Molecular phylogeny of Southeast-Asian Dipterocarps belonging to tribe Dipterocarpeae (family Dipterocarpaceae) based on non-coding sequence data of chloroplast and nuclear DNA

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I. Introduction

I.1 The family Dipterocarpaceae

The name Dipterocarpaceae is based on the name of first genus described as *Dipterocarpus*, which is characterized by perculiar two-winged fruits. Dipterocarpaceae Blume comprises the main timber trees of the tropical rain forests in Southeast Asia. They are one of the 17 families which are widely disjunct among the Africa-Eurasian (-Pacific) regions (Symington 1943; Radford et al. 1974). All dipterocarp species are large trees with alternative entire leaves, and pentamerous flowers (Maury-Lechon and Curtet 1998).

The Dipterocarpaceae family was described very early by Blume (1825) as a family with a close affinity to the Tiliaceae. Many changes in position of this family were proposed, for example the family was moved to order Ochnales (Hutchinson 1959), Theales (Cronquist 1968; Takhtajan 1969) and Malvales (Dalgren 1975; Takhtajan 1997). However, recent studies proved that dipterocarps have a close affinity with the family Sarcolaenaceae and belong to the order Malvales (Marguire and Ashton 1977; APG 1993; Chase et al. 1993; Alverson et al. 1998; Bayer et al. 1999; Dayanandan et al. 1999).

According to Blume (1825), Ashton (1982, 1988), Marguire and Ashton (1977), Vercourt (1989), Villiers (1991) and Londoño (1995), the taxonomical charateristics of Dipterocarpaceae were described as follows: "***trees*** small or large, resinous, usually evergreen, semi-evergreen, rarely deciduous in dry season, usually buttressed. Xylem with aromatic resin in intercellular resin canals. ***Branchlets*** with stipular scars, sometimes annular. ***Leaves*** alternate, simple, margin entire or sinuate, not crenate, terminating ± abruptly at the ± prominent geniculate petiole, penninerved, often with domatia in axils between nerves and midrib or along midrib and (rarely) nerves; tertiary nerves scalanform or reticulate. ***Stipules*** paired, large or small, persistent or fugaceous, leaving small to amplexicaul scars. ***Inflorescence*** paniculate, racemose, rarely cymose, ± regularly, rarely irregularly, branched, terminal or axillary; bracts and bracteoles paired, small or large, persistent or fugaceous. Inflorescences, calyces, petals, ovary, and other parts usually with stellate, squamate, fascicled or free-standing hairs. ***Flowers*** secund or distichous, bisexual, actinomorphic, scented, nodding. ***Calyx*** persistent, five-merous; two to five sepals usually greatly enlarging

into wing-like lobes in fruit; sepals either free to base, imbricate in bud, remaining so or becoming valvate in fruit, or fused at base, forming a cup or tube ± enclosing the fruit, adnate to or free from it. ***Carolla*** five-merous, contorted, base connate or free, usually partially or entirely unicellular hairy. ***Stamens*** 5-110, one to three verticillate or irregular, hypogynous or subperigynous, centrifugal; filaments compressed or filiform, free or connate, frequently cohering with petals on falling; anthers erect, two-celled with (two to) four pollen sacs, introrse or laterally dehiscent; tapetal cells binucleate, pollen grains two-celled at anthesis; connective with short or prominent appendage. ***Ovary*** superior, rarely semi-inferior, slightly immersed in torus, three-, rarely two-locular and rarely many ovuled; style ± thickened at base into a stylopodium, entire or trifid towards apex; stigma obscure or prominent, three- or six-lobed; ovules two (to three) in each loculus, axile, pendulous, or laterally anatropous, bitegmatic with ventral raphe and superior micropyle. ***Fruit*** indehiscent, one-seeded; with woody pericarp and persistent ± aliform sepals; seed exalbuminous; cotyledons fleshy, equal or unequal, applanate or folded or cerebriform, entire or laciniate; radicle directed toward hilum, usually included between cotyledons. ***Germination*** epigeal or hypogeal; pericarp splitting irregularly or along three sutures."

The family Dipterocarpaceae *sensu lato* comprises three subfamilies with, depending on different taxonomies, 15, 16 or 19 genera and 470 to 580 species (or more) which spread across tropical regions of Africa, Asia, and South America. The subfamily Monotoideae presents in Africa and South America with 3 genera and about 40 species, Pakaraimoideae in South America with 1 genus and 1 species, and the largest subfamily, Dipterocarpoideae, in Asia with 13 genera and about 470 species (see Figure 1) (Bancroft 1935; Ashton 1982; Maury-Lechon and Curtet 1998; Symington 2004).

The family Dipterocarpaceae presumably evolved and migrated from the supercontinent Gonwana (100-500 millions years ago) and fully developed during the Tertiary (15-70 millions years ago) (Meijer and Wood 1964). A recent genetic study based on the analysis of *rbc*L sequences (Dayanandan et al. 1999) supported the hypotheses that the Monotoideae can not be separated to form a new family which Kostermans stated in 1978, and that Asian dipterocarps shared a common ancestor with the Sarcolaenaeae, a endemic tree family of Madagascar. The geographical origin of this family was discussed in detail by Ashton (1982) and Maury-Lechon and Curtet (1998).

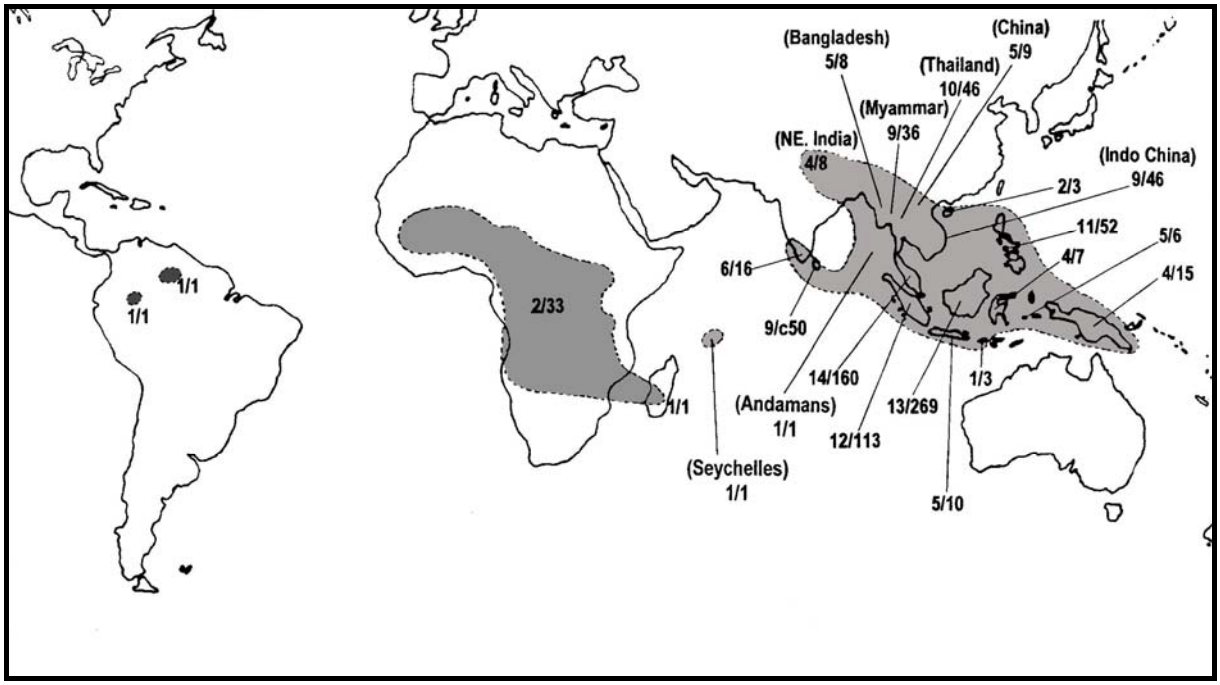

Figure 1. World distribution of Dipterocarpaceae. The first figure, before the slash, indicates the number of genera; and the second, after the slash, the number of species in the area (Symington 2004).

I.2. Subfamily Dipterocarpoideae

The Dipterocarpaceae *sensu stricto* or subfamily Dipterocarpoideae is restricted to Asia. It is the most species-rich subfamily and distinguished from other subfamilies by their resin canals, which are abundant in many tissues, and by details of wood anatomy, pollen, and stamens. Fruit dispersal is by wind or more often by gravity (Ashton 1988).

All the Asian dipterocarps are small or large, resinous, usually evergreen trees, often buttressed and usually developing scaly or fissured bark on large trees, with alternate simple leaves, indistinct leaf venation, stipules large or small, 1-2 layered hypodermis, rays multiserate or mixed uni/multiserate, intercellular resin canals, inflorescence in panicles usually with flowers secund, five-merous (Maury-Lechon and Curtet 1998). Figure 2 shows the shapes of two species of genus *Dipterocarpus*, one of the largest genera of Dipterocarpoideae, namely *Dipterocarpus alatus* and *D. tuberculatus*, respectively.

Figure 2. *Dipterocarpus alatus* (left) and *Dipterocarpus tuberculatus* (right) in Vietnam

The main achitecture models of this subfamily belong to three forms. The Roux's model, which is characterized by continuous growth of stem with plagiotropic branches, is characteristic for many genera such as *Anisoptera, Balanocarpus, Hopea,* most *Shorea, Upuna* and *Vatica*. Rauh's model, a rhythmic growth of the stem with orthotrophic branches, is frequent in *Dipterocarpus* and *Cotylelobium*. While Massart's model, a rhythmic growth of stem with plagiotrophic branches, is observed in some *Shorea* and *Dipterocarpus* species (Halle et al. 1978; Halle 1979).

The family is distributed in the Seychelles, Sri Lanka, India, Southeast Asia to New Guinea, but mostly in Malesia, where it forms the dominant lowland forests (Ashton 1982). The centre of species diversity of this subfamily is in Borneo and surrounding regions where it presumably invaded through Asia by the way of the Indian fragment of the Gondwanaland continent (Ashton 1982, 1988; Maury-Lechon and Curtet 1998). The distribution of Asian dipterocarps is limited by altitude because of the climatic enviromental features and by other natural barriers as large rivers and watersheds (Symington 1943).

The tribes and genera of this subfamily are grouped in different ways as shown in Table 1. A diversity of opinions still exists for generic levels such as in genus *Shorea, Vatica* and *Cotylelobium*, while other genera (*Dryobalanops, Dipterocarpus, Anisoptera* and *Upuna*) are well defined (Maury-Lechon and Curtet 1998).

Ashton (1982) divided the Dipterocarpoideae into the two tribes Dipterocarpeae and Shoreae based on many differences in details of the fruit calyx and wood anatomy. This subfamily comprises 12 genera in Asia and one genus endemic to the Seychelles with around 470 species and at least 100 intraspecific entities. Tribes are also distinguished based on the basic chromosome number which x= 11 for tribe Dipterocarpeae and x= 7 for tribe Shoreae (Jong and Kaur 1979, Somego 1978).

Tribe Dipterocarpeae (or valvate-Dipterocarpi group) consists of 8 genera: *Anisoptera, Cotylelobium, Dipterocarpus, Stemonoporus, Upuna, Vateria, Vateriopsis, Vatica*, which are charaterized by valvate fruit sepals, solitary vessels, scattered resin canals, and a basic chromosome number of x= 11. While the tribe Shoreae (or imbricate-Shoreae group) consists of *Dryobalanops, Hopea, Neobalanocarpus, Parashorea, Shorea* with imbricate fruit sepals, grouped vessels, resin canals in tangential bands, and a basic chromosome number of x= 7.

Dipterocarps in Asia often flower massively and synchronously (Sakai 2002; Kishimoto-Yamada and Itioka 2008). The flowering time in non-seasonal regions does not appear annually, and only extends over a short time of 2-3.5 weeks. The usual time of flowering is from March to April and fruits ripen from September to October (Wood 1956; Chan and Appanah 1980; Ashton et al. 1988). Dipterocarps have bisexual flowers pollinated by a wide variety of insects (Appanah and Chan 1981; Sakai et al. 1999, Bawa 1998). Fruit dispersal can be by wind (fruits with wing-like sepals, see Figure 3) or water (without wing-like sepals or short sepals) supporting their expansion over long distances (Ashton 1982; Bawa 1998). Ashton (1982) reviewed and concluded that the reproductive biology of subfamily Dipterocarpoideae differs in distinct forest formations where they occur. Figure 3 shows some fruits and flowers of dipterocarps.