CHAPTER 9
DISCUSSION – MOLECULAR PHYLOGENETIC ANALYSIS

Sequence analysis

The total length of sequences of ITS+5.8S nuclear ribosomal DNA of *Curcuma* species obtained from this study vary from 578 to 604 bp (table 9) similar to sequences of *Curcuma* deposited in Genbank. *C. longa* has the longest sequence, 604 bp, followed by *C. aeruginosa*, 602 bp. The shortest sequence is taken from *C. parviflora*, 582 bp. Approximately, 70% of characters are conserved. This is higher than those suggested by Soltis *et al*. (1998), 40-60% conserved among flowering plants and also Ngamriabsakul (2004), 48.34% among Zinigiberaceae. This may caused by the more closely related taxa in this study. But the informative characters are enough to show the relationship of these taxa.

Cladistic analysis

Cladistic analysis of 15 species of *Curcuma* in this study resulted in four major clades with *Camptandra*, *Caulokaempferia* and *Hedychium* as basal clades. These three genera were confirmed the sister groups as shown in Kress et al. (2002) and in Ngamriabsakul et al. (2004). The first clade is *C. cochinchinensis* and the genus *Smithatris*. *Smithatris* formed a group with *C. ecomata* in Ngamriabsakul's work but in this study, it is grouped with *C. cochinchinensis* leaving *C. ecomata* from a separated group with *C. bicolor* and *C. flaviflora*. These two groups of *Curcuma* differ from each other in flower shape (closed form of *C. cochinchinensis* vs. open form of *C. ecomata* group) and anther spurs (short filament of *C. cochinchinensis* vs. large, blunt and forward-pointed of *C. ecomata* group).

C. alismatifolia, *C. parviflora* and *C*. sp. (Pitsanulok) form a distinct group with weak bootstrap support. *C. harmandii* show uncertain relationship with this group in strict consensus tree, but in two out of three most parsimonious trees, *C. harmandii* is grouped with the other member of *C. alismatifolia* group. These four species belong to subgenus *Hitcheniopsis*. Valeton (1918) suggested to separate this subgenus from *Curcuma* and established a new genus. The distinguished characters of this group are the absence of anther spurs and stylodes. The genus *Stahlianthus* is the

sister group of the *C. alismatifolia* group as in Kress and Ngamriabsakul's works. *Stahlianthus* has unique inflorescence which is urceolate-shape, formed by two bracts adnate by side to each other, subtending flowers inside.

The last group is the complex of *C. longa* group, *C. petiolata* group, the genus *Hitchenia* and the monotypic genus *Paracautleya*. *C. roscoeana* is the basal clade of this complex. *C. longa* group, core group, with moderate to strong bootstrap support comprises *C. aeruginosa*, *C. amada*, *C. comosa*, *C. longa* and *C. rubescens*. This group belongs to subgenus *Curcuma* (*Eucurcuma* K. Schum.). The genus *Hitchenia* is the sister group. The *C. petiolata* group, represented by *C. petiolata* and *C. roscoeana* were polyphyletic in this analysis. It included the genus *Paracautleya*. But it is clear that this group is separated from the *C. longa* group by the occurrence of the genus *Hitcheniopsis*. The genus *Paracautleya* is monotypic with only one species in southern India. It shares some morphological characters with *Curcuma*, for example sharply pointed anther spurs. More taxa of *C. petiolata* group are needed to clarify the relationship within this group and with the other groups and the other genera. *C. roscoeana* is not fit with petiolata group in an important character, i.e. lack of anther spurs.

Significant morphological characters

The anther spurs is the most important character to separate *Curcuma* into four groups and confirmed by this analysis. Each type of anther spurs is unique for each group, as discussed in taxonomic study, with an exception of two species in *C. longa* group. *C. aurantiaca* and *C. roscoeana* are lacking of anther spurs but the other characters are fitted well with this group.

Stylodes occur in three out of four groups, except *C. alismatifolia* group. *C. aurantiaca* and *C. roscoeana* which have no anther spurs but both of them have stylodes.

It is proved that the position of the inflorescence, especially in *C. longa* group, is not a significant character in classification. From the strict consensus tree (figure 45), *C. amada* and *C. longa* which produce terminal inflorescence appeared among *C. aeruginosa*, *C. comosa* and *C. rubescens* which produce lateral inflorescence. It was reported in some species that this character is not constant

(Verayutdin *et al*., 1996). *C. angustifolia* and *C. latifolia*, for example produced lateral inflorescence first in the late of dry season to early of rainy season. After that, in mid of rainy season it produced terminal inflorescence. The structure of these two kinds of inflorescence is almost identical except that the lower floral bracts of the terminal one is narrower. Horaninov (1862) established section Amphiantha for species produce both types of inflorescences.

Ardiyani (1999) also found that section *Exantha* and *Mesantha* are not supported by cladistic analysis based on ITS-1 sequence. Red patch on lamina is constant for some species, for example *C. latifolia* and *C. rubescens* but is variable in *C. angustifolia*. Indumentum of lower surfaces is pubescent only in two species, *C. elata* and *C. latifolia*. Some characters are not informative in classification but can be used in identification such as rhizome color, red patch along midrib and flower color.

In conclusion, the cladogram from this study shows that *Curcuma* is polyphyletic. It can be divided into four groups, *C. alismatifolia*, *C. cochinchinensis*, *C. ecomata* and *C. longa* group with moderate to strong bootstrap support. Each group is more closely related to another genus than with other groups; *C. cochinchinensis* group and *Smithatris*, *C. alismatifolia* group and *Stahlianthus*, *C. longa* group and *Hitchenia* and *Paracautleya*. *C. ecomata* group does not have any other genus as sister group. *C. cochinchinensis* and *C. ecomata* group are completely separated from the remaining groups and can be separated as two new genera. These group are also supported by morphological characters. The genus *Stahlianthus* is sister of *C. alismatifolia* group. The only problem of this group is the unclear relationship of *C. harmandii* in strict consensus tree. Morphology also supports *C. alismatifolia* group as distinct genus. The core group is *C. longa* group which is monophyletic. It has *Hitchenia* as sister group. *C. petiolata* and *C. roscoeana* are clearly separated from *C. longa* group. Morphologically, they form a distinct group. But they show unclear relationship in strict consensus tree. Sequences of more taxa in this group are needed to clarify the status and the relationship of this group.