specimens from Sulawesi that stored in BO have been identified. Therefore, systematic studies to reveal the Musaceae diversity in Sulawesi need to be done.

The aims of the study were 1) to provide information of the diversity and the distribution of wild banana species in Sulawesi, 2) to provide species description and an identification key and 3) to determine the phylogenetic relationship between the species using morphological characters and ITS region of nrDNA sequences.

2 LITERATURE REVIEW

Taxonomy of Musa L.

APG III (Angiospermae Phylogeny Group) places genus Musa L., together with Ensete Bruce ex Horan and Musella (Franchet) H.W. Li into family Musaceae, the member of Zingeberales (APG 2009). Genus Musa is the largest genus in the family that is firstly established by Linnaeus (1753). Some botanists believe that the name of genus Musa is thought to be derived from the Arabic name for the plant (mouz) which in turn may have been applied in honour of Antonius Musa who is physician to Octavius Agustus, the first emperor of Rome (Hyam and Pankhurst 1995). Whereas the name “banana” is derived from the Arabic banan that means finger (Boning 2006) and was thought to be used in Guinea (West Africa) concomitant with the introduction of the fruit by the Portuguese and then the name spread to the New World (Cheesman 1948a).

The taxonomic complexity at the family level continues down to the genus level and there are inconsistencies in the number of sections and number of species proposed for inclusion in the genus Musa. The first classification made by Sagot (1887) that divided the genus into three groups: (1) giant bananas (type: *M. ensete* J.F. Gmel.), (2) bananas with fleshy fruit and often edible (type: *M. sapientum* L.), (3) ornamental bananas with erect inflorescences and brightly coloured bracts (type: *M. coccinea* Andrews).

In 1893, Baker divided genus Musa into three subgenera: (1) *Physocaulis*, characterized by stem bottle-shaped; flowers many to a bract; petal usually tricuspidate; fruit not edible. (2) *Eumusa*, characterized by stem cylindrical; flower many to a bract; petal ovate-acuminate; bracts green, brown or dull violet; fruit edible. (3) *Rhodoclamys*, characterized by stem cylindrical; flower few to a bract; petals linear; bracts bright-coloured; fruit usually not edible.

Cheesman (1947) elevated the first subgenus to the generic level as the genus *Ensete* and then made new classification. The classification is based on the haploid number of the chromosome that followed by the similarity and the differences on morphological characters. He treated the genus into four sections as follows:

A. Chromosome number x=11

Inflorescence pendent or semi-pendent from the first, the fruits reflexing in development toward the base of the rachis. Flowers many to a bract, in two series. Bracts commonly dull coloured, green, brownish or dull
purple. Pseudostems commonly exceeding three meters high

Section Eumusa

2. Inflorescence erect, or at least at the base, so that the fruits do not reflex in development but point toward the apex of the rachis. Flowers few to a bract, usually in a single serie. Bract brightly coloured, often red. Pseudostems less than three meters high

Section Rhodochlamys

3. Chromosome number x=10

Section Australimusa

4. Seeds cylindrical, barrel-shaped, or top shaped, marked externally by transverse line or groove, above which are warted, tuberculate, or variously patterned, below usually smooth; internally a well-developed perisperm, chamber above the same line, this chamber empty in the ripe seed

Section Callimusa

Although this classification was widely accepted by most botanists, its validity has been questioned, in any case for some sections. Some newly described species which is already count the chromosome number also appeared problematic. Argent (1976) proposed to establish one more section in *Musa*, *gentimusa* (x=7) to include the single species *M. ingens* N.W. Simmonds. Based on morphological and cytological characters, Simmond and Weatherup (1990) and a very low level of consistency among the characters and suggested that section *Eumusa* is heterogeneous and then divided it into two informal subgroups "Eumusa-1" and "Eumusa-2. Recently, molecular data have been used to solve such problems in taxonomy of *Musa*. By using RFLPs data, Gawel and Jarett (1991) and Gawel et al. (1992) found that a molecular phylogeny was inconsistent with traditional classification and they proposed that section *Rhodochlamys* should be merged with section *Eumusa*. Wong et al. (2002), by using AFLP data suggested that section *Rhodochlamys* should be placed in section *Eumusa*, and section *Australimusa* should be merged in section *Callimusa*.

**General Morphology of Musa L.**

*Musa* is a large perennial herb and they grow in clump with rhizome and false areal stem, cylindrical pseudostem that consisted of sheath leaves wrapped together with each other, has a short underground stem (corm). The root system is adventitious spreading out laterally.

Leaves grow from a cigar leaf into a large, blade oblong, arranged parallel and pinnate. There are some variations on base shape of leaves: both side unded; one side rounded, one pointed; both sides pointed (Figure 1). Leaf canal margin open with margins spreading, wide with erect margin, straight with erect margins, margin curved inward or margins overlapping (Figure 2).
Inflorescence springs from the rhizome and emerges at the top of the stem, either erect or pendulous; the immature inflorescence is encased inside bracts that give the appearance of large bud. Bracts are plane or sulcate, revolute or not revolute before falling, imbricate or not imbricate (Figure 3). Flowers produce nectar, on each bract there is one or two rows of flower. Basal flower is female or hemaprodhite, consist of ovary that protected by compound tepal (calyx) and free tepal (corolla), style and staminode; compound tepal essentially tubular but split to the base on the adaxial side, 5 toothed at the apex (3-lobed at the apex with 2 accessory teeth between the main lobes; free tepal inserted within the compound tepal and opposite to it (i.e. in the adaxial position). Male flower have 5 fertile stamen that falling down with the bract. They are reduced to staminodes in female flower. Female and male flowers are morphologically indistinguishable until the inflorescence is about 12 cm long. At this point, the ovary in the male flower fails to develop any further (Simmonds 1959).
Figure 3 Variation on bract. Not imbricate (a); imbricate (b) (Nasution and Yamada 2001)

Fruits are berry, some dehiscent and some are not, with numerous seeds (except in the parthenocarpic form). Each fruit is known as a “finger”. Each cluster of fruits at node is known as a “hand” and the entire collection of hands is known as a “bunch”. The number of hands varies each others. The outer protective layer of each fruit known as the “skin” or “peel” is fusion of the hypanthium (floral receptacle) and outer layer (exocarp) of the pericarp (fruit wall derived from the ovary wall). This peel is easily removed from the fleshly pulp that originates mainly from the endocarp (innermost layer of the pericarp) (Simmonds 1953). During the development of the fruit from the ovary, the tepals, style, and staminodes abscise leaving a characteristic calloused scar at the tip of the fruit. Fruits develop only after pollination. Fruit size depends on the number of seeds and parenchymatous pulp develops around each seed. The growth volume curve is sigmoidal (Simmonds 1953). Wild banana species have little flash and is filled with black or brown seed. The seeds have linear embryos, large amounts of endosperm and a thick hard testa (Ellis et al. 1985).

Distribution of *Musa* L.

The section *Australimusa* is well-known in Brunei Darussalam, Indonesia, Malaysia, Papua New Guinea, and Philippines, while section *Callimusa* can be found in Brunei Darussalam, China, Cambodia, Indonesia, Malaysia, Papua New Guinea, and Vietnam. The biggest section, *Eumusa* is widespread in Australia, Bhutan, Cambodia, China, Eastern and SouthEast India, Indonesia, Japan, Laos, Malaysia, Papua New Guinea, Philippine, Samoa, Sri Lanka, Thailand, and Vietnam, whereas section *Rhodochlamys* grows well in Bangladesh, China, Malaysia, and Thailand (Figure 4).
Indonesia is situated in the centre of origin and diversity of *Musaceae* and has a large number of both wild banana species and cultivated bananas. The bananas widespread in Sumatra, Java, Lesser Sunda Islands, Borneo, Sulawesi, Moluccas, and Papua. Pollefeys *et al.* (2004) made distribution map of *Musa* sections in Indonesia using MGIS (*Musa* Germplasm Information System) and DIVA-GIS. Almost all of the accessions that they used come from Nasution’s (1991) study and the rest come from Musalogue. Because of insufficient geographical data for some of Nasution’s accessions, no entry is available in MGIS for Kalimantan (Borneo) although the expedition recorded wild species in all major islands (Figure 5).
Internal Transcribed Spacer (ITS)

The major ribosomal RNA (rRNA) genes of plants are localized in clusters on highly repeated sequences. Each repeat consists of sequences from 18S, 5.8S and 25S ribosomal subunits and each copy contains a transcribed region that is separated by the long non-transcribed intergenic spacer (IGS). These genes show little sequence divergence between closely related species. Within each repeat, these conserved regions are separated by internal transcribed spacer (ITS) which occur in the following order: 5′–18S–ITS-1–5.8S–ITS-2–26S(or 25S)–3′ that show higher rates of divergence (Figure 6).

![Figure 6 Organization of ITS region of nrDNA (Soltis and Soltis 1998)](image)

The internal transcribed spacers including ITS-1 and ITS-2 regions are part of the nuclear rDNA (nrDNA) transcript but are not incorporated into ribosomes. In particular, the 5.8S rRNA is separated from 18S, the SSU (small ribosomal subunit) rRNA, by the first of two ITSs (ITS-1), and from 25-28S, the LSU (large ribosomal subunit) rRNA, by the second ITS (ITS-2). Sequencing of the ITS region, however, has an exciting potential as a source of nuclear DNA characters for phylogenetic reconstruction in plants. This promise was heightened recently by encouraging results from ITS sequence-based phylogenies of protoctists (Lee and Taylor 1991), apes and humans (Gonzales et al. 1990). White et al. (1990) have taken advantage of polymerase chain reaction (PCR) technology to promote sequencing of nrDNA in fungi. Baldwin (1992) have described the usefulness of these primers for PCR amplification and sequencing of the ITS region in giosperms and also described the utility of ITS DNA sequences as a source of phylogenetic data in the subtribe Madinae of Asteraceae.

ITS regions take a role in the maturation of nuclear rRNAs, bring ITS of S–26S nrDNA have a particularly valuable marker for phylogenetic analysis at
intraspecific level and intergeneric level among angiosperms and other eukaryotes (Baldwin et al. 1995). In general, the ITS region present some advantages of being a multicopy locus (100-200 copies), having a small size (300-800 bp), varying from one taxon to another but highly conserved in size in a given taxon, and making it a preferred diagnostic target for a universal test (Baldwin et al. 1995).

ITS sequences are proven to be valuable for phylogenetic reconstruction in angiosperms, algae, and ferns. Recent work indicates that ITS-1 and ITS-2 sequences are inherently G+C rich in which portions of these regions are quite conserved among angiosperms. Thus, the ITS regions not only possess high information content at lower taxonomic level, but also exhibit conserved sequence patterns and high alignability across angiosperms (Figure 7).

Figure 7  Taxonomic level of utility of nuclear DNA regions used in phylogenetic reconstruction based on angiosperms. The shaded box showing the taxonomic zone that is not presently well covered by nuclear gene sequences; ? refers to genes that have been rarely used; ----- designates the approximate upper or lower limits of applicability (Soltis and Soltis 1998)