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Research Article

Comparative stem anatomy of four taxa of Calycanthaceae Lindl.

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ABSTRACT

The anatomical character is potential value in Calycanthaceae for their taxonomic study. Four species of Calycanthaceae were collected for this experiment. The experiment was done using the resin methods for preparation of the permanent slide for anatomical studies. The anatomical character like two traces of the unilocular vascular bundle, in the primary vascular cylinder, contains four cortical vascular bundles in the stem, the unilocular structure of primary cylinder, the presence of numerous intercellular space in phloem, the presence of oil cell in the form of scatter in *Calycanthus* whereas small size in *Chimonathus*. *Calycanthus* possess boarder pit with circular aperture while *Chimonanthus* possess elliptical. The tracheid is a characteristic feature of the spiral band wider in *Chimonanthus* than that of *Calycanthus* and *Sinocalycanthus*. The noted sclerenchymatous cells are grouped of the colony which is a characteristic feature of *Sinocalycanthus* and *Calycanthus* but in case of *Chimonanthus* is the long chain with the layer of the cell. Collenchymatous cell was circular with an intercellular in *Calycanthus*; ovoid shape with the intercellular in *Chimonanthus* but in *Sinocalycanthus* is elongation with the minor regular shape. The different character of pith cells found in hexagonal and circular shape which is also distinguished feature in Calycanthaceae. The valuable stem anatomical characters are the importance of their function, ontogeny, and phylogeny.

Keywords: Anatomical character; Calycanthaceae; Collenchyma; Sclerenchymatous; Vascular bundle.

1. INTRODUCTION

Calycanthus, *Chimonanthus*, and *Sinocalycanthus* are the genus of Calycanthaceae. *Sinocalycanthus* is native to China. *Sinocalycanthus* is the synonym of *Calycanthus*. The literature reveals that long horticulture forms and varieties due to the long cultivation of history. Calycanthaceae is the small family of the plant with four genera and ten species which is the sister group of Laurales [1-7]. Within Calycanthaceae, the deepest split is between the tropical monotypic tree *Idiospermum australiense* and the temperate shrubs of the rest of the family Calycanthoideae (Calycanthaceae) [8, 9], unique in Laurales are features of the gynoecium: ovule number and placentation differ from all other Laurales, and the seeds in *Idiospermum* have the largest embryos known in angiosperms [5, 10]. Although very old Chinese drawings and Japanese wood figures of *Chimonanthus* are apparently in existence [11], the first drawing to appear in the taxonomic literature was probably that [12, 13]. *Calycanthus floridus* Linneaus [14] recognized only the genus *Calycanthus* with the two species

Calycanthus floridus and *Calycanthus praecox* [13], as well as Lindley [11], considered *Calycanthus praecox* to represent a new genus *Chimonanthus*. Some authors [13, 15, 16] maintained this concept of two genera. Others [18, 19] followed Linneaus in recognizing only one genus. Prantl [18], the other hand, recognized two sections, viz., *Eucalycanthus* and *Chimonanthus*. There has been some confusion in the past concerning the correct names for these genera. However, the designation of *Calycanthus* L. and *Chimonanthus* Lindley as nomina conservanda Lanjouwa [19] has solved this problem. The pertinent nomenclatural information and synonymy have been summarized by Kearney [20] and Rehder [21] for *Calycanthus* and by Rehder and Wilson [13] for *Chimonanthus*. *Chimonanthus* was monotypic until the description of *Chimonanthus nitens* by Oliver [22] based on material from August Henry's collections from central China. With more complete collections, two additional species have been proposed, *Chimonanthus yunnanensis* Smith [23] and *Chimonanthus salcifolius* Hu [24]. The situation is somewhat different in *Calycanthus*. Although *C. occidentalis* of California has been recognized as comprising a distinct and relatively uniform species, the plants of the southeastern United States have been treated as representing from one to as many as six species. Rafinesque [25] represented the latter extreme by stating to the sp. of *Calycanthus* L. only one. In most manuals at least two species *C. floridus* and *C. fertilis* have been recognized based primarily differences in pubescence and leaf shape.

The aim of this study is the histological comparison of the stem of Calycanthaceae for the purpose of discussion and implication of observed anatomical trait for support the classification of the plant.

2. MATERIAL AND METHODS

Altogether four species (Table 1) is collected. The stems were fixed with the FAA (formalin: glacial acetic acid: 50% ethanol, 5:5:90, by volume) from each family mature stem were selected then passed alcohol series after that; alcohol: technovit 7100 resin. Serial section of 5-6 µm thickness was cut using disposable blade knives stuck into glass slides and dried on electrical slide hot plate for Twenty four hour; slides were stained with 0.1% toluidine blue for 60-90 second. After that rinsed with running water, and again dried on the electric hotplate for more than six hours to remove water. The stained slides were then the mounted with Entellen. Four permanent slides were observed under an Olympus BX-50 light microscope (Olympus Co. Japan), Photographs were taken with the digital camera system attached to the microscope and multiple image alignment was done using Photoshop.

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

Taxa	Collection information
Calycanthus occidentalis Hook. & Arn.	Korea, Cultivated at Kangwon University, K. Heo & N. Paudel s.n. 2016 (KWNU)
Chimonanthus praecox Lindl.	Korea, Cultivated at Kangwon University, K. Heo & N. Paudel s.n. 2016 (KWNU)
Chimonanthus salcifolius S.Y. Hu	Korea, Cultivated in Chollipo Arboritum, K. Heo s.n. 2009
Sinocalycanthus chinensis W.C. Cheng & S.Y. Chang	Korea, Cultivated at Kangwon University, K. Heo & N. Paudel s.n. 2016 (KWNU)

3. RESULTS

Epidermis the single-layered outermost composed of tabular parenchyma cells (Table 2) which are compactly arranged without having inter-cellular spaces in *Chimonanthus praecox* (fig. 2C) and *Chimonanthus salcifolius* (fig. 2C). In *Sinocalycanthus chinensis* and *Calycanthus occidentalis* were intercellular space in the epidermal cell (figs. 1C, 2I). Outer walls were cuticularised. Collen-

chymatous cells are a circular shape which is interconnected with each other layer in *Chimonanthus praecox* and *Chimonanthus salcifolius* (figs. 1I, 2I). Sclerenchymatous cells are higher cell grouped in *Sinocalycanthus chinensis* and *Calycanthus occidentalis* whereas in *Chimonanthus praecox*, and *Chimonanthus salcifolius* formation of long 2 layer chain (figs. 1I, 1D) with two traces of unilocular vascular system was noted in all species *Sinocalycanthus chinensis*, *Calycanthus occidentalis*, *Chimonanthus praecox*, and *Chimonanthus salcifolius*. In primary vascular cylinder, four cortical vascular bundles were noted in all species in Calycanthaceae (figs. 1A, 1G, 2A, 2G). A cortical bundle which is later developed in the central bundle in the stem. Especially unilocular system is in the primary vascular cylinder in all species (figs. 1D, 2H, 2C, 2I). *Calycanthus occidentalis* has circular border pits (fig. 1E). In the center, pith is a loosely bound hexagonal structure (fig. 1F) with intercellular. Parenchymatous cell are in circular and ovoid shaped whereas sclerenchymatous cells are in group colony contains thirteen number of cells (fig.1D) Protoxylem vessels with wider cavities with annular thickening towards the epidermal cell (figs. 1G, 1H). Pentagonal intercellular space gap was seen in *Calycanthus occidentalis* (fig 1I). The tracheid possesses spiral band (figs.1E, 1K).

Chimonanthus praecox possess straight chain border pits which are undergoing towards the epidermis (fig.1K) with elliptical aperture. The pith cell circular with intercellular space was noted in *Chimonanthus praecox* (fig. 1L). The parenchymatous cells were rectangular in shape possess the large intercellular space (figs. 1I, 1J).

Chimonanthus salcifolius also possess straight chain bordered pits (fig. 2E). Parenchymatous cells are circular or ovoid shaped with intercellular space (figs. 2C, 2D) the vascular bundle is collateral (figs. 2B) the pith cell are also noted large circular cell with intercellular space (figs. 1L, 2F).

Sinocalycanthus chinensis parenchymatous cells are ovoid with some rectangular shape (figs. 2I, 2J). The vascular bundle is noted four in each quadrangular side (fig. 2G). The cortical bundle is also the lateral side of the stem (fig. 2H). The pitch cells are large with intercellular space with circular as well as hexagonal shape (fig. 2L).

Table 2. Comparative stem anatomical characters of Calycanthaceae.

Taxa	Epidermis	Collenchyma	Parenchyma	Sclerenchyma	Endodermis	Number of vascular bundle	Xylem	Pith
Calycanthus occidentalis Hook.& Arn.	Single layered	Circular shape, loosely bind	Ovoid or circular shape	13-14 cells in the group, scatter	Single layered, parenchymatous cell	4	Protoxylem vessels with wider cavities	Hexagonal shape with intercellular space, loosely bind each other
Chimonanthus praecox Lindl.	Single layered	Ovoid shape, intercellular space	circular shape	2-6 cells in the group, scatter	Single layered, parenchymatous cell	4	Protoxylem vessels with smaller cavities	Circular shape, loosely bind
Chimonanthus salcifolius S.Y. Hu	Single layered	Ovoid shape, intercellular space	Circular shape	2-7 cells in the group, scatter	Single layered, parenchymatous cell	4	Protoxylem vessel with smaller cavities	Circular shape
Sinocalycanthus chinensis W.C. Cheng & S.Y.Chang	Single layered	Elongation shape, intercellular space	Ovoid or elongation shape	14-17 cells in a group, scatter	Single layered, parenchymatous cell	4	Protoxylem vessel with wider cavities	Hexagonal shape interact with each other

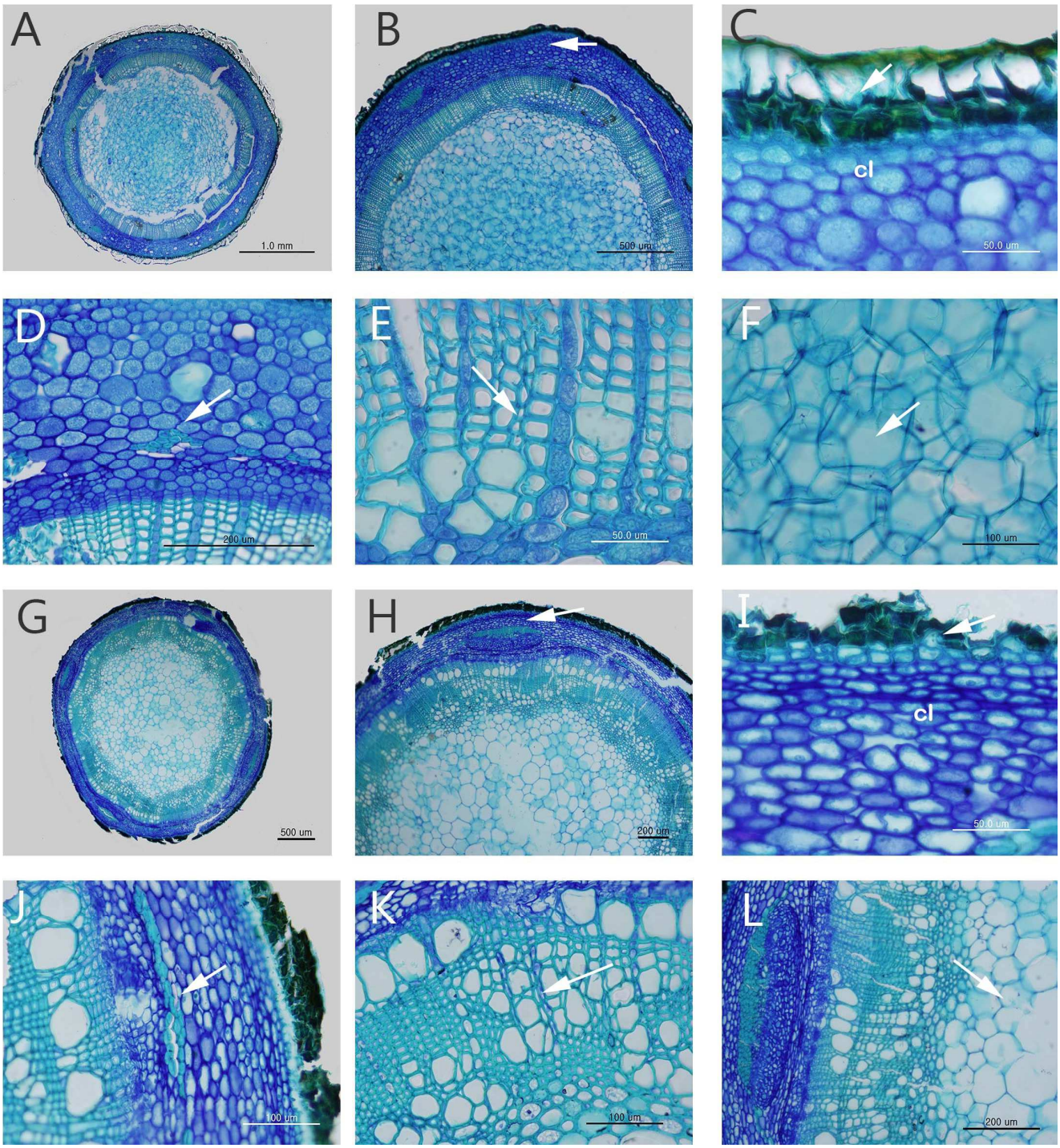

Figure 1. *Calycanthus occidentalis* (**A-F**); **A**. Cross section of young stem, **B**. A detail portion of cross section (arrow head shows the vascular bundle), **C.** Epidermis and collenchyma (arrow head represent epidermis, and cl shows collenchyma), **D.** Sclerenchyma with the colony, **E.** Tracheid and vessel, **F.** Pith. *Chimonanthus praecox* (**G-H**); **G.** Cross section of young stem, **H.** A detail portion of cross section (arrow head shows the vascular bundle), **I.** Epidermis and collenchyma (arrow head represents epidermis, and cl shows collenchyma), **J.** Sclerenchyma with the colony, **K.** Tracheid and vessel, **L.** Pith.

4. DISCUSSION

The stem of the Calycanthaceae is characterized by its quadrangular appearance (figs. 1A, 1H, 2A, 2G). This is usually quite in young stems but less in older ones. The tissues of the mature stem have received a close examination by several researchers. Much of the research has focus on the presence of inverted cortical bundles in the stem.

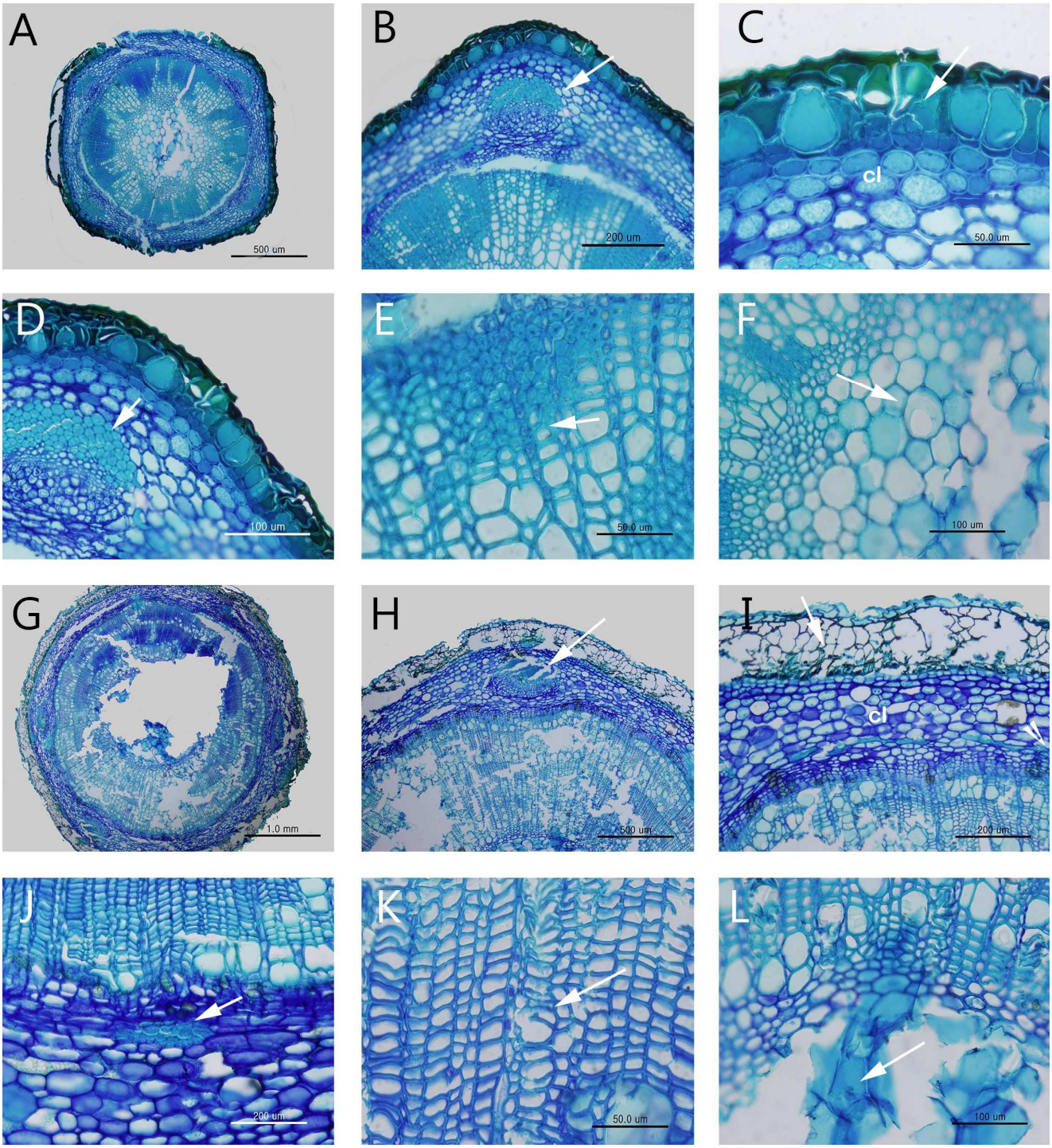

Figure 2. *Chimonanthus salcifolius* (**A-F**); **A**. Cross section of young stem, **B**. A detail portion of cross section (arrow head shows the vascular bundle), **C.** Epidermis and collenchyma (arrow head represent epidermis, and cl shows collenchyma), **D.** Sclerenchyma with colony, **E.** Tracheid and vessel, **F.** Pith; *Sinocalycanthus chinensis* (**G-L**); **G.** Cross section of young stem, **H.** A detail portion of cross section (arrow head shows the vascular bundle), **I.** Epidermis and collenchyma (arrow head represent epidermis, and cl shows collenchyma), **J.** Sclerenchyma with colony, **K.** Tracheid and vessel, **L.** Pith.

The literature has been summarized by Metcalf and Chalk [26], Solereder [27] and in part by Bennett [28] and Quinlan [29]. Quinlan [29] found only slight differences between members of the family. The more important of these works are summarized in the following treatment. The work concerning the vascular system of the family is that of Fahn and Bailey [30] who studied the nodal anatomy of both *Calycanthus* and *Chimonanthus*. They found the family to possess a two-trace, unilacunar vascular

system. Such a nodal structure is now recognized to be primitive among angiosperms and also occurs in the Austrobaileyaceae, Monimiaceae, Annonaceae and Winteraceae among others [31]. They have noted some differences between species of Calycanthaceae in the level of branching and fusion of the vascular bundles of the eustele. This could be utilized as a taxonomic feature which is also supported by our results. In addition to the primary vascular cylinder, four cortical vascular bundles occur in the stem. These were first described [29] and have long drawn the attention of plant anatomists. There has been some disagreement as to their origin and phylogenetic significance and the various descriptions and viewpoints have been summarized by [28] and [29] in a detailed study of the seedling, stated: "Cortical bundles were found to originate from the primary vascular poles of the root after they had diverged from the central cylinder to become the trace to the cotyledon. This is the only point at which the cortical system connects with the central style of the stem. Fahn and Bailey [30] in general agreed with the observations of Bennett. In their study of the eustele as well as the cortical system of the mature stem and seedlings, they found no evidence that the cortical system is a modification of lateral traces of a trilacunar or multilacunar nodal system, as is the apparent case in some dicot families [31], but believed that it is an additional independent system which has been superimposed upon the double trace, unilacunar structure of the primary cylinder. They also indicated that transverse connections between the cortical strands which are present in the nodal region of *Calycanthus* are less well developed in *Chimonanthus.*

A complete and detailed description of the phloem of the Calycanthaceae has been given by Cheadle and Esau [32]. Our results also show *Calycanthus occidentalis*, *Chimonanthus praecox*, *Chimonathus salcifolius* and *Sinocalycanthus chinensis* in which they found the phloem to be very similar and to possess the following characteristics in common: absence of fibers, sclareids, the presence of numerous intercellular spaces, presence of oil cells in varying degrees of abundance, uniseriate to multiseriate rays, sieve elements with thick nacreous walls, and simple sieve plates located laterally or on the end walls in an oblique or transverse manner. Oil cells were found to be in *Calycanthus occidentalis*, and infrequent and usually smaller in *Chimonanthus praecox*, whereas *Chimonanthus salcifolius* and *Sinocalycanthus chinensis* possess big size. In *Sinocalycanthus chinensis* we also demonstrated a more irregular cell pattern with sieve tubes in more markedly isolated strands than in either *Calycanthus occidentalis* or *Chimonanthus praecox* and *Chimonanthus salcifolius.* Characteristics of the secondary wood of the family have been summarized by Metcalf and Chalk [26]. Lemesle [33] in his rather extensive study, reported differences primarily between the two genera in tracheid characteristics.

Those of *Calycanthus and Sinocalycanthus* possess bordered pits with circular apertures while those of *Chimonanthus* possess more elliptical apertures. Lemesle [33] considered the wood of *Calycanthus* to be more primitive, at least in this characteristic. Tracheids are quadrangular in cross section. The internal surface of the tracheids possesses spiral bands which are stated to be slightly wider in *Chimonanthus* than in *Calycanthu*s. We also supported that the majority of the tracheids possess simple oval or oblong perforations although some tracheids may be devoid of the connecting perforations. The vessels are small, polygonal in cross section and possess simple perforations. The fibers are libriform and almost completely devoid of bordered pits in Calycanthaceae.

There was no detail character for the stem in Calycanthaceae. Sclerenchyma possesses chain and colony structure which is a new character for young stem anatomy in Calycanthaceae.

Key to the genera of Calycanthaceae based on the stem anatomy:

1. Sclerenchyma is (14-17) cells group formation of the colony, protoxylem vessel is wider cavity ……..... *Sinocalycanthus*
2. Sclerenchyma is (13-14) cells group formation of colony, protoxylem vessel is wider cavity …….....…. *Calycanthus*
3. Sclerenchyma is arranged in the long chain with two layers, protoxylem vessel is smaller cavity …….....……..... *Chimonanthus*

AUTHOR'S CONTRIBUTION

Both authors have equal contribution. KH: supervisor, research design; sample collection; NP; research design; sample collection, experimental design. All authors have approved the final manuscript.

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TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this article.

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