Morphological Study of the Versatile Anther Group in the Tribe Zingibereae (Zingiberaceae)

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ABSTRACT

Scanning electron micrographs of anther development in *Cautleya spicata* (Sm.) Baker show that the appendages develop from the joint connective tissue where one end of the anther develops first, well before the other turns into the appendages. The anther with appendages is thus basifixed in a mature plant in *Cautleya spicata* while observation of *Curcuma* species reveals that the anther is dorsifixed, and the appendages are derived from the thecae of the anther. Mapping this characteristic of the anther in the six genera that possess versatile anther in Zingiberaceae, namely *Camptandra, Cautleya, Curcuma, Laosanthus, Paracautleya* and *Roscoea*, onto the molecular based phylogeny of the tribe suggests that the dorsifixed versatile anther of the *Curcuma* complex has been lost independently in *Hitchenia*, *Smithatris* and *Stahlianthus*, while the basifixed versatile anther has arisen independently in *Camptandra* and *Cautleya*/*Roscoea*.

Key words: Versatile anther - Zingiberaceae - *Camptandra* - *Cautleya* - *Curcuma* - *Laosanthus* - *Paracautleya* - *Roscoea*

INTRODUCTION

The inflorescence of Zingiberaceae plants is usually thyrse, sometimes with large coloured bracts (1,2). Thyrse is a densely branched inflorescence with the main branch racemose, but the lateral branches cymose (3). The bracts of the inflorescence subtend a short cincinnus of flowers (4). In some taxa, the cincinni are reduced to a single flower, a result of racemes or spike (2). The flowers of Zingiberaceae are zygomorphic or monosymmetric and last only for a day. They are tubular and contain nectar. The most outstanding parts of the flower are the petaloid staminodes. Only one stamen is fertile while the remaining five stamens are transformed or absent. The lip of the flower is composed of the two staminodes of the inner whorl, whereas, if present, the two lateral petaloid staminodes are those of the outer whorl. The anterior member of the outer whorl is always suppressed and absent (5,6).

The two-locular anther is attached to the filament mostly basally and along its whole length. The connective is sometimes produced apically into a structure called anther crest that may be large and petaloid, as in *Kaempferia*. The connective near the joint of the filament and anther is sometimes also structured into a special base that is termed anther appendages or spurs. These anther appendages give the anther versatility. They can be found in *Cautleya*, *Roscoea* and *Camptandra*. In other cases,

the anther is dorsifixed, as found in *Curcuma*, *Laosanthus* and *Paracautleya*, and thus also giving rise to versatile anthers. Unlike the anther appendages in *Cautleya*, *Roscoea* and *Camptandra*; *Curcuma*, *Laosanthus* and *Paracautleya* have anther appendages that are formed from the bases of the thecae of the anther, not distinctly so from the connective, as in the former group. In all, the versatile anther is observed in six genera of the tribe Zingibereae namely: *Cautleya*, *Camptandra*, *Curcuma*, *Laosanthus, Paracautleya* and *Roscoea* (4,7).

This study attempts to understand the ontogeny of the two types of anther appendages using scanning electron microscopy (SEM) and direct observation. The result will be discussed with the phylogenetic findings of the plants. The versatile anther genera in the tribe Zingibereae involve three distinct lineages namely the clade of *Cautleya/Roscoea*, the *Curcuma* complex clade and the separate clade of *Camptandra* (8,9). *Cautleya spicata* representing the clade of *Cautleya/Roscoea*, was studied for the growth and development of the appendages by SEM. *Roscoea* species and *Curcuma* species were observed from fresh material, spirit collection and drawings. *Camptandra*, *Laosanthus* and *Paracautleya* are not in cultivation at the Royal Botanic Garden Edinburgh, only spirit material and drawings were available for observation.

MATERIALS AND METHODS

A plant sample of *Cautleya spicata* was obtained from the cultivated stocks of the Royal Botanic Garden Edinburgh for the scanning electron microscope study. The accession number and the voucher specimen number are RBGE 19590760 and C. Ngamriabsakul 30. Living plant observation was also made on *Roscoea* species, *Curcuma* species in the garden in addition to the spirit collection and available drawings. *Camptandra, Laosanthus* and *Paracautleya* were studied from spirit material and drawings.

The material of *Cautleya spicata* was fixed in FAA (9 parts 70% ethanol: 0.5 parts glacial acetic acid: 0.5 parts formaldyhyde) overnight. Afterwards, the material was passed through a series of increasing concentrations of ethanol to absolute ethanol and finally acetone to dehydrate it (70% ethanol for 15 minutes, 95% ethanol for 10 minutes, 100% ethanol for 5 minutes and 100% acetone for 5 minutes twice). The material was next dried in an Emitech K850 critical point dryer. Dried parts were mounted with carbon discs on 1.25-cm Agar Scientific aluminuim stubs, and further dissected. The stubs were sputter coated with gold-palladium using an Emscope sc500. Specimens were viewed using a Zeiss DSM962 SEM at a working distance of 8-13 mm, and operating at 5 kV. Digital photographs were taken. Phylogenetic findings (8,9) were also used as an additional basis for the evolutionary interpretation.

It was intended that a sample taxon of *Roscoea* would be included in the study. Unfortunately, by the time I started to collect materials, it was discovered that *Roscoea* had already developed inflorescences and flowers. Although, no leaf or a lack of the elongation of pseudostem were observed in *Roscoea*, the inflorescences and the flowers were already well into advanced development. The rates of the inflorescence and floral development in *Roscoea* species are generally faster than those of *Cautleya* species. Vegetative and reproductive growth seem to be concomitant in *Roscoea* whereas *Cautleya spicata* seems to have developed quite a few leaves and a long stem before the maturation of its inflorescence and flowers. Thus only *Cautleya spicata,*

whose stages of inflorescence and floral development were available, was suitable for this development study.

RESULTS

Although it has been observed that the six genera in the tribe Zingibereae i.e. *Cautleya*, *Camptandra*, *Curcuma*, *Laosanthus, Paracautleya* and *Roscoea* possess versatile anthers (4,7), the nature of the versatile anthers has not been given much attention or has not been mentioned at all. It is rather interesting why this characteristic of the anther has managed to escape attention, as it can be observed by the naked eye or with a hand lens. In addition, light microscopy could be used to confirm the character. In this present study, visual inspection was confirmed by SEM that the type of the connection of the filament and the anther in *Cautleya* is basifixed. Observation in *Camptandra, Curcuma, Laosanthus, Paracautleya* and *Roscoea* revealed that the versatile anther of *Camptandra* and *Roscoea* is basifixed whereas it is dorsifixed in *Curcuma, Laosanthus* and *Paracautleya*.

The development of the versatile anthers in *Cautleya spicata* suggests that the appendages were developed at the base of the connection of the anther and the filament. The appendages were observed much later in comparison to the growth of the thecae **(Figures 1-9)**. The thecae were already enlarged and developed when the appendages were initiated. Then later, the connection at the thecae side extended pushing the thecae further away from the appendages **(Figures 10 and 11)**. The dried plant material also gave a clear distinction between the thecae and the appendages **(Figures 7,8,12)**. There appeared to be a groove in the middle and along the appendages whereas the thecae were slightly changed in form.

In *Curcuma* species, the appendages are produced from the base of the thecae compared to the appendages from the connection tissue of *Cautleya* and *Roscoea*. Despite the lack of the appendages in some *Curcuma* species e.g. *C. alismatifolia* Gagnep., *C. harmandii* Gagnep. and *C. parviflora* Wall., the anthers in these species are still versatile because of the dorsifixed attachment of the anthers to the filament. It should be noted here also, that the thecae of some *Curcuma* species are fertile only in part while the thecae in *Cautleya* and *Roscoea* are fertile throughout.

Legend for Figure 1-12. St denotes stigma while Sty = style, Ant = anther, App = appendage, EpiG = epigynous gland, Stm = staminode and Ova = ovary

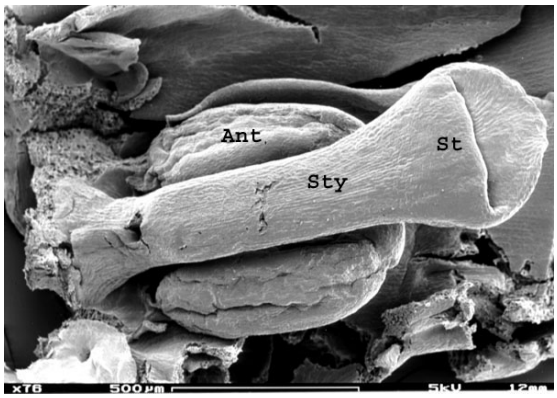
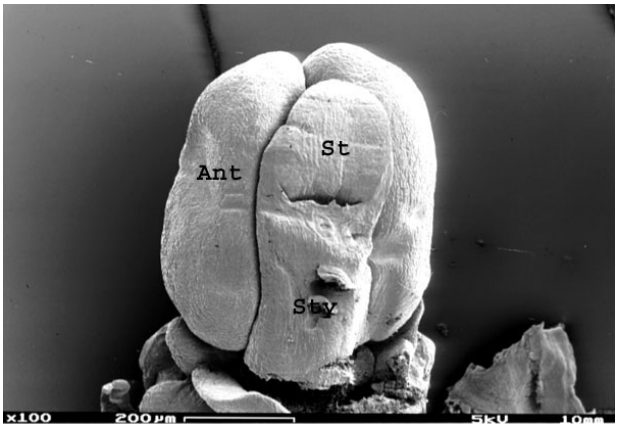


Figure 1.



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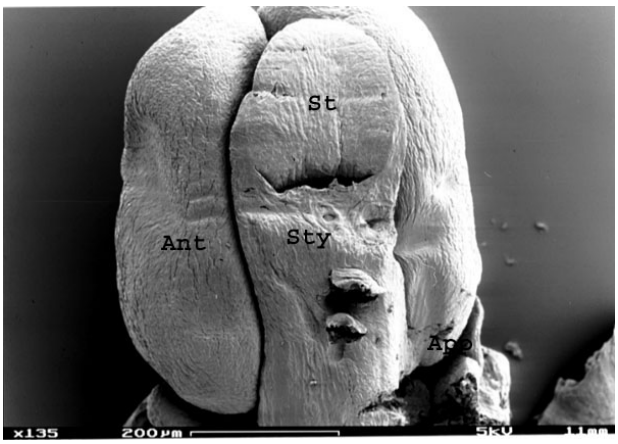

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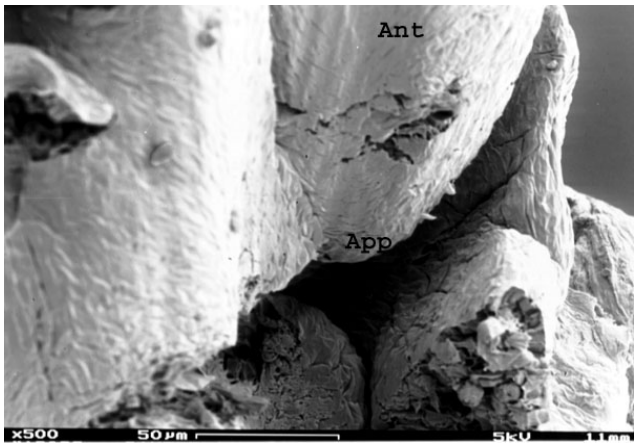

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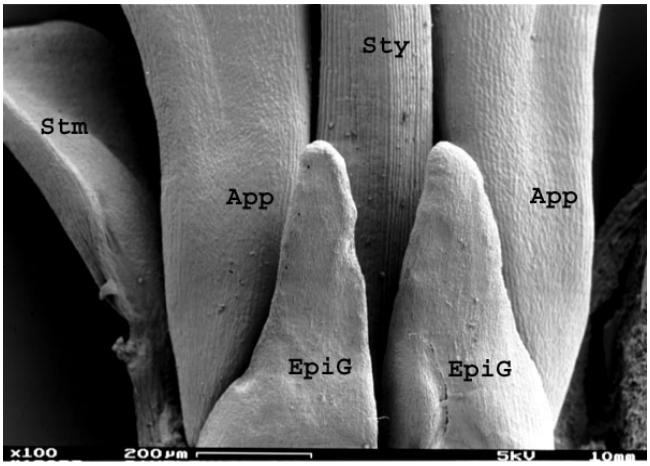

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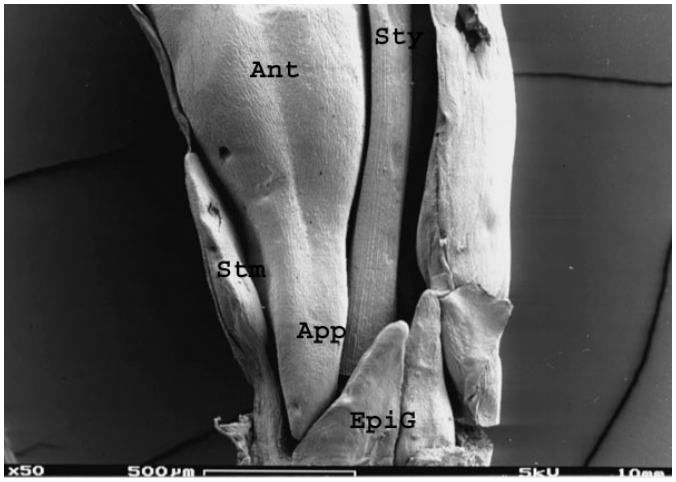

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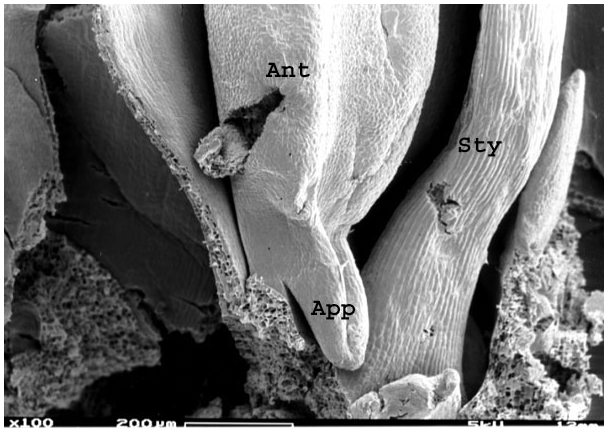

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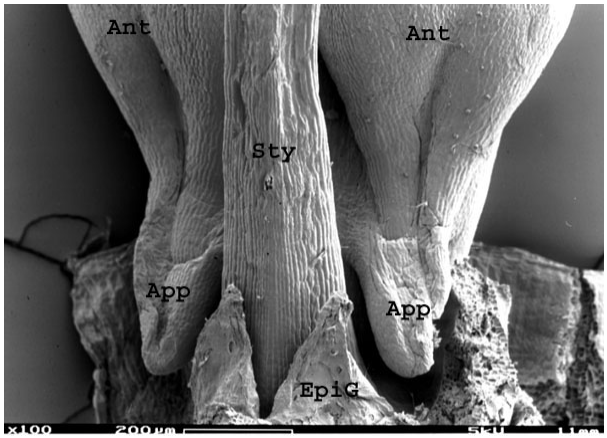

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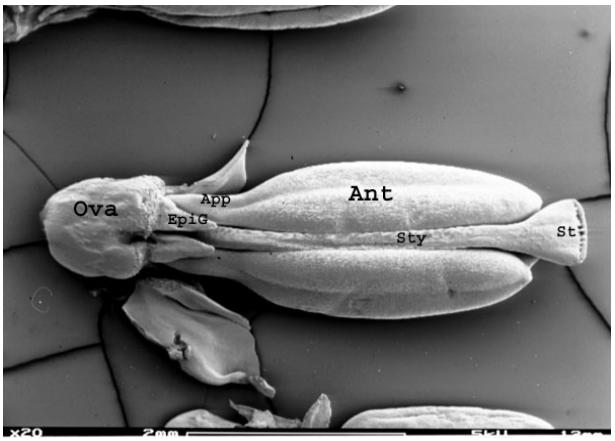

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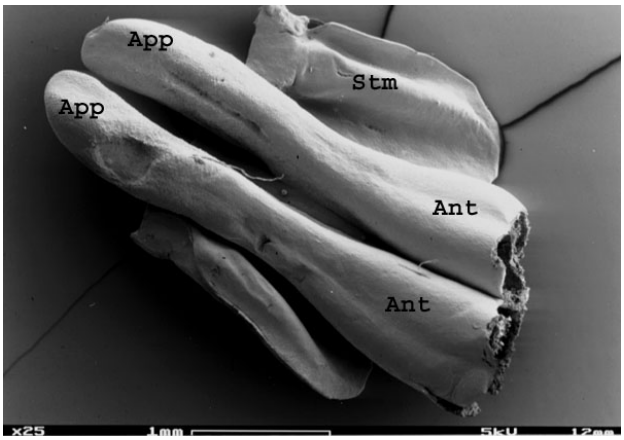

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Figure 11.

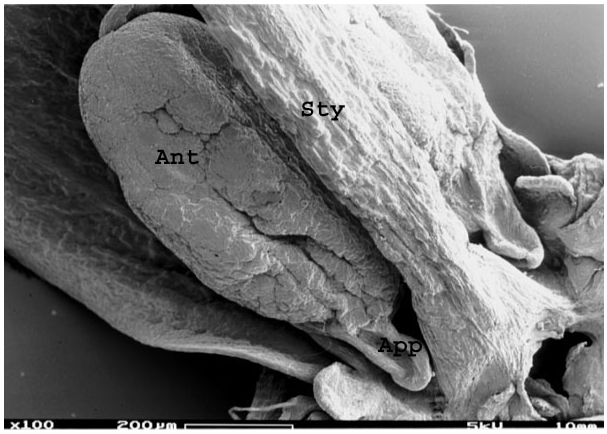

Figure 12.

DISCUSSION

Developmental studies of the inflorescence and flower of Zingiberaceae, especially members of the tribe Zingibereae (formerly Hedychieae), have been carried out by Kirchoff (5,6). The results of these studies reveal that, even in a very short period of time, differences in morphological changes through time (heterochrony) are observed in two closely related species *Hedychium coronarium* König and *H. gardnerianum* Roscoe (10). The study of ontogeny, or the series of developmental processes through time, is of pivotal value to the study of phylogeny and systematics. It may be demonstrated that slight differences in development can lead to dramatic differences in mature organ structures (divergence). On the other hand, different pathways can also lead to invariant mature floral morphology (convergence).

The molecular phylogenetic findings (8,9) suggested that the basifixed versatile anther in the clade of *Cautleya/Roscoea* and *Camptandra* has been derived independently **(Figure 13)**. The convergence of basifixed versatile anther in the two distinct lineages in the tribe Zingibereae may have resulted from adaptation to the similar pollination syndromes in different habitats. Floral structure, including the

anther and the appendages indicate that the pollinators of *Cautleya*, *Roscoea* and *Camptandra* are bee species that forage for the nectar of the flower. However, there is no report of pollination studies in these genera. The pendulous lip of the flower is thought to be the platform for the pollinator to enter and in so doing the appendages will be pushed, bringing down the anther into contact with the back of the pollinator. Fruits of *Roscoea* are often observed at the Royal Botanic Garden Edinburgh where there is probably no true pollinator of *Roscoea* as in its wild habitat. Garden bees may be pollinating the flowers, leading to the formation of fruits. Because *Roscoea* grows as a clump of individuals, possibly other insects or wind may also play a part in the pollination.

The appendages can grow into varying shapes and sizes in *Curcuma* species (11,12) and *Roscoea* species (13,14). Not only are they useful taxonomically, but also may be a clue suggesting the pollinators of the species.

The molecular phylogenetic findings also suggested that the dorsifixed versatile anther of the *Curcuma* complex has been lost independently in *Hitchenia*, *Smithatris* and *Stahlianthus* **(Figure 13)**. These may have further obscured the patterns of morphological changes in the tribe Zingibereae which otherwise would be more revealing for the researchers of Zingiberaceae. Holttum (15) who studied the Zingiberaceae of the Malay Peninsula, however, with meticulous conduct, came to notice the differences of the anther appendages in *Camptandra* (and *Roscoea*) and *Curcuma* as well as suggesting the implication of their function as quoted below.

"In *Camptandra* (and apparently also in *Roscoea*) the pollen-sacs are much produced basally into the sterile appendages which are inclined forwards away from the filament, thus giving a versatile character to the anther. In *Curcuma* also the anther is versatile, being attached usually about the middle of the pollen-sacs, and at the same time there is usually a sterile outgrowth from the back of the base of each pollen-sacs. These outgrowths are usually called spurs, and they function in the same way as the basal appendages in *Camptandra* as a mechanism for cross-pollination. A visiting insect pushes against the spurs on entering the flower, and in so doing brings the pollen-sacs into contact with its back" (15, p. 46-47).

CONCLUSIONS

To summarise, the molecular phylogenetic studies of the tribe Zingibereae (8,9) and morphological observations in this study suggest that there are two types of anther appendages in the tribe Zingibereae. One is derived from the joint of the anther and the filament, as found in basifixed versatile anther of *Cautleya*/*Roscoea* and *Camptandra*. The other is derived from the base of the thecae of the anther, as found in dorsifixed versatile anther of *Curcuma*, *Laosanthus* and *Paracautleya*.

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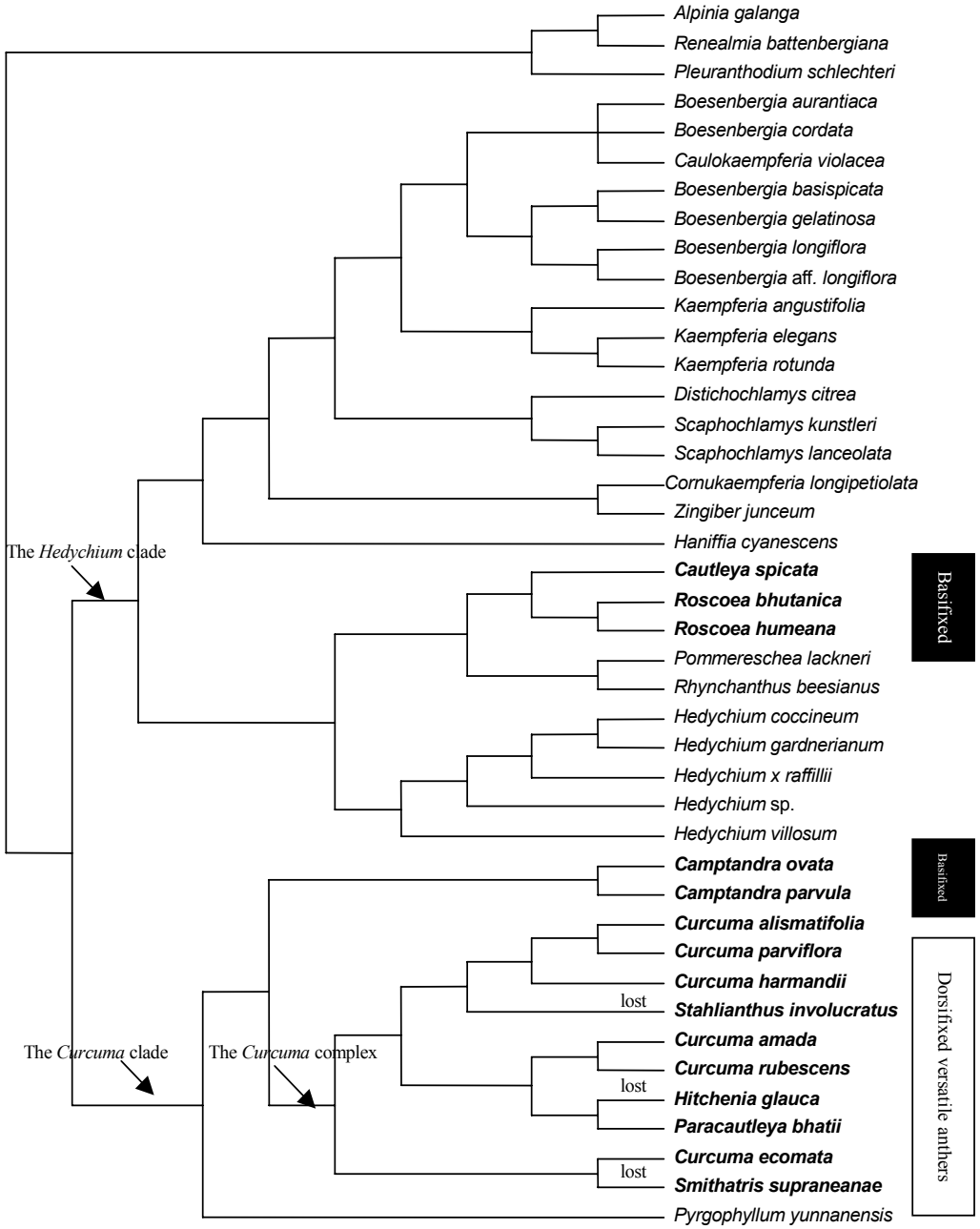

Figure 13. The strict consensus tree of two equally optimal trees resulting from maximum likelihood analysis of 42 taxa ITS data set (9). Versatile anther character i.e. dorsifixed and basifixed is superimposed onto the tree. *Laosanthus* is found nested within the clade of *Stahlianthus* and related *Curcuma* species (Tania, pers. comm.).

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บทคัดย่อ

ฉัตรชัย งามเรียบสกุล

การศึกษาทางสัณฐานวิทยาของพืชกลุ่มที่มีอับเรณูแบบติดไหวได้ของเผ่า Zingibereae (Zingiberaceae)

ภาพถ่ายจากกล้องจุลทรรศน์แบบส่องกราดของการเจริญของอับเรณู *Cautleya spicata* (Sm.) Baker แสดงให้เห็นว่าระยางค์เจริญมาจากเนื้อเยื่อส่วนเชื่อมต่อระหว่างอับเรณูและก้านชูอับเรณู โดยที่อับเรณูมีการเจริญก่อนและเร็วกว่าการเจริญของระยางค์ อับเรณูจึงมีลักษณะการเชื่อมต่อกับก้านชูอับเรณูแบบที่ฐาน ขณะที่ในสกุล *Curcuma* ลักษณะการเชื่อมต่อของอับเรณูและก้านชูอับเรณูเป็นแบบด้านหลังและระยางค์เจริญยื่นออกมาจากพูของอับเรณู เมื่อวางลักษณะการเชื่อมต่อของอับเรณู และก้านชูอับเรณูของหกสกุลที่มีอับเรณูแบบติดไหวได้บนแผนภูมิแสดงสายสัมพันธ์ทางวิวัฒนาการที่ได้จากข้อมูลระดับโมเลกุล พบว่า การเชื่อมต่อของก้านชูอับเรณูและอับเรณูแบบด้านหลัง สูญหายไปในบางหมวดหมู่ของ *Curcuma* complex คือ *Hitchenia, Smithatris* และ *Stahlianthus* ขณะที่การเชื่อมต่อของก้านชูอับเรณูและอับเรณูแบบที่ฐาน เกิดขึ้นอย่างอิสระในสองหมวดหมู่คือ *Camptandra* และ *Cautleya*/*Roscoea*

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