Generic delimitations within the *Sium* alliance (Apiaceae tribe Oenantheae) inferred from cpDNA \(rps16-5'trnK^{(UUU)}\) and nrDNA ITS sequences

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Previous studies of the *Sium* alliance within Apiaceae tribe Oenantheae, based only on nrDNA ITS sequences, revealed that the genera *Sium* and *Berula* may not be monophyletic. To confirm issues of relationships and implement nomenclatural changes, we obtained additional ITS sequences as well as independent data from the cpDNA \(rps16-5'trnK^{(UUU)}\) region which includes the \(rps16\) intron and the spacer region between genes \(rps16\) and \(5'trnK\). We examined 78 accessions of tribe Oenantheae including representatives of all 23 species of the *Sium* alliance (*Afrocarum*, 1 sp.; *Apium* pro parte/ *Helosciadium*, 5 spp.; *Berula*, 1 sp.; *Cryptotaenia*, 4 spp.; *Sium*, 12 spp.). Results of Bayesian analysis and maximum parsimony analyses of partitioned and combined data revealed that the *Sium* alliance is strongly supported as monophyletic. Within this clade, four major subclades are resolved. Three of these subclades comprise species that were traditionally placed in *Cryptotaenia*, *Helosciadium*/*Apium* pro parte, and *Sium* s.s. (9 spp.). A restitution of the genus *Helosciadium*, including all Eurasian species of *Apium* with the exception of *A. graveolens*, the generitype, is supported by both molecular data and morphology. The fourth subclade, *Berula* sensu lato, encompasses all representatives of a widely distributed *B. erecta*, a monotypic African *Afrocarum*, and three members of *Sium* from Africa (*S. repandum* and Saint Helena (*S. bracteatum*, *S. burchelli*). The *Sium* species from Saint Helena form a sister group to African representatives of *B. erecta*. The *Berula* sensu lato clade is recognized at the generic level and these four African/Saint Helena species are transferred into *Berula*. African and North American populations of *B. erecta* are distinct from their Eurasian relatives and are therefore proposed to be treated as separate species (*B. thunbergii* and *B. incisa*, respectively).

**KEYWORDS:** Apiaceae, nomenclature, phylogeny, taxonomy, Umbelliferae

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**INTRODUCTION**

Recent molecular studies have resurrected and radically revised the circumscription of the umbellifer tribe Oenantheae Dumort. to comprise species that share many ecological and morphological characters (Hardway & al., 2004). Its members usually grow in moist to wet habitats and some are even true aquatics. All have fascicled roots, glabrous leaves and stems, and many are characterized by once-pinnate leaves and globose to broadly-ovate corky fruits facilitating dispersal in water. Given the overall similarity of its members, it is astonishing that this group of genera had not been recognized as a natural unit prior to molecular phylogenetic studies. Tribe Oenantheae is therefore an excellent example on how phylogenetic analyses of molecular data can help to reveal previously overlooked or disregarded patterns of morphological and ecological similarity.

Apart from delimiting the boundary of the tribe, molecular studies have also suggested that many of its constituent genera are polyphyletic or paraphyletic. This particularly concerns a group of five genera (*Sium* L., *Afrocarum* Rauschert, *Berula* W.D.J. Koch, *Cryptotaenia* DC., *Apium* L.), subsequently named the *Sium* alliance, that forms a strongly supported clade in all hitherto published analyses (Hardway & al., 2004; Spalik & Downie, 2006, 2007; Zhou & al., 2008).

The genus *Sium* includes twelve species that are widely distributed in Eurasia, North America, and sub-Saharan Africa (Pimenov & Leonov, 1993; Spalik & Downie, 2006). The Eurasian members include *S. frigida*, *S. latifolium*, *S. medium*, *S. ninsi*, *S. serra*, *S. sisarum*, *S. sisaroides*, *S. suave*, and *S. tenue* (the authors of the species names considered in this study are given in the Appendix). *Sium suave* occurs also in North America. In sub-Saharan Africa, the genus is represented by *S. repandum*; two additional species, *S. burchelli* and *S. bracteatum*, occur on the island of Saint Helena.

*Berula* is usually regarded as very closely related to *Sium* and is often synonymized with the latter (Drude,
1897–1898a). At present, only the single species Berula erecta is recognized. It occurs in Europe, western Asia, Africa, and North America and is probably the most widespread umbellifer species excluding weeds. This aquatic umbellifer shows little morphological variation throughout its geographical range and is rarely divided into infraspecific taxa. Western Asian populations, once recognized as a separate species under the invalidly published binomial “B. orientalis Woronow ex Schischk.” (Schischkin, 1950), are not now distinguished from the European nominative taxon (Hedge & Lamond, 1987). Similarly, the African populations that were once classified as B. thunbergii (DC.) H. Wolff are now reduced to the rank of subspecies (Burtt, 1991) or not recognized as distinct from their Eurasian cousins (Townsend, 1989). The North American members of the genus were once distinguished as B. pusilla (Nutt. ex Torr. & A. Gray) Fernald, nom. illeg., or B. incisa (Torr.) G.N. Jones. They differ from their Old World relatives in having somewhat dimorphic leaves (Cronquist, 1961), but modern treatments now generally regard this as a minor difference and the taxon deserving no more than the status of variety.

Eight members of Cryptotaenia show an anomalous distribution pattern that cannot be explained with any common biogeographic scenario (Spalik & Downie, 2007). Two species, Cryptotaenia japonica and C. canadensis, are widespread in eastern Asia and eastern North America, C. africana occurs on mountains of tropical central and eastern Africa, whereas the remaining members are narrow endemics of Tanzania (C. polygama, C. calycina), the Canary Islands (C. elegans), southern Italy (C. thomasii), and the Caucasus (C. flahaultii). The monophyly of Cryptotaenia was long ago questioned (Koso-Poljansky, 1915) and various taxonomic affinities were proposed for its disjunct members (discussed in Spalik & Downie, 2007).

Afrocarum is a morphologically distinct monospecific genus occurring in sub-Saharan Africa; its only member, A. imbricatum, was suggested to be related to Carum (Townsend, 1989).

The genus Apium is characterized by a disjunct distribution with six of its members occurring in western Eurasia and northern Africa, whereas its remaining species occur in southern regions of South America, South Africa, Australia, and New Zealand (Pimenov & Leonov, 1993; Hardway & al., 2004).

The results of molecular systematic studies have overturned traditional assumptions of phylogenetic affinities among these taxa. The genus Sium appeared to be polyphyletic because its African and Saint Helena members, along with Afrocarum, were placed within Berula making the latter paraphyletic. This entire clade, named Berula sensu lato (s.l.), was strongly supported in molecular analyses (Spalik & Downie, 2006). The remaining members of Sium constituted two clades, but their sister group relationship was not apparent. Although the polyphyley of Sium and paraphyly of Berula are not supported by morphological characters, the phylogenetic relationships inferred from molecular data are congruent with the biogeography of its members (Spalik & Downie, 2006). The genus Cryptotaenia was demonstrated to be polyphyletic, with four of its members included in the Sium alliance of tribe Oenantheae, the Macaronesian C. elegans placed among the African/Mediterranean members of Daucus L. (tribe Scandiceae subtribe Daucinae), and the African congeners grouped with African genera Fromnia H. Wolff, Phellolophium Baker, and other representatives of tribe Pimpinelleae (Spalik & Downie, 2007). Similarly, Apium was shown to be polyphyletic. The type of the name, A. graveolens L., applies to a species that is a sister group to Naupraga baleareica Constance & Cannon (Downie & al., 2000a), whereas the remaining Old World members of Apium were placed within the Sium alliance (Downie & al., 2000b; Hardway & al., 2004) suggesting that a restitution of the genus Helosciadium W.D.J. Koch is necessary (Hardway & al., 2004).

The aforementioned inferences were based on analyses of a single marker, the nuclear ribosomal DNA internal transcribed spacer (ITS) region, whose utility for phylogenetic estimation has been questioned (Álvarez & Wendel, 2003). Therefore, nomenclatural changes in the Sium alliance were postponed until confirmation from an independent marker, such as that from the chloroplast genome. The results of phylogenetic analyses of subfamily Apioideae using ITS sequences, however, are generally congruent to those results obtained from other molecular markers, particularly chloroplast DNA (cpDNA) intergenic spacer and intron sequences (Downie & al., 2001).

With Afrocarum and three species of Sium nested within Berula, the present taxonomic treatment of Sium and Berula is untenable. However, when considering formal taxonomic changes at the generic level, several objectives must be taken into account. To promote the stability of the classification, the monophyly of the redefined genera needs to be firmly established. Moreover, formal classification should not only be consistent with the phylogenetic tree but should also be user-friendly. Therefore, the genera have to be well delimited and manageable in size. Both too large and too narrowly defined genera should be avoided (Spalik & al., 2001). The impact of the nomenclatural changes needs also to be considered, particularly if widely used names are subject to change. It is better to change names of several species with limited distributions than to replace a name of a widespread taxon.

In this paper, we present additional data from the chloroplast genome to address the question of generic delimitation within the Sium alliance of Apiaceae tribe Oenantheae. We consider sequence data from the cpDNA
rps16-5′trnK(UUU) region. We examine congruence of relationship inferred by analyses of chloroplast and nuclear DNA sequence data and compare the results against current taxonomic treatments of the aforementioned genera. Subsequently, we propose a new generic treatment for these taxa.

MATERIALS AND METHODS

Taxon sampling. — Seventy-eight accessions were examined for ITS and cpDNA sequence variation (Appendix). The Sium alliance was represented by all 23 species (Afrocarum, 1 sp.; Apium/Helosciadium, 5 spp.; Berula, 1 sp.; Cryptotaenia, 4 spp.; Sium, 12 spp.) that were placed in this clade based on our earlier studies (Hardway & al., 2004; Spalik & Downie, 2006, 2007). Some species, particularly those characterized by a broad geographic distribution (e.g., B. erecta and S. siave) were represented by several accessions from different parts of their ranges. Because in an earlier molecular study representatives of Cicuta L., Oenanthe L., and the North American Endem- ics clade collectively formed a sister group to the Sium alliance (Hardway & al., 2004), we also considered all species of Cicuta, including some infraspecific taxa revealed by molecular studies (Lee & Downie, 2006), a broad representation of Oenanthe that hitherto has not been comprehensively sampled for molecular systematic investigation, and the North American genera Atrema, Neogoegzia, Oxypolis, and Trepocarpus. All trees were rooted with the North American genus Perideridia, as many previous molecular studies of both plastid and nuclear markers supported a sister group relationship between Perideridia and a clade comprised of all other oenanthis genera (Plunkett & al., 1996; Downie & al., 1998; Plunkett & Downie, 1999; Hardway & al., 2004; Spalik & Downie, 2007).

DNA extraction, amplification, and sequenc- ing. — ITS sequences (ITS1, 5.8S rDNA, ITS2) from 10 accessions and cpDNA sequences from 25 accessions were obtained for this study, while the remaining ones had been previously submitted to GenBank (Downie & al., 2004; Hardway & al., 2004; Lee & Downie, 2006; Spalik & Downie, 2006, 2007; Downie & al., 2008). For the new accessions, total genomic DNA was isolated from ca. 20 mg of dried leaf tissue using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.). To obtain the ITS sequences, the purified DNAs were PCR-amplified using either primers “ITS4” and “ITS5” (White & al., 1990) or “N-nc18S10” and “C26A” (Wen & Zimmer, 1996). For some accessions, the ITS 1 and ITS 2 regions were each amplified separately using primers “18S-ITS1-F” and “5.8S-ITS1-R” for ITS 1 and “ITS-3N” and “C26A” for ITS 2 (Spalik & Downie, 2006). Details of the ITS PCR amplifications are provided in Downie & al. (2000a). The cpDNA region includes the rps16 intron, the 3′rps16 exon, and the 3′rps16-5′trnK(UUU) intergenic spacer region (here- after, called rps16-trnK). In Apiaceae tribe Oenantheae, this locus is approximately 1.8 kbp in size and with the exception of the rps16 exon, all regions are noncoding. The rps16 intron has been used widely in phylogenetic studies of both Apiaceae and other angiosperms (Downie & Katz-Downie, 1999; Kelchner, 2002; Shaw & al., 2005), whereas its adjacent intergenic spacer region has not. Recent studies, however, have demonstrated that the 3′rps16- 5′trnK(UUU) intergenic spacer region offers a high level of variation and is appropriate for interspecific phylogenetic study (Lee & Downie, 2006; Calviño & Downie, 2007; Shaw & al., 2007; Downie & al., 2008).

Details of the experimental strategy used to obtain the rps16-trnK cpDNA sequences are presented elsewhere (Downie & Katz-Downie, 1999; Lee & Downie, 2006; Calviño & Downie, 2007). For most accessions, the entire cpDNA region was PCR-amplified and sequenced on both strands in two overlapping parts, using primer pairs “5′exon rps16” and “3′exon rps16” for the intron and “rps16-2” and “trnk” for the spacer region (Lee & Downie, 2006). Each PCR product was electrophoresed in a 1% agarose gel, stained with ethidium bromide, then excised and eluted using either a QIAEX II or a QIAquick Gel Extraction kit (Qiagen). Cycle sequencing reactions were performed using the purified PCR product, AmpliTaq DNA polymerase (Roche Molecular Systems, Alam- eda, California, U.S.A.), and fluorescent Big Dye termina- tors (Applied Biosystems, Foster City, California, U.S.A.). The sequencing products were resolved by electrophoresis using an ABI 3730XL high-throughput DNA capillary sequencer (Applied Biosystems, Foster City, California, U.S.A.). Scans were edited and corrected when necessary. All newly obtained sequences have been deposited in GenBank (Appendix). For the accession of Cryptotaenia flahaultii 2803, only data for the cpDNA rps16 intron were obtained because of difficulties with PCR amplification. For Apium bermejoi and Helosciadium inundatum, only ITS data were available because plant material for these species was no longer available.

Sequence and phylogenetic analyses. — Accessions that yielded identical ITS or cpDNA sequences were represented in the analyses by single terminals. All DNA sequences were aligned using CLUSTAL X (Jeanmougin & al., 1998), with default parameters for gap penalty and extension. Data for ITS and cpDNA were analyzed separately and combined. Prior to the combined analysis, the datasets were tested for incongruence using the method of Farris & al. (1995), as implemented in PAUP* vers. 4.0b10 (Swofford, 1998) using 100 replicate analyses with the maximum number of trees per replicate set to 10,000. Phylogenetic analyses included Bayesian inference using
MrBayes vers. 3.1 (Ronquist & Huelsenbeck, 2003) and maximum parsimony (MP) implemented using PAUP*. Those two accessions for which complete cpDNA rps16-trnK sequences were not available were excluded from substitution-model–based analyses. The substitution model for the Bayesian analysis was selected separately for the ITS and cpDNA portions using the program MrModeltest vers. 2 (Nylander, 2004) and the Akaike information criterion (Akaike, 1974). Bayesian analyses were carried out for 1,000,000 generations with four Monte Carlo Markov chains initiated and a sampling frequency of 100 generations. The initial 10,000 saved trees were discarded and the consensus and posterior probabilities (PP) of particular clades were calculated based on the remaining trees. MP analysis was carried out with gap states treated as missing data, characters unordered, and all character transformations equally weighted. One thousand heuristic searches were initiated with random addition of taxa and tree-bisection-reconnection (TBR) branch swapping. Bootstrap (BS) support was estimated using 1,000 resampled datasets using TBR branch swapping and simple stepwise addition of taxa, saving no more than 1,000 trees per replicate.

**RESULTS**

The sequence characteristics and tree statistics of the three data matrices used in this study (ITS, cpDNA, combined ITS + cpDNA) are presented in Table 1. Data from both genomes yielded similar numbers of parsimony informative positions; these constituted 38% and 11% of aligned positions in the ITS and cpDNA matrices, respectively. For the cpDNA matrix, 9% of aligned sites (185) were ambiguous and were therefore excluded from subsequent analyses. The partition homogeneity test resulted in a P-value of 0.01, therefore the null hypothesis on the congruence of the ITS and cpDNA datasets was rejected. To identify the areas of incongruence, separate MP analyses were performed and the resulting trees compared. Heuristic searches of the ITS sequences resulted in 492 shortest trees of 850 steps each and consistency (CI) and retention (RI) indices of 0.579 and 0.823, respectively. MP analyses of the cpDNA sequences resulted in 2,150 trees of 494 steps each, a CI of 0.749 and a RI of 0.915. Their respective strict consensus trees are compared in Fig. 1. Despite several points of incongruence that concerned mostly nodes with poor bootstrap support, major clades inferred in these analyses are similar. The *Sium* alliance clade (*Afrocarum, Berula, Cryptotaenia, Helosciadium, Sium*) received 82% and 98% BS support in the ITS and cpDNA strict consensus trees, respectively. Within this group, the three clades corresponding to *Berula s.l.*, *Cryptotaenia* and *Helosciadium* received strong BS support. The members of the *Sium* sensu stricto (s.str.) group, i.e., excluding those congeners pertaining to the *Berula* s.l. clade, formed monophyletic sister groups in the ITS trees (BS = 57%), but this affinity was not supported in the cpDNA trees. However, in the cpDNA majority-rule bootstrap consensus tree (not shown), these two clades united in 64% of all trees.

The *Berula* s.l. clade included three moderately to well-supported subclades, two of which encompassed African taxa: *B. erecta* subsp. *thunbergii*, *Afrocarum imbricatum*, and three species hitherto placed in *Sium* (*S. bracteatum*, *S. burchellii*, *S. repandum*). The third subclade comprised Holarctic (Eurasian and North American) representatives of *B. erecta*. The relationships among these three subclades were unresolved in the ITS trees, whereas in the cpDNA trees these African taxa were paraphyletic with respect to the Holarctic subclade. In the ITS trees, accessions of North American *B. erecta* var. *incisa* formed a clade sister group to Palearctic accessions of the same species, whereas in the cpDNA trees the former allied weakly with their Asian representatives (*B. erecta* “orientalis”).

The *Sium* s.str. group comprised two lineages. In the ITS trees, these lineages comprised monophyletic sister groups, although this relationship was supported only weakly (57% BS), whereas in the cpDNA trees they comprised two branches of a five-branched polytomy along with *Berula* s.l., *Cryptotaenia*, and *Helosciadium*. One of these *Sium* clades included the cultivated *S. sarum* and its cousins characterized by tuberous roots that occur generally in the southern Palearctic (Spalink & Downie, 2006). However, the relationships inferred within this clade, particularly the positions of Chinese *S. frigidum* and Japanese *S. serrata*, differed depending on the source of molecular data. In the ITS trees, *S. frigidum*, a
Fig. 1. Comparison of strict consensus trees obtained from maximum parsimony heuristic searches of nrDNA ITS and cpDNA rps16-trnK sequence data for 71 accessions of the Sium alliance and outgroups (see Table 1 for details). Bootstrap values are indicated along branches; those values < 50% are omitted. Major clades are marked with bars and are discussed in the text. NA, North American.
Fig. 2. Strict consensus tree of 864 shortest trees (each of length 1,380 steps) obtained from maximum parsimony heuristic searches of combined nrDNA ITS and cpDNA rps16-trnK sequence data for 74 accessions of the Sium alliance and outgroups (see Table 1 for details). Bootstrap values are indicated along branches; those values < 50% are omitted. Major clades are marked with bars and are the same as presented in Fig. 1. NA, North American.
Fig. 3. Majority-rule consensus tree obtained from Bayesian analyses of combined nrDNA ITS and cpDNA rps16-trnK sequence data for 71 accessions of the Sium alliance and outgroups. Branches are proportional to the GTR + G + I substitution model. Posterior probabilities are indicated along branches. Major clades are marked with bars and are the same as those presented in the previous two figures. NA, North American.
narrow endemic to SW China, is sister group to the clade of *S. ninsi* and *S. tenue*, the latter both narrow endemics of Japan and adjacent coastal area of Asia, whereas *S. serra* is related to *S. sisarum* and its wild relative *S. sisaroides*. In the cpDNA trees, *S. frigidum* is sister group to the clade of *S. sisarum*, *S. sisaroides*, *S. serra*, *S. ninsi*, and *S. tenue*. The other major *Sium* clade included species distributed in the northern Palearctic (Spalik & Downie, 2006): Eurasian *S. latifolium*, central Asiatic *S. medium*, and eastern Asiatic-North American *S. suave*. In both ITS and cpDNA trees, *S. medium* is sister group to the clade of *S. latifolium* and *S. suave*.

The relationships among the members of *Cryptotaenia* were identical in both ITS and cpDNA trees. The eastern North American *C. canadensis* was confirmed as a sister group to eastern Asian *C. japonica*, with *C. thomasi* being their more distant relative. Similarly, the *Helosciadium* clade has identical topology in both ITS and cpDNA trees.

Within the *Sium* alliance, the relationships among its four major clades were unresolved or poorly supported in both ITS and cpDNA trees. In the ITS trees, *Cryptotaenia* is a weakly supported sister group to the *Berula s.l.* clade, with this entire clade rather weakly placed as sister group to the *Sium s.str.* group. Successively basal in the ITS trees is *Helosciadium*, sister group to all aforementioned clades of the *Sium* alliance. In the cpDNA strict consensus tree, all major clades of the *Sium* alliance formed a five-way polytomy.

Maximum parsimony analyses of combined cpDNA and ITS data for 74 terminals (including *Apium bermejoi*, *Cryptotaenia flahaultii*, and *H. inundatum*) resulted in 864 shortest trees of 1,380 steps each and a CI and a RI of 0.629 and 0.858, respectively. The strict consensus of these trees is presented in Fig. 2. This tree is generally congruent to those obtained from separate analyses of data from the two genomes. The *Sium* alliance is strongly supported as monophyletic (BS = 100%) and is a sister group to the North American Endemics clade. Each of the four groups collectively forming this alliance is monophyletic. The *Sium s.str.* clade is weakly supported (BS = 60%), whereas the remaining clades (*Berula s.l.*, *Cryptotaenia*, *Helosciadium*) received 100% BS support each. The relationships among these four clades, however, remained unresolved. Those three accessions for which only ITS or ITS and partial cpDNA data were available grouped with their putative congeners. Caucasian *Cryptotaenia flahaultii* was placed sister group to Italian *C. thomasi*, *Helosciadium inundatum* grouped with *H. crassipes*, and *Apium bermejoi* constituted a trichotomy with *H. nodiflorum* and *H. repens*. The genera *Cicuta* and *Oenanthe* constituted well-supported monophyletic sister groups (BS = 100%).

The points of incongruence between the cpDNA and ITS datasets were resolved in combined analyses in favor of those relationships inferred previously using chloroplast markers. Within *Berula s.l.*, the African species were paraphyletic with regard to the Eurasian taxa. In *Sium s.str.*, within the southern Palearctic clade, *S. frigidum* is a sister group to the remaining members of this clade.

MrModeltest using the Akaike information criterion selected the GTR + G + I model of nucleotide substitution for both ITS and cpDNA datasets. The topology of the Bayesian tree (Fig. 3) was congruent to the strict consensus tree obtained from MP analyses. As before, the relationship among the four major clades of the *Sium* alliance was unresolved.

### DISCUSSION

**Incongruence of ITS and cpDNA data.** — Nuclear ribosomal DNA ITS sequences and non-coding chloroplast loci (introns and intergenic spacers) are among the most commonly used molecular markers for resolving plant phylogeny at low taxonomic levels. Because these sequences are non-coding, they usually provide adequate polymorphisms for resolving phylogenetic relationships among closely related species and genera. However, their widespread utility for phylogenetic inference has been questioned. Such phenomena as extensive sequence variation arising from array duplication events, genomic harbouring of pseudogenes in various states of decay, and incomplete intra- or inter-array homogenization may substantially obscure the phylogenetic signal of ITS sequences (Álvarez & Wendel, 2003). Non-coding chloroplast sequences may also pose problems as mutations in these regions constitute structured, non-random and non-independent events (Kelchner, 2000). While these regions usually evolve more slowly than non-coding nuclear loci, they may contain fast-evolving microsatellite regions that exhibit a high level of homoplasy (Hale & al., 2004). Another source of incongruence between nuclear and chloroplast markers results from hybridization and introgression, and such events are well documented for European oaks (Petit & al., 2002) and European ashes (Heuertz & al., 2006). In umbrellifers, phylogenetic conflict between ITS and cpDNA data resulting from putative hybridization and introgression was reported previously for *Osmorhiza* Raf. (Yoo & al., 2002) and *Cicuta* L. (Lee & Downie, 2006).

Incongruence between ITS and cpDNA datasets has also been detected in the present study. However, as evident from comparisons between the ITS and cpDNA trees (Fig. 1), this incongruence comprises only some rearrangements in otherwise poorly supported nodes. Such rearrangements may introduce ambiguities in biogeographic or comparative analyses as they affect the reconstruction of ancestral states, but they are less important
for taxonomic studies at the generic level so long as the major clades chosen for recognition are firmly supported.

Morphology and monophyly of the Sium alliance. — The affinity of the members of the Sium alliance as inferred from molecular data is only partly corroborated by their morphology. The members of traditionally delimited Berula, Sium, and Helosciadium (Apium pro parte) share a similar ecology and vegetative morphology. These are usually aquatic plants with once-pinnate leaves and spreading rhizomes. Indeed, the European species in a vegetative stage may be very difficult to distinguish (Van Moorssel & Baudewijn, 2000). Berula and Sium have long been regarded as closely related and sometimes even synonymized (Drude, 1897–1898a). In contrast, the species of Cryptotaenia occur in mesic forests and have ternate leaves. Its Italian and Caucasian congeners were placed in the distinct genus Lereschia Boiss. with a suggested relationship to the monospecific Sicilian endemic Petagnaea Caruel (Tutin, 1968b); the latter, however, is now firmly established in Apiaceae subfamily Saniculoideae (Calvino & Downie, 2007). Unfortunately the Sium alliance does not have any obvious morphological synapomorphies. Common characteristics of its members, like fascicled roots and a glabrous epidermis, are plesiomorphies that occur elsewhere in tribe Oenantheae and other genera of Apiaceae. Therefore, it is its inclusive clades rather than the entire alliance that deserve formal taxonomic recognition.

With the majority of umbellifer species not hitherto included in molecular systematic studies, the question remains whether all members of the Sium alliance were indeed considered in this study. Within Apiaceae, generic and tribal boundaries are, for the most part, highly artificial. However, in contrast to other major lineages of Apiaceae redefined on the basis of molecular data, tribe Oenantheae is well defined using both molecular and morphological characters (Hardway & al., 2004). Searching for potential members of the tribe, we examined numerous umbellifers that exhibited at least some traits characteristic for Oenantheae, including a glabrous epidermis, pinnate or tripartite leaves, fascicled roots or corky fruits (Hardway & al., 2004; Spalik & Downie, 2006, 2007). Only three of the taxa examined had all of these characters and two of them have already been confirmed as belonging to tribe Oenantheae upon ITS sequencing (S.R. Downie & al., unpub. data). The third taxon that may be included in the tribe based on morphological features is the monospecific genus Apodicarpum Makino (discussed below).

Cryptotaenia redefined. — All species traditionally placed in Cryptotaenia s.l. are somewhat similar morphologically. Their stems bear many long-pedunculate umbels. Their rays and pedicels are also exceptionally long, usually exceeding the lengths of the flowers or fruits, and they are often uneven. The whole fruiting stem has therefore a paniculate, ‘grass-like’ appearance. The leaflets are usually scarcely divided, with broad lobes. However, given the polyphyletic nature of the genus as traditionally circumscribed, these characters are of poor diagnostic value. Of the eight species hitherto recognized in Cryptotaenia, only four are retained based on molecular systematic study (Spalik & Downie, 2007). These four species, denoted here as Cryptotaenia s.str., exhibit a recticular Holarctic distribution pattern comprising two pairs of sister species. The first pair comprises C. flahautii and C. thomasii that survived in southern Italy and the Caucasus (in the Apennine and Colchis glacial refugia, respectively). The other pair, C. japonica and C. canadensis, represents the classical eastern Asian-eastern North American disjunction pattern (summarized by Wen, 1999, 2001). The affinity of these four species and the exclusion of the other congeners are well supported by morphological data.

Although the four species of Cryptotaenia s.str. occur in mesic rather than watery habitats, they are entirely without any pubescence like the other members of tribe Oenantheae. In contrast, the three African congeners (C. africana, C. calycina, C. polygama) and C. elegans from the Canary Islands have an indumentum, although in the latter it is sparse. The members of Cryptotaenia s.str. have distinctly ternate leaves, with lateral divisions nearly as large as the terminal division. These divisions are not further divided, although in C. japonica and C. canadensis they may be deeply cut. The lateral divisions are then bilobate and the terminal division trilobate. In contrast, C. africana and C. calycina have at least some leaves once-pinnate, sometimes even bipinnate. Only their cauline leaves are ternate and these may superficially resemble those of Cryptotaenia s.str. The basal and lower cauline leaves of C. elegans are always pinnate, the former usually bi- or tri-pinnate. The species also differ in characteristics of the root system. Members of Cryptotaenia s.str. have fascicled roots growing from a creeping rhizome, similar to the other members of tribe Oenantheae (Hardway & al., 2004), whereas the African species have a creeping rootstock and C. elegans has a distinct taproot.

Given these differences in morphology, it is surprising that Cryptotaenia s.l. has survived intact for so long. Koso-Poljansky (1915) considered the African species and C. elegans to be anomalous in the genus. However, he was only able to make limited observations rather than a thorough study and he did not propose alternative placements for them. Since then, no one has attempted a worldwide revision of the genus. Based on phylogenetic analysis of molecular data, the African congeners are clearly related to African Pimpinella, Frommia, Phellolophium of tribe Pimpinelleae, whereas the Canary Islands endemic is placed among members of Scandiceae subtribe Daucinae (Spalik & Downie, 2007).
Restitution of Helosciadium. — The genus *Apium* includes ca. 20–25 species (Pimenov & Leonov, 1993) with a striking amphipolar disjunction pattern. Six species of *Apium* occur in Europe (its centre of endemism in the Northern Hemisphere), of which some extend into western Asia or northern and eastern Africa (Tutin, 1968a; Llorens, 1982; Townsend, 1989). The remaining species are native to southern South America (Maticorena & Quezada, 1985; Martinez, 1999), Juan Fernandez Islands (Johow, 1896), Australasia (Short, 1979; Gardner, 2000), and South Africa (Townsend, 1989; van Wyk & Tilney, 2004). These species are characterized by a glabrous epidermis, lateral umbels, and small oblong fruits. However, this broad treatment of the genus (e.g., Drude, 1897–1898b; Wolff, 1927; Tutin, 1968a), particularly with respect to its European taxa, has not been unanimously accepted. Most European congeners have also been placed in *Helosciadium* (Koch, 1824).

The most recent comprehensive worldwide treatment of *Apium* is that by Wolff (1927), who divided the genus into five sections. Three of these five sections are at present recognized as separate genera: *Niphogoton* Schldl. (Mathias & Constance, 1951), *Cyclospermum* L. (Constance, 1990), and *Apodicarpum* (Hiroe & Constance, 1958). Based on molecular systematic studies, *Niphogoton* is placed among members of tribe Selinaceae Spreng., whereas *Cyclospermum* is a member of tribe Pyramidoptereae Boiss. (C. Calviño, K. Spalik & S. Downie, unpub. data). Our attempts to include the Japanese endemic *Apodicarpum* in molecular studies were unsuccessful because of difficulties with PCR amplifications. The only member of this genus, *A. ikenoi* Makino, shares many morphological characteristics of tribe Oenantheae. Its roots are thickened similarly to those roots of members of *Sium* from the southern Paleartic clade. It is therefore probable that *Apodicarpum* will find its relatives among the members of Oenantheae, but most likely within *Sium* rather than *Helosciadium*.

With the three aforementioned sections excluded, *Apium* is recognized at present as encompassing only two sections: *A*. sect. *Apium* comprising *A. graveolens* and those members from the southern hemisphere, and *A*. sect. *Mauchartia* (DC.) Benth. including those species pertaining to *Helosciadium* (Short, 1979). However, molecular phylogenetic analyses demonstrated that even when restricted to these two sections the genus *Apium* is still polyphyletic. *Apium graveolens*, the generitype, is unrelated to its European relatives and is most closely related to the monospecific genus *Naufragia*, an endemic of the Balearic Islands (Downie & al., 2000a). Its remaining Old World congeners formed a distinct clade alongside *Sium*, *Berula*, and *Cryptotaenia* in tribe Oenantheae (Downie & al., 2000b; Hardway & al., 2004; and this study). The other members of *Apium* sect. *Apium* are related to *A. graveolens* (C. Danderson, K. Spalik & S. Downie, unpub. data). Therefore, the restitution of *Helosciadium* was postulated (Hardway & al., 2004). Of the five members of the *Helosciadium* clade, only the relatively recently described *Apium bermejoi* (Llorens, 1982) has not been formally recognized in the genus. The taxonomic treatment of *Helosciadium* and its morphological circumscription will be published separately (A.C. Ronse, Z.A. Popper, J.C. Preston & M.F. Watson, unpub. data).

Taxonomic treatment of *Berula* s.l. — The present taxonomic treatment of members of the *Berula* s.l. clade is in obvious conflict with the phylogenies inferred herein from molecular data. This clade includes three subclades, two of which comprise African (and Saint Helenean) taxa traditionally placed in *Berula*, *Sium*, and *Afrocarum*, and the third encompasses the Holarctic representatives of the genus (*Berula* s.str.). Therefore, both *Berula* and *Sium* are polyphyletic. Although the Holarctic representatives of *Berula erecta* form a highly supported subclade, the accessions of African *B. erecta* subsp. *thunbergii* arise as a sister group to the Saint Helenean members of *Sium*, whereas *S. repandum* from continental Africa is a sister group to a monospecific *Afrocarum*. Upon the addition of cpDNA data, these African taxa form a paraphyletic group with regard to their Holarctic relatives, whereas in our previous studies a sister group relationship between the African and Holarctic groups was suggested (Spalik & Downie, 2006).

The representatives of traditionally delimited *Berula erecta* show little morphological variation throughout its vast geographical range. The African populations differ from those of Eurasia in the cutting of the leaflets of the cauline leaves. These leaflets are incised and very acute with a narrow commissure and small but obvious calyx teeth (Townsend, 1989), whereas the European representatives have narrower fruits with a broader commissure and calyx teeth that are absent at maturity (Arenas Posada & García Martin, 1993). The Central Asian populations of *B. erecta* have been invalidly described as “*B. orientalis* Woronow ex Schischk.” (Schischkin, 1950). This taxon was supposed to have shorter and narrower leaflets than those of typical *B. erecta*, and finely crenate leaf margins as opposed to the irregularly and deeply toothed or incised margins of the European plants. These differences have not been confirmed by other authors and, at present, the Asian populations of *B. erecta* are not recognized as distinct (Hedge & Lamond, 1987). The North American *B. erecta* var. *incisa* differs from the typical variety in having markedly dimorphic leaves. Submerged,
filiform-dissected leaves are sometimes present, whereas the Old World plants have leaves that are all of the same shape (Cronquist, 1961). Molecular data confirm that the European, Asian and North American populations form more or less distinct subclades, likely a result of their long geographic isolation.

We could not find any obvious morphological or anatomical characters from habit, leaves, flowers and fruits that would separate the African clades from each other and from *Berula* s.str. Therefore, we have decided not to recognize the two subclades of *Berula* s.l. as distinct, separate genera. Such a taxonomic treatment would save the name *Afrocarum*, but the resulting small genera would be difficult to distinguish.

The *Berula* s.l. clade is very strongly supported in all analyses and its members are morphologically similar. So far as the separation of *Sium* s.str. and *Berula* s.l. is concerned, the members of *Berula* usually have incised bracts, whereas those of *Sium* are entire. Formal recognition of the *Berula* s.l. clade at the generic level involves the transfer of four species to *Berula*. The three continental African members of this clade are traditionally placed in separate genera despite their obvious similarities. Among the African umbellifers, these species are easily distinguished by their once-pinnate oblong leaves, fascicled roots, and lack of an indumentum. Transferring them to *Berula* will simplify generic keys and facilitate their recognition. We have chosen, therefore, to recognize the *Berula* s.l. clade at the generic level. The following new combinations are necessary:


Since the African accessions of *B. erecta* form a clade that is a sister group to the Saint Helenean species rather than to the conspecific accessions, the resurrection of *Berula thunbergii* is justified. Because the remaining accessions of *B. erecta* form one clade, they are retained in a single species. However, there is a distinct split between the Eurasian and the North American populations and the sequence variation within each of these populations is relatively low (Spalik & Downie, 2006). We favor, therefore, the restitution of the species *B. incisa* for the North American members of *Berula*.

**Monophyly of *Sium* s.str.** — The monophyly of each of the two inclusive clades of *Sium* s.str. is strongly supported in all analyses. These clades also differ morphologically (Spalik & Downie, 2006). In our previous study based on ITS sequences, the sister group relationship between these two clades was supported only in the distance-based analyses, whereas in some MP trees they formed a paraphyletic group with respect to *Berula* s.l. Upon the inclusion of cpDNA in the present study, the monophyly of *Sium* s.str. is supported in all analyses. Therefore, apart from the exclusion of its African and Saint Helenean members, no further changes in *Sium* are necessary.

With some of its previous members transferred to *Berula*, the morphological delineation of *Sium* becomes somewhat problematic. It is difficult to find any obvious morphological synapomorphies for the genus, although it is easy to distinguish its members from morphologically similar species of *Berula* and *Helosciadium*. All members of *Sium* have the lowest leaflets of similar size to the others, whereas in *Berula* these are smaller or reduced. *Sium* and *Helosciadium* are easy to distinguish based on habit; members of the former are erect plants with terminal umbels, whereas species of the latter are creeping herbs with lateral umbels.

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**LITERATURE CITED**


Appendix. Accessions of Apiaceae tribe Oenantheae and outgroups from which cpDNA and nuclear rDNA ITS data sequences were obtained, with corresponding DNA accession and GenBank reference numbers and voucher information.

TAXON name — DNA accession identifier; voucher information; cpDNA GenBank no., ITS GenBank no.
Appendix. Continued.

**Taxon name** — DNA accession identifier; voucher information; cpDNA GenBank no., ITS GenBank no.

1971; U.S.A., Illinois, Shelby Co., NE of Assumption, Shildneck 12686 (ILL); EF185250, AY246910. *Perideridia kelloggii* (A. Gray) Mathias — 778; U.S.A., California, Sonoma Co., King Ridge Rd, 5 mi N of Cazadero, Ordoff & al. s.n. (UC), cult. University of California Botanical Garden, Berkeley (no. 81.0521); EF185251, U78373. *Sium bracteatum* (Roxb.) Cronk — K177; Saint Helen, material provided by W. Williams (WA); EF367712, AY335982. *Sium burchelli* (Hook. f.) Hemsley. — K178; Saint Helen, material provided by W. Williams (WA); EF367713, AY335983. *Sium frigidum* Hand.-Mazz. — 2337; China, Yunnan, Alden & al. 393 (E 0005284); UE224396, DQ005665. *Sium latifolium* L. — 1632; France, Bas-Rhin, Hultenheim, cult. Botanical Conservatory Mulhouse no. 9466, Herb. Reduron s.n.; EF185266, AY360257. 2256; Denmark, Sjælland, Bromme Lilleso, Petersen & Seberg GPL31 (C); EF185267, AY360258. *Sium medium* Fisch. & C.A. Mey. — 2209; Kyrgyzstan, Khotkor, Konov & Kotschegawa 456 (LE); EF185268, DQ005674. *Sium nissii* L. — K122; Japan, Tohoku distr., Iwatsuki 127 (MO 4253273); EF367714, DQ005678. *Sium repandum* Welw. ex Hiern — K61; South Africa, Transvaal, Kaapche Hoop, Rogers 9101 (G); EF367715, AY353977. 1216; South Africa, van Hoepen 1695 (MO 4348119); EF367716, DQ005680. *Sium serratum* (Franch. & Sav.) Kitag. — K123; Japan, Honshu, Tateishi & al. 14776 (BE 00043297); EF367703, AY353979. *Sium sisaroides* DC. — 132; Turkey, A9 Kars, Davis 46661 (E); EF367718, DQ005688. *Sium sisarum* L. — 53; Spain, cult. UIUC from seeds obtained from Real Jardín Botánico, Downie 33 (ILL); EF185297, AY360260. *Sium suave* Walter — 12; Canada, Montréal, cult. UIUC from seeds obtained from Jardin botanique de Montréal, Downie 388 (ILL); EF185273, AY360260. 1494; U.S.A., Illinois, Vermilion Co., NE of Assumption, Shildneck 12686 (ILL); EF185250, AY246910. *Perideridia americana* (Nutt. ex DC.) Trepocarpus aethusae Nutt. ex DC. — 1871; U.S.A., Illinois, Alexander Co., Horseshoe Lake Conservation Area, Basinger 10891 (ILLS 194558); EF185280, AY360264.