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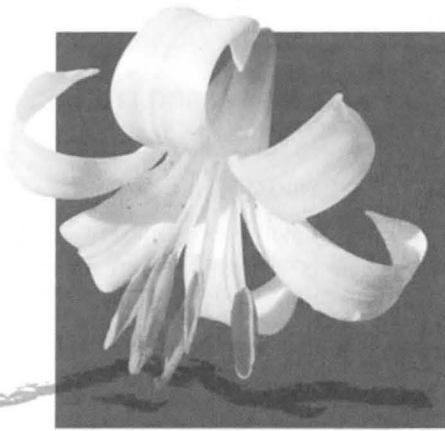


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PHYLOGENY OF IRIDACEAE SUBFAMILY CROCOIDEAE BASED ON A COMBINED MULTIGENE PLASTID DNA ANALYSIS

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ABSTRACT

The phylogeny of Crocoideae, the largest of four subfamilies currently recognized in Iridaceae, has eluded resolution until sequences of two more plastid DNA regions were added here to a previously published matrix containing sequences from four DNA plastid regions. Sister to the core Nivenioideae, the woody *Klattia*, *Nivenia*, and *Witsenia*, Crocoideae are a climax group in Iridaceae, comprising some 995 species, slightly more than half of the total in the family. Synapomorphies of Crocoideae include pollen exine perforate, pollen aperture operculate, ovule campylotropous (or hypotropous), root xylem vessels with simple perforations, cormous rootstock, inflorescence a spike, and plants deciduous. The six DNA region analysis here that includes examples of 27 of the 28 genera of the subfamily shows the southern African *Tritoniopsis* sister to the remaining genera, which resolve into four well-supported clusters (bootstrap support >85%). Each of these major clades is treated as a tribe, the synapomorphies of which are discussed in light of the molecular phylogenetic analyses. Original embryological and seed developmental studies largely support the tribal classification. **Tritoniopsidae** alone has the inner floral bracts not forked apically, and a hypotropous ovule, while this tribe and Watsonieae have axillary corm development. The remaining three tribes have apical corm development, and together with Watsonieae have a campylotropous ovule, and the inner layer of the inner integument crushed at maturity.

Key words: Croceae, family classification, Freesieae, Gladioleae, Iridaceae, phylogeny, plastid DNA sequences, Tritoniopsidae, Watsonieae.

INTRODUCTION

One of four subfamilies of Iridaceae (order Asparagales) currently recognized (Goldblatt 1990, 2001), Crocoideae comprise about 995 species, slightly more than half the total species in the entire family. The subfamily is a climax group within Iridaceae and includes such well known and horticulturally important genera as *Crocasmia* (8 spp.), *Crocus* (ca. 80 spp.), *Freesia* (16 spp.), *Gladiolus* (ca. 260 spp.), *Sparaxis* (15 spp.), and *Watsonia* (ca. 51 spp). Crocoideae (Burnett 1835), better known by the later synonym Ixioidae (Klatt 1866), are morphologically the most sharply differentiated of the four subfamilies of Iridaceae (Goldblatt 1990, 2001; Goldblatt et al. 1991) though the position of the subfamily within Iridaceae is undisputed. The species are currently arranged in 28 genera, which our current level of understanding indicates are all monophyletic. Crocoideae are also the most recently evolved of the subfamilies, probably having begun their radiation after the mid-Oligocene, ca. 30 million years ago (mya) (Goldblatt et al. 2002). Synapomorphies for Crocoideae are numerous (Table 1), and comprise all organs of the plant including the rootstock (a corm), leaf anatomy, inflorescence, flower (ancestrally zygomorphic), ovules, and pollen grains.

In contrast to the range and number of morphological specializations uniting the genera of Crocoideae, paralleled by strong support in molecular analyses of the subfamily using a range of plastid DNA regions (Souza-Chies et al. 1997;

Reeves et al. 2001a, b), understanding of generic relationships has remained poor. Since 1954, when G. J. Lewis proposed a classification of the alliance (as tribe Ixieae), the number of infrafamilial taxa recognized (tribes and subtribes) has varied considerably. The study by Reeves et al. (2001a, b), using four plastid DNA regions, *rbcL*, *rps4*, the *trnL* intron, and the *trnL*-F intergene spacer, failed to produce significant resolution of generic relationships. That study offered strong bootstrap (bs) support (defined here as 85% or more), only for three associations: *Geissorhiza*, *Hesperanthera*, and *Schizostylis* (bs 99%), *Micranthus*-*Thereianthus* (bs 97%), and *Lapeirousia*-*Watsonia* (bs 100%).

Molecular studies of Iridaceae have continued at the Jodrell Laboratory, Royal Botanic Gardens, Kew, partly to try to elucidate the poorly resolved generic phylogeny of Crocoideae, in which there remained little support for generic associations other than those mentioned above (Reeves et al. 2001b). In order to better understand the phylogeny of the subfamily, we obtained samples of all genera of Crocoideae currently recognized. Only one genus, the tropical African *Zygotritonia*, escaped our net. In addition to the four plastid DNA regions used in Reeves et al.'s studies, two more plastid DNA regions, *matK* and *rps16* were amplified and sequenced for an example of each genus.

The phylogeny generated using these data is discussed in terms of morphology and a tribal classification of Crocoideae is developed using combined molecular and morphological data. Embryological and seed developmental data,

Table 1. Morphological and anatomical synapomorphies of Crocoideae (Goldblatt 1990; Goldblatt et al. 1991; Goldblatt and Manning 1995; Rudall 1995).

1. Xylem vessels elements of the roots with simple perforations (shared with Iridoideae)
2. Rootstock a corm with discrete vascular bundles and rooting from below
3. Bladeless cataphylls produced before the first true leaves (the character is consistently associated with a cormous rootstock)
4. Leaf epidermal cells with wavy margins and with multiple papillae
5. Leaf blades (when plane) with a pseudomidrib (excluding *Tritoniopsis*)
6. Mesophyll cells elongating parallel to the leaf axis
7. Inflorescence a spike (sometimes flowers solitary on branches), flowers always and subtended by a pair of bracts (possibly homologous with the spathes enclosing the inflorescence units of Nivenioideae) at the base of the ovary
8. Flower bilaterally symmetric (i.e., zygomorphic)
9. Anther endothecium with spiral thickenings (also developed in Iridoideae)
10. Ovule campylotropous (or hypotropous in *Tritoniopsis*), but anatropous in the outgroup
11. Pollen grains ancestrally with perforate exine
12. Pollen grain apertures monosulcate with one or a pair of longitudinal exine bands forming an operculum (except rarely when grains trisulcate, zonosulcate, spiraperturate, or inaperturate)

taken from ongoing studies (Goldblatt and Manning 1995; Manning and Goldblatt unpubl. data), have provided several significant characters congruent with the molecular phylogenetic analyses.

MATERIALS AND METHODS

Plant Materials and DNA Sequencing

Taxa included in the phylogenetic analyses are listed in Appendix 1 together with voucher information and European Molecular Biology Laboratory (EMBL) nucleotide sequence database accession numbers. Sequences for *rbcL*, *rps4*, and *trnL-F* were obtained from Reeves et al. (2001b) unless otherwise stated in Appendix 1. Two further plastid regions were added to this matrix, the plastid *matK* exon and the *rps16* intron.

Total genomic DNA was extracted using the 2X CTAB method described by Doyle and Doyle (1987) or was obtained from the DNA Bank at the Royal Botanic Gardens, Kew (<http://www.rbgekew.org.uk/data/dnaBank/DnaBank-Form.html> [Nov 2003]).

PCR amplification (denaturation, 94°C for 1 min; annealing, 52°C for 45 sec; extension 72°C for 2 min 30 sec, plus 30 sec for each additional cycle) of the plastid *matK* region was performed on a Perkin-Elmer DNA thermal cycler (Applied Biosystems, Warrington, Cheshire, UK) using the forward primer 19F and the reverse primer -2R according to Cuénoud et al. (2002). In a few cases slightly degraded DNA required the use of the two additional internal primers 540F (5' GATATACTAATACCCCATCCC 3') and 1326R (Cuénoud et al. 2002). A further primer combination of 1330F (5' TTTCGTGTGCTAGAACTTTGG 3') and -2R was also used in a small minority of cases. This was required due to

a region containing a multiple adenine string of variable length at the *matK-trnK* interface, inhibiting accurate sequencing beyond this region in either the forward or reverse direction. Before DNA sequencing, amplified products were cleaned using Concert Rapid PCR Purification System (Life Technologies, Paisley, Scotland, UK). Cycle sequencing followed the protocols described in Goldblatt et al. (2002), with the resuspended samples run on an ABI 377 automated DNA Sequencer or ABI 3100 Genetic Analyzer following manufacturer's protocols (Applied Biosystems).

PCR amplification of the *rps16* intron was carried out as described in Goldblatt et al. (2002). A further forward primer, 58F (5' GTCGAGTCAAGAGCATCTTCA 3'), was used in a small number of cases due to the discovery of a secondary primer binding site that caused amplification of a second unidentified nucleotide region.

Phylogenetic Analyses

Sequences were edited using Sequence Navigator vers. 1.0 and assembled with Autoassembler vers. 1.40 (Applied Biosystems). Alignment was performed by eye in PAUP* vers. 4.0b10 (Swofford 2001). Regions for which unambiguous alignment was not possible were excluded from the analysis. Insertions/deletions (indels) were coded as presence/absence characters using PaupGap vers. 1.12 (Cox 1997), regions with multiple repeats of variable length were excluded from the gap coding as homology was hard to establish.

Phylogenetic analysis was performed using PAUP* vers. 4.0b10. Initial most-parsimonious trees were found from 500 replicates of random taxon addition using equal weights (Fitch 1971) and tree-bisection-reconnection (TBR) branch swapping. The resulting trees were then used to reweight the characters according to their rescaled consistency indices (Farris 1989). The search process was iterated until weights reached equilibrium, following the successive approximation weighting approach of Farris (1969). Internal support for clades was evaluated by performing 1000 bootstrap replicates (Felsenstein 1985), using equal weights and the TBR swapping algorithm with simple taxon addition. A second analysis was performed following the same procedure but excluding the gap coding characters.

RESULTS

Using DNA sequences of the six plastid DNA regions listed above from one species each of 27 genera of Crocoideae, plus 34 other genera of Iridaceae, and four outgroup genera combined in one matrix, together with results from a gap analysis and with successive weighting, we obtained a single most-parsimonious tree (see Table 2 for tree statistics). Without weighting, four trees were obtained, but two of these differed from each other only in outgroup relationships. Thus, only two trees differed with respect to the ingroup. For this paper we present only the Crocoide clade (Fig. 1), obtained by successive weighting, which received 100% bs as sister to the rest of the family. Our study confirmed the sister relationship of Crocoideae with the core genera of Nivenioideae, *Klattia* Baker, *Nivenia* Vent., and *Witsenia* Thunb., established by Reeves et al. (2001a, b). Our results represent the first relatively well-resolved generic phylogenetic tree of Crocoideae.

Table 2. Tree statistics for the combined multigene plastid DNA analysis for members of Iridaceae subfamily Crocoideae. The column for *trnL-F* includes the *trnL* intron.

	Total	<i>rbcL</i>	<i>rps4</i>	<i>trnL-F</i>	<i>matK</i>	<i>rps16</i>	Indels
Total number of characters included	6592	1335	603	1256	1924	1110	364
Number of variable characters	2880	356	233	447	969	512	363
Number of parsimony informative characters	1540	185	121	231	586	273	144
Consistency index (excl. uninformative characters)	0.44	0.37	0.42	0.56	0.47	0.44	0.31
Retention index	0.74	0.64	0.38	0.81	0.77	0.7	0.72
Percentage of steps contributed by each region of the matrix	100	13.6	8.2	12.7	36.5	18.3	10.7

Within the Crocoideae clade, the taxonomically isolated Cape genus, *Tritoniopsis* (24 spp.), is sister to the remaining genera (bs 76%). The latter clade resolves into what is essentially a polytomy that includes several well-supported clades. Most important of these are: *Gladiolus-Melasphaerula* (bs 94%), *Micranthus-Lapeirousia* (bs 85%), *Anomatheca-Xenoscapa* (bs 93%), *Crocus-Radinosiphon* (bs 88%), and *Ixia-Hesperantha* (bs 85%). All five clades are interpreted as having strong support (bs 85% or more). The last two clades together form one clade with moderate support (bs 80%) that includes half the genera of the subfamily and one-third the total species in the family, 13 genera, and over 600 species. Within the *Ixia-Hesperantha* clade, two genera, *Hesperantha* and *Geissorhiza*, make up a clade with bs 100%, sister to the remaining genera, a clade that receives only moderate support (bs 63%).

DISCUSSION

Crocoideae represent an important assemblage within Iridaceae, the circumscription of which has had a long history. First described by Burnett in 1835, and sometimes treated as a tribe rather than a subfamily, the assemblage has been used more or less continuously, although sometimes as Ixiodeae or Ixieae, since its treatment in *Genera Plantarum* (Benthams and Hooker 1883) and in the first edition of Engler and Prantl's *Das Pflanzenfamilien* (Pax 1888). Ironically, in Pax's account Crocoideae s.s. were narrowly circumscribed to include only acaulescent genera, and therefore were treated as separate from the remaining Ixiodeae. Within Crocoideae (in the sense used here), few authors have agreed about generic associations, or a subfamilial (or tribal) classification that implies such natural associations. Lewis (1954), whose understanding of Crocoideae transcended all prior accounts of the subfamily, recognized the assemblage as a tribe, following Diels (1930), and named but did not formally describe, five subtribes. She made few explicit statements about the reasons for her classification, which was largely based on broad morphological similarities among genera. Goldblatt (1971) formalized Lewis's taxonomy, also recognizing the tribe as the highest infrafamilial rank, and added two more subtribes. Later, Goldblatt (1990, 2001) returned to the earlier treatments in recognizing the subfamily, first using the name Ixiodeae for what is now known to be the earlier name, Crocoideae.

Our initial perception of this result was consternation, for few generic associations seemed congruent with some apparently fundamental synapomorphies of various genera of Crocoideae (Table 3). In particular, the highly unusual character state represented by an excluded vasculature trace to

the ovule, found in just eight genera, appeared to have evolved twice in the family, in the *Ixia-Chasmanthe* lineage and in *Crocsmi-Devia* within the *Anomatheca-Xenoscapa* clade. Anatomical examination of examples of seed development (see Fig. 2–12) in all genera with excluded ovule vasculature, however, raises the possibility that the ovule vasculature may be excluded in two different ways, and thus may represent two separate characters (Fig. 10–12). Specifically, in the genera *Dierama*, *Ixia*, *Tritonia*, and *Sparaxis*, the epidermis (Fig. 10, 11) above the ovular trace along the raphe consists of small, thin-walled cells (Goldblatt and Manning 1995). In *Crocsmia* (Fig. 12), however, the epidermis is relatively uniform and has a thick cuticle throughout, including the raphal area. A uniform epidermis over the ovular trace is also characteristic of *Freesia*, in which the raphe is unusually enlarged and the vascular strand is not ultimately pinched off and excluded from the seed body (Goldblatt and Manning 1995).

Phylogenetic Patterns in Crocoideae

This, the first well-resolved phylogenetic tree obtained for Crocoideae (Fig. 1), leaves us with several puzzles, for few of the generic clusters resulting from the DNA sequence analysis appear initially to be congruent with conventional morphological and anatomical characters. In fact, we were so confounded by some generic clusters that we checked for errors by sequencing an additional sample of some genera, including *Chasmanthe*, *Crocsmia*, and *Radinosiphon*. In each case the initial result was confirmed. We review each clade in turn below.

The Tritoniopsis clade.—The relationships of this small southern African genus (24 spp.; Manning and Goldblatt 2001; Manning et al. 2002) have always been uncertain. Lewis (1954) treated the genus (together with *Anapalina* and *Exohebea* R. C. Foster, now synonyms of *Tritoniopsis*) as a separate subtribe, *Exohebeinae*. She regarded the genus (or group of genera) as exceptional in having the outer and inner floral bracts adnate to prophylls and, most significantly, the inner bract is a single structure, longer than the outer bract. In other Crocoideae the inner bract has two major veins, is often two-keeled, and is forked apically or sometimes divided (in *Babiana* sect. *Babiana*) to the base. Another unusual feature is that *Tritoniopsis* has leaves with multiple major veins, whereas most other Crocoideae have leaves with a pseudomidrib unless pleted. Leaves of *Tritoniopsis* also have plesiomorphic leaf anatomy, with undifferentiated marginal epidermal cells and subepidermal marginal sclerenchy-

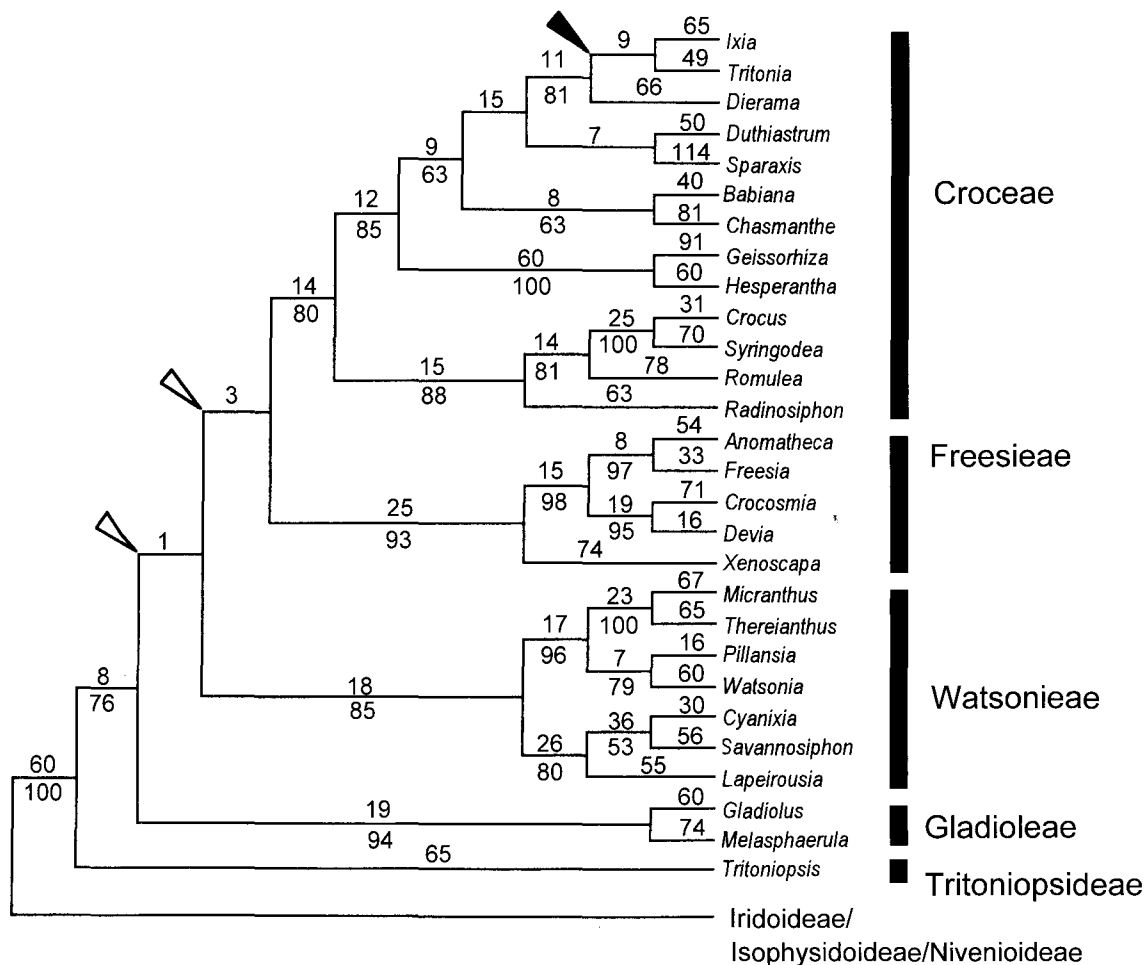


Fig. 1.—The Crocoideae clade from the single most-parsimonious tree obtained after successive weighting. The number of steps after optimization of equally weighted characters is shown above the branches (ACCTRAN optimization) and bootstrap percentages above 50% are shown below the branches. The black arrow indicates the group that collapses in the strict consensus of the equally weighted trees; unfilled arrows indicate additional nodes collapsing in the strict consensus of the equally weighted tree when gap characters are excluded from the analysis. The names indicated on the right-hand side of the tree correspond to the tribal classification proposed in here. Tree statistics are shown in Table 2.

ma associated with a marginal vein (Rudall 1995). The ontogeny of the corm is axillary (Goldblatt and Manning unpubl. obs.). *Tritoniopsis* seedlings have the odd combination of a tubular cotyledonry sheath and one tubular cataphyll (Tillich pers. comm. 2004), which may be derived. That seedling type is otherwise only present in the subfamily in *Crocus* and *Romulea* (Tillich 2003).

The sister relationship of *Tritoniopsis* to the rest of the subfamily makes the nature of the floral bracts seem particularly significant. Additional features of the genus, obtained from a study of seed development (Manning and Goldblatt unpubl. data), show that most Crocoideae have campylotropous ovules (Fig. 6, 7; anatropous in *Melasphaerula*), whereas in *Tritoniopsis* the ovules are hypotropous (Fig. 3, 4). The sister group, Nivenioideae (Manning and Goldblatt 1991), and all other Iridaceae have anatropous ovules (e.g., Fig. 2). The inner integument of *Tritoniopsis* consists of two layers that remain intact as the seed develops (Fig. 5), but in all other Crocoideae examined (not *Chasmanthe* and *Devia*), the inner layer of the inner integument is crushed at maturity (Fig. 8, 9). Crocoideae, excepting *Tritoniopsis*, thus

appear to have at least three derived features, two embryological and one floral bract character that set them apart, and are congruent with the molecular data.

The Gladiolus–Melasphaerula clade.—A close association between the large Afro-Eurasian genus *Gladiolus* (over 250 spp.) and the monotypic southwestern African *Melasphaerula* is one that has not been predicted from morphology, possibly because their corm tunics appear to differ fundamentally as do their ancestral chromosome base numbers. Corms of *Gladiolus* have papery to fibrous tunics and a rounded base, whereas *Melasphaerula* corms have a flat base and more or less woody tunics (Goldblatt and Manning 2000). Basic chromosome number in *Gladiolus* is $x = 15$, but *Melasphaerula* has $x = 10$ (Goldblatt and Takei 1997). *Gladiolus* has derived, winged seeds borne in inflated, ovoid capsules while *Melasphaerula* has apparently unspecialized, ovoid seeds in apomorphic, short, winged capsules; the locules bearing up to three pairs of ovules but usually only two seeds each, evidently another apomorphic feature. The relationships of both *Gladiolus* and *Melasphaerula* have never

Table 3. Major morphological and anatomical characters of genera in Crocoideae. Data from Goldblatt (1990), Goldblatt and Manning (1995), Goldblatt and Takei (1997), and Tillich (2003, pers. comm. 2004). Abbreviations: n/a = not applicable; ? = unknown; +/- = mixed.

Genus	Chromosome base no.	Corm ontogeny	Leaf margins	Midrib present unless blade pleated	Style branches	Seed epidermis outline	Ovule trace	Seedling type
Tritoniopsidae								
<i>Tritoniopsis</i>	?16	axillary	normal	absent	simple	prominent	included	tubular cotyledon with cataphyll
Watsonieae								
<i>Cyanixia</i>	10	axillary	normal	pleated	simple	prominent	included	?
<i>Lapeirousia</i>	?10 (9, 8, 7, 5, 4)	axillary	normal	present or pleated	divided	prominent	included	compact with cataphyll
<i>Micranthus</i>	10	axillary	normal	present	divided	prominent	included	?
<i>Pillansia</i>	20	axillary	normal	absent	simple	prominent	included	?
<i>Savannosiphon</i>	8	axillary	normal	pleated	divided	prominent	included	?
<i>Thereianthus</i>	10	axillary	normal	present	divided	prominent	included	compact with cataphyll
<i>Watsonia</i>	9	axillary	normal	present	divided	prominent	included	compact with cataphyll
<i>Zygotritonia</i>	7	axillary	normal	pleated	none	prominent	included	?
Gladioleae								
<i>Gladiolus</i>	15 (14, 12, 11, 10)	terminal	normal	present	simple	prominent	included	tubular cotyledon
<i>Melasphaerula</i>	10	terminal	normal	present	simple	prominent	included	tubular cotyledon
Freesieae								
<i>Crocospia</i>	11	terminal	epidermis columnar	present or pleated	simple	prominent	excluded	compact with cataphyll
<i>Devia</i>	10	terminal	epidermis columnar	present	simple	prominent	excluded	?
<i>Freesia</i>	11	terminal	epidermis columnar	present	divided	smooth	included	compact with cataphyll
<i>Xenoscapa</i>	11	terminal	epidermis columnar	present	divided	prominent	included	?
Croceae								
<i>Babiana</i>	7	terminal	normal	pleated	simple	smooth	included	compact with cataphyll
<i>Chasmanthe</i>	10	terminal	epidermis columnar	present	simple	smooth	excluded	compact with cataphyll
<i>Crocus</i>	?	terminal	n/a	n/a	+/-	prominent	included	tubular cotyledon with cataphyll
<i>Dierama</i>	10	terminal	normal	present	simple	smooth	excluded	compact with cataphyll
<i>Ixia</i>	10	terminal	normal	present	simple	smooth	excluded	compact with cataphyll
<i>Radinosiphon</i>	15	terminal	normal	present	simple	prominent	included	tubular cotyledon
<i>Romulea</i>	?13 (12, 11, 10, 9)	terminal	normal	present	divided	prominent	included	tubular cotyledon with cataphyll
<i>Sparaxis</i>	10	terminal	epidermis columnar	present	simple	smooth	excluded	compact with cataphyll
<i>Syringodea</i>	6 (11)	terminal	n/a	n/a	simple	prominent	included	?
<i>Tritonia</i>	11 (10)	terminal	epidermis columnar	present	simple	smooth	excluded	compact with cataphyll
<i>Hesperantha</i>	13 (12)	terminal	normal	present	simple	prominent	included	Tubular cotyledon
<i>Geissorhiza</i>	13	terminal	normal	present	simple	prominent	included	?

been satisfactorily understood (Goldblatt and Manning 1998, 2000) so that their association is no more surprising than any other might have been. Notably, however, both genera have specialized seedlings with an elongate tubular cotyledon (rarely a tubular coleoptile). This seedling morphology is otherwise shared in the subfamily only with *Hesperantha* and *Radinosiphon* (Tillich 2003). The two other members of the *Crocus*-*Radinosiphon* clade examined, *Crocus* and *Romulea*, also have a tubular cotyledon, but this is combined

with a tubular cataphyll. In addition, of all Crocoideae examined, the cotyledons of the latter two genera have lost their ability to produce chlorophyll. The association of *Gladiolus* and *Melasphaerula* seems less surprising in light of this information. Members of other clades in the subfamily have a seedling with a compact cotyledon that has one or more cataphylls before the first leaves appear. Seedlings of Nivenioideae, sister to Crocoideae, have a short cotyledon and coleoptile but lack cataphylls.

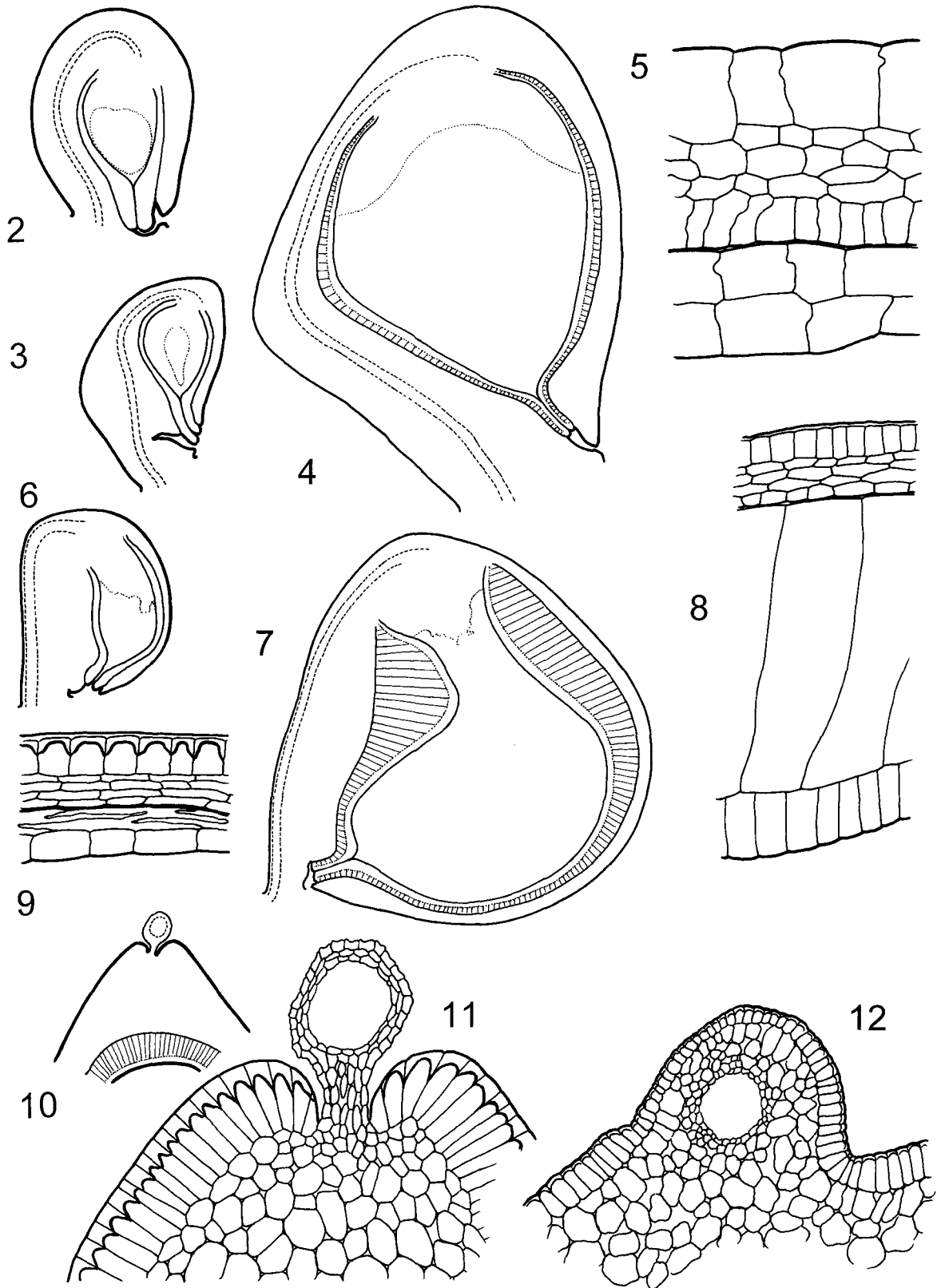


Fig. 2–12.—Ovule and seed coat anatomical characters in Iridaceae.—2. *Moraea spathulata* (L. f.) Klatt (Iridoideae).—3–5. *Tritoniopsis burchellii* (N. E. Br.) Goldblatt (Crocoideae: Tritoniopsidae).—6–11. *Sparaxis grandiflora* (D. Delaroche) Ker Gawl. (Crocoideae: Croceae).—12. *Crocosmia aurea* (Hook. f.) Planch. (Crocoideae: Croceae). (Fig. 2, L. S. ovule, $\times 50$; Fig. 3, L. S. ovule, $\times 70$; Fig. 4, L. S. seed, $\times 55$; Fig. 5, detail of seed coat, $\times 500$; Fig. 6, L. S. ovule, $\times 100$; Fig. 7, L. S. seed, $\times 70$; Fig. 8, 9, detail of seed coat, $\times 400$; Fig. 10, T. S. of raphe, $\times 100$; Fig. 11–12, cellular detail of raphe [vascular trace not shown], $\times 400$. (Fig. 2–12 drawings by John Manning).

The Watsonia clade.—Including the tribes Watsonieae and Pillansieae of Goldblatt (1990, 2001), the *Watsonia* clade receives weak support from morphology. Deeply divided style branches are characteristic of most genera of the *Watsonia* clade, but *Cyanixia* and *Pillansia* have the style branches notched only apically. All the genera in the clade have corms of axillary origin (de Vos 1977; Goldblatt et al. 2003), but that type of corm origin also occurs in *Tritoniopsis* (Table 3), and is likely to be ancestral for Crocoideae, making it plesiomorphic. Similarly, seedlings of the three genera of Watsonieae known, *Lapeirousia*, *Thereianthus*, and *Watsonia*, have a compact coleoptile as in the sister group to Crocoideae, Nivenioideae, but they are specialized in having a cataphyll produced before the first true leaf (Tillich 2003, pers. comm. 2004). Such seedlings, often with more than one cataphyll, are common in Crocoideae but appear derived. *Tritoniopsis* has seedlings with a tubular cotyledonary sheath and one tubular cataphyll (Tillich, pers. comm. 2004).

Moderately strong support for a close relationship between *Micranthus* ($x = 10$), *Thereianthus* ($x = 10$), and *Pillansia* ($x = 20$) (bs 82%) and of *Watsonia* ($x = 9$) and *Lapeirousia* ($x = 10$) (bs 100%) was one of the features of Reeves et al.'s (2001a, b) study, but *Savannosiphon* ($x = 8$) was not associated with either of these two clades. *Cyanixia* (*Babiana socotrana* J. D. Hook.), the remaining member of the *Watsonia* clade sequenced here, was not included in that analysis. In our tree, a sister relationship between *Micranthus* and *Thereianthus* receives strong support, but the two are sister to *Pillansia*–*Watsonia* (bs 79%). This combined clade has 96% bs support.

Among other genera of the clade, *Cyanixia* and *Savannosiphon* have pleated leaves, with associated multiple major veins, as does the tropical African *Zygotritonia*, which we infer belongs here because it also has axillary corm development (Goldblatt et al. 2003). *Zygotritonia* alone of Crocoideae genera has an undivided style. *Cyanixia* ($x = 10$) and *Zygotritonia* ($x = 7$) have derived trisulcate pollen grains (Goldblatt et al. 1991, 2003), possibly a significant shared feature if *Zygotritonia* correctly belongs here. It is probably also noteworthy that *Cyanixia*, *Lapeirousia*, and *Savannosiphon* (together with bs 80%), plus *Zygotritonia*, all occur in tropical Africa. Only *Lapeirousia* has significant representation in western southern Africa as well; all other Watsonieae occur in southern Africa.

Lewis (1954) associated most of these genera in her subtribe Watsoniinae, but included *Anomatheca* and *Freesia* with them, while treating *Pillansia* as the sole member of Pillansiinae. At that time, *Savannosiphon*, which is monospecific, was included in *Lapeirousia*. Lewis did not consider *Zygotritonia* at all in her study.

The Anomatheca–Xenoscapa clade.—The *Freesia*–*Anomatheca* pairing receives strong support in our analysis, increasing our confidence in their union as a single genus; sharing a smooth, glossy seed surface, seeds with an inflated raphe, and divided style branches (Goldblatt and Manning 1995). Unexpectedly, the clade is sister to *Crocospmia*–*Devia*, an association that receives strong support (bs 98%). *Crocospmia* has the same basic chromosome number as *Freesia*, $x = 11$, while *Devia* has $x = 10$, and both *Crocospmia* and *Devia*

have excluded seed vasculature (apomorphic) and seeds with epidermal cell outlines prominent (plesiomorphic). A close relationship between *Crocospmia* and *Devia* was hypothesized by Goldblatt and Manning (1990) when the latter was described, for they share not only the derived seed vasculature character, but the leaves have similar thickened columnar marginal epidermal cells and lack subepidermal marginal sclerenchyma. Six other genera, however, also possess excluded seed vasculature and similar leaf anatomy (Rudall 1995), all members of the *Ixia*–*Chasmanthe* clade excepting *Babiana*. These genera as well as *Babiana* have apomorphic smooth, glossy seeds, and as we have shown, the process of exclusion of the vascular trace to the ovule differs in some details from that in *Crocospmia* (*Devia* is unknown in this respect). This makes it plausible to infer the independent development of the feature.

Historically, *Crocospmia* has always been associated with *Chasmanthe* and secondarily with *Tritonia*, for example, by Lewis (1954), de Vos (1984), and Goldblatt and Manning (2000), mainly due to similarities in floral features (small bracts and orange perianth). Most *Crocospmia* species were first assigned to *Tritonia*, a relationship that similar leaf anatomy, excluded seed vasculature, and shared basic chromosome number, all established much later, seemed to support. In light of molecular evidence, that inferred relationship must now be seen as misleading, and instead the result of convergence. The significance of the seed surface differences between *Crocospmia* and *Tritonia* (and other members of the *Ixia*–*Chasmanthe* clade) were not appreciated, or not even noted. Both the chromosome base of $x = 11$ (Table 3) and leaf margins with columnar marginal epidermal cells are fairly widespread in Crocoideae (Rudall and Goldblatt 1991; Rudall 1995) and the latter feature evidently evolved more than once. It now seems clear that *Crocospmia* is not directly related to *Tritonia* (or to any member of the larger *Ixia* clade, including *Chasmanthe*). The molecular evidence convincingly shows that *Crocospmia* and *Devia* are allied to *Freesia*.

The *Freesia* clade also includes the taxonomically isolated *Xenoscapa*, evidently sister to the remaining members of the clade. The relationships of this genus of two species of southwestern southern Africa, has long been obscure (Goldblatt and Manning 1995), but historically *Xenoscapa* was associated with *Anomatheca*, mainly because of its divided style branches and netted corm tunics. When *Anomatheca* was united with *Freesia*, *Xenoscapa*, then a subgenus of *Anomatheca*, was raised to generic rank. It lacks the two critical specializations of *Freesia*–*Anomatheca* (bs 93%), seeds with a smooth glossy surface and an inflated raphal ridge. *Xenoscapa* does accord with the *Freesia* clade in leaf anatomy and seed epidermal cell outlines prominent, the latter shared only with *Crocospmia* and *Devia* (Goldblatt and Manning 1995, and unpubl. data).

The Crocus–Radinosisiphon clade.—The close relationship of *Radinosisiphon* with the acaulescent crocoide genera, *Crocus*, *Romulea*, and *Syringodea*, is surprising. The tropical and eastern southern African *Radinosisiphon*, with only one or two species, comprises caulescent plants with soft-textured leaves with a well-developed pseudomidrib, symmetric corms with soft, fibrous corm tunics, a flattened, angular stem, and a spike of zygomorphic, long-tubed flowers with

simple style branches (Goldblatt and Manning 2000). The remainder of the clade consists of acaulescent plants with rounded stems, radially symmetrical flowers solitary on short branches (equivalent to single-flowered spikes). Leaves of *Romulea* are fibrotic and distinctive in having a pair of longitudinal grooves on each surface; *Crocus* and *Syringodea* have bifacial leaves. Both *Crocus* and *Romulea* have elaborate style branches, usually deeply divided or multifid, but in *Syringodea* the style branches are, as in *Radinosiphon*, undivided (Table 3). Pollen, fruit, and seed characters are consistent in *Radinosiphon* and *Romulea* but are plesiomorphic within Crocoideae, while *Crocus* and *Syringodea* have derived pollen, either inaperturate or spiraperturate, and seeds. Corms, usually important in assessing relationships in Crocoideae, likewise do not reflect support for this clade: those of *Romulea*, *Syringodea* and some *Crocus* species have woody (or at least non-fibrous) tunics and corms of the former two genera are usually asymmetric, producing roots from lateral or basal ridges.

Seedling characters (Tillich 2003) appear derived. Seedlings of *Radinosiphon* are of the derived *Gladiolus*-type with a tubular, elongated cotyledon while those of *Crocus* and *Romulea* are unique in having the tubular cotyledon combined with a tubular cataphyll. The cotyledons of these two genera have lost their ability to produce chlorophyll. Seedlings of the remaining member of the clade, *Syringodea*, are unknown.

Clearly, the acaulescent *Romulea*–*Syringodea*–*Crocus* clade (all with radially symmetric flowers) must have evolved from an ancestor with an aerial stem and zygomorphic flower (the ancestral feature of Crocoideae), but *Radinosiphon* has never been proposed as their closest living ancestor.

The Ixia–Chasmanthe clade.—An association of the genera of the *Ixia–Chasmanthe* clade (excepting *Babiana*) has long been recognized, though *Crocospia* was always included with them. Lewis (1954), for example, included *Dierama*, *Ixia*, *Sparaxis* (plus its synonyms *Streptanthera* Sweet and *Synnotia*), all $x = 10$, plus *Tritonia* (with its synonym *Montbretopsis* L. Bolus; $x = 11$) with *Chasmanthe* ($x = 10$) and *Crocospia* ($x = 11$), in one lineage, the major portion of her subtribe Ixiinae. De Vos (1982) and Rudall and Goldblatt (1991) showed that, notably excepting *Dierama* and *Ixia*, this cluster of genera plus *Freesia* (including *Anomatheca*; $x = 11$) has similar leaf anatomy. They have columnar marginal epidermal cells with thickened walls and they lack subepidermal marginal sclerenchyma (often associated with a marginal vein). The cluster also receives support from seed morphology: excepting *Freesia*, the vascular trace to the ovule is excluded from the seed coat during development (Goldblatt and Manning 1995, and unpubl. data). Barring *Crocospia*, these genera (Table 3) also have a specialized seed coat, in which the epidermal cell outlines are obscured by heavy thickening of the outer epidermal walls and cuticle.

The congruence of morphology and molecular data is, however, incomplete. In our molecular-based tree the *Ixia–Chasmanthe* clade includes *Babiana* which has leaves with subepidermal marginal sclerenchyma and conventional seed vasculature and the derived chromosome base number of $x = 7$ (Goldblatt and Takei 1997). The seeds have an unusual,

pear shape and this derived form may be associated with suppression of the excluded trace character (seeds of *Freesia* have an inflated raphe that may likewise inhibit the exclusion of the ovular trace). As discussed above, *Crocospia* and its sister genus, *Devia* (not known to Lewis), do not fall in the *Chasmanthe* clade, but are sister to *Freesia* (plus the congeneric *Anomatheca*).

The inclusion of *Babiana* within the *Ixia–Chasmanthe* clade, specifically as sister to *Chasmanthe*, is surprising. The relationships of *Babiana* have always been uncertain, although a close relationship with *Gladiolus* has been suggested because both have style branches apically expanded and green, soft-textured floral bracts (Goldblatt 1996). *Babiana*, a genus of some 80 species, centered in western southern Africa, has apomorphic pear-shaped seeds with a smooth surface, a derived basic chromosome number, pleated leaves, and stems and leaves variously hairy, unusual in Crocoideae. Lewis (1954) was unable to suggest any close allies for the genus, and assigned it to subtribe Babianinae (with *Anaclanthe* and *Antholyza ringens* L.—the latter genus is a nomenclatural synonym of *Gladiolus*). The shared smooth seeds evidently provide the only morphological support for the molecular data that places it in the *Ixia–Chasmanthe* clade. Seedlings with a compact cotyledon and production of one or more cataphylls accords with the clade but, as noted above this seedling type is probably plesiomorphic.

Hesperantha–Geissorhiza.—The sub-Saharan African *Hesperantha* with 79 species (Goldblatt 2003) and the exclusively western southern African *Geissorhiza* with some 85 species, have always been considered closely allied. The clade, comprising the two genera plus *Schizostylis*, now *H. coccinea* (Backh. & Harv.) Goldblatt & J. C. Manning, received strong support (bs 99%) in Reeves et al.'s (2001a, b) analysis using four DNA regions. A close relationship between the two genera is confirmed with strong support in our combined analysis using six DNA regions. The clade is sister to the *Ixia–Chasmanthe* lineage, which has moderate support (bs 63%). That relationship has virtually no morphological support: their constituent genera differ in almost all characters that appear to be taxonomically significant. *Hesperantha* and *Geissorhiza* have leaves with subepidermal marginal sclerenchyma, intact seed vasculature, and seed epidermal cells with prominent outlines. Both *Hesperantha* and *Geissorhiza* have derived corms with roots produced from a lateral ridge and woody tunics (shared with *Romulea* and *Syringodea*), and a basic chromosome number of $x = 13$. In addition, *Hesperantha* has specialized *Gladiolus*-type seedlings (Tillich 2003), also found in *Radinosiphon* (*Geissorhiza* seedlings are unknown). No clear morphological feature appears to corroborate relationships of the *Hesperantha–Geissorhiza* clade despite the strong support for a sister relationship with the *Ixia–Chasmanthe* clade in our molecular-based analysis.

Major Synapomorphies and Convergence in Crocoideae

Important specializations in Crocoideae appear to be the evolution of a campylotropous ovule, the inner layer of the inner integument crushed at maturity, and the apically forked and usually two-veined inner floral bracts. All three features

characterize the genera of Crocoideae, except *Tritoniopsis*. Another is the development of a corm of apical origin, the ontogeny of which is described by de Vos (1977). The feature must be assumed to have evolved once in the higher Crocoideae (*Ixia*–*Radinosiphon*), plus the *Gladiolus*–*Melasphaerula* clade, which has no support for its position between *Tritoniopsis* and the *Watsonia* clade.

Assuming our tree topology (Fig. 1), the most unusual and striking character is an excluded ovular trace in the mature seed, evolved twice, in *Ixia*–*Chasmanthe* and in *Crocospia*–*Devia*. Developmental evidence, discussed above, suggests the feature may have been achieved in two different ways, and so may not represent true homoplasy. A smooth seed surface (cell outlines obscured by heavy cutinization) seems important in the higher crocoids, and may have arisen twice, in *Freesia* (including *Anomatheca*) alone of the *Anomatheca*–*Xenoscapa* clade, and in *Ixia*–*Chasmanthe*.

Derived leaf marginal anatomy may also have arisen twice, in the *Ixia*–*Chasmanthe* clade and *Anomatheca*–*Xenoscapa* (but then reversed in *Babiana* and in *Dierama*–*Ixia*) or it evolved once, but reversed in *Hesperantha*–*Geissorhiza*, *Babiana*, the *Radinosiphon* clade, and *Dierama*–*Ixia*. It is worth mentioning here that two specialized species of *Tritonia*, *T. delpierrei* M. P. de Vos and *T. marlothii* M. P. de Vos, are an additional example of reversal to the plesiomorphic leaf anatomy, assuming they are correctly assigned to genus. Unlike other species of *Tritonia*, they have plesiomorphic leaf anatomy (de Vos 1982). The instability of what appears to be a fundamental anatomical feature, generically constant except in the one example mentioned, is unusual.

A feature not much discussed above, but a striking one, asymmetric corms with roots arising from lateral ridges, may have arisen twice, in *Hesperantha*–*Geissorhiza* and at the base of the *Crocus*–*Romulea* clade, but was then reversed in *Crocus*. The character is associated with woody corm tunics and it may be that one followed the other for they are fully congruent. *Lapeirousia* and *Melasphaerula*, which both have more or less woody tunics, also have roots produced from a ridge, but in their case, the corms are symmetric and bell-shaped and the roots emerge from the lower margin, where the basal and domed portions of the corm tunics are articulated at the weak point in the tunics. Woody corm tunics thus may have arisen four times.

The available molecular evidence suggests that specialized seedlings with an elongate tubular cotyledon arose twice, in *Gladiolus*–*Melasphaerula*, the *Radinosiphon* clade, and *Hesperantha* (*Geissorhiza* is unknown for the character). We do not understand the significance of seedling specializations and find it difficult to comment on this organ, a particularly diverse one in Iridaceae (Tillich 2003).

It may seem strange that there are virtually no floral characters invoked in this discussion of important characters of Crocoideae, a subfamily in which floral diversity is striking (e.g., Goldblatt and Manning 1998). This is because of the high level of homoplasy for flower structure. Flowers of most genera with more than ten species are adapted to a wide range of different pollinating insects and birds (Bernhardt and Goldblatt 2000, 2006). Even *Tritoniopsis* has species that have flowers adapted for pollination by either large-bodied anthophorine bees, large butterflies, night flying moths, long-proboscid flies, sunbirds, or in one instance, the

oil-collecting bee, *Rediviva gigas* (Manning and Goldblatt 2002). All the larger genera of the subfamily display a diversity in their floral features, which in turn are associated with different pollination systems, each with a similar series of floral adaptations for pigmentation and patterning, symmetry, perianth tube length, scent, and nectar characteristics. For example, we have discussed elsewhere the remarkable morphological convergence for sunbird pollinated flowers in the crocoid genera *Babiana*, *Chasmanthe*, *Crocospia*, *Gladiolus*, *Tritoniopsis*, and *Watsonia* (Goldblatt et al. 1999). All we can say with confidence is that a tubular, zygomorphic perianth with unilateral, arcuate stamens, present in *Tritoniopsis* and the majority of other genera, is the ancestral condition for Crocoideae. Floral radial symmetry and symmetrically disposed stamens are derived within the subfamily, and have evolved repeatedly, evidently in response to selection for pollination systems using pollen-collecting female bees (Goldblatt et al. 1998) or hopliine scarab beetles (Goldblatt et al. 2000), or they are associated with suppression of the aerial stem (Goldblatt et al. 1995).

Tribal Classification of Crocoideae

We wonder whether it serves any purpose to adjust the tribal classification of Crocoideae in the absence of any real morphological discontinuities among them. There is no strong reason in terms of biology and geography that demands the formal recognition of tribes. Nevertheless, subfamilial classifications of Crocoideae already exist, and it seems incumbent on us to modify the current classification in the light of strongly supported molecular data (Table 4).

The most recent classification, that of Goldblatt (1990), later modified to a limited extent (Goldblatt 2001), admitted three tribes, Pillansieae (including only the apparently phylogenetically isolated *Pillansia*, which has a paniculate inflorescence), Watsonieae (for genera with a spike, divided style branches, and axillary corm development), and Ixieae, comprising the remaining genera. In view of the results of the Reeves et al. (2001a, b) molecular analysis, which showed *Pillansia* to comprise a clade with *Micranthus* and *Thereianthus*, the latter two genera were added to Pillansieae (Goldblatt 2001). At the same time Goldblatt recognized a more narrowly circumscribed Ixieae (for genera with excluded seed vasculature) and assigned the remaining genera assigned in 1990 to Ixieae to Croceae.

Molecular data now convincingly show that there is no merit in a tribe Pillansieae and we include it in an expanded Watsonieae (Table 4), which in this revised circumscription includes all the genera with axillary corm development except *Tritoniopsis*. Both Watsonieae (except *Pillansia*) and *Tritoniopsis* have plesiomorphic leaf marginal anatomy, with subepidermal sclerenchyma associated with a marginal vein and undifferentiated marginal epidermis. We are reluctant to recognize a separate tribe for *Tritoniopsis* alone, but as the genus is sister to the rest of the subfamily and it differs significantly from the remaining Crocoideae in some fundamental features, there appears no alternative. The tribe Tritoniopsidae is formally described below (Table 4).

For the remaining 19 genera (Table 4), we recognize the three remaining major clades as separate tribes, thus Gladioleae for *Gladiolus* and *Melasphaerula*, Freesieae for *Cro-*

Table 4. Generic classification of the subfamily Crocoideae based on a combination of molecular and non-molecular characters. Based on the classification of Goldblatt (1990, 2001), the arrangement here departs from that system in the inclusion of Pillansieae in Watsonieae, the expansion of Croceae to include *Gladiolus*, *Melasphaerula*, and *Radinosisiphon*, and placement of *Babiana* in Ixieae. Following the genera, the number of species is noted in parentheses. Total: 28 genera, ca. 995 species.

Tribe 1. **Tritoniopsidae** Goldblatt & J. C. Manning, trib. nov.—TYPE: *Tritoniopsis* L. Bolus.

Plantae cormiferi, corno axiliter effecto, tunicis corni fibrosis non reticulatis, caule florente saepe bene evoluto, lamina folii multinervosa atque sclerenchyma subepidermale consociata veni marginalis, inflorente spica bracteis adaxialiter non furcatis abaxialiter grandioribus, rami styli simplice, ovulo hypotropo, seminibus ambitis cellulibus epidermidis prominentibus, nervo ovuli intacto; plantula ignota, $x = ?16$.

Plants with axillary corm development; corm tunics fibrous; flowering stem always well developed; leaf blade with multiple major veins and with subepidermal sclerenchyma associated with a marginal vein; inflorescence as spike, inner bracts not forked apically and exceeding the outer, style branches simple; ovule hypotropous; seed epidermal cell outlines prominent; ovular vascular trace intact; seedling type unknown; $x = ?16$.

Tritoniopsis L. Bolus (incl. *Anapalina* N. E. Br.) (24)—southwestern and southern Cape, South Africa

Tribe 2. **WATSONIEAE** Klatt, *Abh. Naturf. Ges. Halle* **15**: 351 (1882).—TYPE: *Watsonia* P. Miller.

Pillansieae Goldblatt, *Ann. Missouri Bot. Gard.* **77**: 621 (1990), syn. nov.

Plants with corm development axillary; corm tunics fibrous; flowering stem always well developed; inflorescence a spike, the inner bracts forked apically and shorter than the outer; leaf blades with a pseudomidrib (except *Pillansia*), the margins with subepidermal sclerenchyma associated with a marginal vein (lacking in *Pillansia*); style branches (often) deeply divided (not *Cyanixia*, *Pillansia*, *Zygotritonia*); ovule campylotropous; seed epidermal cell outlines prominent; ovular vasculature intact; seedling compact, with cotyledon short and single cataphyll produced before the first leaf; $x = 10$ (9, 8, 7).

Cyanixia Goldblatt & J. C. Manning (= *Babiana socotrana*) (1)—Socotra Is.

Lapeirousia Pourret (42)—tropical and southern Africa

Micranthus (Pers.) Eckl. (3)—southwestern Cape, South Africa

Pillansia L. Bolus (1)—southwestern Cape, South Africa

Savannosisiphon Goldblatt & Marais (1)—south tropical Africa

Thereianthus G. J. Lewis (8)—southwestern Cape, South Africa

Watsonia Miller (51)—southern Africa

Zygotritonia Mildbr. (4)—tropical Africa

Tribe 3. **GLADIOLAE** Dumort., *Fl. Belg.*: 137 (1827).—TYPE: *Gladiolus* L.

Plants with corm development terminal; corm tunics fibrous and reticulate, or of unbroken sub woody layers; flowering stem well developed; leaf blade with pseudomidrib, the margins with a subepidermal sclerenchyma strand; style branches simple, usually apically expanded; inflorescence a spike; ovule campylotropous (anotropous in *Melasphaerula*); seed epidermal cell outlines prominent, ovular vasculature intact; seedling with elongated cotyledon and without cataphylls; $x = 15$ (10 in *Melasphaerula*).

Gladiolus L. (incl. *Anomalesia* N. E. Br., *Homoglossum* Salisb., *Oenostachys* Bullock) (ca. 260)—Africa, Madagascar, Eurasia

Melasphaerula Ker Gawl. (1)—southern Africa

Tribe 4. **Freesieae** Goldblatt & J. C. Manning, trib. nov.—TYPE: *Freesia* Klatt.

Plantae cormiferi, corno apicale effecto, tunicis fibrosis reticulatis, caule florente bene evoluto lamina, lamina folii ensiforme vel lineare atque nervo medio, inflorescentia spica bracteis adaxialiter furcatus abaxialiter grandioribus, rami styli apicibus bifurcatis, ovulo campylotropo, seminibus ambitis cellulibus epidermidis prominentibus vel glabris, nervo ovuli intacto vel excluso; plantulis compactis cataphyllis, $x = 11$ (10).

Plants with corm development terminal; corm tunics fibrous and reticulate; flowering stem well developed; leaf blade with pseudomidrib, margins with columnar epidermal cells and without subepidermal sclerenchyma; style branches apically notched to deeply divided; inflorescence a spike or flowers solitary on branches; ovule campylotropous; seed epidermal cell outlines prominent (smooth in *Freesia*); ovular vasculature excluded or intact (*Freesia*, *Xenoscapa*); seedling compact, with cataphylls, cotyledon not elongated; $x = 11$ (10).

Crocsmia Planch. (8)—tropical and southern Africa, Madagascar

Devia Goldblatt & J. C. Manning (1)—western Karoo, South Africa

Freesia Ecklon (incl. *Anomatheca* Ker Gawl.) (16)—eastern tropical and southern Africa

Xenoscapa Goldblatt & J. C. Manning (2)—southern Africa

Tribe 5. **CROCEAE** Dumort., *Anal. Fam. Pl.*: 57 (1829).—TYPE: *Crocus* L.

Ixieae Dumort., *Obs. Bot.* **58** (1822); Tritonieae Klatt, *Abh. Naturf. Ges. Halle* **15**: 65 (1882).

Table 4. Continued.

Plants with corm development terminal; corm tunics fibrous or woody and then corms usually asymmetric; leaf blade with a pseudomidrib (leaves bifacial in *Crocus*, *Syringodea*), margins either with a subepidermal sclerenchyma strand or with columnar epidermis; flowering stem well developed or subterranean; inflorescence a spike, or flowers solitary on branches; style branches simple (deeply divided in *Romulea*, many *Crocus*); ovule campylotropous; seed epidermal cell outlines smooth or prominent; ovular vasculature intact or excluded; seedling compact with cataphylls, rarely cotyledon elongated and with a tubular cataphyll; $x = ?11$ (15, 14, 13, 10, 7, 6 in various genera).

<i>Babiana</i> Ker Gawl. (incl. <i>Anaclanthe</i> N. E. Br.) (ca. 85)—southern Africa
<i>Chasmanthe</i> N. E. Br. (3)—southwestern Cape, South Africa
<i>Crocus</i> L. (ca. 80)—Europe, Asia, North Africa
<i>Dierama</i> K. Koch (44)—tropical and southern Africa
<i>Duthieastrum</i> M. P. de Vos (1)—southern Africa
<i>Geissorhiza</i> Ker Gawl. (85)—southern Africa, mainly southwestern Cape
<i>Hesperantha</i> Ker Gawl. (incl. <i>Schizostylis</i> Backh. & Harv.) (ca. 80)—sub-Saharan Africa
<i>Ixia</i> L. (ca. 55)—southern Africa
<i>Radinosophon</i> N. E. Br. (2 or more)—south tropical and southern Africa
<i>Romulea</i> Maratti (ca. 90)—Africa–Arabia, Mediterranean Basin, Canary Is., Socotra Is.
<i>Syringodea</i> J. D. Hook. (8)—southern Africa
<i>Sparaxis</i> Ker Gawl. (incl. <i>Synnotia</i> Sweet) (15)—southwestern Cape, South Africa
<i>Tritonia</i> Ker Gawl. (28)—south tropical and southern Africa

cosmia, *Devia*, *Freesia* (including *Anomatheca*), and *Xenoscapa*, and Croceae for the last 13 genera. The major clusters within Croceae may be treated as subtribes, names for which are available (see below). The two genera of Gladioleae have plesiomorphic leaf anatomy and seed surface morphology, but they have a derived seedling type, shared with some genera of Croceae, including *Hesperantha* and *Radinosophon*. Freesiineae have derived leaf anatomy and *Freesia* alone has derived seed surface morphology, while *Crococsmia* and *Devia* have the specialized excluded ovular trace. Croceae are mixed for both leaf anatomy and seed surface morphology. Within Croceae, an excluded ovular trace (evidently convergent with the condition in some Freesiaceae) characterizes a subset of genera, the *Ixia* group, in which that character is linked to derived seed surface morphology.

There are regrettably no clear morphological markers for these three tribes, or for that matter for Watsonieae, although most genera have forked style branches. Gladioleae, Freesiaceae, and Crocoideae are all linked by a second type of corm development, the terminal type. Thus, the utility of this classification is severely limited. We submit, however, that the adoption of a classification well supported by available molecular data, and to some extent by leaf anatomy, embryology, and corm development data, will be a guide to future thinking about relationships and character evolution within Crocoideae. We can suggest no better classification using traditional morphology because of the high level of homoplasy for floral and corm tunic features, so conspicuous at the generic level. Gladioleae have a derived seedling type, shared with some genera of Croceae, including *Hesperantha* and *Radinosophon*.

Within Croceae, three well-supported generic clusters, *Crocus*–*Radinosophon*, *Hesperantha*–*Geissorhiza*, and *Ixia*–*Chasmanthe* stand out. Each has some unusual features. As discussed above, *Crocus*, *Romulea*, and *Syringodea* are acaulescent, and might be recognized as subtribe Crocinae Benth. & Hook. f. (1883) (admittedly *Radinosophon* fits poorly here). *Ixia*–*Chasmanthe* have specialized seeds with smooth epidermal cell outlines and, excepting *Babiana*, an excluded vascular trace; most of these genera also have de-

rived leaf marginal anatomy, but not *Babiana*, *Dierama*, and *Ixia*. These genera can conveniently be treated as Ixiinae G. J. Lewis ex Goldblatt (1971). The remaining *Hesperantha* and *Geissorhiza* fit poorly within either subtribe and are best treated as Hesperanthinae Goldblatt (1971). The two genera have asymmetric corms, woody corm tunics, and the derived base number, $x = 13$, but plesiomorphic leaf anatomy and seeds. There seems little need for such a finely drawn classification, but note that the nomenclature at subtribal level exists for these clades.

We strongly advocate the accumulation of additional molecular data, particularly within genera. This should test the statistical support for the major clades (i.e., the tribes) and would, we hope, reveal any weaknesses in the classification. Until we obtain this information we recommend adoption of the classification outlined in Table 4 as the best available for the present.

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Appendix 1. Voucher, molecular database accession and literature information for all species of Crocoideae included in this study. C. (1993) = Chase et al. (1993), R. (2001) = Reeves et al. (2001a), S. (1997) = Souza-Chies et al. (1997), n/a = not available.

Species	Accession/herbarium voucher or reference	Literature citation/or EMBL accession numbers for new sequences				
		<i>rbcL</i>	<i>trnL-F</i>	<i>rps4</i>	<i>matK</i>	<i>rps16</i>
<i>Anomatheca laxa</i> (Thunb.) Goldblatt	Chase I-1 (K)	C. (1995)	R. (2001)	R. (2001)	AJ579931	AJ578764
<i>Babiana ecklonii</i> Klatt	Goldblatt and Manning 9958 (MO)	R. (2001)	R. (2001)	n/a	AJ579934	AJ578766
<i>B. stricta</i> Ker Gawl.	S. (1997)	n/a	n/a	S. (1997)	n/a	n/a
<i>Chasmanthe aethiopica</i> (L.) N. E. Br.	Chase I-3 (K)	R. (2001)	R. (2001)	R. (2001)	AJ579938	AJ578771
<i>Crocoshia mathewsiana</i> (L. Bol.) Goldblatt ex M. P. de Vos	Goldblatt and Manning 9830 (MO)	AJ577250	AJ577602	AJ577242	AJ580608/9	AJ578773
<i>Crocus nudiflorus</i> Hohen.	S. (1997)	n/a	n/a	S. (1997)	n/a	n/a
<i>C. pulchellus</i> Herb.	Chase I-19 (K)	R. (2001)	R. (2001)	n/a	AJ579914	AJ578774
<i>Cyanixia socotrana</i> (Hook. f.) Goldblatt & J. C. Manning	Lavaranos s. n. (MO)	AJ577247	AJ577603	AJ577241	AJ580762	AJ578768
<i>Devia xeromorpha</i> Goldblatt & J. C. Manning	Snijman and Manning 1194 (MO)	AJ577246	AJ577604	AJ577243	AJ580612/3	AJ578776
<i>Dierama robustum</i> N. E. Br.	Goldblatt and Manning 9536 (MO)	AJ580809	AJ580813	AJ580811	AJ579944	AJ578777
<i>Duthiastrum linifolium</i> (Phill.) M. P. de Vos	Manning 2235 (MO)	AJ577249	AJ577605	AJ577244	AJ579948	AJ578781
<i>Freesia</i> sp.	S. (1997)	n/a	n/a	S. (1997)	n/a	n/a
<i>F. alba</i> Baker	Goldblatt 5293 (MO)	R. (2001)	R. (2001)	n/a	AJ579952	AJ578784
<i>Gladiolus guenzii</i> Kunze	Goldblatt 9052 (MO)	R. (2001)	R. (2001)	R. (2001)	n/a	n/a
<i>G. papilio</i> Hook. f.	Goldblatt and Manning 9841 (MO)	n/a	n/a	S. (1997)	AJ579956	AJ578788
<i>Hesperantha pseudopilosa</i> Goldblatt	Goldblatt and Manning 9677 (MO)	R. (2001)	R. (2001)	R. (2001)	AJ579958	AJ578791
<i>Ixia latifolia</i> D. Delaroche	Goldblatt and Manning 9594 (MO)	R. (2001)	R. (2001)	R. (2001)	AJ579964	AJ578797
<i>Lapeirousia neglecta</i> Goldblatt & J. C. Manning	Goldblatt and Manning 9489 (MO)	R. (2001)	R. (2001)	S. (1997)	AJ579967	AJ578800
<i>Melasphaerula ramosa</i> (Burm. f.) Ker Gawl.	Goldblatt s. n. (MO)	AJ580810	AJ580812	AJ580814	AJ579969	AJ578802
<i>Micranthus junceus</i> N. E. Br.	Chase I-156 (K)	R. (2001)	R. (2001)	R. (2001)	AJ579970	AJ578803
<i>Pillansia templemanni</i> L. Bol.	Bean s. n. (MO)	R. (2001)	R. (2001)	S. (1997)	AJ579978	AJ578811
<i>Radinosiphon leptostachya</i> (Baker) N. E. Br.	Boussard s. n. (MO)	R. (2001)	R. (2001)	R. (2001)	n/a	n/a
<i>R. lomatenensis</i> N. E. Br.	Goldblatt and Porter s. n. 2002 (MO)	n/a	n/a	n/a	AJ579979	AJ578812
<i>Romulea monadelphica</i> (Sweet) Baker	Goldblatt 6230 (MO)	R. (2001)	R. (2001)	n/a	AJ579980	AJ578813
<i>R. revelieri</i> Jord. & Fourr.	Moret 93-88 (P)	n/a	n/a	S. (1997)	n/a	n/a
<i>Savannosiphon euryphylla</i> (Harms) Goldblatt & Marais	Bolnick s. n. (MO)	R. (2001)	R. (2001)	R. (2001)	AJ580625/6	AJ578814
<i>Sparaxis</i> sp.	S. (1997)	n/a	n/a	S. (1997)	n/a	n/a
<i>S. variegata</i> (Sweet) Goldblatt	Goldblatt 2460 (MO)	R. (2001)	R. (2001)	n/a	AJ579984	AJ578817
<i>Syringodea bifucata</i> M. P. de Vos	Davidson 3108 (MO)	R. (2001)	R. (2001)	R. (2001)	AJ579985	AJ578818
<i>Thereianthus racemosus</i> (Klatt) G. J. Lewis	Goldblatt 10454 (K)	R. (2001)	R. (2001)	R. (2001)	AJ579986	AJ578819
<i>Tritonia disticha</i> Baker	Goldblatt and Manning 9545 (MO)	R. (2001)	R. (2001)	R. (2001)	AJ579989	AJ578822
<i>Tritoniopsis unguicularis</i> (Lam.) G. J. Lewis	Goldblatt 9486 (MO)	R. (2001)	R. (2001)	S. (1997)	AJ579990	AJ578823
<i>Watsonia angusta</i> Ker Gawl.	Goldblatt 6904 (MO)	R. (2001)	R. (2001)	S. (1997)	AJ579991	AJ578824
<i>Xenoscapa fistulosa</i> (Spreng. ex Klatt) Goldblatt & J. C. Manning	Chase 11508 (K)	AJ577248	AJ577607	AJ577245	AJ580630/1	AJ578827