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REGULAR PAPER

Phylogenetic relationships among subgenera, species, and varieties of Japanese *Salvia* L. (Lamiaceae)

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Abstract To determine evolutionary relationships among all Japanese members of the genus *Salvia* (Lamiaceae), we conducted molecular phylogenetic analyses of two chloroplast DNA (cpDNA) regions (*rbcL* and the intergenic spacer region of *trnL−trnF*:*trnL−trnF*) and one nuclear DNA (nrDNA) region (internal transcribed spacer, ITS). In cpDNA, nrDNA, and cpDNA+nrDNA trees, we found evidence that all Japanese and two Taiwanese *Salvia* species are included in a clade with other Asian *Salvia*, and Japanese *Salvia* species were distributed among three subclades: (1) *S. plebeia* (subgenus *Sclarea*), (2) species belonging to subg. *Salvia*, and (3) species belonging to subg. *Allagospadonopsis*. At the specific level our findings suggest: a close relationship between *S. nipponica* and *S. glabrescens*, no support for monophyly of *S. lutescens* and its varieties in cpDNA, nrDNA and cpDNA+nrDNA trees, and that *S. pygmaea* var. *simplicior* may be more closely related to *S. japonica* than to other varieties of *S. pygmaea*.

Keywords cpDNA · Lamiaceae · nrDNA · Phylogenetics · *Salvia*

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Introduction

The genus *Salvia* L. (tribe Mentheae) is the largest genus in the Lamiaceae, comprising nearly 1,000 species; *Salvia* has radiated extensively in three regions of the world, Central and South America (500 spp.), and West (200 spp.) and East Asia (100 spp.) (Alziar, 1988–1993). It is distinguished from the other genera in the Lamiaceae by the presence of two aborted posterior stamens and a markedly elongated connective tissue separating thecae of the two expressed stamens, which may act as an effective tool for pollination (e.g., Grant and Grant 1964; Faegri and Van der Pijl 1979; Huck 1992; Crassen-Bockhoff et al. 2003; Reith et al. 2007). The presence of such an unusual stamen structure led taxonomists to believe, until recently, that *Salvia* was monophyletic. However, recent molecular phylogenetic studies have shown that the genus is polyphyletic, with three major lineages and five other genera intercalated within it (Walker et al. 2004; Walker and Sytsma 2007). *Salvia* clade I sensu Walker and Sytsma (2007), which is a sister to *Rosmarinus* and *Perovskia*, contains European, Central African, Southern African and West Asian species, while clade II contains species from the New World. Clade III comprises West Asian, Central Asian, East Asian, Mediterranean, and African *Salvia* species.

Since Thunberg's (1784) first accounts of Japanese *Salvia* (*S. japonica* Thunb.), ten species, eight varieties, and one putative hybrid have been described; they are classified into the subgenera (subg.) *Salvia*, *Allagospadonopsis*, and *Sclarea* (Murata and Yamazaki 1993, Inoue 1997, Hihara et al. 2001). Most of the taxa are endemic to Japan, except in the cases of *S. japonica* (which also occurs in Korea, Taiwan and Central to Southern China) and *S. plebeia* R. Br. (widely distributed in temperate and tropical Asia, and Australia) (Murata and Yamazaki 1993).

Additionally, one of the varieties of *S. nipponica* Miq., var. *formosana* (Hayata) Kudo, is known only from Taiwan (Li and Hedge 1994; Huang and Wu 1998). Therefore, we imagine that almost all Japanese taxa, including the Japanese endemics, speciated in Japan.

Inoue and Ozawa (1998) conducted morphological and allozymic analyses among species of subg. *Salvia*, and argued that *S. glabrescens* (Franch. et Sav.) Makino and *S. nipponica* var. *nipponica* should be considered conspecific, because genetic similarity between them was so high ($I = 0.84$-0.99). They reported genetic differentiation between *S. nipponica* populations in eastern and western Japan. They also suggested that *S. nipponica* var. *kisoensis* K. Imai should be elevated to species rank, because it was found to be genetically differentiated from *S. nipponica* var. *nipponica* and *S. glabrescens.* However, they did not undertake taxonomic or nomenclatural revisions of Japanese *Salvia*. Sudarmono and Okada (2007) conducted cpDNA and nrDNA phylogenetic analyses of selected Japanese and Taiwanese *Salvia.* They considered the speciation process in *S. isensis* Nakai, taking into account a contradiction in the phylogenetic positions of the species studied. Sudarmono and Okada (2008) analyzed additional species and demonstrated that species of subg. *Allagospadonopsis* formed a well-supported clade, that two species of subg. *Salvia* (*S. glabrescens* and *S. nipponica*) belonged together in another clade, and that *S. plebeia*, the sole Japanese species in subg. *Sclarea*, was sister to the clade of subg. *Salvia*. Although their results suggested the possibility of polyphyly among *S. lutescens* (Koidz.) Koidz., *S. nipponica* and *S. glabrescens*; however, their discussion was limited to examining the genetic differentiation among populations of *S. japonica* and related species. Two Japanese *Salvia* (*S. koyamae* and *S. omerocalyx* Hayata) were not included in Sudarmono and Okada's (2008) analyses.

As part of our ongoing investigations into speciation processes of endemic Japanese *Salvia*, we conducted molecular phylogenetic analyses of all taxa of Japanese *Salvia* above variety level not considered by Sudarmono and Okada (2008), i.e., varieties of *S. glabrescens* (var. *glabrescens* and var. *purpreomaculata* (Makino) K. Inoue ex. T. Shimizu), *S. koyamae* Makino, *S. lutescens* var. *stolonifera* (G. Nakai), varieties of *S. nipponica* (var. *kisoensis,* var. *trisecta* (Matsum. ex Kudo) Honda), *S. omerocalyx* (var. *omerocalyx* and var. *prostrata* Satake), *S. pygmaea* Matsum var. *simplicior*, and *S. sakuensis* Naruh. et Hihara.

Materials and methods

DNA extraction, PCR, and DNA sequencing

Total DNA was isolated from 0.7-1.5 g of fresh or silica gel-dried leaves, using a modified version of the 2× cetyltrimethylammonium bromide (CTAB) extraction protocol of Doyle and Doyle (1987). DNA sequences were amplified with *rbcL* 1-1 as the forward primer and *rbcL* NN3-2 as the reverse primer for *rbcL* (Hasebe et al. 1994), FRF as the forward primer and 5FR as the reverse primer for *trnL-F* (Sudarmono and Okada 2007), and ITS5 as the forward primer and ITS4 as the reverse primer for nrDNA ITS (White et al. 1990). The protocol and conditions of the polymerase chain reaction (PCR), purification, and cycle sequencing followed Sudarmono and Okada (2007). To sequence these three regions amplified by PCR, we used an additional pair of internal primers, i.e., 724F, 744R for *rbcL* (Sudarmono 2007), 3RF and 3FR for *trnL-F* (Sudarmono and Okada 2007), and ITS2 and ITS3 (White et al. 1990) for the nrDNA region, including the ITS1-5.8S rDNA-ITS2 region (hereafter, ITS).

Sequence alignment and phylogenetic analysis

Novel sequences were collected for 18 individuals of 12 taxa and the 3 regions. Other sequences were obtained through Genbank. Raw sequences were assembled and edited using the BioEdit software (ver. 5.0.9; Hall 1999). DNA sequences were aligned by multiple alignments using the CLUSTALW 1.83 software package with default settings (Thompson et al. 1994). Alignments of *rbcL* and the intergenic spacer region of *trnL-F* of cpDNA were combined. Gaps were treated as missing data.

We used 32 individuals of 10 species, 13 varieties, 4 formas, and 1 hybrid (*S.* × *sakuensis*) from Japan, and other selected species belonging to clade I (*S. roemeriana* Scheele, *S. sclarea* L., and *S. texana* Torr.), clade II (*S. cedrosensis* Greene, *S. chionopeplica* Epling, *S. clevelandii* (Gray) Greene, and *S. farinacea* Benth.), and clade III (*S. digitaloides* Diels, *S. flava* Forrest ex Diels, *S. glutinosa* L., *S. hians* Royle ex Benth., *S. miltiorrhiza* Bunge, *S. przewalskii* Maxim., *S. trijuga* Diels, and *S. yunnanensis* C. H. Wright) sensu Walker and Sytsma (2007). *Horminum pyrenaicum* L. and *Melissa officinalis* L. were used as outgroups. Materials, accession numbers for the sequences, vouchers, and literature are presented in ESM 1. Three datasets were constructed: (1) cpDNA (=*rbcL*+*trnL-F*) contained 47 individuals from 36 taxa, (2) ITS (hereafter nrDNA) contained 53 individuals from 42 taxa (several not included in cpDNA and cpDNA+nrDNA datasets), and (3) cpDNA+nrDNA contained 47 individuals from 36 taxa.

We analyzed these datasets using three methods. Maximum Parsimony (MP) analysis was performed with the PAUP* 4.0b10 software (Swofford 2003). Heuristic searches were conducted with RANDOM addition, tree-bisection-reconnection (TBR) branch swapping, and MULPARS options. Support for branches was estimated using bootstrap analysis with 1,000 replications (Felsenstein 1985),

through a heuristic search using RANDOM addition and TBR branch swapping. Maximum likelihood (ML) was also conducted with the PAUP* 4.0b10 software (Swofford 2003). We conducted a hierarchical likelihood ratio test using MrModeltest software (ver. 2.3; Nylander 2004) to determine the best-fit model of sequence evolution in the ML analysis. The GTR+G (for cpDNA and nrDNA datasets) and GTR+G+I (for the cpDNA+nrDNA datasets) models were chosen by the analysis. Heuristic searches were used in the analyses to find ML trees with RANDOM sequence addition and TBR branch swapping; we saved all of the best trees at each step (Multrees). Bootstrap analysis under the ML criterion was conducted using "fast" stepwise addition searches with 200 replicates. Additionally, a Bayesian analysis was conducted using the MrBayes software (ver. 3.1.2; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The best fitting substitution model (GTR+G for the cpDNA and nrDNA datasets, and GTR+I+G for the cpDNA+nrDNA dataset) for Bayesian analysis was selected using a series of hierarchical likelihood ratio tests implemented in the MrModeltest software (ver. 2.3). We performed the analysis using the selected model with two simultaneous runs of two million generations with four chains, sampling every 100 generations. Each analysis reached stationarity (the average standard deviation of split frequencies between runs $\leq$0.01) well before the end of the run. Burn-in (=5,000) trees were discarded, and the remaining trees and their parameters were saved. A 50% majority rule consensus tree was constructed. The results of the Bayesian analysis are reported as the posterior probabilities (PP; Huelsenbeck and Ronquist 2001), which are equal to the percentage of trees sampled when a given clade was resolved. Only PP scores in excess of 50% are shown.

We assessed the degree of phylogenetic incongruence between the cpDNA (=*rbcL*+*trnL-F*) and nrDNA (=ITS) datasets of 47 taxa using the incongruence-length difference (ILD) test (Mickevich and Farris 1981; Farris et al. 1994) in the PAUP*4.0 beta10 software, TBR branch swapping, and saving all of the most parsimonious trees.

Results

The features of alignments in the combined cpDNA (*rbcL*+*trnL-F*), nrDNA (ITS), and cpDNA+nrDNA datasets are shown in ESM 2. The G+C contents of ITS regions varied from 59.9% (*S. roemeriana*) to 68.7% (*S. chionopeplica*).

We found that the homogeneity test (ILD test) provided a P value < 0.01 for the combined cpDNA datasets and ITS (hereafter, nrDNA) datasets compared with random partitions. However, variable evolutionary rates and heterogeneity rates of substitution appear to affect the ILD test results, increasing the probability of type I errors (the error of incorrectly rejecting the correct hypothesis of congruence) (Baker and Lutzoni 2002; Darlu and Lecointre 2002). This may also be the case for our datasets since the substitution rate of the cpDNA and nrDNA regions studied were quite different (9.9 vs. 40.8%, respectively; ESM 2); therefore, we decided to combine cpDNA and nrDNA datasets.

The cpDNA data set of 47 individuals from 36 taxa contained 2,139 characters, and 99 of these were parsimony-informative. Parsimony analysis produced eight most parsimonious trees of 265 steps, a consistency index (CI) of 0.834 and a retention index (RI) of 0.901. Likelihood analysis resulted in a ML tree with $-\ln L = 4{,}863.258$. The MP strict consensus, ML, and Bayesian trees had the same topology; the MP tree is shown with bootstrap and PP support in Fig. 1. The species of clade I sensu Walker and Sytsma (2007) were sister to all other *Salvia*, and the species of clade II were sister to the species of clade III plus Japanese and Taiwanese *Salvia*. There were three subclades within the clade: 1. *S. plebeia*, 2. species of Japanese subg. *Salvia* (*S. glabrescens*, *S. koyamae*, *S. nipponica, S. sakuensis*) plus *S. glutinosa* (species of clade III), and 3. Japanese and Taiwanese species of subg. *Allagospadonopsis* plus *S. hians* (species of clade III). The *rbcL* and *trnL-F* sequences in *S. koyamae* and *S. nipponica* var. *kisoensis* were identical, and those of all taxa included in this subclade were also highly similar (99.6–99.9% identical) to one another, except for *S. nipponica* var. *nipponica* from Kumamoto and *S. glutinosa*. The species in subg. *Allagospadonopsis* formed another well-supported subclade, and Taiwanese (=*S. arisanensis* Hayata and *S. hayatana* Makino ex Hayata) and Ryukyu (=*S. pygmaea* var. *pygmaea*) *Salvia* were sister to other species. Individuals of *S. omerocalyx* and *S. lutescens* and its varieties were found in two different subclades: two varieties of *S. omerocalyx* (var. *omerocalyx* and var. *prostrata*) formed a subclade with the *S. lutescens* group (except for *S. lutescens* var. *crenata*), *S. isensis* and *S. ranzaniana* Makino; and a subclade containing *S. japonica*, an individual *S. omerocalyx* plant (specimen from Takeno, Toyooka), *S. lutescens* var. *crenata* (Makino) Murata, and one Ryukyu taxon, *S. pygmaea* var. *simplicior*. *Salvia hians* was sister to the *Allagospadonopsis* clade.

The ITS datasets for 53 individuals in 42 taxa contained 687 characters, and 179 of these were parsimony-informative. Parsimony analysis produced 2,795 most parsimonious trees of 629 steps, a consistency index (CI) of 0.618 and a retention index (RI) of 0.791. Likelihood analysis resulted in a ML tree with $-\ln L = 4{,}263.061$. The MP strict consensus, ML, and Bayesian trees had the same topology, and the MP tree is shown with bootstrap and PP

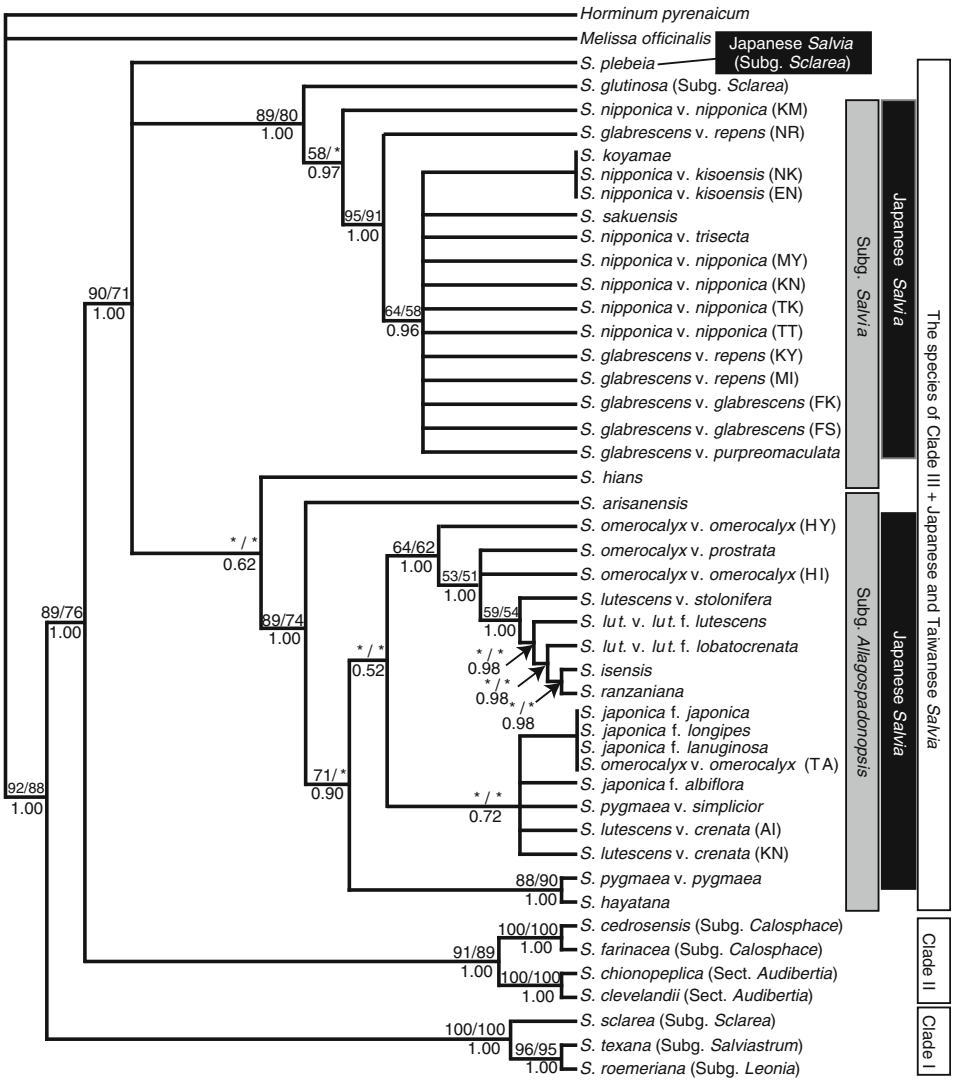

Fig. 1 A strict consensus tree of eight most parsimonious trees derived from the cpDNA (*rbcL* + *trnL-F*) datasets, CI = 0.834, RI = 0.901. MP/ML bootstrap support is shown above branches, and Bayesian PP numbers are shown below. *Asterisk* indicate ≤50% support in selected analyses. Clades I, II, and III are sensu Walker and Sytsma (2007). Subg., subgenus; *S.*, *Salvia; S. lut.* v. *lut*. f. *lutescens*, *S. lutescens* var. *lutescens* f. *lutescens*; *S. lut.* v. *lut* f. *lobatocrenata*, *S. lutescens* var. *lutescens* f. *lobatocrenata*

support in Fig. 2. The species of clade III sensu Walker and Sytsma (2007) plus Japanese and Taiwanese *Salvia* consisted of a strongly supported clade. *Salvia plebeia* was sister to all other species. As in the cpDNA tree, *S. glabrescens* and its varieties, *S. koyamae*, *S. nipponica* and its varieties, and *S. sakuensis* were grouped with relatively low support in a clade with several species of clade III sensu Walker and Sytsma (2007) (=*S. digitaloides*, *S. flava*, *S. glutinosa*, *S. hians*, *S. przewalskii*, *S. trijuga*). The sequences of *Salvia glabrescens*, *S. nipponica* and their varieties were highly similar to each other (95.8–99.2% identical). The species belonging to subg. *Allagospadonopsis* and two Chinese species of clade III sensu Walker and Sytsma (2007), *S. miltiorrhiza* and *S. yunnanensis* (in subg. *Sclarea*) formed a well-supported clade. Within the clade, the two Chinese species were sister to other species, and the species in subg. *Allagospadonopsis* formed a clade with relatively low support. In this clade, *S. lutescens* and its varieties, *S. omerocalyx* and its variety, *S. isensis* and *S. ranzaniana* comprised a subclade with rather low support. Individuals of *S. lutescens* and its varieties were scattered within the subclade, intercalated with *S. isensis*, *S. omerocalyx* and *S. ranzaniana*. Two Taiwanese species, *S. arisanensis* and *S. hayatana*, were sister to this subclade; *S. japonica* and its formas, and *S. pygmaea* and its variety formed a subclade sister to the remaining species of *Allagospadonopisis*.

The combined cpDNA and nrDNA datasets for 47 individuals in 36 taxa contained 2,823 characters, and 285 of these were parsimony-informative. Parsimony analysis produced 26,058 most parsimonious trees of 923 steps, a consistency index (CI) of 0.681 and a retention index (RI) of 0.810. Likelihood analysis resulted in an ML tree with –ln *L* = 9,561.679. The MP strict consensus, ML, and Bayesian trees had the same topology, and the MP tree is shown with bootstrap and PP support in Fig. 3. The species

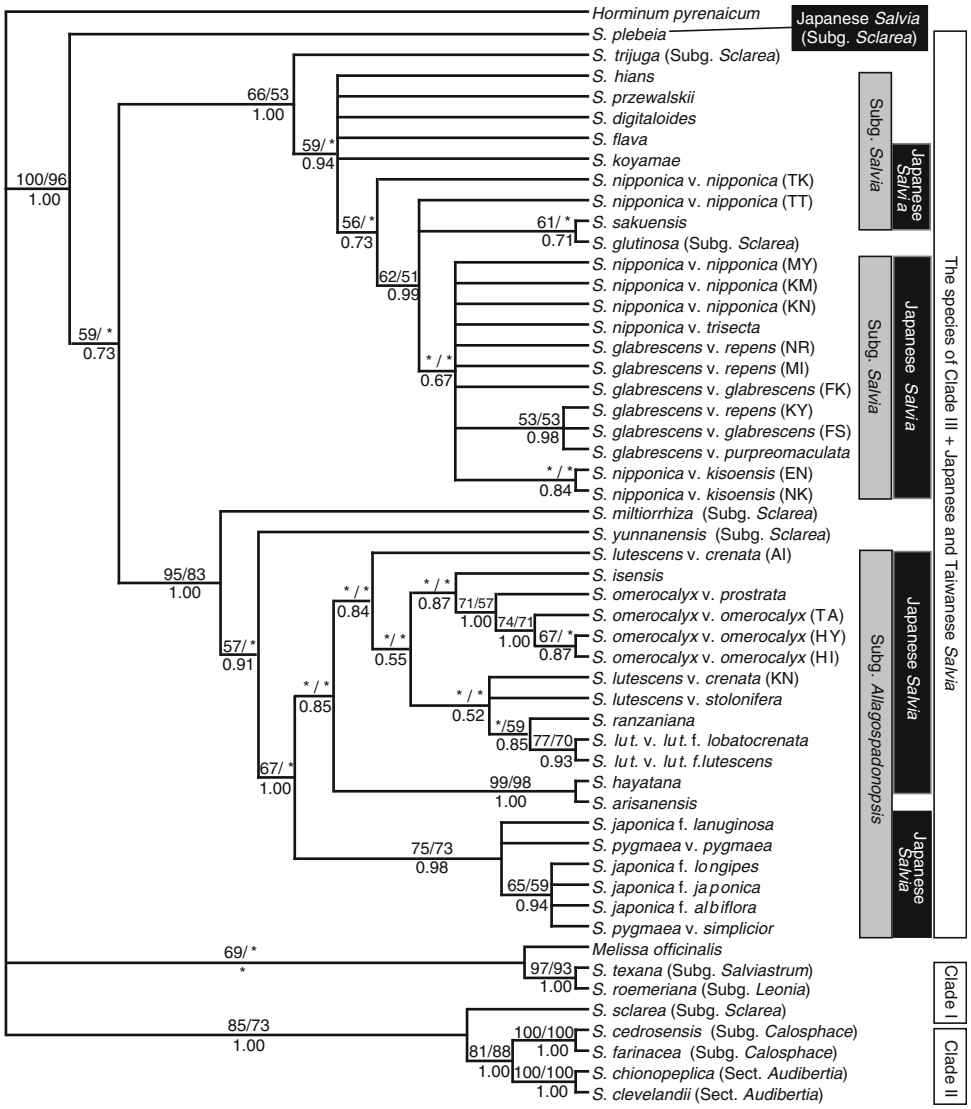

Fig. 2 A strict consensus tree of 2,795 most parsimonious trees derived from the ITS dataset, CI = 0.618, RI = 0.791. MP/ML bootstrap support is shown above branches, Bayesian PP numbers are shown below. *Asterisk* indicate ≤50% support in selected analysis. Clades I, II, and III are sensu Walker and Sytsma (2007). Subg., subgenus; *S.*, *Salvia*, *S. lut.* v. *lut.* f. *lutescens*, *S. lutescens* var. *lutescens* f. *lutescens*; *S. lut.* v. *lut.* f. *lobatocrenata*, *S. lutescens* var. *lutescens* f. *lobatocrenata*

of clade I sensu Walker and Sytsma (2007) were sister to all other *Salvia*, and the species of clade II were sister to the species of clade III plus Japanese and Taiwanese *Salvia*. *Salvia glutinosa* and *S. hians,* species of clade III, comprised a strongly supported clade with Japanese *Salvia* subg. *Salvia*, i.e., *Salvia glabrescens* and its varieties, *S. koyamae*, *S. nipponica* and its varieties, and *S. sakuensis*, and were sister to Japanese *Salvia*. Among Japanese *Salvia, S. nipponica* var. *nipponica* from Kumamoto was sister to the others. *Salvia plebeia* was sister to the species of subg. *Allagospadonopsis. Salvia omerocalyx* and its variety were sister to all the other taxa, and the two Taiwanese *Salvia* were sister to the rest. *Salvia lutescens* and its varieties, *S. isensis* and *S. ranzaniana* comprised a subclade with rather low support, and *S. japonica* and its formas and *S. pygmaea* and its variety formed another subclade.

Discussion

Our phylogenetic analyses of Japanese species, two Taiwanese species, and species of clade I, II, and III sensu Walker and Sytsma (2007) using cpDNA, nrDNA, and a combined dataset showed that all Japanese species and the two Taiwanese species of *Salvia* formed a well-supported clade with the *Salvia* species of clade III (Figs. 1, 2, 3). Thus, Japanese and Taiwanese *Salvia* may belong to clade III, as proposed by Walker and Sytsma (2007).

The results of our molecular phylogenetic analysis generally supported Murata and Yamazaki's (1993) system, which divided Japanese *Salvia* into three subgenera. The species belonging to subg. *Salvia* (*S. glabrescens, S. koyamae, S. nipponica, S. sakuensis*) were included in the same phylogenetic clade, the species of subg. *Allagospadonopsis* formed another clade, and *S. plebeia* (subg.

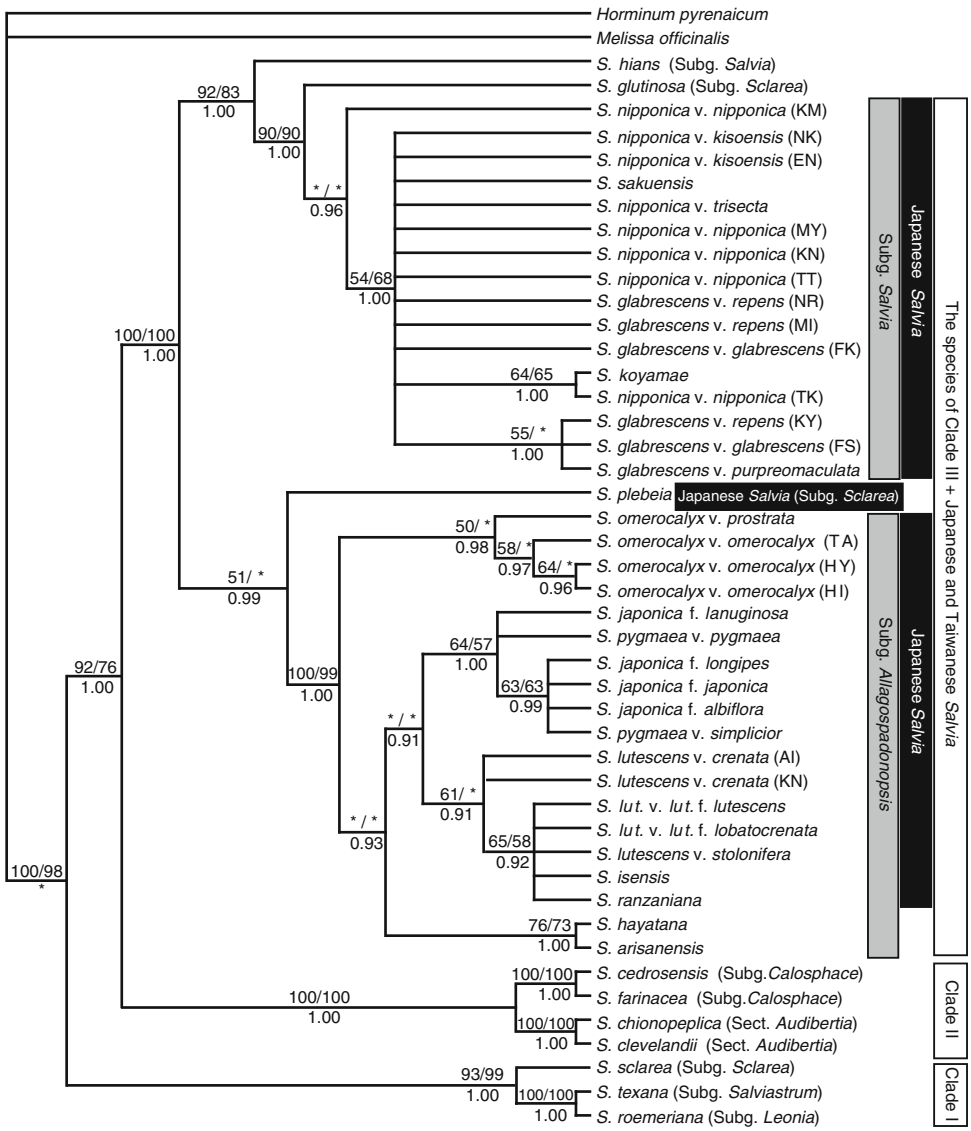

Fig. 3 A strict consensus tree of 26,058 most parsimonious trees derived from the combined cpDNA (*rbcL* + *trnL-F*) and nrDNA (ITS) datasets CI = 0.681, RI = 0.810. MP/ML bootstrap support is shown above branches, and Bayesian PP numbers are shown below. *Asterisk* indicate ≤50% support in that analysis. Clades I, II, and III are sensu Walker and Sytsma (2007). Subg., subgenus; *S., Salvia; S. lut.* v. *lut*. f. *lutescens*, *S. lutescens* var. *lutescens* f. *lutescens*; *S. lut.* v. *lut.* f. *lobatocrenata*, *S. lutescens* var. *lutescens* f. *lobatocrenata*

Sclarea) fell outside both these clades in all cpDNA, nrDNA, and cpDNA+nrDNA trees. However, monophyly of subg. *Salvia* and *Sclarea* were not supported. *Salvia glutinosa*, a member of subg. *Sclarea,* was sister to all the species belonging to subg. *Salvia* in our cpDNA and cpDNA+nrDNA trees (Figs. 1, 3), and formed a subclade together with *S. sakuensis* in the *Salvia* clade of our nrDNA tree (Fig. 2). The monophyly of species belong to subg. *Allagospadonopsis* are supported in cpDNA and cpDNA+nrDNA trees (Figs. 1, 3), however, the BS/PP support of the *Allagospadonopsis* clade in nrDNA tree was weak (Fig. 2). In our nrDNA tree, *S. miltiorrhiza* and *S. yunnanensis* (i.e., subg. *Sclarea* species) formed a well-supported clade that included species of subg. *Allagospadonopsis*. There might remain the possibility of monophyly of subg. *Allagospadonopsis*, but further analyses increasing taxa and data are needed to confirm it.

The status of some species is also questionable. For example, all individuals of *S. nipponica* and *S. glabrescens* (and their varieties) fell into the same unresolved clade in both cpDNA and nrDNA trees, with a few exceptions, because of high similarity of their sequences (99.6–99.8% in cpDNA and 95.5–99.2% in nrDNA). This supports Inoue and Ozawa's (1998) claim that the two species were conspecific. However, a proposal to elevate *S. nipponica* var. *kisoensis* to species rank was not supported by our analysis. Furthermore, a geographic differentiation trend suggested by Inoue and Ozawa (1998) was not apparent in our data, although genetic differentiation might have occurred in some populations, e.g. *S. nipponica* var. *nipponica* from Kumamoto (cpDNA and cpDNA+nrDNA trees Figs. 1, 3) and Tokushima (nrDNA tree, Fig. 2).

Monophyly of *S. lutescens* and its varieties is also problematic. In our cpDNA tree, *S. isensis* and *S. ranzaniana*

were included in the *S. lutescens* clade, while one *S. lutescens* var. *crenata* individual fell into the *S. japonica* clade (Fig. 1). One individual of *S. lutescens* var. *crenata* was sister to *S. isensis*, *S. lutescens* and its varieties, *S. omerocalyx*, and *S. ranzaniana* in the nrDNA tree (Fig. 2). All varieties of *S. lutescens* were gathered into one clade in the cpDNA+nrDNA tree, but at the same time, this clade contained *S. isensis* and *S. ranzaniana* (Fig. 3). Hybridization/introgression may have occurred because the distributions and flowering seasons of *S. japonica* and *S. lutescens* partly overlap. It is clear that taxonomic revision of *S. lutescens* will require more information on morphological characters, habitat, and ecological niches.

Unexpectedly, *S. pygmaea* var. *simplicior* was included in the clade containing *S. japonica* in all cpDNA, nrDNA, and cpDNA+nrDNA trees, suggesting close affinity between the two taxa (Figs. 1, 2, 3). Distributions of *S. japonica* and *S. pygmaea* var. *simplicior* do not overlap; the former is distributed in Honshu, Shikoku, and Kyushu southward to Yaku-shima Island, Taiwan and China, but is absent from the island chain from Amami-oshima Island to Iriomote Island, while *S. pygmaea* var. *simplicior* is found only on Amami-oshima Island and Tokunoshima Island. Thus, hybridization/introgression between these two taxa seems unlikely, at least in recent times. Morphologically, the taxa are readily distinguishable: the calyx tube of *S. japonica* is larger and has long pilose hairs on the upper half of the inner tube, and its leaves are generally radical and cauline; the calyx tube of *S. pygmaea* var. *simplicior* is puberulent on all inner surfaces, and leaves are all radical (Murata and Yamazaki 1993). However, *S. pygmaea* var. *simplicior* has been little studied since it was formally established in 1993 by T. Yamazaki. Further comparative examinations of *S. japonica* and varieties of *S. pygmaea* (var. *pygmaea* and var. *simplicior*) are required to determine taxonomic status.

Our molecular analyses revealed several taxonomic issues warranting additional study in Japanese *Salvia*: the non-monophyletic nature of *S. lutescens* and its varieties, and the close relationships between *S. glabrescens* and *S. nipponica,* as well as between *S. japonica* and varieties of *S. pygmaea.* Further analyses, e.g., using population genetic markers, should provide a better understanding of evolutionary history in Japanese *Salvia*.

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