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Review: Recent status of *Selaginella* (Selaginellaceae) research in Nusantara

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ABSTRACT

Setyawan AD. 2011. Recent status of Selaginella *(Selaginellaceae) research in Nusantara. Biodiversitas 12: 112-124. Selaginella* Pal. Beauv. (Selaginellaceae Reichb.) is a cosmopolitan genus that grows in tropical and temperate regions. Some species of *Selaginella* have widely distribution and tend to be invasive, but the others are endemics or even, according to IUCN criteria, endangered. Nusantara or Malesia (Malay Archipelago) is the most complex biogeographic region and rich in biodiversity. It is one of the biodiversity hotspot of *Selaginella*, whereas about 200 species of 700-750 species are exist. *Selaginella* has been survived for 440 mya without any significant morphological modification, but extinction of tree-shaped species. *Selaginella* have similar morphological characteristics, particularly having heterospore form and loose strobili; and is classified as one genus and one family. However, individual species has high morphological variation caused by different edaphic and climatic factors. Genetic studies indicate high polymorphism among *Selaginella* species. *Selaginella* had been used as complementary and alternative medicines treated to postpartum, menstrual disorder, wound, etc. Biflavonoid – the main secondary metabolites – gives this benefit and is especially used as anti oxidant, anti-inflammatory, and anti cancer in modern pharmaceutical industry. The other metabolites, trehalose, potentially act as molecular stabilizer in biological based industry. Metabolite profiles can also be used to identify *Selaginella* by its species, time and harvest age, and locations. Since most of *Selaginella* grows on moist, organically rich, and well drained soils, which is vulnerable to forest degradation and global warming, it needs more conservation priority. Biosystematics and ethnobotanical researches of Nusantara *Selaginella* is needed to expand taxonomic status and bioprospecting of this bioresources.

Key words: biosystematics, ethnobotany, research, Nusantara, *Selaginella*.

INTRODUCTION

The word *Nusantara* is firstly used officially by the Kingdom of Majapahit, to name their sovereignty including most of Island of Southeast Asia (ISEA) and Malay Peninsula. Later, this archipelago is also named Malesia or Malay Archipelago refers to Malay Language which acts as lingua franca. Now, Nusantara or Malesia is a biogeographic region including Sumatra, Malay Peninsula, Kalimantan, and Java in the western; the Philippines, Sulawesi and the Lesser Sunda Islands in the middle; and Maluku and Papuasia (New Guinea and the Solomons) in the eastern. This archipelago is developed in accordance with the breakup of Gondwana and the end of the last glaciations. In the perspective of Gondwana separation, Asia mainland is withstand in the original place and compose Sunda shelf, whereas another fission, nowadays Australia and New Guinea floating away to the north, compose Sahul shelf, and uplifted islands between two continent. At the end of ice age, the two shelves sink and generate Nusantara as seen nowadays. Sunda, Sahul and the islands between them have different historical evolution and generate different flourish biological diversity. Tropical Nusantara is one of the major mega biodiversity centers of the world, together with tropical South America and tropical Central Africa. Unfortunately, habitat degradation is quite high, both land and oceanic areas, which threatens sustainability of this biological resource.

Selaginella Pal. Beauv. (Selaginellaceae Reichb.) is one of the biological resources of Nusantara. This ancient plants can survive from natural selection without significant morphological modification, and sometimes called spike moss or resurrection plants (especially for incurling-drought ones). This plant has been grown in humid and warm climate regions from the early Carboniferous period, which having dichotomic branch and dimorphic leaves (especially in Nusantara species); and differing from another allied lycophytes by loose, free and open strobili. *Selaginella* is potent medicinal plants, which contain phenolics (flavonoids), alkaloids, as well as terpenoids; however biflavonoid – a dimeric form of flavonoid – is the main bioactive compounds of *Selaginella*, which comprising of 13 compounds, especially amentoflavone and ginkgetin. Biflavonoids have several medicinal properties, especially as antioxidant, anti-cancer, and anti-inflammatory. Since most of Nusantara *Selaginella* naturally grow in humid and cool areas, which threatened by natural degradation and global warming, it insists conservation effort to ensure sustainability supply of this biological resources.

Selaginella has been existed since 440 mya and grown in all biome types, except in Antarctica. This plant generates several characteristics for fading competition, reducing herbivory, pest and disease, optimizing growth and capturing sunlight, etc. *Selaginella* can grow in the most extreme habitat such as in cold tundra and alpine (*S. selaginoides, S. rupestris*) or in drought dessert (*S. lepidophylla, S. sartorii*); however most of them grows in tropical rain forest. Those species are only remaining herbs-form and leaving tree-form; have minute dimorphic leaves, forming bizonoplast (*S. erythropus*) and iridescent schemochromic tydall-blue color (*S. willdenowii*, *S uncinata*); generate more chlorophyll a than b (*S. willdenowii*); and produce several secondary metabolites, mainly bioflavonoid, as well as trehalose, a molecular stabilization of drought conditions. This adaptation induces continuation of *Selaginella* through time, and triggers the diversity. Biosystematics of *Selaginella* is used for analyzing data obtained from morphological, ecological, genetic, biochemical, etc. to assess the taxonomic relationships, especially within an evolutionary framework.

Since research on Nusantara *Selaginella* is rarely conducted, it is needed to review all research progress and compared to global researches. This review explains current condition of Nusantara *Selaginella* which cover all aspect of investigation, such as biogeography, morphology and anatomy, genetics or molecular biology, natural products, ecology, paleobotany, ethnobotany, as well as Nusantara population which can impact on biodiversity. This matter is needed to improve conservation effort and is used to solve biosystematics problems of this bioresources.

PHYLOGENETICS AND CLASSIFICATION

Phylogenetics

Selaginella has approximately occurred since 440 mya (Banks 2009); far before Nusantara reside in the present days location. *Selaginella* is estimated congeneric with genus *Selaginellites* Zeller which nowadays remains to fossil (Fairon-Demaret 1989). Molecular based research indicates that Selaginellaceae also has close relationship to Lycopodiaceae and Isoetaceae (Tryon and Tryon 1982), which is chemotaxonomically indicated by the flavonoid patterns (Voirin and Jay 1978). This family has divergence from Isoetaceae at Upper Devonian period (370 mya) (Korall et al. 1999). Selaginellaceae has only one genus, *Selaginella* (Jermy 1986 1990b; Tryon and Tryon 1982), that consist of 700-750 species (Tryon and Tryon 1982; Jermy 1990). This ascertainment is not only supported by common taxonomic data, but also supported by newest biomolecular data. The word *Selaginella* is firstly introduced by Palisot de Beauvois (1805) as a name of a genus, but previously this word has been used by Linnaeus (1754) to denominate species of *Lycopodium selaginoides* L.; word of *selaginoides* mean like *selago*, an ancient name for *Lycopodium*. The name of this species has been revised and nowadays the valid name is *Selaginella selaginoides* (Heidel and Handley 2006). The use of molecular markers

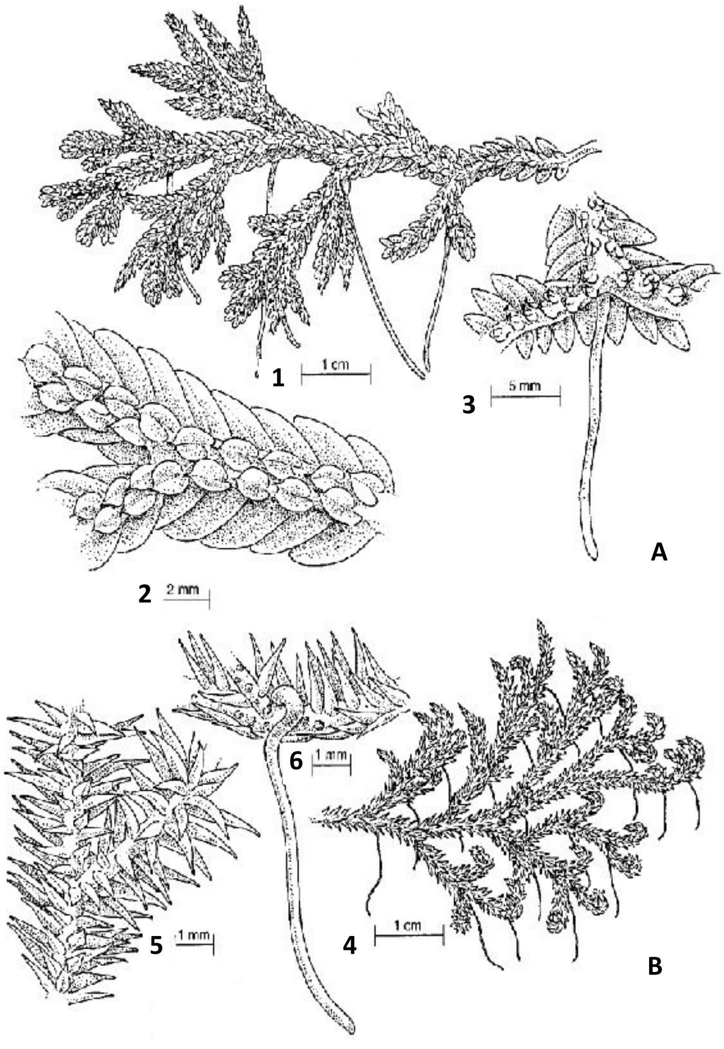

Figure 1. Morphological comparison of anisophyllous and isophyllous of *Selaginella*. All Nusantara *Selaginella* has anisophyllous leaves and including in subgenus *Stachygynandrum*. (1) Habit. (2) Close-up of upper side of vegetative branch shows dimorphic leaves, decussately arranged in four rows. (3) Close-up of branch dichotomy shows rhizophore originating on the ventral side of the branch. While several others has isophyllous leaves such as subgenus *Tetragonostachys*. (4) Habit, note the uncurling vegetative branch tips. (5) Close up of vegetative branch shows uniform spirally arranged leaves. (6) Close-up of branch dichotomy shows rhizophore originating on the dorsal side of the branch (after Korall and Kenrick 2002).

that are relatively resistant to evolution such as cpDNA is very important to clarify phylogenetics of this taxon. This will be strengthened when more species are studied.

Classification

Palisot de Beauvois (1805) classifies Selaginellaceae into four genera, but Spring (1850) unites it all into one genus, *Selaginella*, and then Jermy (1986) divides it into five sub-genera, namely *Selaginella* Pal. Beauv. (2 spp.), *Ericetorum* Jermy (3 spp.), *Tetragonostachys* Jermy (~ 50 spp.), *Stachygynandrum* (Pal. Beauv.) Baker (~ 600 spp.), and *Heterostachys* Baker (~ 60 spp.). The first tree of subgenus has similar leaf measure and shape (isophyllous), while the other two have different leaf measure and shape (anisophyllous). According to Korall and Kenrick (2002), subgenus *Selaginella* and *Tetragonostachys* have monophyletic character, *Stachygynandrum* and *Heterostachys* have polyphyletic character, while character of *Ericetorum* is still unknown. Previously Korall et al. (1999) suggest that subgenus *Selaginella* closely ties to other subgenus though its morphological form rather differing, whereas has leaf whorl and has not rhizophores. Korall and Taylor (2006) indicate that *Selaginella* is monophyletic genus base on *rbcL* map, however at subgenus level it is monophyletic or paraphyletic. Modern and fossil of *Selaginella* species is monophyletic in Selaginellaceae family base on ultrastructure of megaspore. According to Camus (1997), all Nusantara *Selaginella* is member of subgenus *Stachygynandrum,* particularly featured by its dimorphic leaves (Figure 1); until now we have never found any anisophyllus *Selaginella* in the archipelago, so the study on this matter is needed.

Diversity

Nusantara is one of the center researches of world biogeography that is considered as natural biogeographical unit. Nusantara has very high plant diversity and endemicity that is estimated to reach 42,000 species (Roos 1993), whereas 70% is endemic plant. However, most study is only conducted in western part, though many taxa spread in all over areas (Brown et al. 2006). *Selaginella* is fern with cosmopolitan distribution, especially at subtropical and tropical areas (Tryon and Tryon 1982; Jermy 1990; Kramer and Green 1990). This plant can be found in ninth bioregions of Nusantara, which diversity depends on the island width. A lot of species is naturally distributed in all over regions, such as *S. wildenowii*; but several species are spreaded by human introduction, such as *S. plana*. These species originally came from the western part of Nusantara, but now are distributed throughout the archipelago, even to North, Central and South America (Hassler and Swale 2002).

Nusantara has about 200 species of *Selaginella*, amount of total and endemic species in each bioregion as follow: Malaya 32 (6), Sumatra 28 (9), Kalimantan 57 (42), Java 24 (5), Sulawesi 18 (7), Maluku 16 (4), Lesser Sunda Islands (10 (0), Philippine 54 (41), and Papuasia 59 (48) (Camus 1997; Hassler and Swale 2002; Setyawan 2008a). In Bahasa Indonesia, *Selaginella* is called as *"cakar ayam"* (scrawl) referring to the position of scale leaves which adhere to stem which is similar to the scales of chicken foot (Dalimartha 1999); or *"rane"*, an absorption word from Sundanese word (Sastrapradja and Afriastini 1985). Since taxonomy of Nusantara *Selaginella* is still based on old research, such as Alderwereld van Rosenburgh (1915a, b; 1916, 1917, 1918, 1920, 1922) and Alston (1934, 1935a, b, 1937, 1940), it is needed to re-evaluate the taxonomic classification for reducing misidentification and covering new species, new record or new naturalization of non native species. Check list of Papuasian and Kalimantan *Selaginella* diversity have not been conceived yet. But for the Malay Peninsula, the revision has been conducted twice by Wong (1982, 2010). Setyawan (2009) showed that from 40 species of *Selaginella* found throughout Indonesia in his ethnobotanical research, 18 species are not identified and are alleged to be a new species or new records. With more intensive research, it is believed that more unidentified species will be found.

MORPHOLOGICAL AND ANATOMICAL DESCRIPTION

Selaginella is the largest genus of heterospore fern, the sporangium is modified from reproductive leaves on tip of branch that forming loose, free and open groups called strobili (Hitchcock et al. 1969). It has dichotomous branch and minute scale-like leaves that are generally in two different sizes, where median is smaller than lateral ones (Jermy 1990). This morphological characteristic is only little change since Carboniferous period, when this plant lives in tropical wetland (Thomas 1992, 1997).

Nusantara *Selaginella* has generally perennial herbs form (in north hemisphere there are also annual herbs). Stem is dichotomous branch with or without certain pattern (Wong 1982). Stele is generally protostele type, but there is also siphonostele or actino-plectostele. Stem is erect, creeping, climbing, or rosette root. Basal part is usually without branch. Rhizophore which is a *sui generis* organ emerges at ramification or basal rhizome (Jernstedt and Mansfield 1985; Jernstedt et al. 1994), which is combination of stem and root (Goebel 1905). Rhizophore is present or not, leafless, colorless, positively geotropic, elongated, growing downwards from point of bifurcation stem, emerges at ramification stem, entire or only at basal ramification stem. Rhizophores are unique to the *Selaginella* genus. Both rhizophores and roots have dichotomous branch, forming a multibranch rhizophore–root system (Otreba and Gola 2011). Leaf is small, single with single vein, and always has ligulae at base of adaxial leaf (only in the early leaf forming) (Valdespino 1993), some species of Central America have hair on the leaves (Caluff and Shelton 2009), as well as *S. biformis* of Java (Alston 1935a). In Nusantara, vegetative leaf is always dimorphic (isophyllous), and usually lap over in two median lane and two lateral lane; median leaf usually smaller and has different shape from lateral ones (Camus 1997), however the most outstanding *Selaginella* in northern hemisphere namely *S. selaginoides* is anisophyllous ones (Heidel and Handley 2006).

Strobili (set of sporophylls) does not always exist, it usually emerges at branch tip, is generally uncompact and open; cylindrical, longitudinal quadrangle, or flatten, with basal megasporangia and apical microsporangia. Sporophylls or reproductive leaf is rather or very different from vegetative leaf, and generally anisophyllous (*S. rothertii*), or isophyllous (*S. plana*) or resupinat-anisophyllous (*S. alutacia*). It has two spore types (heterospore); microsporophyll has one microsporangium which contains hundreds of microspore; while megasporophyll has one megasporangium containing four megaspores. Microspore is much smaller than megaspore and usually has different color. Sporangium has short petiole, solitary, in sporophyll base, oval or circular, and opening across transversal aperture (Valdespino 1993; Tsai and Shieh 1994; Camus 1997). Megasporangia lays at lowest sporophyll of strobilus, but sometime lays at tip of strobilus, while microsporangia lays above megasporangia (Valdespino 1993). In different habitat, ornamentation of megaspore can harsher or softer (Cronquist et al. 1972).

Schulz et al. (2010) chooses *S. apoda* as an appropriate model species to understand its morphology, anatomy, and life cycle in detail. Development and growth patterns indicate that each segment is an independent module consisting of a section of the stem, leaves, rhizophores, and roots. A comparison of different leaf types (dorsal and ventral leaves, dorsal and ventral sporophylls) shows similar stomata and papillae distributions but type-specific forms and sizes. In the transition from vegetative to reproductive growth, the small dorsal leaves do transition to small dorsal sporophylls bearing microsporangia, and the large ventral leaves do transition to large ventral sporophylls bearing either microsporangia or megasporangia.

Selaginella has various pigmentations, such as blue chromatic, crimson red, variegate, yellow gold, and silver. Morphological diversity and pigmentation are important characteristic in taxonomy of *Selaginella* (Dahlen 1988; Czeladzinski 2003). Shape of stele (Hieronymus, 1901) and structure of sporangium (Horner and Arnott 1963; Fraile and Lap 1981; Quansah 1988) are also used as distinguishing characteristics. Single base megasporangium is used for grouping articulate species (Hieronymus, 1901; Somers, 1978), but the newest research indicates that it indicates no certain group (Korall and Kenrick 2002). Depth research on morphology, anatomy and life cycle of Nusantara *Selaginella* is necessary, as research of Schulz et al. (2010) in *S. apoda*.

MORPHOLOGICAL ADAPTATION AND PLASTICITY

Morphological adaptation

The earliest fossil of Selaginellaceae is *Selaginellites resimus* Rowe (Rowe 1988) coming from early Carboniferous period (440 mya) (Banks 2009); it is a uniform leaf herbs (isophyllous) and grows in tropical wet area (Fairon-Demaret 1989). In the late Carboniferous period (290-323 mya), *Selaginella* that has herb form, dichotomous branching, and small dimorphic leaf begin to widespread and constitute of coal (Thomas 1992, 1997). *Selaginella* is generally abundant in forest floor; its dimorphism leaf is an adaptation to low light intensity (Hebant and Lee 1984). Some species can modify chloroplast to boost exploitation of sunshine by forming bizonoplast, such as *S. erythropus* (Sheue et al. 2007) or non-pigment optical structure of schemochromic tydall-blue iridescent, such as *S. willdenowii* and *S. uncinata* (Fox and Wells, 1971; Hebant and Lee 1984; Lee 2001; Thomas 2010), and *S. singalanensis* (Setyawan 2009), which loose in open place (Hebant and Lee 1984). *S. willdenowii* also adapt to grow under shade, with ratio of chlorophyll a:b equal to 2,2 (Nasrulhaq and Duckett 1991).

This adaptation increases in line with progressive dense of forest canopy through increasing tree fern high at Westphalian period (303-320 mya) (DiMichele et al. 1992). Morphological similarity of fossil and modern *Selaginella* indicate that some tropical species which live in wet area is possibility direct offspring of ancient species from Carboniferous period (Korall et al. 1999), but *rbcL* topology of tropical *Selaginella* is not paraphyletic or basal to species from dry area or sub-tropical climate (Koral and Kenrick 2002). *Selaginella* adaptability that can survive the environmental changes over millions of years need to be examined to determine the superior characteristics of plants.

Morphological plasticity

Species concept of *Selaginella* is hard to determine because of morphological plasticity of some species, resulting difference of edaphic and climatic factors, age, genotypes, etc. Sunshine intensity can cause difference morphological form and chemical content. Soil fertility and humidity can influence forming of root and rhizophores. Ground water content and individual age can also influence morphological appearance. At certain species, old individual or drough cause the color become darker than young ones or humid ones, such as *S. plana* (Lu and Jernstedt 1996). In one species, sometimes there are various shapes and shades of leaves, for example *S. ornata*, so that in the past this species was divided into several species (Setyawan 2009). This morphological variation causes difficulty in classification of *Selaginella*; and a challenge in systematics research, because until now the morpho-species concept is still a major cornerstone in plant systematics.

ECOLOGY

Selaginella is a cosmopolitan genus and grows at various climate and soil types. Some species grow at very extreme climate such as cool alpine or artic circle (*S. selaginoides, S. rupestris*), and also barren and dry desert (*S. lepidophyta, S. sartorii*) (Tryon and Tryon 1982; Zoller 2005), but highest diversity is in tropical area. *Selaginella* grows in all Nusantara bioregions, because it has been existed before the breaking of the great continent of Gondwana. Some species are believed existing in tropical area since late Paleozoic (Korall and Kenrick 2002). Most of *Selaginella* grow under forest canopy and are protected from direct sunshine. It also found at forest floor, marsh, river bank, around waterfall, and water spring. *Selaginella* can grow at marginal soil which is lack of nutrient to lessen competition. This plant is rarely feed by herbivores because contains secondary metabolite that harsh most animals, except for several insect (Mound et al. 1994), wild pig, *Sus barbatus* (Küsters 2001) and snail. Livestock grazing and competitor plants that absorb much water can make environment drier which influence the growth of *Selaginella* (Heidel and Handley 2006). It can lessen the density of *Selaginella*, for example at *S. densa* (van Dyne and Vogel 1967).

Since *Selaginella* is heterosporic fern, the microspore and megaspore must grow in adjacent site to generate microgametophyte and megagametophyte, then fertilizing to generate sporophyte. *Selaginella* megaspore is larger and heavier than other lycophytes, causing tighter distribution. Root system of gametophyte is very shallow and requires water for moving the sperm to egg cell, causing most *Selaginella* grows in moist place (Cronquist et al. 1972). *S. lepidophylla* can withstand in seasonally dry conditions, adapt to extremely arid climates and depend on infrequent wet periods for growth and reproduction (Cantrill and Webb 1998). Megagametophyte is long life autotrophic plant, which can conduct photosynthesis (Cronquist et al. 1972) and absorb nutrient without mycorrhiza (Heidel and Handley 2006), however mycorrhiza can be found in sporophyte (Strobel and Daisy 2003). Spreading mechanism of megaspore and microspore is conducive for cross-breeding, though it is rarely happened in *Selaginella* (Soltis and Soltis 1988). In cultivation, *Selaginella* is multiplied by dissociating mature corps, however it can also use spore (Carolina Biological Supply 1998). It needs moist and porous media, which enable water and air emitting (Heidel and Handley 2006).

Nusantara *Selaginella* can exist and abundantly grow in moist, organically rich, and well drained soils, in shade or half shade, often near streams, a long trail and at the edge of clearing areas, in lowland and mid-montane primary or secondary forest (Winter and Jansen 2003). *S. tamariscina* is only plant grows in dry and rocky habitats found in Wallacea, i.e. the Philippines (Alston 1935b), Sulawesi and Lesser Sunda Islands, and may be also introduced by Chinese in West Kalimantan (ADS 2007-2008, personal observation to BO collections). In dry period, *S. tamariscina* rolls inwards and turns into ball, while *S. plana* turns to darker reddish brown. Study on *Selaginella* community in Nusantara species is rarely conducted, while this is generally conducted in northern hemisphere for *S. selaginoides* and *S. rupestris (=S. sibirica*), including abundance of spores in several paleo-environmental era. They are light-demanding and able to grow in poor soils and in extremely frost (dry and cool) environment. They are commonly found in steppe, open larch and pine forests, and even in grass shrub tundra with shrubby pine (Molozhnikov, 1986; Lozhkin et al. 1993; Baker et al. 2002). During the last glacial maximum (LGM), abundance of the spore indicates increasing open habitat (Tobolski and Ammann 2000; Schirrmeister et al. 2002) and the vegetation is still fairly open and the soil is poorly developed (Heiri et al. 2003). It also suggests that the climate is colder and drier than today (Ikehara 2003), such as cold boreal and sub arctic conditions. According to the pollen spectra, the maximum warming is about 12,000 BP (Schirrmeister et al. 2002), that causes ice melting of LGM and then the climate becomes warm again (Ruddiman 2005). *Selaginella* spores can be used as bioindicator of paleoclimatic in ancient time, whereas the distribution in past and present time can be used for modeling future climate. In Nusantara, there is no research related to the global warming and the existing or losing of *Selaginella*.

MOLECULAR BIOLOGY

Karyomorphology

In the evolutionary history, fern perform several polyploidy and hybridization causing a large chromosome number (Walker 1984), but it is rarely happened in heterosporic fern such as *Selaginella* (Soltis and Soltis 1988; Marcon et al. 2005). Hybridization has been reported between *S. ludoviciana* and *S. apoda* in United States (Somers and Buck, 1975) and between *S. firmula* and *S. laxa* in Fiji (Gardner 1997). Moreover, Hassler and Swale (2002) indicated that only one from 691 species of world *Selaginella* is proven to be hybrids.

Morphological karyotype of *Selaginella* is firstly published by Manton (1950). Chromosome number of *Selaginella* has been investigated on several species of Europe (Reese 1951; Love and Love 1961; Borgen 1975), North America (Tryon 1955; Love and Love 1961, 1976), India (Kuriachan 1963; Loyal 1976, 1984; Ghatak 1977; Vasudeva and Bir 1983), China (Wang et al. 1984; Weng and Qiu 1988), Taiwan (Tsai and Shieh 1983, 1988), Japan (Takamiya 1993), and other countries (Tchermak-Woes and DolezaI-Janish 1959; Zhukova and Petrovsky 1975). Jermy et al. (1967) made extensive investigation on chromosome numbers of 76 species of *Selaginella* collected from the Malay Peninsula, Borneo, New Guinea, Australia, Trinidad, Puerto Rico, and Brazil, as well as on cultivated plants. Those are the only karyomorphological investigation which include Nusantara *Selaginella*. In almost all other Nusantara bioregion, the study of chromosomal morphology of *Selaginella* has never been conducted.

Selaginella has variation of diploid chromosome number, 2n = 14-60, with basic chromosome number is x = 7, 8, 9, 10, 11, and 12 (Kuriachan, 1963; Jermy et al. 1967; Takamiya 1993). This matter originate from x = 9, which later grouping with x = 10, 11, 12, and the other grouping with x = 7, 8 (Takamiya 1993; Mukhopadhyay 1998). Diploid chromosome number in each subgenus is possibility formed through different evolutionary pathway (Takamiya 1993). Variation of chromosome number and rDNA amount and size of *Selaginella* indicate that the chromosome evolution is very complicated, whereas each basic chromosome number emerge twice or more (Marcon et al. 2005).

Isozyme

Research on diversity of *Selaginella* base on isozymic marker for population dynamics or classification is rarely conducted. Several studies are generally related to physiological characteristic. *Selaginella* is known to have two isozymic bands of chorismate mutase able to be measured clearly. This isozyme and other isozymic shikimate have potent as diagnostic characteristic (Woodin et al. 1978). Preliminary study indicate that enzyme system of esterase (EST), peroxidase (PER, PRX), malate dehydrogenase (MDH), aspartate aminotransferase (AAT), and acid phosphatase (ACP) can be used as diagnostic characteristic for more than 20 species of Nusantara *Selaginella* (ADS 2008, data not be shown).

DNA marker

Research on diversity of *Selaginella* based on DNA/RNA marker is relatively still limited. Kolukisanoglu et al. (1995) indicate that *Selaginella* and *Equisetum* emerge earlier than *Psilotum* based on phytochrome gene. This result corresponds to chloroplast gene (Raubeson and Jansen 1992); and supported by ribulose-bisphosphate carboxylase gene (*rbcL*) (Korall et al. 1999; Korall and Kenrick 2002). Korall and Kenrick (2002, 2004) prove that subgenera *Selaginella* and *Tetragonostachys* are monophyletic, *Stachygynandrum* and *Heterostachys* are polyphyletic; while the nature of *Ericetorum* is still unknown yet. This research is relied on chloroplast gene of *rbcL* from 62 species (± 10%), which selected by morphological, ecological and geographical diversity. Besides those above, monophyletic of *Tetragonostachys* is also proved by nuclear internal transcribed spacer (nr ITS) (Therrien et al. 1999; Therrien and Haufler 2000). Research by Korall and Kenrick (2002) with *rbcL* sequence indicates that classification by using DNA marker is not always compatible to morphological characteristic. Some classification can be referred by morphological characteristic, such as existence of rhizophores, growth of rhizophores, and morphology of leaf and stem. Some other can be referred by ecological characteristic such as wet or dry habitat. But the most classification is not related to morphological, ecological, and physiological characteristics. Research on amplified fragment length polymorphism (AFLP) of 10 species of Nusantara *Selaginella* indicated that high polymorphic variation among species (Setyawan 2008b). In addition, Li et al. (2007) indicate that random amplification of polymorphic DNA (RAPD) can be used as marker to differentiate *Selaginella* both species and population from different habitats. Investigation of *Selaginella* diversity base on another DNA marker has not apparently been done.

NATURAL PRODUCTS

Biflavonoid

Major secondary metabolite of *Selaginella* is biflavonoid, which only found at Selaginellales, Psilotales, Gymnosperm (Seigler 1998), several Bryophytes and several Angiosperm (DNP 1992). *Selaginella* also contains alkaloid, phenolic (flavonoid, tannin, saponin), dan terpenoid (triterpene, steroid) (Chikmawati and Miftahudin, 2008; Chikmawati et al. 2008). *S. lepidophylla* is also reported contains volatile oils (Andrade-Cetto and Heinrich 2005); and some species of Japan have ekdisteroid (Takemoto et al. 1967; Hikino et al. 1973; Yen et al. 1974). The different species of *Selaginella* shows different HPLC fingerprint characteristic. The samples of the similar species but collected in different period, different environment or different locations shows certain difference in fingerprints. However, it also generate "main fingerprint peaks", which can be used to evaluate and distinguish the different species or infra species (Li et al. 2007).

Biflavonoid which has been identified from *Selaginella* is only 13 compounds and/or its derivatives, namely amentoflavone, 2',8"-biapigenin, delicatulaflavone, ginkgetin, heveaflavone, hinokiflavone, isocryptomerine, kayaflavone, ochnaflavone, podocarpusflavone A, robustaflavone, sumaflavone, and taiwaniaflavone (Setyawan and Darusman 2008; Setyawan 2011). Some biflavonoid are easily found at various species of *Selaginella*, but the other only found at certain species. Amentoflavone is biflavonoid compound of several *Selaginella*, while sumaflavone is only reported from *S. tamariscina* (Yang et al. 2006; Lee et al. 2008). Preliminary study shows that amentoflavone is found at high content (> 20%) at two of about 35 species of Nusantara *Selaginella*, namely *S. subalpina* and *S. involvens* (ADS 2008, data not be shown).

Biflavonoid of *Selaginella* has various bioactivities, such as antioxidant, anti-inflammatory, anti cancer, antimicrobials (antivirus, antibacterial, anti fungi, anti-protozoan), neuroprotective, vasorelaxant, anti UV-irradiation, antispasmodic, anti allergy, antihaemorrhagic, antinociceptive, etc. Amentoflavone, the commonest biflavonoid of *Selaginella* has bioactivity as antioxidant, anti-inflammatory, anti cancer, antimicrobials, antivirus, vasorelaxant, anti stomachic, anti depression, anxiolityc, and analgesic. Additional group of hydroxylation, methoxylation, methylation, and glycosilation very influence the bioactivity properties (Setyawan and Latifah 2008).

The widely use of *Selaginella* in complementary and alternative medicine is usually conducted in traditional Chinese medicine (TCM). *S. tamariscina* is the most used species in TCM, which supported by high variety of biflavonoid, include amentoflavone, hinokiflavone, 2',8"-biapigenin, isocryptomerine, sumaflavone, and taiwaniaflavone. *S. tamariscina* extract has various usefulness, such as anti cancer, antioxidant, anti-inflammatory, antifungal, anti UV irradiation, anti allergy, vasorelaxant, anti diabetic, and influence reproduction cycle (Setyawan and Latifah 2008). In the present days, study of *Selaginella* biflavonoid and other natural products are still limited to certain species, biflavonoid type, and solvent type.

Trehalose

S. lepidophylla often mentioned as resurrection plant or rose of Jericho able to survive on long drought and recover through rehydration (Crowe et al. 1992), even almost all of body water has been evaporated (van Dijck et al. 2002). Several *Selaginella* have high rate of trehalose, a simple sugar, which is responsibility to endurance of heat stress and drought (Avigad 1982), such as *S. lepidophylla* (Adams et al. 1990; Mueller et al. 1995; Zentella et al. 1995) and *S. sartorii* (Iturriaga et al. 2000). Drought can change pigmentation and fluorescence, but generally can not cause dying (Casper et al. 1993). Trehalose is potent for molecular stabilizer (Roser 1991; Kidd and Devorak 1994). Secondary metabolite content can vary depend on environmental factors such as habitat location, climate, and soil type; harvesting and extraction procedure (Nahrstedt and Butterweck 1997); and also intrinsic factor such as species or variety, part of extracted and age (Setyawan and Latifah 2008). Research on trehalose constituent of Nusantara *Selaginella* species has never been done; considering the high potential as bio-stabilizer in the industry, this research is urgently needed. In general, species that contain trehalose will roll up its leaves in dry conditions. *S. involvens* and *S. tamariscina* estimated to contain this compound.

ETHNOBOTANY

One of the primary factors influencing biodiversity is human culture. Relation of human with plant known as ethnobotany, while if related to market known as economic botany. Ethnobotany means the study of traditional plant use, or the scientific study of the traditional classification and uses of plants in different human societies (Harshberger 1896). Exploration of plant which has medicinal or food usage is the most interesting ethnobotanical study, because this matter becomes the basis for modern development. Since Nusantara has very high variation of culture and plants species, it has various exploitation types, including traditional medicine (jamu) and culinary (Setyawan 2009).

In Southeast Asia, *Selaginella* is generally used as medicine, food, ornaments, and handicrafts (Winter and Jansen 2003). Most *Selaginella* species have not been used as medicinal plants or other purposes of economic potent, and there are at least 10 species that have been used with varying intensity. *S. involvens, S. ornata, S. willdenowii*, and *S. plana* are used as medicinal ingredients. *S. ciliaris, S. singalanensis*, and *Selaginella* sp.1 are used as an ornamental plant. *S. opaca, S. plana* and *S. wildenowii* are used as a vegetable. *S. caudata* and *Selaginella* sp.4 are used as a wrapping of fruits and vegetables from the garden (Setyawan 2009).

Medicinal usage

Selaginella is traditionally used to cure several diseases such as: wound, postpartum, menstrual disorder, skin disease, headache, fever, infection of exhalation channel, infection of urethra, cirrhosis, cancer, rheumatism, bone fracture, etc. Part to be used is entire plant, though it only refers to leaf or herb (Setyawan and Darusman 2008; Setyawan 2009). The usage can be conducted single or combination, fresh or dried, direct eaten or boiled (Dalimartha 1999; Wijayakusuma 2004). This plant has sweet taste and gives warm effect on the body (Bensky et al. 2004). The use of *Selaginella* as medicinal matter is occurred in the entire world. The largest usage is conducted by Chinese, especially for *S. tamariscina, S. doederleinii, S. moellendorffii, S. uncinata*, and *S. involvens* (Chang et al. 2000; Lin et al. 1991; Wang and Wang 2001).

Selaginella is rarely exploited in Nusantara. Traditional *jamu* of Java, the most advanced traditional medication system in Nusantara, uses more cultivated rhizomes and spices than wild herbs or grasses. In Kalimantan, Dayaks of Kayan Mentarang NP, East Kalimantan use *S. plana* to cure hemorrhage (Uluk et al. 2001), while Dayaks of Bukit Baka-Bukit Raya NP, West Kalimantan use *S. magnifica* and some other *Selaginella* to cure headache, fewer, and skin diseases (Caniago and Siebert 1998). In Sabah, Dayaks use *S. argentea* and *S. plana* to treat high fever and headache (Ahmad and Raji 1992). In northern Borneo, dry leaves of *S. padangensis* is smoked like tobacco and is also used as poultice for vertigo and for toothache treatment (Winter and Jansen 2003).

In West Java, Sundanese at Mount Halimun-Salak NP, West Java use several *S. ornata, S. wildenowii, S. involvens*, and *S. intermedia* to cure wound, postpartum, menstrual disorder, and tonic (Nasution, 1993; Harada et al. 2002; Setyawan and Darusman 2008). *S. plana* leaves is drunk in decoction as tonic for treatment postpartum (Harada et al. 2002). *S. intermedia* is given in decoction for stomach-ache and is applied as poultice over the whole body for asthma. In Java, young leaves of *S. ornata* and *S. wildenowii* are eaten as vegetable and also as depurative or stomachic. *S. wildenowii* is also used in decoction as a protective medicine postpartum and as an ingredient of tonic as well as to treat skin disease such as itches and ringworm (Winter and Jansen 2003). In Sumatra and Java, several species of *Selaginella* is used to neutralize poison, fever, purify the blood, menstrual disorder, eczema and postpartum (Warintek 2002).

In Sumatra, Kalimantan, and Malaya, *S. padangensis* is used as poultice for vertigo and for toothache treatment (Winter and Jansen 2003). In Sumatra and Malaya, *S. stipulate* is used in decoction as protective medicines postpartum. In Sumatra, Malaya, and southern Thailand, *S. wallichii* is used in decoction as protective medicines postpartum. In Malaya, *S. wildenowii* is also given internally as an infusion to treat fever and the ashes is used in liniment for backache (Winter and Jansen 2003). In Kedah, Malaya, *Selaginella* is used as tonic to increase body endurance (Abu-Shamah et al. 2000; Batugal et al. 2004). In the Philippines *S. tamariscina* is used to cure wound, hemorrhage resulting stomachic, menstrual disorder, or pile (Pam 2008). In Papua New Guinea, *S. flabellate* is treated to fever and headache (Kambuou 1996). In mainland of Southeast Asia, *S. doederleinii* is used to cure various disease and supplementary food, while in Laos *S. delicatula* is used for sedative (ARCBC 2004).

Food source

In Nusantara, the usage of *Selaginella* for food is very limited, but there are noted that young leaves of *S. plana* can be eaten as raw dishes (Heyne 1927), especially by Sundanese in West Java. While bitter young leaves of *S. willdenowii* is eaten by Javanese within food for medicinal purposes (Ochse 1931). In West Java, *S. plana* is sometimes eaten as dishes from lowland Bogor till around Mount Halimun-Salak, while in the mountainous areas they also eat *S. wildenowii* (Setyawan 2009). In the Philippines, young leaf of *S. tamariscina* can be cooked for vegetable (PAM 2008). In Papua, several *Selaginella* which have the wide leaf is sometimes used to pack sago, fruits, or other crop from forest (Setyawan 2009).

Ornamentals and other usages

In West Java, *S. ornata* and *S. intermedia* is used as ornamental plant in moist and shaded area (Sastrapradja el al. 1979). In India, *S. rupestris* is used as a decorative crop (Khare, 2007). In Taiwan, aboriginal Taiwan use *S. involvens* as ornamental plant, while *S. delicatula* as medicines (EDTA 2009). In Japan, *S. involvens*, *S. tamariscina* and *S. uncinata* is cultivated in garden (Michishita et al 2004). In Western countries, some species of *Selaginella* is very famous as ornamental plant, particularly as below ground vegetation, although it is potent to be invasive, such as *S. erythropus*, *S. kraussiana* (many varieties), *S. moellendorfii*, *S. uncinata*, *S. plana*, etc (Blooming Nursery 2009; Casa Flora 2009; Germania Seed Company 2009). The usage of *Selaginella* for traditional ceremony or rituals is not recorded in Nusantara, while in Gabon, *S. myosurus* is used for the sake of ritual or cultural (Sassen and Wan 2006).

THREAT AND CONSERVATION

Major threat to sustainability of *Selaginella* is habitat conversion and fragmentation. Habitat conversion can cause the totally loss of *Selaginella* due to microclimatic changes, as a result of water way changes, road-works, husbandry, fire-burning, deforestation, and development of recreation area (Heidel and Handley 2006). Habitat fragmentation can induce inbreeding depression that degrading offspring endurance (Barrington 1993), which observed at fragmented spots of *S. selaginoides* communities. However, global warming is the recent threat that can change diversity and sustainability of organism in the longer time period. It might change macro- and microclimatic of an area that impact to plant distribution, migration and extinction, and even invasiveness. *Selaginella* grow at various climatic and soil types, but generally require humidity for better growing and need water for fertilization; its presence in an area becomes indicator of habitat condition, including global warming and global cooling.

Several species of *Selaginella* growing in new habitat, evermore tend to be invasive. Nowadays, *S. plana* that originally from West Nusantara is naturalized in almost entire tropical Asia and America. *S. lepidophylla* of Mexican desert grow in Arabia and exploit for traditional drug (Ambrosiani and Robertsson 1992; Al-Wahaibi 2004). *S. martensii* that imported as ornamental crop has potent to be invasive in Victoria, Australia (Blood 2003). *S. biformis, S. moellendorfii* and *S. uncinata* is recognized as alien species to be established in Japan or found in the Japanese wild (Mito and Uesugi 2004). Global warming has been caused *S. kraussiana* of mountainous African and subtropical Azores (van Leeuwen et al. 2005) to be invasive in several sub-tropical areas, even can also be found in montane zone of Mount Kilimanjaro, Tanzania which initially too cool for the growth (Hemp 2008). *S. kraussiana* that introduced for ornamental crop become invasive in the British Islands (Stokes et al. 2006), the United States (Stapes et al. 2004), Australia (Carr et al. 1992; Groves 2003), and New Zealand (Bannister 1984; Esler 1988; Timmins and Braithwaite 2002; West 2002). This species becomes serious weeds in New Zealand, though sensitive to intense sunshine and drought (Thetford et al. 2006). *S. kraussiana's* ability in adapting new environment is possibly caused by diversity of the chromosomal number, i.e. 2n = 20, 30, 40 (Love et al. 1977; Kenton et al. 1993; Obermayer et al. 2002). Although, *S. kraussiana* which is potential to be invasive in new habitat, is still continuously sold in several Western countries (Blooming Nursery 2009; Casa Flora 2009; Germania Seed Company 2009). In Nusantara, invasion of non native *Selaginella* is not reported yet, though it is possibly occurred due to the introduction of new species for gardening and medicinal purposes.

Several species of *Selaginella* can be used as bioindicator of environmental changes. It usually grows in wet and humid or moist areas such as riparian vegetation of water spring and tributaries, wet escarpment at road side, below ground vegetation in mountainous forest, etc; however, some species can grow in hot and dry areas. Since *S. selaginoides* is a typical plant for cold area, the presence and abundance of it on ancient time indicates the occurrence of global cooling, while tree vegetations decrease, and cooler and barer areas increase (Heusser and Peteet 1988; Baker et al. 1989; Garry et al. 1990; Ambrosiani and Robertsson 1992; Demske et al. 2002). Microspore fossil of *S. selaginoides* can be identified until species level without significant difference with modern species, which still have facultative tetrads spores (Tryon and Lugardon 1991). In northern hemisphere, the abundance of this species is continuously high between 10,000 and 47,000 BP, and it decreases after LGM caused by global warming that initiate a habitat fragmentation and degradation (Heusser and Igarashi 1994). *S. selaginoides* fossil in peat bog and lake sediment can be used as an indicator of historical climate changes in those areas (Heusser and Peteet 1988). The losing of *S. selaginoides* indicates the occurrence of global warming, because it needs cool areas for growing. Nowadays, global warming has been insisted *S. selaginoides* to high mountain and/or high longitude (toward polar circle); and remains in relic habitat (Hornbeck et al. 2003). Some of the best evidence for global climate change has come from biogeography and community ecology, which make document of shifts of species composition and geographical ranges through time. In general, particular ecological communities are expected to move upward in both elevation and latitude (Walther et al. 2002). As with other species, montane and higher-latitude populations are mostly at risk (Root et al. 2003).

Concerning with *Selaginella* conservation, it is not always similar in every Nusantara countries. In Indonesia, there is no protected species of *Selaginella* (Lampiran PP RI No. 7/1999), though field survey shows several species that formerly found in Java and has become the collection of Herbarium Bogoriense (BO). Nowadays, it is difficult to find that species again in nature (Setyawan 2009). In Philippines, *S. atimonanensis* and *S. pricei* are categorized as endangered, while *S. magnifica* and *S. tamariscina* are categorized as vulnerable (DERN RP 2003); these four species are protected, though the last species is common in China and is sold as medicinal plant. In India, *S. adunca, S. cataractum,* and *S. coonoriana* is listed as threatened (RDBIP 2008). In Equador, *S. carinata* is categorized as vulnerable, while *S. sericea* is categorized as near threatened (IUCN 2009). In several states of the USA, *S. selaginoides, S. rupestris, S. apoda, S. eclipse, S. watsonii* are listed as threatened and endangered species, but those are not protected by federal legislature (FWS 2009). In Greenland, *S. rupestris* is also categorized as vulnerable (Jensen and Christensen 2003); while in Nova Scotia it is listed as endangered (Keddy 1978). In Sri Lanka, several *Selaginella* is listed as threatened species of vascular plants, namely *S. calostachya, S. cochleata, S. praetermissa,* and *S. wightii* (Ganashan et al. 1996). In Japan, *S. involvens* is listed as vulnerable by Chiba City (Nakamura and Short 2001). The inventory of Nusantara *Selaginella* is urgently needed for the high habitat destruction and the threat of global warming; in future, it can be used to arrange conservation measures.

SYSTEMATIC PROBLEMS OF *SELAGINELLA*

Since Nusantara is formed from two continental plates bringing different diversity, and tends to insulate and adapts to islands habitat, the diversity and endemicity is very high. This matter is challenge to specify taxonomic concept of species and genus. One of main problems in biosystematics study of *Selaginella* is the importance of more coherent definition to morphological species concept (morpho-species). Some *Selaginella* have very high morphological diversity such as shape-form, size-measure and pigmentation that complicate the classification. This matter is related to intrinsic factor such as age/maturity and genetics and also environmental factors such as climate and soil condition.

In Nusantara, taxonomy of *Selaginella* is generally base on old reference compiled in first-half of 20^{th} century, which require revising because of the possibility of finding new species, introducing alien species, changing species concept, etc. Originally, taxonomy of *Selaginella* is relied on morphological characteristic of herbaria that used for identifying, nomenclaturing, and classifying. However, it has limitation because evolution rate of morphological and genetic (protein, DNA) evidences are not always congruent; phenotype is controlled by many genotypes, and genotypic change is not always directly expressed in phenotype. Other challenge in taxonomy of *Selaginella* is high morphological plasticity caused by climate, soil, biogeographic factors, as well as age and variety of species. This matter causes the importance of genetic research to find out the existence of *Selaginella* diversity more detail. Genetic diversity is needed for further cultivation or breeding improvement. It is also necessary to answer the question how *Selaginella* adapt to environmental changes, and survive for millions of years.

Taxonomy of *Selaginella* can also be strengthened using variability of natural product (secondary metabolite). *Selaginella* has varied pigmentation depends on environmental and intrinsic factors, which especially reflects diversity of natural products. Main secondary metabolite of *Selaginella* is biflavonoid, which is expected to be able to give contribution to taxonomy (chemotaxonomy). This study can also become scientific base to traditional usage of *Selaginella* as traditional medicine and its development as modern drug.

CONCLUSION

Nusantara *Selaginella* has a very high diversity and is prospective natural resources, but research on this species is very limited. A large number of species believed to still deposite in nature, waiting to be discovered. To preserve and reveal the efficiency of this plant, it is necessary to do thorough biosystematics research, accompanied by profound ethnobotany (ethnopharmacology) research. By showing the obvious economic benefits, it is expected to meet the conservation demands to maintain the sustainability of this biological resource.

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