Molecular Phylogenetics of Core Brassicales, Placement of Orphan Genera *Emblingia, Forchhammeria, Tirania*, and Character Evolution

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**Abstract.** Many genera previously placed in the traditionally circumscribed Capparaceae are either unrelated or, more commonly, isolated lineages in the order Brassicales. This study examines the relationships of these genera, analyses were conducted across Brassicales using chloroplast *rbcL*, *ndhF*, and *matK* sequence information. Both maximum parsimony and maximum likelihood analyses place all three genera in the well-supported core Brassicales (Brassicaceae, Capparaceae, Cleomaceae, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, and Tovariaceae). The Asiatic *Tirania* and New World tropical *Forchhammeria* are closely related to two small families, the pan-temperate *Resedaceae* and the Australian *Gyrostemonaceae*. These analyses also indicate a novel placement of *Emblingia* as sister to all remaining members of core Brassicales. Although there is strong support for the relationships among most of these taxa, relationships of Pentadiplandraceae and Tovariaceae are weakly resolved. Thus, the core Brassicales is a biogeographically dispersed lineage that is comprised of many small and morphologically distinct clades plus the large crown group *Brassicaceae* s. lat. Patterns of morphological evolution appear complex, especially in floral morphology and carpel and locule number. Likewise, the evolution of breeding systems within this lineage involves recurrent shifts towards monoecy or dioecy, and possible reversals to bisexuality. Further sampling of Capparaceae tribe Stixeae is critical for any taxonomic recommendation of familial status for these orphan genera.

The pantropical family Capparaceae Jussieu, with up to 45 genera and 900 species, has long presented enormous difficulties related to monophyly and generic placements. Recent molecular and morphological phylogenetic analyses of Capparaceae s. lat. and closely related Brassicales in the order Brassicales indicate that Capparaceae s. lat. as traditionally circumscribed are paraphyletic (Rodman et al. 1993, 1996, 1998; Judd et al. 1994; Hall et al. 2002; Capparaceae s. lat. indicates traditional, paraphyletic familial delimitation). Capparaceae s. lat. comprise two monophyletic groups corresponding to the two major subfamilies, Cleomioideae and Capparoideae, with the Cleomioideae more closely related to Brassicales than to Capparoideae (Hall et al. 2002). These three taxa have been either combined into one family, Brassicales s. lat. (Judd et al. 1994; APG 1998, 2003), or elevated to three separate families, Capparaceae s. str. (equivalent to Capparoideae), Cleomaceae (Airy Shaw 1965), and Brassicales (Hall et al. 2002). In this paper, we use the later classification based on both morphological and molecular grounds (Hall et al. 2002). In addition to challenges of monophyly of Capparaceae s. lat. and issues of familial rank, there have been numerous challenges based on both morphological and molecular evidence for placement of genera within Capparaceae s. lat. (Table 1). Although some of these genera are now removed from Brassicales entirely (e.g., *Caloptrotrocha* and *Phymena* to Caryophyllales), most questionable genera remain in Brassicales but outside Capparaceae or Cleomaceae (e.g., *Pentapiplandra*, Villiers 1973; *Setchellanthus*, Karol et al. 1999). Two prime examples include *Pentapiplandra* and *Tirania*, monotypic genera placed in Capparaceae s. lat. that have been elevated to familial status and are seemingly isolated members of Brassicales (Rodman et al. 1996, 1998).

Recent studies indicate two genera placed in Capparaceae s. lat., *Emblingia* and *Forchhammeria*, represent genera that are closely related to, but not part of, Capparaceae s. lat. *Emblingia* is an enigmatic monotypic genus that was placed in Capparaceae (Pax and Hoffmann 1936), but has been recently aligned with Sapindaceae (Leins in Erdtman et al. 1969; Thorne 1992), Goodeniaceae (Melville and Metcalfe in Erdtman et al. 1969), or Polygalaceae (Erdtman in Erdtman et al. 1969; Cronquist 1981). Based on *rbcL* sequences, *Emblingia* was tentatively linked with Resedaceae in the core Brassicales (Chandler and Bayer 2000), a clade in the order comprising Brassicales, Capparaceae, Cleomaceae, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, and Tovariaceae (Rodman et al. 1993, 1996, 1998; Hall et al. 2002). Chloroplast sequences of *ndhF* and *trnL-trnF* placed the largely Central American *Forchhammeria* within the core Brassicales and as sister to either Resedaceae or Gyrostemonaceae (Hall et al. 2002). The ten species of *Forchhammeria* are relatively nongroup, dioecious shrubs that have been assigned to numerous families by a variety of taxonomists, and their placement in Capparaceae s. lat. has often been regarded as provisional (Hansen 1977). Pax and Hoffmann (1936) placed *Forchhammeria* in the tribe Stixeae with *Neothorelia*, *Stixis*, and *Tirania*. Based on
Table 1. Problematic genera placed in Capparaceae s. lat. (= Capparaceae s. str. and Cleomaceae) by Pax and Hoffmann (1936) and Brandegee (1909). In Erdtman et al. (1969), each of the four authors hypothesizes a different taxonomic placement of *Emblingia*.

<table>
<thead>
<tr>
<th>Genus, traditionally of Capparaceae s. lat.</th>
<th>Subfamily (Tribe) of Pax and Hoffmann (1936)</th>
<th>Other suggested relationships</th>
<th>Current familial classification</th>
<th>Ordinal Classification (APG 1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Buhsia</em> Benge</td>
<td>Buhsioideae</td>
<td></td>
<td>Uncertain</td>
<td>Caryophyllales</td>
</tr>
<tr>
<td><em>Emblingia</em> F. Muell.</td>
<td>Emblingioideae</td>
<td>Polygalaceae, affinity with Goodeniaceae, or affinity to Sapindaceae (Erdtman et al. 1969)</td>
<td>Emblingiaceae (APG 1998; Chandler and Bayer 2000)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Forchhammeria</em> Liebm.</td>
<td>Capparoideae (Stixeae)</td>
<td>Polygalaceae, affinity with Goodeniaceae, or affinity to Sapindaceae (Erdtman et al. 1969)</td>
<td>Near Resedaceae and Gyrostemonaceae, this study</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Koeberlinia</em> Zucc.</td>
<td>Capparoideae (Koeberliniaceae)</td>
<td></td>
<td>Koeberliniaceae (Rodman et al. 1996)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Neothorelia</em> Gagnep.</td>
<td>Capparoideae (Stixeae)</td>
<td>near Geleaceae (Hutchinson 1973)</td>
<td>Uncertain (Kers 2003)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Pentadiplandra</em> Baill.</td>
<td>Pentadiplandroideae</td>
<td>Passifloraceae or Flacourtiaceae (reviewed in Dickison and Miller 1993)</td>
<td>Pentadiplandraceae (Villiers 1973)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Physena</em> Norinha ex Thouars</td>
<td>Capparoideae (Stixeae)</td>
<td></td>
<td>Physenaceae (Morton, Karol, and Chase 1997)</td>
<td>Caryophyllales</td>
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<tr>
<td><em>Setchellanthus</em> Brandegee</td>
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<td></td>
<td>Uncertain (Kers 2003)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Stefania</em> Chiovenda</td>
<td>Buhsioideae</td>
<td></td>
<td>Setchellanthus (Itis 1999)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Stixis</em> Lour.</td>
<td>Capparoideae (Stixeae)</td>
<td></td>
<td>Resedaceae (Hutchinson 1973; not accepted by Kubitzki 2003f)</td>
<td>Brassicales</td>
</tr>
<tr>
<td><em>Tirania</em> Pierre</td>
<td>Capparoideae (Stixeae)</td>
<td></td>
<td>Uncertain (Kers 2003)</td>
<td>Brassicales</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Near Resedaceae and Gyrostemonaceae, this study</td>
<td>Brassicales</td>
</tr>
</tbody>
</table>
FIG. 1. Distributions of `orphan' genera of Capparaceae s. lat. and their putative relatives: Emblingia, Forchhammeria, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, Tirania, and Tovariaceae.

Members of the core Brassicales, other than Brassicaceae s. str., Cleomaceae, and Capparaceae s. str., exhibit widely disparate biogeographical distributions (Fig. 1), although many are found or centered in drier habitats (e.g., Mediterranean biome). The distribution of Forchhammeria is limited to Mexico, Central America, and the West Indies, where seven of the ten species are rare and local, mostly in either dry or seasonally dry areas (Hansen 1977). Other members of tribe Stixeae putatively related to Forchhammeria are restricted to southeast Asia (e.g., Tirania). Like Emblingia, Gyrostemonaceae are endemic to Australia where most species are fire-opportunists or plants of disturbed areas (George 1982). The monotypic Pentadiplandraceae and Tovariaceae (questionably ditypic) are restricted to tropical Africa and America, respectively. Whereas Tovaria in Capparaceae s. lat. based on leaf and wood anatomy (Carlquist 1985; Thorne 1992), this coarse shrub is distinct in its typically octmerous flowers with plurilocular ovaries with axile placentation and developed endosperm (Cronquist 1981; Appel and Bayer 2003), a distinction supported by molecular data (Rodman et al. 1993, 1996, 1998). Gyrostemonaceae and Resedaceae represent the remaining members of the core Brassicales. Gyrostemonaceae comprise five genera and 18 species of dioecious shrubs. Resedaceae comprise approximately six genera and 70–75 species of herbs or small shrubs with variable breeding systems. Examination of habit, morphological characters, and breeding systems of these problematic genera using an explicit phylogenetic framework should provide insight into their evolution and biogeography.

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**Materials and Methods**

**Taxon Sampling.** We sampled widely within the Brassicales (sensu Rodman et al. 1996; Appendix 1) and covered 13 of the 16 families in the order excluding Limnanthaceae, Setchellanthaceae, and Akaniaceae (which has been recently combined with Bretschneidera into one family [Bayer and Appel 2003]). Rodman et al. (1998) did not sample Emblinlingia so this taxon was not included as a member of the order until AFG (1998) and Chandler and Bayer (2000) placed it there. Our sampling includes all families of core Brassicales. Although Capparaceae, Chromaceae, and Brassicaceae represent the three largest clades of the core Brassicales, we sampled only four to five representatives from each family because all three are well supported as monophyletic, and taxa selected represent both the smaller, early diverging lineages and the larger subclades within each family (Hall et al. 2002). A single representative each from Pentadiplandraceae (monotypic) and Tovariaeaceae (monotypic or questionably ditypic [Appel and Bayer 2003]) were available. Resedaceae and Gynostemonaceae had two and three representatives, respectively. Five species of Forchhammeria were sampled (for both rbcL and matK), representing all three subgenera recognized by Hansen (1977). The monotypic Tirania, an additional member of the tribe Stixeae, and Emblinlingia were included in the ndhF and matK data sets. Outside of the core Brassicales, sequences were obtained for six additional families (seven in the rbcL analysis that also samples Salvadoraceae), with one species representing each family. Breit Todoerhneraceae, Koeberliniaceae, and Setchellanthaceae are monotypic and, Cariceae, Morinaceae, and Tropaeoaceae are each monophyletic based on molecular data (Gadek et al. 1992; Andersson and Andersson 2000; Olson 2002a, b). Tropaeolum and Bretschneidera have been identified as sister to the remaining Brassicales (Rodman et al. 1993, 1994, 1996, 1998) and were designated as a monophyletic outgroup. A suite of floral and embryological characters supports Tropaeolum and Bretschneidera as a monophyletic group (Ronse Decraene et al. 2002). For the majority of the 38 taxa sampled, all three genes were obtained from the same DNAs or species (Appendix 1). In rare cases, the sequence was obtained from closely related species (e.g., Capsella in matK).

**Extraction, Amplification, and Sequencing.** Total genomic DNA was extracted from fresh, frozen, silica-dried or herbarium samples using a modified 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 were used to amplify and sequence double stranded DNA (e.g., Hall et al. 2002). The 3′ end of the ndhF gene was amplified using forward primer 972F and reverse primer 2110R from Olmstead et al. (1993) or slightly modified based on Arabidopsis sequences (Hall et al. 2002). Four primers were used to sequence both strands of the ndhF gene: 972F, 1603R, 1518F and 2110R. The rbcL gene was either amplified in one reaction using 5′ and 3′ primers or in two reactions using two primer pairs, 5′/764R and 523F/3′ (see Conti et al. 1997 for details). Four sequencing reactions were conducted using primers 5′, 3′, 523F, 674R, allowing for verification of both strands. Occasionally, other primers (1020F, 1088R, 895R) were used for sequence verification. The matK region was amplified using trnK-710F and matK-1495R primers (Koch et al. 2001). A combination of primers was used to sequence this region: 1E, 1F, 495F, 495R, 1010R, 1010F, 1088R, 1412F, 1412R, and 1495R (Koch et al. 2001). In almost every instance, both strands of each of the three genes were obtained. In the few exceptions where this was not possible, manual proofreading of sequences was done in order to detect any misreads. Sequences were aligned using Sequencher v.3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and alignment was further refined using Se-Al v.2.0a6 (Rambaut 2001) or MacClade v.4.05 (Maddison and Maddison 2002). All regions were codon aligned using the known Arabidopsis sequences. Whenever possible, rbcL sequences were also checked for an extension past the typical termination point, which has been demonstrated to be characteristic of core Brassicales and near relatives (Rodman et al. 1994, 1996; Karol et al. 1999). Previous phylogenetic studies have noted indel events both in ndhF (Hall et al. 2002; Sytsma et al. 2002) and matK (Koch et al. 2001) that can be phylogenetically informative. Indels that were potentially parsimony informative (e.g., shared by two or more but not all taxa) were scored and added to the end of the data set as presence/absence characters following the guidelines of Baum et al. (1994).

**Phylogenetic Analyses.** Variation in DNA sequences was used to reconstruct phylogenetic relationships using maximum parsimony (MP) and maximum likelihood (ML) in PAUP* (v. 4.0b10; Swofford 2002). All three data sets were analyzed individually and in combination. Phylogenetic data sets have been deposited in TreeBASE (study accession number S1000). To explore the possibility of multiple islands of most parsimonious trees, 1000 random addition replicates with Multtrees (save multiple trees) holding five trees at each step and TBR branch swapping were used. All characters were equally weighted and treated as unordered (Fitch 1971). In analyses of ndhF and matK, these data were analyzed with and without scored indels. In addition to standard measures of fit of characters to the trees produced (i.e., consistency index, retention index), the strength of support for individual branches was estimated using the bootstrap (Felsenstein 1985). Bootstrap
analyses used 1000 replicates (simple addition, saving up to 1000 trees per replicate, TBR branch swapping, multtrees, holding 1 tree per step).

Fifty-six maximum likelihood models were explored in individual- and combined-data analyses (Posada and Crandall 1998). To establish which model of DNA substitution best fits the data, this program compares these ML models in a hierarchical testing framework by calculating the likelihood ratio statistic between different models. Likelihood ratio tests and their associated p-value compare alternative models of sequence evolution and improvement in fit with increasing model complexity. A heuristic ML search with TBR branch swapping was then conducted using parameters determined for the best model of sequence evolution.

Combined analyses were conducted with two different sampling schemes. The first included only the 31 taxa for which sequence information existed for all three genes. The one exception is Aethionema, which has a critical relationship of being sister to all other Brassicaceae s. str. (Galloway et al. 1996). Aethionema was represented as a composite terminal taxon based on Aethionema saxatile and Aethionema grandiflora. This approach was justified by the strong sister relationship of the two taxa species with the ndhF analyses. However, this reduced 31 taxon sample excluded some critical taxa, specifically Emblica, one species of Forchhammeria, and Tirania, for which we were not able to obtain sequence data for matK. To evaluate the relationships of these taxa, we conducted parsimony and maximum likelihood analyses on an expanded combined analysis with 35 taxa to include all taxa for which there was missing data (Aethionema, Emblica, Forchhammeria pallida and Tirania).

The incongruence length difference (ILD) test (Farris et al. 1994, 1995), implemented in PAUP* as the partition homogeneity test, was conducted to measure conflict between the three data sets. Although aspects of the ILD test have been criticized (Yoder et al. 2001; Barker and Lutzoni 2002; Darlu and Lecointre 2002), Hipp et al. (2004) have demonstrated that some of these criticisms are invalid and argue that the ILD has merit as a first estimate of combinability of data sets. A three-way test on parsimony-informative characters of 1000 replicates (simple addition, TBR branch swapping) was performed, saving only 1000 trees per replicate. Pair-wise ILD tests were also conducted using the same search strategy above on each pair of data sets.

Alternative a priori taxonomic hypotheses were explored by enforcing topological constraints, using 100 random addition replicates and calculating the number of additional steps required. Alternative relationships suggested by individual, but not the combined, analyses were also examined in similar fashion. As presently no parsimony-based statistical test of such tree differences (a priori vs. a posteriori derived trees) are generally accepted (Goldman et al. 2000), we implemented ML based tests of tree differences using the Shimodaira and Hasegawa (1999) parametric test (SH test). Following guidelines for tree selection in Buckley et al. (2001, 2002), a tree set including a number of the a priori taxonomic hypotheses previously explored plus a posteriori ML trees from individual and combined analyses were evaluated with the SH test in PAUP* with RELL optimization using ML settings for the combined data set.

Character Evolution. Patterns of morphological evolution were assessed in MacClade by mapping characters of interest onto the most parsimonious trees obtained from the combined parsimony analysis. The following six characters were evaluated: 1. habit (a. annual herb, b. perennial herb, c. woody; unordered), 2. carpel number (a. two, b. three, c. three; both ordered and unordered), 3. locule number (a. two, b. three, c. three; both ordered and unordered), 4. sepal number (a. <four, b. four, c. five, d. six, e. irregular [unevenly lobed]; unordered), 5. petal number (a. <four, b. four, c. five, d. six, e. irregular [unevenly lobed]; f. absent; unordered), and 6. breeding system (a. hemaphroditic, b. monoeccious, c. dioecious, d. polygamous; unordered). Morphological data were determined using field studies, herbarium sheets, and literature (Hansen 1977; Cronquist 1981; George 1982, 2003; Rodman et al. 1991; Hufnord 1996; Olson 2002a, b; Ronse Decraene 2002; Ronse Decraene et al. 1998, 2002; Appel and Al-Shehab 2003; Appel and Bayer 2003; Bayer and Appel 2003a-c; Kubitzki 2003a-g). Character states were scored for all species present in the combined analyses of 35 taxa and then mapped onto the maximum likelihood tree from that analysis using the TRACE CHARACTERS function in MacClade, which explores both Deltran and Acteon resolving options. The topologies of the maximum likelihood and maximum parsimony trees differed only with respect to the position of Pentaphylanthes, so both positions were explored when mapping characters. In rare instances, taxa were polymorphic but were scored as having one state (see discussion for details).

RESULTS

Phylogenetic Relationships. Although there are minor differences between individual and combined analyses, there was very strong congruence in topologies (Figs. 2, 3). All analyses support the core Brassicaceae as a monophyletic clade. Koelererinia and Batis form a sister group to the core Brassicaceae (with the exception in rbcL analysis) and Caricaceae and Morinaeae are sister to these two clades. Within the core Brassicaceae, all families and genera with more than one species sampled (Capparaceae, Cleomeae, Gyrostemonaeae, Forchhammeriaeae, and Resedaeaeae) were monophyletic. Brassicaceae s. lat. form a monophyletic lineage with Cleomeae and Brassicaceae s. str. as sister groups. Forchhammeria is consistently sister to Resedaceae, with Gyrostemonaceae sister to these two clades, and is clearly outside the Capparaceae. Although Tirania is most closely related to Resedaceae and Forchhammeria, the precise relationships among these three clades are unresolved. For ease of discussion, we refer to the lineage containing Gyrostemonaceae, Resedaceae, Forchhammeria, and Tirania as the GRFT clade. Emblica is sister to all other core Brassicaceae in ndhF and rbcL analyses (not sampled for matK). Relationships of Tovariaceae and Pentadiplandraceae within the core Brassicaceae vary among all analyses with little support for alternative topologies.

rbcL Analysis. In parsimony analyses of the rbcL sequences of 35 taxa, 48 most parsimonious trees of length 817 on a single island were recovered (Table 2). The aligned length of the region is 1479 of which 242 (16.4%) characters were parsimony informative (Table 2) and no indels were introduced during alignment. Only partial sequences were obtained for Crateva pulmeri, Polanisia dodendranda, and Wilszenia refracta (with sequence lengths of 864, 787, and 851, respectively). There are 14.2% cells missing in this data set. All Gyrostemonaceae share the same c-terminal amino acid sequence inferred from nucleotide sequences as previously reported (Karol et al. 1998). The inferred amino acid at positions 1426–1428 in the rbcL sequences of Emblica, Forchhammeria watsonian, E. sessilifolia, F. sp. nov., and Tirania is aspartic acid (rather than a stop codon that is typical of many dicots), a synapomorphy for the core Brassicaceae (Rodman et al. 1994, 1996; Karl et al. 1999). Unfortunately, for most of these taxa our sequences ended at this codon position so we are
Fig. 2. Phylogram results of parsimony analyses for individual and combined data sets, showing only key relationships within the core Brassicales. Bootstrap values are above branches and for Forchhammeria, Resedaceae, and Gyrostemonaceae the values indicate monophyly for those clades. (A) Consensus of the 48 trees based on analysis of 35 sequences of rbcL. (B) The most parsimonious tree in analyses of 35 ndhF sequences. (C) Consensus of the matK data set of 31 taxa, excluding Tirania and Emlingia. (D) Eighty percent bootstrap consensus of expanded combined analysis (35 taxon sample) of rbcL, ndhF and matK including taxa with missing sequences for matK (Tirania and Emlingia).

not able to examine the extent of the ‘tail.’ The strict consensus of the core Brassicales is shown with bootstrap values indicated (Fig. 2A). There is low support (bootstrap 55%) for the monophyly of the core Brassicales, including Emlingia, and for relationships of Pentadiplandra, Tirania, and Emlingia within the core Brassicales (all < 50% bootstrap). Emlingia is sister to all other core Brassicales whereas Tirania and Pentadiplandra are part of the GRFT clade. Forchhammeria and Tirania are strongly supported as closely related to Resedaceae (bootstrap 98%) with the position of Tirania unresolved. Gyrostemonaceae is weakly supported as sister to Forchhammeria, Tirania, and Resedaceae (bootstrap 61%). Among other members of Brassicales, there is more than one most parsimonious relationship hypothesized for Koeleria.

Hierarchical likelihood ratio tests indicated that the best model of substitution for the rbcL data is TrN + I + G, which allows one substitution rate for transversions, two substitutions rates for transitions, and among site rate heterogeneity is modeled by allowing some sites to be invariant (I) while the rest have rates drawn from a discrete approximation to a gamma distribution with a single shape parameter, alpha (G; Table 2). The heuristic search converged on a single tree (ln L = -6678.34464; tree not shown) that is nearly identical in topology to the consensus of the parsimony analysis. In the ML tree, Tirania is sister to Resedaceae and these two clades are sister to Forchhammeria. Also, within Cleomeaceae, Cleome pilosa and C. viridiflora are sister species whereas in the MP topology C. pilosa is sister to Podandrogyne. The overall topology of the ML tree is similar to analysis of rbcL by Karol et al. (1999), with the exception of the sister relationship of Tirania and Pentadiplandra recovered in Karol et al. (1999).

ndhF Analysis. One most parsimonious tree (length 1283) was recovered in parsimony analysis of 35 ndhF sequences (Fig. 2B). Six indels in lengths of 3, 6 or 9 base pairs were introduced during sequence
alignment. The entire aligned data set is 1097 base pairs in length and 7.74% cells are scored as missing (Table 2). *Aethionema saxatile* is missing approximately one half of the sequence. Excluding or including the scored indels even up to weight of 10 had no effect on resulting topology and only minor effect on relative bootstrap values. The partition homogeneity test indicates *rbcL* and *ndhF* have similar phylogenetic structure (*P* = 0.193). The following three relationships recovered in the *ndhF* analyses differ from *rbcL*: (1) a sister relationship of *Maerua* and *Apophyllum* (Capparaceae; relationships not shown in figure), (2) *Tovaria* as sister to Capparaceae, Cleomaceae plus Brassicaceae, and (3) *Tirania* as sister to a well supported (bootstrap 85%) clade of Resedaceae plus *Forchhammeria*. *Emblingia* is supported (bootstrap 87%) as sister to remaining core Brassicales. The heuristic search under likelihood settings resulted in a single tree (ln L = −8046.93736;
TABLE 2. Characteristics of the three data sets and parsimony and maximum likelihood analyses. 1 Analyses excluding indels. 2 Model selected using hierarchical likelihood ratio tests in Modeltest (Posada and Crandall 1998).

<table>
<thead>
<tr>
<th></th>
<th>rbcL</th>
<th>ndhF</th>
<th>matK</th>
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<th>Combined 2</th>
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<td>Parsimony:</td>
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<td>Parsimony informative sites (%)</td>
<td>242 (16.4%)</td>
<td>342 (31.2%)</td>
<td>458 (29.2%)</td>
<td>988 (23.9%)</td>
<td>1031 (24.9%)</td>
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<td>MP trees (length)</td>
<td>48 (817)</td>
<td>1 (1283)</td>
<td>6 (1582)</td>
<td>2 (3494)</td>
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<td>CI (RI)</td>
<td>0.661 (0.704)</td>
<td>0.633 (0.726)</td>
<td>0.677 (0.760)</td>
<td>0.674 (0.741)</td>
<td>0.656 (0.736)</td>
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<td>ML model12</td>
<td>TrN + I + G</td>
<td>GTR + G</td>
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<td>Likelihood score (-ln L)</td>
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The tree not shown) that is identical to the parsimony tree, except the relationship of Tovaria is unresolved (there is a hard polytomy). The best model of substitution for the ndhF data set is GTR + G that allows for six different substitution rates for all nucleotide pairs and among site rate heterogeneity is approximated by a gamma distribution (Table 2).

matK Analysis. Six most parsimonious trees of length 1582 resulted from analyses of 31 matK sequences (Fig. 2C; Table 2). The aligned length of the data set was 1566 base pairs with five indel events introduced. For some taxa we were not able to obtain complete sequences and 5.44% of the data set is scored as missing. Including or excluding the scored indels altered topology and support of resulting trees. The inclusions of weighted indels dissolve the following sister relationships: (1) Moringa and Carica, and (2) Batis and Koebelitina. These relationships are well supported in other analyses (Figs. 2, 3; Rodman et al. 1993, 1998; Olson, 2002a, b), indicating the indels in matK are homoplasious and justifiably excluded from analyses. The relationships within the core Brassicales and the placement of Forchhammeria are not altered with the inclusion of indels. The resulting topology of the matK parsimony analysis is highly congruent with both ndhF and rbcL analyses (Figs. 2, 3). Tovaria and Pentadiplandra are both unresolved by matK analyses. The ILD test indicates there is similar phylogenetic structure of matK with rbcL (P = 0.684) and with ndhF (P = 0.934). The best model of DNA substitution for the matK data is the transversional model TVM + G in which there are four different transversion rates, one transition rate, and rate heterogeneity is approximated using the gamma distribution. The topology of the single tree resulting from maximum likelihood analyses of the matK data shows a topology (ln L = -10490.42846; tree not shown) in which Pentadiplandra is sister to the Capparaceae, Cleomaceae, and Brassicaceae with Tovaria sister to these two clades.

Combined Analysis. The three-way partition homogeneity test, conducted on the smaller 31 taxon sample, indicated all three data sets have similar phylogenetic structure (P = 0.414). Parsimony analyses on taxa for which sequences were available for all three regions (with the exception of Aethionema treated as a single taxon) resulted in two most-parsimonious trees (length 3494; tree not shown), which differ only in the relationships among species of Forchhammeria. The topology is highly congruent with all individual analyses, and bootstrap support for all branches is increased. The combined analysis with 31 taxa suggests relationships of Tovaria and Pentadiplandra not indicated in the individual analyses. Tovaria is sister to all other core Brassicales (bootstrap 71%) whereas Pentadiplandra is sister to Capparaceae, Cleomaceae, and Brassicaceae (bootstrap 60%). In the expanded combined
analysis with 35 taxa, two trees resulted in which the overall topology is almost identical to the 31 taxon sample tree (Fig. 2D). The two most parsimonious trees differ with respect to Pentadiplandra. One topology is identical to the 31 taxon sample tree topology (e.g., sister to Brassicaceae s. lat.), whereas the other topology indicates Pentadiplandra is sister to the GRFT clade. Tirania is placed in a polytomy with Resedaceae and Forchhammeria, and Emblingia is sister to remaining core Brassicales (Fig. 2D). Relationships of T ovaria and Pentadiplandra are unresolved, although there is weak support (bootstrap 60%) for T ovaria as sister to all core Brassicales other than Emblingia.

The best model of DNA substitution for the combined 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. That model of evolution is the same for both 31 and 35 taxon data sets (Table 2), and the 31 taxon tree is identical to the 35 taxon tree (Fig. 3). Tirania and Emblingia have the same relationships as the rbcL, ndhF, and combined parsimony analyses. The precise placement of Tirania is unresolved by likelihood analyses.

**Discussion**

Data from more than 60 new rbcL, ndhF, and matK sequences, individually and in combination, resolve many relationships within the core Brassicales and help place Emblingia, Forchhammeria, and Tirania. Several results confirm relationships within Brassicales and core Brassicales indicated in previous analyses (Rodman et al. 1993, 1994, 1996, 1998; Hall et al. 2002) but with increased support. Novel phylogenetic results that emerge from these studies include: (1) Emblingia is sister to remaining core Brassicales, (2) Gyrostemonaceae, Resedaceae, Forchhammeria, and Tirania form a well-supported clade within the core Brassicales, and (3) the relationships of Pentadiplandra and T ovaria within the core Brassicales remain unresolved. Mapping of morphological traits onto phylogenetic trees indicates that many of the characters used to classify these taxa are prone to convergent evolution. Although many patterns of morphological evolution remain elusive, some insight is gained on the evolution of floral merosity and breeding systems. Two other orphan genera of Capparaceae, Capparaceae, Cleomaceae, Emblingiaceae, Forchhammeria, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, Tirania, and T ovariae. The GRFT clade of Gyrostemonaceae, Resedaceae, Forchhammeria, and Tirania is a novel lineage established here, with problematic affinities to Pentadiplandra and T ovaria. Ronse Decraene (2002) suggested that racemose inflorescences, (andro) gynophore, extrastaminal nectary, tendency to dissymmetry, reduced stipules, and imbricate sepals and petals unite the core Brassicales. Other characters that are common, but not necessarily synapomorphies, include campylotropous seed orientation (Hufford 1996) with the exception of Forchhammeria (Hansen 1977), anomocytic stomates, and curved embryos (also seen in Koeberlinia).

Emblingia is Sister to Remainder of Core Brassicales. The studies presented here suggest a novel relationship of Emblingia, as sister to remaining core Brassicales. A previous molecular study using rbcL, placed Emblingia in Brassicales as sister to Resedaceae but with low support (bootstrap <50%; Chandler and Bayer 2000). The lack of resolution in their study was likely the result of a very broad sampling across many orders that had been suggested to house Emblingia (Table 1; Chandler and Bayer 2000), limited sampling within core Brassicales, and reliance solely on rbcL sequences. Although Emblingia has many unique morphological features, there are some features in common with other members of core Brassicales: androgynophore (Erdtman et al. 1969; Chandler and Bayer 2000), curved or reniform seeds; tricolporate pollen; stamens usually 4-merous (or 8); and zygomorphic flowers. To our knowledge, no studies have been conducted to examine the presence of mustard oils (glucosinolates) in Emblingia.

Positions of Pentadiplandra and T ovaria are not Resolved. Although there were 1031 parsimony informative characters in the combined 35 taxon analysis, some relationships within core Brassicales are still not resolved. Every analysis suggested different relationships of T ovaria and Pentadiplandra, each with low statistical support (Figs. 2, 3). Since both families are monotypic (T ovaria unquestionably ditypic), increased sampling is not a viable approach for resolving these relationships. Both genera have been classified as Capparaceae in the broad sense by some taxonomists (Pax and Hoffmann 1936; Carlquist 1985; Thorne 1992), although Hutchinson (1973) placed Pentadiplandra near the Celastraceae. Ronse Decraene (2002) suggested a strong affinity of T ovaria and Pentadiplandra based on the presence of plesiomorphic characters within Brassicales such as stipulate leaves, pentamery, diplostemony, and axile placentation. However, none of the analyses presented here indicate a sister relationship of these two genera (but see Rodman et al. 1998; Karol et al. 1999).

Forchhammeria and Tirania are Related to Gyrostemonaceae and Resedaceae. The clade comprising
Gyrostemonaceae, Resedaceae, Forchhammeria, and Tirania (GRFT clade) is moderately (bootstrap 61% in \textit{rbcL}) to strongly supported (bootstrap 94–99% in other analyses). Gyrostemonaceae, here represented by three species, is sister to all remaining members of the clade. Despite the strong support for \textit{Forchhammeria}, \textit{Tirania}, and Resedaceae as a clade, there is no consensus on how these three lineages are related. The combined data presented here indicate a sister relationship of \textit{Forchhammeria} and Resedaceae, a relationship further supported by a unique 9bp \textit{matK} deletion. In a previous analysis using only \textit{trnL-trnF} data (Fig. 3 in Hall et al. 2002), \textit{Forchhammeria} is placed sister to Resedaceae plus Gyrostemonaceae (bootstrap 83%). This analysis, however, was conducted with only one representative from each of the three lineages, and limited sampling may be an issue. In order to evaluate the significance of these differences, the ML based Shi-modaira-Hasegawa test was conducted on combined and the \textit{trnL-trnF} data sets (\textit{trnL-trnF} data set was expanded to include all the same representatives of the GRFT clade, J. Hall unpublished data) under ML parameters determined by ModelTest (Posada and Cran-dall 1998). Our a priori hypotheses included 15 topologies representing all possible relationships among the four members of the GRFT clade. The combined data set rejects all topologies ($P = 0.001$) except those in which Gyrostemonaceae are sister to all other members of the GRFT clade ($P = 0.896–0.914$). In contrast, the \textit{trnL-trnF} data set fails to reject any of the 15 topologies ($P = 0.158–0.569$). These analyses indicate that the sister relationship of \textit{Forchhammeria} and Resedaceae suggested by combined \textit{matK}, \textit{rbcL}, and \textit{ndhF} is more likely than that indicated by the previous \textit{trnL-trnF} analysis, based on fewer taxa.

Although sampling is limited among some members of the GRFT clade, all genera or families with more than one species sampled are strongly supported as monophyletic with clear putative morphological synapomorphies. Resedaceae have a syncarpous gynoe-cium that remains open at the distal end throughout development (Cronquist 1981; Hufford 1996; Kubitzki 2003f), although the family exhibits variation in carpel number. The massive embryos of \textit{Gyrostemonaceae} are pseudo-monocotyledonous (Hansen 1977), where only the outer cotyledon develops into a fat folded nutrient storage structure, completely enveloping the inner, undeveloped cotyledon and with the radicle highly reduced (except \textit{Forchhammeria} sp. nov.). Female flowers of Gyrostemonaceae are apocarpic with carpels adnate to a central column that is often expanded at the top (Cronquist 1981; Hufford 1996). In fact, the unusual syncarp of Gyrostemonaceae appears to be associated with the formation of a large, sterile region in the apex of flowers (Hufford 1996).

Despite the lack of a clear morphological synapomorphy for the GRFT clade, members of the clade have some morphological similarities or trends. Gyrostemonaceae, \textit{Forchhammeria}, and \textit{Ochradenus} (Resedaceae, unsampled in these studies) all have uniseriate perianths, grow in arid habitats, and are anemophilous (Hansen 1977; Hufford 1996). The flowers of \textit{Forchhammeria}, like Gyrostemonaceae, are much reduced with a fused calyx that splits in an irregular fashion. Gyrostemonaceae and \textit{Forchhammeria} are dioecious with highly reduced flowers, presumably adapted for wind pollination. However, many of these features may be the result of convergent evolution due to dioecy and arid habitat. \textit{Tirania} and Resedaceae (excluding \textit{Ochradenus}) are the only members of the clade that have petals. Of some interest is that both genera sampled from the tribe Stixae of Capparaceae s. lat. (Pax and Hoffmann 1936), \textit{Forchhammeria} and \textit{Tirania}, are closely related to one another and Resedaceae. Members of the tribe Stixae have two to multilocular ovaries, axile placentation, three- to five-merous perianths, and well developed stigmas (Pax and Hoffmann 1936; Kers 2003), which support their placement within the GRFT clade (Fig. 4). Precisely how \textit{Forchhammeria} and \textit{Tirania} are related to one another is still unresolved based on \textit{rbcL} and \textit{ndhF} sequence data (Figs. 2, 3). Additional sampling of remaining Stixoeae (i.e., \textit{Neothorelia} and \textit{Stixis}) is warranted based on the clear separation of these two genera from Capparaceae s. lat. and their close relationship.

**Morphological Evolution.** Mapping of character states revealed few clear patterns of morphological evolution within the Brassicales. Figure 4 maps breeding systems and shows selected habit and floral characters (mapping of these characters can be obtained from authors upon request). Moving \textit{Pentadiplandra} as sister to the GRFT clade does not change the reconstruction of any of the characters (typically the branch is equivocal in either relationship) with the exception of breeding systems. Habit is the only morphological character for which there are no equivocal branches in the reconstruction. Woodiness is pleisiomorphic in Brassicales and, based on current sampling, the herbaceous habit has arisen four times: (1) Cleomaceae plus Brassicales, (2) \textit{Oligomeris} (Resedaceae), (3) \textit{Ter-sonia} (Gyrostemonaceae), and (4) \textit{Tropaeolum} (Tropaeo-laceae).

Merosity of both the gynoeccium and perianth is very labile within the Brassicales (Fig. 4). When designated as unordered, the pleisiomorphic state of locule and carpel number is equivocal. When ordered, three carpels are basal within the Brassicales, but the base of the core Brassicales is still equivocal. Within the core Brassicales, two carpels characterize the Capparaceae, Cleomaceae, and Brassicaeae clade in addition to \textit{Forchhammeria} and \textit{Gyrostemon tepperi}. Whether treated as ordered or unordered, the primitive locule number...
for the core Brassicales is equivocal. Within the core Brassicales, two locules is characteristic of both Brassicaceae s.str. and *Forchhammeria*. The evolution of perianth number is equally ambiguous, although merosity characterizes a few clades. Within the core Brassicales, all dioecious plants lack petals and all but one have irregular sepals. There is no clear pattern of sepal number within the GRFT clade, except that in general they have irregular splitting sepals. The equivocal status of many of the characters at the base of the GRFT clade is due to the unresolved nature of *Tirania*.

The ancestral condition of breeding systems for the core Brassicales and Brassicales in the broad sense is bisexual (Fig. 4). Based on this sampling, dioecy evolved unambiguously from the hermaphroditic condition three times (*Carica, Batis, Apophyllum*), and monoeey or polygamy evolved twice (*Cratera, Pentadiplandra*), also unambiguously. The evolution of dioecy within the GRFT clade is ambiguous, as a result of the lack of resolution among *Forchhammeria, Tirania*, and Resedaceae. Alternative topologies of these three taxa were explored. If *Forchhammeria* is sister to *Tirania* plus *Pentadiplandra* and *Resedaceae* to the GRFT clade, then monoecy, dioecy, and hermaphroditism are all possible ancestral conditions. In all other arrangements of *Forchhammeria, Tirania*, and Resedaceae (re-
Biogeographical Relationships. Many members of the core Brassicales and the GRFT clade, in particular, have disjunct distributions (Fig. 1). There is a strong Austral-Asian component in the clade as well as a propensity for Mediterranean habitats. The majority of Gyrostemonaceae are endemic to southwestern Australia (Codonocarpus attenuatus) or throughout central Australia (Codonocarpus cotinifolius) (George 1982, 2003). Resedaceae are primarily in arid regions of the Mediterranean, with outliers from Oligomeris in South Africa and southwestern North America (Kubitzki 2003f). Tirania is endemic to southern Vietnam (Hutchinson 1967; Kers 2003). Forchhammeria is distributed in Mexico, Central America, and the West Indies (Hansen 1977). Hansen (1977) suggested that Forchhammeria was closely related to some African genera of Capparaceae (Bosca and Maefu) and that the current distribution of the genus was the result of long distance dispersal. Given the relationships presented here using chloroplast sequence data, the biogeographical history of Forchhammeria needs to be examined instead relative to the temperate Resedaceae, Australian Gyrostemonaceae, and Asiatic Tirania. These biogeographical relationships are not clearly resolved in the GRFT clade and become increasingly less clear when the distributions of other core Brassicales are considered: Pentadiplandra of tropical west Africa, Tovaria of tropical America, Eningling of Australia, and the worldwide distributed Brassicaceae, Cleomaceae, and Capparaceae. A preliminary molecular clock dating of these lineages (Hall 2003) suggests that the core Brassicales diversified during the late Cretaceous and early Tertiary, but more detailed analyses are needed to understand biogeographical relationships and events within the core Brassicales in the context of continental separations and contacts.

Floral Divergence and Convergence in Core Brassicales. One result of using a molecular phylogenetic framework to address patterns of morphological evolution in the Brassicales is the demonstration of the diversity and lability of floral merosity in the order. Recurring or convergent shifts in sepal, petal, carpel, and locule number are common, especially within the core Brassicales (Fig. 4). In assigning character states to taxa, some of this diversity was necessarily simplified from even greater complexity in floral evolution of Brassicales. Resedaceae were scored as having greater than three carpels (pleisiomorphic condition), although the family varies in carpel number between two and seven: Reseda has three to four whereas Oligomeris has four to five (Cronquist 1981; Kubitzki 2003f). Also, although flowers of Resedaceae are clearly hermaphroditic (pleisiomorphic as scored here), apparently derived unisexual forms occur (Cronquist 1981; Kubitzki 2003f). Tovaria was scored with a merosity number of six, but merosity varies between six and eight (Ronse Decraene 2002).

Patterns of morphological evolution are limited by taxon sampling in the analyses. Although Batis is scored as dioecious, the family is ditypic, and the other species, not sampled in these analyses, is monoecious (Bayer and Appel 2003b). Gyrostemon sp. (probably a new species, see Rodman et al. 1994) was scored based on generic characters, and the typical carpel number for most Gyrostemonaceae is six to eight (Hufford 1996). Gyrostemon tepperi typically has one to two carpels, which likely represents an evolutionary reduction (Hufford 1996). Assuming that this assessment is correct and the reduced carpel number in G. tepperi is derived, an alternative scoring of G. tepperi was implemented with a high carpel number for the family. With this scoring, the GRFT clade (regardless of the position of Pentadiplandra) has a plesiomorphic condition of high carpel number with a reduction in Forchhammeria. In fact, the entire core Brassicales has a plesiomorphic character state of greater than three carpels with two reductions: (1) Forchhammeria and (2) Brassicaceae plus Cleomaceae plus Capparaceae.

Floral dimorphism (dioecy, monoecy, or polygamy) evolved minimally eight times within Brassicales based on current sampling (Fig. 4). This is likely an underestimate of change in breeding systems because there are many unsampled species in Capparaceae and Cleomaceae that are either monoecious or polygamous. Dioecy has evolved four to five times within Brassicales: Carica papaya (Caricaceae), Batis maritima (Bataceae), Apopllyllum (Capparaceae), Gyrostemonaceae, and Forchhammeria. The transition to dioecy is from an hermaphroditic ancestor except for Forchhammeria and Gyrostemonaceae, where the ancestral state is equivocal. Because the sister relationship of Forchhammeria and Gyrostemonaceae is rejected by the Shimodaira-Hasegawa test, two independent origins of dioecy from hermaphroditism are likely. The shifts to dioecy from hermaphroditism differ from the general pattern (Rønner and Ricklefs 1995), but are similar to the pattern of breeding system shifts within monocots (Weiblen et al. 2000). Generally, hypotheses of evolutionary pathways to dioecy include gynodioecious or monoecious intermediates (Charlesworth and Charlesworth 1978; Sytsma et al. 1991; Barrett 2002). The direct transition between hermaphroditism and dioecy may be spurious as transitions can be missed when looking at phylogenetic reconstructions (Weiblen et al. 2000).

Dioecy has been associated with many characteristics in flowering plants including wind pollination, perennial growth, and fleshy fruits (Givnish 1980; Rønner and Ricklefs 1995), which can convolute homology assessment. A change to drier habitats can also result
in subsequent shifts to wind pollination due to loss of pollinators (Sakai et al. 1995). Initial loss of insect pollination may lead to selfing and inbreeding depression, which in turn may favor the spread of females (Charlesworth and Charlesworth 1978). Four of the five dioecious lineages sampled occur in dry or saline habitats suggesting a correlation with water stressed habitats (Fig. 4; see also Sakai et al. 1995). Nonetheless, presence in dry habitats may occur without changes in reproductive systems since the hermaphroditic Resedaceae are distributed in arid regions. Alternatively, if the ancestral condition of the GRFT clade was dioecious, then reversal to hermaphroditism occurs without changes to more mesic habitats. Perianth reduction is often associated with wind pollination, which is central to this hypothesis on the evolution of dioecy (Sakai et al. 1995). Renner and Ricklefs (1995) argued that abiotic pollination favors unisexual flowers (less likely to self pollinate), which facilitates the transition from monoeccy to dioecy. Four of the dioecious groups (Batis, Apophyllum, Forchhammeria, and Gymastermonaceae) lack petals. Developmental studies support independent origins of sexual systems, which correspond to correlations of drier habitat and wind pollination. Flowers of Pentadiplandra and Forchhammeria are unisexual as the result of organ abortion (Hansen 1977; Ronse Decraene 2002), whereas there is no indication of staminal initiation in floral development studies of female flowers of Gymastermon and Tersonia (Gymastermonaceae; Huford, 1996).

**Taxonomic Implications.** As suggested by Kers (2003), Tirania and Forchhammeria should no longer be classified as Capparaceae s. lat. Although we have clearly demonstrated that these taxa belong to the GRFT clade, not the Brassicaceae s. lat. lineage (Capparaceae, Cleomaceae, Brassicaceae), we hesitate to make any formal taxonomic changes at this point in time. First, further sampling of members of tribe Stixaeae that are likely to be part of the GRFT clade based on morphological features are needed. Second, the exact placement of Tirania relative to Forchhammeria has not been established. As argued earlier (Chandler and Bayer 2000), Emblingia is distinct and should be recognized as a monotypic family. Likewise, Pentadiplandra and Tovaria are each morphologically distinct and isolated within the core Brassicales and merit familial status. With additional recognition of a family for Forchhammeria (with or without other Stixaeae), the core Brassicales would be composed of a high number of small families. Despite discouragement of such practice (APG 1998), the core Brassicales is a clade that merits a number of small families as the lineages are phylogenetically isolated, morphologically distinct, and geographically disjunct (see Ittis 1999).

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**Literature Cited**


**APPENDIX 1**

Accession list of taxa and GenBank numbers used in phylogenetic analyses. The same vouchers and DNAs were used in this study, Hall et al. 2002, and Rodman et al. 1993, 1994.

**BRASSICALES**

*Batacea*: *Batis maritima* L. (Ilitis 30500, WIS; *rbcL* [L122438, Rodman et al. 1994]; *ndhF* [AY122403, Hall et al. 2002]; *matK* [AY483219]

*Bretschneideraceae*: *Bretschneidera sinensis* Hems. (Leu & Lin 726, WIS; *rbcL* [M95753, Rodman et al. 1993]; *ndhF* [AY483247]; *matK* [AY483220]

*Caricaceae*: *Carica papaya* L. (WIS Botanical Garden; *rbcL* [M95671, Rodman et al. 1993]; *ndhF* [AY483246]; *matK* [AY483221]

*Koeberliniaceae*: *Koeberlinia spinosa* Zucc. (Al Shehbaz s.n., MO; *rbcL* [L14600, Rodman et al. 1993]; *ndhF* [AY483246]; *matK* [AY483222]

*Moringaceae*: *Moringa oleifera* Lam. (Ilitis 30501, WIS; *rbcL* [L11359, Rodman et al. 1993]; *ndhF* [AY122405, Hall et al. 2002]; *matK* [AY483223]

*Salvadoraceae*: *Salvadora angustifolia* Turrill (*rbcL* [U38532, Rodman et al. 1996])
Tropaeolaceae: Tropaeolum majus L. (rbcL [L14706, Price and Palmer 1993]; Rodman 529, WIS; ndhF [AY122408, Hall et al. 2002]; matK AY483224)

CORE BRASSICALES

Brassicaceae s. str.: Aethionema grandiflorum L. (ndhF [AF64657, Gallego et al. 1998]; matK [AF144354, Koch et al. 2001]); Aethionema saxatile R. Br. (Moore s.n., WIS; rbcL AY483252; matK AY483246); Arabidopsis thaliana Heynh. (rbcL [L91966, Zhu et al. 1997]; ndhF [AY122394, Hall et al. 2002]; matK [AF144348, Koch et al. 2001]); Capsella bursa-pastoris (L.) Medicus (rbcL [D88904, Tsukaya et al. 1997]; ndhF [AY122396, Hall et al. 2002]; matK, C. rubella Reuter [AF144334, Koch et al. 2001]); Nasturtium officinale R.Br. (rbcL [AF020325, Les et al. 1994]; Stahmann 233, WIS; ndhF [AY122399, Hall et al. 2002]; matK AY483225); Standea pinnata (Pursh) Britton (Hall 1, AZ; rclL AY483263; ndhF [AY122401, Hall et al. 2002]; matK AY483226)

Capparaceae s. str.: Apophyllum anomalum F. Muell. (Covry 12044, MO; rclL AY483264; ndhF [AY122356, Hall et al. 2002]; matK AY483227); Capparis hastata Jacq. (Illis 30330, WIS; rclL [M95754, Rodman et al. 1993]; ndhF [AY122366, Hall et al. 2002]; matK AY483228); Creataea palmeri Rose (Hall 105, WIS; rclL AY483265; ndhF [AY122370, Hall et al. 2002]; matK AY483229); Maerua kirki (Oliv.) F. White (Hall 261, WIS; rclL AY483266; ndhF [AY122378, Hall et al. 2002]; matK AY483230)

Cleomaceae: Cleome pilosa Benth. (Illis 30585, WIS; rclL AY483267; ndhF [AY122385, Hall et al. 2002]; matK AY483231); Cleome scutellata (L.) Heynh. (rbcL AY483282; Psidodrogone ciliaris (Standl.) Woodson (Nepokroeff 450, WIS; rclL AY483269; ndhF [AY122393, Hall et al. 2002]; matK AY483233); Polanisia dodonaeana DC (Grette 8603, WIS; rclL AY483270; ndhF AY483251; matK AY483234); Wilsenzenia refracta Engelm. (Vanderpool 1340, OKL; rclL AY483271; ndhF [AY122391, Hall et al. 2002]; matK AY483235)

Gyrostemonaceae: Gyrostemon sp. (Cranfield, FERTH no. 02068672; rclL [L22439 Rodman et al. 1994]; ndhF AY483252; matK AY483236); Gyrostemon tepperi (F. Muell. ex H. Walter) A. S. George (Thompson, 2243, MO; rclL [L22440, Rodman et al. 1994]; ndhF AY483253; matK AY483237); Tersonia cathiflora (Fenzl) A.S.George (Cranfield FERTH no.02068682; rclL [L22441, Rodman et al. 1994]; ndhF [AY122404, Hall et al. 2002]; matK AY483238)

Pentadiplandraceae: Pentadiplandra brazzeana Baill. (rclL [L38533, Rodman et al. 1996]; Hall 263, WIS; ndhF AY483254; matK AY483239)

Resedaceae: Oligomeris lunifolia MacBride; (Ertter 5613, WIS; rclL AY483272; ndhF AY483255; matK AY483240); Reseda lutea L. (Rodman 535, WIS; rclL AY483273; ndhF [AY122406, Hall et al. 2002]; matK AY483241)

Towariaceae: Towaria pendula Ruiz. & Pav. (Smith and Smith 1834, WIS; rclL [M95758, Rodman et al. 1993]; ndhF [AY122407, Hall et al. 2002]; matK AY483242)

ANOMALOUS GENERA

Emblingia calceolifera F. Muell. (rclL [AF146014, Chandler and Bay-er 2000]; Quin 288a; ndhF AY483256)

Forchhammeria pulla Liebmann (Illis 29350a, WIS; rclL AY483274; ndhF [AY122381, Hall et al. 2002])

Forchhammeria sessiliflora Standl. (Coehane 12967, WIS; rclL AY483275; ndhF AY483243)

Forchhammeria sp. nov. (Illis 30784, WIS; rclL AY483276; ndhF AY483258; matK AY483244)

Forchhammeria trifoliata Radlk (Hansen 3002, WIS; rclL AY483277; ndhF AY483259; matK AY483245)

Forchhammeria untonii Rose (Fishbein 3070, WIS; rclL AY483278; ndhF AY483260; matK AY483246)

Tirania purpurea Pierre (Squires 874, GH; rclL AY483279; ndhF AY483261)