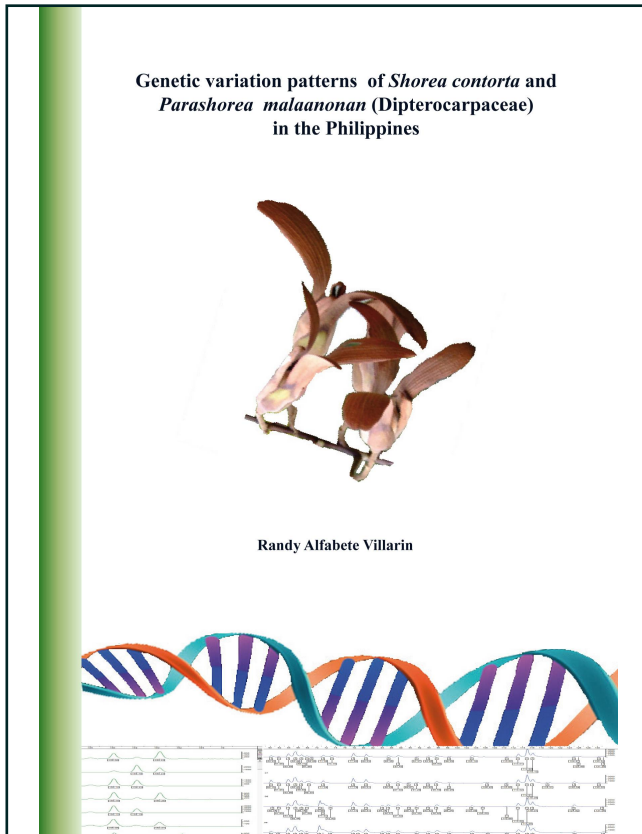




Randy Alfabete Villarín (Autor)

# Genetic variation patterns of *Shorea contorta* and *Parashorea malaanonan* (Dipterocarpaceae) in the Philippines



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## 1 GENERAL INTRODUCTION

The tropical rainforests in Southeast Asia are globally significant and considered to be among the most diverse forests in the world harboring a large proportion of the world's biodiversity (Myers *et al.*, 2000; Davies *et al.*, 2005). The tree communities in this ecosystem are dominated by a species-rich family, the Dipterocarpaceae (Ashton, 1982; Proctor *et al.*, 1983; Newman *et al.*, 1996, 1998; Brearley *et al.*, 2004; Slik *et al.*, 2009). This family is one of the most well-known trees in the tropics (Appanah and Turnbull, 1998) and the species are considered to be most important both from the ecological and economic points of view (Lamprecht, 1989). Dipterocarps occur in the lowland forest with up to 80% of the emergent individuals and 40% understorey trees (Ashton, 1982). In the Philippines, they are mostly found in groups with other species occurring in relatively dense stands (Lomibao, 1973).

During the past century, however, the Philippine forests were vastly denuded due to logging, fuel wood gathering and charcoal making, shifting cultivation and permanent agriculture (Kummer, 1992; FMB-DENR, 1999). There was a rapid loss of the forests from 21M hectare in 1900 to less than 6M hectares in 1996 (DENR, 2002), a scenario considered to be one of the most severe forest destructions in the world (Heaney, 1998). Consequently, dipterocarp populations also decreased. In 1996, for instance, the old growth dipterocarps accounted to 0.8M hectares (DENR, 2002) from 4.99M hectares in 1970 (FAO, 1981a). Such alarming forest loss had increased forest rehabilitation programs from the government and non-government sectors. One of the biggest forest restoration activities happened from 1987 to 1995 through the National Forestation Program, which targeted to reforest 1.4M hectares (Magno, 1994). Another project was based on a Community-Based Forest Management strategy in which the communities were contracted by the government for reforestation and have legal rights to occupy the developed area (Pulhin *et al.*, 2006). A different technology was also introduced, the rainforestation program (Margraf and Milan, 2006), promoting native species in reforestation and thus mimicking a natural rainforest ecosystem. These efforts and the continuous concern for forest rehabilitation accounted for 352,000 ha of plantation forests in the country in 2010 (FAO-FRA, 2011).

*Shorea contorta* and *Parashorea malaanonan* are two of the most dominant native forest tree species used in reforestation. The species are just two of the 45 Dipterocarpaceae species in the Philippines (Ashton, 1982). They grow at most 60 meters in height and could reach up to 2 meters in dbh (De Guzman *et al.*, 1986). Both species are the most common and



widespread emergents of the lowland dipterocarp forest in the Philippines and grow naturally up to 1300 meters altitude (De Guzman *et al.*, 1986; SWCF, 2009). During the early years of the last century, the species were economically important in the country and their timbers were commercially known in the international trade (De Guzman *et al.*, 1986).

This investigation is the first comprehensive population genetic study for dipterocarps of the Philippines, specifically for the two mentioned species. An earlier study conducted by Huesing (2007) for the existing plantations in the Central Philippines revealed nursery owners to have only little awareness or knowledge of genetic requirements of a tree plantation. It is likely that reproductive material used in plantations comes from few, if not single, mother trees. Hence, it could be expected that the genetic structure of planted populations differs strongly from natural populations. Planted populations may exhibit reduced levels of genetic variation and low adapted potentials compared to natural populations.

Knowing variation patterns in planted and natural populations can provide useful data for better planning actions for reinforcement of existing populations, re-introduction, *ex situ* collection or seed collection. Genetic information at planted populations would reveal information on important alleles that were lost in the natural habitat (natural populations). The genetic variation determines the evolutionary potential of trees in populations (Siregar, 2000; Finkeldey and Hattermer, 2007; White *et al.*, 2007; Lundqvist *et al.*, 2008) and the maintenance of adequate genetic variation within and among populations is necessary to accommodate new selection pressures brought about by environmental changes (Ledig, 1988). Populations with high genetic diversity have high chances for a long term survival (Gauli *et al.*, 2009) and those with low level of genetic variation are unlikely to adapt to drastic environmental changes and are more susceptible to become extinct (Boshier and Amaral, 2004). In conclusion, genetic variation is crucial for the long term stability of forest ecosystems (Sharma *et al.*, 2002).



## 2 OBJECTIVES OF THE STUDY

The main objectives of this study were (a) to determine genetic variation within natural populations of *S. contorta* and *P. malaanonan* and to compare results with variation patterns in planted populations; and (b) to assess the geographic patterns of genetic variation within and among natural populations of *S. contorta* and *P. malaanonan*. Genetic variation patterns in populations were further compared at two marker systems, the microsatellites (Simple Sequence Repeats: SSRs) and Amplified Fragment Length Polymorphisms (AFLPs).

Specifically, this study aimed to:

- transfer SSRs developed for *S. leprosula* and *S. curtisii* to *S. contorta* and *P. malaanonan*;
- determine and compare genetic diversity levels within and among (a) Northern and Central populations; and (b) natural and planted populations;
- determine genetic relationships among (a) Northern and Central populations; and (b) natural and planted populations; and
- conduct a comparative analyses between SSRs and AFLPs in *S. contorta* and *P. malaanonan* populations.



### 3 REVIEW OF RELATED LITERATURE

#### 3.1 State of the Philippine Forest

The Philippines is among the few countries in the world that was originally almost completely covered by rainforests (Schulte, 2002). The high biodiversity and endemism in the region had made it into one of the mega diverse countries of the world (Myers, 1988; McNeely *et al.*, 1990; Mittermeier *et al.*, 1999; Myers *et al.*, 2000). In terms of floral composition, the country ranks 23<sup>rd</sup> as most plant species-rich globally and at 7<sup>th</sup> in the Asian region ([www.pnh.com.ph/category/4-Articles/5-Biodiversity-in-the-Philippines-page-1.htm](http://www.pnh.com.ph/category/4-Articles/5-Biodiversity-in-the-Philippines-page-1.htm), 20-11-2012). The Philippine forest harbors about 13000 species of plants, which comprise 5% of the world's total forest (DENR-UNEP, 1997). Further, there were about 3500 species of trees indigenous to the Philippines (Salvosa, 1963) and about 10% of these have economic importance as a timber source (Meniado *et al.*, 1974).

In the beginning of the last century, almost 70% of the country was covered by forests that had vastly reduced to almost 18% in 1999 (Figure 1; ESSC, 1999). In 1972, the actual forest cover was 15.6M ha (BFD, 1972) and had declined to only 5.4M ha in 1997 (FMB, 1997). This remaining forest cover was not purely dominated by timber trees but a mix of different forest types and land uses (Table 1). On the other hand, the recent global forest resource assessment of FAO reported that the extent of forest and other wooded land in the country has been 7.665M ha and 10.128M ha, respectively (FAO-FRA, 2011). This accounted to a percent land area of 26% and 34%, accordingly. Based on forest management and legal status, 1.804M ha and 2.250M ha were forests within protected areas and with management plan, respectively.

The trends in the extent of forests in the country from 1990 to 2010 showed that it increased to at most 0.8% change rate annually. From 1990 to 2000, its annual change rate was 0.8% while 0.76% and 0.73% in 2000-2005 and 2005-2010, respectively (FAO-FRA, 2011).

Table 1. Area of forest types and land uses in the Philippines in 1997.

| Land use/ Forest type                                 | Area (hectares) | % total area |
|---|-----------------|--------------|
| <i>Forest</i>   | 5, 391, 717     | 17.9         |
| Dipterocarp   | 3, 536, 017     | 11.8         |
| Old- growth   | 804,000         | 2.7          |
| Residual  | 2, 731, 117     | 9.1          |
| Pine  | 227, 900        | 0.7          |
| Submarginal(include limestone and ultramafic forests) | 475, 100        | 1.5          |
| Mossy   | 1, 040, 300     | 3.4          |
| Mangrove  | 112, 400        | 0.3          |
| <i>Brushland</i>                                      | 2, 232, 300     | 7.4          |
| <i>Other land use</i>                                 | 22, 375, 983    | 74.6         |

Source: DENR-UNEP, 1997 (cited by Fernando, 2001)

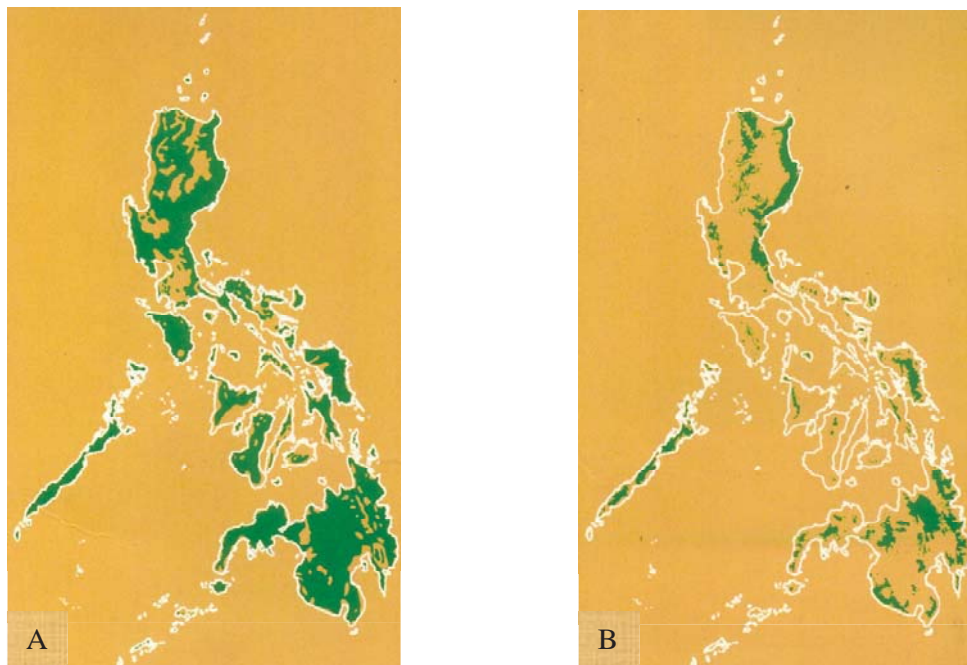


Fig. 1 Forest cover in the country indicating its extent from 1900 (A) to 1999 (B).

Source: ESSC, 1999

### 3.2 Reforestation efforts in the Philippines

The rapid depletion of the forests in the country from the last century (FMB, 1997; ESSC, 1999; DENR, 2002; FAO-FRA, 2011) was considered to be one of the most severe forest destructions in the world (Heaney, 1998). The vast denudation was due to logging, fuel wood gathering and charcoal making, shifting cultivation and permanent agriculture (Fig. 2; Kummer, 1992; FMB-DENR, 1999). It was reported that there were about 20M Filipinos living in upland watershed areas and half of whom were dependent on shifting cultivation for their livelihood (Cruz and Zosa-Feranil, 1998). These activities had contributed to 13M ha of open grassland, of which 5.2M ha need immediate rehabilitation (FAO, 2005). With regard to deforestation rate, the average was 170,000 ha per year from 1969 to 1973 (FDC, 1987) and was expected to increase to about 190,000 to 200,000 ha in the next 20 years (Revilla, 1997). With continued deforestation, the country has been considered as one of the hotspots or areas of concern in the world (McNeely *et al.*, 1990; Myers *et al.*, 2000).



Fig. 2. Fresh illegally-cut dipterocarp in Leyte (A) and the on-going kaingin in Samar (B).  
Source: For. D. Peque (A); Dr. I. Navarrete (B).

To address the deforestation, the government promoted reforestation activities to rehabilitate the degraded areas. Reforestation activities started since 1910 through various projects initiated by the government (Pulhin *et al.*, 2006) but success was poorly seen (Esteban, 2003). Poor species-site matching, inadequate monitoring, corruption and social conflicts were some of the major reasons for such failure (Carandang and Cardenas, 1991; Carandang and Lasco, 1998; Lasco *et al.*, 2001).

In the late 1980s, one of the biggest reforestation activities in the country was the National Forestation Program, in 1987 to 1995, which targeted to reforest 1.4M ha (Magno,

1994). Another initiative started in 1995 through Executive Order 363. This had implemented the national strategy to ensure the sustainable development of the country's forests by a Community-Based Forest Management strategy (Pulhin *et al.*, 2006). It was observed that reforestation used limited number of species, of which 80% were introduced (Pulhin *et al.*, 2006). In the mid 1990s, rainforestation as a scheme to forest rehabilitation was introduced in Leyte. The strategy is mimicking the function of the natural rainforest with social considerations. The activity advocated the use of native species for reforestation and promoted a mixture of fruit and forest trees and agricultural crops like pineapple (Fig. 3). The strategy aimed to restore degraded areas and farms planted with old coconut stands through a highly diverse and economically future-oriented and sustainable tree farm (Margraf and Milan, 2006 as cited by Come, 2010).

The continuous efforts for reforestation in the country had accounted to 352,000 ha of planted forests in 2010 that corresponded to 5% total forest area. It was an increase from 302 000 ha in 1990, 327 000 ha in 2000, and 340 000 ha in 2005. Accordingly, the annual change rates of planted forests were 0.80% from 1990 to 2000, 0.78% from 2000 to 2005, and 0.70% from 2005 to 2010. The report further indicated that 99% of trees in plantations were introduced species (FAO-FRA, 2011).



Fig. 3. One of the fruit trees (A: *Durio zibethinus*) and agricultural crops (B: *Ananas comosus*) planted in rainforestation areas.





### 3.3 The investigated species

#### 3.3.1 Dipterocarpaceae

Blume (1825), Ashton (1982, 1988), Marguire and Ashton (1977) and Vercourt (1989) described species of the family as: **trees** small or large, resinous, usually evergreen and buttressed; **branchlets** with stipular scars, sometimes annular; **leaves** alternate, simple, margin entire or sinuate, not crenate, terminating somewhat abruptly at the usually prominent geniculate petiole, penninerved (in *Dryobalanops* and some *Hopea* nerves indefinitely numerous, dense and slender), often with domatia in axils between nerves and midrib or along midrib and (rarely) nerves; tertiary nerves scalariform or reticulate; **stipules** paired, large or small, persistent or fugacious, leaving small to amplexicaul scars; **inflorescence** paniculate, racemose, rarely cymose, somewhat regularly, rarely irregularly, branched, terminal or axillary; bracts and bracteoles paired, small or large, persistent or fugacious; **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 usually inclosing the fruit, adnate to or free from it; **corolla** five-merous contorted, base connate or free, usually partially or entirely unicellular hairy; **stamens** five to 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 or semi inferior, three to rarely two-locular; **style** usually thickened at the 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, bitegmic with ventral raphe and superior micropyle; **fruits** indehiscent, one-seeded; with woody pericarp and persistent usually aliform sepals; embryosac development of Polygonum type: endosperm of the nuclear type, embryo normal, ripe seeds with or more usually without endosperm; cotyledons equal or more usually unequal and with one more or less enclosing the other, laminar or fleshy, entire or lobed, enclosing the radical; **germination** epigeal or hypogeal; pericarp splitting irregularly or along three sutures.

Dipterocarps usually begin to flower and bear good seeds before their 30<sup>th</sup> year. The age range of the first flowering is 17 to 36 years. In evergreen forests of the central tropics,

flowering time is irregular with a mass production in a short 2-3.5 weeks flowering period (Ashton *et al.*, 1988, Chan and Appanah, 1980). Further, flowering is not simultaneous with other dipterocarps (LaFrankie and Chan, 1991) and also varies in different elevations (Sasaki *et al.*, 1979). Dipterocarps are pollinated by different insect vectors (Appanah, 1981; Appanah and Chan, 1981; Corlett, 2004; Momose *et al.*, 1998; Sakai *et al.*, 1999a). Once developed into seeds, they are short-lived and easily lose their viability, often within three weeks after dispersal. The seeds are usually shed off toward the start of the wet season and germinate three days to two weeks after shedding whether or not they reach the ground. Heavy seed years occur at infrequent intervals normally from five to seven years, rare extreme intervals are two to twenty years, and a few species seed annually (Revilla, 1976; Ng, 1966; Nicholson, 1958; 1960; Wyatt-Smith, 1952 as cited by De Guzman *et al.*, 1981). During fruiting years, the seeds germinating on the ground form a dense mat of seedlings. The seedlings under the forest canopy can tolerate shade and may not show significant growth for years but are capable to shoot up to the canopy when light becomes available as gaps are formed (Whitmore, 1990).



Fig. 4. Physiognomy of a mature *S. contorta* with an almost 2m dbh (A) and a long straight bole (B) in a primary forest in Leyte. Source: For. D. Peque



### 3.3.2 The Philippine dipterocarps

The family Dipterocarpaceae in the country consisted of six genera and 45 species, of which about 47% can only be found within the country (Ashton, 1982). There was, however, a proposal suggesting that the family should be composed of 56 species, with seven species described for the first time as new to science (Gutierrez, 1980 as cited by De Guzman *et al.*, 1981). The dipterocarps of the Philippines are mostly medium-to-large-sized trees, unbranched to a considerable height and usually attain a height of 40-65 meters and a diameter at breast height (DBH) or diameter above buttress (dab) of 60-150 centimeters. Few unusually large trees are reported to attain DBH as large as 300 centimeters. The boles are generally straight and regular (Whitford, 1911; Tamesis and Aguilar, 1951 as cited by De Guzman *et al.*, 1981).

In the Philippines, almost all of the species can be found in the lowland and lower hill dipterocarp forests below 400 meters. The best dipterocarp forests are found along the eastern side of the archipelago where rainfall is highest and evenly distributed throughout the year. The largest blocks of dipterocarp forest occur in the eastern portion of Mindanao. In the Visayas only the island of Samar still has considerable areas under rainforest. Dipterocarps in the island of Palawan are different from those in the rest of the archipelago. A major portion of the forest cover consists of a stunted forest with diameters too small for utilization. It is also noticeable that there is an absence of the entire genus of *Shorea* in the island. In the western sections of the archipelago, the climate is generally drier with a more pronounced dry season. So here, the rainforest is less well-developed and shifting cultivation led to the formation of extensive areas with *Imperata cylindrica* grass, interspersed with some hardy second growth vegetation and only vestiges of the original forest vegetation remain along some creeks (Weidelt and Banaag, 1982).

### 3.3.3 Economic and ecological significance

Dipterocarps are the most important commercial timber species in Southeast Asia. The species account for 80% of timber exports from this region, which provided major source of revenue for the developing countries of the region. The round-wood logs of the species formed 25% of global consumption of tropical hardwood, which was valued at most half a billion US\$ between 2006 and 2007 (ITTO, 2008). In the Philippines, dipterocarps once produced the greatest bulk of commercial wood sold worldwide under the trade name "Philippine Mahogany". It contributed much to the country's log export and provided wood



not only for furniture making but also for local construction in buildings and other infrastructure development. The world famous “Philippine Mahogany” also represented the most important of all the trees found in the Philippine forest (Gavine, 1997).

*Shorea* is the largest and economically most important genus in this family. About 15 species of the Dipterocarpaceae, mainly *Shorea*, produce a nut with an edible fat, similar to that of cocoa, and an excellent substitute for cocoa butter in the manufacture of chocolates and cosmetics (Seibert, 1996). Other non-timber products like nuts and resins (Shiva and Jantan, 1998) have also contributed to the species economic importance.

Ecologically, dipterocarps are mutually associated with mycorrhiza (Lee, 2006; Lee *et al.*, 2008). Mycorrhiza extends roots’ nutrient depletion zones especially for poorly mobile inorganic nutrients like phosphorus that are at low concentrations in many tropical soils (Proctor *et al.*, 1983; Brearley, 2003; Brearley *et al.*, 2004; Paoli *et al.*, 2006). The Southeast Asian rainforests, where these dipterocarps dominated, provided numerous ecosystem services including fresh water management, soil protection and pollination services, maintenance of the region’s biodiversity and the global function as carbon storage (Barlow *et al.*, 2007; Meijaard and Sheil, 2010; Berry *et al.*, 2010; Kettle, 2010).

### **3.3.4 Management system**

In the Philippines, a special system of forest management based on the concept of sustained yield is applied to dipterocarp forest. The system was implemented as early as 1954, which made the country being the first to practice forest management in Southeast Asia (Lasco *et al.*, 2001). The Philippine Selective Logging System (PSLS) aims to retain an adequate number of undamaged and healthy residuals to be utilized in the next cutting cycle or later (Siapno, 1970). PSLS follows four phases that include determination of marking goal, tree marking, residual inventory and timber stand improvement.

PSLS was practiced limitedly since the proclamation of the total log ban in the country’s primary forests in 1992. The system has been recently unused with the implementation of the Executive Order No 23 s. 2011, which proclaimed a total log ban in all forest types (<http://www.gov.ph/2011/02/01/executive-order-no-23-4/> , 20-11-2012).

### 3.3.5 *Shorea contorta* and *Parashorea malaanonan*

#### 3.3.5.1 Distinguishing features

*Parashorea* species were argued to be included within the genus *Shorea* (Symington, 1943) because of their trait similarities. Ashton (1982) suggested a separate classification due to distinct characters between species of the two genera. In the case of *S. contorta* and *P. malaanonan*, distinguishing features can be observed. *S. contorta* has an unequal leaf base with nerves from seven to nine pairs. The leaves vary greatly in size and increase from seasonal to ever wet habitat. *P. malaanonan* has stipules that are deciduous, semi-amplexicaul, and are leaving annular scars. Leaves are glabrous on the under surface, in length about 15 cm. The style is long, exceeding the sepals (De Guzman *et al.*, 1981). Further, *Parashorea* has plicate seedling leaves and globose or verrucose with lenticellate fruit nut (Ashton, 1982).



Fig. 5. Leaf and fruit differences between *S. contorta* (A) and *P. malaanonan* (B).  
coin scale: 2cm; Source: SWCF, 2009



### 3.3.5.2 Habitat

*S. contorta* grows naturally in lowland forests from sea level to about 700 m (De Guzman *et al.*, 1981) while *P. malaanonan* could be found up to 1300 m altitude (SWCF, 2009). Both species were observed to co-occur in most of the natural populations where the samples of this study were collected.

### 3.3.5.3 Phenology

*S. contorta* was observed to flower in March (Laguna, Palawan), April (Laguna) and May (Quezon) while *P. malaanonan* flowers from February to April and is fruiting in May (De Guzman *et al.*, 1981). During collection of leaf samples for this study, all natural populations of both species were not flowering (December 2009 to March in 2010: Central Philippines; January to March 2011: Northern region). Wildlings of the species, however, were observed in both regions.

### 3.3.5.4 Distribution

*S. contorta* is geographically limited within the Philippines. The species is distributed in many provinces of Luzon (Northern Region) from Cagayan to Sorsogon. It is also found in Babuyan, Polillo, Marinduque, Masbate, Samar, Leyte, Negros, and Sibuyan (Central Region). In Mindanao (Southern Region), the species is found in Samal, Polau and Basilan. *P. malaanonan*, on the other hand, is found in many areas of Luzon including Nueva Ecija, Bulacan, Laguna, Quezon, Camarines, Albay and Sorsogon. It is also found in Polillo, Catanduanes, Samar, Leyte, Masbate, Panay and Negros. In the Southern Philippines, it occurs in Surigao, Agusan, Bukidnon, Davao, Zamboanga and Basilan. Unlike the former species, the latter is also growing in Borneo (De Guzman *et al.*, 1981).

### 3.3.5.5 Economic importance and ecological status

*S. contorta* and *P. malaanonan* belong to the “Philippine Mahogany” group specifically categorized as “Light Red Mahogany” (De Guzman *et al.*, 1981). Presently, the two species are depleted and have been considered by the IUCN (2011) as critically endangered. This means that almost 80% of them were already gone in the natural forests.



### 3.4 Genetic investigations in Philippine dipterocarps

Genetic investigations conducted for forest tree resources in the Philippines are scarce. Concerning dipterocarps, a first study aimed to develop a molecular phylogeny by Villarin (2007). The study represented 17 species of six genera of Philippine dipterocarps. Its results were generally consistent with species traditional classification by Ashton (1982). The next study was a cross-amplification for *Shorea* SSRs in *P. malaanonan* based on the interpretation of banding patterns from agarose gel electrophoresis (Abasolo *et al.*, 2009). Four polymorphic *Shorea* SSRs (*Shc04*, *Shc07*, *Shc11* and *Sle290*: Ujino *et al.*, 1998; Lee *et al.*, 2004) were initially tested for genetic diversity in nine individuals.

Further studies were conducted in which few of the Philippine dipterocarps (*Dipterocarpus kerrii*, *Hopea plagata*, *P. malaanonan*, *S. almon*, and *S. contorta*) were included in the study of Rachmayanti (2009). This was an initiative to develop tools to identify dipterocarp wood of different origins by investigating informative DNA markers. Nguyen (2009) included *Anisoptera aurea*, *D. caudatus*, *D. kerrii*, *D. validus* and *Vatica mangachapoi* in his study about the molecular phylogeny of dipterocarps based on non-coding sequence data of chloroplast and nuclear DNA. The study showed a clustering of the Philippine dipterocarps specific to its genera including other species from Indonesia and Vietnam. Just recently, an investigation on genetic variation of *S. guiso* populations (Estoque, 2011) compared genetic diversity between planted and natural populations of the species using microsatellite markers. So far, this present study has been the most comprehensive investigation for population genetics of Philippine dipterocarps in which samples represented populations extending from the Northern to the Central part of the country.

### 3.5 Transferability of SSRs in dipterocarps

The development of microsatellites or simple sequence repeats (SSRs) is comparatively expensive and time-consuming. SSRs are mostly species-specific markers (Chabane *et al.*, 2005). Hence, a simple but informative and cost efficient technique is to cross-amplify or transfer SSR markers from closely related taxa. Some SSRs showed high rates of transferability across species (Gaitán-Solís *et al.*, 2002; Saha *et al.*, 2004, Akkak *et al.*, 2009; Kaneko *et al.*, 2011). They are often transferable among closely related species because of the conservation of DNA sequences within the flanking regions of phylogenetically related taxa (Kijas *et al.*, 1995; Dayanandan *et al.*, 1997). Successful cross-



amplification have been reported in many plants, from short-lived agronomic crops and grasses (e.g., Hempel and Peakall, 2003; Kuleung *et al.*, 2004; Gao *et al.*, 2005; Vigna *et al.*, 2011) to long-lived timber species (e.g., Isagi and Suhandono, 1997; Gozalez-Martinez *et al.*, 2004; Souza *et al.*, 2009).

Transferability of SSRs within Dipterocarpaceae species has been reported by many scientists. SSRs were developed for *S. cordifolia* (Stacy *et al.*, 2001), *S. curtisii* (Ujino *et al.*, 1998), *S. laevis* (Masuda *et al.*, 2010), *S. leprosula* (Lee *et al.*, 2004a; Ng *et al.*, 2009; Ohtani *et al.*, 2012), *S. platyclados* (Ng *et al.*, 2009), *Hopea bilitonensis* (Lee *et al.*, 2004b), *Neobalanocarpus heimii* (Iwata *et al.*, 2000) and *Dryobananops aromatica* (Nanami *et al.*, 2007) and have been successfully cross-amplified to *S. maxwelliana* (Masuda *et al.*, 2010), *S. megistophylla* (Stacy *et al.*, 2001), *S. parvifolia* (Lee *et al.*, 2004a), *S. robusta* (Pandey and Geburek, 2009) and to other species of different genera of the family (Ujino *et al.*, 1998; Ng *et al.*, 2009). In this study, we successfully transferred SSRs developed for *S. leprosula* (Ng *et al.*, 2009) and *S. curtisii* (Ujino *et al.*, 1998) to *S. contorta* and *P. malaanonan* (Manuscript 1: Appendix 1).

### **3.6 Applicability of SSR and AFLP markers in dipterocarps**

Microsatellites as molecular markers based on sequence repeat motifs in the DNA are also named simple sequence repeats (SSRs). They consist of multiple repeats of short sequences (tandem repeats) of one to six bases length (Litt and Luty, 1989; Tautz, 1989; Hancock, 1999). These markers are often used in genetic studies because of their codominant mode of inheritance with a high degree of polymorphism (Litt and Luty, 1989; Weber and May, 1989; Karp *et al.*, 1997), which result from unusually high mutation rates for the nucleotide sequences (Peakall *et al.*, 1998), replication slippage and possibly unequal crossing-over events (Schlotterer and Tautz 1992; Valdes *et al.*, 1993). In addition, SSRs have high genomic abundance both in coding and non-coding regions (Hokanson *et al.*, 1998; Hancock, 1999), with high reproducibility and can be easily amplified by polymerase chain reaction (Byrne *et al.*, 1996; Weising *et al.*, 2005), sometimes even within a highly degraded or ancient DNA (Morgante and Olivieri, 1993). With such various advantages, these markers have been used in a wide array of genetic investigations like estimation of effective population size (e.g., Allen *et al.*, 1995; Stoehr *et al.*, 2008), forensic DNA (Jarne and Lagoda, 1996; Bielikova *et al.*, 2010; Ishida *et al.*, 2012), genetic diversity (e.g., Tahernezhad *et al.*, 2009; Eliades *et al.*, 2011), gene flow and mating system (e.g., Gaiotto, 2003; Imbert





and Lefevre, 2003; Shimono *et al.*, 2011; Feres *et al.*, 2012), genetic variation patterns in populations (e.g., Garcia *et al.*, 2004; Stefanon *et al.*, 2008, Gauli *et al.*, 2009; Eliades *et al.*, 2011), genome mapping and QTL analyses (e.g., Knapik *et al.*, 1998; Chagné *et al.*, 2003; Portis *et al.*, 2012), paternity and kinship analysis (e.g., Queller *et al.*, 1993; Malik *et al.*, 2011, Feres *et al.*, 2012), and phylogenetics (e.g., Thomas *et al.*, 2003; Stenson *et al.*, 2004; Neophytou *et al.*, 2008; Wang *et al.*, 2011).

The Amplified Fragment Length Polymorphism (AFLP) technique is based on the detection of restriction fragments by PCR amplification and the electrophoretic separation of fragments. This marker system was first described by Vos *et al.* in 1995. Unlike SSRs, AFLPs are only dominant markers but have the capacity for the simultaneous screening of many different DNA regions distributed randomly throughout the genome (Müller and Wolfenbarger, 1999). Another advantage of this marker is its applicability even without prior sequence information of a species. This allowed to explore genetic diversity in different organisms, from microbes (Szaluś-Jordano *et al.*, 2012; Ngadze *et al.*, 2012) to higher forms of animals (Huang *et al.*, 2009; Yunguo *et al.*, 2011) and from cultivated short-lived crops (Dong *et al.*, 2000; Culumber *et al.*, 2011; Rampino *et al.*, 2011) to long-lived trees (Cao *et al.*, 2006; Semerikova and Semerikov, 2011).

AFLPs have proven to be useful in assessing genetic variation and taxonomic relatedness in plant species (Winfield *et al.*, 1998; Cao *et al.*, 2006; Miller and Schaal, 2006; Emshwiller *et al.*, 2009; Culumber *et al.*, 2011). The markers have also been used in pathotyping (Wang *et al.*, 2010), kinship (Soleimani *et al.*, 2002), linkage (Dong *et al.*, 2000; Ipek *et al.*, 2005) and QTL analyses (Liu *et al.*, 2010).

In genetic studies for dipterocarps, both markers had already been applied. SSR was used to estimate gene flow in *N. heimii*, inferred from paternity analysis (Konuma *et al.*, 2000). In the same species, the marker was used to assess the degree of selfing and inbreeding depression at the seed and seedling stages (Naito *et al.*, 2005). SSR was also used to investigate genetic variation in *D. aromatica* Gaerfn. F. in Peninsular Malaysia (Lim *et al.*, 2002) and in determining spatial structure and genetic diversity of *S. curtisii*, *S. leprosula*, *S. macroptera* and *S. ovalis* (Ng *et al.*, 2004; 2006). The breeding system and seed dispersal in *S. leprosula* (Nagamitsu *et al.*, 2001) and the genetic diversity in undisturbed and selectively logged forests of *S. curtisii* (Obayashi *et al.*, 2002) were also inferred based on SSRs. Pandey and Geburek (2010) applied the marker to determine genetic differences between continuous and disjunct populations of *S. robusta* in Nepal. In *S. platyclados*, full-sib families were



detected by paternity analyses based on SSRs (Ng *et al.*, 2011). The marker also revealed genetic variation patterns in populations of *S. guiso* (Estoque, 2011).

AFLP markers were already applied to investigate the genetic variation and mating system in *Dipterocarpus* cf. *condorensis* (Luu, 2005). In *S. leprosula* and *S. parvifolia*, it was used to determine genetic diversity within and among populations (Cao *et al.*, 2006). The markers were also used to investigate genetic variation of Indonesian dipterocarps and the relationships of the species in relevance to molecular phylogenies and taxonomic subdivisions (Cao *et al.*, 2006; 2009).