CHAPTER II

THE OVERVIEW OF THE FAMILY ANNONACEAE AND GENUS *GONIOTHALAMUS*

Annonaceae (custard apple family) are a pantropical family of shrubs, trees and lianas. The family is a member of Magnoliales and basally positioned within the angiosperm (Soltis et al., 2000; APG II, 2003). The estimated age of stem Annonaceae according to Wikstrom, Savolainen and Chase (2001) is between 82 and 91 Myr ago. This proposed date is confirmed by Richardson et al. (2004) whose estimated age of the family obtained when calibrating the tree using *Archaeanthus* is 90.6 ± 1.3 Myr ago and placed the family at the stem of Magnoliaceae. The oldest remains of the family are seeds with a perichalazal ring and endosperm with lamelliform rumination that were found in the Maastrichtian of Nigeria (Chesters, 1955) and primitive pollen morphology from Columbia (Sole de Porta, 1971). This evidence suggests that the Annonaceae originated in west Gondwanaland (Richardson et al., 2004). Post-Eocene cooling presumably resulted in disjunctive distributions of taxa between tropical Africa, Asia and the New World. *Goniothalamus-Neostenanthera* African-Southeast Asian (34.9-41.1 ± 2.8 Myr ago) estimated splits could be the result of such a disruption of boreotropical vegetation (Pennington and Dick, 2004). From age estimates for the diversification of species-rich genera, the most rapid radiation seems to have taken place in the Southeast Asian genus *Goniothalamus*, whose crown is estimated at 3.6-4.8 ± 1.5 Myr ago (Richardson et al., 2004).

Although the position of Annonaceae among the flowering plants and their monophyly are not disputed (Doyle et al., 2004), the relationships of the genera within the family are not well understood. Morphological characters that are useful for the delimitation of genera and species are also overlaped at higher taxonomic levels (e.g. tribal level). More or less formal classification based on intuition or phenetic analyses of morphological characters (Hutchinson, 1923, 1964; Sinclair, 1955; Fries, 1959; Walker, 1971; Van Heusden, 1992; Van Setten and Koek-Noorman, 1992; Keßler, 1993; Koek-

Noorman, Van Setten and Van Zuilen, 1997) do not accurately predict relationships among genera in Annonaceae.

The genus *Goniothalamus* is one of the largest and most important genera of the Annonaceae in Asia. Widespread in tropical and subtropical Asia (Figure 2.1), they primarily occur in Malesia but a few species also occur in China, the Indian subcontinent and northern Australia. To date, more than 150 taxa have been described in *Goniothalamus*; about 120 species and 10 subspecies/varieties are currently recognized (Mat-Salleh, 1993; Saunders, 2002, 2003). Most species of *Goniothalamus* are small monocaulous treelets. Hence, they have not been used as major commercial timber forest products. Nevertheless, many of the species have promising medicinal value. They have been widely utilized in traditional medicinal practices. *Goniothalamus* are especially popular among Asian native women as post-natal medicines and abortifacients (Burkill, 1935; Perry, 1980; Mat-Salleh, 1989). The genus is characterized by axillary (or slightly supra-axillary) flowers that are generally pendent. As with most Annonaceae, the flowers have three sepals and two whorls of three petals. The outer petals are typically larger than the inner (although sometimes only slightly so), and the inner petals covers over the reproductive organs, forming a distinctive mitriform dome. The flowers are bisexual, with numerous free stamens and carpels. The stamens have broad apical connectives that are variable in shape, ranging from truncate to apiculate, and thecae that are septate; the pollen is released as tetrads. The apocarpous fruits are taxonomically very important, with variation in size, shape, indument, peduncle and stipe length (Saunders, 2003).

A widely used infrafamilial classification of the Annonaceae was introduced by Bentham and Hooker (1862), in which they placed the genera into five tribes based on the aestivation of calyx and corolla and the structure of the stamen connective. In their classification, the genus *Goniothalamus* was considered a member of the tribe *Mitrephoreae* (whose inner petals curve over the sexual organs forming a dome-shaped "mitriform" structure) together with *Mitrephora*, *Pseuduvaria*, *Friesodielsia*, *Orophea*, *Popowia* and *Neo-uvaria*. In Ridley's *Flora of the Malay Peninsula* (1922), the Annonaceae were grouped into six tribes. *Goniothalamus* was in the tribe *Mitrephoreae*,

along with *Orophea*, *Oxymitra*, *Mitrephora* and *Popowia*. Sinclair's (1955) revision of the Peninsular Malaysian Annonaceae, however, noted that while *Orophea* fits the circumscription of the tribe *Mitrephoreae* by virtue of the characters of its inner petals, some members of this genus also have unusual stamens that associate it with tribe *Miliuseae*. Sinclair further noted that the inner petals of *Mitrephora*, *Popowia* and *Neo-uvaria* are united only at the beginning of flower development and are separated at anthesis. Only flowers of *Goniothalamus*, *Friesodielsia* and *Pseuduvaria* have a true mitriform dome during anthesis. Based on floral characters, Sinclair (1955) suggested that *Friesodielsia* is perhaps the nearest relative of *Goniothalamus*. Members of *Friesodielsia*, however, are exclusively scandent lianas, a habit unknown in *Goniothalamus*, and the leaves are generally small. Furthermore, Sinclair inferred that the closest genus to *Goniothalamus* is probably *Mitrephora*, but this genus has different and unique chartaceous leafy outer petals and the mitriform inner petals are free before anthesis, spreading and generally much smaller than the inner petals of *Goniothalamus*.

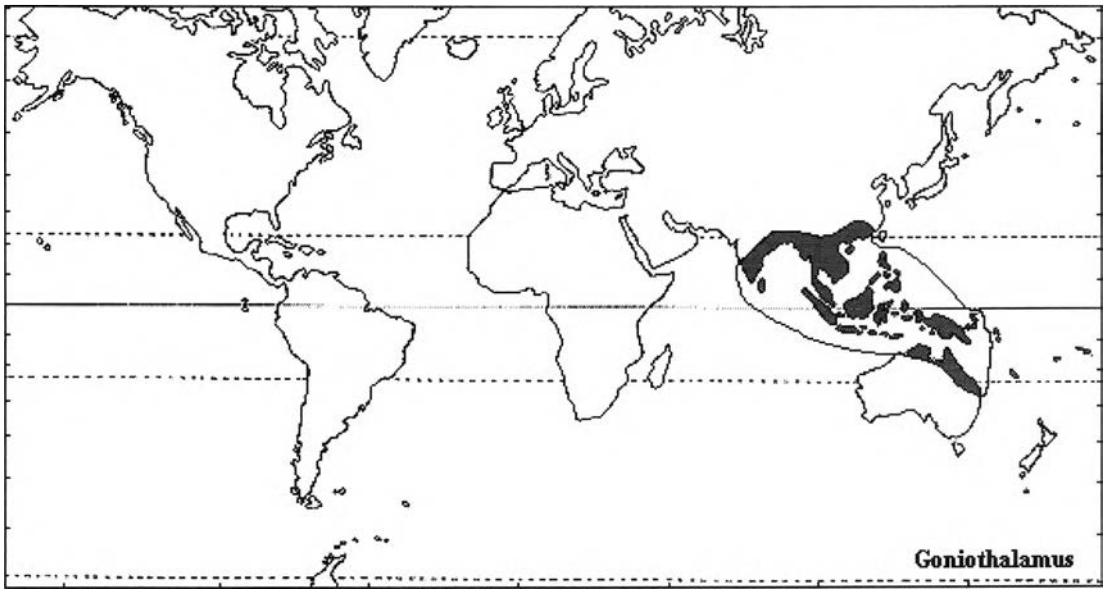

Figure 2.1 The distribution of *Goniothalamus*

Bentham and Hooker's classification (1862) was further modified by Fries (1959). Fries subdivided Bentham and Hooker's tribe into 14 new groups based on additional characters such as the position of the inflorescence, the number and position

of the ovules, fruit types, the presence of the floral bracts and stamen and thecae characters. Walker (1971) used Fries's classification as the basis of an infrafamilial classification of the family, enhanced yet using pollen characteristics. These characters are similar to the pollens of *Anaxagorea*, *Piptostigma*, *Xylopia*, *Fusaea*, *Duckeanthus*, *Cananga*, *Meiocarpidium*, *Neostenanthera* and *Richella*, thus grouped in "the *Fusaea* Subfamily" in Walker's classification. Recently, there have been several morphological and molecular cladistic studies of the angiosperm family Annonaceae, all of which have indicated that *Goniothalamus* is in the "long branch clade" together with *Dasymaschalon*, *Friesodielsia*, *Annona*, *Rollinia*, *Asimina*, *Disepalum*, *Anonidium* and *Neostenanthera* (Doyle and Le Thomas, 1994; Richardson et al., 2004).

There is conflicting opinion about the generic delimitation and relationships among *Goniothalamus* and related genera. On the one hand, it has been suggested that *Goniothalamus* is related to *Pseuduvaria*, *Orophea*, *Oxymitra*, *Mitrephora* and *Popowia* (Sinclair, 1955; Fries, 1959). On the other hand, it has been suggested that *Goniothalamus* is more closely related to *Anaxagorea*, *Piptostigma*, *Xylopia*, *Fusaea*, *Duckeanthus*, *Cananga*, *Meiocarpidium*, *Neostenanthera* and *Richella* (Walker, 1971; Koek-Noorman et al., 1997). However, the molecular phylogenetic of Annonaceae suggested that *Goniothalamus* is closely related to *Anonidium*, *Neostenanthera*, *Disepalum*, *Asimina*, *Annona*, *Rollinia* (Richardson et al., 2004).

The first author to formalize the infrageneric classification of *Goniothalamus* was Boerlage (1899) who proposed the section *Beccariodendron* to accommodate *Beccariodendron grandiflorus* and other multi-ovulate species of *Goniothalamus*. Members of *Goniothalamus* with one or two ovules were grouped into section *Eu-Goniothalamus*. However, Boerlage's classification has not been adopted by later authors. Until Sinclair (1955) subsequently classified the genus into two informal groups using stamen characters, apiculate and convex stamen connectives, because they are easier to be observed in herbarium specimen.

Walker (1971) studied pollen characteristics of nine species of *Goniothalamus*. His report indicated that the pollen of *Goniothalamus* consists of

tetrahedral or tetragonal tetrads, with heteropolar, bilateral, cataulcerate, disc-like concave-convex grains. The grains are comparatively large to very large, averaging 95 µm in diameter. They are microtectate, with no discernible columellae, and wide pitted or otherwise psilate exine. However, the morphology of pollen and tetrads observed in this study did not offer enough diversity to be useful for classification at the species level. The pollen of *Goniothalamus* is more or less homogenous throughout the genus. When Le Thomas (1981a, 1981b) disclosed more remarkable structural diversity of Annonaceae pollen, it was found that all Annonaceae pollen lacks endexine, a feature otherwise found in the monocotyledons. She also found that the reduction of pollen ultrastructure is paralleled by floral reduction and concluded that the family is perhaps finished an evolution.

Bân (1974a) reported that the single most taxonomically important part of the stamen is the connective, especially the tip. In fact, stamen connectives have been widely utilized in traditional classification to divide *Goniothalamus* into subgenera or sections. Thus Bân (1974b) proposed his infra-generic classification. Bân was doing little more than formalizing existing "dichotomous keys" as a supraspecific classification, and that none of his sections/subsections have been assessed to determine whether they are truly monophyletic. He divided the genus into two subgenera using staminal connective shape, *Goniothalamus* (apiculate stamens) and *Truncatella* (truncate stamens). He further proposed four sections, two sections in each subgenus, based on the shapes of the stigma and styles. In addition, he used number of ovules only at the subsection level. However, some species have intermediate stamen characters between two subgenera.

According to the surveys to establish the base chromosome number in the Annonaceae, the genus *Goniothalamus* is not well represented in accounts of chromosome data for the family. Only five papers provide original chromosome counts for the genus. Reports by Ehrendorfer et al. (1968), Sobha and Ramachandran (1979), Okada and Ueda (1984), Sauer and Ehrendorfer (1984) and Morawetz (1988) for *G. grandiflorus*, *G. microphyllus*, *G. opacus*, *G. wynadensis* and *G. australis* show a consistent count of $2n = 16$.

Annonaceae woods presented fine continuous tangential bands of parenchyma rays, occurring with remarkable uniformity and consistency among all genera. In contrast to the rather primitive floral characters, however, woods of Annonaceae seem to attain a high level of anatomical specialization (Wyk and Canright, 1956). Later Blunden and Jewers (1973, 1974a, b) mentioned the anatomical analysis for Bornean *Goniothalamus*, *G. andersonii*, *G. macrophyllus*, *G. malayanus* and *G. velutinus* that their leaf, stem and root anatomical features can be used to differentiate taxa.

Most *Goniothalamus* species are concentrated in West Malesia with high percentage of endemics in Indochina, Borneo, the Philippines and New Guinea. In his revision of Bornean representative, Mat-Salleh (1993) showed that Borneo has a remarkable representation of richness and diversity of the genus. A total of 30 species, including 11 new species, of the genus are currently recognized from Borneo, the largest number represented in any single biogeographic area. Based on inflorescence, inner petal dome, stamen, gynoecium and leaf characteristics, 11 informal species alliances were established for the Bornean species. Twenty-four species are local endemics and 10 Bornean species are known from very few localities. The distribution of these species in Borneo and other areas and the high percentage of endemics suggested that the most of these *Goniothalanus* species are locally evolved.

Mat-Salleh, Lim and Ratnam (2000) studied PCR-DAMD using M13 universal primer to determine generic relatedness between populations of *G. umbrosus*. The result showed that populations of kenerak in Kelantan are genetically close to each other. However, Penang populations were a distinct cluster of their own. In contrary, individuals from Perlis showed close relationship with other individuals from Kelantan. This might indicate that Penang population is not an introduced population but an isolated natural population. Genetic similarity of Perlis-Kelantan populations suggested that *G. umbrosus* is in fact a southern Thailand element, and they moved southward or introduced as a medicinal plant in the east and west coast of Peninsular.