

Pattamon Sangin 2010: Phylogenetic Analysis of Cycad and Sex Identification in *Cycas* using Molecular Markers. Doctor of Philosophy (Bioscience), Major Field: Bioscience, Interdisciplinary Graduate Program. Thesis Advisor: Associated Professor Mingkwan Mingmuang, Ph.D. 138 pages.

Phylogenetic relationships among 43 species within the family Zamiaceae and Stangeriaceae were examined using 4 non-coding regions of chloroplast DNA (*atpB-rbcL*, *psbM-trnD*, *trnL-trnF* and *trnS-trnM*). Maximum likelihood (ML), Maximum parsimony (MP) and Neighbor-joining (NJ) analyses were performed on separated and combined data sets to generate phylogenetic trees. Zamiaceae and Stangeriaceae are not monophyletic entities based on these analyses with *Bowenia* and *Stangeria* embedded and separated within the former family. *Stangeria* was closely related with *Ceratozamia* in MP and NJ trees. *Dioon* was clearly showed as the most basal genus. *Encephalartos* and *Lepidozamia* were closer to each other than *Macrozamia* with these three genera forming a monophyletic group. Furthermore, in confirmation to recent publications, *Chigua* was found to be paraphyletic with *Zamia*. The *trnL-trnF* sequences were more informative than other regions in helping resolve relationships in the cycads. A new, simplified suprageneric classification for the extant cycads was presented whereby two families are recognized. The subfamily Dioonioideae was newly recognized. Further investigation on the infrageneric relationships among 27 species of five *Cycas* sections was made using the same methods. Three data sets were combined (*trnS-trnG*, *psbM-trnD* and *trnL-trnF*) because they did not differ significantly in structure. All three methods showed similar topology which divided *Cycas* into two main clades. The first clade consisted of two sections, *Cycas* and *Indosinenses*, while the other clade contained *Asiorientales*, *Wadeanae* and *Stangerioides*. Base substitution pattern, further revealed that subsection *Rumphiae* of the section *Cycas* could be separated into two groups (*C. rumphii* and *C. edentata*). In addition, *C. taitungensis* was closely related to *C. revoluta* while other species of the section *Wadeanae* (*C. wadei* and *C. curranii*) were closely related which agreed with the morphology. The *trnS-trnG* sequences were more informative than other regions in addressing phylogeny. Microsatellite and minisatellite detected in this region also indicated the high rate of evolution in *Cycas* species. The minisatellite identified in this study is the first report. RAPD and ISSR primers were tested on *Cycas* to identify sex-specific molecular markers. Sixty different RAPD primers and twenty-nine ISSR primers were screened out of which only two RAPD primers (OPB-8 and UBC 485) and three ISSR primers (001, 836 and 864) were found to be polymorphic. These sequences were converted into specific Sequence Characterized Amplified Region (SCAR) markers. However, none of SCAR markers showed sex specific fragment when testing using DNA of individual males and females *Cycas*.

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Thesis Advisor's signature