1997)
Subtribe Ferrariinae				
Ferraria crispa Burm.	Goldblatt & Manning 9732 (I-176), MO	this paper	this paper	this paper
Tribe Tigridieae				
Tigridia pavonia (L.f) DC	M.W. Chase (I-242), K	this paper	this paper	-
Tigridia sp. Juss.	MNHN (EB)	-	-	Souza-Chies *et al.*,(1997)
Cypella aquatilis Ravenna	Castillo (I-202), MO	this paper	this paper	-
Cypella sp. Herb.	Porto Alegre (027)	-	-	Souza-Chies *et al.* (1997)
Cipura campanulata Ravenna	Henrich 143 (I-201), MO	this paper	this paper	this paper
Eleutherine latifolia (Standl.& Will.) Ravenna	Goldblatt 9072 (I-204), MO	this paper	this paper	this paper
Ennealophus euryandrus (Gris.) Ravenna	Solomon 9972 (I-23), MO	this paper	this paper	this paper
Gelasine elongata (R.Grah.) Ravenna	Goldblatt 5925 (I-48), MO	this paper	this paper	this paper

Calydorea pallens Griseb.	Goldblatt 9579 (I-200), MO	this paper	this paper	this paper
Herbertia pulchella Sweet	Goldblatt (I-205), MO	this paper	this paper	this paper
Alophia verucruzana Goldbl. & T.M. Howard	Goldblatt and Howard 9070 (I-199), MO	this paper	this paper	Souza-Chies *et al.* (1997)
Hesperoxiphion peruvianum Bak.	Goldblatt (I-206), MO	this paper	this paper	this paper
Tribe Mariceae				
Neomarica northiana Sprague	Solomon 6950 (I-207), MO	this paper	this paper	-
Neomarica sp. Sprague	Porto Alegre (010)	-	-	Souza-Chies *et al.* (1997)
Trimezia martinicensis (Jacq.) Herb.	Berry 3802 (I-5), MO	this paper	this paper	-
Trimezia stayermarkii R. Foster	Goldblatt 1245 54, MO	-	-	Souza-Chies *et al.* (1997)
Subfamily Ixioideae				
Tribe Pillansieae				
Pillansia templemanni L.Bolus	Bean (I-6), MO	this paper	this paper	Souza-Chies *et al.* (1997)
Tribe Watsonieae				
Lapeirousia neglecta Goldbl.	Goldblatt & Manning 9489 (I-7), MO	this paper	this paper	Souza-Chies *et al.* (1997)
Savannosiphon euryphylla	Bolnick (I-193), MO	this paper	this paper	this paper
Micranthus junceus N.E. Brown	M.W. Chase (I-156), K	this paper	this paper	this paper
Thereianthus racemosus (Klatt) Lewis	Goldblatt 10454 (I-224), K	this paper	this paper	this paper
Watsonia anguta Ker Gawl.	Goldblatt 6904 (I-4), MO	this paper	this paper	this paper
Tribe Ixieae				
Ixia latifolia D. Delaroche	Goldblatt & Manning 9594 (I-184), MO	this paper	this paper	this paper
Chasmanthe aethiopica (L.) N.E. Br.	(I-3)	this paper	this paper	this paper
Freesia alba Bak.	Goldblatt 5293 (I-68), MO	this paper	this paper	-

Freesia sp. Klatt	MNHN (EB)	-	-	Souza-Chies *et al.* (1997)
Geissorhiza heterostyla L.Bolus	Goldblatt & Manning 9668 (I-178), MO	this paper	this paper	this paper
Hesperantha pseudopilosa Goldbl.	Goldblatt & Manning 9677 (I-182), MO	this paper	this paper	this paper
Tritonia disticha Bak.	Goldblatt & Manning 9545 (I-49), MO	this paper	this paper	this paper
Sparaxis variegata (Sweet) Goldbl	Goldblatt 2460 (I-50), MO	this paper	this paper	-
Sparaxis sp. Ker-Gawl.	MNHN (EB)	-	-	Souza-Chies *et al.* (1997)
Babiana ecklonii Klatt	Goldblatt & Manning 9958 (I-172), MO	this paper	this paper	-
Babiana stricta Ker-Gawl	MNHN (S 94-578)	-	-	Souza-Chies *et al.* (1997)
Gladiolus guenzii Kunze	Goldblatt 9052 (I-64), MO	this paper	this paper	
Gladiolus papilio Hook.	Goldblatt & Manning 9841, MO	-	-	Souza-Chies *et al.* (1997)
Radinosiphon leptostachya (Baker) N.E.Br.	Boussard (I-66), MO	this paper	this paper	this paper
Tritoniopsis unguicularis (Lam.) G.J. Lewis	Goldblatt 9486 (I-65), MO	this paper	this paper	this paper
Romulea monodelpha (Sweet) Bak.	Goldblatt 6230 (I-60), MO	this paper	this paper	-
Romulea revelieri Jord. Fourr.	MNHN (Moret 93-88)	-	-	Souza-Chies *et al.* (1997)
Syringodea bifucata M.P. de Vos	Davidson 3108 (I-9), MO	this paper	this paper	this paper
Crocus pulchellus Herb.	M.W. Chase I-19, K	this paper	this paper	-
Crocus nudiflorus Hohen.	MNHN (EB 66-2483bis)	-	-	Souza-Chies *et al.* (1997)
Schizostylis coccinea Backh. & Harv.	Rycroft (I-194), NBG	this paper	this paper	this paper
Anomatheca laxa (Thunb.) Goldbl.	M.W. Chase (I-1), K	Chase *et al.*, (1993)	this paper	this paper
Ixioliriaceae				
Ixiolirion tataricum (Pall.) Herb.	M.W. Chase 489, K	Chase *et al.* (1995)	this paper	this paper
Doryanthaceae				
Doryanthes excelsa Correa	M.W. Chase 188, NCU	Chase *et al.* (1995)	this paper	this paper

Tecophilaeaceae				
Tecophilaea cyanocrocus Leyb.	M.W. Chase 447, K	Chase *et al*. (1995)	this paper	this paper
Walleria mackenzii J. Kirk.	M.W. Chase 1677, K	Brummitt *et al*. (1997)	this paper •	this paper
Zephyra elegans D. Don.	M.W. Chase 1575, K	Brummitt *et al*. (1997)	this paper	this paper
Cyanastraceae		Brummitt *et al*. (1997)	this paper	this paper
Cyanastrum bussei Engl. Jahrb	M.W. Chase 1378, K	Brummitt *et al*. (1997)	this paper	this paper

All gaps were coded as missing.

Amplified double-stranded DNA fragments were purified using «Wizard» mini columns (Promega) and directly sequenced on an ABI 373A automated sequencer using standard dye-terminator chemistry following manafacturers protocols (Applied Biosystems Inc.). For editing and assembly of the complimentary strands «Sequence Navigator» and «Autoassembler» (Applied Biosystems Inc.) were used.

All cladistic analyses were performed using the parsimony alogrithm of the software package PAUP for Macintosh (phylogenetic analysis using parsimony version 3.1.1; Swofford, 1993) on a Power Macintosh 7200/90 with 16MB RAM. The data matrices corresponding to each of the four plastid DNA regions and a combined data matrix of all four were analysed using 1000 replicates of random taxon addition order, tree bisection-reconnection (TBR) branch swapping, with MULPARS on, and all character transformations treated as equally likely (Fitch parsimony; Fitch, 1971). To minimise the time spent searching sub-optimal «islands» (Maddison, 1991), a limit of ten trees were saved from each replicate. Characters were reweighted according to their corresponding RC values and after each round of reweighting a heuristic search with simple taxon addition was performed. When the tree length remained the same in two successive rounds these were the finite trees. The final successive (SW) weights were then applied to either all trees or a random large subset (more than 2000) of the initial trees collected during branch swapping for a second heuristic search with random taxon addition TBR branch swapping and MULPARS on. Internal support was assessed using 1000 bootstrap replicates (Felsenstein, 1985) and the SW weights. Only those groups of greater than 50% frequency were reported.

RESULTS

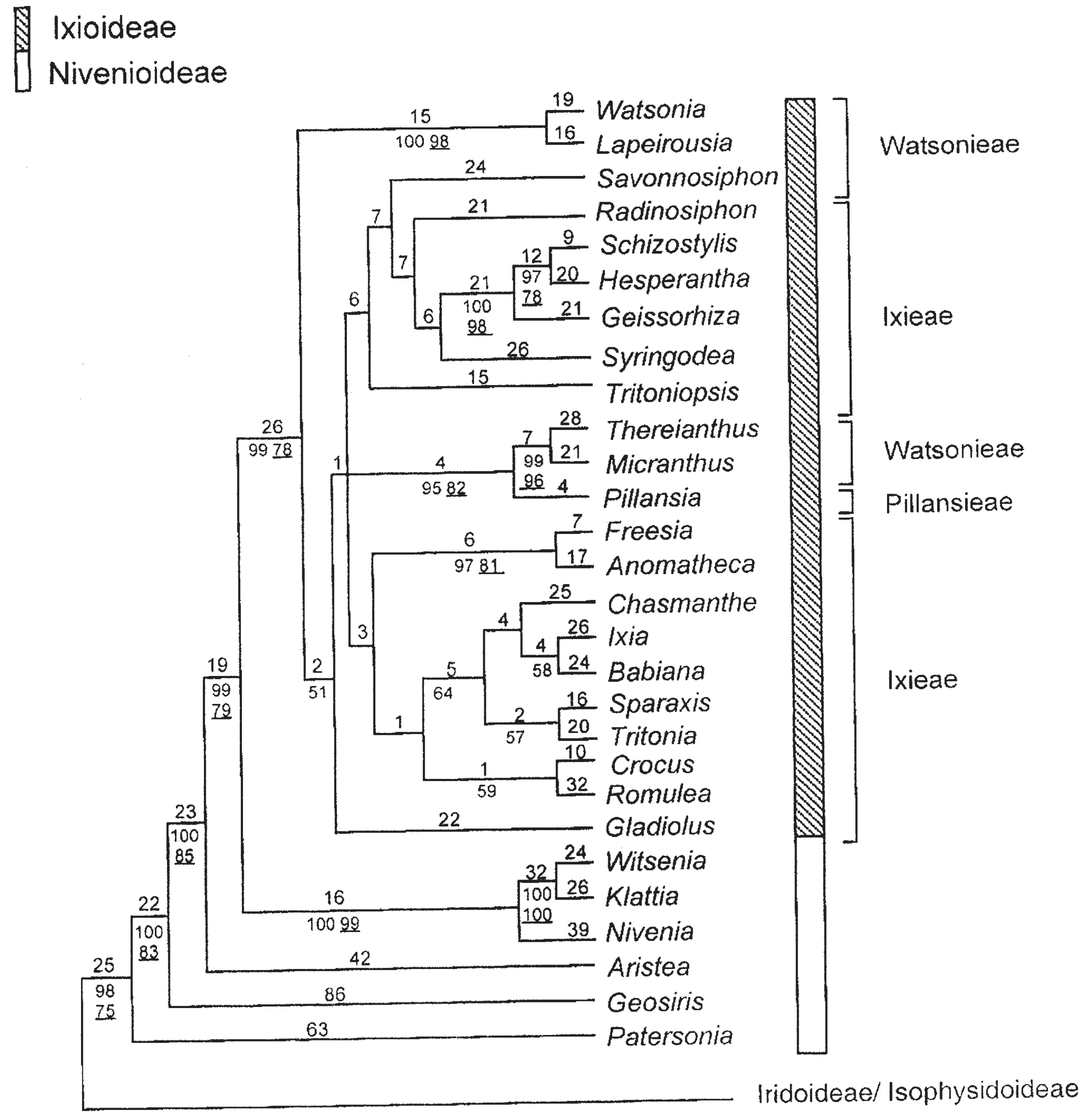
Since all analyses produced highly congruent patterns, we present here only the best supported, the combined analysis. The combined analysis of the three plastid DNA regions included 3232 characters. Parsimony analysis with equal weights produced 244 equally parsimonious trees of 2464 steps with consistency index (CI) = 0.58 and retention index (RI) = 0.73. Applying successive weights resulted in three equally parsimonious trees of 1021582 steps with CI = 0.86 and RI = 0.90. These trees were a subset of the Fitch trees (i.e. they also had 2464 steps).

The combined tree demonstrates excellent support for subfamily *Ixioideae*, however several polytomies occur within the subfamily. The delimitation of tribes *Watsonieae, Pillansieae* and *Ixieae sensu* Goldblatt (1990) are not supported. However, some groupings are well resolved with high bootstrap support, notably the alliance of two genera of *Watsonieae*, *Thereianthus* and *Micranthus,* with the monogeneric subfamily *Pillansieae*. *Watsonia* and *Lapeirousia* (*Watsonieae*) form a supported clade, as do three genera of *Ixieae*, *Schizostylis*, *Hesperantha* and *Geissorhiza*. *Freesia* and *Anomatheca* (*Ixieae*) are also well supported as sister taxa. *Ixioideae* form the most derived clade with a paraphyletic *Nivenioideae*. Within this paraphyletic grade three genera, (*Witsenia*, *Klattia* and *Nivenia*) form a monophyletic group. *Iridoideae* are monophyletic, with tribes *Irideae, Tigridieae, Mariceae* and *Sisyrinchieae* resolved in all trees. However, *Bobartia* and *Diplarrhena* (both often placed in *Sisyrinchieae*), occupy positions outside *Sisyrinchieae*. *Bobartia* is sister to *Irideae*, and *Diplarrhena* is sister to the rest of *Iridoideae*. *Isophysis* is the sister taxon to the rest of the family.

DISCUSSION

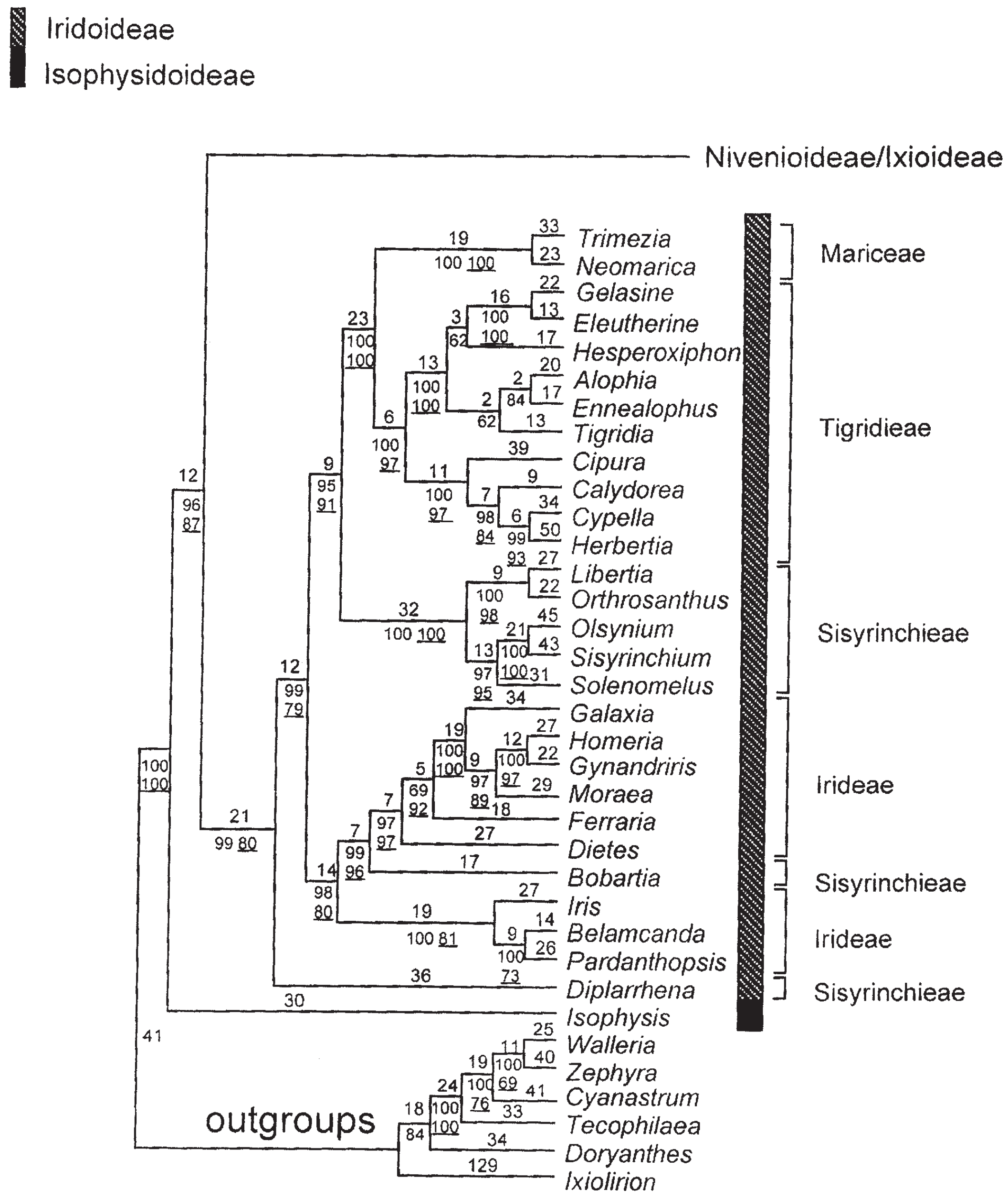
Previous cladistic analyses of non-molecular characters (Goldblatt, 1990; Rudall, 1994) have identified four distinct groups within *Iridaceae.* To date, no evaluation has been performed to evaluate the robustness of these clades and hence the two phylogenies represent somewhat conflicting hypotheses which must be equally accepted as possible explanations of *Iridaceae* phylogeny. In contrast, the tree presented here has been evaluated for the level of support attributable to each of the individual clades. The taxonomic implications of the combined tree are discussed below.

Affinities of the monotypic genus *Isophysis* vary considerably among classification systems, and its inclusion in *Iridaceae* is controversial largely due to its possession of a superior ovary. *Isophysis* does, however, share with the rest of the family at least two synapomorphies: presence of styloid calcium oxalate crystals (Goldblatt *et al*., 1984) and flowers with three stamens. These characters have led recent authors to include *Isophysis* in *Iridaceae*: tribe *Isophysideae* (Hutchinson, 1934) and subfamily *Isophysidoideae* (Goldblatt *et al*., 1984; Dahlgren *et al*., 1985). The precise placement of *Isophysis* within *Iridaceae* has also been disputed (Goldblatt, 1990; Rudall, 1994). In the combined molecular analysis *Isophysis* belongs in a position as sister to the remainder of *Iridaceae*, as Goldblatt (1990) suggested. *Isophysis* is unambiguously placed in all combined trees, and bootstrap support for the *Iridaceae* clade excluding *Isophysis* is high.



A

Figure 1 - One of the three most parsimonious trees found with successive weighting. Fitch lengths are shown above the branches. Bootstrap values achieved with successive weights and with Fitch weights (underlined) are shown below the branches. The taxonomic scheme is that of Goldblatt (1990).



B

Figure 1 (cont)

The placement of *Isophysis* as sister to *Iridaceae* in Goldblatt's analysis (1990) may be explained somewhat by his choice of outgroups which all possess a superior ovary. If *Isophysis* is the earliest diverging genus of *Iridaceae*, this could imply that the superior ovary is the ancestral state for the family. Subsequent to Goldblatt's analysis, the *rbc*L monocot analysis (Chase *et al*., 1995) placed *Iridaceae* within the 'lower' asparagoids in a grade including *Doryanthaceae* and *Ixioliriaceae*, with *Tecophilaeaceae* (including *Cyanastraceae*) as their sister group. These closest relatives, as implied by the *rbc*L tree, all possess an inferior ovary and are the families from which outgroup taxa have been chosen for this molecular analysis. Therefore, since both ingroup and outgroup taxa possess an inferior ovary, the superior ovary of *Isophysis* must be regarded as an autapomorphy and is thus uninformative.

The six genera included in *Nivenioideae sensu* Goldblatt (1990) are represented in this analysis. The monophyly of *Nivenioideae* is not supported in any of the molecular analyses; instead the subfamily comprises a paraphyletic grade which collectively forms a clade with subfamily *Ixioideae*. Within paraphyletic *Nivenioideae*, the three shrubby Cape genera *Witsenia*, *Klattia* and *Nivenia* form a well supported clade (100% bootstrap) in the combined analysis. The inclusion of the Madagascan saprophyte *Geosiris* in the *Nivenioideae-Ixioideae* clade is consistent in all of the molecular analyses and confirms its proper status within, and as a member, of the family (Goldblatt *et al*., 1987). In the combined tree, the Australian genus *Patersonia* represents the sister group of the *Nivenioideae-Iridoideae* clade.

Coherence of *Nivenioideae* as a monophyletic group has been questioned by previous authors (Goldblatt, 1990) because, in addition to its broad geographical distribution, only three non-molecular characters define the subfamily: binate rhipidia, a blue perianth, and a fugacious flower. The last two are also found in *Iridoideae*, and none of these characters can be assessed robustly by outgroup comparison.

The delimitation of the largely African subfamily *Ixioideae* is in accordance with most systems of classification of *Iridaceae* which have consistently accepted its existence as a distinct group within the family (tribe Ixieae of Bentham and Hooker, 1883; Diels, 1930). *Ixioideae* are well defined by both morphological and anatomical characters, but relationships within this subfamily remain ambiguous due to the lack of divergence demonstrated by the plastid DNA regions used in this analysis. Several sub-familial groupings do emerge in the combined analysis. Tribe *Watsonieae* are split into two well supported groups with the exception of *Savannosiphon*. One of these groups, comprising *Theiranthus* and *Micranthus*, appears to be associated with the monogeneric tribe *Pillansieae*. These three taxa are embedded in the partly unresolved tribe *Ixieae*.

Sampling for *Ixioideae* may be improved but it does appear that the plastid regions used to reconstruct this phylogeny do not exhibit enough variation to resolve the relationships within this apparently rapidly radiating subfamily. Normally, improved resolution may be achieved by sequencing more variable nuclear DNA regions, for example the internal transcribed spacer region (ITS). However, within many genera of *Iridaceae,* ITS rDNA appears to exist in a series of highly divergent repeats at different chromosomal locations (Chase *et al*., unpubl.). This makes the ITS region difficult to use for phylogenetic reconstruction.

Subfamily *Iridoideae sensu* Goldblatt (1990) emerges as a monophyletic group in the combined analysis with bootstrap support of 99%. Many tribal groupings are also well resolved and supported, and in the main part are in accordance with those outlined by Goldblatt (1990). The exceptions to the tribal groupings *sensu* Goldblatt (1990) are the placement of two members of tribe *Sisyrinchieae*: *Bobartia* and *Diplarrhena*. In Goldblatt's scheme, *Bobartia* is the only South African member of the tribe, and molecular data place *Bobartia* in a clade with representatives of the South African tribe Irideae, a position which has been previously considered but not supported by any prior cladistic analysis (Goldblatt & Rudall, 1992). *Diplarrhena* is unusual within *Iridaceae* as it only possess two stamens whereas three stamens is uniform for the rest of the family (Rudall and Goldblatt, this volume). The combined molecular analysis positions *Diplarrhena* as sister to the remainder of *Iridoideae*.

Based upon the combined parsimony analysis of the three plastid DNA regions the following taxonomic recommendations are appropriate:

(i) Combination of *Nivenioideae* and *Ixioideae* into a larger *Ixioideae* with tribes *Patersonieae, Geosirieae, Aristeae, Nivenieae* (including *Nivenia*, *Witsenia* and *Klattia*) and *Ixieae* (including all the current *Ixieae, Watsonieae* and *Pillansieae*). Since only fairly insignificant characters delimit the groupings in the present Ixioideae there is no strong argument for recognition of tribes *Watsonieae* and *Pillansieae*.

(ii) In subfamily *Iridoideae Diplarrhena*, as a distinct species within the group, should be assigned to its own tribe *Diplarrheneae* (Rudall and Goldblatt, this volume). *Bobartia* should be included in tribe *Irideae* rather than *Sisyrinchieae*.

In conclusion, this study strongly supports combining data for systematic inference when more than one data set is available. Combining consensus trees would not resolve the positions of *Isophysis*, *Diplarrhena* and *Patersonia* for example. Further work should include the combination of non-molecular characters with DNA sequence data as this may provide greater resolution within *Ixioideae* in particular.

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