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ACADEMIC PRESS

Molecular Phylogenetics and Evolution xxx (2002) xxx–xxx

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.academicpress.com

Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions

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Received 2 February 2002; received in revised form 22 April 2002

Abstract

Phylogenetic analyses of four plastid DNA regions, the *rbcL* exon, *trnL* intron, *trnL–trnF* intergenic spacer, and *rps16* intron from each of 73 species in the African genus *Moraea* (Iridaceae: Irideae) including accessions of all major species clusters in the genus, show *Moraea* to be paraphyletic when *Barnardiella*, *Galaxia*, *Hexaglottis*, *Homeria* (all southern African), and *Gynandriris* (Eurasian as well) were recognized as separate genera. There are several small, isolated species clusters at the basal nodes of the tree that are all restricted to the winter-rainfall zone of southern Africa (the Greater Cape floral kingdom) and a few, highly derived, large species groups that have radiated extensively within the winter-rainfall zone. Mapping of floral traits shows that an *Iris*-type flower is ancestral in *Moraea*. Floral changes are associated with shifts in pollination systems, either from passive pollen deposition on long-tongued bees foraging for nectar to active pollen collection by female bees foraging for pollen, fly, or hopliine scarab beetle pollination. Dating the nodes of the phylogenetic tree using non-parametric rate smoothing with a calibration point derived from broad dating of the angiosperms indicates that the divergence between *Moraea* and its sister genus *Ferraria* occurred about 25 mya in the early Miocene. The early radiation of *Moraea* took place against a background of aridification and the spread of open habitats, such as desert, shrubland, and fynbos. © 2002 Elsevier Science (USA). All rights reserved.

1. Introduction

The Cape flora is one of the richest temperate flora, with 9000 species of which approximately 70% are endemic (Goldblatt and Manning, 2000a; Linder, 1991). The species to genus ratio is one of the highest, even exceeding those of oceanic islands such as Hawaii and New Zealand (Goldblatt, 1978; Goldblatt and Manning, 2000a). Despite the considerable literature produced on the subject (Cowling et al., 1992; Cowling and Hilton-Taylor, 1998), the reasons for this amazingly high level of diversity are not understood and would benefit greatly from phylogenetic interpretation. We include here a phylogenetic study of one of the largest South African plant genera, the peacock irises *Moraea* (Iridaceae). Circumscription of the Old World and largely sub-Saharan African *Moraea* (Iridaceae: Irideae), a genus of nearly 200 species of herbaceous geophytes, has long been confused. Early treatments included species with corms of a single-internode, bifacial leaves, and *Iris*-like flowers with petaloid style branches, each of which bears a prominent crest. Species with similar corms and leaves but stellate flowers that have simple, narrow to filiform style branches often with minute crests were included in *Galaxia* (15 species), *Hexaglottis* (six species), *Homeria* (32 species), or *Roggeveldia* (two species). Several species with a tubular extension of the ovary have been included in *Iris* (ca. 250 species) or *Gynandriris* (nine species; Dykes, 1913; Goldblatt, 1981), whereas *Barnardiella* (one species) has stellate flowers without style crests and a tubular extension of the ovary. Cytology and crossing experiments showed that the distinction between *Moraea* and *Homeria* could not consistently be upheld (Goldblatt, 1980a). Some species

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PII: S1055-7903(02)00235-X

that have stellate flowers without petaloid style branches and crests shared unique karyotypes and vegetative morphology with some species of *Moraea*. Anatomical studies of *Moraea* and allied genera (Rudall and Goldblatt, 1993) also showed that *Moraea* as then circumscribed was paraphyletic. Exactly how the various species of *Moraea* and these other genera were related remained uncertain because of morphological parallelism. By 1993, however, it was clear that *Barnardiella*, *Galaxia*, *Gynandriris*, *Hexaglottis*, *Homeria*, and *Roggeveldia* were nested in *Moraea* (Rudall and Goldblatt, 1993), and in 1998 Goldblatt transferred species of these genera to *Moraea*, the oldest name at generic rank.

This transfer brought the total species in *Moraea*, already a large genus with over 150 species, to 196. We have undertaken a molecular study to provide support for the hypothesis that *Moraea* was paraphyletic and determine relationships of species of the erstwhile genera now included in *Moraea*. We present here the results of DNA sequence analysis of four DNA regions, the *rbcL* exon, the *rps16* intron, the *trnL* intron, and the *trnL–trnF* intergenic spacer, for each of 73 species of *Moraea*. Outgroup genera were the remaining members of tribe Irideae, as established by Reeves et al. (2000, 2001).

A second aspect of this research has been to investigate the geographical patterns in *Moraea*. About 150 *Moraea* species (more than 75% of the total) and its sister genus *Ferraria* are centered in the southern African winter-rainfall zone (the Greater Cape flora region; Jürgens, 1991, 1997), although both have species in southern tropical Africa, and in the case of *Moraea* there is substantial representation in summer-rainfall eastern southern Africa north to Ethiopia, with outliers in the Mediterranean–Middle East. The southern African winter-rainfall zone extends along the southwestern coast and near interior of Namibia south through Namaqualand and the western Karoo to the southern coast of South Africa near Port Elizabeth. This relatively small area has a remarkably rich flora of some 12,000 vascular plant species. Of these, about 9000 species (over 67% endemic) occur in the area traditionally regarded as the Cape flora region (Goldblatt and Manning, 2000a), making it the richest temperate flora in the world. This flora is well known for its diversity of succulents and geophytes, of which *Moraea* is a conspicuous member.

2. Materials and methods

2.1. Plant material, DNA extraction, PCR, and sequencing

Taxa, voucher information and accession numbers of the DNA sequences are listed in Table 1. Total DNA from fresh, silica gel-dried leaves (0.1–0.3 g tissue) or seeds (< 0.1 g) was extracted using the 2× CTAB method (Doyle and Doyle, 1987) and subsequently purified through a cesium chloride gradient (1.55 g/ml) or with QIAquick silica columns (Qiagen, Crawley, West Sussex, UK) according to the manufacturer's protocol for PCR products. Four plastid DNA regions were amplified and sequenced. The *rbcL* exon was amplified by PCR (28–30 cycles, 1 min denaturation at 95 °C, 30 s annealing at 50 °C, 1 min extension at 72 °C, 7 min final extension) using Master Mix (2.5 mM $MgCl_2$; ABgene, Epsom, Surrey, UK) and primers cited in Reeves et al. (2001). With a few seed samples (some up to 30 years old) only relatively small amounts of DNA were retrieved; in this case *rbcL* was amplified in half pieces using the primer pairs cited in Reeves et al. (2001). The *rps16* intron was amplified using the same protocol as for *rbcL* but with the PCR primers of Oxelman et al. (1996). The *trnL–trnF* region (*trnL* intron and *trnL–trnF* intergenic; hereafter *trnL-F*) was amplified as one piece in most taxa using the "c" and "f" primers of Taberlet et al. (1991); for the seed samples, we used the primer pairs "c/d" and "e/f." Before sequencing, the amplification products were purified using QIAquick (Quiagen, Crawley, West Sussex, UK) or Concert (Life Technologies, Paisley, Scotland, UK) columns. Cycle sequencing (26 cycles, 10 s denaturation at 96 °C, 5 s annealing at 50 °C, 4 min extension at 60 °C) with BigDye Terminators (v2.0; Applied Biosystems, Warrington, Cheshire, UK) was performed in 5 μl volumes on the purified PCR products and then precipitated with ethanol. Both strands were sequenced using the amplification primers (plus internal primers of Reeves et al., 2001, for *rbcL*), which provided 80–90% overlapping and complementary pairs of sequences for each DNA region. The resuspended samples were run on an Applied Biosystems. Three-hundred and seventy seven automated DNA sequencer following the manufacturer's protocols. Contigs were edited using Sequence Navigator (Applied Biosystems, Warrington, Cheshire, UK) and assembled using Autoassembler (Applied Biosystems). Each base position was checked for agreement of the two strands.

2.2. Alignment and phylogenetic analyses

DNA sequences were aligned manually because no alignment program takes into consideration the different categories of change in plastid spacer regions (e.g., homopolymer regions, direct and indirect repeats, unique repeats, etc.), which need to be treated differently; therefore automated alignments are usually unsatisfactory (see review in Kelchner, 2000). Some regions of the three non-coding regions were difficult to align unambiguously or comprised nucleotide repeats that have been shown to vary within species (e.g., see Fay and Cowan, 2001; Vendramin et al., 1996), so these were excluded from the analysis (300 bp in total). Insertions/

Table 1
List of taxa with geographic distribution, voucher information, and GenBank/EMBL accession numbers for each DNA region

Species	Section or subgenus (of *Moraea*)	Geographic distribution[a]	Voucher	*rbcL*	*trnL–trnF* region	*rps16* intron
Moraea albicuspa Goldblatt	*Vieusseuxia*	ESA	Goldblatt 11238 MO	AJ307084	AJ307241	AJ307162
M. alpina Goldblatt	*Polyanthes*	ESA	Goldblatt 11230 MO	AJ307085	AJ307242	AJ307163
M. alticola Goldblatt	*Grandiflora*	ESA	Goldblatt and Nänni 11231a MO	AJ307086	AJ307243	AJ307164
M. angusta (Thunberg) Ker Gawler	*Monocephalae*	NWC, SWC, LBC	Goldblatt 10794 NBG	AJ307087	AJ307244	AJ307165
M. autumnalis (Goldblatt) Goldblatt	*Homeria*	NWC	Viviers s.n. MO	AJ307088	AJ307245	AJ307166
M. barnardiella Goldblatt	*Galaxia*	SWC	Goldblatt 2499 NBG	AJ307089	AJ307246	N/A
M. bella Harms	*Grandiflora*	STA	Spurrier 608 MO	AJ307090	AJ307247	AJ307167
M. bifida (L. Bolus) Goldblatt	*Homeria*	NAM, NWC	Goldblatt 5593 NBG	AJ307091	AJ307248	AJ307168
M. bituminosa (L. f.) Ker-Gawl.	*Visciramosa*	NWC, SWC, LBC, SEC	Goldblatt 10795 NBG	AJ307092	AJ307249	AJ307169
M. brevistyla (Goldblatt) Goldblatt	*Vieusseuxia*	ESA	Nänni 144 NBG	AJ307093	AJ307250	AJ307170
M. brittenniae (L. Bolus) Goldblatt	*Homeria*	SEC	Bayliss s.n. MO	AJ307094	AJ307251	AJ307171
M. carsonii Baker	*Polyanthes*	STA	Goldblatt 7544 MO	AJ307095	AJ307252	AJ307172
M. cedarmonticola Goldblatt	*Homeria*	NWC	Goldblatt 3871 NBG	AJ307096	AJ307253	AJ307173
M. ciliata (L. f.) Ker-Gawl.	*Acaules*	NAM, NWC, SWC, LBC, KMC, SEC, KAR, RV	Goldblatt and Manning 9672b NBG	AJ307097	AJ307254	AJ307174
M. collina Goldblatt	*Homeria*	SWC	Goldblatt 10241a MO	AJ307098	AJ307255	AJ307175
M. cookii (L. Bolus) Goldblatt	*Homeria*	NWC, KMC, RV, ESA	Goldblatt and Manning 9672b MO	AJ307099	AJ307256	AJ307176
M. dracomontana Goldblatt	*Vieusseuxia*	ESA	Goldblatt 11239 MO	AJ307100	AJ307257	AJ307177
M. elliotii Baker	*Polyanthes*	ESA, NSA, STA	Nänni 137 NBG	AJ307101	AJ307258	AJ307178
M. falcifolia Klatt	*Acaules*	NAM, KAR, RV	Goldblatt s.n. MO	AJ307102	AJ307259	AJ307179
M. flaccida Sweet	*Homeria*	SWC	Goldblatt s.n. MO	AJ307103	AJ307260	AJ307180
M. fugax (D. Delaroche) Jacq.	*Moraea*	NAM, NWC, SWC, LBC	Steiner 1814 NBG	AJ307104	AJ307261	AJ307181
M. galpinii (Baker) N.E. Brown	*Grandiflora*	ESA, NSA	Goldblatt 11251 MO	AJ307105	AJ307262	AJ307182
M. garipensis Goldblatt	*Moraea*	NAM	Goldblatt 7153 NBG	AJ307106	AJ307263	AJ307183
M. gawleri Spreng.	*Moraea*	NAM, NWC, SWC, LBC, KMC, SEC	Goldblatt and Manning 9591 MO	AJ307107	AJ307264	AJ307184
M. graniticola Goldblatt	*Moraea*	NAM	Lavranos 20007 MO	AJ307108	AJ307265	AJ307185
M. herrei (L. Bolus) Goldblatt	*Moraea*	NAM	Goldblatt and Manning 11372 MO	AJ307109	AJ307266	AJ307186
M. hesperantha (Goldblatt) Goldblatt	*Gynandriris*	RV	Goldblatt 4371 NBG	AJ307110	AJ307267	AJ307187
M. huttonii (Baker) Oberm.	*Grandiflora*	ESA	Esterhuysen s.n. NBG	AJ307111	AJ307268	AJ307188
M. inclinata Goldblatt	*Polyanthes*	ESA	Goldblatt and Nänni 11226 NBG	AJ307112	AJ307269	AJ307189
M. inconspicua Goldblatt	*Visciramosa*	NAM, NWC, SWC, LBC, SEC	Manning 2197 NBG	AJ307113	AJ307270	AJ307190
M. incurva G.J. Lewis	*Vieusseuxia*	NWC	Hansford s.n. NBG	AJ307114	AJ307271	AJ307191
M. lewisiae (Goldblatt) Goldblatt	*Hexaglottis*	NAM, NWC, SWC, LBC, SEC, RV	Goldblatt 11036A MO	AJ307115	AJ307272	AJ307192
M. lugubris (Salisb.) Goldblatt	*Moraea*	NWC, SWC	Goldblatt and Manning 11032 MO	AJ307116	AJ307273	AJ307193
M. lurida Ker-Gawl.	*Vieusseuxia*	SWC	Goldblatt 11036 MO	AJ307117	AJ307274	AJ307194
M. luteoalba (Goldblatt) Goldblatt	*Galaxia*	NWC	Goldblatt 7221 MO	AJ307118	AJ307275	AJ307195
M. macgregorii Goldblatt	*Moraea*	NWC	Goldblatt 3097 NBG	AJ307119	AJ307276	AJ307196
M. melanops Goldblatt and J.C. Manning	*Galaxia*	SWC	Goldblatt and Nänni 10249 MO	AJ307120	AJ307277	AJ307197

Table 1 (*continued*)

Species	Section or subgenus (of *Moraea*)	Geographic distribution[a]	Voucher	*rbcL*	*trnL–trnF* region	*rps16* intron
M. miniata Andrews	*Homeria*	NAM, NWC, SWC, LBC, KAR	Goldblatt 5124 MO	AJ307121	AJ307278	AJ307198
M. minor Ecklon	*Homeria*	NWC, SWC	Goldblatt 4085a MO	AJ307122	AJ307279	AJ307199
M. muddii N.E. Br.	*Gandiflora*	ESA, NSA, STA	Goldblatt 11250 NBG	AJ307123	AJ307280	AJ307200
M. namaquamontana Goldblatt	*Moraea*	NAM	Goldblatt 7374 MO	AJ307124	AJ307281	AJ307201
M. natalensis Baker	*Polyanthes*	ESA, NSA, STA	Nänni 136 NBG	AJ307125	AJ307282	AJ307202
M. neglecta G.J. Lewis	*Monocephalae*	NWC, SWC, LBC	Goldblatt s.n. MO	AJ307126	AJ307283	AJ307203
M. ochroleuca (Salisb.) Drapiez	*Homeria*	NWC, SWC	Goldblatt 6096 MO	AJ307127	AJ307284	AJ307204
M. papilionacea (L. f.) Ker-Gawl.	*Moraea*	NWC, SWC	Goldblatt and Nänni 10254 NBG	AJ307128	AJ307285	AJ307205
M. patens (Goldblatt) Goldblatt	*Homeria*	NWC	Goldblatt 5594 NBG	AJ307129	AJ307286	AJ307206
M. pilifolia Goldblatt	*Galaxia*	NAM, NWC	Goldblatt and Manning 10970 MO	AJ307130	AJ307287	AJ307207
M. polyanthos L. f.	*Polyanthes*	LBC, KMC, SEC	Chase I-221 K	AJ307131	AJ307288	AJ307208
Moraea polystachya (Thunb.) Ker-Gawl.	*Polyanthes*	KMC, KAR	Nänni 150 NBG	AJ307132	AJ307289	AJ307209
M. pritzeliana Diels	*Gynandriris*	NWC	Goldblatt 7403 NBG	AJ307133	AJ307290	AJ307210
M. pubiflora N.E. Brown	*Vieusseuxia*	NSA	Goldblatt 11252 MO	AJ307134	AJ307291	AJ307211
M. radians (Goldblatt) Goldblatt	*Homeria*	SWC	Goldblatt 5903 NBG	AJ307135	AJ307292	AJ307212
M. ramosissima (L. f.) Ker-Gawl.	*Moraea*	NWC, SWC, LBC, KMC, SEC	Goldblatt 11037 MO	AJ307136	AJ307293	AJ307213
M. regalis Goldblatt and J.C. Manning	*Vieusseuxia*	KMC	Vlok 345 MO	AJ307137	AJ307294	AJ307214
M. rigidifolia Goldblatt	*Moraea*	NAM	Goldblatt 7016 MO	AJ307138	AJ307295	AJ307215
M. rivulicola Goldblatt and J.C. Manning	*Vieusseuxia*	NAM	Goldblatt and Manning 9710 NBG	AJ307139	AJ307296	AJ307216
M. schimperi (Hochst.) Pic.-Serm.	*Grandiflora*	STA, NTA	Goldblatt 4529 NBG	AJ307140	AJ307297	AJ307217
Moraea serpentina Baker	*Moraea*	NAM, NWC	Hall 3361 NBG	AJ307141	AJ307298	AJ307218
M. sisyrinchium (L.) Ker-Gawl.	*Gynandriris*	MME	Chase I-107 K	AJ307142	AJ307299	AJ307219
M. spathulata (L. f.) Klatt	*Grandiflora*	SEC, ESA, NSA, STA	Goldblatt 10872 MO	AJ307143	AJ307300	AJ307220
M. thomasiae Goldblatt	*Vieusseuxia*	LBC, KMC	Goldblatt 2422 MO	AJ307144	AJ307301	AJ307221
M. tricuspidata (L. f.) G.J. Lewis	*Vieusseuxia*	NWC, SWC, LBC, SEC	Goldblatt and Manning 11038 MO	AJ307145	AJ307302	AJ307222
Moraea trifida R.C. Foster	*Vieusseuxia*	ESA, NSA	Nänni 132 NBG	AJ307146	AJ307303	AJ307223
M. tripetala (L. f.) Ker-Gawl.	*Vieusseuxia*	NWC, SWC, LBC, KMC, RV	Goldblatt 10982 MO	AJ307147	AJ307304	AJ307224
Moraea tulbaghensis L. Bolus	*Vieusseuxia*	NWC, SWC	Goldblatt and Manning 9364 MO	AJ307148	AJ307305	AJ307225
M. umbellata Thun.	*Homeria*	SWC	Goldblatt 11040 MO	AJ307149	AJ307306	AJ307226
M. unguiculata Ker-Gawl.	*Vieusseuxia*	NAM, NWC, SWC, LBC, SEC	Goldblatt and Manning 10786 MO	AJ307150	AJ307307	AJ307227
M. vegeta L.	*Moraea*	NWC, SWC	Goldblatt s.n. NBG	AJ307151	AJ307308	AJ307228
M. ventricosa Baker	*Grandiflora*	STA	Lovett 4621 MO	AJ307152	AJ307309	AJ307229
M. verdickii De Wildman	*Grandiflora*	STA	Bidgood *et al.* 3795 K	AJ307153	AJ307310	AJ307230
M. verecunda Goldblatt	*Polyanthes*	NWC	Goldblatt 7404 MO	AJ307154	AJ307311	AJ307231
M. vigilans Goldblatt and J.C. Manning	*Vieusseuxia*	ESA	Goldblatt and Manning 11046 NBG	AJ307155	AJ307312	AJ307232
M. villosa (Ker-Gawl.) Ker-Gawl.	*Vieusseuxia*	NWC, SWC	Goldblatt 6275 MO NBG	AJ307156	AJ307313	AJ307233
Belamcanda chinensis (L.) DC.			Chase I-171 K	AJ307078	AJ307235	AJ307158
Bobartia gladiata (L. f.) Ker-Gawl.			Goldblatt 9490 MO	AJ307079	AJ307236	AJ307159
Dietes robinsoniana (F. Muell.) Klatt			Pickard 3377 MO	AJ307080	AJ307237	N/A
Pardanthopsis dichotoma (Pall.) Lenz			Chase I-155 K	AJ307157	AJ307314	AJ307234

Table 1 (*continued*)

Species	Section or subgenus (of *Moraea*)	Geographic distribution[a]	Voucher	*rbcL*	*trnL–trnF* region	*rps16* intron
Ferraria crispa Burm.		SWC, LBC, KMC	Goldblatt and Manning 9732 MO	AJ307081	AJ307238	N/A
F. uncinata Sweet		NAM, NWC, SWC	Goldblatt and Manning 11034 MO	AJ307082	AJ307239	AJ307160
Iris forrestii Dykes			Chase 140 K	AJ307083	AJ307240	AJ307161

Geographic centers, based on Goldblatt and Manning (2000a,b) with eastern southern African and tropical Africa defined below, are as follows: NAM, Namaqualand including southwestern Namibia; NWC, Northwest Center of the Cape floral region (CFR); SWC, Southwest Center of the CFR; LBC, Langeberg Center of CFR; KMC, Karoo Mountain Center of CFR; SEC, Southeast Center of CFR; KAR, Great Karoo; RV, Roggeveld Center; ESA, eastern southern Africa (including eastern southern Africa from Port Elizabeth to northern KwaZulu-Natal); NSA, northern southern Africa (including northern KwaZulu-Natal to the Limpopo River); STA, south tropical Africa (the Limpopo River to the Tanzanian border); NTA, north tropical Africa (from the southern Tanzanian border to Ethiopia and West Africa); MME, Mediterranean–Middle East.

[a] Nam, Namaqualand; NWC, Northwest Cape; SWC, Southwest Cape; LBC, Langeberg Cape; KMC, Mt. Karoo Cape; SEC, Southeast Cape; KAR, Karoo; RV, Roggeveld; ESA, Eastern South Africa; NSA, Northern South Africa; STA, South tropical Africa; NTA, North tropical Africa; MME, Mediterranean Middle East.

deletions (indels) were coded as presence/absence characters using PaupGap 1.12 (Cox, 1997). Indels were considered as homologous when they shared exactly the same matrix position, and nested indels were treated as separate characters. Matrices are available electronically from VS and MWC (v.savolainen@kew.org and m.chase@kew.org).

Phylogenetic analysis was performed using PAUP*4.0b2a (Swofford, 1998). Initial most-parsimonious trees were obtained from 1000 replicates of random taxon addition using equal weights (Fitch, 1971) and tree bisection-reconnection (TBR) branch swapping, with only 10 trees held at each step to reduce the time spent in swapping on large or suboptimal islands of trees. All trees collected in these replicates were then used as starting trees in another search to find all trees at this shortest length or up to the pre-set limit of 15,000, at which time all of these trees were swapped to completion. For the combined analysis only, these trees were then used to reweight the characters according to their rescaled consistency indices (Farris, 1989), and new searches as described above were performed using the reweighted matrix until weights reached equilibrium. This ''successive approximation weighting'' (SW) approach in tree search reduces the disturbing effect, if any, of unstable taxa (Farris, 1969). To evaluate the internal support of each clade, 500 bootstrap replicates (Felsenstein, 1985) were performed with equal weights and the TBR swapping algorithm with simple addition of taxa and only 10 trees held at each step to reduce the time spent swapping in each replicate. We report only those bootstrap percentages (BS) greater than 50% that are consistent with the strict consensus tree.

Bootstrap analyses were conducted on two matrices, *rbcL* (the coding matrix) and *trnL-F* and *rps16* combined (the noncoding matrix). We did not analyze each of the two introns and the intergenic spacer separately because each of these short regions did not contain enough variable sites to avoid sampling error effects. We then directly combined all data because the individual analyses did not display evidence of incongruent groups with high bootstrap support (85% or greater); however, we also did incongruence tests for each pair of partitions using the 'partition homogeneity test' from Farris et al. (1995) and implemented in PAUP*. Such tests are known to fail (Reeves et al., 2001; Yoder et al., 2001), so they should not be considered proof of incongruence and following the recommendations of Wiens (1998) direct combination should be carried out even though tests may have indicated incongruence was present. Chromosome numbers were optimized on the tree using MacClade 3.07 (Maddison and Maddison, 1992). Flower types and pollinators (when known) are mapped next to species names in Fig. 5.

The ancestral area analysis of Bremer (1992) was performed using PAUP* to study the geographic origin

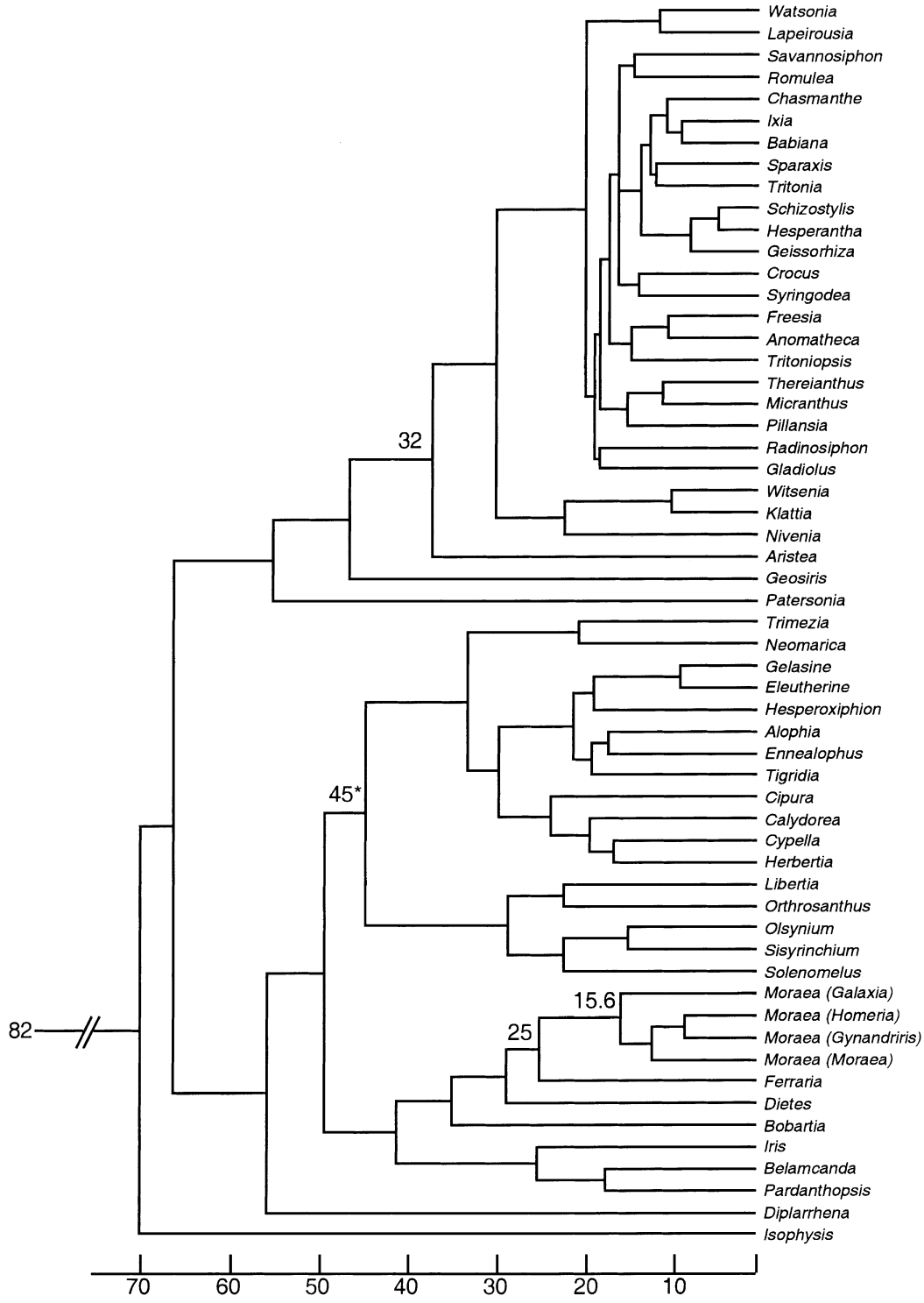

Fig. 1. Chronogram of Iridaceae after non-parametric rate smoothing on the phylogenetic tree from Reeves et al. (2000, 2001), which was calibrated with the root node of Iridaceae and their outgroups at 82 mya (Wikström et al., 2001); the scale is in mya (see text for details). Note that the node separating Neotropical/Australasian Sisyrinchieae/Tigridieae (of subfamily Iridoideae) is at 45 mya (indicated by a star, see Reeves et al., 2000, 2001, for details). This implies long distance dispersal between South America and Africa because the separation of these continents is dated between 105 mya (Deacon et al., 1992) and 70 mya (Pitman et al., 1993).

of this plant group; distributions for all species are indicated in Table 1; only *Ferraria* was used as outgroup for this last analysis. *Iris* was specified as ultimate outgroup (Goldblatt, 1990; Reeves et al., 2000, 2001; Souza-Chies et al., 1997), and sequences of *Bobartia*, *Dietes*, and *Ferraria* were included as additional outgroups.

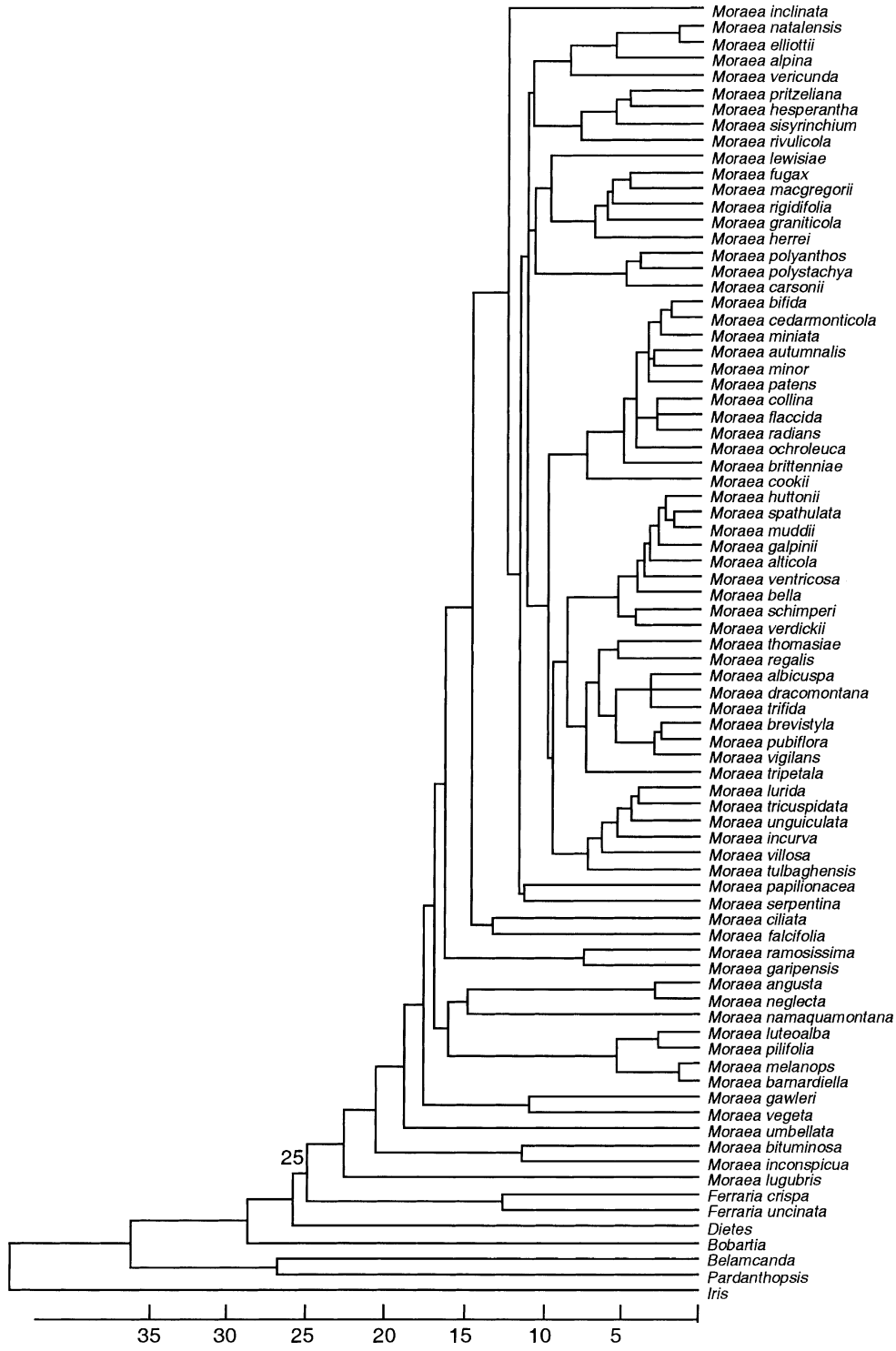

Fig. 2. Chronogram of *Moraea* and related genera after non-parametric rate smoothing on the phylogenetic presented in Fig. 5, calibrated with the split between *Moraea* and *Ferraria* at 25 mya; the scale is in mya (see text for details).

2.3. Molecular clock

A molecular clock was rejected ($P < 0.001$, data not shown) by the likelihood ratio test (Felsenstein, 1988), so we used the non-parametric rate smoothing (NPRS) method of Sanderson (1997) implemented in TreeEdit (v1.0 alpha 4-61, written by A. Rambaut and M. Charleston; http://evolve.zoo.ox.ac.uk/software/TreeEdit), which accounts for rate heterogeneity across lineages to produce an ultrametric tree. Maximum

Table 2
Statistics of the DNA matrix (all calculations were made using one of the most parsimonious SW trees)

Statistics	*rbcL* exon	*trnL–trnF* region	*rps16* intron	Indels
Total number of characters included	1335	1028	882	216
Number of variable characters	220 (16.5%)	246 (23.9%)	214 (24.2%)	216
Number of parsimony-informative characters	107 (8.0%)	129 (12.5%)	111 (12.6%)	99 (46.9%)
Consistency index (excl. uninformative characters)	0.45	0.59	0.54	0.50
Retention index	0.71	0.82	0.76	0.70
Percentage of steps contributed by each region of the matrix	25.6%	25.2%	21.8%	27.4%

parsimony (MP) and maximum likelihood (ML) branch lengths were optimized onto the trees. For ML branch lengths, we used the HKY85 model of DNA evolution (Hasegawa et al., 1985; empirical base frequencies and transition/transversion ratio estimated from the data using PAUP*) with a gamma distribution accounting for heterogeneity among sites (Yang, 1993; alpha shape parameter estimated from the data); the HKY85 model of DNA evolution is a good compromise between complexity of the model and computer time requirements compared to the more complex general-time reversible model of evolution. To transform relative time into absolute ages, we needed a calibration point; we used the date for the root node of Iridaceae and their closest relatives, 82 mya, from the study of Wikström et al. (2001) and applied this to the phylogenetic tree of Iridaceae from Reeves et al. (2000, 2001). Wikström and co-workers performed NPRS analyses using the large angiosperm three-gene matrix from Soltis et al. (1999) and Soltis et al. (2000). Using fossils as calibration points, Wikström and co-workers calculated the ages and error estimates for over 75% of all angiosperm families, and these figures were largely in agreement with the fossil record (Wikström et al., 2001). The 82 mya calibration provided a minimum age of 25 mya for the split between *Moraea* and *Ferraria* (MP; using ML: 26 mya), which we applied in our more detailed phylogenetic tree (see Figs. 1 and 2) as a new calibration point. To compute an error estimate for the root node of *Moraea*, we reapplied the NPRS procedure to 100 bootstrapped matrices (Reeves et al., 2000, 2001) obtained using PHYLIP 3.573c (Felsenstein, 1993).

3. Results

3.1. rbcL versus noncoding analyses

The length of the *rbcL* gene included in the analysis was 1335 positions, of which 220 (16.5%) positions were variable and 107 (8.0%) were potentially informative (Table 2). The length of the *rbcL* trees was 395 steps with a consistency index (CI excl. uninformative characters) of 0.45 and a retention index (RI) of 0.71. The bootstrap consensus tree based on the *rbcL* sequences alone (Fig. 3) is poorly resolved, and *Ferraria*, taxonomically isolated *Moraea lugubris*, and the rest of *Moraea* forms a trichotomy, sister to *Dietes* (BS 65%). The two species of *M.* subgenus *Visciramosa* (*M. bituminosa*, *M. inconspicua*) represent an isolated, early clade (BS 84%) of the genus, sister to a poorly resolved remainder. Among this large cluster, species of *M.* section *Homeria* (*M. cookii–M. bifida*) cluster together (BS 63%), as do the four species of *M.* section *Galaxia* in the analysis (*M. luteoalba–M. barnardiella*; BS 98%). Of the remaining small clades of two or three species, two bear mention. These are *M. angusta–M. neglecta*, which represent *M.* subgenus *Monocephalae* (BS 92%) and *M. ramosissima–M. garipensis*, a closely related species pair of *M.* section *Moraea*. The clade that includes *M. polystachya*, *M. polyanthos*, and *M. carsonii* represent allied species of *M.* section *Polyanthes* (BS 94%), as does the *M. natalensis–M. elliotii–M. alpina* (BS 54%). Other presumed members of this section are scattered among the unresolved species. The large *M.* subgenus *Vieusseuxia* likewise has species scattered among this unresolved group, as well as two clades receiving bootstrap support, *M. albicuspa–M. dracomontana–M. trifida* (BS 71%) and *M. brevistyla–M. pubiflora–M. vigilans* (BS 59%). The three species of *M.* section *Gynandriris* included in the analysis, *M. hesperantha*, *M. pritzeliana*, and *M. sisyrinchium* fall together as a weakly supported clade (BS 59%).

Each of the noncoding regions has a similar number of variable sites to those found for *rbcL*: *trnL-F* had 246 (23.9%) of which 129 (12.5%) were potentially informative; and the *rps16* intron had 214 (24.2%) of which 111 (12.6%) were potentially informative. In spite of being a "conserved coding region" the level of variability in *rbcL* compares favorably with both of these putatively more variable plastid regions. In the three non-coding regions, there were 216 indels, of which 99 (46.9%) were potentially informative (Table 2). The noncoding regions and the indels had a higher CI than did *rbcL*, but the RI was of all components was similar (Table 2). The bootstrap consensus tree derived from the non-coding analysis (Fig. 4) is similar to the *rbcL* bootstrap consensus tree in most respects but better resolved. Here *Ferraria* is sister to *Moraea* (BS 86%). *Moraea umbellata*, *M. lugubris*, and *M. namaquamontana* are unresolved, but the strongly supported clades representing *M. ramosissima–M. garipensis*, *M.* subge-

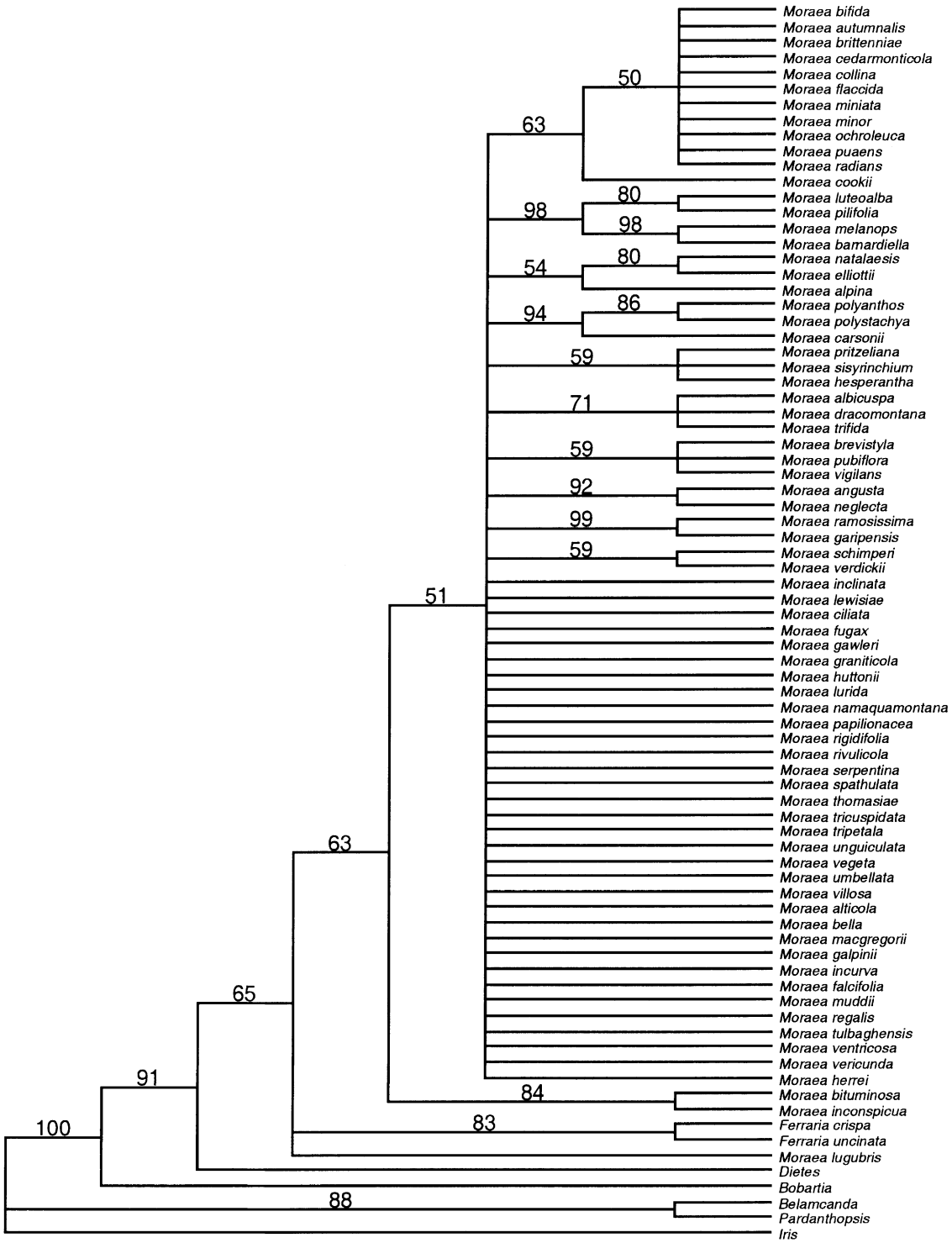

Fig. 3. Bootstrap consensus tree based on the *rbcL* exon only, bootstrap percentages above or equal to 50% are indicated above the branches.

nus *Visciramosa*, and *M.* subgenus *Monocephalae* each have BS 100%, as do the four species of *Galaxia*. Other notable clades are *M.* section *Homeria* (BS 64%), which as in the *rbcL* tree has *M. cookii* as sister to the rest; and the large and morphologically distinctive *M.* subgenus *Grandiflora* (*M. spathulata–M. ventricosa*; BS 74%). The two resolved clades of *M.* subgenus *Vieusseuxia* of the *rbcL* tree are now united (*M. albicuspa–M. vigilans*) (BS 79%). These species represent all of the eastern southern African members of the subgenus in the analysis. Cape species of this subgenus are partly resolved, with *M. lurida–M. incurva* falling as one clade (BS 87%) but other members of the subgenus are either unresolved or placed with species believed on the basis of morphology

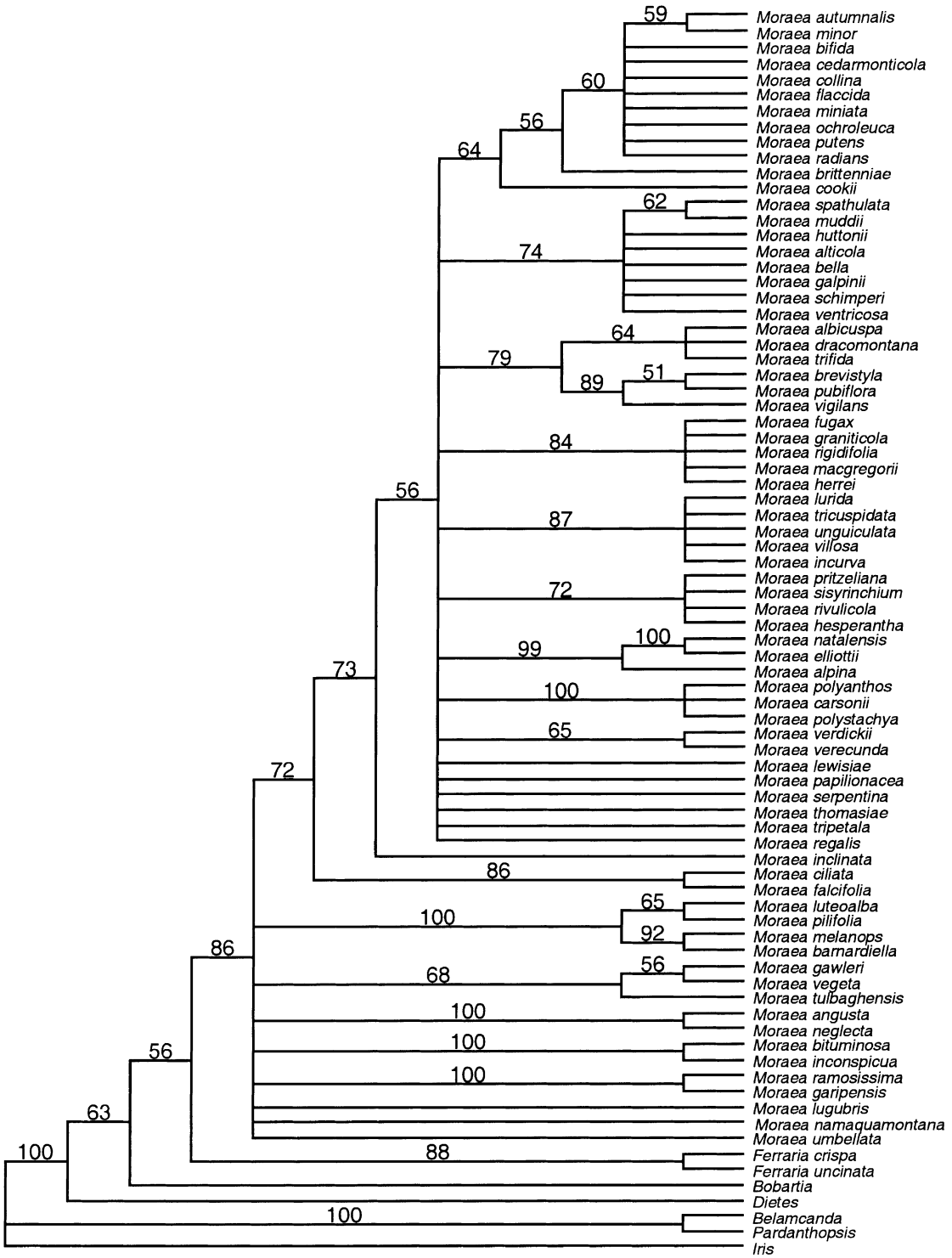

Fig. 4. Bootstrap consensus tree based on the noncoding data set only, bootstrap percentages above or equal to 50% are indicated above the branches.

and chromosome cytology to be unrelated: *M. rivulicola* within *M*. section *Gynandriris*, and *M. tulbaghensis* with two species of *M*. section *Moraea* (*M. gawleri* and *M. vegeta*).

There is no evidence of "hard" incongruence (a topology strongly supported by one matrix that is strongly supported in a different arrangement from another matrix) between the trees based on two sets of data. All partition homogeneity tests were significant ($P < 0.01$), but in many cases "incongruence tests" have indicated that separate matrices should not be directly combined in spite of much improved overall results produced by

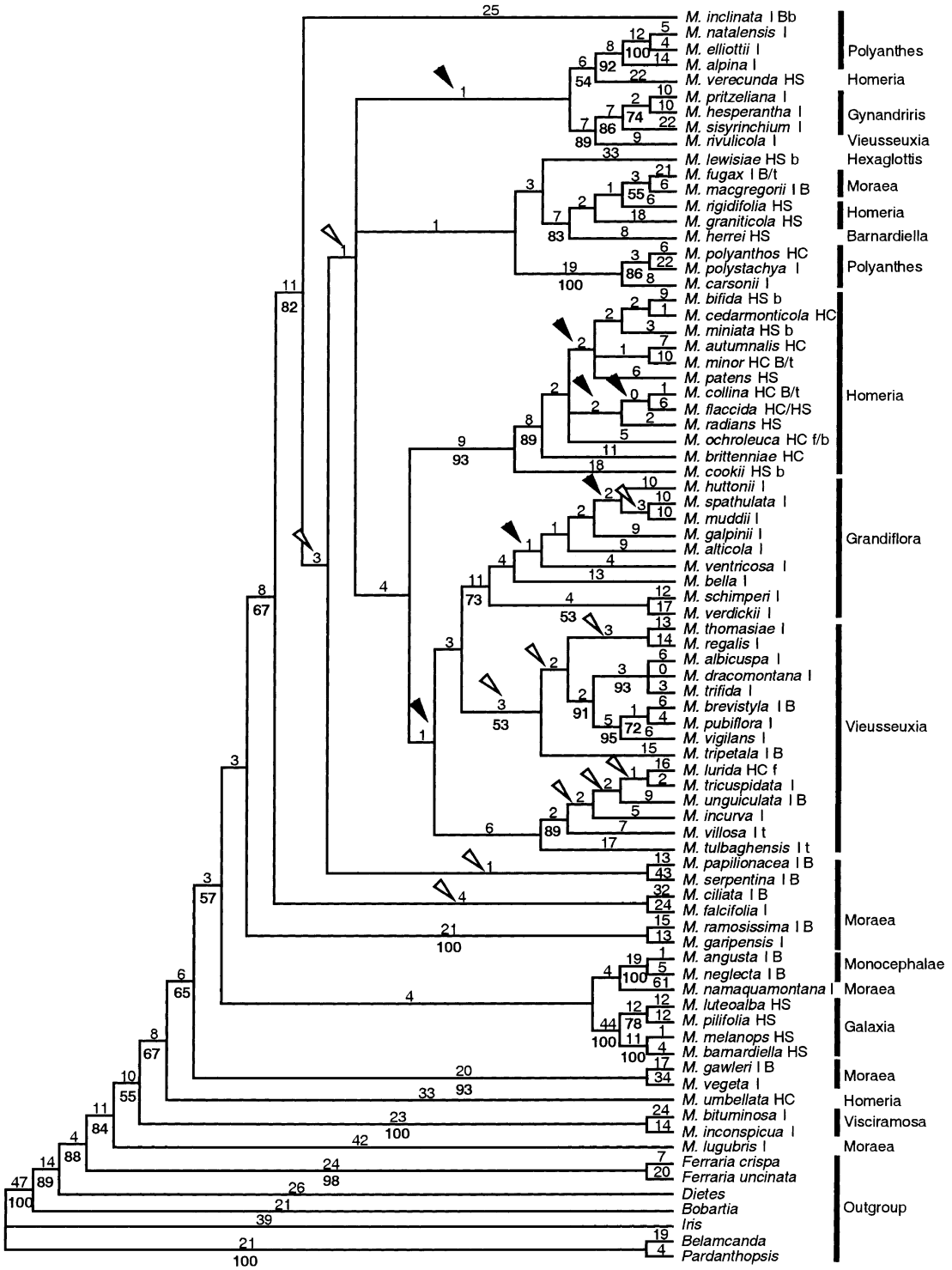

Fig. 5. One of the 980 most parsimonious trees found after successive weighting. Number of steps after optimization of equally weighted characters are shown above the branches (ACCTRAN optimization) and bootstrap percentages above 50% are shown in bold below the branches. Black arrows indicate the groups collapsing in the strict consensus of the successively weighted trees; unfilled arrows indicate additional nodes collapsing in the strict consensus of the equally weighted trees. Flower typesf: HC, *Homeria* flower of the cup type; HS, *Homeria* with star type flower; I, *Iris* flower type. Pollinators (when known): B, nectar-collecting bees; b, pollen-collecting bees; t, beetles; f, flies.

direct combination (see Soltis et al., 1998; Reeves et al., 2001; Wiens, 1998; Yoder et al., 2001). Well-supported groups (BS > 70%) in one analysis that either disappear or show lower bootstrap percentages when directly combined with other data are better evidence of incongruence than any of the available tests (Eldenãs and

Linder, 2000; Reeves et al., 2001; Wiens, 1998); in this sense, all partitions in this study are combinable.

3.2. Combined analysis

The combined matrix contained 3461 characters of which only 446 were potentially informative. The initial equally weighted analysis resulted in 140 trees of 1593 steps with CI of 0.46 (excl. uninformative characters) and RI of 0.71; these trees were used to reweight characters. Successive weighting (SW) resulted in 980 most parsimonious trees of SW length = 77,439,131 (equally weighted length 1596 steps, CI excluding uninformative characters = 0.46, RI = 0.71, SW CI excluding uninformative characters = 0.46, and SW RI = 0.71). It is noteworthy that we obtained a greater number of most parsimonious SW trees than with equal weights (980 versus 140), which is paradoxical when SW is expected to reduce the disturbing effects of unstable taxa (Farris, 1969). Such an increase would imply that the resolution obtained with the equally weighted data is not consistent with the base positions providing most of the signal, such that local, equally most parsimonious rearrangements are more numerous after SW. We show one of the SW trees (Fig. 5) with equally weighted branch lengths (ACCTRAN optimization); equally weighted bootstrap percentages are given below the branches, and arrowheads indicate groups not present in the strict consensus trees of both the equally weighted (filled arrowheads) and SW (unfilled arrowheads) analyses.

As stated above (see Section 2), Fig. 1 presents the chronogram for Iridaceae after NPRS: the split between *Moraea* and *Ferraria* is given at 25 mya using MP (ML: 26 mya); the distribution of ages depicted from the analysis of the bootstrapped matrices provides an error estimate of 2.15 mya for this date. It is noteworthy that despite the fact that they are based on different sets of genes and taxa, the NPRS angiosperm tree from Wikström et al. (2001) used here as calibration and the Iridaceae tree (Reeves et al., 2001, and this paper; Fig. 1) are consistent: the split between *Gladiolus* and *Aristea* is dated at 32 mya in the former and 37 mya in the latter. Finally, the date for the root node of *Moraea* is 15.6 mya (MP; using ML: 17 mya; Fig. 1), but this only applies for the species of *Moraea* included in the Iridaceae tree of Reeves et al. (2000, 2001); this becomes older once more species have been added and when the split at 25 mya between *Moraea* and *Ferraria* is used as a new calibration point in this extended *Moraea* tree (Fig. 2).

Trees derived from the combined analysis are well resolved. *Ferraria* (BS 98%) and *Moraea* (BS 84%) remain sister to *Dietes* (BS 88%), *M. lugubris* is sister to the rest of *Moraea*, and *M.* subgenus *Visciramosa* (BS 100%) is sister to the remainder of *Moraea*. *Moraea* section *Galaxia* (100% BS) here is sister to a clade that includes *Moraea angusta–M. neglecta* (*M.* subgenus *Monocephalae*) and the taxonomically and geographically isolated *M. namaquamontana*, but this topology has BS < 50%. The four species of *M.* section *Galaxia* in our study (of a total 14 in the genus) form two clades, each with strong support (BS 100% and 78%, respectively). The *M. garipensis–ramosissima* clade (BS 100%) and a second two-species clade, *M. ciliata–M. macgregorii* are sister to a large clade composed of the rest of the genus (BS 82%). Nodes separating *M. inclinata* and the *M. papilionacea–M. serpentina* clade, each in turn sister to the rest, have no BS < 50% and collapse in the strict consensus of the equally weighted trees. The remaining species form a trichotomy of unsupported clades. One of these comprises species of *M.* subgenus *Vieusseuxia*, *M.* subgenus *Grandiflora*, and *M.* section *Homeria*; the second includes some members of section *Polyanthes* and some of section *Moraea* plus *M. herrei* (previously the genus *Barnardiella*) and *M. lewisiae* (previously the genus *Hexaglottis*), and a third comprises species of *M.* section *Polyanthes* and those of *M.* section *Gynandriris* plus *M. rivulicola*. The three species of section *Gynandriris* (out of nine in the section) form a well-supported clade (BS 86%) and the *Gynandriris–M. rivulicola* clade has 89% bootstrap support.

The *Homeria* section (*M. cookii–M. bifida*; BS 93%) is sister to *M.* subgenera *Grandiflora* plus *Vieusseuxia*, but this clade has no support < 50% and collapses in the strict consensus of the SW trees. *Moraea* subgenus *Grandiflora* is monophyletic (BS 73%) and sister to one of two clades of *M.* subgenus *Vieusseuxia* (BS 53%). The other clade of the subgenus, *M. tulbaghensis–M. lurida* (BS > 50%) is sister to *M.* subgenus *Grandiflora* and the other portion of *M.* subgenus *Vieusseuxia*.

4. Discussion

On a molecular systematics basis, the use of *rbcL* for a species level phylogeny of *Moraea* would a priori seem unlikely to be a useful contribution, but it is clear from Table 2 that *rbcL* provides as much information as any of the other components of this analysis (in the combined analysis, *rbcL* provided 25.6% of steps on the combined tree). Most researchers would never consider including *rbcL* when low levels of variability are expected; this pattern of comparable or greater variability in *rbcL* than plastid spacer/introns has also been found in Arecaceae (Asmussen and Chase, 2001). None of the regions alone provided enough variation to be used alone, but the combination provided a framework to address other topics (see below).

What is clearly missing from this study is a nuclear DNA region(s), but the internal transcribed spacers (*nrITS*; Baldwin et al., 1995) were tried and not found suitable in *Moraea*, as was also found in several other genera of Iridaceae (including genera in both Iridoideae

and Ixioideae; Chase et al., unpubl.). Each accession has multiple NORs per chromosome complement (L. Hansen, pers. comm.), and PCR products from these contain a mixture of three or more apparently functional ITS copies (they form typical stem/loop structures and have intact 5.8S gene sequences with no indels or substitutions). No single copy type occurs across all species so designing PCR primers specific to a single copy type does not solve the problem. Nuclear protein-coding regions should be useful, but so far we have been unable to routinely use several previously published ones. Our plans are to include one or more nuclear DNA regions in this project, but for now we have focused on producing a well-resolved and supported plastid DNA tree. Although incongruence between nuclear and plastid-based DNA sequences occur, many studies have demonstrated congruence between plastid and nrITS (e.g., Whitten et al., 2000; Williams et al., 2000), and we expect a plastid tree to be a useful tool for evolutionary studies in this genus.

4.1. Systematics

The most striking aspect of our results is the inclusion in *Moraea* of species that until recently (Goldblatt, 1998) were referred to *Barnardiella*, *Galaxia*, *Gynandriris*, *Hexaglottis*, and *Homeria*. These other genera had been considered to be separate on the bases of their divergent floral morphologies, but they share with *Moraea* a consistent set of vegetative features, including corms of a single internode, corm production from a lateral bud, well-developed fibrous corm tunics, and bifacial, channeled (rarely centric) leaves without a midrib.

The four species of *M*. section *Galaxia* (BS 100%, see Fig. 5 hereafter for BS) are highly divergent from all species of *Moraea* and have no single particularly close relative among the *Moraea* species here sampled. *Moraea* section *Galaxia* is sister to a clade that includes *M. angusta*, *M. neglecta* (*M*. subgenus *Monocephalae*), and the taxonomically and geographically isolated *M. namaquamontana*, but this topology has BS $< 50\%$. The morphology of the latter set of species shows no obvious synapomorphies with *M*. section *Galaxia*. The four species of *M*. section *Galaxia* in our study (of a total 14 in the section) form two clades, each with strong bootstrap support (100% and 78%, respectively), and they represent the two main lineages of *M*. section *Galaxia* (Goldblatt, 1979a), one with yellow (or white flowers) and fringed style branches (*M. luteoalba* and *M. pilifolia*) and the other with pink or lilac flowers and undivided style branches (*M. barnardiella* and *M. melanops*). Evidently *M*. section *Galaxia* represents an early specialization of the *Moraea* lineage, derived in its acaulescent habit, single-flowered inflorescence units (Goldblatt and Manning, 2000b), perianth tube, subequal tepals, and filament column. Apart from the perianth tube, the flower is of the *Homeria*-type, with spreading, subequal tepals, a prominent staminal column, and reduced style crests. Such flowers have developed repeatedly in *Moraea*, most notably in the section *Homeria* but also elsewhere in the genus; we discuss the adaptive significance of this flower type below.

Species once included in genus *Homeria* comprise yellow-flowered *M. umbellata*, in an isolated position, one large clade for the yellow- or pink-flowered *M*. section *Homeria* sensu Goldblatt (1981, BS 93%), and blue-flowered *M. polyanthos*, which is sister to *M. polystachya* (BS 86%). *Moraea verecunda*, *M. herrei*, and *M. rigidifolia*, which also have a *Homeria*-type flower with a blue perianth but were never included in that genus (except for *M. herrei*), consistently fall in two different clades. *Moraea verecunda* is sister to the eastern southern African *M. alpina*, *M. elliotii*, and *M. natalensis* (all blue-flowered but with conventional, broad style branches bearing prominent crests) although this clade has weak support (BS 53%). *Moraea herrei* and *M. rigidifolia* fall in a clade (BS 83%) of species of the southern African west coast that includes, among others, *M. fugax* and *M. graniticola*. *M. herrei* was the only species included in *Barnardiella* (Goldblat, 1976b), and no other member of this clade has its peculiar beaked, tubular ovary (the defining feature of *Barnardiella* as well as of *M*. section *Gynandriris*). A second defining feature of *M. herrei*, and the one that distinguishes it from section *Gynandriris*, is the *Homeria*-type flower, a syndrome that has evolved repeatedly in *Moraea*.

The *Gynandriris* clade has just three species (of nine in the genus; BS 86%), significantly including both species from the Mediterranean (*Moraea sisyrinchium*) and the southern African winter-rainfall zone (*M. pritzeliana* and *M. hesperantha*). The beaked, tubular ovary was viewed as a putative synapomorphy in both *Gynandriris* and *Barnardiella*, but clearly evolved independently. It is puzzling that *M*. section *Gynandriris* is sister to *M. rivulicola* (BS 89%), because this species of *M*. subgenus *Vieusseuxia* has a morphology that unambiguously places it in that subgenus, which is characterized by apomorphic flowers with a long-lasting perianth, tricuspidate inner tepals, and a single foliage leaf (Goldblatt and Manning, 1995). Members of *M*. section *Gynandriris* usually have two leaves (or potentially two leaves), a fugaceous perianth (both plesiomorphic features, but here viewed as reversals), derived membranous spathes, and a sessile ovary with a sterile, tubular beak. Another sample of *M. rivulicola* from a second population gave the same result, so human error cannot explain this topology; plastid DNA capture would appear to be an unlikely explanation because no natural hybrids between these taxa are known. This result needs further investigation.

Another unexpected result of the analysis is the apparently anomalous position of the eastern southern

African *M. inclinata*, which morphology indicates should be most closely allied to *M. natalensis* (e.g., Goldblatt, 1986). Not only does it fall outside the clade with *M. natalensis*, but it lies outside all of the clades of non-Cape species; additional material for these species would help to clarify these patterns.

The single species of *M.* section *Hexaglottis* in the analysis is sister to the *M. fugax–M. herrei* clade (Fig. 5), a relationship that has BS > 50%. A possible relationship of members of *M.* sections *Hexaglottis* and *Homeria* suggested by Goldblatt (1987) on the basis of similar karyology ($x = 6$) is not reflected in the combined, successively weighed tree, but we do not yet have any clearer perspective on the relationships of members of *M.* section *Hexaglottis*, which must await the addition of more species of that alliance. The flowers of *M.* section *Hexaglottis* have spreading subequal tepals like in *M.* section *Homeria* but lack a staminal column. Instead, the filaments are short and united for half their length; the style divides into six filiform arms that extend horizontally between the anther bases.

Our molecular data convincingly show the hypothesized paraphyly of *Moraea* as previously circumscribed (Goldblatt, 1976a, 1986). *Barnardiella*, *Galaxia*, *Gynandriris*, *Homeria*, and *Hexaglottis* should not be treated as separate genera. Relying upon morphological evidence, Goldblatt (1998) transferred all these species to *Moraea*, a move corroborated by the analyses presented here. In addition, this analysis contributes significantly to an understanding the phylogeny of *Moraea*. *Moraea* subgenus *Grandiflora* (Fig. 5) is monophyletic (BS 73%) and nested in a larger group, all of which have $x = 6$. *Moraea* subgenus *Vieusseuxia* forms two separate clades (BS 53% and > 50%, respectively). Within the first of the two *Vieusseuxia* clades, all the eastern southern African species of the subgenus (Goldblatt, 1986) fall in one group (BS 91%), sister to the widespread winter-rainfall species *M. tripetala*, a clear signal of a single origin of the non-Cape members of *M.* subgenus *Vieusseuxia*, all but one species of which are included in this study. *Homeria* sensu Goldblatt (1980a, 1981) is also monophyletic (BS 93%), but its evident sister relationship to subgenera *Grandiflora* and *Vieusseuxia* (Fig. 5) has weak support (BS > 50%).

4.2. Biogeography

Results from the ancestral area analysis according to Bremer's method (1992) show that the highest gain to loss ratios are for the northwestern and southwestern centers of the Cape floral region (0.92 and 0.83, respectively), indicating the highest probability for these areas to be ancestral (Table 3). They are followed by Namaqualand and the Langeberg center of the Cape flora (gain/loss ratios 0.42 and 0.40, respectively). Therefore phylogenetic analysis of plastid DNA provides evidence for a southern origin of *Moraea*. All basal nodes within *Moraea* are occupied by species occurring in the southern African winter-rainfall zone and thus within the Greater Cape flora region (the traditional Cape flora region plus Namaqualand/southwestern Namibia). Note that ancestral analyses would be affected by incomplete sampling at the base of the tree (Bremer, 1992), but we have thoroughly sampled species and geographical groups within the genus, and additional as yet unsampled species are unlikely to alter these geographical patterns. Significantly, all species of the genus with the putative ancestral chromosome number, $x = 10$ (or $x = 9$ or 8 in the case of section *Galaxia* and *M. papilionacea*; Fig. 6) occur within this area. Although *Moraea* is well represented outside the Greater Cape flora, the non-Cape species fall in just a few clades well nested within clades of Cape species. Notable among the former are *M.* subgenus *Grandiflora* and the single lineage within *M.* subgenus *Vieusseuxia* mentioned above. Other extra-Cape clades are *M. carsonii* and presumably its allies in tropical Africa (Goldblatt, 1977) not yet included in our analysis, the *M. natalensis–M. alpina* clade, and some species of *Gynandriris*, such as *M. sisyrinchium* (Mediterranean).

Table 3
Ancestral area analysis following Bremer (1992); a higher gain to loss ratio indicates a higher probability to be an ancestral area; geographic distribution for each species is indicated in Table 1

	Gains	Losses	Gains/losses
Northwest Cape	22	24	0.92
Southwest Cape	20	24	0.83
Namaqualand	13	31	0.42
Langeberg Cape	14	35	0.40
Mt. Karoo Cape	8	21	0.38
Southeastern Cape	10	31	0.32
Eastern South Africa	7	24	0.29
Southern tropical Africa	6	25	0.24
Roggeveld	6	26	0.23
Northern South Africa	5	31	0.16
Karoo	4	30	0.13
Mediterranean/Middle East	1	18	0.06
Northern tropical Africa	1	21	0.05

4.3. Cytology

Cytology of *Moraea* is relatively well known (Goldblatt, 1971, 1976a, 1979b, 1981) and remarkably variable. Whereas most genera of Iridaceae are conservative for chromosome number, haploid numbers in *Moraea*, exclusive of direct polyploidy, are $n = 10, 9, 8, 7, 6, 5$, and 4, with $n = 6$ followed by $n = 10$ by far the most common. The north temperate genus *Iris* shows a similarly variable pattern of chromosome numbers. The only count for the problematic *M. inclinata* (see above), $n = 11$, may represent hypopolyploidy, and its closest relatives, inferred from morphological similarity (*M. elliotii* and *M. natal-*

ensis), have $n = 6$. Elsewhere it has been suggested (Goldblatt, 1971, 1976a; Goldblatt and Takei, 1997) that the ancestral base number for *Moraea* is $x = 10$, the same base number found in *Bobartia*, *Dietes*, and *Ferraria*, genera that closest to *Moraea* in the phylogenetic tree (Figs. 5 and 6). This leads to the conclusion that much of the early evolution of *Moraea* took place with little or no change in chromosome number. Only the isolated *M. papilionacea* and the *Galaxia* group among the lower clades have derived base numbers, $x = 9$ (or 8, or 7 in a few species or populations).

The derived base number of $x = 6$ characteristic of all members of subgenera *Grandiflora* and *Vieusseuxia*, and sections *Hexaglottis*, *Homeria*, *Polyanthos*, and *Gynandriris*, appears to have multiple origins and much of the adaptive radiation in *Moraea* has occurred in groups with this base number. The existence of few species with base numbers intermediate between $x = 10$ and $x = 6$, none on clades leading to groups with $n = 6$, seems to indicate rapid diversification after the change in number. As in other groups, this could reflect the attainment of a presumably adaptive gene arrangement (Stebbins, 1950).

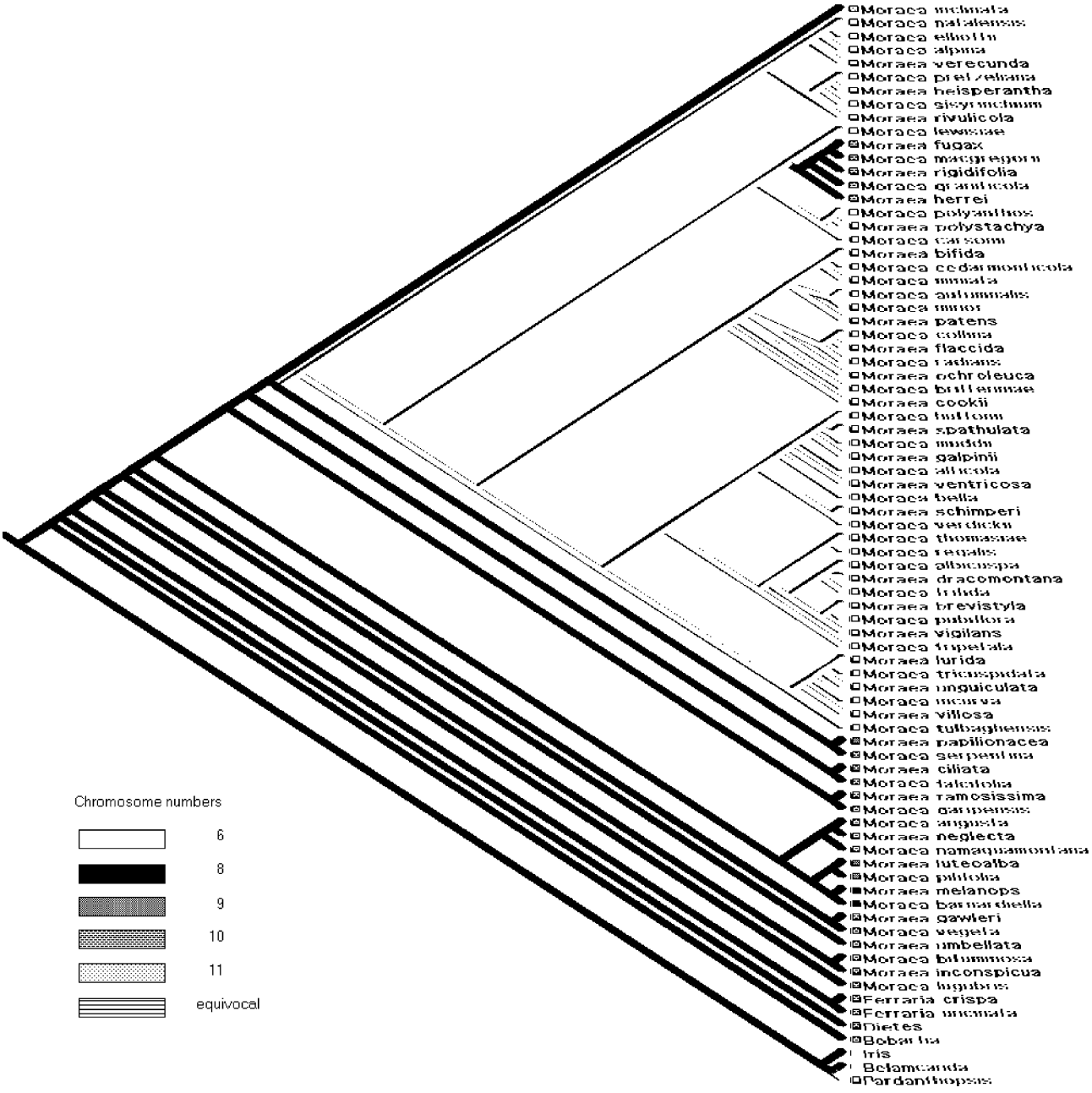

Fig. 6. Phylogenetic tree from Fig. 5 on which chromosome numbers are mapped (ACCTRAN optimization; see text for details).

4.4. Adaptive shifts in Moraea

The basic vegetative form of *Moraea* was established early in its evolution. All species have a structurally identical single-internode, astelic corm of axillary origin that produces roots from the base of the apical bud (Goldblatt, 1986, 1990). Leaves of all species are bifacial (rarely centric) but without a midvein, a derived development in Iridaceae, in which a unifacial leaf is ancestral (Goldblatt, 1990). Both the bifacial leaf and the corm of a single internode are synapomorphies for the genus. Specialization has proceeded along a path of vegetative reduction, resulting in a decrease in leaf number from several and indeterminate in number to consistently one leaf (*M*. subgenera *Grandiflora* and *Vieusseuxia*) and reduction in the number of branches (stems of *M*. subgenus *Grandiflora* are consistently unbranched) or in the loss of an aerial stem (the section *Galaxia*, *M. ciliata* and its immediate allies, and *M. falcifolia*).

A more conspicuous radiation has been floral. The basic *Moraea* flower (Goldblatt, 1986, 1998) is like that of *Iris* and consists of clawed tepals, with members of the outer whorl larger than the inner and bearing a conspicuous nectar guide at the base of the limb (Fig. 5). Whereas the tepals are united in a tubular hypanthium in *Iris*, they are free in *Moraea*, but in both genera as well as in *Dietes* and *Ferraria* the slender style divides at about mid-filament level into tangentially flattened, tepal-like branches terminating in a pair of erect appendages, the style crests. Each stamen is pressed against the abaxial surface of the opposed style branch. The stigma is a transverse band of tissue at the abaxial base of the style crests, lying just above the apex of the anther. This radially symmetric but complex flower has been compared to a meranthium of three, separate functional units (Müller, 1883; Proctor et al., 1996), each consisting of an outer tepal and the opposed stamen, style branch, stigma, and crests. Viewed in this way, each unit may be interpreted as a bilabiate structure with the tepal claw–style branch forming a gullet and the tepal limb a landing platform for the insect visitor. The anthers are usually concealed in such flowers, and the reward to visitors is nectar produced from perigonal nectaries at the base of the outer tepal claw (or in the case of *Iris*, on the walls of the perianth tube). Such flowers are usually pollinated by large, long-tongued bees foraging for nectar (Goldblatt et al., 1989, unpublished data, see Fig. 5). Pollen deposition is passive and on the dorsal part of the head and thorax as the bee probes the gullet in search of nectar.

The major shift away from this classic *Iris*-type flower in *Moraea* has been the reduction of the style branches and crests so that the anthers and their pollen are visible from outside the flower, and simultaneously the distinction between the outer and inner tepals is lost. Both whorls may bear nectaries and nectar guides, and the tepal claws are modified in one of two ways. They may be broadened to form a common cup enclosing the stamens or reduced so that the anthers are held well outside the flower and prominently displayed on a filament column (star type). Both variants of this reduced flower have been called the *Homeria*-type (Fig. 5). These floral modifications have long been believed to have been derived independently (Goldblatt, 1980a, 1998) and to be apomorphic, despite their apparently unspecialized nature compared to the complex, ancestral *Iris*-type flower. Our analysis confirms the multiple origins of the *Homeria*-type flower and its derived status within *Moraea* (Fig. 5).

The adaptive stimulus for the floral shift is believed to be a switch in pollination strategy. Whereas the bilabiate floral unit of the *Moraea* flower promotes passive pollen deposition on large bees of either sex, the *Homeria*-type flower may be specialized for a range of different pollinators depending on factors such as scent characteristics, perianth coloring, accessibility to nectar, and disposition of the anthers and pollen. The conspicuously displayed anthers and pollen of, for example, *M. bifida*, *M. herrei*, *M. rigidifolia*, *M. miniata*, and *M. verecunda* are classic bee flowers in which pollen for nest provisioning is the reward for female bees (Fig. 5). Bees visiting such flowers are often short-tongued, and pollen is actively collected (Goldblatt and Bernhardt, 1999). Bees encompass species of several families including Halictidae, Megachilidae, Melittidae, as well as Apidae, whereas bees visiting the bilabiate floral units of *Moraea* are almost always long-tongued members of Apidae (bee taxonomy according to Roig-Alsina and Michener, 1993, in which Anthophoridae are included in Apidae).

Other pollination strategies in *Moraea* include classical sapromyophily, i.e., fly pollination by Muscidae and Scathophagidae with lapping mouth parts (Faegri and van der Pijl, 1979). This strategy includes bowl-shaped flowers with aminoid or rotting odors and nectar easily reached by these insects, which have short probosces adapted for lapping rather than sucking. Sapromyophily is found most frequently in section *Homeria* (Fig. 5). Prime examples of this pollination strategy are *M. lurida* (*M*. subgenus *Vieusseuxia*) and *M. ochroleuca* (section *Homeria*), the tepals of which form a wide bowl, and nectar is produced not from discrete nectaries but is exuded from veins along the tepal surface (Goldblatt et al., 1999), a striking example of parallel evolution. Other fly-pollinated species of section *Homeria* have discrete nectaries at the tepal bases.

Hopliine scarab beetle pollination is associated with the presence of dark nectar guides contrasting with paler colors, absence of scent and nectar, and a disc-like flower that provides a broad unobstructed surface for beetle assembly, mate selection, and copulation (Goldblatt et al., 1998; Steiner, 1998). This system is most conspicuously developed in, for example *M. villosa,*

M. tulbaghensis (*M.* subgenus *Vieusseuxia*), and *M. elegans* (*Homeria* group; Fig. 5).

Comparison of the known or inferred pollination systems in the *Moraea* species included here shows a pattern of multiple origins of each of the derived pollination systems. As we add more species, we hope to compare all ecological aspects of immediately related species to gain some understanding of the factors that may have led to species diversification. Central to this question will be comparison of floral and pollinator divergence, habitat, and geography of terminal clades. What we offer here is just a preliminary overview of pollination strategies, and we suggest that such shifts have contributed to species diversity in *Moraea*.

4.5. Dating the radiation of Moraea

Any attempt to apply dates to the radiation of a family such as Iridaceae, in which there is no fossil record, must be considered highly speculative. Here we make a first effort to do this using the NPRS method with DNA sequences. We applied the conservative estimate of 82 mya from Wikström et al. (2001) to the root node of Iridaceae and their outgroups based on the generic phylogenetic tree of Reeves et al. (2000, 2001, Fig. 1). This yielded a date of circa 25 mya (early Miocene) for the divergence of the sister pair, *Moraea* and *Ferraria*. We then applied this date to our phylogenetic tree of *Moraea* (Fig. 2). The mid-Tertiary was a time of climatic deterioration across Africa, which by the Late Oligocene saw the development of a seasonally dry climate and the spread of grassland and savanna at the expense of forest (Axelrod and Raven, 1979; Coetzee, 1993). This pattern of climatic change would have favored the evolution of herbaceous forms with adaptations to survive annual periods of aridity. *Ferraria* and *Moraea* are seasonal perennials with underground corms (geophytes), thus admirably adapted to such conditions, whereas their ancestors would have been more like *Dietes* and the primitive members of the *Iris* clade, which favor forested habitats and are rhizomatous evergreen taxa.

Continued deterioration of the African climate during the later Oligocene and into the Miocene (24–5.5 mya) saw the evolution of the major clades of *Moraea* as Africa became drier, and continental uplift resulted in the spread of increasingly dry habitats (e.g., Coetzee, 1993; King, 1962). The development of the proto-Benguela current off the coast of southwestern Africa by the mid-Miocene (14 mya: Siesser, 1978) was the direct result of the spread of the Antarctic ice sheet, precipitated by the separation of South America from Antarctica–Australasia at this time and the establishment of a circum-Antarctic ocean current. Southwestern Africa became increasingly drier at this time, a period that must have seen the evolution of the proto-Cape and Namib floras, with their wealth of succulent and geophytic forms (Goldblatt, 1997; Goldblatt and Manning, 2000a). Among the latter was, we assume, *Moraea*, the early diversification of which occurred against this background during the Miocene. According to the dates inferred from our phylogenetic tree, the main clades of the genus evolved before the end of the Miocene.

The wealth of species of *Moraea* at the southwestern tip of Africa, however, is the direct result of events during the Pliocene when severe seasonal aridity off the southwestern African coast followed the strengthening of the Benguela current and accompanying summer drought (Coetzee, 1993; Hendey, 1981) saw the origin of the present climate there. This process would have caused local extinction of the less drought-adapted species of *Moraea*, which presumably existed inland from the coast. A few taxonomically isolated species remain in sheltered sites in Namibia and the adjacent Richtersveld of South Africa. Here *M. garipensis* and *M. namaquamontana* survive in a few sites, watered by summer fog and occasional winter storms. To the south, closer to the warm Indian Ocean and the cyclonic westerlies, which produce winter precipitation, aridity must have been and still is less pronounced, and the climate is Mediterranean, with wet, mild winters and hot, but not entirely dry summers. Geophytes with habits like those of *Moraea* and *Ferraria* have radiatated extensively along the coast and immediate interior of South Africa in the area that today has such a distinctive flora that Takhtajan (1986), relying solely upon intuition and without having a stated set of criteria, has termed it a "plant kingdom," one of just six he recognized. Like all floras, individual component species are present for many different reasons having arrived at different times, and the term "plant kingdom" appears to us to imply that these are some form of evolutionary unit, which they are not. For example, *Protea* (Proteaceae) would appear to have begun its radiation 35 mya ago (Reeves, 2001), significantly before *Moraea* (ca. 25 mya; Fig. 2). In contrast, *Phylica* (Rhamnaceae), a characteristic shrubby genus of the Cape flora may only have diverged from *Nesiota-Trichocephalus* 13 mya, and its major radiation occurred at the end of the Miocene, ca. 7 mya ago (Richardson et al., 2001a,b).

Nonetheless, the Cape flora is amazingly diverse and rich in species (9000), of which approximately 17% can be considered geophytes, plants with bulbs, corms, or other underground storage and perennating organs (Goldblatt and Manning, 2000a). The Pliocene (5.5 mya) saw the final establishment of the Sahara and the Namib deserts, the creation of the great Namib sand sea, and the development of the southern African Mediterranean zone (Coetzee, 1993; Pitman et al., 1993) The adaptive radiation of *Moraea* and initial events leading to the current species-richness of the Cape flora clearly occurred against this geological background and

with which our preliminary dating of the radiation of the genus is broadly consistent. The diversification of *Phylica* (Richardson et al., 2001b) is inferred on the basis of a molecular clock calibrated by the presence of endemic taxa on oceanic islands, the Mascarenes and St. Helena, for which dates of origin are known (these two dates corroborate each other; one is for *Nesiota-Trichocephalus*, which is sister to *Phylica*, and the other is for *P. nitida*, a species deeply imbedded in *Phylica*). The massive radiation of the 100-plus *Phylica* species of woody shrubs present at the Cape today began at the end of the Miocene (ca. 7 mya), but most extant species date from the late Pliocene (ca. 1–2 mya).

The occurrence of *Moraea* outside the southern African winter-rainfall zone and the Greater Cape flora appears to be a relatively recent phenomenon. As we mentioned above the few non-Cape clades are nested high up the tree, and all have sister taxa in the Cape Region. The events precipitating this migration could have been continental uplift in southeastern Africa (Coetzee, 1993), where the Drakensberg Range now reaches over 3500 m in some places. Topographical elevation in what would otherwise be a hot, wet climate again seems to have favored the radiation of geophytic forms as well as grasses at the expense of forest that is not suited to the cold winters of the southern African highlands and survives locally only in sheltered sites. It is no coincidence that *Moraea* species outside the Cape Region are concentrated in montane and highland habitats. Finally, we hope that a complete molecular phylogenetic study of *Moraea*, where we intend to sample all species of this genus, will cast light on the reasons for high species diversity in genera that are characteristic of the Cape flora.

Acknowledgments

We thank Ingrid Nänni and Jim Clarkson for their help in collecting material, Anette de Bruijn and Jeffrey Joseph for their technical assistance, Elenor Kuntz and Becky Greene who provided unpublished outgroup sequences, and Peter Linder and an anonymous reviewer for their comments on the manuscript. This study was partially supported by the National Geographic Society (USA), the Royal Society (UK), the Royal Botanic Gardens, Kew, and the Swiss National Science Foundation (31-52378.97).

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