



Molecular phylogeny of tribe Stachydeae (Lamiaceae subfamily Lamioideae)

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ABSTRACT

Although tribe Stachydeae (Lamiaceae) is considered monophyletic, relationships within the tribe are still poorly understood. The complexity of Stachydeae includes paraphyletic genera, considerable morphological plasticity, a range of ploidy levels, and presumably frequent natural hybridization. We performed parsimony and Bayesian phylogenetic analyses of nuclear (ribosomal ITS) and plastid (*trnL intron*, *trnL-trnF spacer*, *rps16* intron) DNA sequence data from a taxonomically and geographically broad sampling of the tribe to identify major evolutionary lineages and to test taxonomic hypotheses within this largest of all lamioid tribes. We included 143 accessions corresponding to 121 species, representing both Old and New World species, and all 12 recognized genera of tribe Stachydeae. Both nuclear and plastid data corroborate monophyly of the tribe, with *Melittis* as sister to all remaining Stachydeae. For the latter well-supported clade, we suggest the phylogenetic name Eurystachys. Within Eurystachys, although monophyly is supported by both nuclear and plastid data for several named and unnamed groups, the majority of recognized taxa appear to be para- or polyphyletic. The taxon compositions of most subclades are congruent between the plastid and nuclear tree topologies, whereas their relative phylogenetic placements are often not. This level of plastid–nuclear incongruence suggests considerable impact of hybridization in the evolution of Stachydeae.

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1. Introduction

The focal group of the present study, tribe Stachydeae Dumort., belongs to subfamily Lamioideae of the Lamiaceae. Scheen et al. (2010) recently established an updated tribal classification of the Lamioideae based on a comprehensive plastid phylogeny. Currently, ten lamioid tribes are recognized (Scheen et al., 2010; Bendiksby et al., 2011a), of which Stachydeae is the largest and probably the most taxonomically challenging. The complexity of Stachydeae includes: (1) paraphyletic genera, (2) considerable morphological plasticity, (3) a range of ploidy levels, and (4) frequent natural hybridization events (Feld and Koenen, 1913; Wilcock and Jones,

1974; Lindqvist and Albert, 2002; Scheen et al., 2010; Bendiksby et al., 2011a; Salmaki et al., 2012b; Roy et al., 2013).

Tribe Stachydeae, or some of its component genera, have previously been the subject of molecular phylogenetic investigations (e.g., Lindqvist and Albert, 2002; Lindqvist et al., 2003; Barber et al., 2002, 2007; Scheen et al., 2010; Bendiksby et al., 2011a; Roy et al., 2013). The study performed by Lindqvist and Albert (2002) revealed that the Hawaiian genera *Haplostachys* (A. Gray) Hillebr., *Phyllostegia* Benth., and *Stenogyne* Benth. as well as the genera *Prasium* L., *Phlomidoschema* (Benth.) Vved. and *Sideritis* L. are phylogenetically nested within the large genus *Stachys* L. This paraphyly of *Stachys* was corroborated by Scheen et al. (2010) who also demonstrated that the Asian genera *Chamaesphacos* Schrenk ex Fisch. & C.A. Mey., *Suzukia* Kudô and *Thuspeinanta* T. Durand are embedded within *Stachys*, and that the monotypic genus *Melittis* L. represents the phylogenetic sister to all other Stachydeae. Bendiksby et al. (2011a) added *Hypogomphia* Bunge to the list of taxa nested within *Stachys*. Thus, currently, tribe Stachydeae

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encompasses 12 genera and ca. 470 species (see Table 1). Most of the genera contain few species and four are monotypic (i.e., *Chamaespachos*, *Melittis*, *Phlomidoschema* and *Prasium*). In contrast, two genera are very species-rich: *Stachys*, with about 275 species, and *Sideritis*, with approximately 125 species (Harley et al., 2004). Scheen et al. (2010) found no non-molecular synapomorphies for this diverse tribe, but listed the following characteristics as common among its members: calyx campanulate or weakly 2-lipped, calyx lobes often spiny, throat often hairy and corolla strongly 2-lipped. However, these characteristics are also very common in the rest of the subfamily. The following differences are more informative but far from consistent: the nutlets in this tribe are usually apically rounded, whereas they are apically truncate in many other members of the subfamily; and most members of the genus *Stachys* differ from the rest of the subfamily in having the anterior pair of stamens bending outwards after pollination.

The published plastid phylogenies (Lindqvist and Albert, 2002; Scheen et al., 2010; Bendiksby et al., 2011a) depict a major subdivision of Stachydeae (excl. *Melittis*) into two strongly supported subclades, each including *Stachys* as well as non-*Stachys* species. One clade comprises the Hawaiian endemic labiates, *Suzukia*, all New World (NW) and a few Old World (OW) *Stachys* species. The second clade includes OW species of *Stachys* as well as the remaining genera. Only a few taxa of Stachydeae have been subjected to molecular phylogenetic investigations using nuclear DNA sequence data (i.e., Lindqvist and Albert, 2002; Lindqvist et al., 2003; Barber et al., 2002, 2007; Roy et al., 2013). The major dichotomy noted in plastid phylogenies was not evident in the nuclear tree of Lindqvist and Albert (2002) and Roy et al. (2013). Plastid versus nuclear incongruence has also been detected in *Sideritis* (Barber et al., 2002, 2007). Incongruence between tree topologies inferred from different genomes has been revealed in other molecular studies

within the Lamiaceae (e.g., Bräuchler et al., 2010; Menthinae; Bendiksby et al., 2011b; *Lamium*), and it is quite common in many groups of plants (see e.g., Albach and Chase, 2004; Kim and Donoghue, 2008; Tu et al., 2008; Pelser et al., 2010). Potential causes for such topological conflicts are extensively investigated, and four major causes for topological conflicts have been suggested (Zou and Ge, 2008): (1) stochastic errors (sampling errors), arising from low numbers of taxa or informative characters; (2) systematic errors, such as substitution rate variation or long-branch attraction; (3) biological factors, such as hybridization; and (4) incomplete lineage sorting. Together, these phenomena make it more difficult to reconstruct the evolutionary history of certain plant lineages.

Untangling the evolutionary history of large and taxonomically complex groups can be a challenging undertaking. The species-rich and nearly cosmopolitan genus *Stachys* is clearly in need of taxonomic revision. *Stachys* consists of annual and perennial herbs and subshrubs that exhibit extensive variation in morphological and cytological characters (Mulligan and Munro, 1989), but some species are also highly polymorphic and vaguely delimited. Consequently, the genus is regarded as notoriously difficult with respect to its taxonomy. The most comprehensive classification of *Stachys* was proposed by Bhattacharjee (1980) and is commonly used as a framework for systematic treatments in the genus (e.g., Rechinger, 1982; Harvey and Demissew, 1994; İlçim et al., 2008; Pool, 2007). Bhattacharjee (1980) recognized two major subgenera: *Betonica* L. and *Stachys*, the latter including 19 sections and 22 subsections (Table 2). However, plastid and nuclear ribosomal DNA data suggest that subgenus *Betonica* is isolated from *Stachys* and all other Stachydeae and thus should be recognized as a distinct genus (Lindqvist and Albert, 2002; Scheen et al. 2010).

So far Bhattacharjee's (1980) classification has not received much support by molecular data (Lindqvist and Albert, 2002; Scheen et al., 2010; Bendiksby et al., 2011a). However, these molecular investigations included relatively few species (up to ca. 15% of the tribe and 15% of *Stachys*) and were skewed with respect to taxonomic and geographic coverage, leaving many taxonomic and evolutionary questions unanswered. Because *Stachys*, as currently circumscribed, is paraphyletic with members scattered throughout tribe Stachydeae, it becomes necessary to include a taxonomically and geographically broad sampling of the entire tribe and appropriate lamiod outgroup taxa, to reveal the evolutionary history of *Stachys*.

In the present study, a comprehensive phylogeny of Stachydeae is presented using a broad and balanced taxon sampling and both nuclear and plastid DNA sequence data. Because taxonomic and evolutionary conclusions can be flawed if para- or polyphyly remains undetected, we considered it an important first step to establish a broad phylogeny of Stachydeae that can guide taxon selection for future, more in-depth studies of subgroups. More specifically, our goals are to use the molecular phylogeny to: (1) test taxonomic hypotheses (assess monophyly of taxa), (2) identify major evolutionary lineages within tribe Stachydeae, and (3) reveal nuclear-plastid incongruence that may have resulted from past hybridization events.

2. Materials and methods

2.1. Taxon sampling

Leaf material for DNA extraction was removed from herbarium specimens held at the following herbaria: A, B, BISH, BO, C, KUN, LPA, M, MSB, NY, O, ORT, S, TARI, TEX, TUH, UPS, UNA, US, and WU, or in several cases (especially species distributed in Iran) from silica dried leaves. All taxon names in the present study follow *World Checklist of Lamiaceae and Verbenaceae* (Govaerts et al.,

Table 1

List of genera of Stachydeae sensu Scheen et al. (2010) and Bendiksby et al. (2011a), their type species, total number of species per genus, proportion sampled for this study, and their geographic distributions.

Genus	Type species	# Of species	Proportion sampled	Distributional range
<i>Chamaespachos</i> Schrenk ex Fisch. and C.A. Mey.	<i>C. ilicifolius</i>	1	1/1	West and Central Asia
<i>Haplostachys</i> (Gray) Hillebr	<i>H. haplostachya</i>	5	1/5	Hawaiian islands
<i>Hypogomphia</i> Bunge	<i>Hy. turkestanica</i>	3	1/3	West and Central Asia
<i>Melittis</i> L.	<i>M. melissophyllum</i>	1	1/1	Europe and West Asia
<i>Phlomidoschema</i> (Benth.) Vved.	<i>P. parviflorum</i>	1	1/1	West and Central Asia to North West India
<i>Phyllostegia</i> Benth.	<i>Ph. mollis</i>	34	2/34	Pacific
<i>Prasium</i> L.	<i>Pr. majus</i>	1	1/1	Macaronesia and Mediterranean
<i>Sideritis</i> L.	<i>Si. hirsuta</i> ^a	ca. 125	25/125	Europe, Macaronesia to China
<i>Stachys</i> L.	<i>S. sylvatica</i>	ca. 275	83/275	Cosmopolitan
<i>Stenogyne</i> Benth.	<i>St. rugosa</i>	20	1/20	Hawaiian islands
<i>Suzukia</i> Kudô	<i>Su. shikikunensis</i>	2	2/2	Temperate East Asia
<i>Thuspeinanta</i> T.Durand	<i>T. persica</i>	2	2/2	West and Central Asia to Pakistan

^a The database "Index Nominum Genericorum [ING]" lists *Si. hyssopifolia* as the type of the genus incorrectly.

Table 2

Infrageneric classification of the two largest genera of tribe Stachydeae, *Stachys* [sensu Bhattacharjee (1980) and Krestovskaya (2003, 2006, 2007)] and *Sideritis* [sensu Castro and Nuñez (1994) and Pérez et al. (1992)], showing the proportion of species sampled from each section and subsection, and listing the species sampled per section. Abbreviations: B. = *Betonica*; NW = New World; OW = Old World; S. = *Stachys*; Si. = *Sideritis*.

Genera	Subgenera	Sections	Subsections	Proportion sampled	Species sampled
<i>Stachys</i>					
	OW <i>Stachys</i>	<i>Ambleia</i>			
			<i>Brevibracteolatae</i>	12/38 8/32	<i>S. yemenensis</i> (unplaced to subsection) <i>S. aegyptiaca</i> , <i>S. dregeana</i> , <i>S. hildebrandtii</i> , <i>S. hyssopoides</i> , <i>S. inflata</i> , <i>S. schetschgleevii</i> , <i>S. subaphylla</i> , <i>S. turcomanica</i>
			<i>Burchelliana</i>	1/1	<i>S. burchelliana</i>
			<i>Flavescentes</i>	2/3	<i>S. aurea</i> , <i>S. lamarckii</i>
			<i>Fruticosae</i>	-/1	-
		<i>Aucheriana</i>		2/7	<i>S. acerosa</i> , <i>S. glutinosa</i>
		<i>Campaniastrum</i>		3/7	
			<i>Arvenses</i>	2/4	<i>S. arabica</i> , <i>S. arvensis</i>
			<i>Ocymastrum</i>	1/3	<i>S. ocymastrum</i>
		<i>Candida</i>		2/6	<i>S. chrysanthia</i> , <i>S. saxicola</i>
		<i>Corsica</i>		1/1	<i>S. corsica</i>
		<i>Eriostomum</i>		10/33	<i>S. graeca</i> (unplaced to subsection) <i>S. alpina</i> , <i>S. balansae</i> , <i>S. byzantina</i> , <i>S. cretica</i> , <i>S. ehrenbergii</i> , <i>S. germanica</i> , <i>S. heraclea</i> , <i>S. persica</i>
			<i>Germanicae</i>	8/25	
			<i>Libanotiae</i>	-/2	-
			<i>Spectabiles</i>	1/5	<i>S. spectabilis</i>
		<i>Fragilicaulis</i>		5/21	
			<i>Fragiles</i>	3/10	<i>S. benthamiana</i> , <i>S. kurdica</i> , <i>S. megalodonta</i>
			<i>Multibracteolatae</i>	2/11	<i>S. kermanshahensis</i> , <i>S. nephrophylla</i>
		<i>Infrarosularis</i>		1/6	<i>S. rupestris</i>
		<i>Mucronata</i>		1/1	<i>S. mucronata</i>
		<i>Neurocalyx</i>		1/1	<i>S. neurocalycina</i>
		<i>Olisia</i>		10/27	
			<i>Annuae</i>	3/7	<i>S. annua</i> , <i>S. maritima</i> , <i>S. pubescens</i>
			<i>Distantes</i>	2/3	<i>S. bombycina</i> , <i>S. distans</i>
			<i>Rectae</i>	4/12	<i>S. arenaria</i> , <i>S. atherocalyx</i> , <i>S. recta</i> , <i>S. tetragona</i>
			<i>Rosulatae</i>	-/4	-
			<i>Spinosa</i>	1/1	<i>S. spinosa</i>
		<i>Pseudosideritopsis</i>		-/1	-
		<i>Roseostachys</i>		-/1	-
		<i>Satureoides</i>		-/4	-
		<i>Setifolia</i>		2/3	<i>S. menthoides</i> , <i>S. setifera</i>
		<i>Stachys</i>		4/7	
			<i>Circinatae</i>	2/4	<i>S. circinata</i> , <i>S. durandiana</i>
			<i>Sylvaticae</i>	2/3	<i>S. palustris</i> , <i>S. sylvatica</i>
		<i>Swainsoniana</i>		6/11	<i>S. mollissima</i> (unplaced to subsection) <i>S. canescens</i> , <i>S. menthifolia</i>
			<i>Decumbentes</i>	2/6	<i>S. ionica</i> , <i>S. spruneri</i> , <i>S. swainsonii</i>
		<i>Thamnostachys</i>		3/4	
			<i>Fruticulosae</i>	2/5	<i>S. araxiana</i> , <i>S. fruticulosa</i>
			<i>Paulinae</i>	-/1	-
		<i>Trinerves</i>		1/1	<i>S. trinervis</i>
		<i>Zietenia</i>		1/1	<i>S. lavandulifolia</i>
		Unplaced to section		7	<i>S. aculeolata</i> , <i>S. aethiopica</i> , <i>S. alpigena</i> , <i>S. grandifolia</i> , <i>S. natalensis</i> , <i>S. nigricans</i> , <i>S. reptans</i>
	NW <i>Stachys</i>	Unplaced to section		11/60	<i>S. albens</i> , <i>S. bullata</i> , <i>S. debilis</i> , <i>S. eriantha</i> , <i>S. floridana</i> , <i>S. grandidentata</i> , <i>S. lamiooides</i> , <i>S. latidens</i> , <i>S. macraei</i> , <i>S. pilosa</i> , <i>S. tenuifolia</i>
	<i>Betonica</i>				
		<i>Betonica</i>		2/10	<i>B. officinalis</i>
		<i>Macrostachys</i>		1/5	<i>B. scardica</i>
<i>Sideritis</i>					
	<i>Marrubiastrum</i>			13/24	
		<i>Creticae</i>		2/3	<i>Si. cretica</i> , <i>Si. macrostachys</i>
		<i>Empedocleopsis</i>		2/3	<i>Si. gomerae</i> , <i>Si. nutans</i>
		<i>Marrubiastrum</i>		9/18	<i>Si. barbellata</i> , <i>Si. canariensis</i> , <i>Si. candicans</i> , <i>Si. cytosiphon</i> , <i>Si. dasygynaphala</i> , <i>Si. infernalis</i> , <i>Si. kuegleriana</i> , <i>Si. ortoteneriffae</i> , <i>Si. soluta</i>
	<i>Sideritis</i>			12/91	
		<i>Burgsdorffia</i>		1/2	<i>Si. romana</i>
		<i>Empedoclea</i>		4/38	<i>Si. syriaca</i> , <i>Si. scardica</i> , <i>Si. clandestina</i> , <i>Si. perfoliata</i>
		<i>Hesiodia</i>		1/1	<i>Si. montana</i>
		<i>Sideritis</i>		6/50	<i>Si. antiatlantica</i> , <i>Si. endresii</i> , <i>Si. glauca</i> , <i>Si. incana</i> , <i>Si. leucantha</i> , <i>Si. tragoriganum</i>

2012) except for species of *Betonica*, which are still placed in synonymy with *Stachys* therein (but see Scheen et al., 2010).

Our sampling of tribe Stachydeae (the ingroup) covers both its morphological and geographical range. Stachydeae is represented by 143 accessions of 121 species (ca. 26% of all species) and all 12 genera (Table 1). Specimens of *Stachys* and *Sideritis*, which are

by far the largest genera of the tribe, were selected in order to include as many as possible of the infrageneric groups (Table 2). Representative species of three sections and four subsections of subgenus *Stachys* (sensu Bhattacharjee (1980) and Krestovskaya (2003, 2006, 2007)) were omitted because no appropriate material was available or attempts to amplify DNA failed. The outgroups

were selected based on results from previous phylogenetic studies of subfamily Lamioideae (Scheen et al., 2010; Bendiksby et al., 2011a) and included 11 accessions representing the lamioid genera *Ballota* L., *Betonica*, *Galeopsis* L., *Lamium* L., *Phlomis* L., and *Phlomoides* Moench. DNA was extracted anew from 82 specimens, of which 57 represent new species in GenBank, and 419 DNA sequences were produced for the present study. A list of all accessions analyzed, including voucher information and GenBank accession numbers, is given in Table 3 (data generated specifically for the present study are indicated with an asterisk).

2.2. DNA extraction, amplification, and sequencing

DNA was extracted following protocols presented in previous studies on Lamioideae (Barber et al., 2007; Scheen et al., 2010; Bendiksby et al., 2011a; Salmaki et al., 2012a). We PCR-amplified and sequenced the nuclear ribosomal ITS (nrITS) and the plastid *trnL* intron, *trnL*-F intergenic spacer (collectively referred to as the *trnL*-F region hereafter) and *rps16* intron. The molecular work was performed in several different laboratories, and only procedures not described in the earlier studies will be described here. We amplified the nrITS region as described by Salmaki et al. (2012a). The plastid DNA regions were amplified as described by Scheen et al. (2010) and Bendiksby et al. (2011a). For PCR amplification of the nrITS and the *rps16* intron from some old herbarium specimens, we used Phusion® polymerase (New England Biolabs, Ipswich, Massachusetts, USA) and/or followed the modified procedure described by Bendiksby et al. (2011c). The genetic regions were sequenced using the same primers as in the PCR reactions.

2.3. Data matrix composition

All sequences were aligned using ClustalW 1.8 (BCM Search Launcher, USA) with manual adjustments in BioEdit v. 5.0.6 (Hall, 1999). Ambiguously aligned characters and mononucleotide repeat units were excluded prior to analyses (see Table 4). The excluded regions are indicated on the matrices, which are available from the first author upon request. Unambiguously aligned gaps were coded using the “simple indel coding” of Simmons and Ochoterena (2000) as implemented in SeqState (Müller, 2005). We constructed and analyzed four datasets (Table 4): (1) the nrITS, (2) the *trnL*-F region, (3) the *rps16* intron, and (4) the concatenated plastid markers (ptDNA: *trnL*-F region and *rps16* intron).

2.4. Phylogenetic analyses

We analyzed the data using both Maximum Parsimony and Bayesian phylogenetic analyses. The genetic markers were first analyzed separately in order to test the existence of conflicting signals. Congruent datasets were concatenated prior to final analyses. The final datasets were analyzed twice, with and without indels coded.

The parsimony analyses were carried out using PAUP* v. 4.0b10 (Swofford, 2003) with all characters unordered and equally weighted, heuristic searches with random sequence addition, TBR branch swapping, 50 random-addition-sequence replicates, and MAXTREES set to 20,000. Parsimony bootstrapping (Felsenstein, 1985) was performed using the following settings: hsearch addseq = random, nchuck = 10, chuckscore = 1, nreps = 50, bootstrap nreps = 5000. In addition, the parsimony analyses of the same datasets were carried out using NONA (Goloboff, 1999) in combination with WinClada v. 1.0 (Nixon, 1999) applying the heuristic search option with 2000 replications and MAXTREES set to 5000, and otherwise default settings. Parsimony jackknife analysis (Farris et al., 1996) was performed with 1000 replicates and otherwise default settings.

The Bayesian analyses were conducted using the program MrBayes version 3.1 (Ronquist and Huelsenbeck, 2003; Huelsenbeck et al., 2002). Prior to analysis, the model of nucleotide substitution for each dataset was selected using the Akaike information criterion (AIC) in jModelTest (Posada, 2008). We used the F81 (restriction site) model for the indel matrix. The priors on state frequencies, rates and variation across sites were estimated automatically from the data assuming no prior knowledge about their values. From different random starting trees, two simultaneous searches, each comprising four Markov chains (one cold and three heated), were conducted for 20 (nrITS) and 10 (ptDNA) million generations, with sampling every 1000th generation. Convergence on a common phylogenetic topology by two separate Bayesian searches was checked using the methods described by Torke and Schaal (2008): similarity in log likelihood scores at stationarity, similarity in consensus tree topologies, and a final average standard deviation of split frequencies (ASDSF) for simultaneous searches below 0.01. We discarded as burnin the number of trees of each run prior to the level where stationarity had been reached. The remaining trees were summarized into a 50% majority-rule consensus tree with the posterior probabilities (PP).

2.5. Testing significance of incongruence

The congruence across the three plastid markers, and between nrITS data and the combined ptDNA dataset, was tested using the incongruence length difference (ILD) test (Farris et al., 1995), as implemented in PAUP*. The ILD test was conducted with 1000 replicates, each with 10 random addition sequence replicates, TBR branch swapping, and keeping no more than 100 trees per random addition replicate. Following Cunningham (1997), a significance level of $P = 0.01$ was adopted for this test. Congruence between datasets was also assessed by visual comparison of tree topologies and clade support values.

3. Results

We found no significant incongruence among the separate plastid phylogenies ($P = 0.412$), whereas the level of incongruence between plastid and nuclear data was highly significant ($P = 0.00173$). We therefore concatenated the plastid regions prior to final analyses and analyzed the nuclear data separately. Information about the sequence alignments, selected models of nucleotide substitution, the ASDSF at termination of the Bayesian analyses, and parsimony tree statistics are summarized in Table 4. The four independent Bayesian analyses of each dataset showed MCMC convergence for all parameters. Pairwise comparisons between tree files of each run showed no significant difference in the PP of all splits for paired MCMC analyses.

Results from the Bayesian analyses were largely congruent with results from the parsimony analyses, both with and without indels coded. Since the results from the Bayesian analyses and all analyses with indels coded had the best topological resolution and overall highest branch support, only results from the indel coded Bayesian analyses are presented (Figs. 1 and 2).

The backbone resolution of the ingroup (i.e., Stachydeae; clade A) in the nrITS tree (Fig. 1) is poorly supported and consists of groups with relatively short branches, whereas in the ptDNA tree (Fig. 2), the backbone resolution receives support from both Bayesian and parsimony analyses. In all, 21 groups of at least two species of Stachydeae are consistently supported by both nuclear and plastid data (Fig. 3a: clades A–U; Section 3.1 below). Their interrelationships, however, are often not congruent between the nuclear and plastid topologies (Figs. 1–3a; Section 3.2 below). Three supported clades in the nuclear tree (Fig. 1: clades V–X) are not

Table 3

Alphabetical list of Stachydeae accessions included, voucher information and GenBank accession numbers. The specimens from which DNA was extracted for the present study and newly generated sequences are indicated with an asterisk (after taxon name and GenBank accession number, respectively). n.a. = sequence not available.

Species	Geographic source	Collector, number, herbarium	GenBank accession number			
			trnL intron	trnL-trnF spacer	rps16 intron	ITS
<i>Outgroup</i>						
<i>Ballota</i> L.						
<i>Ballota acetabulosa</i> (L.) Benth.	Greece	M. Bendiksby and A.-C. Scheen, 04-012, O	EU138442	EU138365	EU138296	–
<i>Ballota hirsuta</i> Benth.	Morocco	D. Podlech, 53328, M	–	–	–	JN680359
<i>Ballota nigra</i> L.	Iran	Y. Salmaki et al., 39813, TUH	–	–	–	JN680358
<i>Ballota pseudodictamnus</i> (L.) Benth.	Greece	M. Bendiksby and A.-C. Scheen, 04-020, O	EF546935	EF546857	EU138295	–
<i>Ingroup</i>						
<i>Betonica</i> L.						
<i>Betonica officinalis</i> L.	Cult.	C. Lindqvist and V.A. Albert, 357, UNA	AF502056	FJ854224	FJ854109	KF529533
<i>Betonica scardica</i> Griseb.	Greece	J. Ugelvig and L.S. Christiansen, 1048, C	FJ854326	FJ854229	FJ854114	KF529534
<i>Galeopsis</i> L.						
<i>Galeopsis ladanum</i> subsp. <i>angustifolia</i> (Ehrh. ex Hoffm.) Gaudin	Norway	E. Dahl s.n. 26.08.1979, O	EF546939	EF546862	FJ854035	KF529535
<i>Galeopsis pubescens</i> Besser	Poland	T. Tacik and M. Sychowa, 366, O	EF546940	EF546863	FJ854036	KF529536
<i>Lamium</i> L.						
<i>Lamium album</i> L.	Norway	M. Bendiksby, 05-014, O	JF779961	JF779961	JF780035	KF529537
<i>Lamium galeobdolon</i> (L.) L.	Norway	M. Bendiksby, 05-016, O	JF779994	JF779994	JF780068	KF529538
<i>Phlomis</i> L.						
<i>Phlomis fruticosa</i> L.	Greece	E. Julin s.n., 1985, UPS	FJ854294	FJ854180	FJ854063	KF529539
<i>Phlomis bruguieri</i> Desf.	Iran	Y. Salmaki et al., 39423, TUH	KF529766	KF529856	KF529658	JN680366
<i>Phlomoides</i> Moench						
<i>Phlomoides tuberosa</i> (L.) Moench (1)	Kyrgyzstan	Fyatov and Kuzgechov, 259, LE	–	–	–	JN680387
<i>Phlomoides tuberosa</i> (2)	Cult.	C. Mathiesen and J.M. Taylor, 88, O	FJ854295	FJ854181	FJ854064	–
<i>Chamaespachos</i> Schrenk ex Fisch. & C.A.Mey.						
<i>Chamaespachos ilicifolius</i> Schrenk	Iran	K.H. Rechinger, 50961, C	FJ854269	FJ854156	FJ854023	KF529540
<i>Haplostachys</i> (A.Gray) Hillebr.						
<i>Haplostachys haplostachya</i> (A.Gray) H.St.John	Hawaii	S. Perlman, 14328, NY	AF502029	FJ854166	FJ854039	KF529541
<i>Hypogomphia</i> Bunge						
<i>Hypogomphia bucharica</i> Vved. *	Uzbekistan	A. Vvedensky, AV6427, C	KF529767	KF529857	KF529660	KF529542
<i>Hypogomphia turkestanica</i> Bunge	C Asia	O. Paulsen, 275, C	HQ911703	HQ911774	HQ911634	KF529543
<i>Melittis</i> L.						
<i>Melittis melissophyllum</i> L. (1)	Cult.	M. Bendiksby, 09-010, O	HQ911702	HQ911773	HQ911633	KF529544
<i>Melittis melissophyllum</i> (2)	Hungary	M.E. Steiner et al., 1127, UPS	EF546929	EF54849	FJ854051	KF529545
<i>Phlomidoschema</i> (Benth.) Vved.						
<i>Phlomidoschema parviflorum</i> (Benth.) Vved.	Afghanistan	J.S. Andersen and I.C. Petersen, 394, C	FJ854293	FJ854179	FJ854062	KF529546
<i>Phyllostegia</i> Benth.						
<i>Phyllostegia velutina</i> (Sherff) H.St.John	Hawaii	V.A. Albert et al., HI03-061, O	HQ911704	HQ911775	HQ911635	KF529547
<i>Phyllostegia waimeae</i> Wawra*	Hawaii	V.A. Albert et al., HI03-027, O	KF529768	KF529858	KF529661	KF529548
<i>Prasium</i> L.						
<i>Prasium majus</i> L. (1)	Greece	A. Strid et al., 39091, C	AF502035	KF529859	KF529662	KF529549
<i>Prasium majus</i> (2)	Spain	M. Thulin, 5752, UPS	FJ854300	FJ854187	FJ854072	KF529550
<i>Sideritis</i> L.						
<i>Sideritis antiatlantica</i> (Maire) Rejdali	Morocco	ASG s.n., ORT	AF335663	KF529860	KF529663	AF335625
<i>Sideritis barbellata</i> Mend.–Heuer	Spain (La Palma Cl)	J. Barber, 262, TEX	DQ900796	KF529861	KF529664	DQ900750
<i>Sideritis canariensis</i> L.	CI-E1 Hierro	J. Barber, 257, TEX	AF335644*	KF529862	KF529665	F335605
<i>Sideritis candicans</i> Aiton	Portugal (Madeira)	J. Barber, 230, TEX	AF335645	KF529863	KF529666	AF335606
<i>Sideritis clandestina</i> (Bory & Chaub.) Hayek	Cult.	J. Barber, 286, TEX	AF335655	KF529864	KF529667	AF335616
<i>Sideritis cretica</i> L.	Tenerife	J. Barber, 213, TEX	DQ900801	KF529865	KF529668	DQ900755
<i>Sideritis cystosiphon</i> Svent.	Tenerife	J. Barber, 222, TEX	DQ900802	KF529866	KF529669	DQ900756
<i>Sideritis dasynaphala</i> (Webb & Berthel.) Clos	I-Gran Canaria	ASG, 18691, LPA	DQ900803	KF529867	KF529670	DQ900757
<i>Sideritis endressii</i> Willk. ssp. <i>emporitana</i>	Portugal (Cabo Espichel)	J. Barber, 282, TEX	KF529769	KF529868	KF529671	AF335627
<i>Sideritis glauca</i> Cav.	Spain (Alicante)	J. Barber, 248, TEX	KF529770	KF529869	KF529672	AF335630
<i>Sideritis gomeriae</i> Bolle	Spain (Cl-La Gomera)	J. Barber, 256, TEX	AF335647	FJ854192	FJ854077	AF335608
<i>Sideritis incana</i> L.	Spain (Valencia)	J. Barber, 246, TEX	AF335673	KF529962	KF529674	AF335634
<i>Sideritis infernalis</i> Bolle	C I-Tenerife	J. Barber, 225, TEX	DQ900808,	KF529870	KF529675	DQ900762
<i>Sideritis kuegleriana</i> Bornm.	C I-Tenerife	J. Barber, 16767, ORT	DQ900809	KF529871	KF529676	DQ900763
<i>Sideritis leucantha</i> Cav.	Spain (Murcia)	J. Barber, 249, TEX	AF335675	KF529961	KF529677	AF335636
<i>Sideritis macrostachys</i> Poir.	C I-Tenerife	J. Barber, 254, TEX	AF335648	FJ854194	FJ854079	AF335609
<i>Sideritis montana</i> L. (1)	Balkan Peninsula	J. Barber, 212, TEX	AF335651	KF529872	KF529679	AF335612
<i>Sideritis montana</i> (2)*	Cult.	M. Bendiksby, MB09-002, O	KF529771	KF529873	KF529680	KF529551
<i>Sideritis nutans</i> Svent.	C I-La Gomera	J. Barber, 201, TEX	DQ900813	KF529874	KF529681	DQ900767
<i>Sideritis oroteneriffae</i> Negrin & P.Pérez var. <i>oroteneriffae</i>	C I-Tenerife	J. Barber, 207, TEX	DQ900815	KF529875	KF529682	DQ900769

(continued on next page)

Table 3 (continued)

Species	Geographic source	Collector, number, herbarium	GenBank accession number			
			<i>trnL</i> intron	<i>trnL-trnF</i> spacer	<i>rps16</i> intron	ITS
<i>Sideritis perfoliata</i> L.	Cult.	J. Barber, 294, TEX	AF335657	KF529876	KF529683	AF335618
<i>Sideritis romana</i> L. (1)	Italy	J. Barber, 209, TEX	AF335653	FJ854196	FJ854081	AF335614
<i>Sideritis romana</i> (2)*	Greece (Skiros)	S. Snogerup and B. Snogerup, 16918, UPS	KF529772	KF529877	KF529684	KF529552
<i>Sideritis scardica</i> Griseb.	Cult.	J. Barber, 211, TEX	AF335658	KF529878	KF529686	AF335619
<i>Sideritis soluta</i> Clos	C I-Tenerife	J. Barber, 217, TEX	AF335650	KF529879	KF529687	AF335611
<i>Sideritis syriaca</i> L. (1)	Greece	J. Barber, 210, TEX	AF335659	FJ854197	FJ854082	AF335620
<i>Sideritis syriaca</i> (2)	Greece	M. Bendiksby and A.-C. Scheen, 04-008, O	FJ854304	FJ854198	FJ854083	KF529553
<i>Sideritis tragopogonum</i> Lag.	Spain (Lagasca)	J. Barber, 241, TEX	AF335678	KF529880	KF529688	AF335639
<i>Stachys</i> L.						
<i>Stachys acerosa</i> Boiss. (1)*	Persia	K. H. Rechinger, 47425, C	KF529773	KF529881	KF529689	KF529554
<i>Stachys acerosa</i> (2)*	Iran	Y. Salmaki and S. Zarre, 38881, TUH	KF529774	KF529882	KF529690	KF529555
<i>Stachys aculeolata</i> Hook.f.	Kenya	Y.B. Harvey et al., 7, C	FJ854305	FJ854199	FJ854084	KF529556
<i>Stachys aegyptiaca</i> Pers. (1)*	Egypt	–, 50085, MSB	KF529775	KF529883	KF529691	KF529557
<i>Stachys aegyptiaca</i> (2)*	Palestine	M. Zohary & J. D'Angelis, 570, UPS	KF529776	KF529884	KF529692	KF529558
<i>Stachys aethiopica</i> L.	Mozambique	B. Pettersson, 2146, UPS	FJ854307	FJ854201	FJ854086	KF529559
<i>Stachys albens</i> A.Gray*	California	M.B. Dunkle, 4088, UPS	KF529777	KF529885	KF529693	KF529560
<i>Stachys alpigena</i> T.C.E.Fr.	Ethiopia	O. Ryding, 2133, UPS	FJ854309	FJ854204	FJ854089	KF529561
<i>Stachys alpina</i> L. subsp. <i>alpina</i> *	Germany	M. Sichler, 82394, M	KF529778	KF529886	KF529694	KF529562
<i>Stachys annua</i> (L.) L.*	Turkey	M. Nydegger, 42679, MSB	KF529779	KF529887	KF529695	KF529563
<i>Stachys arabica</i> Hornem.	Palestine	I. Gruenberg, 685, UPS	FJ854312	FJ854207	FJ854092	KF529564
<i>Stachys araxiana</i> Kopell.*	Turkey	M. Nydegger, 46587, MSB	KF529780	KF529888	KF529696	KF529565
<i>Stachys arenaria</i> Vahl*	Morocco	D. Podlech, 48272, MSB	KF529781	KF529889	KF529697	KF529566
<i>Stachys arvensis</i> (L.) L. (1)*	Germany	W.L. Dietrich, 6143, M	KF529782	KF529890	KF529698	KF529567
<i>Stachys arvensis</i> (2)	Canary Islands	N. Lundqvist, 8157, UPS	FJ854313	FJ854209	FJ854094	KF529568
<i>Stachys atherocalyx</i> K.Koch*	Iran	Y. Salmaki et al., 39801, TUH	KF529783	–	KF529699	KF529569
<i>Stachys aurea</i> Benth.*	South Africa	–, 9826, M	KF529784	KF529891	KF529700	KF529570
<i>Stachys balansae</i> Boiss. & Kotschy*	Iran	–, 36532, TUH	KF529785	KF529892	KF529701	KF529571
<i>Stachys benthamiana</i> Boiss.*	Iran	Y. Salmaki and S. Zarre, 35897, TUH	KF529786	KF529893	KF529702	KF529572
<i>Stachys bombycinia</i> Boiss.*	Turkey	R. Ulrich, 1997, M	KF529787	KF529894	KF529703	KF529573
<i>Stachys burchelliana</i> Launert (1)*	Namibia	I. Örtendahl, 51a, UPS	KF529788	KF529895	KF529704	KF529574
<i>Stachys burchelliana</i> (2)*	South Africa	B. De Winter, 1955, M	KF529789	KF529896	KF529705	KF529575
<i>Stachys bullata</i> Benth.*	California	H. K. Sharpsmith, 4271, UPS	KF529790	KF529897	KF529706	KF529576
<i>Stachys byzantina</i> K.Koch (1)	Cult.	C. Lindqvist and V.A. Albert, 356, UNA	AF502046	FJ854211	FJ854096	KF529577
<i>Stachys byzantina</i> (2)*	Iran	Y. Salmaki et al., 36528, TUH	KF529791	KF529898	KF529707	KF529578
<i>Stachys canescens</i> Bory & Chaub.*	Greece	M. Nydegger, 32600, MSB	KF529792	KF529899	KF529708	KF529579
<i>Stachys chrysanthia</i> Boiss. & Heldr.*	Greece	W. Lippert, 21312, M	KF529793	KF529900	KF529709	KF529580
<i>Stachys circinata</i> L'Hér *	Spain	M. Thulin, 5733, UPS	KF529794	KF529901	KF529710	KF529581
<i>Stachys corsica</i> Pers.*	Corsica	B. Jonsell, 1428, UPS	KF529795	KF529902	KF529711	KF529582
<i>Stachys cretica</i> L.	Greece	A. Strid et al., 42603, C	FJ854316	FJ854215	FJ854100	KF529583
<i>Stachys debilis</i> Kunth	Ecuador	C. Jativa and C. Epling, 242, US	FJ854317	FJ854216	FJ854101	KF529584
<i>Stachys distans</i> Benth.*	Lebanon	H. Roessler, 5211, M	KF529796	KF529903	KF529712	KF529585
<i>Stachys dregeana</i> Benth.*	Southern Africa, E.	P. B. Phillipson, 682, UPS	KF529797	KF529904	KF529713	KF529586
<i>Stachys durandiana</i> Coss.*	Cape	D. Podlech, 43312, MSB	KF529798	KF529905	KF529714	KF529587
<i>Stachys ehrenbergii</i> Boiss.*	Morocco	C. Bräuchler, s.n., M	KF529799	KF529906	KF529715	KF529588
<i>Stachys eriantha</i> Benth.*	Lebanon	J. H. Beaman, 1970, UPS	KF529800	KF529907	KF529716	KF529589
<i>Stachys floridana</i> Shuttlew. ex Benth.*	Mexico	W.T. Gillis, 6444, UPS	KF529801	KF529908	KF529717	KF529590
<i>Stachys fruticulosa</i> M.Bieb. (1)*	USA, Florida	Y. Salmaki et al., 36506, TUH	KF529802	KF529909	KF529718	KF529591
<i>Stachys fruticulosa</i> (2)*	Iran	T. Heideeman, s.n., 22.04.1936, O	KF529803	KF529910	KF529719	KF529592
<i>Stachys germanica</i> subsp. <i>heldreichii</i> (Boiss.) Hayek*	Greece	A. Strid, 40194, C	KF529804	KF529911	KF529720	KF529593
<i>Stachys glutinosa</i> L.*	Corsica	H. Merxmüller and W. Lippert, 31239, M	KF529805	KF529912	KF529721	KF529594
<i>Stachys graeca</i> Boiss. & Heldr. (<i>acutifolia</i>)	Greece	K.H. Rechinger, 16887, US	FJ854306	FJ854200	FJ854085	KF529595
<i>Stachys grandidentata</i> Lindl.	Chile	W.J. Eyerdam, 10081, US	FJ854318	FJ854217	FJ854102	KF529596
<i>Stachys grandifolia</i> E.Mey.*	Southern Africa, E.	P.B. Phillipson, 1546, UPS	KF529806	KF529913	KF529722	KF529597
<i>Stachys heraclea</i> All.*	Cape	Elias, s.n., 01.07.1910, C	KF529807	KF529914	KF529723	KF529598
<i>Stachys hildebrandtii</i> Vatke*	Spain	O. Ryding et al., 2215, UPS	KF529808	KF529915	KF529724	KF529599
<i>Stachys hyssopoides</i> Burch. ex Benth.	Ethiopia	E. Retief, 1080, US	FJ854319	FJ854218	FJ854103	KF529600
<i>Stachys inflata</i> Benth.*	South Africa	Y. Salmaki, 35910, TUH	KF529809	KF529916	–	KF529601
<i>Stachys ionica</i> Halász*	Iran	O. Ryding, 2294, UPS	KF529810	KF529917	KF529725	KF529602
<i>Stachys kermanshahensis</i> Rech.f.*	Greece, Corfu	Y. Salmaki and S. Zarre, 36522, TUH	KF529811	KF529918	KF529726	KF529603
<i>Stachys kurdica</i> Boiss. & Hohen. (1)*	Iran	Y. Salmaki and S. Zarre, 35877, TUH	KF529812	KF529919	KF529727	KF529604
<i>Stachys kurdica</i> (2)*	Iran	Y. Salmaki and S. Zarre, 36513, TUH	KF529813	KF529920	KF529728	KF529605
<i>Stachys lamarckii</i> Benth.*	South Africa	P.H. Cloks, 18973, M	KF529814	KF529921	KF529729	KF529606

Table 3 (continued)

Species	Geographic source	Collector, number, herbarium	GenBank accession number			
			<i>trnL</i> intron	<i>trnL-trnF</i> spacer	<i>rps16</i> intron	ITS
<i>Stachys lamiooides</i> Benth.*	Ecuador	E. Asplund, 17092, US	KF529815	KF529922	KF529730	KF529607
<i>Stachys latidens</i> Small*	USA, N. Carolina	O. Ryding, 2012, UPS	KF529816	KF529923	KF529731	KF529608
<i>Stachys lavandulifolia</i> Vahl (1)*	Iran	Y. Salmaki, 36521, TUH	KF529817	KF529924	KF529732	KF529609
<i>Stachys lavandulifolia</i> (2)	Iran	J.S. Andersen, and A.G. Jensen, 7032, C	AF502053	FJ854219	FJ854104	KF529610
<i>Stachys macraei</i> Benth.*	Chile	Kunkel, 152, C	KF529818	KF529925	KF529733	KF529611
<i>Stachys maritima</i> Gouan	Romania	I. Gergely 3362, US	FJ854321	FJ854222	FJ854107	KF529612
<i>Stachys megalodonta</i> Hausskn. & Bornm.*	Iran	Y. Salmaki and S. Zarre, 25190, TUH	KF529819	KF529925	KF529734	KF529613
<i>Stachys menthifolia</i> Vis.*	Peninsula (Balkan Pen.)	Collector, 11156, M	KF529820	KF529927	KF529735	KF529614
<i>Stachys menthoidea</i> Kotschy & Boiss.*	Turkey	Duncan and Tait, 191, M	KF529821	KF529928	KF529736	KF529615
<i>Stachys mollissima</i> Willd.*	Greece	J. Damboldt, 1974, M	KF529822	KF529929	KF529737	KF529616
<i>Stachys mucronata</i> Sieber ex Spreng. (1)*	Greece	T. Raus, 8347, C	KF529823	KF529930	KF529738	KF529617
<i>Stachys mucronata</i> (2)*	Greece	R. Ulrich, 1990, M	KF529824	KF529931	KF529739	KF529618
<i>Stachys natalensis</i> Hochst.*	Swaziland	O. Ryding, 2332, UPS	KF529825	KF529932	KF529740	KF529619
<i>Stachys nephrophylla</i> Rech.f.	Iraq	K.H. Rechinger, 11250, WU (Typus)	FJ854322	FJ854223	FJ854108	KF529620
<i>Stachys neurocalycina</i> Boiss.*	Palestine	–, 13511, MSB	KF529826	KF529933	KF529741	KF529621
<i>Stachys nigricans</i> Benth.*	Swaziland	O. Ryding, 2347, UPS	KF529827	KF529934	KF529742	KF529622
<i>Stachys ocytomastrum</i> (L.) Briq.*	Spain	P. Garin, 17388, MSB	KF529828	KF529935	KF529743	KF529623
<i>Stachys palustris</i> L. (1)*	Germany	–, 80–2081, UPS	KF529829	KF529936	KF529744	KF529624
<i>Stachys palustris</i> (2)*	Norway	M. Bendiksby, 04–37, O	KF529830	KF529937	KF529745	KF529625
<i>Stachys persica</i> S.G.Gmel. ex C.A.Mey. (1)*	Iran	–, 71718, TARJ	KF529831	KF529938	KF529746	KF529626
<i>Stachys persica</i> (2)	Persia	K.H. Rechinger, 43403, C	KF529832	KF529939	KF529747	KF529627
<i>Stachys pilosa</i> Nutt.	USA	H. Hapeman, s.n., 1938, UPS	FJ854311	FJ854206	FJ854091	KF529628
<i>Stachys pubescens</i> Ten.*	Azerbaijan	K.H. Rechinger, 41292, C	KF529833	KF529940	KF529748	KF529629
<i>Stachys recta</i> L. subsp. <i>recta</i> (1)	Austria	P. Schönswetter, 2517, WU	FJ854323	FJ854226	FJ854111	KF529630
<i>Stachys recta</i> subsp. <i>subcrenata</i> (Vis.) Briq. (2)	Croatia	G. Schneeweiss et al., 6268, WU	FJ854330	FJ854233	FJ854118	KF529631
<i>Stachys reptans</i> Hedge*	Madagascar	M. Thulin et al., 10280, UPS	KF529834	KF529941	KF529749	KF529632
<i>Stachys rupestris</i> Montbret & Aucher*	Turkey	M. Nydegger, 42299, MSB	KF529835	KF529942	KF529750	KF529633
<i>Stachys saxicola</i> Coss. and Balansa*	Morocco	J. Lid, s.n., 21.04.1926, O	KF529836	KF529943	–	KF529634
<i>Stachys setifera</i> C.A.Mey. subsp. <i>setifera</i>	Iran	J.S. Andersen and I.C. Petersen, 115, C	FJ854328	FJ854231	FJ854116	KF529635
<i>Stachys setifera</i> subsp. <i>iranica</i> (Rech.f.) Rech.f.*	Iran	Y. Salmaki and S. Zarre, 36502, TUH	KF529837	KF529944	KF529751	KF529636
<i>Stachys schtschegleevii</i> Sosn. ex Grossh.*	Iran	Y. Salmaki et al., 39838, TUH	KF529838	KF529945	KF529752	KF529637
<i>Stachys spectabilis</i> Choisy ex DC.*	Iran	Y. Salmaki and S. Zarre, 36529, TUH	KF529839	KF529946	KF529753	KF529638
<i>Stachys spinosa</i> L.	Greece	M. Bendiksby and A.-C. Scheen, 04–022, O	FJ854329	FJ854232	FJ854117	KF529639
<i>Stachys spruneri</i> Boiss.*	Greece, Attikis	A. Strid. et al., 26691, C	KF529841	KF529948	KF529754	KF529640
<i>Stachys subaphylla</i> Rech.f.*	Iran	Y. Salmaki et al., 36868, TUH	KF529842	KF529949	KF529755	KF529641
<i>Stachys swainsonii</i> Benth	Greece	A. Strid et al., 39692, C	AF502062	FJ854234	FJ854119	KF529642
<i>Stachys sylvatica</i> L. (1)*	Border Italy/France	M. Bendiksby and A. Tribsch, 06–011, O	KF529843	KF529950	KF529756	KF529643
<i>Stachys sylvatica</i> (2)*	Cult.	C. Lindqvist and V.A. Albert, 358, UNA	AF502063	FJ854235	FJ854120	KF529644
<i>Stachys tenuifolia</i> Willd.*	E. Canada/ E: USA	Athles, 84653, C	KF529845	KF529952	KF529757	KF529645
<i>Stachys tetragona</i> Boiss. & Heldr.*	Greece	–, 3990, M	KF529846	KF529953	KF529758	KF529646
<i>Stachys trinervis</i> Aitch. & Hemsl. (1)*	Iran	Y. Salmaki and S. Zarre, 36765, TUH	KF529847	KF529954	KF529759	KF529647
<i>Stachys trinervis</i> (2)*	Afghanistan	K.H. Rechinger, 33325, C	KF529848	KF529955	KF529760	KF529648
<i>Stachys turcomanica</i> Trautv. (1)*	Iran	Y. Salmaki et al. 38080, TUH	KF529849	KF529956	KF529761	KF529649
<i>Stachys turcomanica</i> (2)*	Iran	Y. Salmaki et al., 38080, TUH	KF529850	KF529957	KF529762	KF529650
<i>Stachys yemenensis</i> Hedge*	Yemen	M. Thulin et al., 8165, UPS	KF529851	KF529958	KF529763	KF529651
<i>Stenogyne</i> Benth.						
<i>Stenogyne bifida</i> Hillebr.*	Hawaii	V. Albert et al., HI03–32, O	KF529852	KF529959	KF529764	KF529652
<i>Suzukia</i> Kudo						
<i>Suzukia luchuensis</i> Kudô (1)*	Japan	S. Kobayashi , 1926, A	KF529853	KF529960	KF529765	KF529653
<i>Suzukia luchuensis</i> (2)	Japan	S. Tawada and S. Hatusima, 18179, US	FJ854331	FJ854237	FJ854122	KF529654
<i>Suzukia shikikunensis</i> Kudô	Taiwan	C–C. Liao et al., 564, A	FJ854332	FJ854238	FJ854123	KF529655
<i>Thuspeinanta</i> T. Durand						
<i>Thuspeinanta brahuica</i> (Boiss.) Briq.	Iran	K.H. and F. Rechinger, 4701, US	FJ854333	FJ854239	FJ854125	KF529656
<i>Thuspeinanta persica</i> (Boiss.) Briq.	Iraq	K.H. Rechinger, 9604, S	FJ854334	FJ854240	FJ854126	KF529657

present in the plastid tree (Fig. 2), and five supported clades in the plastid tree (Fig. 2): clades Y–Å¹) are not present in the nuclear tree (Fig. 1).

3.1. Clades and taxa supported by both nuclear and plastid DNA

Both the nrITS (Fig. 1) and ptDNA (Fig. 2) data recover a monophyletic Stachydeae (clade A) with *Melittis* (clade B; Fig. 4a) as sister to all remaining Stachydeae (clade C) and *Betonica* (type: *Betonica officinalis* L.; Fig. 4b) forming a clade with *Galeopsis*

¹ Our labelling follows the Norwegian alphabet in which the last three letters (27–29) are Å, Ø and Å.

Table 4

Various sequence alignment information and tree statistics. Abbreviations: ASDSF = average, standard deviation of split frequencies, bp = base pairs, CI = consistency index (Kluge and Farris, 1969), GTR = general time reversible, G = gamma distribution, I = proportion invariant sites, MPT = most parsimonious tree, RC = rescaled consistency index and RI = retention index (Farris, 1989), TVM = transversion model.

	Plastid markers			Nuclear marker
	<i>trnL</i> –P ^a region	<i>rps16</i> intron	Combined pDNA	ITS
Number of accessions	155	153	155	155
Aligned length (bp)	944 ^a	974	1918	672
Excluded characters (bp)	9	36	10	–
Constant characters (bp)	798	801	1599	441
Parsimony informative characters, ingroup only (bp)	105	127	232	165
Number of coded indels	53	45	98	128
Number of MPTs	864	5000	5000	5000
CI of MPTs	0.79	0.74	0.75	0.44
RI of MPTs	0.94	0.92	0.93	0.8
RC of MPTs	0.743	0.681	0.698	0.352
Length of MPTs	368	425	805	1420
Selected substitution model	GTR + G	GTR + G	GTR + G	GTR + I + G ^b
ASDSF at termination	–	–	0.007133	0.0049

^a One exceptionally large deletion (122 bp long) occurred in the *trnL*–F region of clade B (Fig. 1). Most other indels were 10 bp long or shorter (rarely to 20–25 bp).

^b jModelTest selected TVM + I + G as the best-fit model for the ITS dataset. This model of evolution, characterized by a five-parameter nucleotide substitution rate matrix, is not currently available in MrBayes. Instead, we used the model that was selected as the second best: the parameter rich GTR + I + G.

(outgroups) outside of tribe Stachydeae. Clade D comprises all included accessions of *Stachys* sections *Eriostomum* (Hoffmanns. & Link) Dumort. and *Mucronata* R. Bhattacharjee, and is strongly supported by both nuclear and plastid data (Figs. 1 and 2). Clade E comprises the five genera *Haplostachys*, *Phyllostegia*, *Stachys*, *Stenogyne* and *Suzukia*. *Suzukia* and the Hawaiian labiates (*Haplostachys*, *Phyllostegia* and *Stenogyne*; Fig. 4d–g) form monophyletic subgroups present in both the nuclear and plastid topologies (Figs. 1 and 2: clades F and G, respectively). Representatives of the genus *Stachys* in clade E include all NW and some OW species (Figs. 1–3b), including the type of the genus (i.e., *Stachys sylvatica* L.; Fig. 4c). Among the NW *Stachys* species, only *S. grandidentata* Lindl. and *S. macraei* Benth. form a supported clade in both gene trees (Figs. 1 and 2: clade H). A group of western Mediterranean *Stachys* species as well as a group of all the African species in clade E also form clades in both gene trees (Figs. 1–3b: clades I and J, respectively). With the present taxon sampling, *Stachys* sections *Stachys*, *Campaniastrum* (Habrl.) Reichb. and *Corsica* R. Bhattacharjee are confined to clade E, whereas some members of the sections *Candida* R. Bhattacharjee and *Olisia* Dumort. also occur outside of clade E, the latter mainly so (Figs. 1 and 2).

Three multiple-species clades of *Sideritis* (type: *Si. hirsuta* L.: Fig. 4i) are strongly supported in both the nuclear and plastid trees (Figs. 1 and 2: clades K–M) and correspond to named taxa (Table 2). Thus, the genus *Sideritis* appears para- or polyphyletic based on nrITS (Fig. 1) and ptDNA (Fig. 2) data, respectively.

Clades N–U cluster in the same part of the trees (Figs. 1 and 2) together with several monotypic taxa (*Prasium*, *Phlomidoschema*, and *Stachys* sections *Infrasularis* R. Bhattacharjee, *Neurocalyx* R. Bhattacharjee, *Trinerves* Krestovsk., and *Zietenia* (Gled.) Benth.), and all or parts of three multi-species taxa (e.g., *Stachys* sections *Ambleia* Benth., *Aucheriana* R. Bhattacharjee, and *Olisia*). Clades N, P and T correspond to named taxa (see Table 2). *Stachys* section *Ambleia* is confined to this part of the trees (clades X and Å; Figs. 1 and 2, respectively), but appears polyphyletic in both gene-trees. Five of the nine N–U clades are supported by both nuclear and plastid data but do not represent named taxa: *S. subaphylla* Rech. f. (section *Ambleia*) as sister to *S. trinerves* Aitch. & Hemsl. (section

Trinerves; clade O); *S. acerosa* Boiss. (section *Aucheriana*) as sister to clade R (clade Q); African *Ambleia* (clade R); a clade comprising the three genera *Chamaesphacos*, *Hypogomphia* and *Thuspeinanta* (clade S; CHT clade; Fig. 4k and l); and a sister-relationship between *S. kermanshahensis* Rech. f. and *S. nephrophylla* Rech. f. (both members of section *Fragilicaulis* R. Bhattacharjee; clade U).

Overall, monophly is supported by both nuclear and plastid data for the following multi-species taxa (Figs. 1 and 2; Table 2): *Suzukia* (clade F); *Stachys* sections *Setifolia* R. Bhattacharjee (clade N; type: *S. setifera* C.A.Mey.: Fig. 4j), *Thamnostachys* Kapeller (clade P; i.e. subsection *Fruticosae* R. Bhattacharjee) and *Fragilicaulis* (clade T); *Sideritis* subgenus *Marrubiastrum* (Moench) Mendoza-Heuer (clade M; type: *Si. canariensis* L.; Fig. 4h); and the sections *Empedoclea* (Rafin.) Benth. (clade K) and *Sideritis* (clade L) under subgenus *Sideritis*. The following taxa appear non-monophyletic as currently circumscribed (Figs. 1 and 2; and Table 2): *Stachys* sections *Ambleia* (in clades X/Å), *Aucheriana* (in clades Q and V/Y), *Campaniastrum* (in clade E), *Candida* (clades I and Z/Å), *Eriostomum* (clade D), *Olisia* (clades C, Å), *Stachys* (in clade E), and *Swainsoniana* R. Bhattacharjee (clade V/Z); and *Sideritis* subgenus *Marrubiastrum* sections *Creticae* P.Pérez & Negrín, *Empedoceopsis* Huynh and *Marrubiastrum* (clade M). The following twelve clades are supported by both nuclear and plastid data but do not represent named taxa: C, D, E, G, H, I, J, O, Q, R, S, and U.

3.2. Gene tree incongruence and geography

Several taxa or clades exhibit inconsistent positions between the nuclear and plastid topologies. In fact, the interrelationships among most of the clades A–U described above are inconsistent across gene trees (Figs. 1–3a). Clade D is poorly supported as sister to clade E in the nuclear tree (Fig. 1) and largely unresolved with respect to groups of *Sideritis* and *Stachys* in the plastid tree (Fig. 2: in clade Y). The inter-relationships of the subclades F–J in clade E are highly inconsistent between the nuclear and plastid results (Figs. 1–3a). Likewise, phylogenetic relationships within and among clades K–M and the two monotypic sections *Hesiodia* (Moench) Benth. and *Burgsdorffia* (Moench) Briq. of subgenus *Sideritis*, as well as the interrelationships among clades N–U, are inconsistent across gene trees (Figs. 1–3a).

The following taxa/clades group with different sets of species in the two gene trees (Figs. 1 and 2): *Suzukia* (clade F), the Hawaiian labiates (clade G), members of the sections *Stachys* (in clade E), *Olisia* (in clade C) and *Setifolia* (clade N) of *Stachys*, the sections *Burgsdorffia*, *Hesiodia* and *Empedoclea* (clade K) of subgenus *Sideritis*, the monotypic genus *Prasium* (Fig. 4m), and several species of *Stachys* (e.g., *S. glutinosa* L., *S. lamiooides* Benth., *S. pubescens* Ten., *S. rupestris* Phil.). Also, two accessions of *S. lavandulifolia* Vahl (section *Zietenia*) behave differently in the two gene trees; they form a clade in the nrITS tree (Fig. 1) but are polyphyletic in the plastid tree (Fig. 2).

The species compositions of two clades are nearly the same across the two gene trees (Figs. 1 and 2): clades V and Z, and clades X and Å, respectively. The inconsistent positions of *S. glutinosa* and *S. pubescens* cause the difference between clades V and Z, and the different phylogenetic position of section *Empedoclea* (clade K) of subgenus *Sideritis* causes the difference between clades X and Å.

Some clades occurring only in the plastid tree show a geographic component. Clade Å comprises only OW species, with clade Y showing a western tendency and clade Ø an eastern and southern tendency (Fig. 3b). Clades with a geographic component in both trees tend to be more strongly supported in the plastid tree (Figs. 1 and 2: e.g., clades I, H and R).

4. Discussion

This study represents the most comprehensive molecular phylogenetic investigation of the lamioid tribe Stachydeae to date.

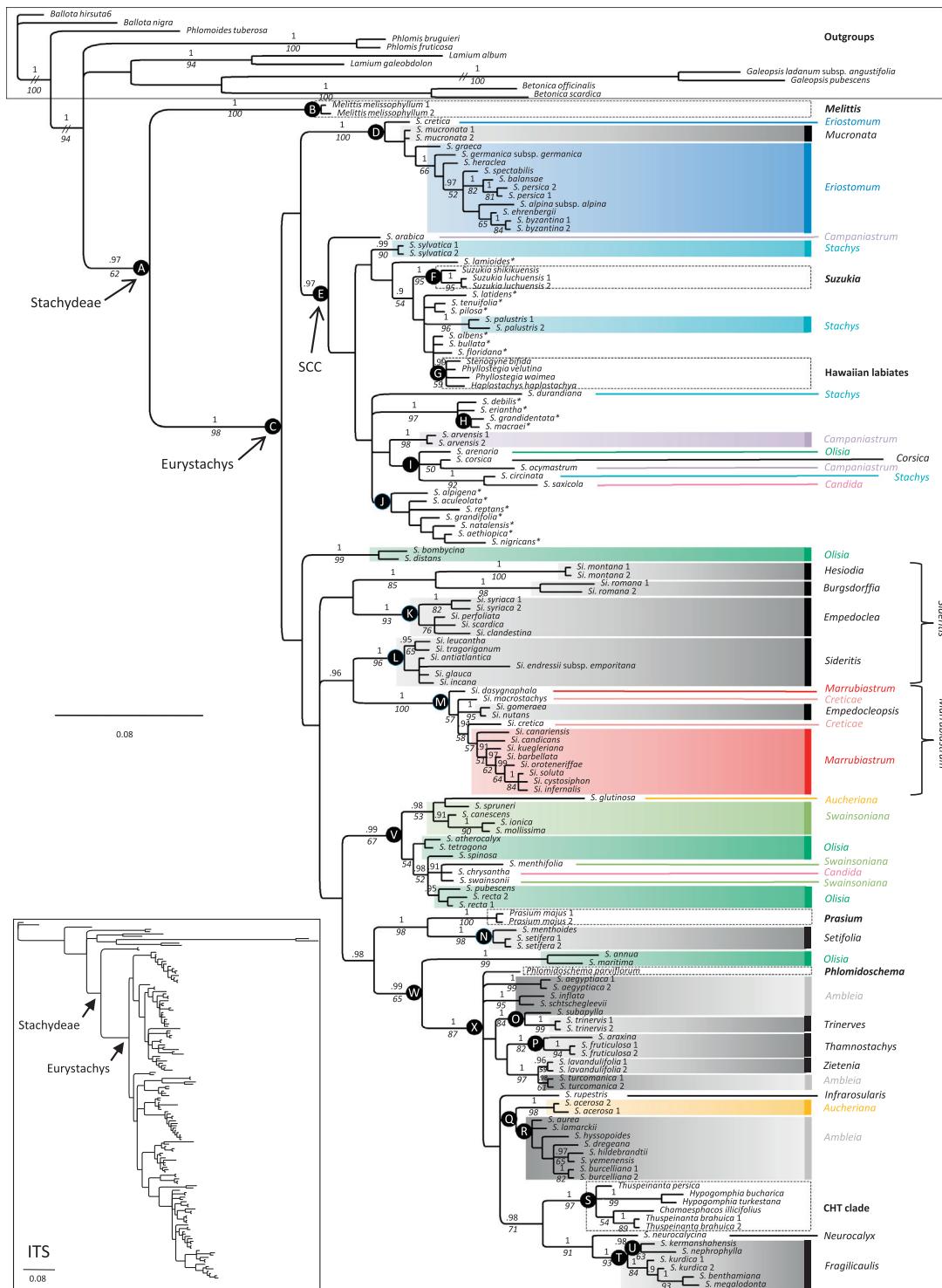


Fig. 1. The Bayesian 50% majority rule consensus phylogram based on the indel coded nrDNA (ITS1 + 5.8S + ITS2) data with 155 accessions (of which 11 are outgroup terminals) and 672 aligned characters. Bayesian posterior probability values ≥ 0.9 are reported above branches. Maximum parsimony jackknife values $\geq 50\%$ are reported in italics below branches. Three branches (indicated with double slashes) are manually shortened to reduce the size of a broad figure. Branches are drawn to scale in the inset tip-free tree. Multiple accessions of the same species are numbered according to Table 3. The sections of *Stachys* and *Sideritis* (see Table 2) are indicated to the right: the non-monophyletic sections are variously coloured; monophyletic sections are in black. The remaining ten genera of Stachydeae (see Table 1) are indicated by bold text and stippled boxes. *Stachys* species unplaced at the section level are indicated with an asterisk. The two *Sideritis* subgenera, *Sideritis* and *Marrubiastrum*, are indicated with braces. Clades discussed in the text are marked with capital letters, and those that correspond between datasets/figures are given the same letter. Abbreviations: SCC = Stachys Core Clade, *S.* = *Stachys*, *Si.* = *Sideritis*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Our molecular results, based on both nuclear (Fig. 1) and plastid (Fig. 2) DNA sequence data, show that *Betonica* is extraneous in *Stachys* (and even in Stachydeae), that Stachydeae is monophyletic and encompasses 12 lamioid genera (listed in Table 1), that

Melittis melissophyllum L. (Fig. 4a) is sister to remaining Stachydeae, and that the large genus *Stachys* is paraphyletic with respect to all genera of Stachydeae except *Melittis*. These results corroborate previous findings that were based on investigations

with more limited taxonomic and geographic coverage (Lindqvist and Albert, 2002) and only plastid data (Scheen et al., 2010; Bendiksby et al., 2011a). However, the inclusion of 55 additional

species of *Stachys* in the present study reveals several clades not previously evident. The use of both nuclear and plastid data on the exact same set of species has enabled us to identify several

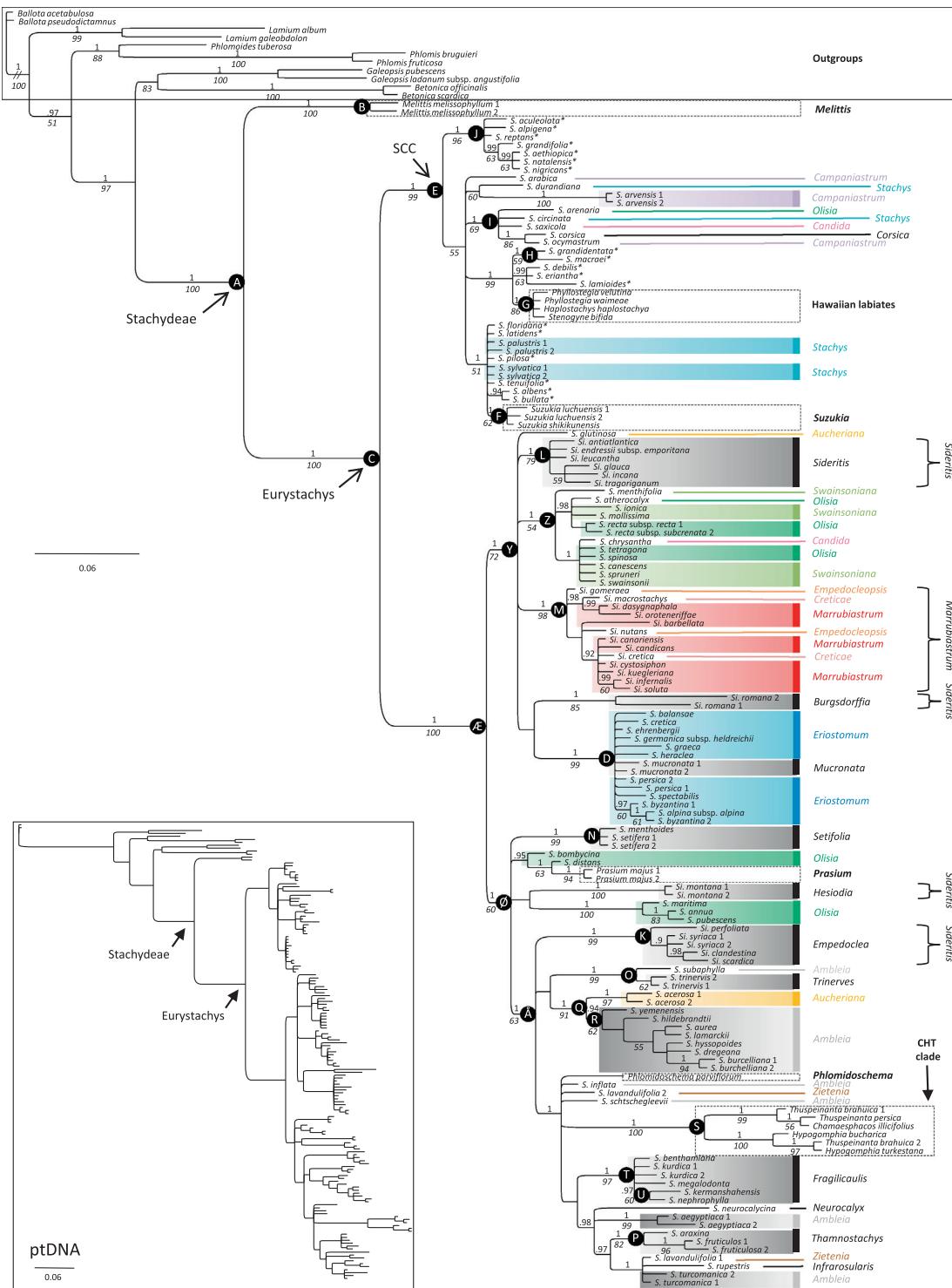


Fig. 2. The Bayesian 50% majority rule consensus phylogram based on the indel coded concatenated matrix of the plastid *trnL*-F region and *rps16* intron, i.e., the ptDNA matrix with 155 accessions (of which 11 are outgroup terminals) and 1918 aligned characters. Bayesian posterior probability values ≥ 0.9 are reported above branches. Maximum parsimony jackknife values $\geq 50\%$ are reported in italics below branches. One branch (indicated with double slashes) is manually shortened to reduce the size of a broad figure. Branches are drawn to scale in the inset tip-free tree. Multiple accessions of the same species are numbered according to Table 3. The infra-generic sections of *Stachys* and *Sideritis* (see Table 2) are indicated to the right: the non-monophyletic sections are variously coloured; monophyletic sections are in black. The remaining ten genera of Stachydeae (see Table 1) are indicated by bold text and stippled boxes. *Stachys* species unplaced at the section level are indicated with an asterisk. The two *Sideritis* subgenera, *Sideritis* and *Marrubiastrum*, are indicated with braces. Clades discussed in the text are marked with capital letters, and those that corresponded between datasets/figures are given the same letter. Abbreviations: SCC = *Stachys* Core Clade, S. = *Stachys*, Si. = *Sideritis*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

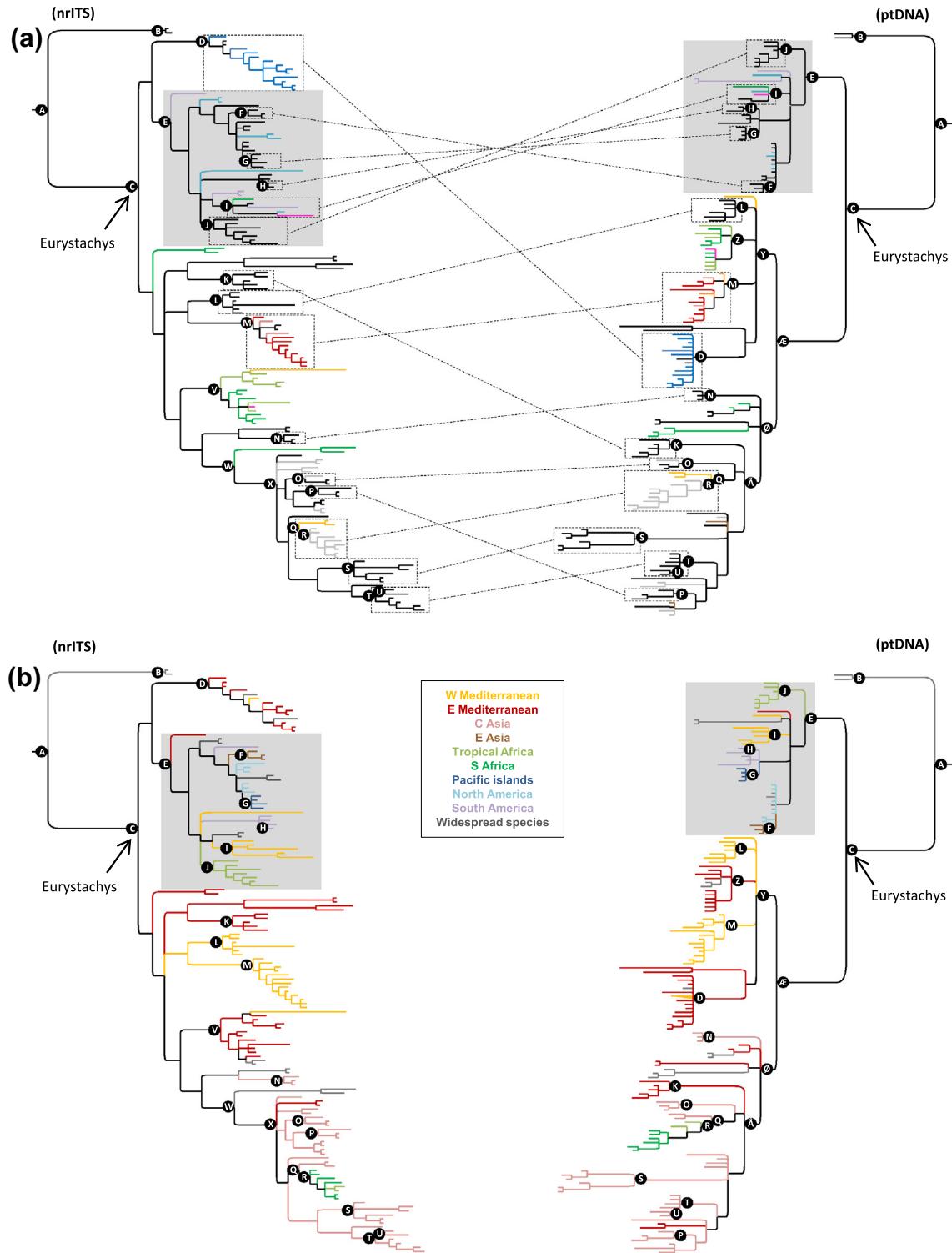


Fig. 3. The Bayesian tree topologies of Stachydeae (clade A) presented in Fig. 1 (left: ITS) and Fig. 2 (right: ptDNA) with various information superimposed. The 21 groups that are monophyletic in both tree topologies are indicated with capital letters (A–T; groupings of multiple accessions of the same species not indicated). Stachys Core Clade is indicated with gray shading. (a) Visualization of the nuclear-plastid DNA congruence/incongruence of tribe Stachydeae. Relative positions of the smaller clades are indicated with stippled boxes and lines. The infrageneric taxonomic complexity of the two largest genera, *Stachys* and *Sideritis*, is also visualized by coloured branches corresponding to the non-monophyletic sections (colouring as in Figs. 1 and 2). (b) Center of geographic distribution of each species, according to the "World Checklist of Selected Plant Families" (Govaerts et al., 2012), indicated with differently coloured branches. The Mediterranean region is broadly defined to include the area from the Canary Islands to western Turkey, Syria and Jordan, and from central Europe to northern Africa. Taxa that could not be attributed to only one of the pre-defined geographic areas were coded as widespread (gray). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

instances of nuclear-plastid incongruence, as well as to identify evolutionary lineages within tribe Stachydeae that are supported by unlinked sources of data. Moreover, our careful selection of

species, so as to include the type species of all currently recognized taxa, allows for a preliminary evaluation of the current taxonomy.



Fig. 4. Photographs of selected species of Stachydeae (photographer in brackets): (a) *Melittis melissophyllum* (from Walter Obermayer), (b) *Betonica officinalis* (from Yasaman Salmaki), (c) *Stachys sylvatica* (from Yasaman Salmaki), (d) *Haplostachys haplostachya* (from Gerald D. Carr), (e) *Phyllostegia ambigua* (from Gerald D. Carr), (f) *Phyllostegia glabra* (from Gerald D. Carr), (g) *Stenogyne kamehamehae* (from Gerald D. Carr), (h) *Sideritis canariensis* (from Manuel Luis Gil González), (i) *Sideritis hirsuta* (from Andreas Borns), (j) *Stachys setifera* (from Yasaman Salmaki), (k) *Chamaesphacos ilicifolius* (from Tulkin Sotvoldievich Tillaev), (l) *Hypogomphia bucharica* (from Eugene A. Davkaev), (m) *Prasium majus* (from Linda Young).

4.1. Major evolutionary lineages and taxonomy in light of the molecular results

In the nrITS tree (Fig. 1), monophly is supported for the following ten multi-species taxa: *Betonica* (in outgroups), Stachydeae (clade A), *Suzukia* (clade F), the sections *Empedoclea* (clade K) and *Sideritis* (clade L) of subgenus *Sideritis*, subgenus *Marrubiastrum* (clade M), *Sideritis* section *Empedocleopsis* of subgenus *Marrubiastrum*, *Stachys* sections *Setifolia* (clade N) and *Thamnostachys* (subsection *Fruticosae*; clade P) and *Stachys* section *Fragilicaulis* (clade T). The same taxa, except *Sideritis* section *Empedocleopsis*, are monophyletic in the plastid tree (Fig. 2). All other multi-species taxa appear para- or polyphyletic, and taxonomic polyphyly is most prevalent in the plastid topology (Fig. 2). In addition, some of the unnamed clades within Stachydeae (clade A) that are supported by both plastid and nuclear data (Figs. 1–3a) could

potentially represent taxonomically valid groups. Moreover, some of these clades also represent major evolutionary lineages that could be investigated further largely independently of the remainder of Stachydeae.

4.1.1. Clades A–C

Although Scheen et al. (2010) could not identify any non-molecular features that characterize all or most members of the diverse tribe Stachydeae, the congruence between our plastid and nuclear data now supports its status as a monophyletic group without doubt (Figs. 1 and 2; clade A). In the nuclear tree (Fig. 1), the relationship between the *Betonica*–*Galeopsis* clade and Stachydeae remains unsettled, whereas in the plastid tree (Fig. 2), these groups represent strongly supported sister taxa. Morphological implications of this result are discussed by Scheen et al. (2010). The monotypic genus *Melittis* (clade B) represents a highly distinct

evolutionary lineage within tribe Stachydeae, both molecularly (Figs. 1 and 2) and morphologically. *Melittis melissophyllum* is a perennial herb distributed in eastern Europe and western Asia. It clearly differs from the rest of Stachydeae in having the calyx strongly 2-lipped (Harley et al., 2004) and appearing mostly 3-dentate or with an entire or irregularly dentate upper lip, and the nutlets irregularly reticulate with large meshes on the surface.

The remainder of Stachydeae represents a strongly supported monophyletic group (Figs. 1 and 2: clade C) comprising 11 genera, three of which are monotypic (*Chamaesphacos*, *Phlomidoschema* and *Prasium*). Among the eight non-monotypic genera of highly variable size (see Table 1), monophyly is supported only for *Suzukia* (Figs. 1 and 2: clade F). The plastid results of Scheen et al. (2010) also depicted taxonomic disarray at the infra-generic levels in both *Stachys* and *Sideritis*. Our inclusion of nuclear data and additional relevant species shows that the majority of infrageneric taxa are indeed para- or polyphyletic (Figs. 1 and 2). Extensive paraphyly above, at, and below genus level in clade C of tribe Stachydeae poses major challenges to traditional rank-based nomenclature. More data are needed before sound taxonomic conclusions can be drawn. However, in order to facilitate scientific communication about the large and strongly supported clade C, we propose the phylogenetic name Eurystachys, following the draft *PhyloCode* (Cantino and de Queiroz, 2006).

Eurystachys Y. Salmaki & M. Bendiksby, new clade name.

Comments on name: There is no pre-existing scientific name for this crown clade. We chose the name Eurystachys because *Stachys*, the largest component genus, is paraphyletic with respect to the remaining 10 genera of the clade. *Eury-* is Greek and means broad, wide, or widespread. The name Eurystachys has apparently never been published with a description and therefore does not qualify as a pre-existing name. A second candidate name, Holostachys (*Holo-* means whole, entire, or all in Greek), was not chosen because it could readily be confused with the already existing genus name *Holostachyum*.

Definition (branch-modified node-based with an internal qualifier): the most inclusive crown-clade containing *Stachys sylvatica* but not *Melittis melissophyllum*.

Composition: *Chamaesphacos* Schrenk ex Fisch., *Haplostachys* (A. Gray) Hillebr., *Hypogomphia* Bunge, *Phlomidoschema* (Benth.) Vved., *Phyllostegia* Benth., *Prasium* L., *Sideritis* L., *Stachys* L., *Steno-gyne* Benth., *Suzukia* Kudô, *Thuspeinanta* T. Durand.

References phylogeny: Salmaki et al. (this study: Figs. 1 and 2). See also Lindqvist and Albert (2002), Scheen et al. (2010) and Bendiksby et al. (2011a).

Synonymy: The clade has been referred to as *Stachys* sensu lato in previous works (Lindqvist and Albert, 2002; Scheen et al., 2010; Bendiksby et al., 2011a).

4.1.2. Clade D

Clade D, comprising all accessions of *Stachys* section *Eriostomum* and monotypic section *Mucronata*, is well supported in both trees (Figs. 1 and 2). Morphologically, clade D forms a comparatively distinctive and well-defined group that can be characterized by having the outer surface of the upper corolla lip covered with long, soft, simple hairs. The clade also differs from most other *Stachys* in having longer bracteoles, and it may also be characterized by the base chromosome number $n = 15$ ($2n = 30$; Martin et al., 2011). *Stachys* section *Eriostomum* appears to be non-monophyletic as currently circumscribed. However, it can easily be made monophyletic, and still remain morphologically well defined, by inclusion of section *Mucronata*.

4.1.3. Clade E (Stachys Core Clade [SCC]) and the subclades F–J

Clade E is the second largest monophyletic group within Stachydeae that is consistent between the nuclear (Fig. 1) and

plastid (Fig. 2) topologies. This morphologically and geographically diverse clade largely corresponds to the ‘*Stachys* sensu stricto’ clade in Lindqvist and Albert (2002). Clade E cannot readily be distinguished morphologically from the remainder of Eurystachys and receives limited support by our nuclear data (Fig. 1). The corresponding clade was recovered in both the plastid and the two nuclear gene trees of Roy et al. (2013), despite a somewhat different and broader NW taxon sampling therein. Some incongruence was detected between the two nuclear ribosomal markers used by Roy et al. (2013; i.e., ETS and 5S-NTS). Yet, monophyletic groups corresponding to clade E occur in all available plastid and nuclear gene-trees (Lindqvist and Albert, 2002; Scheen et al., 2010; Bendiksby et al., 2011a; Roy et al., 2013). We therefore suggest use of the informal name Stachys Core Clade (SCC) for clade E, defined as the largest crown clade that contains *S. sylvatica* (the type of genus *Stachys*; Fig. 4c) but not *S. persica* S.G.Gmel. ex C.A.Mey. and *Sideritis candicans* Aiton.

Subclades within SCC (clade E) that are supported by both gene trees (Figs. 1 and 2: clades F–J) generally receive higher support from the plastid data (Fig. 2), except for *Suzukia* (Figs. 1 and 2: clade F), which receives the strongest support by the nuclear data (Fig. 1). The latter is characterized by creeping plants and racemose inflorescences. Subclades comprising the genus *Suzukia* (clade F), the three Hawaiian labiate genera (*Haplostachys*, *Phyllostegia* and *Stenogyne*; clade G; Fig. 4d–g), and a group of tropical to southern African *Stachys* species (clade J) were recovered also in gene trees by Roy et al. (2013), and clade G, in particular, is thoroughly discussed therein. Subclade H (Figs. 1 and 2), including the two Chilean species *S. grandidentata* and *S. macraei*, does not occur in the trees by Roy et al. (2013). It should be noted that vouchers for *S. macraei* in the two studies are not the same. Future studies should aim to include multiple accessions of this and closely related species.

Subclade I (Figs. 1 and 2) has not previously been identified and comprises the following five western Mediterranean *Stachys* species attributed to five different sections: *S. arenaria* Vahl, *S. corsica* Pers., *S. ocytmastrum* (L.) Briq., *S. circinata* L'Hér., and *S. saxicola* Coss. & Balansa. It is difficult to characterize the group on morphological synapomorphies. As far as is known, the species agree in sharing characteristics that are very common in *Stachys*, such as simple hairs and minute to obsolete bracteoles. Within clade I, a close relationship is inferred between *S. corsica* and *S. ocytmastrum*, which also share the same diploid chromosome number, $2n = 18$ (Goldblatt and Johnson, 2006), so far not documented elsewhere in the tribe.

African *Stachys* species forming subclade J are taxonomically unplaced at the section level. This clade also appeared, although represented by fewer species, in the trees presented by Scheen et al. (2010), Bendiksby et al. (2011a), and Roy et al. (2013). All these species differ from the other tropical to southern African *Stachys* in our study (cf. Figs. 1 and 2: clade R) in having only simple hairs instead of mainly branched hairs. It is worth noting that the diploid chromosome number of *S. aculeolata* Hook.f. is $2n = 52$ (Goldblatt and Johnson, 2006), unique among ‘counted’ members of Stachydeae, and should thus be investigated as a potential synapomorphy for clade J.

4.1.4. Clades K–M (*Sideritis*)

Three multiple-species clades of *Sideritis* (Figs. 1 and 2: clades K–M) are strongly supported and consistent across gene trees, and correspond well with current taxonomy (see Table 2): the endemic Macaronesian subgenus *Marrubiastrum* (clade M); subgenus *Sideritis* section *Empedoclea* (clade K); and subgenus *Sideritis* section *Sideritis* that usually differs from the rest of the genus in having the bracts coarsely toothed or spinose instead of entire (clade M). Clades K–M corroborate the molecular phylogenetic results

by Barber et al. (2007). However, with our present expanded sampling of Stachydeae taxa, the genus appears paraphyletic or polyphyletic based on nrITS (Fig. 1) and ptDNA (Fig. 2) data, respectively. Presence of short stamens that are included in the corolla tube, rather than exserted, is a character that has been used historically to distinguish *Sideritis* from *Stachys* (Scheen et al., 2010; compare Fig. 4c with h and i), but setting a border on this state separating *Sideritis* from *Stachys* is not always possible.

4.1.5. Clades N–U

Stachys section *Setifolia* (Figs. 1 and 2: clade N) is characterized by having long rhizomes and spinescent floral leaves. Members of this clade occur along streams, mostly on sandy substrates. All data strongly support *S. subaphylla* (section *Ambleia*) as sister to two accessions of *S. trinervis* (the monotypic section *Trinerves*; Figs. 1 and 2: clade O), adding to the polyphyly of section *Ambleia* (subsection *Brevibracteolatae* Krestovsk. in particular). Although all members of *Stachys* section *Ambleia* occur in the N–U part of the trees (Figs. 1 and 2), they are far from forming monophyletic groups corresponding to the infrageneric classification (see Table 2). Both *S. subaphylla* and *S. trinervis* have narrow leaves with branched hairs, but these two characteristics also occur in the neighbouring clades. *Stachys* section *Thamnostachys* (represented here by subsection *Fruticulosae*; clade P) consists of dwarf shrubs with 1-flowered cymes and inflated fruiting calyces that grow on reddish clay.

Stachys acerosa of section *Aucheriana* appears to be closely related to a group of Arabian to tropical and southern African species of *Stachys* section *Ambleia* (Figs. 1 and 2: clade Q), rendering both sections non-monophyletic. Whereas *S. acerosa* has simple hairs only, the members of clade R agree with the North African to Central Asian members of the same section (*Ambleia*) in having branched hairs. Because the latter species emerge elsewhere in the tree, this indumentum character conflicts with our phylogeny, but the character otherwise appears to be rather informative. The presence of branched hairs can be used to distinguish the members of clade R from the other tropical to southern African species included here (Figs. 1 and 2: cf. clade J).

The *Chamaephacos-Hypogomphia-Thuspeinanta* (CHT) clade (Figs. 1 and 2: clade S), also identified by Bendiksby et al. (2011a), is comparatively morphologically distinct. The group is primarily characterized by having narrow nutlets and consists of annuals with 1–3-flowered cymes. *Stachys* section *Fragilicaulis* (clade T) is characterized by the fragile base of the flowering stems and occurrence in saxicolous habitats (Bhattacharjee, 1980).

Interspecific relationships near the tips of the trees are rarely consistent (Figs. 1 and 2), but one example occurs within *Stachys* section *Fragilicaulis*: a grouping of *S. kermanshahensis* and *S. nephrophylla* (clade U). Whereas these two species belong to subsection *Multibracteolatae* R. Bhattacharjee (emend. Rechinger, 1982), the rest of the clade T belongs to subsection *Fragiles* Boiss. ex Rech. f. According to Bhattacharjee (1980), subsection *Multibracteolatae* differs from subsection *Fragiles* in having the bracteoles larger and more herbaceous, and also having the nutlets obovoid and apically smooth rather than oblong and apiculate. However, these characteristics are rather common in the rest of *Stachys*. Hence, *Multibracteolatae* (emend. Rechinger, 1982) appears to be monophyletic, while the phylogenetic status of the other subsection remains uncertain.

4.1.6. Clades V–Å

Several groups almost, but not entirely, correspond between the plastid and nuclear topologies. For example, the mostly eastern Mediterranean clade V in the nuclear tree (Fig. 1) corresponds to clade Z in the plastid tree (Fig. 2), if *S. glutinosa* (*Stachys* section *Aucheriana*) and *S. pubescens* (*Stachys* section *Olisia*) are removed from the latter. *Stachys* sections *Candida*, *Olisia* and *Swainsoniana*

are the main components of clade V/Z, and the lattermost is restricted to this clade. The members of clade V/Z for which chromosome counts are available all have $2n = 34$, but so does *Olisia* subsection *Distantes* R. Bhattacharjee, which is not part of clade V/Z.

Another example of close correspondence is related to the East Mediterranean to Asian and tropical to South African clade X in the nuclear tree (Fig. 1), which corresponds to clade Å in the plastid tree (Fig. 2) if the eastern Mediterranean *Sideritis* section *Empedoclea* (clade K) is excluded from the latter (Fig. 3b). The X/Å clades have a largely Central Asian distribution. The clade is morphologically diverse, comprising six/five genera, corroborating previous plastid results (Bendiksby et al., 2011a), and eight different sections of *Stachys* (Figs. 1 and 2: clades X and Å, respectively). It is worth mentioning that most of the Stachydeae with branched hairs (*Phlomidoschema* and *Stachys* sections *Ambleia* and *Zietenia*) emerge in this clade (X/Å), but within the clade the character of presence or absence of such hairs appears to be rather homoplastic.

4.2. Gene tree incongruence

Although the nuclear data corroborate the plastid data when it comes to the composition of the clades A–U, the interrelationships among or within these clades are often not congruent (Figs. 1–3a), or clades that are strongly supported in one gene-tree are largely unresolved in the other. Plastid–nuclear incongruence among some members of Stachydeae has previously been revealed by Lindqvist and Albert (2002), Barber et al. (2002, 2007), and Roy et al. (2013). Although the taxon sampling in the earlier studies was limited, it was clear that some major, strongly supported clades in the plastid tree were not present in the nuclear tree and vice versa.

Hybridization is assumed to be prevalent in Stachydeae (e.g., Bomble, 2013; Nuñez and Castro, 1990) and is therefore a plausible cause of the plastid–nuclear conflicts revealed in the present study (Fig. 3a). Hybridization seems to be particularly common in *Stachys*, especially in its main diversification center (i.e., the Iranian highlands; Salmaki et al., 2012b). By selecting a taxonomically (morphologically) and geographically broad sampling of Stachydeae, and by using DNA regions with an adequate level of nucleotide substitution, we have tried to avoid the stochastic and systematic errors described by Zou and Ge (2008). Hence, the plastid–nuclear incongruent patterns identified herein are most likely caused by horizontal gene transfer (introgression/hybridization), in which case the nuclear phylogeny will be the more reliable, or by incomplete lineage sorting, which may affect both genomes (e.g., Joly et al., 2009; Riesenbergs, 1997; Sang and Zhong, 2000).

It is interesting that the strongly supported dichotomous branching into clades E and Å rather deep in the plastid tree (Fig. 2) is not corroborated by nuclear data (Fig. 1). This is likely caused by the inconsistent placement of clade D (Fig. 3a) which comprises the sections *Eriostomum* and *Mucronata* of *Stachys*. Clade D is sister to clade E in the nuclear tree (Fig. 1), whereas in the plastid topology (Fig. 2) the clade is largely unresolved with respect to groups of *Sideritis* and *Stachys* in clade Y. However, due to a lack of support for the backbone resolution in the nuclear tree (Fig. 1), there remains a possibility that clade Å could be real. More data is needed to illuminate this.

The inconsistent inter-relationships among subclades of the *Stachys* Core Clade (SCC; clade E) across gene trees (Figs. 1–3a) suggest a complex evolutionary history. For example, the Hawaiian labiates (clade G) group with *Suzukia* (clade F) and a few OW and all included North American *Stachys* species in the nuclear tree (Fig. 1), whereas they occur in a strongly supported clade together with all included South American *Stachys* species in the plastid tree (Fig. 2). This suggests that the Hawaiian labiates (clade G) have a

hybrid origin. In addition, the inconsistent position of the South American *S. lamiooides* indicates past hybridization. As stated by Roy et al. (2013), untangling the evolutionary history of the SCC (clade E) may require use of low copy nuclear loci that are, as opposed to ribosomal DNA, not subject to concerted evolution.

The interrelationships among clades K–M and the two monotypic sections *Hesiodia* and *Burgsdorffia* of subgenus *Sideritis*, as well as among species within the K–M clades, are not consistent across gene trees (Figs. 1–3a). For example, in the nrITS topology (Fig. 1), clades K–M and sections *Hesiodia* and *Burgsdorffia* are largely unresolved (i.e., no supported resolution) with respect to all other subclades of Eurystachys (clade C), whereas in the ptDNA tree, these clades fall into different supported subclades (Fig. 2: clades Y, Ø and Å). Thus, whereas the nuclear results (Fig. 1) leave open the possibility that *Sideritis* represents a monophyletic genus, the plastid results (Fig. 1) render it highly polyphyletic. Incongruent plastid–nuclear results in *Sideritis* were also shown by Barber et al. (2002, 2007). This large genus, with a circum-Mediterranean center of species diversity, is characterized by having the stamens included in the corolla tube, the corolla tube shorter than the calyx, and the posterior corolla lip almost flat (Harley et al., 2004). In spite of some overlap in the differential characters, *Sideritis* appears to be rather distinctive, suggesting that the genus may be monophyletic. As previously recognized (Barber et al., 2002, 2007), the morphology of *Sideritis* seems to be more consistent with nuclear data (Fig. 1) than with the plastid data (Fig. 2), whereas relationships based on plastid data appear to have a geographical component (Fig. 3b) – a pattern also observed in other groups of the Lamiaceae (Albaladejo et al., 2005; *Phlomis*; Bräuchler et al., 2010; *Menthinae*; Bendiksby et al., 2011b; *Lamium*). Correlations between each of the inferred topologies and biogeography or morphological traits are evident, but less strong, in our present study (Fig. 3a, and b).

Although clades N–U cluster in the same part of the trees (Figs. 1 and 2), the taxon composition of this part of the tree varies across gene trees, and so do the interrelationships of the clades N–U (Figs. 1–3b). The strongly supported sister-relationship between *Prasium majus* L. and *Stachys* section *Setifolia* (clade N) in the nuclear tree (Fig. 1) is neither recovered nor strongly contradicted, in the plastid trees of the present (Fig. 2) and previous (Scheen et al., 2010; Bendiksby et al., 2011a) studies. The *Prasium*–*Setifolia* clade was previously supported, however, by nuclear ribosomal 5S-NTS sequences data (Lindqvist and Albert, 2002; referred to as the “PRAS” clade). Contrary to nuclear data (Fig. 1), *Prasium* groups with *S. distans* Benth. (section *Oisia*) in the plastid phylogeny (Fig. 2). This hard plastid–nuclear incongruence suggests that the monotypic *Prasium* may have originated from a past hybridization event. Morphologically, *P. majus* differs from members of *Stachys* sect. *Setifolia* in having a suffrutescent (rather than herbaceous) life form, in lacking spinescent floral leaves, and the presence of fleshy nutlets.

The nuclear tree provides support for a sister-relationship between the CHT-clade (clade S) and *Stachys* sections *Neurocalyx* and *Fragilicaulis* (Fig. 1), whereas in the plastid tree (Fig. 2), the CHT-clade falls out largely unresolved in clade Å. Within the CHT-clade (clade S), *Hypogomphia* is strongly supported as monophyletic in the nuclear tree (Fig. 1) but is paraphyletic in the plastid tree (Fig. 2). As mentioned by Harley et al. (2004), the genus is morphologically well defined by having the anterior pair of stamens reduced to staminodes.

Stachys pubescens is sometimes regarded as a synonym of *S. annua* (L.) L. (Govaerts et al., 2012; Salmaki et al., 2012b). Our plastid data support a close relationship between *S. pubescens* and *S. annua* (Fig. 2: in clade Ø), in a strongly supported clade that also includes *S. maritima* L. In the nuclear tree, however, the same accession of

S. pubescens instead emerges as sister to two accessions of *S. recta* L. (Fig. 2: within clade V). It should be noted that, irrespective of gene tree, the species groups with members of *Stachys* section *Oisia*. This hard nuclear-plastid incongruence is a first hint at hybrid origin of *S. pubescens* and should be investigated further. Inconsistent positions of *Stachys rupestris* (section *Infrarolularis* R. Bhattacharjee; in clades X/Å) also merit further investigations. The divergent phylogenetic placements of the two accessions of *Stachys lavandulifolia* (section *Zietenia*) was not a surprise to us, as this species has been shown to hybridize extensively with *S. inflata* Benth. (section *Ambleia*; Salmaki et al., 2012b). Based on the present data, the correct phylogenetic position of *S. lavandulifolia* is therefore as sister to *S. turcomanica* Trautv. (section *Ambleia*). The strongly supported grouping of *S. lavandulifolia* and *S. turcomanica* may merit naming.

4.3. Paraphyletic *Stachys* – a huge challenge to classification

The large and paraphyletic genus *Stachys* is a major challenge to classification. Our results suggest two alternatives: (1) a broad circumscription of *Stachys* (*Stachys* s.l.) to include all genera attributed today to Stachydeae except *Melittis* (the lumping approach); or (2) retention of monophyletic genera and describing several new ones to encompass groups of *Stachys* not affiliated with its type (the splitting approach). Both alternatives are challenging. The lumping approach would result in one very large genus comprising about 470 species. Moreover, new combinations would be needed for about 194 species (Table 1) and new epithets required for a considerable portion of these, since several species of the various genera share the same epithet. Another serious problem would be the loss of information about morphologically highly distinct groups of species to which one would wish or need to refer. Establishing a subgeneric classification within a broadly circumscribed *Stachys* might seem as a solution to the latter problem; in fact, no subgeneric classification exists, as no update has been undertaken since the exclusion of *Betonica*. However, establishing a subgeneric classification of an expanded *Stachys* would expose us to a new set of challenges, the same as those of the splitting approach. The splitting approach would include division of Eurystachys (Figs. 1 and 2: clade C) into a much higher number of genera than at present, the exact number depending on strictness to monophyly versus avoidance of monotypic taxa. A splitting approach, regardless of taxonomic level, would require more in-depth morphological and molecular investigations of many groupings of Eurystachys to reach a natural classification with support from multiple sources of data.

5. Conclusions

This international collaboration is a first contribution to a long-needed phylogenetic investigation of tribe Stachydeae on a global scale. Our identification of both major lineages and taxa of possible hybrid origin may guide selection of taxa and topics for further research. As such, the present study may be viewed as preliminary – as a useful starting point for future more in-depth studies.

Apart from describing Eurystachys (clade C) following the PhyloCode, and giving clade E the informal name *Stachys Core Clade*, we have not devised a satisfactory way to transform the taxonomy of Stachydeae into a more ‘natural’ classification at this point. Clearly, more studies of various kinds, are needed in order to draw the taxonomic conclusions required to reach the desired taxonomic update of Stachydeae. A micro-morphological approach (e.g., Abu-Asab and Cantino, 1992, 1994; Ryding, 1994; Salmaki et al., 2008a,b, 2009, 2011) at a global scale may represent a promising supplement to the more traditionally applied macro-morphological investigations.

Identification and confirmation of past hybridization events and untangling allopolloid origins of taxa will also be important, both for taxonomic evaluations and for understanding the evolutionary processes that have shaped this alliance. The molecular markers selected for the present study appear to exhibit an appropriate level of nucleotide variation for biosystematic studies at genus level and below in Stachydeae. The nrITS region proved surprisingly easy to work with given the heterogeneous properties of Stachydeae, such as the huge differences in chromosome counts, suggesting an evolutionary history involving frequent cytological changes. However, although several nuclear-plastid conflicts are uncovered at different levels in the phylogeny of Stachydeae, other single- or low-copy markers are needed for untangling the presumably reticulate evolution of the taxa concerned. We anticipate a revival of karyotype investigations (e.g., Martin et al., 2011; Mulligan and Munro, 1989) and increased use of low-copy nuclear genes (e.g., Bendiksby et al., 2011b,d) in future investigations of Stachydeae.

Based on our molecular results, it seems justifiable to study (in more detail) the following clades more or less independently of the remaining of Stachydeae: C, D, E, M, Q, V/Z, and X/A. However, although we have pursued including species typical for each taxon, the high level of para- and polyphyletic taxa in our molecular results strongly indicates that unsampled species cannot reliably be placed in the correct clade based on morphology alone. Sequencing of the same DNA regions used herein will allow for placing new data in the phylogenetic framework of the present study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.07.024>.

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