2007

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PHYLOGENY AND HISTORICAL ECOLOGY OF *RHODOCOMA* (RESTIONACEAE) FROM THE CAPE FLORISTIC REGION

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ABSTRACT

A macroevolutionary analysis of macroecological relationships in *Rhodocoma* revealed a complex history of rapid ecological divergence, as well as genetic isolation via shifts in flowering times. The rate and extent of divergence observed among even the youngest of species pairs indicated that the selective forces driving these processes are strong enough to effect substantial amounts of ecological change in relatively short periods of time, and are potentially important factors promoting the origin and persistence of species diversity not only in *Rhodocoma,* but also the African Restionaceae as a whole. These results also suggest that the rate and extent of ecological differentiation can vary between lineages, and this may be a consequence of variations in the intensities of selective regimes or phylogenetic constraints that different lineages experience. Investigation into the nature of this differentiation revealed that much of it has occurred along altitudinal gradients, but in tandem with substantial shifts in other ecological parameters such as rainfall and fire survival. This multidimensionality of ecological differentiation increases the number of possible combinations of ecological parameters and may allow for a more precise partitioning of niche space.

Key words: ancestor reconstruction, Cape Floristic Region, historical ecology, niche conservatism, Restionaceae, *Rhodocoma.*

INTRODUCTION

Rhodocoma Nees consists of eight species of African Restionaceae (Linder 2002). The African Restionaceae comprise a large (19 genera; 350 spp.) and ecologically important clade of graminoid, dioecious, wind-pollinated perennials largely restricted to the Cape Floristic Region (CFR) (Goldblatt 1978) of southern Africa (Linder 1991, 2002). As a genus, *Rhodocoma* is distributed primarily in the eastern half of the CFR and is distinguished from other African Restionaceae by the combination of pendulous male spikelets, single-flowered female spikelets, and trilocular capsules (Linder 1984, 1991; Linder and Vlok 1991). More than ten years ago, Linder and Vlok (1991) concluded that patterns of biogeography and ecological variation in *Rhodocoma* were consistent with some form of sympatric speciation, driven by selective forces across steep ecological gradients. Based on a cladistic analysis of morphological and anatomical characters, sister species were shown to be either sympatric or parapatric, and distinguished by ecological (chiefly habitat) factors. However, the limited character sampling and absence of two subsequently described species limited the strength of these earlier conclusions. Here we present the results of a reinvestigation of phylogenetic relationships in *Rhodocoma,* incorporating these two additional species and newly acquired DNA sequence data. Using an approach similar to that developed by Linder and Hardy (2005), we also quantify patterns of ecological differentiation in the genus and reevaluate the potential influence of ecological factors in *Rhodocoma* diversification. Using this combined macro-evolutionary and macroecological approach, we explore the potential role of ecology in not only driving cladogenesis, but also in promoting the maintenance of species richness in *Rhodocoma.*

MATERIALS AND METHODS

Taxon Sampling

The eight recognized species of *Rhodocoma* were each represented by a single sample. The immediate outgroup to *Rhodocoma* is not certain. The family level analyses of Linder (1984), Eldenäs and Linder (2000), and Linder et al. (2000) have resolved the genus *Thamnochortus* Berg. as sister to *Rhodocoma,* a relationship supported by their sharing numerous morphological attributes, including the possession of pendulous male inflorescences. Accordingly, outgroup sampling included 10 of the 32 species of *Thamnochortus.* However, preliminary results from an ongoing phylogenetic study of the African Restionaceae as a whole indicate that while *Thamnochortus* and *Rhodocoma* are closely related, they may not be sister taxa (Hardy and Linder unpubl. data), and additional outgroup taxa were chosen in accordance with these results. A complete list of these species and their vouchers is presented in Table 1.

Character Sampling

DNA sequences were generated from the plastid regions completely spanning the *trn*L intron through the *trn*L–*trn*F intergenic spacer (Taberlet et al. 1991), the complete gene encoding *rbc*L (Chase and Albert 1998), and the complete *atp*B–*rbc*L intergenic spacer (Manen et al. 1994; Chiang and Schaal 2000; Cuénoud et al. 2000), as well as *mat*K and the flanking *trn*K intron (Hilu and Liang 1997). Total DNA was

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Table 1.   Species sampled for this study.

Taxon	Voucher information[a]	(GenBank accession numbers)
Rhodocoma alpina H. P. Linder & Vlok	*Linder, Hardy, and Moline 7603*	(AY640389)
Rh. arida H. P. Linder & Vlok	*Linder et al. 7414*	(AY640390; AY690747; AY690785)
Rh. capensis Nees ex Steud.	*Linder et al. 7248*	(AY640391; AY690748; AY690786)
Rh. foliosa (N. E. Br.) H. P. Linder	*Linder et al. 7280*	(AY640392)
Rh. fruticosa (Thunb.) H. P. Linder	*Linder et al. 7609*	(AY640393; AY690749; AY690787)
Rh. gigantea (Kunth) H. P. Linder	*Linder et al. 7401*	(AY640394; AY690750; AY690788)
Rh. gracilis H. P. Linder & Vlok	*Linder et al. 7400*	(AY640395; AY881396; AY881470; AY881616)
Rh. vleibergensis H. P. Linder ined.	*Linder et al. 7426*	(AY640396; AY690751; AY690789)
Thamnocortus erectus (Thunb.) Mast.	*Linder et al. 7364*	(AY640397; AY690771; AY690809)
T. fruticosus Berg.	*Linder et al. 7594*	AY640398; AY690755; AY690793)
T. gracilis Mast.	*Linder et al. 7333*	(AY640399; AY690757; AY690795)
T. karooica H. P. Linder	*Linder et al. 7283*	(AY640400; AY690758; AY690796)
T. levynsiae Pillans	*Linder et al. 7345*	(AY640401; AY690759; AY690797)
T. lucens Poir.	*Linder 7147*	(AY640402; AY690774; AY690812)
T. nutans (Thunb.) Pillans	*Linder et al. 7350*	(AY640403; AY690761; AY690799)
T. obtusus Pillans	*Linder et al. 7285*	(AY640404; AY690775; AY690813)
T. paniculatus Mast.	*Linder et al. 7310*	(AY640405; AY690762; AY690800)
T. pulcher Pillans	*Linder et al. 7338*	(AY640406; AY690765; AY690803)
Calopsis burchellii (Mast.) H. P. Linder	*Linder et al. 7393*	(AY640385; AY690743; AY690782)
C. viminea (Rottb.) H. P. Linder	*Linder et al. 7200*	(AY640386; AY690744; AY690783)
Restio insignis Pillans	*Linder 7144*	(AY640387; AY690745; AY690784)
Re. similis Pillans	*Linder et al. 7324*	(AY640388; AY690746; AY690820)

[a] All collections made in Cape Floristic Region, South Africa. All vouchers deposited in the herbarium (Z) at the Institute of Systematic Botany, University of Zurich, Switzerland.

isolated from silica-gel-dried culms using the Dneasy® Plant Mini Kit (QIAGEN, Inc.,Valencia, California, USA). Sequences for *trn*L–F were obtained as described in Eldenäs and Linder (2000). The two regions spanning the contiguous *atp*B–*rbc*L spacer plus *rbc*L, as well as *mat*K and the flanking *trn*K intron, were each amplified from a single polymerase chain reaction using the primers designated in Table 2. Sequences were generated using standard methods for automated sequencing, using the primers designated in Table 2.

Phylogenetic Analysis

Raw sequence data files were analyzed with the ABI Prism™ 377 Software Collection vers. 2.1 (Applied Biosys-

tems, Inc., Foster City, California, USA). Contigs were constructed in Sequencher™ (Gene Codes Corporation, Ann Arbor, Michigan, USA) and alignments were performed using the default alignment parameters in CLUSTAL_X (Thompson et al. 1997), followed by manual adjustment by eye with the criterion of minimizing inferred evolutionary events, where insertions/deletions (indels) and base substitutions were equally weighted. These sequences were assembled into a single matrix in WinClada vers. 1.00.08 (Nixon 2002). Indels were coded at the end of the matrix as unordered binary or multistate characters. The data matrix used in the analysis is available from the authors, and at the following website: *http://www.treebase.org* (accession #SN1741). Parsimony searches were conducted with both PAUP* vers. 4.0

Table 2.   Sequencing and PCR primers used for the *trn*K–*mat*K and *atp*B–*rbc*L regions.

Name	Locus	Direction	Sequence	Utility	Reference
Ar_F1c	*atp*B–*rbc*L	forward	5′-CCAGCACGGGCCGTATAATTTG-3′	PCR and sequencing	C. Hardy and P. Moline, Univ. Zurich
Ar_R1a2	*atp*B–*rbc*L	reverse	5′-CCTGGTTGAGGAGTTACTCGGAAT-3′	Sequencing	C. Hardy and P. Moline
1f	*atp*B–*rbc*L	reverse	5′-ATGTCACCACAAACAGAAAC-3′	Sequencing	Asmussen and Chase (2001)
636f	*atp*B–*rbc*L	forward	5′-GCGTTGGAGAGATCGTTTCT-3′	Sequencing	Asmussen and Chase (2001)
797r	*atp*B–*rbc*L	reverse	5′-CCGTTAAGTAGTCGTGCA-3′	Sequencing	C. Hardy and P. Moline
rbcL rev	*atp*B–*rbc*L	reverse	5′-TCCTTTTAGTAAAAGATTGGGCCGAG-3′	PCR and sequencing	Asmussen and Chase (2001)
mk_F1	*trn*K–*mat*K	forward	5′-AAGACYRCGACTGATCCT-3′	PCR and sequencing	A. Kocyan pers. comm.
matk-r4	*trn*K–*mat*K	reverse	5′-CGCGTCAACAATACTTCT-3′	Sequencing	P. Moline
mk_B4	*trn*K–*mat*K	reverse	5′-CCTATAGAAGTGGATTCGTTC-3′	Sequencing	C. Hardy
mk_A2	*trn*K–*mat*K	forward	5′-CCAAAGTCAAAAGAGCAATTG-3′	Sequencing	C. Hardy
matk-r2	*trn*K–*mat*K	reverse	5′-GGGACATCCTATTAGTAAA-3′	Sequencing	P. Moline
mk_B2	*trn*K–*mat*K	reverse	5′-CGAGCCAAAGTTCTAGCACAC-3′	Sequencing	C. Hardy
matk-f2	*trn*K–*mat*K	forward	5′-CCATTATTCCTCTCATTG-3′	Sequencing	P. Moline
mk_R1	*trn*K–*mat*K	reverse	5′-CATTTTTCATTGCACACGRC-3′	PCR and sequencing	A. Kocyan pers. comm.

Table 3. Ecological parameters coded. Altitude, Average annual rainfall, Rainfall seasonality (0 = winter rain; 1 = all year), Fire survival mode (0 = resprouting; 1 = reseeding), Groundwater availability (0 = none; 1 = seeps), Bedrock (0 = TMS; 1 = shale; 2 = granite; 3 = silcrete; 4 = enon conglomerate; 5 = cave sandstone; 6 = limestone; 7 = acid coastal sand; 8 = alkaline sand), and Soil rockiness (0 = none; 1 = stony; 2 = bedrock).

	Altitude (km)	Average annual rainfall (mm)	Rainfall seasonality	Fire survival mode	Ground water availability	Bedrock	Soil rockiness
Calopsis burchellii	0.25–0.35	600–1000	1	0	0	3	1
C. viminea	0.05–1.4	200–600	0, 1	0	0	0, 2, 3, 6, 7	1
Restio insignis	1.2–1.8	600–1200	0	1	0	0	1
Re. similis	0.05–1.5	600–1200	0	—	0	0	0, 1
Rhodocoma alpina	1.6–2.0	900–1200	1	0	0	0	1
Rh. arida	0.5–0.8	200–300	1	1	0	0, 4	2
Rh. capensis	0.9–1.5	500–1000	0, 1	0, 1	1	0, 1	0, 2
Rh. foliosa	0.3–0.9	700–1000	1	0	0	0	1
Rh. fruticosa	0.2–1.6	400–900	0, 1	0	0	0, 3, 5	1
Rh. gigantea	0.1–0.6	700–1000	1	1	0	0, 1	1
Rh. gracilis	0.3–0.6	700–1000	1	1	0	0	0, 2
Rh. vleiberensis	1.0–1.6	400–800	0	1	0	0	1, 2
Thamnochortus erectus	0.01–0.2	400–1000	0, 1	0	0	8	0, 1
T. fruticosus	0.0–1.2	400–1000	0, 1	0	0	0, 2, 3, 6	0
T. gracilis	0.1–0.6	400–1000	0	0	0	0	1
T. karooica	0.45–1.0	100–400	1	1	0	0	1
T. levynsiae	0.5–1.0	800–1000	0	—	0	0	2
T. lucens	0.1–1.0	200–1200	0	0	0	0	1
T. nutans	0.9–1.0	1000–200	0	0	0	0	2
T. obtusus	0.01–0.2	400–600	0	—	0	7	0
T. paniculatus	0.01–0.2	20–600	0, 1	1	0	6	1
T. pulcher	0.03–0.5	600–1200	0	0	0	0	1

for Macintosh (Swofford 2002) using the "Branch and Bound" option, and the heuristic options in NONA vers. 1.6 (Goloboff 1993), run as a daughter process from WinClada. In NONA, 1000 tree searches were conducted, with each search initiated with the generation of a Wagner tree, using a random taxon entry sequence, and followed by tree-bisection-reconnection (TBR) swapping on the Wagner tree, with one shortest tree retained and subjected to branch swapping. All most-parsimonious trees accumulated during these searches then were subjected to TBR swapping, including swapping on all trees propagated during this phase of the search, with up to 10,000 trees retained and swapped. Bootstrap support values (Felsenstein 1985) were obtained using NONA spawned as a daughter process in WinClada using 1000 replicates with 100 TBR searches each, holding one tree per TBR search. Percentages were then based on the strict consensus tree of each of the 1000 replicates (i.e., the "strict-consensus" bootstrap sensu Soreng and Davis 1998).

Branch lengths within the genus *Rhodocoma* were estimated using maximum likelihood (ML). To do this, a modified matrix was constructed including only sequences from *Rhodocoma,* including all sites in the aligned sequence data set and excluding indel characters. ModelTest vers. 3.06 (Posada and Crandal 1998) was then employed in tandem with PAUP* to choose an adequately parameter-rich ML model from a possible 56 models of sequence evolution that returns the highest likelihood score not significantly different from the scores returned for more complex models. Although ModelTest vers. 3.06, by default, evaluates models based on a neighbor-joining tree, the topology for *Rhodocoma* obtained through parsimony analysis of the combined data set was used. This required modifying the "modelblock" file

accompanying ModelTest by deleting the first command block, which commands PAUP* to construct a neighbor-joining tree. Then with the *Rhodocoma* matrix and parsimony tree already opened and executed in PAUP*, the modified "modelblock" file is executed. The model selected by ModelTest (the general time reversible [GTR] model of nucleotide substitution, with six substitution types) was then used to perform a likelihood ratio (LR) test (Felsenstein 1981) to assess rate heterogeneity in *Rhodocoma.* Using PAUP*, likelihoods with and without assuming a clock were calculated for the single most-parsimonious topology obtained for *Rhodocoma* from the principal analysis. As the LR test did not reject the clock ($X^2 = 2.00$, $P < 0.01$), ML branch lengths were estimated under the assumption of a clock, using the same parameters as above. The ultrametric tree obtained was viewed and printed from TreeView vers. 1.6.6 (Page 1996).

Macroecological Parameters

The set of ecological parameters used by Linder and Hardy (2005) was somewhat modified and scored for each species (Table 3). The selection of parameters and their definitions were limited by the available data for each species, and to those adequately characterized for the Cape Floristic Region. These parameters are "proxy" parameters for the multitude of environmental variables that may be biologically significant, and so are similar to the Ellenberg indicator values (Ellenberg 1974) used in Europe. Precise quantitative measurements are not available for most species of the Cape flora.

Bedrock.—Species of *Rhodocoma* are restricted to soils derived from the following types of bedrock: sandstone (Table Mountain Sandstone or TMS), shale, silcrete, enon conglomerates, and cave sandstone (sandstone restricted to the Drakensberg, finer textured and potentially more nutrient rich than TMS). Some species of outgroup taxa may also be found on granites, limestones, or acid or alkaline sands near the coast. These soils have a characteristic particle size, pH, nutrient profile, and conductivity (Lambrechts 1979), and so the types recognized are proxy estimates of soil nutrients, water retention, and conductivity. Although these soil categories are crude, they appear to characterize different vegetation types in the CFR, and the boundaries between them are usually sharp.

Altitude.—Altitudinal variation among the species of *Rhodocoma* ranges from near sea level to 2000 m. Altitude may predict diurnal and annual temperature ranges, pan-evaporation, insolation, and UV-B radiation.

Groundwater availability.—In the CFR with its dry and hot summers, groundwater availability may be important to facilitate growth in the summer, particularly for shallow-rooted monocots (Higgins et al. 1987). We recognize three habitats, defined on the basis of groundwater availability: (i) well-drained soils, (ii) seepages (which are saturated with water for only certain periods of the growing season), and (iii) stream banks, from which plants have continuous access to groundwater. Plants of *Rhodocoma* occur in either of the first two.

Soil rockiness.—Soil rockiness is an indirect descriptor of soil depth and moisture availability during periods without rain. Rockless soils in the CFR are generally deep, sandy substrates and occur along the coastal plains and on sandy plateaus on the mountains, which may be waterlogged in winter and dry in the summer. Stony soils generally occur on mountain slopes. These substrates are of generally deeper soils, but with a profile broken by stones of various sizes. The regions beneath the stones provide pockets of moisture that plant roots can access, even during the arid summer months when other soils, such as those of the sandy plains, are dry. The third category comprises the very shallow soils over bedrock.

Average annual rainfall.—Patterns of rainfall in the CFR, particularly in the western Cape, are highly variable and gradients are very steep (Campbell 1983). Totals range from nearly 2000 mm per year in the highest elevations of the mountains immediately facing the coast, to less than 200 mm on the inland slopes of the interior mountain ranges such as the Swartberg and Cederberg (Goldblatt and Manning 2000). Although correlated along certain transects with other parameters such as altitude, Campbell (1983) has shown that these correlations break down in other parts of the Cape, due to complex interactions with other parameters such as aspect and proximity to the coast. These data for *Rhodocoma* are primarily estimates based on the rainfall isohyet maps of the Trigonometrical Survey of South Africa.

Rainfall seasonality.—The seasonal distribution of rainfall varies dramatically from the winter rainfall (dry summer) regions in the west, to the all-year rainfall on the south coast, and the summer rainfall (dry winter) region in the east (Campbell 1983, and references cited therein). *Rhodocoma* is distributed primarily in regions of all-year rainfall, although *Rh. fruticosa* and *Rh. capensis* extend into the winter rainfall regions of the west, to which *Rh. vleibergensis* is restricted.

Fire survival mode.—Much of the CFR experiences fire at least once every 30 years (Van Wilgen 1987) and this phenomenon has been implicated as an important factor (via disturbance) affecting local extinctions, suppressing competitive exclusion and, consequently, promoting speciation in the CFR (Cowling 1987). Moreover, Schutte et al. (1995) demonstrated that plants of many non-geophytic species exhibit one of two distinct mechanisms to survive or to reestablish populations after such fires, and that these differences may be linked to various other life-history traits of biological and ecological significance to the species. Thus, fire survival represents another variable in which ecological differentiation may occur. In some species, all plants are killed by fire, and the population has to be reestablished from seed. In other species, at least some, and often all, individuals survive fire as roots, rootstocks, or rhizomes, and resprout from these. Particular species of *Rhodocoma* (and Restionaceae, in general) fit into one of these two types, although *Rh. capensis* is polymorphic for these features. The degree to which the geographic pattern of these differences in fire survival is influenced by geographic patterns of fire frequency is unknown, as accurate and comprehensive fire frequency data are lacking.

Discovering and Interpreting Patterns of Ecological Differentiation

Ecological similarity.—Ecological similarity was measured with the Jaccard coefficient, as implemented in NTSYSpc (Rohlf 1998). The Jaccard coefficient was chosen because it assesses similarity on the basis of shared occurrences in particular habitats or for particular variables, and it does not take shared absences from other habitats or variables into account. Thus, species are more *similar* if they overlap more along certain environmental gradients or for certain ecological variables. One-hundred percent overlap results in a Jaccard value of 1, and no overlap results in a value of 0. Because Jaccard calculations require binary data, each state (habitat or the like) of discretely coded environmental and ecological characters was coded as a separate binary character reporting the presence or absence of the particular species in the given habitat or for the given variable. As discussed by Linder and Hardy (2005), this enables us to account for several species that are polymorphic for certain environmental parameters (e.g., bedrock type). To make continuously variable characters compatible with Jaccard calculations, such characters were subdivided into increments, each of which was coded as a separate binary character as above. Altitude, for example, was subdivided into 50 m increments. Average annual rainfall into 50 mm/year increments. This method of coding allows for the coding of ranges in such characters. However, parameters such as altitude and rainfall have ordered axes and for these the Jaccard coefficient is not really a measure of similarity, as it does not take order into account. Thus two species dis-

tributed along an altitudinal gradient at 0–100 m and 101–200 m, respectively, receive a pairwise Jaccard coefficient of 0 because, although close, they do not overlap along that gradient.

To avoid biasing a Jaccard calculation with undue influence from parameters with more states (e.g., altitude with 40 states of 50 m increments, vs. fire survival mode with two states), Jaccard similarities were calculated for each parameter separately. These were then summed for each species pair, and the average pairwise similarity was calculated. The resulting similarity matrix was then represented in three-dimensional space by a non-metric multidimensional scaling (NMDS), as implemented in NTSYSpc, using the output from a principal coordinates analysis of that same similarity matrix as an initial configuration matrix.

Ecological, genetic, and phylogenetic distances.—Pairwise ecological distances between species of *Rhodocoma* were taken as one minus the pairwise Jaccard similarity $(1 - J)$. Pairwise genetic distances were calculated in PAUP* using the ''uncorrected (p) distance,'' which is the total number of differences divided by the total length of the comparison (i.e., the length of the aligned sequences plus indel characters). As some measure of the relationship between distance and phylogenetic relatedness, the significance of the difference between the average distance values between sister vs. non-sister species was tested by means of a *t*-test, as implemented in Microsoft® Excel 2000. This was done separately for both genetic distance and ecological distance. The correlation between Jaccard ecological distance and genetic distance matrices was tested by means of a Mantel Test, as implemented in NTSYSpc. Tests were conducted with 9999 randomizations.

Ancestral ecology reconstructions.—Hypotheses of ancestral ecologies (habitat plus fire survival mode) were constructed using optimization procedures. Reconstructions utilized the entire tree (i.e., outgroup plus ingroup). Because individual species may be ''polymorphic'' for categorical ecological parameters and occur in ranges along the axes of the continuously variable parameters altitude and rainfall, it is necessary to apply reconstruction methods that account for these phenomena. For the categorical parameters rainfall seasonality, fire survival mode, groundwater availability, bedrock, and soil rockiness, Polymorphism Coding (as per Hardy and Linder [2005], and introduced by Maddison and Maddison 1992) was employed. This procedure is to code the possible polymorphisms as separate states along with monomorphic states in a single multistate character. A stepmatrix is then employed to assign the desired transition costs (steps) between the states. We assigned equal costs for gains and losses of states (Fig. 1) and then determined the most-parsimonious solutions using Sankoff optimization (Sankoff and Rousseau 1975) using Mesquite vers. 1.0 (Maddison and Maddison 2003). However, because the maximum allowable number of states in Mesquite is 56, the ecological parameter bedrock, with its eight states, could not be optimized algorithmically because the number of possible combinations of these eight component states exceeds 56. Thus, Sankoff optimization for bedrock was carried out manually (Felsenstein 2004:13–15, 67–69).

For altitude and average annual rainfall, MaxMin Coding

	a	b	c	a b	a c	b c	a b c
[a]	.	2	2	1	1	3	2
[b]	2	.	2	1	3	1	2
[c]	2	2	.	3	1	1	2
[ab]	1	1	3	.	2	2	1
[ac]	1	3	1	2	.	2	1
[bc]	3	1	1	2	2	.	1
[abc]	2	2	2	1	1	1	.

Fig. 1.—Stepmatrix employed for Polymorphism Coding of three-state (a, b, c) categorical parameters (e.g., soil rockiness). Equal costs for both gains and losses of states.

(Hardy and Linder 2005) was employed. This procedure involves coding the maximum and minimum of each species' observed range as two separate characters. Both values are optimized independently to internal nodes and the ancestral ranges inferred to lie between the two values. Optimization of these MaxMin-coded ranges employed linear parsimony (LP) as implemented in Mesquite. LP minimizes the total change throughout the tree. As LP tends to reconstruct many branches with no change, in favor of relatively large amounts of change on few branches, LP may incorporate an implicit model of stabilizing selection with occasional adaptive shifts (Losos 1999).

Following the optimization of ecological parameters to internal nodes, a new Jaccard similarity matrix including both the terminal nodes (extant species) and internal nodes (ancestors) was constructed for *Rhodocoma*. For altitude and rainfall, ranges of equally parsimonious maxima or minima were sometimes provided. In these cases, the average value of the maximum or minimum as appropriate for a node was scored in the Jaccard matrix. As above for the extant species only, the resulting similarity matrix was then represented in 3-dimensional space by NMDS, again using the output from a principal coordinates analysis of that same similarity matrix as an initial configuration matrix. This produces a hypothesis of ecological relationships between both extant and ancestral species. This also allows for inferences of the extent and trajectories of ecological divergences. Although there is uncertainty associated with ancestral reconstructions in general (Frumhoff and Reeve 1994; Maddison 1995; Donoghue and Ackerly 1996; Schluter et al. 1997; Cunningham et al. 1998; Sharkey 1999), these uncertainties do not distort the ecological relationships represented between extant species.

RESULTS

Phylogeny

The sequenced portion of the *trn*L–F region comprises 1077 aligned bases spanning the complete *trn*L intron, *trn*L 3′ exon, and the *trn*L–*trn*F intergenic spacer. Of these, there were 46 cladistically informative nucleotide sites in addition to seven informative indel characters coded at the end of matrix as separate binary or unordered multistate characters. The sequenced region spanning the *atp*B–*rbc*L spacer consisted of 983 aligned bases, comprising 101 base pairs (bp) of the 5′-end of *atp*B plus 882 bp of intergenic spacer. Of

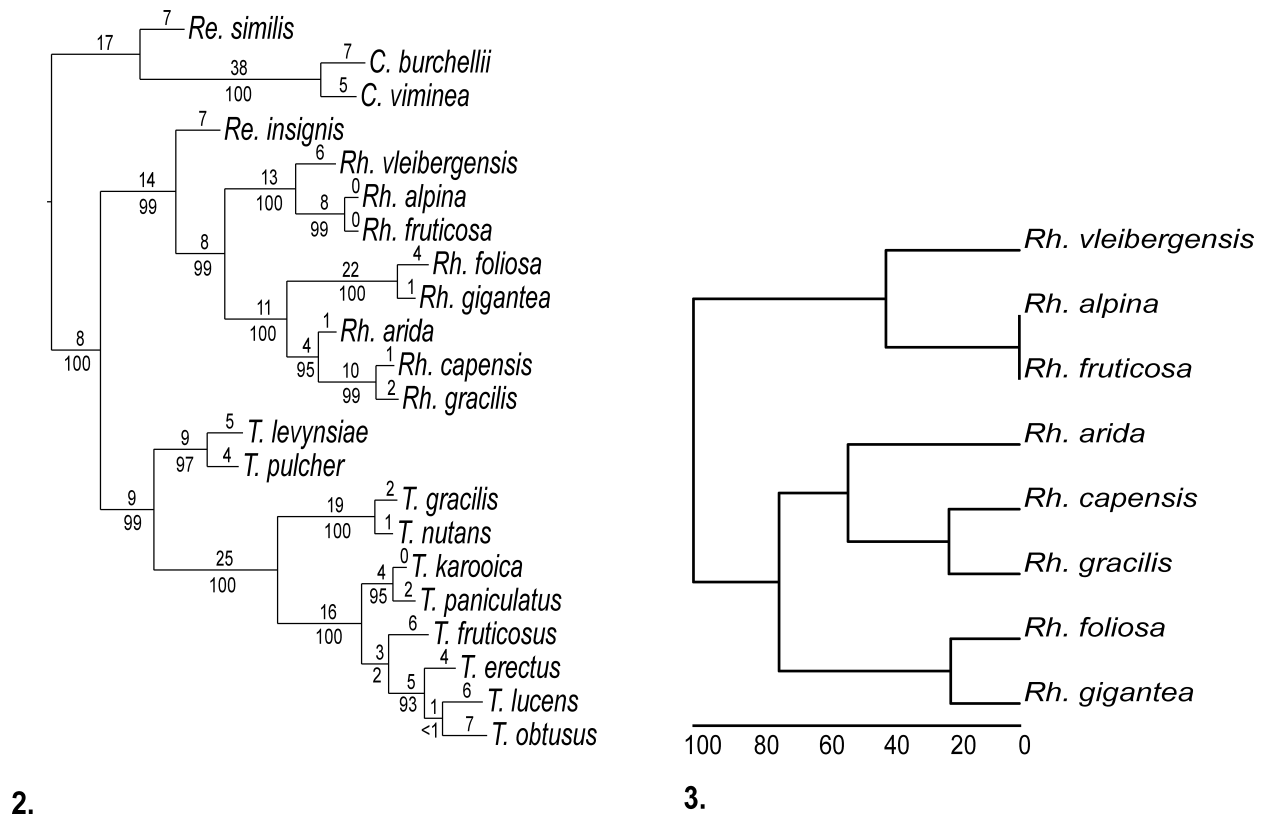**2.**   **3.**

Fig. 2–3.—The most-parsimonious cladogram with (2) and without (3) the outgroup.—2. Branch lengths (ACCTRAN) shown above the branches, bootstrap percentages below (L = 322, CI = 0.78, RI = 0.90).—3. *Rhodocoma* chronogram; scale of 100 is arbitrary.

these, there were 26 cladistically informative nucleotide sites in addition to four informative indel characters. Complete sequences for *rbc*L were 1437 bp in length from which came 18 informative substitution characters and no length variation. Additionally, a contiguous 61 bp region downstream of *rbc*L (part of the same amplification product) was also sequenced, from which came three informative substitution characters and no length variation. The sequenced region spanning the entire *mat*K gene and the flanking regions of the *trn*K intron comprised 2493 aligned bases, with 114 informative substitution characters and nine informative indels. Thus, the combined molecular matrix comprises 6051 aligned bases and 207 cladistically informative substitution characters and 20 informative indel characters. A simultaneous analysis in both NONA and PAUP* resulted in the same single most-parsimonious tree (Fig. 2; L = 322, CI = 0.78, RI = 0.90).

This analysis strongly supports the monophyly of both *Rhodocoma* and *Thamnochortus*. The rooting depicted in Fig. 2 is based on a larger, Restionaceae-wide analysis. As sampled here, *Restio insignis* is resolved as sister to *Rhodocoma* and this clade is sister to *Thamnochortus*. Within *Thamnochortus* the clade of *T. levynsiae* and *T. pulcher* is sister to all other species of *Thamnochortus*. The next diverging lineage is the clade of *T. gracilis* and *T. nutans*. The next diverging lineage is the clade of *T. karooica* and *T. paniculatus,* which is sister to the clade (*T. fruticosus* (*T. obtusus* (*T. erectus, T. lucens*))).

Rhodocoma is fully resolved with well-supported nodes. *Rhodocoma vleibergensis* is sister to *Rh. alpina* and *Rh. fru-*

ticosa. This clade is sister to the clade of ((*Rh. foliosa, Rh. gigantea*) (*Rh. arida* (*Rh. capensis, Rh. gracilis*))).

As there are no data (e.g., fossils) with which to calibrate the molecular clock estimates, the ultrametric tree for *Rhodocoma* (Fig. 3) was fitted onto an axis of relative time, with the scale of 0 (present) to 100 (the ancestral node for *Rhodocoma*). This shows that the three most recent speciation events are represented by the three sister-species pairs (*Rh. alpina–Rh. fruticosa, Rh. capensis–Rh. gracilis,* and *Rh. foliosa–Rh. gigantea*).

Macroecology

The macroecological analysis was restricted to *Rhodocoma,* for which all species were sampled and all nodes robustly resolved. The NMDS ordination of the average Jaccard values of the eight *Rhodocoma* species is depicted in Fig. 4. Distances between any two spheres representing species reflect the degree of overlap in the ecological parameters scored: nearer spheres represent species that overlap more along the component environmental gradients or ecological characters than do species represented by more distant spheres. These results demonstrate that closely related species do not necessarily occupy the closest points in the ordination and sister species do not generally exhibit greater ecological overlap than non-sister species (Fig. 5; *t*-test, *P* = 0.35). This is in contrast to a strong positive correlation between genetic distance and phylogenetic distance (e.g., Fig. 6; *P* < 0.05). Because genetic distance is a good predictor of phylogenetic distance, and because the likelihood
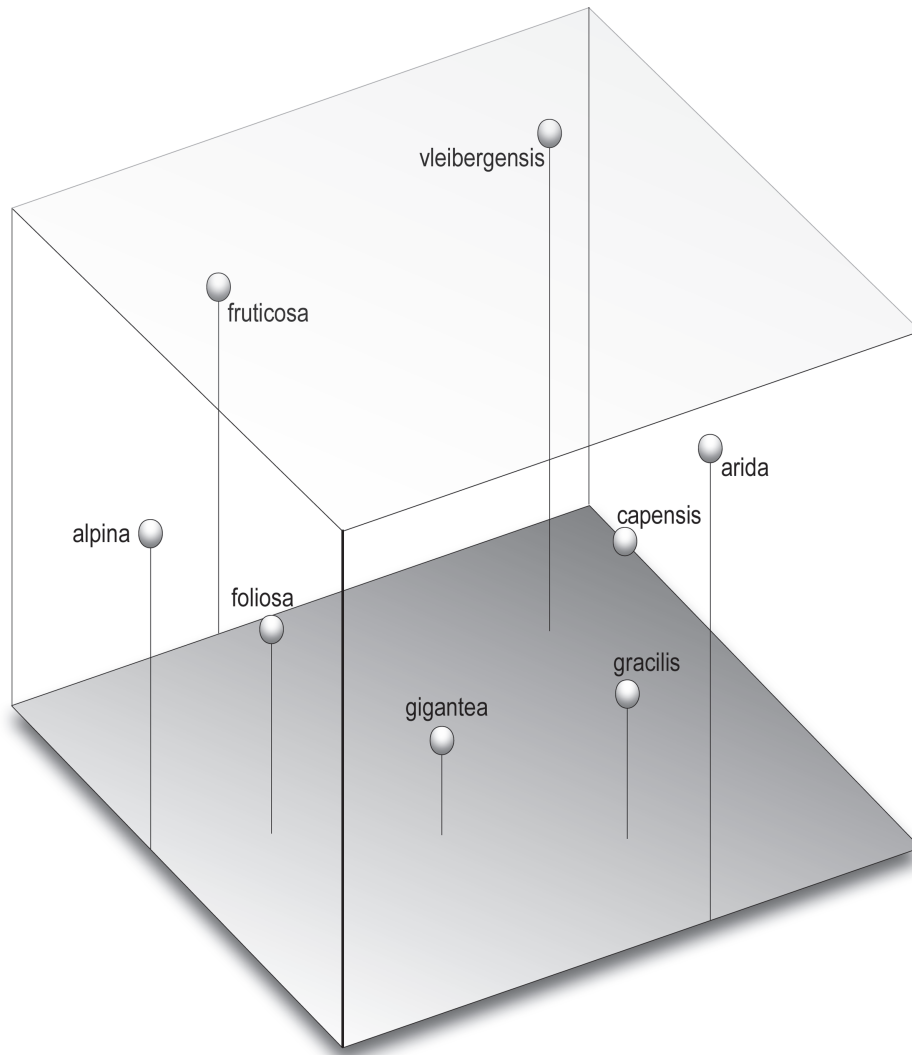
Fig. 4.—Non-metric multidimensional scaling of average ecological Jaccard values for the extant species of *Rhodocoma*. Species represented by spheres and listed by their specific epithets only.
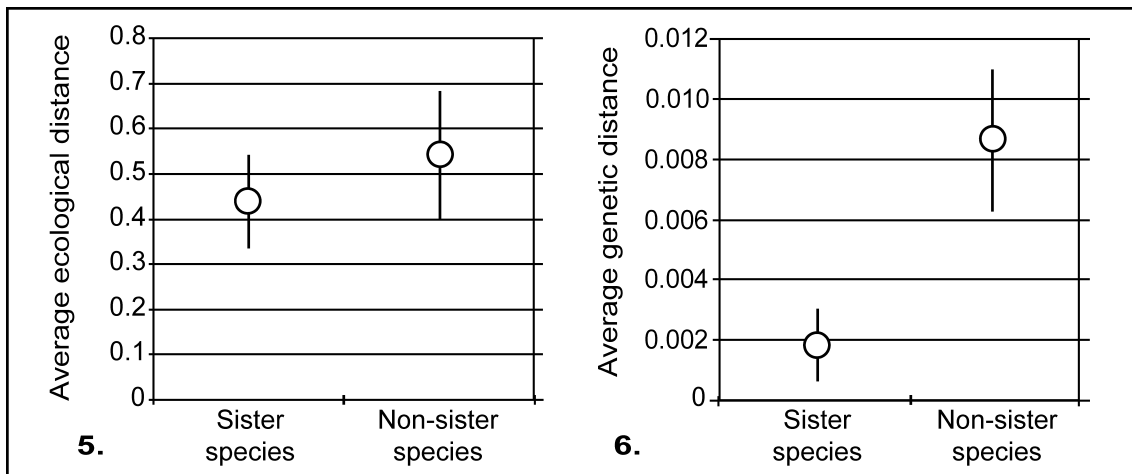
Fig. 5–6.—Relationship between phylogenetic relatedness and average ecological (5) or genetic (6) distance. Vertical lines about each point represent standard deviations.
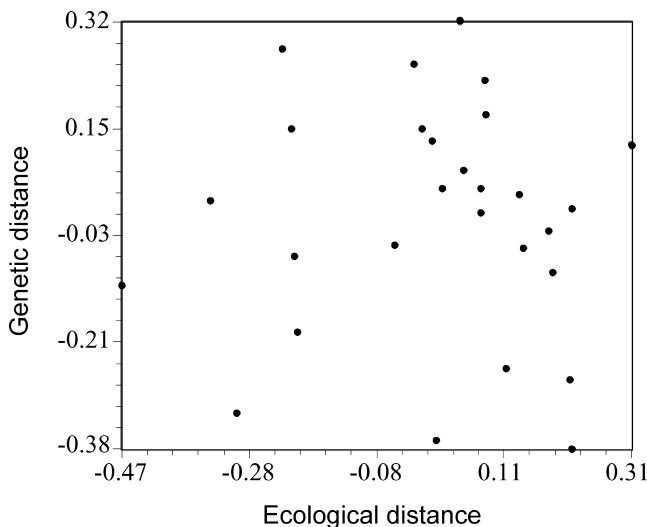
Fig. 7.—Relationship between genetic distance and ecological distance: a plot of pairwise genetic distances against pairwise ecological distances for each species pair in *Rhodocoma*.

ratio test did not reject the assumption that genetic distance is proportional to time since divergence (i.e., sequence evolution has been clock-like), a plot of pairwise ecological distances against pairwise genetic distances (Fig. 7) graphically depicts the lack of any correlation between ecological distance and phylogenetic distance or time since divergence. A Mantel Test confirms this ($r = -0.081$; $P = 0.366$). An analysis of the individual ecological parameters contributing to differentiation of the most recently diverged species (i.e., sister species) reveals that differentiation has occurred along multiple ecological axes (Fig. 8).

Historical Ecology

Using optimization procedures, hypotheses of ancestral ecologies (habitat plus fire survival mode) were constructed (Fig. 9) and a Jaccard ecological similarity matrix for both ancestral and extant species was produced. Based on this Jaccard matrix, a combined NMDS ordination of both ancestral (internal) and extant (terminal) nodes (Fig. 10, 11) was performed, allowing the inference of the relative extent and trajectory of ecological differentiation among not only sister species, but all lineages through time.

The ancestral species to all *Rhodocoma* may have been a reseeding species distributed along middle elevations on well-drained and stony sandstone-derived soils in regions with 500–1000 mm of rain per year, distributed either predominantly during winter months (DELTRAN) or more evenly throughout the year (ACCTRAN). The ACCTRAN option depicts a habitat similar to that along the coastal mountains of the present day southern Cape. The basal split within the genus appears to have occurred primarily along an altitudinal gradient, with the *Rh. vleibergensis–Rh. alpina* lineage remaining at upper-middle to high elevations (node 4), and the lineage leading to the remainder of *Rhodocoma* contracting its range to middle elevations below 900 m (node 2). In the latter lineage, the cladogenic event represented by node 2 is associated with a shift from stony soils of medium depth to shallow soils over bedrock (the *Rh. arida–Rh. gracilis* lineage; node 3). The cladogenic event represented by node 3 is associated primarily with a shift by the *Rh. arida* lineage into a much lower rainfall regime (presently occurring in the Little Karroo), concomitant with the expansion by the *Rh. capensis–Rh. gracilis* lineage from shallow soils over bedrock, onto deeper, rockless soils. The cladogenic events represented by nodes 4, 5a, and 5b are associated with the evolution of the resprouting fire survival mode from re-
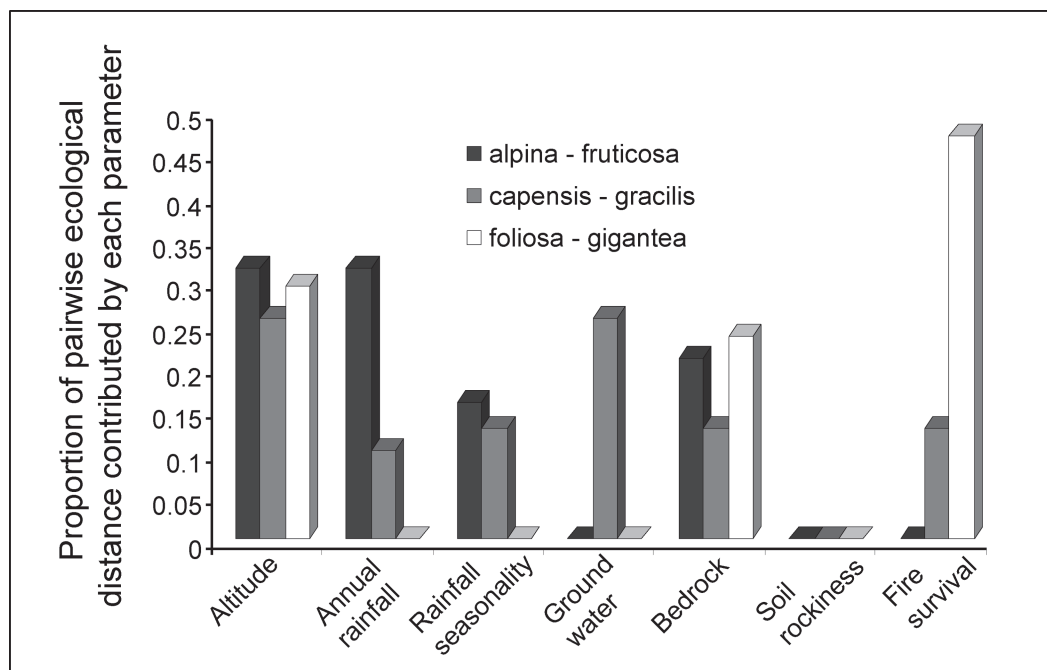

Fig. 8.—Proportion of total ecological distance between sister species in *Rhodocoma* contributed by each parameter.
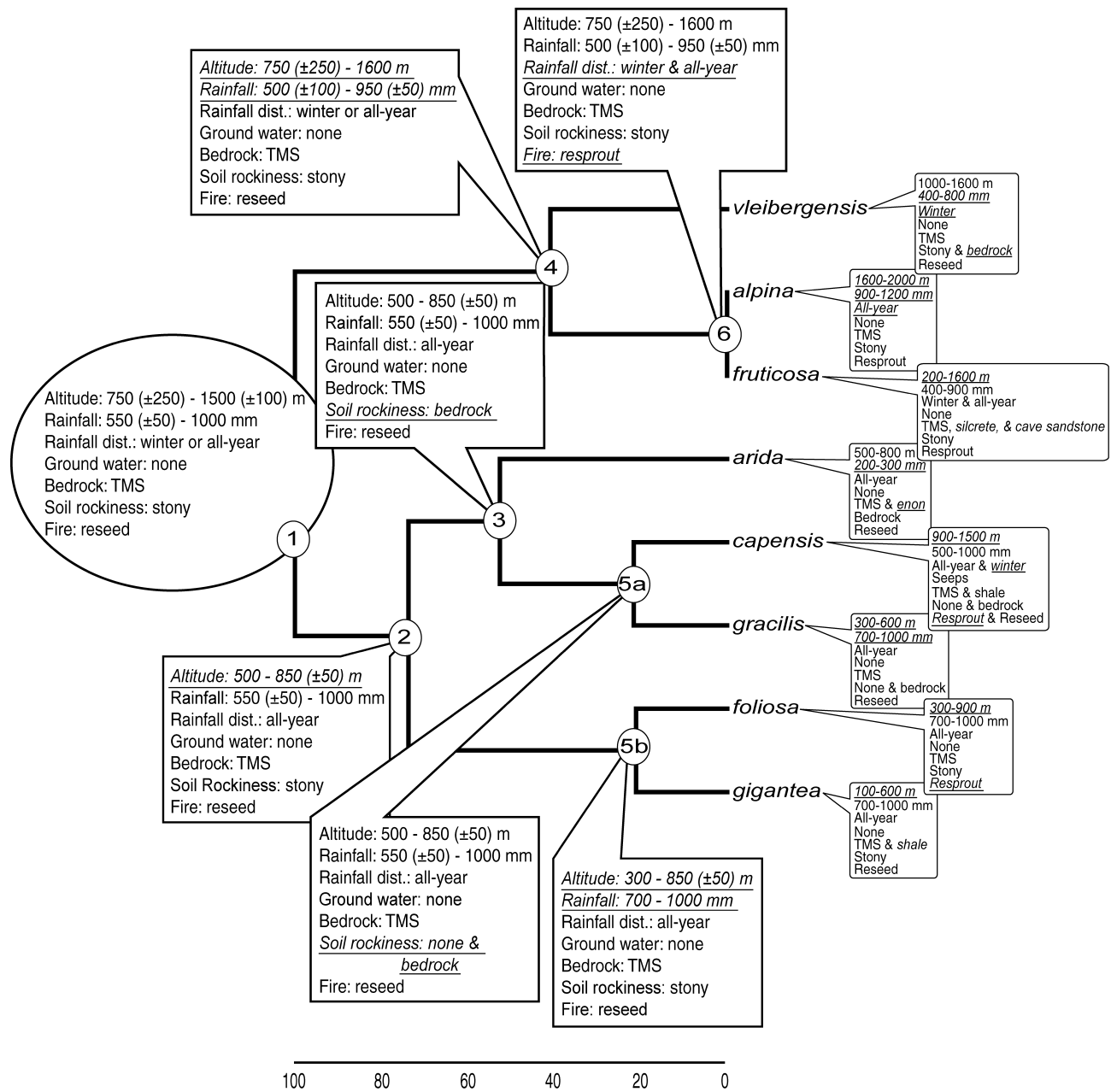
Fig. 9.—Ancestral ecological reconstructions in *Rhodocoma*. Reconstructions utilized the entire cladogram (including the outgroup), but are shown here for *Rhodocoma* only. Inferred state changes in italics and underlined. States separated by an "&" indicates that both states are present in that taxon or ancestor. States separated by an "or" indicates ambiguity (sensu ACCTRAN/DELTRAN) in the optimization procedure as to which state is reconstructed as optimal for that particular ancestor. Nodes numbered chronologically; the oldest (1) represents the ancestral node for *Rhodocoma*. TMS = Table Mountain Sandstone. Scale of 100 arbitrary.

seeding ancestors independently in the *Rh. alpina–Rh. fruticosa, Rh. capensis,* and *Rh. foliosa* lineages, successively. Concomitant with a shift in fire survival mode, there is an incomplete partitioning along an altitudinal gradient between node 5b descendants *Rh. foliosa* and *Rh. gigantea.* The subsequent cladogenic event represented by node 5a is associated with the evolution of *Rh. capensis* into exclusively moist, seepage habitats, thereby releasing it from a direct reliance on rainfall for water for at least part of the year and, concomitant with this, expanding to encompass a wider range of—including drier—rainfall regimes. All of the most

recent speciation events leading to extant sister species (including that represented by node 6) are associated with partitioning along altitudinal gradients, as well as expansions onto soils derived from additional types of bedrock such as silcrete, shale, and enon conglomerate.

DISCUSSION

Phylogeny

Precise comparison of the *Rhodocoma* topology obtained here and that obtained by Linder and Vlok (1991) is impos-
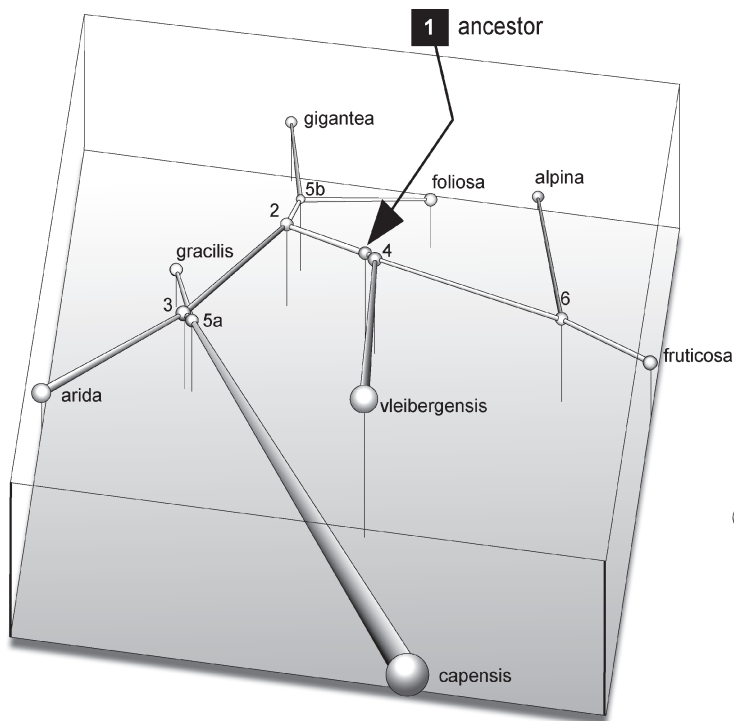
10. ACCTRAN   **11. DELTRAN**

Fig. 10–11.—Non-metric multidimensional scaling of average Jaccard values for the extant species (terminal nodes) and internal (ancestral) nodes of *Rhodocoma*. Extant species listed by their specific epithets only; internal nodes numbered chronologically from the ancestral node, as obtained from the chronogram in Fig. 3.—10. ACCTRAN optimization of rainfall seasonality (ancestor and node 4 optimized to "all-year rainfall").—11. DELTRAN optimization of rainfall seasonality (ancestor and node 4 optimized to "winter rainfall").
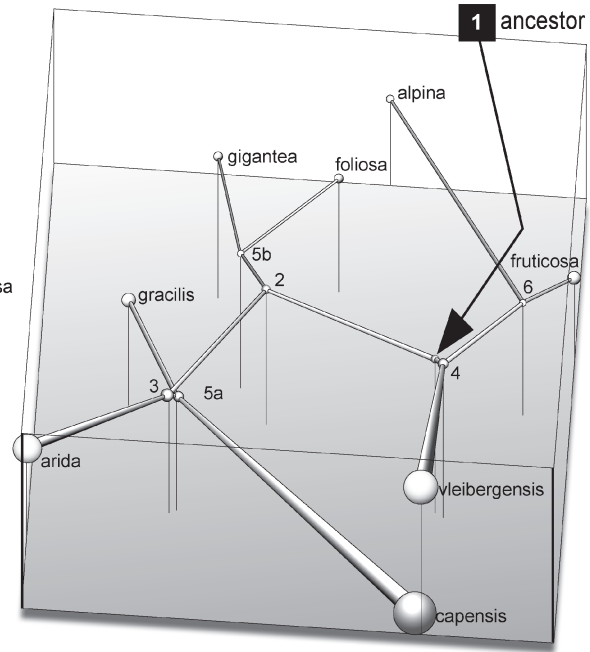
sible because *Rh. vleibergensis* and *Rh. foliosa* (described or assigned species status after 1991) were not represented in the study of Linder and Vlok. Even accounting for this sampling discrepancy, the topologies from both studies appear to be generally incongruent. A decision as to which topology is more strongly supported is not straightforward, as both studies have their potential weaknesses. The analysis of Linder and Vlok was based on fewer than 15 morphological characters, whereas the current analysis was based on ca. 6000 aligned nucleotide bases and over 230 informative characters. Although the current analysis provides more strongly supported nodes in terms of bootstrap and raw character support measures, it is compromised by the interpretive complications arising when working only with plastid sequences due to the potential for not detecting phenomena such as introgression and lineage sorting (Doyle 1992). The current analysis, however, has a complete sampling of the extant species of *Rhodocoma,* and so the details of the topological discrepancies will not be discussed and the topology of Linder and Vlok will not be considered further.

One noteworthy aspect of the topology from the current analysis is the position of *Restio insignis* as sister to *Rhodocoma,* the implications of which are twofold. The first, that the genus *Restio* Rottb. is not monophyletic, is not surprising given that the genus of ca. 89 species is recognized primarily on the basis of symplesiomorphic characters (Linder 1991) and that the combined molecular and morphology-based cladistic analysis of the African Restionaceae by Eldenäs and Linder (2000), which included seven *Restio* species, suggested that the genus may indeed be polyphyletic.

The second implication is that *Thamnochortus* and *Rhodocoma* may not be sister clades, thereby calling into question a long held view of relationships (e.g., Linder 1984; Linder et al. 2000; Eldenäs and Linder 2000), as well as the homology of the pendulous male spikelets, the culm anatomy with scattered cavities, and the pollen aperture shape shared by both. Regarding each of these questions, however, it seems prudent to reserve judgment until a better taxonomic sampling of the genus *Restio,* as well as nuclear data, are obtained.

This study provides strong support for the monophyly of *Rhodocoma* (Fig. 2), with this clade having been recovered in 99% of the 1000 bootstrap replicates. Curiously, however, the initial morphological support for *Rhodocoma* was weak. Linder (1984) separated *Rhodocoma* from *Restio* because it shared the above-mentioned synapomorphies with the highly distinctive *Thamnochortus,* but support for the monophyly of *Rhodocoma* was limited to a seed coat character. An additional autapomorphy for the genus is the reduction to just one flower per pistillate spikelet, although this transition has occurred independently elsewhere in Restionaceae (Linder 2002).

The basal split in *Rhodocoma* is inferred to be that between the clades (*Rh. vleibergensis* (*Rh. alpina, Rh. fruticosa*)) and ((*Rh. gigantea, Rh. foliosa*) (*Rh. arida* (*Rh. capensis, Rh. gracilis*))), each clade being recovered in 100% of the bootstrap replicates. The former clade may be characterized as generally occurring at higher altitudes (>1000 m) than the latter, although one member of the former (*Rh. fruticosa*) is highly variable and also extends into lower el-

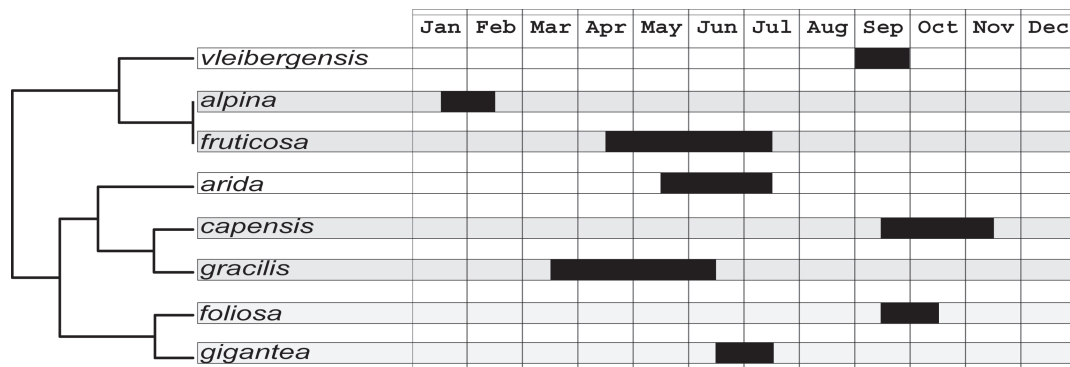|	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
vleibergensis									■			
alpina		■										
fruticosa				■	■	■						
arida					■	■						
capensis										■	■	
gracilis				■	■	■						
foliosa										■		
gigantea							■					

Fig. 12.—Range of flowering times recorded for species of *Rhodocoma*. Determined from observations in the field by the authors, in addition to herbarium specimen data from the Bolus (BOL) and Zurich (Z) herbaria. Species abbreviated by their specific epithets.

evations. There are no known morphological characters supporting the monophyly of the clade (*Rh. vleibergensis* (*Rh. alpina, Rh. fruticosa*)), resulting from the membership of *Rh. vleibergensis.* Indeed on herbarium sheets *Rh. vleibergensis* is difficult to distinguish from *Rh. arida,* a member of the other clade, although these two species are easily distinguished in the field based on habit and geography (Linder 2002). The loss of a sheath mucro would unambiguously support the monophyly of the second clade ((*Rh. gigantea, Rh. foliosa*) (*Rh. arida* (*Rh. capensis, Rh. gracilis*))), although parallel losses of the mucro would also have to be hypothesized to have occurred within *Thamnochortus* and in *Rh. fruticosa* (Linder 2002).

In two out of three cases, the sister species resolved here are concordant with expectations. *Rhodocoma foliosa* is resolved as sister to *Rh. gigantea,* the former being a parapatric segregate of the latter and distinguished by minor differences in spikelet size, habit, and ecology (Linder 2002). Previous reviews treated these two as conspecific (Linder 1991). Both species occur on the wet, south-facing slopes, or cool valleys of the coastal mountains in the southern Cape and are distinguished from others in the genus by their greater stature and the clusters of sterile branches at the nodes of fertile branches. The position of *Rh. alpina* as sister to *Rh. fruticosa* is consistent with the former's status as a higher altitude segregate of the latter, with larger spathes and shorter, stouter culms (Linder and Vlok 1991). Although *Rh. alpina* is parapatric to nearly sympatric with *Rh. fruticosa,* their differing flowering times (summer vs. autumn, respectively; Fig. 12) may aid in maintaining their specific boundary. The only sister species relationship that is unexpected is that between *Rh. gracilis* and *Rh. capensis,* but perhaps only because of the autapomorphic morphology and ecology of the latter. *Rhodocoma capensis* is unusual in the genus because of its huge tussock-forming habit, dense whorls of fertile branches, and unusually diffuse inflorescence structure. It is also the only species to have made the transition from well-drained soils to seepages where constant access to groundwater is provided for extended periods throughout the year.

Historical Ecology

According to Linder and Vlok (1991), the differentiation of sister species and lineages in *Rhodocoma* along ecologi-

cal, rather than geographical gradients provided evidence for the importance of ecological speciation (Schluter 2000) in the genus. Indeed, these same criteria are commonly used to make inferences of ecological speciation (e.g., Linder 1985; Schliewen et al. 1994; Shaw et al. 2000; Johannesson 2001). Where sister lineages are sympatric or parapatric and specific boundaries are maintained despite the potential for gene flow provided by their geographical proximity to one another, it is plausible that the differential selective forces associated with any interspecific ecological differences may have been strong enough to override the homogenizing effects of gene flow and affect speciation. For example, the three species in Linder and Vlok's clade (*Rh. alpina* (*Rh. arida, Rh. fruticosa*)) are distributed in ''rings'' around each other, with *Rh. arida* being widespread in the Little Karoo, *Rh. fruticosa* occurring on the slopes of the mountains surrounding the Little Karoo, and *Rh. alpina* occurring at the highest altitudes on these mountains. These three nested sets of sister lineages were not only parapatric, but geographically enveloped each other, making a model of allopatric speciation difficult to conceive and identifying their differentiation along temperature and moisture gradients as possible agents of the speciation events from which they came. In the topology obtained in the current analysis, however, the ''center'' of the ring, *Rh. arida,* is no longer sister to the parapatric *Rh. alpina* and *Rh. fruticosa,* so this particular scenario of ecological, sympatric speciation is no longer applicable.

In the topology of *Rhodocoma* obtained here, two of the three pairs of sister species (*Rh. alpina* and *Rh. fruticosa*; *Rh. foliosa* and *Rh. gigantea*) are parapatric, and the members of both pairs are differentiated along ecological gradients (*Rh. alpina* and *Rh. fruticosa* primarily along the linked gradients of altitude and rainfall, whereas *Rh. foliosa* and *Rh. gigantea* differ primarily in fire survival mode). Additionally, each of these sets of sister species are distinguished by differences in flowering time; e.g., spring vs. autumn in the case of *Rh. foliosa* and *Rh. gigantea,* and summer vs. autumn in the case of *Rh. alpina* and *Rh. fruticosa* (Fig. 12). The evolution of prezygotic reproductive barriers (such as flowering time shifts) is one plausible response to strong differential selection among sympatric or parapatric populations or species (Grant 1966; Levin 1978), and therefore is compatible with an ecological model of speciation or spe-

cies maintenance (Ehrlich and Raven 1969; Andersson 1990). Although each member of the third sister species pair of *Rh. capensis–Rh. gracilis* also flowers in a different season and occurs in a habitat substantially different from the other's in terms of groundwater availability and altitude (Table 3; Fig. 9), these species are allopatric (Linder and Vlok 1991) and so there is no evidence that these species diverged in sympatry. This is not to say that ecological factors and the attendant selective forces did not have a prominent role in the evolution of each of these latter two species, but only that there is no evidence that these forces operated, or that their divergence was initiated, in sympatry or parapatry.

Regardless of the driving forces and geography of speciation in *Rhodocoma,* it is evident that the history of diversification in the genus has been one of rapid ecological differentiation among sister lineages, and genetic isolation via shifts in flowering times. Substantial ecological divergence among even the youngest of species pairs indicates that the selective forces driving these processes are strong enough to effect substantial amounts of change in relatively short periods of time, and are potentially important factors promoting the origin and persistence of species diversity not only in *Rhodocoma,* but also the African Restionaceae as a whole. The speed and extent to which sister lineages have diverged ecologically can be appreciated in part by the observation that phylogenetic or genetic distances are not reliable indicators of ecological distance (Fig. 5–7), but this can only be fully understood with some estimate of the relative trajectories and nature of ecological divergence events (Fig. 10, 11). Such an estimate reveals that the historical ecology of *Rhodocoma* is complex and, on the whole, directionless such that at any time the ecological trajectories of some lineages are convergent, in terms of net Jaccard distance, with those of other initially divergent lineages. While for some time there may be a positive correlation between ecological distance and time during the divergence of two sister lineages, the overall pattern of ecological differentiation for the clade as a whole may mask this correlation.

A further implication of the high rates and overall pattern of ecological evolution in *Rhodocoma* is that the concept of phylogenetic niche conservatism (Harvey and Pagel 1991; Holt and Gaines 1992; Holt 1996) is not supported. An adequate evaluation of this concept probably eludes us here not only because a single and unifying concept of the ''niche'' may not be tenable, but also because we do not presume to have accurately portrayed the niches of these species. At best we have quantified just a few niche dimensions, with a strong emphasis on habitat. Nevertheless, niche conservatism is consistently conceptualized in terms of the tendency for adaptation in ecological dimensions to be slow and evolutionarily conservative such that closely related species are more ecologically similar than distantly related species. Given the seven ecological parameters analyzed here, this is clearly not the case for *Rhodocoma.* While similar results have been found elsewhere (e.g., Rice et al. 2003), studies that support niche conservatism are in the majority by far (e.g., Peterson et al. 1999; Ackerly 2003 and references cited therein).

An additional discovery concerning the historical ecology of *Rhodocoma* concerns the nature of change. Consistent with what was demonstrated for several other Cape clades by Linder and Hardy (2005), diverging lineages in *Rhodocoma* have consistently differentiated along multiple environmental/ecological axes. A comparison between sister species, for example, reveals that although these most recent divergence events primarily involved differentiation along altitudinal gradients, substantial shifts also occurred along rainfall gradients and in fire survival (Fig. 8). A consequence of this multidimensional nature of macroecological differentiation is an increase in the number of apparent yet, from a plant perspective, more precisely defined habitats. As suggested by Linder and Hardy (2005), this ability to more finely partition ecological space by exploiting multiple ecological dimensions may be an attribute that distinguishes species-rich from species-poor clades. Comparative studies of both species-rich and species-poor clades are needed to test this hypothesis.

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