American Journal of Botany 93(5): 747–761. 2006.

THE EVOLUTIONARY HISTORY OF *SIUM* SENSU LATO (APIACEAE): DISPERSAL, VICARIANCE, AND DOMESTICATION AS INFERRED FROM ITS rDNA PHYLOGENY[1]

KRZYSZTOF SPALIK[2,4] AND STEPHEN R. DOWNIE[3]

[2]Department of Plant Systematics and Geography, Warsaw University, Aleje Ujazdowskie 4, 00-478 Warszawa, Poland; and
[3]Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA

The biogeographic history of *Sium* sensu lato (s.l.) (including *Sium* sensu stricto [s.s.], *Afrocarum*, and *Berula*) was inferred using a nuclear rDNA ITS sequence phylogeny and dispersal–vicariance analysis. One hundred accessions were analyzed, including 86 specimens of *Sium* s.l., representing all taxa throughout their geographical range. The phylogenetic analyses showed that *Afrocarum* and the African and St. Helena species of *Sium* were nested within *Berula* forming the *Berula* s.l. clade. The remaining species of *Sium* constituted a weakly supported *Sium* s.s. clade. The cultivated *S. sisarum* is closely related to the western Asian *S. sisaroideum*. The divergence of *Sium* s.s. from *Berula* s.l., estimated under the assumption of a molecular clock and using a substitution rate of 0.61% per million years, took place around 10.4 million years ago. Dispersal–vicariance analysis suggested that the biogeographic pattern of this group resulted from simple vicariance and eventual migration to adjacent areas rather than from long-distance dispersal. The ancestral area of *Sium* s.l. was probably western Palearctic. The dispersal of *Berula* to North America occurred ca. 3 million years ago, possibly by island hopping. The migration of *S. suave* to North America took place ca 0.6 million years ago through Beringia.

Key words: *Afrocarum*; Apiaceae; *Berula*; biogeography; rDNA ITS; phylogeny; *Sium*; Umbelliferae.

Vicariance is generally regarded as a null hypothesis in modern historical biogeography, contrary to the traditional point of view that considered dispersal from the centers of origin as the major factor determining biogeographic patterns (Nelson, 1978; Bremer, 1992; Ronquist, 1997). In most cases, vicariance is a simpler explanation of species distributions than long-distance dispersal, hence traditional dispersal biogeography was described by Nelson (1978) as "a science of the improbable." A classic example of the vicariance scenario was the biogeographical history of the southern hemisphere with the sequential breakup of Gondwana causing vicariant division of its biota (Raven and Axelrod, 1972). However, recent analyses show that dispersal did play an important role in shaping distributional patterns of some groups of organisms. Dispersal–vicariance analysis (DIVA) of 54 animal and 19 plant taxa of presumed Gondwanian origin revealed that, while animal data are generally congruent with the area cladogram, the pattern of plant distribution is better explained by long-distance dispersal (Sanmartín and Ronquist, 2004). Moreover, divergence dating of some groups considered as typical examples of Gondwana-fragmentation vicariance—for instance, chameleons (Raxworthy et al., 2002), cichlids (Vences et al., 2001), and the plant genus *Exacum* L. (Yuan et al., 2005)—revealed that lineage divergence occurred much later than the tectonic events claimed responsible for the vicariance.

Dispersal has also been reinstated as an important factor shaping biogeographic patterns in the northern hemisphere. The area relationship of this region was reticulate rather than branching (Enghoff, 1996), with the Turgai Straits dividing the Palearctic and land bridges crossing the Atlantic and Pacific Oceans throughout part of the Cenozoic (Tiffney and Manchester, 2001). Recent analyses from different plant phylogenies indicate that the historical biogeography of the Holarctic was complex and cannot be explained by a simple vicariance model (Wen, 1999; Donoghue et al., 2001; Wen, 2001; Xiang and Soltis, 2001).

The exceptionally wide distributional ranges of aquatic plants are traditionally explained by long-distance dispersal by migratory birds, although the vicariance scenario cannot readily be excluded (Les et al., 2003 and references therein). Several hydrophytes, including Nympheales Dumort., constitute early branching lineages of angiosperms, hence their disjunct distribution patterns may be relictual, rather than resulting from long-distance dispersal. Their morphological similarity may have been preserved due to stabilizing selection in aquatic habitats. However, for most disjunct hydrophytes analyzed, Les et al. (2003) found divergence times far too recent to implicate continental drift as a major factor determining these disjunctions. Apparently, more data are needed to estimate the relative role of dispersal in shaping plant biogeographic patterns.

Progress in historical biogeography is also limited by a poor knowledge of species phylogeny. When analyzing eastern Asian–eastern North American disjunct distribution patterns among plants, Wen (1999, 2001) demonstrated that several presumed disjunct species-pairs are not sister taxa. The case of *Osmorhiza* Raf. (Apiaceae) is particularly illustrative. Molecular data have shown that *O. aristata* (Thunb.) Rydb., the only Asian member of this otherwise New World genus, is sister to its remaining congeners (Downie et al., 2000a; Wen et al., 2002; Yoo et al., 2002), whereas morphology placed it alongside the North American *O. longistylis* (Torr.) DC. and *O. claytonii* (Michx.) C.B. Clarke (Lowry and Jones, 1984).

---

[1] Manuscript received 17 May 2005; revision accepted 13 February 2006.

The authors thank V. Williams (St. Helena), J.-P. Reduron (Mulhouse, France), M. Watson (Edinburgh, Scotland), A. A. Oskolski (St. Petersburg, Russia), and the curators of E, G, ILL, ILLS, LE, MO, RM, UC, and WA for providing us with material, C. Calviño, D. Katz-Downie, J. H. Paik, and F. Sun for laboratory and/or bibliographic assistance, and two anonymous reviewers for comments on the manuscript. This research was supported by NSF grant DEB 0089452 to S.R.D. and by KBN grant 6 P04C 039 21 to K.S.

[4] Author for correspondence (e-mail: spalik@biol.uw.edu.pl)

The similarity of these three species was believed to be apomorphic, and they were placed in a single section; at present, this similarity is regarded as plesiomorphic and retained by stabilizing selection (Downie et al., 2000a; Wen et al., 2002).

The historical biogeography of *Sium* L. and related genera (Apiaceae) is subject to the aforementioned problems. The phylogeny of the group is uncertain, with several species of dubious affinity, and their distribution patterns are not easily reconciled with any simple vicariance scenario.

The genus *Sium* L. belongs to the umbellifer tribe Oenantheae Dumort. (Hardway et al., 2004). Formerly identified as the "*Oenanthe* clade" (Plunkett et al., 1996; Downie et al., 1998; Plunkett and Downie, 1999; Downie et al., 2000b, 2001), this tribe encompasses species that often live in moist to wet habitats, including some that are even true aquatics. Currently, about 12 species are recognized in *Sium* (Mukherjee and Constance, 1993; Pimenov and Leonov, 1993), although nearly 150 specific names have been published (World Umbellifer Database, http://rbg-web2.rbge.org.uk/URC/Nomenclature/). Most species are Eurasian, one occurs in Africa south of the Sahara (*S. repandum*; the taxonomic authorities are given in the Appendix), two are endemic to St. Helena Island (*S. burchellii* and *S. bracteatum*), and one is widespread in eastern Asia and North America (*S. suave*). The major center of endemism is eastern Asia, where five species occur (*S. frigidum*, *S. ninsi*, *S. tenue*, *S. serra*, and *S. suave*). The genus *Sium* also includes a neglected pot herb, *S. sisarum*, once widely cultivated for its thickened roots that are rich in carbohydrates. The cultivated form is generally regarded as related to wild *S. sisaroideum* that occurs in the Middle East (Schischkin, 1950a; Peşmen, 1972; Hedge and Rechinger, 1987). However, several botanists including Linnaeus and A. P. de Candolle regarded eastern Asia as the place of *S. sisarum* domestication and indicated *S. ninsi* as its wild ancestor (Rostafiński, 1884). Hiroe and Constance (1958) followed this treatment and synonymized *S. ninsi* with *S. sisarum*.

The genus *Berula* W. D. J. Koch is generally regarded as closely related to *Sium* and is sometimes synonymized with it (Drude, 1897–1898). Presently, *Berula* is monotypic, although several species had been previously recognized. The European populations constituted *B. erecta* sensu stricto (s.s.), the nomenclatural type of the genus. The plants from Asia were recognized as "*B. orientalis* Woronow ex Schischk." (Schischkin, 1950b). This taxon denoted by an invalidly published binomial (as it was not accompanied by a Latin diagnosis) is now sunk without a trace in *B. erecta* (Hedge and Lamond, 1987). The African members of *Berula*, once classified as *B. thunbergii* (DC.) H. Wolff, are now reduced to the rank of subspecies (Burtt, 1991). The North American congeners were placed in *Berula pusilla* (Nutt. ex Torr. & A. Gray) Fernald, nom. illeg., or *B. incisa* (Torr.) G. N. Jones, currently regarded as synonymous to *B. erecta*. Such a broad treatment of *B. erecta* has been generally accepted because this taxon varies little morphologically throughout its geographical range. Cronquist (1961) noted that the North American plants, in contrast to their Old World cousins, have markedly dimorphic leaves. However, he considered this difference minor and therefore recognized the North American plants as var. *incisa*. When establishing subsp. *thunbergii*, Burtt (1991) admitted that the African and Eurasian plants differ only slightly in leaf lobe incision. Such a broad circumscription makes *B. erecta* presumably the most widespread umbellifer species in the world (excluding weedy umbellifers).

The results of preliminary phylogenetic analyses of tribe Oenantheae are surprising in that they reveal clades uniting species of common geographic origin rather than of similar morphology (Hardway et al., 2004). In these analyses, neither *Berula* nor *Sium* were monophyletic. Instead, a weakly supported clade encompassing the African accessions of *B. erecta*, *S. repandum* and its two congeners endemic to St. Helena Island (*S. bracteatum* and *S. burchellii*), and the monotypic African genus *Afrocarum* Rauschert is evident. The remaining members of *Sium* formed two clades that were weakly supported sister groups in some analyses. However, the results were hardly conclusive, and the monophyly of *Berula* might actually be supported upon expanded sampling. Additionally, the relationship between the African clade and the non-African members of *Sium* has remained unresolved. These analyses did not include several species of *Sium* endemic to central and eastern Asia (*S. medium*, *S. ninsi*, *S. serra*, and *S. tenue*). The position of *S. serra* is particularly dubious. In the *rbcL* analysis of Kondo et al. (1996), this species was placed along other members of Oenantheae, whereas in the new edition of the *Flora of China* it has been retained in *Pimpinella* L. (Pu and Watson, 2005a). The identity of Chinese *S. frigidum* has been recently questioned, and its affinity to *Sinocarum* H. Wolff ex R. H. Shan & F. T. Pu has been postulated (Valiejo-Roman et al., 2002; Pimenov and Kljuykov, 2003).

The major objective of this paper is to infer the phylogenetic relationships among members of *Sium* sensu lato (s.l.), i.e., *Sium* s.s. and its presumed relatives, *Berula* and *Afrocarum*, and to analyze the dispersal–vicariance history of the group. We are particularly interested in the phylogeography of the previously identified African clade and the Asian members of *Sium*. In order to reconcile the phylogeny of *Sium* s.l. with the geological history of the Holarctic, we estimate the timing of major divergence events, particularly those involving transcontinental disjunctions. An additional objective is to identify the wild ancestor of *S. sisarum*.

The nuclear rDNA internal transcribed spacer (ITS) region is among the most popular molecular markers used at low taxonomic levels. However, its use in phylogenetic studies has been also strongly criticized (Álvarez and Wendel, 2003). These criticisms include orthology/paralogy conflation, compensatory base change, and problems in alignment due to indel accumulation; however, these mostly refer to large-scale analyses and may not occur when dealing with relatively recent radiations. An additional disadvantage of this region for phylogeographic studies is the possibility of occasional recombination. In this paper, we also discuss the utility of the ITS region in inferring phylogenetic relationships of umbellifers at low taxonomic levels.

MATERIALS AND METHODS

Taxon sampling—One hundred accessions were examined for ITS (ITS 1, 5.8S rDNA, ITS 2) sequence variation (Appendix). The *Sium* s.l. group was represented by 14 species: *Afrocarum imbricatum*, *Berula erecta*, and 12 species of *Sium*. Sampling of those ingroup members that are characterized by a broad geographic distribution—*B. erecta* and *S. suave*—was comprehensive. Because *B. erecta* was polyphyletic in our earlier analyses, 26 accessions from different parts of its range were considered. *Sium suave*, having a North American–eastern Asian disjunction, was represented by 17 samples. We also included *Cryptotaenia japonica*, *C. canadensis*, and representatives of

Helosciadium W. D. J. Koch, that in our previous analyses formed a clade with *Sium* and *Berula* (Hardway et al., 2004). Outgroups included other members of tribe Oenantheae: *Cicuta virosa*, *Neogoezia macvaughii*, *N. planipetala*, *Oenanthe fistulosa*, and *Oxypolis occidentalis*.

DNA extraction, amplification, and sequencing—ITS sequences from 76 accessions were obtained or updated for this study, one unpublished accession was obtained from GenBank, while the remaining ones have already been published (Downie et al., 1998, 2000b; Hardway et al., 2004). For the new accessions, total genomic DNA was isolated from ca. 20 mg of dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). The purified DNAs were PCR-amplified using either primers "ITS4" and "ITS5" (White et al., 1990) or "N-nc18S10" and "C26A" (Wen and Zimmer, 1996). For some accessions, the ITS 1 and ITS 2 regions were each amplified separately using the following pairs of primers, three of which were newly constructed: 18S-ITS1-F (5′-TAGAGGAAGGAGAAGTCGTAACA-3′) and 5.8S-ITS1-R (5′-TTCTGCAATTCACACCAAGTAT-3′) for ITS 1, and ITS-3N (5′-CGATGAAGAACGTAGCGAAATG-3′) and C26A primers for ITS 2. Details of the PCR amplifications are provided in Downie et al. (2000a). Each PCR product was electrophoresed in a 1% agarose gel, stained with ethidium bromide, then excised and eluted using either the QIAEX II or QIAquick gel extraction kits (Qiagen). Cycle sequencing reactions were performed using the purified PCR product, Ampli*Taq* DNA polymerase (Roche Molecular Systems, Alameda, California, USA), and fluorescent Big Dye terminators (Applied Biosystems, Foster City, California, USA). The products were resolved by electrophoresis using an ABI 377A automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Scans were edited and corrected when necessary. All newly obtained ITS sequences have been deposited in GenBank as contiguous ITS 1, 5.8S, and ITS 2 data (Appendix). For one accession (*S. repandum* K61), sequence data were unavailable for 5.8S rDNA and ITS 2, despite our repeated but unsuccessful efforts to amplify these regions via PCR.

Sequence and phylogenetic analyses—The DNA sequences were aligned using CLUSTAL X (Jeanmougin et al., 1998) with default parameters for gap penalty and extension (the matrix has been deposited with TreeBase, study accession number S1469, matrix accession number M2646). Hardway et al. (2004) reported that tree topologies obtained from maximum parsimony analyses changed when different cost matrices were used in sequence alignment. However, this concerned only fast-evolving members of the "North American endemics clade," whereas the relationships among *Berula*, *Afrocarum*, and the African *Sium* species remained the same upon consideration of different gap costs. Uncorrected pairwise nucleotide differences were determined using PAUP* version 4.0b10 (Swofford, 2003).

Phylogenetic analyses included neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods, implemented using PAUP*. For the NJ analysis, we used several substitution models. Bootstrap analysis (Felsenstein, 1985) was performed for 1000 pseudoreplicates. MP analysis was carried out with gap states treated as missing data, characters unordered, and all character transformations equally weighted. Heuristic MP searches were replicated 100 000 times with random stepwise addition of taxa, tree-bisection-reconnection (TBR) branch swapping, and saving multiple trees (MulTrees). Bootstrap support was estimated using 1000 resampled data sets, saving no more than 10 trees per replicate. Bremer (1994) support values were obtained using AutoDecay 4.0 (Eriksson, 1998). The substitution model for the ML analysis was selected using the program Modeltest version 3.6 (Posada and Crandall, 1998) and the Akaike information criterion (Akaike, 1974). A heuristic search using random stepwise addition and TBR branch swapping was implemented using PAUP*. One hundred bootstrap replicate analyses were conducted using heuristic searches with the ML parameters inferred by Modeltest.

All trees were rooted with representatives of *Cicuta*, *Neogoezia*, *Oenanthe*, and *Oxypolis*. In our previous analyses, these genera constituted a clade that was sister to a monophyletic group containing *Sium*, *Berula*, *Afrocarum*, *Cryptotaenia*, and *Helosciadium* (Hardway et al., 2004).

Estimation of divergence times—Previous phylogenetic analyses revealed that the "North American endemics clade" (of which *Neogoezia* Hemsl. is a member) had an accelerated nucleotide substitution rate when compared to all other branches of Oenantheae (Hardway et al., 2004). Therefore, for the analysis of divergence times, we used a reduced matrix including only the *Sium* s.l. group with *Helosciadium* serving as the outgroup. Only ITS1 and ITS2 sequences were used to apply the substitution rates obtained by others for these same two regions (Sang et al., 1994, 1995; Wendel et al., 1995; Wen, 1999). Additional MP searches were performed, and one of the shortest trees was arbitrarily chosen for further analyses. A substitution model was selected using Modeltest 3.6. Branch lengths were estimated using PAUP* and the ML method, with and without the assumption of a molecular clock. The molecular clock assumption was verified using the likelihood ratio test (Felsenstein, 1988).

Because there are no fossils of Apiaceae that may be used to calibrate the molecular clock, to calculate divergence times we used the rate of 0.61% substitution per million years (my) inferred for the Juan Fernandez Island endemic *Dendroseris* D. Don (Asteraceae, Sang et al., 1994). The report on the presence of pollen attributed to *Berula* in lower Miocene deposits from Méndez, Mexico (Palacios Chávez and Rzedowski, 1993) that could have been used for dating the dispersal of *Berula* to America is erroneous. *Berula* pollen is distinctly elliptic in outline, whereas that illustrated in Palacios Chávez and Rzedowski (1993) is equatorially constricted (K. Bińka, Warsaw University, personal communication).

Biogeographic analyses—The biogeographic scenario for *Sium* s.l. was reconstructed using a simplified, fully resolved MP tree obtained from the reduced data matrix. The terminals were 24 lineages revealed by the phylogenetic analyses, including all species and infraspecific groups within *B. erecta*, *S. latifolium*, and *S. suave*. We choose a tree that was congruent to the majority-rule consensus of shortest trees from the MP searches. *Helosciadium* was used as the outgroup. The following 15 unit areas were defined: western Europe (A), eastern Europe (B), western Siberia (C), Middle East (D), central Asia (E), Russian Far East (F), China (G), Korea and adjacent areas of Russia (H), Japan (I), Arabia (J), eastern Africa (K), southern Africa (L), central and western Africa (M), St. Helena (N), and North America (O). Although this delimitation was in some cases imprecise and a more detailed division would be advantageous, 15 is the maximum number of areas allowed by the program DIVA (Ronquist, 1996).

The dispersal–vicariance analysis was performed using DIVA version 1.1 (Ronquist, 1996, 1997). Each distribution area was coded as a separate character, and two optimizations were done: first, with an unconstrained number of unit areas for each ancestral node, and second, with this number restricted to six (i.e., one more than the highest number of distribution areas occurring among the ingroup taxa). From among the scenarios inferred, we highlight those reconstructions that contained geographically adjacent areas. If such reconstructions did not occur, we considered those that minimized long-distance disjunctions.

RESULTS

Sequence analysis—The following 20 groups of accessions had identical ITS sequences. As such, each group was represented by a single terminal taxon in the phylogenetic analyses and was denoted on the trees by their respective parenthetical number:

(1) *Afrocarum imbricatum* K151 and 1444; (2) *Berula erecta* subsp. *erecta* "*B. orientalis*" 2810, 2811, and E117; (3) *B. erecta* subsp. *erecta* "*B. orientalis*" E110, E114, and E115; (4) *B. erecta* subsp. *erecta* 2253 and 2257; (5) *B. erecta* subsp. *thunbergii* K82, K116, and E119; (6) *B. erecta* var. *incisa* 503 and 1144; (7) *Sium bracteatum* K177, MW, and *S. burchellii* K178; (8) *S. latifolium* K179, 1632, 2256, and E; (9) *S. latifolium* K180, K215, 2806, 2807, and E129; (10) *S. medium* 2342 and K59; (11) *S. medium* 2808 and 2809; (12) *S. medium* K58 and E127; (13) *S. ninsi* K60 and K122; (14) *S. repandum* K126 and K61 (ITS 1 only); (15) *S. sisarum* 97, 83, 53, and 311; (16) *S. sisaroideum* 2340, E124, E125, E126, and E130; (17) *S. suave* 12, 1655, and 2013; (18) *S. suave* 1656, 1808, 1811, 1815, 1965, and 1968; (19) *S. suave* K184, 2804, and 2805; (20) *S. tenue* K63, K125, and E134.

The final matrix comprised 59 sequences. Forty-two insertion–deletion events (indels) were introduced to align them. The indels were generally unambiguous. In a few cases,

Table 1. Sequence characteristics of the ITS 1, 5.8S rDNA, and ITS 2 regions, separately and combined, for the 59 representatives of *Sium* s.l. and outgroups used in this study.

Nucleotide sites	ITS 1	5.8S rDNA	ITS 2	Entire region
No. total aligned positions	222	164	241	627
No. constant	111	149	99	359
No. autapomorphic	30	3	45	78
No. parsimony informative	81	12	97	190
No. of alignment gaps	16	3	23	42

alternative alignments were also possible, but this did not affect the phylogenetic results because these positions were uninformative. Thirty-eight indels were 1 bp in size, two indels were 2 bp in size, and two other indels were 3 bp and 20 bp in size. The 20 bp indel was synapomorphic for all members of the genus *Helosciadium*.

The alignment of sequences resulted in a matrix of 627 characters, of which 190 (30.3%) were parsimony informative (Table 1). Uncorrected pairwise sequence divergence values were low. The mean value for the *Sium* s.l. group was 5.6%, with a maximum divergence value of 10.2% between *S. frigidum* 109 and *A. imbricatum* K132.

ITS sequence heterogeneity was detected in several accessions, as evidenced by polymorphisms in otherwise perfect chromatograms. If the level of noise in the chromatogram was high, we treated such double peaks as uncertainties. Most North American accessions of *B. erecta* (i.e., nos. 503, 1144, 1816, 1530, 2157, 1111) had an A/T polymorphism at position 98 in the aligned matrix. The remaining accessions of this species had an A in this position. For *B. erecta* 1111, two haplotypes differing in the presence of two 1-bp indels were found (a shorter sequence was used in the phylogenetic analyses). The first of these indels was an insertion of G within a run of GGGGGG, starting at position 70 in the aligned matrix. This indel also occurred in accession 2157. The second indel was an insertion of an A within a run of AAA starting at position 216 in the aligned matrix (at the end of the ITS 1 region). A C/T polymorphism was found at position 175 in *S. medium* K58 and E127. The other accessions of this species have either C (nos. 2342 and K59) or T (nos. 2808 and 2809) in this position.

Phylogenetic analyses—Parsimony searches resulted in 33 408 shortest trees, each of 507 steps (consistency index [CI] excluding uninformative characters of 0.641; retention index [RI] of 0.890). These trees constituted nine islands with the major one comprising 33 399 trees. In all MP trees, five major monophyletic groups are apparent within the ingroup (Fig. 1). These are (1) *Helosciadium*, which includes all accessions of this genus; (2) *Cryptotaenia*, which encompasses *C. japonica* and *C. canadensis*; (3) *Berula* s.l., which includes all accessions of this genus, as well as *Afrocarum imbricatum*, *Sium repandum*, *S. bracteatum*, and *S. burchellii*; (4) a clade encompassing *Sium latifolium*, *S. medium*, and *S. suave*; because these species generally occur in the northern Holarctic, we refer to this group as the "northern Holarctic clade;" (5) a clade encompassing *Sium sisarum*, *S. sisaroideum*, *S. frigidum*, *S. ninsi*, *S. serra*, and *S. tenue*; because all members of this clade are distributed in the Palearctic, usually farther south than the members of the previous clade, we refer to this group as the "southern Palearctic clade."

In 99.6% of the trees obtained from MP searches (and in the majority-rule consensus tree, Fig. 1), the northern Holarctic and southern Palearctic clades form a monophyletic group. Because this group encompasses nearly all members of *Sium* as this genus is currently recognized, we named it the "*Sium* s.s. group." In the majority-rule consensus tree, this group is sister to the *Berula* s.l. clade, which collectively comprise the *Sium* s.l. group (Fig. 1).

Within the aforementioned monophyletic groups, several subclades are apparent. Each of the member species of the northern Holarctic clade—*S. suave*, *S. latifolium*, and *S. medium*—is monophyletic. Additionally, within *S. suave*, two sister subclades are distinguished in the majority of trees, encompassing the Far East Asian and the North American accessions of this species, respectively. Among the nine accessions of *S. latifolium* examined, two ITS haplotypes were detected: *S. latifolium* (8) encompassing all accessions from western Europe, and *S. latifolium* (9) including all accessions from eastern Europe and Asia. The relationships among *S. latifolium*, *S. medium*, and *S. suave* remain unresolved. In 43% of bootstrap searches, *S. latifolium* and *S. suave* were supported as sister taxa, whereas *S. latifolium* and *S. medium* formed a clade in 29% of all MP trees.

Within the southern Palearctic clade, three eastern Asian species, *S. tenue*, *S. ninsi*, and *S. frigidum*, formed a strongly supported clade, with the first two of these being sister taxa. The position of the fourth eastern Asian member of this clade, *S. serra*, is unresolved; in the majority of trees, this species grouped with *S. sisarum* and *S. sisaroideum. Sium sisarum* formed a polytomy with *S. sisaroideum.*

The *Berula* s.l. clade includes three major subclades, two of which comprise the African taxa and the third comprising the Holarctic accessions of *B. erecta*. This third subclade is divided into North American and Eurasian branches, with the latter separated into three groups: European, western Asian, and central Asian. The African representatives of *B. erecta* form a sister group to the two species of *Sium* from St. Helena, whereas *Afrocarum imbricatum* is sister to *S. repandum*. These two subclades of African taxa, previously identified as a weakly monophyletic African clade (Hardway et al., 2004), were sister groups in 33% of the trees obtained from MP searches.

The NJ trees obtained using different models of nucleotide substitutions (not shown) have similar topologies to the MP majority-rule consensus tree. In most NJ trees, the two clades encompassing the African taxa form a trichotomy with the *Berula* s.s. clade, although in some trees (e.g., those obtained using Kimura's two-parameter substitution model) they were sister taxa. *Sium medium* was sister species to *S. latifolium*.

The Akaike information criterion of Modeltest selected the SYM + G substitution model for the ML analysis. Maximum likelihood searches resulted in two trees (–ln likelihood value of 3719.74) differing only in a minor rearrangement within the *S. medium* clade. In these trees (not shown), the *Sium* s.l. group was not monophyletic. Instead, the *Helosciadium* clade was sister to the *Sium* s.s. clade, whereas this entire clade was sister to the *Berula* s.l. group. However, such a relationship did not receive strong bootstrap support, with the *Helosciadium*-*Sium* s.s. clade occurring only in 32% of the trees obtained from resampled data. *Sium medium* and *S. latifolium* were sister taxa, as they were in the trees resulting from NJ analyses.

Divergence time estimation—For a reduced data set (ITS 1 and 2 only for *Sium* s.l. and *Helosciadium*), the Akaike

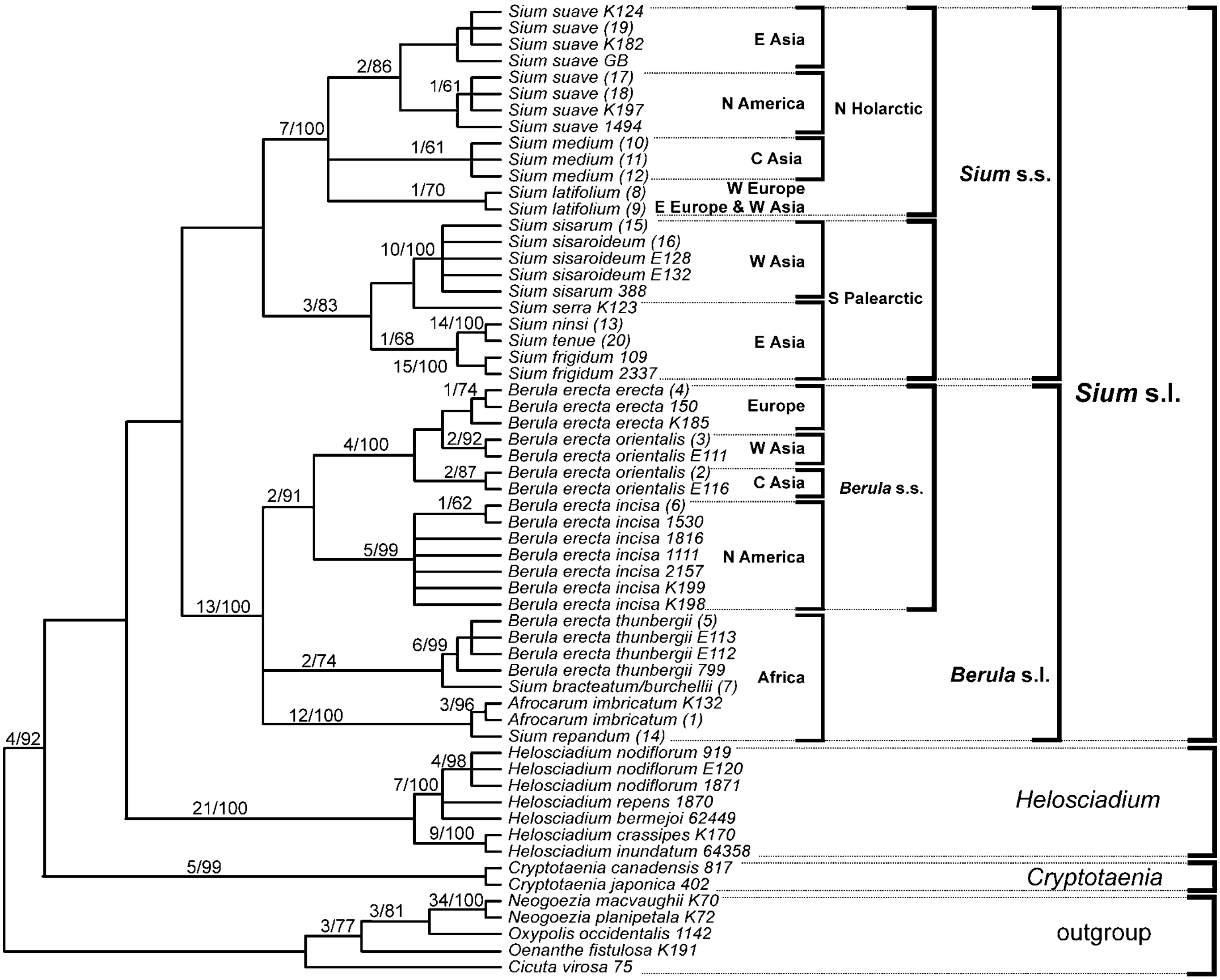

Fig. 1. Majority-rule consensus of 33 408 shortest trees (each of length 507 steps; CI = 0.641, excluding uninformative characters; RI = 0.890) obtained from maximum parsimony heuristic searches using a 59-sequence data set of *Sium* s.l., *Helosciadium*, *Cryptotaenia*, and outgroups. Decay indices/bootstrap values for the clades supported in the strict consensus tree are indicated along branches. Major clades and subclades marked with brackets are discussed in the text.

information criterion of Modeltest selected the GTR + G substitution model. In the ML trees derived from this model, the relationships inferred were similar to those obtained from the larger data set: the *Sium* s.l. clade was not monophyletic and the *Sium* s.s. formed a clade with *Helosciadium*. However, such a topology was not supported by ML bootstrap analyses, which resulted in a majority-rule consensus tree similar to that obtained from MP searches. Therefore, for the divergence time estimation, we chose one of the shortest trees obtained from the MP analysis.

Maximum parsimony heuristic searches resulted in 3168 shortest trees, each of 275 steps. These trees were congruent to those obtained from the larger matrix. For the divergence time estimation, we chose a tree that had a topology similar to those obtained from both the distance and the majority-rule consensus of ML bootstrap analyses. The –ln likelihood values of this tree, under the assumption of a molecular clock and without a constant ratio constraint, are 2306.89 and 2273.90, respectively. The likelihood ratio test value is 65.98, which is not significant ($P = 0.065$). Therefore, the assumption of constant substitution rate is not rejected.

Although the African group within *Berula* s.l. was monophyletic in the chosen tree, it collapsed into two clades when branch lengths were estimated (Fig. 2). Assuming a substitution rate of 0.61% per million years, the main split into the *Sium* s.s. clade and the *Berula* s.l. clade is estimated at ca. 10.4 million years ago (mya). The separation of the *Sium* s.s. clade into its northern and southern branches took place ca. 8.3 mya. The radiation of the southern Palearctic clade is dated to 6.6 mya, whereas the divergence of the northern Holarctic clade occurred around 1.9 mya. The North American subclade of *S. suave* diverged ca. 0.6 mya. Within the *Berula* sensu lato

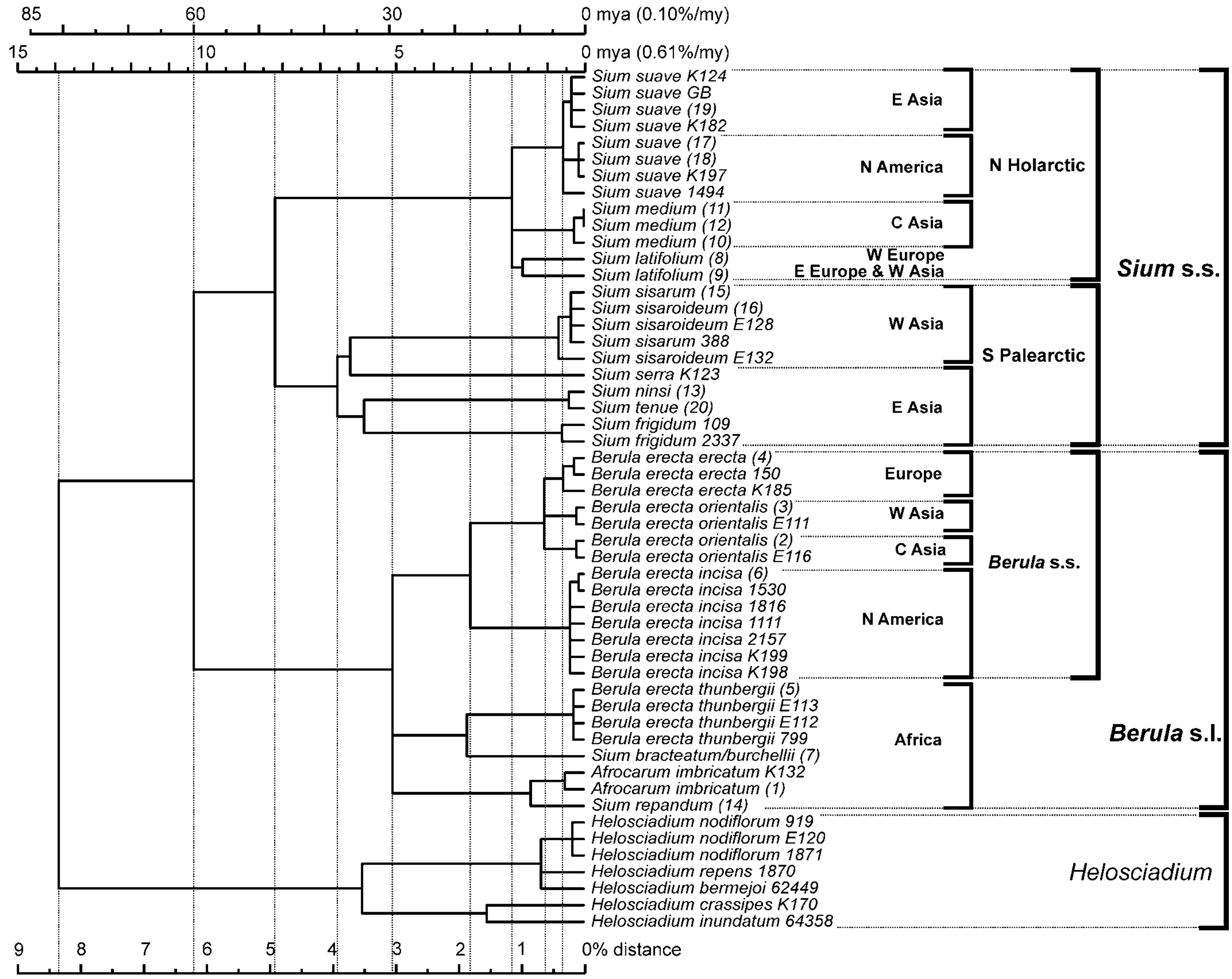

Fig. 2. One of 3168 shortest trees resulting from maximum parsimony analysis of *Sium* s.l. with *Helosciadium* as the outgroup. Branch lengths (scale bar at bottom of figure) were estimated with the maximum likelihood method using the GTR + G substitution model under the assumption of a molecular clock. Divergence times were assessed using either a substitution rate of 0.61%/my (lower time scale at top of figure) or dating the divergence of *Sium* s.l. at 60 mya and with a substitution rate of 0.10%/my (upper time scale at top of figure). Vertical dotted lines mark the position of major divergence events. Bracketed clades and subclades are the same as those in Fig. 1.

clade, the African lineages separated around 5.1 mya. The colonization of St. Helena took place ca. 3.1 mya. The dispersal of Berula to North America took place ca. 3 mya. The split of Eurasian *B. erecta* into European, western Asian, and central Asian populations occurred ca. 1 mya.

Alternatively, we calibrated the main division into the *Sium* s.s. clade and the *Berula* s.l. clade with the opening of the Turgai Straits during the mid-Paleocene, ca. 60 mya. The substitution rate inferred using this calibration is 0.10% per my. In this scenario, the division of the major clades within *Sium* s.s. took place around 48 mya, whereas the radiation of the southern Palearctic clade occurred around 38 mya. The radiation within the *Berula* s.l. clade started ca. 30 mya. The North American branches of *B. erecta* and *S. suave* diverged from their Old World counterparts 17 and 3.5 mya, respectively.

Biogeographic analyses—Because relationships within the *Berula* s.l. clade are partly unresolved, for the dispersal–vicariance analysis we arbitrarily assumed that the African members of this clade form a monophyletic group. The DIVA program did not provide unambiguous reconstructions of ancestral areas of *Sium* s.l. and most included clades. In the unconstrained analysis, the ancestral area was a summary of all individual areas: a result expected with vicariance as the null biogeographic hypothesis (Ronquist, 1996, 1997). In this reconstruction, 24 dispersals were required to explain the present distribution of the taxa.

With the maximum number of individual unit areas set to six, the best DIVA reconstruction required 31 dispersals. In this reconstruction, six alternative ancestral areas were inferred. The number of alternative reconstructions for particular nodes varied between 1 and 31 (Fig. 3). The reconstructions con-

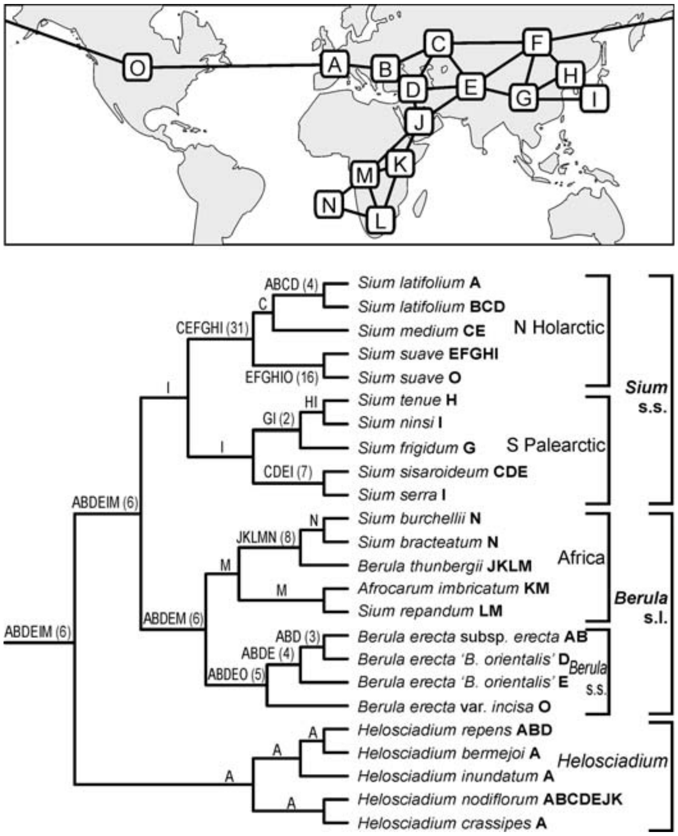

Fig. 3. A dispersal–vicariance scenario as reconstructed by DIVA with the maximum number of area units set to six. A–O denote respective area units (illustrated in map and further described in the Materials and Methods section). For the nodes with more than one ancestral area reconstruction inferred (see numbers in parentheses), one arbitrarily chosen reconstruction is shown that comprised either geographically adjacent area-units or minimized long-distance dispersal (see links on the map). Major clades within the *Sium* s.l. are marked with brackets.

taining only adjacent areas were not found for all nodes. The ancestral area reconstructions for *Sium* s.l. included western Eurasia (areas A, B, D, and E), but also Japan (I), central and western Africa (M), and/or North America (O). For the *Berula* s.l. clade, the reconstructions contained western Eurasia (A, B, D, and E), central and western Africa (M), and/or North America (O). Both unconstrained and constrained analyses indicated central and western Africa (M) as the place of origin of the African clade. The colonization of St. Helena (N) occurred from Africa. The dispersal of *Berula* to North America (O) occurred from western Eurasia (unless North America was part of the ancestral area of *Sium* s.l.).

Both dispersal–vicariance analyses suggested that Japan was the place of origin of *Sium* s.s., as well as the place of origin of the southern Palearctic clade. This latter clade differentiated in eastern Asia and spread into western Asia. As many as 31 reconstructions of the ancestral area of the northern Holarctic clade were inferred: all contained western Siberia (C) and at least one of the other Asian area units. This ancestor was divided into eastern *S. suave* and western *S. latifolium*/*S. medium*. The latter subsequently split into western *S. latifolium* and eastern *S. medium*. The colonization of North America by *S. suave* occurred from eastern Asia.

DISCUSSION

The utility of the ITS region at low taxonomic levels—The ITS region constitutes one of the most popular noncoding loci used in phylogenetic analyses at low taxonomic levels. The advantages of this region are (1) the presence of multiple copies facilitating PCR amplification even if the DNA sample is degraded; (2) a high rate of nucleotide substitution; (3) a relatively small size facilitating sequencing (ca. 600 bp in Apioideae, including 5.8S rDNA); (4) highly conserved flanking regions (rDNA), allowing the use of universal primers; and (5) a high number of sequences available in databases allowing for immediate comparisons. Downie et al. (2001) compared several data sets used to infer phylogeny of Apiaceae and concluded that only restriction site analysis rivaled ITS sequencing in yielding the greatest number of informative characters.

The use of ITS sequences for inferring plant phylogeny has also been subject to criticism. Homoplasy has been shown to be higher in ITS than in other data sets due to orthology/paralogy conflation, compensatory base change, and problems in alignment due to indel accumulation (Álvarez and Wendel, 2003). Such phenomena as extensive sequence variation arising from array duplication events, genomic harboring of pseudogenes in various states of decay, and incomplete intra- or interarray homogenization may substantially reduce the utility of the ITS region for phylogenetic analysis. Álvarez and Wendel (2003) suggested using single- or low-copy genes instead. However, such loci have yet to be examined within Apiaceae.

Several of the aforementioned problems have also been encountered in phylogenetic studies of Apiaceae. For *Osmorhiza*, the results of ITS phylogenetic analyses were inconsistent with those obtained from chloroplast introns, probably as a result of a hybridization event (Yoo et al., 2002). ITS sequence divergence is too high for an analysis of the entire family. Sequences obtained from members of subfamilies Hydrocotyloideae, Saniculoideae, and several early branching members of subfamily Apioideae cannot be reliably aligned with those of core Apioideae (Downie et al., 2001). Divergent paralogous ITS sequences has been detected for some South African umbellifers (C. Calviño [University of Illinois] and S. R. Downie, unpublished data). In this paper, we report minor sequence heterogeneity for *S. medium* and North American *B. erecta* that, however, does not affect the phylogenetic analyses.

In spite of these objections, the ITS region seems to be the best marker for phylogenetic analyses of Apiaceae at low taxonomic levels at the present time. Multicopy regions like ITS can successfully be amplified even using somewhat degraded DNA samples from herbarium specimens. At the infrageneric level, the accumulation of indels in the ITS region is usually insignificant and the alignment is unambiguous, whereas the paralogs may readily be identified due to their sequence divergence.

The estimation of divergence time under different ITS substitution rates—In the absence of sufficient paleontological evidence, the choice of substitution rate is somewhat arbitrary. A variety of substitution rates for nuclear ITS rDNA has been already inferred (Suh et al., 1993; Sang et al., 1994, 1995; Wendel et al., 1995). For Winteraceae, the inferred rate was relatively low, 0.032–0.057%/my (Suh et al., 1993); rates reported for other taxa were an order of magnitude higher, 0.27–0.78% (Suh et al., 1993; Sang et al., 1994, 1995; Wendel et al., 1995; Les et al., 2003). The calibration of these estimates relies either on presumed vicariance events, like the separation of New Zealand from eastern Gondwana as in the case of Winteraceae (Suh et al., 1993) or from comparisons with other molecular markers with known divergence rates (Wendel et al., 1995). Particularly valuable are those estimates that concern endemics of oceanic islands of known age, such as the Juan Fernandez Islands (Sang et al., 1994, 1995) because the calibration point is less arbitrary. Among those, a substitution rate of 0.39% estimated based on sequence variation in *Dendroseris* (Sang et al., 1994) is most commonly cited (e.g., Wen, 1999). However, Sang et al. (1994) considered this value as the slowest possible rate and suggested that a rate of 0.61% is more likely, which is also closer to that inferred for *Robinsonia* DC. (Sang et al., 1995) and *Gossypium* L. (Wendel et al., 1995). In their study of the biogeography of hydrophytes, Les et al. (2003) used a relatively low rate of 0.27%/my. Apart from ITS sequences, for 12 pairs of species they also used other molecular markers with known substitution rates: *trnK* intron, *rbcL* (synonymous), *matK* (synonymous), and *rpl16* intron. In 11 of these cases, the divergence times inferred from ITS were higher than those estimated using the other markers. To check which of the substitution rates for ITS gives divergence times that better approximate other estimates, we reanalyzed the data of Les et al. (2003) by comparing the values inferred from ITS using different substitution rates with those obtained from other sequences. Mean (and median) percentage differences between the divergence times estimated from ITS using 0.27, 0.39, 0.61, and 0.78% substitution rates and those inferred from other sequences were 155 (110), 77 (47), 15 (−4), and −9 (−15)%, respectively. The substitution rate of 0.27% chosen for ITS by Les et al. (2003) gives divergence time estimates on average 2.5 times higher than those obtained from other molecular markers. Of the four substitution rates analyzed, that of 0.61%/my is the best choice.

Another question is the accuracy of calibrating the molecular clock, particularly in those cases when the calibration point is a geological event claimed to be responsible for the vicariance. Such calibration may substantially underestimate the substitution rate if the major factor shaping the biogeographic pattern is dispersal rather than vicariance. The present distribution of several plant taxa of presumed Gondwanian origin is better explained by long-distance dispersal than Gondwana-fragmentation vicariance (Sanmartín and Ronquist, 2004). In several groups considered as typical examples of this vicariance, including chameleons (Raxworthy et al., 2002), cichlids (Vences et al., 2001), and *Exacum* (Yuan et al., 2005), divergence dating based on a molecular clock is in serious disagreement with that estimated from the land breakup. This discrepancy may explain the very low ITS substitution rate inferred for Winteraceae (Suh et al., 1993). The molecular clock for Winteraceae was calibrated based on isolation of New Zealand *Pseudowintera* from the Old World genera by continental drift. However, the ancestor of *Pseudowintera* may have been a late colonizer to New Zealand rather than a Gondwanian relic, and the ITS substitution rate for Winteraceae may be similar to those occurring in other taxa.

Using a substitution rate of 0.61%/my, the first divergence in *Sium* s.l. took place around 10.4 mya. This date coincides with the Tertiary cooling of the climate. Subsequent climatic oscillations of the late Tertiary and Quaternary may have introduced the barriers necessary for vicariance. However, if we question the accuracy of this rate and consider known barriers in the past that may have been responsible for this vicariance, we get a different story. In the early Tertiary, the Palearctic was divided by the Turgai Straits (Tiffney and Manchester, 2001). Using this as a calibration point for the divergence between *Berula* s.l. and *Sium* s.s., the substitution rate is ca. 0.10%, a rather low estimate but not that low as to be rejected in favor of other values. However, such a scenario is in disagreement with the results of DIVA. If the opening of the Turgai Straits had been responsible for the first division within *Sium* s.l., then the ancestral areas of *Berula* s.l. and *Sium* s.s. would have been western Eurasia and central Eurasia, respectively. DIVA, however, postulates, that the ancestral area for *Sium* s.s. was Japan.

Phylogenetic relationships and vicariance within Sium s.l.—The monophyly of *Sium* s.l. is not unequivocally corroborated by our analyses because *Helosciadium* is nested within *Sium* s.l. in some MP and ML trees. However, in the majority of trees obtained from MP searches and in all trees obtained using distance methods, *Helosciadium* was sister to the *Sium* s.l. clade, and this entire group was sister to *Cryptotaenia*. Such a topology better explains the general morphology and ecology of these taxa than alternative scenarios. *Sium* s.l. and *Helosciadium* include mostly water plants that are morphologically similar. With their succulent, simple pinnate leaves, these species are difficult to distinguish when in the vegetative stage (van Moorsel and Baudewijn, 2000). In contrast, *Cryptotaenia* occurs in mesic habitats and has ternate leaves. The monophyly of *Helosciadium* is strongly supported by a synapomorphic 20-bp deletion in the ITS region. Therefore, we favor the results of those analyses that support the sister relationship between *Sium* s.l. and *Helosciadium*. However, one cannot exclude that the similarity seen in *Sium* is plesiomorphic and that the genus is paraphyletic with respect to *Berula* and *Helosciadium*.

Because DIVA does not assume any area relationships, the reconstructed ancestral areas may be disjunct. Indeed, such ancestral areas were inferred for *Berula* s.l., *Sium* s.l., and *Helosciadium* + *Sium* s.l. They comprise southwestern Eurasia (A, B, D, and E), central and western Africa (M) and, in the case of *Sium* s.l. and *Helosciadium* + *Sium* s.l., Japan (I) (Fig. 3). These disjunctions are not accounted for in DIVA reconstructions; to explain them one has to postulate either two additional long-distance dispersals from southwestern Eurasia to Africa and Japan, respectively, or gradual migrations and eventual extinctions in the connecting areas. Extinctions never occur in DIVA reconstructions when using the default parameter values because there are always more parsimonious solutions without them (F. Ronquist, personal communication cited in Xiang and Soltis, 2001).

The ancestor of *Sium* s.l. is reconstructed as a widespread taxon. In DIVA performed without any constraints on area-unit number, the ancestral area of *Sium* s.l. includes all individual units. Such a result is to be expected because DIVA assigns a cost only for dispersal and not for vicariance (Ronquist, 1996, 1997). Vicariance is generally considered the null hypothesis in historic biogeography, although the resulting conclusion that the ancestors should generally have broader distributions than their living descendants is somewhat disturbing (Bremer, 1992; Ronquist, 1994). Additionally, a vicariance event in a widespread species may leave a confusing phylogenetic signal in the genetic structure of the vicariants. Gene splitting predates population splitting, and lineage sorting is one reason why a gene tree may differ from a species tree (Nei, 1987; Avise, 2004). An individual gene tree topology approaches the species tree topology only if in the daughter taxa single lineages become fixed before the next divergence. The probability that an allele becomes fixed between successive splits depends on the effective population size and the time between these events. The more widespread the taxon and the less time passing from one divergence event to another, the less likely the gene trees will reflect the species phylogeny. Therefore, we are reluctant to strictly adhere to the results of DIVA and instead treat them as suggestions of general pattern rather than detailed solutions. In particular, the disjunct ancestral areas of the *Berula* s.l., *Sium* s.l. and *Sium* s.l. + *Helosciadium* clades seem to be rather improbable, because such a disjunction would have immediately created a barrier to gene flow. Therefore, we favor a scenario assuming southwestern Eurasia as the ancestral area of the group, with subsequent long-distance dispersals to Japan (or more generally, eastern Asia) and central Africa that at the same time initiated vicariance.

The differentiation of major lineages within the *Sium* s.l. group started ca. 10 mya, which coincided with the global cooling of the climate and initiation of a glaciation period with its concomitant floristic changes. For instance, around 11–12 mya temperate elements start to appear in the palynoflora of northern Latin America (Graham, 1999). The evolutionary history of *Sium* s.l. must have been much more complex than that reconstructed by DIVA and possibly included migrations southwards as the ice sheet approached, isolations in refugia during glacial maximums, and postglacial expansions. Associated bottleneck effects could have reduced the genetic variation providing consistency between the species phylogeny and the gene tree. A good example is *S. latifolium* which, despite its present widespread distribution, is represented by only two ITS haplotypes (western European and eastern European/western Asian) that presumably reflect its survival in as many refugia during the last glacial maximum. The separation of Eurasian *B. erecta* into three distinct clades may also be attributed to the isolation of these populations during the Ice Ages. The divergence of *S. suave* into Asian and North American branches (with one exception) also occurred in the Quaternary. Several European and Holarctic species show similar phylogeographic patterns, with distinct clades that diverged during the Quaternary and, presumably, survived in different glacial refugia (Hewitt, 2004).

Southwestern Eurasia presumably constituted the ancestral area of *Sium* s.l. with the first split into a western (or southwestern) *Berula* s.l. clade and an eastern (or northeastern) *Sium* s.s. clade. The former radiated into Europe, the Middle East, and Africa, and through transatlantic dispersal colonized North America (Fig. 4), whereas the latter radiated in Eurasia, with one species colonizing North America via transpacific dispersal (Fig. 5).

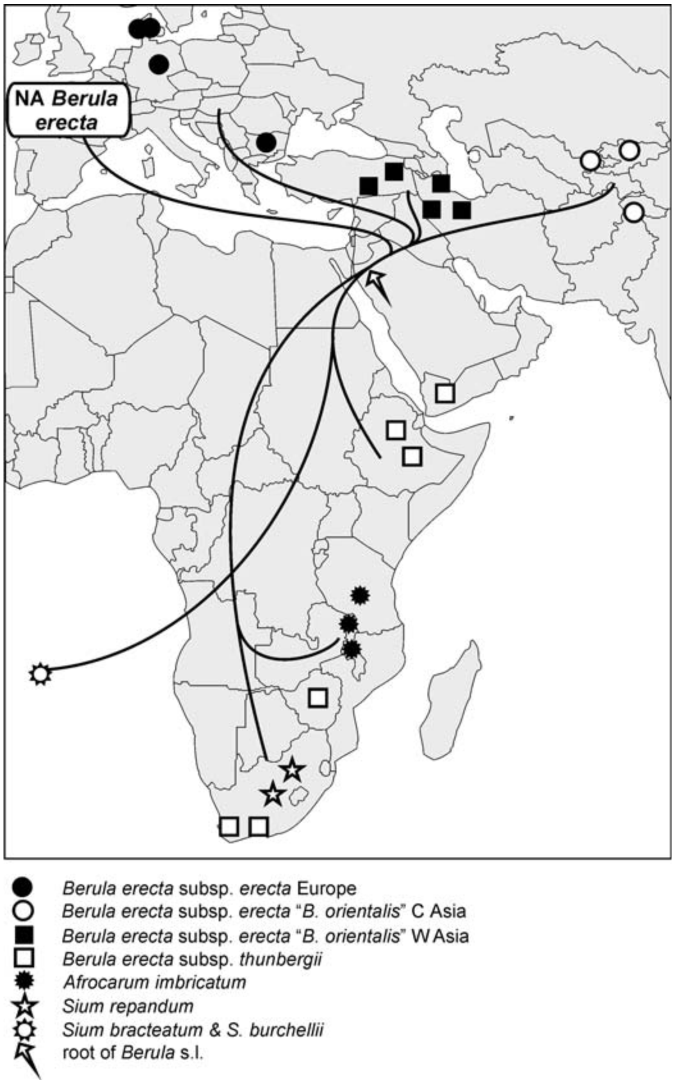

Fig. 4. A phylogeny of *Berula* s.l. traced on a map of the distribution of accessions sampled for this study. North American accessions of *B. erecta* var. *incisa* ("NA *Berula erecta*") were omitted.

The Berula s.l. clade and the morphological stasis of Berula erecta—The *Berula* s.l. clade, encompassing all accessions of *Berula*, *Afrocarum*, and the African and St. Helena members of *Sium*, is very strongly supported in all analyses. Although these results are in conflict with current taxonomic treatments, they are consistent with the geographic distributions of the included accessions (Fig. 4). The only relatively good morphological character that distinguishes members of the *Berula* s.l. clade from those of the *Sium* s.s. clade is bract incision. In Eurasian and North American representatives of *Berula*, usually at least one bract in the primary umbel (and sometimes also in the higher order umbels) is bi- or trilobate. In African *B. erecta*, *S. repandum*, and *A. imbricatum*, at least some bracts are incised. In contrast, the bracts of *Sium* s.s. are usually entire.

The Eurasian, North American, and African umbellifers hitherto classified in *Berula erecta* are so similar that infraspecific taxa are only rarely recognized. In the account of *B. erecta* for *Flora Zambeziaca*, Cannon (1978) commented that characters used by previous authors did not appear to justify the separation of the African populations from the more widely distributed members. Burtt (1991) recognized the African populations as subsp. *thunbergii* based on the cutting of the leaflets of the cauline leaves: jagged and very acute in

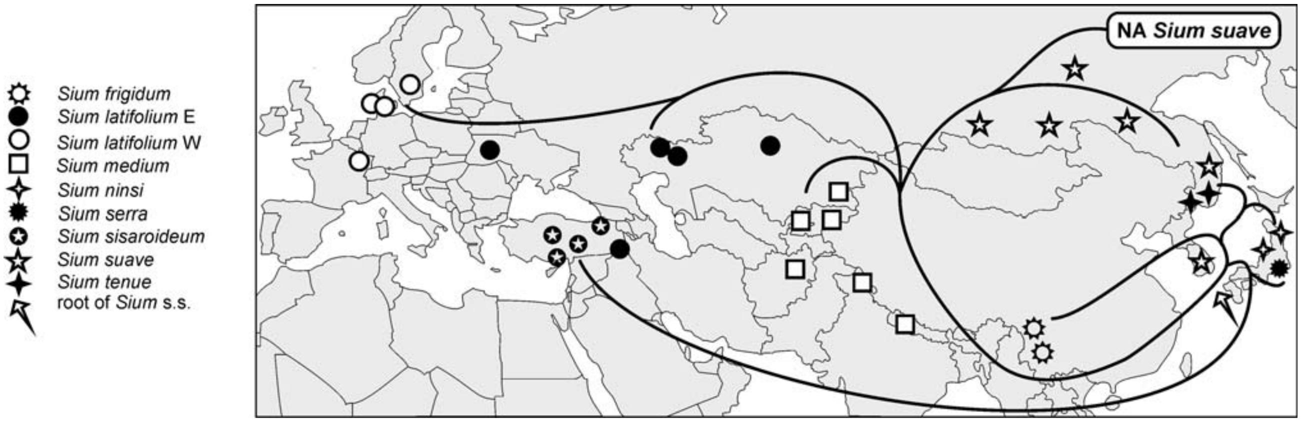

Fig. 5. A phylogeny of *Sium* s.s. traced on a map of the distribution of accessions sampled for this study. North American accessions of *S. suave* ("NA *Sium suave*") were omitted. E and W denote eastern and western ITS haplotypes of *S. latifolium*, respectively.

the north, more regularly and less deeply dentate in the south. However, intermediate specimens are common. These taxa also differ in their fruit structure. The African representatives have fruits that are relatively broad and almost round in outline, with a narrow commissure and small but obvious calyx teeth (Townsend, 1989). Their European congeners have narrower fruits with a broader commissure, but are deprived of calyx teeth at maturity (Arenas Posada and García Martín, 1993). The North American plants constitute var. *incisa*, and differ from the typical variety in having markedly dimorphic airborne leaves. Submerged, filiform-dissected leaves are also sometimes present, whereas the Old World plants have leaves all of the same shape (Cronquist, 1961).

The dispersal of *Berula* to North America is dated to 3 mya. During that time, the continuity of the North Atlantic land bridge had already been broken and the seasonality of the climate had increased; however, the migration of temperate taxa could still have occurred through island hopping (Tiffney and Manchester, 2001). The similarity of African and Eurasian-North American members of *Berula* is likely plesiomorphic. Therefore, *Berula erecta* is recognized as paraphyletic rather than polyphyletic, with *Afrocarum* and three species of *Sium* nested within. Such a case of morphological stasis in early branching lineages coupled with rapid character evolution in terminal branches has already been reported in *Osmorhiza*. The species traditionally placed in section *Osmorhiza* and sometimes reduced to subspecific rank due to their morphological similarities (Lowry and Jones, 1984) constitute the first branches off the phylogenetic tree. In contrast, the only member of the morphologically distinct *Osmorhiza* subgenus *Glycosma* occupied one of the terminal branches (Downie et al., 2000a; Wen et al., 2002; Yoo et al., 2002). It is noteworthy that for both genera this scenario followed a transcontinental dispersal: from Asia to North America in the case of *Osmorhiza* and from Eurasia to Africa in the case of *Berula*. Some invaders have retained their morphological and ecological characteristics due to stabilizing selection, whereas other lineages have undergone rapid evolution adapting to different habitats.

Schischkin (1950b) treated central Asian populations of *B. erecta* as a separate species, *B. orientalis* Woronow ex Schischk., claiming that they differed from the type in having shorter and narrower leaflets. Additionally, their leaf margins were described as finely crenate, whereas in the European plants they are irregularly and deeply toothed or incised. However, Hedge and Lamond (1987) included these central Asian specimens in *B. erecta* s.l., sinking Schischkin's *B. orientalis* into synonymy. The geographic distribution of *B. erecta* in Eurasia includes Europe, the Middle East and central Asia, reaching Kashmir to the southeast and the Lake Balkhash region to the northeast (Schischkin, 1950b; Hedge and Lamond, 1987; Mukherjee and Constance, 1993). Having examined a set of specimens from Europe, the Middle East, and central Asia we do not see any difference in leaf shape, size, and denticulation among them, despite their distinctiveness in the phylogenetic analyses. As their separation was recent, only ca. 1 mya, morphological differences are not yet apparent.

The African group and the evolution of St. Helena jellicos—With increased sampling from our previous study (Hardway et al., 2004), the monophyly of the group encompassing the African members of *B. erecta*, *Afrocarum imbricatum*, *Sium repandum*, and the two St. Helena endemics (*S. burchellii* and *S. bracteatum*) is not supported nor excluded. Moreover, we could not find any obvious morphological characters from habit, leaves, flowers, and fruits that would separate the African group from *Berula* s.s.

Although *Afrocarum imbricatum* was once regarded as related to *Carum* (Townsend, 1989) rather than to *Berula* or *Sium*, its placement in Oenantheae is not surprising because it has many characters typical for this tribe. It has a woody rhizome with numerous fibrous rootlets and occasionally sends out hypogeal stolons. Its leaves are simple pinnate, resembling those of *Berula*, although the leaflets are smaller, firmer, and more densely arranged. *Afrocarum imbricatum* occurs in "drier" habitats than does African *B. erecta*. The former occurs in bogs or swamps, especially along rivers, and also in damp montane grassland, whereas the latter is aquatic or semiaquatic (Townsend, 1989). The affinity of the St. Helena members of *Sium* to *Berula* rather than to their congeners is also not surprising. Their leaves with broad and rounded leaflets are more similar to those of *Berula* than to *Sium*. *Sium repandum*, however, due to its narrow leaflets and fruits with solitary vittae in each vallecula resembles its other congeners rather than *Berula*.

The St. Helena endemics, *Sium burchellii* and *S. bracteatum*, locally known as jellicos, are morphologically similar, differing only with respect to their growth habit and minor fruit characteristics. This morphological similarity, in conjunction with their identical ITS sequences, suggests that a single species should be recognized. However, when grown side by side from seed, these taxa maintain their distinctive characteristics (Cronk, 2000). In his analysis of the endemic flora of St. Helena, Cronk (1987) identified the African members of *Sium* as being the closest relatives to the St. Helena jellicos, giving *S. thunbergii* (=*B. erecta*) as an example. *Berula erecta* has a very wide distribution in Africa, from Sudan and Ethiopia through eastern Africa to Congo, Angola, and South Africa (Cannon, 1978). Our data also indicate that the populations of *Berula* from Arabia are related to the African members of this genus rather than to their European or Asian representatives. Due to the limited availability of material for molecular studies, only five samples of *B. erecta* from Africa were sequenced. Along with the single examined accession of *B. erecta* from Yemen, they form a clade that is sister to the jellicos. If the jellicos had been derived from the closest continental population of *B. erecta*, they should have been nested within this clade. Because they are not, the ancestor of jellicos presumably diverged before the spread of *B. erecta* in Africa. This spread was recent, as their ITS haplotypes diverged less than 0.5 mya. Moreover, the same haplotypes occur in Ethiopia and South Africa.

Cronk (1987) considered several migration routes into St. Helena. Because some of the endemic species of this island have their closest relatives in South Africa and the Mascarenes, he speculated that seeds arrived by means of oceanic currents (i.e., the Agulhas and Benguela Currents, respectively). However, it is unlikely that the fruits of *Berula*, in spite of their adaptation to floating, would survive such a long journey in salt water. The ancestor of jellicos may have been brought to the island by vagrant waterfowl. African moorhens [*Gallinula chloropus* (L.)] and cattle egrets [*Bulbulcus ibis* (L.)] are sporadic visitors to St. Helena (Basilewsky, 1970). Aquatic plants represent a disproportionately large number of angiosperm taxa characterized by broad distributions, including intercontinental disjunctions (Les et al., 2003). Long-distance dispersal by migratory birds seems to be the best explanation for this phenomenon. However, many of these taxa, including Nympheales and *Ceratophyllum,* are evolutionarily old and continental drift provides an alternative explanation for these disjunctions. Recently, Les et al. (2003) estimated divergence times for these disjunct taxa using molecular data. They found that most of these times are far too recent to implicate continental drift as a major determinant of these discontinuous distributions.

The St. Helena jellicos are edible. The stems of *S. bracteatum* were once collected and eaten by islanders as a raw green vegetable like celery (Cronk, 2000). In contrast, Townsend (1989) cited an annotation from a herbarium sheet of an African *B. erecta* that described the plant as extremely poisonous, causing death of cattle within 1 h. The Natural Resources Conservation Service Plants Database (http://plants.usda.gov) characterizes North American *B. erecta* as slightly toxic. This species contains many more phenolic compounds (coumarins) than other aquatic macrophytes (Smolders et al., 2000), and it is very rarely grazed by invertebrate herbivores (Sand-Jensen and Madsen, 1989; Rowell and Blinn, 2003). Through long-distance dispersal, it is possible that the ancestor of jellicos escaped from its herbivores. The ability to produce phenolic compounds may have been no longer essential for its survival and, therefore, it was successively lost. Additionally, the seed protection structures in the fruits of jellicos, such as vittae (resin-containing canals), are much reduced when compared to those of their closest relatives. Vittae surround the endosperm and not only provide mechanical protection, but also contain active compounds toxic to insects (Berenbaum, 1990). These canals are numerous and very well developed in the fruits of Eurasian *B. erecta*, forming a continuous layer around the seed (Hedge and Lamond, 1987; Arenas Posada and García Martín, 1993), and while these canals are less numerous in the African specimens (Townsend, 1989), they are also tightly arranged around the seed. However, in the jellicos, the vittae are either two (*S. burchellii*) or three (*S. bracteatum*) in each furrow and are relatively small (Cronk, 2000). Similar reductions in fruit defensive structures and chemical protection following long-distance dispersal and escape from fruit predation have been reported previously for two other umbellifer genera, *Chaerophyllum* and *Pastinaca* (Spalik et al., 2001; Zangerl and Berenbaum, 2005).

Southern Palearctic species of Sium—The six species of *Sium* comprising the southern Palearctic clade are distributed in Eurasia usually more to the south than members of the northern Holarctic clade (Fig. 5). *Sium sisarum* is known only from cultivation, while its wild form, *S. sisaroideum*, occurs in the eastern Mediterranean and Irano-Turanian regions. The remaining members of this clade, *S. serra*, *S. frigidum*, *S. tenue*, and *S. ninsi*, are distributed in eastern Asia. *Sium serra* and *S. ninsi* occur in Japan (Hiroe and Constance, 1958), *S. frigidum* grows in the montane regions of southwestern China (Pimenov and Kljuykov, 2003), and *S. tenue* is reported from the Ussuri region in the Russian Far East (Schischkin, 1950a). Although the members of this clade differ considerably in habit, ranging from dwarf montane plants (*S. frigidum*) to tall robust herbs (*S. sisarum*), they share one common characteristic: fascicled tuberous roots. The other members of *Sium* s.l. also have fascicled roots, but they are not swollen. However, this character is homoplastic within the tribe as swollen roots also occur in North American *Perideridia*, as well as in several species of *Oenanthe*.

Sium sisarum was once widely cultivated in Asia and Europe for its roots, which are rich in carbohydrates, but the introduction of the potato initiated its demise (Rostafiński, 1884). It differs from the wild *S. sisaroideum* in having longer and thicker roots and shorter stems. These two taxa are regarded either as representing a single species with two varieties, *S. sisarum* var. *sisarum* and var. *lancifolium* (M. Bieb.) Thell. (Peşmen, 1972), or two separate species (Hedge and Rechinger, 1987; Pimenov and Kljuykov, 2002). Our data confirm that these two taxa are very closely related, suggesting that the cultivated plant has been derived from *S. sisaroideum*. Therefore, we favor the recognition of a single species with two varieties.

Sium ninsi occurs in Korea (Lee, 1985, 1996) and Japan (Hiroe and Constance, 1958; Ohwi, 1965; Ohba, 1999). Interestingly, Japanese authors treat this taxon as conspecific with *S. sisarum*, probably because both have swollen roots. However, such roots are characteristic for the entire clade, whereas the leaf morphology of these species is different. The upper cauline leaves of *S. ninsi* are distinctly ternate with the petiole longer than the leaf blade, whereas the cauline leaves of *S. sisarum* are pinnate, similar to most other congeners. Based on the results of the phylogenetic analyses, *S. ninsi* is very closely related to *S. tenue*. These two species are nearly identical, with *S. tenue* having somewhat narrower leaf divisions. Schischkin (1950a) described *S. tenue* as an endemic of the Ussuri region. Gorovoy and Ulanova, in the label of exsiccata of the flora of the Soviet Union no. 5981, wrote that this species also occurs in NE China. It is, however, not listed in the old edition of the *Flora of China* (Wang, 1985; Pu et al., 1992), nor is it included in the new one (Pu and Watson, 2005b). If collected without underground parts, *S. tenue* is easily confused with *S. suave* because both have narrow leaf divisions. It differs from the latter in having smaller umbels (up to 5 cm diameter) with fewer umbellets (ca. 10) and fewer bracts. However, we have only examined a few specimens of this taxon. Another species of *Sium* with tuberous roots, *S. heterophyllum* Y. N. Lee, nom. illeg., was described from two localities in Korea (Lee, 1996). The plant illustrated in this account is in fact the cultivated *S. sisarum*.

Sium serra was originally described by Franchet and Savatier (1879) in *Pimpinella*. However, these authors remarked that this placement was not certain because they had not seen ripe fruits. They also noted the similarity of this species to certain members of *Sium* and *Cryptotaenia japonica*. The species was transferred to *Sium* by Kitagawa (1941), and this treatment was subsequently adopted in standard Japanese floras (Ohwi, 1965; Ohba, 1999). However, several taxonomic studies (Hiroe and Constance, 1958; Pu, 1985; Pu and Watson, 2005a) retained this species in *Pimpinella* despite its striking similarity to *Sium*. This similarity is reflected in its simple pinnate leaves, lobes with finely serrate margins in the airborne leaves and dissected in the submerged leaves, and fascicled, thickened roots. In phylogenetic analyses using *rbcL* sequences, *S. serra* grouped with other members of Oenantheae (Kondo et al., 1996). Our results confirm the placement of this species in *Sium*.

The identity of *S. frigidum* is subject to controversy. This species was described by Handel-Mazzetti (1933) from a montane region in NW Yunnan, China. It is a dwarf plant reaching up to 20 cm and resembles its congeners in having simple pinnate leaves, thickened fascicled roots, and vertical rhizomes with adventitious roots (Pimenov and Kljuykov, 2003). Shan in Pu (1993) transferred this species to *Chamaesium* H. Wolff, a genus of dwarf montane perennials with simple pinnate leaves. Having examined the type of *S. frigidum* at WU, Pimenov and Kljuykov (2003) synonymized this species with another obscure Chinese species, *Apium ventricosum* H. Boissieu. An accession of *S. frigidum*/*A. ventricosum* examined by Valiejo-Roman et al. (2002) was related neither to *Sium* nor to *Apium* L. but to *Sinocarum* H. Wolff ex R. H. Shan & F. T. Pu. In contrast, our results indicate that *S. frigidum* clearly belongs within *Sium*. We have examined the typical gathering of this species, *Handel-Mazzetti 7684* (E), and we can confirm that it is conspecific with specimens *FED 109* and *Alden et al. 595* used in this study. The Handel-Mazzetti specimens are preserved with underground parts: one plant shows a distinct rhizome with adventitious roots, whereas another bears fascicled, tuberous roots. Immature fruits are also present and these resemble those illustrated in Pu et al. (1992), although the filiform ridges are not as prominent (but they may develop at fruit maturity). The fruit dissection published by Pu et al. (1992) also falls within the variation seen in *Sium* fruits, with its distinct ridges presumably due to thickened vascular bundles. Pimenov and Kljuykov (2003) questioned the accuracy of this drawing, and in their own drawing the fruit has no ridges and the vascular bundles are inconspicuous. Therefore, it is probable that they are referring to another species that is distantly related to *Sium*.

We also sequenced the ITS region from an accession of *Chamaesium paradoxum*, the nomenclatural type of *Chamaesium*, to test its affinity to *Sium*. *Chamaesium paradoxum* is unrelated to *Sium* or to any other member of tribe Oenantheae but constitutes one of the early branches of the apioid phylogenetic tree (K. Spalik and S. R. Downie, unpublished data). Because we have not seen the type of *Apium ventricosum*, we cannot confirm that this specimen is conspecific with *S. frigidum*. However, whatever specific epithet this species will bear, its correct placement is beyond any doubt in *Sium*.

Northern Holarctic clade—The northern Holarctic clade of *Sium* includes only three species, *S. latifolium*, *S. medium*, and *S. suave*. We have included in *S. medium* those accessions from India and Nepal that are traditionally placed in *S. latijugum* C.B. Clarke because these two taxa most likely represent a single species (Pimenov and Kljuykov, 2002); they were previously separated based on geographic distribution and political borders rather than by any substantive morphological difference. Schischkin (1950a) treated *S. medium* as an endemic of central Asia, with its distribution extending from the Irtysh region of western Siberia through the Lake Balkhash area to Tien Shan. *Sium latijugum* is distributed in the western Himalayas from Himachal Pradesh to Pakistan and northern Afghanistan (Hedge and Rechinger, 1987; Mukherjee and Constance, 1993). Both are relatively small plants (30–80 cm high) compared to their closest relatives, *S. latifolium* and *S. suave*, which are more robust (70–120 cm high). Our analyses confirm that both taxa are very closely related and that a single species, *S. medium*, should be recognized.

The two other members of this clade, *S. latifolium* and *S. suave*, are characterized by relatively broad geographic distributions. Both show very distinct phylogeographic patterns (Fig. 5) that may have been shaped by Quaternary climatic oscillations (Hewitt, 2004). The genetic variation in *S. latifolium* is poorer than that shown by *S. suave*, suggesting a stronger bottleneck effect in the former. The split between Asian and North American populations of *S. suave* is very recent indicating a late migration of this species into North America. The conditions for dispersal through the Bering land bridge were optimal in the Eocene and then deteriorated due to increasing seasonality and opening of the Bering Straits (Tiffney and Manchester, 2001). However, many temperate and Arctic species migrated during the late Tertiary and Quaternary, and Beringia was a major refugium for some Arctic species through the recent Ice Ages (Hewitt, 2004).

Taxonomy of Berula and Sium—With *Afrocarum* and three species of *Sium* nested within the *Berula* s.l. clade, the present taxonomic treatments of *Sium* and *Berula* are untenable. Although the *Berula* s.l. clade is very strongly supported in all analyses, the monophyly of *Sium* has not been confirmed. Additional data are therefore necessary before any nomenclatural changes are proposed.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

Álvarez, I., and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

Arenas Posada, J. A., and F. García Martín. 1993. Atlas carpológico y corológico de la subfamilia Apioideae Drude (Umbelliferae) en España peninsular y Baleares. *Ruizia* 12: 1–245.

Avise, J. C. 2004. Molecular markers, natural history, and evolution. Sinauer, Sunderland, Massachusetts, USA.

Basilewsky, P. 1970. La faune terrestre de l'Île de Sainte-Hélène (première partie). Vertébrés. *Annales du Musée Royal de l'Afrique Centrale, Tervuren, serie 8* 181: 77–130.

Berenbaum, M. R. 1990. Evolution of specialization in insect–umbellifer associations. *Annual Review of Entomology* 35: 319–343.

Bremer, K. 1992. Ancestral areas: a cladistic reinterpretation of the center of origin concept. *Systematic Biology* 41: 436–445.

Bremer, K. 1994. Branch support and tree stability. *Cladistics* 10: 295–304.

Burtt, B. L. 1991. Umbelliferae of southern Africa: an introduction and annotated check-list. *Edinburgh Journal of Botany* 48: 133–282.

Cannon, J. F. M. 1978. Umbelliferae. *In* E. Launert [ed.], Flora zambesiaca, vol. 4, 555–621. Flora Zambesiaca Managing Committee, London, UK.

Cronk, Q. C. B. 1987. The history of endemic flora of St. Helena: a relictual series. *New Phytologist* 105: 509–520.

Cronk, Q. C. B. 2000. The endemic flora of St. Helena. Anthony Nelson, Oswestry, UK.

Cronquist, A. 1961. *Berula* Hoffm. *In* C. L. Hitchcock and A. Cronquist [eds.], Vascular plants of the Pacific North West, vol. 3, 519. University of Washington Press, Seattle, Washington, USA.

Donoghue, M. J., C. D. Bell, and J. Li. 2001. Phylogenetic patterns in northern hemisphere plant geography. *International Journal of Plant Sciences* 162: S41–S52.

Downie, S. R., D. S. Katz-Downie, and K. Spalik. 2000a. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 87: 76–95.

Downie, S. R., G. M. Plunkett, M. F. Watson, K. Spalik, D. S. Katz-Downie, C. M. Valiejo-Roman, E. I. Terentieva, A. V. Troitsky, B. Y. Lee, J. Lahham, and A. El-Oqlah. 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. *Edinburgh Journal of Botany* 58: 301–330.

Downie, S. R., S. Ramanath, D. S. Katz-Downie, and E. Llanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. *American Journal of Botany* 85: 563–591.

Downie, S. R., M. F. Watson, K. Spalik, and D. S. Katz-Downie. 2000b. Molecular systematics of Old World Apioideae (Apiaceae): relationships among some members of tribe Peucedaneae sensu lato, the placement of several island endemic species, and resolution within the apioid superclade. *Canadian Journal of Botany* 78: 506–528.

Drude, C. G. O. 1897–1898. Umbelliferae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, vol. 3, 63–250. Wilhelm Engelmann, Leipzig, Germany.

Enghoff, H. 1996. Historical biogeography of the Holarctic: area relationships, ancestral areas, and dispersal of non-marine animals. *Cladistics* 11: 223–263.

Eriksson, T. 1998. AutoDecay, version 4.0 (program distributed by the author). Department of Botany, Stockholm University, Stockholm, Sweden.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Felsenstein, J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics* 22: 521–565.

Franchet, A., and L. Savatier. 1879. Enumeratio plantarum in Japonia sponte crescentium. F. Savy, Paris, France.

Graham, A. 1999. The Tertiary history of the northern temperate element in the northern Latin America biota. *American Journal of Botany* 86: 32–38.

Handel-Mazzetti, H. F. 1933. Umbelliferae. *In* H. F. Handel-Mazzetti [ed.], Symbolae sinicae, vol. 7, no. 3, 706–730. Springer, Wien, Austria.

Hardway, T. M., K. Spalik, M. F. Watson, D. S. Katz-Downie, and S. R. Downie. 2004. Circumscription of Apiaceae tribe Oenantheae. *South African Journal of Botany* 70: 393–406.

Hedge, I. C., and J. M. Lamond. 1987. *Berula* Hoffm. *In* I. C. Hedge, J. M. Lamond, and K. H. Rechinger [eds.], Umbelliferae, *in* K. H. Rechinger [ed.], Flora iranica, vol. 162, 305–306. Akademische Druck- und Verlagsanstalt, Graz, Austria.

Hedge, I. C., and K. H. Rechinger. 1987. *Sium* L. *In* I. C. Hedge, J. M. Lamond, and K. H. Rechinger [eds.], Umbelliferae, *in* K. H. Rechinger [ed.], Flora iranica, vol. 162, 303–304. Akademische Druck- und Verlagsanstalt, Graz, Austria.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society, London, B* 359: 183–195.

Hiroe, M., and L. Constance. 1958. Umbelliferae of Japan. *University of California Publications in Botany* 30: 1–144.

Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson. 1998. Multiple sequence alignment with CLUSTAL X. *Trends in Biochemical Sciences* 23: 403–405.

Kitagawa, M. 1941. Miscellaneous notes on Apiaceae (Umbelliferae) of Japan and Manchuria (IV). *Journal of Japanese Botany* 17: 557–562.

Kondo, K., S. Terabayashi, M. Okada, C. Q. Yuan, and S. N. He. 1996. Phylogenetic relationship of medicinally important *Cnidium officinale* and Japanese Apiaceae based on *rbcL* sequences. *Journal of Plant Research* 109: 21–27.

Lee, T. B. 1985. Illustrated flora of Korea. Hyangmoonsa, Seoul, Korea.

Lee, Y. N. 1996. Flora of Korea. Kyo-Hak, Seoul, Korea.

Les, D. H., D. J. Crawford, R. T. Kimball, M. L. Moody, and E. Landolt. 2003. Biogeography of discontinuously distributed hydrophytes: a molecular appraisal of intercontinental disjunctions. *International Journal of Plant Sciences* 164: 917–932.

Lowry, P. P., and A. G. Jones. 1984. Systematics of *Osmorhiza* Raf. (Apiaceae, Apioideae). *Annals of the Missouri Botanical Garden* 71: 1128–1171.

Mukherjee, P. K., and L. Constance. 1993. Umbelliferae (Apiaceae) of India. American Institute of Indian Studies and Oxford and IBH Publishing, New Delhi, India.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York, USA.

Nelson, G. 1978. From Candolle to Croizat: comments on the history of biogeography. *Journal of the History of Biology* 11: 269–305.

Ohba, H. 1999. Umbelliferae. *In* K. Iwatsuki, D. E. Boufford, and H. Ohba [eds.], Flora of Japan, vol. 2c, 268–303. Kodansha, Tokyo, Japan.

Ohwi, J. 1965. Flora of Japan. Smithsonian Institution, Washington, D.C., USA.

Palacios Chávez, R., and J. Rzedowski. 1993. Estudio palinológico de las floras fosiles del Miocene inferior y principios del Mioceno medio de la región de Pichucalco, Chiapas, Mexico. *Acta Botánica Mexicana* 24: 1–96.

Pesmen, H. 1972. *Sium* L. *In* P. H. Davis [ed.], Flora of Turkey and the east Aegean Islands, vol. 4, 365–366. Edinburgh University Press, Edinburgh, Scotland.

Pimenov, M. G., and M. V. Leonov. 1993. The genera of the Umbelliferae. Royal Botanic Gardens, Kew, UK.

Pimenov, M. G., and E. V. Kljuykov. 2002. Zontichnye (Umbelliferae) Kirgizii. KMK Scientific Press, Moscow, Russia.

Pimenov, M. G., and E. V. Kljuykov. 2003. What is *Sium frigidum* Hand.-Mazz. (Umbelliferae)? *Feddes Repertorium* 114: 350–357.

Plunkett, G. M., and S. R. Downie. 1999. Major lineages within Apiaceae subfamily Apioideae: a comparison of chloroplast restriction site and DNA sequence data. *American Journal of Botany* 86: 1014–1026.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1996. Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. *Systematic Botany* 21: 477–495.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

PU, F. 1985. *Pimpinella* L. *In* R. H. Shan and M. L. Sheh [eds.], Flora Reipublicae Popularis Sinicae, vol. 55, no. 2, 67–114. Academia Sinica, Beijing, China.

PU, F. 1993. Umbelliferae. *In* W. T. Wang and S. G. Wu [eds.], Vascular plants of the Hengduan Mountains, vol. 1, 1276–1355. Science Press, Beijing, China.

PU, F., M. L. SHEH, AND S. L. LIOU. 1992. *Sium* L. *In* R. H. Shan and M. L. Sheh [eds.], Flora Reipublicae Popularis Sinicae, vol. 55, no. 3, 246, 249–250. Academia Sinica, Beijing, China.

PU, F., AND M. F. WATSON. 2005a. *Pimpinella* Linnaeus. *In* M. She, F. Pu, Z. Pan, M. F. Watson, J. F. M. Cannon, I. Holmes-Smith, E. V. Kljuykov, L. R. Phillippe, and M. G. Pimenov [eds.], Apiaceae, *in* Flora of China Editorial Committee [ed.], Flora of China, vol. 14, 93–104. Missouri Botanical Garden, St. Louis, Missouri, USA.

PU, F., AND M. F. WATSON. 2005b. *Sium* Linnaeus. *In* M. She, F. Pu, Z. Pan, M. F. Watson, J. F. M. Cannon, I. Holmes-Smith, E. V. Kljuykov, L. R. Phillippe, and M. G. Pimenov [eds.], Apiaceae, *in* Flora of China Editorial Committee [ed.], Flora of China, vol. 14, 115–116. Missouri Botanical Garden, St. Louis, Missouri, USA.

RAVEN, P. H., AND D. I. AXELROD. 1972. Plate tectonics and Australasian paleobiogeography. *Science* 176: 1379–1386.

RAXWORTHY, C. R., M. R. J. FORSTNER, AND R. A. NUSSBAUM. 2002. Chameleon radiation by oceanic dispersal. *Nature* 415: 784–787.

RONQUIST, F. 1994. Ancestral area and parsimony. *Systematic Biology* 43: 267–274.

RONQUIST, F. 1996. DIVA version 1.1. Computer program and manual available at website http://ftp.uu.se or http://ftp.systbot.uu.se [Accessed on March 1, 2005].

RONQUIST, F. 1997. Disperal–vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* 46: 195–203.

ROSTAFIŃSKI, J. 1884. Kucmerka (*Sium sisarum*) pod względem geograficzno-botanicznym i historyi kultury. Drukarnia Uniwersytetu Jagiellońskiego, Cracow, Poland.

ROWELL, K., AND D. W. BLINN. 2003. Herbivory on a chemically defended plant as a predation deterrent in *Hyalella azteca*. *Freshwater Biology* 48: 247–254.

SAND-JENSEN, K., AND T. V. MADSEN. 1989. Invertebrates graze submerged rooted macrophytes in lowland streams. *Oikos* 55: 420–423.

SANG, T., D. J. CRAWFORD, S.-C. KIM, AND T. F. STUESSY. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez islands: evidence from sequences of the ITS regions of nuclear ribosomal DNA. *American Journal of Botany* 81: 1494–1501.

SANG, T., D. J. CRAWFORD, T. F. STUESSY, AND M. O. SILVA. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany* 20: 55–64.

SANMARTÍN, I., AND F. RONQUIST. 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* 53: 216–243.

SCHISCHKIN, B. K. 1950a. *Sium* L. *In* B. K. Schischkin [ed.], Flora SSSR, vol. 16, 458–467. Izdatelstvo Akademii Nauk SSSR, Moscow, Russia (formerly USSR).

SCHISCHKIN, B. K. 1950b. *Berula* Hoffm. *In* B. K. Schischkin [ed.], Flora SSSR, vol. 16, 466–468. Izdatelstvo Akademii Nauk SSSR, Moscow, Russia (formerly USSR).

SMOLDERS, A. J. P., L. H. T. VERGEER, G. VAN DER VELDE, AND J. G. M. ROELOFS. 2000. Phenolic contents of submerged, emergent and floating leaves of aquatic and semiaquatic macrophyte species: why do they differ? *Oikos* 9: 307–310.

SPALIK, K., A. WOJEWÓDZKA, AND S. R. DOWNIE. 2001. The evolution of fruit in Scandiceae subtribe Scandicinae (Apiaceae). *Canadian Journal of Botany* 79: 1358–1374.

SUH, Y., L. B. THIEN, H. E. REEVE, AND E. A. ZIMMER. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *American Journal of Botany* 80: 1042–1055.

SWOFFORD, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA

TIFFNEY, B. H., AND S. R. MANCHESTER. 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences* 162: S3–S17.

TOWNSEND, C. C. 1989. Umbelliferae. *In* R. M. Polhill [ed.], Flora of tropical east Africa, 1–127. AA Balkema, Rotterdam, Netherlands.

VALIEJO-ROMAN, C. M., E. I. TERENTIEVA, T. H. SAMIGULLIN, AND M. G. PIMENOV. 2002. nrDNA ITS sequences and affinities of Sino-Himalayan Apioideae (Umbelliferae). *Taxon* 51: 685–701.

VAN MOORSEL, R. C. M. J., AND O. BAUDEWIJN. 2000. *Apium*, *Berula* en *Sium*. *Gorteria* 26: 244–245.

VENCES, M., J. FREYHOF, R. SONNENBERG, J. KOSUCH, AND M. VEITH. 2001. Reconciling fossils and molecules: Cenozoic divergence of cichlid fishes and the biogeography of Madagascar. *Journal of Biogeography* 28: 1091–1099.

WANG, T. S. 1985. *Sium* L. *In* R. H. Shan and M. L. Sheh [eds.], Flora Reipublicae Popularis Sinicae, vol. 55, no. 2, 155–158. Academia Sinica, Beijing, China.

WEN, J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annual Review of Ecology and Systematics* 30: 421–455.

WEN, J. 2001. Evolution of eastern Asian–eastern North American biogeographic disjunctions: a few additional issues. *International Journal of Plant Sciences* 162: S117–S122.

WEN, J., P. P. LOWRY, J. L. WALCK, AND K. O. YOO. 2002. Phylogenetic and biogeographic diversification in *Osmorhiza* (Apiaceae). *Annals of the Missouri Botanical Garden* 89: 414–428.

WEN, J., AND E. A. ZIMMER. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6: 167–177.

WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995. An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient, cryptic intergenomic introgression. *Molecular Phylogenetics and Evolution* 4: 298–313.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

XIANG, Q.-Y., AND D. E. SOLTIS. 2001. Dispersal–vicariance analyses of intercontinental disjuncts: historical biogeographical implications for angiosperms in the northern hemisphere. *International Journal of Plant Sciences* 162: S29–S39.

YOO, K. O., P. P. LOWRY, AND J. WEN. 2002. Discordance of chloroplast and nuclear ribosomal DNA data in *Osmorhiza* (Apiaceae). *American Journal of Botany* 89: 966–971.

YUAN, Y.-M., S. WOHLHAUSER, M. MÖLLER, J. KLACKENBERG, M. W. CALLMANDER, AND P. KÜPFER. 2005. Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian Ocean resulted from long distance dispersal and extensive radiation. *Systematic Biology* 54: 21–34.

ZANGERL, A., AND M. BERENBAUM. 2005. Increase in toxicity of an invasive weed after reassociation with its coevolved herbivore. *Proceedings of the National Academy of Sciences, USA* 102: 15529–15532.

APPENDIX. Plant accessions from which nuclear ribosomal DNA ITS sequences were obtained. Accession numbers of newly obtained sequences start with the letter D; those that were updated for this study are marked with an asterisk. Herbarium acronyms: E = Royal Botanic Garden Edinburgh, G = Conservatoire et Jardin botaniques de la Ville de Genève; ILL = University of Illinois, Urbana-Champaign; ILLS = Illinois Natural History Survey; JEPS = Jepson Herbarium, Berkeley; LE = V.L. Komarov Botanical Institute, St. Petersburg; MO = Missouri Botanical Garden; RM = Rocky Mountain Herbarium, University of Wyoming; UC = University of California, Berkeley; WA = Warsaw University, Warszawa.

Taxon—DNA identifier; GenBank accession number; Country of origin, *Source* (Herbarium).

Afrocarum imbricatum **(Schinz) Rauschert**—K132; AY360228; Tanzania, *Kayombo & Kayombo 217* (MO 04672352). K151; DQ005640; Malawi, *Pawek 8513* (MO 2214486). 1444; AY360229; Malawi, *Pawek 5442* (MO 2189561).

Berula erecta **(Huds.) Coville subsp. *erecta* var. *erecta***—150; U79607; Germany, *Downie 150* (ILL). 2253; AY360230; Denmark, *Petersen GPL28* (ILL). 2257; AY360231; Denmark, *Seberg OSA 486* (ILL). K185; DQ005641; Bulgaria, *Vihodcevsky s.n.* (WA). ***B. erecta*** **subsp. *erecta* "*B. orientalis* Schischk."**—2810; DQ005650; Uzbekistan, *Tsvelev* (LE). 2811; DQ005651; Kyrgyzstan, *Konnov et al. 394* (LE). E110; DQ005652; Iran, Azerbaijan, *Bot. Dep. Univ. Tehran 266* (E). E111; DQ005653; Iran, Kurdestan, *Runemark & Mozaffarian 27446* (E). E114; DQ005654; Turkey, B9 Van, *Davis 46001* (E). E115; DQ005655; Turkey, Adapazari, *Davis & Coode 36264* (E). E116; DQ005657; Iran, Arak, *Jardine 764B* (E). E117; DQ005656; Pakistan, *Burtt 677* (E). ***B. erecta*** **subsp. *erecta* var. *incisa* (Torr.) Cronquist**—503; DQ005647; USA, California, *Raiche and Zadnik RR50099* (UC); cult. UC Bot. Gard., Berkeley, no. 85.0288 (ILL). 1144; DQ005643; USA, California, *Sanders 6612* (UC 1535388). 1816; DQ005645; USA, Nebraska, *Shildneck C-6829* (ILLS 153954). K199; DQ005649; USA, Nevada, *Tiehm & Williams 6703* (UC 1481205). 1111; DQ005642; USA, Wyoming, *Nelson 29396* (RM). 1530; DQ005644; USA, California, *Oswald 2171* (UC 1532346). 2157; DQ005646; USA, Nevada, *Holmgren & Holmgren 4577* (ILL). K198; DQ005648; USA, Wyoming, *Evert 5084* (UC 1534163). ***B. erecta*** **subsp. *thunbergii* (DC.) B.L.Burtt**—799; U78369; Ethiopia, *Constance pers. coll. C-2453* (UC). K82; *AY353975; Zimbabwe, *Bayliss 10592* (MO 3258774). K116; *AY353976; Ethiopia, *Ash 859* (MO 2298256). E112; DQ005658; South Africa, *Balter 1135* (E). E113; DQ005659; South Africa, *Balter 933* (E 00110387). E119; DQ005660; Yemen, *Heckel & Wood Y1215* (E).

Cicuta virosa **L.**—75; U78372; Finland; *Downie 75* (ILL). ***Cryptotaenia canadensis*** **(L.) DC.**— 817; U79613; USA, Illinois, *Downie 817* (ILL). ***C. japonica*** **Hassk.**—402; AY360236; China; *Downie 402* (ILL).

Helosciadium bermejoi **(L. Llorens) Popper & M.F. Watson**—62449; *AY353979; Spain, Balearic Islands, cult. RBG Edinburgh, no. 19962449 (E). ***H. crassipes*** **W.D.J. Koch ex Rchb.**—K170; AY360239; France, Corse, *Reduron* (ILL). ***H. inundatum*** **(L.) W.D. J. Koch**—64358; *AF164822; Italy, Sicily, *Davis & Sutton 64358* (E). ***H. nodiflorum*** **(L.) W.D.J. Koch**—919; AF164823; Jordan, *Lahham & El-Oqlah 18* (Yarmouk Univ. Herb.). 1871; AY360240; France, *Reduron s.n.* (ILL). E120; DQ005661; Saudi Arabia, *Collenette 7289* (E). ***H. repens*** **(Jacq.) W.D.J. Koch**—1870; AY360241; France, cult. Cons. bot. Mulhouse, no. 9463, *Reduron s.n.* (ILL).

Neogoezia macvaughii **Constance**—K70; DQ005662; Mexico, *Breedlove 35768* (MO 3238958). ***N. planipetala*** **Hemsl.**—K72; DQ005663; Mexico, *Tenorio & Flores 16030* (MO 4036088).

Oenanthe fistulosa **L.**—K191; DQ005664; Poland, Nowe Warpno near Szczecin (WA 075167). ***Oxypolis occidentalis*** **J.M. Coult. & Rose**—1142; AY360254; USA, California, *Follette s.n.* (JEPS).

Sium bracteatum **(Roxb.) Cronk**—MW; *AY353981; St Helena; material provided by M. Watson (E). K177; *AY353982; St Helena; material provided by V. Williams (WA). ***S. burchellii*** **(Hook. f.) Hemsl.**—K178; *AY353983; St. Helena, material provided by V. Williams (WA). ***S. frigidum*** **Hand.-Mazz.**—109; *AF164842; China, Yunnan, *FED 109* (E). 2337; DQ005665; China, Yunnan, *Alden et al 595* (E 00003284). ***S. latifolium*** **L.**—E; *AY353984; Denmark, *Davis s.n.* (E). 1632; AY360257; France, cult. Cons. bot. Mulhouse, no. 9466, *Reduron s.n.* (ILL). 2256; AY360258; Denmark, *Petersen & Seberg GPL31* (ILL). K179; DQ005669; Sweden, *Sylven s.n.* (WA). K180; DQ005670; Ukraine, Czernihov distr., *Verschkovskii s.n.* (WA). K215; DQ005671; Kazakhstan, Uralskaja obl., *Klinkova s.n.* (MO). 2806; DQ005666; Kazakhstan, Akmolinsk (Astana) obl., *Grubov 53* (LE). 2807; DQ005667; Kazakhstan, Uralskaja obl., *Bjalt* (LE). E129; DQ005668; Turkey, C10 Hakkari, *Davis 45787* (E). ***S. medium*** **Fisch. & C.A. Mey.**—K58; DQ005676; India, W Ladakh, *Hartmann 3130* (G 349468). K59; DQ005677; Kyrgyzstan, *Pimenov et al. 703* (G 211675). E127; DQ005675; Afghanistan, *Podlech 12634* (E). 2342; DQ005672; Nepal, *Polunin et al. 4866* (E 00041833). 2808; DQ005673; Kazakhstan, Alma-Ata obl., *Tasekeev & Novikova 471* (LE). 2809; DQ005674; Kyrgyzstan, Kotshkor, *Konnov & Kotshgareva 456* (LE). ***S. ninsi*** **L.**—K60; DQ005679; Japan, Honshu, *Togasi 1710* (G). K122; DQ005678; Japan, Tohoku distr., *Iwasaki 127* (MO 4253273). ***S. repandum*** **Welw. ex Hiern**—K61; AY353977 (ITS-1 only); South Africa, *Rogers 9101* (G). K126; DQ005680; South Africa, *van Hoepen 1695* (MO 4348119). ***S. serra*** **(Franch. & Sav.) Kitag.**—K123; DQ005681; Japan, Honshu, *Tateishi et al. 14776* (MO 3883493). ***S. sisaroideum*** **DC**—2340; DQ005682; Turkey, Maras, *Davis et al. 20412* (E 00158000). E124; DQ005683; Turkey, C6 Adana, *Darrah 670* (E). E125; DQ005684; Turkey, C6 Adana, *Darrah 651* (E). E126; DQ005685; Turkey, C6 Hatay, *Darrah 625* (E). E128, DQ005686; Turkey, Zonguldak, *Davis & Coode 38988* (E). E130; DQ005687; Turkey, C6 Hatay, *Darrah 571* (E). E132; DQ005688; Turkey, A9 Kars, *Davis 46661* (E). ***S. sisarum*** **L.**—97; U78370; Hungary, cult., *Downie 97* (ILL). 311; AY360259; France, cult., *Downie 311* (ILL). 388; AY360260; Canada, cult., *Downie 388* (ILL). 53; AY360261; Spain, cultivated, *Downie 53* (ILL). 83; AY360262; Finland, cultivated, *Downie 83* (ILL). ***S. suave*** **Walter**—12; AY360263; Canada, *Downie 12* (ILL). 1494; DQ005689; USA, Illinois, *Morris et al. 849* (ILLS 182643). 1655; DQ005690; USA, Illinois, *Simon et al. 436* (ILLS 192873). 1656; DQ005691; USA, Illinois, *Masi & Epting 580* (ILLS 192171–1). 1808; DQ005692; USA, Illinois, *Hill et al. 28584* (ILLS 195322). 1811; DQ005693; USA, Illinois, *Phillippe 22699* (ILLS 181623). K124; DQ005700; Russia, Siberia, Yakutsk region, *McNeal et al. 697* (MO). K182; DQ005701; Russia, Siberia, Nertschynsk, *Karo 505* (WA). K184; DQ005702; Russia, Siberia, prov. Irkutsk, *Malcev 2646* (WA). 2804; DQ005698; Russia, Siberia, Amurskaja oblast, *Lipatova & Petrova* (LE). 2805; DQ005699; Russia, Siberia, Primorje, *Ovsiannikova* (LE). K197; DQ005703; USA, Montana, *Halse 4803* (UC 1606457). 1815; DQ005694; USA, Illinois, *Feist 77* (ILLS 194650). 1965; DQ005695; USA, Illinois, *Smith 1404-b* (ILLS 175302). 1968; DQ005696; USA, Illinois, *Hill & Tessene 28800* (ILLS 190100). 2013; DQ005697; USA, Illinois, *Phillippe 22880* (ILLS 183654). GB; AY548213; Submitted to GenBank by Choi, Choi, and Jung, South Korea. ***S. tenue*** **Kom.**—E134; DQ005704; Russia, Siberia, Primorje, *Pimenov & Kljuykov 213* (E). K63; DQ005706; Russia, Siberia, Primorje, *Ulanova 5981* (G 234160). K125; DQ005705; Russia, Siberia, Primorje, *Ulanova 5981* (MO 4376176).