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Convergent evolution of a complex fruit structure in the tribe Brassiceae (Brassicaceae)[1]

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- *Premise of study:* Many angiosperms have fruit morphologies that result in seeds from the same plant having different dispersal capabilities. A prime example is found in the Brassiceae (Brassicaceae), which has many members with segmented or heteroarthrocarpic fruits. Since only 40% of the genera are heteroarthrocarpic, this tribe provides an opportunity to study the evolution of an ecologically significant novelty and its variants.
- *Methods:* We analyzed nuclear (*PHYA*) and plastid (*matK*) sequences from 66 accessions using maximum parsimony, maximum likelihood, and Bayesian inference approaches. The evolution of heteroarthrocarpy and its variants was evaluated using maximum parsimony and maximum likelihood ancestral state reconstructions.
- *Key results:* Although nuclear and plastid phylogenies are incongruent with each other, the following findings are consistent: (1) *Cakile*, *Crambe*, *Vella*, and *Zilla* lineages are monophyletic; (2) the Nigra lineage is not monophyletic; and (3) within the *Cakile* clade, *Cakile*, *Didesmus*, and *Erucaria* are paraphyletic. Despite differences in the *matK* and *PHYA* topologies at both deep and shallow nodes, similar patterns of morphological evolution emerge. Heteroarthrocarpy, a complex morphological trait, has evolved multiple times across the tribe. Moreover, there are convergent transitions in dehiscence capabilities and fruit disarticulation across the tribe.
- *Conclusions:* We present the first explicit analysis of fruit evolution within the Brassiceae, which exemplifies evolutionary lability. The repeated loss and gain of segment dehiscence and disarticulation suggests conservation in the genetic pathway controlling abscission with differential expression across taxa. This study provides a strong foundation for future studies of mechanisms underlying variation in dispersal capabilities of Brassiceae.

Key words: ancestral state reconstruction; Brassicaceae; dehiscence; fruit evolution; segmentation; silique.

Evolutionary biologists frequently use phylogenetic hypotheses to address the number of times specific traits have evolved within a clade of interest. Repeated evolution of traits leads to fundamental inquiries on the selective advantages of particular morphologies (Schluter and Nagel, 1995), biogeographical context of selective environments (Wiens et al., 2006), whether some traits represent key innovations leading to species diversification (Cubas, 2004), and how developmental and genetic mechanisms underlying traits can distinguish between parallelisms and convergences (Abouheif and Wray, 2002; Yoon and Baum, 2004; Scotland, 2011). Fruit morphology is particularly promising for investigations regarding the independent origins of a morphological trait. Moreover, because seed dispersal is essential for plant reproduction and adaptation, fruit type is an important ecological trait.

A prime example of a potentially labile trait is heteroarthrocarpy in the Brassicaceae. Heteroarthrocarpic fruits are two-segmented with each segment containing seeds or rudimentary ovules (Appel, 1999). Heteroarthrocarpy and/or having conduplicate cotyledons characterize species of the tribe Brassiceae and do not occur elsewhere in the family (Gómez-Campo, 1980, 1999; Al-Shehbaz, 1985; Warwick and Sauder, 2005). In fact, Brassiceae is highly unusual in the Brassicaceae because it is the only tribe whose traditional circumscription is supported by molecular data (Warwick and Sauder, 2005; Bailey et al., 2006; Beilstein et al., 2006, 2008; Warwick et al., 2010). Not all members of the tribe have the heteroarthrocarpic fruit type, however, and a wide range variation of fruit morphology exists (Gómez-Campo, 1980; Al-Shehbaz, 1984; Al-Shehbaz et al., 2006). Approximately 40% of described genera have heteroarthrocarpic fruits, whereas the remaining genera have fruits without segments (hereafter referred to as non-heteroarthrocarpic). Across the tribe, examination of heteroarthrocarpy has typically been conducted via surveys at the generic level; however, few genera are supported as monophyletic in molecular-based phylogenies (e.g., Warwick and Sauder, 2005), suggesting this trait may have a complicated evolutionary history. No studies have explicitly examined the distribution of this novel fruit type across species in a phylogenetic context, although heteroarthrocarpy has

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been proposed as both ancestral (Appel, 1999) and derived (Gómez-Campo, 1980).

The significant variation in fruit morphology observed across the tribe may reflect different modes of passive seed dispersal. Non-heteroarthrocarpic members have typical brassicaceous siliques: two-valved capsules with persistent placental tissue (replum). At maturity, the valves detach from the replum along the valve margin, then all seeds are released unprotected into the environment (Fig. 1A). In contrast, heteroarthrocarpic fruits are modified with a joint, a structure that laterally bisects the fruits into two segments (Fig. 1B). In many heteroarthrocarpic species, the joint forms a novel separation layer such that the distal segment separates and is dispersed independently of the proximal segment, a phenomenon referred to as disarticulation. While the distal segment is always indehiscent, the proximal segment may or may not dehisce, depending on the species (Fig. 1B). When indehiscent, the seeds within the proximal segment may or may not be dispersed when the maternal plant senesces. These differences in morphology result in three major variants of heteroarthrocarpic fruits (Fig. 1B): (1) proximal segment dehiscent and no disarticulation, (2) completely indehiscent fruits that disarticulate, and (3) proximal segment dehiscent with disarticulation. There are also some variations on these three major themes, including a form of type 2 in which the proximal segment is seedless, and thus all seeds are contained in the dispersed, indehiscent distal segment. All types of heteroarthrocarpy result in seeds from the same plant being released into the environment in different manners, a characteristic of many angiosperms that has important ecological consequences (Venable, 1985; Imbert, 2002). Thus, the joint of heteroarthrocarpic fruits is an innovation that allows a significant shift in seed dispersal (Rodman, 1974; Donohue, 1998). Seeds from dehiscent segments are dispersed independently without any protection from the pericarp, while seeds in indehiscent segments are protected but then are dispersed as a unit. The proportion of seeds in each type of segment influences the proportion of seeds that are dispersed at all and the proportion that are dispersed freely vs. in a protected propagule.

A phylogenetic framework of Brassiceae is essential for evaluating the evolution of this unusual fruit type and its variants. Relationships within the Brassiceae have been examined using both morphological (Gómez-Campo, 1980; Al-Shehbaz, 1985) and molecular data (Warwick et al., 1992; Warwick and Black, 1993, 1994, 1997; Yang et al., 1999; Crespo et al., 2000; Warwick and Sauder, 2005). These studies resulted in the identification of seven major lineages, *Cakile*, *Crambe*, Nigra, *Savignya*, Rapa/Oleracea, *Vella*, and *Zilla*, although three genera remain unplaced [*Henophyton* Coss. & Durieu, *Pseuderucaria* (Boiss.) O. E. Schulz, and *Orychophragmus* Bunge; reviewed in Warwick and Hall, 2009]. Analyses reveal that most genera are not monophyletic, which makes it challenging to assess the distribution of fruit types using traditionally described genera. Although previous work has greatly clarified the boundaries of the tribe and placement of taxa into lineages, there is limited resolution within and among genera and lineages.

Among the seven lineages, the *Cakile* clade is of particular interest due to variation in types of heteroarthrocarpy and a tractable number of species. This lineage, also referred to as subtribe Cakilinae, includes four genera: *Cakile* Mill. (7 spp.), *Crambella* Maire (1 sp.), *Didesmus* Desv. (2 spp.), and *Erucaria* Gaertn. (6 spp.) (Warwick and Black, 1997). All purported members of the *Cakile* lineage have heteroarthrocarpic

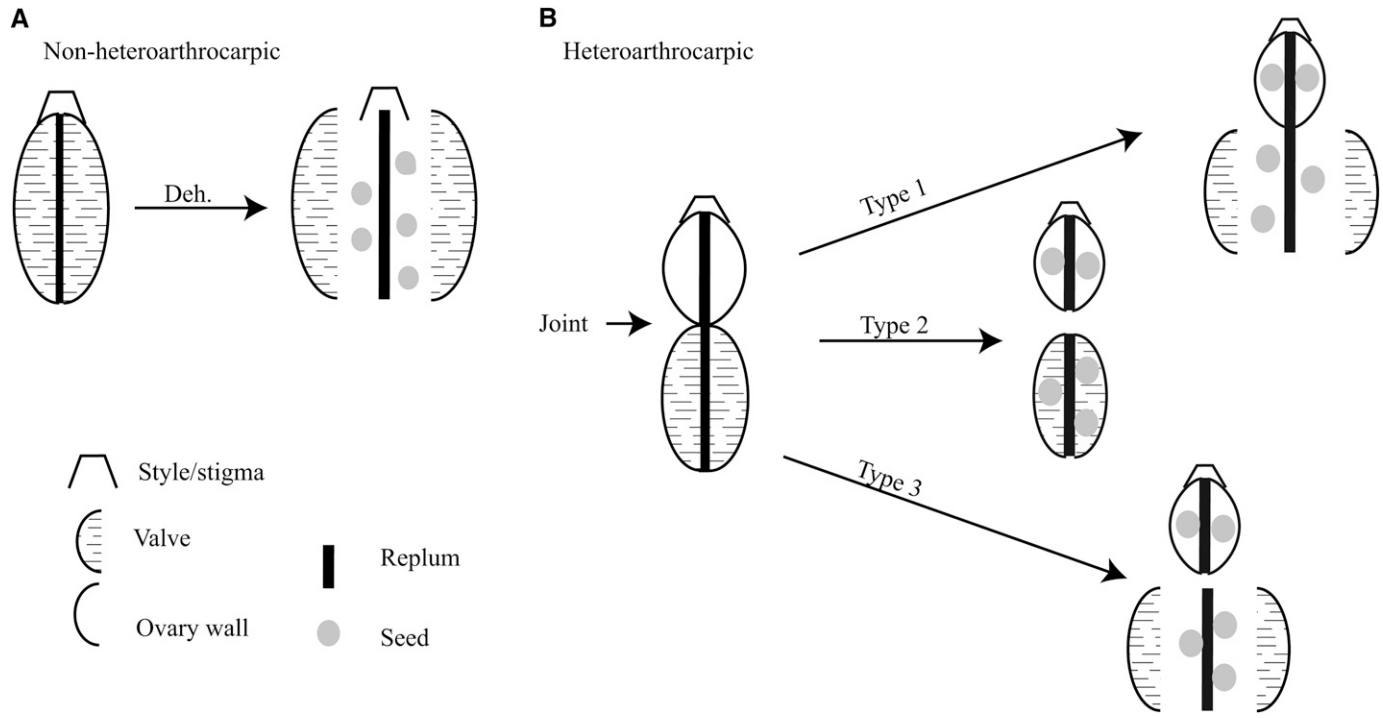

Fig. 1. Fruit maturation and seed dispersal in Brassiceae. (A) Non-heteroarthrocarpic taxa have fruits in which all seeds are dispersed freely into the environment, whereas (B) heteroarthrocarpic fruits release seeds in three distinct manners. Type 1 = proximal segment dehiscent, no disarticulation; type 2 = completely indehiscent fruit with disarticulation; type 3 = proximal segment dehiscent with disarticulation. Arrows designate fruit maturation and subsequent dehiscence and do not imply evolutionary relationships. Deh. = Dehiscence

fruits and display variation in heteroarthrocarpy that is also observed across the tribe. Furthermore, focusing on a small group of morphologically variable taxa allows for more complete sampling, thereby reducing effects that incomplete taxon sampling can have on ancestral state reconstruction (Salisbury and Kim, 2001; Heath et al., 2008; but see Li et al., 2008). Such an examination of fruit evolution is complemented by developmental (Hall et al., 2006), ecological (Barbour, 1970; Keddy, 1980, 1981; Dudley, 1996a, b), dispersal (Payne and Maun, 1981; Maun and Payne, 1989; Donohue, 1997, 1998), and systematic (Rodman, 1974) studies of species in this lineage. In addition, taxonomic sampling of the *Cakile* lineage has been variable across previous phylogenetic analyses highlighting outstanding systematic problems. In the most extensively sampled analysis of the entire tribe to date, support for monophyly of the group that contains the two species, *Cakile maritima* Scop. and *Erucaria hispanica* (L.) Druce, was low (58% bootstrap, Warwick and Sauder, 2005). A previous phylogenetic study based on chloroplast restriction data (Warwick and Black, 1997) sampled eight species (two *Cakile*, four *Erucaria*, one *Didesmus*, and *Crambella*). This analysis supported lineage monophyly, but relationships among species were unresolved. In addition, generic boundaries of the various genera have also been questioned. *Reboudia* Coss. & Durieu is a synonym of *Erucaria*, and Appel and Al-Shehbaz (2003) proposed that *Didesmus* is likely another.

The monophyly of the tribe, combined with remarkable variation in fruit morphology, provides a unique opportunity to evaluate evolution and potential lability of a novel fruit trait within Brassicaceae. Fruit morphology is homoplastic across the family (Koch et al., 2003) and, as such, often not examined in close detail. We present a phylogenetic study of Brassiceae with emphasis on the Cakilinae using both nuclear, phytochrome A (*PHYA*), and chloroplast sequence, maturase K (*matK*), information. The primary goals of this study are to determine the number of times heteroarthrocarpy has evolved within the tribe and to assess evolutionary patterns across variants of heteroarthrocarpy, with emphasis on the *Cakile* lineage.

MATERIALS AND METHODS

***Taxon sampling*—**Using previous studies as a framework (Warwick and Black, 1993, 1994, 1997; Francisco-Ortega et al., 1999; Warwick and Sauder, 2005), we sampled broadly across the tribe to incorporate a range of fruit morphologies. At least two representatives were sampled from all seven identified lineages with the exception of the *Savignya* lineage. Unplaced genera *Henophyton* and *Pseuderucaria* were also included. Within the *Cakile* lineage, all species were sampled except *Cakile geniculata* (B. L. Rob.) Millsp., *Erucaria crassifolia* Delile, *E. rostrata* (Boiss.) A. W. Will ex Greuter & Burdet, and *E. uncata* Boiss. Sixty-six accessions were included in the analyses (Appendix 1) including two outgroups, *Sisymbrium altissimum* L. (tribe Sisymbrieae) and *Stanleya pinnata* (Pursh) Britton (tribe Thelypodieae) chosen based on family-wide phylogenetic studies (Koch et al., 2001; Bailey et al., 2006; Beilstein et al., 2006, 2008). *Arabidopsis thaliana* (L). Heynh. (GenBank accession L21154) and *Armoracia rusticana* G. Geartn., B. Mey., & Scherb. (GenBank AB036762) were used as additional outgroups for some *PHYA* analyses that were conducted to establish number of gene copies (see phylogenetic inference section).

***DNA extraction, amplification, cloning, and sequencing*—**All plants were grown from seed in the Department of Organismal and Evolutionary Biology greenhouses at Harvard University. The majority of seeds were obtained from the seed stock of the late C. Gómez-Campo, but additional *Cakile* material was obtained. Vouchers were taken from greenhouse-grown materials and deposited in Harvard University Herbaria (HUH). Total DNA was extracted using Qiagen DNeasy (Qiagen, Germantown, Maryland, USA).

Low-copy nuclear genes can be informative since they often evolve at faster rates than chloroplast genes. The phytochrome gene family is well characterized (Clack et al., 1994; Mathews and Sharrock, 1997; Mathews, 2010), sequence variation has been valuable in resolving relationships in a range of plant families (e.g., Poaceae, Mathews and Sharrock, 1996; Orobanchaceae, Bennett and Mathews, 2006; Malpighiaceae, Davis, 2002), and *PHYA* has been used previously within the Brassicaceae (Beilstein et al., 2008). Amplification of a 1.2-kb region of *PHYA* used degenerate primers and a TripleMaster enzyme that includes a proofreading component (Eppendorf, Westerbury, New York, USA). Primers a230f (GACTTTGARCCNGTBAAGCCTTAYG) and a832.3 (RTTCCAYTCNGARCACCANCC) resulted in amplification that included most of the first exon, all of the first intron, and a small portion of the second exon. Primer sequences were kindly provided by Sarah Mathews (Harvard University). Appropriately sized PCR products were gel-extracted using a MinElute Gel extraction kit (Qiagen). Cloning procedures were essentially those of Mathews et al. (2000). *PHYA* fragments were ligated into PGEM-T Easy vectors (Promega, Madison, Wisconsin, USA) and incubated overnight at 4°C. XL1-Blue *E. coli* competent cells (Stratagene, La Jolla, California, USA) were transformed with ligation products and incubated overnight at 37°C. Colonies were PCR-screened using vector-based primers m13f (GTTTTCCCAGTCACGAC) and sp6 (GATTTAGGTGACACTATAG) to confirm that colonies had appropriately sized inserts. Colonies containing appropriately sized inserts were cultured overnight in a nutrient broth, and plasmid DNA was isolated using Eppendorf Minipreps kits (Eppendorf). Four to six clones per accession were sequenced with the m13f primer, and then sequences were confirmed as *PHYA* using a BLASTn search. Once clones were identified as *PHYA*, sequencing primers used were m13f, sp6, and three internal primers (320f, 444F, 788R). One to six clones were sequenced per accession (averaging four per accession). Sequences were obtained with an ABI model 3100 or 3700 automated sequencer (Applied Biosystems, Foster City, California, USA) and edited with the program Sequencher 3.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). Alignments are available in Appendix S1 and S2 (see Supplemental Data with online version of this article).

The *matK* region, including the *trnK* intron, has been shown to be informative and effective at resolving relationships at the familial level (Johnson and Soltis, 1994, 1995; Koch et al., 2001; Samuel et al., 2005; Hall, 2008). The *matK* region was amplified using *trnK*-710F and *trnK*-2R primers (Koch et al., 2001). A number of primers were used to sequence including *trnK*-710F, *trnK*--2R, 495R, 495F, 1010F, 1010R, and 1089R (Koch et al., 2001). In almost every instance, both strands were sequenced using a 3700 automated sequencer (Applied Biosystems) and edited with the program MacVector v8.1.1 (MacVector, Cary, North Carolina, USA). In the few exceptions where it was not possible to sequence both strands, manual proofreading of sequences was done to detect any possible misreads. The alignment is available in online Appendix S3.

***Phylogenetic inference*—**Phylogenetic hypotheses were estimated using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Parsimony heuristic searches were conducted on individual data sets in the program PAUP* 4.0b10 (Swofford, 2000) using the following search parameters: 100 random addition replicates, hold 10 trees per replicate, and tree-bisection-reconnection (TBR) branch swapping. Parsimony bootstrap (BS) was conducted to assess support for branches (1000 replicates, TBR swapping, simple addition replicates, saving no more than 1000 trees per replicate). Maximum likelihood analyses were conducted on coding regions of the two data sets. The best model of sequence evolution was determined using the Akaike information criterion (AIC) as implemented in the program MrModelTest ver. 2 (Nylander, 2004). Maximum likelihood analyses were conducted using the program GARli v0.95 (Zwickl, 2006) starting from random trees, using 10 000 generations per search, and estimating model parameters. Values for ML BS were determined by conducting 100 replicates of the ML search. Bayesian analyses were conducted in the program MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001). Two independent Bayesian analyses were run under default priors for one million generations, sampling every 100 generations. Runs were stopped when the average standard deviation of split frequencies was 0.009. Convergence between runs was also confirmed by verifying that potential scale reduction factor (PSRF) values were close to 1.0. Initial analyses of the *PHYA* data set revealed that the two runs had poor mixing and were slow to converge; thus, for these analyses, the heating temperature was set to 0.1 (default is 0.2). A majority rule tree excluding 25% burn-in was visualized in the program Mesquite ver. 2.72 (Maddison and Maddison, 2009) to obtain posterior probability

(PP) values. Visual inspection of topologies from independent analyses of the chloroplast and nuclear data sets revealed substantial differences between the two topologies. Branches with moderate to strong support (≥70% BS) in one topology that were contradicted by the alternative data set were used as constraints for topology tests (see topology tests below). Of the 19 branches in conflict, eight were found to be significantly different (online Appendix S4). As such, data were not combined in any analysis.

***Morphological characters and ancestral state reconstruction*—**By overlaying morphological traits onto the molecular-based phylogenetic hypotheses, we could assess evolution of fruit features. For the majority of species, fruit characteristics were scored from multiple individuals grown in the Harvard University Greenhouses. Character-state information was also obtained and/or verified from herbarium specimens housed at HUH and from literature (Edgecombe, 1970; Tackholm, 1974; Jafri, 1977; Hedge and Lamond, 1980; Beckett, 1993; Rollins, 1993; Short, 1994; Stace, 1997; Chaudhary, 1999). We focused on major characteristics that alter seed packaging and potentially influence dispersal (see online Appendix S5 for data matrix). Characters were scored across all Brassiceae: (1) heteroarthrocarpy—absent, present; (2) dehiscence—fully dehiscent (i.e., non-heteroarthrocarpic), partially indehiscent (distal segment indehiscent, proximal dehiscent), indehiscent; and (3) fruit disarticulation—non-heteroarthrocarpic, no disarticulation (i.e., no joint abscission), and disarticulation (i.e., joint abscission). Species were scored as heteroarthrocarpic if seeds or ovules were present above the distal portion of the valve margin (i.e., septum and ovary extended beyond the tip of valve tissue).

Ancestral state reconstructions were explored using MP and ML approaches in Mesquite ver. 2.72 (Maddison and Maddison, 2009). Because different topologies can result in different reconstructions, we account for this phylogenetic uncertainty (Reeb et al., 2004; Tripp and Manos, 2008; Arnold et al., 2009) by conducting reconstructions over a portion of the posterior distribution of trees from each data set's Bayesian analysis (the last 500 trees per run for a total of 1000 trees). Using the trace characters over trees command in Mesquite ver. 2.72 (Maddison and Maddison, 2009), reconstructions were made on the majority rule BI tree by counting trees with a uniquely best state. In parsimony reconstructions, characters were treated as unordered, and the number of steps of character change was minimized. Two alternative weightings, 1 : 1 and 10 : 1, of heteroarthrocarpy gain to loss were considered. Given the multiple modifications in heteroarthrocarpic fruits when compared to non-heteroarthrocarpic (Hall et al., 2006), weighting the loss of this fruit type over its gain seemed unlikely and was not examined. Alternative-step matrices were also used for both the dehiscence and disarticulation characters. Since loss-of-function mutations in a few genes can lead to indehiscent fruit in *Arabidopsis* (Dinneny and Yanofsky, 2004), the transition from dehiscent to indehiscent may have a simple genetic basis in Brassicaceae. In contrast, the shift from an indehiscent to dehiscent valvular portion may be less likely. Considering this, we weighted the transition from partial to complete dehiscence as 1 and the change in state from indehiscent to dehiscent as 10. Since the joint is a novel feature not seen in other Brassicaceae fruits, we examined the transition between disarticulation at the joint and no-disarticulation at the joint. Initial parsimony reconstructions indicated that the shift from disarticulation to no-disarticulation was more frequent. Thus, a 10 : 1 weighting for the transition from no-disarticulation to disarticulation was examined to assess alternative patterns.

Likelihood methods determine ancestral states by maximizing the probability of the observed data, taking into account the model of character evolution and differences in branch lengths (Schluter et al., 1997). Likelihoods of the data were calculated using equal rate of gain and loss, known as the one-parameter Markov *k*-state model (mk1). Likelihoods of ancestral reconstruction are indicated as proportions with a particular state being most likely for a node with a threshold of significance at two (Schluter et al., 1997). That is, when the difference of log likelihood was two or higher, the lower valued state was rejected and the higher value state was designated as the most likely state for that branch.

***Topology tests*—**Alternative phylogenetic trees were evaluated using the non-parametric Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999). Maximum likelihood analyses were conducted enforcing topological constraints in PAUP* 4.0b10 using model parameters as specified by MrModeltest, heuristic search using a neighbor-joining tree as the starting tree, and TBR branch swapping. The SH test was then run in PAUP* 4.0b10 to determine whether trees constrained to match an alternative topology are significantly different from the ML tree obtained in unconstrained analyses (10 000 bootstrap replicates, RELL method). Constraints were specified according to the alternative topology such that the clade had to be present, but relationships within the constrained clade were not specified. Two sets of constraints were used (online Appendix S4). The first set examined potential conflict between data sets by comparing branches with greater than 70% BS support in either *PHYA* or *matK* trees that were not observed in trees recovered from analysis of the other data set. To assess morphological evolution of heteroarthrocarpy, we also constrained the topology such that all heteroarthrocarpic taxa were monophyletic. This tests the hypothesis of Gómez-Campo (1980), who suggested a single, derived origin to the fruit type.

RESULTS

***PHYA*—**Analyses were conducted on different combinations of terminal taxa and sequence data. Initial *PHYA* analyses that were conducted on all clones (227 terminal sequences) indicate that two copies are present and represent distinct clades as observed in MP and ML analyses (Appendix S6). The second copy of *PHYA* was found in seven taxa: *Enarthrocarpus lyratus* (Forssk.) DC., *Eruca vesicaria* (L.) Cav., *Erucaria cakiloidea* (DC.) O. E. Schulz, *Erucastrum abyssinicum* (A. Rich.) O. E. Schulz, *Erucastrum gallicum* (Willd.) O. E. Schulz, *Morisia monanthos* (Viv.) Asch., and *Schouwia purpurea* (Forssk.) Schweinf. The disparity in sequencing the second copy suggests that we were preferentially recovering one copy (hereafter referred to as copy 1) or one copy is being preferentially lost. Since we are able to distinguish between the two copies based on phylogenetic analyses and distinct nucleotide motifs in the intron (see also Beilstein et al., 2008), we focused all subsequent analyses on copy 1. In the vast majority of samples, all clones from a single accession were strongly supported (BS ≥ 95%) as monophyletic in both MP and ML analyses. We concluded that nucleotide variation among clones is minimal and, as such, is allelic or due to PCR error and chose a single clone to represent each taxon (as per Beilstein et al., 2008). There were two exceptions to the monophyly of clones from a single accession: (1) clones from *Crambe abyssinica* Hochst. ex. R. E. Fr. suggest a recent duplication event within the genus *Crambe* L., and (2) clones from many *Cakile* accessions do not form distinct monophyletic clades. We believe that the lack of distinction among clones of *Cakile* (with the exception of all clones from *C. arabica* Velen. & Bornm.) in the broad analysis is due to limited variation among clones. As a result, a single clone was chosen to represent each species of the *Cakile* lineage in all subsequent analyses. Only one *PHYA* clone of copy 1 was sequenced for the following taxa: *Cakile lanceolata* (Willd.) O. E. Schulz (population mc5), *Diplotaxis assurgens* (Delile) Gren., *Erucastrum gallicum*, and *Psychine stylosa* Desf. For *Eruca vesicaria*, only copy 2 was recovered, so this taxon was not included in any subsequent *PHYA* analysis.

For the single-clone analyses, 58 taxa were included. Because the intron was highly variable and difficult to align, this region was excluded from all analyses. The aligned length of exons was 1786 bp. An additional 36 bp were excluded from analyses due to ambiguous alignment. There were 480 parsimony-informative characters. The MP analyses resulted in seven trees of length 2645 (consistency index [CI] = 0.346, retention index [RI] = 0.544). Based on the AIC, the most appropriate model of evolution for these data is the GTR + I + Γ, which allows for an independent rate of substitution for all nucleotide pairs (GTR). To model among-site heterogeneity, some sites were allowed to be invariant (I), while the rest had rates drawn from a discrete approximation (the alpha parameter of the gamma shape distribution). The ML analysis resulted in a single tree with –ln L = 15 886.407. The ML tree is presented (Fig. 2) as MP, ML, and Bayesian analyses resulted in similar tree topologies (see Figs. 4 and 5 for the Bayesian consensus tree).

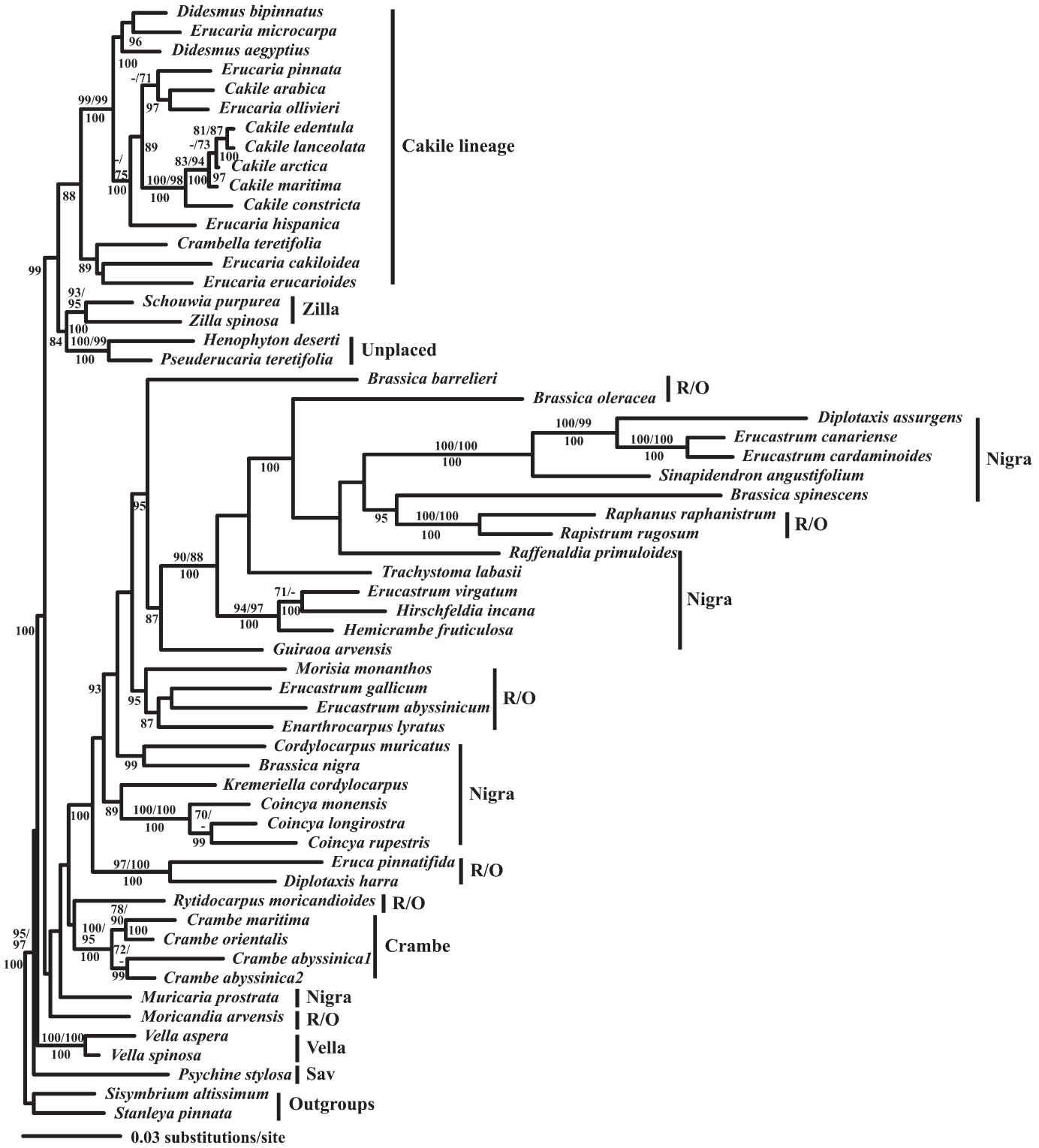

Fig. 2. Maximum likelihood tree of Brassiceae based on *PHYA* sequences. Bootstrap values are indicated above branches (maximum likelihood/maximum parsimony) and Bayesian posterior probabilities are below. Branch lengths are proportional to the number of changes. Lineages are indicated; R/O = Rapa/Oleracea lineage; Sav = *Savignya* lineage.

Whereas support values are high for some terminal clades, the backbone of the *PHYA* phylogeny is largely unsupported (Fig. 2). Of the six lineages for which more than one member was sampled, four are supported as monophyletic: (1) *Cakile* lineage (PP 88%) but with no bootstrap support for the inclusion of *Crambella teretifolia* (Batt. & Trab.) Maire, *Erucaria*

cakiloidea, and *E. erucarioides* (Coss. & Durieu) MüllBerol.; (2) *Zilla*, represented by *Zilla spinosa* (L.) Prantl. and *Schouwia purpurea* (BS 93% ML, 95% MP; PP 100%); (3) *Crambe,* represented by *Crambe abyssinica*, *C. maritima* L., and *C. orientalis* L. (BS 100% ML, 95% MP; PP 100%); and (4) *Vella*, represented by *Vella aspera* Pers. and *V. spinosa* Boiss. (BS 100% ML, 100% MP; PP 100%). Neither the Rapa/Oleracea nor the Nigra lineage is monophyletic (Fig. 2). For genera for which more than one species was sampled, only *Coincya* Rouy, *Crambe*, and *Vella* L. are monophyletic. Within the *Cakile* clade, the monophyly of all *Cakile* species excluding *C. arabica* is strongly supported (BS 100% ML, 98% MP; PP 100%). *Erucaria* and *Didesmus* are not monophyletic.

***matK*—**The aligned length of the *matK* data set from 60 taxa is 1860 bp, of which 341 bp are non-coding. Similar results were obtained when the non-coding region was included (not shown) or excluded in MP analyses. In the coding region, there were 190 parsimony informative characters. The MP analyses resulted in 8474 trees of length 622 from two islands (CI = 0.746; RI = 0.813). The best model of sequence evolution based on AIC was also GTR + I + Γ. The ML analysis resulted in a single tree with –ln L = 6182.4256. The ML tree is shown (Fig. 3) because MP, ML, and Bayesian analyses resulted in similar topologies (see Figs. 4 and 5 for the Bayesian consensus tree).

The cpDNA phylogeny shows greater resolution at deeper nodes than the nuclear topology (Fig. 3). There is strong support for the *Zilla* lineage as sister to all remaining members of the Brassiceae (BS 99% ML, 96% MP; PP 100%). A clade comprising members of the *Cakile*, *Crambe*, Nigra, Rapa/Oleracea, and unplaced genera is moderately supported (BS 89% ML; PP 100%). Four lineages are strongly supported in these analyses: (1) *Cakile* (BS 99% ML, 93% MP; PP 100%); (2) *Crambe* lineage (BS 97%; PP 100%); (3) *Vella* lineage (BS 100%; PP 100%); and (4) *Zilla* lineage (BS 100% ML, 99% MP; PP 100%). There is moderate support (BS 78% ML, 71% MP; PP 100%) for the Rapa/Oleracea lineage. However, the Nigra lineage is not resolved as monophyletic with these data because *Coincya* is part of an unresolved polytomy and *Muricaria prostrata* (Desf.) Desv. is sister to *Crambe*.

Ancestral character reconstruction*—Evolution of heteroarthrocarpy*—In both *PHYA* and *matK* topologies, ML and equal weight MP reconstructions resulted in similar patterns in the evolution of heteroarthrocarpy (only ML shown, Fig. 4). These reconstructions indicate that non-heteroarthrocarpic fruits are ancestral for the tribe with multiple transitions to heteroarthrocarpic fruits. In the *PHYA* topology (Fig. 4A), heteroarthrocarpy evolved at least two times independently: (1) in the *Cakile* lineage with no reversals to non-heteroarthrocarpy and (2) in the clade inclusive of *Brassica barrelieri* (L.) Janka and *Eruca pinnatifida* (Desf.) Pomel (PP 100%) with possibly three to five reversals to non-heteroarthrocarpy. Similar trends are seen in *matK* reconstructions, with heteroarthrocarpy ancestral for the *Cakile* and *Crambe* lineages as well as derived clades within the Nigra and Rapa/Oleracea lineages (e.g., *Coincya*; Fig. 4B). In contrast to equal-weight reconstructions, when the gain of heteroarthrocarpy is weighted 10 times greater than its loss in MP reconstructions, heteroarthrocarpy is the ancestral state in both *PHYA* and *matK* topologies (data not shown). A single origin of heteroarthrocarpy requires at least 10 (*PHYA*) to 14 (*matK*) losses across the Brassiceae with current sampling.

Evolution of dehiscence and disarticulation—Evolutionary patterns of dehiscence and disarticulation are ambiguous based on reconstructions on both *PHYA* and *matK* topologies. Reconstructions of dehiscence on both trees result in many equivocal ancestral states, obscuring clear patterns across the tribe (Fig. 5). In the *PHYA* topology, full indehiscence is the most probable ancestral state for the *Crambe* lineage and the *Cakile* lineage, excluding *Crambella*, *Erucaria cakiloidea*, and *E. erucarioides*. Based on *matK*, full indehiscence also arose within *Cakile* lineage, but the *Crambe* lineage is ambiguous. Partial indehiscence is ancestral for *Coincya*. Depending on the topology, there is a second clade with a partially indehiscent ancestor: *Erucastrum virgatum* C. Presl to *Hemicrambe fruticulosa* Webb for *PHYA* or *Erucastrum elatum* (Ball) O. E. Schulz to *H. fruticulosa* for *matK*. Completely indehiscent and partially dehiscent taxa are scattered throughout both phylogenies contributing to ambiguous ancestral states. If the loss of dehiscence is weighted lower than the gain of dehiscence (1 : 10), then reconstructions on either topology result in many losses of dehiscence. However, the precise number of losses is difficult to determine due to many equivocal nodes (data not shown). With both *matK* and *PHYA* topologies, most branches with potential transitions between fruit disarticulation and no-disarticulation are equivocal with equal weighting (Appendix S7). However, within the *Cakile* clade, there are two independent shifts from disarticulation to no-disarticulation regardless of the method of reconstruction: *Erucaria erucarioides* and *E. hispanica*.

DISCUSSION

This study represents the first explicit phylogenetic evaluation of heteroarthrocarpy and its variants across the Brassiceae. Heteroarthrocarpy is a notable example of seed heteromorphism, a trait observed across angiosperms in which seeds produced by an individual plant have different dispersal capabilities (Imbert, 2002). Although chloroplast and nuclear data produce substantially different topologies, the following conclusions can be made: (1) the *Cakile*, *Crambe*, *Vella*, and *Zilla* lineages are monophyletic; (2) heteroarthrocarpy has been gained and lost many times across the tribe; and (3) changes in dehiscence capabilities and fruit disarticulation frequently occur and are best evident in the *Cakile* lineage.

***Disagreement between chloroplast and nuclear data sets*—**Although the nt and cpDNA trees share a number of clades, considerable topological incongruence is present (Figs. 2, 3; Appendix S4). Differences in topologies are observed at both deep (e.g., Nigra and Rapa/Oleracea lineages) and shallow (e.g., within the *Cakile* lineage) nodes. Some of the incongruence observed between the *PHYA* and *matK* topologies likely reflects either the inability of data to resolve some relationships or analytical issues. In contrast, other differences may reflect biological processes such as hybridization or incomplete lineage sorting (Wendel and Doyle, 1998).

Hybridization may explain incongruence of the deeper nodes in the Brassiceae phylogenies presented here, although this process is usually more relevant for species level inquiries. Importantly, comparative genomic studies indicate that most members of the Brassiceae are likely paleopolyploids with a hexaploid ancestor (Lysak et al., 2005, 2007; Parkin et al., 2005). In addition, hybridization is documented among *Brassica* L., *Diplotaxis*

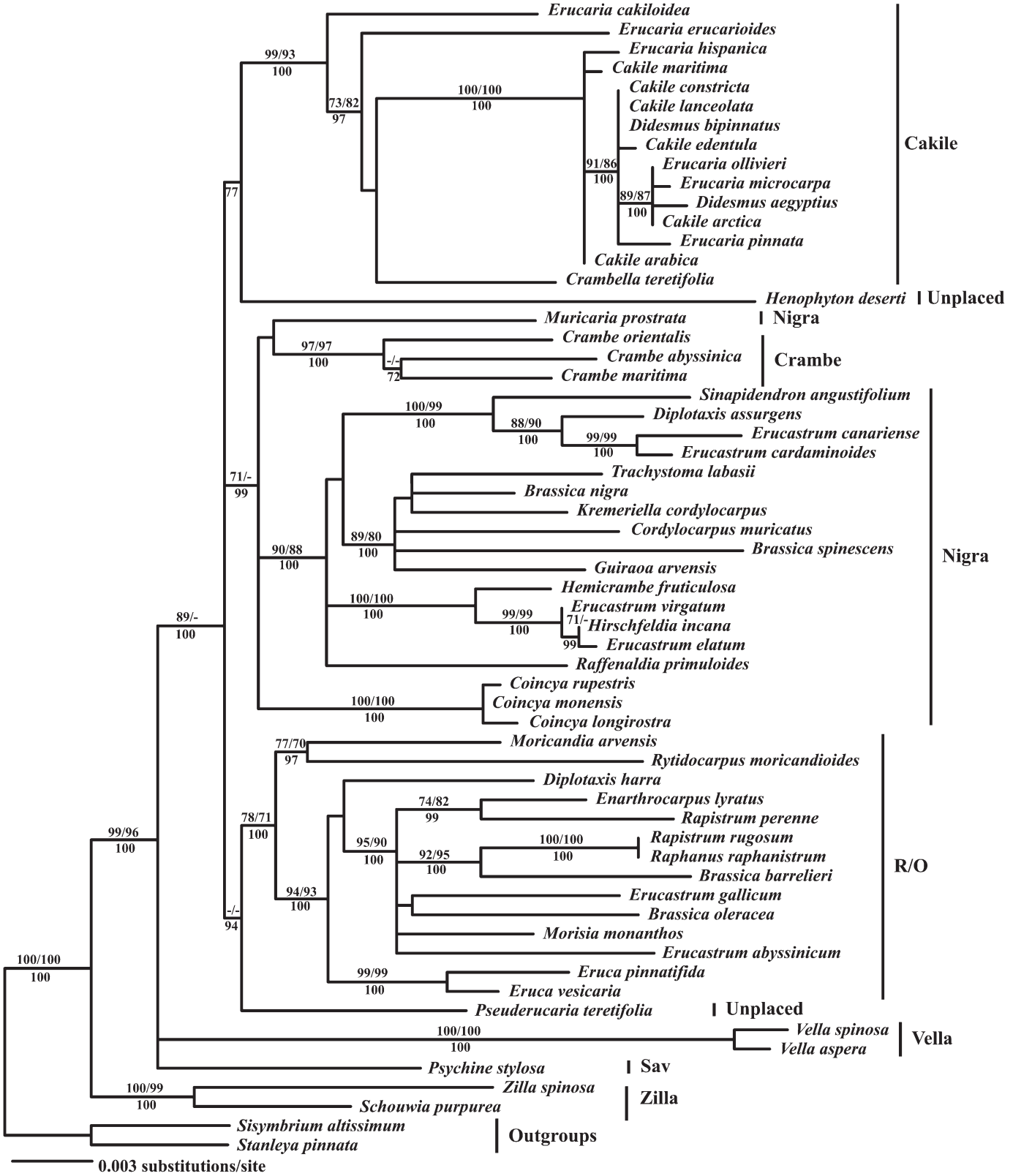

Fig. 3. Maximum likelihood tree of Brassiceae based on *matK* sequences. Bootstrap values are indicated above branches (maximum likelihood/maximum parsimony) and Bayesian posterior probabilities are below. Branch lengths are proportional to the number of changes. Lineages are indicated; R/O = Rapa/Oleracea lineage; Sav = *Savignya* lineage.

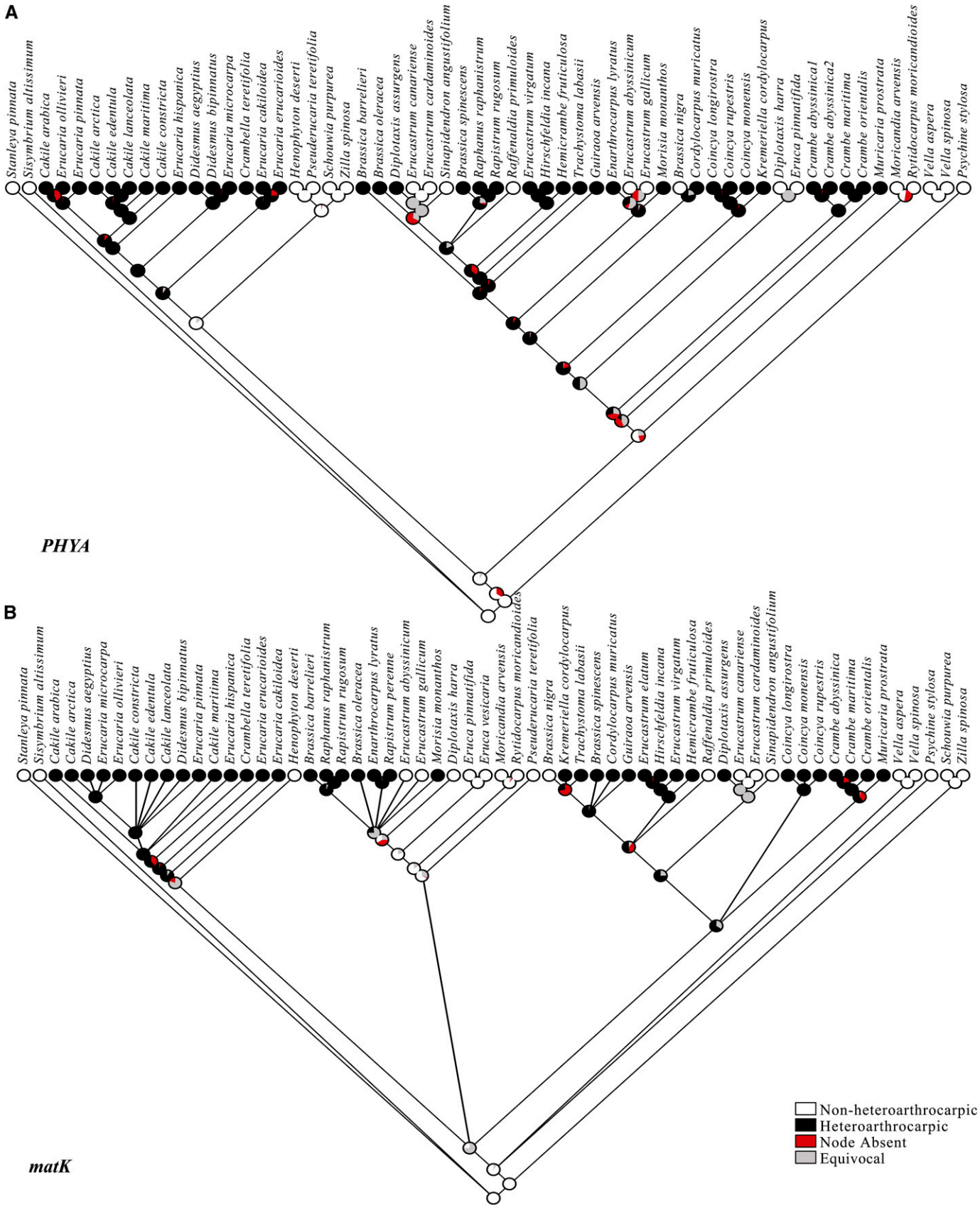

Fig. 4. Evolution of heteroarthrocarpy across the majority rule Bayesian consensus tree based on (A) *PHYA* and (B) *matK* sequences. Pie charts represent reconstruction results on 1000 Bayesian trees and show the proportion of maximum likelihood reconstructions in which a given ancestral state was significant.

DC., and *Erucastrum* C. Presl (reviewed in Warwick et al., 2009). Furthermore, *Brassica carinata* A. Braun, *B. juncea* (L.) Czern., *B. napus* L., *Diplotaxis muralis* (L.) DC., *Erucastrum elatum*, and *E. gallicum* are all known polyploids (Warwick and Black, 1993; Warwick and Anderson, 1997a, b; Warwick et al., 2009). These data imply that hybridization events have been prominent in the evolution of Brassiceae.

Incongruence observed within the *Cakile* lineage may be due to incomplete lineage sorting, hybridization, or a combination of these two processes. Although incongruence can also be due to sampling or analytical error, taxon sampling is likely not involved here. *Cakile* is likely a young genus whose evolution and speciation was influenced by recent glaciation (Rodman, 1974). Thus, discrepancies between the chloroplast and nuclear genome may be due to the retention of ancient polymorphisms. However, hybridization may be a culprit in this lineage as well. Artificial crosses among all species of *Cakile* are successful (Rodman, 1974), data that are supported by intermediate natural populations in the eastern United States (Rodman, 1974) and Australia (Cody and Cody, 2004). Successful crosses were reported among species of *Erucaria* (Harberd, 1972). Furthermore, artificial crosses between *Cakile* and *Erucaria*, *Cakile* and *Didesmus*, and *Didesmus* and *Erucaria* have been successful (C. Willis, unpublished data; S. I. Warwick and L. D. Black, unpublished data). Additional data are needed to discern among these processes and resolve species relationships.

Phylogeny of Brassiceae—In agreement with previous analyses, both nuclear and chloroplast analyses presented here support the monophyly of the *Cakile* lineage (Warwick and Black, 1997; Warwick and Sauder, 2005), the *Crambe* lineage (Francisco-Ortega et al., 1999; Warwick and Sauder, 2005), the *Vella* lineage (Warwick and Black, 1994; Crespo et al., 2000; Warwick and Sauder, 2005), and the *Zilla* lineage (Warwick and Black, 1994; Warwick and Sauder, 2005). Chloroplast-based phylogenies presented here (Fig. 3) and elsewhere (Warwick and Black, 1991, 1993, 1997) suggest the Rapa/Oleracea lineage is monophyletic in contrast to nuclear analyses (Fig. 2; Warwick and Sauder, 2005). A similar pattern was observed with the Nigra lineage (Warwick and Black, 1991, 1997; Warwick and Sauder, 2005), although the chloroplast data presented here do not support the monophyly of this lineage (Fig. 3).

The *matK* data set provided better resolution along the backbone of the Brassiceae tree than *PHYA*, although relationships among some lineages remain unclear. Chloroplast data strongly support the *Zilla* lineage as sister to all other members of the tribe (Fig. 3). This represents a novel relationship uncovered by these data, which should be confirmed with additional data sets. Analyses of the nuclear data place the *Savignya* lineage as sister to all Brassiceae, with the *Zilla* lineage as the next diverging lineage. However, there is no support for these short branches (Fig. 2). The cpDNA topology indicates the *Cakile*, *Crambe*, Nigra, Rapa/Oleracea lineages and unplaced genera are closely related, another relationship contradicted by the *PHYA* data. As observed with the nuclear phylogeny, previous analyses were not able to resolve deep relationships within the tribe (Warwick and Sauder, 2005). The lack of resolution along the backbone suggests that markers chosen to date do not have appropriate variation or the tribe has recently radiated.

Relationships within the Cakilinae—Based on analyses presented here and elsewhere (Warwick and Black, 1997), the *Cakile* lineage includes *Cakile*, *Crambella*, *Didesmus*, and *Erucaria* (Figs. 2, 3). Although *matK* data and chloroplast restriction data (Warwick and Black, 1997) strongly support the monophyly of these genera combined (Fig. 3), *PHYA* data do not (Fig. 2). Both cp and ntDNA support a core group of taxa: *Cakile*, *Didesmus*, *Erucaria microcarpa* Boiss., *E. ollivieri* Maire, and *E. hispanica*. The inclusion of *Crambella teretifolia*, *E. cakiloidea*, and *E. erucarioides* is only indicated by cpDNA (Figs. 2, 3). Although Schulz (1936) included only *Cakile* and *Erucaria* in the subtribe Cakilinae, Warwick and Black (1997) extended the tribe to include *Crambella* and *Didesmus* noting similarities in habit, habitat, and flower color.

Within the *Cakile* lineage, no genus is supported as monophyletic based on either *matK* or *PHYA* data, not considering the monotypic *Crambella*. Nuclear data strongly support the monophyly of *Cakile* excluding *C. arabica* (Fig. 2). This relationship is noteworthy as Rodman (1974) tentatively suggested *C. arabica* might be the ancestral species in the genus based on the observation that *C. arabica* is the only species distributed in inland deserts. All other species in *Cakile* are strand plants distributed along beaches. The placement of *C. arabica* in the *matK* topology is less clear as this taxon is part of a polytomy. Moreover, these data strongly contradict the monophyly of *Cakile* (Fig. 3). Both *PHYA* and *matK* analyses indicate that *Erucaria* and *Didesmus* are also paraphyletic (Figs. 2, 3). However, subsuming *Didesmus* into *Erucaria* as suggested by Appel and Al-Shehbaz (2003) would still not result in a monophyletic *Erucaria*. The reinstatement of *Reboudia*, currently a synonym of *Erucaria* including *E. erucarioides*, *E. microcarpa*, and *E. pinnata* (Viv.) Täckh. & Boulos, is also not a tenable taxonomic solution because these taxa do not represent a monophyletic group (Figs. 2, 3). More detailed phylogenetic studies with different markers are needed to ascertain species relationships and generic boundaries within the *Cakile* lineage.

Multiple origins of heteroarthrocarpy in Brassiceae—Unweighted parsimony and ML reconstructions assign non-heteroarthrocarpic fruits as ancestral for the tribe. This ancestral state leads to multiple origins of heteroarthrocarpy and several reversals to non-heteroarthrocarpic fruits across the tribe (Fig. 4), although the incorporation of phylogenetic uncertainty precludes a precise assessment of the number of changes. This pattern is consistent regardless of which phylogenetic hypothesis was considered. Constraining all heteroarthrocarpic taxa as monophyletic was rejected by both data sets according to the SH test ($P < 0.001$; Appendix S4). Given the complexities of heteroarthrocarpy, we explored alternative weightings in parsimony reconstructions. To achieve an ancestral state of heteroarthrocarpy for the tribe, its gain was weighted 10 : 1 to loss. This reconstruction also results in lability because a high number of losses were invoked to explain character distribution across either *PHYA* or *matK* topologies. The combination of these results contradicts Gómez-Campo's (1980, p. 29) prediction that this fruit type is a "notable phylogenetic achievement", that probably arose only once in the family.

To examine the feasibility of repeated evolutionary changes between heteroarthrocarpy and non-heteroarthrocarpy, we must first consider their developmental bases. Studies conducted on the heteroarthrocarpic species *Cakile lanceolata* and *Erucaria erucarioides* provide a developmental explanation of this unusual fruit type and variants (Hall et al., 2006), although data on additional species are clearly needed. Whereas the ovary, as defined by the presence of replum and ovules, is homologous between the heteroarthrocarpic and non-heteroarthrocarpic fruit

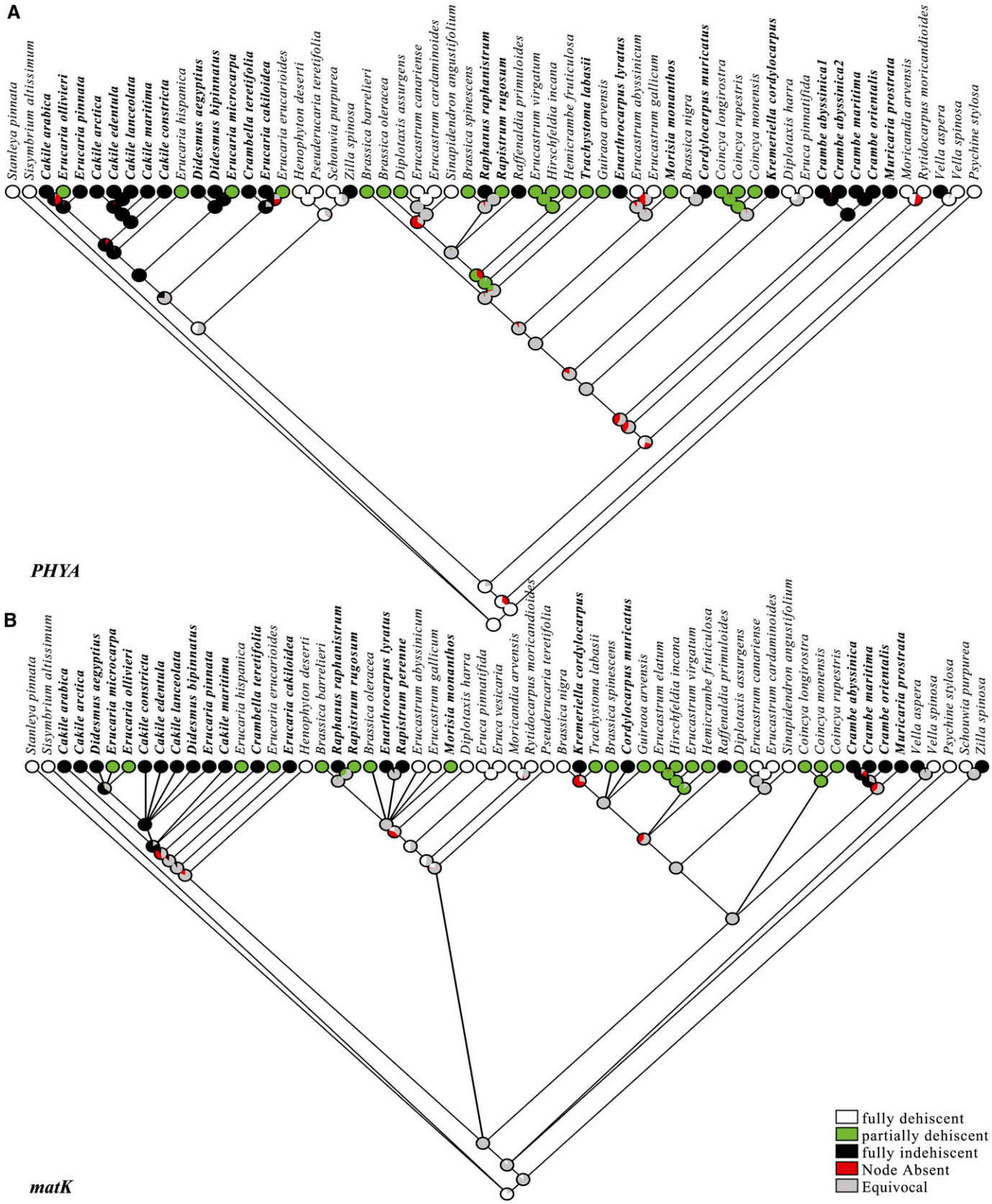

Fig. 5. Evolution of dehiscence across the majority rule Bayesian consensus tree based on (A) *PHYA* and (B) *matK* sequences. Pie charts represent reconstruction results on 1000 Bayesian trees and show the proportion of maximum likelihood reconstructions in which a given ancestral state was significant. Boldface names represent taxa with heteroarthrocarpic fruits with disarticulation of the joint.

types, the valves are not. In non-heteroarthrocarpic fruits, the valve tissue is equivalent to the entire ovary wall, but in heteroarthrocarpic fruit, only the ovary wall of the proximal segment corresponds to valves of a typical silique, regardless of whether the valves dehisce at maturity (Hall et al., 2006). Distal segments comprise both ovary and stylar components, and the ovary wall in this segment does not differentiate into valvular tissue. In other words, while the valve encompasses the entire ovary wall in non-heteroarthrocarpic fruits, it has been reduced to only a portion of the ovary wall in heteroarthrocarpic fruits. The joint is a complex structure that combines the distal extent of the valve margin with internal proliferation of mesocarp tissue. Disarticulation entails further modification of this structure to form an abscission zone that transversely bisects the entire fruit. Thus, the evolution of heteroarthrocarpy involves rearrangement of valves relative to the replum, accompanied by other morphological changes including basal-apical patterning and reduction in the number of seeds (Hall et al., 2006). The finding of multiple gains of heteroarthrocarpy in the Brassiceae presented here represents convergence in the sense that the same phenotype has arisen more than once (Scotland, 2011). This pattern provides the opportunity to determine whether the independent origins of heteroarthrocarpy have the same genetic basis and, thus, represents a true parallelism (sensu Scotland 2011).

Is it realistic for heteroarthrocarpy to have been gained and/or lost multiple times given the many morphological differences between heteroarthrocarpic and typical siliques? It is possible that the valve-margin genetic pathway, which is well characterized in *Arabidopsis* (Dinneny and Yanofsky, 2004; Dinneny et al., 2005), has been recruited and/or modified within the Brassiceae. This hypothesis is testable using developmental genetic studies that address whether different origins of heteroarthrocarpy are the result of convergence or parallelism. However, distinguishing between these phenomena does not explain why this unusual fruit type is only present within the Brassiceae or why multiple origins of heteroarthrocarpy in the tribe are likely. One possibility is that an evolutionary innovation permitting the disassociation of valve and replum tissue occurred specifically in this lineage. That is, the ability to modify or reposition the valve–replum boundary may be a "developmental enabler" (Donoghue, 2005) in the Brassiceae that allowed for repeated changes in fruit morphology. For instance, genome duplication may have facilitated genetic dissociation within the fruit developmental program, thereby allowing greater flexibility.

Another important aspect is the ecological impacts of the morphology. Dimorphic fruits convey different dispersal capabilities in proximal vs. distal segments of *Cakile* (Payne and Maun, 1981; Maun and Payne, 1989) and may represent a bet-hedging strategy (Imbert, 2002). Not only are the seeds different sizes between the two segments (Rodman, 1974; Maun and Payne, 1989), but the distal and proximal segments are adapted to long- and short-distance dispersal, respectively (Rodman, 1974; Payne and Maun, 1981). For instance, *Cakile* has a corky pericarp, a unique feature in the tribe, which facilitates water dispersal. Specifically, the distal segment performs better than the proximal in water dispersal. Other factors relating to the potential selective advantage of heteroarthrocarpy, such as the protection of seeds, and the biogeographic context of environments where heteroarthrocarpy is present, must also be evaluated to thoroughly examine why a particular trait has evolved multiple times within a clade (Wiens et al., 2006).

Evolutionary lability in types of heteroarthrocarpy—The evolution of heteroarthrocarpy entails altering the position of the valve margin to be associated only with the basal portion of the ovary, loss of dehiscence, and disarticulation. Ancestral state reconstructions were unable to determine whether disarticulation precedes or follows loss of dehiscence. Regardless, variation in types among closely related taxa is the rule. For example, within the Nigra (excluding *Coincya* and *Muricaria prostrata*) and Rapa/Oleracea lineages of the *matK* topology and the *Brassica oleracea* L. to *Hemicrambe fruticulosa* of the *PHYA* topology, most possible fruit morphologies are present (non-heteroarthrocarpic, fully dehiscent, partially dehiscent, disarticulation, and no disarticulation). The *Cakile* lineage represents an important example since all members are heteroarthrocarpic, and it likely represents an independent origin based on unweighted parsimony and ML reconstructions (Fig. 4). Thus, key differences in dehiscence and disarticulation are recent modifications in types of heteroarthrocarpy. Most members of the lineage have completely indehiscent fruits that disarticulate, although there is lability in proximal segment dehiscence and disarticulation. Two taxa, *Erucaria erucarioides* and *E. hispanica*, have fruits that do not undergo disarticulation, whereas four taxa (*E. erucarioides*, *E. hispanica*, *E. microcarpa*, and *E. ollivieri*) have dehiscent proximal segments. Trait reconstructions imply these variations in morphology are independent. While constraining all taxa with dehiscent proximal segments as monophyletic is rejected by both *matK* and *PHYA* ($P = 0.03$), the monophyly of taxa without disarticulation is not ($P = 0.16$ *matK*; $P = 0.27$ *PHYA*). These patterns indicate that loss and gain of these characteristics is more frequent than may have been predicted a priori.

There are two hypotheses to explain patterns of the evolution implied by the reconstructions. We might first consider that the reconstructions do not accurately reflect evolutionary history (Wiens et al., 2007). Although unweighted parsimony and ML reconstructions suggest that the transition from indehiscence to dehiscence is more likely, other genetic evidence implies losing dehiscence is easier than gaining it. Using *Arabidopsis* as an example, loss of function in any of the following genes results in indehiscent fruits: *SHATTERPROOF1* and *SHATTERPROOF2*, *ALCATRAZ,* or *INDEHISCENT* (reviewed in Ferrandiz, 2002; Dinneny and Yanofsky, 2004; Dinneny et al., 2005). It should be noted that overexpression of *FRUITFULL* also results in loss of dehiscence in *Arabidopsis* (Ferrandiz et al., 2000) and *Brassica juncea* (Ostergaard et al., 2006). It has been argued previously that the apparently simple genetic regulation of fruit dehiscence would likely result in the independent loss of dehiscence in taxa across Brassicaceae (Koch et al., 2003). Even though the argument can be made that loss of a trait is easier to achieve than a gain, this does not mean that the frequencies of evolutionary transitions must reflect this (Donoghue et al., 1998). Thus the alternative hypothesis is that unweighted parsimony and ML reconstructions reflect evolutionary history. Repeated gains of dehiscence in the *Cakile* lineage, for example, suggest that the genetic pathway is maintained through its use in other developmental pathways. Both dehiscence and disarticulation involve the formation of an abscission zone, specifically by contrasting nonlignified and lignified cells (Hall et al., 2006). Furthermore, there are no heteroarthrocarpic taxa that have completely indehiscent fruits without disarticulation, which indicates that some kind of abscission zone is consistently formed across all variants of heteroarthrocarpy. Thus, lability between types of heteroarthrocarpy may reflect differential

expression of otherwise conserved genetic pathways in the fruit. A key test of this hypothesis would be to compare the genes involved in the formation of the abscission zone(s) involved in valve dehiscence to those involved in disarticulation. This model predicts that such pathways can be turned on and off in different positions relatively easily, which may represent the developmental basis for the high degree of variation observed within the tribe.

***Conclusions*—**Heteroarthrocarpy is one of many examples in which fruits have been modified such that seeds from the same plant may be dispersed differently, although these phenomena of seed and fruit heteromorphisms appear to be restricted to a limited number of lineages (Imbert, 2002). For example, Asteraceae, Amaranthaceae, and Fabaceae have genera that exhibit different fruit morphologies on the same plant (reviewed in Imbert, 2002), and dispersal heteromorphism is common among cleistogamous taxa (reviewed in Culley and Klooster, 2007). Furthermore, the repeated gain and loss of segmentation is also observed with the loment fruit type in the Fabaceae (Lavin et al., 2001). Although lomented fruits are not heteromorphic, per se, the parallel between lomented legumes and heteroarthrocarpic silique reveals that other lineages have a propensity for repeated evolution of a complex fruit morphology. More generally, independent origins of complex transitions in dispersal mode are known across the angiosperms. For example, berries have evolved independently across angiosperms (Lorts et al., 2008) as well as within families (Clausing et al., 2000; Knapp, 2002).

After previous molecular-based phylogenies revealed the extreme homoplasy of fruit characteristics in Brassicaceae, explicit examination of fruit evolution was avoided or, when incorporated, used to highlight discordance between fruit morphology and phylogeny. Although the distribution of heteroarthrocarpy is not helpful for discerning absolute relationships within Brassiceae, investigation of the evolution of heteroarthrocarpy and its variants across the tribe has been informative. Heteroarthrocarpy has been gained and lost multiple times, a pattern that reflects the repeated origin and loss of a complex fruit morphology. Moreover, this lability is extended to variants of heteroarthrocarpy. The apparent flexibility in dehiscence and disarticulation suggests that the pathway for generating an abscission zone can be differentially expressed in these taxa. Our results also indicate that investigations of the genetic pathways involved in dehiscence and disarticulation could shed light on the genetic basis of this evolutionary lability.

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APPENDIX 1. Taxon sampling, GenBank accessions and voucher information for the present study. All vouchers from greenhouse grown material were deposited at the Harvard University Herbaria (HUH). Underlined *PHYA* GenBank accession numbers represent the sequences used in the single-clone analyses.

Taxon; GenBank accession(s): *PHYA*; *matK*; Seed stock # or population designation, Collection locale of seed stock.

Brassica barrelieri (L.) Janka; JN584725–JN584726; JN584950; 1160-67, S. Guadarrama, C. Spain. ***Brassica nigra*** (L.) W.D.J. Koch; JN584727–JN584729; JN584951; 0049-67, Vejer de la Frontera, Cádiz, S. Spain. ***Brassica oleracea*** L. var. *acephala* DC.; JN584730–JN584734; JN584952; 2861-77, Origin unknown. ***Brassica spinescens*** Pomel; JN584735–JN584738; JN584953; 1800-70, Cap Falcon, Oran, Algeria.

Cakile arabica Velen. & Bornm.; JN584739–JN584742; JN584954; North Summan 5918. ***Cakile arctica*** Pobed.; JN584743–JN584745; JN584955; BORG 8A. ***Cakile constricta*** Rodman; JN584746–JN584750; TX27. ***Cakile constricta***; JN584751–JN584754; JN584956; BS1. ***Cakile edentula*** (Bigelow) Hook. subsp. *edentula*; JN584755–JN584758; MB9. ***Cakile edentula*** subsp. *edentula*; JN584759–JN584761; JN584957; KIT1r. ***Cakile edentula*** subsp. *harperi* (Small) Rodman; JN584762–JN584764; TI8. ***Cakile lanceolata*** (Willd.) O.E.Schulz; JN584765; MC5. ***Cakile lanceolata*** subsp. *fusiformis* (Greene) Rodman; JN584766–JN584768; JN584958; GK1. ***Cakile lanceolata*** subsp. *lanceolata*; JN584769–JN584772; BPK2. ***Cakile maritima*** Scop.; JN584773–JN584776; CAR21. ***Cakile maritima***; JN584772–JN584780; JN584959; 1396-67, coastal sands of Pontevedra, NW Spain.

Coincya longirostra (Boiss.) Greuter & Burdet; JN584781–JN584783; JN584960; 1175-67, acid rocks, Despeñaperros, N. Jaén, C.Spain. ***Coincya monensis*** (L.) Greuter & Burdet; JN584784–JN584787; JN584961; 4429-75, sand dunes near Wallasey, U.K. ***Coincya rupestris*** Porta & Rigo ex Rouy.; JN584788–JN584789; JN584962; 1577-68, calc. rocks, Alcaraz, Albacete, S.E Spain. ***Cordylocarpus muricatus*** Desf.; JN584790–JN584792; JN584963; 1137-68, roadsides, Beni Snassen Mts., N.E. Morocco. ***Crambe abyssinica*** Hochst. ex R.E. Fr.; JN584793–JN584797; JN584964; 1397-67, Inst. Nac. Invest. Agrarias, Madrid, Spain. ***Crambe maritima*** L.; JN584798–JN584800; JN584965; 0510-67, B.G. Strasbourg, France. ***Crambe orientalis*** L.; JN584801–JN584804; JN584966; 3696-75, E. Tehran, Iran. ***Crambella teretifolia*** (Batt. & Trab.) Maire; JN584805–JN584808; JN584967; 1971-71, dry pastures near Taourirt, N.E. Morocco.

Didesmus aegyptius (L.) Desv.; JN584809–JN584811; JN584968; 7320-86, Athalassas, near Nicosia, Cyprus. ***Didesmus bipinnatus*** (Desf.) DC.; JN584812–JN584815; JN584969; 1853-70, between Biskra and Bou Saada, Algeria. ***Diplotaxis assurgens*** (Delile) Gren.; JN584816; JN584970; 1120-67, Beni Mellal, E. Marrakech, Morocco. ***Diplotaxis harra*** (Forssk.) Boiss.; JN584817–JN584818; JN584971; 1831-70, arid slopes, M'Chedallah, Alger, Algeria.

Enarthrocarpus lyratus (Forssk.) DC.; JN584819–JN584822; JN584972; 1206-68, B.G.Madrid, Spain. ***Eruca pinnatifida*** (Desf.) Pomel; JN584823–JN584824; JN584973; 1471-68, Ksabi, near Midelt, C. Morocco. ***Eruca vesicaria*** (L.) Cav.; JN584825–JN584831; JN584974; 3750-77. ***Erucaria cakiloidea*** (DC.) O.E. Schulz; JN584832–JN584837; JN584975; 3738-75, Quasr-el-Shirin, W. Iran. ***Erucaria erucarioides*** (Coss. & Durieu) MüllBerol.; JN584838–JN584843; JN584976; 1944-71, arid stony plains near Béchar, W. Algeria. ***Erucaria hispanica*** (L.) Druce; JN584844–JN584847; JN584977; 2055-72, supplied by J. Harberd (Univ. of Leeds, U. Kingdom). ***Erucaria microcarpa*** Boiss.; JN584848–JN584850; JN584978; 4620-77, Sands near Lejandria, Egypt. ***Erucaria ollivieri*** Maire; JN584851–JN584853; JN584979; 2983-74, arid plains, Zreouila, S. Agadir, Morocco. ***Erucaria pinnata*** (Viv.) Täckh. & Boulos; JN584854–JN584857; JN584980; 1851-70, near Biskra city, Algeria. ***Erucastrum abyssinicum*** (A. Rich.) O.E. Schulz; JN584858–JN584861; JN584981; 0430-67, B.G. Copenhagen Denmark. ***Erucastrum canariense*** Webb & Berthel.; JN584862–JN584865; JN584982; 5305-79, Lanzarote Isl., Canary, Spain. ***Erucastrum cardaminoides*** (Webb ex H. Christ) O.E. Schulz; JN584866–JN584868; JN584983;1070-68, Buenavista, N.W. Tenerife Island Spain. ***Erucastrum elatum*** (Ball) O.E. Schulz; JN584984; 4127-76. ***Erucastrum gallicum*** (Willd.) O.E. Schulz; JN584869–JN584871; JN584985; 1209-69, B.G. Leipzig, E. Germany. ***Erucastrum virgatum*** C.Presl subsp. *baeticum* (Boiss.) Gómez-Campo; JN584872–JN584875; JN584986; 5364-79, Monda, Málaga., S. Spain.

Guiraoa arvensis Coss.; JN584876–JN584878; JN584987; 1550-68, road embank., Campello, Alicante, S.E Spain.

Hemicrambe fruticulosa Webb; JN584879–JN584880; JN584988; 2232-73, cliffs, Jbel Moussa, Ceuta, N. Morocco. ***Henophyton deserti*** (Coss. & Durieu) Coss. & Durieu; JN584881–JN584884; JN584989; 1945-71, roadsides, bet. Ouargla and Gardaia, Algeria. ***Hirschfeldia incana*** (L.) Lagr.-Foss.; JN584885–JN584890; JN584990; 2024-71, cult. fields near Béjar, W. Spain.

Kremeriella cordylocarpus (Coss. & Durieu) Maire; JN584891–JN584895; JN584991; 1142-67, Beni Snassen Mts., N.E. Morocco.

Moricandia arvensis (L.) DC.; JN584896–JN584897; JN584992; 0863-66, near Jumilla, Murcia, SE Spain. ***Morisia monanthos*** (Viv.) Asch.; JN584898–JN584904; JN584993; 3816-75, roadsides near Sasari, Sardinia, Italy. ***Muricaria prostrata*** (Desf.) Desv.; JN584905–JN584906; JN584994; 1855-70, between Djelfa and Bou Saada, Algeria.

Pseuderucaria teretifolia (Desf.) O.E.Schulz; JN584907–JN584910; JN584995; 1844-70, arid fields near Biskra, Algeria. ***Psychine stylosa*** Desf.; JN584911; JN584996; 1458-68, abandoned fields near Msoum, Morocco.

Raffenaldia primuloides Godr.; JN584912–JN584914; JN584997; 4386-76,Col de Tamrhemt, High Atlas, Morocco. ***Raphanus raphanistrum*** L.; JN584915–JN584917; JN584998; 1509-68, coastal dunes near Sanúcar, Cádiz, S. Spain. ***Rapistrum perenne*** (L.) All.; JN584999; 1404-68, B.G. Cluj, Romania. ***Rapistrum rugosum*** (L.) All.; JN584918–JN584920; JN585000; 1527-68, as a weed, Alcalá Henares, Madrid, C. Spain. ***Rytidocarpus moricandioides*** Coss.; JN584921–JN584924; JN585001; 0708-67, B.G. Parfs, France.

Schouwia purpurea (Forssk.) Schweinf.; JN584925–JN584927; JN585002; 5780-81, from Wadi Kharit, Egypt. ***Sinapidendron angustifolium*** (DC.) Lowe; JN584928–JN584929; JN585003; 3620-75, litoral cliffs. Cámara de lobos, Madeira, Portugal. ***Sisymbrium altissimum*** L.; JN584930–JN584932; JN585004; 1724-69, Oakzanitas, near San Diego, California, USA. ***Stanleya pinnata*** (Pursh) Britton; JN584933–JN584935; JN585005; 1735-69, seleniferous soils, Joshua Tree desert, Calif., USA.

Trachystoma labasii Maire; JN584936–JN584938; JN585006; 3014-74, near Beni Mellal, C. Morocco.

Vella aspera Pers.; JN584939–JN584941; JN585007; 1587-68, near Caspe, Zaragoza, Spain. ***Vella spinosa*** Boiss.; JN584942–JN584945; JN585008; 2007-71, S. Nevada, S. Spain.

Zilla spinosa (L.) Prantl.; JN584946–JN584949; JN585009; 0731-67, plains near Tamanrasset, Hoggar Massif, S. Algeria.