Molecular phylogeny and taxonomy of the genus *Lamium* L. (Lamiaceae): Disentangling origins of presumed allotetraploids

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Abstract This is the first comprehensive molecular investigation of the genus *Lamium* L. We have addressed phylogenetic relationships and presumed allopolyploid speciation by use of nuclear (NRPA2, 5S-NTS) and chloroplast (*matK*, *psbA-trnH*, *rps16*, *trnL*, *trnL-F*, *trnS-G*) DNA sequence data. Nuclear and chloroplast data were incongruent, and nuclear data showed better correlation with morphology. Bayesian and parsimony phylogenetic results show that (1) *Lamium galeobdolon* is sister to all remaining *Lamium* species; (2) *Wiedemannia* is nested within *Lamium*; (3) *L. amplexicaule* is polyphyletic; (4) most tetraploids are of hybrid origin; (5) *L. amplexicaule* var. *orientale* is allotetraploid; and (6) Mennema's (1989) infrageneric classification is not corroborated by molecular data. Based on the molecular results, and taking morphology into account, we suggest resurrection of two species: *L. aleppicum* and *L. paczoskianum*.

Keywords 5S-NTS; allopolyploidy; classification; cpDNA; *Lamium*; molecular phylogenetics; NRPA2; speciation

■ INTRODUCTION

Lamium L. is the type of the family name Lamiaceae (the deadnettle/mint family) and subfamily Lamioideae. The genus is native to the temperate and subtropical regions of Europe, Asia, and Northern Africa, although a few species have been introduced to other parts of the world. Most of the species are characterised by short and toothed lateral lobes of the lower lip of the corolla and a broad and emarginate mid-lobe. However, species with other corolla lip shapes have also been included in the genus (see below). Based on a molecular phylogenetic survey of subfamily Lamioideae, Scheen & al. (2010) established tribe Lamieae to encompass *Lamium* s.str. and taxa that have sometimes been assigned to the separate genera *Lamiastrum* Heist. ex Fabr. and *Wiedemannia* Fisch. & C.A. Mey. A close relationship to *Stachyopsis* Popov & Vved. and *Eriophyton* Benth. s.l. was identified in a follow-up study by Bendiksby & al. (2011a), who subsumed these two genera into tribe Lamieae.

Typical *Lamium* species, such as the type of the generic name, *L. purpureum* L., and *L. album* L., have been included in most of the literature on the genus except in a few old works (e.g., Willdenow, 1787; Opiz, 1852; Fourreau, 1869: 134–135), while the generic classifications of several less typical species have varied, also in recent literature. For example, *L. multifidum* L. was originally described as a *Lamium* species but was moved early on to *Wiedemannia* (Bentham, 1848). *Wiedemannia* was distinguished from *Lamium* by the slightly 2-lipped calyx, with an entire upper lip and a 4-lobed lower lip (Fischer & Meyer, 1838). However, Krause (1903) and Ryding (2003) included the two species of *Wiedemannia* (*W. multifida* (L.) Benth., *W. orientalis* Fisch. & C.A. Mey.) in *Lamium*, and their classification was adopted by Harley & al. (2004) and Govaerts & al. (2010).

Lamium galeobdolon (L.) L. has been variably included in *Lamium* or placed in a separate genus called either *Lamiastrum* or *Galeobdolon* Adans. (a younger homotypic synonym of *Lamiastrum*). Harley & al. (2004) and Govaerts & al. (2010) included *L. galeobdolon* in *Lamium*, whereas Mossberg & al. (1992), Ryding (2006), and Stace (2010) placed the species in *Lamiastrum*. This species can easily be distinguished from other *Lamium* species by having subequal, triangular, and acute lobes of the lower lip of the corolla. Clearly, the generic position of this species is not settled.

As mentioned by Mennema (1989), many authors have used *Lamium* as a repository for several extraneous East Asian labiates with uncertain generic positions. Some of these species are still placed in *Lamium* by Govaerts & al. (2010). However, based on molecular phylogenetic evidence, Bendiksby & al. (2011a) recently transferred *L. nepalense* Hedge, *L. staintonii* Hedge, and *L. tuberosum* Hedge (incl. *L. gilongensis* H.W. Li) to the genus *Eriophyton*, and *L. chinense* Benth., *Galeobdolon kwangtungense* C.Y. Wu, *G. szechuanense* C.Y. Wu, and *G. yangsoense* Y.Z. Sun to the genus *Matsumurella* Makino. Ying's (1991) species description and photograph show that also the Taiwanese species, *L. taiwanense* S.S. Ying, appears to be extraneous in *Lamium*. All these species differ from *Lamium* in having prominent and rounded side-lobes of the lower lip of the corolla.

Infrageneric classifications were presented by Bentham (1832–1836, 1848) and Briquet (1895–1897). Mennema's (1989)

infrageneric classification resembles these old classifications. He recognised the following three subgenera: (1) subg. *Lamium*, comprising species with hairy anthers; (2) subg. *Orvala* (L.) Briq., with the single species *L. orvala* L. that has glabrous anthers; and (3) subg. *Galeobdolon* (Adans.) Asch., with *L. galeobdolon* and *L. flexuosum* Ten. that also have glabrous anthers. *Lamium* subg. *Galeobdolon* is supposed to differ from subg. *Orvala* in having the bracteoles spreading to recurved and more aristate at the apex, but these differences are found to be vague and hardly consistent. The group is probably unnatural, as the two species strongly differ in the shape of the lower lip of the corolla. Due to this difference, Ball (1972) and Pignatti (1982) retained *L. flexuosum* in *Lamium* and placed *L. galeobdolon* in *Lamiastrum*.

Within subg. *Lamium*, Mennema (1989) discerned the following three sections: (1) sect. *Lamium*, which comprises species with bracteoles and a straight corolla tube (*L. bifidum* Cirillo, *L. confertum* Fr., *L. garganicum* L., *L. glaberrimum* (K. Koch) Taliev, *L. purpureum* sensu Mennema, 1989); (2) sect. *Lamiotypus* Dumort., which comprises species with bracteoles and a sigmoid corolla tube that is abruptly dilated and ventrally saccate (*L. album*, *L. galactophyllum* Boiss. & Reut., *L. maculatum* (L.) L., *L. moschatum* Mill., *L. tomentosum* Willd.); and (3) the new section *Amplexicaule* Mennema, which includes species that lack bracteoles (*L. amplexicaule* L., *L. eriocephalum* Benth., *L. macrodon* Boiss. & A. Huet).

The number of accepted *Lamium* species varies considerably in the literature. Bentham (1848) and Briquet (1895–1897) recognised 35 and 38 species, respectively; similar, narrow species circumscriptions were applied by Mill (1982) and Gorschkova (1954). In his monograph, Mennema (1989) treated many of the earlier species as subspecies and varieties and reduced the number of species to 16. Since Mennema (1989), other authors have resurrected some of the species that he reduced and some new species have been described. Mennema's (1989) classification and most of the subsequent modifications were accepted by Govaerts & al. (2010), but their database was not updated based on more recent changes. Whereas Mennema (1989) included *L. hybridum* Vill. in *L. purpureum*, and divided it into three varieties (var. *hybridum* (Vill.) Vill., var. *incisum* (Willd.) Pers., var. *moluccellifolium* Schum.), Stace (2010) and Pujadas Salvà (2010) retained *L. hybridum* as a species and did not divide it into infraspecific taxa. Following Stace (2010), Pujadas Salvà (2010) and Bendiksby & al. (2011a), and excluding *L. taiwanense*, we consider *Lamium* to comprise 24 species, 15 subspecies, and 9 varieties.

Lamium has the chromosome base number $x = 9$. Most other genera of the subfamily Lamioideae have other base numbers, but $x = 9$ has also been recorded in *Synandra* and *Macbridea* (Cantino, 1985) as well as in some *Leonurus* and *Marrubium* species (Fedorov, 1969). According to Mennema (1989), *Lamium* comprises mostly diploid taxa ($2n = 18$): *L. album* subsp. *album* and subsp. *barbatum* (Siebold & Zucc.) Mennema, *L. amplexicaule* var. *amplexicaule*, *L. bifidum*, *L. flexuosum*, *L. galeobdolon* subsp. *flavidum* (F. Herm.) Á. Löve & D. Löve and subsp. *galeobdolon*, *L. garganicum* subsp. *corsicum* (Gren. & Godr.) Mennema, subsp. *garganicum* and subsp. *striatum* (Sm.) Hayek, *L. maculatum, L. moschatum, L. orvala, L. purpureum* var. *purpureum* and *L. tomentosum*. However the following four taxa are reported to be tetraploids ($2n = 36$): *L. confertum*, *L. galeobdolon* subsp. *argentatum* (Smejkal) J. Duvign. and subsp. *montanum* (Pers.) Hayek, and *L. hybridum* (as *L. purpureum* var. *incisum*). The tetraploid taxa are presumed to have allopolyploid origins. Bernström (1955) performed crossing experiments with some *Lamium* species. His crossings between *L. amplexicaule* and *L. purpureum* resulted in allotetraploid hybrid plants that were morphologically highly similar to *L. confertum*. Additional crossings between *L. purpureum* and *L. bifidum* produced allotetraploid hybrid plants that resembled *L. hybridum*. These results strongly suggest that *L. confertum* is an allotetraploid hybrid between *L. amplexicaule* and *L. purpureum*, and *L. hybridum* an allotetraploid hybrid between *L. purpureum* and *L. bifidum*. Statements that the second parental species of *L. hybridum* should be *L. moschatum* seem to be based on an erroneous citation of Bernström's paper in Ball (1972). Furthermore, Dersch (1964) suggested that the tetraploid *L. galeobdolon* subsp. *montanum* may have originated from hybridization between the two diploid subspecies, subsp. *galeobdolon* and subsp. *flavidum*. This suggestion is supported by Mennema's (1989: 37–39) morphological measurements; the tetraploid subspecies is more or less intermediate between the diploids in all measured characters. The fourth tetraploid, subsp. *argentatum*, is morphologically more similar to subsp. *galeobdolon*. The ploidy level of *L.* × *holsaticum* E.H.L. Krause is unknown, but the taxon is commonly believed to be a hybrid between *L. album* and *L. maculatum* as it seems to be morphologically intermediate between these two species.

Low-copy nuclear genes may be useful for disentangling reticulate evolutionary relationships that involve hybrid origin of polyploid species, especially when the polyploidization event occurred relatively recently and both paralogs are intact and present in the polyploid genome (e.g., Brysting & al., 2007; Fortune & al., 2008; Mason-Gamer, 2008). Past events of chloroplast capture (via hybridization) can be identified from incongruent nuclear versus chloroplast phylogenies (e.g., Rieseberg & al., 1996; Frajman & Oxelman, 2007). Chloroplast DNA sequences provide information about only one of the parental genomes (the maternal if the chloroplast is maternally inherited, as is assumed to be the case in most, but not necessarily all, angiosperm groups), and may thus be used to identify the organellar parent in an allopolyploidization event.

The aim of our study was to explore phylogenetic relationships in the genus *Lamium* and disentangle the origins of the presumed allotetraploids by the use of nuclear and chloroplast DNA sequence data. Specifically, we wanted to test: (1) whether *Lamium* s.str. remains monophyletic when *L. galeobdolon* is excluded from the genus; (2) whether the two species previously assigned to the genus *Wiedemannia* are phylogenetically nested within *Lamium*; (3) whether the tetraploid *Lamium* species have hybrid origins as suggested from the literature (see above); and (4) whether Mennema's (1989) infrageneric classification is corroborated by molecular data.

■ MATERIAL AND METHODS

The circumscription of *Lamium* and the names of the taxa in the present study follow the "World Checklist of Lamiaceae and Verbenaceae" (Govaerts & al., 2010), with the following exceptions: (1) *L. taiwanense* and the species transferred to *Eriophyton* or *Matsumurella* by Bendiksby & al. (2011a) are excluded, and (2) *L. hybridum* is accepted at species rank, and *L. purpureum* var. *hybridum*, var. *incisum*, and var. *moluccelli-folium* are treated as synomyms of *L. hybridum*.

Taxon sampling. — We generated DNA sequences that encode the second-largest subunit of the low-copy nuclear RNA polymerase I (NRPA2; following the 4-letter subunit nomenclature of nuclear RNA polymerases as registered with The Arabidopsis Information Resource and also used in several recent studies, e.g., Marcussen & al., 2010, and Brysting & al., 2011), the nuclear ribosomal 5S non-transcribed spacer (5S-NTS), and six chloroplast DNA regions (cpDNA; *matK*, *psbA-trnH* spacer, *rps16* intron, *trnL* intron, *trnL-trnF* spacer, and *trnS-trnG* spacer). As ingroup, we included 79 accessions representing 19 species and 10 taxa below species level. We could not obtain material of the following five species: *L. caucasicum* Grossh., *L. gevorense* (Gómez Hern.) Gómez Hern. & A. Pujadas, *L. glaberrimum*, *L. tschorochense* A.P. Khokhr., and *L. vreemanii* A.P. Khokhr. We analyzed three datasets (see below) separately; two nuclear and one chloroplast. In the NRPA2 analysis, we used as outgroup four accessions from equally many species of *Galeopsis*. In the 5S-NTS and cpDNA analyses, we used as outgroup four accessions of three lamioid genera (*Eriophyton*, *Roylea*, *Stachyopsis*), which have been shown to be closely related to *Lamium* (Bendiksby & al., 2011a). The voucher specimens are held at the following herbaria: A, C, GH, O, S, UPS, US, and WU (Appendix).

DNA extraction. — We crushed 10–30 mg of leaf tissue from 73 herbarium specimens and 6 silica-dried samples (all ingroup; outgroup DNA extracts were available from a previous study) in 2 mL plastic tubes with two tungsten carbide beads in each for 2×1 min at 30 Hz on a mixer mill (MM301, Retsch GmbH & Co., Haan, Germany). We extracted total DNA from the crushed samples using the E.Z.N.A SP Plant DNA Mini Kit (Omega Bio-tek, Norcross, Georgia, U.S.A.) according to the manufacturer's manual. We performed the DNA elution twice in the same tube and used the first eluate in the second elution step. We have deposited all DNA aliquots used in the present study in the DNA/tissue collection at Natural History Museum, Oslo (O).

PCR amplification and DNA sequencing. — We amplified DNA in 25 µL reactions using the AmpliTaq DNA polymerase buffer II kit (Applied Biosystems, Foster City, California, U.S.A.) containing 0.2 mM of each dNTP, 0.04% bovine serum albumin (BSA), 0.01 mM tetramethylammonium chloride (TMACl), 0.4 µM of each primer, and 2 µL unquantified genomic DNA. We performed all amplifications in a GeneAmp PCR System 9700 (Applied Biosystems) using the following cycling conditions: 95°C for 10 min, 31 (cpDNA, 5S-NTS) or 34 (NRPA2) cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 1 min, followed by 72°C for 10 min and hold forever at 10°C. For DNA extracts that would not amplify using the above described approach, we performed nested-PCR or the replicate procedure described in Bendiksby & al. (unpub.).

Initially, we amplified NRPA2 from four distantly related diploid *Lamium* species following the nested PCR procedure and degenerate primers described in Popp & Oxelman (2004). We cloned the products using the TOPO-TA cloning kit (Invitrogen Dynal AS, Oslo, Norway) following the manufacturer's manual but using only half of the recommended volumes. We picked and amplified 8 to 16 colonies and sequenced four to eight products. From conserved exon regions in the resultant NRPA2 matrix, we developed a set of non-degenerate NRPA2 primers (L-A2F/R; Table 1). NRPA2 appears to be single-copy in Lamium, because we were able to amplify PCR products from diploid species directly using the L-A2F/R primer pair. For amplification of parental homoeologs in the presumed tetraploids, we developed additional internal primer pairs (Table 1). For example, to specifically amplify, in separate reactions, each parental NRPA2 homoeolog of *L. hybridum,* we made species-specific primers based on sequences of the presumed parental species, *L. purpureum* (L-pur-A2F/R) and *L. bifidum* (L-bif-A2F/R). We tested the specificity of the primers by performing PCR on multiple *Lamium* species. Because of intra-taxon nucleotide variation in flanking regions of NRPA2 in *L. galeobdolon* subsp. *galeobdolon* and subsp. *flavidum*, we could not design specific primers for these taxa, and we sought for homoeologs in the tetraploid subsp. *argentatum* and subsp. *montanum* by cloning PCR products amplified by the non-degenerate L-A2F/R primers.

We amplified the 5S-NTS region with the forward primer 5S-30 (5′ GGATCCCATCAGAACTCCG 3′; Bendiksby, 2002) and a non-degenerate version of PII from Cox & al. (1992) as the reverse primer (5′ TGCGATCATACCAGCACTAA 3′). Due to extensive intragenomic DNA sequence variation, the 5S-NTS region required cloning prior to sequencing. We cloned (as described in Scheen & al., 2008, or using the TOPO-TA cloning kit as described above) and sequenced a subset of taxa included in the other datasets (see Appendix). We amplified 8 to 16 clones for each accession, and sequenced products with insert (up to 12).

Table 1. Primers used for amplifying NRPA2.

Primer name	Sequence (5′–3′)
L-A2F	CTCATGCATTTCCTTCTAGGATGAC
L-A2R	GCCAATAAATATTTCGCATGTCAGC
L-alb-A2F	GCTACTTTTTGGTCTGGGTAGA
L-alb-A2R	CTCTACACCATGATAGTTGAAC
L-mac-A2F	ACTACTTTTTTGGCCTGGGTAGT
L-mac-A2R	CTCTACACCATGATAGTTGAAG
L-pur-A2F	ATGTTAAGGTAGCATTGCCAAATG
L-pur-A2R	GTTGAAGCCACGTTCAACCAACA
L-bif-A2F	ATGTTAAGCTAGCATCGACAAATG
L-bif-A2R	GTTAAACCCACGTGCAATCAACT
L-amp-A2F	GTGTTAAGCTAGCATCGCCAAATA
L-amp-A2R	GTTGAACCCACGTGCGACCAACT

We amplified the *matK* gene either as one fragment or as two shorter fragments as described in Bendiksby & al. (2011a) using primers developed for the same study. Likewise, *rps16* was either amplified as one fragment using the primer combination rpsF and rpsR2R (Oxelman & al., 1997), or as two shorter fragments as described in Bendiksby & al. (2011a). Also the *trnL* intron and the *trnL-F* spacer was amplified either as one fragment (hereafter referred to as the *trnL-F* region) using the primers c and f, or as two shorter fragments using the primers c and d, or e and f, respectively (Taberlet & al., 1991). When long fragments did not amplify successfully, assumingly due to low-quality template, we attempted to amplify shorter fragments. We amplified the remaining chloroplast regions as single fragments using the following primers: psbAF and trnHR (*psbA-trnH*; Sang & al., 1997), and trnS^{GSU} and trnG^{UCC} (*trnS-G*; Hamilton, 1999).

We purified the PCR products using 2 µL 10-times diluted ExoSAP-IT (USB Corporation, Santa Clara, California, U.S.A.) to 8 µL PCR product, incubating at 37°C for 45 min followed by 15 min at 80°C. Prepared amplicons for sequencing contained: 9 µL 0–30× diluted purified PCR product (depending on product strength) and 1 µL of 10 µM primer (the same primers as used in the PCR). Cycle sequencing was performed by the ABI laboratory staff at the Centre for Ecological and Evolutionary Synthesis, Department of Biology, University of Oslo, using the ABI BigDye Terminator sequencing buffer and v.3.1 Cycle Sequencing kit (Applied Biosystems). Sequences were processed on an ABI 3730 DNA analyser (Applied Biosystems). We assembled and edited the sequences using SEQUENCHER v.4.1.4 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). We have deposited all new sequences in GenBank, and accession numbers are listed in the Appendix.

Alignment and phylogeny reconstructions. — We aligned the sequences manually using BioEdit v.7.0.9.0 (Hall, 1999). In order to check for incongruencies between gene trees, we compared strict consensus trees from preliminary parsimony phylogenetic analyses (see below) of the six genetic regions (*trnL-F* region analyzed as one unit). For selecting optimal models of nucleotide substitution for the various markers we used the Akaike information criterion with an empirical correction for small sample sizes (AICc), as implemented in MrAIC (Nylander, 2004), together with PHYML (Guindon & Gascuel, 2003). We coded indels and added them to the matrices as additional, unordered characters (0 or 1). For this, we used the simple indel coding of Simmons & Ochoterena (2000) as implemented in the program SeqState (Müller, 2005). We analyzed datasets both with and without coded indels using maximum parsimony and Bayesian inference phylogenetic methods.

We performed parsimony analyses using TNT v.1.1 (Goloboff & al., 2003) applying the traditional search option with equal character weights, gaps treated as missing (replaced with question marks prior to analysis), 1000 random entry order replicates saving 10 trees per replicate, and tree bisection reconnection (TBR) branch swapping. We performed parsimony bootstrapping with 2000 replicates.

We performed Bayesian inference phylogenetic analyses using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with the priors set according to the output of MrAIC. We determined posterior probabilities by running one cold and three heated chains for six million generations in parallel mode, saving trees every 1000th generation. When coded indels were included, we analysed them as a separate unlinked partition with a binary model. We repeated the analyses twice to check their convergence for the same topology. To test whether the Markov Chain converged, we monitored the standard deviation of split frequencies (SDSF), which should fall below 0.01 when comparing two independent runs. We discarded as burn-in the generations prior to the point where the analysis reached stationarity and summarized the remaining trees as a 50% majority-rule consensus tree.

We also analyzed a reduced NRPA2 alignment, which included only one accession when more accessions of the same species were part of a monophyletic clade in the NRPA2 tree. For this, we used MrBayes and the same settings as outlined above. We used the resulting 50% majority rule consensus tree as input tree file in the computer software PADRE (Lott & al., 2009) for construction of an allopolyploid species network from a multilabelled tree.

We ran the MrAIC and MrBayes analyses on the Bioportal server, University of Oslo, Norway (http://www.bioportal.uio.no).

■ RESULTS

We obtained DNA extracts of sufficient quality for amplifying and sequencing both chloroplast and nuclear DNA regions from all samples included (collected between 1853 and 2006; Appendix). Preliminary parsimony analyses indicated incongruence between the nuclear and chloroplast data, whereas the nuclear regions (NRPA2, 5S-NTS) and all chloroplast datasets were largely congruent, respectively. Several paralogous 5S-NTS sequences precluded concatenation of the two nuclear DNA regions. Therefore, we concatenated the chloroplast regions prior to final analyses (referred to as cpDNA hereafter). Thus, we analyzed three datasets: (1) the NRPA2 matrix of 65 accessions; (2) the 5S-NTS matrix of 38 accessions; and (3) the partitioned concatenated cpDNA matrix of 82 accessions, of which four accessions represented the outgroup taxa in each dataset. The three datasets and the resultant Bayesian genealogies are available from TreeBase (http://treebase.org) using the identifier S11382.

NRPA2. — We obtained only one NRPA2 sequence type from clones of diploid *Lamium* species using the degenerate NRPA2 primers described in Popp & Oxelman (2004). We amplified successfully and sequenced directly NRPA2 from all diploid species using the *Lamium* specific primers (L-A2F/R). The species-specific primer pairs amplified only the species that we had designed them for and homoeologs from the tetraploid(s) to which they had contributed their genomes. Thus, the *L. purpureum*-specific primers (L-purA2F/R) successfully amplified NRPA2 from both *L. confertum* and *L. hybridum*, and no other *Lamium* species. We obtained a second NRPA2 homoeolog from *L. confertum* using the *L. amplexicaule*-specific primers

(L-ampA2F/R) and from *L. hybridum* using the *L. bifidum*-specific primers (L-bifA2F/R). We could amplify and sequence DNA from *L.* × *holsaticum* using the *L. maculatum*-specific primers (L-macA2F/R), whereas no PCR product was obtained when we used the *L. album*-specific primers (L-albA2F/R). By cloning and sequencing *L. galeobdolon* subsp. *argentatum* and subsp. *montanum*, we detected two different NRPA2 types. We detected two distinct NRPA2 types also in sequenced clones from *L. amplexicaule* var. *orientale* (Pacz.) Mennema.

The NRPA2 sequences ranged in length from 692 to 964 basepairs (bp), of which the longest fragments (*L. amplexicaule* var. *aleppicum* (Boiss. & Hausskn.) Bornm. 1, *L. maculatum* 1, and *L. moschatum* 3) contained long autapomorphic inserts (237 bp, 211 bp, and 107 bp, respectively). These inserts, as well as a 346 bp long insert in *Galeopsis*, contributed to the rather long final NRPA2 alignment of 1899 bp. We identified a total of 112 indels, and numbers of parsimony-informative characters were 275 and 211 for the datasets with and without coded indels, respectively. With coded indels, the number of most parsimonious trees (MPTs) was six, and rescaled consistency (RC) and homoplasy (HI) indices were 0.75 and 0.18, respectively. Without coded indels, 1788 MPTs were found with RC and HI of 0.74 and 0.19, respectively. Because the analysis with coded indels generated fewer MPTs and provided a better resolved phylogeny (not shown) that contained less homoplasy, all results described in the following were obtained from the NRPA2 dataset with coded indels. We performed the Bayesian analysis under the HKY+G model. Resultant consensus phylogenies from parsimony and Bayesian analyses were congruent but resolved to different extents. The 50% majority-rule consensus tree obtained from the Bayesian analysis is presented with both posterior probabilities and parsimony bootstrap support for branches in Fig. 1.

5S-NTS. — The intragenomic 5S-NTS sequence variation was extensive in all 38 accessions that we cloned and sequenced. There seemed to be two main paralogs (labelled a and b in Fig. 2) in all diploid species, but none of the 261 sequences were identical, and they ranged in length from 139 to 411 bp. For most taxa, monophyly of the two main paralogs was not inferred, but in some cases, the two main paralogs of a taxon (e.g., *L. galeobdolon*, *L. orvala*, *L. flexuosum*, "*Wiedemannia*" [i.e., *L. multifidum* and *L. orientale* (Fisch. & C.A. Mey.) E.H.L. Krause], *L. amplexicaule* var. *amplexicaule*, and all outgroup taxa) or a group of taxa (e.g., clade E [see below]) were sistergroups. Within each main paralog, multiple paralogous sequences from the same accession were sometimes paraphyletic with respect to those of closely related species. We obtained three or four main paralogs/homoeologs (labelled a to c/d in Fig. 2) from the tetraploid species *L. hybridum* and *L. confertum*, respectively.

The aligned region was 456 bp long, and we identified 118 indels. Numbers of parsimony informative characters were 437 and 370 for the datasets with and without coded indels. The numbers of MPTs exceeded 5000 both with and without coded indels, and RC and HI were 0.36/0.35 and 0.59/0.61, respectively. As coding of indels decreased the amount of homoplasy on the tree and increased the support for some branches in otherwise congruent topologies, all results described in the following were obtained from the 5S-NTS dataset with coded indels. We performed the Bayesian analysis under the HKY+I+G model. Resultant consensus phylogenies from parsimony and Bayesian analyses were congruent but resolved to different extents. The 50% majority rule consensus tree obtained from the Bayesian analysis is presented in Fig. 2 (tree with terminals available from TreeBase: S11382).

cpDNA. — Max/min sequence lengths of the various chloroplast regions were: *matK* 1147/1132 bp; *psbA-trnH* 596/308 bp; *rps16* 900/888 bp; *trnL-F* 890/846 bp; and *trnS-trnG* 758/507 bp, and lengths of the aligned regions were (with trimmed ends): *matK* 1141 bp; *psbA-trnH* 468 bp; *rps16* 906 bp; *trnL-F* 901 bp; and *trnS-trnG* 711 bp. The *psbA-trnH* spacer was the most variable region but also the most homoplastic one and difficult to align. The concatenated cpDNA matrix was 4127 bp long, and we identified 137 indels. Numbers of parsimony informative characters were 421 and 330 for the datasets with and without coded indels. With coded indels, the number of MPTs was 480, and RC and HI were 0.72 and 0.27, respectively. Without coded indels, 12 MPTs were found with RC and HI of 0.81 and 0.15, respectively. Thus, contrary to NRPA2 and 5S-NTS, coding of indels increased the amount of homoplasy on the tree as well as the number of MPTs. This was also reflected in consensus topologies, which were better resolved for the dataset without coded indels. Therefore, all results described in the following were obtained from the cpDNA dataset without coded indels. We performed the partitioned Bayesian analyses under the GTR+G model for all regions except *psbA-trnH*, for which we used GTR+G+I. Resultant consensus phylogenies from parsimony and Bayesian analyses were congruent but resolved to different extents, although resolution and support were generally high in both. The 50% majority rule consensus tree obtained from the Bayesian analysis is presented with parsimony bootstrap support for branches in Fig. 3.

Phylogenies. — The topologies of the obtained NRPA2 and 5S-NTS genealogies were largely congruent (Figs. 1, 2), whereas the cpDNA genealogy (Fig. 3) was incongruent with respect to the nuclear data (Figs. 1–2). For example, monophyly of *L. galeobdolon* was supported by both nuclear and chloroplast datasets (Figs. 1–3: clade A), but the phylogenetic position of *L. galeobdolon* within *Lamium* varied between the nuclear and chloroplast trees. The nuclear data rendered *L. galeobdolon* sister to a strongly supported group of all remaining *Lamium* species (Figs. 1, 2: clade B). In the cpDNA tree (Fig. 3), however, *L. galeobdolon* appeared along with *L. flexuosum* and *L. orvala* in an unresolved and poorly supported clade, whereas a clade comprising all accessions of *L. album* and *L. tomentosum* (referred to as the *album-tomentosum* group hereafter; clade C) obtained a position as phylogenetic sister to all remaining *Lamium* species. Monophyly of the *album-tomentosum* group was strongly supported also in the nuclear trees (Figs. 1, 2: clades C, C1 and C2, respectively). In the NRPA2 tree, the *album-tomentosum* group formed a supported clade together with a monophyletic "*Wiedemannia*" (Fig. 1: clade D). This relationship was not upheld in the 5S-NTS tree (Fig. 2); the two main paralogs (a, b) of "*Wiedemannia*" grouped with high support, whereas the *album-tomentosum* main paralogs occurred

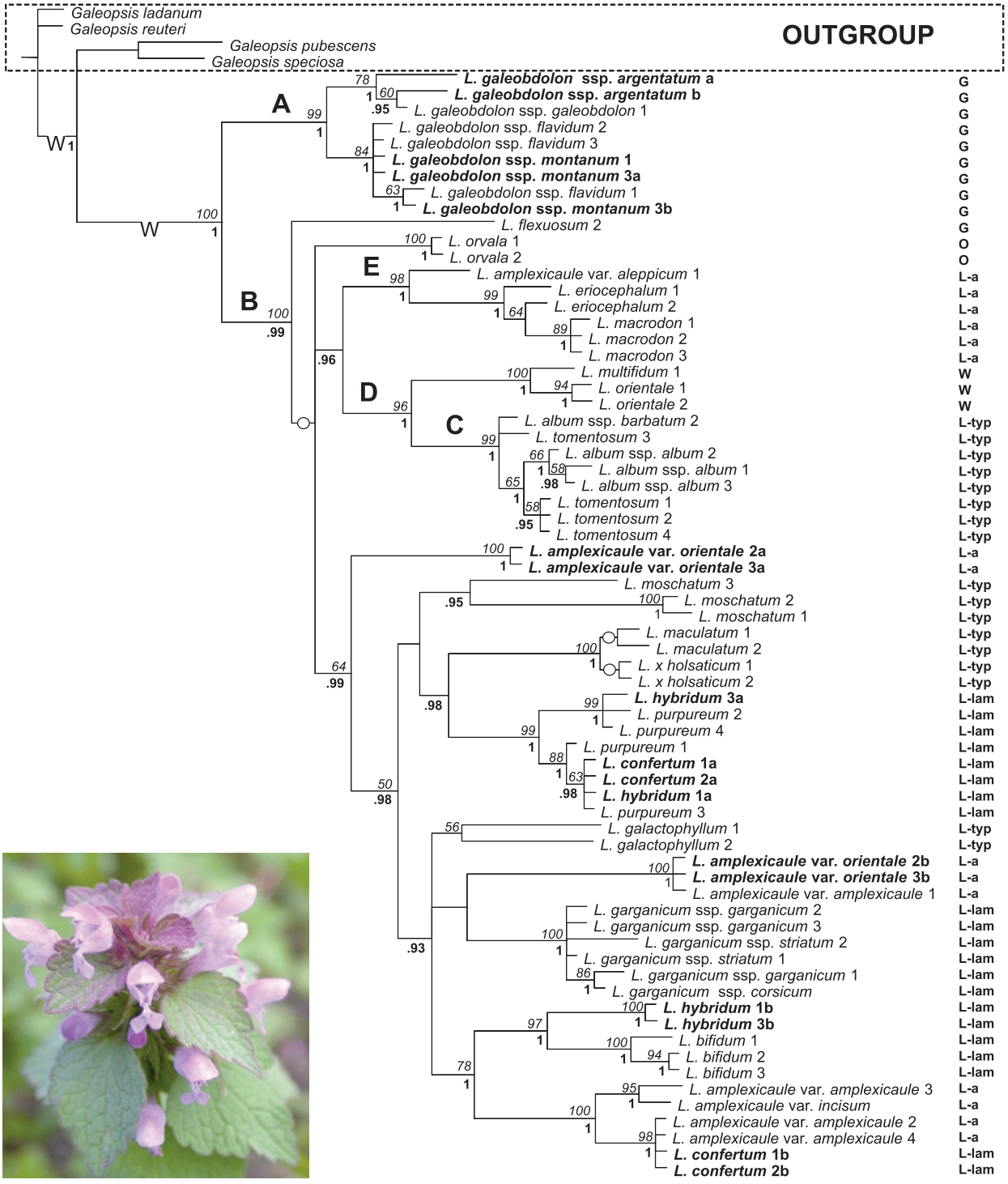

Fig. 1. The 50% majority-rule consensus phylogram from a partitioned Bayesian analysis of a NRPA2 matrix with 65 accessions and coded indels. All generations prior to the point when the SDSF fell permanently below 0.01 (0.003884 at termination) were discarded as burn-in. Bayesian posterior probability (PP) values above 0.95 are reported in bold face below branches, and parsimony bootstrap support (BS) values above 50% are reported in italics above branches. Branches that collapsed in the parsimony strict consensus tree are marked with a white circle. Multiple accessions of the same species are numbered according to the Appendix. Taxa shown to be tetraploid are in bold and different homoeologs of the same accession are labelled a and b. Clades discussed in the text are marked with capital letters; those that correspond between datasets are given the same letter. Two branches (indicated with a zigzag line) were manually shortened to reduce the size of a broad figure. Abbreviations to the right refer to Mennema's (1989) infrageneric classification: G = subg. *Galeobdolon*; L = subg. *Lamium*; O = subg. *Orvala*; a = sect. *Amplexicaule*; lam = sect. *Lamium*; typ = sect. *Lamiotypus*. Moreover, W = "*Wiedemannia*". Inset picture of *Lamium purpureum*, the type of the generic name *Lamium* (photograph by the first author; picture colored in the online version).

Fig. 2. The 50% majority-rule consensus phylogram from a Bayesian analysis of a matrix with multiple 5S-NTS sequence paralogs from 38 accessions and coded indels. All generations prior to the point when the SDSF fell permanently below 0.01 (0.008821 at termination) were discarded as burn-in. Groups of taxa contained within supported clades are indicated to the right. Clades discussed in the text are marked with capital letters; those that correspond between datasets are given the same letter. The two main paralogs in diploids are labelled a and b, and paralogs/homoeologs in tetraploids are labelled a–d. Taxa shown to be tetraploid are in bold (except the *L. galeobdolon* tetraploids). One branch (indicated with a zigzag line) was manually shortened to reduce the size of a broad figure. Abbreviations and branch support as in Fig. 1. Inset picture of *Lamium maculatum* (photograph by the first author; picture colored in the online version).

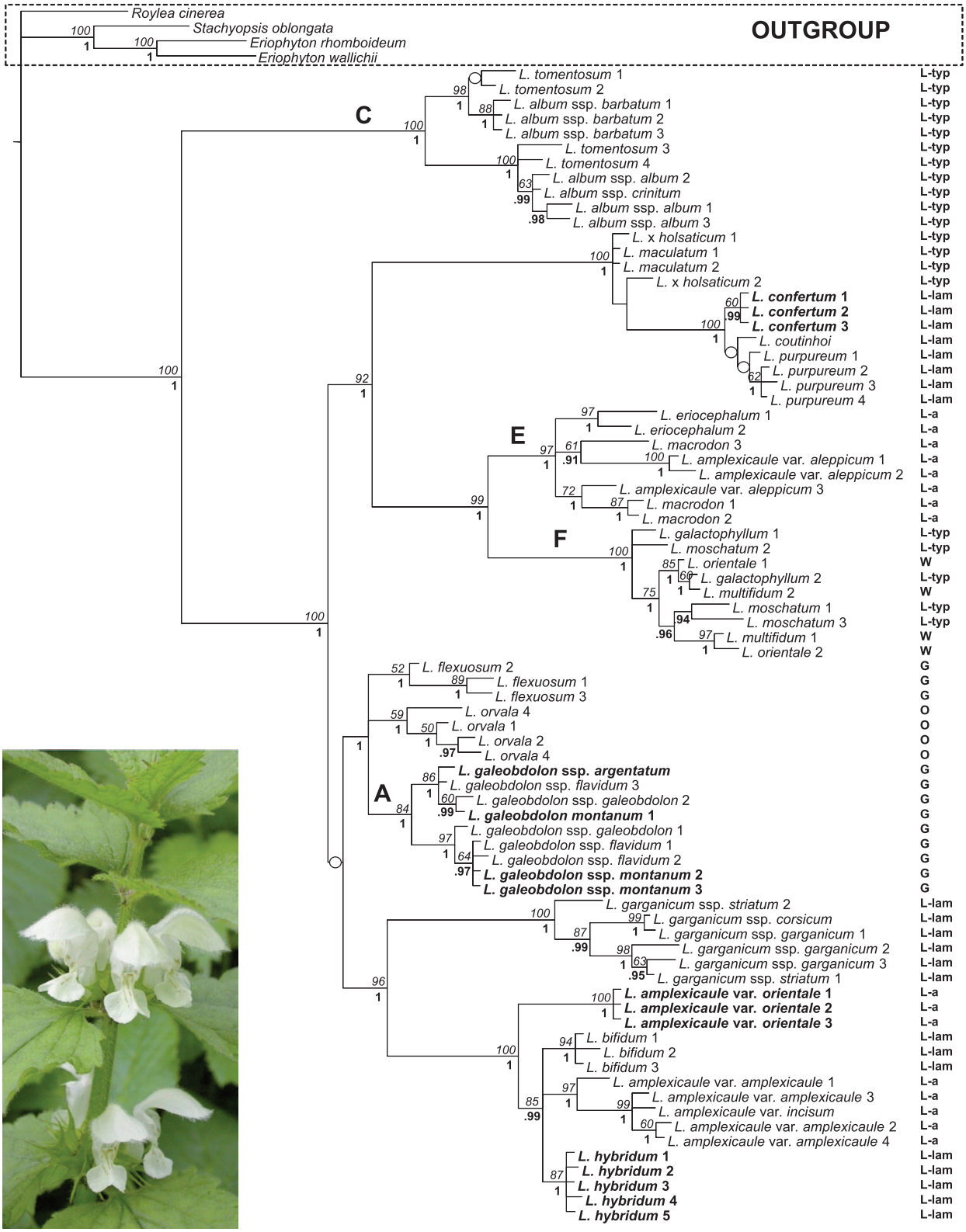

Fig. 3. The 50% majority-rule consensus phylogram from a partitioned Bayesian analysis of a concatenated matrix of six chloroplast regions (*matK*, *psbA-trnH*, *rps16* intron, *trnL*-intron, *trnL-F*, *trnS-G*) and 82 accessions. All generations prior to the point when the SDSF fell permanently below 0.01 (0.006782 at termination) were discarded as burn-in. Multiple accessions of the same species are numbered according to the Appendix. Species known to be tetraploid are in bold. Clades discussed in the text are marked with capital letters; those that correspond between datasets are given the same letter. Abbreviations and branch support reported as in Fig. 1. Inset picture of *Lamium album* (photograph by the first author; picture colored in the online version).).

in different places on the tree, and none of them grouped with the "*Wiedemannia*" clade. In the cpDNA phylogeny (Fig. 3), accessions of the two "*Wiedemannia*" species positioned between accessions of *L. galactophyllum* and *L. moschatum*, and this group of four species (clade F) received strong support. Clade F (Fig. 3) was not supported by the nuclear data (Figs. 1, 2); rather accessions grouped according to circumscribed taxa (i.e., *L. galactophyllum*, *L. moschatum*, and "*Wiedemannia*", respectively). However, in the 5S-NTS tree (Fig. 2), the b paralogs of *L. galactophyllum* and *L. moschatum* grouped with some support, but the monophyletic "*Wiedemannia*" was not part of this clade. Thus, all three datasets supported a phylogenetic placement of "*Wiedemannia*" within *Lamium*, although its phylogenetic position within *Lamium* remains uncertain (Figs. 1–3). Sister to clade F was a strongly supported group comprising *L. amplexicaule* var. *aleppicum*, *L. eriocephalum*, and *L. macrodon* (Fig. 3: clade E). This group existed and received strong support also in the nuclear phylogenies (Figs. 1, 2: clade E).

In the nuclear phylogenies (Figs. 1, 2), multiple accessions mostly grouped according to species, except that the *L. amplexicaule* varieties were spread out through the trees. Non-monophyly of *L. amplexicaule* was corroborated by the cpDNA tree (Fig. 3). Species monophyly was generally poorer in the cpDNA tree (Fig. 3) as compared to the nuclear trees (Figs. 1, 2). However, some congruent patterns could be identified between the three datasets (Figs. 1–3): (1) monophyly of *Lamium* (as currently circumscribed and based on the taxa included); (2) monophyly of several of the species within the genus (e.g., *L. bifidum*, *L. galeobdolon*, *L. garganicum*, *L. orvala*, and *L. purpureum*); (3) a close relationship between *L. amplexicaule* var. *amplexicaule* and *L. bifidum* (sistergroup relationship if the allotetraploids are ignored); (4) a close relationship between *L. maculatum* and *L. purpureum* (sistergroup relationship if the allotetraploids are ignored); (5) a close relationship between *L. amplexicaule* var. *aleppicum*, *L. eriocephalum*, and *L. macrodon*; (6) a monophyletic *album-tomentosum* group; and (7) a colse relationship between *L. maculatum* and *L.* × *holsaticum*.

Network. — The PADRE reconstruction of allopolyploid relationships based on a reduced NRPA2 alignment identified altogether six genome mergers (Fig. 4: 1–6), of which most corresponded to previous hypotheses of hybrid origins for tetraploid species within the genus: (1) *L. confertum* combined one diploid genome from *L. amplexicaule* var. *amplexicaule* and one from *L. purpureum*; (2–3) *L. hybridum* combined one

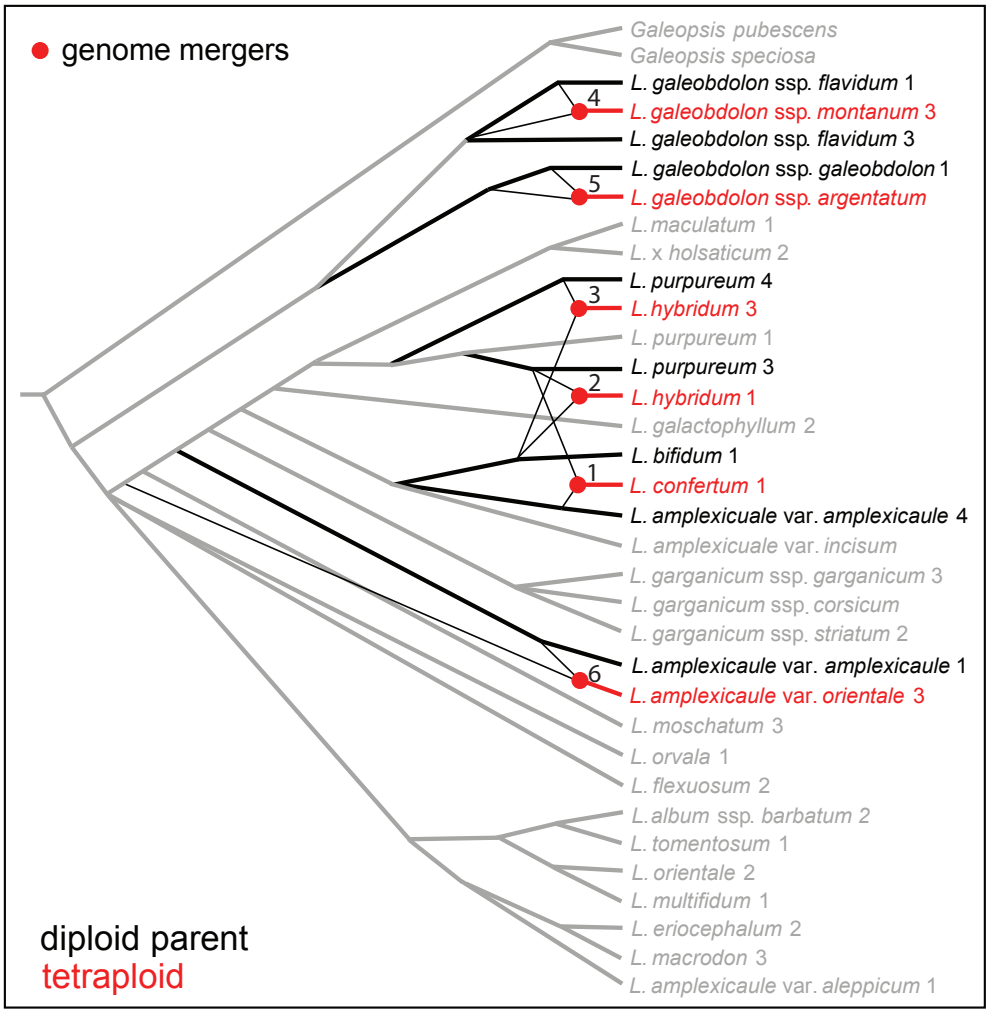

Fig. 4. The PADRE reconstruction of reticulate evolution and allopolyploid relationships within the genus *Lamium* based on the 50% majority-rule consensus tree from a Bayesian analysis of a NRPA2 matrix with 34 accessions and coded indels. Genome mergers are shown as filled dark gray (red in online version) circles at line junctions and numbered according to the sequence in which they are mentioned in the text. Accessions are numbered according to the Appendix.

diploid genome from *L. purpureum* and one from *L. bifidum*, and two different *L. purpureum* genotypes were obviously involved in the origin of the two *L. hybridum* accession included in the analysis; (4) *L. galeobdolon* subsp. *montanum* combined two diverged diploid genomes of subsp. *flavidum*; (5) *L. galeobdolon* subsp. *argentatum* combined two diverged diploid genomes of subsp. *galeobdolon*; and, (6) *L. amplexicaule* var. *orientale* combined one diploid genome from var. *amplexicaule* 1 and another diploid genome from a distant, but not identified, diploid parent. The presumed *L. album* × *maculatum* hybrid, *L.* × *holsaticum*, was close to *L. maculatum* in all trees and the network (Figs. 1–4).

■ DISCUSSION

Circumscription and species classification of the lamioid genus *Lamium* has varied through time. For example, *Lamium galeobdolon* and "*Wiedemannia*" have been variously classified as parts of *Lamium* or placed in separate genera (e.g., Bentham, 1848; Krause, 1903; Ryding, 2003). Moreover, *Lamium* has served as a respository for several species that are clearly extraneous to the genus (Mennema, 1989; Ryding, 2003). A recent molecular phylogenetic investigation of subfamily Lamioideae (Bendiksby & al., 2011a) corroborated the extraneousness of these species in *Lamium*, and a *Lamium* s.str. was identified, which is the target group of the present study. This group largely corresponds with the sum of taxa included in Mennema's (1989) and Ryding's (2003) morphological investigations of the genus.

Phylogeny and taxonomy. — We aimed at revealing phylogenetic relationships in *Lamium* using nuclear and chloroplast DNA sequence data. Our phylogenetic and taxonomical conclusions are predominantly based on results from the nuclear data, because largely congruent phylogenetic relationships were obtained from the two unlinked nuclear regions (NRPA2 and 5S-NTS; Figs. 1, 2) and because they correspond better with our perception of relatedness from morphology than do the cpDNA data (Fig. 3).

In his taxonomic revision of *Lamium*, Mennema (1989: 19) presented an "intuitive phylogenetic tree" of the genus without explaining how he arrived at that hypothesis. It is also problematic that the infrageneric classification he proposed in the same publication did not correspond to monophyletic groups of his phylogeny (Mennema, 1989: 19). Ryding (2003) performed a cladistic analysis of the genus based on morphological characters and received a different tree topology. In Ryding (2003), all the included *Lamium* species except *L. galeobdolon* formed a supported clade, and the two species previously assigned to *Wiedemannia* were nested within *Lamium*. Only one of Mennema's (1989) infrageneric taxa, the monotypic subg. *Orvala*, received support from our molecular data (i.e., multiple accessions of *L. orvala*; Figs. 1–3).

In our molecular trees (Figs. 1–3), *Lamium*, as circumscribed according to Bendiksby & al. (2011a), comprises a strongly supported clade on the basis of the taxa included herein. However, *L. galeobdolon* is morphologically very distinct, and we wanted to assess whether the remainder of *Lamium* would maintain monophyletic if *L. galeobdolon* was excluded. This is suggested by our nuclear data (Figs. 1, 2); the morphologically divergent *L. galeobdolon* (clade A) forms a sistergroup to a strongly supported clade comprising all remaining *Lamium* species (including *L. flexuosum*; clade B). Hence, based on the nuclear data, *Lamium* forms a monophyletic group irrespective of whether *L. galeobdolon* is included or not. Moreover, the exclusion of the divergent *L. galeobdolon* would render *Lamium* much more homogeneous and easier to define. Core-*Lamium* (Figs. 1, 2: clade B) can be distinguished from other Lamioideae in having the side-lobes of the lower lip of the corolla shorter and mostly dentate, and differ from most other Lamioideae in having the mid-lobe broader. Thus, *L. galeobdolon* may deserve to be circumscribed in a separate genus on the account of being very distinct. In spite of this, we hesitate to place *L. galeobdolon* in a separate genus (*Lamiastrum*) because monophyly of the rest of *Lamium* is not supported by the cpDNA data (Fig. 3). Monotypic taxa such as *Lamiastrum* may also be considered redundant in classification. The large clade of *Lamium* including *L. galeobdolon* is strongly supported by molecular data (Figs. 1–3; see also Bendiksby & al., 2011a) and may be supported by the presence of an elaiosome at the base of the nutlets (Gams, 1927; Bouman & Meeuse, 1992). Unfortunately, available data on this character is incomplete. It is often difficult to observe the elaiosomes in dried plant materials, such as herbarium specimens.

As mentioned above, the two species *L. multifidum* and *L. orientale* have been variably placed in *Lamium* or in a separate genus *Wiedemannia*. We wanted to test, by use of molecular data, Ryding's (2003) claim that *Wiedemannia* constitutes a subgroup of *Lamium*. Our molecular results corroborate his morphology-based conclusion; all our molecular data place "*Wiedemannia*" phylogenetically nested within *Lamium* (Figs. 1–3).

Lamium aleppicum Boiss. was originally described as a species, but was reduced to a variety under *L. amplexicaule* by Bornmüller (1907). All our molecular data (Figs. 1–3) show that *L. amplexicaule* is polyphyletic and that var. *aleppicum* does not group together with other *L. amplexicaule* varieties. Mennema (1989) mentioned that var. *aleppicum* differs from the other varieties in having narrower leaves. We found that the range of variation in ratio of leaf length/leaf width is (1.2–)1.3–2.7 in var. *aleppicum*, viz. 0.6–1.2(–1.3) in the rest of the species. The slight overlap in range of variation only applies to a few extreme leaves, and the plants that we examined can be divided into distinct groups based on average leaf shape. Mennema (1989) also mentioned that var. *aleppicum* has 2.50–3.25 mm long nutlets, while the other varieties have 2.00–2.75 mm long nutlets. *Lamium amplexicaule* var. *aleppicum* further tends to differ in having a faint grayish-bluish tint of the leaves. Hence, based on our molecular data and support from morphology, we propose that *L. aleppicum* should be resurrected as a species.

Allopolyploid origins. — We wanted to test whether the four tetraploid *Lamium* species have hybrid origins as suggested from the literature. As expected, two NRPA2 homoeologs and mostly four 5S-NTS main paralogs/homoeologs were obtained from all four tetraploids (the pattern in the 5S-NTS data from

the *L. galeobdolon* tetraploids was less clear), and the supported sister relationships were congruent and informative about the parentage (Figs. 1, 2, 4). Moreover, the organellar contributor to each tetraploid genome could be confirmed by our cpDNA results (Fig. 3). The two NRPA2 homoeologs obtained from *L. confertum* grouped with *L. purpureum* and *L. amplexicaule,* respectively (Figs. 1, 4), and *L. purpureum* was inferred as the organellar parent (Fig. 3). However, as *L. amplexicaule* is polyphyletic as currently circumscribed (Figs. 1, 3), it should be emphasized that var. *amplexicaule* was the second contributor to the tetraploid genome of *L. confertum*. The two NRPA2 homoeologs obtained from *L. hybridum* grouped with *L. bifidum* and *L. purpureum,* respectively (Figs. 1, 4), and *L. bifidum* was inferred as the organellar parent (Fig. 3). Hence, the presumed parentage of these two tetraploids is hereby confirmed.

The tetraploid *L. galeobdolon* subsp. *montanum* is morphologically intermediate between the diploid subsp. *galeobdolon* and subsp. *flavidum* (see Mennema's histograms, 1989), supporting Dersch's (1964) view that subsp. *montanum* originated from an allopolyploidization between subsp. *galeobdolon* and subsp. *flavidum*. However, both of the divergent NRPA2 homoeologs obtained from subsp. *montanum* emerged in the clade of subsp. *flavidum*, indicating that subsp. *montanum* may have originated from subsp. *flavidum* alone (Figs. 1, 4). Likewise, both NRPA2 homoeologs of the tetraploid subsp. *argentatum* grouped with subsp. *galeobdolon*, suggesting that it may have originated from the diploid subsp. *galeobdolon* alone. It should be noted, however, that variation at the nucleotide level was found within both subsp. *galeobdolon* and subsp. *flavidum*, and a more comprehensive sampling of these taxa is needed to identify with more certainty the parental genomes contributing to subsp. *montanum* and subsp. *argentatum*.

As mentioned by Mennema (1989), *L.* × *holsaticum* is commonly believed to be a hybrid between *L. album* and *L. maculatum*. The taxon does indeed seem to be morphologically intermediate between these two species. As *L.* × *holsaticum* has not had chromosomes counted, the ploidy level of this taxon remains unknown. We did not obtain PCR products from *L.* × *holsaticum* accessions using the *album*-specific NRPA2 primer pair, whereas the *maculatum*-specific primers generated PCR product that could be sequenced directly. PCR products were also obtained and could be sequenced directly using the less specific L-A2F/R primer pair. Finally, sequencing 8 to 16 clones of these NRPA2 products revealed only one NRPA2 type in each of the two accessions included of this taxon, suggesting no additional genome-contributor to *L.* × *holsaticum*. In all genealogies (Figs. 1–3), *L.* × *holsaticum* is close to *L. maculatum*. Hence, we found no molecular evidence that could support a hybrid origin of *L.* × *holsaticum*. The taxon may represent a diploid variety of *L. maculatum* or, if later shown to be polyploid, an autotetraploid of the same species. Because of our strong evidence against *L.* × *holsaticum* being of hybrid origin, the '×' before the species epithet should be removed. However, it is more uncertain whether the taxon is suffiently distinct to be treated as a species. More studies are needed before a well-founded decision about its taxonomic status can be made.

The presence of two highly divergent NRPA2 copies in each accession of *L. amplexicaule* var. *orientale* is interpreted as evidence for tetraploidy and an alloploid origin of this taxon. One of the homoeologs emerged close to one accession of var. *amplexicaule*, while the other emerged in a more isolated part of the tree (Figs. 1, 4), suggesting that the variety constitutes a hybrid between var. *amplexicaule* and a divergent, but not sampled, second parent. This scenario was corroborated by the 5S-NTS data (Fig. 2). It should be noted, however, that the var. *amplexicaule* accession with which var. *orientale* grouped (var. *amplexicaule* 1; Fig. 1) was to some degree divergent, both genetically and morphologically, from the remaining accessions of the variety. As such, it appears to represent a distinct lineage of *L. amplexicaule* that may deserve to be recognized taxonomically after a more thorough investigation of additional samples. The probable allopolyploid origin of var. *orientale* (Fig. 4) suggests that it should be treated as a different species, not the least in order to be consistent with the way other allopolyploid taxa within *Lamium* have been treated. At the rank of species it should be known by the name *L. paczoskianum* Vorosh. However, it is problematic that var. *orientale* is morphologically very similar to *L. amplexicaule* var. *incisum* Boiss., which emerges along with the remaining accessions of var. *amplexicaule* (Figs. 1, 3). According to Mennema (1989), the best diagnostic character of var. *orientale* is the corolla being 3.5–4.0 times, instead of ca. 2.5 times, longer than the calyx, but this character hardly seems to be consistent. Hence, it is with some hesitation that we propose resurrection of the species *L. paczoskianum*.

Infrageneric classification. — Finally, we wanted to test whether Mennema's (1989) infrageneric classification is corroborated in whole or in part by molecular data. Monophyly of the monotypic subg. *Orvala* is corroborated, whereas subg. *Galeobdolon* is paraphyletic or polyphyletic, and subg. *Lamium* is neither contradicted nor supported by our molecular data (Figs. 1–3). Monophyly of subg. *Lamium* is cladistically supported by morphology (Ryding, 2003). Thus, the joint data of molecular and morphological characters would probably identify a monophyletic, although not strongly supported, subg. *Lamium*. However, all but three species (*L. galeobdolon*, *L. orvala*, *L. flexuosum*) would belong to subg. *Lamium*, which, in our view, renders Mennema's (1989) infrageneric classification redundant. Mennema's (1989) three sections within subg. *Lamium* are all para- or polyphyletic in our molecular trees (Figs. 1–3). Therefore, we suggest that Mennema's (1989) infrageneric classification should be abandoned.

Clades of some diploid taxa that were present in all molecular trees (Figs. 1–3; e.g., *L. album* and *L. tomentosum*; *L. amplexicaule* var. *amplexicaule* and *L. bifidum*; *L. maculatum* and *L. purpureum*; and, *L. amplexicaule* var. *aleppicum*, *L. eriocephalum*, and *L. macrodon*) could potentially have formed grounds for new infrageneric groupings. However, as no large monophyletic groups were identified that received strong support by both molecular (present study) and non-homoplastic morphological synapomorphies (Ryding, 2003), and most of the species would remain unplaced, no new infrageneric classification is proposed.

Nuclear-chloroplast incongruence. — The incongruence found between the nuclear and the chloroplast (cpDNA) genealogies is substantial (Figs. 1–3). For example, *L. galeobdolon* holds a strongly supported position as sister to all remaining *Lamium* taxa in the nuclear trees (Figs. 1, 2), whereas the *album-tomentosum* group holds such a position in the cpDNA tree (Fig. 3). Also, clade F (*L. galactophyllum*, *L. moschatum*, "*Wiedemannia*") receives strong support in the cpDNA tree (Fig. 3), whereas this group does not exist in the nuclear trees (Figs. 1, 2). Topological incongruence between genealogies of unlinked genes is quite common, particularly in plants where hybridization and introgression are frequent and might result in incongruent patterns between nuclear and chloroplast data (e.g., Rieseberg & Soltis, 1991; Rieseberg & al., 1996). Even though chloroplast capture through introgression might account for many or even most cases of incongruent nuclear and cytoplasmic gene trees (Tsitrone & al., 2003), similar patterns may result from other processes such as differential lineage sorting of ancestral polymorphisms in chloroplast and nuclear genes (Comes & Abbott, 2001) or evolutionary convergence (homoplasy; Davis & al., 1998), and to settle the relative importance of different mechanisms is a huge challenge (Pfeil & al., 2005; Frajman & al., 2009).

The grouping of *L. galactophyllum*, *L. moschatum* and "*Wiedemannia*" in the cpDNA phylogeny (Fig. 3: clade F) could be a result of introgression and chloroplast capture between these taxa, which occur more or less in sympatry; a requirement for introgression and hybridization to occur. Moreover, the taxa of clade E (*L. eriocephalum*, *L. macrodon*, *L. amplexicaule* var. *aleppicum*), which group with clade F in the cpDNA tree (Fig. 3) but not in the nuclear trees (Figs. 1, 2), have the same centre of distribution as those of clade F. Both the extent of the incongruence, as well as the sympatry of the taxa involved, speak in favour of an introgression hypothesis. Likewise, the shifting positions of *L. galeobdolon* (clade A) and the *album-tomentosum* group (clade C) in the nuclear versus cpDNA trees (Figs. 1–3) are most likely due to introgression.

Non-monophyly of some morphologically rather distinct species, such as *L. tomentosum* and *L. album* in both the NRPA2 and the cpDNA phylogenies (Figs. 1, 3), and *L. eriocephalum* in the NRPA2 tree (Fig. 1), may be better explained by incomplete sorting of ancestral polymorphisms, as introgression mostly affects the chloroplast genome, and the patterns of incongruence do not correlate with geographical distributions.

Notes on paralogy and phylogenetic utility of the nuclear DNA regions.— NRPA2 is a single-copy gene located on chromosome 1 in *Arabidopsis thaliana* Schur (The Arabidopsis Genome Initiative, 2000). It was reported as single copy also in *Silene* (Popp & Oxelman, 2004, 2007). However, duplication of the NRPA2 gene may have occurred in some plant lineages, e.g., *Heliosperma* (Frajman & al., 2009). Because sequenced clones from amplification products using the degenerate NRPA2 primers described by Popp & Oxelman (2004) produced only one sequence type from diploid *Lamium* species, NPRA2 is most likely single-copy in *Lamium*. Also in a second lamioid genus, *Galeopsis*, the NRPA2 gene was shown to be single-copy (Bendiksby & al., 2011b). Because of the ease with which we could amplify and sequence NRPA2 directly, we anticipate that this DNA regions will be increasingly used in future phylogenetic investigations.

The nuclear ribosomal 5S-NTS, on the other hand, occurs in multiple inter- and intragenomic paralogs in *Lamium* (Fig. 2; tree with terminals available from TreeBase: S11382). Among these, we could identify two main paralogs. Two main paralogous copies of 5S-NTS have been found also in *Brassaiopsis* (Araliaceae; Mitchell & Wen, 2005), and several studies have reported plants with two 5S rDNA FISH sites (Dhar & al., 2006; Wolny & Hasterok, 2009).

In addition to a high number of substitutions, the inter- and intragenomic differences in *Lamium* 5S-NTS include also a high number of insertions and deletions (indels). It seems, therefore, that a complex combination of duplications, indels, and restricted concerted evolution has been involved in the evolution of the 5S rDNA family in *Lamium*. Similar results have been reported from a wide range of taxa (e.g., Campo & al., 2009; Morgan & al., 2009), whereas for the genus *Alibertia* (Rubiaceae), no paralogous loci were found (e.g., Persson, 2000). Obviously, the molecular evolution of 5S-NTS varies between taxa, which is also our own experience from extensive 5S-NTS cloning and sequencing of additional lamioid taxa (Bendiksby & al., unpub.).

Due to the complex and, between taxa, inconsistent molecular evolution of 5S-NTS, the genetic region has by some been regarded as unsuitable for phylogenetic inference (e.g., Sajdak & al., 1998; Pornpongrungrueng & al., 2009). However, the congruence between our 5S-NTS and NRPA2 results supports the utility of this region for phylogenetic inference, at least in *Lamium*. Also in Machaerantherinae (Asteraceae), 5S-NTS seemed to hold a phylogenetic signal despite of extensive inter- and intragenomic sequence variation (Morgan & al., 2009). In fact, publications most often report 5S-NTS to perform well. This may, however, be due to the success-bias of published data. A comprehensive molecular evolutionary investigation of the 5S rDNA family across taxonomic groups is clearly warranted.

■ CONCLUSIONS

Our molecular investigations brought new knowledge about phylogenetic relationships and allopolyploid speciation within the medium-sized Eurasian genus *Lamium*. The results also provide a striking example of incongruence between nuclear versus chloroplast genealogies. The parental-specific primer approach used for the single-copy NRPA2 may prove useful for other groups as well. Despite a seemingly unlimited number of 5S-NTS paralogs within all species investigated, the 5S-NTS seems to hold some potential as a phylogenetic marker within this group. Future studies should aim at including the five *Lamium* species as well as additional subspecific taxa that we were not able to obtain for the present study. Moreover, usage of more variable molecular markers might provide a phylogeny with more resolution and support for larger groupings.

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■ LITERATURE CITED

Ball, P.W. 1972. *Lamium* and *Lamiastrum*. Pp. 147–149 in: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.M., Walters, S.M. & Webb, D.A. (eds.), *Flora Europaea*, vol 3. Cambridge: Cambridge University Press.

Bendiksby, M. 2002. A molecular evolutionary study suggests a highly divergent plant lineage and recently evolved species in *Rafflesia* (Rafflesiaceae). M.Sc. Thesis, University of Oslo, Norway.

Bendiksby, M., Thorbek, L.B., Scheen, A.C., Lindqvist, C. & Ryding, O. 2011a. An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. *Taxon* 60: 471–484.

Bendiksby, M., Tribsch, A., Borgen, L., Trávníček, P. & Brysting, A.K. 2011b. Allopolyploid origin of *Galeopsis* tetraploids — revisiting Muntzing's (1932) classical textbook example using molecular tools. *New Phytol*. DOI: 10.1111/j.1469-8137.2011.03753.x.

Bentham, G. 1832–1836. *Labiatarum genera et species*. London: James Ridgway and Sons.

Bentham, G. 1848. Labiatae. Pp. 27–603 in: Candolle, A. de (ed.), *Prodromus systematis naturalis regni vegetabilis*, vol. 12. Paris: Masson.

Bernström, P. 1955. Cytogenetic studies on relationships between annual species of *Lamium*. *Hereditas* 41: 1–122.

Bornmüller, J.F.N. 1907. Plantae Straussianae – sive enumeratio plantarum a Th. Strauss annis 1889–1899 in Persia occidentali collectarum, pars III. *Beih. Bot. Centralbl., Abt. 2* 22: 102–142.

Bouman, F. & Meeuse, A.D.J. 1992. Dispersal in Labiatae. Pp. 193–202 in: Harley, R.M. & Reynolds, T. (eds.), *Advances in labiate science*. Kew: Royal Botanic Gardens.

Briquet, J. 1895–1897. Labiatae. Pp. 183–375 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol 4. Leipzig: Engelmann.

Brysting, A.K., Mathiesen, C. & Marcussen, T. 2011. Challenges in polyploid phylogenetic reconstruction: A case story from the arctic-alpine *Cerastium alpinum* complex. *Taxon* 60: 333–347.

Brysting, A.K., Oxelman, B., Huber, K.T., Moulton, V. & Brochmann, C. 2007. Untangling complex histories of genome mergings in high polyploids. *Syst. Biol.* 56: 467–476.

Campo, D., Machado-Schiaffino, G., Horreo, J.L. & Garcia-Vazquez, E. 2009. Molecular organization and evolution of 5S rDNA in the genus *Merluccius* and their phylogenetic implications. *J. Molec. Evol.* 68*:* 208–216.

Cantino, P.D. 1985. Chromosome studies in subtribe Melittidinae (Labiatae) and systematic implications. *Syst. Bot.* 10: 1–6.

Comes, H.P. & Abbott, R.J. 2001. Molecular phylogeny, reticulation, and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). *Evolution* 55: 1943–1962.

Cox, A.V., Bennett, M.D. & Dyer, T.A. 1992. Use of the polymerase chain-reaction to detect spacer size heterogeneity in plant 5S-rRNA gene clusters and to locate such clusters in wheat (*Triticum aestivum* L). *Theor. Appl. Genet.* 83: 684–690.

Davis, J.I., Simmons, M.P., Stevenson, D.W. & Wendel, J.F. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: An example from the monocotyledons using mitochondrial *atpA* sequences. *Syst. Biol.* 47: 282–310.

Dersch, G. 1964. Zur Cytologie und Taxonomie der Goldnessel (*Lamium galeobdolon* (L.) L.). *Ber. Deutsch. Bot. Ges.* 76: 351–359.

Dhar, M.K., Friebe, B., Kaul, S. & Gill, B.S. 2006. Characterization and physical mapping of ribosomal RNA gene families in *Plantago*. *Ann. Bot.* 97: 541–548.

Fedorov, A. (ed.). 1969. *Chromosome numbers of flowering plants*. Leningrad: Academy of Science of the USSR.

Fischer, F.E.L. & Meyer, C.A. 1838. *Wiedemannia*. Pp. 51–52 in: Fischer, F.E.L., Meyer, C.A. & Trautvetter, E.R. von (eds.), *Animadversiones Botanicea: Index seminum, quae Hortus Botanicus Imperialis Petropolitanus pro mutua commutatione offert*, vol 4. St. Petersburg.

Fortune, P.M., Pourtau, N., Viron, N. & Ainouche, M.L. 2008. Molecular phylogeny and reticulate origins of the polyploid *Bromus* species from the section *Genea* (Poaceae). *Amer. J. Bot.* 95: 454–464.

Fourreau, J.-P. 1869. Catalogue des plantes du cours du Rhone. *Ann. Soc. Linn. Lyon*, ser. 2, 17: 89–200.

Frajman, B., Eggens, F. & Oxelman, B. 2009. Hybrid origins and homoploid reticulate evolution within *Heliosperma* (Sileneae, Caryophyllaceae)—a multigene phylogenetic approach with relative dating. *Syst. Biol.* 58: 328–345.

Frajman, B. & Oxelman, B. 2007. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (Sileneae, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molec. Phylog. Evol.* 43: 140–155.

Gams, H. 1927. Labiatae. Pp. 2255–2548 in: Hegi, G. (ed.), *Illustrierte Flora von Mittel-Europa*, vol 5, reprint 1964. Munich: Hanser.

Goloboff, P.A., Farris, J.S. & Nixon, K. 2003. TNT: Tree analysis using new technology, version 1.0. Program and documentation, available at http://www.zmuc.dk/public/phylogeny/tnt.

Gorschkova, S.G. 1954. [*Lamium* and *Galeobdolon*]. Pp. 124–140 in: Schishkin, B.K. (ed.), *Flora of the USSR*, vol. 21. Moscow, Leningrad: Akademii Nauk SSR.

Govaerts, R., Paton, A., Harvey, Y. & Navarro, T. 2010. World checklist of Lamiaceae and Verbenaceae. Kew, Richmond: The Board of Trustees of the Royal Botanic Gardens. http://www.kew.org/wcsp/lamiaceae/ (accessed 10 Oct 2010).

Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95–98.

Hamilton, M.B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molec. Ecol.* 8: 521–523.

Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Grayer, R., Harley, M.M., de Kok, R., Krestovskaya, T., Morales, R., Paton, A.J., Ryding, O. & Upson, T. 2004. Labiatae. Pp. 167–275 in: Kubitzki, K. & Kadereit, J.W. (eds.), *The families and genera of vascular plants*, vol. 7. Berlin, Heidelberg: Springer.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Krause, E.H.L. (ed.). 1903. *J. Sturms Flora von Deutschland*, ed. 2, vol. 11. Stuttgart: Lutz.

Lott, M., Spillner, A., Huber, K.T. & Moulton, V. 2009. PADRE: A package for analyzing and dsiplaying reticulated evolution. *Bioinformatics* 25: 1199–1200.

Marcussen, T., Oxelman, B., Skog, A. & Jakobsen, K.S. 2010. Evolution of plant RNA polymerase IV/V genes: Evidence of subneofunctionalization of duplicated *NRPD2/NRPE2*-like paralogs in *Viola* (Violaceae). *B.M.C. Evol. Biol.* 10: 45. DOI: 10.1186/1471-2148-10-45.

Mason-Gamer, R.J. 2008. Allohexaploidy, introgression, and the complex phylogenetic history of *Elymus repens* (Poaceae). *Molec. Phylog. Evol.* 47: 598–611.

Mennema, J. 1989. A taxonomic revision of *Lamium* (Lamiaceae). *Leiden Bot. Ser.* 11: 1–198.

Mill, R.R. 1982. [*Lamium* to *Galeobdolon*]. Pp. 126–151 in: Davis,

P.H. (ed.), *Flora of Turkey and the East Aegean Islands*, vol. 7. Edinburgh: Edinburgh University Press.

Mitchell, A., Wen, J. & Hoot, S.B. 2005. Phylogeny of *Brassaiopsis* (Araliaceae) in Asia based on nuclear ITS and 5S-NTS DNA sequences. *Syst. Bot.* 30: 872–886.

Morgan, D.R., Korn, R.L. & Mugleston, S.L. 2009. Insights into reticulate evolution in Machaerantherinae (Asteraceae: Astereae): 5S ribosomal RNA spacer variation, estimating support for incongruence, and constructing reticulate phylogenies. *Amer. J. Bot.* 96: 920–932.

Mossberg, B., Stenberg, L. & Ericsson, S. 1992. *Den nordiska floran*. Stockholm: Wahlström & Widstrand.

Müller, K. 2005. SeqState—primer design and sequence statistics for phylogenetic DNA data sets. *Appl. Bioinformatics* 4: 65–69.

Nylander, J.A.A. 2004. MrAIC.pl. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Opiz, F.M. 1852. *Seznam rostlin kveteny Ceské.* Praha: v Kommissi u Fr. Řiunáce.

Oxelman, B., Liden, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206*:* 393–410.

Persson, C. 2000. Phylogeny of the Neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. *Amer. J. Bot.* 87: 1018–1028.

Pfeil, B.E., Schlueter, J.A., Shoemaker, R.C. & Doyle, J.J. 2005. Placing paleopolyploidy in relation to taxon divergence: A phylogenetic analysis in legumes using 39 gene families. *Syst. Biol.* 54: 441–454.

Pignatti, S. 1982. *Flora d'Italia,* vol. 2. Bologna: Edagricole.

Popp, M. & Oxelman, B. 2004. Evolution of a RNA polymerase gene family in *Silene* (Caryophyllaceae)—Incomplete concerted evolution and topological congruence among paralogues. *Syst. Biol.* 53: 914–932.

Popp, M. & Oxelman, B. 2007. Origin and evolution of North American polyploid *Silene* (Caryophyllaceae). *Amer. J. Bot.* 94: 330–349.

Pornpongrungrueng, P., Borchsenius, F. & Gustafsson, M.H.G. 2009. Relationships within *Blumea* (Inuleae, Asteraceae) and the utility of the 5S-NTS in species-level phylogeny reconstruction. *Taxon* 58: 1181–1193.

Pujadas Salvà, A.J. 2010. *Lamium*. Pp. 180–196 in: Morales, R., Quintanar, A., Cabezas, F., Pujadas Salvà, A.J. & Cirujano, S. (eds.), *Flora Iberica*, vol. 12. Madrid: Real Jardín Botánico.

Rieseberg, L.H. & Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evol. Trends Pl.* 5: 6–83.

Rieseberg, L.H., Whitton, J. & Linder, C.R. 1996. Molecular marker incongruence in plants hybrid zones and phylogenetic trees. *Acta Bot. Neerl.* 45: 243–262.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Ryding, O. 2003. Reconsideration of *Wiedemannia* and notes on the circumscription of *Lamium* (Lamiaceae). *Bot. Jahrb. Syst.* 124: 325–335.

Ryding, O. 2006. Lamiaceae. Pp. 497–519 in: Frederiksen, S., Rasmussen, F.N. & Seberg, O. (eds.), *Dansk flora*. Copenhagen: Gyldendal.

Sajdak, S.L., Reed, K.M. & Phillips, R.B. 1998. Intraindividual and interspecies variation in the 5S rDNA of coregonid fish. *J. Molec. Evol.* 46: 680–688.

Sang, T., Crawford, D.J. & Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot.* 84: 1120–1136.

Scheen, A.-C. & Albert, V.A. 2009. Molecular phylogenetics of the *Leucas* group (Lamioideae; Lamiaceae). *Syst. Bot.* 34: 173–181.

Scheen, A.C., Bendiksby, M., Ryding, O., Mathiesen, C., Albert, V.A. & Lindqvist, C. 2010. Molecular phylogenetics, character evolution, and suprageneric classification of Lamioideae (Lamiaceae). *Ann. Missouri Bot. Gard.* 97: 191–217.

Scheen, A.-C., Lindqvist, C., Fossdal, C.G. & Albert, V.A. 2008. Molecular phylogenetics of tribe Synandreae, a North American lineage of lamioid mints (Lamiaceae). *Cladistics* 23: 1–16.

Simmons, M.P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49: 369–381.

Stace, C. 2010. *New Flora of the British Isles*, ed. 3. Cambridge: Cambridge University Press.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.

Tsitrone, A., Kirkpatrick, M. & Levin, D.A. 2003. A model for chloroplast capture. *Evolution* 57: 1776–1782.

Willdenow, C.L. 1787. *Florae berolinensis prodromus secundum systema etc.* Berlin: Impensis Wilhelmi Viewegii.

Wolny, E. & Hasterok, R. 2009. Comparative cytogenetic analysis of the genomes of the model grass *Brachypodium distachyon* and its close relatives. *Ann. Bot.* 104: 873–881.

Ying. S.S. 1991. A new species of genus *Lamium* (Labiatae) from Taiwan. *Mem. Coll. Agric. Natl. Taiwan Univ.* 31: 22–23, 35.

Appendix. Information about the specimens used in this study (outgroup taxa separately at the end): taxon names, voucher information, country of origin, year of collection, and GenBank accession numbers for DNA sequence data. Accessions marked with collection year in bold italics were extracted from silica-dried leaf material. All other accessions were extracted from herbarium specimens. Multiple accessions from the same species are numbered consecutively. Chromosome numbers (2*n*) were obtained from IPCN. GenBank accession numbers of the two NRPA2 homoeologs in tetraploids are separated by a slash. When submitted separately, accession numbers of the *trnL* intron and the *trnL-F* spacer are separated by a slash. All 5S-NTS paralogs from a single voucher have consecutive GenBank accession numbers; only first and last reported (hyphened). Seven 5S-NTS paralogs (superscript-numbered) that were shorter than 200 bp (and therefore not accepted in GenBank) are reported in their entire length at the end of the Appendix. Missing data are indicated with N/A.

INGROUP/OUTGROUP: specimen-1, voucher, origin, year, NRPA2, 5S-NTS, *trnL-F* region (intron and intergenic spacer), *rps16* intron, *trnS-trnG* intergenic spacer, *psbA-trnH* intergenic spacer, *matK*; specimen-2, etc.

INGROUP: ***Lamium album*** L., 1. *A. Elven s.n.*, 02.07.1995 (O), Norway, 1995, JF780191, JF780258–JF780269, JF779959, JF780033, JF779882, JF780110, N/A; 2. *F. Wischmann s.n.*, 26.06.1998 (O), Norway, 1998, JF780193, N/A, JF779960, JF780034, JF779883, JF780111, N/A; 3. *M. Bendiksby 05-014* (O) , Norway, ***2005,*** JF780194, N/A, JF779961, JF780035, JF779884, JF780112, JF779864; ***L. album*** L. subsp. ***barbatum*** (Siebold & Zucc.) Mennema*,* 1. *G. Murata & H. Koyama 75* (WU), Japan, 1963, N/A, N/A, JF779962, JF780035, JF779885, JF780113, N/A; 2. *H. Smith 6513* (S), China, 1924, JF780192, JF780335–JF780345, JF779963, JF780037, JF779886, JF780114, N/A; 3. *N. Satomi 15258* (S), Japan, 1954, N/A, N/A, JF779964, JF780038, JF779887, JF780115, N/A; ***L. album*** L. subsp. ***crinitum*** (Montbret & Aucher ex Benth.) Mennema*, J. Bornmüller 7947* (WU), Iran, 1902, N/A, N/A, EF546932/EF546854, FJ854044, JF779888, JF780116, N/A; ***L. amplexicaule*** L.*,* 1. *D. Albach 233* (WU), Turkey, 2000, JF780234, N/A, JF779968, JF780042, JF779892, JF780120, N/A; 2. *J.I. Båtvik 102* (O), Norway, 1998, JF780196, N/A, JF779969, JF780043, JF779893, JF780121, N/A; 3. P.W. Leenhouts 3568 (O), Netherland, 1979, JF780201, N/A, JF779970, JF780044, JF779894, JF780122, JF779865; 4. R. Elven 280241 (O), Norway, 2001, JF780202, JF780462–JF780470, JF779971, JF780045, JF779895, JF780123, N/A; ***L. amplexicaule*** L. var. ***aleppicum*** (Boiss. & Hausskn.) Bornm.*,* 1. *G. Samuelsson 4942* (S), Lebanon, 1933, JF780190, N/A, JF779965, JF780039, JF779889, JF780117, N/A; 2. *O. Stapf 209* (WU), Iraq, 1888, N/A, JF780441–JF780450, JF779966, JF780040, JF779890, JF780118, N/A; 3. *Th. Pichler s.n.*, anno 1882 (WU), Iran, 1882, N/A, JF780487–JF780494, JF779967, JF780041, JF779891, JF780119, N/A; ***L. amplexicaule*** L. var. ***incisum*** Boiss.*, H. Helbaek 383* (C), Iraq, 1955, JF780195, N/A, JF779972, JF780046, JF779896, JF780124, JF779866; ***L. amplexicaule*** L. var. ***orientale*** (Pacz.) Mennema*,* 1. *C. Roth s.n.*, April 1903 (S), SW Russia, 1903, N/A, N/A, JF779973, JF780047, JF779897, JF780125, N/A; 2. *G. Kleopow 5000* (S), SW Russia, 1925, JF780197/JF780198, N/A, JF779974, JF780048, JF779898, JF780126, N/A; 3. *P. Oksiuk s.n.*, 19.5.1929 (S), Ukraina, 1929, JF780199/JF780200, JF780451–JF780454, JF779975, JF780049, JF779899, JF780127, N/A;

Appendix. Continued.

L. bifidum Cirillo, 1. *A. Latzel s.n.*, 29.3.1909 (UPS), Croatia, 1909, JF780203, JF780270–JF780276, JF779976, JF780050, JF779900, JF780128, N/A; 2. *M. Bendiksby 05-021* (O), Italy, ***2005*****,** JF780204, N/A, JF779977, JF780051, JF779901, JF780129, JF779867; 3. *W. Till s/n 4396* (WU), Italy, 2001, JF780205, N/A, JF779978, JF780052, JF779902, JF780130, N/A; ***L. confertum*** Fr., 1. *I. Holtan s.n.*, 5.6.1998 (O), Norway, 1998, JF780206/JF780207, JF780346–JF780354, JF779979, JF780053, JF779903, JF780131, N/A; 2. *I. Segelberg 23857* (S), Faroe Isl., 2003, JF780208/JF780209, JF780360–JF780368, JF779980, JF780054, JF779904, JF780132, N/A; 3. *R. Elven 90453* (O), Norway, 1994, N/A, N/A, JF779981, JF780055, JF779905, JF780133, N/A; ***L. coutinhoi*** J.G. García, *A. Fernandes 4151* (UPS), Portugal, 1952, N/A, N/A, JF779982, JF780056, JF779906, JF780134, N/A; ***L. eriocephalum*** Benth., 1. *A. Strid & al. 23887* (C), Turkey, 1984, JF780210, JF780277–JF780287[1], JF779983, JF780057, JF779907, JF780135, N/A; 2. *Gerolle 340* (WU), Cicily, 1895, JF780211, N/A, JF779984, JF780058, JF779908, JF780136, N/A; ***L. flexuosum*** Ten., 1. *Gröbner s.n.*, 3.6.1968 (C), Italy, 1968, N/A, JF780326–JF780329, JF779985, JF780059, JF779909, JF780137, JF779868; 2. *H. Lindberg 3722* (S), Morocco, 1926, JF780212, N/A, JF779986, JF780060, JF779910, JF780138, N/A; 3. *I. Segelberg s.n.*, 13.5.1962 (S), Italy, 1962, N/A, JF780369–JF780372[2], JF779987, JF780061, JF779911, JF780139, N/A; ***L. galactophyllum*** Boiss. & Reut., 1. *E. Bourgeau 223* (WU), Armenia, 1862, JF780213, N/A, JF779988, JF780062, JF779912, JF780140, N/A; 2. *E. Koenig s.n.,* 7.6.1904 (WU), Turkey, 1904, JF780214, JF780310–JF780318, JF779989, JF780063, JF779913, JF780141, N/A; ***L. galeobdolon*** (L.) L., 1. *M. Bendiksby 05-016* (O), Norway, ***2005*****,** JF780220, N/A, JF779994, JF780068, JF779918, JF780146, JF779869; 2. *N. Orderud 236911* (O), Norway, 1998, N/A, JF780423–JF780429, JF779995, JF780069, JF779919, JF780147, N/A; ***L. galeobdolon*** (L.) L. subsp. ***argentatum*** (Smejkal) J. Duvign., *H. Nielsen s.n.*, 22.7.1989 (C), Sweden, 1989, JF780215, JF780216, JF780334, JF779990, JF780064, JF779914, JF780142, N/A; ***L. galeobdolon*** (L.) L. subsp. *flavidum* (F. Herm.) Á. Löve & D. Löve, 1. *G. Kleesadl 405* (WU), Austria, 1995, JF780217, JF780319–JF780325[3], JF779991, JF780065, JF779915, JF780143, N/A; 2. *G. & E. Gölles 365* (WU), Austria, 1988, JF780218, JF779992, JF780066, JF779916, JF780144, N/A; 3. *X. Giraldez & al. 2189* (C), Italy, 1990, JF780219, JF780505–JF780511, JF779993, JF780067, JF779917, JF780145, N/A; ***L. galeobdolon*** (L.) L. subsp. ***montanum*** (Pers.) Hayek, 1. *M. Bendiksby 05-015* (O), Norway, ***2005*****,** JF780221, N/A, JF779996, JF780070, JF779920, JF780148, JF779870; 2. *W. Möschl & H. Pittoni s.n.,* 11.5.1980 (C), Austria, 1980, N/A, JF780495–JF780496, JF779997, JF780071, JF779921, JF780149, N/A; 3. *W. Till s.n.*, 24.5.1998 (WU), Austria, 1998, JF780222/JF780223, JF780497–JF780504, FJ854282/FJ854170, FJ854043, JF779922, JF780150, HQ911456; ***L. garganicum*** L., 1. *A. Tribsch & M. Bendiksby 06-017* (O), Italy/France, ***2006*****,** JF780224, JF780288–JF780294, JF779999, JF780073, JF779924, JF780152, JF779872; 2. *E. Hörandl & al. 4754* (WU), Turkey, 1992, JF780225, N/A, JF780000, JF780074, JF779925, JF780153, N/A; 3. *S. & B. Snogerup 15184* (UPS), Greece, 1998, JF780227, N/A, JF780001, JF780075, JF779926, JF780154, N/A; ***L. garganicum*** subsp. *corsicum* (Gren. & Godr.) Mennema, *D.C. Forsyth Major s.n.*, 15.5.1884 (UPS), Corse, 1884, JF780226, JF780301–JF780309, JF779998, JF780072, JF779923, JF780151, JF779871; ***L. garganicum*** subsp. ***striatum*** (Sm.) Hayek, 1. *Mittelmeer Exkusion 39* (WU), Corfu, 1985, JF780229, JF780409–JF780418, JF780002, JF780076, JF779927, JF780155, N/A; 2. *Rawi 8699* (US), Iraq, 1947, JF780228, N/A, JF780003, JF780077, JF779928, JF780156, N/A; ***L. hybridum*** Vill., 1. *J.E. Eriksen s.n.*, 2.7.2000 (O), Norway, 2000, JF780230/JF780231, JF780384–JF780390[4,5], JF780006, JF780080, JF779931, JF780159, N/A; 2. *J.E. Palmér s.n.*, May 1905 (UPS), Sweden, 1905, N/A, N/A, JF780007, N/A, JF779932, JF780160, N/A; 3. *K.A. Lye 23936* (O), Norway, 2000, JF780232/JF780233, N/A, JF780008, JF780081, JF779933, JF780161, N/A; 4. *Kerner 397* (WU), Germany, 1875, N/A, N/A, JF780009, JF780082, JF779934, JF780162, N/A; 5. *M. & M. Malzéville 2436* (WU), France, 1908, N/A, N/A, JF780010, JF780083, JF779935, JF780163, N/A; ***L. macrodon*** Boiss. & A. Huet., 1. *E. Zederbauer s.n.*, May 1902 (WU), Turkey, 1902, JF780235, N/A, JF780011, JF780084, JF779936, JF780164, N/A; 2. *E. Zederbauer s.n.*, June 1902 (WU), Turkey, 1902, JF780236, N/A, JF780012, JF780085, N/A, JF780165, N/A; 3. *P. Sintenis 15477* (WU), Armenia, 1894, JF780237, JF780455–JF780461, JF780013, JF780086, JF779937, JF780166, N/A; ***L. maculatum*** (L.) L., 1. *H. Aun 10148* (O), Denmark, 1955, JF780238, JF780330–JF780333, JF780014, JF780087, JF779938, JF780167, N/A; 2. *R. Elven 90722* (O), Norway, 1994, JF780239, N/A, JF780015, JF780088, JF779939, JF780168, N/A; ***L. moschatum*** Mill., 1. *O. Hedberg & al. 6673* (UPS), Rhodos, 1978, JF780242, N/A, JF780016, JF780089, JF779940, JF780169, N/A; 2. *S. Linder s.n.*, 4.11.1912 (UPS), Palestine, 1912, JF780240, JF780471–JF780474[6,7], JF780017, JF780090, JF779941, JF780170, JF779873; 3. *Strid & Mikkelsen 34608* (C), Greece, 1993, JF780241, JF780479–JF780481, JF780018, JF780091, JF779942, JF780171, N/A; ***L. multifidum*** L., 1. *HDP s.n.*, May 1853 (O), Armenia, 1853, JF780243, N/A, JF780019, JF780092, JF779943, JF780172, N/A; 2. *J. & F. Bornmüller 14536* (S), Turkey, 1929, N/A, JF780373–JF780378, FJ854335/FJ854241, FJ854128, JF779944, JF780173, HQ911457; ***L. orientale*** (Fisch. & C.A. Mey.) E.H.L. Krause, 1. *O. Schwarz 1264* (S), Turkey, 1933, JF780244, N/A, JF780020, JF780093, JF779945, JF780174, JF779874; 2. *T.Å. Tengwall 374* (S), Turkey, 1936, JF780245, JF780482–JF780486, JF780021, JF780094, JF779946, JF780175, N/A; ***L. orvala*** L., 1. *A. Tribsch 111165* (O), Slowenia, ***2006*****,** JF780246, N/A, N/A, N/A, N/A, N/A, N/A; 2. *E. Folkeson s.n.*, 15.5.1972 (S), Italy, 1972, JF780247, N/A, JF780022, JF780095, JF779947, JF780176, N/A; 3. *I. Segelberg s.n.*, 17.7.1965 (S), Italy, 1965, N/A, N/A, JF780023, JF780096, JF779948, JF780177, N/A; 4. *M. Thulin 1722* (UPS), Slovenia, 1972, N/A, JF780419–JF780422, JF780024, JF780097, JF779949, JF780178, N/A; 5. *N. Lundqvist 7702* (UPS), Croatia, 1972, N/A, N/A, JF780025, JF780098, JF779950, JF780179, JF779875; ***L. purpureum*** L., 1. *J.P. Bernard 80-035* (O), Canada, 1980, JF780248, N/A, JF780026, JF780099, JF779951, JF780180, JF779876; 2. *N. Orderud s.n.*, 18.7.1999 (O), Norway, 1999, JF780249, JF780430–JF780436, JF780027, JF780100, JF779952, JF780181, N/A; 3. *O. Pedersen s.n.*, 25.5.1998 (O), Norway, 1998, JF780250, N/A, JF780028, JF780101, JF779953, JF780182, N/A; 4. *P.W. Leenhouts 3358* (O), Netherlands, 1978, JF780251, N/A, JF780029, JF780102, JF779954, JF780183, JF779877; ***L. tomentosum*** Willd., 1. *A. Dogadova & T. Kolessnikova 7385* (S), Caucasus, 1961, JF780252, N/A, JF780030, JF780103, JF779955, JF780184, N/A; 2. *E. Hörandl & F. Hadacek s.n.*, 27.7.1988 (WU), Georgia, 1988, JF780253, N/A, JF780031, JF780104, JF779956, JF780185, N/A; 3. *J. & A. Bornmüller s.n.,* 17.7.1902 (S), Iran, 1902, JF780254, JF780379–JF780383, JF780032, JF780105, JF779957, JF780186, N/A; 4. *J. Klackenberg 820620-27* (S), Russia, 1982, JF780255, JF780399–JF780408, EF546933/EF546855, EU138293, JF779958, JF780187, HQ911459; *L.* × *holsaticum* E.H.L. Krause, 1. *Cufodontis s.n.*, 23.4.1953 (WU), Garden material, 1953, JF780256, JF780295–JF780300, JF780004, JF780078, JF779929, JF780157, N/A; 2. *Wettstein s.n.*, anno 1890 (WU), Hungary, 1890, JF780257, N/A, JF780005, JF780079, JF779930, JF780158, N/A. OUTGROUP: ***Eriophyton rhomboideum*** (Benth.) Ryding, *T. Thomson s.n.*, anno 1848–1849 (C), Tibet, 1848, N/A, JF780391–JF780398, HQ911684/HQ911754, HQ911615, JF779880, JF780108, HQ911461; ***Eriophyton wallichii*** Benth., *Stainton & al. 7748* (UPS), Nepal, 1954, N/A, JF780475–JF780478, FJ854277/FJ854164, FJ854034, JF779881, JF780109, HQ911462; ***Galeopsis ladanum*** L., *M. Bendiksby & A. Tribsch 06-083* (O), Italy, ***2006*****,** JF780188, N/A, N/A, N/A, N/A, N/A, N/A; ***Galeopsis pubescens*** Besser, *A. Tribsch & M. Bendiksby 06-043* (O), Italy/France, ***2006*****,** JF746449, N/A, N/A, N/A, N/A, N/A; ***Galeopsis reuteri*** Rchb. f., *M. Bendiksby & A. Tribsch 06-040* (O), Italy, ***2006*****,** JF780189, N/A, N/A, N/A, N/A, N/A; ***Galeopsis speciosa*** Mill., *T. Berg 04-001* (O), Norway, ***2004*****,** JF746479, N/A, N/A, N/A, N/A, N/A, N/A; ***Roylea cinerea*** (D. Don) Baill., *O. Polunin & al. 837* (UPS), Nepal, 1952, N/A, JF780437–JF780440, EU138450/EU138373, EU138290, JF779878, JF780106, HQ911454; ***Stachyopsis oblongata*** (Schrenk) Popov & Vved., I. *Roldugin & V. Fissjun 5394* (C), Kazachstan, 1964, N/A, JF780355–JF780359, HQ911686/HQ911757, HQ911616, JF779879, JF780107, HQ911463.

1 CCGGAAATTCCGTTCAACTATATAGTTGACCACATCGACGGGCCGGGAACGAGCTTCGTGTTGATATGTTGTGGCCCGCGTGACTCATTACG
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2 ACCCCTTTTTGCCCCAGTTTTCCTTTTCGGCCATTTTTTGTGCTTCTTCTTGAGTATATTTTTTTGATATGCTGTGGCCCGCGTAACTCATTAC
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3 ACCCCTTTTTGCCTCTTTTTTTCTACTCGTCTCTCCTCACCCGTCCATTTTTTTTTCTCTTTGCATTGAACACCTTCAGAATTCAAACCCAA
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4 ACGTCGTCGGGCCGGGAACGAGCTTCGTTTTGATATATTGTGGCCCGCGTGACTCATTACGAGTCGAAAGTTATGCCATTTTGAATTTTA
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5 ACCCCTTTTTGCCCCCATTTTCAACTCTCCTTCCTTTTCGATCTTTTTTTTTTGTTTCTTGAGTTCAAACACCGTAGGAATTTGTTCCT
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6 TTTTGCCCCCATTTTCTACTCGTCTTCCTTTCCGGTCACCATTTTTTTTTTCATAAGTTTAAACAACGTAAGAATTTGTTCCTCAAACCCAATAAT
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7 TCGAATTTGGTCGTTTTCGAGGCGAAACGGCCTTTTTTTGGCCCAAAAATTTCGTTCATTGGGCAAGCCAAATGACGATGTCGTCGGGCC
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GTGGGTTGGGCATAAATGC