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In Silico Phylogenetic Analysis of Lamiaceae Based on ITS, *matK*, and *rbcL* DNA Barcodes

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Abstract

Lamiaceae, widely used as herbal medicine, is increasingly vulnerable to adulteration driven by market demand, compromising product safety and efficacy. Prevention is challenging due to morphological similarities; thus, DNA-based phylogenetics offer an alternative for accurate species authentication. However, Lamiaceae phylogenetics remain complicated by inconsistencies between morphological and DNA data. This study reconstructed Lamiaceae phylogeny using partial ITS, *matK*, and *rbcL* barcodes to evaluate their potential application in species authentication and adulteration prevention. Sequences for 52 species across 11 genera (*Spathodea campanulata*: outgroup) were obtained from NCBI GenBank, aligned, and trimmed. Four maximum parsimony (MP) trees were constructed in MEGA 11 (three single-barcode, one concatenated). The concatenated dataset was also analyzed by maximum likelihood (ML). Tree robustness was evaluated with bootstrapping, consistency index (CI), and retention index (RI). *matK* had the longest mean sequence (785.6 bp), *rbcL* the highest homology (83.5%), and ITS the most parsimony-informative sites (40.3%). MP trees exhibited moderate homoplasy (mean CI = 0.63) but strong synapomorphic signal (mean RI = 0.83). Individual barcodes produced similar genus groupings, yet misplaced several species. Concatenation corrected these positions across MP and ML trees, resolving six robust monophyletic clades (bootstrap >70%), broadly consistent with earlier phylogenies: *Callicarpa; Scutellaria; Clerodendrum, Lamium, and Stachys; Salvia; Thymus, Origanum, and Mentha; Orthosiphon* and *Ocimum*. Topological discrepancies with prior studies likely reflect differences in barcode choice and taxon sampling. Concatenated barcodes improved phylogenetic resolution in Lamiaceae, producing clades that identify potential adulterants and guide DNA marker development for species authentication and adulterant detection.

Keywords: *DNA barcodes; Lamiaceae; molecular phylogenetics; species authentication*

1. Introduction

Lamiaceae, comprising approximately 7000 species across 236 genera [1], is the largest family in the order Lamiales [2]. Owing to the presence of therapeutic essential oils, 13% of its species are considered medicinal plants [3]. The genus *Salvia* is among the oldest medicinal plants with antibacterial, antioxidant, and anti-inflammatory properties [4]. Additionally, notable species like *Lavandula angustifolia* and *Origanum vulgare* are extensively used in cosmetics and culinary applications [5].

Increasing research on the benefits of herbal medicine over conventional treatments has driven global demand [6], resulting in the adulteration of Lamiaceae species. For instance, peppermint oil (*Mentha piperita*) is often adulterated with menthol oil from *Mentha arvensis* [7], while *Origanum vulgare* has been substituted with *Origanum majorana* [8]. In some cases, the high market demand has driven some species to the brink of extinction due to exploitation [6].

The unique biochemical profiles of Lamiaceae [7] suggest that adulteration compromises both the effectiveness and safety of herbal medicines. However, prevention efforts remain challenging due to morphological similarities among species, particularly in powdered form [9]. For example, products containing *Scutellaria* may pose health risks if adulterated with the hepatotoxic genus *Teucrium* [10]. Given the limitations of morphological identification, a DNA-based phylogenetic approach offers a reliable alternative for more accurate species authentication, as DNA sequences are less susceptible

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to environmental factors. Using DNA barcodes—short, standardized DNA sequences—in molecular phylogenetics has proven effective in estimating evolutionary relationships [11].

However, Lamiaceae phylogenetics has remained complex and unresolved [12]. A study on *Thymus* struggled to delineate species boundaries due to high morphological similarity [13]. Morphological convergence has also occurred across different lineages, as seen in staminal characters: the four stamens of the tribe Mentheae (subfamily Nepetoideae) independently reduced to two in the subtribes Salviinae and Menthinae [14]. Hybridization and polyploidy have also led to genomic alterations, creating complex phylogenetic networks and blurring taxonomic boundaries, as observed in *Mentha, Stachys*, and *Ocimum* [15-17]. A study on *Salvia* further highlighted discrepancies between morphology- and DNA-based phylogenies, with the former supporting its monophyly and the latter suggesting paraphyly [18].

Many phylogenetic studies on Lamiaceae have relied on a single genomic source, either nuclear or chloroplast DNA, limiting phylogenetic resolution. For example, researchers have studied *Scutellaria* using only chloroplast sequences [19], and the most comprehensive Lamiaceae phylogeny, covering 78% of genera, also relied on chloroplast markers [12]. This underscores the need to combine DNA barcodes to improve phylogenetic accuracy [20]. Standard plant barcodes include the chloroplast regions maturase-K (*matK*) and ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*), with the nuclear internal transcribed spacer (ITS) recommended as a supplementary marker [6, 20]. To address this need, the present in silico study incorporated Lamiaceae species beyond those found in Indonesia by retrieving sequences from GenBank. This study aimed to obtain phylogenetic insights based on partial ITS, *matK*, and *rbcL* barcodes to support species authentication and prevent adulteration in Lamiaceae herbal products.

2. Methodology

2.1. Collection of Lamiaceae ITS, matK, and rbcL Sequences

Partial DNA sequences from 52 Lamiaceae species across 11 genera were obtained, with *Spathodea campanulata* (Bignoniaceae) as the outgroup and sister taxon [21] (Table 1). The chosen species were relevant to previous studies addressing phylogenetic challenges and adulteration in Lamiaceae. Partial sequences were retrieved from the National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using species names and barcode regions. The ITS dataset included partial ITS1 and ITS2 regions and the complete 5.8S sequence. ITS was included to complement plastid markers for its strong discriminatory ability at the species level and its recommendation as a core plant barcode [22]. The fast-evolving *matK* and highly conserved *rbcL* markers were selected as the standard barcode pair [20] to resolve recent and older phylogenetic relationships. All sequences were saved in FASTA format to create the DNA database.

2.2. Bioinformatics Analysis

DNA sequences were aligned separately for each barcode using the ClustalW [23] algorithm implemented in *Molecular Evolutionary Genetics Analysis* (MEGA) v11.0.13 [24] under default parameters. Sequences were trimmed at the 5' and 3' ends so that all taxa within each marker had equal length, using the shortest sequence in the dataset as a reference. This ensured length uniformity within barcodes while maintaining length differences among ITS, *matK*, and *rbcL*.

Phylogenetic analyses were conducted using the maximum parsimony (MP) model to evaluate the relative performance of ITS, *matK*, and *rbcL*, both individually and in combination. Four MP trees were constructed in MEGA under default settings: one from each barcode and the concatenated dataset. MP was selected as a computationally efficient method that infers topology based on the parsimony principle, or the minimum number of evolutionary changes [25]. This model is appropriate for relatively small datasets of closely related sequences [26], as in this study. To address the long-branch attraction associated with MP and increase phylogenetic resolution, the concatenated dataset was also used to construct a maximum likelihood (ML) tree in MEGA using the Tamura-Nei substitution model [27]. Node support for both models was evaluated with 1000 bootstrap replicates, with higher percentages indicating stronger branch support [28].

The robustness of the MP trees was further assessed using the consistency index (CI) and retention index (RI), which were calculated by MEGA after the MP analysis. These indices quantify phylogenetic signals by indicating how closely character similarities among taxa reflect their evolutionary relationships, with the signal reduced by homoplasy [29]. CI values approaching 1 indicate low homoplasy [29], whereas RI values close to 1 reflect a higher proportion of synapomorphies [30].

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Table 1. Partial Lamiaceae ITS, *matK*, and *rbcL* sequences from the NCBI GenBank.

No.	Genus	Species	Accession Number		
			ITS	*matK*	*rbcL*
1.	*Orthosiphon*	*Orthosiphon aristatus*	FJ593403	LC456391	MW789616
2.		*Orthosiphon stamineus*	AY506663	KM658969	MH069809
3.	*Thymus*	*Thymus serpyllum*	KR150171	MF350183	MK105914
4.		*Thymus caespititius*	GU381457	HM850802	HM850398
5.		*Thymus vulgaris*	AY506646	OP243225	MN972464
6.		*Thymus quinquecostatus*	EU556524	LC618903	LC618880
7.		*Thymus mongolicus*	MH808603	MN433407	MN185199
8.	*Ocimum*	*Ocimum basilicum*	MW150025	MF694868	ON755091
9.		*Ocimum tenuiflorum*	MW150027	MF468149	JN114828
10.		*Ocimum gratissimum*	MW150026	MH552359	MW150006
11.	*Mentha*	*Mentha piperita*	KY072944	KX783716	JQ230988
12.		*Mentha spicata*	GU381394	KC571807	KU499887
13.		*Mentha arvensis*	KY072946	MG224998	HQ590183
14.		*Mentha aquatica*	KR611529	KP172053	KC584892
15.		*Mentha suaveolens*	GU381395	KP172057	MG223550
16.		*Mentha longifolia*	KR611531	HQ902745	ON755102
17.		*Mentha canadensis*	KY072951	MT929800	JN407303
18.	*Salvia*	*Salvia splendens*	MF622186	KX783777	ON755108
19.		*Salvia przewalskii*	MH808595	MN433404	JQ934026
20.		*Salvia officinalis*	KJ584196	HE967482	ON755112
21.		*Salvia miltiorrhiza*	MT039859	FJ513168	JQ934009
22.		*Salvia rosmarinus*	OQ165223	MH552339	MT931624
23.		*Salvia fruticosa*	KJ584194	HQ902726	HM590078
24.		*Salvia plebeia*	KU563788	MH660151	JQ934021
25.	*Clerodendrum*	*Clerodendrum cyrtophyllum*	KP092826	KJ888428	KJ939237
26.		*Clerodendrum japonicum*	KP092847	MK551817	GQ436521
27.		*Clerodendrum bungei*	EU591963	MH659049	JQ618463
28.		*Clerodendrum colebrookianum*	KX079329	MK551754	MK241954

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No.	Genus	Species	Accession Number		
			ITS	*matK*	*rbcL*
29.	*Callicarpa*	*Callicarpa dichotoma*	KP092811	LC680459	LC694383
30.		*Callicarpa americana*	ON820115	MF350069	KY626890
31.		*Callicarpa macrophylla*	KP092818	OP032135	KF443315
32.		*Callicarpa kochiana*	KP092816	OP032127	KJ688019
33.		*Callicarpa giraldii*	FJ593347	OP032121	MH657300
34.	*Lamium*	*Lamium album*	JX893229	MN311840	FJ395588
35.		*Lamium amplexicaule*	MN718246	MN433402	OL434812
36.		*Lamium galeobdolon*	KF529538	ON286905	JN891020
37.	*Origanum*	*Origanum majorana*	JX162957	KX783725	JQ230991
38.		*Origanum vulgare*	AY506647	MF694869	ON755119
39.		*Origanum onites*	JX163054	HQ902752	HQ902807
40.		*Origanum dictamnus*	EU252137	FR719089	FR720564
41.	*Stachys*	*Stachys sylvatica*	KF529644	JN895511	ON755118
42.		*Stachys palustris*	KF529624	JN894812	HE574636
43.		*Stachys floridana*	KF529590	OL434945	HQ644074
44.		*Stachys recta*	KF529631	KJ204541	KJ746271
45.		*Stachys arvensis*	KF529568	HM850806	MG224452
46.		*Stachys cretica*	KF529583	HQ902708	HQ902776
47.	*Scutellaria*	*Scutellaria baicalensis*	MH711530	MH660079	KT280158
48.		*Scutellaria lateriflora*	MK356052	MG225186	HQ590266
49.		*Scutellaria indica*	MH808599	FJ513171	MN167869
50.		*Scutellaria barbata*	MF193539	FJ513170	FJ513144
51.		*Scutellaria viscidula*	MF193526	HQ676587	HQ676583
52.		*Scutellaria rehderiana*	JX893232	HQ676588	FJ513147
53.	Outgroup: *Spathodea* (Bignoniaceae)	*Spathodea campanulata*	MF616608	MF476853	MT933895

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3. Results and Discussion

3.1. Characteristics of Lamiaceae Nuclear and Chloroplast DNA Barcodes

Table 2 summarizes the characteristics of the partial DNA barcodes used for reconstructing Lamiaceae phylogeny across a global dataset. Among the barcodes, *matK* had the longest average sequence length (785.6 base pairs), whereas *rbcL* showed the highest homology (83.5%), indicating a slower evolutionary rate. Homology refers to traits in organisms that arise from common ancestry [31] and are often conserved due to consistent inheritance, which explains the reduced variability observed in *rbcL*. Halmschlag et al. [32] also found *rbcL* to be less variable than ITS and *matK* in their phylogeny of 89 Lamiaceae species from converted land in Sumatra, Indonesia. The conserved nature of *rbcL* likely reflects its essential role in encoding Rubisco, a key enzyme in photosynthesis and plant adaptation [33].

The highest proportion of parsimony-informative (Pi) sites was observed in ITS (40.3%), while *rbcL* showed the lowest (7.6%). Comparable values were found by Halmschlag et al. [32], with 34% In ITS and 10% in *rbcL*. This consistent trend may be explained by differences in substitution rates, as the nuclear genome evolves approximately ten times faster than the chloroplast genome, where homologous recombination maintains genomic integrity [34]. Pi sites represent inherited mutations that infer shared ancestry and close evolutionary relationships among organisms [35]. In multiple alignments, they highlight unique sequences within specific taxa, thereby increasing the resolution of phylogenetic reconstructions. The low number of Pi sites in *rbcL* further supports its conserved nature within Lamiaceae. Overall, the dataset exhibited higher homology than Pi sites, indicating low levels of nucleotide substitution in Lamiaceae.

3.2. Lamiaceae Phylogenetics Based on Partial Nuclear and Chloroplast DNA Barcodes

Four maximum parsimony (MP) phylogenetic trees were constructed from ITS (Figure 1), *matK* (Figure 2), *rbcL* (Figure 3), and the concatenated dataset (Figure 4). A maximum likelihood (ML) tree was also inferred from the combined dataset to improve phylogenetic resolution and mitigate long-branch attraction associated with MP (Figure 5). Bootstrap values are presented on the branches of each tree, while the consistency and retention indices (CI and RI) are summarized in Table 3. MP tree topologies are assumed to reflect the principle of parsimony, which favors the minimum evolutionary changes [25].

As a quantitative indicator of phylogenetic signals, the MP trees exhibited an average CI of 0.63 and an average RI of 0.83. The relatively low CI may indicate the presence of homoplasy, which reduces phylogenetic signal through convergent evolution [35]. Long-branch attraction is also assumed to promote homoplasy, as the MP algorithm may incorrectly group distant taxa that have accumulated more mutations [36]. High RI values, however, indicate synapomorphic signals in the DNA sequences, providing evolutionary information for MP analysis [37]. In this study, RI values were close to 1, with 0.75 in ITS as the lowest, suggesting that many synapomorphies were retained in the Lamiaceae DNA sequences. Therefore, although the MP trees showed relatively low CI values, the consistently higher RI values supported the robustness of the MP model in recovering reliable evolutionary relationships for this dataset.

Bootstrap support was assessed with 1000 replicates, where values of ≥70% are associated with a 95% probability of the true evolutionary relationships [38]. The MP trees based on individual barcodes showed lower support, with 44% of nodes in the *rbcL* tree, 68% in the *matK* tree, and 76% in the ITS tree exceeding 70%. In contrast, 88% of nodes in the combined

Table 2. Characteristics of the Lamiaceae partial DNA barcodes.

DNA Barcodes	Sample Size	Length Range (bp)	Average Length ± SD (bp)	Post-trimming Length (bp)	Homology (%)	Parsimony -informative (Pi) Sites (%)
ITS	53	478 - 699	606.5 ± 39.4	530	44.5	40.3
matK	53	679 - 866	785.6 ± 35.6	614	33.5	29.0
rbcL	53	523 - 907	633.5 ± 71.8	462	83.5	7.6
ITS + *matK* + *rbcL*	159	478 - 907	675.2 ± 94.2	1606	51.6	26.6

*Note: bp = base pair

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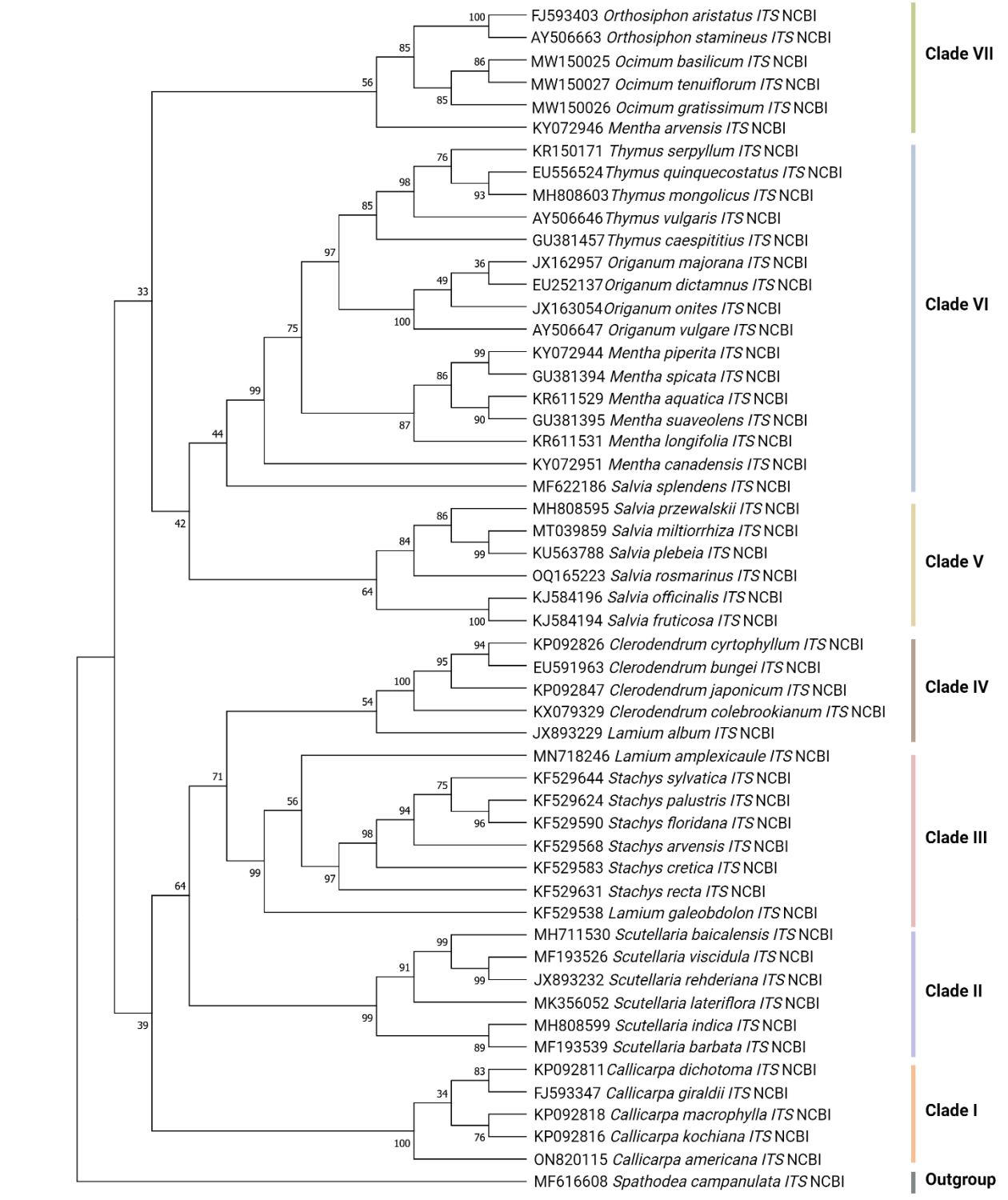

Figure 1. Maximum parsimony phylogenetic tree based on the partial ITS barcode. The numbers on the branches represent bootstrap values from 1000 replicates.

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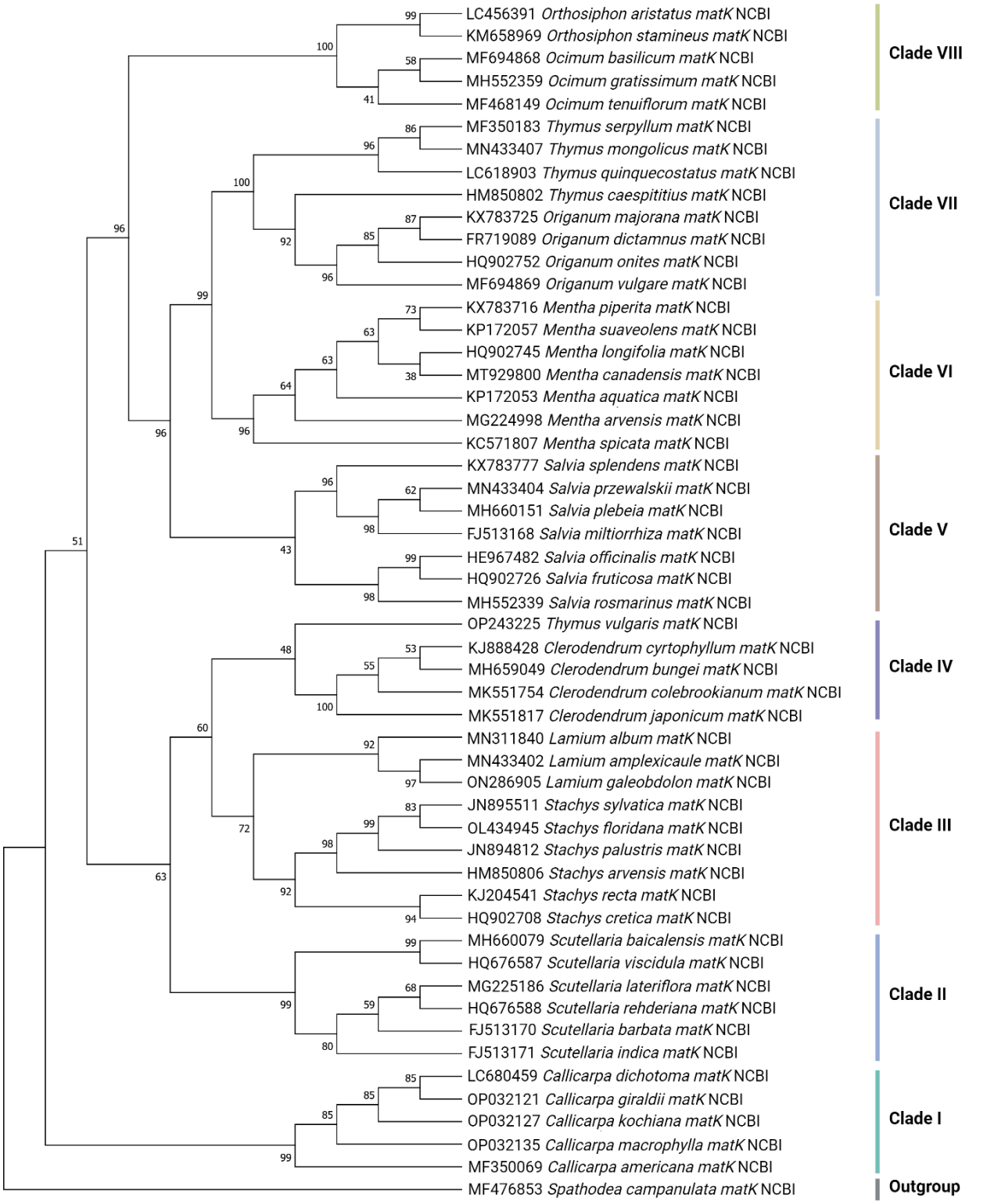

Figure 2. Maximum parsimony phylogenetic tree based on the partial *matK* barcode. The numbers on the branches represent bootstrap values from 1000 replicates.

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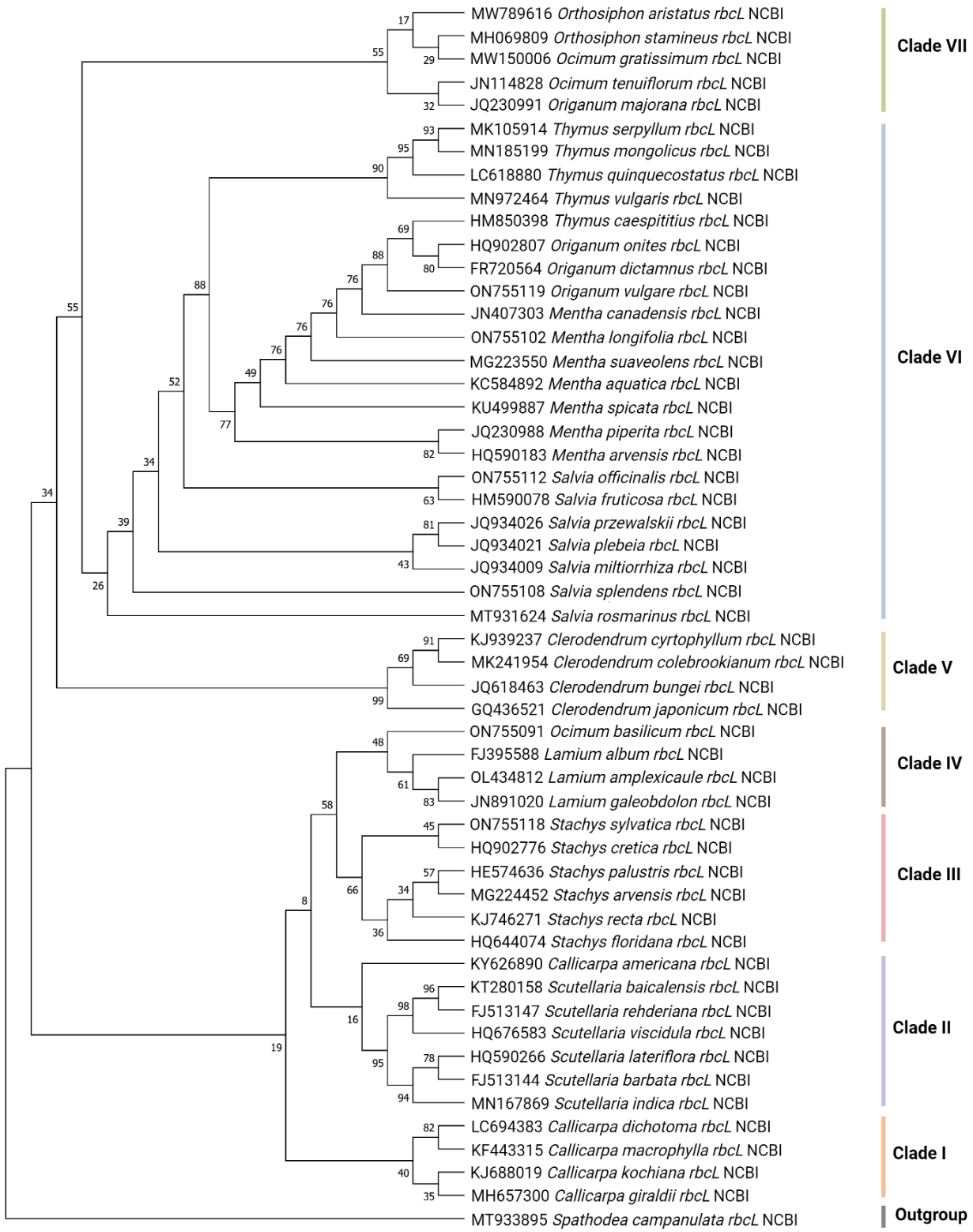

Figure 3. Maximum parsimony phylogenetic tree based on the partial *rbcL* barcode. The numbers on the branches represent bootstrap values from 1000 replicates.

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tree were over 70%. Similarly, 92% of nodes in the ML tree were above 70%, displaying congruence between both models.

Individual barcodes demonstrated similar groupings for closely related genera. Consistent clusterings were observed between *Orthosiphon* and *Ocimum*; *Thymus, Origanum,* and *Mentha*; and *Lamium* with *Stachys*. However, three grouping patterns were not recovered in the *rbcL* tree: (i) *Salvia* as the sister group to *Thymus, Origanum*, and *Mentha*; (ii) *Clerodendrum* as sister to *Lamium* and *Stachys*; and (iii) *Clerodendrum, Lamium*, and *Stachys* as sister to *Scutellaria*. These results suggest conserved sequences in the dataset, as reflected by the higher homology relative to Pi sites. Despite these similarities, individual barcodes did not consistently place every species with its correct genus.

In contrast, combining the three barcodes resolved these inconsistencies, grouping Lamiaceae into six robust monophyletic clades in both the MP and ML trees (Figures 4 and 5). Minor differences remained in the placement of one to two *Origanum, Scutellaria*, and *Callicarpa* species. Nevertheless, higher bootstrap values and consistent overall topologies across both trees indicated that the improvement resulted from more informative characters, which increased branch support and phylogenetic resolution [39]. Therefore, only the combined trees are discussed further.

Five clades were classified into four monophyletic subfamilies—Nepetoideae, Ajugoideae, Lamioideae, and Scutellarioideae—consistent with one of the most extensive phylogenetic studies of Lamiaceae [40]. Nepetoideae is sister to the lineage comprising Ajugoideae, Scutellarioideae, and Lamioideae [5]. *Callicarpa* was separated from the rest of Lamiaceae in Clade I, and together with the four subfamilies, form the phylogenetic backbone of Lamiaceae [12].

3.2.1 Callicarpa: Incertae sedis

Callicarpa (Clade I) is considered an *incertae sedis* taxon due to its unresolved phylogenetic placement within Lamiaceae [40]. Previous research based on five chloroplast DNA identified *Callicarpa* as the sister group to the subfamily Prostantheroideae [12]. In contrast, analysis incorporating chloroplast and mitochondrial DNA placed it as a sister to the rest of the family [41]. The present study also maintains its *incertae sedis* status due to incomplete sampling and limited genomic representation, which have contributed to inconsistencies in its phylogenetic position. In the MP tree, *Callicarpa* diverged from the main branches leading to the remaining clades, while in the ML tree, it diverged from Clades II and III. Morphologically, *Callicarpa* is characterized by its peltate or capitate stigma and drupes containing four stony pyrenes [42].

3.2.2 Subfamily Nepetoideae

Nepetoideae encompassed several genera distributed across clades IV – VI. As the largest subfamily within Lamiaceae, it comprises approximately 118 genera divided into three tribes: Mentheae, Ocimeae, and Elsholtzieae [12, 40]. Clades IV and V formed a monophyletic clade within Mentheae, while clade VI belonged to Ocimeae.

Clade IV contained the paraphyletic genus *Salvia* (subtribe Salviinae), which is characterized by two fertile stamens and a distinctive staminal lever mechanism in pollination [43]. *Salvia* was initially classified as monophyletic based on its staminal morphological traits [18]. However, a DNA-based phylogenetic analysis has redefined it as part of a larger clade that includes multiple genera, revealing that some *Salvia* species are more closely related to other genera than each other [44]. Consequently, *Salvia* and its related genera are now recognized as a paraphyletic group.

Clade V was one of the largest clades, comprising *Thymus, Origanum,* and *Mentha* of the subtribe Menthinae. Members of this group are distinguished by reticulate pollen grains and a circular abscission scar [45]. In the study, *Thymus* and *Origanum* each formed monophyletic groups clustered as sister clades, with *Mentha* recovered as their closest relative. However, earlier chloroplast DNA analysis reported *Thymus* as paraphyletic to *Origanum*, pointing to possible introgression rather than strict shared ancestry [46]. Furthermore, *Mentha* was recovered here as paraphyletic, contrary to previous work identifying it as monophyletic using ITS*, trnK*, and *trnL-trnF* barcodes [46].

Clade VI included the monophyletic genera *Orthosiphon* and *Ocimum*, members of the tribe Ocimeae identified by dorsifixed anthers [47]. This close relationship has also been supported by previous studies [48]. Although *Orthosiphon* has been extensively investigated for its therapeutic essential oils, phylogenetic research remains relatively scarce compared to other genera, and available DNA sequence data in GenBank are limited. Thus, this study did not resolve additional evolutionary relationships within *Orthosiphon*, yielding results consistent with Sudarmono et al. [49]. Meanwhile, *Ocimum* was classified as monophyletic, despite earlier research suggesting polyphyly [47], likely due to the absence of clear synapomorphies and high morphological similarities among species [50, 17]. Overall, discrepancies between findings of the current and prior studies may reflect differences in DNA barcodes and the scope of taxon sampling.

3.2.3 Subfamily Ajugoideae and Lamioideae

Clade III comprised the genera *Clerodendrum, Lamium,* and *Stachys. Clerodendrum* was recovered as monophyletic, forming a sister relationship with the latter genera. It is a member of the subfamily Ajugoideae, the third largest in Lamiaceae, consisting of 23 genera [12]. Previous phylogenetic analysis of Ajugoideae using four chloroplast markers classified it into four clades [51], later revised into tribal status, with *Clerodendrum* placed within Clerodendreae [5]. Although previously considered polyphyletic based on

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plastid data [52], nuclear DNA has redefined *Clerodendrum* as monophyletic, except for two Australian and Indian Ocean species more closely related to the genus *Volkameria* [53]. The same study also identified three major lineages, including an Asian-Australian clade [53]. Although the present sampling included only a subset of the Asian clade, the combined ITS, *matK*, and *rbcL* sequences recovered an evolutionary pattern consistent with Satthaphorn et al. [53]. These barcodes thus provide valuable information for reconstructing phylogenetic relationships within *Clerodendrum*. Synapomorphies of this genus include brightly colored accrescent calyces and protandrous reproductive organs [40,52].

Lamium and *Stachys* are categorized within Lamioideae, in the tribes Lamieae and Stachydeae, respectively [54]. Members of this subfamily are characterized by tricolpate pollen grains and spatulate embryos [40]. This analysis resolved *Lamium* as non-monophyletic, with *L. album* separated from a clade containing *L. amplexicaule* and *L. galeobdolon*. Bendiksby et al. [41], using nuclear and chloroplast DNA from 79 *Lamium* samples, generally supported the monophyly of the genus, though *L. galeobdolon* demonstrated variable placements, and *L. amplexicaule* appeared polyphyletic in the chloroplast analysis. *Lamium* is divided into three subgenera, with *L. album* and *L. amplexicaule* belonging to subgenus *Lamium*, which is further subdivided into different sections (*L. album* in *Lamiotypus* and *L. amplexicaule* in *Amplexicaule*) [55]. The non-monophyly of *Lamium* observed here may reflect these section-level divergences within subgenus *Lamium* and the uncertain position of *L. galeobdolon*, which has been assigned within the genus or to separate genera [41].

Stachys was found to form a monophyletic group, with members of Stachydeae often characterized by campanulated calyces and strongly two-lipped corollas [54]. However, it is recognized as a paraphyletic genus including several smaller genera nestled within [54]. As a result, this taxonomic complexity complicates the phylogenetic placement of *Stachys*, as its species are distributed across Stachydeae.

The present study grouped *Lamium* and *Stachys* as sisters to *Clerodendrum* in Clade III, with Lamioideae and Ajugoideae forming a sister group to Scutellarioideae. This topology differs from Scheen et al. [54], who reported Lamioideae as more closely related to Scutellarioideae. Another large-scale analysis using four chloroplast markers [56] revealed similar findings. Such topological incongruence among studies is likely caused by variations in DNA barcodes and sampling. Expanding taxon representation remains one of the most effective strategies to improve the accuracy of Lamiaceae phylogeny, particularly at the subfamily level, as demonstrated in this case [57].

3.2.4 Subfamily Scutellarioideae

Clade II consisted of a single genus, *Scutellaria*, within the subfamily Scutellarioideae. It formed a monophyletic group, consistent with the findings of a previous study using three chloroplast DNA regions [19]. *Scutellaria* is distinguished by a unique morphological structure called the scutellum, a projecting appendage on the upper lip of the bilabiate calyx [58]. Based on inflorescence and bract characteristics, *Scutellaria* has been classified into two subgenera, subg. *Scutellaria* and subg. *Apeltanthus* [59]. In Salimov et al. [19], these subgenera were divided into three clades: subg. *Apeltanthus* formed a clade with several species of subg. *Scutellaria*, while the other two clades consisted solely of subg. *Scutellaria*. Thus, the latter subgenus was inferred to be paraphyletic to subg. *Apeltanthus*. However, this paraphyly was not detected in the present study, as sampling only included species from subg. *Scutellaria*.

This study identified Lamioideae and Ajugoideae as the sister group to Scutellarioideae, in contrast to several earlier DNA-based analyses mentioned in the previous subsection. Morphological evidence provides additional support for a closer relationship between Lamioideae and Scutellarioideae. In particular, calyx structure and xylem tissue characteristics indicate that species in both subfamilies generally possess a higher density of fibre cells, tracheids, and vessels in the calyx tube compared to those in other Lamiaceae subfamilies, especially Nepetoideae [60]. These findings are consistent with the Lamiaceae phylogenetic backbone proposed by Li et al. [12], supporting also a closer evolutionary relationship between Lamioideae and Scutellarioideae than with Ajugoideae.

3.3. Lamiaceae Phylogenetics: Species Authentication and Adulteration Prevention

The use of plants in the treatment of various diseases has long been integral to human health. Approximately 80% of the global population relies on plants as their primary source of traditional healthcare, largely due to their perceived lower side effects compared to conventional medicines [61, 62]. However, the increasing demand for plant-based remedies has also led to rising cases of adulteration, including within the Lamiaceae family. The phylogenetic reconstruction of Lamiaceae using the concatenated dataset presented in this study provides a valuable framework for addressing these issues, particularly in species authentication to prevent adulteration.

The tree topologies derived from the combined sequence revealed relationships among taxa that support accurate species authentication. These phylogenies can highlight closely related species that are potential adulterants, thereby guiding the development of DNA markers for

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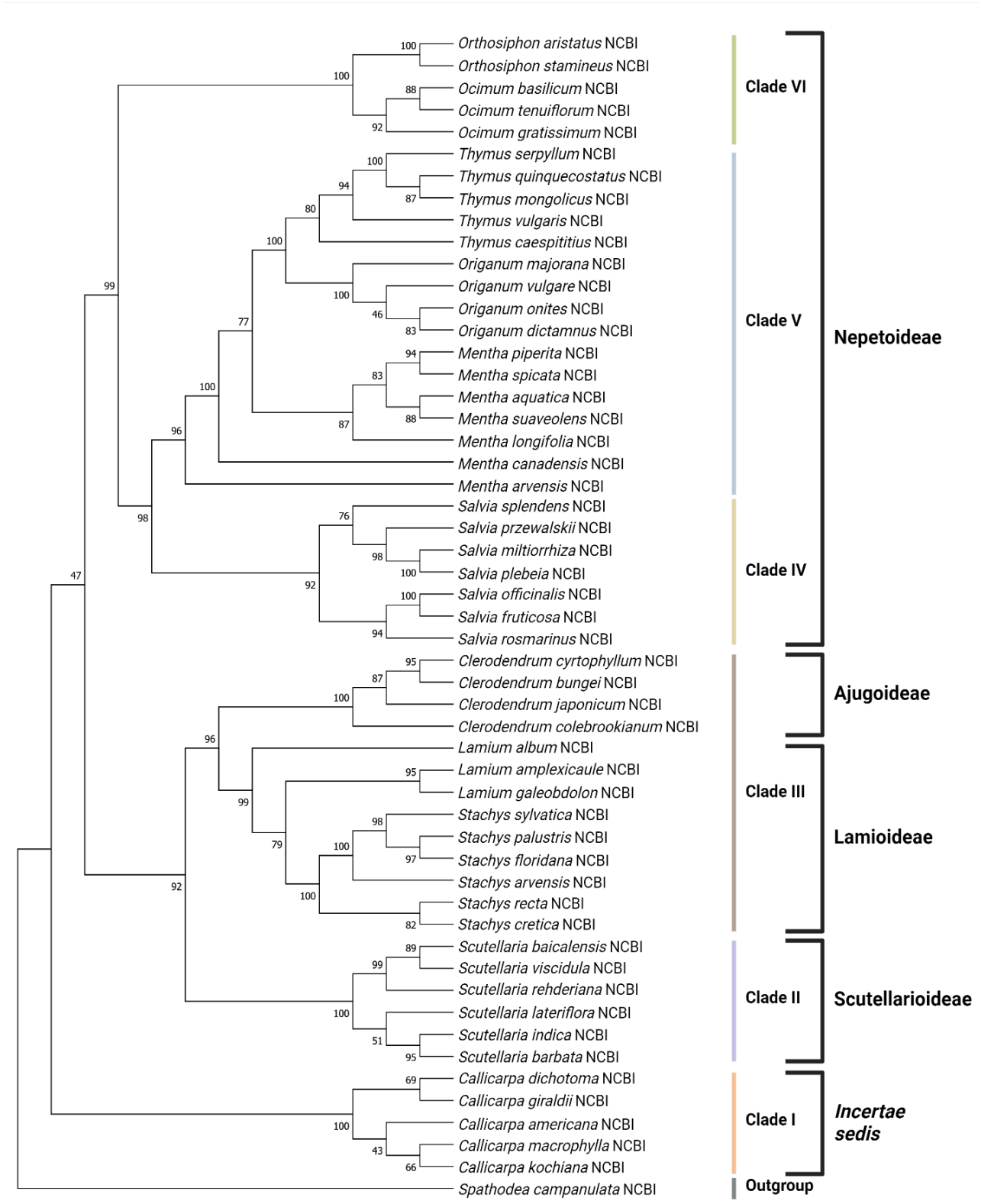

Figure 4. Maximum parsimony phylogenetic tree based on the concatenated partial ITS, *matK*, and *rbcL* barcodes. The numbers on the branches represent bootstrap values from 1000 replicates. The bolded words on the far right indicate subfamilies and an *incertae sedis* taxon.

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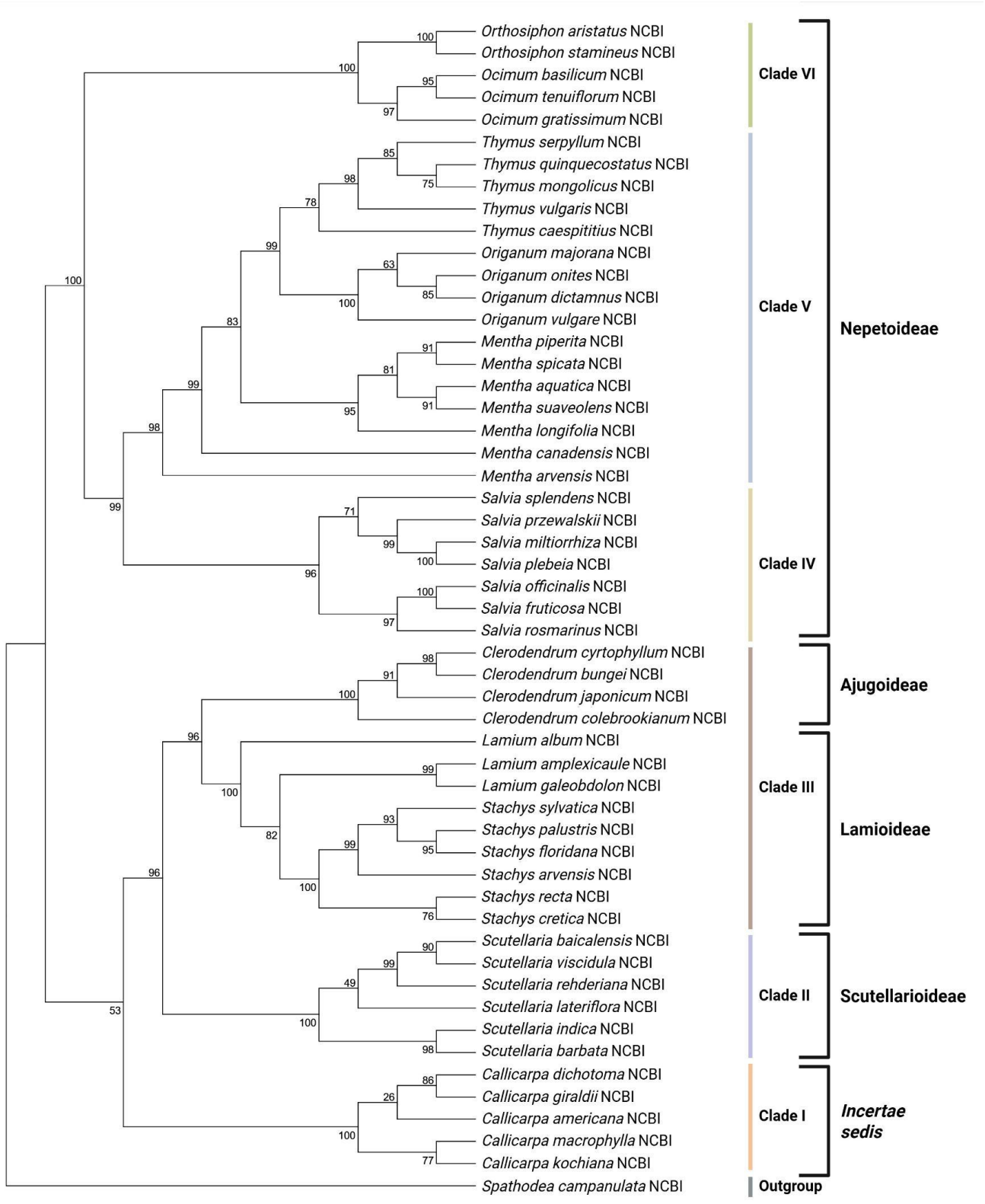

Figure 5. Maximum likelihood phylogenetic tree based on the concatenated partial ITS, *matK*, and *rbcL* barcodes. The numbers on the branches represent bootstrap values from 1000 replicates. The bolded words on the far right indicate subfamilies and an *incertae sedis* taxon.

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Table 3. Consistency and retention indices of Lamiaceae maximum parsimony phylogenetic trees.

DNA Barcodes	Consistency Index (CI)	Retention Index (RI)
ITS	0.489	0.755
matK	0.803	0.913
rbcL	0.649	0.867
ITS + *matK* + *rbcL*	0.586	0.803

reliable identification of authentic species and their likely substitutes in raw materials and finished products. Such authentication is crucial, as adulteration may compromise the purity and efficacy of herbal products [63]. Furthermore, considering that different Lamiaceae taxa possess distinct secondary metabolite profiles, adulteration may also introduce toxic compounds that raise safety concerns [64].

The issue is exemplified by *Scutellaria baicalensis*, widely used in traditional cancer treatments for its wogonin content [65]. High market demand has resulted in the adulteration of its commercial products with *S. rehderiana* [66]. Similarly, peppermint oil from *Mentha piperita*, which contains menthone and menthofuran that are beneficial against oxidative stress and inflammation [67, 68], is frequently substituted with menthol oil from *M. arvensis* [7]. These substitutions may involve differences in chemical compositions and affect therapeutic efficacy. Therefore, DNA-based phylogenetic analysis enables reliable species authentication and adulterant detection.

4. Conclusion

In the present study, individual partial DNA barcodes (ITS, *matK*, and *rbcL*) consistently grouped closely related genera, confirming their ability to reconstruct relatively accurate Lamiaceae phylogenies. Nevertheless, several species were separated from their respective genera. Concatenation of the three barcodes resolved these discrepancies, yielding improved phylogenetic resolution further supported by the ML tree model. The combined analysis clustered Lamiaceae into six well-supported monophyletic clades, largely consistent with previous studies, although some differences in phylogenetic placement were observed. These findings highlight the utility of multilocus DNA barcodes for clarifying evolutionary relationships in Lamiaceae and supporting species authentication and adulteration prevention in herbal products.

Future research should expand taxon sampling, including but not limited to more *incertae sedis* genera and geographically diverse species. Incorporating longer DNA sequences, additional markers such as mitochondrial apocytochrome b (*COB*), and diverse phylogenetic tree models may further refine phylogenetic resolution.

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