MISSOURI BOTANICAL GARDEN

PHYLOGENY, MORPHOLOGICAL EVOLUTION, AND SPECIATION OF ENDEMIC BRASSICACEAE GENERA IN THE CAPE FLORA OF SOUTHERN AFRICA[1]

Klaus Mummenhoff,[2]
Ihsan A. Al-Shehbaz,[3]
Freek T. Bakker,[4]
H. Peter Linder,[5]
and Andreas Mühlhausen[6]

ABSTRACT

Heliophila (ca. 73 spp.), the ditypic *Cycloptychis* and *Thlaspeocarpa*, and the monotypic *Schlechteria*, *Silicularia*, *Brachycarpaea*, and *Chamira* are endemic to the Cape region of South Africa, where they are the dominant genera of Brassicaceae. They may be regarded as the most diversified Brassicaceae lineage in every aspect of habit, leaf, flower, and fruit morphology. The characters used in the separation of these genera and their species, especially fruit type (silique vs. silicle), dehiscence (dehiscent vs. indehiscent), compression (latiseptate vs. angustiseptate), and cotyledonary type (spirolobal, diplecolobal, twice conduplicate), have been used extensively in the delimitation of tribes. The relationship and taxonomic limits among these genera are unclear and controversial.

The present ITS study demonstrates the monophyly of tribe Heliophileae, with *Chamira* as sister clade. The other five smaller genera above are nested within two of the three main lineages of *Heliophila*, to which they should be reduced to synonymy. The current study reveals parallel evolution of fruit characters often used heavily in the traditional classification schemes of the family. However, the arrangement of species into three main clades largely corresponds with the distribution of morphological characters (e.g., habit, leaf shape, seed structure, inflorescence type, and presence/absence of basal appendages on the pedicels, petals, and staminal filaments) not adequately accounted for in previous studies. Estimation of divergence times of the main lineages of *Heliophila* is in agreement with recent estimations in other plant groups, all of which date the diversification against a background of aridification in the Pliocene and Pleistocene. Species of one main clade are perennial, microphyllous shrubs/subshrubs typically restricted to poor sandstone soils in the southwestern and western parts of the Cape Floristic Region of South Africa. Species of the other two clades are predominantly annuals that grow in more arid regions of Namibia and Namaqualand, as well as in the above sandstone areas of the Cape Region. The adaptive significance of various floral structures is discussed in terms of their possible role in the rapid diversification within *Heliophila*.

Key words: Brachycarpaea, Cape flora, Cape Floristic Region, Chamira, Cycloptychis, Heliophila, Heliophileae, ITS, phylogeny, radiation, Schlechteria, Silicularia, speciation, Thlaspeocarpa, trnL-F.

Seven genera of Brassicaceae, *Heliophila* (73 spp.), *Cycloptychis* (2 spp.), *Schlechteria* (1 sp.), *Silicularia* (1 sp.), *Thlaspeocarpa* (2 spp.), *Brachycarpaea* (1 sp.), and *Chamira* (1 sp.), are endemic to southern Africa (for author names of these genera and their species, see Table 1 and Appendix 1). Most species occur in the winter-rainfall area of the western Cape Floristic Region (CFR), where they represent the dominant Brassicaceae. Although several classification systems (Table 1) have been proposed (e.g., Hayek, 1911; Schulz, 1936; Janchen, 1942), the relationships among these genera remain unresolved. In a recent re-evaluation of the group, Appel and Al-Shehbaz (1997) placed the first six genera in the tribe Heliophileae and retained *Chamira* in the monotypic Chamireae. They

Table 1. Classification systems for South African endemic Brassicaceae genera. Dash (—) = taxon not recognized.

	Candolle (1821)	Sonder (1846)	Prantl (1891)	Hayek (1911)	Schulz (1936)	Appel & Al-Shehbaz (1997)
Chamira Thunb.	Heliophileae	Chamireae	Thelypodieae–Chamirinae	Chamireae	Chamireae	Chamireae
Heliophila L.	Heliophileae	Heliophileae	Thelypodieae–Heliophilinae	Heliophileae	Heliophileae	Heliophileae
Thlaspeocarpa C. A. Sm.	—	—	Thelypodieae–Heliophilinae	Heliophileae	Heliophileae	Heliophileae
Cycloptychis E. Mey.	—	—	Thelypodieae–Heliophilinae	Heliophileae	Heliophileae	Heliophileae
Brachycarpaea DC.	Brachycarpeae	—	Thelypodieae–Heliophilinae	Heliophileae	Lepidieae–Brachycarpaeinae	Heliophileae
Schlechteria Bolus	—	—	—	Alysseae–Lunariinae	Lunarieae	Heliophileae
Silicularia Compton	—	—	—	—	—	Heliophileae

characterized the Heliophileae by having dipleco-lobal cotyledons (elongated and twice transversely folded). This uncommon cotyledonary type is also known in three Australian species of *Lepidium* L. sect. *Monoploca* (Bunge) Prantl subsect. *Diploploca* Hewson (Hewson, 1981; Mummenhoff et al., 2001), making this character homoplastic.

The Heliophileae may be regarded as the most diversified Brassicaceae lineage in every aspect of habit, flower, and fruit morphology (Table 2; compiled from Appel & Al-Shehbaz, 1997). It has flowers with enormous diversity in size ranging from the largest in the family (petals to 25 mm long in *Brachycarpaea juncea* and *Cycloptychis virgata*) to nearly the smallest (petals ca. 1.2 mm long in *Heliophila pectinata*). *Heliophila* has a wide range of flower color, including blue, a color otherwise known in Brassicaceae only in the unrelated Himalayan *Solms-laubachia* Muschl. (Al-Shehbaz & Yang, 2001). Furthermore, the flowers usually have appendages on the basal portions of petals and/or staminal filaments. Several species (e.g., *H. africana*) produce beaked fruits similar to those of the tribe Brassiceae, whereas others have siliques more than 12 cm long (e.g., *H. scoparia*) or minute silicles only about 2 mm in diameter (e.g., *H. patens*). Fruit shape is quite variable (linear, lanceolate, oblong, elliptic, ovoid, or globose), whereas fruit compression in *Heliophila* varies from latiseptate (flattened parallel to the septum) to terete, and in *Brachycarpaea* it is angustiseptate (fruit flattened at a right angle to the septum) (Figs. 1, 2). Fruits may be dehiscent (*Heliophila*), indehiscent and woody (*Silicularia*), samaroid (*Thlaspeocarpa*), or even schizocarpic and with a distinct (*Cycloptychis*) or rudimentary carpophore (*Brachycarpaea*). These fruit characters (e.g., silique versus silicle, dehiscent vs. indehiscent, latiseptate vs. angustiseptate), which are used in the separation of species and genera within Heliophileae, have been used extensively in the delimitation of tribes (see Al-Shehbaz, 1984). Within *Heliophila* one finds ephemeral to perennial herbs, shrubs, and lianas to 3 m tall (*H. scandens*). Apart from previous controversial classification systems discussed by Appel and Al-Shehbaz (1997), nothing is known about the phylogenetic relationships within the Heliophileae.

The most striking features of the CFR are the high level of endemism and the remarkable species richness. Species richness, estimated at about 9000 species in an area of 90,000 km² (Goldblatt & Manning, 2000), is comparable to those of the most diverse equatorial areas, and species endemism of ca. 70% is similar to that found on islands (Linder, 2003). In a recent review on the origin of the Cape

Table 2. Comparison of the six genera of the tribe Heliophileae. Data compiled from Appel and Al-Shehbaz (1997).

| Character	Cycloptychis	Heliophila	Schlechteria	Silicularia	Thlaspeocarpa	Brachycarpaea
Number of species	2	73	1	1	2	1
Habit	shrubs or subshrubs	herbs, subshrubs, shrubs, or lianas	subshrubs	subshrubs	herbs	subshrubs
Appendage of staminal filament	absent	present or absent	absent	absent	present	absent
Style	long or short	long to obsolete	obsolete	short to obsolete	obsolete	short to obsolete
Ovule number	2	2 to 70	2 to 4	2	1 or 2	2
Orientation of fruiting pedicels	erect	ascending to recurved	recurved	recurved	recurved	ascending
Fruit shape	elliptic or ovate	orbicular to linear	elliptic	ovate to orbicular	elliptic to orbicular	oblate, subdidymous
Fruit compression	latiseptate	latiseptate to terete	latiseptate	latiseptate	latiseptate	angustiseptate
Fruit (dehiscence)	dehiscent	dehiscent	indehiscent	indehiscent	indehiscent	dehiscent
Carpophore	present	absent	absent	absent	absent	absent
Septum	present	present	absent	absent	absent	present
Valve texture	leathery or woody	membranous to leathery	thin leathery	± woody	thin leathery	woody
Valve sculpture	reticulate-rugose or prominently veined	smooth	smooth	reticulate rugose	with slightly raised veins	smooth to rugose
Seed shape	oval	oblong to orbicular or globose	oblong to oval	orbicular to oval	orbicular	globose
Seed wing	present or absent	present or absent	absent	absent	present	absent
Cotyledons	diplecolobal	diplecolobal	diplecolobal	diplecolobal	diplecolobal	spirally twisted
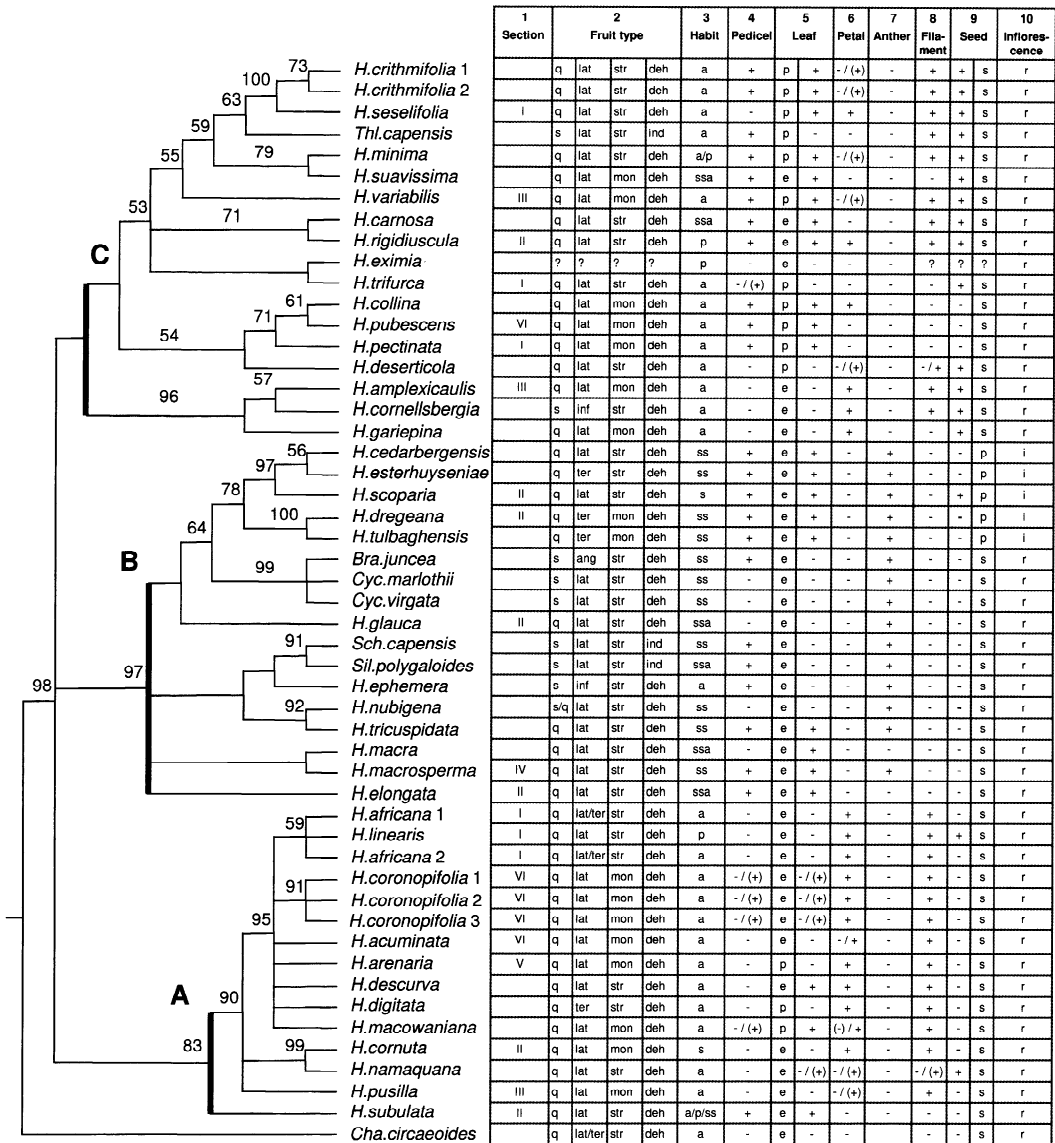
Figure 1.   Strict consensus ITS tree with the distribution of morphological data in *Heliophila* and related genera. *Cleome spinosa* (Cleomaceae), *Aethionema saxatile*, *Alliaria petiolata*, *Rorippa amphibia*, and *Cardamine matthioli* (Brassicaceae) served as the outgroup. The consensus tree is based on 14,560 equally parsimonious reconstructions found after heuristic search; jackknife support > 50% (10,000 replicates) are indicated at the branches. Taxon abbreviation: *H.* = *Heliophila*, *Thl.* = *Thlaspeocarpa*, *Bra.* = *Brachycarpaea*, *Cyc.* = *Cycloptychis*, *Sch.* = *Schlechteria*, *Sil.* = *Silicularia*, *Cha.* = *Chamira*.

[1] **Sectional classification of Sonder (1846):** I = *Orthoselis* subsection 1: herbaceous species, II = *Orthoselis* subsection 2: shrubby species, III = *Ormiscus*, IV = *Lanceolaria*, V = *Pachystylum*, VI = *Leptormus*. *Heliophila* species not characterized by Roman numerals were not recognized by Sonder (1846) or they represent other genera. [2] **Fruit types: relative fruit length:** q = silique, s = silicle; **orientation and degree of the fruit compression:** lat = latiseptate, ang = angustiseptate, ter = terete, inf. = inflated; **fruit margin:** mon = moniliform (fruits deeply constricted between the seeds), str = fruits with straight margins; **fruit opening:** deh = dehiscent, ind = indehiscent. [3] **Habit:** a = annual herb, p = perennial herb, s = shrub, ss = subshrub, ssa = subshrub, but annual shoots arising from a woody crown. [4] **Pedicel:** + = with two small bracts, − = without two small bracts. [5] **Leaf:** p = pinnately divided, e = entire; − = leaves exstipulate, + = leaves subtended by two minute stipules. [6] **Petal:** − = without basal appendages, + = with basal appendages. [7] **Anther:** + = presence of apicula, − = apicula absent. [8] **Filament:** + = with basal appendages, − = without basal appendages. [9] **Seed:** − = wingless or with a very narrow margin, + = distinct wing; p = papillate, s = smooth. [10] **Inflorescence:** r = raceme, i = intercalary inflorescence. Data compiled from Marais (1970), Bean (1990), and Goldblatt and Manning (2000) and the authors' studies of specimens at MO.

flora, Linder (2003) suggested that the high level of endemism might be the consequence of ecological and geographical isolation of the CFR. He also suggested that the species richness might be the consequence of radiation that started between 18 and 8 million years ago, which might be accounted for by the diverse limitations to gene flow (e.g., dissected landscapes, pollinator specialization), as well as by a climatically and topographically complex environment (e.g., altitudinal variation, soil types) allowing numerous niches and resulting in highly fragmented distribution ranges.

Studies are needed to provide (i) phylogenetic data demonstrating the monophyly of diversifying lineages in the CFR, (ii) more molecular clock estimates to accurately date the radiation, and (iii) species-level phylogenies to detect sister-species relationships to study speciation. The current study addresses these needs by providing a robust phylogeny of the tribe Heliophileae based on the nuclear ITS region of 55 of its 80 species. We used nonparametric rate smoothing (NPRS) of Sanderson (1997), as well as a "forced" (global) clock approach to date the diversification of Heliophileae by implementing fossil data and published calibration points (Wikström et al., 2001) and a range of published rDNA ITS substitution rates. In addition, the pattern of species distribution in relation to their ecology and phylogeny was investigated in an attempt to get insights into speciation mode in this lineage.

MATERIAL AND METHODS

MORPHOLOGY

All species of *Brachycarpaea, Cycloptychis, Heliophila, Silicularia, Schlechteria,* and *Thlaspeocarpa* have been critically evaluated morphologically. The characters studied, all considered to be taxonomically important (see Marais, 1970), include habit; presence vs. absence of staminal appendages; ovule number per ovary; orientation of fruiting pedicels; fruit shape, dehiscence, compression, presence vs. absence of carpophore, development of the septum, valve texture, and valve sculpture; seed shape and presence vs. absence of wing; and cotyledonary type. These are listed in Table 2 and Figure 1 and need no further details.

DNA EXTRACTION, GENE AMPLIFICATION, AND SEQUENCING

Plant material, locality information, voucher details, and GenBank accession numbers are given in Appendix 1. *Cleome spinosa* (Cleomaceae), *Aethi-*onema saxatile, Alliaria petiolata, Rorippa amphibia,* and *Cardamine matthioli* were chosen as outgroups on the basis of previous molecular phylogenetic studies (Koch et al., 2001). Methods for DNA extraction, PCR amplification of the rDNA ITS and cpDNA *trn*L-F regions, BigDye terminator labeled sequencing, sequence assembly and alignment are described in Bowman et al. (1999) and Mummenhoff et al. (2001, 2004).

DATA ANALYSIS

The aligned sequences were subjected to both parsimony and Bayesian analysis, using PAUP*4.0b10 (PPC/Altivec) and MrBayes 3 (Ronquist & Huelsenbeck, 2003) implemented on a Macintosh G4 computer. Jackknife analysis was carried out using PAUP* with settings so as to emulate Parsimony Jackknifer (Farris et al., 1996): percentage of characters deleted in each replicate = 37, "fast" stepwise addition, and "Jac" re-sampling method. Heuristic searches involved TBR branch swapping, MULPARS, and collapse branches when maximum length is zero. Starting trees were generated by 1000 cycles of random addition sequence holding 3 trees at each step, and keeping no more than 1000 trees > length 904 steps. Trees resulting from this search were then used as starting trees in a subsequent search during which they were swapped to completion as far as possible.

Bayesian analysis was performed on the ITS alignment using settings derived from ModelTest analysis (Posada & Crandall, 1998): the maximum likelihood model employed 6 substitution types ("nst = 6") and rate variation across sites was modeled using a gamma distribution (rates = "gamma"), while invariant sites were also assumed. The Markov chain Monte Carlo search was run with 4 chains, one of which "cold," for 1,000,000 generations, with trees being sampled every 100 generations. After discarding the first 10% of trees as "burnin," Bayesian search results were summarized by 50% majority rule consensus and the posterior probability values ("clade credibilities") are indicated at the branches (Fig. 2).

Likelihood ratio testing was performed using the Bayesian 50% majority rule consensus tree in order to check for a molecular clock. An ultrametric tree was then produced from the Bayesian consensus tree using both NPRS as implemented in the program "r8s" version 1.06 (beta) described by Sanderson (1997), as well as by "forcing" a clock in PAUP*. The Heliophileae ITS tree with branch lengths in six decimals was saved and input in r8s, in which the following branch length format settings

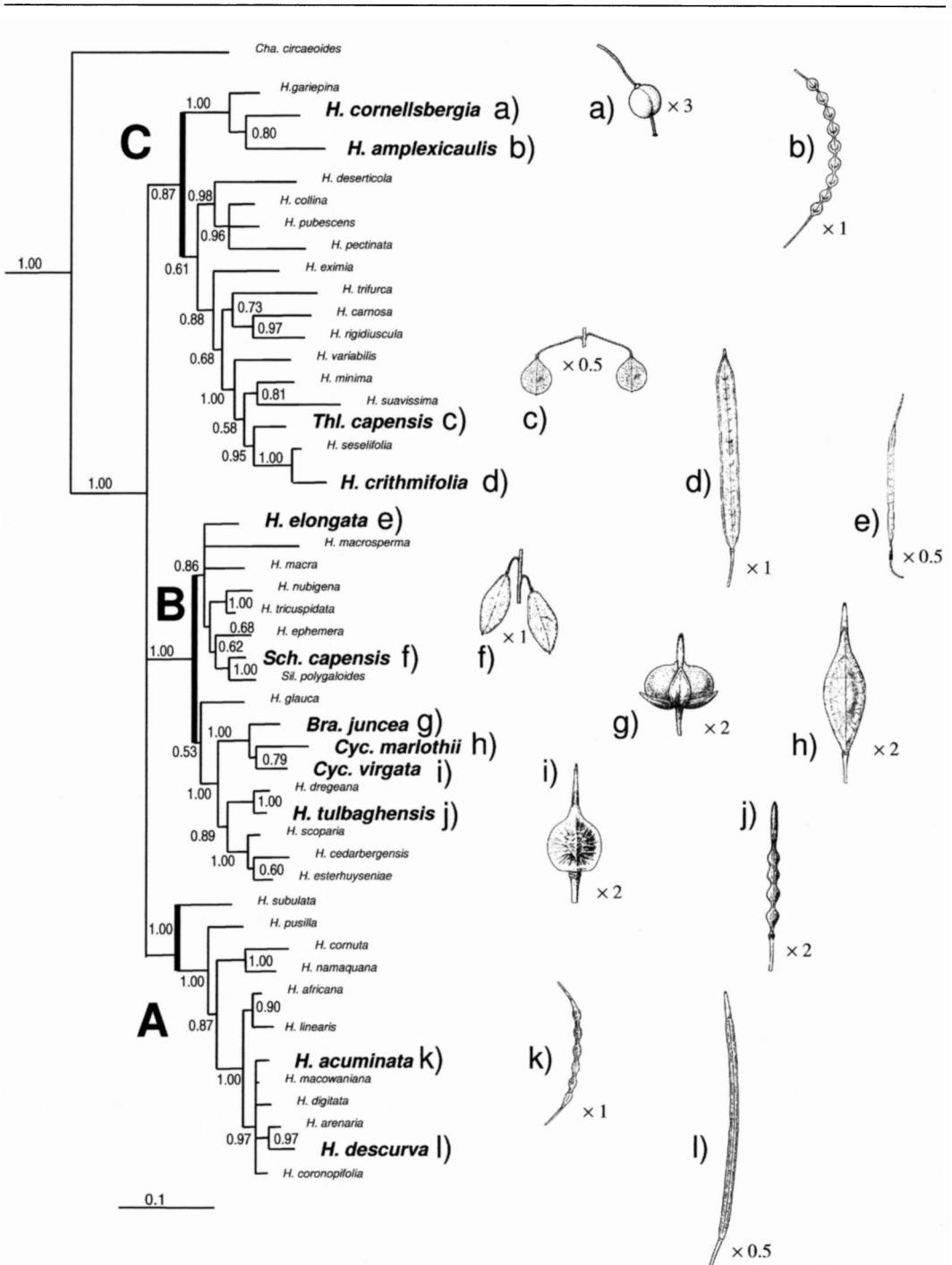Figure 2. Bayesian consensus tree with mean branch length and a posteriori probabilities, and the distribution of fruit types among the main clades of *Heliophila* s.l. Taxon abbreviation follows Figure 1. *Cleome spinosa* (Cleomaceae), *Aethionema saxatile, Alliaria petiolata, Rorippa amphibia,* and *Cardamine matthioli* (Brassicaceae) served as the outgroup. Illustrated species written in larger bold font.

were used: "length = persite, nsites = 498, ultra-
metric = no, round = yes"; divergence times of
other than the fixed node (see below) were esti-
mated using the settings: "method = NPRS, algo-
rithm = POWELL," and "set num_time_guesses =
10" in order to ensure optimal exploration of so-
lution space.

In order to estimate error around node ages, we
performed 100 replications of constrained jackknife
analysis of the data matrix, saving each tree under
the topological constraints of the Bayesian consen-
sus tree. In this way, identical topology trees are
produced with variation in branch lengths reflecting
"substitutional noise" as picked up by the re-sam-
pling procedure. All hundred trees were then an-
alyzed in r8s with respect to their A, B, and C
nodes, using the settings above. Age estimates
around these nodes were summarized using the
"profile" command.

In addition, we prepared a "forced (global)
clock" tree in PAUP*. In this approach an ultra-
metric tree is produced by assuming overall clock-
like behavior of the data, under otherwise the same
likelihood model as the one arrived at in the Bayes-
ian analysis described above. Forced clock trees
were made in- and excluding the outgroup *Cleome
spinosa*.

As an alternative to the calibration using the
dates from Wikström et al. (2001), we also cali-
brated our ultrametric trees by applying a range of
published rDNA ITS substitution rates. We took the
rates published for *Dendroseris* D. Don (Asteraceae)
from Sang et al. (1994), i.e., 3.9×10^{-9} subst./site/
yr, and *Soldanella* L. (Primulaceae) from Zhang et
al. (2001), i.e., 8.3×10^{-9} subst./site/yr, to repre-
sent the currently known range of substitution rate
for this region in angiosperm eudicots. Ultrametric
tree node height H is the result of accumulated
substitutions per site along two evolutionary lines;
therefore height = substitution rate $r \times$ twice di-
vergence time T, and T can be calculated as $H/2r$.
Rate calibration was performed in TreeEdit v.
1.0a10 (Rambaut & Charlston, 2001) by dividing
the ultrametric node heights by twice the substi-
tution rate, and multiplying by the number of sites
used (n = 498) in order to arrive at node heights
in millions of years.

We used fruit fossil data of *Rorippa* Scop. (2.5–
5 million years old; Mai, 1995) to constrain the
clade containing *Rorippa* and *Cardamine* L. at 2.5–
5 mya minimally. These genera have been shown
to be sister taxa in several phylogenetic studies
(e.g., Mummenhoff et al., 2001, 2004).

ECOLOGICAL OPTIMIZATIONS

We followed the species-level taxonomy of Ma-
rais (1970) because it is the only comprehensive
account of the Heliophileae. However, he did use
a rather broad species concept and recognized
many infraspecific taxa occupying ecologically het-
erogeneous habitats. The ecological attributes of
the species, and their distribution ranges, were tak-
en primarily from Marais (1970). In addition, the
herbarium holdings of BOL and PRE were surveyed
for collection data, though their identities were not
verified because most of them were studied by Mar-
ais and are well curated. Altitudinal data were
checked against the ranges in Germishuizen and
Meyer (2003) and Goldblatt and Manning (2000).

Species were scored for the following four envi-
ronmental parameters:

1. Distribution ranges (Roggeveld, Drakensberg,
Eastern Cape, Southern Cape, Swartruggens, cen-
tral Cape mountains, Cedarberg-Nieuwoudtville,
Kamiesberg, Gordonia, Richtersveld, Namib).
These ranges follow a combination of topographical
features of southern Africa that often provide nat-
ural boundaries to species distribution, and this
theme is used in combination with actual climatic
zones (see Fig. 3).

2. Vegetation type (woodland, forest, grassland,
Karoo, thicket, Namaqua Broken Veld, succulent
Karoo, Renosterveld, Strandveld, Fynbos). These
types are largely structural rather than floristic. Ka-
roo refers to the shrubby to grassy semi-arid sum-
mer-rainfall vegetation of the central uplands of
southern Africa, whereas succulent Karoo is a very
succulent shrubby winter-rainfall semidesert vege-
tation (Rutherford & Westfall, 1986). Namaqua
Broken Veld is a taller shrubland characteristic of
the granitic escarpment of Namaqualand (Acocks,
1975). Renosterveld, strandveld, and fynbos are
typical vegetation types of the Cape Floristic Re-
gion. The first is a widespread shrubland on richer
soils dominated by *Elytropappus rhinocerotis* (L. f.)
Less. (Asteraceae). Strandveld is a non-pyrophytic
woody shrubland of coastal dunes, and fynbos is
the typical pyrophytic heath vegetation of oligotro-
phic soils dominated by Restionaceae, Proteaceae,
and Ericaceae.

3. Bedrock/substrate types (sandstone, shale,
granite, coastal sand, acidic sand, limestone, Karoo
shale). These categories represent the substrate of
a general area, rather than a specific microhabitat,
and as such can be inferred from geological maps.
Sandstone, shale, limestone, coastal sand, and
acidic sand are the major substrate types of the
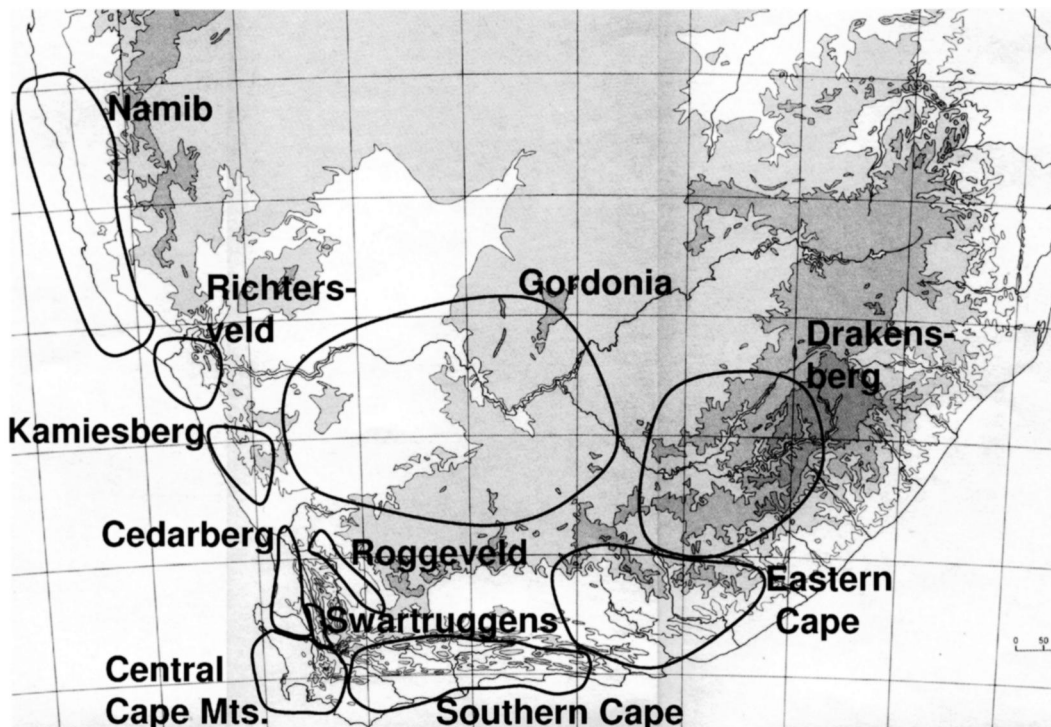Cape Floristic Region. Karoo shale refers to the

Figure 3. Distribution areas of *Heliophila* species and related genera in outlines. The distributional categories follow a combination of the topographical features of southern Africa, which often provide natural boundaries to distribution ranges, used in combination with actual distributions and zones of climatic change (see Goldblatt & Manning, 2000; Cowling & Heijnis, 2001).

fine-grained sedimentary rock that is widespread throughout the Karoo basin and also forms the pediments of the Drakensberg. Much of the Namaqualand escarpment is granitic.

4. Soil type (stony-gravel, gravel, sand, calcrete, loam, boulders, clay). This indicates the substrate at the microhabitat scale, and is recorded largely from herbarium notes.

The environmental attributes of the internal cladogram nodes were inferred using DIVA optimization (Ronquist, 1997). This optimization was developed to track changes in the distributions of clades, assuming that vicariance and sympatric speciation were the "normal" situation, by attaching a cost to dispersal, but not to sympatric speciation or vicariance. This method does not enforce monomorphy at internal nodes, and it allows ancestors to remain polymorphic for individual attributes. As such it is the ideal approach for inferring the evolutionary history of environmental attributes (Linder & Hardy, 2005; Hardy & Linder, 2005). It is implemented in the software DIVA (Ronquist, 1996). In the biogeographical analysis the number of possible results obtained by DIVA was very large. Therefore, a maximum of four areas were as-

signed to any ancestral node reconstruction. This impacted the reconstruction of the basal but not the more terminal nodes. In order to use DIVA, a fully resolved tree was needed, and one of the maximally parsimonious trees was arbitrarily chosen for this analysis. The environmental attribute information for some species was incomplete; in these cases the species were scored the same as their sister species.

RESULTS

PHYLOGENETIC ANALYSIS

The ITS alignment, including the outgroups (*Cleome spinosa* (Cleomaceae), *Aethionema saxatile*, *Alliaria petiolata*, *Rorippa amphibia*, and *Cardamine matthioli* (Brassicaceae)), contained 57 taxa and 473 characters of which 195 were parsimony informative. Using the above-mentioned settings, parsimony searches yielded 14,560 MPTs 904 steps long (CI = 0.52, RI = 0.69) of which the strict consensus is shown in Figure 1. In the Bayesian analysis and after the MCMC had finished, 1000 out of the 10,000 trees sampled were discarded as burnin "trees." Model parameter values had con-

Table 3. Date estimates of the Heliophileae clade and its comprising subclades A, B, and C based on different approaches; numbers indicate millions of years. See text for further details.

Calibration	Heliophileae	Node		
		A	B	C
Wikström et al.[1]				
NPRS[2]	4.2/4.6	3.4/3.7	2.9/3.3	3.9/4.3
NPRS jackknife[3]	3.7–5.4	2.8–4.5	2.2–3.9	n.a.
Forced clock[4]	1.9	1.3	1.0	1.7
rDNA ITS rates[5]				
non-ultrametric distances	2.7–5.8	1.1–2.3	1.5–3.2	2.2–3.8
Forced clock[4]	2.3–4.9	1.6–3.3	1.3–2.7	2.1–4.4

[1] The Brassica/Cleome clade was dated 22 mya (from Wikström et al., 2001).

[2] Tree made ultrametric using NPRS; additional calibration by constraining the Rorippa/Cardamine clade to be minimally 2.5/5.0 mya.

[3] Estimated on jackknife re-sampled branch lengths under NPRS.

[4] Tree made ultrametric assuming a global clock.

[5] (3.9–8.3) × 10^{-9} subst./site/year (from Sang et al., 1994; Zhang et al., 2001).

verged at TL = 3.15 (± 2.67), r(C↔T) = 5.15 (±0.82), r(C↔G) = 0.74 (± 0.03), r(A↔T) = 2.22 (± 0.21), r(A↔G) = 2.98 (± 0.31), r(A↔C) = 1.42 (± 0.11), pi(A) = 0.27, pi(C) = 0.23, pi(G) = 0.22, pi(T) = 0.29, α = 1.08, and pinvar = 0.12. Parsimony and Bayesian tree topologies were largely congruent.

The cpDNA *trn*L intron region data set included 370 base pairs, of which 57 were variable characters and only 13 were phylogenetically informative. Due to the lack of informative characters, both parsimony and Bayesian analysis of this data set resulted in a poorly resolved tree topology. Eight cpDNA lineages were found that were all present (except two species) in the ITS toplogy as well. However, their relationships and the main lineages A, B, and C could not be resolved. Thus, the discussion hereafter is based on the ITS data only.

In all phylogenetic trees, one robust main clade with three sublineages A through C is recognized (Figs. 1, 2). These three clades form a trichotomy in the strict consensus tree and the Bayesian tree, and they comprise all *Heliophila* species along with the genera *Thlaspeocarpa* (clade C), *Brachycarpaea*, *Cycloptychis*, *Schlechteria*, and *Silicularia* (clade B). Within clade B, *Brachycarpaea juncea*, *Cycloptychis marlothii*, and *C. virgata* form a well-supported monophyletic group (jackknife support 99%), whereas *Schlechteria capensis* and *Silicularia polygaloides* form the other. *Chamira* is a sister to the main lineage, referred to hereafter as *Heliophila* s.l.

TIME ESTIMATES

The Bayesian tree was tested for clocklike behavior of the data using likelihood ratio testing.

With all model parameters estimated and excluding some of the outgroup taxa, i.e., *Cleome spinosa* and *Aethionema* R. Br., a molecular clock was rejected ($P < 0.01$); therefore, the ingroup tree was made ultrametric using the NPRS method as implemented in r8s, as well as by applying a global clock in PAUP* ("forced clock," see below). Node ages were then estimated in the following ways: the age of the entire tree including *Cleome spinosa* and *Aethionema* was fixed at 22 (± 2) mya, as this is the age estimated by Wikström et al. (2001) for their *rbc*L-based Brassica/Cleome clade. The dates in Wikström et al. (2001) may be severe underestimates of clade ages, possibly due to the "thin" taxon sampling in that study (Wikström et al., 2003). Therefore, actual dates obtained here for the *Heliophila* ITS tree may be too young as well, although it is not clear by how much. Moreover, as no further outgroup ITS sequences could be aligned to the *Heliophila* matrix, the only option was to fix the entire tree rather than apply a maximum constraint. In addition, we simultaneously applied a minimum age constraint of 2.5–5 mya for the clade containing *Rorippa amphibia* and *Cardamine matthioli*, as this corresponds to the age of *Rorippa* fruit fossils (range 2.5–5 mya; Mai, 1995). The r8s analysis described above estimated the age of the Heliophileae clade to be 4.2–4.6 mya, whereas the constrained jackknife analyses yielded a range of 3.7–5.4 mya for the same node, and subclades A and B were estimated to be 2.8–4.5 and 2.2–3.9 mya, respectively. The confidence analysis for clade C could not be completed because the clade was not present in a large proportion of jackknife trees (for all dating results, see Table 3).

The "forced clock" approach yielded ultrametric trees with significantly shorter branches within the Heliophileae s.l. lineage than those obtained with NPRS. This phenomenon has been observed before (Barraclough & Reeves, 2005; Linder & Hardy, 2004; Linder et al., 2005), and it is not fully understood at the moment. In order to assess the possible influence of the relatively long *Cleome spinosa*-ingroup branch, we repeated the forced clock analysis excluding this outgroup to prevent < 0.5% increase of branch length in the ingroup (not shown). "Forced clock" ultrametric tree was then analyzed in TreeEdit using the "scale tree" option, scaling the total "node height" to 22 mya. This yielded 1.9 mya for the Heliophileae clade and between 1.0 and 1.7 mya for the main three subclades A, B, and C.

An application of a range of published rDNA ITS substitution rates on the forced clock ultrametric branch lengths (see above) yielded the age 2.3–4.9 mya for the Heliophileae clade. An age estimate of 2.7–5.8 mya for the Heliophileae clade resulted from the application of substitution rates to the (non-ultrametric) GTR+I+Γ distances observed among the rDNA ITS sequences. This estimate is in agreement with the "ultrametric" estimates (maximum distance $D = 22.43\%$ between *Cycloptychis marlothii* and *Heliophila suavissima*; $D =$ substitution rate $r \times$ number of sites $n \times$ twice divergence time T, and therefore T can be calculated as $D/2rn$; using either the 3.9×10^{-9} or 8.3×10^{-9} subst./site/yr for rDNA ITS rate, and with n being 498, this approach yields an age estimate for the tribe at 2.7–5.8 mya).

ECOLOGICAL OPTIMIZATIONS IN *HELIOPHILA*

The optimization to the basal node or even basal three nodes using DIVA is generally poorly resolved, as no sensible outgroup could be used. Therefore, an interpretation of the basal nodes was avoided in this study, and instead we report the situation within the three main clades.

The distributional optimization (Fig. 4) shows that clades A and B are centered in the Cedarberg–Nieuwoudtville area, with *H. cornuta* being the only widespread species. Several species are found further to the east in the central southern Cape mountains, and this is especially evident in clade B. Clade C is centered in the Richtersveld, with outliers reaching south to the Roggeveld, and east to the Drakensberg (*H. carnosa–H. rigidiuscula*). The soils optimization (Fig. 5) shows that sandy soils are generally preferred, especially in clades A and C. Clade B is remarkable for its preference for

boulders and ledges, and occasionally for gravelly soils. Clade B is almost entirely perennial, with a single annual species; clades A and C are largely annual, but perennial lineages evolved seven times (Fig. 6).

The vegetation type optimization (Fig. 7) shows clades A and B centered in fynbos, with outliers in renosterveld, strandveld, and also the surrounding arid vegetation types. Clade C is more common in Karooid vegetation, but with a wider range of outliers than clades A and B — these include grassland, succulent karoo, fynbos, renosterveld, Namaqua Broken Veld, among others. The rock types (Fig. 8) are simple, with clades A and B primarily found on sandstone (with outliers on granite, shale, coastal and acidic sand). Clade C is primarily found on Karoo shale.

DISCUSSION

TAXONOMIC CONSIDERATIONS

Tribal level

Phylogenetic relationships within the Brassicaceae have been a source of considerable controversy, and the most frequent problems are associated with the tribal classification and delimitation of genera, including some larger ones such as *Alyssum* L., *Arabis* L., *Brassica* L., *Draba* L., *Heliophila*, *Lepidium*, and *Sisymbrium* L. (Koch et al., 2003, and references therein). These problems are mostly the result of a heavy reliance on potentially homoplastic fruit characters (Koch et al., 2003).

The Heliophileae were considered by Appel and Al-Shehbaz (1997) as a natural, well-defined tribe based on a single synapomorphy, diplecolobal cotyledons. One of its genera, *Brachycarpaea*, has spirally coiled cotyledons, a feature interpreted by them as derived from diplecolobal ones. The rather unexpected occurrence of diplecolobal cotyledons in three Australian species of *Lepidium* sect. *Monoploca* subsect. *Diploploca* (Hewson, 1981) was shown by Mummenhoff (unpublished) to have evolved independently from that of the Heliophileae. The monotypic *Chamira* has persistent, twice longitudinally folded cotyledons that act as the major photosynthetic organ. These features were used by Appel and Al-Shehbaz (1997) to maintain the genus in a unigeneric tribe, Chamireae. The present ITS study supports the recognition of the Heliophileae as a monophyletic group, with *Chamira* as sister, underlining the potential broad-scale phylogenetic utility of molecular markers in the Brassicaceae. The evaluation of Chamireae as a distinct

Figure 4. Distribution optimizations. Where many possible optimizations were indicated at an internal node, only the most widespread (that includes all areas of the other, more limited optimizations) is indicated. This is the most conservative possible route. Where only two, different optimizations are indicated, they are separated by a backslash. Area codes are: a—Roggeveld; b—Drakensberg; c—Eastern Cape; d—Southern Cape; e—Swartruggens; f—central Cape mountains; g—Cedarberg-Nieuwoudtville; h—Kamiesberg; i—Gordonia; j—Richtersveld; k—Namib. H. = *Heliophila*.

Figure 5. Soil optimizations. Where many possible optimizations were indicated at an internal node, only the most polymorphic (that includes all soils of the other, more limited optimizations) is indicated. This is the most conservative possible route. Where only two, different optimizations are indicated, they are separated by a backslash. Soils codes are: a—stony-gravel; b—gravel; c—loam; d—calcrete; e—boulders; f—clay; g—sand. H. = *Heliophila*.
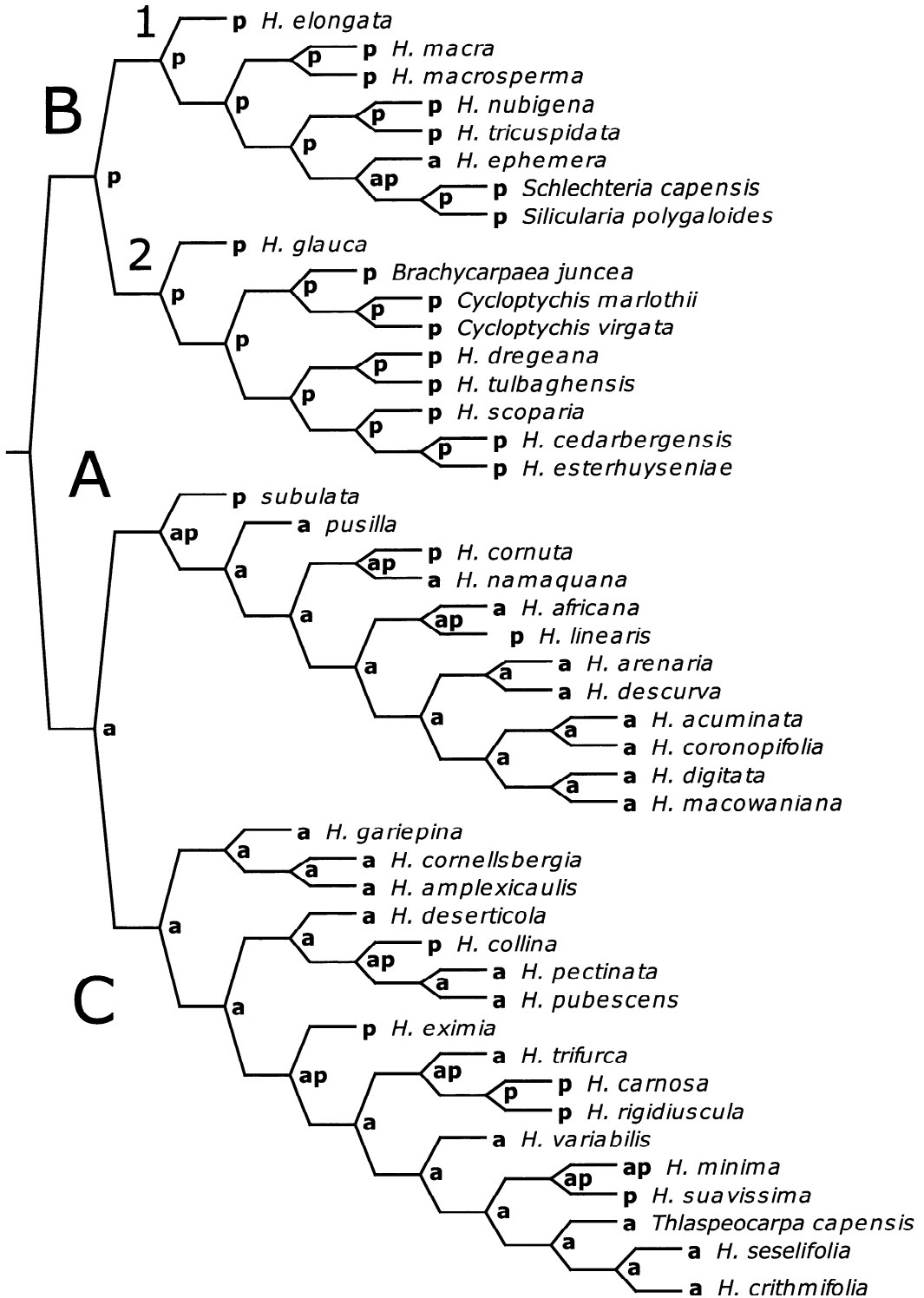
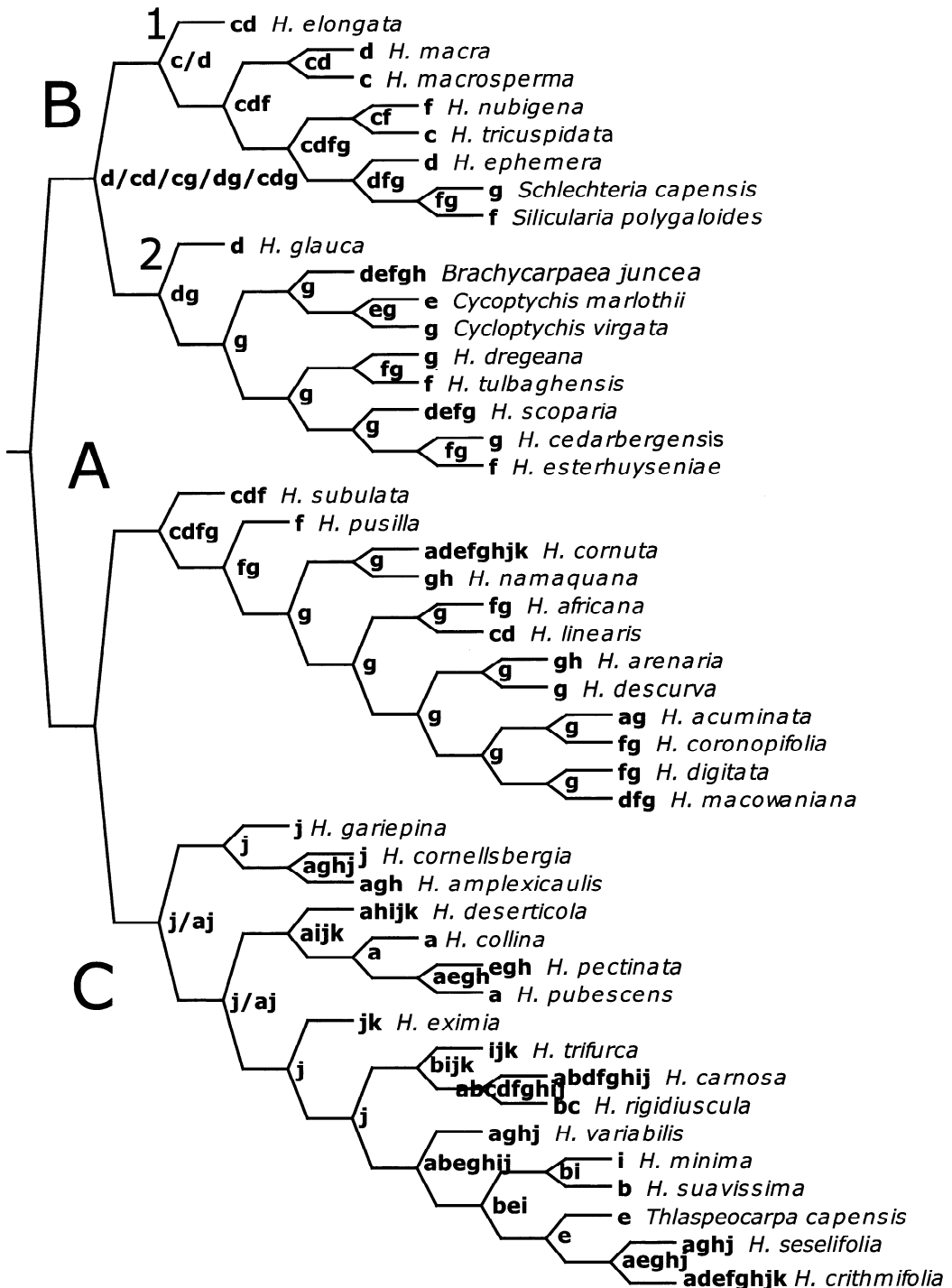Figure 6.   Habit optimizations. Habit codes are: a—annual; p—perennial. H. = *Heliophila*.
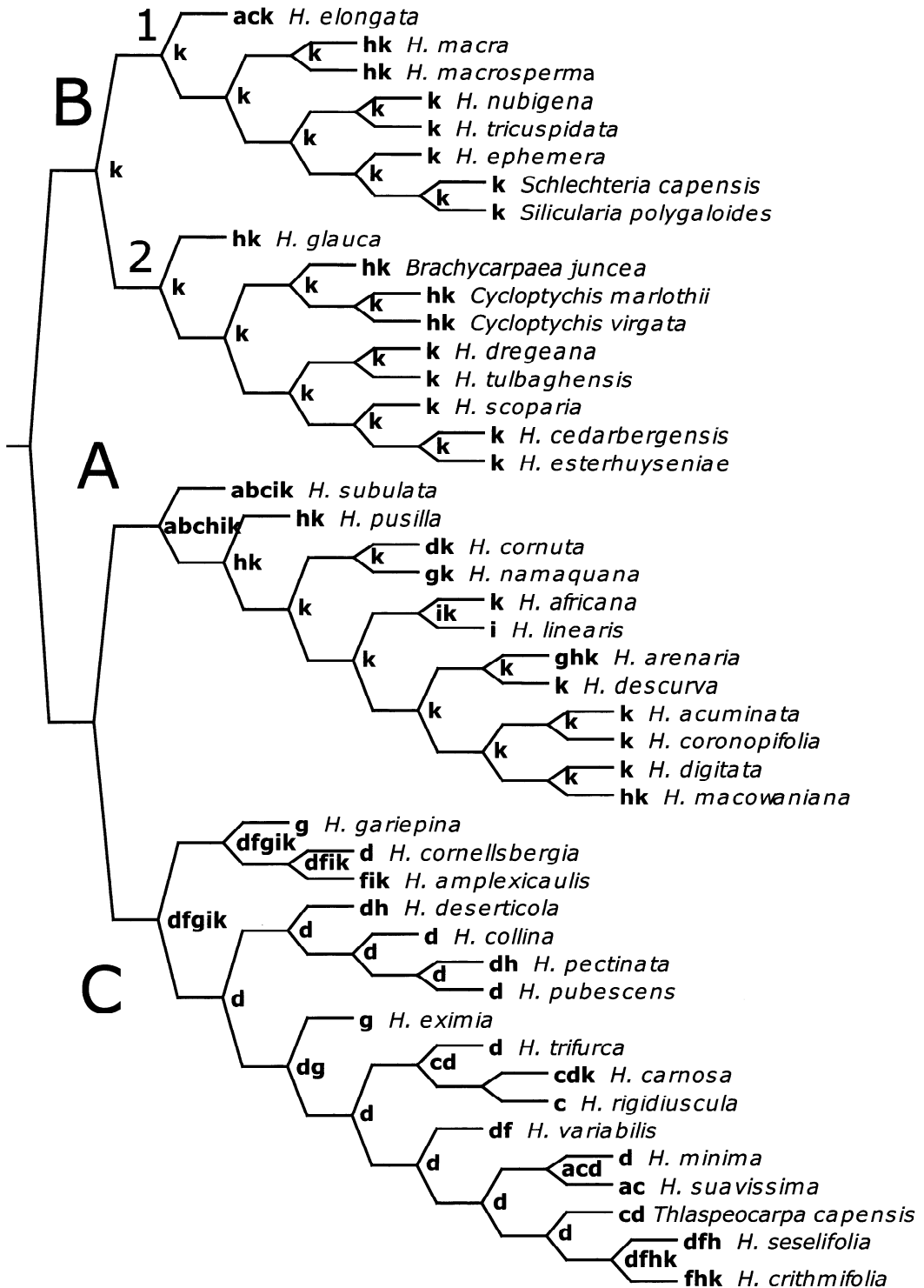
Figure 7. Vegetation optimizations. Where many possible optimizations were indicated at an internal node, only the most polymorphic (that includes all vegetation types of the other, more limited optimizations) is indicated. This is the most conservative possible route. Where only two, different optimizations are indicated, they are separated by a backslash. Vegetation codes are: a—woodland; b—forest; c—grassland; d—Karoo shrublands; f—Namaqua Broken Veld; g—succulent Karoo; h—Renosterveld; i–Strandveld; k—Fynbos. H. = *Heliophila*.

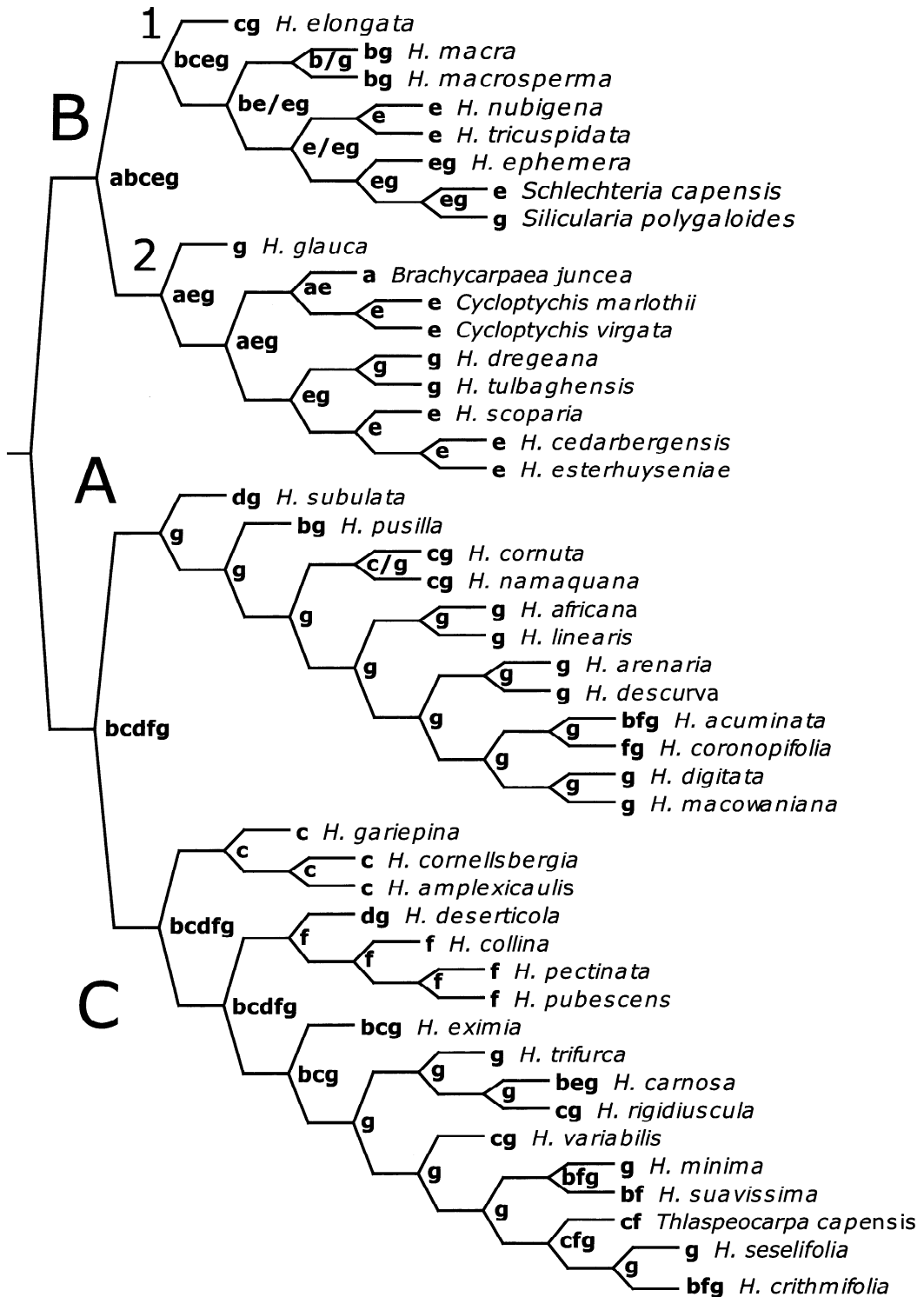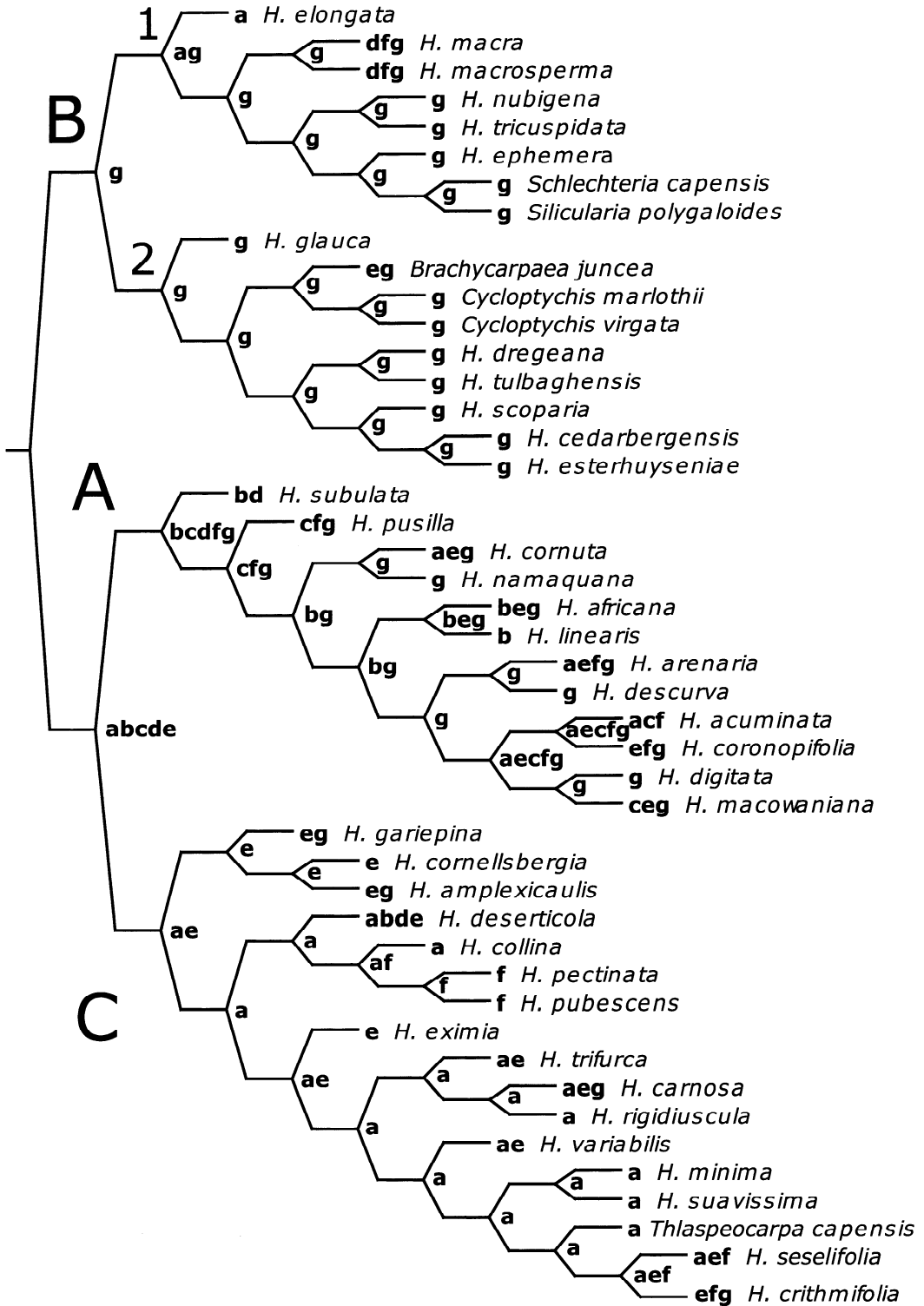Figure 8.   Bedrock/substrate optimizations. Where many possible optimizations were indicated at an internal node, only the most polymorphic (that includes all bedrock types of the other, more limited optimizations) is indicated. This is the most conservative possible route. Where only two, different optimizations are indicated, they are separated by a backslash. Bedrock codes are: a—Karoo Shale; b—coastal sand; c—acidic sand; d—limestone; e—granite; f—shale; g—sandstone. H. = *Heliophila*.

tribe must await a comprehensive family-wide phy-
logenetic analysis.

Generic limits within Heliophileae

Large genera of Brassicaceae (e.g., *Lepidium*) are
usually well characterized by distinct fruit types
but exhibit considerable variation in habit (Mum-
menhoff et al., 2001). By contrast, *Heliophila* s.l.
shows both extreme variation in habit and striking
multitude of fruit shapes (Figs. 1, 2, Table 2). As
many as nine smaller genera have been segregated
from *Heliophila* (for synonymy, see Marais, 1970),
all of which represent morphological extremes con-
nected by intermediates in almost every conceiv-
able character combination (Appel & Al-Shehbaz,
1997). Although different classification schemes of
the Heliophileae have been proposed, the tribal
disposition of and systematic relationships among
component genera are unknown, and no phyloge-
netic concept has been put forward so far. All five
smaller genera assigned by Appel and Al-Shehbaz
(1997) to this tribe (Table 1) are well nested in two
of the three clades of *Heliophila* in the molecular
trees (Figs. 1, 2). *Thlaspeocarpa capensis* is includ-
ed in clade C, whereas *Brachycarpaea juncea* to-
gether with the two *Cycloptychis* species, and *Sili-
cularia polygaloides* along with *Schlechteria
capensis* form two monophyletic groups within clade
B, respectively. The phylogenetic position of these
taxa in the molecular tree is also supported mor-
phologically. The two species of *Cycloptychis* clear-
ly form a monophyletic assemblage characterized
by the presence of a carpophore, a feature not
found elsewhere in the Heliophileae. *Cycloptychis*
is easily distinguished from *Heliophila* by its schiz-
ocarpic, erect, appressed fruits with carpophores,
and sculptured, thick, leathery or woody fruit
valves (Table 2). *Brachycarpaea* is well defined by
its angustiseptate fruits and spirally coiled cotyle-
dons, but it strikingly resembles *Cycloptychis* in
habit, flower size and morphology, schizocarpic
fruits (though with a rudimentary carpophore) with
one-seeded mericarps, apiculate anthers, and
smooth seeds. Therefore, these remarkable morpho-
logical similarities are in complete agreement with
the molecular analysis (Figs. 1, 2).

The remaining three genera, *Thlaspeocarpa*, *Sil-
icularia*, and *Schlechteria*, form a group character-
ized by lacking the septum and by having indehis-
cent fruits on recurved pedicels, but the features
used in the separation of these three taxa (i.e., sta-
minal appendages, habit) are also found in *Helio-
phila* and *Cycloptychis*. Therefore, it is not surpris-
ing to find *Thlaspeocarpa* in clade C as a relative

to some *Heliophila* species (e.g., *H. crithmifolia, H.
seselifolia*), all of which are annual herbs with ap-
pendaged staminal filaments.

Schlechteria and *Silicularia* are closely related,
and they form a subclade in clade B, closest to *H.
ephemera, H. nubigena*, and *H. tricuspidata*. Except
for *H. ephemera*, these taxa are all perennial sub-
shrubs. Bean (1990) stated that *H. ephemera* differs
from the rest of *Heliophila* by having inflated fruits,
spongy-walled seeds, coarsely tuberculate leaves,
and intercalary racemes. Upon a critical study of
the type material of *H. ephemera*, we conclude that
the inflorescence is a typical rather than intercalary
raceme. Interestingly, some *Heliophila* species (e.g.,
*H. cedarbergensis, H. esterhuyseniae, H. scoparia, H.
dregeana, H. tulbaghensis*), *Schlechteria*, and *H.
ephemera* share in clade B the apiculate anthers
and one or a few seeds per locule. However, except
for the presence of basal appendages on the fila-
ments and their absence on petals, species of lin-
eage B are not characterized by a consistent char-
acter pattern (Fig. 1).

The five smaller genera (*Brachycarpaea, Cyclop-
tychis, Schlechteria, Silicularia, Thlaspeocarpa*)
nested in *Heliophila* s. str. are distinguishable by a
combination of (rather than unique) morphological
characters all found within the *Heliophila* clades B
and C. In our opinion, the maintenance of these
genera as distinct from *Heliophila* would mean that
a paraphyletic *Heliophila* has to be recognized, a
position we do not support. Therefore, these five
genera have been reduced to synonymy of *Helio-
phila* (Al-Shehbaz & Mummenhoff, 2005).

Infrageneric classification and taxonomic status of component genera of tribe Heliophileae

Previous infrageneric classification schemes in
Heliophila (Candolle, 1821; Sonder, 1846) relied
heavily on fruit morphology. However, the most re-
cent taxonomic treatment of the genus (Marais,
1970) did not recognize any sections. Recent mo-
lecular studies in the Brassicaceae clearly demon-
strated convergence in almost every fruit character
(Koch et al., 2003, and references therein), and the
current study further supports that conclusion. Al-
though Sonder (1846) did not have knowledge of
all species included in this study, none of his sec-
tions represents a monophyletic group. Instead,
species of his sections are uniformly distributed
among all three main clades in the molecular tree
(Figs. 1, 2). Thus, the fruit types used by Sonder
to distinguish sections are not synapomorphies and
evidently evolved independently in the different
lineages of *Heliophila*.

The molecular splitting of *Heliophila*, together with the nested *Brachycarpaea*, *Cycloptychis*, *Schlechteria*, *Silicularia*, and *Thlaspeocarpa*, in three clades (A–C in Figs. 1, 2) correlates quite well with the distribution of morphological characters. These character combinations (see below) were not adequately accounted for in previous taxonomic treatments.

Species of clade A are typically annual herbs (rarely perennials, as in *Heliophila subulata* and *H. linearis*) with "exstipulate," entire leaves (*H. arenaria*, *H. digitata*, and *H. macowaniana* have pinnately lobed leaves), basally appendaged staminal filaments and frequently also petals (*H. subulata* has unappendaged filaments and petals), and smooth, often wingless seeds (*H. linearis* and *H. cornuta* have winged seeds).

Optimization of different habits (Figs. 1, 6) shows that species of clade B are shrubs/subshrubs (only *Heliophila ephemera* is herbaceous) with exclusively unappendaged staminal filaments and petals, simple leaves, wingless seeds (only *H. scoparia* with winged seeds), and apiculate anthers (*H. macra* and *H. elongata* with unapiculate anthers). Within clade B, a terminal, well-supported (78% jackknife value) subclade, which includes *H. cedarbergensis*, *H. esterhuysensiae*, *H. scoparia*, *H. dregeana*, and *H. tulbaghensis*, is readily distinguished from the entire Heliophileae by having intercalary inflorescences (rather than typical racemes) and a generally papillate (vs. smooth) seed coat.

Species of clade C exhibit a mosaic of the character states also occurring in clades A and B. All species of this clade have smooth seeds, racemes, and latiseptate fruits (*Heliophila cornellsbergia* has inflated fruits). The vast majority of members of this clade are annuals, but *H. carnosa* and *H. suavissima* are perennial subshrubs that produce annual shoots from a woody crown. Optimization on the cladogram indicates that these morphological deviations represent secondary reversals. Furthermore, the development of the seed wing seems to be a primitive feature in the clade, and wingless seeds evolved independently in a subclade including *H. collina*, *H. pubescens*, and *H. pectinata*, as well as in *H. arenosa*. The clade also shows a mosaic pattern in the presence of small "bracts" at the base of pedicels, minute "stipules" at the leaf base, as well as in the development of basal appendages on the petals and stamens.

The small appendages at the pedicel bases in the Heliophileae were interpreted as "stipules" or otherwise completely reduced "bracts" (Marais, 1970; Appel & Al-Shehbaz, 2003), but functionally they probably represent extrafloral nectaries. Further studies of Bean (1990) revealed their presence in *Chamira* and all other genera of the Heliophileae except *Cycloptychis*. Such "bracts" are typically found in nearly all species of clades B and C, and their absence in a few species of these clades most likely represents parallel losses. It further appears that the absence of "bracts" is diagnostic for all species of clade A except *Heliophila subulata*.

The recognition of monotypic and ditypic genera in the Heliophileae mirrors several other cases in the Brassicaceae where differences in fruit characters often are overemphasized at the expense of taxonomically more useful other characters. Koch et al. (2003) listed several such examples, including *Twisselmannia* Al-Shehbaz versus *Tropidocarpum* Hook., *Thlaspi* L. versus *Alliaria* Scop., and *Lepidium* versus *Coronopus* Zinn. Ideally, a critical evaluation of the taxonomic status of the *Heliophila* s.l. clades (e.g., generic vs. subgeneric level) should await more sequence data from other markers and more extensive sampling of the ingroups and outgroups.

Morphological character evolution

Despite the incompleteness of our study, one can safely make some generalizations regarding the evolution of certain features (Fig. 1). For example, intercalary inflorescences and papillate seeds apparently evolved only once within a terminal subclade of clade B that includes *H. cedarbergensis*, *H. esterhuyseniae*, *H. scoparia*, *H. dregeana*, and *H. tulbaghensis*. On the other hand, apiculate anthers also probably evolved once at the base of clade B, with reversals in both *H. macra* and *H. elongata*. By contrast, the evolution of indehiscent, one- or two-seeded fruits occurred independently in *Thlaspeocarpa* of clade C and at least twice in clade B: in the subclade containing *Brachycarpaea* and *Cycloptychis* as well as that including *Schlechteria*, *Silicularia*, and *H. ephemera*. Wingless seeds appear to be basal in each of clades A and B, and it is likely that winged seeds might be basal in clade C. It seems that the shift from one seed type to another occurred several times within *Heliophila* s.l. Finally, taxa with entire leaves appear to be basal in the entire genus *Heliophila* s.l., and pectinate or pinnately lobed leaves with filiform or narrowly linear segments evolved secondarily in clades A and C (Fig. 1).

Fruit diversity within Heliophileae has no match anywhere in the Brassicaceae. For example, fruit length ranges from long siliques to minute silicles, fruit compression varies from latiseptate, terete, to angustiseptate, and upon maturity, the fruits may

be dehiscent, indehiscent, or even schizocarpic (Marais, 1970; Appel & Al-Shehbaz, 1997, 2003). The result of mapping fruit morphologies onto the phylogenetic trees (Fig. 2) suggests that fruit characters are quite variable even within each of the three main clades of *Heliophila*. On the basis of the present molecular studies, one can trace fruit evolution from latiseptate and unconstricted (smooth) to moniliform siliques (e.g., *H. dregeana* and *H. tulbaghensis* in clade B); from latiseptate silicles to schizocarpic (*Cycloptychis*) and angustiseptate-didymous (*Brachycarpaea*); and from latiseptate and dehiscent siliques to indehiscent silicles (*Thlaspeocarpa*). Furthermore, it appears that schizocarpic fruits in the Heliophileae evolved once in the subclade including *Brachycarpaea* and *Cycloptychis*. The occurrence in each of the three *Heliophila* subclades of the same fruit types demonstrates convergent/parallel evolution (Fig. 2). For example, moniliform fruits evolved independently in each of clades A, B, and C, though they appear to have evolved once within clade B in a well-supported (100% bootstrap) subclade that includes *H. dregeana* and *H. tulbaghensis* (Fig. 1).

It is important to note that fruit dehiscence and relative length, both of which are major diagnostic characters for generic delimitation in the traditional systems of Hayek (1911) and Schulz (1936), are controlled in *Arabidopsis thaliana* by the single MADS-box genes *SHATTERPROOF* and *FRUITFUL* (Liljegren et al., 2000; Ferrandiz et al., 2000). Such a relatively simple control of fruit morphology (dehiscence/indehiscence, relative length) would easily explain the rapid and independent evolution of multiple fruit types within various genera of the Brassicaceae, including *Heliophila*. One suspects that such simple inheritance controlling drastic morphological differences might hold true for the other characters addressed above.

We think it likely that the number of genes controlling the remarkable differences in fruit morphology are relatively few, thus allowing rapid evolutionary changes independent of other aspects of morphology. The high degree of sequence similarity between several species of *Heliophila* and members of the five smaller genera (*Brachycarpaea*, *Cycloptychis*, *Schlechteria*, *Silicularia*, and *Thlaspeocarpa*) strongly emphasizes the apparent rapidity with which drastic changes in fruit morphology can sometimes occur in the family, thus leading to classifications or generic delimitations that obscure rather than clarify evolutionary relationships. As suggested by Koch et al. (2003), future molecular studies would most likely reveal that the vast majority of monotypic and oligotypic genera of the

Brassicaceae should be reduced to synonymy of larger genera, and our present study strongly supports that hypothesis.

In conclusion, we have demonstrated that the heavy reliance on fruit characters alone may well lead to erroneous taxonomic results and that such characters should be critically evaluated in light of the molecular and other morphological data (Koch et al., 2003, and references therein). A critical evaluation of morphology in *Heliophila* and allies reveals that the individual characters vary in their value for systematic classification (see above). As demonstrated by Bailey et al. (2002) for the Halimolobine Brassicaceae, it appears that a combination of morphological characters is potentially useful in classification, and as demonstrated above, we believe that this also holds within Heliophileae.

Cytology

Hardly anything is known about the chromosome numbers in the Heliophileae. The four species studied, all with $2n = 20$, are *Heliophila africana* (as *H. pilosa*), *H. amplexicaulis*, *H. crithmifolia*, and *H. linearis* (Jaretzky, 1932; Manton, 1932). However, these counts were based on material grown in botanical gardens, and no vouchers are available to verify the identity of the taxa involved.

DATING THE RADIATION

Using different approaches to calibrate our trees (i.e., the 22 mya for the Brassica/Cleome clade from Wikström et al. (2001), or a range of published angiosperm rDNA ITS substitution rates (see Results)), we arrive at age estimations of 2–5 mya for the proliferation of the Heliophileae (see Table 3). The NPRS smoothing algorithm consistently estimates older ages, but not more than around 5 mya. Given that the dates from Wikström et al. (2001) may be far too young, our age estimation for the Heliophileae proliferation could be up to 10 mya maximally. On the other hand, the observation that our published rDNA ITS substitution rate-based estimate is remarkably similar to the "forced clock"-derived estimation, combined with the notion that NPRS tends to overestimate ages, suggests a more recent age of 2–5 mya. If accurate, the amazing range of morphological variation observed in Cape *Heliophila* species would thus have been generated within a Pliocene–Pleistocene time frame. Such an estimate suggests a considerably faster rate compared with radiations in other Cape genera, such as *Pelargonium* L'Hér. (Geraniaceae) and *Phylica* L. (Rhamnaceae), for both of which Miocene age was suggested (Bakker et al., 2005; Richardson et

al., 2001), and Restionaceae, for which an Oligocene age was proposed (Linder & Hardy, 2004).

Our estimate would also constitute the most recent Cape radiation reported so far, which is much earlier than the 8–32 mya range given by Linder (2003) and Linder and Hardy (2004) for the Cape Floral Region crown clade proliferations. Most of the Cape-clade radiation dates are based on NPRS-derived ultrametric trees, and several rely on the Wikström et al. (2001) dates for calibration. At the moment there does not appear to be an indication of simultaneous radiation in these lineages. Clearly, a meta-analysis needs to be conducted in which calibration and ultrametricity are arrived at in a concerted approach.

ECO-GEOGRAPHICAL EVOLUTION IN *HELIOPHILA*

The three major clades of *Heliophila* s.l. can be largely characterized in terms of distribution patterns and habit (Figs. 4, 6). Distribution patterns also reflect rainfall gradients, vegetation type, and bedrock types. Our crude data make it impossible to achieve a finer resolution of the eco-geographical patterns.

Clade B, which exclusively includes shrubs or subshrubs (except for the herbaceous *Heliophila ephemera*), is almost completely restricted to the CFR (Cedarberg-Nieuwoudtville, Swartruggens, central Cape mountains, southern Cape), with an ancestral distribution optimized as restricted to the CFR (Fig. 4). Its species mostly grow on soils derived from Table Mountain sandstone (Fig. 8), very often among boulders or on rock ledges (Fig. 5), and in fynbos and renosterveld (Fig. 7). Fire is a regular occurrence in fynbos vegetation, with a frequency of 5–50 years (van Wilgen, 1987), and several species show adaptations to survive after fire by resprouting (Schutte et al., 1995). The associations with rock might also indicate habitats somewhat protected from fire. The distribution pattern suggests a rainfall range of 300–2000 mm, with dry summers. The optimizations indicate an ancestral occurrence on sandstone, and in fynbos, with several expansions on shale soils and into renosterveld vegetation. Furthermore, clade B can be divided into an eastern subclade (clade B1 in Fig. 4), the species of which grow in areas that receive more summer rain and contain several Eastern Cape species. Clade B2 in Figure 4, which is centered more to the north, has no representatives in the summer-wet Eastern Cape, but its species grow in the arid Swartruggens that receive no rain in the summer and very little in the winter. The only annual species, *H. ephemera*, is not ecologically different from the other members of clade B.

Clade A, which consists largely of annuals (Fig. 6), is also centered in the CFR with an ancestral distribution optimized as restricted to the CFR (Fig. 4). It has a wider range of outliers than clade B (three species reach the Kamiesberg and two the Roggeveld mountains); these are interpreted as dispersal from the CFR. Although most species show the typical CFR syndrome (i.e., occurring in fynbos heathy pyrophytic vegetation and growing on sandstone-derived soils, Figs. 7, 8), the ancestral situation is uncertain, and the optimizations indicate that the basal nodes are polymorphic. The two coastal species of *Heliophila* belong to this clade, and they occur on limestone or coastal dunes, mostly on alkaline sand, and in non-pyrophytic strandveld. These represent two invasions of this habitat by *H. subulata* and *H. linearis*.

Clade C, also including mostly annuals (Fig. 6), is centered on the Richtersveld in Karooid vegetation, with an ancestral distribution optimized to be either the Richtersveld or the Richtersveld and the Roggeveld. Its species grow on sandy, loamy, or clayey soils derived from shale or granite (Figs. 5, 8). These areas are generally arid, have hot dry summers and short winters, and receive less than 400 mm of annual rainfall. They are generally rich in annuals, possibly due to a combination of very harsh summers and relatively rich soils (Pienaar & Nicholas, 1988) that allow rapid growth in the short, winter-spring favorable season.

The three Drakensberg species (*Heliophila carnosa*, *H. rigidiuscula*, and *H. suavissima*) are nested in this group C, and placed in two subclades. These species indicate two range extensions from the Richtersveld to the east (Fig. 4), as well as a change from annual to perennial habit (Fig. 6). In addition, they indicate a shift from sparse winter-rainfall (less than 300 mm annually and without frost), to wet summer-rainfall areas with frequent winter snow. It is a remarkable ecological range extension also matched by *Pentaschistis airoides* Stapf and several other grasses (Linder & Ellis, 1990). Possibly the harsh alpine environment of the Drakensberg has similarities to the harsh semidesert environment of the Namaqualand escarpment. The other two Drakensberg *Heliophila* species, *H. alpina* and *H. formosa*, were not included in this study, and it is unclear whether they also group with the above three species. The only other species from KwaZulu-Natal included in this analysis is the coastal *H. elongata*, a species that also grows in the Eastern Cape and that groups basally into Clade B1 in Figure 4.

Four species, *Heliophila eximia*, *H. deserticola*, *H. trifurca*, and *H. crithmifolia*, show an expansion into the arid Namib desert in which none is endemic (Fig. 4). Of these, *H. eximia* is a perennial, a habit that goes against the general trend of the annual *Heliophila* species found in seasonally arid areas. However, this species is found only in southern Namibia, and it might be considered as a member of the Richtersveld biogeographical region.

The Roggeveld is biogeographically interesting because it forms the border between the CFR and the Gordonia region. There are no species of clade B in this region, while clade A is represented by its most widespread species, *Heliophila cornuta*, as well as *H. acuminata*, which is also found in the adjacent Cedarberg. Clade C is represented by 8 of its 17 species in the region, which can be regarded as the center for clade C.

Evidently, the perennial species are associated with wetter or at least summer-wet areas, while the annuals occupy the more arid western half of the subcontinent. If the annual habit is assumed to be primitive in *Heliophila* s.l., then the perennial habit (Fig. 6) evolved independently at least seven times and was lost once (*H. ephemera*). The habit seems to be tightly correlated with macro-ecology and phylogeny, but it is unclear whether or not a change of habit allowed the species to be established in the Drakensberg region.

ADAPTIVE SHIFTS/FACTORS EXPLAINING THE RADIATION IN *HELIOPHILA*

Goldblatt and Manning (2000) and Linder (2003) suggested that the remarkable species richness in the CFR might be the consequence of diverse limitations to gene flow (e.g., dissected landscapes, pollinator specialization, long flowering times allowing much phenological specialization) and a complex environment providing a diversity of selective forces (e.g., geographical climatic variation, altitudinal variation, different soil types, regular fires). Due to the lack of experimental and observational studies on the adaptive significance of various structures, morphology will be used to infer the various functions of these structures. This approach should result in a predictive framework on which experimental studies can be based.

In addition to the diversity in flower color and size (see the introductory paragraphs), the differences in inflorescence and flower structure obviously are linked to different pollinators or pollination strategies (Johnson, 1996; Linder, 2003, and references therein; Perret et al., 2003; Bakker et al., 2005). The inflorescence in *Heliophila* species

is typically a raceme, but it is intercalary in five species of clade B (Fig. 1), which means that the terminal flower is overtopped by new leafy growth of the central axis (Marais, 1970; Bean, 1990). In these intercalary inflorescences the growth recommences after a first flush of flowers, thus potentially lengthening flowering time and allowing specialization. The majority of Brassicaceae have flowers with visible nectaries and easily accessible nectar for the visiting insects, such as short-proboscid flies, wasps, or beetles (Knuth, 1898; Schultze-Motel, 1986). Species of many genera have tubular (e.g., *Matthiola* R. Br.) or internally appendaged flowers (*Heliophila*) in which the nectar is hidden, and their flowers are pollinated by long-proboscid insects such as bees, bumblebees, moths, and butterflies (Knuth, 1898; Kugler, 1955; Schultze-Motel, 1986; Procter et al., 1996). Both appendaged and unappendaged flowers are found in *Heliophila*, and species of clades A and C are typically characterized by basal appendages on the petals and staminal filaments (Fig. 1).

Schulz (1931) suggested that the floral basal appendages in *Heliophila* are associated with the nectaries and often hide them. We suggest that the basal appendages of petals and filaments, which allow insects with longer rather than shorter prosboces to access nectar, play a selective role that may promote interspecific reproductive isolation and therefore may increase speciation rates and diversification. Unfortunately, hardly anything is known about the floral biology of *Heliophila* species, and extensive field studies would most likely provide pollination data valuable to understanding the adaptive radiation of the genus in South Africa.

The leaves and pedicels of many species of clades B and C are basally subtended by small appendages interpreted as "stipules" or "bracts" (Marais, 1970; Appel & Al-Shehbaz, 2003) but functionally may represent extrafloral nectaries. If nectaries, they might attract insects and prevent pollination between neighboring flowers of the same inflorescence. Ants are poor cross-pollinators and are attracted to extrafloral nectaries (Chauhan, 1979), but it is unknown if they play any such role in *Heliophila*. In some species of clade A (e.g., *H. linearis*, *H. namaquana*, *H. africana*), Schulz (1931) described swollen clavate styles in the ripe fruits. Although the adaptive significance of these structures is unknown, Schulz (1931) suggested that they might represent a food offer to herbivores to prevent seed damage when snipped off.

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Appendix 1.   Collection data and GeneBank accession numbers of South African endemic Brassicaceae species studied.

Species[1]	Provenance[2] / Herbarium-source[3] / Collector[4]	ITS I	ITS II
		Gen Bank accession number	
Heliophila acuminata (Eckl. & Zeyh.) Steud.	South Africa, SW, Malmesbury district, Klein Swartfontein, near Moorreesburg, 33°08′S, 18°39′E / PRE / *Acocks 24401*	AJ863603	AJ864807
H. africana 1 (L.) Marais	South Africa, SW, Cape Town, 33°52′S, 18°31′E / NBG / *Steiner 1975*	AJ863602	AJ864808
H. africana 2 (L.) Marais	South Africa, Cape Peninsula, Olifantbos Bay / OSBU / *Neuffer 9402*	AJ863601	AJ864809
H. amplexicaulis L.f.	South Africa, NW, SW, KM / cultivated, Botanic Garden Paris, France	AJ863611	AJ864810
H. arenaria Sond.	South Africa, NW, Clanwilliam Distr., between Nardouws Pass and Brakvlei, 33°10′S, 18°55′E / PRE / *Marais 1436*	AJ863600	AJ864811
H. carnosa (Thunb.) Steud.	South Africa, N, Ceres Div., Baviaansberg, 33°08′S, 19°37′E / BOL / *Esterhuysen 29796*	AJ863599	AJ864805
H. cedarbergensis Marais	South Africa, NW, Clanwilliam Distr., Langeberg, Central Cedarberg Mountains / BOL / *Esterhuysen 35055*	AJ863607	AJ864812
H. collina O. E. Schulz	South Africa, NW, Calvinia, Nieuwoudtville Reserve, 31°22′S, 19°07′E / NBG / *Perry & Snijman 2199*	AJ863598	AJ864813
H. cornellsbergia B. J. Pienaar & Nicholas	South Africa, NW, Richtersveld, Cornellsberg in Stinkfontein Mts. Southern slopes to a neck S of top / PRE / *Oliver, Tölken & Venter 715*	AJ863576	AJ864803
H. cornuta Sond.	South Africa, Ceres Div., Zwartruggens, Groenfontein, near Stompiesfontein, 33°05′S, 19°19′E / BOL / *Bean & Trinder-Smith 2686*	AJ863597	AJ864804
H. coronopifolia 1 L.	South Africa, SW, Caledon, foot of Hoys Koppie, Hermanus, 34°25′S, 19°14′E / MO / *Williams 982*	AJ863596	AJ864814
H. coronopifolia 2 L.	Cultivated / Botanical Garden Halle, Germany	AJ863592	AJ864817
H. coronopifolia 3 L.	Cultivated / Botanical Garden Aarhus, Denmark	AJ863588	AJ864824
H. crithmifolia 1 Willd.	South Africa, NW, in between Clanwilliam and Wuppertal, road side, 32°13′S, 19°10′E / OSBU / *Neuffer 9258*	AJ863577	AJ864799
H. crithmifolia 2 Willd.	South Africa, LB, KM, South Cape district, Oudtshoorn, next to turnoff to Blossoms, 33°33′S, 22°11′E / BOL / *Vlok 1048*	AJ863595	AJ864806
H. descurva Schltr.	South Africa, Cedarberg Mts., near Wuppertal, 32°16′S, 19°13′E / OSBU / *Neuffer 9253*	AJ863575	AJ864815
H. deserticola Schltr.	Namibia, Oranjemund, 5 km N of Oranje near Sendelingsdrif, 28°09′S, 16°53′E / MO / *Giess & Müller 14375*	AJ863594	AJ864798
H. digitata L.f.	South Africa, Clanwilliam, Farm Suurfontein / NBG / *Whitehead s.n.*	AJ863593	AJ864816
H. dregeana Sond.	South Africa, Cold Bokkeveld, Elands Kloof at Twee Rivieren / MO / *Goldblatt 2578*	AJ863606	AJ864818
H. elongata (Thunb.) DC.	South Africa, forests near Knysna, 34°01′S, 23°03′E / MO / *Lavranos 6203*	AJ628255	AJ628256
H. ephemera P. A. Bean	South Africa, Oudtshoorn Prov., Swartberg, northern slopes, 4 km E of Blouberg on Botha's track / MO / *Viviers, Vlok & Bean 1551*	AJ628257	AJ628258
H. esterhuyseniae Marais	South Africa, Caledon Prov., Franschhoek Mountains, Roesbos Peak, 33°54′S, 19°08′E / BOL / *Esterhuysen 29422*	AJ628259	AJ628260
H. eximia Marais	South Africa, N, Richtersveld, Kodaspiek, main ridge SE of Beacon and up to summit, 28°32′S, 17°05′E / PRE / *Oliver, Tölken & Venter 390*	AJ863591	AJ864819
H. gariepina Schltr.	South Africa, N, Richtersveld, Kodaspiek, main ridge SE of Beacon and up to summit, 28°32′S, 17°05′E / PRE / *Oliver, Tölken & Venter 476*	AJ863590	AJ864820

Appendix 1. Continued.

Species[1]	Provenance[2] / Herbarium-source[3] / Collector[4]	Gen Bank accession number	
		ITS I	ITS II
H. glauca Burch. ex DC.	South Africa, Ladismith Div., Anysberg, N slopes, 33°30'S, 20°46'E / MO / *Esterhuysen 32859*	AJ863610	AJ864821
H. linearis (Thunb.) DC.	South Africa, Mossel Bay, between Outeniquastrand and Tergniet, 34°03'S, 22°12'E / BOL / *Vlok 1949*	AJ863589	AJ864822
H. macowaniana Schltr.	South Africa, road from Clanwilliam and Cape Town, 20.5 km from Clanwilliam, road side, 32°21'S, 18°56'E / PRE / *Marais 1447*	AJ863587	AJ864825
H. macra Schltr.	South Africa, Caledon Div., Hermanus, Fernkloof Nature Reserve, above Northcliff, 34°25'S, 19°14'E / MO / *Robertson 134*	AJ863609	AJ864826
H. macrosperma Burch. ex DC.	South Africa, Albany, Fish River Pass, 33°17'S, 26°47'E / MO / *Bayliss 2257*	AJ863608	AJ864827
H. minima (Stephens) Marais	South Africa, Richmond Distr., 31 km NW of Merriman Station, 30°59'S, 23°33'E / PRE / *Acocks 24430*	AJ863586	AJ864828
H. namaquana Bolus	South Africa, Clanwilliam Dist., Pienaarsvlakte between Krom River and Matjiesriver / BOL / *Bean 1345*	AJ863585	AJ864802
H. nubigena Schltr.	South Africa, Worcester Dist., Keeromsberg / BOL / *Esterhuysen 33671*	AJ863613	AJ864829
H. pectinata Burch. ex DC.	South Africa, Ceres-Clanwilliam road via Groot, turnoff to farm Kleinveld, S of Blinkberg Pass / PRE / *Marais 1416*	AJ863584	AJ864830
H. pubescens Burch. ex DC.	South Africa, Calvinia, Kleinfontein, Agterkop, Pk. Middelpos, 32°01'S, 20°04'E / PRE / *Hanekom 2138*	AJ863583	AJ864831
H. pusilla L.f.	South Africa, Caledon, Hermanus, Die Duine, 34°25'S, 19°14'E / PRE / *Williams 1264*	AJ863582	AJ864832
H. rigidiuscula Sond.	South Africa, Mkambati, road to Lupatana, 31°19'S, 29°57'E / MO / *Hutchings 727*	AJ863572	AJ864797
H. scoparia Burch ex DC.	South Africa, Worcester Dist., Hex Rivier Mts., Audensberg, 33°28'S, 19°34'E / BOL / *Esterhuysen 28193*	AJ863605	AJ864833
H. seselifolia Burch. ex DC.	South Africa, Williston Dist., Snyderspoort through the Basterberge, 31°20'S, 20°55'E / STE / *Thompson 3161*	AJ863581	AJ864801
H. suavissima Burch. ex DC.	South Africa, Orange Free State, Glen Landboukollege / PRE / *PC & L. Zietsman s.n.*	AJ863574	AJ864834
H. subulata Burch. ex DC.	South Africa, Riversdale, Stillbaai, ridge below reservoir, 34°22'S, 21°26'E / STE / *Bohnen 5223*	AJ863580	AJ864835
H. tricuspidata Schltr.	South Africa, Caledon Dist., Jonas Kop, Langeberg, NW of Genadendal, 34°01'S, 19°36'E / BOL / *Esterhuysen 32705*	AJ863612	AJ864836
H. trifurca Burch. ex DC.	South Africa, Namaqualand, road from Garies via eastern mountains to Springbok, 30°07'S 17°59'E / OSBU / *Neuffer 9280*	AJ863604	AJ864800
H. tulbaghensis Schinz	South Africa, Paarl Dist., path from Fransch Hoek Pass towards Paardekoop, near head of valley, 33°54'S, 19°09'E / BOL / *Esterhuysen 35692*	AJ863579	AJ864837
H. variabilis Burch. ex DC.	South Africa, Richtersveld, top of Hellskloof, road going to Springbokvlakte / STE / *Nicholas 2511*	AJ863578	AJ864838
Brachycarpaea juncea (Bergius) Marais	South Africa, Clanwilliam Dist., Berg Road / NBG / *Barker 10426*	AJ862707	AJ862708
Chamira circaeoides (L.f.) Zahlbr.	South Africa, Malmesbury Dist., Bokbaai, 33°34'S, 18°21'E / BOL / *Bean & Viviers 1901*	AJ862719	AJ862720
Cycloptychis marlothii O. E. Schulz	South Africa, Ceres Dist., Zwartruggens, Groenfontein, near Stompiesfontein in rugged refugium in TMS ridge, 32°59'S, 19°01'E / BOL / *Bean & Trinder-Smith 2687*	AJ862709	AJ862710
Cycloptychis virgata (Thunb.) E. Mey. ex Sond.	South Africa, Piketberg Dist., Piketberg Mts. near Piketberg, 32°53'S, 18°43'E / NBG / *Barker 10341*	AJ862711	AJ862712

Appendix 1.   Continued.

Species[1]	Provenance[2] / Herbarium-source[3] / Collector[4]	Gen Bank accession number	
		ITS I	ITS II
Schlechteria capensis Bolus	South Africa, Clanwilliam Dist., Wolfberg, N Cedarberg, 32°28'S, 19°08'E / BOL / *Esterhuysen 29984*	AJ862715	AJ862716
Silicularia polygaloides (Schltr.) Marais	South Africa, Ceres Dist. / BOL / *Leighton 2288*	AJ862713	AJ862714
Thlaspeocarpa capensis (Sond.) C. A. Sm.	South Africa, Sutherland, Voelfontein, valley S of farm house on road to mountain, 32°23'S, 20°39'E / MO / *Goldblatt 6319*	AJ862717	AJ862718
Alliaria petiolata (M. Bieb.) Cavara & Grande	Campus area University of Osnabrück, Germany, 52°16'N, 7°59'E	AJ862703	AJ862704
Aethionema saxatile (L.) R. Br.	Cultivated, Botanical Garden of the University of Osnabrück, Germany, 86 30 096 14	AJ862697	AJ862698
Rorippa amphibia (L.) Besser	Germany, Cologne / *M. Koch s.n.*	AF078025	AF078524
Cardamine matthioli Moretti	Slovakian Republic, Slovenské Rudohorie Mountains, near Brezno, 48°44'N, 19°51'E / OSBU / *Franzke 11 / 6*	AF077985	AF077986
Cleome spinosa Jacq.	Cultivated, Botanical Garden of the University of Osnabrück, Germany	AY254535	AY254535

[1] Nomenclature follows Jordaan (1993).

[2] Phytogeographic centers are given by abbreviation in capital letters (Goldblatt & Manning, 2000).

[3] BOL: Bolus Herbarium, University of Cape Town, South Africa; MO: Missouri Botanical Garden, Saint Louis, Missouri, U.S.A.; OSBU: Herbarium University of Osnabrück, Germany; PRE: National Botanical Institute, Pretoria, South Africa; STE: National Botanical Institute, Stellenbosch, South Africa; NBG: Compton Herbarium, National Botanical Institute, Claremont, South Africa.

[4] Collector with collection number.