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Comparative pollen morphology of Calycanthaceae for their taxonomic implication

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ABSTRACT: The pollen morphology of four species of Calycanthaceae is described based on the observation of scanning electron microscopy and light microscopy. All pollens are monad, large grain category. The pollen grain was elliptic with disulcate. Surface ornamentation is smooth. Pollen grains of each species are from the genus *Sinocalycanthus*, *Calycanthus*, *Chimonanthus* and *Idiospermum*. The basic shape of the pollen grains is elliptical. The pollen is spheroid in Calycanthaceae except in *Idiospermum*, which represent boat-shape. The circular shape was in polar views in *Chimonanthus* but equatorial shape in *Sinocalycanthus*. *Idiospermum* and *Chimonanthus* were smooth exine with micro-perforation but rugose exine in *Sinocalycanthus* and *Chimonanthus*.

Keywords: Light microscopy; Ornamentation; Scanning electron microscopy; Shape and size; Symmetry.

1. INTRODUCTION

Calycanthaceae is distributed in East Asia and North America region, as well as North and South temperate zones have been gradually formed [1]. Calycanthaceae is sister of Laurales [2-4]. In the APG IV system, Calycanthaceae was placed in the Laurales [5].

Pollen morphological characters are regarded as additional tool for solving the taxonomic problem of the family, generic, or species levels. The long evolutionary history makes pollen morphology (symmetry, shape, aperture, pattern and exine configuration) a very conservative feature, considering phylogenetic trait useful for the taxonomic assessment of the plant [6, 7].

Literatures show that the some pollen studies were conducted [8-14]. However, only a few investigations have been on the pollen morphology of some taxa of Calycanthaceae. The classification of the different cultivar of *Chimonanthus praecox* has presented as 2-lobed circular in polar views, and exine ornamentation indicated that white wintersweet most advanced and, the pollen grain size of cultivar are smaller, length and width of colpus, difference in detail ornamentation are the identified characters of the *Chimonanthus praecox* [14], and furrow like pollen grain in *Chimonanthus praecox* [15]. The pollen morphology of the previous study of all genera was not detailed for the phylogeny of Calycanthaceae.

Therefore, the main objective of the present study is to provide a detail account of the pollen morphology of four genera, and to assess the utility of the palynological data for classification and phylogeny of Calycanthaceae.

2. MATERIALS AND METHODS

Plant materials were growing in KWNU field except *Idiospermum australiense*. Voucher specimens were deposited at Kangwon National University (Table 1). Pollen morphological study was included both light microscopy and scanning electron microscopy.

Table 1. Collection information of genus and species used in present study.

Taxa	Collection information
<i>Calycanthus occidentalis</i> Hook. & Arn.	Korea. Cultivated at Kangwon National University, K. Heo & N. Paudel <i>s.n.</i> 2016 (KWNU)
<i>Chimonanthus praecox</i> (L.) Link	Korea. Cultivated at Kangwon National University, K. Heo & N. Paudel <i>s.n.</i> 2016 (KWNU)
<i>Idiospermum australiense</i> S.T. Blake	Australia. Central Coast, Cultivated in Royal Botanical Garden, Sydney, R.G. Coveny <i>s.n.</i> 1994 (KWNU)
<i>Sinocalycanthus chinensis</i> W.C.Cheng & S.Y.Chang	Korea. Cultivated at Kangwon National University, K. Heo & N. Paudel <i>s.n.</i> 2016 (KWNU)

2.1. Light microscopy

Mature flower buds were collected (Table 1). Collected flowers were fixed with FAA. Pollen preparations were made by the acetolysis [16].

Anthers were transferred with alcohol into in 1.5 ml centrifuge plastic tube. After that, anther was crush with the forceps then collected fine pollen dust. In addition, decanted then added 5ml freshly prepared acetolysis mixture (9:1; acetic anhydride and glacial sulphuric acid). After that, the centrifuge tube was put in water bath at 70°C. The mixture is allowed to remain in the hot water until the mixture attain dark brown color, centrifuge and decant water and a few drops of distilled water 3-4 times, centrifuge, decant water and add a few drops of dilute glycerin and keep aside for slide preparation after that observed under the BX-50 light microscope (Olympus Co. Japan).

2.2. Scanning electron microscopy

The pollen samples were collected in 1.5 ml centrifuge plastic tube. Pollens were passed ethanol series for dehydration. In addition, decanted pollen sample was passed with ethanol: isoamyl. Then, a few drops of Hexamethyldisilazane (HMDS) were added for dry of pollen sample for 1hr afterward two drops of sample were used for mounting. SEM image was carried out from KBSI, Chuncheon at EHT = 5 kV, and multiple image alignment done using photoshop CS6.

The pollen terminology was followed from Put et al. [17] for light microscopy, Hesse et al. [18] and Halbritter et al. [19] for scanning electron microscopy.

3. RESULTS

The detail description of four genera of Calycanthaceae was following and in Table 2.

3.1. *Calycanthus occidentalis*

Pollen monad, heteropolar, polar axis/equatorial ratio elliptic, oblate in polar view, equatorial diameter, disulcate, sculpture reticulate to perforate, radial symmetry in polar view, bilaterally symmetry in equatorial views, pollen are large grain category (Figs. 1A-C).

3.2. *Idiospermum australiense*

Pollen monad, heteropolar, boat shaped somewhat elliptic, pollen grains are dry, polar exist polar/ equatorial ratio oblate, bilaterally symmetry in polar and equatorial views, annuals connected with micro-reticulate, exist in single grain, exine ornamentation is perforate (Figs. 1D-F).

3.3. *Chimonanthus praecox*

Pollen monad, micro-reticulate, spheroid, bilaterally symmetry in polar and equatorial views, disulcate, elliptic or porate-elliptic, verrucate-rugose, annulus, aperture membrane are ornamented, operculate, ectoaperture, diculpus, annulus, circular in polar views, elliptic in equatorial views, exist in single grain, pollen have 2-colporate. The exine is rugulose with coarse ornamentation, large grain category, smooth exine with micro-perforation connected as reticulate tectum (Figs. 1G-I).

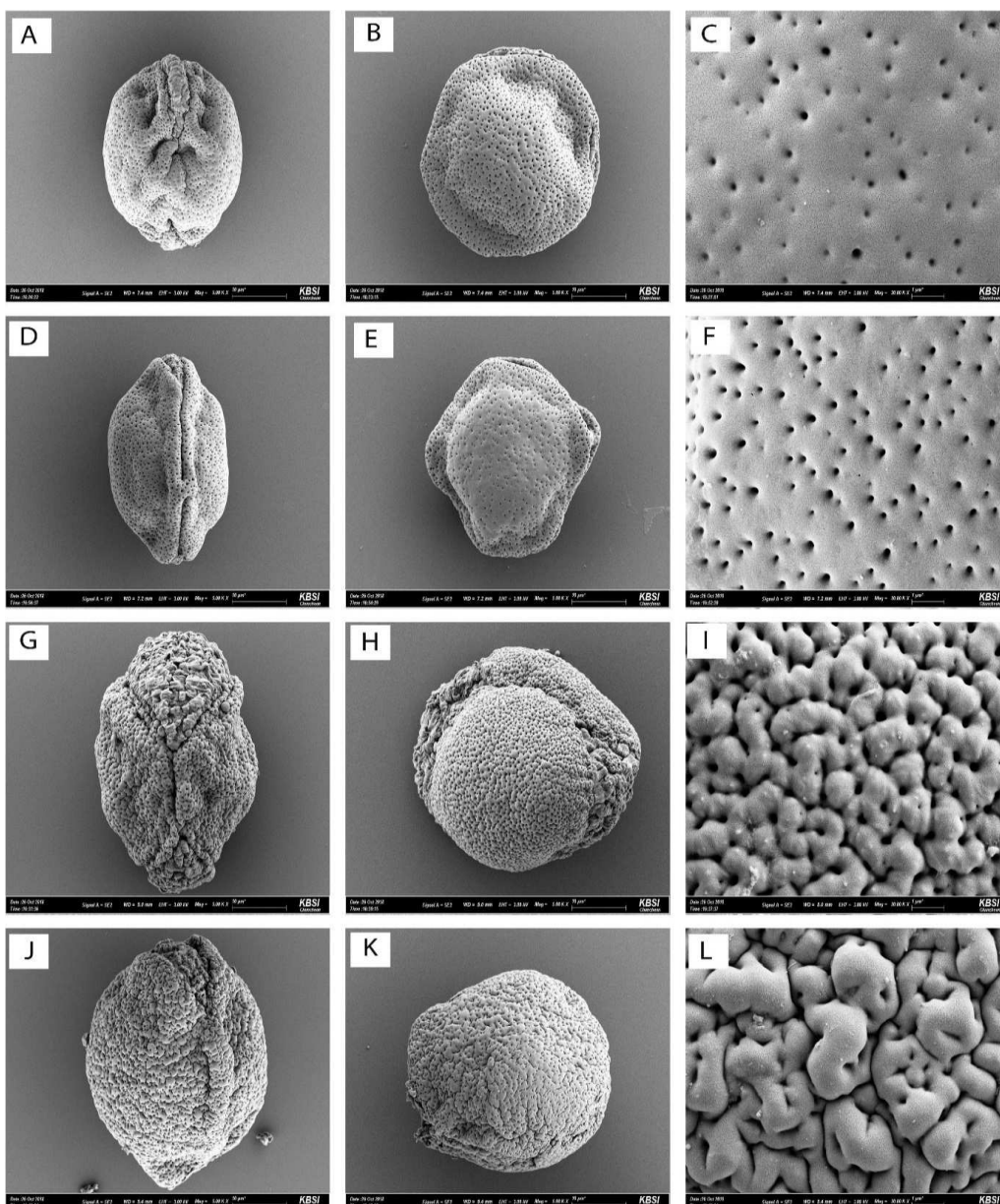


Figure 1. Equatorial view, polar view and sculpture detail of pollen grains of Calycanthaceae under the scanning electron microscopy. A-C, *Calycanthus occidentalis*; D-F, *Idiospermum australiense*; G-I, *Chimonanthus praecox*; J-L, *Sinocalycanthus chinensis*.

Table 2. Pollen morphological characters of four taxa of Calycanthaceae [8-12].

Taxa	Shape	Size (µm)	Pollen size class	P/E ratio	Pollen wall	Aperture		Sculpture	Opercula	Symmetry	Ornamentation
						Type	Position				
<i>Calycanthus occidentalis</i>	Elliptic	60-70	Large grain	1.2	Semi-tectate	Disulcate	Longitudinal	Reticulate to perforate	Nano overculate to Sublayer and microreticulate	Bilaterally	Smooth sexine with microperforatio
<i>Chimonanthus praecox</i>	Elliptic	70-80	Large grain	1.5	Tactate	Disulcate	Longitudinal	Rugose	Granulate sublayer and reticulate supralayer	Bilaterally	Reticulate with rugose
<i>Idiospermum australiense</i>	Elliptic to boat shaped	60-75	Large grain	1.25	Semi-tectate	Disulcate	Longitudinal	Reticulate to perforate	Nanooverrucate to granulate sublayer and perforate supralayer	Bilaterally	Smooth sexine with microperforation
<i>Sinocalycanthus chinensis</i>	Elliptic	80-100	Large grain	1.3	Tactate	Disulcate	Longitudinal	Rugose	Nonoverculate to granulate sublayer and microreticulate supralayer	Bilaterally	Reticulate with rugose

3.4. *Sinocalycanthus chinensis*

Pollen monad, heteropolar, micro-perforation ornamentation, spheroid, bilaterally symmetry in polar and equatorial views, disulcate, elliptic or porate-elliptic, verrucate rugose, annulus, connected as micro-reticulate tectate, ectoculpus, pollen exine ornamentation, exist into single grain, two colpi, The exine is rugulose with coarse ornamentation, large grain category, smooth exine with micro-perforation, connected as micro-reticulate tectum (Figs. 1J-L).

4. DISCUSSION

Present study shows distinctive variations in pollen morphology of the *Sinocalycanthus*, *Calycanthus*, *Chimonanthus* and *Idiospermum*. The combined molecular analysis provided a hypothesis of phylogeny of Calycanthaceae, which is largely extent published. Based on data of molecular analysis, *Calycanthus* and *Sinocalycanthus* are placed in same genus [2].

The study of pollen morphology, the dissimilarities characters were observed in Calycanthaceae. In present analysis, the *Calycanthus* and *Sinocalycanthus* were different genus due to the sculpture character. The pollen shape of the *Calycanthus occidentalis* was elliptic. In addition, *Sinocalycanthus chinensis* was spheroid shape in polar view, bilaterally equatorial views. The exine is rugose in *Sinocalycanthus chinensis* whereas psilate in *Calycanthus occidentalis*.

Pollen grains are psilate, disulcate, and globes in *Calycanthus* [11]. In observation, pollen exine was marked rugose and perforate in *Sinocalycanthus chinensis* and *Calycanthus occidentalis*. Walker [8, 9] suggested that the exine is columellate and both of tectum and foot layer are relatively thick and homogenous to much thinner, where it partly fused irregular size granules. Results were support that in *Calycanthus occidentalis* exine was psilate. The *Calycanthus* has disulcate pollen [10]. The results further focused on pollen of *Idiospermum australiense* was boat shaped. The result shows that the all genus have different characters in shape and size. Samson [12] observed that the end exine varies in thickness and consist of several layer of tangentially aligned lamellae or granules in both aperture and non-aperture regions. In addition, the intine is two-layered. In observation, the exine is thicker layer in *Chimonanthus praecox* and *Sinocalycanthus chinensis* than that of *Calycanthus occidentalis* and *Idiospermum australiense*. Renner [20] analyzed and stated that the *Idiospermum* was a member of Calycanthaceae based on the sequence data and morphological characters. From the pollen data, *Idiospermum* also is in Calycanthaceae due to the similarities pollen characters like as large grain, monad, equatorial views shows slightly similar to the other taxa of the family and, one the important perforate hole in *Calycanthus occidentalis* and *Idiospermum australiense* than other taxa of Calycanthaceae.

According to the Li and Li [21], *Chimonanthus* may be an earliest branch from pre-Calycanthaceae, which almost all primitive characters of the ancestor, while *Sinocalycanthus* will closely related to the *Calycanthus*. Both *Calycanthus* and *Sinocalycanthus* are intermediate genera from primitive to advance taxa in Calycanthaceae but *Idiospermum* is the most advance in the family. However, *Sinocalycanthus* and *Calycanthus* are very different due to the pollen morphological characters somewhat is similar but the shape size, symmetry, ornamentation is different with rugose exine. The result was opposed for the molecular phylogenetic analysis formulated by Zhou et al. [2].

From the pollen observation, the *Sinocalycanthus* and *Calycanthus* are the separate genus each other. This information helps the classification of the genera within the Calycanthaceae. A little bit controversy will

be minimized by pollen data. From the pollen observation, *Sinocalycanthus*, *Calycanthus*, *Chimonanthus*, and *Idiospermum* are recognized as independent genus.

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

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

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