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Cladistics analysis of Calycanthaceae on the basis of morphological and anatomical characters

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ABSTRACT: Cladistics analysis was carried out to find the correct phylogenetic relationship of the four genera of Calycanthaceae. Morphological and anatomical information from all aspect of the data were considered for the analysis. *Siparuna guianensis* (Siparunaceae) and *Cinnamomum malabatrum* (Lauraceae) were considered as out-group. Characters were selected mainly for reasonable argument of the similarity. Character-state transformation and discrimination of the genera were decided based upon the out-group comparison method. PAUP* (ver. 4.0) program was used for the dataset analysis and to make phylogenetic tree. The genera split into two separate genera *Idiospermum* + *Chimonanthus* and *Sinocalycanthus* + *Calycanthus*. *Chimonanthus* + *Idiospermum* are supported strict consensus tree with f-value calculation. Furthermore, *Sinocalycanthus* and *Calycanthus* are separated genera. Therefore, Calycanthaceae be redefined wide circumscriptions of the characters. The detailed investigation of the cladistics analysis revealed that the *Sinocalycanthus* and *Calycanthus* are the sole genus.

Keywords: Calycanthaceae; Characters and characters state; Cladistics; Out-group; Phylogeny.

1. INTRODUCTION

The major split into four genera, *Sinocalycanthus*, *Calycanthus*, *Chimonanthus* and *Idiospermum*, phylogenetic relationships with in the between genera remained problematic and classification schemes currently is used have been widely debated. Staedler et al. [1, 2] were added such characters based on the floral morphology to help for phylogeny of Calycanthaceae. Graybeal [3] found that when the total number of characters is held constant, accuracy is much higher if the characters are distributed across a larger number of taxa, has explored the effects on phylogenetic accuracy, resolution, and clade support of adding taxa and/or characters. Graybeal [3] also stated that denser species sampling greatly improves the ability of analysis to reconstruct phylogeny. Paudel and Heo [4-7] also characterized the morphological and anatomical aspect on Calycanthaceae.

These above long standing controversies over the relationship of four genera of Calycanthaceae allow to putting a different approach on this matter. The characteristics of the Calycanthaceae reveals different debates of different researches. Hence, the primary purpose of this study was to come across the correct phylogenetic relationships of these genera.

2. MATERIALS AND METHODS

This study was based on a secondary data, literature survey and leaf, stem, seed morphology and anatomy. Characters or character state (Table 1) which unique to the individual genera were not considered for analysis. Characters were considered after that prepared data matrix for the cladistics analysis (Table 2). *Siparuna guianensis* and *Cinnamomum malabatrum* were considered as out-group.

Table 1. Characters and character states used for cladistic analysis of Calycanthaceae [1, 2, 8-13].

No.	Characters	Character states
1	Habit	Tree (0) / shrubs (1)
2	Leaf shape	Ovate (0) / elliptic (1) / lanceolate (2)
3	Leaf color	Reddish brown (0) / green (1)
4	Leaf duration	Deciduous (0) / evergreen (1)
5	Wax layer in leaf	Absent (0) / present (1)
6	Crystal in mesophyll	Absent (0) / present (1)
7	Shape of the vascular bundle	U-shaped (0) / V-shaped (1)
8	Stomata frequency	Low (0) / high (1)
9	Hypodermis	Not-developed (0) / well-developed (1)
10	Trichomes in adaxial surface	Absent (0) / present (1)
11	Tepals color	White (0) / red (1) / yellow (2)
12	Terminal bud	Ovoid (0) / globular (1) / ovate (2)
13	Anther	Pubescent (0) / glabrous / (1)
14	Filament	Long (0) / short (1)
15	Flower shape	Narrow (0) / broad (1) / pitcher (2)
16	Lower ovule shape	Hood shaped (0) / elongated (1) / ovoid (2)
17	Fruit length	Large (0) / small (1)
18	Fruit shape	Ovate (0) / concave (1) / ovoid (2)
19	Testal cell shape	Polygonal (0) /sub-polygonal (1) / irregular (2)
20	Thickness of mesocarp	Thick (0) / thin (1)
21	Fruit surface	Rough (0) / smooth (1)
22	Number of cotyledons	Two (0) / three or four (1)
23	Shape of the parenchyma cell	Ovoid (0) / circular (1) / elongation (2)
24	Sclerenchyma cell formation	Long chain (0) /aggregate (1)
25	Pith cell shape	Hexagonal (0) / circular (1)
26	Pollen shape	Boat-shaped (0) / elliptic (1)
27	Pollen wall	Tectate (0) /semi-tectate (1)
28	Foot layer	Thick (0) / thin partly fused (1)
29	Pollen surface	Perforate (0) / rugulate (1)

3. RESULTS

Out of 29, total parsimony informative characters are identified for this cladistic analysis of four genera of Calycanthaceae. Maximum parsimony analysis is produced best trees with rearrangement trial. Consistency index (CI) 0.64, homoplasy index (HI) 0.60, retention index (RI) 0.61, rescale consistency index (RC) 0.39, F value 40, F-ratio is 0.44. The strict consensus tree is given in Fig. 1.

Table 2. Data matrix used for present cladistics analysis of Calycanthaceae.

No.	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	SG	0	0	1	0	?	?	0	0	?	?	0	0	?	?	0	?	0	0	?	?	0	0	0	0	0	?	?	?	0
2	CM	0	2	0	1	1	?	?	0	0	?	0	0	?	?	0	?	0	1	?	0	?	0	0	?	1	0	?	?	0
3	CO	1	1	1	0	0	0	1	0	0	1	1	2	0	0	0	1	1	1	0	0	0	0	0	1	0	1	1	1	0
4	CF	1	1	1	0	0	1	0	1	0	1	2	1	1	0	0	1	0	0	1	0	0	0	1	0	1	1	?	0	1
5	CL	1	1	1	2	0	1	0	1	0	1	2	1	1	0	0	1	0	2	1	1	0	0	1	0	1	1	?	0	1
6	CN	1	1	1	0	0	0	0	1	0	1	0	1	1	1	0	2	0	2	1	0	0	0	1	0	1	1	?	0	1
7	CP	1	1	1	0	0	1	0	1	0	1	2	1	1	1	0	1	0	2	1	1	0	0	1	0	1	1	0	1	1
8	CS	1	1	0	1	0	1	0	1	0	1	2	1	1	1	0	1	0	2	0	1	0	0	1	0	1	1	?	0	1
9	CY	1	0	0	0	0	0	0	1	0	1	2	1	1	1	0	1	0	2	1	1	0	0	2	0	1	1	?	0	1
10	CZ	1	1	1	1	0	0	0	1	0	0	2	1	1	1	0	1	0	2	1	1	0	0	2	0	1	1	?	0	1
11	IA	0	1	1	0	0	0	0	1	1	0	0	1	?	0	2	0	0	2	1	0	1	1	1	0	?	0	1	0	0
12	SC	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	1	0	2	1	0	0	0	1	1	1	0	1	1

Abbreviations; SG = *Siparuna guianensis*; CM = *Cinnamomum malabatrum*; CO = *Calycanthus occidentalis*; CF = *Chimonanthus fragrans*; CL= *Chimonanthus luteus;* CN = *Chimonanthus nitens*; CP = *Chimonanthus praecox*; CS = *Chimonanthus salicifolius*; CY = *Chimonanthus yunnanensis*; CZ = *Chimonanthus zhejingenensis*; IA = *Idiospermum australiense*; SC= *Sinocalycanthus chinensis*.

Sinocalycanthus chinensis and *Calycanthus occidentalis* are split in sole genus with same origin. *Idiospermum* split from the phylogeny tree. Among the genera, *Idiospermum australiense* and *Chimonanthus* clade were supported the close relationship between them. Parsimony of the *Sinocalycanthus chinensis* and *Calycanthus occidentalis* were similar (Fig. 1).

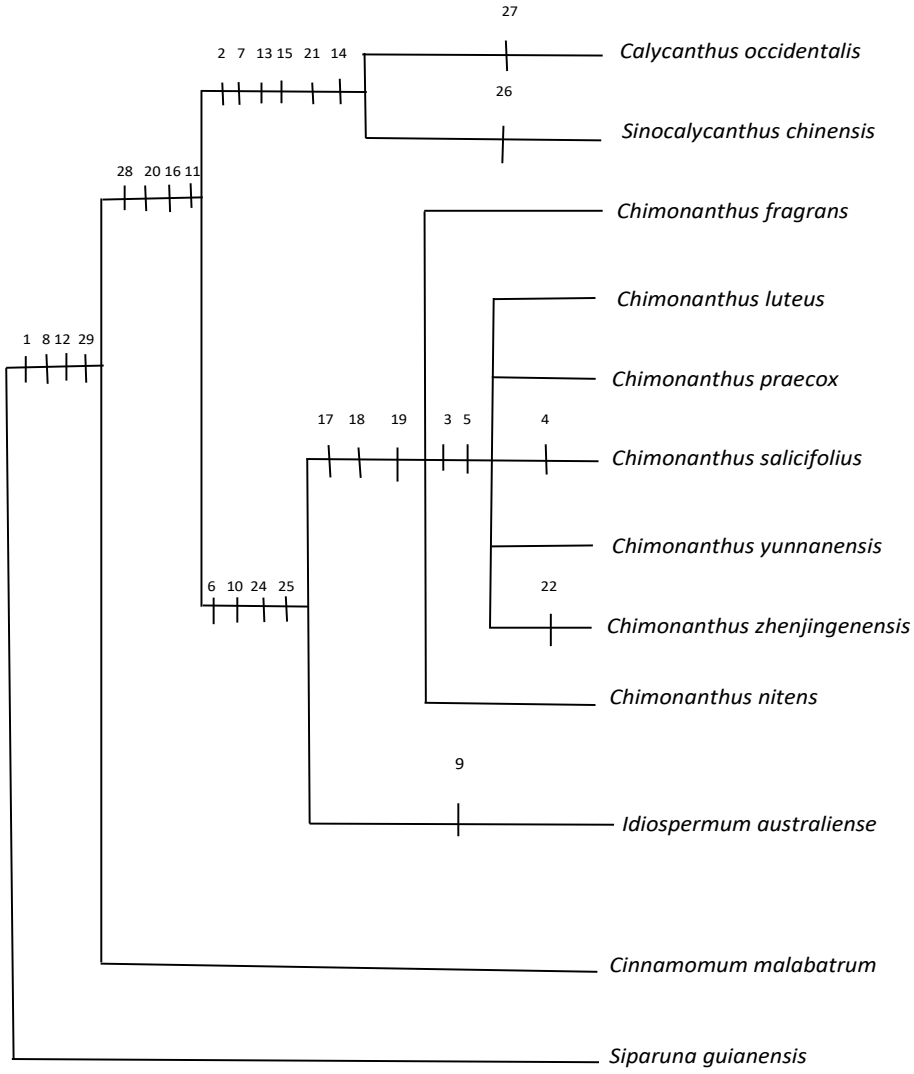

Figure 1. Strict consensus tree based on morphological characters (CI = 0.64, RI = 0.61).

4. DISCUSSION

Calycanthaceae is characterized by putative synapomorphies including per carpel, disulculate columellate pollen, lack of large nectary gland and stamen filament bases [14]. This family has mainly 10 species. Species of *Calycanthus* are distributed in North America, with *Calycanthus floridus* is in east and *Calycanthus occidentalis* in west. Both *Chimonanthus* and *Sinocalycanthus* are endemic to China; The former compromise five species and the later a single species *Sinocalycanthus chinensis*. *Idiospermum* a monotypic genus segregated from *Calycanthus* [15] occurs the rain forest of Queensland, Australia. Some people prefer the recognize *Idiospermum* as its own family [15-18] whereas other include in the Calycanthaceae [10, 19-22]. Phylogenetic studies suggest a sister relationship of *Idiospermum* to the remaining Calycanthaceae; thus, whether or not recognize it as separate family may be issue of taste [14, 23].

Based on morphological characters, *Chimonanthus* is sister to a clade containing *Sinocalycanthus* and *Calycanthus* [12]. Based on karyomorphological analysis, Li and Li [12] suggest that *Sinocalycanthus* is more primitive than species of *Calycanthus.* The restriction fragment length polymorphism data shows that the two North American species from a clade that is sister to *Sinocalycanthus* [24]. In this study, *Sinocalycanthus* and *Calycanthus* are closely related. Furthermore, *Chimonanthus* and *Idiospermum* are also be related to each other. Unique morphological and anatomical characters make the genera as in different but considered as the single family Calycanthaceae.

Cladistics analysis of Calycanthaceae has been conducted. The results of this study lead to the conclusion that *Sinocalycanthus* and *Calycanthus* are obviously close. Furthermore, these result effectively verified the relationship of *Sinocalycanthus* and *Calycanthus* as a member of Calycanthaceae. It can be seen that the *Sinocalycanthus* is paraphyletic the group of *Idiospermum* and *Chimonanthus* is closely related to the advanced group hence they are the monophyletic group. Although the Calycanthaceae have unique feature and maintained in common character has accounted and presented the data as a single family.

The morphological and anatomical characters are the generic delamination of the Calycanthaceae. The great consultancy of seed coat feature has observed in Calycanthaceae. In addition, the pollen morphology is different between the *Sinocalycanthus* and *Calycanthus*. From the results, *Sinocalycanthus* and *Calycanthus* are sole genus each other. Also, the *Idiospermum* must include in Calycanthaceae.

Key to the genera of Calycanthaceae based on leaf, stem, pollen, fruit and seed coat

1. Crystal absent in leaf, hypodermis well-developed …….... *Idiospermum*
1. Crystal present in leaf, hypodermis not developed …… 2
2. Trichome absent in adaxial Surface ……...… *Sinocalycanthus*
2. Trichome present in adaxial Surface 3
3. Pollen surface perforate ……..… *Calycanthus*
3. Pollen surface regulate ……..……..…… *Chimonanthus*

Authors' Contributions: NP managed literature, search data, Conduct experiment and wrote the draft. KH designed and supervised the study. Both authors have approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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