

## Molecular Phylogenetic Analysis of Anacardiaceae in Thailand

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### Introduction

All of the species of Anacardiaceae in Thailand have been morphologically studied. In order to make natural classification of Anacardiaceae, which reflect the evolutionary history, it needs to know its intrafamilial phylogenetic relationships. Due to recent progress of molecular techniques, to determine and compare nucleotide sequences of peculiar gene have become the shortest and most reliable way to know the phylogeny. For plant systematics, *rbcL* gene has been most widely used. In this study I analyzed this gene of Anacardiaceae and discussed the phylogenetic relationships among the genera. Along with the molecular phylogenetic tree obtained, evolution of characters that have been considered as systematically important was interpreted. Finally, the phylogenetic grouping of Thai Anacardiaceae is proposed as a working hypothesis for constructing a phylogenetic system of the whole family.

### Materials and Method

Sixteen species representing sixteen different genera of four tribes (Mangifereae, Spondieae, Rhoideae and Semecarpeae) of Anacardiaceae in Thailand were collected from natural populations (Table 1). Identification of the materials was based on the keys and descriptions newly provided for the Flora of Thailand. Two species of Aceraceae was used as the outgroup, i.e. *Acer mono* Maxim. and *A. carpinifolium* Sieb. et Zucc., collected in the Botanical Gardens, University of Tokyo. The status of Aceraceae as the outgroup of Anacardiaceae has been supported by the *rbcL* molecular tree of Angiosperm families by Chase et al. (1993).

Total DNA were extracted from leaf materials using CTAB method (Doyle & Dolye, 1987). Then *rbcL* fragments were amplified using PCR method. To amplify *rbcL* of Anacardiaceae, widely-applicable primers for higher plants, developed in the laboratory, Botanical Gardens, University of Tokyo, were used.

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Amplified *rbcL* fragments were purified using GeneClean II and are determined its sequence using Autocycle sequencing kit and ALF autosequencer (Pharmacia). The sequences obtained are analyzed using Genetyx program package. Then, molecular phylogenetic trees were made by maximum-parsimony method using branch and bound option of PAUP program package.

## Results and discussion

### 1. Character evolution

The trees produced from *rbcL* sequences resulted in two equally parsimonious trees at a length of 166 steps with a consistency index of 0.572 and a retention index of 0.660. Fig. 1 shows the strict consensus tree of them. *Parishia* appears the earliest lineage of the Anacardiaceae. A terminal clade with *Lannea* and *Choerospondias* is differentiated next, then a clade with *Spondias* and *Dracontomelon*, *Camptosperma*, and a clade with *Holigarna* and *Semecarpus* follow. The remaining eight genera are divided into two monophyletic clades; one consisting of *Gluta*, *Swintonia*, *Bouea*, *Mangifera*, *Melanochyla*, and another consisting of *Pentaspadon*, *Rhus* and *Buchanania*.

The tree clearly shows that the Engler's system is not totally supported phylogenetically. In Fig. 1, Engler's four tribes are shown in different colours. Rhoideae in red colour are divided into three distant parts so that Rhoideae is a polyphyletic group. Situations are same for Mangifereae in green and Semecarpeae in blue. Both of them are polyphyletic group. Spondieae in yellow is not monophyletic group, but a typical paraphyletic group. *Spondias* and *Dracontomelon*, and *Lannea* and *Choerospondias* are respectively closely related, but these two clusters are very distantly related. Spondieae is, therefore, an artificial taxon. Consequently, none of Engler's tribes were natural groups.

To understand evolutionary history, it is important procedure to consider the evolution of each characters. According to the phylogenetic relationships indicated by the DNA analysis, evolution of each characters are considered as follows:

**Compound Leaves** (Fig. 2): Dissection of leaves in an important taxonomic character in Anacardiaceae where both simple and compound (pinnate) leaves occur. As compound leaves are developed later stage of leaf ontogeny, it is likely to be considered as advanced character state. From the distribution of simple and compound leaves in Anacardiaceae, maximum parsimonious reconstruction of leaf evolution tells that compound leaves are primitive state and simple leaves evolved only once at the blue box. Compound leaves reversely evolved from simple leaves at the red box, at the base of the clade between *Pentaspadon* and *Rhus*. If a compound leaf is a primitive character state, sharing of this does not necessarily indicate monophyly. A paraphyletic group Spondieae, indicated in yellow colour in Fig. 2, was a group defined by this character state. *Pentaspadon*, *Rhus* and *Parishia*, included in the tribe Rhoideae by Engler (1883), share compound leaves but origin of the compound leaves are different. At the time of Engler, there was no way to recognize the difference.



**Hypanthium** (Fig. 3): Engler's tribe Semecarpeae, including *Melanochyla*, *Holigarna* and *Semecarpus*, was characterized by having a hypanthium, which is the cup-like receptacle formed with the calyx and floral axis. The present molecular tree, however, suggests that hypanthium of *Melanochyla* is of different origin from those of *Holigarna* and *Semecarpus*. In flower stage, the hypanthium of the two groups looks similar to each other but show completely different morphology in fruit state. In *Semecarpus* and *Holigarna* the hypanthium developed into a distinct, fleshy hypocarp, while in *Melanochyla* the hypanthium does not expand at all. This fact well corresponds to the molecular result which shows parallel evolution of the hypanthium.

**Number of carpels** (Fig. 4): The number of carpels is also an important taxonomic character of Anacardiaceae. Fig. 4 shows the distribution of the three different state of this character (1, 3 and 5) and their changes in the course of evolution. It indicates that the number of carpels parallelly decreased from 5 to 3 at the yellow boxes and 3 to 1 at the red boxes, respectively. A reverse change, from 3 to 5, happened once at the green box (*Buchanania*).

**Sex expression and number of stamens** (Fig. 5): The sex expression and number of stamens are not constant in Anacardiaceae. Fig. 5 shows the evolution of these two characters. Changes of sex expression is indicated with circles, and those of stamen numbers are indicated with boxes.

The molecular tree suggests that dioecy, most commonly found in this family, is the primitive character state. The monoecy, which are found in *Mangifera*, *Bouea*, *Swintonia* and *Gluta*, evolved once from dioecy at the orange circle. Hermaphrodite which indicated in red circles, evolved four times parallelly. It occurred once from dioecy at the common base of *Spondias* and *Dracontomelon*, and three times from monoecy in *Gluta*, *Pentaspadon* and *Buchanania*.

The number of stamens in Anacardiaceae is basically as much or twice as much as the petals. The character states are named here as single if the number is as much as the petals, or double if it is twice as much. Fig. 5 shows that decrease from double to single happened twice at the blue boxes, and the reverse evolution from single to double happened in the green and yellow boxes, respectively. It is notable that all of hermaphrodite groups have doubled stamens and three parallel changes in sex to hermaphrodite associate with changes in stamen number from single to double. The correlation of these two character states may suggest advantage in pollination.

## 2. New grouping of Anacardiaceae in Thailand

Based on the molecular tree, in considering character evolution, a new grouping recognizing eight monophyletic groups of Anacardiaceae in Thailand is proposed (Fig. 6).

*Parishia* group consisting of *Parishia* is the earliest lineage of the Anacardiaceae. Morphologically, this group has at least three apomorphic characters, three carpels reduced from five carpels (Fig. 4), stamens as much as petals (Fig. 5), and coarsely reticulate pollen ornamentation (Fig. 7). It is notable that the former two characters are homoplasies which parallelly occur in the later differentiated groups. This group has *Anacardium*-type endocarp (Wannen & Quinn 1990).



*Lannea* group including *Lannea* and *Choerospondias* and *Spondias* group including *Spondias* and *Dracontomelon* share primitive characters, i.e. pinnate leaves, five carpels and stamens twice as much as petals. *Spondias* group is hermaphrodite, while *Lannea* group still remains in dioecious condition. This group has *Spondias*-type endocarp (Wannen & Quinn 1990).

*Camptosperma* group consisting of *Camptosperma* has an ovary consisting of a single carpel. This is a homoplasious apomorphy. This group has *Spondias*-type endocarp (Wannen & Quinn 1990).

*Semecarpus* group consisting of *Semecarpus* and *Holigarna* is characterized a unique apomorphic character, a hypanthium which develop into hypocarp. This group has *Anacardium*-type endocarp (Wannen & Quinn 1990).

*Pentaspadon* group includes *Pentaspadon*, *Rhus* and *Buchanania*. This is a rather unnatural group morphologically and no synapomorphy were found so far. The endocarp is *Anacardium* type in *Rhus* and *Spondias* type in the other two (Wannen & Quinn 1990).

*Melanochyla* group consisting of *Melanochyla* has hypanthium but it does not develop into distinct hypocarp. This group has *Anacardium*-type endocarp (Wannen & Quinn 1990).

*Mangifera* group include *Gluta*, *Swintonia*, *Bouea* and *Mangifera*. This group is considered to have a common ancestor which were monoecious and an ovary consisting of a single carpel. In this group, *Gluta* and *Swintonia* have a synapomorphy, wings on fruits derived from petals. This group has *Anacardium*-type endocarp (Wannen & Quinn 1990).

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**Table 1.** A list of samples for the examination of *rbcL* gene sequence.

Taxon	Locality	Voucher Specimens (in BKF)
<i>Bouea macrophylla</i>	Huai Ru Nat. Park, Takuapa, Phangnga	Chayamarit 561
<i>Gluta velutina</i>	Ngao Waterfall, Ranong	Chayamarit 553
<i>Melanochyla bracteata</i>	Peat swamp forest, Takbai, Narathiwat	Chinarunchai 1
<i>Camptosperma coriaceum</i>	Peat swamp forest, Takbai, Narathiwat	Chinarunchai 2
<i>Semecarpus albescens</i>	Doi Suthep, Chiang Mai	Pooma s.n.
<i>Holigarna helferi</i>	Khao Phra Teao, Talang, Phuket	Chayamarit 570
<i>Drimycarpus racemosus</i>	Mae Cheam, Doi Inthanon, Chiang Mai	Chayamarit 595
<i>Mangifera indica</i>	Bangkapi, Bangkok	Chayamarit 673
<i>Swintonia floribunda</i>	Ngao Waterfall, Ranong	Chayamarit 549
<i>Parishia insignis</i>	Peat swamp forest, Takbai, Narathiwat	Chinarunchai 3
<i>Rhus javanica</i> var. <i>chinensis</i>	Doi Suthep, Chiang Mai	Chayamarit 596
<i>Spondias bipinnata</i>	Huai Keao Arboretum, Chiang Mai	Chayamarit 610
<i>Pentaspadon velutinus</i>	Khao Chong Botanical Garden, Trang	Ting-nga s.n.
<i>Lannea coromandelica</i>	Doi Inthanon, Chiang Mai	Chayamarit 601
<i>Dracontomelon dao</i>	Khao Phra Teao, Talang, Phuket	Chayamarit 562
<i>Choerospondias axillaris</i>	Doi Suthep, Chiang Mai	Chayamarit 593



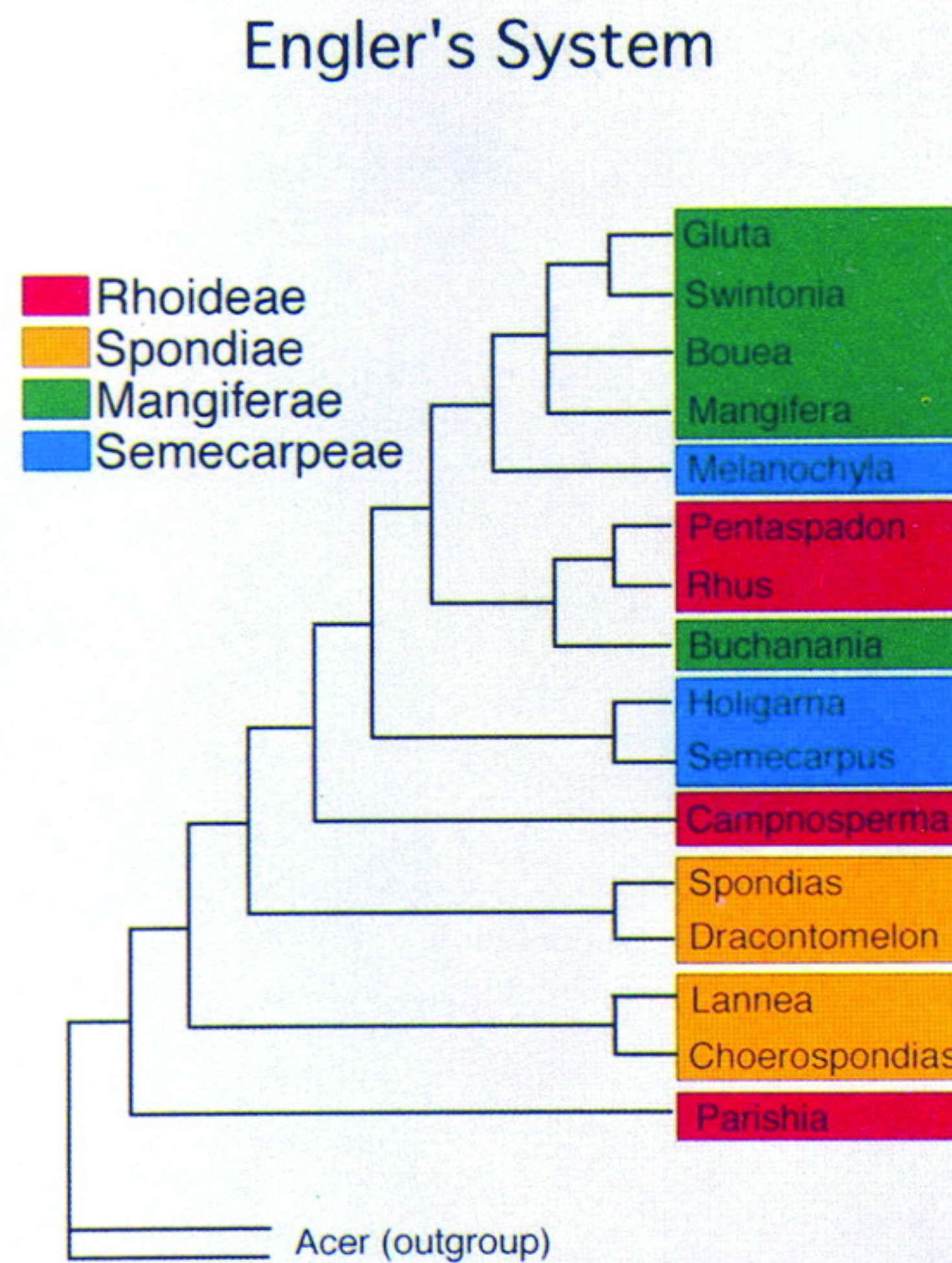


Figure 1. The strict consensus tree based on *rbcL* sequences for 16 genera of Anacardiaceae in Thailand and two outgroup taxa. Engler's 4 tribes are shown in different colours: red=Rhoideae, yellow=Spondieae, green=Mangiferae, blue=Semecarpeae



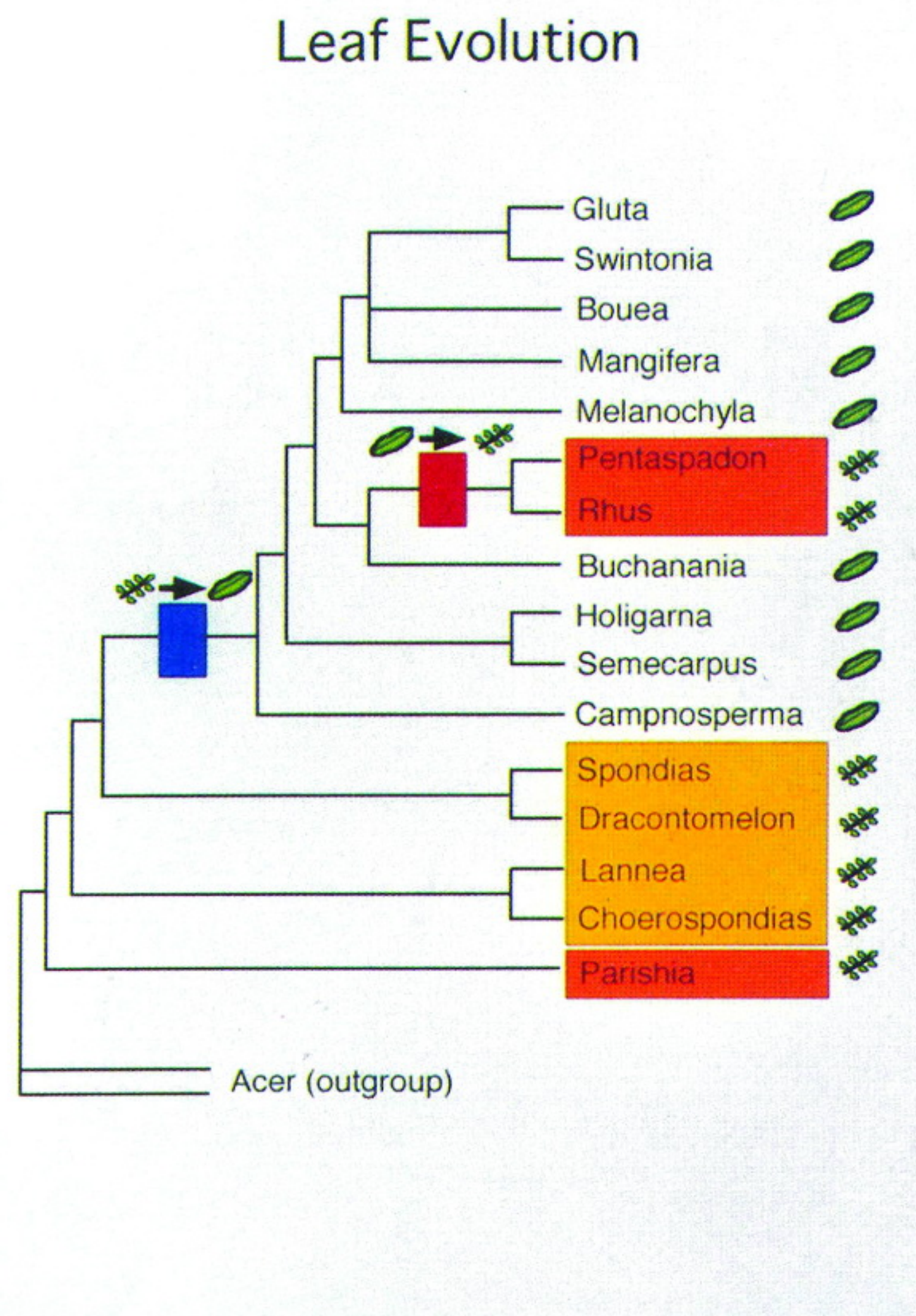


Figure 2. The evolution of compound leaves in Anacardiaceae. Change from compound to simple leaves and reverse change from simple to compound leaves are indicated with blue and red boxes, respectively. Compound leaves reversely evolved from simple leaves in red box. Engler's tribe Spondieae and Rhoideae are shown in yellow and orange, respectively. Spondieae is a paraphyletic and Rhoideae is polyphyletic.



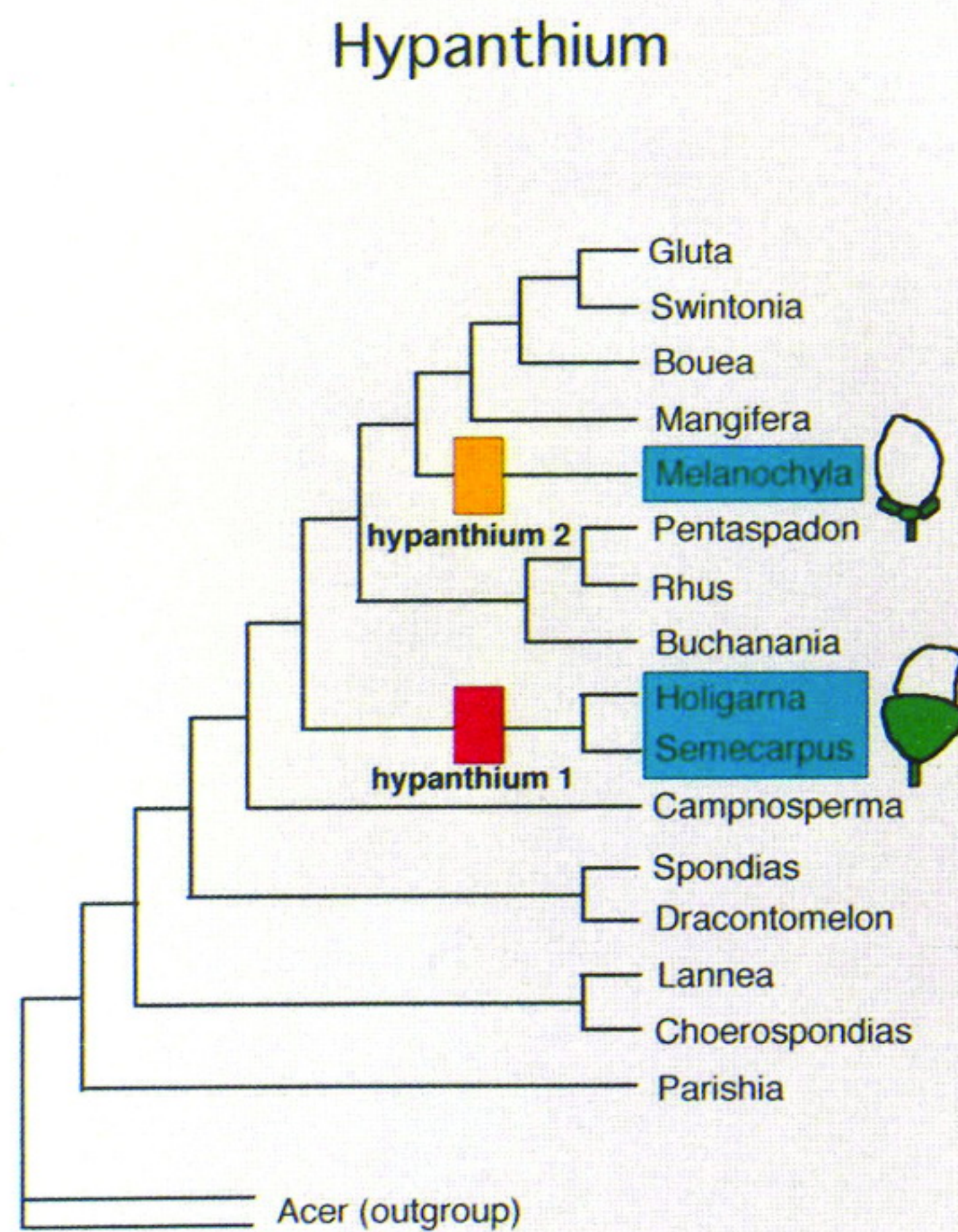


Figure 3. The evolution of hypanthium in Anacardiaceae. Engler's tribe Rhoideae is shown in blue. The hypanthium 1, which developed into a fleshy hypocarp, shown with red box, and the hypanthium 2, which does not expand in fruit, shown in yellow box, are suggested to have different origin. Rhoideae is a polyphyletic group.



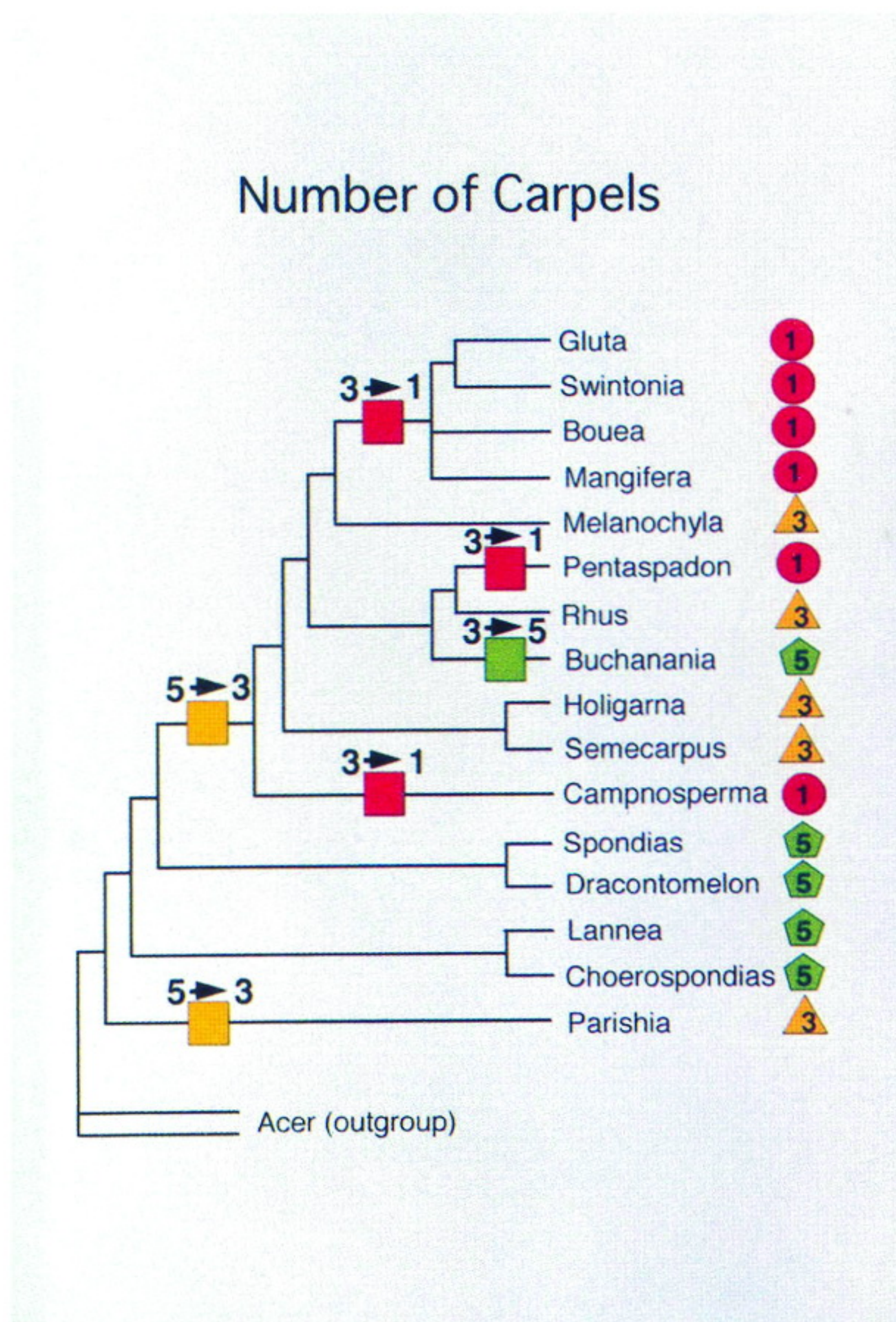


Figure 4. The evolution of the number of carpels in Anacardiaceae. Carpel numbers are shown in different colour at the terminal ends. Number of carpels parallelly decreased from five to three (in yellow box) and three to one (in red box). The reverse change from three to five (in green box) occurred once.



## Sex Expression & Stamen Number

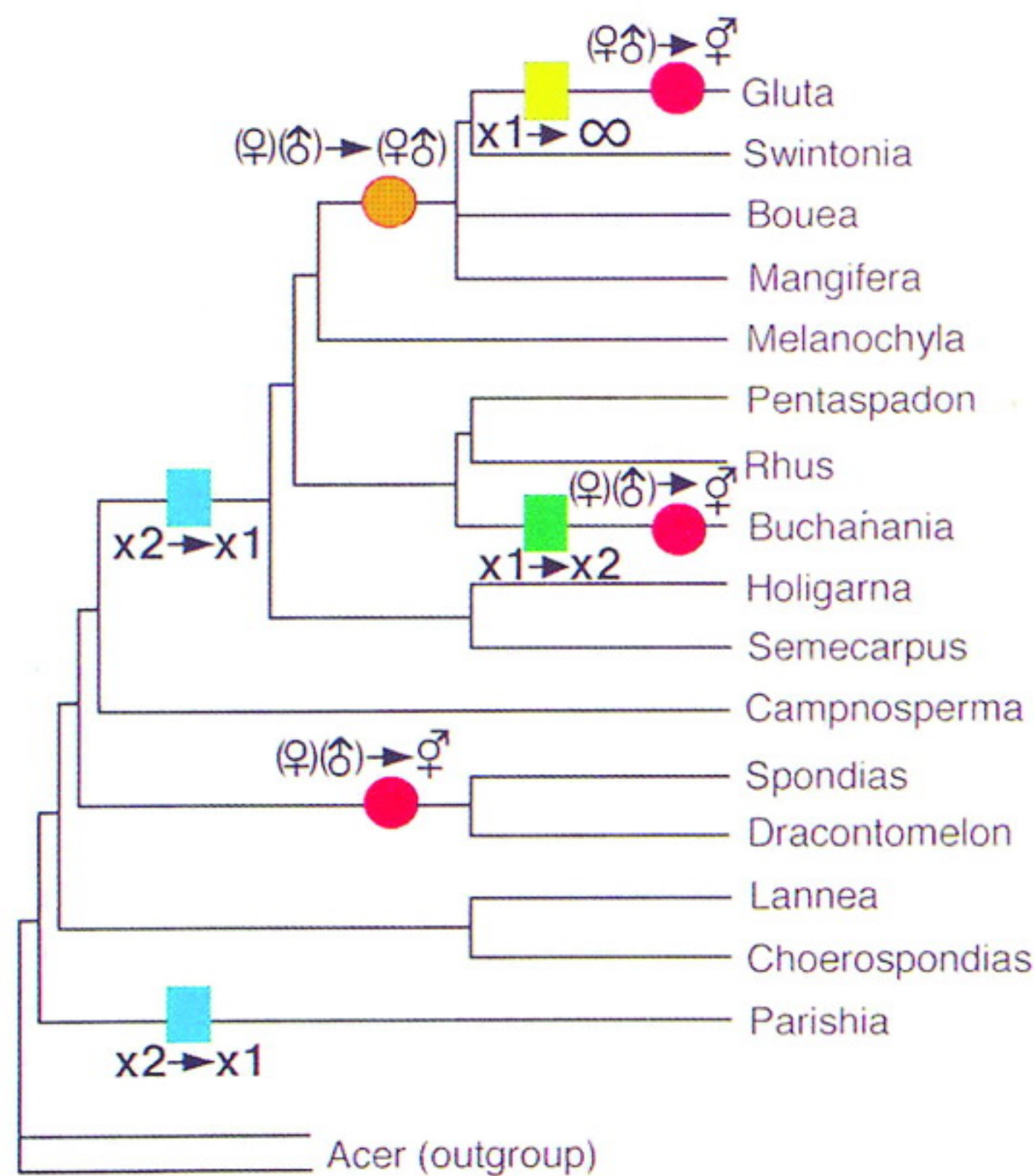


Figure 5. The evolution of sex expression and number of stamens. Sex expression and number of stamens are shown with circles and boxes, respectively. Change from dioecy to monoecy (in orange) happened once. Hermaphrodite (in red) happened three times parallelly; once from dioecy (in *Spondias* and *Dracontomelon* clade) and three times from monoecy (in *Gluta*, *Pentaspadon* and *Buchanania*). Stamens decreased from double (= twice as much as the petals) to single (= as much as petals) happened twice in blue box, and reverse from single to double are shown in green (i.e. without exception) respectively. It is notable that the change from monoecy to hermaphrodite is always associated with increase in stamen numbers.



## New Grouping

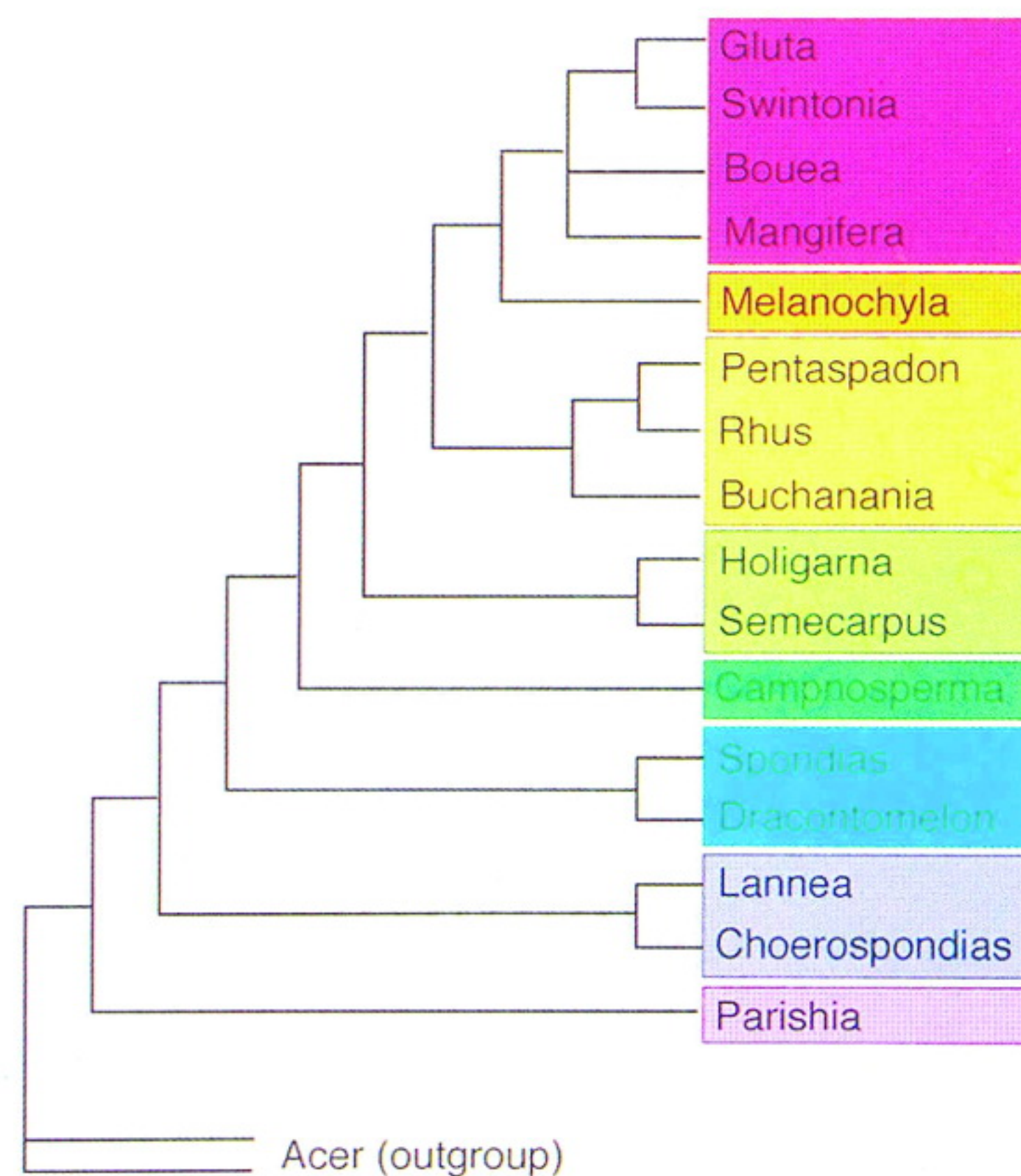


Figure 6. A new grouping of Anacardiaceae in Thailand shown on the strict consensus tree in different colours: *Gluta* group in red; *Melanochyla* group in dark yellow; *Pentaspadon* group in pale yellow; *Semecarpus* group in pale green; *Campnosperma* group in dark green; *Spondias* group in blue; *Lannea* group in purple; and *Parishia* in pink.



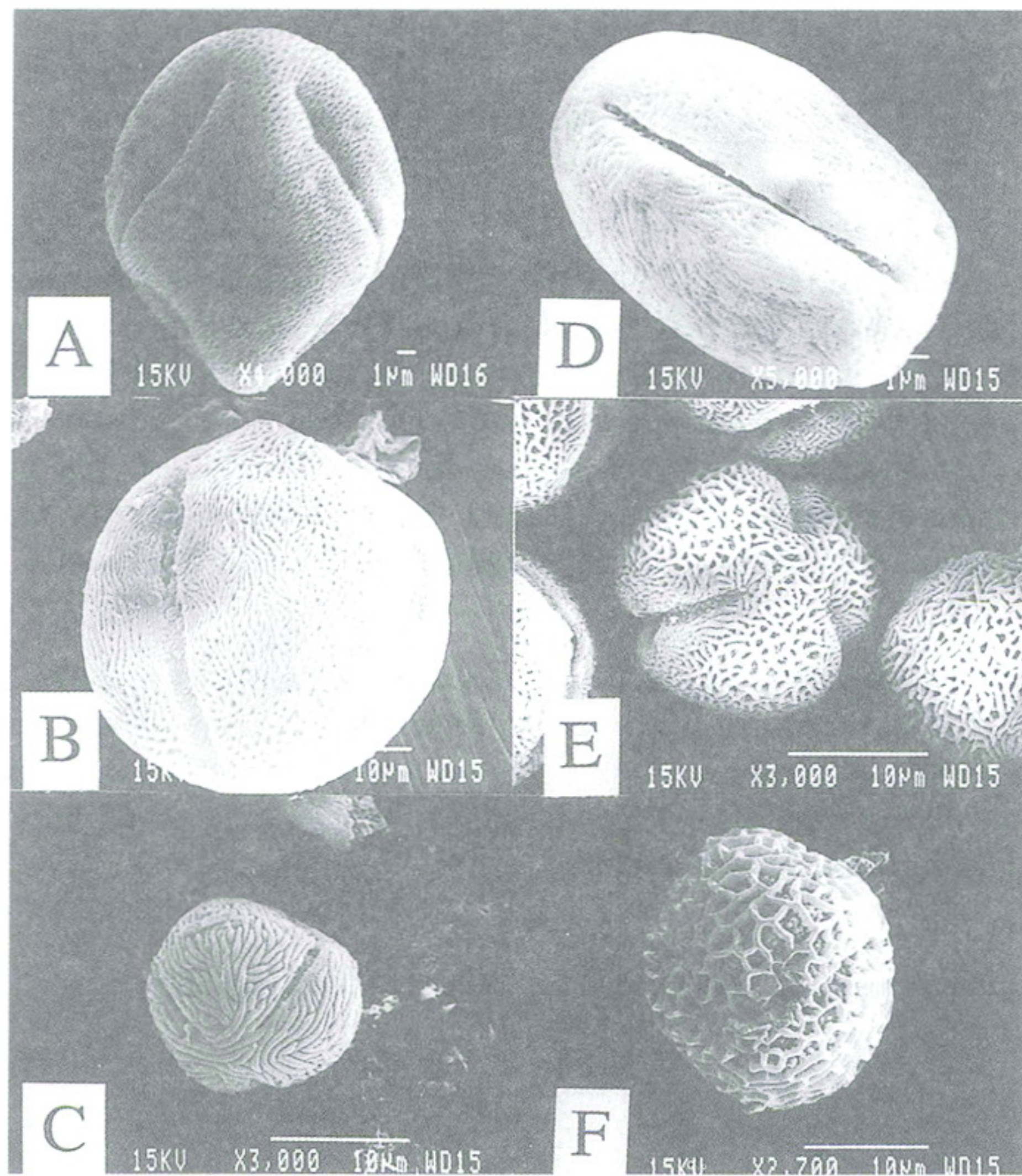


Figure 7. Pollen grains of Anacardiaceae. A: *Gluta usitata* (Adisai 320); B: *Semecarpus curtisii* (Beusekom et. al. 4657); C: *Campnosperma coriaceum* (Niyomdham 605); D: *Dracontomelon laoticum* (Schmid s.n.); E: *Lannea coromandelica* (Hansen, Seidenfaden & Smitinand 11268); F: *Parishia insignis* (Niyomdham 851).