
PHYLOGENY OF SAURURACEAE BASED ON MORPHOLOGY AND FIVE REGIONS FROM THREE PLANT GENOMES¹

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ABSTRACT

Phylogenetic relationships of the six extant species of four genera of the Saururaceae are resolved based on sequence data: 18S and ITS from the nuclear genome; *rbcL* and *trnL-F* from the chloroplast genome; and *matR* from the mitochondrial genome. *Zippelia begoniaefolia*, from a genus of Piperaceae, is used as an outgroup. Results are presented in separate and combined analyses of sequence data. Forty-nine morphological characters reconstruct the phylogeny in this family, again using *Zippelia* as outgroup. Whether the topologies of Saururaceae are based on individual genomic data sets, the combined DNA sequence data sets, morphological data sets, or the combined DNA sequence and morphological data sets, all are closely congruent. In all analyses, the monophyly of *Saururus* and *Gymnotheca*, respectively, is strongly supported, and the sister relationship between *Gymnotheca* and *Saururus* is well supported. In the analysis of nuclear DNA data sets, *Anemopsis* is the sister group to all other Saururaceae, with *Houttuynia* then sister to *Saururus* and *Gymnotheca*, and with *Saururus* sister to *Gymnotheca*; however, in the analyses of the other data sets, *Anemopsis* is the sister group of *Houttuynia*, and the *Anemopsis-Houttuynia* clade lies sister to the *Saururus-Gymnotheca* clade. The result that the *Anemopsis-Houttuynia* clade comprises the sister group of *Saururus-Gymnotheca* clade is novel and differs from previous phylogenetic opinion.

Key words: *Anemopsis*, genomes, *Gymnotheca*, *Houttuynia*, morphology, multigene data, phylogeny, Saururaceae, *Saururus*.

Saururaceae are a core member of the paleoherbs (Tucker & Douglas, 1996) and are an ancient family with six species in the four relictual genera *Saururus*, *Gymnotheca*, *Anemopsis*, and *Houttuynia* (Liang, 1995). These are all perennial herbs with simple flowers that bear bracts without perianths. Saururaceae have an East Asian–North American disjunction, with *Anemopsis californica* Hook. & Arn. and *Saururus cernuus* L. in North America, *Houttuynia cordata* Thunb., *Gymnotheca chinensis* Decne., *Gymnotheca involucrata* Pei, and *Saururus chinensis* (Lour.) Baill. in East Asia. Due to their basal systematic position and interesting geographical pattern of distribution, Saururaceae have been of much phylogenetic interest, although they are a small family including six species. Current view-

points on the phylogeny of Saururaceae diverge, based on gross morphology, cytology, and floral morphogenesis. Wu and Wang (1957) included *Saururus*, *Circaeocarpus*, *Anemopsis*, *Gymnotheca*, and *Houttuynia* in Saururaceae and thought that *Circaeocarpus*, *Anemopsis*, *Gymnotheca*, and *Houttuynia* derived directly from *Saururus* one after another. Later, they (Wu & Wang, 1958) realized that the recently published *Circaeocarpus* (Wu & Wang, 1957) was in fact a member of Piperaceae, and *Circaeocarpus saururoides* C. Y. Wu and *Zippelia begoniaefolia* Blume ex Schult. & L. H. Schult. were synonymous. Considering their biogeography, Wu (1984) later thought *Anemopsis* and *Houttuynia* to be products of a vicariance event, and *S. chinensis* and *S. cernuus* were products of another

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Table 1. List of species and vouchers** for Saururaceae and its outgroup (Piperaceae).

Species	Origin	Collection no.	18S	ITS
Ingroup				
<i>Anemopsis californica</i> Hook. & Arn.	U.S.A. Louisiana.	Liang 97017	AF197576*	AF21592
<i>Gymnotheca chinensis</i> Decne.	China. Yunnan: Xuanwei.	Meng 99003	AY032643	AF20362
<i>Gymnotheca involucrata</i> Pei	China. Sichuan: Emei.	Liang 97015	AY032644	AF20362
<i>Houttuynia cordata</i> Thunb.	China. Guizhou: Xingyi.	Meng 99301	AY032645	AF20362
<i>Saururus cernuus</i> L.	U.S.A. Louisiana.	Liang 97016	AY032646	AF22330
<i>Saururus chinensis</i> (Lour.) Baill.	China. Yunnan: Mengla.	Meng 99525	AY032647	AF21592
Outgroup				
<i>Zippelia begoniifolia</i> Blume ex Schult. & L. H. Schult.	China. Yunnan: Menglun.	Meng 99715	AY032648	AF20363

** All vouchers are deposited at KUN.

* Obtained from GenBank.

vicariance event. Based on basic chromosome numbers of *Saururus*, *Anemopsis*, and *Houttuynia*, Okada (1986) proposed that *Anemopsis* and *Houttuynia* were derived from *Saururus*. Lei et al. (1991) supported Okada's opinion and thought that *Gymnotheca* was the more derived taxon based on chromosome number. Based on a cladistic analysis of morphological and ontogenetic characters, Tucker et al. (1993) inferred that *Saururus* was the first to diverge from the ancestral Saururaceae, followed by *Gymnotheca*, with *Houttuynia* and *Anemopsis* as sister taxa. Combining data from gross morphology, anatomy, embryology, palynology, cytology, and flower development (Liang & Tucker, 1990; Liang, 1991, 1992, 1994, 1995), Liang (1995) proposed that the ancestors of Saururaceae were divided into two lineages: *Gymnotheca*–*Anemopsis* and *Saururus*–*Houttuynia*.

In spite of the fact that certain Saururaceae, such as *Saururus*, *Anemopsis*, and *Houttuynia*, have been represented in recent studies of higher-level phylogenetic relationships within the angiosperms (e.g., Chase et al., 1993; Soltis et al., 1997; Mathews & Donoghue, 1999; Qiu et al., 1999; Soltis et al., 2000), further investigation into the molecular systematics of Saururaceae has been needed to determine phylogenetic relationships within this family of basal angiosperms. Our phylogenetic assessment of Saururaceae is based on five genic regions from all three plant genomes: the 18S ribosomal RNA gene and ITS spacer (including 5.8S) from the nuclear genome; *rbcL* and *trnL-F*, its intron and gene spacer, from the chloroplast genome; and *matR* from the mitochondrial genome. Forty-nine morphological characters were also selected for phylogenetic analysis (Appendix 1). These morphological characters were comprised of subsets from gross morphology, anatomy, embryology, palynology,

cytology, and flower development. Generally, 18S, *rbcL*, and *matR* genes have been used to reconstruct higher-level phylogeny, such as relationships among orders, families, or distantly related genera (e.g., Chase et al., 1993; Soltis et al., 1997; Qiu et al., 1999), while ITS and *trnL-F* have commonly been used for genera, species, and lower-level questions (Baldwin et al., 1995; Bayer & Starr, 1998). We selected these five gene regions because taxa in this family likely diverged at diverse points in time.

MATERIALS AND METHODS

PLANT MATERIALS

All six species of the ingroup, *Anemopsis californica*, *Gymnotheca chinensis*, *G. involucrata*, *Houttuynia cordata*, *Saururus cernuus*, *S. chinensis*, and one designated outgroup, *Zippelia begoniaefolia* of Piperaceae, were collected from natural populations. Vouchers are deposited in the herbarium of Kunming Institute of Botany (KUN), Chinese Academy of Sciences, Kunming (see Table 1). The GenBank accession numbers of all relevant sequences are included.

DNA EXTRACTION, PCR, AND SEQUENCING

Genomic DNA was extracted from silica-gel-dried or fresh leaves using a modified CTAB procedure (Doyle & Doyle, 1987). PCR amplifications were conducted at a thermocycler (Perkin-Elmer 9600). It consisted of initial denaturization at 94°C (4 min.), followed by 35 cycles of 94°C denaturization (1 min.), 55°C annealing (1 min.), and 72°C extension (90 sec.), with a final extension for 7 min. at 72°C. The 18S primers used for amplification and sequencing were 5' CTAGAGCTAATA-

CGTGCAAC 3' (121F) and 5' GATAAGGTTCA-GTGGACTTC 3' (1692R). The primers of ITS, *rbcL*, *trnL-F*, and *matR* followed White et al. (1990), Feng et al. (1998), Taberlet et al. (1991), and Meng et al. (2002), respectively. PCR products were separated with 1.5% agarose TAE gel and were purified using Wizard PCR Preps DNA Purification System. Sequencing reactions were performed using PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Inc.). The products of sequencing reaction were electrophoresed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Inc.), which performs capillary electrophoresis and can ensure accurate base stretch above 1200 bp in one sequencing (guidebook about sequencing from Applied Biosystems, Inc.). Each studied DNA segment is sequenced twice from two ends in opposite directions.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

Contiguous DNA sequences were compiled using Seq^{Ed} (Applied Biosystems, Inc.). All sequences were aligned using Clustal X (Thompson et al., 1997) and Mega 2b3 (Sudhir et al., 2000). Maximum parsimony analyses were performed using PAUP 4.0 b10 (Swofford, 2001). We used the branch-and-bound search option with furthest addition sequence. Gaps were treated as missing data. A bootstrap analysis was performed with 1000 replicates (Felsenstein, 1985).

COMBINED DNA DATA ANALYSIS

We first analyzed individual genomic data sets after combining 18S and ITS to represent nrDNA, combining *rbcL* and *trnL-F* to represent cpDNA, and finally using *matR* DNA to represent mitochondrial genomic data. All DNA sequence data were then combined into one matrix to analyze. These combined data sets were analyzed using the same settings as the individual genomic data sets. After the phylogenetic tree was reconstructed from the combined DNA data sets, the matrix of all DNA sequences was re-analyzed with characters reweighted according to rescaled consistency indices (RC) (Farris, 1989).

Separate matrixes of each genomic data set as well as the matrix of combined DNA data sets are available upon request from the corresponding author.

MORPHOLOGICAL DATA ANALYSIS

Forty-nine morphological characters were selected to reconstruct the phylogeny in this family (Appendix 1). These characters were derived from herbarium specimens and literature (e.g., Liang & Tucker et al., 1990, 1995; Liang, 1991, 1992, 1994, 1995; Tucker, 1975, 1980, 1981, 1982a, b, 1985; Tucker et al., 1993; Lei et al., 1991; Carlquist et al., 1995; Meng & Liang, 1997). Six characters were vegetative; 3 were from stem anatomy; 15 were from floral morphology; 10 were from floral anatomy; 5 were from pollen; 8 were from embryology; and 2 were from cytology. Within this morphological character matrix (Appendix 2), 37 characters were treated as binary and 12 as multi-state. The analysis of Saururaceae was conducted using PAUP 4.0 b10 (Swofford, 2001). All characters were first equally treated as weighted and unordered. Other settings are the same as those for the molecular data. After a morphological phylogenetic tree was reconstructed using the above setting, the morphological matrix was also analyzed with characters reweighted according to rescaled consistency indices.

ANALYSIS OF THE COMBINED DNA AND MORPHOLOGICAL DATA

After replacing A, G, C, T from DNA sequence with 0, 1, 2, 3, respectively, we combined all DNA data sets with the morphological one into a common matrix and re-analyzed. All characters were unordered and equally weighted. Other settings in PAUP 4.0 were the same as those for the combined molecular data.

RECONSTRUCTION OF CHARACTER EVOLUTION

Using the program WinClada v. 0.9.99m 7.5 beta (Nixon, 1999), we analyzed the morphological matrix again and recovered a phylogenetic tree topology similar to that from the PAUP 4.0 analysis. The distribution of each morphological character was then analyzed to investigate the evolution of each morphological character in Saururaceae. The combination of this heuristic search, 1000 replications, with one starting tree per replication, using a multiple TBR and TBR search strategy, with zero random seed, and a slow optimization, was used in the maximum parsimony analysis.

RESULTS

NUCLEAR GENOME DATA ANALYSIS

The alignment of 7 sequences resulted in a matrix of 2279 aligned positions, of which 195 were

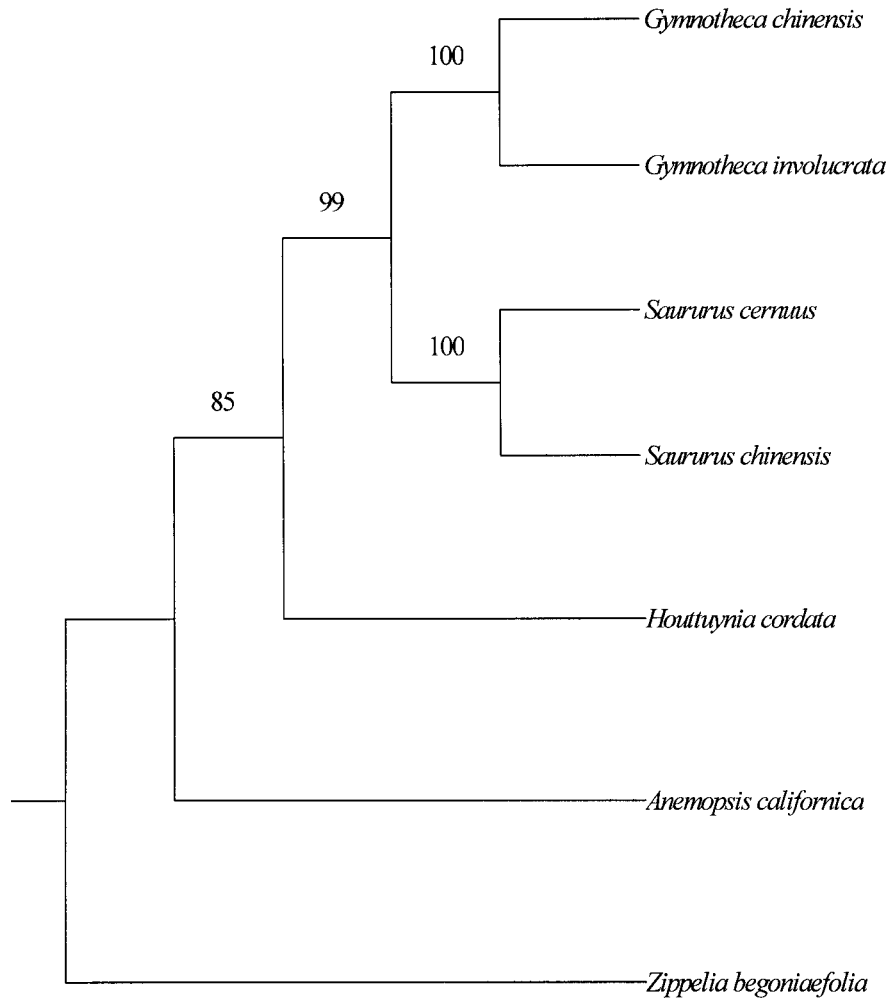


Figure 1. The single most parsimonious tree for Saururaceae based on nuclear genome data. Tree length = 434, CI = 0.8963, RI = 0.7384, and RC = 0.6618. Bootstrap values are found above branches.

variable and uninformative but 137 were parsimony-informative. Our percentage of phylogenetic-informative sites was 6%. The uncorrected sequence divergence ranged from 0.09% to 6.517% within Saururaceae and from 9.233% to 10.11% between the outgroup and the ingroups. Pairwise distance comparisons of all data sets, including the individual genomic DNA data sets, the combined DNA data sets, and the morphological data sets, are available from the corresponding author.

A single most parsimonious tree of 434 steps was obtained from nrDNA (18S and ITS), with CI = 0.8963, RI = 0.7384, and RC = 0.6618 (Fig. 1). The monophyly of *Saururus* (100% BS; bootstrap percentage) and *Gymnotheca* (100% BS) was strongly supported. *Anemopsis californica* was the sister group of other Saururaceae. *Houttuynia cor-*

data was sister to the *Saururus*–*Gymnotheca* clade (85% BS). *Saururus* was sister to *Gymnotheca* (99% BS).

CHLOROPLAST GENOME DATA ANALYSIS

Sequence alignment yielded 2400 bp, 166 of which were variably uninformative and 76 of which were parsimony-informative. The percentage of phylogenetic-informative sites was 3.167%. The uncorrected sequence divergence ranged from 0.043% to 2.543% among Saururaceae sampled and from 7.846% to 8.38% between the outgroup in Piperaceae and the ingroup taxa.

A single most parsimonious tree of 270 steps was yielded for cpDNA data sets (CI = 0.9444, RI = 0.8315, and RC = 0.7853; Fig. 2). The monophyly

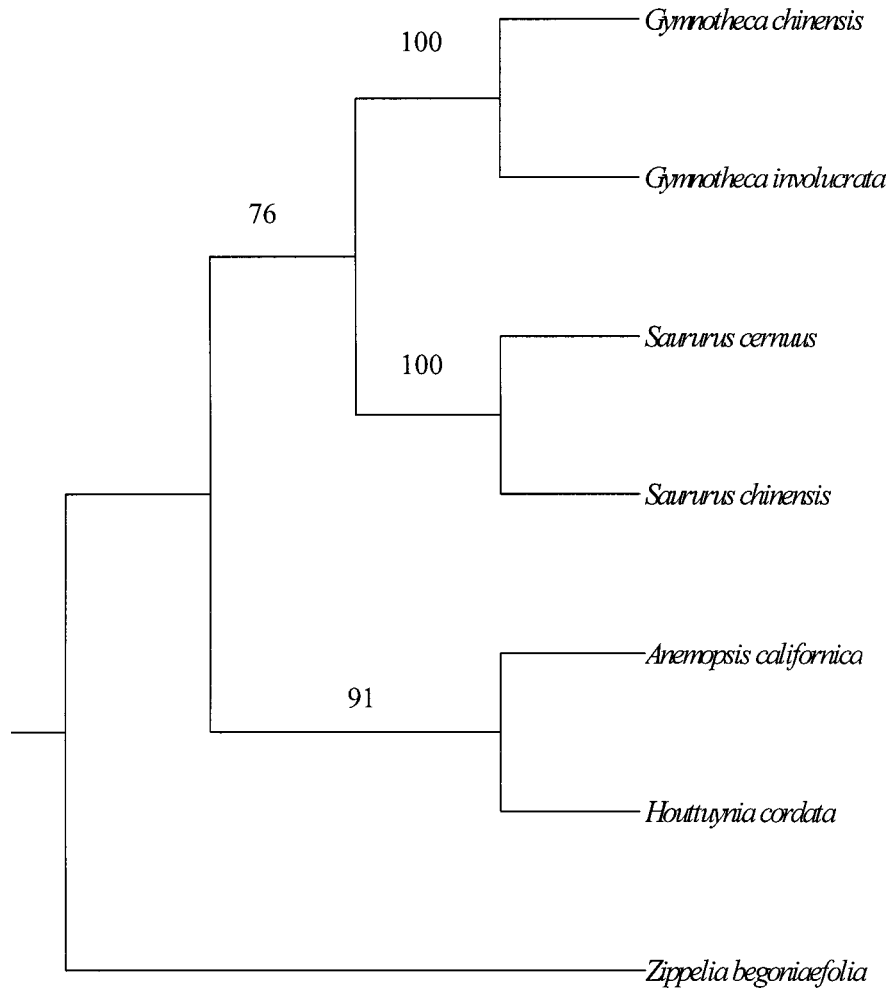


Figure 2. The single most parsimonious tree for Saururaceae based on chloroplast genomic data. Tree length = 270, CI = 0.9444, RI = 0.8315, and RC = 0.7853. Bootstrap values are found above branches.

of *Gymnotheca* (100% BS) and *Saururus* (100% BS) was strongly supported. Moreover, *A. californica* was the sister group of *H. cordata* (91% BS), and the *Anemopsis*–*Houttuynia* clade was sister to the *Saururus*–*Gymnotheca* clade. The sister relationship of *Saururus* and *Gymnotheca* was supported (76% BS).

MITOCHONDRIAL GENOME DATA ANALYSIS

Sequence alignment yielded 1777 bp, 59 of which were at variable sites and 19 at parsimony-informative sites. The percentage of phylogenetically informative sites was 1.07%. The uncorrected sequence divergence ranged from 0 to 1.753% among Saururaceae sampled and from 1.439% to 2.064% between the outgroup and the ingroup taxa.

A single most parsimonious tree of 63 steps was

yielded for mitochondrial genomic data (*matR*), and the topology of the tree matches Figure 2, with CI = 0.9524, RI = 0.8696, and RC = 0.8282. The monophyly of *Gymnotheca* (99% BS) and *Saururus* (99% BS) was resolved with strong internal support. *Anemopsis californica* was the sister group of *H. cordata* (73% BS). The *Anemopsis*–*Houttuynia* clade was sister to the *Saururus*–*Gymnotheca* clade. *Saururus* was sister to *Gymnotheca* (74% BS).

COMBINED MOLECULAR DATA ANALYSIS

After all molecular data were combined, there were 6456 bp in the matrix: 633 of them were variable, 232 were parsimony-informative. The percentage of phylogenetic-informative sites was 3.59%. The uncorrected sequence divergence ranged from 0.111% to 3.365% among included

Saururaceae and from 6.712% to 7.073% between the outgroup and the ingroups.

A single most parsimonious tree of 775 steps was obtained for the combined molecular data sets, with the topology of the tree as in Figure 2, with CI = 0.9084, RI = 0.75, and RC = 0.6813. The monophyly of *Saururus* (100% BS) and *Gymnotheca* (100% BS) was strongly supported. *Anemopsis californica* was the sister group of *H. cordata* (52% BS), and the *Anemopsis–Houttuynia* clade sister to the *Saururus–Gymnotheca* clade. *Saururus* was then sister to *Gymnotheca* (100% BS).

A stable topology was generated after the matrix of combined DNA data sets was re-analyzed once with characters reweighted according to RC (base weight = 2). The topology was still identical to the previous one (Fig. 2). However, the following parameters and bootstrap values were much higher: tree length = 1276, CI = 0.9953, RI = 0.9839, and RC = 0.9792. Again, the monophyly of *Saururus* (100% BS) and *Gymnotheca* (100% BS), and sister-group relationships between *Saururus* and *Gymnotheca* (100% BS), and between *Anemopsis* and *Houttuynia* (100% BS), were strongly supported.

MORPHOLOGICAL DATA ANALYSIS

Of the 49 characters considered, 19 were variable-uninformative and 29 were parsimony-informative. The percentage of phylogenetic-informative characters was 59.18%. The uncorrected character divergence ranged from 2.041% to 61.702% within the Saururaceae and from 53.061% to 63.83% between the outgroup and the ingroups.

A single most parsimonious tree of 71 steps was produced for the morphological matrix, and the topology of the tree was again congruent with Figure 2, with CI = 0.8451, RI = 0.7442, and RC = 0.6289. The monophyly of *Saururus* (100% BS) and *Gymnotheca* (83% BS) was strongly supported, with *A. californica* sister to *H. cordata* (72% BS), and *Anemopsis–Houttuynia* sister to *Saururus–Gymnotheca*. The sister relationship between *Saururus* and *Gymnotheca* was weakly supported (57% BS).

After the morphological matrix was re-analyzed, with characters reweighted according to RC (base weight = 2), a stable topology was obtained. This also resembled previous topologies. However, the following parameters were much higher: tree length = 101, CI = 0.9703, RI = 0.94, and RC = 0.9121. Again, the monophyly of *Saururus* (100% BS) and *Gymnotheca* (95% BS), and sister-group relationships between *Saururus* and *Gymnotheca*

(93% BS), and between *Anemopsis* and *Houttuynia* (99% BS), were strongly supported.

ANALYSIS OF THE COMBINED DATA SETS OF DNA AND MORPHOLOGY

After the molecular and the morphological data were combined, there were 6505 bp in the matrix, 681 of which were variable and 261 of which were parsimony-informative. The percentage of phylogenetic-informative sites was 4.01%. The uncorrected sequence divergence ranged from 0.157% to 3.799% among Saururaceae sampled and from 7.137% to 7.479% between the outgroup and the ingroups.

A single most parsimonious tree of 846 steps was obtained for the combined molecular and morphological data sets, and the topology of the tree corresponded to Figure 2: CI = 0.9031, RI = 0.7492, and RC = 0.6766. The monophyly of *Saururus* (100% BS) and *Gymnotheca* (100% BS) was strongly supported. *Anemopsis californica* was the sister group of *H. cordata* (82% BS), with *Anemopsis* and *Houttuynia* sister to *Saururus* and *Gymnotheca*. *Saururus* was the sister group of *Gymnotheca* (100% BS).

ANALYSIS OF MORPHOLOGICAL CHARACTERS

A phylogenetic tree was obtained when we analyzed the morphological matrix using WinClada v. 0.9.99m 7.5 beta, with its topology corresponding to Figure 2. After analyzing the distribution of each character and its state on the phylogenetic tree, characters 3, 12, 14, 16, 19, 28, 30, 33, 35, 36, and 39 were realized as homoplasious, with the other characters homologous in Saururaceae (Fig. 3). A “homoplasious character” means that its diverse states are due to convergent, parallel, or reverse evolution and not due to inheritance from a common ancestor. Such a character still contributes to constructing the phylogenetic tree in a cladistic analysis (see Fig. 3), but it is prone to mislead if overweighted in building a phylogeny.

DISCUSSION

THE PHYLOGENY OF SAURURACEAE

In all analyses, the monophyly of *Saururus* and *Gymnotheca* is resolved with high bootstrap support. The combined analysis of molecular data and morphological data strongly supports the monophyly of *Saururus* and *Gymnotheca*, and the sister-group relationships between *Anemopsis* and *Houttuynia*, between *Gymnotheca* and *Saururus*, and between the *Anemopsis–Houttuynia* clade and the

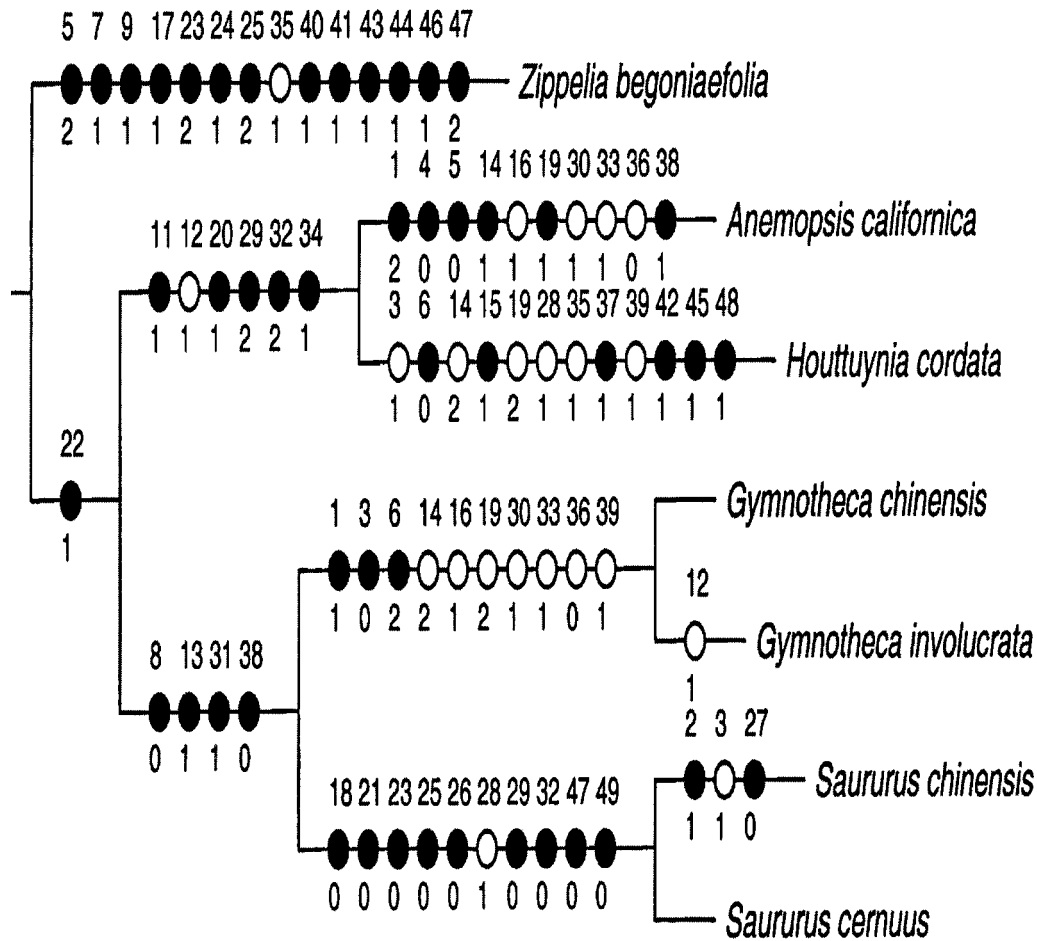


Figure 3. Distribution of morphological character states. The numbers above branches indicate characters; the numbers below branches refer to corresponding character states. Solid black circles represent homologous characters with empty circles representing homoplasious characters.

Gymnotheca–*Saururus* clade. Similarly, strong support is seen separately from analyses of chloroplast genomic data, mitochondrial genomic data, morphological data, and combined DNA data. Departure occurs in our analysis of the nuclear genome data sets (18S, ITS): *Anemopsis* is the sister group of all other plants of Saururaceae, with *Houttuynia* then sister to *Saururus* and *Gymnotheca*, and *Saururus* sister to *Gymnotheca* (Fig. 1). These results are surprising and differ from all the other phylogenetic opinions on Saururaceae based on morphological data (Wu & Wang, 1957, 1958; Okada, 1986; Lei et al., 1991; Tucker et al., 1993; Liang, 1995).

Our results disagree with the phylogenetic tree of Saururaceae of Wu and Wang (1957, 1958), but partly confirm their relationships as seen by Wu (1984), who proposed that *Anemopsis* and *Houttuynia*

may be vicariant genera, and *S. chinensis* and *S. cernuus* may be vicariant species. Vicariant genera and species may be interpreted as “sister groups” in a phylogenetic sense because whether they are two vicariant genera or two vicariant species, they are from an immediate common ancestor. The sister-group relationships between *Anemopsis* and *Houttuynia*, and between *S. chinensis* and *S. cernuus* are well supported in our study. Our results are not congruent with Okada (1986) and Lei et al. (1991), who considered *Saururus* as the basal genus of Saururaceae, and *Anemopsis* and *Houttuynia* to be derived from an ancient *Saururus*. Lei et al. (1991) further suggested that *Gymnotheca* was most distantly derived from any ancestral *Saururus*, as was supported by Tseng (1982) in the *Flora Republicae Popularis Sinicae* and by Xia and Brach (1999) in the *Flora of China*. In terms of the close

relationship of *Anemopsis* and *Houttuynia*, our results partly agree with Tucker et al. (1993), who generated a tree similar to our combined molecular tree (compare Fig. 2 herein and fig. 5 in Tucker et al., 1993), but with low bootstrap values; they treated *Saururus* as the first derived genus in Saururaceae and believed that *Saururus* bore many plesiomorphies. Tucker et al. (1993) used *Magnolia*, *Cabomba*, *Chloranthus*, *Lactoris*, and *Saruma* as outgroups of Saururaceae and Piperaceae. According to the present understanding of angiosperm phylogeny (APG, 1998), *Chloranthus*, *Cabomba*, and *Magnolia*, lying too distant from Saururaceae, may not be the best choices for outgroups of Saururaceae, although *Lactoris* and *Saruma* may serve as outgroups of Saururaceae (Parkinson et al., 1999; Graham & Olmstead, 2000; González & Rudall, 2001). However, Piperaceae are preferable to *Lactoris* and *Saruma* to function as the outgroup of Saururaceae (APG, 1998; Mathews & Donoghue, 1999; Qiu et al., 1999; Soltis et al., 2000). Also at issue is the interpretation of character 20 in Tucker et al. (1993: 621), whether a pair of stamens originated from separate primordia or from a common primordium. The stamens of *Houttuynia* have been confirmed to originate from separate primordia (Tucker, 1981; Liang, 1995). However, this character was variably coded in Tucker et al. (1993). When we correct for this and re-analyze, using Piperaceae as outgroup, the topology resembles Figure 2, and the bootstrap supports rise.

Liang (1995) weighted the rhizomatous character to support the monophyly of Saururaceae. She (1995: 261) treated “stoloniferous” and “separate initiation of bract-flower” as synapomorphies that supported the sister relationship of *Gymnotheca* and *Anemopsis*, and treated “common primordium initiation of bract-flower” as a synapomorphy for *Saururus* and *Houttuynia*. However, the ontogeny of the bract-flower in Saururaceae was homoplasious.

SELECTION OF THE OUTGROUP IN THE PHYLOGENETIC RECONSTRUCTION OF SAURURACEAE

Hennig (1966) pointed out that a sister group is the preferred outgroup, and one of the main tasks of phylogenetic analysis is to look for these. What then is the sister group of Saururaceae? Hutchinson (1959) and Cronquist (1981) both treated Piperaceae, Saururaceae, and Chloranthaceae in Piperales. Melchior (1964) circumscribed Saururaceae, Piperaceae, Chloranthaceae, and Lactoridaceae in Piperales. For Dahlgren (1983), Thorne (1983), and Takhtajan (1987), Piperales were restricted to Saururaceae and Piperaceae, although Takhtajan

(1997) further distinguished Peperomiaceae from Piperaceae. Chase et al. (1993) supported the sister relationship between Piperaceae and Saururaceae in *rbcL* analysis, as did Hoot et al. (1999), including *atpB*, *rbcL*, and 18S. Additional support was provided by Mathews and Donoghue (1999) using duplicate phytochrome genes (PHYA and PHYC), from Qiu et al. (1999) for *rbcL*, *atpB*, 18S, *matR*, and *atp1*, spanning three genomes, as well as Soltis et al. (2000) from *atpB*, *rbcL*, and 18S. In conclusion, the sister relationship between Piperaceae and Saururaceae has been well established (Tucker et al., 1993; Wu et al., 1998; APG, 1998; Mathews & Donoghue, 1999; Qiu et al., 1999; Soltis et al., 2000). Piperaceae are the sister group of Saururaceae, confirmed not only by morphology but also molecular systematics, and therefore the better outgroup for study.

IS SAURURUS THE SISTER GROUP OF THE REST OF SAURURACEAE?

Previous authors (Wu & Wang, 1957, 1958; Okada, 1986; Lei et al., 1991; Tucker et al., 1993; Liang, 1995) postulated that the ancestral Saururaceae were similar to extant *Saururus* in having free carpels, free stamens, and superior ovaries. *Saururus* was considered to have the following ancestral features: a stamen number of 6 (character 15, Appendix 1); stamens free (character 16) and hypogynous (character 14); carpels superior (character 19) and free (character 21); stamens and carpels free and not adnate (character 18); and placenta marginal (character 23). These morphological characters and others in Appendix 1 were scored following traditional opinion about morphological character evolution. Nonetheless, our analysis herein differs from previous phylogenetic trees based on morphology (Wu & Wang, 1957, 1958; Okada, 1986; Lei et al., 1991; Tucker et al., 1993; Liang, 1995), in that the *Anemopsis*–*Houttuynia* clade lies sister to the *Gymnotheca*–*Saururus* clade. We think the difference is due to the following three reasons at least. First, our morphological characters exceed others, in that 49 from diverse sources were considered. Okada (1986) and Lei et al. (1991) mainly used chromosome numbers to reconstruct the phylogeny of Saururaceae. Tucker et al. (1993) used 35 characters restricted to morphology and ontogeny. Second, the method of analysis is different. Wu and Wang (1957, 1958), Okada (1986), Lei et al. (1991), and Liang (1995) did not use cladistic analysis, whereas Tucker et al. (1993) and our study did. Third, certain morphological characters are homoplasious in Saururaceae, e.g., characters 14

(stamen position), 16 (stamen fusion), and 19 (ovary position) (Fig. 3). However, hypogynous stamens (our character 14), free stamens (our character 16), and superior ovary (our character 19) were over-weighted by previous researchers (Wu & Wang, 1957, 1958; Liang, 1995). In our included analyses, *Saururus* is not the first derived genus within Saururaceae (Figs. 1, 2). Our analysis is supported by the following documents. Tutupalli and Chaulal (1975) studied the constituents of essential oils of *A. californica*, *H. cordata*, and *S. cernuus*. They pointed out that each species in Saururaceae had its own characteristic essential oil type, and that *Saururus* was not the least specialized genus according to its chemosystematics. After comparing wood and stem anatomy of *A. californica*, *H. cordata*, and *S. cernuus*, Carlquist et al. (1995) thought that *Anemopsis* possessed likely ancestral character states, such as relatively abundant secondary growth and tracheids. Buddell and Thieret (1997) put *Anemopsis* before *Saururus* when they described Saururaceae in the *Flora of North America*.

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APPENDIX I

MORPHOLOGICAL CHARACTERS AND THEIR CHARACTER STATE CODES.

Vegetative

1. Stem: erect (0), stolon (1), short stem with one node (2).
2. Terminal leaf of stem in reproductive period: green (0), white (1).
3. Tomentum on lamina: none (0), restricted to underside (1), on both sides (2).
4. Leaf venation: pinnate (0), palmate (1).
5. Secondary venation: none (0), dichotomous (1), not dichotomous (2).
6. Areoles: incomplete (0), incomplete or imperfect (1), imperfect or perfect (2).

Stem Anatomy

7. Number of stem vascular cylinders: 1 (0), 2 (2).
8. Fiber in stem: discontinuous (0), continuous (1).
9. Perforation plate type in vessel members: scalariform (0), simple (1).

Floral Morphology

10. Floral symmetry: radial (0), dorsiventral or zygomorphic (1).
11. Abnormal regular flower: none (0), present (1).
12. Color of inflorescence involucre: green (0), not green, showy (1).

13. Flower-bract stalk: absent (0), present (1).
 14. Stamen position: hypogynous (0), perigynous (1), epigynous (2).
 15. Number of stamens: 6 (0), 3 (1).
 16. Stamen fusion: free (0), connate (1).
 17. Anther dehiscence: stomium along entire length of anther (0), predominantly in proximal position (1), in distal position (2).
 18. Adnation of stamens and carpels: free (0), partial fusion (1).
 19. Ovary position: superior (0), perigynous (1), inferior (2).
 20. Number of carpels: 4 (0), 3 (1), 1 (2).
 21. Carpel adnation: free (0), fully adnate (1), single carpel (2).
 22. Style presence: none (0), present (1).
 23. Placenta: marginal (0), parietal (1), basal (2).
 24. Ovules per carpel: greater than or equal to 3 (0), less than 1 (1).

Floral Anatomy

25. Number of carpel vascular bundles: 2 (0), coadnate (1), 1 (2).
 26. Vascular bundle fusion of stamens and carpels: free (0), partial fusion (1).
 27. Fusion of adaxial and abaxial carpel bundle: free (0), partial fusion (1).
 28. Genesis of bract-flower: discrete bract and flower initiation (0), common primordial initiation (1).
 29. Genesis order of carpels: middle primordium first (0), bilateral primordium first (1), simultaneous appearance or single or common primordium (2).
 30. Genesis of stamens: discrete primordium (0), common primordium (1).
 31. Genesis ordering of stamens: bilateral stamens first (0), middle stamens first (1).

32. Genesis pattern of median sagittal stamens: in pair (0), adaxial axis first (1), no adaxial or abaxial stamen (2).
 33. Genesis pattern of bilateral stamen pair: discrete primordium (0), common primordium (1).
 34. Median sagittal carpels: adaxial and abaxial carpels (0), adaxial only (1).

Pollen

35. Germinal aperture: anasulcate (0), anasulcate and an-trichotomosulcate (1), inaperturate (2).
 36. Small verruculae at the edge of foveolae of pollen tectum: absent (0), present (1), narrow belt of granule on tectum (2).
 37. Microspore genesis: simultaneous (0), successive (1).
 38. Type of minor tetrad: bilateral symmetry, T shape and + shape (0), bilateral symmetry and + shape (1), bilateral symmetry (2).
 39. Pollen abortion: absent (0), present (1).

Embryology

40. Layers of ovule integument: two (0), outer layer present but degraded (1), only inner layer present (2).
 41. Micropyle: both inner and outer integuments (0), inner integument only (1).
 42. Nucellus: crassinucellate (0), tenuinucellate (1).
 43. Functional megaspore: from micropylar or chalazal megaspore (0), from megaspore tetrad (1).
 44. Embryo sac: *Polygonum* type (0), *Drusa* or *Peperomia* type (1), *Fritillaria* type (2).
 45. Apomixis: absent (0), present (1).
 46. Perisperm: cellular type (0), nuclear type (1).
 47. Fruit type: capsule (1), berry (2).

Cytology

48. Ploidy: diploid (0), polyploid (1).
 49. Base chromosome number: 11 (0), not 11 (1).

Appendix 2. The matrix of coded morphological characters.

Taxon/Characters	1111111111222222222233333333334444444444 1234567890123456789012345678901234567890123456789
<i>Zippelia begoniaefolia</i>	0021211111000000110010212110100100110201101101201 1
<i>Anemopsis californica</i>	20200?0?0011010101111110111021021100010000000101 1
<i>Houttuynia cordata</i>	0011100100110210012111101111200201111210010010111 1
<i>Saururus chinensis</i>	0111110001001000000001000001001000010000000000000
<i>Saururus cernuus</i>	0021110001001000000001000011001000010000000000000
<i>Gymnotheca chinensis</i>	1001120001001201012011101110111110000010000000101
<i>Gymnotheca involucrata</i>	1001120001011201012011101110111110000010000000101