

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 three of these orphan genera, *Emblingia*, *Forchhammeria*, and *Tirania*, in the context of a focused analysis of the core Brassicales. In order to assess 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 merosity 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 bisexual. Further sampling of Capparaceae tribe Stixee 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 Brassicaceae 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, Cleomoideae and Capparoideae, with the Cleomoideae more closely related to Brassicaceae than to Capparoideae (Hall et al. 2002). These three taxa have been either combined into one family, Brassicaceae 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 Brassicaceae (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., *Calyptrotheca* and *Physena* to Caryophyllales), most questionable genera remain in Brassicales but outside Capparaceae or Cleomaceae (e.g., *Pentadiplandra*, Villiers 1973; *Setchellanthus*, Karol

et al. 1999). Two prime examples include *Pentadiplandra* and *Tovaria*, 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 Hoffman 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 only on *rbcl* sequences, *Emblingia* was tentatively linked with Resedaceae in the core Brassicales (Chandler and Bayer 2000), a clade in the order comprising Brassicaceae, 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 nondescript, 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 Stixee 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).¹ In Erdtman et al. (1969), each of the four authors hypothesizes a different taxonomic placement of *Emblingia*.

Genus, traditionally of Capparaceae s. lat.	Subfamily (Tribe) of Pax and Hoffmann (1936)	Other suggested relationships	Current familial classification	Ordinal Classification (APG 1998)
<i>Bahsia</i> Benge	Buhsioideae		Uncertain	Caryophyllales
<i>Calyptrotheca</i> Gilg.	Calyptrothecoideae	Portulacaceae (Nyananyo 1986)	Near Didiereaceae (Appelquist and Wallace 2001)	Brassicales
<i>Emblingia</i> F. Muell.	Emblingioideae	Polygalaceae, affinity with Goodeniaceae, or affinity to Sapindaceae (Erdtman et al. 1969)	Emblingiaceae (APG 1998; Chandler and Bayer 2000)	Brassicales
<i>Forchhammeria</i> Liebm.	Capparoideae (Stixeeae)	Euphorbiaceae, Sapindaceae, Oleaceae, Malvaceae (Hansen 1977)	Near Resedaceae and Gyrostemonaceae, this study	Brassicales
<i>Koerberlinia</i> Zucc.	Capparoideae (Koerberlineae)		Koerberliniaceae (Rodman et al. 1996)	Brassicales
<i>Neohorelia</i> Gagnep.	Capparoideae (Stixeeae)		Uncertain (Kers 2003)	Brassicales
<i>Pentadiplandra</i> Baill	Pentadiplandroideae	Near Celestraceae (Hutchinson 1973)	Pentadiplandraceae (Villiers 1973)	Caryophyllales
<i>Physena</i> Norrinha ex Thouars	Capparoideae (Stixeeae)	Near Passifloraceae or Flacourtiaceae (reviewed in Dickison and Miller 1993)	Physenaceae (Morton, Karol, and Chase 1997)	Brassicales
<i>Setchellanthus</i> Brandegee	N/A		Setchellanthaceae (Iltis 1999)	Brassicales
<i>Stefania</i> Chiovenda	Buhsioideae		Resedaceae (Hutchinson 1973; not accepted by Kubitzki 2003f)	Brassicales
<i>Stixis</i> Lour.	Capparoideae (Stixeeae)		Uncertain (Kers 2003)	Brassicales
<i>Tiranita</i> Pierre	Capparoideae (Stixeeae)		Near Resedaceae and Gyrostemonaceae, this study	Brassicales

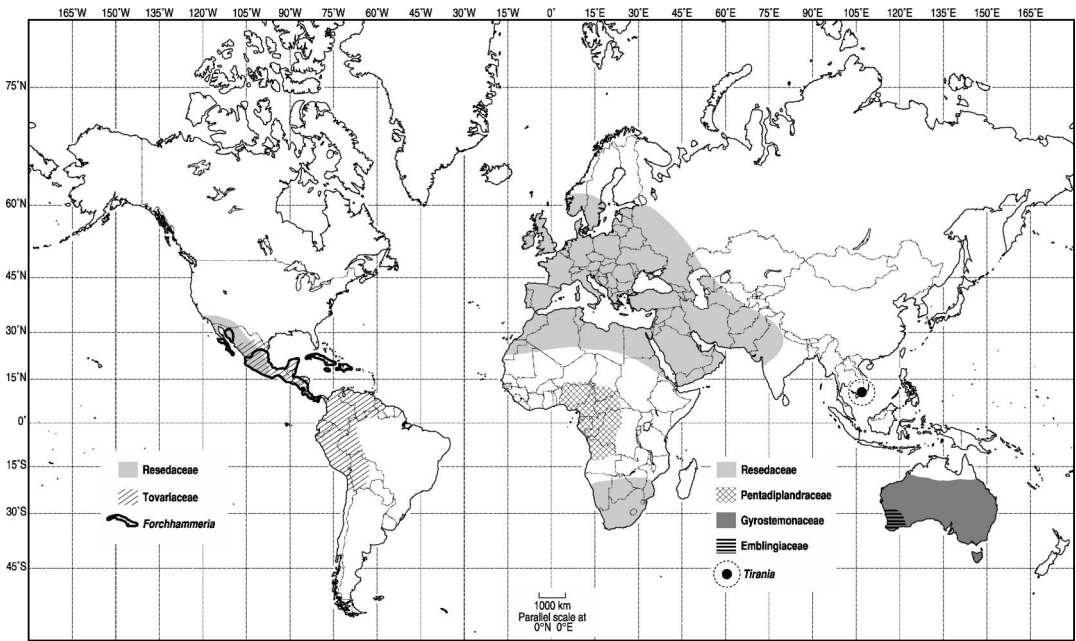


FIG. 1. Distributions of 'orphan' genera of Capparaceae s. lat. and their putative relatives: *Emblingia*, *Forchhammeria*, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, *Tirania*, and Tovariaceae.

differences in perianth number and number of locules from Capparaceae s. lat., Kers (2003) excluded all members of Stixeeae from his recent generic level treatment of Capparaceae s. lat. Further phylogenetic studies within the core Brassicales are thus warranted, as only one species of *Forchhammeria* was sampled and putative relatives of *Forchhammeria*, such as *Tirania*, in the tribe Stixeeae have not been sampled.

Perhaps more importantly from evolutionary and biogeographical perspectives, the isolated genera and small families of the core Brassicales exhibit remarkable variation in habit (annual, perennial herb or shrub, climbing shrub), floral structure (carpel, locule, stamen, and perianth number), breeding systems (bisexual, monoecious, dioecious, polygamous), and disjunct areas of endemism (Fig. 1). *Emblingia* is a small shrub possessing five sepals, two petals, a gynophore, and a multi-carpellate fruit with replum. The unique pollen of *Emblingia* has short colpi with rounded ends and exine that thickens close to the apertures such that the apertural areas bulge out (Erdtman et al. 1969; Kubitzi 2003a). The ovary in *Forchhammeria* flowers is bicarpellate and bilocular with each locule usually containing two seeds early in development. However, only one of the seeds usually develops, giving the appearance of being unilocular (Hansen 1977). The petals of *Tirania* have a ligulate rim similar to *Pentadiplandra* (Kers 2003). *Pentadiplandra* is a monoecious, climbing shrub possessing pentamerous flowers with more than three carpels and locules. Although some authors include *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.

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 Stixeeae 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 *To-*

varia is found in cloud forests (Appel and Bayer 2003), *Pentadiplandra* grows in savannas, rain forests, and secondary growth (Ronse Decraene 2002; Bayer and Appel 2003a). Only Resedaceae have a widespread distribution restricted to the more arid parts of the Northern Hemisphere, mostly in the Old World but with a few species in southwestern United States and northern Mexico. A more detailed phylogenetic analysis of the core Brassicales may provide insights into understanding their peculiar biogeographical patterns.

Presented here is a phylogenetic analysis of the Brassicales with emphasis on the core Brassicales using chloroplast sequence information from three coding regions. *ndhF* (Alverson et al. 1999; Hall et al. 2002; Sytsma et al. 2002), *rbcl* (Alverson et al. 1998; Conti et al. 1996, 1997, 2002; Rodman et al. 1996, 1998; Sytsma et al. 2002), and *matK* (Koch et al. 2001; Sytsma et al. 2003; Wilson et al. 2001) have been shown to be informative at the infrafamilial level in Brassicales and related orders Malvales, Sapindales, and Myrtales. In order to address questions on relationships among families of the core Brassicales and the placement of *Emblingia*, *Forchhammeria*, and *Tirania*, we added approximately 60 new sequences to the Rodman et al. (1996, 1998) *rbcl*, Koch et al. (2001) *matK*, and our own (Hall et al. 2002) *ndhF* data sets. With increased sampling of taxa within the core Brassicales and combined sequence information from three gene regions, we should be able to improve phylogenetic resolution. This study has three major goals: (1) determine relationships of lineages within the core Brassicales, specifically relationships among Gyrostemonaceae, Resedaceae, Tovariaceae, and Pentadiplandraceae; (2) determine the placement of three of the unusual genera of Capparaceae s. lat., *Forchhammeria*, *Tirania*, and *Emblingia*, within the core Brassicales; and (3) elucidate patterns, based on these molecular phylogenetic estimates, of evolution in floral morphology and breeding systems within core Brassicales.

MATERIALS AND METHODS

Taxon Sampling. We sampled widely within the Brassicales (sensu Rodman et al. 1998; 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 2003b]). Rodman et al. (1998) did not sample *Emblingia* so this taxon was not included as a member of the order until APG (1998) and Chandler and Bayer (2000) placed it there. Our sampling includes all families of core Brassicales. Although Capparaceae, Cleomaceae, 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 Tovariaceae (monotypic or questionably ditopic [Appel and Bayer 2003]) were available. Resedaceae and Gyrostemonaceae 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 Stixeeae, and *Emblingia* were included in the *ndhF* and *rbcl* 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. Bretschneideraceae, Koerberliniaceae, and Setchellanthaceae are monotypic and, Caricaceae, Moringaceae, and Tropaeolaceae 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, 1318F 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' / 674R 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: 1F, 16F, 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 Multrees (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, multrees, holding 1 tree per step).

Fifty-six maximum likelihood models were explored in individual and combined data using Modeltest v.3.06 (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. 1998). *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 *Emblingia*, 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*, *Emblingia*, *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 Lutizoni 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 REL 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. >3; both ordered and unordered), 3. locule number (a. two, b. three, c. >3; 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. hermaphroditic, b. monoecious, 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; Hufford 1996; Olson 2002a, b; Ronsse Decraene 2002; Ronsse Decraene et al. 1998, 2002; Appel and Al-Shehbaz 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 Acctran resolving options. The topologies of the maximum likelihood and maximum parsimony trees differed only with respect to the position of *Pentadiplandra*, 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 Brassicales as a monophyletic clade. *Koerberlinia* and *Batis* form a sister group to the core Brassicales (with the exception in *rbcl* analysis) and Caricaceae and Moringaceae are sister to these two clades. Within the core Brassicales, all families and genera with more than one species sampled (Capparaceae, Cleomaceae, Gyrostemonaceae, *Forchhammeria*, and Resedaceae) were monophyletic. Brassicaceae s. lat. form a monophyletic lineage with Cleomaceae 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. *Emblingia* is sister to all other core Brassicales in *ndhF* and *rbcl* analyses (not sampled for *matK*). Relationships of Tovariaceae and Pentadiplandraceae within the core Brassicales 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 *Cratogeomys palmeri*, *Polanisia dodecandra*, and *Wislizenia 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 *Emblingia*, *Forchhammeria watsonii*, *F. 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 Brassicales (Rodman et al. 1994, 1996; Karol et al. 1999). Unfortunately, for most of these taxa our sequences ended at this codon position so we are

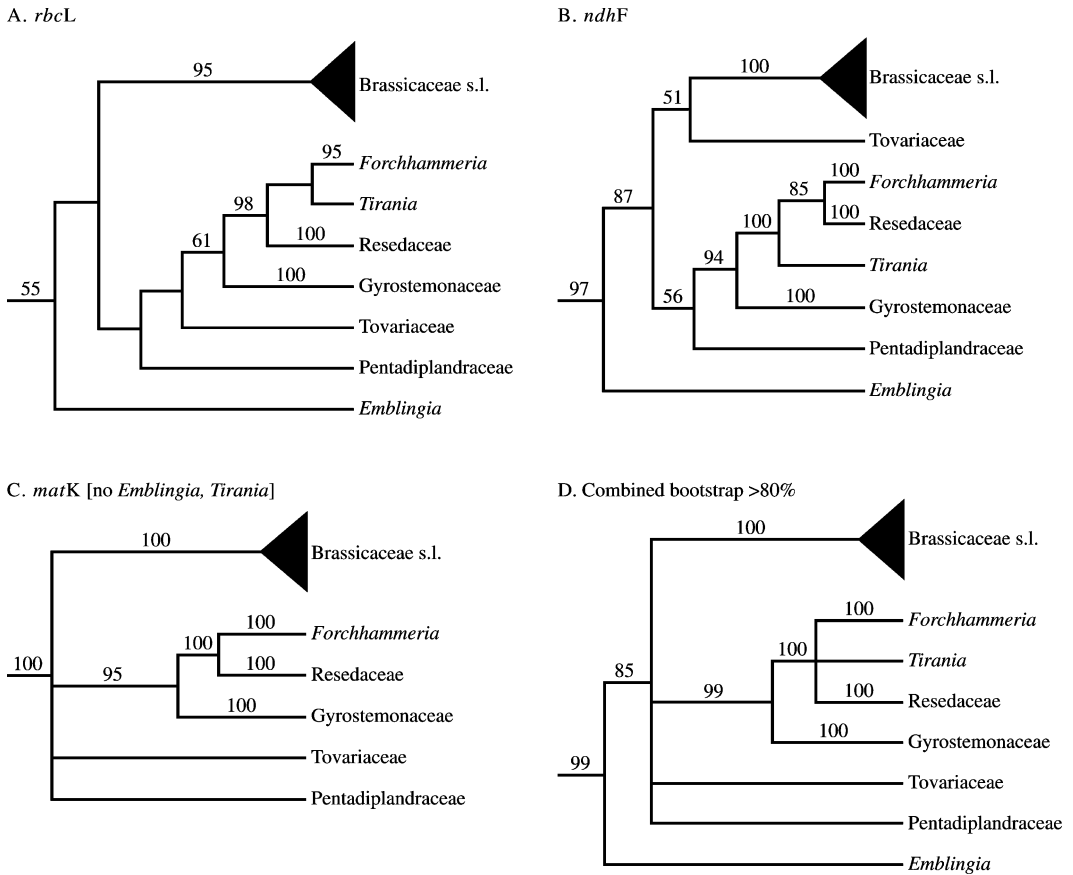


FIG. 2. Phylogram results of parsimony analyses for individual and combined data sets, showing only key relationships within the core Brassicales. Bootstrap values are above branches and for *Forchhammeria*, *Resedaceae*, and *Gyrostemonaceae* the values indicate monophyly for those clades. (A) Consensus 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 *Emblingia*. (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 *Emblingia*).

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 *Emblingia*, and for relationships of *Pentadiplandra*, *Tovaria*, and *Emblingia* within the core Brassicales (all <50% bootstrap). *Emblingia* is sister to all other core Brassicales whereas *Tovaria* 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 *Koerberlinia*.

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 *Cleomaceae*, *Cleome pilosa* and *C. viridiflora* are sister species whereas in the MP topology *C. pilosa* is sister to *Podandroyne*. 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 *Tovaria* 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

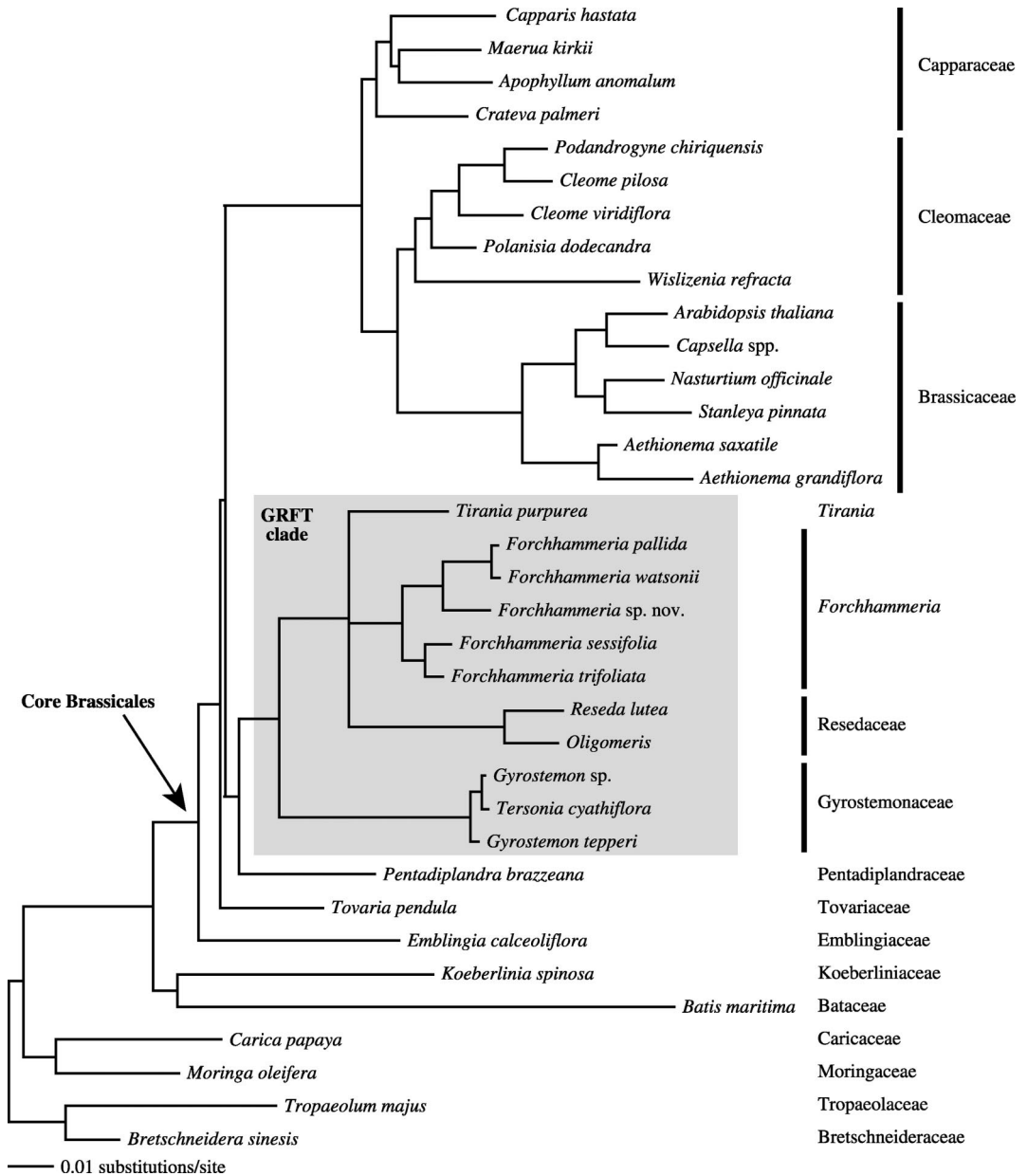


FIG. 3. The maximum likelihood tree of the 35 taxa combined *rbcl*, *ndhF*, and *matK* analysis including *Tirania* and *Emblingia* under the TVM + I + G model of DNA substitution. The topology of this tree is almost identical to the maximum parsimony analyses with the exception of position of *Pentadiplandra*. Branch lengths are proportional to the number of changes along each branch. GRFT clade in shaded box.

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 re-

covered 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).

	<i>rbcl</i>	<i>ndhF</i> ¹	<i>matK</i> ¹	Combined 1	Combined 2
Number of taxa	35	35	31	31	35
Aligned length	1479	1097	1566	4142	4142
Parsimony:					
Parsimony informative sites (%)	242 (16.4%)	342 (31.2%)	458 (29.2%)	988 (23.9%)	1031 (24.9%)
MP trees (length)	48 (817)	1(1283)	6 (1582)	2 (3494)	2 (3660)
CI (RI)	0.661 (0.704)	0.633 (0.726)	0.677 (0.760)	0.674 (0.741)	0.656 (0.736)
Maximum likelihood:					
ML model ²	TrN + I + G	GTR + G	TVM + G	TVM + I + G	TVM + I + G
Likelihood score (-ln L)	6678.34464	8046.93736	10490.42846	24508.01206	25390.54641
Base frequencies					
A	0.2845	0.3416	0.3272	0.3143	0.3147
C	0.1886	0.1291	0.1463	0.1571	0.1577
G	0.2397	0.1322	0.1453	0.1745	0.1723
T	0.2872	0.3972	0.3812	0.3540	0.3553
Substitution rates					
A-C	1.0000	1.4130	1.5828	1.4784	1.4329
A-G	1.9635	2.1857	2.1531	2.1438	2.1309
A-T	1.0000	0.2872	0.3107	0.4356	0.4261
C-G	1.0000	2.1703	1.6964	1.4891	1.4609
C-T	2.6116	1.8780	2.1531	2.1438	2.1309
G-T	1.0000	1.0000	1.0000	1.0000	1.0000
Proportion invariant sites (I)	0.5418	0	0	0.2603	0.2906
Gamma distribution shape parameter (G)	0.7781	0.6765	1.1023	0.9474	1.0425

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 *Koerberlinia*. 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 *Tovaria* and *Pentadiplandra* are unresolved, although there is weak support (bootstrap 60%) for *Tovaria* 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 taxa 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 *Tovaria* 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 s. lat., *Stixis* and *Neothorelia*, which have been placed in the tribe Stixeeae, clearly need to be sampled.

Monophyly of the Core Brassicales. The core Brassicales are a strongly supported clade (bootstrap 100% in combined analyses) comprising Brassicaceae, Capparaceae, Cleomaceae, Emblingiaceae, *Forchhammeria*, Gyrostemonaceae, Pentadiplandraceae, Resedaceae,

Tirania, and Tovariaceae. The GRFT clade of Gyrostemonaceae, Resedaceae, *Forchhammeria*, and *Tirania* is a novel lineage established here, with problematic affinities to *Pentadiplandra* and *Tovaria*. 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 *Koerberlinia*).

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 Tovaria 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 *Tovaria* and *Pentadiplandra*, each with low statistical support (Figs. 2, 3). Since both families are monotypic (*Tovaria* questionably 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 *Tovaria* 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 *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 *Forchhammeria*, *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 *Forchhammeria* and Resedaceae, a relationship further supported by a unique 9bp *matK* deletion. In a previous analysis using only *trnL-trnF* data (Fig. 3 in Hall et al. 2002), *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 Shimodaira-Hasegawa test was conducted on combined and the *trnL-trnF* data sets (*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 Crandall 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 *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 *Forchhammeria* and Resedaceae suggested by combined *matK*, *rbcL*, and *ndhF* is more likely than that indicated by the previous *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 gynoecium 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 *Forchhammeria* 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 *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 syncarpy 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 synapo-

morphy for the GRFT clade, members of the clade have some morphological similarities or trends. Gyrostemonaceae, *Forchhammeria*, and *Ochradenus* (Resedaceae, unsampled in these studies) all have uniseriate perianths, grow in arid habitats, and are anemophilous (Hansen 1977; Hufford 1996). The flowers of *Forchhammeria*, like Gyrostemonaceae, are much reduced with a fused calyx that splits in an irregular fashion. Gyrostemonaceae and *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. *Tirania* and Resedaceae (excluding *Ochradenus*) are the only members of the clade that have petals. Of some interest is that both genera sampled from the tribe Stixeeae of Capparaceae s. lat. (Pax and Hoffmann 1936), *Forchhammeria* and *Tirania*, are closely related to one another and Resedaceae. Members of the tribe Stixeeae have two to multilocular ovaries, axile placentation, three- to five-merous perianth, and well developed stigmas (Pax and Hoffmann 1936; Kers 2003), which support their placement within the GRFT clade (Fig. 4). Precisely how *Forchhammeria* and *Tirania* are related to one another is still unresolved based on *rbcL* and *ndhF* sequence data (Figs. 2, 3). Additional sampling of remaining Stixeeae (i.e., *Neothorelia* and *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 *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 plesiomorphic in Brassicales and, based on current sampling, the herbaceous habit has arisen four times: (1) Cleomaceae plus Brassicaceae, (2) *Oligomeris* (Resedaceae), (3) *Tersonia* (Gyrostemonaceae), and (4) *Tropaeolum* (Tropaeoaceae).

Morosity of both the gynoecium and perianth is very labile within the Brassicales (Fig. 4). When designated as unordered, the plesiomorphic 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 Brassicaceae clade in addition to *Forchhammeria* and *Gyrostemon tepperi*. Whether treated as ordered or unordered, the primitive locule number

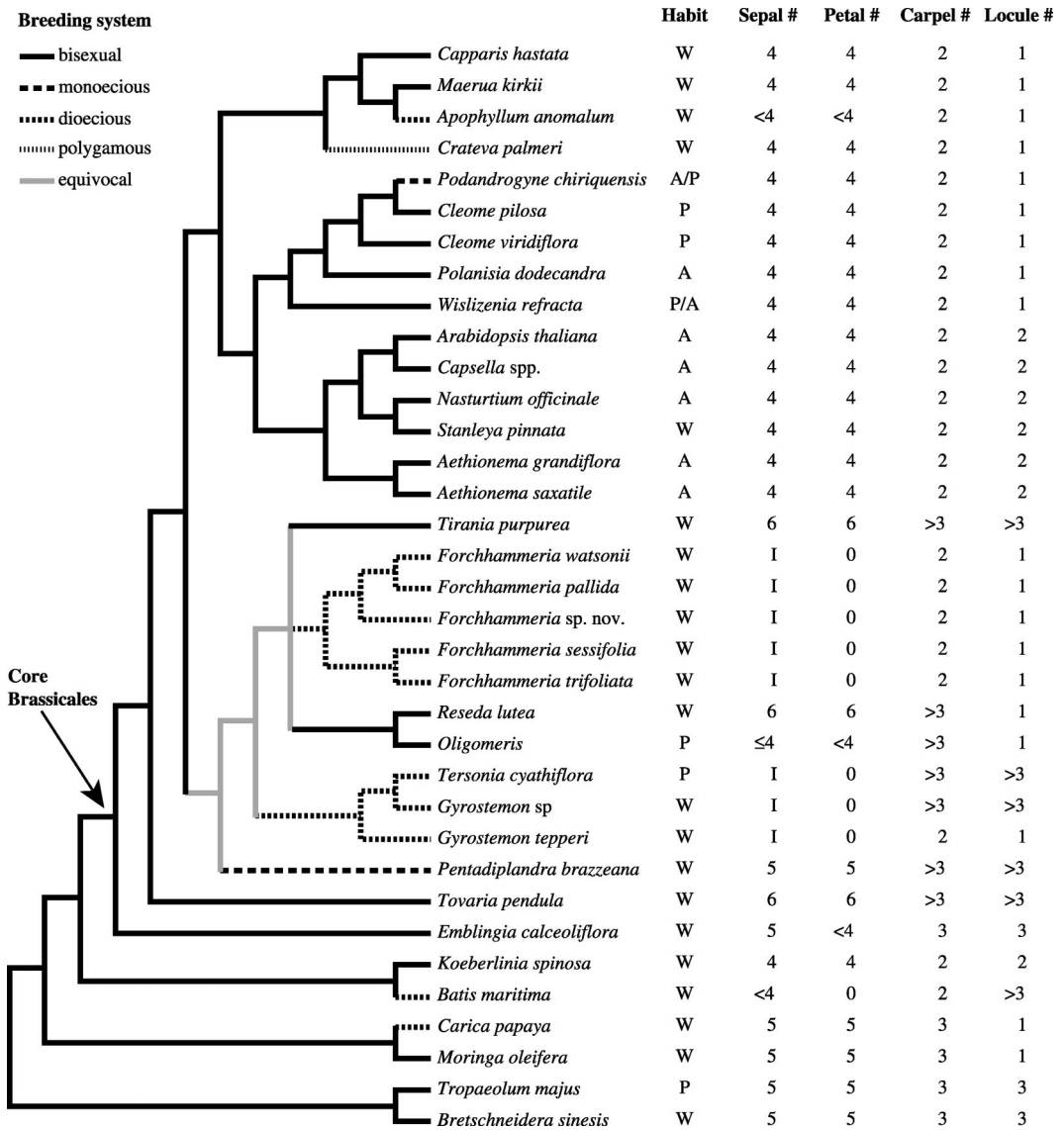


FIG. 4. Optimization mapping of breeding systems on the maximum likelihood tree from the combined 35 taxa analysis. Character states for habit, sepal number, petal number, carpel number, and locule number are given. W = woody perennial, P = herbaceous perennial, H = annual herbaceous, I = irregular.

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 monoecy or polygamy evolved twice (*Crateva*, *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 Resedaceae and *Pentadiplandra* is sister to the GRFT clade, then monoecy, dioecy, and hermaphroditism are all possible ancestral conditions. In all other arrangements of *Forchhammeria*, *Tirania*, and Resedaceae (re-

ardless of placement of *Pentadiplandra*, the ancestral condition is hermaphroditism with two independent origins of dioecy.

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, occur in eastern 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 (*Boscia* and *Maerua*) 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 biogeographic 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, *Emblingia* 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 (plesiomorphic 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 (plesiomorphic as scored here), apparently de-

rived 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), *Apophyllum* (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 (Renner 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; Renner 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 monoecy to dioecy. Four of the dioecious groups (*Batis*, *Apophyllum*, *Forchhammeria*, and *Gyrostemonaceae*) 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 *Gyrostemon* and *Tersonia* (*Gyrostemonaceae*; Hufford, 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 Stixeeae 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 Stixeeae), 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 Iltis 1999).

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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

Bataceae: *Batis maritima* L. (Itis 30500, WIS; *rbcL* [L22438, Rodman et al. 1994]; *ndhF* [AY122403, Hall et al. 2002]; *matK* AY483219)
 Bretschneideraceae: *Bretschneidera sinensis* Hemsl. (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* AY483248; *matK* AY483221)
 Koerberliniaceae: *Koerberlinia spinosa* Zucc. (Al Shehbaz s.n., MO; *rbcL* [L14600, Rodman et al. 1993]; *ndhF* AY483249; *matK* AY483222)

Moringaceae: *Moringa oleifera* Lam. (Itis 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 grandiflora* L. (*ndhF* [AF064657, Galloway et al. 1998]; *matK* [AF144354, Koch et al. 2001]); *Aethionema saxatile* R. Br. (Moore s.n., WIS; *rbcl* AY483262; *ndhF* AY483250); *Arabidopsis thaliana* (L.) Heynh. (*rbcl* [U91966, 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); *Stanleya pinnata* (Pursh) Britton (Hall 1, AZ; *rbcl* AY483263; *ndhF* [AY122401, Hall et al. 2002]; *matK* AY483226)

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

Cleomeae: *Cleome pilosa* Benth. (Iltis 30585, WIS; *rbcl* AY483267; *ndhF* [AY122385, Hall et al. 2002]; *matK* AY483231); *Cleome viridiflora* Schreb. (Solomon s.n., MO; *rbcl* AY483268; *ndhF* [AY122386, Hall et al. 2002]; *matK* AY483232); *Podandrogyne chiriquensis* (Standl.) Woodson (Nepokroeff 450, WIS; *rbcl* AY483269; *ndhF* [AY122393, Hall et al. 2002]; *matK* AY483233); *Polanisia dodecandra* DC (Grette 8603, WIS; *rbcl* AY483270; *ndhF* AY483251; *matK*

AY483234); *Wislizenia refracta* Engelm. (Vanderpool 1340, OKL; *rbcl* AY483271; *ndhF* [AY122391, Hall et al. 2002]; *matK* AY483235)

Gyrostemonaceae: *Gyrostemon* sp. (Cranfield, PERTH no. 02068672; *rbcl* [L22439 Rodman et al. 1994]; *ndhF* AY483252; *matK* AY483236); *Gyrostemon tepperi* (F. Muell. ex. H. Walter) A. S. George (Thompson, 2243, MO; *rbcl* [L22440, Rodman et al. 1994]; *ndhF* AY483253; *matK* AY483237); *Tersonia cyathiflora* (Fenzl) A.S. George (Cranfield PERTH no.02068682; *rbcl* [L22441, Rodman et al. 1994]; *ndhF* [AF122404, Hall et al. 2002]; *matK* AY483238)

Pentadiplandraceae: *Pentadiplandra brazzeana* Baill. (*rbcl* [U38533, Rodman et al. 1996]; Hall 263, WIS; *ndhF* AY483254; *matK* AY483239)

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

Tovariaceae: *Tovaria pendula* Ruiz. & Pav. (Smith and Smith 1834, WIS; *rbcl* [M95758, Rodman et al. 1993]; *ndhF* [AY122407, Hall et al. 2002]; *matK* AY483242)

ANOMALOUS GENERA

Emblingia calceoliflora F. Muell. (*rbcl* [AF146014, Chandler and Bayer 2000]; Quinn 288a; *ndhF* AY483256)

Forchhammeria pallida Liebmann (Iltis 29350a, WIS; *rbcl* AY483274; *ndhF* [AY122381, Hall et al. 2002])

Forchhammeria sessilifolia Standl. (Cochrane 12967, WIS; *rbcl* AY483275; *ndhF* AY483257; *matK* AY483243)

Forchhammeria sp. nov. (Iltis 30784, WIS; *rbcl* AY483276; *ndhF* AY483258; *matK* AY483244)

Forchhammeria trifoliata Radlkofer (Hansen 3002, WIS; *rbcl* AY483277; *ndhF* AY483259; *matK* AY483245)

Forchhammeria watsonii Rose (Fishbein 3070, WS; *rbcl* AY483278; *ndhF* AY483260; *matK* AY483246)

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