Taxonomic study on Japanese *Salvia* (Lamiaceae): Phylogenetic position of *S. akiensis*, and polyphyletic nature of *S. lutescens* var. *intermedia*

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Abstract
Both *Salvia akiensis* and *S. lutescens* (Lamiaceae) are endemic to Japan. *Salvia akiensis* was recently described in 2014 in the Chugoku (= SW Honshu) region, and each four varieties of *S. lutescens* distributed allopatrically. Among varieties in *S. lutescens*, var. *intermedia* show a disjunctive distribution in the Kanto (=E Honshu) and Kinki (= W Honshu) regions. Recent field studies of *S. lutescens* var. *intermedia* revealed several morphological differences between the Kanto and Kinki populations. Here, I evaluated these differences among *Salvia lutescens* var. *intermedia* and its allies with morphological analysis and molecular phylogenetic analyses of nuclear ribosomal DNA (internal and external transcribed spacer regions) and plastid DNA (*ycf1-rps15* spacer, *rbcL*, and *trnL-F* sequences). Both morphological analysis and molecular phylogenetic analyses showed that *S. lutescens* var. *intermedia* from the Kinki region and var. *lutescens* were closely related to each other. However, var. *intermedia* from the Kanto region exhibited an association with *S. lutescens* var. *crenata* and var. *stolonifera*, which also grew in eastern Japan, rather than var. *intermedia* in the Kinki region. These results indicated that *S. lutescens* var. *intermedia* is not a taxon with a disjunctive distribution, but a combination of two or more allopatric taxa. Present study also suggested that *S. akiensis* was most closely related to *S. omerocalyx*.

Keywords
cpDNA, Lamiaceae, nrDNA, Phylogenetics, *Salvia akiensis*, *Salvia lutescens*
Introduction

The genus *Salvia* L. (tribe Mentheae) is the largest genus in Lamiaceae; it comprises nearly 1,000 species. *Salvia* has radiated extensively into three regions of the world: Central and South America (500 spp.), West Asia (200 spp.), and East Asia (100 spp.) (Alziar, 1988–1993). In Japan, twelve species, eight varieties, and one putative hybrid have been described since Thunberg’s (1784) first account. The genus was classified into three subgenera (subg.), including *Allagospadonopsis* Briq., *Salvia*, and *Sclarea* (Moench) Benth, (Hihara et al. 2001, Inoue 1997, Murata and Yamazaki 1993, Takano et al. 2014). Most of the taxa are endemic to Japan, with the exception of *S. japonica* Thunb., *S. nipponica* Miq. and *S. plebeia* R. Br. (Murata and Yamazaki 1993).

There are four varieties known in *S. lutescens* (Koidz.) Koidz.: var. *crenata*, var. *intermedia*, var. *lutescens*, and var. *stolonifera* (Murata 1952, Yonekura and Kajita 2003 onwards). Fukuoka and Kurosaki (1982) noticed distribution of each taxon does not overlap and clarified that the distribution of var. *crenata* on the Japan Sea side of Central to Northern Honshu, var. *stolonifera* on the Pacific side of Central Honshu, var. *lutescens* around the Suzuka Mountain range (Mie Pref., W Honshu), and the disjunctive distribution of var. *intermedia* in the Kanto (E Honshu) and Kinki regions (W Honshu) based on herbarium works.

Takano and Okada (2011) conducted molecular phylogenetic analyses of Japanese *Salvia* and found that the species were distributed among three subclades: (1) *S. plebeia* (subg. *Sclarea*), (2) subg. *Salvia*, and (3) subg. *Allagospadonopsis*. They also found four varieties of *S. lutescens* that did not form a monophyletic group; instead, they were dispersed among several clades in phylogenetic trees, based on both plastid DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) data, and their topologies were not concordant with each other. In addition, they became paraphyletic in the phylogenetic trees based on combined cpCNA and nrDNA data (Takano and Okada 2011). Furthermore, during a recent field survey, I noticed that *S. lutescens* var. *intermedia* in the Kanto and Kinki regions had different morphological characteristics. The basal part of the anther connective was generally glabrous in the Kanto population, but it was pilose in the Kinki population. Also, in the Kanto population, the stalk of the inflorescence declinated toward the ground after flowering, and it typically became proliferous; in contrast, in the Kinki population, the inflorescence grew erect, and it was never proliferous.

Recently, a new species of Japanese *Salvia*, *S. akiensis* A.Takano, T.Sera et Kurosaki has been described from Shimane and Hiroshima Prefectures (Takano et al. 2014). At the moment, this species shows disjunctive distribution, ca. 40 km away from each, and the habitat is also very different between Hiroshima and Shimane: it grows among bamboo by roadsides and on slopes below evergreen mixed forests and plantations in Shimane (Sakoda et al. 2014), but it is found in moist, shallow soil on rock walls by streams in deciduous forests in Hiroshima (Takano et al. 2014). Therefore, it may wonder if the species be monophyletic. Takano et al. (2014) discussed relationships among *S. akiensis*, *S. isensis* Nakai ex H.Hara, *S. lutescens* and *S. omerocalyx* Hayata.
based on morphological characters, but molecular phylogenetic position of S. akiensis remains unclear.

As a step toward taxonomic revision of variety of S. lutescens and to confirm monophyly and phylogenetic position of S. akiensis, morphological and molecular phylogenetic analyses were conducted. Takano and Okada (2011) followed the Murata and Yamazaki (1993) system in which treated var. intermedia as a forma f. lobatocrenata and var. lutescens as f. lutescens, however, here I follow the Murata (1952) system (=Y-list, Yonekura and Kajita 2003 onwards), and each infraspecific taxon of S. lutescens is treated as a variety.

**Materials and methods**

**Morphological analyses on S. lutescens in herbaria**

Murata (1952) studied morphological variations in the plants of subgen. Allagospadonopsis in Japan and found hairiness, number or shape of leaflets, presence/absence of glandular hairs were so variable and could not be used as diagnostic characters. Diagnostic characters separated each variety of S. lutescens are indumentums of the basal part of the anther connective and floral color (Nakai 1950, Murata 1952). Among varieties, var. lutescens shows pale yellow flowers and pilose at the base of anther connective, var. intermedia shows deep violet corolla and pilose at the base of anther connective, var. crenata does purple corolla and glabrous base of anther connective. Floral color and indumentums of var. stolonifera is same as var. crenata, however, var. stolonifera extends its stolon after anthesis (Nakai 1950). Since it is difficult to know exact floral color by examining dry specimens, the indumentums at the base of the anther connective were observed for glabrousity in selected specimens, which bore at least several flowers. A total of 89 specimens of S. lutescens var. intermedia, including its syntypes, of the 34 specimens are from Kanto region and 55 from Kinki, were examined in the following herbaria: the Museum of Nature and Human Activities, Hyogo (HYO); the Kanagawa Prefectural Museum (KPM); Kyoto University (KYO); Tokyo Metropolitan University (MAK), the Osaka Museum of Natural History (OSA), and The University of Tokyo (TI) (Appendix 1). Additionally, all the specimens of S. lutescens var. lutescens including its holotype at KYO were examined on the same characters, since no information on that character is available.

**DNA extraction, PCR, and DNA sequencing**

The protocols for DNA extraction, polymerase chain reaction (PCR), purification, and DNA sequencing were described previously by Takano and Okada (2011). The PCR conditions and the PCR and sequencing primers for rbcL, the trnL-F intergenic spacer region of cpDNA (trnL-F), and the internal transcribed spacer (ITS) region of nuclear
ribosomal DNA (nrDNA) were also described previously by Takano and Okada (2011). To amplify in the ycf1-rps15 spacer region found in cpDNA (ycf1-rps15), 5711f and rps15r (both from Drew and Sytsma 2011) were used as PCR primers in PCR assays, and ETS-bdf1 (Drew and Sytsma 2011) and 18S-E (Baldwin and Marks 1998) were used to amplify the external transcribed spacer (ETS) sequence from 18S-26S ribosomal DNA. The four PCR primers were also used for sequencing. The PCR conditions for amplifying the two loci were: denaturation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min.

Sequence alignment and phylogenetic analysis

Raw sequence data were assembled and edited manually, with BioEdit software (ver. 7.2.5 Hall 1999).

DNA sequences were aligned with the CLUSTALW 1.83 software package, with default settings and multiple alignments (Thompson et al. 1994). Alignments of the rbcL, trnL-F, and ycf1-rps15 sequences of cpDNA, and the ITS and ETS sequences of nrDNA were combined. Gaps were deleted.

Compared to Takano and Okada (2011), the ETS (Baldwin and Marks 1998) and ycf1-rps15 of cpDNA (Dong et al. 2015) were newly sequenced for all samples. Further, two individuals of S. akiensis and three of S. lutescens var. intermedia, three of S. lutescens var. crenata, and one each of S. isensis, S. japonica var. japonica, S. lutescens var. lutescens, and S. plebeia were newly added for the analysis. The sampling sites of S. lutescens group were shown in Fig. 1. A total of 36 individuals of Salvia were used, including all the Salvia taxa from Japan and one Taiwanese Salvia (S. arisanensis Hayata). Salvia polystachya M. Martens et Galeotti and S. plebeia were selected as outgroup; the former species belonged to clade II sensu Maria and Classen-Bockhoff (2014), which became a sister to group IV and contained the East Asian Salvia; the latter species became a sister to a species of the subgenus Allagospadonopsis and Salvia (Hu 2015). Materials, accession numbers for the sequences, vouchers, and references to the literature are presented in Table 1. The sampling sites for the varieties of S. lutescens are shown in Fig. 1.

The incongruence length difference (ILD) test (Farris et al. 1994) was used to evaluate congruence between the chloroplast and the nuclear data sets. 100 replications were performed using PAUP*4.010b (Swofford 2002). As the ILD test (P < 0.01) suggested incongruence between the two datasets, and the topologies also exhibited discordance, I performed separate analyses for the cpDNA and the nrDNA data. Maximum Likelihood (ML) and Bayesian inference (BI) were used. Nucleotide substitution model parameters were determined for each partition by gene was evaluated with KAKUSAN 4.0 (Tanabe 2007), and the corrected Akaike information criterion (AICc) (Sugiura 1978) was used for model selection. For the cpDNA partitions KAKUSAN suggested the HKY85 (rbcL) and GTR+G (trnL-F, ycf1-rps15spacer) models, and the HKY85 model for ETS and GTR+G model for ITS for the nrDNA partitions. The ML
analyses were completed using TREEFINDER version March 2011 (Jobb et al. 2004). A replicated (500 iterations) partitioned analysis was performed with bootstrap (1000 rounds) using AICc separated model for nrDNA dataset and AICc proportional model for cpDNA dataset. Bayesian evolutionary analysis using partitioned datasets were run in BEAST v.1.8.3 (Drummond et al. 2012, Heled and Drummond 2010) with 20 million Markov Chain Monte Carlo (MCMC) iterations, under an uncorrelated relaxed clock (Drummond et al. 2006), Yule process of speciation with a random starting tree for each partition. Convergence of the chains was checked using the program Tracer 1.6 (Rambaut et al. 2014). High effective sample sizes were observed for all parameters (posterior ESS values > 200 for the combined analyses). Maximum clade credibility trees with divergence times means and 95% highest probability densities (HPDs) were produced using Tree Annotator (Drummond et al. 2012).

Results

Morphological characteristics

Among the 89 specimens of *S. lutescens* var. *intermedia* examined, 52 specimens from the Kinki region were pilose at the base of the anther connective (Fig. 2), and no speci-
| Name | Pop. Code | rbcL | trnL-F | ycf1-rps15 | ETS | ITS | Voucher / References |
|------|-----------|------|--------|------------|-----|-----|---------------------|
| S. akiensis | A.Takano, T.Sera et Kurosaki | LC124176 | LC124188 | LC060729 | LC060835 | LC060730 | A.Takano and N.Kurosaki with T.Sera 130606-1 (HYO) |
| S. arisanensis | Hayata | LC124177 | LC124189 | LC060731 | LC060836 | LC060731 | M.Sakoda et al. 1 (HYO, KYO) |
| S. glabrescens | (Franch. et Sav.) Makino | LC124178 | LC124190 | LC060732 | LC060831 | LC060731 | A.Takano 140622-2 (HYO) |
| var. glabrescens | | | | | | | Originally from Owariasahi city, Aichi Pref. |
| var. repens | (Koidz.) Kurosaki | LC124179 | LC124191 | LC060733 | LC060834 | LC060734 | A.Takano 140606-1 (HYO) |
| S. isensis | Nakai ex Hara | LC124180 | LC124192 | LC060735 | LC060835 | LC060735 | A.Takano 140606-1 (HYO) |
| var. albiflora | Hiyama | LC124181 | LC124193 | LC060736 | LC060836 | LC060736 | A.Takano 140606-1 (HYO) |
| var. japonica | Osaka | LC124182 | LC124194 | LC060737 | LC060837 | LC060737 | A.Takano 140606-1 (HYO) |
| var. longipes | (Nakai) Sugimoto | LC124183 | LC124195 | LC060738 | LC060838 | LC060738 | A.Takano 140606-1 (HYO) |
| S. koyamae | Makino | LC124184 | LC124196 | LC060739 | LC060839 | LC060739 | A.Takano 140606-1 (HYO) |
| S. lutescens | Koidz. | LC124185 | LC124197 | LC060740 | LC060840 | LC060740 | A.Takano 140606-1 (HYO) |
| var. crenata | (Makino) Murata | LC124186 | LC124198 | LC060741 | LC060841 | LC060741 | A.Takano 140606-1 (HYO) |
| var. intermedius | (Makino) Murata | LC124187 | LC124199 | LC060742 | LC060842 | LC060742 | A.Takano 140606-1 (HYO) |
| var. lutescens | Koidz. | LC124190 | LC124200 | LC060743 | LC060843 | LC060743 | A.Takano 140606-1 (HYO) |

**Table 1.** Genbank accession numbers, and voucher specimens/references used in this study. Newly sequenced data are shown bold.
| Name                  | Pop. Code        | rbcL   | trnL-F | ycf1-rps15 | ETS       | ITS       | Voucher / References       |
|----------------------|------------------|--------|--------|------------|-----------|-----------|-----------------------------|
| var. stolonifera G.Nakai | AB541139         | AB541153 | LC060551 | LC060847   | AB541125  | Takano and Okada (2011)    |
| S. nipponica Miq.    |                  |        |        |            |           |           |                             |
| var. nipponica       | TOKU (Tokushima) | AB541132 | AB541146 | LC060552   | LC060848  | AB541118  | Takano and Okada (2011)    |
|                      | KUMA (Kumamoto)  | AB541127 | AB541141 | LC060553   | LC060849  | AB541113  | Takano and Okada (2011)    |
| var. kisoensis Imai  | NAK              | AB541136 | AB541150 | LC060554   | LC060850  | AB541122  | Takano and Okada (2011)    |
| S. omerocalyx Hayata |                  |        |        |            |           |           |                             |
| var. omerocalyx      | HI (Hidaka, Hyogo)| AB353204 | AB353196 | LC060555   | LC060851  | AB353200  | Takano and Okada (2011)    |
|                      | HYO (Yabu, Hyogo)| AB353205 | AB353197 | LC060556   | LC060852  | AB353201  | Takano and Okada (2011)    |
| var. prostrata Satake| AB541138         | AB541152 | LC060557 | LC060853   | AB541124  | Takano and Okada (2011)    |
| S. pygmaea Matsum.   |                  |        |        |            |           |           |                             |
| var. pygmaea         | AB295072         | AB295083 | LC060558 | LC060854   | AB295094  | Sudarmono and Okada (2007) |
| var. simplicior Hatus. ex T.Yamaz. | AB541140 | AB541154 | LC060559 | LC060855   | AB541126  | Takano and Okada (2011)    |
| S. mizumiana Makino  | AB287373         | AB287374 | LC060560 | LC060856   | AB287375  | Sudarmono and Okada (2007) |
| S. ×sakwensis Naru. et Hihara | AB541129 | AB541143 | LC060561 | LC060857   | AB541116  | Takano and Okada (2011)    |
| Outgroup             |                  |        |        |            |           |           |                             |
| S. plebeia R.Br.     | KIZU             | AB295073 | AB295084 | LC060563   | LC060858  | AB295095  | Sudarmono and Okada (2007) |
|                      | KAMI             | LC124187 | LC124199 | LC060562   | LC060859  | LC060738  | A.Takano and N.Kurosaki 090607-2 (HYO) |
| S. Pohystachya M.Martens et Galeotti | AY570435 | JF301399 | JF289067 | JF301334   | JF301356  | Drew and Systma (2011)     |
Photographs of *S. lutescens* var. *intermedia* flowers. **a** Flower of *A. Takano 140806-4-2* (HYO), from Mt. Mikuni, Susono-shi, Shizuoka Pref. (Kanto region). Arrows indicate the base of the anther connective. No hairs are visible. **b** Flower of *A. Takano 140813-1* (HYO), from Mt. Yamatokatsuragi, Gose-shi, Nara Pref. (Kinki region). The red open circle indicates the base of the anther connective. White hairs are visible.

Ten specimens collected from the Kanto region had at least one, but less than 10 hairs. Twenty-four specimens from the Kanto region (Fig. 2) and three specimens from the Kinki region (Y. Kato s.n. [KYO], T. Kobayashi 23369 [KYO], and A. Takano 140821-1 [HYO]) were glabrous at the base of the anther connective. However, a duplicate of T. Kobayashi 23369 (KYO) examined at HYO was pilose at the base of the anther connective (Appendix 1).

Totally, 18 specimens of *S. lutescens* var. *lutescens* were deposited at KYO and examined, 13 of these had pilose at the base of the anther connective (Appendix 1). Four of these had no flowers, and only one specimen, M. Hara s.n., collected from Mt. Takami, Maze-Mura, Iinan-gun, Mie Pref. showed glabrousity.

**Phylogenetic positions of Japanese taxa in the genus Salvia**

A likelihood analysis using the concatenate cpDNA datasets (*rbcL*+*trnL-F*+*ycf1-rps15* spacer) for 36 individuals in 23 taxa resulted in a ML tree with $-\ln L = 5295.264$. The ML and Bayesian trees had similar topology; the Bayesian maximum clade credibility tree is shown with ML bootstrap (ML-BS) and Bayesian posterior probability (BI-PP) in Figure 4. The Japanese and Taiwanese species of subg. *Allagospadonopsis* formed a well supported clade (ML-BS/BI-PP, 100/0.97). Two subclades were found in the subg. *Allagospadonopsis* clade: (1) *S. japonica* + *S. pygmaea* + one *S. akiensis* + *S. arisanensis* + five individuals of *S. lutescens* in E Japan subclade, and (2) one *S. akiensis* (S1), two *S. isensis*, *S. lutescens* in Kinki + *S. ranzaniana* + two *S. lutescens* in the Kanto region.
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![Figure 3](image.png)

*Figure 3.* A graph shows the number of specimens examined indumentums at the base of anther connective.

+ *S. omerocalyx*. The latter group of taxa, minus the *S. omerocalyx* (HYO), consisted of a strongly supported subclade, with high ML-BS/BI-PP values (98/0.99). *S. lutescens* in E Japan were scattered between both subclades, but the *S. lutescens* in the Kinki region consisted a cluster with the weak support (--/0.70).

A concatenate nrDNA datasets (ETS+ITS) yielded a ML tree with $-\text{lnL} = 3789.114$. The ML and Bayesian trees had similar topology; the Bayesian maximum clade credibility tree is shown with ML-BS and BI-PP in Figure 5. The Japanese and Taiwanese species of subg. *Allagospadonopsis* formed a well supported clade (ML-BS/BI-PP, 100/1.00). There were four subclades in the *Allagospadonopsis* clade: (1) *S. lutescens* group in E Japan + *S. isensis* (ML-BS/BI-PP, --/0.69), (2) *S. lutescens* in Kinki + *S. ranzaniana* (ML-BS/BI-PP, 61/0.57), (3) *S. arisanensis* + *S. omerocalyx* + *S. akiensis* (ML-BS/BA-PP, 76/0.99), and (4) *S. lutescens* var. *crenata* + *S. japonica* + *S. pygmaea* (ML-BS/BA-PP, 58/0.97). Thus, *Salvia lutescens* and its allies apparently became polyphyletic. *Salvia ranzaniana* became a sister group to *S. lutescens* in the Kinki region but the ML-BS /BA-PP support was weak (61/0.57). *Salvia isensis* became a sister group to *S. lutescens* in the Kanto region with strong ML-BS/BA-PP support (86/1.00). *Salvia akiensis* formed a strongly supported subclade with *S. omerocalyx* group (89/1.00).
Figure 4. The Bayesian maximum clade credibility tree derived from plastid DNA (concatenate dataset of rbcL, trnL-F, ycf1-rps15). ML-bootstrap/Bayesian PP numbers are shown near the corresponding branch. Thick lines denote a clade that was strongly supported, with ML-bootstrap and/or Bayesian-PP greater than 95%. ML: maximum likelihood; PP: posterior probability.

Discussion

This study suggests that S. lutescens var. intermedia is polyphyletic. Four individuals of var. intermedia, two from the Kanto and two from the Kinki region fell into different subclades in both molecular phylogenetic trees using cpDNA and nrDNA datasets, although the two from the Kinki region were always in the same subclade (Figs 4, 5). The plants of var. intermedia from the Kanto region (Tanzawa and Mt.Mikuni) fell into the same subclade in the nrDNA tree together with var. crenata, var. stolonifera,
Figure 5. The Bayesian maximum clade credibility tree derived from nuclear ribosomal DNA (concatenate dataset of ETS and ITS). ML-bootstrap/Bayesian-PP numbers are shown above or below the corresponding branch. Thick lines denote a clade that was strongly supported with ML-bootstrap and/or Bayesian-PP values greater than 95%. ML: maximum likelihood; PP: posterior probability.

and S. isensis whereas they fell into different subclades in the cpDNA tree. Such a contradiction might indicate that var. intermedia from the Kanto region have multiple origins, or might have originated via hybridization or introgressive gene flow between neighbouring taxa (e.g., Sudarmono and Okada 2007). The discordance between nrDNA and cpDNA data is common in the mint family (Trusty et al. 2004, Moon et al. 2010, Drew and Sytsma 2013, Deng et al. 2015), and chloroplast-based phylogeny often does not reflect their morphological relationships, which can be explained by
chloroplast capture (Rieseberg and Soltis 1991). Morphological analysis also supports the contention that var. intermedia is polyphyletic, as the specimens of var. intermedia studied showed in the indumentums at the base of the anther connective: pilose in the plants from the Kinki region, and glabrous in the plants from the Kanto region (Fig. 3). Therefore it is clear that var. intermedia from the Kinki region and the taxon from the Kanto region are different entities, suggesting that var. intermedia is not a taxon that shows disjunctive distribution, but is instead admixture of two or more biological entities. Additionally, as mentioned in introduction, after flowering the stalk of the inflorescence becomes declinate to ground and usually proliferous in case of the plants from the Kanto region, but never become declinate in the plants from the Kinki region. The indumentums at the base of anther connective is effective to select pollinators to avoid intrusion of insects which could not be effective pollinators (R.Classen-Bockhoff pers. Comm.) However, pollinators of var. intermedia in the Kinki and the Kanto region are not different (=Bombus (Diversobombus) diversus diversus, some Halictidae, and Syrphidae. Takano 2017). Habitat is also similar: half-shaded, on mesic soils along streamlet on the forest floor of deciduous forests. They might have begun to be diverged from each other after long geographical isolation.

On the contrary, present morphological and molecular phylogenetic analyses indicated that S. lutescens var. lutescens and var. intermedia from the Kinki region are closely related to each other. In molecular phylogenetic analysis, they formed a cluster in both cpDNA- and nrDNA trees, though ML-BP/BI-PP support was not strong in cpDNA tree. The morphological study revealed var. lutescens is pilose at the base of the anther connective: therefore, S. lutescens var. intermedia in the Kinki region share the same morphological status with var. lutescens. The distribution of var. lutescens is very near to that of var. intermedia in the Kinki region (Mie, Shiga, Nara Pref.s.), although var. lutescens and populations of the Kinki regions of var. intermedia have never been found to grow together.

Salvia lutescens var. intermedia in the Kanto region may be more closely related to var. crenata and var. stolonifera. Murata (1952) mentioned that the base of anther connective is glabrous in var. stolonifera and var. crenata. The present study revealed that var. intermedia in the Kanto region shares this character with those two taxa. Salvia lutescens var. intermedia in the Kanto region formed a strongly supported suclade with var. crenata, var. stolonifera and S. isensis in nrDNA phylogenetic tree. In the cpDNA phylogenetic tree, S.lutescens var.intermedia from the Kanto region (Mt.Mikuni) was included in the subclade containing S. akiensis, S. japonica, S. lutescens var. crenata, and S. pygmaea whereas S. lutescens var. intermedia (Tanzawa) formed a subclade with var. stolonifera and was included in the subclade containing S. akiensis, S. omerocalyx, S. ranzaniana, and S.lutescens var. intermedia from the Kinki + S. isensis. These findings suggest a close relationship among var. crenata, var. stolonifera, and var. intermedia from the Kanto region. Var. intermedia from the Kanto region may belong to var. stolonifera and var. crenata. The identity of var. intermedia and other varieties of S. lutescens are needed to be re-evaluated, and further study is necessary towards revision of varieties of S. lutescens.
The phylogenetic analyses also suggest that *S. akiensis* comprises a monophyletic group, as indicated by nrDNA tree, and that most of the species allied to *S. akiensis* was the *S. omerocalyx* group. *Salvia akiensis* and *S. omerocalyx* group comprised a subclade in nrDNA (ML-BS/BI-PP: 89/1.00). These two taxa did not form a subclade in cpDNA, but it may be of introgression/chloroplast capture /hybridization as mentioned above. In contrast, *S. akiensis* and *S. omerocalyx* share following characters: bearing the largest flowers among species in the subg. *Allagospadonopsis*, flower from May to June, and exhibit gynodioecy (Takano 2013; Takano et al. 2014). These characters are assumed to be synapomorph.

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Appendix I

Specimens examined *Salvia lutescens* var. *intermedia*. And var. *lutescens*.

*Salvia lutescens* var. *intermedia*

Specimens glabrous at base of anther connective (27 sheets)

**KANTO Region. Yamagata Pref.**: Mt. Kushigata, *S. Kigawa s.n.*, July 10, 2001 (KPM NA0151444, NA015445). **Ibaragi Pref.**: Tsukuba, *C. Owatari s.n.*, July 8, 1895 (syntype, TI); Ibidem, *C. Owatari s.n.*, July 25, 1895 (syntype, TI). **Gunma Pref.**: Mt. Akagi, *H. Hara s.n.*, July 12, 1928 (TI); Akagi, Shikisimadori, Chubu, *H. Hara s.n.*, 11 July 1928 (TI), Kouzuke, Tone, Yunogoya-daira, *H. Hara s.n.*, 13 July, 1928(TI); Joshu, Tone-gun, Tokura, *S. Saito 145* (TI). **Kanagawa Pref.**: Hakone, *S. Ohkubo s.n.*, July 26, 1881 (syntype TI); Ibidem, unknown collector, July 26, 1881 (syntype, TI); ibidem, *S. Tamaki s.n.*, July 14, 1914 (TI); Minami pass, Hakone, *T. Makino 62582* (KYO); Yoduku, Yamakita-cho, *T. Katsumayama et al.*, July 23, 2005 (KPM NA0124794); Mikuniyama-rindo, Hakone, *Ioue et al.*, June 18, 1998 (KPM NA0112995); Tanzawa-Ohyama, *T. Nishio 1489* (KPM); Summit of Mt. Kintoki, Hakone, *S. Kigawa s.n.*, July 3, 1980 (KPM NA1020531); Ishigoya-Ochiai, Kiyokawa-mura, *H. Takahashi 20565* (KPM); Ishigoya-Ochiai, Kiyokawa-mura, *H. Takahashi 20565* (KPM); Minesaka Pass to Myojin Pass, Yamakita-cho, *S. Mori 20536* (KPM); Kaminokawa, Tsukui-machi, *S. Kigawa 20559* (KPM); Kurokura, Yamakita-machi, *M. Furuse 45371* (KPM); Ogawadani, Yamakita-cho, *A. Takano 140622-2* (HYO). **Shizuoka Pref.**: Mt.Mikuni, Fukayoshi, Susono-shi, *A. Takano 140806-4-1* (HYO); ibidem, *A. Takano 140806-4-2* (HYO).

**KINKI Region. Nara Pref.**: Yoshino, Yamato, *Y. Kato s.n.*, Aug. 8. 1936 (KYO). **Hyogo Pref.**; Taki-gun, Nishiki-cho, *T. Kobayashi 23369* (KYO). **Shiga Pref.**: Ikadachi-tochu, *A. Takano 140821-1* (HYO).

Specimens showed one to several hairs at base of anther connective (10 sheets)

**KANTO Region. Pref.Sizuoka**: Mt. Higane, Prov. Izu, *S. Shimazu s.n.*, July 18, 1920(KPM); **Pref.Kanagawa**: Sirogane rindo, Yigawara-cho, *Y. Hasegawa 14263* (KPM); Ogawadani-rindo, Yamakita-cho, *T. Katsumaya s.n.*, Aug. 22, 1995 (KPM NA0100397); Marudake, Hakone, *M. Tashiro s.n.*, July 18, 1956 (KPM NA0157166); Tounomine, Hakone, *T. Deguchi 80495* (KPM); Tougadake, Yamakita-cho, *S. Kigawa s.n.*, July 3, 1980 (KPM NA1020531); Ishigoya-Ochiai, Kiyokawa-mura, *H. Takahashi 20564* (KPM), Mt.Ohmuro, Tsukui-machi, *S. Kigawa s.n.*, June 10, 1979 (KPM NA1020566); Hayatogawa Rindo, Tsukui-machi, *S. Kigawa 20541* (KPM), Youkizawa, Yamakita-machi, *S. Kigawa 20567* (KPM).
Specimens which showed long pilose at base of anther connective (52 sheets)

KINKI Region. Kyoto Pref.: Raku[hoku, Obara, Otonashi W.F., S.Hajacava s.n., Aug. 1896 (TI), Kyoto, Obara, T.Tsuyama s.n., Sep. 7, 1934 (TI); Kiyotaki-Takao, Ukyo-ku, Kyoto, S.Tsugaru & T.Takahashi 26448 (KYO); Mt.Hyotankuzure-yama, near Ohara, G.Nakai 5401 (KYO); ibidem, G.Nakai s.n., July 25, 1951 (KYO); Kadono-gun, Nakagawa-mura, M.Tagawa 887 (KYO, two sheets); Maesaka Takanomine to Shimosugisaka, S.Okamoto s.n., July 14, 1932 (KYO), Bodai W.F., Nakagawa, G.Nakai 6305 (KYO); Mt. Kibune, unknown collector, June 28, 1921 (KYO); Kyoto-shi, Nakagawa to Bodai no Taki, M.Hutoh 10515 (OSA); ibidem, M.Hutoh 9264 (OSA); ibidem, M.Hutoh 10528 (OSA); ibidem, M.Hutoh 3465 (OSA); Mt.Hiei, S.Tanaka s.n., June 8, 1928 (TI); Kosei River, Ten-kawa Village, K.Seto 44248 (OSA); Mt. Omine to Mt.Sanjogadake, H.Hara 4683 (TI); en route from Wasamata hut to Mt.Nihon-dake, Kamikitayama-mura, M.Okamoto 1966 (OSA); Mt.Daifugendake, T.Kodama 10833 (OSA); Shonoiwaya-Mt.Wasamata Kamikitayama-mura, K.Kodama 14356 (OSA); Irinami, Yamato, S.Sakaguchi s.n., June, 1930 (KYO); Mt.Ohmine, S.Sakaguchi s.n., Aug. 4, 1931 (KYO); en route from Mt.Sanjo to Mt.Daihugen, T.Kodama s.n., Aug. 6, 1959 (KYO); Mt.Sanjo, G.Koizumi s.n., July 13, 1922 (KYO); Mt.Yamatokatsuragi, Gose, A.Takano 140819-1 (HYO). Osaka Pref.: Mt.Izumi-katsuragi, S.Nakanishi s.n., July 30, 1968 (OSA); Ibidem, T.Nakajima s.n., Aug.21, 1952 (OSA); Ibidem, C.Satonaka s.n., July 12, 1981 (OSA). Wakayama Pref.: Ryujin-Mura, Koya, T.Nakajima s.n., July 31, 1931 (two sheets, TI); Doro Hacho, G.Nakai 5213 (KYO); Ibidem, T.Kodama s.n., May 30, 1951 (OSA); ibidem, M.Hori s.n., May 30, 1951 (OSA); Hidaga-gun, Ooze, S.Sakaguchi s.n., July 27, 1932 (KYO); Mt. Sukuyama, Katsuragi-cho, Ito-gun, K.Seto 29839 (KYO, OSA); Mt. Kurosawa, Sayiki-mura, Y.Ogawa s.n., Aug.30, 1950 (KYO). Shiga Pref.: Otsu, N.Takemura s.n., June 1901 (Lectotype MAK); Omi, Tochu, M.Tagashi 1205 (TI); Tochu to Ikadachi, M.Umebayashi 737 (KYO); Mt. Hiei, G.Murata 11415 (KYO); Ukawa, Shiga-cho, M.Tanimoto s.n., June 9, 1973 (KYO); Benzaiten to Sakamoto, Mt.Hiei, S.Tanaka s.n., June 30, 1932 (KYO). Mie Pref.: Wada, Kiwa-cho, Minami-murogun, H.Takahashi 21040 (KYO); Taki-gun, Miyagawa-mura, Shimomate (cult.), K.Seto 17303 (OSA).

Salvia lutescens var. lutescens

Specimens which showed long pilose at base of anther connective (13 sheets)

Mie Pref.: Itaya, Kata, Kameyama, S.Tsugaru & T.Sawada 34155(KYO); Notoyama, Suzuka-gun, T.Hattori s.n., Aug. 5, 1928 (KYO); Kozu-mura, Naga-gun, G.Nakai
4772 (KYO); Shinzan kokuyu-rin, Iinan-gun, Z. Tashiro s.n., 4 Aug., 1934 (KYO), Mt. Gozaisyo, G. Koizumi s.n., 11 Jun. 1922 (KYO); Kozu-mura, Myouga-gun, (cult. at KYO) G. Nakai 5402 (KYO); Ibidem, G. Nakai 4773 (KYO); Nagaishi-dani, Mt. Kamagadake, Komono-cho, Mie-gun, N. Fukuoka 4948 (KYO); Onsen-do, Mt. Gozaisho, G. K. & S. F. s.n., June 1922 (KYO, holotype).

**Shiga Pref.:** Nasugahara, Ohara-Mura, Kouga gun, G. Koizumi s.n., 2 July, 1939 (KYO), Kurotaki, Tsuchiyama-cho, Koga-gun, T. Murase 47897 (KYO); Koga-gun, Suzuka-Pass, H. Koyama & N. Fukuoka 55 (KYO); **Nara Pref.:** Kamide, Momomata, Mitsue-mura, Uda-gun, K. Kawabata 9994 (KYO)