Journal of King Saud University – Science 35 (2023) 102398

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Insights into the phylogenetic relationship of the lamiids orders based on whole chloroplast genome sequencing

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ARTICLE INFO

Article history:
Received 25 April 2022
Revised 26 August 2022
Accepted 22 October 2022
Available online 30 October 2022

Keywords:
Basal lamiids
Chloroplast genome
Core lamiids
Phylogenetic relationship
Systematics

ABSTRACT

Lamiids, an asterid clade consists of over 40,000 species distributed among eight orders, Icacinales, Garryales and Metteniusales, known informally as "basal lamiids", Boraginales, Gentianales, Lamiales, Solanales, and Vahliales, known informally as "core lamiids". Over recent years, different phylogenetic studies have clarified the formation of lamiids, however, the relationships among the orders remain unresolved. The whole chloroplast genome sequences of 49 taxa have been downloaded from GenBank (NCBI) and used to evaluate the evolutionary relationships of lamiids, and particularly to test the relationships among the main lineages of lamiids. The phylogenetic tree resulting from using Maximum Parsimony and Bayesian Inference were with identical topologies and provide good support for the following relationships, Lamiales as sister to Solanales, and Boraginales as sister to Gentianales together forming the core lamiids. In basal lamiids, the result support Garryales as sister to Metteniusales, while Icacinales was found to be the immediate sister to the other orders in the core lamiids. Our results may improve our understanding of the relationships between the orders of lamiids.

1. Introduction

Asterids is the largest group in eudicots and comprising two clades, campanulids and lamiids (Zhang et al., 2020; Li et al., 2021). The lamiids are the largest, most species-rich and most diverse clade of asterids, with estimates ranging between 40,000 to 50,000 species, representing 15% of eudicots (Refulio-Rodriguez and Olmstead, 2014; Yang et al., 2020). According to the Angiosperm Phylogeny Group (APG IV, 2016), the clade is composed of eight orders, Icacinales (Include Icacinaceae and Oncothecaceae), Garryales and Metteniusales, referred to informally as "basal lamiids". The rest of the lamiids include Boraginales, Gentianales, Lamiales, Solanales, and Vahliales, known informally as "core lamiids". Different phylogenetic studies strongly favoured the monophyly of the clade (Albach et al., 2001; Soltis et al., 2011; Refulio-Rodriguez and Olmstead, 2014). The lamiids has been referred to by several names including asterids I, euasterids I or lamiidae (Takhtajan, 1987; Chase et al., 1993, APG I, 1998. Olmstead et al., 2000; Soltis et al., 2000, APG II, 2003; Soltis et al., 2011). Multiple characters of lamiids have been inferred as ancestral states such as woody habit, superior ovaries, unitegmic ovules, trilacunar nodes, scalariform perforation plates, presence of iridoids cellular endosperm and opposite leaves (Stull et al., 2018; Wang et al., 2022). However, considerable morphological differences within the lamiids clade, the non-molecular synapomorphies are uncertain (Stull et al., 2015).

The formation of lamiids has been clarified by numerous phylogenetic studies during the last few decades. However, the relationships among the orders of lamiids remain unclear or inconsistent. Firstly, the evolutionary relationships amongst the core lamiids group, Boraginales, Gentianales, Lamiales, Solanales, and Vahliales are contradictory (Fig. 1 A-G). Based on one nucleus gene (*18S*) and three chloroplast genes (*atpB*, *ndhF* and *rbcL*) Albach et al. (2001) (Fig. 1-A) have found support for the relationships (((Lamiales–Boraginales), Vahliales), Gentianales–Solanales) with strong Maximum Parsimony (MP) support. Bremer et al. (2002) (Fig. 1-B) recovered a different topology (((Lamiales–Solanales), Boraginales–Vahliales), Gentianales) using six plastid genes (*rbcL, ndhF, matK, trnL, trnV* and *rps16)*, however, the MP support was low. Qiu et al. (2010) (Fig. 1-C) based on four mitochondrial marks (*atp1*, *matR*, *nad5*, and *rps3*) obtained different relationships

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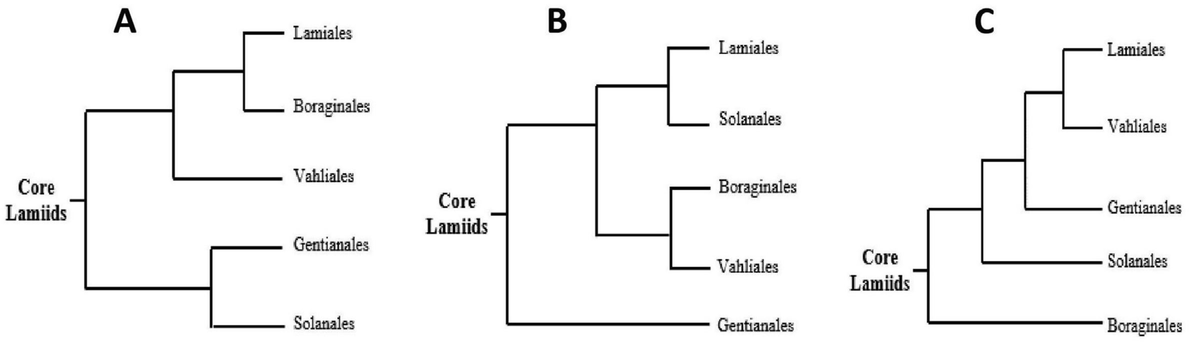
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

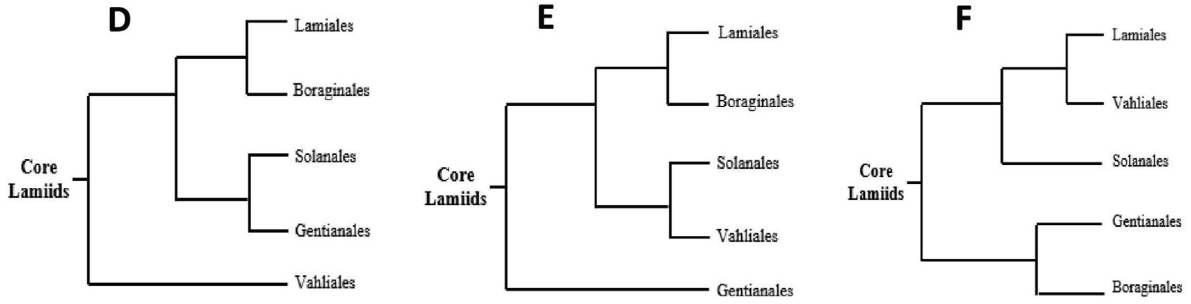
https://doi.org/10.1016/j.jksus.2022.102398
1018-3647/



Albach et al. 2001, using four plastid genes.

Bremer et al. 2002, using six plastid genes.

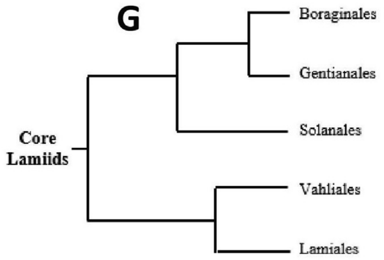
Qiu et al. 2010, using four mitochondrial marks.



Soltis et al. 2011, using 17 genes from mitochondrion, nucleus and plastid genomes.

Refulio-Rodriguez and Olmstead 2014, using one mitochondrial gene and nine plastid genes.

Stull et al. 2015, using 73 plastid genes.

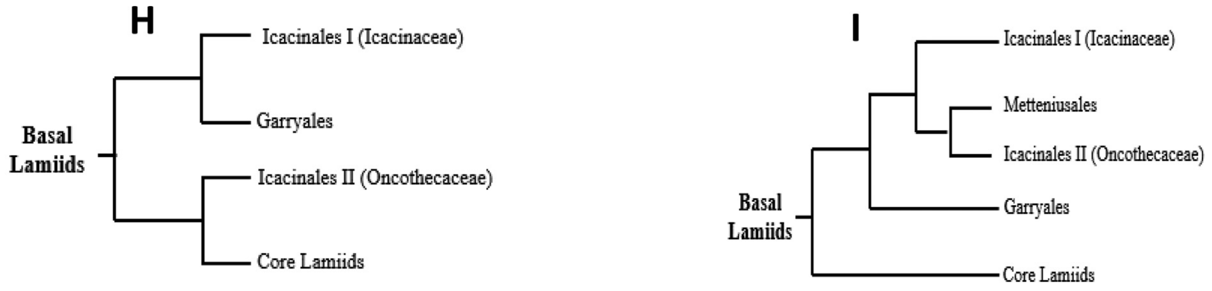


Zhang et al. 2020, using 213 transcriptomes/genomes.

Fig. 1. Seven different topologies from previous phylogenetic studies show different results in identifying the evolutionary relationship among the core lamiids.

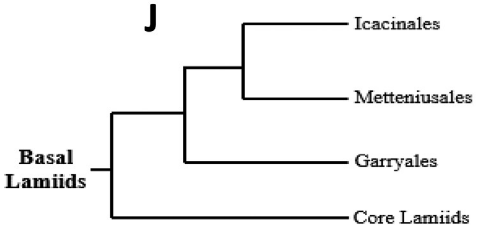
((((Lamiales–Vahliales), Gentianales), Solanales), Boraginales) but with low Maximum Likelihood (ML) support. Soltis et al. (2011) (Fig. 1-D) recovered the pattern (((Boraginales–Lamiales), Solanales–Gentianales), Vahliales) using 17 genes including two nucleus genes (*18S* and *26S rDNA*), eight plastid genes (*rbcL, rpoC2, rps16, rps4, psbBTNH, ndhF, matK, and atpB*), and four mitochondrial marks (*rps3, nad5, matR, and atp1*) with strong ML support. Refulio-Rodriguez and Olmstead (2014) (Fig. 1-E) using one mitochondrial gene (*rps3*) and nine chloroplast genes (*trnV-atpE, trnL-F, rps16, rps4, rbcL, psbBTNH, ndhF, matK and atpB*) have recovered (((Boraginales–Lamiales), Solanales–Vahliales), Gentianales) with moderate ML support but strong Bayesian Inference (BI) support. Stull et al. (2015) (Fig. 1-F) have recognized a different topology (((Lamiales – Vahliales), Solanales), Gentianales–Boraginales) based on 73 protein-coding genes, with strong BI support, however, ML support was low. Zhang et al. (2020) (Fig. 1-G) recovered (((Boraginales–Gentianales), Solanales), Vahliales–Lamiales) based on 213 transcriptomes/genomes, with strong ML support.

Secondly, the relationships between the other three lamiids orders, Icacinales, Garryales, and Metteniusales are controversial. Soltis et al. (2011) (Fig. 2-H) recovered the pattern ((Icacinaceae–Garryales), Oncothecaceae) with moderate ML support, however, Metteniusales order was not included. Refulio-Rodriguez and Olmstead (2014) (Fig. 2-I) recovered a different relationship (((Icacinaceae), Metteniusales–Oncothecaceae), Garryales) with weak BI, ML and MP support. Stull et al. (2015) (Fig. 2-J) have found support for the relationships ((Icacinales–Metteniusales), Garryales) with relatively strong ML support. Moreover, Metteniusales



Soltis et al. 2011, using 17 genes from mitochondrion, nucleus and plastid genomes

Refulio-Rodriguez and Olmstead 2014, using one mitochondrial gene and nine plastid gene regions.



Stull et al. 2015, using 73 plastid genes.

Fig. 2. Three different topologies from previous phylogenetic studies show different results in identifying the evolutionary relationship among the basal lamiids.

was found to be the immediate sister to the core lamiids in Gonzalez et al. (2007), Icacinales by Soltis et al. (2011), and it was Garryales in Refulio-Rodriguez and Olmstead (2014) and Stull et al. (2015).

Generally, as reviewed above, the relationships among the major lineages of lamiids have suffered from instability. Most of the phylogenetic studies that covered the evolutionary relationships between the orders of the lamiids were based on a few markers or genes (including chloroplast, nucleus and mitochondrial DNA). Using a single or a few genes may lead to different results in identifying the evolutionary relationship among species in comparison to chloroplast (cp) genome sequencing, which is based on the whole genome (Yao et al., 2020). Since the plastid genome approach has the capacity to resolve evolutionary relationships among some complex taxa (Huang et al., 2020). The plastid genome has been extensively used in plant phylogenetic studies (Adachi et al., 2000). The chloroplast genome of angiosperms species highly conserved in term of structural, gene content and arrangement (Fonseca et al., 2022). In this work, we sought to cover the relationships among the major lineages of lamiids reported in previous studies and determine the closest relatives of each order using chloroplast genome sequences data. Systematics is discussed in light of the phylogenetic analyses.

2. Materials and methods

2.1. Samples sequences

The chloroplast genome sequences of 49 taxa, 47 that represent all major lineages identified to date within lamiids (except Vahliales; the cp genome of this order was not available) and two taxa represent campanulids clade as outgroup were downloaded from Genbank - NCBI (Table 1). MAFFT v.7 was used to align all of the downloaded cp genome sequences (Katoh and Standley, 2013).

2.2. Phylogenetic analysis - Maximum Parsimony (MP)

Maximum parsimony PAUP version 4.0b10 was used to analyze the aligned sequences (Felsenstein, 1978). The heuristic searches were assessed with 100,000 replicates of branch swapping, tree bisection reconnections and random taxon addition. Non-parametric bootstrap analysis was determined with 1,000 replicates to evaluate branch support.

2.3. Phylogenetic analysis - Bayesian inference (BI)

MrBayes version 3.2.6 (Ronquist et al., 2012) was used to perform Bayesian inference, and the best substitution model (GTR + G) was identified using the Akaike information criterion (AIC) in jModelTest version 3.7. (Posada, 2008). MrBayes was run for 1,000,000 generations with two separate Markov chain Monte Carlo (MCMC) analyses, sampling and printing every 500 generations. Both constructed trees from (MP) and (BI) were edited and visualized using FigTree version 1.4.4.

3. Results

The topologies resulting from the Maximum Parsimony and Bayesian analyses were virtually identical. The phylogenetic tree is presented in Fig. 3 with bootstrap (BS) and posterior probability (PP) support values. The basal lamiids together with core lamiids formed a strongly supported clade (BS = 100, PP = 1).

3.1. Phylogenetic Inference: Relationships within basal lamiids

The first clade to diverge in basal lamiids consists of Garryales and Metteniusales were recovered as a sister but with strong support only from PP (BS = 62, PP = 1), while Icacinales was found to be the immediate sister to the core lamiids.

Table 1
The list of taxa that were downloaded from Genbank - NCBI with names of Order, Family, Species and GenBank accession.

	Order	Family	Species	Accession Number
1	Garryales	Garryaceae	*Aucuba obcordata*	NC_056113
2	Icacinales	Icacinaceae	*Iodes cirrhosa*	NC_036304
3	Metteniusales	Metteniusaceae	*Pittosporopsis kerrii*	MK488090
4	Boraginales	Boraginaceae	*Arnebia euchroma*	NC_053782
5	Boraginales	Boraginaceae	*Borago officinalis*	NC_046796
6	Boraginales	Boraginaceae	*Lithospermum erythrorhizon*	NC_053783
7	Boraginales	Boraginaceae	*Onosma fuyunensis*	NC_049569
8	Boraginales	Ehretiaceae	*Ehretia dicksonii*	MZ555766
9	Gentianales	Apocynaceae	*Alstonia scholaris*	NC_057091
10	Gentianales	Apocynaceae	*Apocynum venetum*	NC_053902
11	Gentianales	Apocynaceae	*Periploca forrestii*	NC_056319
12	Gentianales	Apocynaceae	*Vincetoxicum hainanense*	NC_051946
13	Gentianales	Gelsemiaceae	*Gelsemium elegans*	MH327990
14	Gentianales	Gentianaceae	*Eustoma exaltatum*	MK991810
15	Gentianales	Gentianaceae	*Exacum affine*	NC_056993
16	Gentianales	Gentianaceae	*Fagraea fragrans*	NC_057263
17	Gentianales	Gentianaceae	*Gentiana manshurica*	NC_053840
18	Gentianales	Loganiaceae	*Mitrasacme pygmaea*	NC_050922
19	Gentianales	Loganiaceae	*Mitreola yangchunensis*	NC_050923
20	Gentianales	Rubiaceae	*Cinchona officinalis*	MZ151891
21	Gentianales	Rubiaceae	*Dunnia sinensis*	MN883829
22	Gentianales	Rubiaceae	*Emmenopterys henryi*	NC_036300
23	Lamiales	Acanthaceae	*Acanthus ilicifolius*	MW752129
24	Lamiales	Bignoniaceae	*Tanaecium tetragonolobum*	KR534325
25	Lamiales	Carlemanniaceae	*Silvianthus bracteatus*	NC_047484
26	Lamiales	Gesneriaceae	*Boea hygrometrica*	NC_016468
27	Lamiales	Lamiaceae	*Lamium takeshimense*	MN240520
28	Lamiales	Lentibulariaceae	*Genlisea violacea*	NC_037083
29	Lamiales	Linderniaceae	*Torenia benthamiana*	NC_045273
30	Lamiales	Mazaceae	*Mazus xiuningensis*	NC_056340
31	Lamiales	Oleaceae	*Olea europaea*	MT182986
32	Lamiales	Paulowniaceae	*Paulownia elongata*	MK805127
33	Lamiales	Plantaginaceae	*Plantago depressa*	NC_041161
34	Lamiales	Scrophulariaceae	*Verbascum phoeniceum*	MN893301
35	Lamiales	Verbenaceae	*Verbena officinalis*	MW348926
36	Solanales	Convolvulaceae	*Convolvulus arvensis*	NC_054224
37	Solanales	Convolvulaceae	*Cressa cretica*	NC_035516
38	Solanales	Convolvulaceae	*Evolvulus alsinoides*	MN548282
39	Solanales	Convolvulaceae	*Ipomoea ramosissima*	NC_041205
40	Solanales	Solanaceae	*Capsicum lycianthoides*	NC_026551
41	Solanales	Solanaceae	*Datura stramonium*	MT610897
42	Solanales	Solanaceae	*Hyoscyamus niger*	KF248009
43	Solanales	Solanaceae	*Lycium ferocissimum*	MN866909
44	Solanales	Solanaceae	*Nicandra physalodes*	MN165114
45	Solanales	Solanaceae	*Nicotiana attenuata*	MG182422
46	Solanales	Solanaceae	*Physalis minima*	NC_048515
47	Solanales	Solanaceae	*Solanum anguivi*	NC_039611
48	Aquifoliales	Helwingiaceae	*Helwingia chinensis*	MZ504968
49	Apiales	Araliaceae	Kalopanax septemlobus	NC_022814

3.2. Phylogenetic Inference: Relationships within core lamiids

The core lamiids formed a strongly supported clade (BS = 100, PP = 1). Lamiales and Solanales were recovered as sister with strong support (BS = 85, PP = 1), while Boraginales and Gentianales were recovered as a sister but with strong support only from PP (BS = 65, PP = 1).

4. Discussion

4.1. Phylogeny of basal lamiids

The first clade to diverge consists of Garryales and Metteniusales (Fig. 3). This finding contrasts with Refulio-Rodriguez and Olmstead (2014) and Stull et al. (2015), which they have suggested that Metteniusales is sister to Icacinales. The results also indicate that Icacinales are the immediate sister to the other orders within the core lamiids. This is accordant with Soltis et al. (2011).

4.2. Phylogeny of core lamiids

Lamiales and Solanales are recovered as sisters, consistent with Bremer et al. (2002) and Stull et al. (2015). The second clade consists of Boraginales and Gentianales. This finding is consistent with Stull et al. (2015) and Zhang et al. (2020). However, several previous phylogenetic studies identified different relationships between the orders within core lamiids in comparison to our findings. For example, Lamiales was recovered as sister to Boraginales in Albach et al. (2001), Soltis et al. (2011) and Refulio-Rodriguez and Olmstead (2014), as sister to Vahliales and Gentianales in Qiu et al. (2010). Also, Solanales was recovered as sister to Gentianales in Albach et al. (2001) and Soltis et al. (2011), as sister to Vahliales in Refulio-Rodriguez and Olmstead (2014), as sister to Boraginales and Gentianales in Zhang et al. (2020). In addition, Boraginales was recovered as sister to Lamiales in Albach et al. (2001), Soltis et al. (2011) and Refulio-Rodriguez and Olmstead (2014), as sister to Vahliales in Bremer et al. (2002), the earliest branch to diverge and sister to all other orders in Qiu et al.

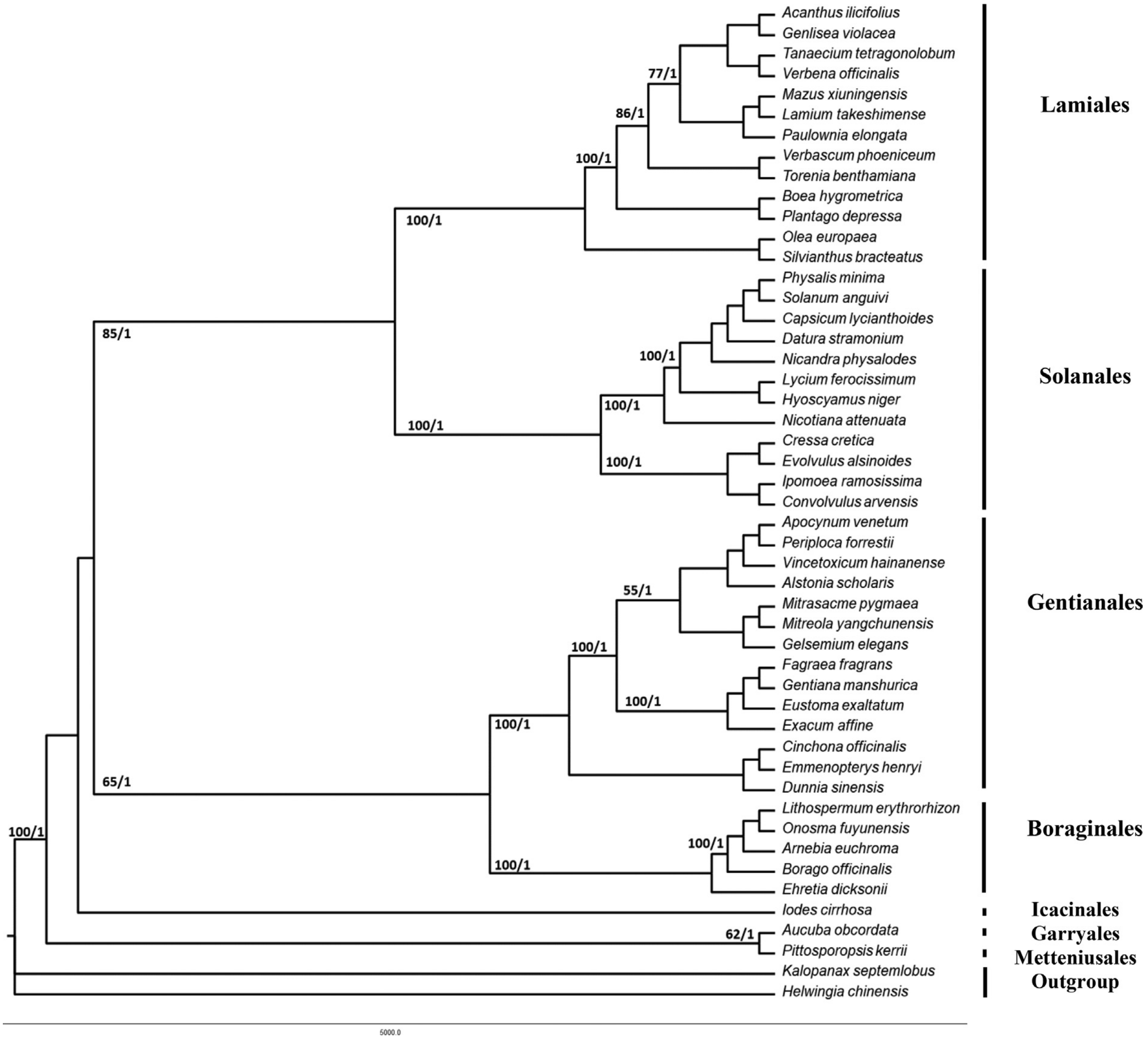


Fig. 3. Phylogenetic tree reconstruction of the 49 species based on the whole plastid genomes using Maximum parsimony (MP) and Bayesian inference (BI) analyses; the tree illustrate the relationships among the major lineages of lamiids and the figures in the branch nodes represent the total of bootstrap (BS)/posterior probability (PP).

(2010). Moreover, Gentianales was recovered as sister to Solanales in Albach et al. (2001) and Soltis et al. (2011), the earliest branch to diverge and sister to the all other orders in Bremer et al. (2002) and Refulio-Rodriguez and Olmstead (2014), as sister to Lamiales and Vahliales in Qiu et al. (2010).

5. Conclusion

Larger-scale phylogenetic studies of angiosperms have not definitively determined the relationships among the major lineages of lamiids. Most of the molecular analyses that covered the evolutionary relationships within the lamiids were based on a few markers or genes. Using whole chloroplast genome sequencing gives a more reliable result in identifying the evolutionary relationship among species in comparison to the use of a few genes. In this study, we used 49 whole chloroplast genome sequences, 47 taxa that represent all major lineages identified to date within lamiids except Vahliales; the cp genome of this order was not available, while two taxa represent campanulids clade as outgroup. In basal lamiids clade, our results suggest that Garryales as sister to Metteniusales, while Icacinales is the immediate sister to the other orders within the core lamiids. In core lamiids, Lamiales was found to be sister to Solanales, and Boraginales as sister to Gentianales. This finding increases our understanding of relationships among lineages of lamiids. However, these relationships need more fully investigated with additional sequence data and taxa, especially the chloroplast genome sequence of Vahliales taxa, which was not sampled in the present study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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