Molecular Systematics of the Nepetoideae (Family Labiatae): Phylogenetic Implications from rbcL Gene Sequences

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rbcL, Phylogeny, Labiatae, Nepetoideae, Nucleotide Sequences

Total DNA was extracted from 41 species (20 genera) of the subfamily Nepetoideae (family Labiatae). Using rbcL-specific primers, the rbcL gene was amplified by polymerase chain reaction (PCR) and sequenced directly. RbcL sequences were evaluated with character state (maximum parsimony; PAUP) and distance methods (neighbour-joining; MEGA). In agreement with classical systematics all taxa studied cluster within the Nepetoideae and are clearly distinguished from members of the subfamily Lamioideae. A number of distinctive clades are apparent within the Nepetoideae: I – *Collinsonia,* II – *Lavandula,* III – *Agastache, Glechoma,* IV – *Satureja, Hyssopus, Dracocephalum,* V – *Nepeta,* VI – *Hormium,* VII – *Prunella,* VIII – *Melissa, Ocimum,* IX – *Monarda, Mentha,* X – *Origanum, Thymus,* XI – *Salvia,* XII – *Rosmarinus,* and XIII – *Perovskia.* At least five main branches representing the clades I, II, III to VII, VIII, and IX to XIII respectively, can be distinguished within the Nepetoideae studied. They might be considered representing the tribes (according to Cantino, 1992) Elsholtzieae (I), Lavanduleae (II), and Mentheae (III–XIII). The tribe Mentheae needs to be subdivided into at least three main groups (clades III–VII, VIII and IX–XIII). *Majorana hortensis* which is often classified as *Origanum hortensis* does not cluster with *Origanum* and deserves a generic status of its own.

Introduction

The family Labiatae (or Lamiaceae) covers more than 4000 species which are grouped in approximately 220 genera (Hedge, 1992). It is considered to be one of the most highly evolved plant families with regard to floral structures. Many labiate species produce essential oils and are therefore used by man as perfumes, flavourings, foods or medicinal drugs.

The classification of the Labiatae is still a matter of debate and John Lindley (Professor of Botany at University College, London) already had pointed out in 1829 "... we are indepted to our friend Mr. Bentham, by whom Labiatae have been made a particular study, and to whom we confidently look for rescueing them from a state of confusion, that has gradually been increasing since the days of Linnaeus, until it has become the disgrace of Botany". After early attempts by Bentham (1832–1836) and Briquet (1895–1897) to classify the Labiatae, more recent versions came from Junell (1934), Erdtmann (1945), and Wunderlich (1967). It became evident at least that the Labiatae are closely related to the paraphyletic Verbenaceae (Cantino, 1992; Cantino *et al.,* 1992; Cantino and Sanders, 1986; Olmstead *et al.,* 1992). Erdtman (1945) subdivided them into two major groupings: the Lamioideae and Nepetoideae.

Whereas the Lamioideae are characterized by tricolpate, binucleate pollen, albuminous seeds, spatulate embryos and the presence of iridoid glycosides, the Nepetoideae have hexacolpate, trinucleate pollen, exalbuminous seeds, investing embryos and the presence of volatile terpenoids, mainly monoterpenes (Erdtman, 1945; Wunderlich, 1967; Harley and Reynolds, 1992).

Although many chemical, morphological and biological characters have been determined for the taxa within the subfamily Nepetoideae, the phylogenetic relationships between tribes, subtribes and genera are still far from being clear and unambiguous (Hedge, 1992).

Molecular techniques are being used increasingly in plant systematics (Soltis *et al.,* 1992). Besides restriction site analysis of cpDNA, the use of nucleotide sequences has become an even more powerful

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approach. Informative marker genes include, besides others, the chloroplast gene rbcL (coding for the large subunit of rubisco) and nuclear genes, such as rDNA genes (Soltis *et al.*, 1992; Hillis and Moritz, 1990; Hoelzel, 1992; Avise, 1994). RbcL sequences were recently used for an overall evaluation of higher plant phylogeny (Chase *et al.*, 1993) demonstrating their usefulness for studies in plant systematics and plant phylogeny. These data and those from Olmstead *et al.* (1992), have already outlined the position of the Labiatae within the Asteridae and in relation to the Verbenaceae (Chase *et al.*, 1993; Olmstead *et al.*, 1992).

In this communication we have amplified and sequenced the rbcL gene of 41 taxa (of 20 genera) belonging to the subfamily Nepetoideae. Sequence data were used to determine the phylogenetic relationships between these taxa, which are often exploited for medicinal, culinary and flavouring purposes.

Materials and Methods

DNA isolation

DNA was isolated from fresh material by using the CTAB method (Doyle and Doyle, 1990). The sources of the plant material concerned is documented in Kaufmann (1994).

Polymerase chain reaction (PCR) and DNA sequencing

Primer sequences used for PCR and direct sequencing are given in Table I. A 1420 base pair (bp) portion of the rbcL gene was amplified using 1 µg of total DNA as target, 25 pmol each of primers N and R, 1.5 mM $MgCl_2$ and 2 units Taq polymerase (Promega). After initial denaturation (2 min at 94 °C), 30 cycles of 30 s at 94 °C, 30 s at 45 °C and 60 s at 72 °C were performed on a Biometra thermocycler. After 30 cycles the reaction temperature was maintained at 72 °C for 4 min and then lowered to 4 °C for further storage. PCR products were run on a 1% agarose gel, excised and extracted using the Qiaex gel purification kit (Diagen). After elution, the amplified DNA was precipitated with isopropanol and sodium acetate. The pellet was redissolved in 7.5 µl H_2O. Direct sequencing of the double-stranded DNA was carried out by the chain termination method (Sambrook *et al.*, 1989) at 37 °C using a ^{35}S-dATP as a radioactive marker and Sequenase 2.0 (USB) or T7 polymerase (Pharmacia) according to the distributor's specifications. Primer OF, 2F, 3F, 1R, RF and NR were used as sequencing primers (Table I) to obtain overlapping sequences. Alternatively, in a few instances PCR products were cloned using the T/A cloning kit (Promega) and sequenced subsequently. Products of the sequencing reactions were separated on a 6% polyacrylamide/7 M urea gel by electrophoresis at 65 W. After drying, the gel was exposed to an X-ray film for 3–4 days. About 300–400 nucleotides were readable per sequencing run.

Sequence analysis

Sequences were aligned with the rbcL gene of *Nicotiana tabacum* (Chase *et al.*, 1993). Phylogenetic trees were reconstructed using the maximum parsimony method (phylogeny program PAUP 3.1.1. (Swofford, 1993) and the neighbour-joining method (Saitou and Nei, 1987) (program package MEGA (Kumar *et al.*, 1993)). In the neighbour-joining analyses genetic distances were calculated based on the Kimura 2-parameter model. With PAUP, heuristic algorithms were employed. Bootstrap analysis was performed to obtain confidence estimates for each furcation. Sequences are deposited at the EMBL-sequence library. (EMBL accession numbers: Z37381–Z37383; Z37386–Z37395; Z37404–Z37408; Z37413–Z37435; Z37446; Z37450 and Z37474.)

Results and Discussion

rbcL sequences

DNA was amplified by PCR using rbcL-specific primers from 41 taxa belonging to 20 genera of

Table I. Primer sequences used for PCR and direct sequencing of rbcL genes.

PCR-primers	
	5' 3'
RBCL-N (1):	ATGTCACCACAAACAGAAACTAAAGC
RBCL-R (1420):	TATCCATTGCTGGGAATTCAAATTTG
Sequencing primers	
RBCL-OF (174):	GCCGAATCTTCTACTGGTAC
RBCL-2F (426):	TGCTTATGTTAAAACTTTCC
RBCL-3F (635):	TGCGTTGGAGAGACCGTTTC
RBCL-1R (1207):	GGGTGCCCTAAAGTTCCTCC
RBCL-RF (1023):	ACTTTAGGTTTTGTTGATTT
RBCL-NR (428):	TTATCTCTCTCAACTTGGAT

Table II. RbcL gene nucleotide sequences of 41 species of the Nepetoideae (1368 bp). Only variable sites are illustrated. . = nucleotide identical to that in the first line.

	11	111	111	111	111	222	222	222	222	222	222	223	333	333	333	333	333	334	444	444	444	444	444	444	444	455	555	555	555	555	555	666	666	666	666	666	666
	344	558	888	902	334	455	666	678	011	333	455	666	677	789	990	011	134	466	666	789	990	112	222	333	455	677	888	899	900	002	345	567	888	999	001	222	445	566	677	778
	659	342	478	680	587	901	257	879	759	256	667	146	712	314	562	956	732	916	789	880	396	890	457	234	103	245	035	615	814	588	732	542	078	679	098	147	581	724	923	572
Nicotiana tabacum	ATG	AAG	GCC	GAC	ATA	CTG	GCC	GCT	AAA	GCG	GCG	CGG	TGT	TAT	GCT	CAG	ATC	TGT	AAC	GCG	CAG	ATC	CCG	TGT	TTG	GAA	TAT	GAT	TTC	CTT	GTC	CCA	CGA	TTC	TTG	CAA	ATT	AGG	AAC	TAG
Agastache foeniculum	.G.	...	A.G	AC.	..G	...	...	...	G.G	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	G.T	A..	.A.	CC.	TG.	...	..C	..T	.C.	...	TG.	...	.C.	C..	.CG	.CC	G..	..A	...
Agastache mexicana	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	A..	.A.	CC.	TG.	...	..C	..T	.C.	...	TG.	...	.C.	C..	.CG	.CC	GA.	..A	...
Agastache rugosa	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	G.T	A..	.A.	.C.	TG.	...	..C	..T	.C.	...	TGC	...	.C.	C..	.CG	.CC	G..	..A	...
Ajuga reptans	.G.	...	ATG	AC.	.CG	...	...	A..	G..	...	A.A	..C	CC.	..C	TG.	...	..T	..A	..T	A.A	T..	...	GT.	.A.	.C.	TG.	...	..C	C.T	..G	.C.	TA.	...	...	C..	TCG	G..	...	..A	...
Collinsonia canadensis	.G.	...	A.G	ACT	..G	...	...	...	G..	...	..A	..C	CA.	..C	TG.	...	..T	..A	..T	A.A	T..	..T	A..	.A.	.C.	.G.	...	A.C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	...	...	...
Dracocephalum grandiflorum	.G.	...	ATG	AC.	..G	...	...	...	GC.	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	ATA	T..	..T	...	CA.	.CA	TG.	...	..C	..T	.C.	...	TG.	..G	...	C..	.CG	..C	G..	..A	...
Dracocephalum moldavica	.G.	...	ATG	AC.	..G	...	...	...	GC.	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	ATA	T.C	..T	...	CA.	.CA	TG.	...	..C	..T	.C.	...	TG.	..G	...	C..	.CG	..C	G..	..A	..T
Dracocephalum ruyschiana	.G.	...	ATG	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	ATA	TG.	..T	...	CA.	.CA	TG.	...	..C	..T	.C.	...	TG.	..G	...	C..	.CG	..C	G..	.GA	..T
Glechoma hederacea	.G.	...	ATG	AC.	..G	...	...	...	G..	...	..A	TTC	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	TG.	...	..C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	G..	..A	..T
Hormium pyrenaicum	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	.G.	...	..C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	...	..A	...
Hyssopus officinalis	.G.	...	ATG	AC.	..G	TG.	A..	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	ATA	T..	..T	...	CA.	.CA	TG.	...	..C	..T	.C.	...	TG.	..G	...	C..	.CG	..A	G..	G.A	...
Lavandula angustifolia	.G.	...	A.G	AC.	..G	...	...	...	G..	...	.AA	..C	CCC	..C	TG.	T..	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	...	...	..C	.AT	TC.	...	.G.	...	...	C.A	.CG	..C	...	..A	..T
Lavandula lanata	.G.	...	A.G	AC.	..G	..C	...	...	G..	...	.AA	..C	CCC	..C	TG.	T..	..T	..A	..T	A..	T..	..T	...	.A.	.C.	...	...	.CC	.AT	.C.	...	.G.	...	...	C.A	.CG	.CC	...	..A	..T
Lavandula latifolia	.G.	...	A.G	AC.	.CG	...	...	...	G..	...	.AA	..C	CCC	..C	TG.	T..	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	...	...	..C	..T	.C.	...	.G.	...	...	C.A	.CG	..C	...	..A	..T
Lavandula latifolia "nana alba"	.G.	...	A.G	AC.	..G	...	...	...	G..	...	.AA	..C	CCC	..C	TG.	T..	..T	..A	..T	A.A	...	...	...	.A.	.C.	...	...	..C	..T	TC.	...	.G.	...	...	C.A	.CG	...	...	..A	..T
Lavandula stoechas	.G.	...	A.G	AC.	..G	...	...	...	G..	...	.AA	..C	CCC	..C	TG.	T..	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	...	...	..C	..T	TC.	...	.G.	...	...	C.A	.CG	...	...	..A	..T
Majorana hortensis	.G.	...	A.G	AC.	.CG	...	..G	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	.AC	.C.	.G.	...	..C	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	.T.
Melissa officinalis	.G.	G..	A.G	AC.	..G	...	..G	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	...	.AC	.C.	.G.	...	..C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	...	..A	.T.
Mentha longifolia	GG.	...	A.G	AC.	.CG	...	.A.	...	G..	CGC	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.GG	...	...	..T	.C.	...	.G.	...	..G	G..	.CG	.CC	...	..A	..T
Mentha l. capensis	GG.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	.GT	A.A	T..	..T	GT.	...	.C.	.G.	...	...	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	..T
Mentha rotundifolia	GG.	...	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	...	..C	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	..T
Monarda didyma	GGC	..C	A.G	AC.	.CG	...	...	C..	G..	...	..A	T.C	CCC	..C	TGC	...	.GT	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	...	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	..T
Monarda fistulosa	GGC	..C	A.G	AC.	.CG	...	...	C..	G..	...	..A	T.C	CCC	..C	TG.	...	.GT	..A	..T	A.A	T..	..T	GT.	...	.C.	TG.	...	...	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	..T
Monarda menthaefolia	GG.	..C	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	.GT	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	...	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	..T
Nepeta cataria	.G.	...	A.G	AC.	G.G	...	...	..C	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	G.T	...	.A.	.G.	.G.	...	..C	..T	.CC	...	TG.	...	...	C..	.CG	.CC	...	...	...
Nepeta faassenii	.G.	...	A.G	AC.	G.G	...	...	A.C	G..	...	..A	T.C	CCC	..C	TG.	.GA	GGT	..A	..T	A.A	T..	G.T	...	.A.	.G.	.G.	...	..C	G.T	.CA	...	TG.	...	...	C..	.CG	.CC	...	..A	..T
Nepeta tuberosa	.G.	...	A.G	AC.	G.G	...	...	..C	G..	...	..A	T.C	CCC	.GC	TG.	...	..T	A.A	C.T	A.A	T..	G.T	...	.A.	.G.	.G.	...	..C	..T	.CC	...	TG.	...	C..	CC.	.CG	.CC	...	...	C..
Ocimum basilicum	.G.	.G.	A.G	AC.	..G	.C.	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	.CA	..T	A.A	T..	.C.	...	.AC	.C.	.G.	...	..C	..T	...	...	TG.	G..	...	C..	.CG	.CC	G..	..A	.T.
Origanum laevigatum	GG.	...	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	..C	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	...
Origanum vulgare	GG.	...	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	TG.	..T	GT.	...	.C.	.G.	...	...	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	..T
Perovskia abrotanoides	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	..C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	...	..A	...
Prunella grandiflora	.G.	...	ATG	AC.	..G	...	...	AG.	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	.G.	...	...	..T	.C.	A..	TG.	...	...	C..	.CG	.CC	..A	...	...
Prunella hyssopifolia	.G.	...	ATG	AC.	..G	...	...	AG.	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	A..	..T	...	.A.	.C.	.G.	...	...	..T	.C.	A..	TG.	...	...	C..	.CG	.CC	..A	...	...
Prunella vulgaris	.G.	...	ATG	AC.	..G	...	...	AG.	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	...	.A.	.C.	.G.	...	...	..T	.C.	A..	TG.	...	...	C..	.CG	.CC	..A	...	...
Rosmarinus officinalis	.G.	...	A.G	AC.	.C.	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	ATA	T..	..T	GT.	...	.C.	.G.	C.C	..C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	...	..A	...
Salvia officinalis	.G.	...	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	.G.	..C	..T	.C.	...	TG.	.A.	...	C..	.CG	.CC	...	..A	...
Salvia sclarea	.G.	...	ATG	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	..C	..T	.C.	...	TG.	...	...	C..	.CG	.CC	...	..A	...
Satureja hortensis	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	..T	G.C	.A.	.C.	TG.	...	..C	..T	.C.	...	TG.	...	...	C..	.CG	..C	G..	..A	...
Satureja montana	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	G.T	G.C	.A.	.C.	TG.	...	..C	..T	.C.	...	.G.	...	...	C..	.CG	.CC	G..	..A	...
Satureja thymbra	.G.	...	A.G	AC.	..G	...	...	...	G..	...	..A	T.C	CCC	..C	TG.	...	..T	..A	..T	A.A	T..	G.T	A.C	.A.	.C.	TG.	...	..C	..T	.C.	...	TG.	...	.C.	C..	.CG	.CC	G..	..A	...
Thymus alsinoides	GG.	...	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	C.C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	...	..T	.C.	..T	.G.	...	...	C..	.CG	.CC	...	..A	...
Thymus vulgaris	GG.	...	A.G	AC.	.CG	...	...	...	G..	...	..A	T.C	CCC	C.C	TG.	...	..T	..A	..T	A.A	T..	..T	GT.	...	.C.	.G.	...	...	..T	.C.	...	.G.	...	...	C..	.CG	.CC	...	..A	...

Table II. (Continued).

	667 891 867	777 344 201	777 455 739	777 688 335	777 889 792	788 900 316	888 011 836	888 123 854	888 334 670	888 444 148	888 566 901	888 667 459	888 922 717	899 334 360	999 455 508	999 667 030	999 778 232	11 900 811 414	111 000 222 039	111 000 333 256	111 000 446 070	111 000 677 612	111 000 799 725	111 111 011 001	111 111 222 367	111 111 334 358	111 111 467 070	111 111 889 980	111 112 990 580	111 222 002 670	111 222 344 697	111 222 567 605	111 233 778 100	111 333 900 785	111 333 000 613	111 333 111 458	111 333 222 567	111 333 334 078	111 333 445 581	111 333 566 696	111 333 778 903	11 33 89 160	32
	GAC	AGC	ACA	GCT	CGC	GTA	TGG	GCT	GCG	GCA	AAT	TCC	GTC	CGT	ACA	GTG	TCT	TTA	CAG	CTG	TGG	CTC	TCT	ATT	AAG	CAC	TAA	CCG	GTA	TAA	CTT	TCA	CTC	CAA	TGC	GTT	CAG	AGA	TCG	GAC	GGC	TGA	AG
Nicotiana tabacum	...	G..	GG.	.A.	.T.	...	.AA	..C	.T.	...	...	CTT	.CG	.T.	T.C	.C.	..G	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	..G	...	AC.	...	T.C	.G.	.C.	..C	...	...	GCT	G.C	CTA	..T	T.T	C.G	.A
Agastache foeniculum	...	G..	GG.	.A.	.T.	...	.AA	..C	.T.	...	...	CTT	.CG	.T.	T.C	.C.	..G	.AG	.TA	...	A.A	A..	...	.C.	G..	AT.	..G	...	AC.	...	T.C	.G.	.C.	..C	...	...	GCT	G.C	CTA	..T	T.T	C.G	.A
Agastache mexicana	...	G..	GG.	.A.	.T.	...	.AA	..C	.T.	...	...	CTT	.CG	.T.	T.C	.C.	..G	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	..G	...	AC.	...	T.C	.G.	.C.	..C	...	...	GCT	G.C	CTA	..T	T.T	C.G	.A
Agastache rugosa	...	...	..G	.A.	.T.	...	.AA	.TC	C..	...	...	CTT	A.G	.TC	T.C	A..	..G	.A.	ATA	...	AA.	A..	...	..C	...	AT.	.GG	...	...	...	..C	.G.	.C.	..C	...	.C.	GCT	GAC	.T.	..T	T..	C.G	.A
Ajuga reptans	A..	G..	.G.	.A.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	..G	.AG	TTA	...	A..	A.A	...	.CC	GT.	ATT	..G	.T.	AC.	...	.C.	.G.	.C.	.GC	...	...	ACT	G.C	CTA	..T	T..	C.G	.A
Collinsonia canadensis	.C.	GT.	G..	.A.	.T.	..G	.AA	..C	.T.	T..	...	CTT	A.G	.TC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	GT.	AT.	.GG	..T	ACC	...	T.C	.GG	.C.	..C	...	.CC	GCT	G.C	CTA	..T	T..	C.G	GA
Dracocephalum grandiflorum	.C.	GT.	G..	.A.	.T.	..G	..A	..C	.T.	...	...	CTT	A.G	.TC	T.:	.C.	C..	.AG	.TA	...	A.A	A..	.T.	.CC	G..	AT.	.G.	..T	ACC	..G	T.C	.GG	.CG	..C	...	.CC	GCT	G.C	CTA	..T	T..	C.G	GA
Dracocephalum moldavica	.C.	GT.	G..	.A.	.T.	...	.AA	..C	.T.	TT.	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A.A	AC.	C..	.CC	GT.	AT.	.GG	..T	AC.	...	T.C	.G.	.C.	..C	...	.CC	GCT	G.C	CTA	..T	T..	C.G	GA
Dracocephalum ruyschiana	.G.	G..	GG.	AA.	.T.	...	.AA	..C	.T.	...	...	CTT	ACA	.TC	T.C	.C.	...	.AG	.TA	...	A..	A..	...	.CC	G..	AT.	.CG	...	AC.	...	T.C	.G.	.CG	..C	...	...	GCT	G..	CTA	..T	T.T	CCG	.A
Glechoma hederacea	...	G..	G..	.A.	.T.	...	.AA	..C	.T.	T..	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	..G	...	AC.	...	..C	.G.	.C.	.GC	...	...	GCT	G.C	CTA	..T	T.T	C.G	.A
Hormium pyrenaicum	.C.	G..	G..	.A.	.T.	...	.AA	..C	.T.	T..	...	CTT	A.G	.TC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	.GG	..T	AC.	...	T.C	.GG	TC.	..C	...	..C	GCT	G.C	CAA	..T	T.T	C.G	GA
Hyssopus officinalis	...	G..	...	.A.	.T.	.C.	.A.	..C	.T.	T..	GC.	GTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A..	A..	...	.CC	GT.	AT.	...	...	AC.	...	..C	...	TC.	.GC	...	...	GCC	..C	CTA	..T	T..	C..	.A
Lavandula angustifolia	...	G..	...	.A.	.T.	.C.	.A.	..C	.T.	T..	...	GTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A..	A..	...	.CC	GT.	AT.	...	...	AC.	...	..C	...	.C.	.GC	...	...	GCC	..C	CTA	..T	T..	C..	.A
Lavandula lanata	...	G..	...	.A.	.T.	.C.	.A.	..C	.T.	T..	...	GTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A..	A..	...	.CC	GT.	AT.	...	...	AC.	...	..C	...	..C	.GC	...	...	GCC	..C	CTA	..T	T..	C..	.A
Lavandula latifolia	...	G..	...	.A.	.T.	.C.	.A.	..C	.T.	T..	...	GTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A..	A..	...	.CC	GT.	AT.	...	...	AC.	...	..C	...	TC.	.GC	...	...	GCC	..C	CTA	..T	T..	C..	.A
Lavandula latifolia "nana alba"	...	G..	...	.A.	.T.	.C.	.A.	..C	.T.	T..	...	GTT	ACG	.TC	T.C	.C.	...	.AG	.TA	...	A..	A..	...	.CC	GT.	AT.	...	...	AC.	...	..C	...	TC.	.GC	...	...	GCC	..C	CTA	..T	T..	C..	.A
Lavandula stoechas	...	G..	...	.A.	.T.	.C.	.AA	..C	.T.	T..	..C	GTT	ACG	.TC	T.C	.C.	...	CAG	.TA	...	A..	A..	...	.CC	GT.	AT.	...	...	AC.	...	..C	...	TC.	.GC	...	...	GCC	..C	CTA	..T	T..	C..	.A
Majorana hortensis	...	G..	GG.	A.C	.T.	A..	.AA	..C	.T.	...	...	CTT	ACG	.TC	TG.	.C.	...	.AG	.TA	T..	A.A	A..	...	.CC	G..	ATT	..G	...	AC.	AT.	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Melissa officinalis	...	G..	GG.	AAC	.T.	A..	.AA	..C	CT.	...	...	CTT	ACG	.TC	TG.	.C.	..G	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	..G	...	AC.	...	..C	.G.	.C.	.GC	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Mentha longifolia	...	G..	GGG	AA.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	.G.	.AG	.TA	T..	A.A	A..	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	AG.	..A	AC.	GCT	G.C	CTA	..T	T..	C.G	.A
Mentha l. capensis	...	G..	G.G	AA.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	AC.	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	.AG	.C.	GCT	G.C	CT.	.TT	TA.	C.G	.A
Mentha rotundifolia	...	G..	GGG	AA.	.T.	.C.	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	A..	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	...	.C.	GCT	G.C	CTA	..T	T..	C.G	.A
Monarda didyma	...	G..	G..	AA.	.TG	C..	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	AC.	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	C..	...	GCT	G.C	CTA	..T	T..	C.G	.A
Monarda fistulosa	...	G..	G..	AA.	.TG	C..	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	AC.	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	C..	...	GCT	G.C	CTA	..T	T..	C.G	.A
Monarda menthaefolia	...	G..	G..	AA.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	AC.	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	C..	...	GCT	G.C	CTA	..T	T..	C.G	.A
Nepeta cataria	...	G..	G..	.A.	.T.	...	.AA	.TC	.TA	T..	...	CTT	ACG	TTC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	GCC	G..	AT.	..G	T..	AC.	...	T.C	.G.	.C.	.GC	...	...	GCT	G.C	CTA	..T	T.T	C.G	.A
Nepeta faassenii	...	G..	G..	.A.	.T.	...	.AA	.TC	.TA	T..	...	CTT	ACG	TTC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	..G	T..	AC.	...	TCC	.G.	.C.	.GC	...	...	GCT	G.C	CTA	..T	T.T	C.G	.A
Nepeta tuberosa	...	G..	G..	.A.	TT.	...	.AA	.TC	.TA	T..	...	CTT	ACG	TTC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	..G	T..	AC.	...	T.C	.G.	.C.	.GC	...	...	GCT	G.C	C.A	..T	T.T	C.G	.A
Ocimum basilicum	..T	G..	GG.	.A.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	TG.	.C.	..G	.A.	ATA	...	A.A	A..	...	.CC	G..	AT.	..G	...	AC.	...	..C	.G.	.CG	.GC	C..	...	GCT	G.C	CTA	..T	T..	C.G	.A
Origanum laevigatum	...	G..	GG.	A..	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	TG.	A.A	A..	...	.C.	GG.	AT.	..G	...	AC.	...	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Origanum vulgare	...	G..	GG.	A..	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	TG.	A.A	AC.	...	.C.	GG.	AT.	..G	...	AC.	...	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Perovskia abrotanoides	...	G..	GG.	AA.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	A..	C..	.CC	G..	AT.	.GG	...	AC.	...	..C	.G.	.CG	.GC	...	...	GCT	G.C	CTA	C.T	T..	C.G	.A
Prunella grandiflora	...	G..	G..	.A.	.T.	...	ACA	A.C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	TTA	...	A.A	A..	...	.CC	G..	AT.	.GG	...	AC.	...	..C	.G.	.C.	.G.	...	...	.GT	G.C	CTA	..T	T.T	C.G	.A
Prunella hyssopifolia	...	G..	G..	.A.	.T.	...	ACA	A.C	.T.	...	...	CTT	ACG	.TC	T.C	.CA	...	.AG	TTA	...	A.A	A..	...	.CC	G.A	AT.	.GG	...	AC.	...	..C	.G.	.C.	.G.	...	...	.GT	G.C	CTA	..T	T.T	C.G	.A
Prunella vulgaris	...	G..	G..	.A.	.T.	...	ACA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	TTA	...	A.A	A..	...	.CC	G..	AT.	.GG	...	AC.	...	..C	.G.	.C.	.G.	...	...	.GT	G.C	CTA	..T	T.T	C.G	.A
Rosmarinus officinalis	...	G..	GG.	AA.	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	..G	.TA	T.A	A.A	A..	...	.CC	G..	AT.	..G	...	AC.	...	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Salvia officinalis	...	G..	GG.	AA.	.T.	.C.	.AA	..C	.T.	..G	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	AA.	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Salvia sclarea	...	G..	GG.	AA.	.T.	.C.	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	.C.	...	.AG	.TA	T..	A.A	AC.	..C	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	TCG	.G.	...	...	GCT	..C	CTA	..T	T..	C.G	.A
Satureja hortensis	...	G.T	G..	.A.	.T.	...	.AA	..C	.T.	T..	...	CTT	A.G	.TC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	CGG	..T	AC.	...	T.C	CGG	TC.	AGC	...	.CC	GCT	G.C	CTA	..T	T.T	C.G	GA
Satureja montana	...	G.T	G..	.A.	.T.	...	.AA	..C	.T.	T..	...	CTT	A.G	.TC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	CGG	..T	AC.	...	T.C	CGG	TC.	AGC	...	..C	GCT	G.C	CTA	..T	T..	C.G	GA
Satureja thymbra	...	G..	G..	.A.	.T.	...	.AA	..C	.T.	T..	...	CTT	A.G	.TC	T.C	.C.	...	.AG	.TA	...	A.A	A..	...	.CC	G..	AT.	CGG	..T	AC.	...	T.C	CG.	TC.	AGC	...	...C	GCT	G.C	CTA	..T	T.T	C.G	GA
Thymus alsinoides	...	G..	GG.	A..	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	...	...	.AG	.TA	T..	A.A	AC.	...	.CC	GG.	ATT	..G	..T	AC.	G..	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A
Thymus vulgaris	...	G..	GG.	A..	.T.	...	.AA	..C	.T.	...	...	CTT	ACG	.TC	T.C	...	...	.AG	.TA	T..	A.A	AC.	...	.CC	GG.	ATT	..G	...	AC.	...	..C	.G.	.CG	.G.	...	...	GCT	G.C	CTA	..T	T..	C.G	.A

the subfamily Nepetoideae and from *Prostanthera nivea, Westringia rosmariniformis* and *Ajuga reptans* which served as outgroups. No deletions, insertions or inversions of nucleotides or sequence elements were observed. In Table II the variable nucleotide sites (as compared to *N. tabacum*) of all rbcL sequences are documented.

Sequence variation within a species was almost negligible when PCR products were sequenced directly. Cloned PCR products of two individuals from the same species could differ by 2 or 3 bases, due to misincorporation during PCR or to UV-induced mutations (PCR products were excised from agarose gels using ethidium bromide and UV light). In order to make sure that the sequences in Fig. 1 are representative for a particular genus and to eliminate possible errors due to technical or biological problems, such as hybridization, we have directly sequenced the PCR products of one or two individuals of related species from every genus whenever possible.

Base composition and mode of substitutions

Base composition at first, second and third positions of codons is outlined in Table III. Guanine is significantly enhanced in the first position, but underrepresented in the third codon position. The opposite can be seen for thymine (uracil), being low in the first but high in the third position. The second position is mostly constrained and less variable than first or third positions according to the standard deviations of the means (Table II). A similar conclusion was drawn for other chloroplast

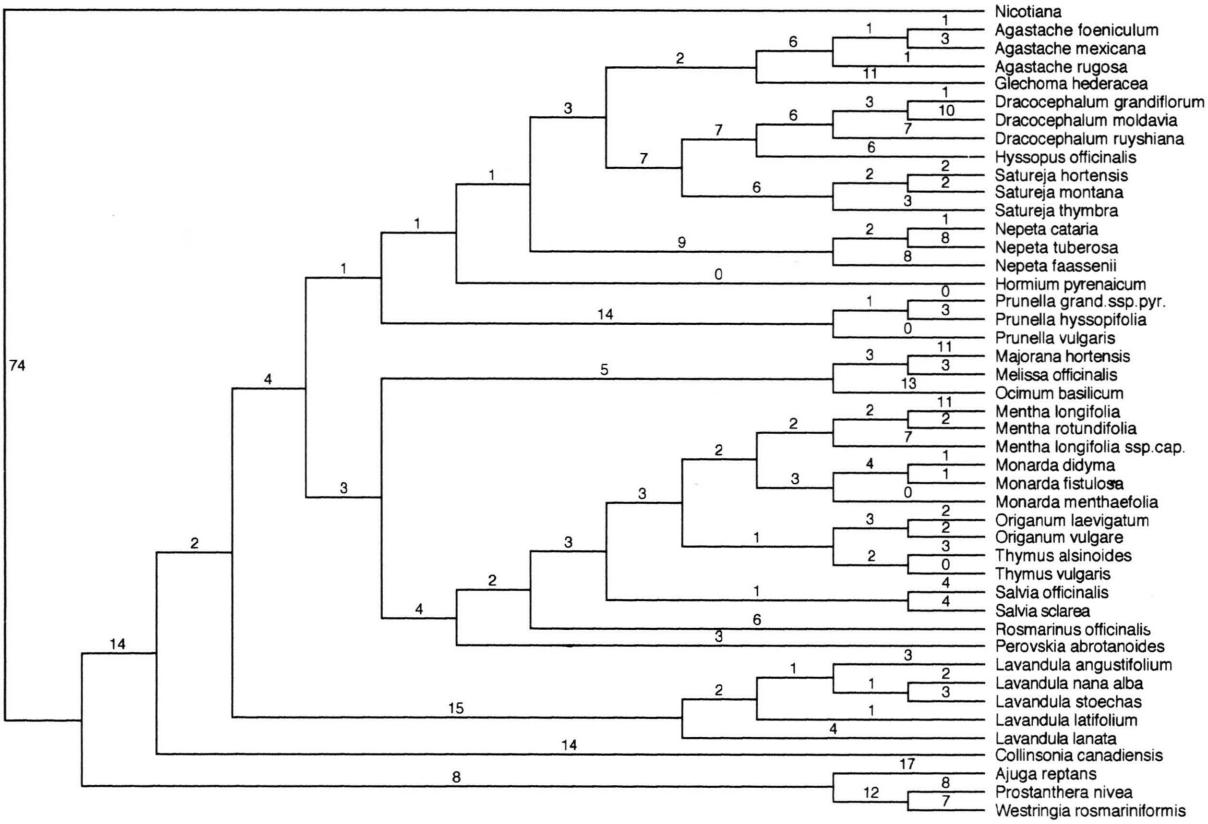

Fig. 1. Reconstruction of a phylogenetic tree of the Nepetoideae by maximum parsimony. The unweighted maximum parsimony analysis was performed using *Nicotiana* as outgroup. Two equally parsimonius trees were obtained which differed only with respect to the grouping within the genus *Agastache.* All other furcations were identical. Tree length: 446 steps; consistency index: 0.641; retention index: 0.774. Sequence additions such as "simple as is" or "random" had no influence on tree topology. Results are illustrated as a cladogram in which numbers refer to nucleotide substitutions between taxa.

Table III. Base composition in rbcL sequences from the Nepetoideae. The analysis was performed for first, second and third codon positions independently. Values in per cent (%).

Species	First				Second				Third			
	A	T	C	G	A	T	C	G	A	T	C	G
Mean ± S.D.	23.6	18.3	19.6	38.6	29.8	26.3	22.6	21.3	28.1	41.1	16.8	14.0
	0.26	0.23	0.35	0.36	0.21	0.17	0.21	0.14	0.60	0.44	0.35	0.54

genes (Albert *et al.*, 1992). Codon usage is biased: A RSCU value of >1.5 (relative synonymous codon usage) can be seen for UCU, CCU, CGU, AUU, ACU, GGA, GUA, GUU, GGU, GAU and GCU which would explain the dominance of G in the first codon position and that of T (U) in the third position. Codons CCG, UCG, AGG, ACG, AAG, GUC, GUG, GGC and GAC have a RSCU value lower than 0.5 explaining the low abundance of G and C in the third position.

Of the 1368 basepairs available per taxon (primer sequences were excluded) 196 positions were variable within the data set; 49 sites were variant at the first, 41 at the second and 106 at the third position. 111 characters were parsimony informative: 26 of them were at the first, 14 at the second and 71 at the third position. When the nucleotide sequences are converted into protein sequences we obtain 456 amino acids of which 79 are variant and 35 phylogenetically informative. These data are in agreement with results from other rbcL studies and reflect the fact that the third codon position is often degenerate (Soltis *et al.*, 1990). The resulting silent mutations are especially helpful for phylogenetic reconstructions since they are not adaptive as morphological characters.

On the other hand, the total of 79 amino acids changes is indicative that the Nepetoideae represent a phylogenetically old group. The coefficient between transitions/transversions (transitions are nucleotide substitutions from A to G or C to T and *vice versa* whereas transversion are changes from pyrimidine to purines and *vice versa, i.e.* G to C or T and A to C or T) is 1.8 (range 1.0 to 3.0), also suggesting the advanced state of evolution of this group. Young taxa are usually rich in transitions and high transition/transversion ratios are the consequence (Avise, 1994).

Phylogenetic analyses

As can be seen from Table IV sequences within a genus were closely related in most instances. Pairwise distances in intrageneric comparisons accounted for 0.5 ± 0.3% (mean ± S.D.) base substitutions whereas they varied from 1.2% to 2% in comparisons between genera and from 2.0 to 2.8% for those between subtribes and tribes. The subfamilies Lamioideae (*sensu* Erdtman) and Nepetoideae differ by 3.8 ± 0.26% which is the largest distance encountered in our data matrix.

The phylogenetic relationships between members of the Nepetoideae were reconstructed using the maximum parsimony and the neighbour-joining method. Sequences of 41 taxa from the Nepetoideae and 3 of the Lamioideae (*sensu* Erdtman) *(Ajuga, Prostanthera, Westringia)* were analyzed using *Nicotiana* as an outgroup. The tree-length distribution of 1000 random trees is significantly skewed ($g1 = -0.62$, $p < 0.01$) indicating that our data contain a significant phylogenetic signal (Hillis and Huelsenbeck, 1992).

Members of the two subfamilies Nepetoideae and Lamioideae (*sensu* Erdtman, 1945) are always separated in two main clades (Fig. 1, 2) independent of the choice of outgroups, character weights, distance algorithms or methods of tree reconstruction. For many genera more than two OTUs were analyzed. With the exception of the genus *Salvia* (Kaufmann and Wink, in preparation) all other genera appear to be monophyletic.

The phylogenetic trees of the Nepetoideae reconstructed by maximum parsimony (MP) and neighbour-joining (NJ) show a high degree of congruence (Fig. 1, 2). Weighted and unweighted parsimony had no significant influence on tree topologies. Considering MP and NJ trees a few differences appear: Whereas *Prunella, Hormium* and *Nepeta* form neighbouring but separated clades in MP trees, they cluster in a common clade in NJ

Table IV. Pairwise genetic distances between members of the Nepetoideae based on 1420 nucleotides of the rbcL gene (compare Fig. 1). Below diagonal: numbers of nucleotide substitutions; above diagonal: proportion of nucleotide positions differing between taxa (1.0 = 100%).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 *Agastache foeniculum*	-	0.003	0.002	0.020	0.021	0.026	0.022	0.013	0.011	0.022	0.027	0.026	0.025	0.027	0.027	0.022	0.016	0.027	0.025
2 *Agastache mexicana*	4	-	0.004	0.020	0.021	0.026	0.022	0.013	0.011	0.022	0.027	0.026	0.025	0.027	0.027	0.022	0.016	0.027	0.025
3 *Agastache rugosa*	3	5	-	0.019	0.020	0.025	0.021	0.013	0.010	0.021	0.027	0.025	0.025	0.027	0.026	0.021	0.015	0.027	0.024
4 *Collinsonia canadensis*	29	29	28	-	0.030	0.036	0.031	0.023	0.016	0.032	0.026	0.025	0.025	0.024	0.026	0.025	0.021	0.029	0.027
5 *Dracocephalum grandiflorum*	30	30	29	42	-	0.008	0.008	0.020	0.018	0.009	0.030	0.030	0.028	0.030	0.029	0.031	0.025	0.034	0.030
6 *Dracocephalum moldavica*	37	37	36	52	11	-	0.014	0.022	0.024	0.015	0.035	0.035	0.033	0.035	0.034	0.033	0.029	0.037	0.033
7 *Dracocephalum ryschiana*	31	31	30	43	11	20	-	0.020	0.018	0.014	0.029	0.029	0.027	0.029	0.029	0.032	0.026	0.034	0.028
8 *Glechoma hederacea*	19	19	18	33	29	32	28	-	0.013	0.021	0.027	0.025	0.025	0.027	0.026	0.022	0.018	0.026	0.023
9 *Hormium pyrenaicum*	15	15	14	23	25	34	26	18	-	0.017	0.018	0.017	0.016	0.018	0.018	0.016	0.011	0.022	0.018
10 *Hyssopus officinalis*	31	31	30	45	13	22	20	30	24	-	0.031	0.032	0.029	0.030	0.029	0.032	0.026	0.036	0.032
11 *Lavandula angustifolia*	39	39	38	37	43	50	42	38	26	44	-	0.006	0.004	0.004	0.005	0.030	0.027	0.034	0.029
12 *Lavandula lanata*	37	37	36	35	43	50	42	36	24	46	8	-	0.005	0.007	0.008	0.029	0.025	0.032	0.028
13 *Lavandula latifolia*	36	36	35	34	40	47	39	35	23	41	5	7	-	0.004	0.004	0.027	0.025	0.030	0.027
14 *Lavandula latifolia "nana alba"*	39	39	38	37	43	50	42	38	26	43	6	10	5	-	0.004	0.030	0.027	0.034	0.029
15 *Lavandula stoechas*	38	38	37	36	42	49	41	37	25	42	7	11	6	5	-	0.029	0.026	0.033	0.029
16 *Majorana hortensis*	32	32	31	35	45	48	46	32	24	46	44	42	39	44	43	-	0.011	0.020	0.019
17 *Melissa officinalis*	23	23	22	29	36	41	37	26	16	37	38	36	35	38	37	15	-	0.026	0.023
18 *Mentha longifolia*	40	40	39	42	50	54	49	38	32	52	49	47	44	49	48	28	38	-	0.014
19 *Mentha l. capensis*	35	35	34	38	43	47	40	33	25	45	42	40	39	42	41	26	33	19	-
20 *Mentha rotundifolia*	28	28	27	31	38	42	37	26	20	40	35	33	30	35	34	17	26	13	11
21 *Monarda didyma*	36	36	35	39	46	50	43	34	26	48	43	41	38	43	42	24	33	23	17
22 *Monarda fistulosa*	34	34	33	39	44	48	41	32	26	46	43	41	38	43	42	24	33	23	17
23 *Monarda menthaefolia*	31	31	30	34	41	45	38	29	21	43	38	36	33	38	37	20	29	18	12
24 *Nepeta cataria*	22	24	21	32	34	43	35	27	13	33	37	35	34	37	36	34	27	42	36
25 *Nepeta faassenii*	28	30	27	38	40	47	39	31	19	39	41	39	38	41	40	40	33	46	40
26 *Nepeta tuberosa*	29	31	28	39	41	50	42	34	20	39	44	42	41	44	43	41	34	48	43
27 *Ocimum basilicum*	30	30	29	37	44	49	45	35	23	46	47	45	44	45	46	28	17	48	44
28 *Origanum laevigatum*	26	24	25	31	38	44	39	26	18	40	37	35	32	37	36	15	24	19	17
29 *Origanum vulgare*	30	28	29	35	42	46	37	28	22	44	39	37	34	39	38	19	28	19	15
30 *Perovskia abrotanoides*	24	24	23	30	35	40	34	23	16	36	38	36	35	38	37	21	22	27	23
31 *Prunella grandifloria*	28	28	27	31	36	42	37	28	17	35	41	39	38	41	40	34	29	40	34
32 *Prunella hyssopifolia*	31	31	30	34	39	45	40	31	20	38	44	42	41	43	43	37	32	43	37
33 *Prunella vulgaris*	27	27	26	30	35	41	36	27	16	34	40	38	37	40	39	33	28	39	33
34 *Rosmarinus officinalis*	26	26	25	32	37	42	38	26	18	38	40	38	35	40	39	19	24	25	23
35 *Salvia officinalis*	28	28	27	30	40	46	40	28	20	42	39	37	34	39	38	18	26	22	20
36 *Salvia sclarea*	28	28	27	31	38	44	37	26	20	38	35	35	30	35	34	19	26	23	19
37 *Satureja hortensis*	25	25	24	39	20	29	25	28	19	17	39	41	36	39	38	38	32	42	38
38 *Satureja montana*	25	25	24	37	22	31	27	28	19	21	37	37	34	37	36	34	30	40	36
39 *Satureja thymbra*	18	20	17	36	24	33	27	26	17	23	39	39	36	39	38	37	30	43	39
40 *Thymus alsinoides*	32	32	31	35	42	48	41	32	24	44	43	41	38	43	42	18	30	21	17
41 *Thymus vulgaris*	31	31	30	34	43	49	42	31	23	45	42	40	37	42	41	18	29	20	16

Table IV. (Continued).

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
1 *Agastache foeniculum*	0.020	0.025	0.024	0.022	0.015	0.020	0.020	0.021	0.018	0.021	0.017	0.020	0.022	0.019	0.018	0.019	0.020	0.018	0.018	0.013	0.022	0.022
2 *Agastache mexicana*	0.020	0.025	0.024	0.022	0.017	0.021	0.022	0.021	0.017	0.020	0.017	0.020	0.022	0.019	0.018	0.019	0.020	0.018	0.018	0.014	0.022	0.022
3 *Agastache rugosa*	0.019	0.025	0.023	0.021	0.015	0.019	0.020	0.020	0.018	0.020	0.016	0.019	0.021	0.018	0.018	0.018	0.019	0.017	0.017	0.012	0.022	0.021
4 *Collinsonia canadensis*	0.022	0.028	0.028	0.025	0.023	0.027	0.028	0.027	0.022	0.025	0.022	0.022	0.025	0.022	0.023	0.021	0.022	0.028	0.027	0.026	0.025	0.025
5 *Dracocephalum grandiflorum*	0.027	0.032	0.031	0.029	0.024	0.028	0.029	0.031	0.027	0.029	0.025	0.025	0.027	0.025	0.026	0.027	0.027	0.014	0.015	0.017	0.029	0.030
6 *Dracocephalum moldaviaca*	0.029	0.035	0.034	0.032	0.030	0.033	0.035	0.034	0.031	0.032	0.028	0.029	0.032	0.029	0.029	0.032	0.031	0.020	0.022	0.023	0.034	0.034
7 *Dracocephalum ryschiana*	0.026	0.030	0.029	0.027	0.025	0.027	0.029	0.032	0.027	0.026	0.024	0.026	0.028	0.025	0.027	0.027	0.026	0.018	0.019	0.019	0.029	0.029
8 *Glechoma hederacea*	0.018	0.024	0.022	0.020	0.019	0.022	0.024	0.025	0.018	0.020	0.016	0.020	0.022	0.019	0.018	0.019	0.018	0.020	0.020	0.018	0.022	0.022
9 *Hormum pyrenaicum*	0.014	0.018	0.018	0.015	0.009	0.013	0.014	0.016	0.013	0.015	0.011	0.012	0.014	0.011	0.013	0.013	0.014	0.013	0.013	0.012	0.017	0.016
10 *Hyssopus officinalis*	0.028	0.034	0.032	0.030	0.023	0.027	0.027	0.032	0.028	0.031	0.025	0.025	0.027	0.024	0.027	0.029	0.027	0.012	0.015	0.016	0.031	0.032
11 *Lavandula angustifolia*	0.025	0.030	0.030	0.027	0.026	0.029	0.031	0.033	0.026	0.027	0.027	0.029	0.031	0.028	0.028	0.027	0.025	0.027	0.026	0.027	0.030	0.029
12 *Lavandula lanata*	0.023	0.029	0.029	0.025	0.025	0.027	0.029	0.032	0.025	0.026	0.025	0.027	0.029	0.027	0.027	0.025	0.025	0.029	0.026	0.027	0.029	0.028
13 *Lavandula latifolia*	0.021	0.027	0.027	0.023	0.024	0.027	0.029	0.031	0.022	0.024	0.025	0.027	0.029	0.026	0.025	0.023	0.021	0.025	0.024	0.025	0.027	0.026
14 *Lavandula latifolia "nana alba"*	0.025	0.030	0.030	0.027	0.026	0.029	0.031	0.032	0.026	0.027	0.027	0.029	0.030	0.028	0.028	0.027	0.025	0.027	0.026	0.027	0.030	0.029
15 *Lavandula stoechas*	0.024	0.029	0.029	0.026	0.025	0.028	0.030	0.032	0.025	0.027	0.026	0.028	0.030	0.027	0.027	0.026	0.024	0.027	0.025	0.027	0.029	0.029
16 *Majorana hortensis*	0.013	0.018	0.018	0.015	0.025	0.029	0.029	0.020	0.011	0.014	0.015	0.025	0.027	0.024	0.014	0.013	0.014	0.027	0.025	0.027	0.013	0.013
17 *Melissa officinalis*	0.018	0.023	0.023	0.020	0.019	0.023	0.024	0.012	0.017	0.020	0.015	0.020	0.022	0.020	0.017	0.018	0.018	0.022	0.021	0.021	0.021	0.020
18 *Mentha longifolia*	0.010	0.017	0.017	0.013	0.030	0.033	0.035	0.034	0.014	0.014	0.020	0.029	0.031	0.028	0.018	0.015	0.017	0.030	0.029	0.031	0.015	0.015
19 *Mentha l. capensis*	0.008	0.012	0.012	0.008	0.025	0.028	0.030	0.031	0.012	0.011	0.016	0.024	0.026	0.023	0.016	0.013	0.013	0.027	0.025	0.027	0.012	0.011
20 *Mentha rotundifolia*	–	0.010	0.010	0.006	0.022	0.025	0.027	0.026	0.006	0.007	0.011	0.022	0.024	0.021	0.010	0.006	0.007	0.023	0.022	0.024	0.008	0.008
1 *Monarda didyma*	14	–	0.001	0.004	0.026	0.027	0.031	0.031	0.011	0.010	0.017	0.024	0.026	0.023	0.015	0.013	0.013	0.029	0.026	0.028	0.011	0.011
2 *Monarda fistulosa*	14	2	–	0.004	0.026	0.027	0.031	0.031	0.011	0.010	0.017	0.024	0.026	0.023	0.015	0.013	0.013	0.027	0.025	0.027	0.011	0.011
3 *Monarda menthaefolia*	9	5	5	–	0.022	0.024	0.027	0.028	0.008	0.006	0.013	0.021	0.023	0.020	0.012	0.009	0.009	0.025	0.022	0.025	0.008	0.007
4 *Nepeta cataria*	31	37	37	32	–	0.008	0.006	0.025	0.020	0.023	0.019	0.018	0.020	0.018	0.020	0.021	0.022	0.020	0.020	0.017	0.025	0.024
5 *Nepeta faassenii*	35	38	38	34	11	–	0.013	0.029	0.025	0.026	0.023	0.022	0.025	0.022	0.025	0.025	0.026	0.024	0.024	0.021	0.029	0.028
6 *Nepeta tuberosa*	38	44	44	39	9	18	–	0.029	0.025	0.028	0.024	0.023	0.025	0.022	0.025	0.026	0.027	0.025	0.025	0.022	0.029	0.029
7 *Ocimum basilicum*	37	44	44	40	35	41	42	–	0.025	0.027	0.023	0.026	0.028	0.025	0.025	0.025	0.026	0.028	0.027	0.027	0.029	0.028
8 *Origanum laevigatum*	8	16	16	11	29	35	36	35	–	0.003	0.010	0.020	0.022	0.020	0.008	0.008	0.008	0.023	0.020	0.022	0.007	0.006
9 *Origanum vulgare*	10	14	14	9	33	37	40	39	4	–	0.013	0.022	0.024	0.021	0.011	0.010	0.010	0.026	0.023	0.025	0.007	0.006
10 *Perovskia abrotanoides*	16	24	24	19	27	33	34	33	14	18	–	0.019	0.021	0.018	0.008	0.011	0.011	0.020	0.019	0.020	0.014	0.013
1 *Prunella grandifloria*	31	34	34	30	26	32	33	37	29	31	27	–	0.002	0.001	0.020	0.020	0.020	0.022	0.022	0.021	0.023	0.022
2 *Prunella hyssopifolia*	34	37	37	33	29	35	36	40	32	34	30	3	–	0.003	0.022	0.023	0.022	0.025	0.025	0.023	0.025	0.025
3 *Prunella vulgaris*	30	33	33	29	25	31	32	36	28	30	26	1	4	–	0.020	0.020	0.020	0.022	0.022	0.020	0.022	0.022
4 *Rosmarinus officinalis*	14	22	22	17	29	35	36	35	12	16	12	29	32	28	–	0.009	0.010	0.023	0.022	0.022	0.013	0.012
5 *Salvia officinalis*	10	19	19	14	31	37	38	37	12	15	16	31	34	30	14	–	0.006	0.025	0.023	0.024	0.011	0.010
6 *Salvia sclarea*	10	18	18	13	31	37	38	37	12	14	16	29	32	28	14	8	–	0.023	0.022	0.022	0.010	0.009
7 *Satureja hortensis*	33	41	39	36	28	34	35	40	33	37	29	32	35	31	33	34	33	–	0.003	0.005	0.026	0.027
8 *Satureja montana*	31	37	35	32	28	34	35	38	29	33	27	32	35	31	31	34	31	4	–	0.005	0.023	0.024
9 *Satureja thymbra*	34	40	38	35	24	30	31	38	32	36	28	30	33	29	32	33	32	7	7	–	0.025	0.026
10 *Thymus alsinoides*	12	16	16	11	35	41	42	41	10	10	20	33	36	32	18	14	14	37	33	36	–	0.002
1 *Thymus vulgaris*	11	15	15	10	34	40	41	40	9	9	19	32	35	31	17	13	13	38	34	37	3	–

trees. The same is true for the *Agastache/Glechoma* pair. A bootstrap analysis was performed with a reduced set of 20 taxa (one per genus) of the Nepetoideae to obtain confidence limits for each furcation (Fig. 2).

A number of distinctive clades became apparent in MP and NJ phylogenetic reconstructions although not all of them are supported by high bootstrap values (Fig. 2): I – *Collinsonia,* II – *Lavandula,* III – *Agastache, Glechoma,* IV – *Satureja, Hyssopus, Dracocephalum,* V – *Nepeta,* VI – *Hormium,* VII – *Prunella,* VIII – *Melissa, Ocimum,* IX – *Monarda, Mentha,* X – *Origanum, Thymus,* XI – *Salvia,* XII – *Rosmarinus,* and XIII – *Perovskia.* At least five main branches representing the clades I, II, III to VII, VIII, and IX to XIII respectively, can be distinguished within the Nepetoideae studied. They might be considered representing the tribes (*sensu* Cantino, 1992) Elsholtzieae (I), Lavanduleae (II), and Mentheae (III–XIII). The tribe Mentheae needs to be subdivided into at least three main groups (clades III–VII, VIII and IX–XIII).

Majorana hortensis has been treated as a distinctive genus by many authors (Briquet, 1895–1897; Hegi, 1927); others have classified it as a member of the genus *Origanum.* Our rbcL data (sequences from two different accessions were identical) imply that *Majorana* differs significantly from *Origanum:* 19 base substitutions were recorded between these taxa accounting for 1.4%, which is a typical distance between related genera. Thus, *Majorana* should be treated as a genus of its own. In MP and NJ trees *Majorana* clusters together with *Melissa* and *Ocimum.* However, the *Ocimum/Melissa/Majorana* cluster is only weakly supported by the bootstrap analysis (Fig. 2).

Prunella had been classified as a member of the subfamily Stachydeae by Bentham (1832–1836) which was disputed already on morphological characteristics (Briquet, 1895–1897; Wunderlich, 1967). According to our rbcL data, *Prunella* is a true member of the tribe Mentheae as defined by Cantino (1992).

Rosmarinus had been classified by Briquet (1895–1897) as a member of the Ajugoideae, which was certainly incorrect as stated already by other taxonomists (Wunderlich, 1967). *Rosmarinus* is apparently integrated in the tribe Mentheae.

Lavandula has been treated as a distinct subfamily (Briquet, 1895–1897) or tribe (Wunderlich, 1967; Cantino, 1992) whereas Bentham (1832–1836) classified this taxon as a subtribe of the Ocimoideae. If *Ajuga* or *Prostanthera* are

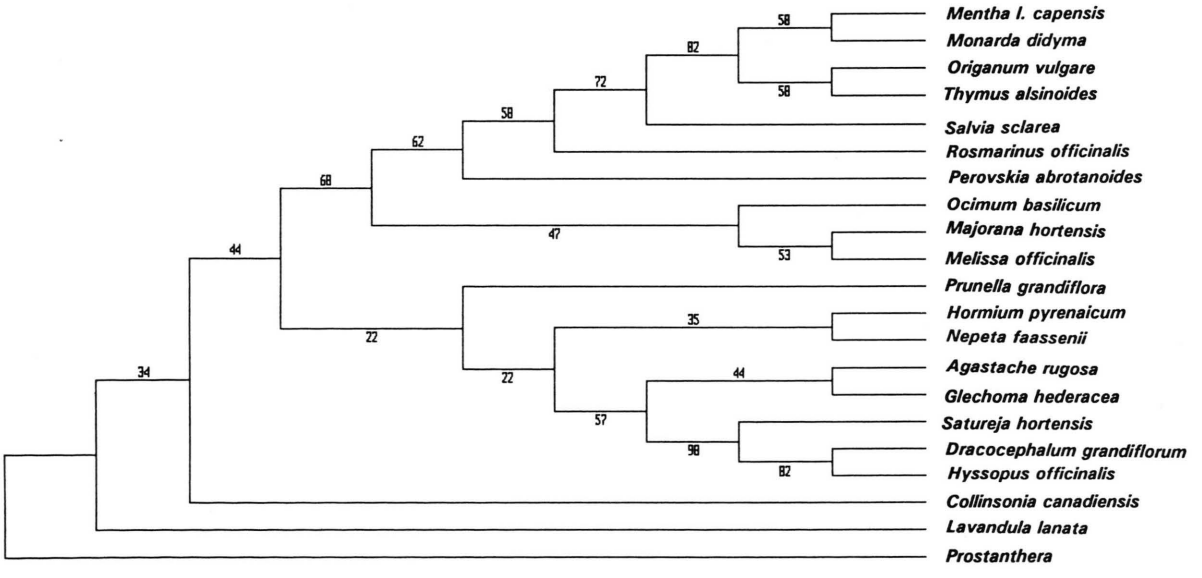

Fig. 2. Bootstrap analysis of the Nepetoideae sequence data. Conditions: neighbour-joining; number of replicates: 200; Kimura 2-parameter model. In order to reduce calculation time, only one representative OTU per genus was chosen with *Prostanthera* as an outgroup which was better suited than *Ajuga* or *Westringia* (Kaufmann, 1994).

chosen as outgroups *Lavandula* becomes a tribe within the subfamily Nepetoideae as illustrated in Fig. 2; however, if a more distant outgroup, such as *Verbena*, was selected *Lavandula* (together with *Collinsonia*) clusters as a subgroup of the Mentheae (Kaufmann, 1994). We explain this discrepancy by a 14% loss of phylogenetically informative sites when *Verbena* is included.

Ocimum constitutes an ambiguous taxon and has been classified as a unique tribe (Bentham, 1832–1836; Cantino, 1992) or even subfamily (Briquet, 1895–1897). According to our rbcL nucleotide data, *Ocimum* together with *Melissa* and *Majorana* either clusters as a clade within the Mentheae or forms a distinct tribe if other outgroups such as *Verbena* are employed. Nucleotide differences between *Ocimum* and other taxa of the Mentheae account for 2.7 ± 0.5% which would support a tribal status.

Taxa which are found in clades III to XIII share a common ancestor. All these taxa, except *Ocimum* have been classified as members of the Mentheae by Cantino (1992) and Cantino *et al.* (1992). Within the tribe Mentheae (*sensu* Cantino, 1992) the rbcL data show that at least three major groupings are present (clades III–VII, VIII and IX–XIII) which do not correlate with former classifications that were based on flower, pollen, embryo or seed morphology. Since our data derive from one gene only, further research (*e.g.*, the analysis of nuclear genes) must show whether these groupings which always appear when reconstructing the phylogeny by either parsimony or distance methods deserve a special taxonomic status, *e.g.* as subtribes.

The correlation of molecular data with morphological and chemical characters (mucilage, monoterpenes, sesquiterpenes, iridoids, diterpenes, flavonoids, rosmarinic acid) is under study in our laboratory and will be discussed together with our rbcL data of the Lamioideae (M. Kaufmann and M. Wink, in preparation).

J. Lindley might still suggest that the classification of the Labiatae is "the disgrace of Botany", but we are sure that the degree of "disgrace" has been diminished due to the recent progress in taxonomical, chemical and molecular research (Cantino, 1992; Cantino *et al.*, 1992; Cantino and Sanders, 1986; Harley and Reynolds, 1992; Olmstead *et al.*, 1992). But much work needs still to be done to understand the probably complicated evolution and history of this particular plant family.

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