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PHYLOGENY OF CAPPARACEAE AND BRASSICACEAE BASED ON CHLOROPLAST SEQUENCE DATA[1]

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Capparaceae and Brassicaceae have long been known to be closely related families, with the monophyly of Capparaceae more recently questioned. To elucidate the relationship between Brassicaceae and Capparaceae as well as to address infrafamilial relationships within Capparaceae, we analyzed sequence variation for a large sampling, especially of Capparaceae, of these two families using two chloroplast regions, *trnL-trnF* and *ndhF*. Results of parsimony and likelihood analyses strongly support the monophyly of Brassicaceae plus Capparaceae, excluding *Forchhammeria*, which is clearly placed outside the Brassicaceae and Capparaceae clade and suggest the recognition of three primary clades—Capparaceae subfamily (subf.) Capparoideae, subf. Cleomoideae, and Brassicaceae. Capparaceae monophyly is strongly contradicted with Cleomoideae appearing as sister to Brassicaceae. Two traditionally recognized subfamilies of Capparaceae, Dipterygioideae and Podandrogynoideae, are embedded within Cleomoideae. Whereas habit and some fruit characteristics demarcate the three major clades, floral symmetry, stamen number, leaf type, and fruit type all show homoplasy. Clades within Capparoideae show a biogeographical pattern based on this sampling. These results are consistent with several alternative classification schemes.

Key words: Brassicaceae; Brassicales; Capparaceae; Capparales; classification; *ndhF*; phylogenetics; *trnL-trnF*.

Capparaceae are a medium-sized family of approximately 40–45 genera and 700–900 species, whose members present considerable diversity in habit, fruit, and floral features (Cronquist, 1981; Heywood, 1993; Mabberley, 1997). ''The flowers of Capparaceae are ecologically versatile and aesthetically exciting'' (Endress, 1992). Floral variation in Capparaceae includes both actinomorphy and zygomorphy, a wide range of stamen number (1–>250), and pronounced basal intercalary elongation zones that may produce gynophores, androgynophores, and elongated stamens (Endress, 1992). Bees, hummingbirds, hawkmoths, and bats are involved in pollination (Endress, 1992). Capparaceae are pantropical in distribution, being most conspicuous in tropical seasonally dry habitats.

It is universally agreed, except for Hutchinson (1967), that Capparaceae and Brassicaceae are closely related (Iltis, 1957; Al-Shehbaz, 1973, 1984; Dahlgren, 1975; Takhtajan, 1980; Cronquist, 1981; Hauser and Crovello, 1982; Rodman et al., 1993, 1996, 1998; Rollins, 1993). Brassicaceae are generally thought to be derived from or share a common ancestor with Capparaceae subfamily Cleomoideae, linked through the putatively basal Brassicaceae tribe Stanleyeae (Airy Shaw, 1965; Takhtajan, 1980) or Thelypodieae (Iltis, 1957; Al-Shehbaz, 1973). Capparaceae (and Brassicaceae) are placed in the Capparales, in which all families containing mustard oils form a monophyletic clade with one exception (*Drypetes* in Euphorbiaceae; Rodman et al., 1993, 1996, 1998). The Capparales are most closely related to either the Malvales or Sapindales (Rodman et al., 1993, 1996, 1998; APG, 1998) and have been placed in the Rosid II clade (APG, 1998) as the Brassicales (see Judd, Sanders, and Donoghue, 1994, for arguments on the priority of the name Brassicaceae over Capparaceae, and thus usage of Brassicales rather than Capparales).

The status of Capparaceae as a monophyletic family has been questioned (Rodman et al., 1993, 1996, 1998; Judd, Sanders, and Donoghue, 1994). Phylogenetic analyses using both morphological and molecular data indicate that *Cleome* (Capparaceae, subf. Cleomoideae) is more closely related to Brassicaceae than to *Capparis* (Capparaceae, subf. Capparoideae) (Fig. 1; Judd, Sanders, and Donoghue, 1994; Rodman et al., 1996, 1998). Rodman et al. (1993, 1996, 1998) sampled one species from each of the major subfamilies in their studies on plants containing mustard oils and found *Cleome* more closely related to Brassicaceae (Fig. 1a). Judd, Sanders, and Donoghue (1994) conducted a morphological cladistic analysis to test the monophyly of Capparaceae, sampling six species of Capparaceae and two species of Brassicaceae. Their data set of ten taxa and 16 characters suggested that Capparoideae form a paraphyletic grade sister to a monophyletic Cleomoideae plus Brassicaceae (Fig. 1b; Judd, Sanders, and Donoghue, 1994). Based on these analyses, the two families have been merged into one family: the Brassicaceae sensu lato (s.l.) (APG, 1998). For the purposes of this study, we refer to Brassicaceae and Capparaceae as separate families with their traditional limits to facilitate ease of discussion.

The family Capparaceae is in clear need of more detailed phylogenetic study. Variation in habit and a bewildering array of floral and fruit forms contributed to making Capparaceae a ''trash-basket'' family in which many unrelated plants were placed. The most comprehensive taxonomic treatment to date was conducted by Pax and Hoffmann (1936) in which they recognized 45 genera (20 monotypic) placed in eight subfamilies. Many genera placed in Capparaceae by Pax and Hoffmann subsequently have been elevated to familial level or placed in unrelated families. Calyptrothecoideae is now placed in the Portulacaceae based on morphological evidence (Nyananyo, 1986) or sister to Didiereaceae based on molecular data (Applequist and Wallace, 2000, 2001). *Physena* (Cappa-

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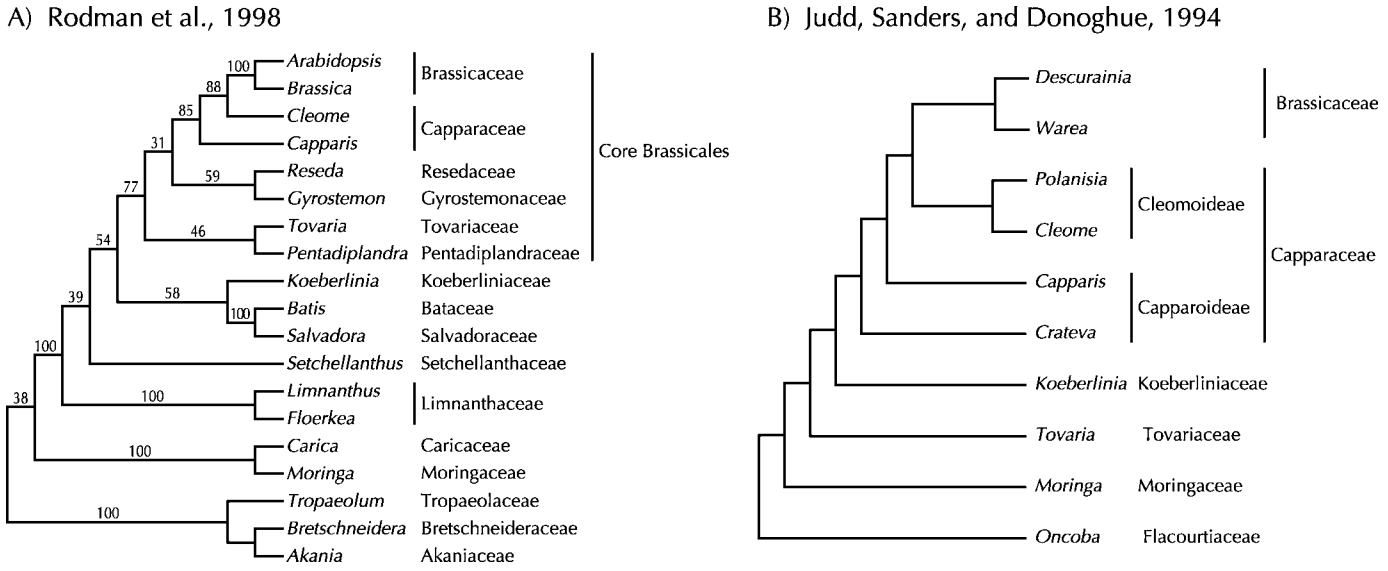

Fig. 1. Previous hypotheses on relationships between Capparaceae and Brassicaceae based on molecular and morphological data. (A) Modified Rodman et al. (1998) maximum parsimony tree is based on combined *rbcL* and 18S sequence data, showing only Brassicales (bootstrap values above branches). Relationships of families most closely related to Capparaceae and Brassicaceae are poorly supported. (B) Judd, Sanders, and Donoghue (1994) results are based on morphological analyses of ten taxa using 16 characters to determine the relationship between Capparaceae and Brassicaceae, using Flacourtiaceae as an outgroup. Capparaceae and Capparoideae are both paraphyletic and authors argue that the two families should be defined as Brassicaceae. Judd, Sanders, and Donoghue (1994) classified *Tovaria* and *Koeberlinia* as Capparaceae.

roideae) was elevated to familial status and placed in the Caryophyllales based on *rbcL* data (Morton, Karol, and Chase, 1997). Three other genera, *Koeberlinia*, *Pentadiplandra*, and *Setchellanthus*, have been elevated to familial status, but remain in the Brassicales (Rodman, 1991a; Rodman et al., 1996, 1998; Iltis, 1999; Karol et al., 1999).

The two major subfamilies of Capparaceae, Cleomoideae and Capparoideae, are quite distinct and have even been elevated to familial status by some authors (Airy Shaw, 1965; Hutchinson, 1967). Capparoideae (about 25 genera/440 species) are typically woody (shrubs to small trees) and have dehiscent or indehiscent fruits, which are fleshy. Cleomoideae (about 8 genera/275 species) are generally herbaceous and have dehiscent fruits with repla. In both subfamilies the type genus is by far the largest and houses the majority of the species: *Cleome* (200 species) and *Capparis* (150–200 species). This imbalance suggests that plants with extreme morphological traits may have been segregated into smaller genera making larger genera paraphyletic. The larger Capparoideae are further divided into four tribes by Pax and Hoffmann (1936) and into three tribes by Hutchinson (1967; equivalent to his Capparaceae). Although there is some agreement among these classification systems, most aspects of Capparaceae relationships remain unresolved. No explicit, family-wide phylogenetic hypothesis exists for Capparaceae (including possible relationships to Brassicaceae), and only a limited number of hypotheses on generic relationships have been proposed.

Brassicaceae are easily recognized by the cruciform corolla, tetradynamous stamens, and characteristic silique fruit type. In addition to these floral and fruit features, there is strong molecular evidence supporting Brassicaceae as a monophyletic group (Price, Palmer, and Al-Shehbaz, 1994; Galloway, Malmberg, and Price, 1998). Although Brassicaceae is one of few families of higher plants to have been recognized as such throughout recorded history (Rollins, 1993), intergeneric relationships within the family remain difficult and unresolved (Al-Shehbaz, 1984). Many of the tribal relationships within the family are unnatural (Al-Shehbaz, 1984; Price, Palmer, and Al-Shehbaz, 1994; Koch, Haubold, and Mitchell-Olds, 2001). Although the majority of flowers in Brassicaceae conform to the same basic floral formula, there are deviations (Endress, 1992; Rollins, 1993; Bowman et al., 1999). Several putative basal members of Brassicaceae share floral features (presence of a gynophore and lack of the tetradynamous stamens) and the woody habit with Capparaceae. There has been considerable debate whether these shared features indicate shared ancestral states or convergent evolution (Al-Shehbaz, 1973; Cronquist, 1981; Rollins, 1993). In addition, two monotypic genera, *Dipterygium* and *Puccionia*, have migrated in placement between Brassicaceae (Hutchinson, 1967) and Capparaceae (Pax and Hoffmann, 1936; Hedge, Kjaer, and Malver, 1980, and citations within). Both genera have been placed in Capparaceae, subf. Dipterygioideae, based on chemical data (Hedge, Kjaer, and Malver, 1980; Lüning, Kers, and Seffers, 1992).

Presented here is a phylogenetic analysis of Capparaceae and Brassicaceae with an emphasis on the family Capparaceae using chloroplast DNA sequence information from a coding region, *ndhF* (*ndhF* encodes a subunit of the NADP dehydrogenase enzyme in the chloroplast), and a non-coding region, *trnL-trnF* (*trn* indicates tRNA genes). Both *ndhF* (e.g., Olmstead and Reeves, 1995; Alverson et al., 1999; Givnish et al., 2000; McDade et al., 2000; Clausing and Renner, 2001; Davis, Anderson, and Donoghue, 2001; Sytsma et al., 2002) and *trnL-trnF* (e.g., McDade et al., 2000; Sweeney and Price, 2000; Bruneau et al., 2001; Davis, Anderson, and Donoghue, 2001; Sytsma et al., 2002) have been shown to be informative and effective at resolving relationships at the familial level. Thus, combining information from two plastid regions should provide resolution within and among these two families. In particular, the Capparaceae was widely sampled; almost all subfamilies and tribes described by Pax and Hoffmann (1936)

were included in the study. This study was undertaken to address four primary goals: (1) determine the precise relationship between Capparaceae and Brassicaceae; (2) test the monophyly of traditionally described subfamilies and clarify the nature of paraphyly within Capparaceae; (3) elucidate patterns of infrageneric relationships within Capparaceae; and (4) re-evaluate morphological characters previously used to delimit Capparaceae and Brassicaceae and thus address the issue of familial classification for this group.

MATERIALS AND METHODS

Taxon sampling—The taxa and voucher information used in this analysis have been listed on the Botanical Society of America website (Appendix 1, http://ajbsupp.botany.org/v89/). Sampling within Capparaceae was widespread at the generic level. In almost all instances (with the exceptions of using two published *ndhF* sequences from Galloway, Malmberg, and Price [1998]), the same species and voucher were used for both chloroplast regions. We were unable to produce completely overlapping taxon sampling for both chloroplast data sets due to inability to amplify *ndhF* in a few taxa and difficulty in aligning *trnL-trnF* sequences outside the Capparaceae and Brassicaceae families. We sampled 39 species from 24 genera of Capparaceae for the *ndhF* region and 42 species from 24 genera for the *trnL-trnF* region; 37 species were in common. This coverage samples broadly within the two largest subfamilies (Cleomoideae and Capparoideae) and most of the remaining subfamilies described by Pax and Hoffmann (1936). The sampling does not include subfamilies already eliminated from Capparaceae by previous workers: Emblingioideae (Erdtman et al., 1969; Chandler and Bayer, 2000), Calyptrothecoideae (Nyananyo, 1986; Applequist and Wallace, 2000, 2001), and Pentadiplandroideae (Villiers, 1973; also supported by Rodman et al., 1998), although Pentadiplandraceae was used as an outgroup. The monotypic Buhsioideae was not sampled due to lack of availability of tissue. All tribes of Capparoideae described by Pax and Hoffmann (1936) and Hutchinson (1967; equivalent to his Capparaceae) were sampled. With the exception of *Haptocarpum*, all genera of Cleomoideae described by Pax and Hoffmann (1936) were sampled, taking into account taxonomic changes. Multiple representatives were used for genera containing more than ten species whenever possible.

The same nine species of Brassicaceae were sampled in both analyses (Appendix 1, http://ajbsupp.botany.org/v89/). Additionally, the partial sequence of *Aethionema grandiflora* (Galloway, Malmberg, and Price, 1998) was added to give a total of 10 *ndhF* sequences, and *Aethionema saxatile* and *Thlaspi arvense* were added to give a total of 11 *trnL-trnF* sequences. Although the sampling of this family is limited, we included putative basal members based on morphological (e.g., *Stanleya* in tribe Stanleyeae based on Takhtajan [1980]) and molecular data (e.g., *Aethionema* based on Galloway, Malmberg, and Price [1998]). In addition, both morphological (Al-Shehbaz, 1984; Rollins, 1993) and molecular data (Galloway, Malmberg, and Price, 1998) strongly support the monophyly of Brassicaceae. Thus, this sampling of Brassicaceae should be sufficient to resolve relationships between Brassicaceae and Capparaceae.

Outgroup selection—The nearest relatives of Brassicaceae and Capparaceae have been examined previously using *rbcL* and 18S nrDNA sequence analysis (Rodman et al., 1993, 1996, 1998). These analyses establish a strongly supported clade of core Brassicales: Capparaceae, Brassicaceae, Resedaceae, Gyrostemonaceae, Tovariaceae, and Pentadiplandraceae (Fig. 1a). Representatives of all families from the core Brassicales except Pentadiplandraceae were included in the *ndhF* analyses. In addition, Bataceae, Tropaeolaceae, and Moringaceae were added as they are closely related to, but not part of, the core Brassicales (Fig. 1a; Rodman et al., 1996, 1998). Tropaeolaceae, representing the clade most distant from the core Brassicales in this analysis, was used as the outgroup in the *ndhF* analyses. For the *trnL-trnF* and combined *ndhF*/*trnL-trnF* analyses, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, and Tovariaceae were used as a monophyletic outgroup; their monophyly (except for the unsampled Pentadiplandraceae) is supported by the *ndhF* analysis. In all analyses *Forchhammeria* (Capparoideae) falls within the outgroup and, therefore, for tree rooting and representation, this taxon was designated as an outgroup for *trnL-trnF* and combined analyses (see below).

Extractions, amplification, and sequencing—Total genomic DNA was extracted from fresh, frozen, silica, or herbarium samples using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987; Smith et al., 1991) or DNeasy Plant Mini Kits (Qiagen, Valencia, California, USA). Standard polymerase chain reaction (PCR) and cycle sequencing techniques (Givnish et al., 2000) were used to amplify and sequence double-stranded DNA. The *trnL-trnF* region was amplified using ''c'' and ''f'' primers (Taberlet et al., 1991) to obtain a product including the *trnL* intron, the 3′ *trnL* exon, and the intergenic spacer between the 3′ *trnL* exon and *trnF* gene of the chloroplast genome. Four cycle sequencing primers were used for the *trnL-trnF* region: ''c,'' ''d,'' ''e,'' and ''f'' (Taberlet et al., 1991) allowing for verification of both strands. The 3′ end of the *ndhF* gene was amplified using forward primer 972F and reverse primer 2110R from Olmstead, Sweere, and Wolfe (1993) or slightly modified based on GenBank sequences of *Arabidopsis* (972F GTCTCAACTCGGTTATATGATG, 1603R GAATAGTATTGTCTGATTCATGCGG, and 2110R CCACCTATATATTTTGTTACTTCTCC). For some problematic taxa, the 3′ end of *ndhF* was amplified in two parts using the primer pairs 972F/1318R and 1318F/2110R. In a few instances, amplification was only possible using 972F and 1955R leaving the last 100 base pairs (bp) scored as missing for those taxa. Four primers were used to sequence both strands of the *ndhF* gene: 972F, 1603R, 1318F, and 2110R. Occasionally other primers (1603F, 1318F, 1655F, and 1955R) were used for sequence verification. All PCR products were cleaned using QiaQuick PCR purification kits (Qiagen). Sequences were generated on an ABI Prism 377 automated DNA sequencer.

Sequences were aligned by eye initially using Sequencer version 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and then in Se-Al version 2.0a6 (Rambaut, 2001). As noted by Taberlet et al. (1991) and confirmed by others for different plant groups (e.g., McDade and Moody, 1999; Sytsma et al., 2002), the *trnL-trnF* region has a relatively high frequency of insertions and/or deletions (indels). Indels that were potentially parsimoniously informative (e.g., shared by two or more taxa) were scored and added to the end of the data sets as presence/absence characters. Gaps that overlap were considered to be nested and treated as a single, multistate character (Simmons and Ochoterena, 2000). In some sequences there were large indels (around 300 bp) in regions of *trnL-trnF* in which there were smaller indels in other sequences. These smaller indels were scored, and taxa possessing the large indel were coded as unknown. Areas of ambiguous alignment or containing poly-n strings in *trnL-trnF* were excluded from all analyses. Indels in *ndhF* were scored in the same fashion and codon aligned in Se-Al using the known *Arabidopsis ndhF* sequence.

Phylogenetic analysis—Variation in DNA sequences was used to reconstruct phylogenetic relationships using PAUP* (Swofford, 2000). To explore the possibility of multiple islands of most parsimonious trees (Maddison, 1991), 1000 random taxon addition sequences with Multrees (save multiple trees) and TBR (tree bisection and reconnection) branch swapping were used to search for most-parsimonious trees. All characters were equally weighted and treated as unordered (Fitch, 1971). Given there were multiple indels in both data sets, all data were analyzed with and without scored indels. In addition to standard measure of fit of characters to the trees produced (i.e., consistency index, retention index), the strength of support for individual branches was estimated using bootstrap (Felstenstein, 1985) and decay analysis (Bremer, 1988). Bootstrap analysis used 1000 replicates (simple addition, saving up to 1000 trees per replicate, TBR branch swapping, Multrees) on the individual and combined data sets. In order to obtain decay values for branches, trees up to two steps longer were obtained using the same heuristic algorithm employed in the Fitch analysis. Decay values for branches still retained in strict consensus trees after these relaxed parsimony runs were obtained using reverse topological constraint with 100 random taxon addition sequences for each search, as suggested by Swofford (1993) and implemented by Baum, Sytsma, and Hoch (1994). Decay analyses were only performed on

TABLE 1. Characteristics of the three data sets.

Characteristic	*ndhF*	*trnL-trnF*	Combined
General			
Number taxa in analysis	55	57	49
Raw length	982–1000	640–959	n/a
Aligned length	1027	1300	2327
Parsimony			
Variable sites (%)	508 (49)	616 (47)	808 (38)
Parsimony informative sites (%)	318 (31)	218 (16)	472 (22)
Maximum likelihood			
Model chosen with hLRT	GTR + G[a]	TVM + I + G[b]	TVM + I + G[b]
Base frequencies			
A	0.3373	0.3604	0.3461
C	0.1304	0.1650	0.1479
G	0.1308	0.1510	0.1447
T	0.4014	0.3235	0.3613
Substitution rates			
A-C	1.3321	1.0049	1.1492
A-G	2.0420	1.3539	1.5525
A-T	0.2098	0.2516	0.2170
C-G	1.9235	1.0846	1.3451
C-T	1.7815	1.3539	1.5525
G-T	1.0000	1.0000	1.000
Proportion invariant sites (I)	0	0.2091	0.2192
Gamma distribution shape parameter (G)	0.7059	2.1897	1.3747

[a] General time reversible with an estimated gamma distribution shape parameter.
[b] Transversional model with an estimated proportion invariant sites and gamma distribution shape parameter.

the combined *ndhF* and *trnL-trnF* data set. The number of extra steps required to force taxa together based on previous systematic hypotheses was obtained by enforcing topological constraints with 100 random addition sequences. The significance of differences between constrained and unconstrained trees was tested using the Templeton (1983) nonparametric tree score option in PAUP*.

Because searches were not completed for *trnL-trnF* data under the above search parameters due to excessive numbers of most parsimonious trees, an alternative search strategy was employed. An initial heuristic search of 100 random addition replicates was conducted, with ten trees saved per replicate. The resulting consensus tree was then used as a backbone constraint to search for trees not consistent with the initial trees. This search strategy should detect that there are no shorter trees and that the strict consensus tree reflects all most parsimonious trees, even though all equal length trees have not been found (Catalán, Kellogg, and Olmstead, 1997).

Since not all species were sampled for both *trnL-trnF* and *ndhF* analyses, a reduced data set was analyzed in a combined analysis including only species with sequences for both regions. The incongruence length difference test (Farris et al., 1994, 1995) implemented as the partition homogeneity test in PAUP* was employed to measure conflict between the two reduced data sets. One thousand replicates were performed on parsimony-informative characters using TBR branch swapping algorithm (simple addition sequence, Multrees, steepest descent) with the maximum number of trees retained for each replicate limited to 1000. This procedure may reduce the chance of finding most parsimonious topologies, but Farris et al. (1994) note that exact tree lengths are not critical to the test. Because of the trivial amount of incongruence seen in topologies of the resulting individual analyses and between the data sets as demonstrated by the partition homogeneity tests, no other test assessing congruence was done.

Maximum likelihood analyses were conducted on the individual and combined data sets as implemented in PAUP*. With individual and combined data sets 56 maximum likelihood models were explored using Modeltest version 3.06 (Posada and Crandall, 1998). Modeltest compares 56 models of DNA substitution in an hierarchical testing framework by calculating the likelihood ratio statistic between different models to establish which model best fits the data. The likelihood ratio statistic and its associated *P* value are calculated using a chi-square distribution in order to reject or fail to reject different models of DNA substitution. One of the most parsimonious trees (randomly chosen) was used as the starting tree when running Modeltest instead of a neighbor-joining tree (default in Modeltest). An heuristic maximum likelihood search with TBR branch-swapping was then conducted using parameters determined for the best model of sequence evolution.

Morphological character state mapping—Patterns of morphological evolution were assessed with selected characters by overlaying them onto one of the most parsimonious trees obtained from the combined analysis using MacClade 4.0 (Maddison and Maddison, 2000); the tree was chosen based on the criteria of greatest topological agreement with the maximum likelihood tree. Characters were scored based on previous cladistic studies on the two families (Judd, Sanders, and Donoghue, 1994), literature (Woodson, 1948; Iltis, 1957, 1958, 1959; Elffers, Graham, and DeWolf, 1964; Jacobs, 1964, 1965; Haddade, 1965; Villiers, 1973; Hewson, 1982; Kers, 1986, 1987; Rollins, 1993; Vanderpool, 1993; Chamberlain and Lamond, 1996; Ruiz-Zapata and Iltis, 1998), herbarium specimens, and field studies. Characters analyzed included (1) habit—herbaceous annual, herbaceous perennial, woody perennial; (2) floral symmetry—actinomorphic, zygomorphic; (3) stamen number—<6, 6, 7–15, >15 and <50, >50; (4) leaf type—simple/pinnatifid, palmately compound; (5) fruit type—indehiscent and fleshy, dehiscent and fleshy, dehiscent with replum, dehiscent with replum and false septum, indehiscent and dry, and dehiscent, not fleshy; and (6) biogeography—northern Old World temperate, Old World tropical (Africa, Madagascar, and Asia), New World temperate, New World tropical, and Australia. All multistate characters were treated as unordered. Characters were scored for the individual species used in the molecular analysis. Character state evolution was reconstructed using the TRACE CHARACTERS function in MacClade.

RESULTS

Analysis of ndhF—The *ndhF* data set had an aligned length of 1027 bp of which 318 bp (31%) were potentially parsimoniously informative (Table 1). Individual sequence length

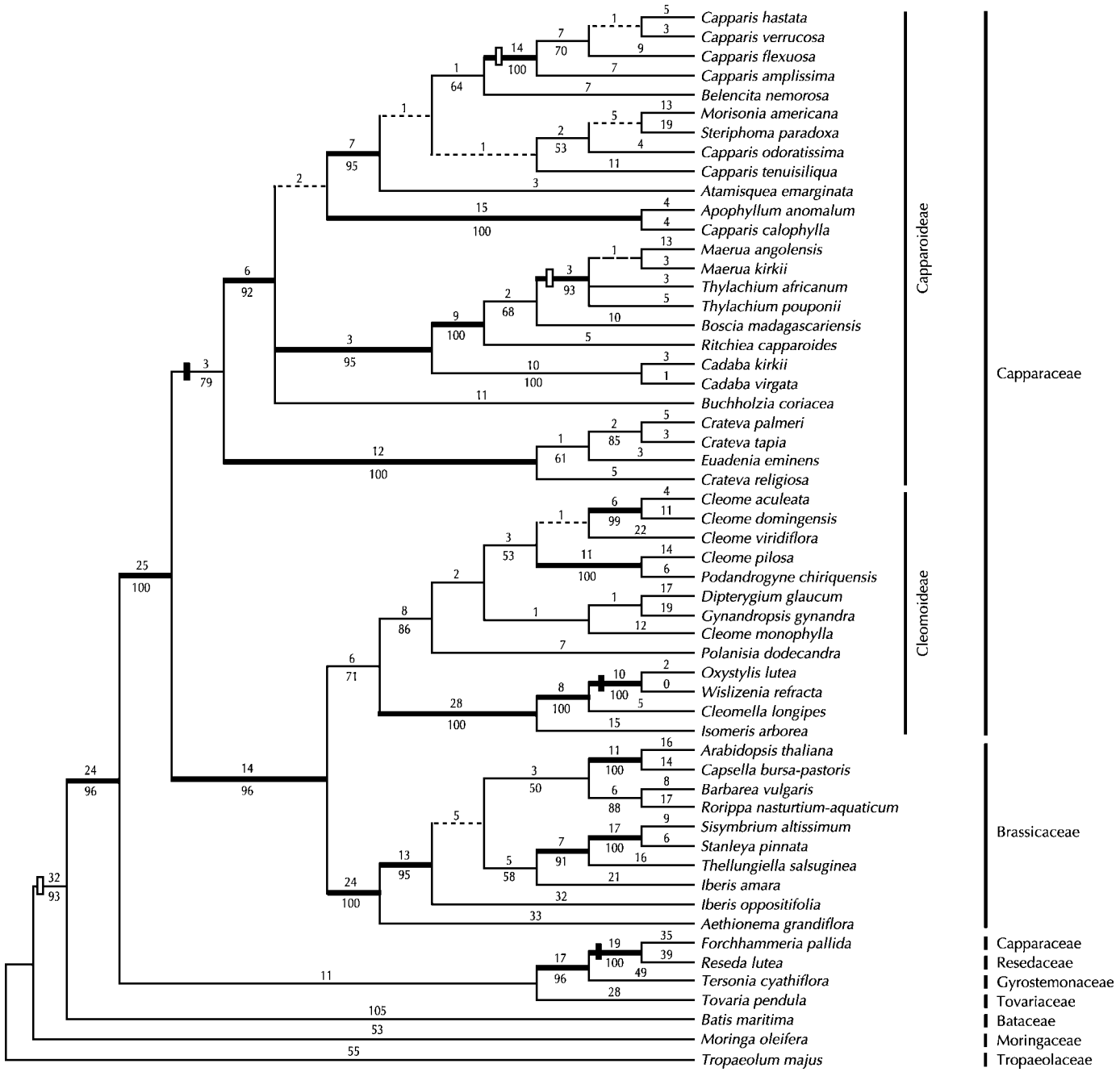

Fig. 2. One of 4499 most-parsimonious trees (randomly chosen) from analyses of the 55-taxa *ndhF* data set. *Tropaeolum* was designated as the outgroup. The trees are 1255 steps long. Branch lengths are provided above each branch, and bootstrap percentages >50% are given below. Branches with bootstrap support greater than 90% are in boldface type. Branches that collapse in the consensus tree are indicated with a dashed line. The four indels are indicated by boxes; closed boxes indicate the three indels with a single origin and open boxes indicate the one indel requiring parallel or reversal events (Appendix 2, http://ajbsupp.botany.org/v89/).

ranged from 982 bp (*Moringa oleifera*) to 1000 bp (*Batis maritima*). Fitch parsimony analyses of all 55 *ndh*F sequences found 4499 trees distributed in two islands (the first island contained 4498 trees, the second island is represented by one tree) of length 1255 (consistency index [CI] = 0.613, retention index [RI] = 0.740, rescaled consistency index [RC] = 0.453). One of the most parsimonious trees (from the larger island) is shown (Fig. 2) with branch lengths and support values indicated. Branches that collapse in the strict consensus tree are indicated by dashed lines. Four indels of 3, 6, or 9 bp in length (Appendix 2, http://ajbsupp.botany.org/v89/) were introduced during alignment. These were excluded from analyses but were mapped onto the tree (Fig. 2). Including or excluding the indels had no effect on the topology of the resulting cladograms or relative support of internal branches. The close sister family relationship between Brassicaceae and Capparaceae is well supported (100% bootstrap). Three major clades are recovered: Brassicaceae, Capparaceae subf. Cleo-

moideae, and Capparaceae subf. Capparoideae. As expected, Brassicaceae form a very well-supported monophyletic clade with 100% bootstrap value. Surprisingly, the two large subfamilies of Capparaceae are also moderately well-supported monophyletic clades (>70% bootstrap). Within the subfamily clades, many parts of the tree remain unresolved. Capparaceae appear to be paraphyletic with Cleomoideae as sister to Brassicaceae with strong support (Fig. 2, 96% bootstrap) and with Capparoideae sister to these two.

Hierarchical likelihood ratio tests suggested that the optimal model for these data is the GTR + G model, which allows for independent rates of substitution for all nucleotide pairs and allows rate heterogeneity among sites to be approximated by a gamma distribution with a single shape parameter, alpha (Table 1). The single tree found under this model (ln L = −8188.83676, tree not shown) has a nearly identical topology to the parsimony trees. In the resulting maximum likelihood tree, *Tovaria* is sister to Capparaceae plus Brassicaceae instead of sister to Resedaceae, Gyrostemonaceae, and *Forchhammeria*. Generally, branches that are poorly resolved in the maximum parsimony analyses (bootstraps <50%) are polytomies in the maximum likelihood tree.

Beyond Brassicaceae and Capparaceae, the topologies of the trees produced from *ndhF* sequence data are congruent with those of the Rodman et al. (1998) analysis of Brassicales (Fig. 1a). *Forchhammeria* (traditionally of Capparaceae subf. Capparoideae) appear in the core Brassicales as sister to Resedaceae, but outside the Capparaceae plus Brassicaceae clade. A Templeton test comparing trees with *Forchhammeria* placed within the Brassicaceae and Capparaceae clade (length 1278) to the most parsimonious trees (length 1255) indicates significant differences between these topologies ($P = 0.0002$). There is support for Gyrostemonaceae being sister to *Forchhammeria* and Resedaceae (96% bootstrap). These *ndhF* data support the placement of Gyrostemonaceae, Resedaceae, and Tovariaceae as closely related to the Capparaceae plus Brassicaceae clade and thus validate their use as outgroups for the *trnL-trnF* and combined analyses.

Analysis of trnL-trnF—The aligned length of *trnL-trnF* data set was 1300 bp in length with 199 bp removed from analysis due to ambiguous alignment and poly-n strings. There were 218 (17%) parsimoniously informative characters (Table 1). Raw sequence length ranged between 640 bp (*Stanleya pinnata*) and 959 bp (*Capparis verrucosa*). The initial analysis of the 57 taxa in which only ten trees per replicate were saved resulted in 960 trees of length 780 (Fig. 3; CI = 0.718, RI = 0.790, RC = 0.567). When the consensus tree from this analysis was used as a constraint tree in a subsequent analysis, only trees of length one step longer (781) were recovered. Thus, the consensus tree probably adequately represents the phylogenetic structure in all most parsimonious trees. Sixteen indels, varying from 4 to 307 bp in length, were scored (Appendix 2, http://ajbsupp.botany.org/v89/) and were mapped onto one of the most parsimonious trees (Fig. 3). The most striking is a deletion of 307 bp found in *Iberis oppositifolia*, *Iberis amara*, *Sisymbrium altissimum*, *Stanleya pinnata*, *Thellungiella salsuginea*, and *Thlaspi arvense* (Appendix 2, http://ajbsupp.botany.org/v89/). Including or excluding the scored indels had no effect on topologies or relative support of branches. Even when the scored indels were given a weight of ten there were still no significant changes or increased resolution in topology. The *trnL-trnF* trees are similar to the *ndhF* trees, recovering the same major three clades with moderate to high support: Brassicaceae (99% bootstrap), Cleomoideae (88% bootstrap), and Capparoideae (72% bootstrap). As with the *ndhF* results, Capparaceae is paraphyletic with Cleomoideae sister to Brassicaceae rather than to Capparoideae (78% bootstrap). Within each of these three clades, the resolved clades, although fewer, are those seen with *ndhF*.

The best model of DNA substitution for the *trnL-trnF* data set is TVM + I + G (transversional model) in which there are four different transversion rates, one transition rate, and among-site rate heterogeneity is modeled by allowing some sites to be invariant while the rest have rates drawn from a discrete approximation to a gamma distribution (Table 1). The heuristic search converged on a single optimal tree (ln L = −6156.6878, tree not shown), which is similar to the most parsimonious trees (Fig. 3), but more resolved. Three relationships recovered in the maximum likelihood analysis, but not in maximum parsimony are (1) a *Dipterygium* and *Gynandropis* (Cleomoideae) clade consistent with *ndhF* analysis, (2) a clade of *Capparis frondosa*, *C. hastata*, *C. tenuisiliqua*, and *C. tomentosa* (Capparoideae), and (3) a clade comprising all Brassicaceae except *Aethionema saxatile*.

Combined ndhF and trnL-trnF analysis—The partition homogeneity test indicated *ndhF* and *trnL-trnF* data sets have similar phylogenetic structure ($P = 0.34500$), but only if *Tersonia* of Gyrostemonaceae is excluded. Because the position of Gyrostemonaceae within the outgroup clade is irrelevant for relationships within and among the clades of Capparaceae and Brassicaceae, combining data is warranted. Parsimony analysis of the combined *ndhF* and *trnL-trnF* data for 49 taxa produced 36 trees of length 1609 (Fig. 4; CI = 0.689, RI = 0.790, RC = 0.544) that are consistent with trees obtained from the individual analyses (Figs. 2–3), especially with those of the *ndhF* analysis. The only area of conflict between the individual analyses is the relative placement of *Ritchiea* and *Boscia*, which swap positions between the two trees. The combined analysis supports the *trnL-trnF* topology, albeit weakly. Branches that were moderately to well supported in individual analyses have increased support in this analysis. Thus all analyses support the paraphyly of Capparaceae with Cleomoideae sister to Brassicaceae and with the anomalous *Forchhammeria* (Capparaceae) placed with the outgroup families Resedaceae and Gyrostemonaceae. Constraining the placement of *Forchhammeria* within the Capparaceae and Brassicaceae clade resulted in a tree 26 steps longer, which is significantly different (Templeton test, $P = 0.0005$).

The maximum likelihood tree (ln L = −12 285.285 56) under the preferred TVM + I + G model has a nearly identical topology to one of the most parsimonious trees (Fig. 5). Although the model of DNA evolution is the same as that for analysis of the *trnL-trnF*, the substitution rates and gamma shape parameter estimates are different between the two analyses (Table 1). There are two trichotomies in the resulting maximum likelihood tree in areas that are also poorly resolved in the parsimony analyses (Figs. 4–5). The maximum likelihood analysis of the combined data set supported *Apophyllum* and *Capparis calophylla* as sister to all Capparoideae excluding the *Crateva* clade; this relationship is consistent with both individual maximum likelihood analyses.

Patterns of morphological evolution—The evolution of particular morphological characters was explored by mapping

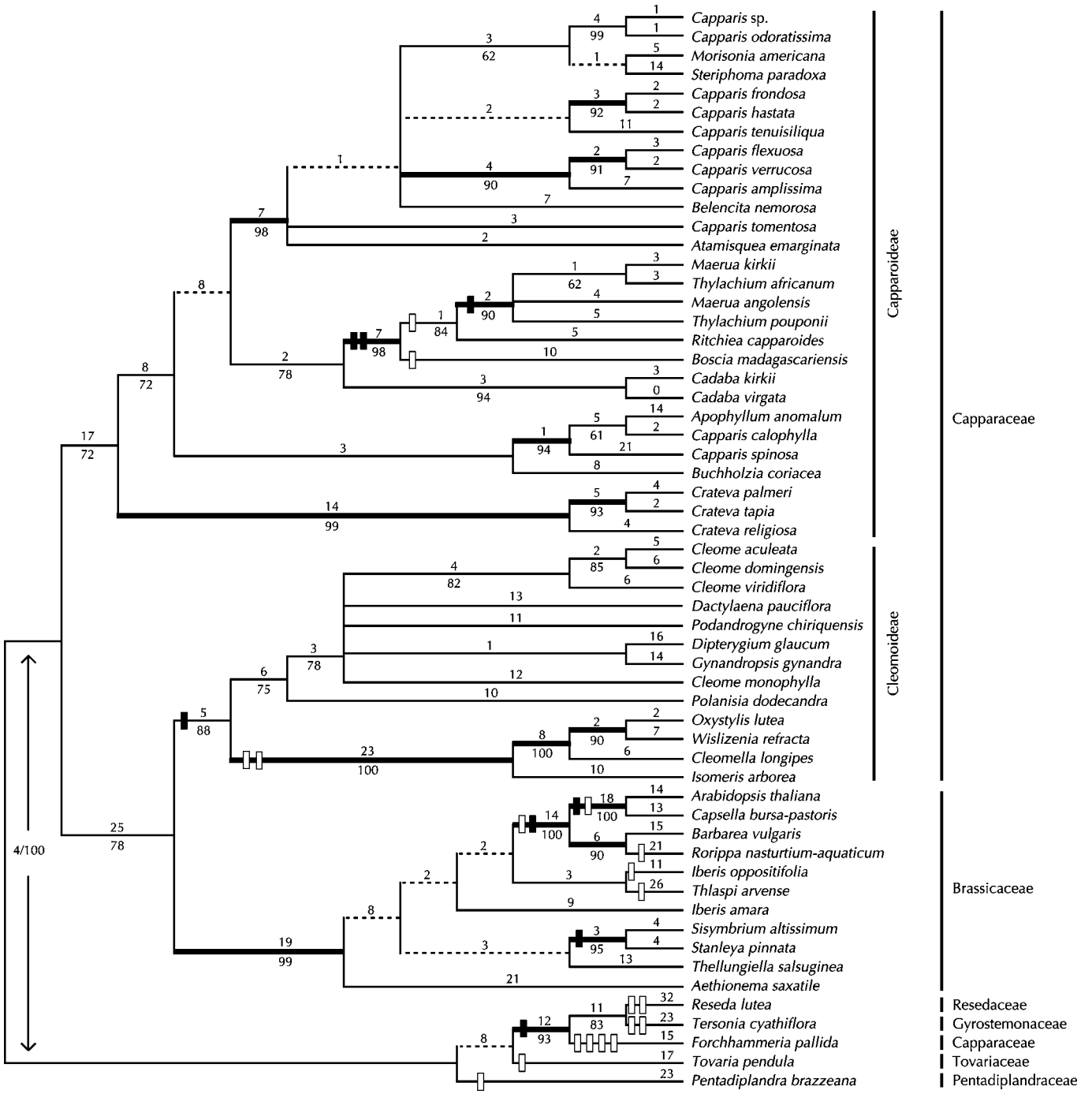

Fig. 3. One of the 960 most-parsimonious trees (randomly chosen) from analyses of the 57-taxa *trnL-trnF* data set. *Tovaria*, *Tersonia*, *Reseda*, *Pentadiplandra*, and *Forchhammeria* were used to root the tree. The trees are 780 steps long. Bootstrap values >50% are below branches. Branches with bootstrap values >90% are in boldface type. The 16 indels are indicated by boxes; closed boxes indicate single origin indels, and open boxes are indels requiring parallel or reversal events (Appendix 2, http://ajbsupp.botany.org/v89/).

character state changes onto one of the most parsimonious trees from the combined analysis (Fig. 6a–f). This tree was selected because it is the most-parsimonious tree that is most topologically congruent with the maximum likelihood analysis of the combined data (Figs. 4–5). The woody habit is plesiomorphic within the core Brassicales and the herbaceous habit is synapomorphic for the Brassicaceae plus Cleomoideae clade (Fig. 6a). Two, clearly parallel, instances of reversions to the woody habit occur in the ancestors of *Isomeris arborea* (Cleomoideae) and *Stanleya pinnata* (Brassicaceae). Floral actinomorphy is plesiomorphic for Brassicaceae and Capparaceae, with independent evolution of zygomorphy occurring at least three times in Capparoideae in *Cadaba*, *Steriphoma*, and *Crateva* (Fig. 6b). Floral zygomorphy is ancestral in Cleomoideae with a reversal to actinomorphy in *Dipterygium*.

Three characters, stamen number, leaf type, and fruit type

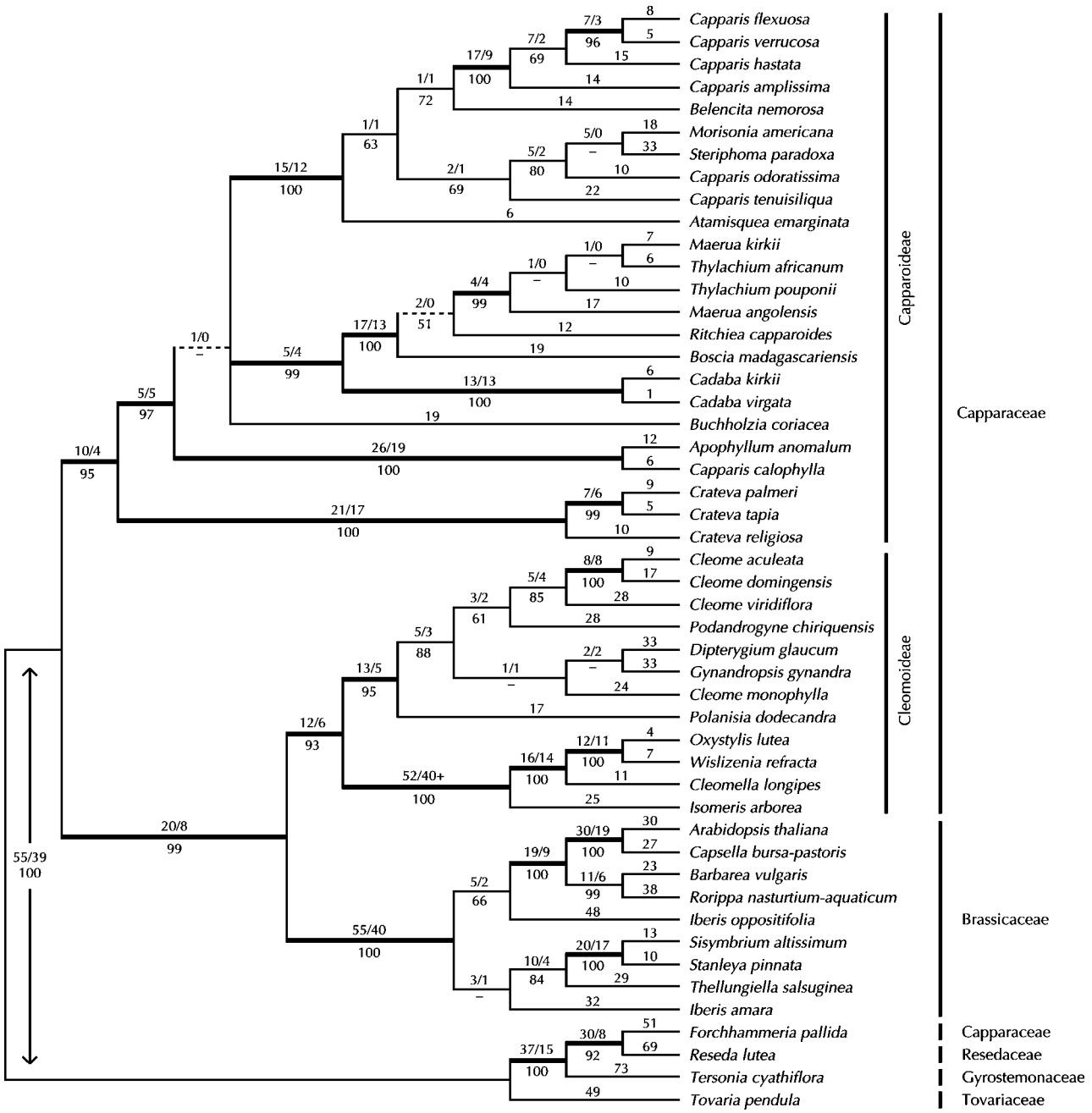

Fig. 4. One of 36 most-parsimonious trees from the 49-taxa combined *ndhF* and *trnL-trnF* data set. *Tovaria*, *Tersonia*, *Reseda*, *Pentadiplandra*, and *Forchhammeria* were designated as outgroups. The tree is 1609 steps long. Branch lengths/decay indices are provided above each branch, and bootstrap percentages are given below if >50%. Branches with bootstrap support >90% are in boldface type. Branches that collapse in the consensus tree are indicated with a dashed line.

(Fig. 6c–e), exhibit more complex changes in form than are evident with habit or floral symmetry. The basal number of stamens for the Brassicaceae and Capparaceae clade appears to be 7–15 (Fig. 6c). In the Cleomoideae and Brassicaceae clade there is a reduction in overall stamen number culminating in one fertile stamen in *Dactylaena* of Cleomoideae (*trnL-trnF* analyses). Among Capparoideae there are multiple increases and decreases in stamen number. The plesiomorphic condition of leaf type for the Capparaceae and Brassicaceae is equivocal (Fig. 6d). Brassicaceae and Cleomoideae are characterized by simple/pinnatifid and palmately compound leaves, respectively. Capparoideae usually have simple leaves, although some clades (especially the genus *Crateva*) have palmately compound leaves. Indehiscent fleshy fruits are plesiomorphic for Capparaceae and Brassicaceae (Fig. 6e). Characteristic capsules with a replum and false septum evolved within Brassicaceae and are distinct from fruits of Cleomoideae, which lack a false septum. Within Cleomoideae there is an independent origin of the dry indehiscent fruit in *Dipterygium*.

Biogeographical distribution of the three families is not completely congruent with phylogenetic relationships (Fig. 6f), but two clades in Capparoideae are associated with ge-

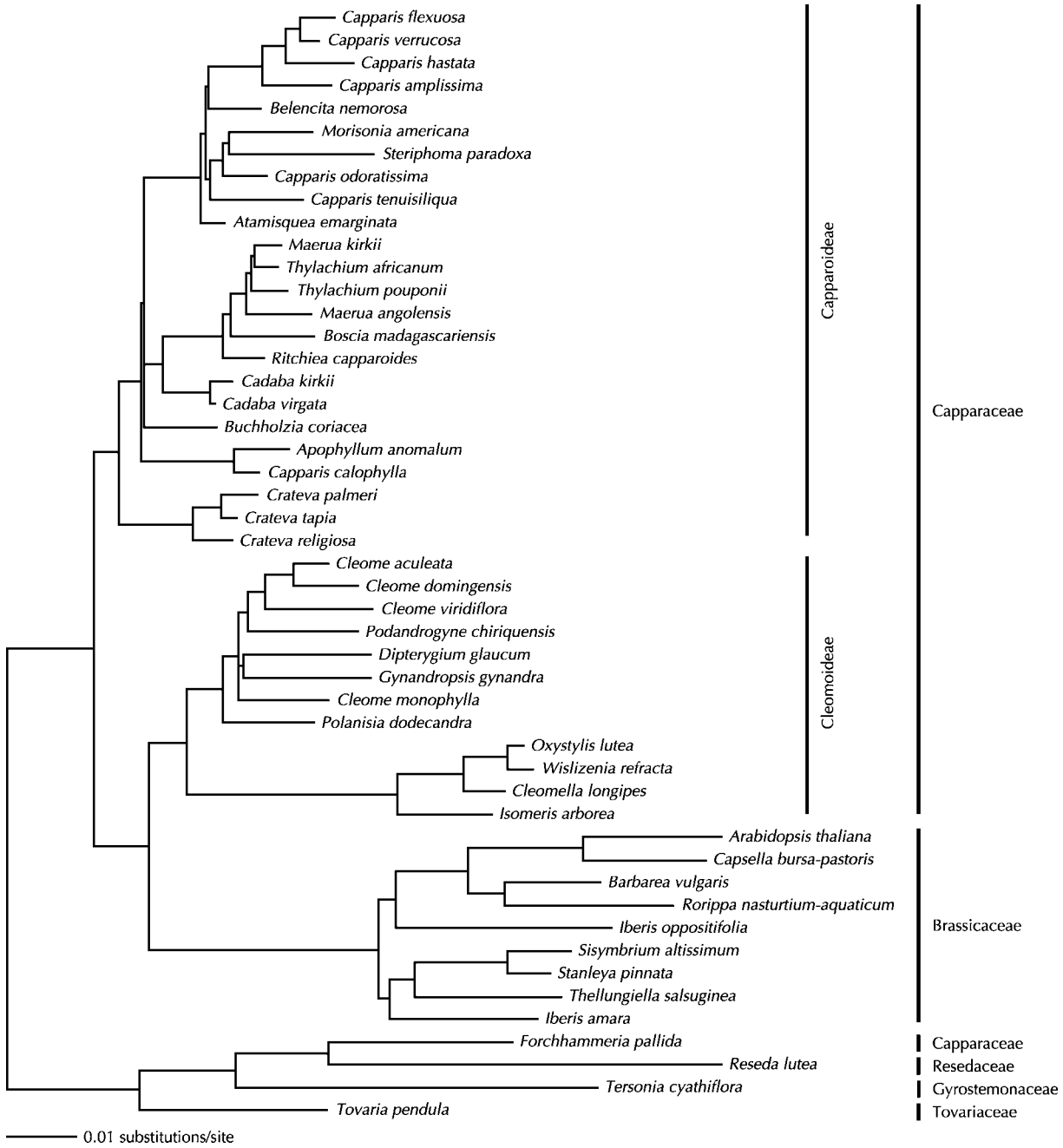

Fig. 5. The resulting maximum likelihood analysis tree of *ndhF* and *trnL-trnF* data sets combined (ln L = −12 285.285 56) under the TVM + I + G model. The topology is almost identical to one of the most parsimonious trees (Fig. 4) with the exception of two areas in the maximum likelihood where relationships between taxa are unresolved (see text). Branch lengths are proportional to the number of base changes along each branch.

ography in addition to one in Cleomoideae. Within Capparoideae, there is a New World clade (all New World representatives of *Capparis*, *Belencita*, *Morisonia*, *Steriphoma*, and *Atamisquea*) and a mostly African Old World clade (*Maerua*, *Thylachium*, *Ritchiea*, *Boscia*, and *Cadaba*). Although the most-parsimonious tree depicted suggests a grade of Old World tropical members in Capparoideae and thus implies a paleotropical origin for the group, the relationships among these clades are not resolved in parsimony analyses. In addition, this biogeographical pattern is complicated by the pantropical genus *Crateva* being sister to the rest of the Capparoideae clade and Old World *Capparis tomentosa* (only sampled *trnL-trnF*) nested within an otherwise New World clade. Within Cleomoideae, there is a well-supported New World temperate clade of *Cleomella*, *Isomeris*, *Oxystylis*, and *Wislizenia*. Relationships among the rest of Cleomoideae are not well enough resolved for biogeographic interpretations.

DISCUSSION

The sampling of Capparaceae presented here is considerably larger than any molecular study to date and is the first to in-

tentionally sample the diversity of the family with respect to Brassicaceae. Four significant groups were omitted from analysis due to lack of availability of tissue: (1) the monotypic Iranian genus *Buhsia* from Buhsioideae, likely to be embedded within Cleomoideae, (2) two Asiatic genera, *Poilanedora* and *Borthwickia*, that were not classified by Pax and Hoffmann (1936) but placed in Hutchinson's (1967) tribe Capparideae, (3) Asiatic genera *Stixis* and *Tirania*, Stixeae (Pax and Hoffman, 1936), and (4) the recently described *Dhofaria*, hypothesized to be closely related to *Apophyllum* (Miller, 1988). Although the data presented here reflect the limitations imposed by the taxon sampling, a better understanding of the phylogenetics of Capparaceae and their relationships to Brassicaceae is emerging. Three primary conclusions result from this molecular study: (1) Capparaceae subf. Cleomoideae are sister to Brassicaceae and not to Capparaceae subf. Capparoideae, thus making the Capparaceae paraphyletic; (2) *Forchhammeria* (previously in Capparaceae subf. Capparoideae) is not part of Capparaceae, being instead an isolated lineage associated with Resedaceae; (3) relationships within both subfamilies of Capparaceae, while not yet completely resolved, show the nonmonophyly of tribes and large genera, such as *Capparis*. The phylogenetic information presented here sheds light on the group's evolution and is compatible with several alternative classification schemes.

Relationship between Brassicaceae and Capparaceae—DNA sequence data from *ndhF* and *trnL-trnF* support the monophyly of Capparaceae plus Brassicaceae (bootstrap 100% in all analyses; Figs. 2–4) within the core Brassicales as seen with previous research (Rodman et al., 1993, 1996, 1998; Karol et al., 1999). Synapomorphies for Capparaceae plus Brassicaceae include (1) glucosinolates derived from methionine (Rodman, 1991b), (2) glucosinolates biosynthesized via long carbon chain extension (Rodman, 1991b), (3) sinapine present (Rodman, 1991b), and (4) vacuolar and utricular cisternae of endoplasmic reticulum (Judd et al., 1999).

The emergence of two well-supported clades of Capparaceae corresponding to the two subfamilies, Capparoideae (bootstrap 72–95%) and Cleomoideae (bootstrap 71–93%), is unexpected. As indicated by previous analyses (Rodman et al., 1993, 1996, 1998; Judd, Sanders, and Donoghue, 1994), Capparaceae as currently delimited are paraphyletic with a monophyletic Cleomoideae more closely related to Brassicaceae (bootstrap 78–99%; Figs. 1–5). However, the precise nature of the paraphyly is unexpected in that, rather than a paraphyletic grade of many lineages as previously suggested based on morphological data (Judd, Sanders, and Donoghue, 1994), there are two well-supported lineages representing the two major subfamilies. Unexpectedly, given the very diverse morphological characteristic of floral, fruit, and vegetative features and contrary to previous morphological analyses (Judd, Sanders, and Donoghue, 1994), Capparoideae form a well-supported clade (bootstrap of 94% in the combined analysis). This monophyly is based on sampling 24 species representing all tribes and 15 of 18 genera placed in the subfamily by Pax and Hoffmann (1936). Capparoideae are distinguished from Cleomoideae and Brassicaceae by plesiomorphic features of woody habit and often fleshy indehiscent fruits that usually lack the presence of a replum. One synapomorphy for the clade is an unique six-bp insertion in the *ndhF* sequences (Fig. 2).

The close relationship between Cleomoideae and Brassicaceae is supported by (1) the presence of a thickened repla in the dehiscent (and silique-like) fruit, in which the valves break away from the persistent replum (although some species of Capparoideae also possess repla), (2) herbaceous habit (Judd, Sanders, and Donoghue, 1994), and (3) reduction in the number of stamens. Cleomoideae is monophyletic with the inclusion of the remaining subfamilies described by Pax and Hoffmann that were sampled in this study. The small Dipterygioideae (represented by *Dipterygium* in our study) is allied with *Cleome* within Cleomoideae. The placement of this genus in some lineage of Capparaceae is supported by the presence of methyl-glucosinolate, a compound that is known from Capparaceae but not Brassicaceae (Hedge, Kjaer, and Malver, 1980; Lüning, Kers, and Seffers, 1992). Floral characteristics that ally *Dipterygium* in Cleomoideae include six stamens of equal length (not tetradynamous) and the presence of a short gynophore. Podandrogynoideae is also allied with *Cleome*, which is in agreement with previous suggestions that it does not warrant subfamilial rank (Woodson, 1948; Iltis and Cochrane, 1989). Characteristics that have been used as synapomorphies for Cleomoideae include palmately compound leaves and zygomorphic flowers (Judd, Sanders, and Donoghue, 1994; Judd et al., 1999). In light of the well-supported relationships of *Crateva* and *Euadenia* (*ndhF*) as sister to the rest of Capparoideae, these characters can no longer serve as unambiguous synapomorphies for Cleomoideae.

Phylogenetic relationships of genera within Brassicaceae are beyond the scope of this paper and are currently being studied by other researchers (e.g., Sweeney and Price, 2000; Koch, Haubold, and Mitchell-Olds, 2001; Mummenhoff, Brüggemann, and Bowman, 2001). The relationships among species presented here are congruent with other analyses of the family, especially with Galloway, Malmberg, and Price (1998), for which there exists the greatest sampling overlap. *Aethionema grandiflora* is sister to all other Brassicaceae in the *ndhF* analyses (not sampled in the *trnL-trnF* or combined analyses; however *A. saxatile* is sister to all other Brassicaceae in the maximum likelihood analysis of *trnL-trnF*), as seen previously (Price, Palmer, and Al-Shehbaz, 1994; Galloway, Malmberg, and Price, 1998). The close relationship of *Sisymbrium* and *Stanleya* and their position well within the Brassicaceae are significant (Price, Palmer, and Al-Shehbaz, 1994; Galloway, Malmberg, and Price, 1998). *Stanleya* belongs to the traditionally putative basal tribe of Thelypodieae (Al-Shehbaz, 1984), which was proposed to be intermediate between Cleomoideae and Brassicaceae (Airy Shaw, 1965). *Stanleya pinnata* is a woody species found in the western United States in which the stamens are not tetradynamous, suggesting this characteristic is secondarily derived within Brassicaceae.

Relationships of Forchhammeria—In all three data sets, *Forchhammeria* (the only representative of tribe Stixeae, Capparoideae) is excluded from the Capparaceae-Brassicaceae lineage. *Forchhammeria* is most closely related to Resedaceae in the *ndhF* and combined analyses (100% and 92% bootstrap, respectively), or to both Resedaceae and Gyrostemonaceae in the *trnL-trnF* analysis (93% bootstrap). The exact relationships of *Forchhammeria* and other members of the tribe Stixeae within the core Brassicales are currently under further study.

Relationships within Capparaceae—Whereas these data provide robust support for the relationships between Brassicaceae and the two subfamilies of Capparaceae, there is less supported resolution within the terminal clades. Capparoideae

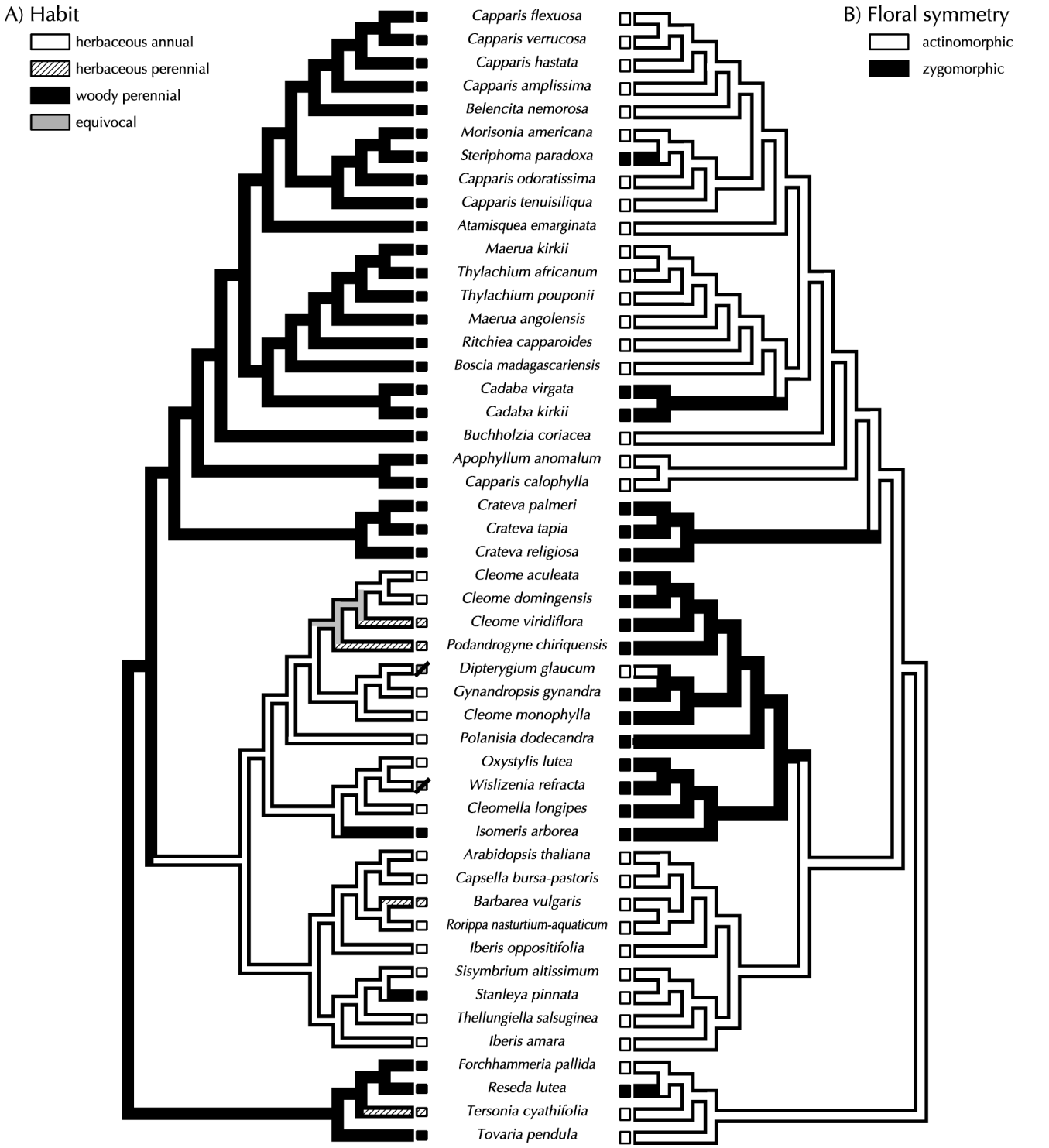

Fig. 6. Overlays of morphological and geographical characters on one of the most parsimonious trees from the combined analyses (Fig. 4). Taxa with polymorphic character states are indicated by a diagonal bar. (A) Habit: herbaceous annual, herbaceous perennial, woody perennial; (B) Floral symmetry: actinomorphic, zygomorphic; (C) Stamen number: <6, 6, 7–15, >15 and <50, >50; (D) Leaf type: simple/pinnatifid, palmately compound, (E) fruit type: indehiscent and fleshy, dehiscent and fleshy, dehiscent with replum, dehiscent with replum and false septum, indehiscent and dry, and dehiscent, not fleshy; (F) Biogeography: northern Old World (OW) temperate, OW tropical (Africa, Madagascar, and Asia), New World (NW) temperate, NW tropical, and Australia.

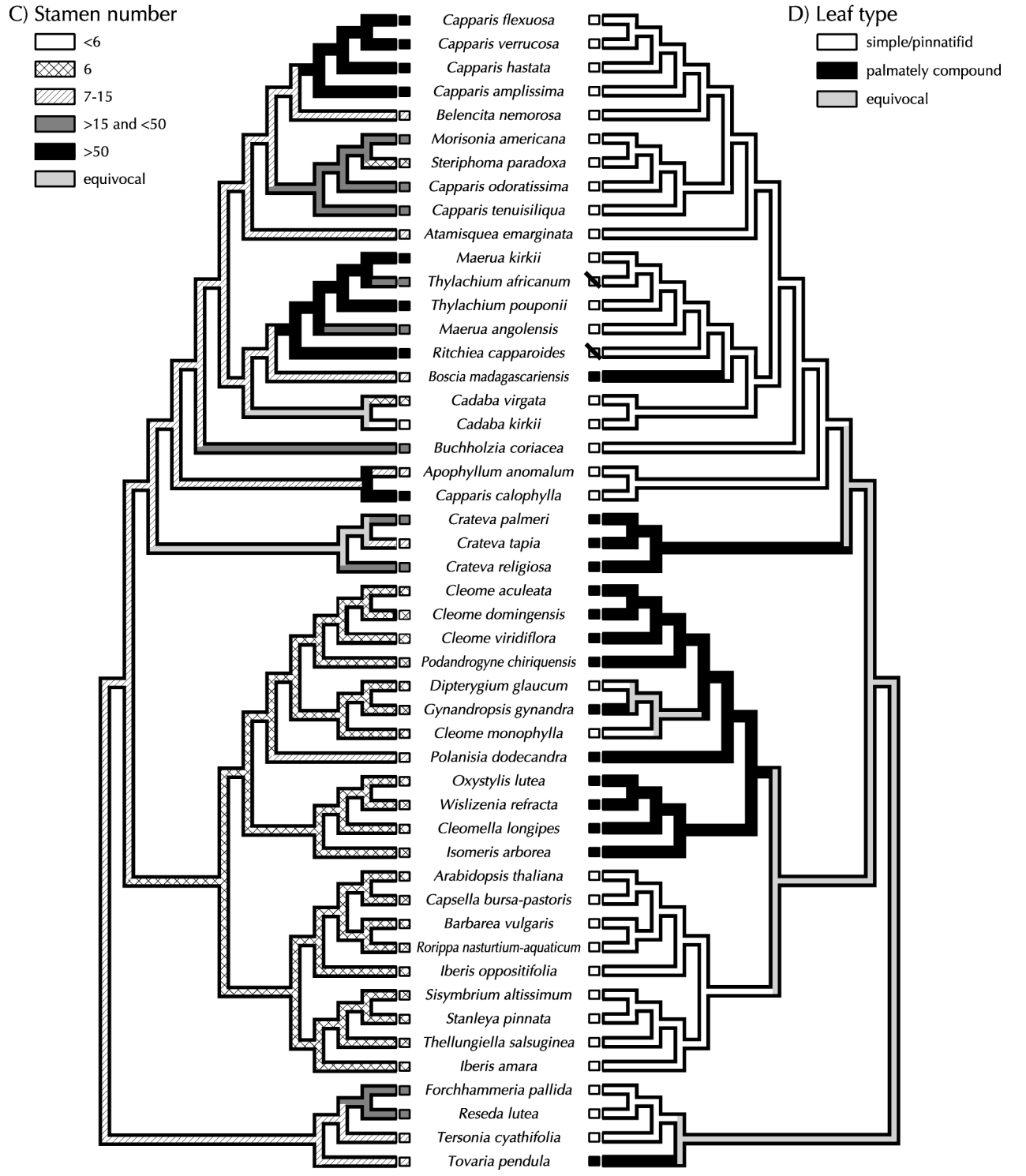

Fig. 6. Continued.

are unresolved but comprise five well-supported clades: (1) *Crateva*, (2) *Boscia*, *Cadaba*, *Maerua*, *Ritchiea*, and *Thylachium*, (3) *Buchholzia*, (4) *Apophyllum* and *Capparis calophylla*, (5) *Atamisquea*, *Belencita*, remaining *Capparis*, *Morisonia*, and *Steriphoma*. With the exceptions of *Maerua* and *Thylachium*, which belong to tribe Maerueae, all these genera belong to the tribe Capparideae of Pax and Hoffmann (1936). There is no correspondence of these clades to any of Hutchinson's (1967) tribes (Appendix 1, http://ajbsupp.botany.org/v89/). The traditional tribes in Capparoideae described by Pax and Hoffmann (1936) divided Capparoideae into Capparideae, with rounded buds, stellate or simple hairs, scales, seldom with

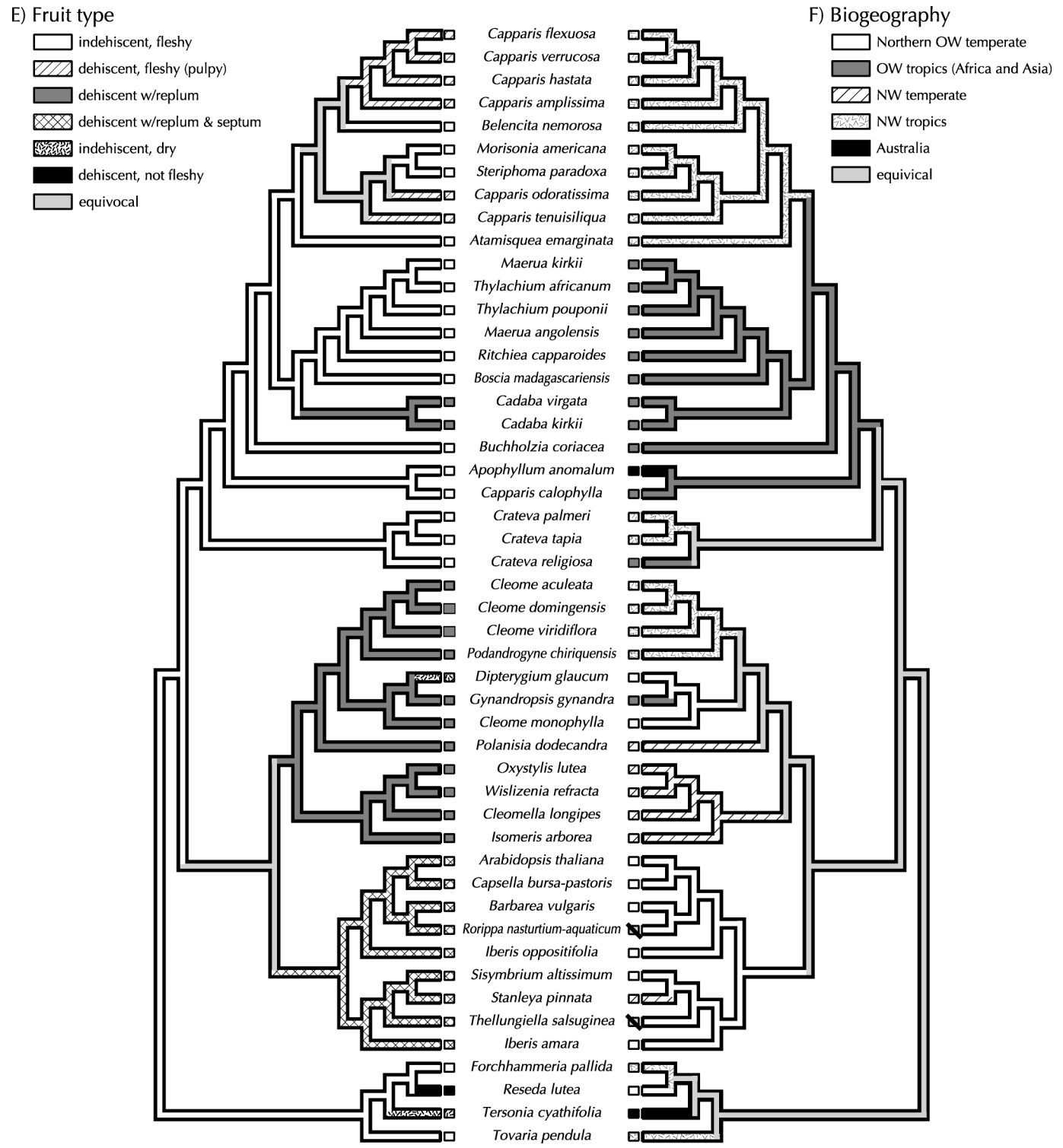

Fig. 6. Continued.

glandular hairs, and no sap (16 genera); Maerueae, with elongated cylindrical buds (3 genera); and Stixeae, with flowers that typically have six sepals (5 genera). Similarly, Hutchinson (1967; Appendix 1, http://ajbsupp.botany.org/v89/) divided Capparoideae (equivalent to his Capparaceae) into Cappardeae, with bisexual flowers and more than two ovules (25 genera); Cadabeae, with bisexual apetalous flowers and more than two ovules (8 genera); and Apophylleae, with unisexual flowers with one or two ovules (2 genera). In all cases where we sampled more than one genus from any of the tribes recognized by the authors, monophyly was contradicted. Hutchinson's classification is particularly unsatisfactory because it

splits two genera, *Maerua* and *Cadaba*, into different tribes because they are polymorphic with respect to the presence/absence of petals. The failure of traditional tribal classifications suggests that the traditionally important morphological characters are more homoplasious than previously considered.

Within Capparoideae there is a surprising association between phylogeny and biogeographical distributions (Fig. 6f). Capparaceae are a primarily tropical group with many genera of Capparoideae confined to the Old World. *Crateva* (with *Euadenia* imbedded within it based on *ndhF*), traditionally placed in the tribe Capparideae, forms a well-supported sister group to the remaining species (Figs. 2–5). *Crateva*, comprising approximately nine species, has a pantropical distribution (except Australia), whereas *Euadenia*, with three species, is endemic to central and western Africa. The close relationship of *Euadenia* and *Crateva* has been suggested based on similarities of habit and floral characteristics (Jacobs, 1964). In addition, *Cladostemon*, unsampled in this study, has a floral morphology suggesting a close relationship with *Euadenia* (DeWolf, 1962; Elffers, Graham, and DeWolf, 1964). The clade of *Crateva* includes species with zygomorphic flowers and palmately compound leaves, characteristics also present in Cleomoideae. DeWolf (1962) suggested that *Crateva* is the most primitive Capparaceae and suggested that all African Capparaceae were derived from within it. However, the hypothesis that *Crateva* has a close relationship to other African genera, *Ritchiea*, *Euadenia*, and *Cladostemon* (Jacobs, 1964) or *Thylachium*, *Maerua*, and *Boscia* (DeWolf, 1962) is not supported by these molecular data.

Of the five genera for which more than one species is sampled (*Capparis*, *Maerua*, *Thylachium*, *Cadaba*, and *Crateva*), only *Cadaba* is supported as monophyletic (bootstrap 94–100%). *Cadaba* is well supported as a natural genus based upon the presence of a large, adaxial gland in the flowers. *Capparis* are not monophyletic with New World *Capparis* (with the exception of Old World *C. tomentosa* in *trnL-trnF* analysis) related to other New World genera such as *Atamisquea*, *Belencita*, *Morisonia*, and *Steriphoma* and forming a group with strong support (100% bootstrap). *Capparis calophylla* and *C. spinosa* (type species of Capparaceae and only sampled in *trnL-trnF* analysis) are more closely related to *Apophyllum*, a genus endemic to Australia, than to other *Capparis*. Constraining *Capparis* to be monophyletic with the combined data set increases the length of the tree by 40 steps, which is significantly different (Templeton test, $P = 0.0001$). The polyphyly of *Capparis* is not surprising, having been suggested previously based on morphological evidence. Hutchinson (1967) proposed dividing up *Capparis* into smaller entities based mostly on calyx variation. Likewise, the possibility of Old World and New World *Capparis* being unrelated has been suggested previously (DeWolf, 1962; Elffers, Graham, and DeWolf, 1964). Unless *Capparis* were expanded to include a large portion of Capparoideae, nomenclatural changes of this genus are needed.

Within Cleomoideae there are two well-supported clades: (1) the North American endemic *Cleomella*, *Oxystylis*, *Wislizenia*, and *Isomeris* and (2) *Cleome*, *Dactylaena*, *Dipterygium*, *Gynandropis*, *Podandrogyne*, and *Polanisia*. The first clade has been studied, both with morphological (Iltis, 1957; Keller, 1979) and molecular (Vanderpool, Elisens, and Estes, 1991) approaches. These genera share a significant number of floral and vegetative features, including the possession of inconspicuous stipules, which are only found in a few other species of Cleomoideae (Iltis, 1957). In addition, there is specialization in fruit type found in these genera, with the replum of *Oxystylis* and *Wislizenia* so reduced that a bilocular fruit is produced. *Polanisia*, generally considered to be closely related to *Cleome* (Iltis, 1958), are supported as sister to the remainder of the second clade (Figs. 2–5). Relationships within this clade are not well resolved, with the only strongly supported relationship being (*Cleome viridiflora*, *Cleome aculeata*, *Cleome domingensis*). When the combined data set topology was constrained to force a monophyletic *Cleome*, the increase in tree length of two steps was not significant (Templeton test, $P = 0.7539$). However, in the *ndhF* analysis the sister relationship of *Cleome pilosa* and *Podandrogyne chiriquensis* is strongly supported (bootstrap 100%; Fig. 2), suggesting *Cleome* is not monophyletic. Although there is clearly a wide range of floral diversity in Cleomoideae, there tend to be fewer stamens (<15) than in Capparoideae (6–250).

Character evolution and biogeography of Capparaceae and Brassicaceae—*Habit*—Habit shows a striking congruence with one of the most parsimonious trees in the combined *ndhF* and *trnL-trnF* analyses (Fig. 6a). All Capparoideae are woody, which is the plesiomorphic condition for Brassicaceae plus Capparaceae clade. The herbaceous habit is found in Cleomoideae and Brassicaceae with the exception of two woody species: *Isomeris arborea* (Cleomoideae) and *Stanleya pinnata* (Brassicaceae), which are clearly parallel origins.

Leaves—The basal condition of leaf type within the Capparaceae and Brassicaceae clade is equivocal (Fig. 6d). Some species of *Cadaba*, *Maerua*, *Thylachium*, and *Podandrogyne*, which were not sampled in these analyses, are palmately compound. In addition, species of *Ritchiea* and *Thylachium* have both simple and compound leaves on the same plant, indicating plasticity of this character. It is also possible that some of these simple leaves would be more accurately described as unifoliate. This interpretation also holds for some species of Cleomoideae, which have superficially simple leaves. *Cleome* section (sect.) *Physostemon* includes mostly unifoliate species, which is clearly a reduction (Iltis, 1959). There is a shift to simple/pinnatifid leaves in the Brassicaceae clade. Because species of *Crateva* have palmate leaves (with the exception of the unsampled *C. simplicifolia*) and leaf type is equivocal at the base of the clade, palmate leaves cannot be supported as a synapomorphy for Cleomoideae (contra Judd, Sanders, and Donoghue, 1994; Judd et al., 1999).

Zygomorphic flowers—The propensity for the zygomorphic condition in Capparaceae is evident in the multiple origins (Fig. 6b) and morphological differences in the flowers of the zygomorphic species. Cleomoideae are characterized by zygomorphic flowers, which has been used as one potential synapomorphy for the clade (Judd et al., 1999). The mature flowers of Cleomoideae typically have all four petals oriented upwards. Under current sampling, zygomorphy also arises at least three times in Capparoideae in *Cadaba*, *Steriphoma*, and *Crateva* (*Euadenia* as well in the *ndhF* analysis). Judd, Sanders, and Donoghue (1994) scored *Crateva* as actinomorphic, but in our studies we score all *Crateva* species as zygomorphic—the upper petals of *Crateva* are larger in size and petals typically take a horizontal position (reminiscent of Cleomoideae). The genus *Euadenia* (part of the *Crateva* clade in the *ndhF* analysis) has clearly dimorphic petals, with the upper

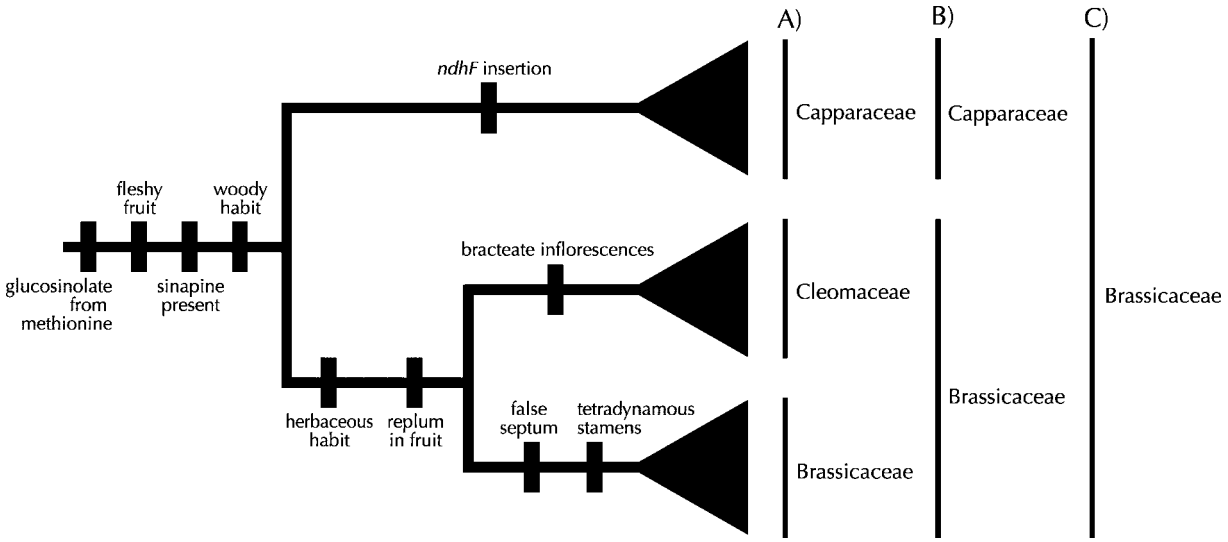

Fig. 7. Three scenarios for family recognition in the Brassicaceae and Capparaceae clade consistent with the molecular data. Some distinguishing morphological characters are placed to represent synapomorphies for particular clades. (A) All three clades are separate families: Capparaceae, Cleomaceae, and Brassicaceae. (B) Cleomoideae are subsumed in Brassicaceae, and Capparaceae remain a separate family. (C) All three clades are in one broadly defined Brassicaceae (sensu APG, 1998).

pair significantly larger than the lower. The flowers of *Cadaba* are distinctive, with a very prominent adaxial gland as well as an often curved androphore, sepals that are in two unequal series, and petals that may be oriented towards one side. The flowers of *Steriphoma* have fused, irregularly splitting sepals, and the stamens curve upward. Other members of the family have a subtler zygomorphy expressed by the sterilization of upper stamen (many species of *Cleome*), only one fertile stamen (*Dactylaena*), stamens distinctly curved (some species of *Cleome*), unequal calyx (many species of *Capparis*), or unequal corolla (some species of *Capparis*). Although the current sampling indicates all Brassicaceae are actinomorphic, zygomorphy appears in ten genera of Brassicaceae with eight of these genera expressing zygomorphy in the stamens, one genus zygomorphic in the petals, and one genus zygomorphic in both the petals and the stamens (Endress, 1992). The most parsimonious explanation (and the most likely based on difference in zygomorphic Capparaceae) is an ancestral actinomorphic flower in Capparaceae plus Brassicaceae with independent origins of zygomorphy. Clearly, the multiple origins of zygomorphy within Capparoideae and Cleomoideae need to be evaluated in more detail.

Stamens—Stamen number is extremely variable within Capparaceae, and a clear pattern of stamen number evolution within Capparaceae and Brassicaceae is elusive. There has been debate regarding whether the six-staminate condition of Cleomoideae and Brassicaceae is derived (Erbar and Leins, 1997a, b; Ronse Decraene and Smets, 1997) or primitive (Endress, 1992) within Brassicales. Erbar and Leins (1997a, b) hypothesized a sequence of transitions from the fascicled stamen development of *Reseda* (Resedaceae) and a broad ring primordia (interpreted as fused fascicles) of *Capparis* to a simple and fixed stamen number of six found in Brassicaceae and Cleomoideae. The data presented here suggest that an intermediate stamen number of 7–15 is the plesiomorphic condition for the Brassicaceae and Capparaceae clade with multiple and independent increases and decreases in stamen number occurring in Brassicaceae and both subfamilies of Capparaceae (Fig. 6c). The homology of the six-stamen condition between Cleomoideae and Brassicaceae needs to be examined further. Some members of Cleomoideae (e.g., *Cleome spinosa*) display the same stamen initiation pattern of many Brassicaceae in which petal primordia initiate first followed by the two transversal stamens and then the four median stamens (Erbar and Leins, 1997a, b). However, the stamens of other species of Cleomoideae develop in a zig-zag (*Cleome violacea*, Erbar and Leins [1997a]) or unidirectional (*Polanisia dodecandra*, Erbar and Leins [1997b]) pattern. Even within Brassicaceae, which displays a remarkable uniformity in number and position of floral parts, there are differences in sequences of development of stamen and petal organs (Erbar and Leins, 1997b). For example, within *Lepidium* (Brassicaceae) there are parallel reductions to two and four stamens (Bowman et al., 1999).

Fruit—Fruit type has long been an important taxonomic character for distinguishing Capparaceae and Brassicaceae. Indehiscent, fleshy fruits are plesiomorphic in the Capparaceae and Brassicaceae and the dominant fruit type of Capparoideae (Fig. 6e). Brassicaceae and Cleomoideae both have dehiscent capsules with a replum, a synapomorphy shared by these two clades (Judd, Sanders, and Donoghue, 1994; Judd et al., 1999). The fruit of *Podandrogyne* curls during opening, which Pax and Hoffmann (1936) interpreted as replum absent and, thus, led them to elevate this genus to subfamilial status. However, as suggested previously (Woodson, 1948; Iltis and Cochrane, 1989), *Podandrogyne* has a replum that is clearly homologous to that of *Cleome*. Fruits of Capparoideae may also have repla and homology of this structure to repla found in Cleomoideae and Brassicaceae needs to be further addressed. Although *Cadaba* (Capparoideae) are usually scored as having the same fruit type as *Cleome*, the fruits of *Cadaba* are fleshy by containing a pulp, which is unlike fruits of *Cleome*. There is a persistent yet obscure replum in some species of *Capparis* (Ruiz-Zapata and Iltis, 1998), which were scored as lacking a replum for the purposes of this study. Within Brassicaceae a false septum

has evolved that results in a two-locular fruit. Although dehiscence has evolved in parallel in the two subfamilies of Capparaceae, the dehiscent fruits of Capparoideae are almost always fleshy in nature in contrast to the often-dry fruits of Cleomoideae and Brassicaceae.

Taxonomic implications for classification: one, two, or three families?—The combination of chloroplast and nuclear data presented here and elsewhere (Rodman et al., 1998) strongly supports the monophyly of Brassicaceae and Capparaceae and its division into three primary clades, Capparaceae subf. Capparoideae, subf. Cleomoideae, and Brassicaceae, with strong support for a sister relationship of the latter two. How then should families be recognized in this group? In previous morphological (Judd, Sanders, and Donoghue, 1994) and molecular (Rodman et al., 1993, 1996, 1998) analyses, perhaps due to a more limited taxon sampling, these relationships were not well resolved. Consequently the combination of the two families into one (Brassicaceae sensu lato) was logical and was advocated by the APG (1998) classification system. However, the criterion of monophyly permits the recognition of either one family (Brassicaceae), two families (Brassicaceae representing both Brassicaceae and Cleomoideae, and Capparaceae representing only Capparoideae), or even three families (Brassicaceae, Cleomaceae, and Capparaceae; Fig. 7). The phylogenetic relationships inferred here with increased sampling of Capparaceae shows that family rank is not about monophyly per se but rather is ''a matter of ranking and pragmatics of family identification'' (Clausing and Renner, 2001, p. 494). Subsuming all of Capparaceae or just Cleomoideae within an expanded Brassicaceae obscures the many clear morphological characters that leave Brassicaceae a practical and cohesive family. Additionally, the two subfamilies of Capparaceae, Cleomoideae and Capparoideae, have already been elevated to familial status by previous taxonomists (Airy Shaw, 1965; Hutchinson, 1967). Based on the strong bootstrap support for the three lineages and the identification of a suite of morphological features (although not necessarily individually) that defines each, we advocate the break up of Brassicaceae s.l. (sensu APG, 1998) and the recognition of three well-supported monophyletic families: Capparaceae, Cleomaceae, and Brassicaceae.

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