

BUNSAWAT ET AL: CHLOROPLAST PHYLOGENY OF *MENTHA*

**Phylogenetics of *Mentha* (Lamiaceae): Evidence from Chloroplast DNA Sequences**

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**ABSTRACT.** Phylogenetic relationships in *Mentha* were inferred from DNA sequences of the chloroplast (cp) *rpl16* intron and *trnL-trnF* region. The objectives were to test monophyly of *Mentha* and each of its sections and assess relationships among *Mentha* species. Based on cpDNA data, *Mentha* is strongly supported as monophyletic. The suggestions that *M. cervina* and *M. cunninghamii* should be placed in other genera gain no support from this analysis. *Mentha cervina* is sister to *M. gattefossei*, and the New Zealand endemic, *M. cunninghamii*, is allied with species from Australia (*M. australis*, *M. diemenica*, *M. satureioides*). No recognized section with more than one species sampled forms a monophyletic group. Section *Pulegium* may be monophyletic, but the third member of this section, *M. grandiflora*, was not sampled. Chloroplast DNA sequences of the putative allopolyploids *M. canadensis* and *M. spicata* suggest that *M. arvensis* and *M. longifolia*, respectively, may be their maternal parents.

An understanding of the systematics of *Mentha* (Lamiaceae; Nepetoideae; Mentheae) has been extremely challenging and its current status remains uncertain (Harley 1972; Harley and Brighton 1977; Chambers and Hummer 1994; Rösch, et al. 2002; Tucker and Naczi, in press). Taxonomic difficulty may be attributed to a high incidence of polyploidy, variation in base chromosome number, diverse morphology, vegetative propagation, and frequent interspecific hybridization (Morton 1956; Harley and Brighton 1977; Gobert et al. 2002; Tucker and Chambers 2002). Several *Mentha* taxa, such as *M. spicata* (spearmint) and *M. x piperita* (peppermint), have considerable economic importance. Shoots and leaves of mints are used as condiments in food, and their essential oil components are processed into flavorings and fragrance elements for use in a variety of products (Chambers and Hummer 1994; Khanuja et al. 2000; Srivastava et al. 2002). Mint essential oils also have other applications that include medicinal, antibacterial, antifungal, and insecticidal activity (e.g., Arakawa et al. 1992; Dikshit and Husain 1994; Franzios et al. 1997; Villaseñor et al. 1997; Imai et al. 2001).

Taxonomic treatments of *Mentha* have recognized 13-18 species (Briquet 1897; Harley and Brighton 1977; Tucker and Naczi, in press). Several species are widely distributed and most occur in Europe and Asia (Briquet 1897; Harley 1972), yet six species are found in Australia and Tasmania, *M. cunninghamii* is a New Zealand endemic, and North American *M. canadensis* is the only species native to the New World (Gleason and Cronquist 1991; Tucker and Naczi, in press). According to the IUCN (World Conservation Union) Red List of Threatened Plants, two *Mentha* species are listed among the rarest plants in the world (Walter and Gillett 1998). *Mentha gattefossei* is restricted to Morocco, and *M. requienii* is known only from four Mediterranean islands: Caprera, Corsica, Monte Cristo, and Sardinia.

Although monophyly of subfamily Nepetoideae (Cantino and Sander 1986; Cantino 1992; Wagstaff et al. 1995, 1998) and tribe Mentheae sensu Cantino et al. (1992) are strongly supported (Trusty et al., in press), phylogenetic relationships within these groups remain unclear as does circumscription of some large genera (Wagstaff et al. 1995). Consequently, monophyly of *Mentha* is questionable because several species have sometimes been placed in other presumably closely related genera such as *Micromeria*, *Satureja* and *Thymus* (Briquet 1897; Tucker and Naczi, in press). Because circumscription of *Mentha* species can be ambiguous, many new taxa have been described. Moreover, several species (notably *M. longifolia*) have been divided into multiple subspecies. Tucker and Naczi (in press) recognized 19 subspecies of *M. longifolia* ranging from western Europe to the Himalayas along with three subspecies in southern Africa. However, they qualified their treatment by stating that this “is a temporary solution until the full variation can be examined in detail, with plants grown in a common environment”.

The circumscription and infrageneric classification of *Mentha* has been problematic. *Mentha* has been divided into four to six groups (Table 1). Briquet (1897) recognized five sections within two *Mentha* subgenera and *M. cervina* was placed in the genus *Preslia* Opiz. Harley and Brighton (1977) also divided *Mentha* into five sections, whereas Tucker and Naczi (in press) separated *Mentha* into four sections and excluded *M. cunninghamii*. The diverse morphology of *Mentha* makes it difficult to identify a single diagnostic trait; thus, the genus has traditionally been identified using a suite of features. Tucker and Naczi (in press), for example, use the following characters to define *Mentha*: “stamens 4, more or less equal, filaments naked, anthers with parallel distinct thecae, more or less actinomorphic calyx, weakly 2-lipped corolla, and subellipsoidal nutlets with rounded apex.” Gleason and Cronquist (1991) present a similar

set of characteristics to define *Mentha*. Consequently, it has been even more difficult to identify diagnostic characters for each *Mentha* section. Tucker and Naczi (in press) do not directly identify morphological traits that are synapomorphic for their sections, and the only discernable pattern is that sect. *Mentha* is much more variable than sections *Eriodontes*, *Pulegium*, and *Tubulosae*.

Several studies have tried to assess relationships in *Mentha*. As a result of economic interests, the primary goal has been to identify and characterize commercially important mint taxa (Rösch et al. 2002). For example, Khanuja et al. (2000) and Fenwick and Ward (2001) used Randomly Amplified Polymorphic DNA (RAPD) markers to assess relationships in six and three taxa respectively, and Gobert et al. (2002) used Amplified Fragment Length Polymorphism (AFLP) to examine relationships and hybridization among section *Mentha* taxa. Gobert et al. (2003) also used sequences of chloroplast and nuclear DNA to infer relationships among species of sect. *Mentha*. The most comprehensive phylogenetic analysis of *Mentha* to date (Tucker and Naczi, in press) included all recognized species and was based on essential oil chemistry, morphology, and chromosome data.

*Mentha* species possess up to five different base chromosome numbers (Harley and Brighton 1977; Chambers and Hummer 1994). Although most species have a base chromosome number of  $x = 12$  (Table 2), *M. requienii* has  $x = 9$  and *M. gattefossei*, *M. japonica*, and *M. pulegium* likely have  $x = 10$ . In *M. cervina* ( $2n = 36$ ), the base chromosome number may be  $x = 12$ , though it has also been considered as  $x = 18$  (Makarov and Reznikova 1972; Harley and Brighton 1977). Polyploidy is common in *Mentha* with only five diploid species; the remaining species range from triploid to decaploid (Table 2).

Hybridization in *Mentha* occurs frequently, but only in sect. *Mentha* do hybrids occur naturally (Morton 1956; Harley and Brighton 1977). Two species, *M. canadensis* and *M. spicata*, are thought to be ancient stabilized allopolyploids. *Mentha canadensis* has been hypothesized to be a hybrid between *M. arvensis* and *M. longifolia* (Tucker and Chambers 2002), and *M. spicata* likely originated from a cross involving *M. longifolia* and *M. suaveolens* (Harley and Brighton 1977; Gobert et al. 2002).

In this study, our objectives were to test monophyly of *Mentha* and each of its recognized sections and assess relationships among *Mentha* species. We generated DNA sequences from the chloroplast (cp) *rpl16* intron and *trnL-trnF* region. The *trnL-trnF* region includes the *trnL* intron and *trnL-trnF* intergenic spacer. Sequences of the *rpl16* intron and the *trnL-trnF* region have shown promise for investigating interspecific relationships in angiosperms in general (e.g., Small et al. 1998) and in Lamiaceae (Oliveira et al. 2002; Walker et al. 2002; Trusty et al., in press).

#### MATERIALS AND METHODS

***Plant Samples.*** We included 15 *Mentha* species representing all recognized sections (Table 2). Samples were received as cuttings from the United States Department of Agriculture-Agricultural Research Service, National Clonal Germplasm Repository (USDA-ARS, NCGR) in Corvallis, OR and established as plants in the WKU Biology Department greenhouse. Morphological vouchers have been deposited in the Western Kentucky University herbarium (WKU).

***Molecular Methods.*** Total cellular DNA was isolated from fresh young leaves using a modified CTAB protocol (Doyle and Doyle 1987). Polymerase Chain Reaction (PCR) amplification generally followed Taberlet et al. (1991) for the *rpl16* intron and *trnL-trnF* region.

PCR products were electrophoresed in 0.8% agarose gels, then excised and purified using a QIAquick gel extraction kit (Qiagen, Inc., Valencia, CA). Each DNA region was sequenced with two to four primers: F71, R1516 (see Small et al. 1998), MF2, and MR2 (this study) for the *rpl16* intron and c, d, e, and f for the *trnL-trnF* region (Taberlet et al., 1991). Samples were sequenced using an ABI PRISM™ Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and then electrophoresed in an ABI 310 Genetic Analyzer. DNA sequences were edited and aligned visually using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, MI). DNA sequences have been deposited in GenBank (see Table 2).

***Outgroup Selection and Phylogenetic Analyses.*** Trusty et al. (in press) performed separate and combined phylogenetic analyses of DNA sequences of the chloroplast *trnL-trnF* and nuclear ribosomal internal transcribed spacer (ITS) regions including 33 Mentheae genera. Based on their combined analysis, *Mentha* is sister to a clade comprising 14 Mentheae genera with *Micromeria*, *Thymus*, *Origanum*, and *Satureja* closely related. However, analysis of cpDNA alone did not resolve the sister group of *Mentha* (Trusty et al., in press). Therefore, we selected *Satureja* as the outgroup; we also included single accessions of three other closely related Mentheae genera (*Acinos*, *Micromeria*, and *Thymus*). Sequences of the *rpl16* intron and *trnL-trnF* region were analyzed in combination.

Both Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were performed using PAUP\* 4.0b10 (Swofford 1998). For the MP analyses, we used the Branch-and-Bound algorithm with all characters equally weighted and gaps treated as missing data. Support for individual clades was evaluated by bootstrap (BS) analysis (Felsenstein 1985), with 500 replicates, and decay (DI) analysis using AutoDecay 4.0 (Eriksson 1998). Model Test 3.06 (Posada and Crandall 1998) and the hierarchical likelihood ratio tests (hLRT) criterion were used

to ascertain an appropriate evolutionary model for ML analysis. For the cpDNA data set we used the HKY + G model. ML searches were conducted using the heuristic algorithm with 100 replicates of random stepwise-addition of taxa and TBR branch swapping; bootstrapping was conducted with 200 replicates. Aligned data matrices (< 1% missing data) and phylogenetic trees have been deposited in TreeBASE (<http://www.treebase.org>).

## RESULTS

***Phylogenetic Relationships of Mentha.*** The MP and ML analyses of the combined cpDNA data yielded nearly identical trees topologically with equivocal levels of nodal support. Parsimony analysis recovered 10 trees of length 147 (strict consensus in Fig. 1). Excluding uninformative sites, the consistency index (CI) is 0.783 and the retention index (RI) is 0.863. *Mentha* is strongly supported (98% BS, 6 DI) as monophyletic. Within *Mentha*, seven clades with bootstrap and decay support are resolved in the cpDNA strict consensus tree. Four species from Australia and New Zealand (*M. australis*, *M. diemenica*, *M. satureoides*, and *M. cunninghamii*) form a well-supported clade (96% BS, 3 DI). Though unresolved in the MP phylogeny, the three former species (all Australian) share a 5-bp insertion in the *rpl16* intron suggesting a close affinity; this result is consistent with the ML tree (not shown). The clade of Australian-New Zealand species is weakly supported as sister to other *Mentha* species.

The remaining *Mentha* species appear in one large clade including *M. pulegium* + *M. requienii* (97% BS, 4 DI, plus an 8-bp deletion in *rpl16*), *M. longifolia* + *M. spicata* (98% BS, 4 DI), *M. suaveolens*, and a clade of six species (70% BS, 1 DI) comprising *M. aquatica*, *M. arvensis*, *M. canadensis*, *M. cervina*, *M. gattefossei*, and *M. japonica*. This latter lineage is divided into two subclades with *M. cervina* + *M. gattefossei* sister to the others. Three insertion/deletion mutations further support *M. cervina* + *M. gattefossei*: a 1-bp deletion and a 4-

bp insertion in *rpl16*, and a 1-bp insertion in *trnL-trnF*. The clade (79% BS, 2 DI) of *M. aquatica*, *M. arvensis*, *M. canadensis*, and *M. japonica* also gains support from a 1-bp insertion in *rpl16* and a 4-bp insertion in *trnL-trnF*.

## DISCUSSION

**Monophyly of *Mentha*.** Chloroplast DNA sequences demonstrate that all *Mentha* species sampled belong to a single well-supported clade. This result is therefore consistent only with Harley and Brighton's (1977) concept of *Mentha*. Conversely, our molecular results conflict with the classifications of both Briquet (1897) and Tucker and Naczi (in press). Briquet (1897) treated *Mentha cervina* as *Preslia cervina* (L.) Fresen., but in our study there is no support for placing *M. cervina* in a separate genus (Figs. 1, 2). *Mentha cervina* is weakly supported as sister to *M. gattefossei*, nested well within the *Mentha* clade. Analysis of morphological, karyological, and chemical characters by Tucker and Naczi (in press) also showed that *M. cervina* and *M. gattefossei* were sister species. Their analysis further suggested that *M. cunninghamii* was outside the clade containing the other *Mentha* species and more closely allied with *Micromeria brownei* (Swartz) Benth. Consequently, Tucker and Naczi (in press) excluded *M. cunninghamii* from *Mentha*. In contrast, our data place *M. cunninghamii* in a well-supported clade with three Australian species, suggesting that *M. cunninghamii* is appropriately included in *Mentha*.

**Classification and Phylogenetic Relationships Among *Mentha* Species.** In considering the interspecific relationships within *Mentha*, none of the infrageneric taxa traditionally recognized with more than one species sampled, except for subg. *Pulegium* sensu Briquet (1897) and possibly sect. *Pulegium* sensu Tucker and Naczi (in press), form monophyletic groups based

on cpDNA sequences (Fig. 1). Thus, these data are inconsistent with existing classifications and imply revision. Each *Mentha* section sensu Tucker and Naczi (in press) is discussed.

Tucker and Naczi (in press) included five species in sect. *Eriodontes* and two species in sect. *Tubulosae*. In our phylogeny (Fig. 1), *M. australis* and *M. satureioides* (both sect. *Eriodontes*) form a strongly supported clade with *M. diemenica* (sect. *Tubulosae*) and *M. cunninghamii*. The close affinity of the four species above--from Australia, Tasmania, and New Zealand--is consistent with established biogeographic patterns (Swenson and Bremer 1997; Alice and Campbell 1999) and with base chromosome number (all are  $x = 12$ ). The remaining sampled members of sect. *Eriodontes*, *M. cervina* and *M. gattefossei*, are sister species with low bootstrap and decay support; yet, three insertion/deletion events strengthen this result. However, these two species occur in a clade that does not contain the four Australian-New Zealand species. Support values for the two ancestral nodes including the *M. cervina* + *M. gattefossei* subclade are also low. Therefore, it is plausible that these two species could form the sister group to the clade containing the Australian-New Zealand species. This outcome indicates that sect. *Eriodontes* sensu Tucker and Naczi (in press) may be polyphyletic. Our data do support the results of Tucker and Naczi (in press) and the suggestion of Harley and Brighton (1977), though not reflected in their classification, of a possible close relationship between *M. cervina* and *M. gattefossei* based on the morphological and ecological similarity of these two species. Both *M. cervina* and *M. gattefossei* are Mediterranean in distribution: southern Europe and northern Africa. Moreover, Chambers and Hummer (1994) noted that *M. gattefossei* had an intermediate morphology between *M. cervina* and *M. pulegium*. Homoplasy in morphological features of *M. gattefossei* and *M. pulegium* may be responsible for the apparent incongruence between Harley and Brighton's (1977) classification and our molecular phylogenies. Our sampling of sect.

*Tubulosae* included only one (*M. diemenica*) of the two species recognized by Tucker and Naczi (in press). Consequently, the monophyly and taxonomic status of sect. *Tubulosae*, and its relationship to the other *Mentha* species, cannot be evaluated in this study.

The seven sect. *Mentha* species do not appear monophyletic. Chloroplast DNA unite *M. aquatica*, *M. arvensis*, *M. canadensis*, and *M. japonica* in a clade that is sister to *M. cervina* + *M. gattefossei* (both sect. *Eriodontes*). Given the phylogenetic position of *M. canadensis*, it appears that *M. arvensis* may be its maternal parent; *M. longifolia* is the other putative parent. The remaining three sect. *Mentha* species are depicted as two lineages: *M. suaveolens* and *M. longifolia* + *M. spicata* (Fig. 1). The sister group relationship of the latter two species suggests that *M. longifolia*, rather than *M. suaveolens*, is the maternal parent of *M. spicata*.

Two of the three species of sect. *Pulegium* (*M. pulegium* and *M. requienii*) were sampled in this study. In the cpDNA phylogeny (Fig. 1), *M. pulegium* is strongly supported as sister to *M. requienii*, consistent with the classifications of both Briquet (1897) and Tucker and Naczi (in press). Thus, sect. *Pulegium* may be monophyletic, but the third member, *M. grandiflora*, needs to be sampled to test this hypothesis.

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## LITERATURE CITED

- ALICE, L. A. and C. S. CAMPBELL. 1999. Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *American Journal of Botany* 86: 81-97.
- ARAKAWA, T. M., SHIBATA, K. HOSOMI, T. WATANABE, Y. HONMA, K. KAWASUMI, and Y. TAKEUCHI. 1992. Anti-allergic effects of peppermint oil, chicle, and Jetutong. *Journal of the Food Hygienic Society of Japan* 33: 569-575.
- BRIQUET, J. 1897. *Preslia, Mentha*. Pp. 317-324 in *Die Natürlichen Pflanzenfamilien IV 3a*, eds. A. Engler and K. Prantl. Leipzig: Wilhelm Engelmann.
- CANTINO, P. D. 1992. Evidence for a polyphyletic origin of the Labiatae. *Annals of the Missouri Botanical Garden* 79: 361-379.
- and R. W. SANDER. 1986. Subfamilial Classification of Labiatae. *Systematic Botany* 11: 163-185.
- , R. M. HARLEY, and S. J. WAGSTAFF. 1992. *Genera of Labiatae: status and classification*. Pp. 27-37 in *Advances in Labiate Science*, eds. R. M. Harley and T. Reynolds. Richmond: Royal Botanic Gardens Kew.
- CHAMBERS, H. L. and K. E. HUMMER. 1994. Chromosome counts in the *Mentha* collection at the USDA-ARS National Clonal Germplasm Repository. *Taxon* 43: 423-432.
- DIKSHIT, A. and A. HUSAIN. 1976. Antifungal action of some essential oils against animal pathogens. *Fitoterpia* 55: 171-176.
- DOYLE, J. J. and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

- ERIKSSON, T. 1998. AutoDecay Version 4.0. Bergius Foundation. Stockholm: Royal Swedish Academy of Sciences.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- FENWICK, A. L. and S. M. WARD. 2001. Use of random amplified polymorphic DNA markers for cultivar identification in mint. *HortScience* 36: 761-764.
- FRANZIOS, G. M. MIROTSOU, E. HATZIAPOSTOULOU, J. KRAL, Z. G. SCOURAS, and P. MAVRAGANI-TSIPIDOU. 1997. Insecticidal and genotoxic activities of mint essential oils. *Journal of Agricultural Food Chemistry* 45: 2690-2694.
- GLEASON, H. A. and A. CRONQUIST. 1991. *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, 2<sup>nd</sup> ed. New York: The New York Botanical Garden.
- GOBERT, V., S. MOJA, M. COLSON, and P. TABERLET. 2002. Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. *American Journal of Botany* 89: 2017-2023.
- , M. WINK, P. TABERLET, and S. MOJA. 2003. Complementarity of molecular data for understanding phylogenetic relationships in the section *Mentha*. Abstract P16 of the 8<sup>th</sup> Evolutionary Biology meeting at Marseille, France (<http://evolution.luminy.univ-mrs.fr/index.html>).
- HARLEY, R. M. 1972. *Mentha*. Pp. 183-186 in *Flora Europaea* vol. 3, eds. T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb. Cambridge: Cambridge University Press.
- and C. A. BRIGHTON. 1977. Chromosome numbers in the genus *Mentha* L. *Botanical Journal of the Linnean Society* 74: 71-96.

- IMAI, H., K. OSAWA, H. YASUDA, H. HAMASHIMA, T. ARAI, and M. SASATSU. 2001. Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. *Microbios* 106 (S1): 31-39.
- KHANUJA, S. P. S., A. K. SHASANY, A. SRIVASTAVA, and S. KUMAR. 2000. Assessment of genetic relationships in *Mentha* species. *Euphytica* 111: 121-125.
- MAKAROV, V. V. and S. A. REZNIKOVA. 1972. Numbers of chromosomes in the genus *Mentha* L. *Byulleten' Moskovskogo obshchestya ispytatelei prirody* 77: 133-141.
- MORTON, J. K. 1956. The chromosome numbers of the British *Menthae*. *Watsonia* 3: 244-252.
- OLIVEIRA, L., R. B. HUCK, P. S. SOLTIS, and D. E. SOLTIS. 2002. Molecular phylogeny and biogeography of *Dicerandra* (Lamiaceae), a genus endemic to the Southeastern United States. Botany 2002 Abstracts published by *American Journal of Botany*. Botany 2002 meeting.
- POSADA, D. and K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- RÖSCH, P., W. KIEFER, and J. POPP. 2002. Chemotaxonomy of mints of genus *Mentha* by applying Raman spectroscopy. *Biopolymers* 67: 358-361.
- SMALL, R. L., J. A. RYBURN, R. C. CRONN, T. SEELANAN, and J. F. WENDEL. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear *ADH* sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany* 85: 1301-1315.
- SRIVASTAVA, R. K., A. K. SINGH, A. KALRA, V. K. S. TOMAR, R. P. BANSAL., D. D. PATRA, S. CHAND, A. A. NAQVI, S. SHARMA, and S. KUMAR. 2002. Characteristics of menthol

- mint (*Mentha arvensis*) cultivated on industrial scale in the Indo-Gangetic plains.  
*Industrial Crops and Products* 15: 189-198.
- SWENSON, U. and K. BREMER. 1997. Pacific biogeography of the Asteraceae genus *Abrotanella* (Senecioneae, Blennospermatinae). *Systematic Botany* 22: 493-508.
- SWOFFORD, D. L. 1998. PAUP\* - Phylogenetic Analysis Using Parsimony, v4.0b10.  
Sunderland: Sinauer Associates.
- TABERLET, P., L. GIELLY, G. PAUTOU, and J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.
- TRUSTY, J., R. G. OLMSTEAD, D. J. BOGLER, A. SANTOS-GUERRA, and J. FRANCISCO-ORTEGA. In press. Using molecular phylogenies from nuclear and chloroplast genes to test a connection of the Macronesian genus *Bystropogon* (Lamiaceae) to the New World and to elucidate the phylogenetic relationships within the Mentheae tribe. *Systematic Botany*.
- TUCKER, A. O. and H. L. CHAMBERS. 2002. *Mentha canadensis* L. (Lamiaceae): a relict amphidiploid from the lower tertiary. *Taxon* 51: 703-718.
- and R. F. C. NACZI. In press. *Mentha: an overview of its classification and relationships*. Pp. xx-xx in *Mints: the genus Mentha*, ed. B. M. Lawrence. London: Taylor and Francis.
- VILLASEÑOR, I. M., D. P. ABERION, and J. S. ANGELADA. 1997. Anticarcinogenicity and antiteratogenicity potential of the antimutagenic chloroform leaf extract from *Mentha cordifolia* Opiz. *Philippine Journal of Science* 126: 207-213.
- WAGSTAFF, S. J., R. G. OLMSTEAD, and P. D. CANTINO. 1995. Parsimony analysis of cpDNA restriction site variation in subfamily Nepetoideae (Labiatae). *American Journal of Botany* 82: 886-892.

- , L. HICKERSON, R. SPANGLER, P. A. REEVES, and R. G. OLMSTEAD. 1998. Phylogeny in Labiatae s. l., inferred from cpDNA sequences. *Plant Systematics and Evolution* 209: 265-274.
- WALKER, J. B., K. J. SYTSMA, and M. WINK. 2002. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and subf. Nepetoideae. Botany 2002 Abstracts published by *American Journal of Botany*. Botany 2002 meeting.
- WALTER, K. S. and H. J. GILLET. 1998. *1997 IUCN Red list of threatened plants*. International Union for Conservation of Nature and Natural Resources (IUCN). Cambridge.

TABLE 1. Three global classification schemes of *Mentha*.

Briquet (1897)	Harley & Brighton (1977)	Tucker and Naczi (in press)
Subg. <i>Menthastrum</i> Coss.	Sect. <i>Audibertia</i> (Benth.) Briq.	Sect. <i>Eriodontes</i> Benth. in DC.
Sect. <i>Verticillatae</i> L.	<i>M. requienii</i> Benth.	<i>M. australis</i> R.Br.
<i>Eriodontes</i> Benth.		<i>M. cervina</i> L.
<i>M. cunninghamii</i> Benth.	Sect. <i>Eriodontes</i> Benth.	<i>M. gattefossei</i> Maire
<i>M. satureioides</i> Br.	<i>M. cunninghamii</i> Benth.	<i>M. laxiflora</i> Benth.
<i>M. repens</i> (Hook.) Briq.	<i>M. satureioides</i> R.Br.	<i>M. satureioides</i> R.Br.
<i>M. serpyllifolia</i> Benth.		
	Sect. <i>Mentha</i>	Sect. <i>Mentha</i>
<i>Tubulosae</i> Briq.	<i>M. aquatica</i> L.	<i>M. aquatica</i> L.
<i>M. diemenica</i> Spreng.	<i>M. arvensis</i> L.	<i>M. arvensis</i> L.
<i>M. australis</i> Benth.	<i>M. longifolia</i> (L.) Huds.	<i>M. canadensis</i> L.
	<i>M. microphylla</i> C. Koch	<i>M. dahurica</i> Fisch. ex Benth.
<i>Grandiflorae</i> Briq.	<i>M. spicata</i> L.	<i>M. japonica</i> (Miq.) Makino
<i>M. grandiflora</i> Benth.	<i>M. suaveolens</i> Ehrh.	<i>M. longifolia</i> (L.) L.
		<i>M. spicata</i> L.
<i>Laxiflorae</i> Briq.	Sect. <i>Preslia</i> (Opiz) Harley	<i>M. suaveolens</i> Ehrh.
<i>M. laxiflora</i> Benth.	<i>M. cervina</i> L.	
<i>Arvensis</i> Benth.	Sect. <i>Pulegium</i> (Miller) DC.	Sect. <i>Pulegium</i> (Mill.) Lam. & DC.
<i>M. arvensis</i> L.	<i>M. gattefossei</i> Maire	<i>M. grandiflora</i> Benth.
	<i>M. pulegium</i> L.	<i>M. pulegium</i> L.
Sect. <i>Capitatae</i> L.	<i>M. micrantha</i> (Benth.)	<i>M. requienii</i> Benth.

Schost-Desjat.

*M. aquatica* L.

Sect. *Spicatae* L.

*Silvestres* Malinv.

*M. viridis* L.

*M. longifolia* Huds.

*Rotundifoliae* Malinv.

*M. microphylla* C. Koch

*M. rotundifolia* L.

Subg. *Pulegium* (Mill.) Lam. &

DC.

Sect. *Eupulegia* Briq.

*M. pulegium* L.

Sect. *Audibertiae* Briq.

*M. requienii* Benth.

Genus *Preslia* Opiz

*P. cervina* (L.) Fres.

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Sect. *Tubulosae* (Briq.) Tucker

*M. diemenica* Spreng.

*M. repens* (Hook. f.) Briq.

TABLE 2. *Mentha* accessions and outgroups sampled; for information on outgroups, see Trusty et al (in press). Infrageneric classification of *Mentha* follows Tucker and Naczi (in press). Ploidy level and base chromosome number are based on Chambers and Hummer (1994) and Tucker and Naczi (in press). Source information includes USDA-ARS, NCGR plant introduction number, geographic origin, and morphological voucher (vouchers of *Mentha* accessions have been deposited at WKU). GenBank accession numbers are *rpl16/ trnL-trnF*.

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### *Mentha*

Sect. *Eriodontes*: *M. australis*, 6x/ 12 (PI 617498, Australia, Bunsawat 2002-43) XXXXXX/  
 XXXXXX; *M. cervina*, 2x, 3x/ 12, 18 (PI 557634, southern Europe, Bunsawat 2003-01)  
 XXXXXX/ XXXXXX; *M. gattefossei*, 4x/ 10 (PI 557639, Morocco, Bunsawat 2002-27)  
 XXXXXX/ XXXXXX; *M. satureioides*, 12x/ 12 (PI 617500, Australia, Bunsawat 2003-02)  
 XXXXXX/ XXXXXX.

Sect. *Mentha*: *M. aquatica*, 8x/ 12 (PI 557572, Germany, Bunsawat 2002-53) XXXXXX/  
 XXXXXX; *M. arvensis*, 6x, 8x/ 12 (PI 557918, Europe, Bunsawat 2002-25) XXXXXX/  
 XXXXXX; *M. canadensis* ‘Brazil 701’, 8x/ 12 (PI 277803, Bunsawat 2002-58) XXXXXX/  
 XXXXXX; *M. japonica*, 5x/ 10 (PI 617475, Japan, Bunsawat 2003-03) XXXXXX/  
 XXXXXX; *M. longifolia*, 2x, 4x/ 12 (PI 557755, Europe, Bunsawat 2002-38) XXXXXX/  
 XXXXXX; *M. spicata*, 3x, 4x/ 12 (PI 557885, unknown, Bunsawat 2002-40) XXXXXX/  
 XXXXXX; *M. suaveolens*, 2x/ 12 (PI 557911, France, Bunsawat 2002-31) XXXXXX/  
 XXXXXX.

Sect. *Pulegium*: *M. pulegium*, 2x/ 10 (PI 557771, OR-USA, Bunsawat 2002-36) XXXXXX/  
XXXXXX; *M. requienii*, 2x/ 9 (PI 557781, England, Bunsawat 2002-30) XXXXXX/  
XXXXXX.

Sect. *Tubulosae*: *M. diemenica*, 10x/ 12 (PI 617482, Australia, Bunsawat 2002-23) XXXXXX/  
XXXXXX.

Unclassified: *M. cunninghamii*, 6x/ 12 (PI 617481, New Zealand, Bunsawat 2002-19)  
XXXXXX/ XXXXXX.

Outgroups: *Acinos alpinus* (L.) Moench. XXXXXX/ AY506594; *Micromeria hyssopifolia*  
Webb. & Berthel. XXXXXX/ AY506612; *Satureja hortensis* L. XXXXXX/ AY506611;  
*Thymus vulgaris* L. XXXXXX/ AY506613.

FIG. 1. Strict consensus of 10 equally parsimonious trees based on combined analysis of cpDNA *rpl16* intron and *trnL-trnF* region sequences (CI = 0.783, RI = 0.863). Outgroup species are shown in uppercase. Numbers above branches are bootstrap values ( $\geq 50\%$ ) and numbers below branches are decay values. Sectional classification follows Tucker and Naczi (in press).

