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Phylogeny of *Isodon* (Schrad. ex Benth.) Spach (Lamiaceae) and Related Genera Inferred from Nuclear Ribosomal ITS, *trnL–trnF* Region, and *rps16* Intron Sequences and Morphology

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***Abstract*—**Phylogenetic analyses of *Isodon* and related genera using the nuclear ribosomal internal transcribed spacer (nrITS), cpDNA regions (*trnL–trnF* region and *rps16* intron), and morphological data are presented. The results clarify the relationships among *Isodon* and its putative related genera and the question of the monophyly of *Isodon* and its relationship with *Siphocranion*. *Siphocranion* is a monophyletic genus and the sister clade to the remaining species; the new subtribe **Siphocranioninae** of tribe Ocimeae is described to accommodate it. *Isodon*, as currently circumscribed, is only monophyletic if *Skapanthus oreophilus* is included in it. *Isodon* forms a distinct subtribe Isodoninae within tribe Ocimeae and three strongly supported subclades are identified within the genus. In addition, a close relationship between *Hanceola* and *Hyptis* is indicated in the combined nrITS + cpDNA data set with Bayesian inference and this is also supported in morphological analyses, but more studies are needed to confirm this relationship. It is evident that subtribe Hanceolinae is polyphyletic as currently defined, so subtribe Hanceolinae is restricted to accommodate *Hanceola* alone. The relationships between *Isodon* and other genera within tribe Ocimeae are still unresolved: the newly erected subtribe **Isodoninae** is a monophyletic group representing a distinct lineage in subtribe Ociminae. The relationships among the species of the largest subclade C of *Isodon* are still poorly known. Additional studies of this group with additional data and more intensive taxon sampling might help to resolve these issues.

Keywords*—Hanceola*, *Isodon,* Labiatae, Lamiaceae, morphology, nrITS, Ocimeae, phylogeny, *rps16* intron, *Siphocranion*, *trnL–trnF*.

Isodon (Schrad. ex Benth.) Spach (Lamiaceae: tribe Ocimeae Dumort sensu Cantino et al. 1992; Harley et al. 2004) contains ca. 100 species distributed predominantly in tropical and subtropical Asia, with the center of diversity in southwestern China with outliers in tropical Africa (Wu and Li 1977; Codd 1984; Li 1988; Li and Hedge 1994). *Isodon* is separated from other members of tribe Ocimeae on the basis of a combination of morphological characters: bracteolate cymes, 4/1-bilabiate corolla limb, equally or subequally 5-toothed or 3/2-bilabiate calyx, and free filaments inserted at the base of the corolla tube (Li 1988). Although none of these characters is unique to *Isodon*, this combination of features nevertheless separates *Isodon* from other Lamiaceae.

Due to the high level of morphological variation, the delimitation and the infrageneric classifications of the genus have been obscure and controversial (Kudo 1929; Nakai 1934; Morton 1962; Hara 1972; Codd 1984; Li 1975, 1988; Murata 1975; Wu and Li 1977; Keng 1978; Ryding 1993a, b; Paton and Ryding 1998). Several segregate genera (i.e. *Amethystanthus* Nakai, *Homalocheilos* Codd, *Rabdosiella* Codd, *Skapanthus* Wu and Li) have been split from *Isodon*, but with varying degrees of recognition by subsequent authors (Nakai 1934, Handel-Mazzetti 1936, 1939; Morton 1962; Codd 1968, 1984; Ryding 1993b; Paton and Ryding 1998). *Rabdosiella* was divided, with the African species *R. calycina* (Benth.) Codd restored to *Plectranthus calycinus* Benth., while the Asian species *R. ternifolia* (D. Don) Codd was returned to *Isodon* Sect. *Pyramidium* as *I. ternifolius* (D. Don) Kudo (Ryding 1993b).

Similarly, *Plectranthus macranthus* Hook. f., whose taxonomic affinities were uncertain, was transferred to *Hancea* Hemsl. (= *Hanceola* Kudo) by Dunn (1913, 1915) and *Isodon* by Kudo (1929). Wu (1959) moved *P. macranthus* into the monotypic genus *Siphocranion* Kudo (1929), but the genus was later reduced to synonymy within *Isodon* (Hara 1972; Murata 1975) and *Plectranthus* (McKean 1982) before being resurrected by Li (1988).

Four sections (or species groups) have been recognized within *Isodon* even when the genus was included within *Plectranthus* (Bentham 1832–1836; Bentham and Hooker 1876; Briquet 1895-1897; Wu and Li 1977; Li 1988). Unfortunately, the characters used for this subclassification (including erect or reflexed fruiting calyx, dense or loose inflorescence, length of the calyx teeth, saccate or calcarate on the upper side near the base of corolla tube) have been shown to be ambiguous and thus unsuitable for infrageneric classification. Several major taxonomic treatments regarding the infrageneric classifications and taxonomic position of *Isodon* and related genera are summarized in Fig. 1.

Isodon has long been thought to be closely related to *Plectranthus* (Kudo 1929; Wu and Li 1977; Keng 1978; Cramer 1981; Li 1988), but this point of view has changed as new evidence has become available. *Isodon* was more closely related to *Hyptis* based on cpDNA analyses (Wagstaff et al. 1995; Paton and Ryding 1998) and to three other Asiatic genera *Hanceola*, *Siphocranion,* and *Skapanthus* based on pericarp structure and bracteole presence in the inflorescence (Ryding 1992, 1993a; Paton and Ryding 1998; Harley et al. 2003, 2004; Paton et al. 2004). *Siphocranion* and *Hanceola* were separated as monotypic and oligotypic genera respectively, and placed within Satureieae-Pogostemoninae (Kudo 1929), but although Wu (1959) recognized similarities between these two genera, he placed *Hanceola* within subfamily Ocimoideae (= Ocimeae sensu Cantino et al. 1992) as a distinct tribe Hanceoleae and *Siphocranion* in tribe Pogostemoneae,

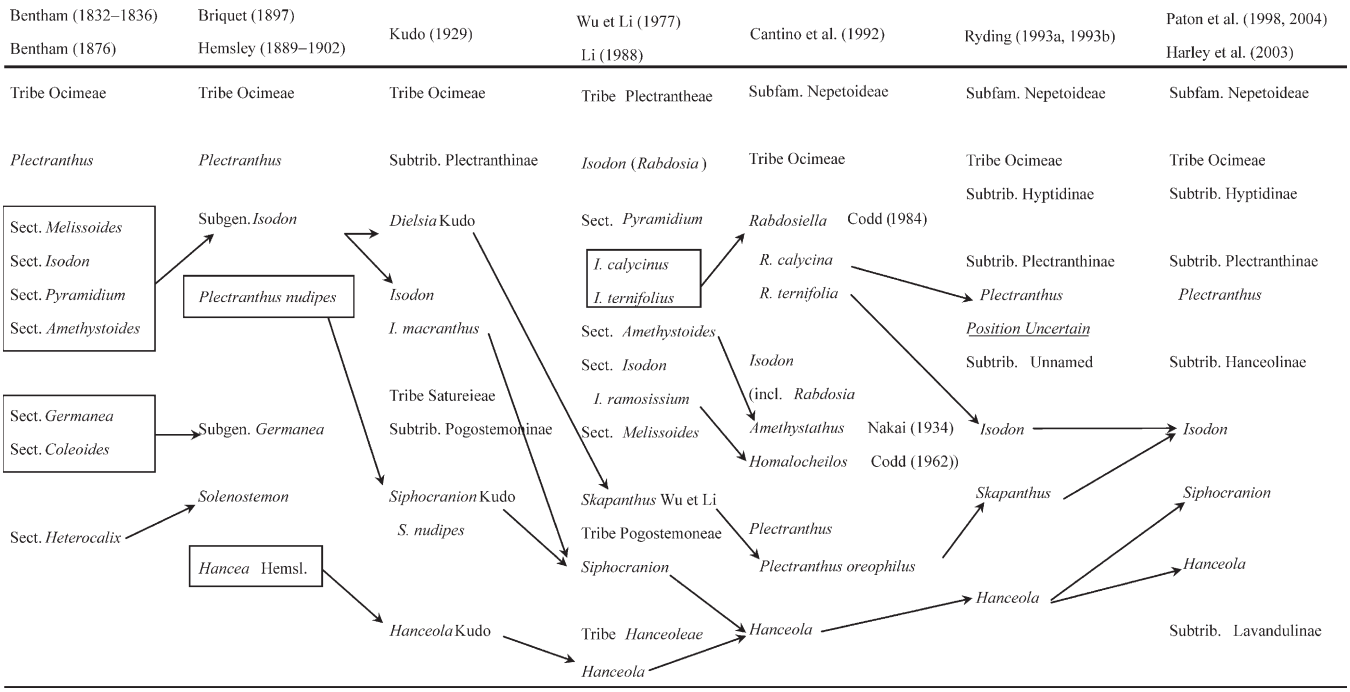

FIG. 1. Previous taxonomic treatments of *Isodon* and related genera (Ocimeae, Lamiaceae).

suggesting that these two Asiatic genera were links between Pogostemoneae and Ocimoideae. However, their recent separation, with *Pogostemon* placed in subfamily Lamioideae, but *Siphocranion* and *Hanceola* into tribe Ocimeae within subfamily Nepetoideae (Harley et al. 2004), indicates that previously postulated affinities are not supported.

Though the monophyly of *Isodon* (excl. *I. calycinus* as Ryding 1993b) was supported by the study of Paton et al. (2004), sampling of *Isodon* in that study was restricted to five species, representing only two out of the four currently recognized sections, and problematic taxa such as *Skapanthus oreophilus* (Diels) C. Y. Wu & H. W. Li, *Siphocranion macranthum* (Hook. f.) C. Y. Wu and *S. nudipes* (Hemsl.) Kudo were not included in their analyses. Accordingly, more comprehensive sampling is required to confirm the monophyly of *Isodon* and to elucidate its infrageneric relationships, as well as the relationships between *Isodon* and related genera. In addition, the subtribal status of Hanceolinae and its circumscription need to be tested to clarify the placement of these Asiatic genera within Ocimeae.

Accordingly, the main aim of this study is to generate a robust phylogeny for *Isodon* and related genera using molecular and morphological data. The results of this phylogenetic study will subsequently be used to (1) test the monophyly of *Isodon* and its infrageneric groups, (2) evaluate the phylogenetic relationships between *Isodon* and related genera, (3) elucidate the phylogenetic placement of *Hanceola*, *Siphocranion* and *Skapanthus* and (4) re-evaluate subtribal circumscriptions within Ocimeae.

Materials and Methods

Taxon Sampling—Nomenclature of *Isodon* in this study largely follows Li (1988) except for the treatment of *Plectranthus calycinus* Benth. which follows Ryding (1993b) and *Isodon taliensis* (C. Y. Wu) H. Hara which is here retained as a distinct species, whereas Li (1988) reduced it as a synonym of *I. setschwanensis* (Hand.-Mazz.) H. Hara. Forty-one samples from 39 species (including four varieties), representing taxa from all four recognized sections of *Isodon*, were included in the analysis (Appendix 1). To evaluate the relationships of *Isodon* and its allies, five species were sampled from putative subtribe Hanceolinae, including the genera *Hanceola*, *Siphocranion*, and *Skapanthus,* as well as representative species of tribe Ocimeae: *Lavandula* (Lavandulinae), *Ocimum* (Ociminae), *Orthosiphon* (Ociminae), *Eriope,* and *Hyptis* (both Hyptidinae), as well as *Coleus*, *Plectranthus*, *Pycnostachys*, *Tetradenia,* and *Thorncroftia* (all Plectranthinae). In addition, to test the monophyly of Ocimeae the species from *Callicarpa, Clinopodium, Collinsonia, Elsholtzia*, *Mentha*, *Nepeta, Perilla,* and *Salvia* were chosen as outgroup taxa on the basis of the work by Paton et al. (2004).

Molecular Analyses—Total DNA was extracted from fresh or silica gel-dried leaves and herbarium specimens using a 3 × CTAB protocol modified from Doyle and Doyle (1987) and the DNA samples were purified using an Omega Purification Kit® (Omega Bio-tek Inc., Norcross, Georgia). The ITS region including the 5.8S gene was then amplified with the primers ITS4 and ITS5m of White et al. (1990) and Sang et al. (1995), using the following protocol: 35 cycles, 45 sec denaturation at 94°C, 45 sec annealing at 50°C or 39°C, 1.5 min extension at 72°C, and further extension for 10 min.

The chloroplast *trnL* gene, intron and *trnL–trnF* intergenic spacer were amplified using the c and f primers according to the protocol described by Taberlet et al. (1991) with the PCR program: 35 cycles, 45 sec denaturation at 94°C, 45 sec annealing at 50°C, 1.5 min extension at 72°C, and 10 min final extension. The *rps16* intron was amplified using the primers *rps*F and *rps*R2 (Oxelman et al. 1997), with the same PCR program as for the *trnL–trnF* region except for an annealing temperature of 57°C.

Amplification products with single bands (most cases) were detected using 1.5% agarose gel then cleaned with the Omega Purification Kit®, whereas a few with multiple bands were separated using the Omega Gel Extraction Kit®. In a few cases, two bands of ITS products were identified and were so similar in length that they could not be segregated with agarose electrophoresis. These two-banded products were incorporated into the pMD18-T Vector (TaKaRa, Shiga, Japan), and at least five colonies for each sample were selected for sequencing, with the subsequently included band confirmed by BLAST searching.

Sequence primers for ITS were selected from White et al. (1990) and Sang et al. (1995), with those for *trnL* and *trnL–F* from Taberlet et al. (1991) and Oxelman et al. (1997). In addition, ITS3m* (CGATACTTGGT GTGAATTGCAG) and ITS2m* (CTGCAATTCACACCAAGTATCG) were designed as a forward/reverse primer pair for internal sequencing when other amplification primers failed. An ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit® with Big Dye Terminator ver. 3.1® was used for cycle sequencing with standard program; the products

were then sequenced after purification on an Applied Biosystems 377 automated DNA sequencer (PE Biosystems, Foster City, California). All sequences were checked with BLAST to rule out the possibility of amplifying nonspecific products.

Sequence data sets for each marker from both the cpDNA and nrITS sequences were aligned in Clustal X (Thompson et al. 1997) or MUSCLE (Edgar 2004) to produce an initial alignment; this was followed by manual adjustment in BioEdit (Hall 1999) or MEGA 4.0 (Tamura et al. 2007) to create the nexus file. All cpDNA and nrITS sequences analyzed in this study were deposited in GenBank under the accession numbers provided in Appendix 1, and the aligned data matrix was submitted to TreeBASE (study number S2431).

Maximum parsimony (MP) analyses of the cpDNA, nrITS, and the combined molecular data set (cpDNA/nrITS matrices) (excluding uninformative sites) were performed with PAUP* version 4.0b10 (Swofford 2003) using heuristic searches, with the MULTREES option off, tree-bisection-reconnection (TBR) branch swapping, and 1,000 random addition sequences with 10 trees held at each step during stepwise addition. In all analyses indels were treated as missing data and all characters were unordered and weighted equally. To estimate the level of homoplasy, the consistency index (CI, Kluge and Farris 1969) and retention index (RI, Farris 1989) were calculated. Taxa that were common to both the cpDNA and nrITS datasets were incorporated into a combined cpDNA + nrITS matrix. In *Ocimum basilicum* L., the two data sets were from different individuals of the same species and combined for the third data set. There were no ITS sequences available for *Hyptis leptostachys* Epling, *H. suaveolens* (L.) Poit., *Lavandula minutolii* Bolle, *L. rotundifolia* Benth., *Plectranthus albicalyx* S. Suddee, *P. calycinus* Benth., or *P. thyrsoideus* (Baker) Mathew, but because of their importance for elucidating the phylogenetic placements of *Hanceola*, *Isodon*, *Skapanthus*, and *Siphocranion* within tribe Ocimeae, they were included in the combined molecular data set with the ITS data coded as missing. Support for clades was calculated via bootstrap analyses (Felsenstein 1985) using 1,000 replicates, with the remaining parameters identical to those used in the parsimony analysis.

The partition homogeneity test (ILD; Farris et al. 1994) was calculated to determine the incongruence between the chloroplast and the nuclear datasets as implemented in WinClada ver. 1.00.08 (Nixon 2002) running NONA ver. 2.0 (Goloboff 1999). Parameters in the ILD test were: 1,000 replications, 5 mul reps/replication, 2 trees to hold/mul rep, and 10 trees for hold* with default optional settings.

The appropriate models of molecular evolution to be used in Bayesian inference (BI) of phylogeny (under AIC) were selected for each data partition using the program Modeltest Version 3.06 (Posada and Crandall 1998). The model GTR + I + G was chosen for nrITS and combined molecular data (cpDNA + nrITS), while GTR + G for cpDNA. All data sets were analyzed by Bayesian methods with MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001) using the default settings of the program. One million generations were run with four Markov Chain Monte Carlo (MCMC) chains, and a tree was saved every 100 generations. The trees from the MrBayes analysis were imported to PAUP* version 4.0 beta 10 (Swofford 2003), discarding the trees sampled during the "burn-in" of the chain (Huelsenbeck and Ronquist 2001; the first 1,000 trees) to only include trees after stationarity was reached. Inspection of the plots of generation vs. log probability suggests that stationarity was reached within the designated burn-in (data not shown). A majority rule consensus tree was produced, showing nodes with posterior probability of 50% or more. For both bootstrap and Bayesian support values, levels ≥ 50% were defined as strong (≥ 90%), moderate (70–89%) or weak (50–69%).

***Morphological Analyses*—**Morphological data were gathered from field collections, herbarium specimens and the published literature. Twenty five morphological characters were chosen for MP analysis, and 17 characters (1–8, 10–13, 15, 17, 20, 24–25) for character mapping (Appendix 2 and supplemental on-line Appendix 1), because of their traditional importance at the generic and sectional level in *Isodon*.

Maximum parsimony analyses based on the morphological character matrix (supplemental on-line Appendix 1) were performed in NONA ver. 2.0 (Goloboff 1999), run within WinClada 1.00.08 (Nixon 2002), employing a heuristic search (maxtree = 1,000; mult*n = 1,000; hold/ = 10; and multiple TBR + TBR mult*max* branch-swapping in effect). All characters were unordered, weighted equally, and *Callicarpa giraldii* Hesse ex Rehder was the outgroup taxon. Bootstrap support values were calculated from 1,000 resamplings holding 10 trees per run and TBR off.

Seventeen morphological character state distribution patterns were then mapped onto the Bayesian tree obtained from the cpDNA + nrITS combined analysis using MacClade 4.08 (Maddison and Maddison 2005) to determine which features were phylogenetically informative and might help to clarify the morphological relationships between *Isodon* and other genera in tribe Ocimeae, as well as its infrageneric relationships.

Results

***Molecular Analyses*—**The characteristics of the cpDNA (*trnL–trnF* region and *rps16* intron) and nrITS sequences of *Isodon* and related genera, and the cpDNA and nrITS partitions of the combined datasets, as well as the aligned matrices of the cpDNA and nrITS datasets are summarized in Table 1.

***Chloroplast DNA*—**The cpDNA included 91 sequences of which 57 are new to this study. The inclusion of previously published sequences is to better infer the phylogenetic relationships between *Isodon* and other genera within Ocimeae on the basis of representative and continuous sampling.

The MP analyses of the cpDNA sequences data yielded 1,722 equally parsimonious trees that were 519 steps long (L) and had a consistency index (CI) of 0.6532 and a retention index (RI) of 0.8765 (Fig. 2, right). The Bayesian consensus tree (Fig. 2, left) was compared with the 50% majority rule consensus tree of all most parsimonious cladograms. Bayesian and parsimony-derived topologies were largely congruent with little discrepancy within the internal branch; however, the former had higher support for the main lineages and the internal nodes (Fig. 2). All Ocimeae taxa were grouped together with moderate support (PP = 0.82) in the BI analysis, forming three, separate, highly supported clades (Fig. 2): the *Siphocranion* clade (Clade III, BS = 100%, PP = 1.00); the *Lavandula* clade (Clade II, BS = 100%, PP = 1.00) and the large

Table 1. Sequence characteristics of *Isodon* and related genera used in this study.

Markers	Matrix	Length range (*Isodon*)	Length range (other genera)	G+C content range (*Isodon*)	G+C content range (other genera)	Aligned length	Variable characters	Parsimony informative characters
*trn*L/F	*trn*L/F + *rps*16	785–841	740–876	36.46%–37.42%	35.73%–37.58%	1,050	251	123 (11.71%)
*rps*16 intron	*trn*L/F + *rps*16	789–812	732–826	32.93%–33.84%	32.10%–35.25%	959	263	142 (14.81%)
ITS	ITS	565–584	556–627	61.95%–66.15%	61.55%–68.43%	695	339	247 (35.54%)
ITS1	ITS	193–197	172 (87)–232	62.37%–68.72%	61.67%–71.36%	258	167	117 (16.83%)
5.8S	ITS	163 or 164	163 or 164	54.27%–55.49%	54.60%–56.10%	164	13	7 (1.007%)
ITS2	ITS	206–226	204 (195)–237	65.61%–71.82%	59.49%–76.47%	273	159	123 (17.70%)
ITS	*trn*L/F + *rps*16 + ITS	565–584	556–622	61.95%–66.15%	59.42%–68.43%	676	303	185 (27.37%)
ITS1	*trn*L/F + *rps*16 + ITS	193–197	172–228	62.37%–68.72%	62.04%–71.36%	252	143	79 (11.69%)
5.8S	*trn*L/F + *rps*16 + ITS	163 or 164	164	55.21–55.49%	54.87%–56.10%	164	10	4 (0.5917%)
ITS2	*trn*L/F + *rps*16 + ITS	206–226	204–237	65.61%–71.82%	59.49%–76.47%	260	150	102 (15.09%)
trnL/F	*trn*L/F + *rps*16 + ITS	793–849	802–884	36.59%–37.55%	35.87%–37.00%	975	183	82 (8.410%)
*rps*16 intron	*trn*L/F + *rps*16 + ITS	789–812	732–815	32.93%–33.84%	33.29%–34.16%	918	202	92 (10.02%)

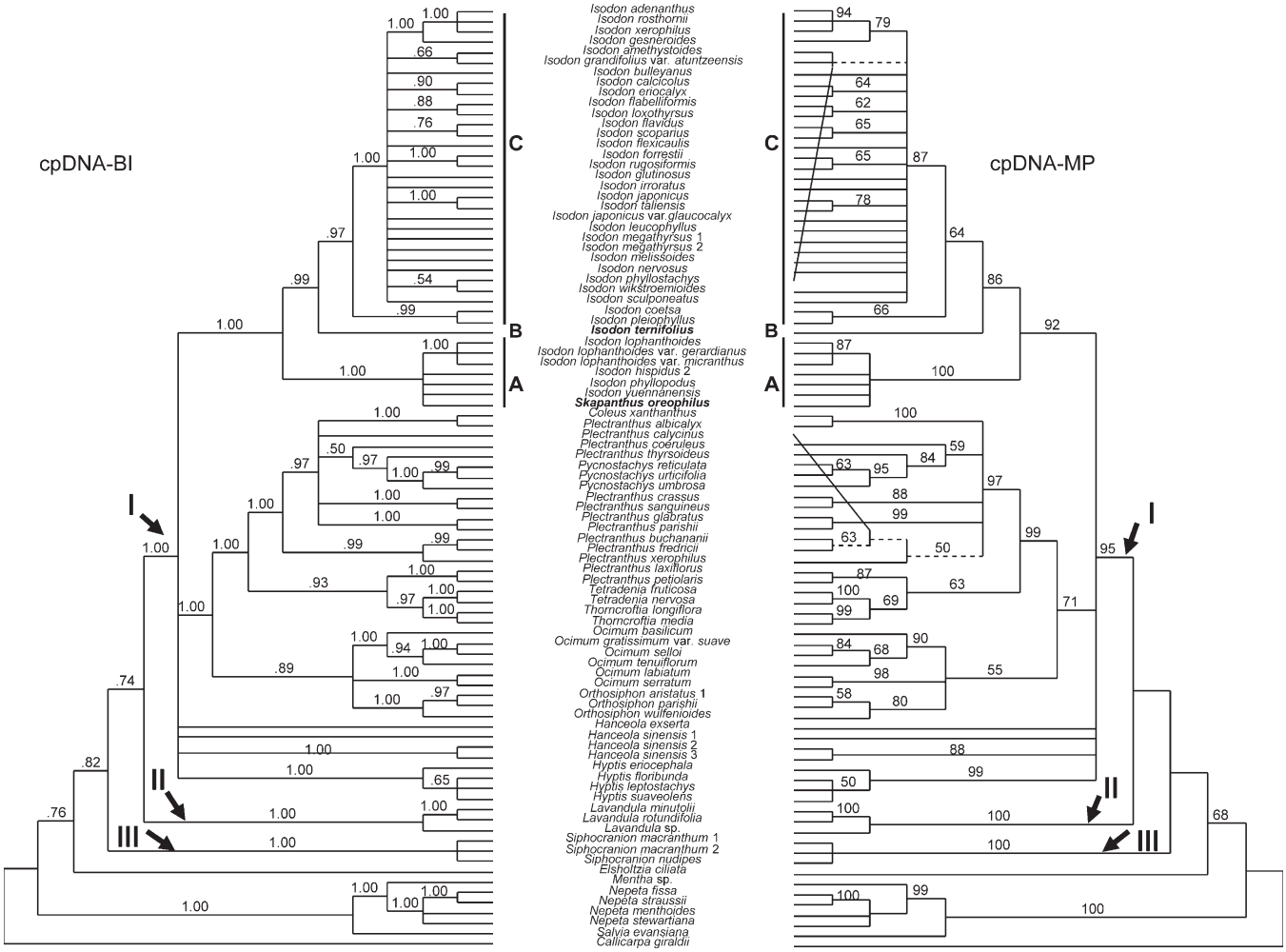

FIG. 2. Left: Bayesian consensus of 9001 trees derived from the analyses of cpDNA sequence data, Bayesian posterior probability values greater than 50% are shown above branches. Right: Majority consensus tree derived from the MP analyses of cpDNA sequence data, Bootstrap values greater than 50% are shown above branches, Dashed lines indicated those branches that collapsed in strict consensus tree. **I** to **III** indicate well supported clades within tribe Ocimeae; A–C represent lineges within *Isodon*.

Ociminae – Plectranthinae – Hyptidinae – Hanceolinae (excl. *Siphocranion*) clade (Clade I, BS = 95%, PP = 1.00).

Within Clade I, members of the subtribes Plectranthinae (BS = 99%, PP = 1.00), Ociminae (BS = 55%, PP = 0.89) and Hyptidinae (BS = 99%, PP = 1.00) formed separated groups, and Plectranthinae and Ociminae were sisters (BS = 71%, PP = 1.00). In contrast, Hanceolinae were not monophyletic. *Skapanthus* was deeply nested within *Isodon* (clade A, BS = 100%, PP = 1.00), while the species of *Hanceola* form a polytomy with the clades representing *Isodon* (incl. *Skapanthus*), Hyptidinae and Plectranthinae + Ociminae. All studied *Isodon* taxa, together with the species of *Skapanthus*, formed a strongly (BS = 92%, PP = 1.00) supported clade and three well-supported lineages were recognized within the genus (A: BS = 100%, PP = 1.00; B: *I. ternifolius* (W. Smith) Kudo; and C: BS = 64%, PP = 0.97).

NUCLEAR RIBOSOMAL ITS—The sequenced nrITS region comprised a total of 74 sequences (63 new) and representatives of subtribes Plectranthinae, Ociminae, and Hyptidinae. The MP analysis of the nrITS data set resulted in 116 equally parsimonious trees (L = 1080, CI = 0.4704, RI = 0.7360) and a 50% majority rule consensus tree was calculated to compare against the Bayesian consensus tree (Fig. 3). The Ocimeae taxa were grouped as a clade with relatively strong support in the Bayesian analysis (PP = 0.92) albeit with < 50% bootstrap value in the most parsimonious analysis. Within the Ocimeae clade, two species of *Siphocranion* formed a strongly supported clade (BS = 100%, PP = 1.00), as well as the sampled species of *Lavandula* (BS = 100%, PP = 1.00). These two genera were nested together with only little support (BS < 50%, PP = 0.69) in both analyses, and were then sister to the other larger clade including the remaining species of Ocimeae which was only moderately supported (PP = 0.80) by Bayesian inference. Within this Ocimeae clade, the taxa were separated further into two clades; one of which was only weakly supported with PP value 0.58, while the other (the *Isodon* clade) was strongly supported (BS = 98%, PP = 1.00).

The weakly supported clade (PP = 0.58) defined in the Bayesian analysis included all the sampled species from the subtribe Plectranthinae (BS = 97%, PP = 1.00), the genus *Hanceola* (BS = 96%, PP = 1.00), the subtribe Hyptidinae (BS = 99%, PP = 1.00) and the subtribe Ociminae. Most of these were supported in both MP and Bayesian analyses respectively, except for the subtribe Ociminae clade which was only supported in the Bayesian analysis (PP = 0.90). Another difference using MP was that these taxa were instead successively related to the *Isodon* clade, but without bootstrap support.

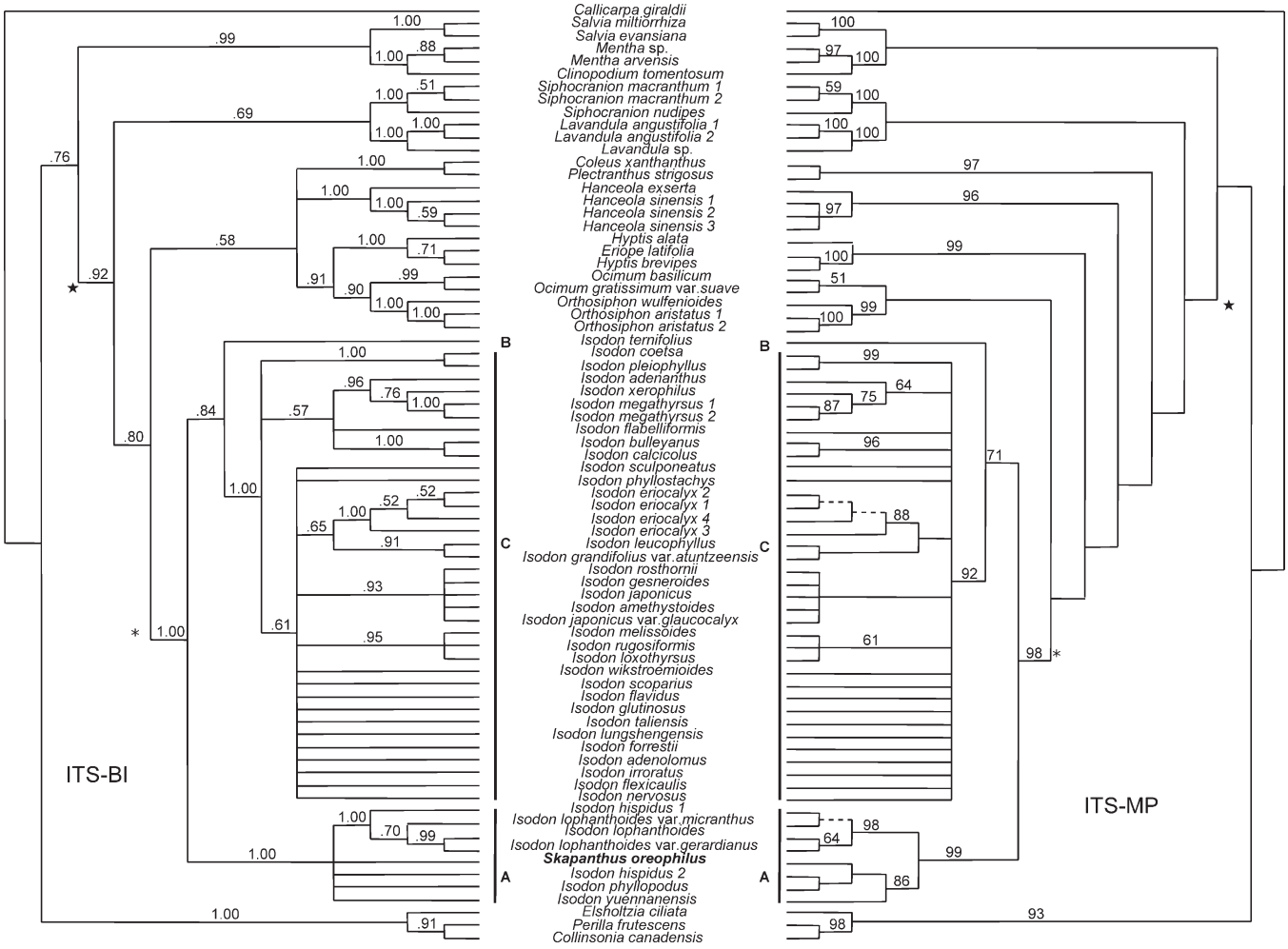

FIG. 3. Left: Bayesian consensus of 9001 trees derived from the analyses of nrITS sequence data, Bayesian posterior probability values greater than 50% are shown above branches. Right: Majority consensus tree derived from the MP analyses of nrITS sequence data, Bootstrap values greater than 50% are shown above branches, Dashed lines indicated those branches that collapsed in strict consensus tree. A star indicates the Ocimeae clade, an asterisk the *Isodon* clade. A–C represent lineages within *Isodon.*

Within the *Isodon* clade, there were two well supported clades (A: BS = 99%, PP = 1.00; and C: BS = 92%, PP = 1.00) and a solitary species branch: B (*I. ternifolius*). *Isodon ternifolius* was sister to clade C in both Bayesian and MP analyses with moderate support (BS = 71%, PP = 0.84). Furthermore, *Skapanthus orephilus* was nested within clade A, forming a polytomy in the Bayesian analysis, which included *I. hispidus*, *I. phyllopodus*, *I. yuennanensis,* and a well supported clade (BS = 98%, PP = 1.00) representing *Skapanthus orephilus,* and *I. lophanthoides* and its varieties.

COMBINED CPDNA + NRITS—The ILD test indicated that the two partitions (cpDNA and nrITS) were significantly incongruent ($p = 0.006$). Nevertheless, because of reported problems with the ILD test (Yoder et al. 2001; Darlu and Lecointre 2002; Baker and Lutzoni 2002), the complexity of tracing the sources of heterogeneity or the resolution of the combined analyses could not be addressed fully here (Soltis and Soltis 1998). However, as the present study is to clarify the phylogenetic relationships between *Isodon* and other genera within tribe Ocimeae, we decided to analyze the combined cpDNA and nrITS data sets as an alternative explanation which were then compared to the results from separate datasets and examined any novel relationships derived from the combined analysis.

The MP analyses of the combined cpDNA + nrITS matrix with 2,569 aligned sites and 359 (13.97%) potentially parsimonious informative characters produced 447 equally parsimonious trees (L = 959, CI = 0.5766, RI = 0.7732). The monophyly of tribe Ocimeae is supported with high support (PP = 0.99) in the Bayesian consensus tree (Fig. 4, left). In addition, three recognized clades (the *Siphocranion* clade, the *Lavandula* clade and the subtribe Plectranthinae + Ociminae + Hyptidinae + Hanceolinae excl. *Siphocranion* complex) were also supported by high bootstrap values (88–100%), and by full posterior probability (PP = 1.00). *Siphocranion* and *Lavandula* were sisters in a clade below a polytomy representing the remainder of the tribe in the Bayesian majority consensus tree (Fig. 4, left). The separation of *Siphocranion macranthum* and *Isodon* was also strongly supported (BS = 100, PP = 1.00).

The large group of the remaining ingroup taxa divided into three groups which were strongly or moderately supported by Bayesian analyses (Fig. 4, left). Within *Isodon*, three clades identified by the individual analyses also received strong support here, as did the inclusion of *Skapanthus oreophilus* within *Isodon*. In the Bayesian analysis, Plectranthinae and Ociminae were grouped as sister taxa with strong support (PP = 1.00) and *Hanceola* and *Hyptis* were sister taxa (PP = 0.77), however

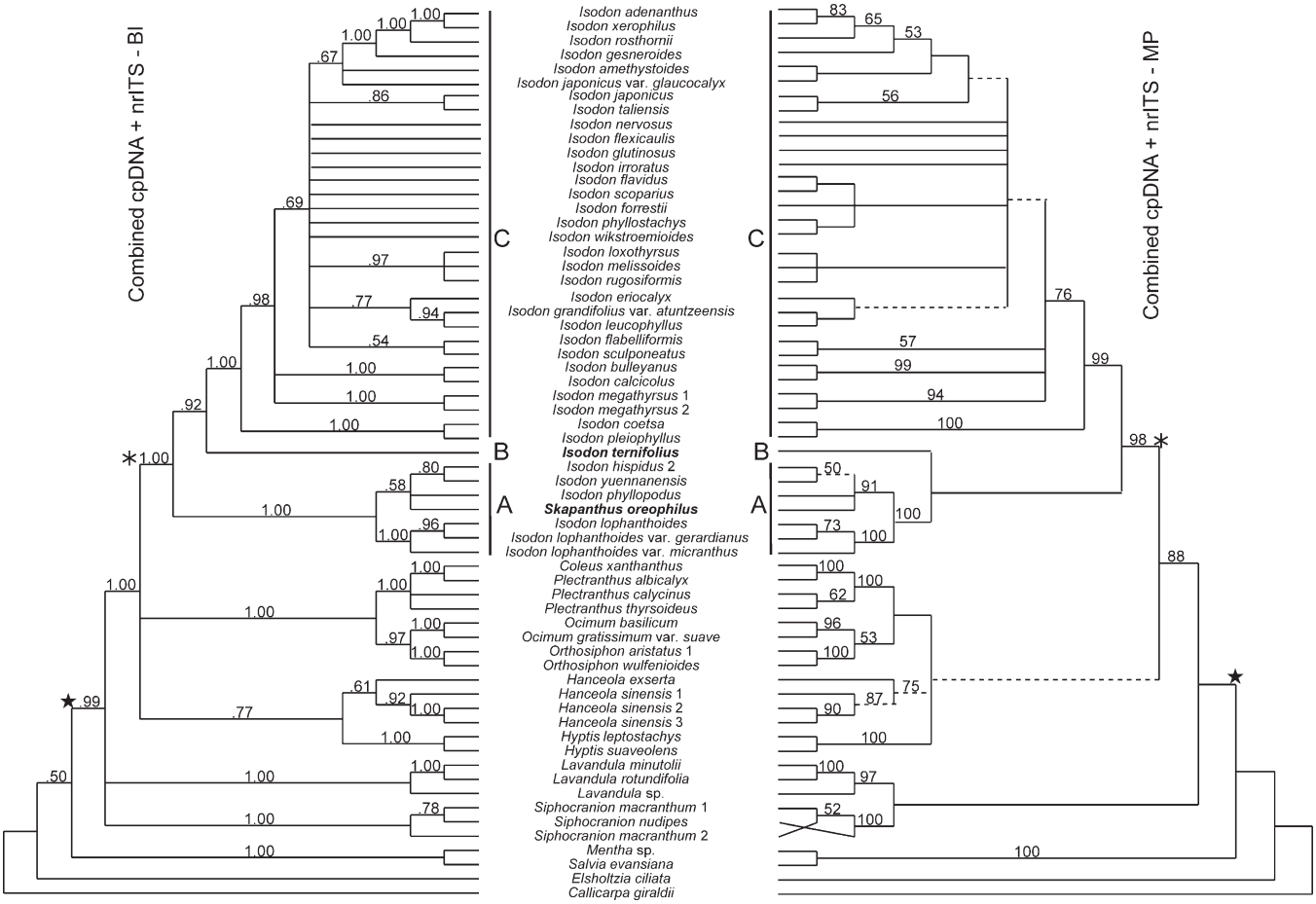

FIG. 4. Left: Bayesian consensus of 9001 trees derived from the analyses of cpDNA + nrITS sequence data, Bayesian posterior probability values greater than 50% are shown above branches. Right: Majority consensus tree derived from the MP analyses of cpDNA + nrITS sequence data, Bootstrap values greater than 50% are shown above branches, Dashed lines indicated those branches that collapsed in strict consensus tree. A star indicates the Ocimeae clade, an asterisk the *Isodon* clade.

neither of the groups received bootstrap support in the parsimony analysis (BS < 50%).

Morphological Analyses—The MP analyses of the morphological matrix (Appendix 2 and supplemental on-line Appendix 1) generated 10,000 equally parsimonious trees (L = 80, CI = 0.37, RI = 0.75, Fig. 5). The Ocimeae were monophyletic (BS = 78%) with the morphological synapomorphies of dorsifixed, synthecous anthers (characters 2 and 13; see also Paton et al. 2004). *Hanceola*, with *Hyptis* nested within it (BS = 52%) were sister to the remainder, with the *Lavandula* clade (BS = 52%), consisting of *Orthosiphon* intermixed with *Lavandula* as the second clade within Ociminae, sister to *Ocimum*. The remaining clade consisted of a basal *Siphocranion* grade (BS = 50%), below Plectranthinae and *Isodon* (incl. *Skapanthus*) clades (BS = 50%). Within the *Isodon* clade there were two major subclades: one defined by leaves with brown abaxial glands and including *Skapanthus oreophilus* as a terminal element; the second defined on basally saccate or spurred upper corolla bases and with *Plectranthus calycinus* as a highly derived, terminal taxon.

Seventeen phylogenetically informative characters were selected and mapped onto the Bayesian cladogram inferred from the combined cpDNA + nrITS analyses (Fig. 6). Synthecous anthers (Character 2), dorsifixed anthers (Character 13) and reflexed stamens (Character 11) can be used to delimit the tribe Ocimeae, even though the latter character was thought previously to be plesiomorphic (Paton et al. 2004). However, finding unique morphological character state synapomorphies that support the molecular clades is extremely difficult, if not impossible, at least for the data available.

Isodon can be distinguished by the possession of: (a) a paniculate inflorescence (Character 1) and not or an only slightly enlarged abaxial disc lobe (Character 15), with *Siphocranion* displaying a raceme-like inflorescence and a conspicuously enlarged lobe; (b) filament proximal attachment within the corolla tube of a conspicuous abaxial disc lobe, concave lower corolla lip, and never a 1/4-bilabiate fruiting calyx as occurs in *Plectranthus*; and (c) the presence of bracteoles in the inflorescence (absent in *Ocimum* and *Orthosiphon*). In addition, 2/3-bilabiate corolla limbs (Character 6) are peculiar to *Hanceola* and *Hyptis,* with the rest of the Ocimeae displaying a 4/1-bilabiate pattern.

Within *Isodon*, fruiting calyx shape (Characters 5, 24) and inflorescence character (Character 25) although currently used to subdivide *Isodon* are distributed sporadically across the tree without any correspondence to subgroups within the genus. In contrast, the presence of abaxial leaf glands and abietane quinones (Characters 4, 17) separate Clade A from the other members of the genus, while verticillate leaves (Character 3) occur only in *I. ternifolius* (Clade B).

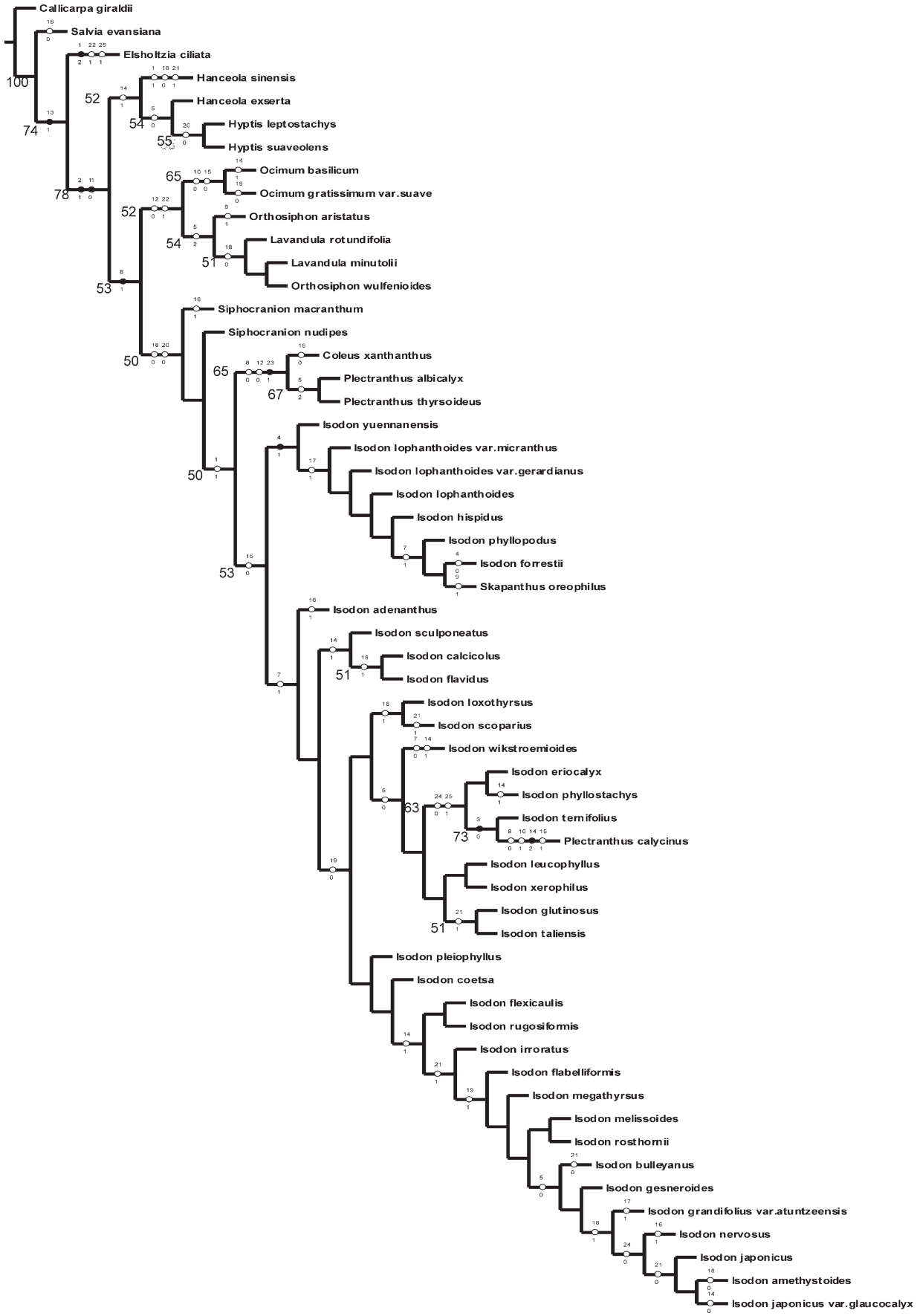

FIG. 5. Randomly selected equally most parsimonious tree from 10,000 trees derived by heuristic analysis of morphological data set (L = 80, CI = 0.37, RI = 0.75). Characters for each are shown above, with their states below; hollow circles represent homoplasious characters and filled symbols represent unique synapomorphies. Bootstrap values greater than 50% at nodes are indicated at branch angles.

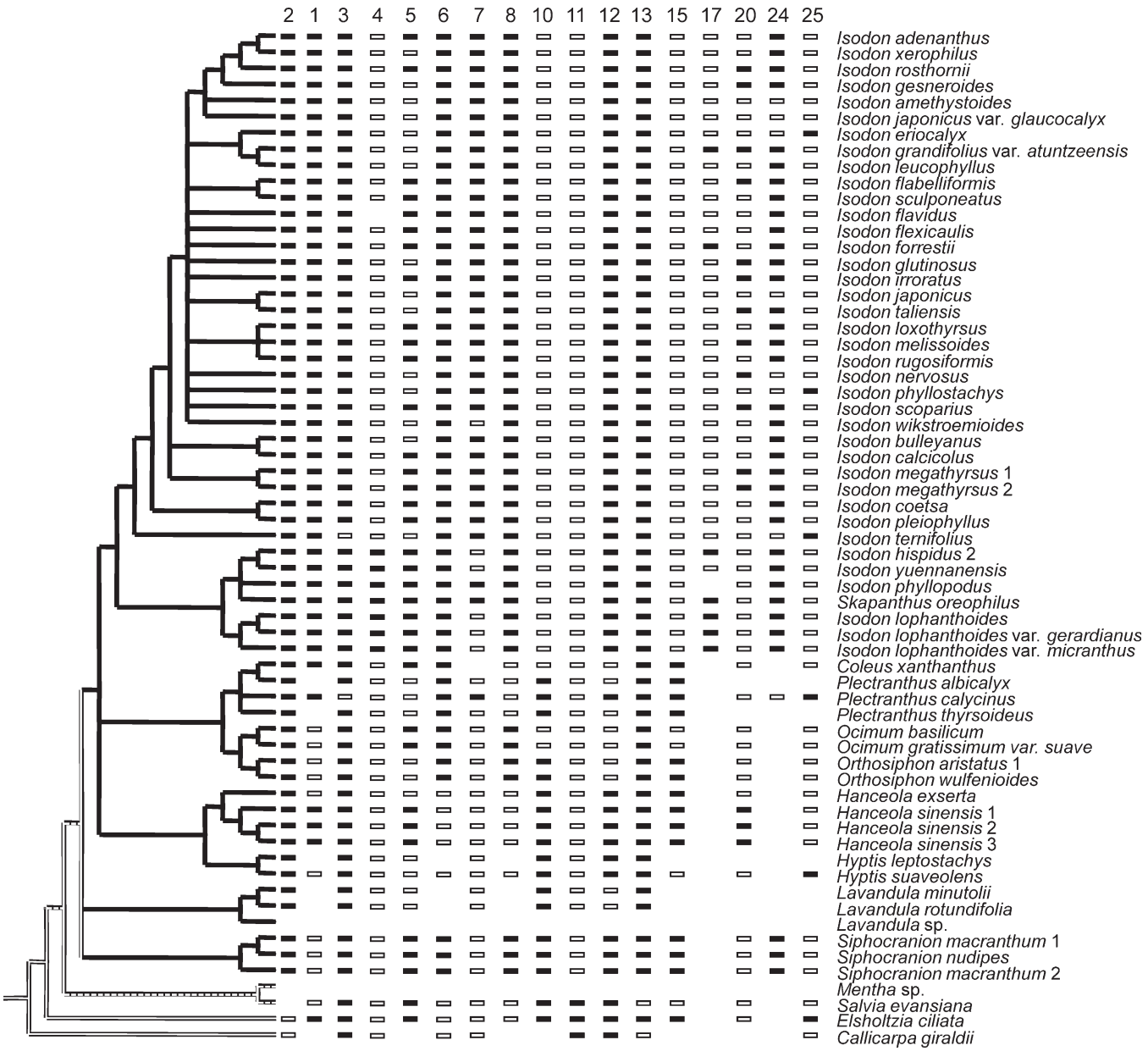

FIG. 6. Selected morphological characters mapped on the Bayesian consensus tree derived from cpDNA + nrITS combined data set. The distribution of the characters is shown with numbered columns of boxes (corresponding to the characters marked with an asterisk in APPENDIX 2).

In contrast, however, no characters were observed in this study to define the species-rich Clade C, which shows a relatively high level of variation in terms of corolla tube (long or short, basally saccate, or spurred upper corolla bases) and fruiting calyx features (5-sub- or equal lobes or 3/2-bilabiate), as well as inflorescence density and indumentum characters. Furthermore, the presence of abaxial leaf glands and abietane quinones (Characters 4, 17) within *Skapanthus* are features otherwise found in Clade A, suggesting that they have evolved convergently.

Discussion

Delimitation and Evolution of Isodon—Species of *Isodon* and *Skapanthus* formed a group with strong support in all analyses (Figs. 2–4). The recognition of the monotypic genus *Skapanthus* was controversial, as it was based on the single character of a corolla limb with a deeply trifid upper lip and the middle lobe widened and notched (Wu and Li 1977), but which could also be interpreted as an unequally 4-lobed upper lip (Paton and Ryding 1998). However, *Skapanthus* also shares affinities with species from *Isodon*, including calyx structure, the basal insertion of the stamens in the corolla tube, the absence of a finger-like abaxial disc lobe and the presence of red-brown glands in the lower surface of the leaves (Wu and Li 1977; Li and Hedge 1994). In our analyses, all of these characters were phylogenetically informative, circumscribing *Isodon* and helping to clarify its relationships to allied genera (Fig. 6 characters 4, 5, 10 and 15). Given that *Skapanthus oreophilus* was nested deeply within *Isodon* in all our analyses (Figs. 2–5), we concur with Paton and Ryding's (1998) decision to merge *Skapanthus* into *Isodon* (as done by Harley et al. 2004).

Accordingly, based on our results and the previous studies of Ryding (1993b) and Paton and Ryding (1998), we delimited

Isodon sensu Li (1988) as a monophyletic genus, but including the Chinese endemic *Skapanthus oreophilus*.

In order to circumscribe *Isodon* completely, the two African species *I. ramosissimus* (Hook. f.) Codd (= *Plectranthus ramosissmius* Hook. f. or *Homalocheilos ramosissimum* (Hook. f.) J. K. Morton) and *Isodon schimperi* Morton (1998) (= *Plectranthus schimperi* Vatke) should also be discussed, even though they were not available for inclusion in the present study. They differ in possessing posterior stamens inserted at the mouth of the corolla tube (Morton 1962; Codd 1984), while the Asian species have posterior stamens inserted in the proximal half (Fig. 6, character 10). Although the two African *Isodon* species have posterior stamens attached generally higher in the corolla tube than the Asian ones, they are not at the base of the anterior lobe and contiguous with the anterior stamens, as is the case for *Plectranthus*; the 3/2-bilabiate calyx and inflorescence are also typical of *Isodon*, not *Plectranthus.* Although neither African species was available for inclusion in this analysis, there is no strong evidence to suggest that they are not members of *Isodon* (Morton 1998; Harley et al. 2004). However, their distribution in Africa, which is a center of diversity for *Plectranthus* (Morton 1962; Codd 1984; Harley et al. 2004; Paton et al. 2004) and more distal attachment of its posterior stamens suggest the affinities of these two African species required further study.

Relationships within Isodon—*Isodon* is traditionally divided into four sections: *Amethystoides*, *Isodon*, *Melissoides,* and *Pyramidium*, but these were not supported either by the single or combined datasets (Figs. 2–4). For example *Isodon hispidus* (Benth.) Murata, *I. lophanthoides* (Buch.-Ham. ex D. Don) H. Hara plus its varieties, *I. phyllopodus* (Diels) Kudo and *I. yuennanensis* (Hand.-Mazz.) H. Hara (section *Isodon*) were grouped with *Skapanthus* (Clade A in Figs. 2–4), while the majority of section *Pyramidium* and the remainder of section *Isodon* were nested variously with members of sections *Amethystoides* and *Melissoides* (Clade C in Figs. 2–4). In contrast, *I. ternifolius* (D. Don) Kudo (section *Pyramidium*) was isolated (B in Figs. 2–4) and placed as sister to either *Isodon* Clade A (Fig. 3) or C (Figs. 2 and 4).

In the morphological analyses, floral structure, particularly the calyx and inflorescence structure, did not concur with molecular groups (Fig. 6, characters 1, 5, 6, 24 and 25), despite their importance in previous classifications of the genus. Instead, leaf arrangement (verticillate vs. opposite) and abaxial leaf glands were phylogenetically informative, delimiting the single species branch *Isodon ternifolius*: branch B (Fig. 6, character 3) and clade A (Fig. 6, character 4) respectively.

The verticillate species *Isodon calycinus* had been thought to be closely related to *I. ternifolius.* However, it was returned to *Plectranthus* by Ryding (1993b) based on the presence of bracteoles in the cymes, 1/4-bilabiate calyx lobes, the distal insertion of stamens within the corolla tube and a strongly enlarged abaxial disc lobe; all of which are considered to be defining features for *Plectranthus*. Moreover, recent molecular analyses supported this transfer (Paton et al. 2004).

The relationships among the species within Clade C are not well resolved, and although some of these species are morphologically different, the variation of the molecular sequences studied are otherwise quite low, representing short branch lengths (data not shown here). The resulting topology may therefore represent a hard polytomy and/or be evidence for a recent, rapid radiation in southwest China, where most of these species are distributed.

Phytochemical investigation of more than 50 *Isodon* species (Sun et al. 2001) found that abietane quinones were usually present within those species with abaxially densely gland-dotted leaves, at least in China (species with this character from outside China have not been investigated). In contrast, even though the genus is generally rich in diterpenoids (Lin et al. 1991; Sun et al. 2001), abietane quinones were absent in the *Isodon* species forming clades B and C, except for *Isodon flavidus* (Hand.-Maz.) H. Hara. We hypothesize that secondary metabolites might help elucidate the infrageneric phylogeny of *Isodon*, but future studies using broader sampling of both taxa and phytochemicals are required.

Relationships Between Isodon and Allied Genera—Although *Isodon* was recognized as distinct by Spach (1840) nevertheless many species have been placed within *Plectranthus* (Bentham 1832–1836; Bentham and Hooker 1876; Briquet 1895-1897), and these two genera have traditionally been regarded as closely related (Kudo 1929; Codd 1968, 1975; Wu and Li 1977; Keng 1978; Cramer 1981). However, this view has changed recently, following evidence from pericarp structure (Ryding 1993a), other morphological characters (Paton and Ryding 1998; Harley et al. 2004) and molecular studies (Wagstaff et al. 1995; Suddee 2001; Paton et al. 2004). In a parsimony analysis of cpDNA restriction site variation (Wagstaff et al. 1995), *Isodon* grouped with *Hyptis*, indicating a relationship and based in part on previous studies (Ryding 1993a). However, Suddee (2001) accepted the recognition of subtribe Hanceolinae, as defined by Paton and Ryding (1998) to include *Hanceola*, *Isodon* (incl. *Skapanthus*) and *Siphocranion*. Moreover, Suddee (2001) suggested that *Isodon* (the only sampled genus representing subtribe Hanceolinae), was sister to the rest of tribe Ocimeae, based on the analyses of cpDNA sequences, with *Lavandula* recognized as a distinct tribe (Lavanduleae) closely related to Ocimeae. This point of view was later partially accepted by Harley et al. (2003, 2004), who concluded that several Asiatic genera including *Hanceola* (either incl. *Siphocranion* or as a distinct genus), *Isodon* and *Skapanthus* were all closely related and together represented a new subtribe (Hanceolinae) within tribe Ocimeae.

Nevertheless, close affinities between *Isodon*, *Hanceola,* and *Siphocranion* were not supported in our results, with *Siphocranion* and *Hanceola* instead forming a separate clade within tribe Ocimeae, both in the separate nrITS and combined cpDNA + nrITS analyses (Figs. 3 and 4).

Furthermore, a single clear sister taxon for *Isodon* was not identified here, agreeing with the study of Paton et al. (2004). Nevertheless, all the sampled *Isodon* species (incl. *Skapanthus*) formed a strongly supported monophyletic lineage separate from the clades containing other genera and subtribes (which were also well supported), suggesting that *Isodon* (incl. *Skapanthus*) represents a distinct subtribe within tribe Ocimeae. Morphological synapomorphies such as the basal insertion of stamens in the corolla tube (Fig. 6, character 10) and the absence of enlarged, finger-like abaxial disc lobes (Fig. 6, character 15) further support the need for a new subtribe Isodoninae, sister to the Ociminae + Plectranthinae and *Hanceola* + *Hyptis* clades (Fig. 4).

Phylogenetic Placement of Siphocranion—One interesting finding is the position of the Sino-Himalayan genus *Siphocranion* (Figs. 2–4). Our results show that the only two species of the genus form a very strongly supported clade, rejecting the reduction of *Siphocranion* as a synonym of *Hanceola* by Cantino et al. (1992) and Ryding (1993a), suggesting

instead a relatively weak, possible sister-group relationship between the *Siphocranion* and *Lavandula* (Figs. 3–4).

The transferral of *Plectranthus macranthus* Hook. f. into Kudo's (1929) previously monotypic *Siphocranion* by Wu (1959) is also supported. *Plectranthus macranthus* is similar to *S. nudipes* in distal leaf arrangement, the possession of two-flowered, verticillate, raceme-like terminal inflorescences with small bracts, a subequally five-toothed, broadly campanulate calyx in flower, which is strongly dilated, nerved, and conspicuously 3/2-bilabiate in fruit, a broader upper-lip, straight and narrow corolla tube, and included stamens with glabrous filaments.

Wu (1959) placed his expanded *Siphocranion* within tribe Pogostemoneae, suggesting a sister-group relationship to *Hanceola* with these two genera as the link between Pogostemoneae (= Pogostemonoideae: Elsholtzeae sensu Cantino et al. 1992) and Ocimoideae (= Ocimeae sensu Cantino et al. 1992). Wu and Li (1977) considered that *Siphocranion* was most probably related to *Hanceola,*whereas Li (1988) regarded it to be closely related to *Isodon* and *Plectranthus*. However, none of these relationships is supported by our analyses, with *Siphocranion* instead showing affinities to *Lavandula* or all the remaining members of tribe of Ocimeae.

Siphocranion is characterized by the combination of a raceme-like inflorescence (consisting of sessile one to three flowered cymes), a long tubular corolla which is neither saccate nor calcarate above the adaxial base, a shortly four-limbed upper lip with an entire lower lip, and stamens inserted at, or near the mouth of the corolla tube (Li 1988; Li and Hedge 1994) (Fig. 6, characters 1, 6, 7 and 10). Despite these characters occurring variously within Ociminae and Plectranthinae (other than *Isodon* and *Hanceola*) and therefore being plesiomorphic or at least homoplasious, the combination of these features nevertheless easily characterizes the genus. In contrast, the presence of bracteoles in the inflorescence, which was once regarded as a synapomorphy for these Asian genera, seems to represent instead parallel or convergent evolution, or even reversal, but this will require additional analyses (Fig. 6), as well as wider taxon sampling and a better resolved phylogeny within tribe Ocimeae. The possession of a more or less erect corolla tube distinguishes *Siphocranion* from most Ocimeae, instead making it resemble species of *Elsholtzia* (Elsholtzeae).

Hanceola and Hyptis—The Asian genus *Hanceola* and the American genus *Hyptis* form a clade in the combined nrITS + cpDNA Bayesian analysis, albeit without strong support (PP = 0.77, Fig. 4), and they also share features such as a long corolla tube with 2/3-bilabiate corolla limbs, hairy stamen filaments, apical insertion of posterior stamens in the corolla tube and the presence of bracteoles in the inflorescence (Wu 1959; Li and Hedge 1994), but these characters also occur elsewhere such as in *Elsholtzia* (Fig. 6) and are thus homoplasious. The 2/3-bilabiate corolla limbs are unusual within Ocimeae, in which most taxa are 4/1-bilabiate, thus might represent a synapomorphy for the two genera, even though this character state is homoplasious and potentially plesiomorphic within the wider Lamiaceae where it is common.

As general conclusions, the study used DNA sequence data and morphological characters to investigate the phylogeny of *Isodon*, *Hanceola*, *Siphocranion,* and related genera in this study. *Siphocranion* is monophyletic and sits either as sister to *Lavandula*, or in a polytomy with *Lavandula* and Ocimeae. *Isodon*, if expanded to include the nested *Skapanthus oreophilus*, forms a distinct subtribe (Isodoninae) within tribe Ocimeae, sister to the remainder. The phylogenetic analyses identified three lineages within *Isodon,* but morphological synapomorphies only supported two of the clades, with no obvious features to define the largest, most poorly-resolved lineage, indicating that further studies are needed. Similarly, the position of the two (unsampled) African *Isodon* species needs to be tested, in particular whether they group with the Asian species or fall elsewhere within Ocimeae.

A relationship between *Hanceola* and *Hyptis* was also indicated in the combined Bayesian nrITS + cpDNA analysis; however, more studies are needed to confirm this. As a result, subtribe Hanceolinae is maintained and restricted to accommodate *Hanceola* alone, awaiting new evidence to determine its phylogenetic placement relative to Hyptidinae and Ocimeae generally.

Similarly, the relationships of *Isodon* within tribe Ocimeae remain unresolved, with the position of subtribe Isodoninae uncertain relative to the Ociminae + Plectranthinae and *Hanceola* + *Hyptis* clades. Accordingly, further studies of Ociminae using additional characters and more extensive taxon sampling are required to resolve the issues raised by this study, both for generic/subtribal relationships as well as species evolution within *Isodon*.

Taxonomic Treatment

We propose here two new subtribal classifications of Ocimeae based on the combined molecular (Fig. 4 left) and morphological analyses (Fig. 5), as well as the results of previous studies. It is evident that subtribe Hanceolinae as currently defined is polyphyletic and as a result, is here restricted to *Hanceola,* with *Skapanthus* transferred to a new subtribe with *Isodon,* and *Siphocranion* similarly forming a second new subtribe, both within tribe Ocimeae.

Subtribe **Isodoninae** J. S. Zhong, J. Li, & H. W. Li, subtrib. nov.—TYPE: *Isodon* (Schrad. ex Benth.) Spach

Only genus: *Isodon* (Schrad. ex Benth.) Spach

Paniculae terminales vel axillares; inflorescentiae bracteolatae; dentes calycis subaequales vel in 3/2-bilabiati; corollae 4/1-bilabiatae; stamina declinata, prope basin tubi corollae inserta, filamenta dorsifixa, antherae syntheceae; discus antice inconspicueve dilatatus vel non dilatatus.

Subtribe **Siphocranioninae** J. S. Zhong, J. Li, & H. W. Li, subtrib. nov.—TYPE: *Siphocranion* Kudo

Only genus: *Siphocranion* Kudo

Cymis sessilibus terminales vel axillares; dentes calycis subaequale vel in 3/2-bilabiati; corollae 4/1-bilabiatae, erectae; stamina supra medium tubi corollae inserta, declinata, filamenta dorsifixa, antherae syntheceae; discus antice conspicue dilatatus.

Subtribe Hanceolinae (C. Y. Wu) A. J. Paton, Ryding, & Harley, Kew Bulletin 58: 487. 2003. p.p. excl. *Isodon*, *Siphocranon* & *Skapanthus*. Hanceoleae C. Y. Wu, Acta Phytotax. Sin. 8: 58. 1959.—TYPE: *Hanceola* Kudo

Only genus: *Hanceola* Kudo

Inflorescentiae bracteolatae; dentes calycis subaequales; corollae 2/3-bilabiatae; stamina declina, filamenta dorsifixa, antherae syntheceae; discus antice conspicue dilatatus.

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Appendix 1. Voucher information and GenBank accession numbers (in the following order: *trnL–F* region, *rps16* intron, and ITS) of the taxa sampled in this study. Accession numbers beginning with AJ are from Paton et al. (2004), with AY from Trusty et al. (2004), DQ from Walker and Sytsma (2007), EF from Kersten and Knoess (unpubl. data). Voucher specimens are deposited in the following herbaria: Xishuangbanna Tropical Botanical Garden Herbarium of CAS (HITBC); Kunming Institute of Botany Herbarium of CAS (KUN). Those regions not sampled for a taxon are represented by a dash. [*Four ITS sequences from *Isodon eriocalyx* were obtained, three of them from cloning; **This species was reduced into *Isodon setschwanensis* (Hand.-Mazz.) H. Hara by Li (1988).]

***Ingroup sequences*—*Isodon* sect. *Pyramidium* (Benth.) H. W. Li:** *I. eriocalyx* (Dunn) Kudo*, Yunnan, China, *Zhong ZJS 03* (HITBC), FJ593422, FJ593302, FJ593361 - FJ593364; *I. phyllostachys* (Diels) Kudo, Yunnan, China, *Zhong & Li 0133* (HITBC), FJ593444, FJ593324, FJ593388; *I. ternifolius* (D. Don) Kudo, Yunnan, China, *Zhong 0009* (HITBC), FJ593451, FJ593331, FJ593395; ***Isodon* sect. *Amethystoides* (Benth.) H. W. Li:** *I. amethystoides* (Benth.) H. Hara, Fujian, China, *Zhong & Li 2007023* (HITBC), FJ593418, FJ593298, FJ593357; *I. japonicus* (Burm. f.) H. Hara, Henan, China, *Zhang 103* (KUN), FJ593432, FJ593312, FJ593375; *I. japonicus* var. *glaucocalyx* (Maxim.) H. W. Li, Beijing, China, *Zhong 2006021* (HITBC), FJ593433, FJ593313, FJ593376; *I. nervosus* (Hemsl.) Kudo, Sichuan, China, *Zhong 2007065* (HITBC), FJ593442, FJ593322, FJ593386; ***Isodon* sect. *Isodon*:** *I. adenolomus* (Hand.-Mazz.) H. Hara, Yunnan, China, *Lin s. n.* (KUN), —, —, FJ593356; *I. bulleyanus* (Diels) Kudo, Yunnan, China, *Zhong & Li 0035* (HITBC), FJ593419, FJ593299, FJ593358; *I. calcicolus* (Hand.-Mazz.) H. Hara, Yunnan, China, *Zhong & Li 0164* (HITBC), FJ593420, FJ593300, FJ593359; *I. coetsa* (Buch.-Ham. ex D. Don) Kudo, Yunnan, China, *Lin s. n.* (KUN), FJ593421, FJ593301, FJ593360; *I. flabelliformis* (C. Y. Wu) H. Hara, Yunnan, China, *Zhong & Li 0016* (HITBC), FJ593423, FJ593303, FJ593303; *I. flavidus* (Hand.-Mazz.) H. Hara, Yunnan, China, *Lin 081* (KUN), FJ593424, FJ593304, FJ593366; *I. flexicaulis* (C. Y. Wu & H. W. Li) H. Hara, Sichuan, China, *Lin 032* (KUN), FJ593425, FJ593305, FJ593367; *I. forrestii* (Diels) Kudo, Yunnan, China, *Zhong & Li 0114* (HITBC), FJ593426, FJ593306, FJ593368; *I. gesneroides* (J. Sinclair) H. Hara, Sichuan, China, *Lin 035* (KUN), FJ593427, FJ593307, FJ593369; *I. glutinosus* (C. Y. Wu & H. W. Li) H. Hara, Yunnan, China, *Zhong & Li 0178* (HITBC), FJ593428, FJ593308, FJ593370; *I. grandifolius* (Hand.-Maz.) H. Hara var. *atuntzeensis* (C. Y. Wu) H. W. Li, Yunnan, China, *Zhong & Li 0194* (HITBC), FJ593429, FJ593309, FJ593371; *I. hispidus* (Benth.) Murata (1), Yunnan, China, *Lin s. n.* (KUN), —, —, FJ593372; *I. hispidus* (Benth.) Murata (2), Yunnan, China, *Zhong & Li 0057* (HITBC), FJ593430, FJ593310, FJ593373; *I. irroratus* (Forrest ex Diels) Kudo, Yunnan, China, *Zhong & Li 0071* (HITBC), FJ593431, FJ593311, FJ593374; *I. leucophyllus* (Dunn) Kudo, Yunnan, China, *Zhong & Li 0183* (HITBC), FJ593434, FJ593314, FJ593377; *I. lophanthoides* (Buch.-Ham. ex D. Don) H. Hara, Yunnan, China, *Zhong & Li 0061* (HITBC), FJ593435, FJ593315, FJ593378; *I. lophanthoides* var. *gerardianus* (Benth.) H. Hara, Guangdong, China, *Zhong 005* (HITBC), FJ593436, FJ593316, FJ593379; *I. lophanthoides* var. *micranthus* (C. Y. Wu) H. W. Li, Yunnan, China, *Lin 045* (KUN), FJ593437, FJ593317, FJ593380; *I. loxothyrsus* (Hand.-Mazz.) H. Hara, Yunnan, China, *Zhong & Li 0186* (HITBC), FJ593438, FJ593318, FJ593381; *I. megathyrsus* (Diels) H. W. Li (1), Yunnan, China, *Lin-Fugong 2* (KUN), FJ593439, FJ593319, FJ593383; *I. megathyrsus* (Diels) H. W. Li (2), Sichuan, China, *Zhong 2007057* (HITBC), FJ593440, FJ593320, FJ593384; *I. phyllopodus* (Diels) Kudo, Yunnan, China, *Lin 054* (KUN), FJ593443, FJ593323, FJ593387; *I. pleiophyllus* (Diels) Kudo, Yunnan, China, *Lin-Lushui 3* (KUN), FJ593445, FJ593325, FJ593389; *I. rosthornii* (Diels) Kudo, Sichuan, China, *Zhong 2007051* (HITBC), FJ593446, FJ593326, FJ593390; *I. rugosiformis* (Hand.-Mazz.) H. Hara, Yunnan, China, *Zhong 2006008* (HITBC), FJ593447, FJ593327, FJ593391; *I. scoparius* (C. Y. Wu & H. W. Li) H. Hara, Yunnan, China, *Lin 050* (KUN), FJ593448, FJ593328, FJ593392; *I. sculponeatus* (Vaniot) Kudo, Yunnan, China, *Zhong-West Hill 001* (HITBC), FJ593449, FJ593329, FJ593393; *I. taliensis* (C. Y. Wu) Hara**, Yunnan, China, *Zhong & Li 0078* (HITBC), FJ593450, FJ593330, FJ593394; *I. wikstroemioides* (Hand.-Mazz.) H. Hara, Yunnan, China, *Zhong & Li 0201* (HITBC), FJ593452, FJ593332, FJ593396; *I. xerophilus* (C. Y. Wu & H. W. Li) H. Hara, Yunnan, China, *Lin 015* (KUN), FJ593453, FJ593333, FJ593397; *I. yuennanensis* (Hand.-Mazz.) H. Hara, Yunnan, China, *Zhong & Li 0172* (HITBC), FJ593454, FJ593334, FJ593398; ***Isodon* sect. *Melissoides* (Benth.) H. W. Li:** *I. adenanthus* (Diels) Kudo, Yunnan, China, *Zhong & Li 0039* (HITBC), FJ593417, FJ593297, FJ593355; *I. lungshengensis* (C. Y. Wu & H. W. Li) H. Hara, Guangxi, China, *Jiang 017* (KUN), —, —, FJ593382; *I. melissoides* (Benth.) H. Hara, *Lin 083* (KUN), China, FJ593441, FJ593321, FJ593385;

Subtribe Hanceolinae A. J. Paton et al.: *Hanceola exserta* Y. Z. Sun, Fujian, China, *Zhong & Li 2007022* (HITBC), FJ593413, FJ593293, FJ593350; *H. sinensis* (Hemsl.) Kudo (1), Sichuan, China, *KUN 0216151* (KUN), FJ593414, FJ593294, FJ593351; *H. sinensis* (Hemsl.) Kudo (2), Sichuan, China, *Zhong 2007054* (HITBC), FJ593415, FJ593295, FJ593352; *H. sinensis* (Hemsl.) Kudo (3), Sichuan, China, *Zhong 2007064* (HITBC), FJ593416, FJ593296, FJ593353; *Siphocranion macranthum* (Hook. f.) C. Y. Wu (1), Xizang, China, *KUN 0821407* (KUN), FJ593463, FJ593343, FJ593406; *S. macranthum* (Hook. f.) C. Y. Wu (2), Sichuan, China, *Zhong 2007061* (HITBC), FJ593464, FJ593344, FJ593407; *S. nudipes* (Hemsl.) Kudo, Yunnan, China, *KUN 0821428* (KUN), FJ593465, FJ593345, FJ593408; *Skapanthus oreophilus* (Diels) C. Y. Wu & H. W. Li, Yunnan, China, *Zhong & Li 0107* (KUN), FJ593466, FJ593346, FJ593409;

Subtribe Hyptidinae Endl.: *Eriope latifolia* Mart. ex Benth., —, —, DQ787416; *Hyptis alata* Shinners, —, —, DQ667346; *H. brevipes* Poit., Mexico, *KUN 0216173* (KUN), —, —, FJ593354; *H. eriocephala* Benth., AJ505450, AJ505338, —; *H. floribunda* Briq. ex Micheli, AJ505451, AJ505339, —; *H. leptostachys* Epling, AJ505452, AJ505340, —; *H. suaveolens* (L.) Poit., AJ505453, AJ505341, —;

Subtribe Lavandulinae Endl.: *Lavandula angustifolia* Mill. (1), Yunnan, China, *Zhong 2006022* (HITBC), —, —, FJ593399; *L. angustifolia* Mill. (2), —, —, EF437225; *L. minutolii* C. Bolle, AJ505462, AJ505348, —; *L. rotundifolia* Benth., AJ505463, AJ505349, —; *Lavandula* sp., Guangdong, China, *Zhong 020* (HITBC), FJ593455, FJ593335, FJ593400;

Subtribe Plectranthinae Endl.: *Coleus xanthanthus* C. Y. Wu & Y. C. Huang, Yunnan, China, *Lin 094* (KUN), FJ593411, FJ593291, FJ593348; *Plectranthus albicalyx* S. Suddee, AJ505498, AJ505376, —; *P. buchananii* Bak., AJ505501, AJ505379, —; *P. calycinus* Benth., AJ505502, AJ505380, —; *P. coeruleus* (Gürke) Agnew, AJ505503, AJ505381, —; *P. crassus* N. E. Br., AJ505504, AJ505382, —; *P. fredricii* (G. Taylor) A. J. Paton, AJ505505, AJ505384, —; *P. glabratus* (Benth.) Alston, AJ505508, AJ505387, —; *P. laxiflorus* Benth., AJ505510, AJ505389, —; *P. parishii* Prain, AJ505511, AJ505390, —; *P. petiolaris* Benth., AJ505512, AJ505391, —; *P. sanguineus* Britten, AJ505513, AJ505392, —; *P. strigosus* Benth., —, —, AY506662; *P. thyrsoideus* (Bak.) Mathew, AJ505533, AJ505405, —; *P. xerophilus* Codd, AJ505515, AJ505394, —; *Pycnostachys reticulata* (E. Mey.) Benth., AJ505516, AJ505395, —; *P. umbrosa* (Vatke) Perkin, AJ505517, AJ505396, —; *P. urticifolia* Hook., AJ505518, AJ505397, —; *Tetradenia fruticosa* Benth., AJ505519, AJ505398, —; *T. nervosa* Codd, AJ505520, AJ505399, —; *Thorncroftia longifolia* N.E. Br., AJ505521, AJ505401, —; *T. media* Codd, AJ505522, AJ505400, —;

Subtribe Ociminae (Dumort.) Schmidt: *Ocimum basilicum* L., Yunnan, China, *Zhong 0006* (HITBC), FJ593458, FJ593338, —; *O. basilicum* L., —, —, DQ667240, *O. gratissimum* L. var. *suave* (Willd.) Hook. f., Yunnan, China, *Zhong 0007* (HITBC), FJ593459, FJ593339, FJ593402; *O. labiatum* (N.E.Br.) A. J. Paton, AJ505471, AJ505356, —; *O. selloi* Benth., AJ505542, AJ505419, —; *O. serratum* (Schtr.) Miq., AJ505472, AJ505357, —; *O. tenuiflorum* L., AJ505473, AJ505358, —; *Orthosiphon aristatus* (Blume) Miq. (1), Yunnan, China, *Zhong 0008* (HITBC), FJ593460, FJ593340, FJ593403; *O. aristatus* (Blume) Miq. (2), —, —, EF421427; *O. parishii* Prain, AJ505475, AJ505359, —; *O. wulfenioides* (Diels) Hand.-Mazz., Yunnan, China, *Zhong 2006009* (HITBC), FJ593461, FJ593341, FJ593404;

Outgroups*—*Callicarpa giraldii Hesse ex Rehder, Yunnan, China, *Zhong 0012* (HITBC), FJ593410, FJ593290, FJ593347; *Clinopodium tomentosum* (Kunth) Govaerts., —, —, DQ017559; *Collinsonia canadensis* L., —, —, DQ667248; *Elsholtzia ciliata* (Thunb.) Hyland, Yunnan, China, *Zhong 0010* (HITBC), FJ593412, FJ593292, FJ593349; *Mentha arvensis* L., —, —, DQ667325; *Mentha* sp., Guangdong, China, *Zhong 014* (HITBC), FJ593456, FJ593336, FJ593401; *Nepeta fissa* C. A. Mey, AJ505430, AJ505323, —; *N. menthoides* Boiss. & Buhse, AJ505431, AJ505324, —; *N. stewartiana* Diels, Yunnan, China, *Zhong & Li 0117* (HITBC), FJ593457, FJ593337, —; *N. straussii* Hausskn. & Bornm., AJ505433, AJ505326, —; *Perilla frutescens* (L.)

Britt., —, —, DQ667246; *Salvia evansiana* Hand.-Mazz., Yunnan, China, *Zhong & Li 0158* (HITBC), FJ593462, FJ593342, FJ593405; *Salvia miltiorrhza* Bunge, —, —, DQ667334.

Appendix 2. Morphological characters and character states used for MP analysis and character mapping. * Selected morphological characters mapped on the Bayesian tree from the cpDNA + nrITS combined data set.

1.* Inflorescence— raceme = 0; panicle = 1; spike = 2. **2.* Anthers**— parallel or convergent = 0; synthecous = 1. **3.* Leaf arrangement**— whorls of 3 or 4 = 0; opposite = 1. **4.* Leaves**— absent of red brown glandular abaxially = 0; present of brown glandular abaxially = 1. **5.* Shape of fruit calyx**— 5-toothed equal or subequal = 0; 3/2 bilabiate = 1; 1/4 bilabiate = 2. **6.* Shape of corolla**— 2/3 bilabiate = 0; 4/1 bilabiate = 1; 1/3 bilabiate or other types = 2. **7.* Upper side of corolla tube near base**— not dilated = 0; saccate or spurred = 1. **8.* Shape of lower lip of the corolla**— deeply boat-shape = 0; concave = 1; spreading = 2. **9. Shape of upper lip of the corolla when lobes more than 1**— lobes subequal = 0; lobes unequal = 1. **10.* Attachment of posterior stamens**— base to mid = 0; mid to throat = 1; not base to mid or mid to throat = 2. **11.* Stamen position**— reflexed = 0; ascending or spreading or projected = 1. **12.* Bracteoles**— absent = 0; present = 1. **13.* Anthers attachment**— adnate or basifixed or versatile = 0; dorsifixed = 1. **14. Indumentum of stamen filaments**— glabrous = 0; hairy = 1; glandular = 2. **15.* Size of abaxial disc lobe**— not or slight enlarged = 0; strongly enlarged = 1. **16. Nutlet indumentum**— glabrous = 0; hairy in various ways = 1. **17.* Abietane quinones**— absent = 0; present = 1. **18. Stamen**— included = 0; exerted = 1. **19. Habit**— subshrub or shrub = 0; herb = 1. **20.* Lower lip of corolla**— narrow at base = 0; not narrow at base = 1. **21. Teeth of fruiting calyx**— less than 1/2 length of fruiting calyx tube = 0; more than 1/2 length of fruiting calyx tube = 1. **22. Spinescent apex of calyx teeth**— absent = 0; present = 1. **23. Filaments**— separate = 0; connate = 1. **24.* Fruiting calyx**: erect = 0; reflexed = 1. **25.* Inflorescence**: loose = 0; dense = 1.