

博士論文

論文題目

**Phylogenetic analysis of pitcher plants *Nepenthes* with molecular evolution of the key digestive enzyme nepenthesin**

(食虫植物ウツボカズラの系統解析と消化酵素ネペンテシンの分子進化)

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## Abstract

### Introduction

*Nepenthes* is a genus of carnivorous plant that belongs to the monotypic family Nepenthaceae (Jebb & Cheek, 1997). The genus *Nepenthes* currently contains 120 species, which are primarily distributed across Southeast Asia. Molecular phylogenetic analysis of this genus had been performed by Meimberg *et al.* (2001) based on the DNA sequence of the plastid *trnK* intron. However, subsequent studies using translocated copies of *trnK* intron and PTR1 low copy gene as phylogenetic markers, demonstrated a topological incongruence; thus, it was considered that the phylogeny of the *trnK* intron did not actually represent the true phylogeny of *Nepenthes* (Meimberg *et al.*, 2006; Meimberg & Heubl, 2006). Therefore, further phylogenetic study of this genus is required to clarify its evolutionary trends and to establish its infra-generic taxonomy.

*Nepenthes* specifically evolved to inhabit marginal environments with nutrient deficiency (Ellison, 2006; Osunkoya *et al.*, 2007; Schultze *et al.*, 1997). *Nepenthes* have adapted the trapping process to live and grow in rainy tropical habitats (Di Giusto *et al.*, 2008; McPherson, 2009). They augment their nutrient uptake by carnivory with their pitchers (McPherson, 2009). Morphological adaptations, including wetness-dependent peristome, encourage the prey to fall into the pitcher (Bauer & Federle, 2009; Bauer *et al.*, 2011; Bohn & Federle, 2004; Gaume & Di Giusto, 2009). Then the body of the prey is drowned by the pitcher fluid (Di Giusto *et al.*, 2008; Gaume & Forterre, 2007) and subsequently digested by enzymes within the pitcher fluid (McPherson, 2009).

Nepenthesins are major constituents of *Nepenthes* digestive enzymes (Hatano & Hamada, 2008). Athauda *et al.* (2004) purified nepenthesin I and II from *N. distillatoria*, and determined their partial internal sequences. However, the molecular genomic study of nepenthesin-encoded genes, in addition to their structure-function relationships, have never been conducted. The structure of nepenthesin-encoded genes might be related to the functioning of genes in response to environmental stress, as the habitat characteristics where *Nepenthes* live. Therefore, the objectives of my study are to examine the molecular phylogeny of *Nepenthes* with the molecular evolution of the *Nep2* gene, which expresses the nepenthesin II digestive enzyme in pitcher fluid.

### Phylogenetic analysis of Nepenthaceae based on ITS nrDNA sequences

In total, 57 ITS sequences from 56 *Nepenthes* species were analyzed. Phylogenetic analysis were conducted using 4 different methods: Maximum parsimony, Bayesian analysis, Neighbor Joining, and Maximum Likelihood. Bootstrap values were calculated from 1000 replicates. Character states for distribution areas were mapped onto 1 of the MPTs by using the MacClade program (v4.06), with ACCTRAN optimization (Maddison & Maddison, 2003), to study the phytogeography of *Nepenthes* in Southeast Asia. In addition, the character states for the peristome of upper pitchers were mapped to study the evolutionary trends of morphological characteristics of the genus *Nepenthes*.

Using *A. robertsoniorum* and *D. muscipula* as outgroups, 2 basal branches and 8 subclades were recognized in the phylogenetic tree. These 8 subclades were designated subclades I–VIIB, and were supported by bootstrap values more than 80%, except subclade II which supported by bootstrap values of 61%, 66%, and 62% (MP/NJ/ML). The Bayesian posterior probability of each subclade was 1.00, except for subclade I, which had a Bayesian posterior probability of 0.99.

The 2 basal taxa were *N. pervillei* from Seychelles and *N. madagascariensis* from Madagascar. Subclade I comprised species distributed in New Guinea and outlying areas (Australia, India, and Sri Lanka); and a widely distributed species. Subclade II contained a species from New Caledonia, a species from Misool Island, and 2 widely distributed species. Subclade III consisted of a species endemic to Sulawesi and a species distributed in Sulawesi and Borneo. Subclade IV and V, both contained species that were restricted to the Philippines and a species endemic to Borneo at the basal position. Subclade VI contained only species from Borneo. Subclade VIIA comprised exclusively 4 species from Peninsular Malaysia and Indochina, a species endemic to Borneo, and a species distributed in Borneo and Sumatra at the basal position. Subclade VIIB comprised 13 *Nepenthes* species from Sumatra with 1 species that was also distributed in Java.

There were positional differences of species between the present ITS analysis and previous *trnK* intron analysis (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006) that may be caused by chloroplast capture phenomenon (Meimberg & Heubl, 2006; Tsitrone *et al.*, 2003),

concerted evolution (Wendel *et al.*, 1995a), and intergenomic introgression (Wendel *et al.*, 1995b) of nrDNA genes. Judging from the distribution patterns and phylogenetic relationships, it is possible that *Nepenthes* have expanded from the eastern region to the western region of Southeast Asia. The migration of *Nepenthes* may be influenced by different climates since the western region has relatively higher annual precipitation and wetter conditions than the eastern region (Heaney, 1991; McPherson, 2009). The tree topology suggested that *Nepenthes* had diverged in Borneo, resulting in the evolution of many local species and could have also migrated to adjacent areas. Therefore, Borneo could be a secondary center of diversification for *Nepenthes* in the Sunda Shelf of Southeast Asia.

The evolutionary trends of the peristomes in this genus with 3 character states: narrow (<10 mm), intermediate (10–20 mm) and broad (>20 mm), suggested that the narrow peristome is the plesiomorphic state in the genus *Nepenthes*. The intermediate and broad peristomes likely evolved at least 7 and 8 times, respectively, in this genus, whereas the narrow peristome evolved again at least 4 times after the widening of the peristome. These results suggested that repeated evolution of these 3 peristome characteristics occurred in this genus. Judging from the tree topology, the evolutionary trends of the peristomes revealed the limitedness of Danser's system of classification for *Nepenthes*.

### **Molecular and adaptive evolution of the *Nep2* gene from the carnivorous plant *Nepenthes***

In the present study, 29 DNA sequences of *Nep2* gene have been successfully amplified for the first time. The *Nep2* genes from 29 *Nepenthes* species varied in length between 1314–1317 bps, but mostly 1317 bp. All of the DNA sequences of the *Nep2* genes show no introns. Intronless *Nep2* gene will produce its protein product rapidly for digesting the trapped prey. This rapid production of nepenthesin II enzyme may help to avoid putrefaction of trapped prey, which resulted in an accumulation of ammonium that may harm the pitcher to die (McPherson, 2009). Thus, *Nep2* gene is supposed to have adapted specifically to produce nepenthesin II digestive enzymes rapidly by removing its introns during the course of molecular evolution. All of the enzyme of nepenthesin II examined contain 12 cysteine residues which would form 6 disulphide bonds expected to contribute greatly to the stability of the enzyme (Athauda *et al.*, 2004) and allows the protein to be resistant to protease degradation (Selitrennikoff, 2001). These structures suggest that nepenthesin II enzyme can remain in the pitcher fluid without digestion (Hatano & Hamada, 2008), which is indicated by the 85% of the original activity after 30 days at pH 3 (Athauda *et al.*, 2004).

In this study, *Nep2* gene suggestively experienced significant positive selection on *N. sumatrana*, a species inhabits the lowest altitude habitats (0-800 m) amongst Sumatran endemic species, which most of them are highland species (McPherson, 2009). Adaptation to lowland habitats in Sumatra would be influenced by multiple physiological factors. For instance, at the physiological level, an obvious adaptive phenotype is the development of unusual 2 types of lower pitchers of *N. sumatrana*. The function of both types of lower pitchers are to trap creeping insects (McPherson, 2009). Most of the nitrogen sources of some *Nepenthes* species inhabit lowland habitats were provided by ants (Adam, 1997; Bazile *et al.*, 2012; Moran & Moran, 1998; Moran *et al.*, 2001). Therefore, the 2 distinct types of lower pitchers of *N. sumatrana* are mirrored by the strategy employed to obtain nutrients from the trapped prey, and the abundance of nutrient uptake should be correlated to the fitness of the species in the lowland habitats.

### **Conclusion**

*Nepenthes* are highly adaptable plants and actively respond to the environmental conditions and availability of the prey in their habitats, by changes in phenotypic and genotypic characteristics. These changes are correlated to the fitness of the plants and are supposed under strong selective pressure of nutrient and environment that characterize the habitats of *Nepenthes* and would influence the distribution of *Nepenthes* species in Southeast Asia.

# Chapter I

## General Introduction

### *Pitcher plants of the world*

Pitcher plants are carnivorous, exhibit leaf extensions which have evolved to attract, trap, and digest prey for nutritional uptake (Clarke, 2006; McPherson, 2009). The products of digestion are then absorbed through the walls of the pitchers and transported to other parts of the plant to support growth and reproduction (Clarke, 2006; Moran *et al.*, 2001; Schultze *et al.*, 1997). Unlike some other carnivorous plants, pitcher plants do not have any moving parts. Instead, they rely upon the slippery surfaces and pitcher fluid to trap their prey (Bauer & Federle, 2009; Bauer *et al.*, 2011; Bohn & Federle, 2004; Clarke, 2006; Gaume & Di Giusto, 2009).

The pitcher plants consist of 7 genera, which are distributed throughout parts of the Americas, Asia, and Australia. Of the 5 American genera, 2 are bromeliads (1 species of *Catopsis* and at least 1 species of *Brocchinia*), while the remainder all belong to the family Sarraceniaceae (*Heliamphora* from Venezuela and Guyana; *Sarracenia* and *Darlingtonia*, both from north America) (Clarke, 2006). The monotypic *Cephalotus* (Cephalotaceae) grows only in south-western Australia, whereas the largest genus, *Nepenthes* (monotypic family Nepenthaceae) is distributed from northern Australia throughout Southeast Asia to southern China (Clarke, 2006; McPherson, 2009). Outlying species of *Nepenthes* occur in Sri Lanka, India, the Seychelles, Madagascar, and New Caledonia, while the vast majority occur on the islands of Borneo and Sumatra (Clarke, 2006, 2007; Krutzsch, 1988; McPherson, 2009).

Pitcher plants, and *Nepenthes* in particular, include the largest of all carnivorous plants (Clarke, 2006; McPherson, 2009). Although the smallest pitchers on mature plants may be less than 2 ml in volume, the largest may exceed 2000 ml (Clarke, 2006). Just as existence of a great variety in pitcher size and shape, considerable range of prey for pitcher plants trap. The commonest prey are ants (Adam, 1997; Bazile *et al.*, 2012; Moran & Moran, 1998; Moran *et al.*, 2001), but many other types of insects are trapped (Bauer *et al.*, 2008; Bohn & Federle, 2004; Gaume *et al.*, 2002), and some of the larger pitchers may even trap vertebrates such as rats and lizards (Clarke, 2006; McPherson, 2009). No other carnivorous plants can boast catches of this size (Clarke, 2006).

Since *Nepenthes* are the most spectacular of all carnivorous plants with largest traps and the only carnivorous plants that mainly distributed in Southeast Asia with outlying distribution areas range as far as Madagascar in the western, India in the northern, and New Caledonia in the eastern, the study of this genus will give valuable information of their mechanism to digest their prey and their phytogeography in Southeast Asia.

### ***Phylogenetic relationship of Nepenthes***

The genus *Nepenthes* currently contains 120 species, which are primarily distributed across Southeast Asia, particularly on the islands of Sumatra (with 37 species, of which 29 species are endemic), Borneo (with 36 species, of which 29 species are endemic), and the Philippines (with 21 species, of which 20 species are endemic) (McPherson, 2009). Molecular and phytochemical data, along with certain anatomical features, show that *Nepenthes* is closely related to sticky carnivorous plants (such as *Aldrovanda*, *Drosera*, *Drosophyllum*, and *Triphyophyllum*), which belong to the families of Droseraceae and Dioncophyllaceae (Ellison & Gotelli, 2001; Heubl *et al.*, 2006;

McPherson, 2009).

Molecular phylogenetic analysis of this genus had been performed by Meimberg *et al.* (2001) based on the DNA sequence of the plastid *trnK* intron. On the basis of the results of this study, the authors assumed that the colonization of *Nepenthes* in Southeast Asia initiated in India, since the Indian endemic species *N. khasiana* is a sister taxon to all Asian *Nepenthes* taxa. However, subsequent comparative analysis between the *Nepenthes trnK* intron and its translocated copies demonstrated a topological incongruence; thus, it was considered that the phylogeny of the *trnK* intron might be misleading, and that did not actually represent the true phylogeny of *Nepenthes* (Meimberg *et al.*, 2006; Meimberg & Heubl, 2006). Meimberg & Heubl (2006) then introduced PTR1 (peptide transferase 1), a nuclear low copy gene, as a phylogenetic marker; however, this gene still had weaknesses, with certain taxa holding different positions in the tree topology compared to previous works. Therefore, the origin and diversification of the genus *Nepenthes* remained unclear (Meimberg & Heubl, 2006); consequently, further phylogenetic study of this genus is required to clarify its evolutionary trends and to establish its infra-generic taxonomy.

### ***Plant structure***

*Nepenthes* are perennial shrubs or lianas that exhibit variable structure, shape, and size among species (Albert *et al.*, 1992; McPherson, 2009). In particular, the leaves of *Nepenthes* species are highly divergent. All leaves of healthy *Nepenthes* plants may bear pitchers that serve as the traps into which animal prey or detritus may fall and be subsequently digested (Clarke, 2006; McPherson, 2009). The pitchers develop from the tendril, which is an extension of the leaf midrib that appears from the tip of the lamina. The pitchers are hollow, watertight vessels. The pitcher opening is called an orifice, and is

surrounded by a peristome, which is a stiff structure with an inwardly curved rim (Clarke, 2006; McPherson, 2009).

All *Nepenthes* species produce two types of pitchers called lower and upper pitchers, except for *N. campanulata*, which produces just 1 type of pitcher. Young *Nepenthes* plants initially produce lower pitchers, which are also called terrestrial pitchers. The lower pitchers develop from a tendril, which attaches to the front of the pitcher on the same side as the orifice. Pitcher shape changes as the climbing stem is produced by older *Nepenthes* plants. The pitchers produced from leaves along this climbing stem are the upper pitchers, in which the tendril attaches to the back of the pitcher on the side where the lid meets the rear of the orifice (Di Giusto *et al.*, 2008; Gaume & Di Giusto, 2009; McPherson, 2009; Moran, 1996). The morphology of lower and upper pitchers are presented in Fig. I-1 and Fig. I-2, respectively.

### ***Habitat types and altitudinal distribution of Nepenthes***

*Nepenthes* inhabit a wide range of habitat types with nutrient poor soils. McPherson (2009) introduced 9 habitat types of *Nepenthes*, including lowland evergreen rainforest, peat swamp forest, heath forest, cliff habitats, mangrove forest, montane forest and scrub, seasonally dry sandfields, seasonally dry grasslands, and degraded and recovering habitats. Most *Nepenthes* species occur in more than one suitable habitat type. The plant may grow terrestrially on the ground, climb on adjacent vegetation as epiphytes, or live as lithophytes on bare rock or cliff edges (Clarke, 2006; Jebb & Cheek, 1997; McPherson, 2009).

Based on their altitudinal distribution, *Nepenthes* are generally divided into two groups, namely lowland and highland species. The lowland species generally distributed from sea level to 1000 m altitude, whereas the highland species occur above 1000 m



altitude. Lowland *Nepenthes* live in environments with warm to hot daytime and nighttime temperature, lower precipitation and humidity than the highland regions, mild winds and grow vigorously in direct sunlight. While highland *Nepenthes* commonly grow in environments with colder temperature, higher precipitation and humidity than the lowland species, tolerant with shaded conditions by canopy or clouds would be required (Clarke, 2007; McPherson, 2009).

### ***Anatomical adaptations and trapping process***

Most *Nepenthes* species live in perhumid regions with high precipitation and high humidity, which facilitates the optimal growth of *Nepenthes* plants (Bauer *et al.*, 2009; Gaume & Forterre, 2007). All *Nepenthes* species may survive brief drought conditions for up to a few days (McPherson, 2009) by acquiring moisture from the soil (Damayanti *et al.*, 2011) and from fluid inside the pitchers (McPherson, 2009). Extended periods of drought are expected to have lethal effects on all *Nepenthes* species (Kruttsch, 1988). However, some species from Indochina (*N. kampoiana*, *N. kongkandana*, and *N. smilesii*) have the ability to survive in seasons of low precipitation in a given year by developing specialized water storage organs in their roots (Cheek & Jebb, 2009; McPherson, 2009).

*Nepenthes* have adapted the trapping process to live and grow in rainy tropical habitats (Di Giusto *et al.*, 2008; McPherson, 2009). Their trapping process evolving from sticky carnivorous plants (Albert *et al.*, 1992; Gaume & Di Giusto, 2009; McPherson, 2009). The sticky leaves covered by stalked mucilaginous glands transformed into pitchers, in which the glands are sunken into watertight pitchers on the surface to protect and retain the digestive enzymes from being washed away by rainwater. This feature allows *Nepenthes* to live in habitats with high precipitation (Di Giusto *et al.*, 2008; Bonhomme *et*

*al.*, 2011). Subsequently, the pitcher-pitfall trap replaced the sticky-flypaper trap; however, similar glands were retained as shown by the comparison of *Nepenthes* and *Drosera* (Albert *et al.*, 1992; Heubl *et al.*, 2006).

Pitchers of most *Nepenthes* species have a well-developed peristome, the surface of which is characterized by a highly regular microstructure composed of first and second order radial ridges (Bauer & Federle, 2009). The microstructure, combined with hydrophilicity, renders the peristome completely hydrophylic (i.e., wettable) compared to most other plant surfaces (Bohn & Federle, 2004). Water droplets spread rapidly, and form homogeneous thin films under humid conditions (Bauer & Federle, 2009), which make the peristome extremely slippery for insects (Bauer *et al.*, 2008; Bohn & Federle, 2004; Gaume *et al.*, 2002). When the peristome is wet, the fluid film prevents the tarsal adhesive pads of insects from making close contact with the surface, which is similar to the aquaplaning of a car tyre on a wet road (Bauer *et al.*, 2008; Bauer & Federle, 2009).

The pitchers of *Nepenthes* resemble the corolla of flowers with their system of attracting insects (Biesmeijer *et al.*, 2005; Di Giusto *et al.*, 2010; Ellison & Gotelli, 2001; Joel *et al.*, 1985; Joel, 1988). The attraction system of *Nepenthes* pitchers exhibits clear floral traits, including nectar, fragrance, and visual orientation cues to a nectar source on the peristome. These traits are used by *Nepenthes* as baits to attract prey (Moran & Clarke, 2010). Once the prey has been attracted to the bait, it then falls down into the pitcher fluid, which drowns the prey body, often digesting it in a few hours through the combined effects of enzymes, infauna, and fluid acidity (Clarke, 2007).

### ***Digestive enzymes of pitcher fluid***

The digestive enzymes of pitcher fluid are secreted from specialized glands located

on the interior surface of the pitcher (McPherson, 2009). Many enzymes have been identified from *Nepenthes* pitcher fluid, including lipases (Tokes *et al.*, 1974), acid and alkaline phosphatases, phosphoamidases, esterases (Higashi *et al.*, 1993) chitinases, thaumatin like-protein,  $\beta$ -1,3-glucanase,  $\beta$ -D-xylosidase, and nepenthesin I and II (Hatano & Hamada, 2008). Nepenthesins are proteases, which are major constituents of *Nepenthes* digestive enzymes (Hatano & Hamada, 2008). Athauda *et al.* (2004) purified nepenthesin I and II from *N. distillatoria*, and determined their partial internal sequences. The authors first cloned the cDNAs, and then deduced the complete amino acid sequences of the enzymes from *N. gracilis*.

However, the molecular genomic study of nepenthesin-encoded genes, in addition to their structure-function relationships, have never been conducted. The structure of nepenthesin-encoded genes might be related to the functioning of genes in response to environmental stress, as the habitat characteristics where *Nepenthes* live. The structure of nepenthesin-encoded genes might also be associated with the physiological role of enzymes that have high stability in the pitcher fluid. Therefore, I was interested in studying the molecular evolution of nepenthesin-encoded genes, which express the nepenthesin digestive enzymes in the pitcher fluid.

### ***The aim of the thesis***

The objectives of my study are to examine the molecular phylogeny of *Nepenthes* with the molecular evolution of the *Nep2* gene, which expresses the nepenthesin II digestive enzyme in pitcher fluid. In the present thesis, I first clarify the phylogenetic relationship of *Nepenthes*, and discuss the phylogeography of *Nepenthes* in Southeast Asia, along with the evolutionary trends of the morphological characteristics (i.e., peristome) inferred from the

phylogeny. For the study of molecular evolution of the *Nep2* gene, I present the structural feature of the gene of 29 *Nepenthes* species and discuss the structure-function relationships of the gene. Moreover, I perform test of positive selection on the *Nep2* gene by using statistical analysis based on the relative abundance of synonymous and nonsynonymous substitutions. On the basis of the results of this study, I discuss the correlation between physical properties of nepenthesin II (i.e., isoelectric point) and the number of habitat types that occupied by *Nepenthes* and I also discuss the adaptations of *Nepenthes* to nutrient and environmental stress.

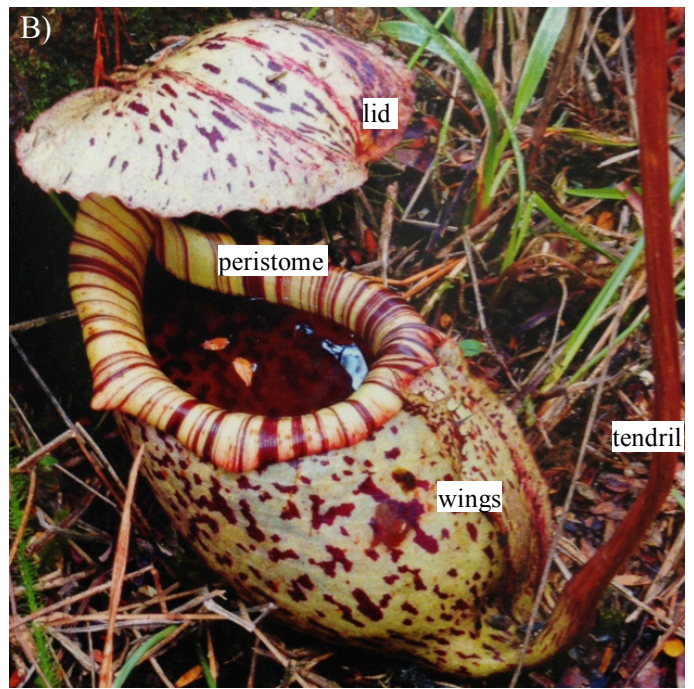
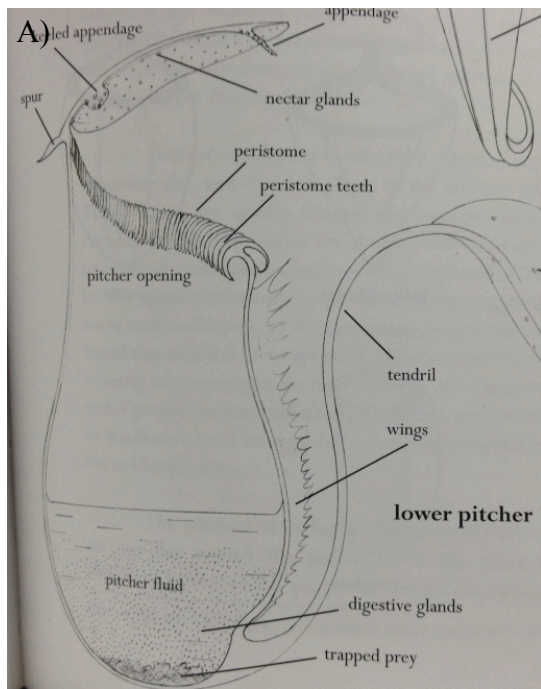


Figure I-1. Morphology of *Nepenthes* lower pitcher. (A) Schematic representation, (B) A lower pitcher of *N. burbidgeae* (McPherson, 2009)

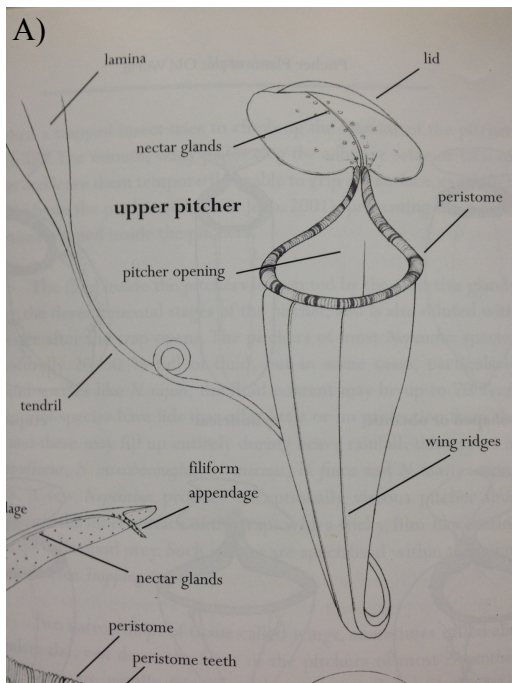


Figure I-2. Morphology of *Nepenthes* upper pitcher. (A) Schematic representation, (B) An upper pitcher of *N. burbidgeae* (McPherson, 2009)

## Chapter II

### Phylogenetic Analysis of Nepenthaceae

#### Based on Internal Transcribed Spacer (ITS) nrDNA Sequences

##### 2-1. Introduction

###### *The monotypic family Nepenthaceae*

Nepenthaceae is a monotypic family of carnivorous pitcher plants comprising the genus *Nepenthes*, which is widely distributed in the Asian tropics, mainly in Southeast Asia and the Sunda Shelf region. The vast majority of species grow in moist regions and are obviously absent from temperate, arid, and polar regions of the world. The distribution area spreads throughout the Old World tropics, as far as Madagascar (*N. madagascariensis* and *N. masoalensis*), Seychelles (*N. pervillei*), and Sri Lanka (*N. distillatoria*) in the western region; India (*N. khasiana*) in the northern region, Australia (*N. tenax* and *N. rowanae*) in the southern region and New Caledonia (*N. vieillardii*) in the eastern region (Clarke, 2007; Krutzsch, 1988; McPherson, 2009). On the islands or mainland where they live, *Nepenthes* are commonly absent from areas with severe seasonal drought, i.e., the north and east Sumatra, south Sulawesi, east Java and the Lesser Sunda islands. It is generally thought that intolerance to drought has limited the distribution of the genus across Madagascar, mainland Asia, and continental Australia (McPherson, 2009).

Although most extant *Nepenthes* species are distributed to the west of the Wallace line, the distinction between Asian and Wallacean species is not clear. Some species, such as *N. ampullaria*, *N. gracilis*, *N. mirabilis*, and *N. tentaculata*, occur in both regions of

Asia and Wallacea (McPherson, 2009). The distribution of the genus has been attributed to biogeographic factors occurring both recently and in the past, including the connection of the Sunda Shelf islands caused by sea level drop and isolation of the islands caused by increased sea levels, changes in global climate, and the ability of *Nepenthes* species to disperse and colonize new habitats (Clarke, 2006; Danser, 1928; McPherson, 2009; Meimberg *et al.*, 2001).

### ***Internal Transcribed Spacer (ITS)***

In the present study, I used nucleotide sequences of the internal transcribed spacer (ITS) to resolve phylogenetic relationships within the genus *Nepenthes*. The characteristics of the ITS region, with its small size, highly conserved flanking regions, and fast evolutionary rate, have made this nuclear ribosomal DNA (nrDNA) sequence a valuable marker for phylogenetic analysis (Baldwin *et al.*, 1995). In addition, ITS sequences from *N. ventricosa* and *N. alata* have been studied to evaluate the potential value of ITS for phylogenetic reconstruction; these studies suggested that the ITS regions of these species have many variable characteristics that are potentially informative for resolving *Nepenthes* phylogeny (Alejandro *et al.*, 2008). However, ribosomal DNA genes are present in high copy numbers and may therefore be subjected to directional concerted evolution (Wendel *et al.*, 1995a) or intergenomic introgression (Wendel *et al.*, 1995b).

The objectives of this study were to clarify the phylogenetic relationships of *Nepenthes* based on ITS nucleotide sequences, to study the phytogeography of *Nepenthes* in Southeast Asia, and to trace the evolutionary trends of taxonomically important characteristics (i.e., peristomes) of the genus.

## 2-2. Materials and Methods

### *Plant materials*

I analyzed 57 samples from 56 *Nepenthes* species in total, representing all geographical areas (Table II-1). Of these species, 7 were found within the last 10 years (Akhriadi *et al.*, 2009; Cheek & Jebb, 2009; Lee *et al.*, 2006; McPherson 2009) and have never been used for phylogenetic studies (i.e., *N. chaniana*, *N. lingulata*, *N. mindanaoensis*, *N. naga*, *N. platychila*, *N. thai*, and *N. vogelii*). Two samples from distantly distributed *N. mirabilis* were also examined. The first was distributed in Bengkulu of Sumatra (named *N. mirabilis1*), and the second was distributed in West Kalimantan of Borneo (named *N. mirabilis2*). *Ancistrocladus robertsoniorum* (Ancistrocladaceae, Genbank: GQ443551) and *Dionaea muscipula* (Droseraceae, Genbank: AB675913) were used as outgroups because these 2 families have been recognized as sister groups to Nepenthaceae on the basis of macromolecular characteristics using nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* sequences (Albert *et al.*, 1992; Cuenoud *et al.*, 2002; Hilu *et al.*, 2003).

### *Amplification and sequencing*

Total DNA was extracted from silica-dried leaf samples with a Qiagen DNeasy Mini Plant Kit (Qiagen) following the manufacturer's protocol. Amplification was performed using a set of primers, AITS1 (5'-AGAAGTCCACTGAACCTTATC-3') and AITS4 (5'-CGCTTCTCCAGACTACAATTC-3'), which are specific for angiosperm, do not co-amplify fungal DNA, and have been used to amplify the ITS region from Ancistrocladaceae (Meimberg *et al.*, 2010). The amplification reaction for the ITS region included Ex-Taq buffer and Ex-Taq DNA polymerase (Takara Bio). The polymerase chain



reaction (PCR) protocol consisted of an initial 90-s predenaturation at 96°C; 30 cycles of 20-s at 96°C (denaturation), 40-s at 58.5°C (annealing), and 40-s at 72°C (extension); and a final 7-min extension at 72°C.

The PCR products were cleaned using the Wizard SV Gel and PCR Clean Up System (Promega) and were used for autocyclus sequencing reactions following the manufacturer's (Beckman Coulter) instructions. Autocycle sequencing products were cleaned by ethanol precipitation. Both forward and reverse sequences were analyzed with a CEQ8000 automated sequencer (Beckman Coulter), using the same primers as for PCR. A set of internal primers, AITS2R (5'-TGCGTTCAAAGACTCGATGG-3') and AITS3F (5'-GAAGAACGTAGCGAAATGCG-3'), was designed to achieve better analysis of the ITS. ITS regions from 7 species samples (*N. ampullaria*, *N. hirsuta*, *N. rowanae*, *N. danseri*, *N. neoguineensis*, *N. papuana*, and *N. tentaculata*) were difficult to sequence directly and were therefore cloned into the pGEM-T Easy vector (Promega) based on the manufacturer's protocol. The recombinant plasmids were sequenced using the same primers as for direct sequencing. Multiple cloning sequences from colonies of each sample were aligned and edited manually, and 1 sequence for each sample was chosen for phylogenetic analysis. The sequence of the ITS region from each sample was used for phylogenetic analysis, and all sequences were deposited in DDBJ/EMBL/Genbank (Table II-1).

### ***Phylogenetic analysis***

DNA sequences obtained from the ITS region were aligned with ClustalX. Phylogenetic analysis involving the maximum parsimony (MP) method was performed using the PAUP (Phylogenetic Analysis using Parsimony) program, version 4.0b10

(Swofford, 2002). Data were analyzed by the heuristic search method with the tree bisection-reconnection (TBR) branch swapping and MulTrees options on and stepwise addition with simple addition sequences using 1 reference taxon (*N. thai*). All of the most parsimonious trees (MPTs) were saved. All characters were equally weighted and unordered (Fitch, 1971), and gaps were treated as missing data. In order to evaluate the internal support of clades, bootstrap analysis (Felsenstein, 1985) was conducted using 1000 replicates in a heuristic search with the TBR branch swapping and MulTrees options off. The number of steps, consistency indices, and retention indices (Farris, 1989) were calculated with 1 of the MPTs in each analysis using the TREE SCORES command in PAUP\*. For comparison, I also performed phylogenetic analysis using 3 different methods.

(1) Bayesian analysis with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) by using the GTR+I+G model, which was selected from the Modeltest (Posada, 2008) as the optimum model for sequence evolution based on AIC criterion. Four chains were run for 1,000,000 generations and were sampled every 100 generations to yield a posterior probability distribution of 10,000 trees. The first 2,500 trees were discarded as burn in. To check whether the MCMC chain was long enough to reach convergence, the trace files resulted from the Bayesian analysis were opened in the Tracer program (v1.5), and their effective sample size (ESS) statistics were calculated for values higher than 100.

(2) The neighbor joining (NJ) method (Saitou & Nei, 1987) with MEGA version 5.05 (Tamura *et al.*, 2011) by using the Tamura 3-parameter model (Tamura, 1992). Bootstrap values were calculated with 1000 replicates.

(3) The maximum likelihood (ML) method with the Treefinder program (Jobb *et al.*, 2004) by using the GTR+G model, which was suggested based on AIC criterion by the “propose model” analysis implemented in the Treefinder program. Bootstrap values were

calculated with 1000 replicates.

### ***Reconstruction of character states***

To study the phylogeography of *Nepenthes* in Southeast Asia, I mapped the character states for distribution areas onto 1 of the MPTs by using the MacClade program (v4.06), with accelerated character transformation (ACCTRAN) optimization (Maddison & Maddison, 2003). In addition, I also mapped the character states for the peristome of upper pitchers to study the evolutionary trends of morphological characteristics of the genus *Nepenthes*. The morphometric data of the peristomes of upper pitchers were taken from McPherson (2009).

All but 1 of the *Nepenthes* species examined in this study showed pitcher dimorphism. *N. campanulata* produced only 1 type of pitchers. However, I still included the data for its pitchers peristomes, since the pitchers developed from leaves further up the stem of mature plants showing tendril attachment similar to typical *Nepenthes* upper pitchers. In this study, I classified peristomes as having 3 character states: narrow (<10 mm), intermediate (10–20 mm) and broad (>20 mm).

For comparison, ancestral state of peristome was also reconstructed by the Bayes Multistate module of Bayes Traits v1.0 (Pagel *et al.*, 2006) with maximum likelihood method. For maximum likelihood estimation, the default setting was used. In addition, The MP ancestral state reconstruction of peristome was conducted by the Mesquite v2.75 (Maddison & Maddison, 2006).

## **2-3. Results**

### ***Molecular phylogeny of Nepenthes***

I obtained 50 ITS sequences by direct sequencing from 49 *Nepenthes* species. Different multiple cloning sequences were obtained from recombinant plasmid sequencing from 2 individual colonies from each of *N. ampullaria*, *N. papuana*, and *N. tentaculata*; 3 individual colonies from *N. neoguineensis*; and 4 individual colonies from each of *N. hirsuta*, *N. rowanae*, and *N. danseri*. Different sequences obtained from each sample were aligned and edited manually, and 1 sequence from each sample was chosen for phylogenetic analysis.

The alignment of 59 entire ITS sequences provided an 859-bp-long matrix. Sequence length variations resulting from insertions and deletions were found among *Nepenthes* species. The aligned ITSs, which comprised a total of 859 characters, contained 261 (30%) constant characters, 222 (26%) parsimony-uninformative variable characters, and 376 (44%) parsimony-informative characters. The analysis resulted in 108 MPTs with a length of 1,416 steps and had consistency (CI) and retention (RI) indices of 0.638 and 0.652, respectively. The strict consensus tree reconstructed with 1000 bootstrap replicates by the parsimony method is shown in Fig. II-1. The trees obtained from the Bayesian analysis, NJ and ML methods were essentially consistent with the tree obtained from the parsimony method. Therefore, I chose the parsimony strict consensus tree as the representative topology for this study.

Using *A. robertsoniorum* and *D. muscipula* as outgroups, I could recognize 2 basal branches and 8 subclades in the phylogenetic tree (Fig. II-1). These 8 subclades were designated subclades I–VIIB. Corresponding to the tree topology, the 2 basal taxa were *N. pervillei* from Seychelles and *N. madagascariensis* from Madagascar. From the bootstrap analysis conducted using the MP, NJ, and ML methods, subclade I was supported by bootstrap values of 89%, 99%, and 92%, respectively. Subclade I comprised species

distributed in New Guinea (*N. papuana*, *N. neoguineensis*, and *N. danseri*); Waigeo Island located near the northwest coast of New Guinea (*N. danseri*); and outlying areas, namely Australia (*N. rowanae*), India (*N. khasiana*), and Sri Lanka (*N. distillatoria*). The subclade also contained a species (*N. ampullaria*) that has a distribution area across Peninsular Malaysia, Sumatra, Borneo, and New Guinea. Subclade II was supported by bootstrap values of 61%, 66%, and 62%, respectively. Subclade II contained a species from New Caledonia (*N. vieillardii*), a species from Misool Island located near the west coast of New Guinea (*N. sp. Misool*), and 2 nonendemic species (*N. mirabilis* and *N. gracilis*) distributed across Borneo, Sumatra, Sulawesi, Peninsular Malaysia, and Indochina, as well as the Philippines, New Guinea, Moluccas, and northern Australia for *N. mirabilis*. Subclade III (bootstrap values of 100%, 100%, and 99%, respectively) consisted of a species endemic to Sulawesi (*N. glabrata*) and a species distributed in Sulawesi and Borneo (*N. tentaculata*). Subclade IV (bootstrap values of 90%, 87%, and 88%, respectively) contained species that were restricted to the Philippines and a species endemic to Borneo (*N. campanulata*) at the basal position. Subclade V had bootstrap supports of 100%, 99%, and 99%, respectively, also consisted of species that were restricted to the Philippines and a species endemic to Borneo (*N. hirsuta*) at the basal position. Subclade VI had bootstrap supports of 85%, 84%, and 85%, respectively, and contained only species from Borneo. Subclade VIIA was supported by bootstrap values of 92%, 86%, and 98%, respectively. This subclade comprised exclusively 4 species from Peninsular Malaysia and Indochina (*N. smilesii*, *N. sanguinea*, *N. alba*, and *N. thai*), a species endemic to Borneo (*N. macrovulgaris*), and a species distributed in Borneo and Sumatra (*N. reinwardtiana*) at the basal position. Subclade VIIB was supported by bootstrap values of 96%, 93%, and 97%, respectively. This subclade comprised 13 *Nepenthes* species from Sumatra with 1 species

(*N. spathulata*) that was also distributed in Java. The Bayesian posterior probability of each subclade was 1.00, except for subclade I, which had a Bayesian posterior probability of 0.99.

### ***Character state reconstruction***

The character state reconstruction of *Nepenthes* for distribution areas and peristomes, based on 1 of the 108 MPTs is presented in Fig. II-2 and Fig. II-3, respectively. Figure II-2 shows the evolutionary trends of distribution areas of *Nepenthes*, which describes the radiation of *Nepenthes* in some islands and the migration of *Nepenthes* to adjacent islands or to the mainland of Southeast Asia. Figure II-3 shows the evolutionary trends of the peristome of the upper pitchers and the comparison between the characteristics of the peristomes with the classification system of the genus by Danser (1928). Figure II-4 shows the ancestral state reconstruction of peristomes with ancestral condition at each node.

## **2-4. Discussions**

### ***Phylogenetic relationships in the genus Nepenthes***

In the present study of ITS DNA analysis (Fig. II-1), *N. pervillei* was found to be the most basal taxon within the genus. This species can be distinguished from all other species by its seeds, which lack the appendages typical of most *Nepenthes* species. In addition, *N. pervillei* also has some unusual characteristics, contrary to the common morphological features of *Nepenthes*, such as nontwining tendrils that emerge slightly from the upper pitcher; similar types of upper and lower pitchers; black, short, ovoid, or truncate seeds; and obconic fruit (Meimberg *et al.*, 2001; McPherson 2009). The basal position of

this species was consistent with that reported in previous studies of *Nepenthes* phylogeny using the *trnK* intron (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006). The second basal taxon of this study, *N. madagascariensis*, was also consistent with the *Nepenthes trnK* intron phylogeny (Meimberg *et al.*, 2001; Meimberg *et al.*, 2006) and with a subsequent study of the *Nepenthes* phylogeny by Meimberg and Heubl (2006), using the *trnK* intron and PTR1 markers. *N. madagascariensis* has the distinctive ability to grow vertically on its own stem up to 1.5 m tall, without intertwining with surrounding vegetation (McPherson, 2009).

In my study, the New Guinean species (*N. papuana*, *N. neoguineensis*, and *N. danseri*) were included in subclade I with species from outlying areas, including Australia (*N. rowanae*), India (*N. khasiana*), Sri Lanka (*N. distillatoria*), and the widely distributed species *N. ampullaria*. The positions of the New Guinean species *N. neoguineensis* and *N. danseri* in the same subclade as species from India (*N. khasiana*) and Sri Lanka (*N. distillatoria*) were contradictory to previous studies of the *Nepenthes trnK* intron phylogeny. *N. neoguineensis* was included in the same subclade as other New Guinean species but was separated from *N. danseri*, as well as from the species *N. khasiana* and *N. distillatoria*, which were distributed in outlying areas. *N. danseri* was indicated as 1 of the basal polytomies, *N. distillatoria* was 1 of the most basal taxa, and *N. khasiana* was positioned between the isolated western species and the Indo-Malayan taxa and was a sister taxon to all other *Nepenthes* taxa that were predominantly distributed in Southeast Asia (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006).

As previously mentioned, the species *N. rowanae* (Australia), *N. khasiana* (India) and *N. distillatoria* (Sri Lanka), found in outlying areas, were included in the same subclade, while 1 species from New Caledonia (*N. vieillardii*) was positioned in different

subclade. This species was included in subclade II with 2 widely distributed species (*N. gracilis* and *N. mirabilis*) and an undescribed species (*N. sp. Misool*). The position of *N. vieillardii* was contradictory to that reported in previous studies of the *Nepenthes trnK* intron phylogeny, where the species was a basal taxon of internal clades (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006). The positions of the widely distributed species *N. ampullaria* in subclade I as well as of *N. gracilis* and *N. mirabilis* in subclade II of the tree topology as determined in my study were also different from those reported in previous studies of the *Nepenthes trnK* intron phylogeny, where these 3 widely distributed species were included in the same subclade as Bornean species (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006).

Despite the different positions of some species in subclades I and II with previous studies of *Nepenthes* phylogeny, these species share similar morphological characteristics including acute leaf apex, orbicular or partly orbicular pitcher lids, and partly cylindrical lower pitcher form, except for *N. ampullaria*, which has a distinctive urceolate shape (McPherson, 2009). Moreover, the pitcher morphology of *N. mirabilis* was shared by 3 species from outlying areas (*N. khasiana*, *N. distillatoria*, and *N. vieillardii*), which similarly lack putative apomorphic characteristics (McPherson, 2009). Another widely distributed species, *N. gracilis* was closely related to an endemic species from Misool Island (*N. sp. Misool*). Due to the lack of information on *N. sp. Misool*, only a few morphological similarities could be observed between these 2 species, including a sessile and decurrent leaf base and small pitchers with narrow peristomes (McPherson, 2009).

The positions of the 2 species included in subclade III, *N. glabrata* and *N. tentaculata*, were in accordance with those reported in previous studies of the *Nepenthes* phylogeny from *trnK* intron analysis as well as PTR1 analysis, where they belong to the



Hamata group (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006). This result suggests that the Hamata group is also supported by another nuclear marker (the ITS region). The position of the Philippine species *N. truncata* in subclade IV separated from other Philippine species (*N. alata*, *N. ventricosa*, *N. burkei*, *N. merrilliana*, and *N. bellii*) in subclade V, was also in accordance with that reported in previous *trnK* intron analysis, where *N. truncata* was also separated into different clade from the other 5 Philippine species (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006).

The inclusion of *N. hirsuta*, a species endemic to Borneo, as the basal and sister taxon to the Philippine species in subclade V, was contradictory with previous *trnK* intron analysis, where *N. hirsuta* was included in a subclade with the widely distributed species (*N. gracilis*, *N. mirabilis*, and *N. ampullaria*) and other Bornean species (*N. mapuluensis* and *N. bicalcarata*) (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006). *N. hirsuta* and the Philippine taxa in subclade V share similar characteristics, including acute or obtuse leaf apex, raceme inflorescence, and lack of an appendage on the lids of lower and upper pitchers (except for *N. alata*, which sometimes bears a triangular-shaped appendage up to 10 mm long on the lower surface of the lids of lower pitchers) (McPherson, 2009).

Seven Bornean species included in subclade VI (*N. veitchii*, *N. clipeata*, *N. stenophylla*, *N. faizaliana*, *N. ephippiata*, *N. fusca*, and *N. burbridgeae*) had positions similar to those reported in previous *trnK* intron analysis, where they were included in the same clade. In contrast, 2 other Bornean species (*N. villosa* and *N. rajah*) were separated together in a different clade (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006). Three species included in subclade VIIA (*N. reinwardtiana*, *N. macrovulgaris*, and *N. sanguinea*) had positions similar to those reported in previous *trnK*

intron analysis, where they were included in the same subclade. The Sumatran species included in subclade VIIB are also had positions similar to those reported in previous *trnK* intron analysis, where they were included in the same subclade (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006).

The positional differences of species between the present ITS analysis and previous *trnK* intron analysis (Meimberg *et al.*, 2001; Meimberg & Heubl, 2006; Meimberg *et al.*, 2006) may be caused by the difference in the markers used. ITS is located in nuclear genome, whereas the *trnK* intron is located in chloroplasts, which are maternally inherited and may lead to the chloroplast capture phenomenon. This phenomenon is considered the main reason for the incongruence of nuclear markers and plastid phylogenies (Meimberg & Heubl, 2006; Tsitrone *et al.*, 2003). In addition, concerted evolution (Wendel *et al.*, 1995a) and intergenomic introgression (Wendel *et al.*, 1995b) of nrDNA genes may also have affected this incongruence.

### ***Distribution of Nepenthes in Southeast Asia***

Danser (1928) proposed that *Nepenthes* originated in the southern hemisphere. The present results, demonstrating recognition of *N. pervillei* from the Seychelles and *N. madagascariensis* from Madagascar as the basal species, along with species from New Guinea and the surrounding islands (Misool and Waigeo islands) and species from the outlying areas (India, Sri Lanka, Australia, and New Caledonia) in subclade I and II (Fig. II-2), are concordant with this hypothesis. This hypothesis was also corroborated by the discovery of an ambiguous pollen from the Kerguelen islands located in the South Indian Ocean; this pollen appears very similar to *Nepenthes* pollen, but was classified as *Droseridites spinosus* (Meimberg *et al.*, 2001).

The widely distributed species *N. ampullaria* had a close relationship with the New Guinean species *N. neoguineensis* in subclade I. Other widely distributed species, i.e., *N. gracilis* and *N. mirabilis* (represented by 2 species that were distantly distributed in West Kalimantan of Borneo and Bengkulu of Sumatra), also had a close relationship with an undescribed species from Misool Island (*N. sp. Misool*) in subclade II, which included a species endemic to New Caledonia (*N. vieillardii*) as the basal taxon in this subclade. Judging from these distribution patterns and phylogenetic relationships, it is possible that these 3 widely distributed species may have expanded from the eastern region to the western region of Southeast Asia. This hypothesis was corroborated by similarities in the morphology, altitudinal distribution, and habitat types of these 3 widely distributed species, which were shared by species from New Guinea, Australia, and New Caledonia (McPherson, 2009). Moreover, the existence of *N. gracilis* and *N. mirabilis* in Sulawesi could support a relationship between species from Sulawesi and New Guinea and a relationship with Australian species since *N. mirabilis* is also distributed in northern Australia.

The 2 species distributed in Sulawesi and included in subclade III (*N. glabrata* and *N. tentaculata*) were related to species from the Sunda Shelf of Southeast Asia (Fig. II-2). The existence of *N. tentaculata* in Sulawesi and the Borneo islands suggests an important link for *Nepenthes* species distribution across Southeast Asia from eastern to western regions. The flora of Sulawesi has a close link with the Australian-New Guinean phytogeographic region, which is indicated by the floral exchange between these 3 areas (van Welzen *et al.*, 2011). Therefore, Sulawesi may have been a “cross bridge” for the migration of *Nepenthes* from the eastern region of the Sahul Shelf to the western region of the Sunda Shelf. The migration of *Nepenthes* may be influenced by different climates since

the western region has relatively higher annual precipitation and wetter conditions than the eastern region (Heaney, 1991; McPherson, 2009), especially in monsoon-affected areas with seasonal droughts, including the Lesser Sunda Islands, southern Sulawesi, southern New Guinea, and northern Australia (Heaney, 1991; van Welzen *et al.*, 2011).

The scenario of *Nepenthes* expansion from eastern to western regions of Southeast Asia is different to that of Meimberg *et al.* (2001). They suggested the scenario that *Nepenthes* started colonization to Southeast Asia from an ancient Indian stock to Malay Peninsula, and subsequently to Indochina and the Malay Archipelago, based on their result that the Indian species was sister to the Southeast Asian species, although they suspended the final conclusion. Consequently, the scenario of Meimberg *et al.* (2001) considered that expansion of this genus occurred in an opposite direction, that was from western region to eastern region in Southeast Asia. However, this topology was not supported by the PTR1 tree, where *N. khasiana* made a clade with the species of Sulawesi and Madagascar (Meimberg & Heubl 2006).

Judging from the tree topology (Fig. II-2), *Nepenthes* probably migrated from Borneo to the Philippines. This scenario is supported by the existence of 2 Bornean species, *N. campanulata* and *N. hirsuta*, at the basal positions of subclades IV and V, respectively, which include the Philippine species. The involvement of subclade IV suggested the presence of a migration path through Mindanao, since *N. campanulata*, a species that was originally discovered in east Kalimantan of Borneo (McPherson 2009), was a sister species to 3 species from Mindanao (*N. mindanaoensis*, *N. truncata*, and *N. copelandii*). In contrast, the involvement of subclade V suggested the presence of a migration path through Palawan, since *N. hirsuta* which is distributed in Brunei and Sarawak (McPherson, 2009), was a sister species of a species distributed on all the major

islands of the Philippines (*N. alata*) and other distinctly distributed Philippine species. From Palawan, *Nepenthes* probably migrated to Luzon (*N. ventricosa*) and Mindoro (*N. burkei*), as well as to Mindanao, which was marked by its endemic species, *N. bellii* and *N. merrilliana*. This scenario of *Nepenthes* migration from Borneo to Philippines is also different from that of Meimberg *et al.* (2001), which suggested an opposite direction of migration, that was from Philippines to Borneo.

The tree topology shown in subclade VI–VIIB (Fig. II-2) suggested that *Nepenthes* had diverged in Borneo, resulting in the evolution of many local species and could have also migrated to adjacent areas. In subclade VIIA, 2 Bornean species, *N. reinwardtiana* and *N. macrovulgaris*, were located in the basal position as sisters to Indochina species (*N. reinwardtiana* is also known to be distributed in Sumatra). All the species of subclade VIIB were distributed in Sumatra, and my result suggested that an ancestor species had migrated from Borneo, diverged in Sumatra, and subsequently migrated to Java (*N. spathulata* is also known to be distributed in Java). Judging from the distribution pattern described above, it can be assumed that Borneo could be a secondary center of diversification for *Nepenthes*, allowing *Nepenthes* species to radiate within the Sunda Shelf of Southeast Asia.

### ***Morphology of the peristome***

Some morphological characteristics in *Nepenthes* species are polymorphic, and each of them have more than 1 form or shape within the different species. Thus, it is quite difficult to evaluate the evolutionary trends of morphological characteristics from this genus. In the present study, I have tried to analyze some of these morphological characteristics, such as indumentum, inflorescence, lamina, leaf apex and base, lower and

upper pitcher forms, and the lid and peristome of pitchers. Among these characteristics, I found that the peristomes of the upper pitchers were consistent within a single species, except for those in 4 species (*N. mindanaoensis*, *N. alata*, *N. ovata*, and *N. densiflora*) and were relatively well correlated with the grouping of *Nepenthes* species within the 8 subclades.

The peristomes have been used in taxonomical studies (Danser, 1928) to distinguish between related species (Cheek & Jebb, 2009; Lee *et al.*, 2006; McPherson, 2009; Robinson *et al.*, 2009) and to determine the existence of new species (Cheek & Jebb, 2009; Lee *et al.*, 2006; Robinson *et al.*, 2009). In the taxonomical study by Danser (1928), 6 species groups were proposed based on morphological and phytogeographical features, namely *Vulgatae*, *Montanae*, *Nobiles*, *Regiae*, *Insignes* and *Urceolatae*. *Vulgatae* was thought to represent species with the simplest forms, some of which are widely distributed. *Montanae* was described as having specialized characteristics from *Vulgatae*, with the main distribution being in the mountains of Peninsular Malaysia, Sumatra and Java. *Regiae* consisted of species with uniquely formed pitchers that are mainly distributed in Borneo. *Nobiles* was suggested to have an intermediate morphology between *Montanae* and *Regiae* species. Similar to *Regiae*, *Insignes* was thought to also have uniquely formed pitchers, but with less aberrant morphologies. The last group, *Urceolatae* was distinguished by the presence of urceolate lower pitchers.

Figure II-3 shows the evolutionary trends of the peristomes in this genus. The peristomes of the 2 basal species as well as species included in subclades II, III, and VIIA were all narrow. This may suggest that the narrow peristome is the plesiomorphic state in the genus *Nepenthes*. In addition, most species in subclades I and IV also possessed narrow peristomes. A Philippine species (*N. bellii*) in subclade V and some Sumatran species in

subclade VIIB also had narrow peristomes on their pitchers, while the Bornean species in subclade VI and most species in subclades V and VIIB, as well as 3 species in subclade I (*N. neoguineensis*, *N. ampullaria*, and *N. rowanae*), had intermediate and broad peristomes. The intermediate and broad peristomes likely evolved at least 7 and 8 times, respectively, in this genus, whereas the narrow peristome evolved again at least 4 times after the widening of the peristome. These results suggested that repeated evolution of narrow, intermediate, and broad peristomes occurred in this genus.

In the taxonomical study by Danser (1928), narrow peristomes were defined as an ancestral characteristics of the *Vulgatae* group. This is concordant with the current results suggesting that *N. pervillei* and *N. madagascariensis* were basal species and that the tree topology of subclades I and II consisted of species from the outlying areas (*N. distillatoria*, *N. khasiana*, and *N. vieillardii*), a New Guinean species (*N. papuana*), and 2 widely distributed species (*N. gracilis* and *N. mirabilis*). All these species were assigned by Danser (1928) to the *Vulgatae* group (Fig. II-3). Although *N. neoguineensis* in subclade I possesses an intermediate peristome, Danser (1928) assigned this species into the *Vulgatae* group. Other species with narrow peristomes in 3 different subclades were also assigned by Danser (1928) to the *Vulgatae* group, including *N. tentaculata*, *N. reinwardtiana*, and *N. tobaica*, which were categorized in subclades III, VIIA, and VIIB, respectively. Since representatives of the *Vulgatae* group appeared scattered in different subclades, the group that bears narrow peristomes is obviously polyphyletic. *N. sanguinea*, a Sumatran species with narrow peristomes, was not assigned by Danser (1928) to the *Vulgatae* group. Instead, it was assigned to the *Montanae* group. Thus, the characteristic of narrow peristomes was not sufficient to independently determine the division of *Nepenthes* species.

The intermediate peristome could be defined as a characteristic of the *Nobiles*

group of the *Nepenthes* genus, while the broad peristome could be defined as a characteristic of the *Regiae*, *Insignes* and *Urceolatae* groups (Danser 1928). *N. ampullaria*, which has broad peristomes, was assigned by Danser (1928) to the distinct *Urceolatae* group (Fig. II-3). All the Philippine species included in subclade V, which share intermediate (*N. ventricosa*) and broad peristomes (*N. burkei* and *N. merrilliana*), were assigned by Danser (1928) to the *Insignes* group, while the other Philippine species included in subclade IV (*N. truncata*), which possesses broad peristomes, was assigned by Danser (1928) to the *Regiae* group. The Bornean species included in subclade VI, which possess intermediate and broad peristomes, were assigned by Danser (1928) to 2 different groups: *Insignes* (*N. villosa*) and *Regiae* (*N. rajah*, *N. veitchii*, *N. stenophylla*, *N. clipeata*, *N. ephippiata*, *N. fusca*, and *N. burbidgeae*), whereas the other Bornean species included in subclade V (*N. hirsuta*), which possesses intermediate peristomes, was assigned by Danser (1928) to the *Nobiles* group. Similarly, a Sumatran species included in subclade VIIB (*N. spectabilis*) was also assigned to the *Nobiles* group. Since the representatives of the *Insignes*, *Regiae*, and *Nobiles* groups appeared scattered in different subclades, the 3 groups that shared intermediate and broad peristomes, were obviously polyphyletic. Judging from the tree topology (Fig. II-3), the evolutionary trends of the peristomes in the 3 character states revealed the limitedness of Danser's system of classification for *Nepenthes*.

The ancestral condition for peristomes in *Nepenthes* was reconstructed in different states at each node (Fig II-4). The likelihood of narrow peristome at node 31, 31, 33 and 34 of subclade VIIA exceeded the likelihood of other states. This is concordant to the character states of narrow peristome of species in subclade VIIA. The likelihood of intermediate peristome at node 2 of subclade I; node 7, 8, 9 10 of subclade II; node 12, 13,



and 14 of subclade IV; subclade 15, 16, 17, 18, and 19 of subclade V; and node 39, 41, 42, 43, 44, and 45 of subclade VIIB, exceeded the likelihood of other states. This implies that the character states of peristome of all species in subclade II, subclade IV, subclade V, and most species in subclade VIIB originated from the intermediate peristome. The likelihood of broad peristome at node 4 and 5 of subclade I, and node 37 and 38 of subclade VIIB exceeded the likelihood of other states. This implies that ancestral state of peristome of some species in subclade I and subclade VIIB originated from broad peristome. The ancestral peristome of subclade II (node 10), subclade IV (node 14) and subclade V (node 19) were reconstructed as intermediate peristome (Fig II-4). This implies that the narrow and broad peristomes in those subclades originated from the intermediate peristome.

Table II-1. Plant materials examined in the study of *Nepenthes* phylogeny. The materials were collected from the Indonesian Carnivorous Plant Society (*Komunitas Tanaman Karnivora Indonesia/KTKI*), Bogor Botanical Garden (BBG), Indonesia; and Kyoto Botanical Garden (KBG), Japan.

Species	Source	Voucher	Genbank accession no. of ITS
<i>Nepenthes adnata</i> Tamin & M.Hotta ex Schlauer	KTKI	B11110901	AB675864
<i>Nepenthes alata</i> Blanco	KTKI	C14110901	AB675865
<i>Nepenthes alba</i> Ridl.	KTKI	E05021001	AB675866
<i>Nepenthes ampullaria</i> Jack	BBG	KRB27100901	AB675914
<i>Nepenthes bellii</i> K.Kondo	KTKI	A11110901	AB675868
<i>Nepenthes burbidgeae</i> Hook.f. ex Burb	KTKI	B14110901	AB675869
<i>Nepenthes burkei</i> Mast.	KTKI	E05021002	AB675870
<i>Nepenthes campanulata</i> Sh.Kurata	KTKI	B14110902	AB675871
<i>Nepenthes chianiana</i> C.Clarke, Chi.C.Lee & S.McPherson	KTKI	B11110902	AB675872
<i>Nepenthes clipeata</i> Danser	KTKI	D07110901	AB675873
<i>Nepenthes copelandii</i> Merr. ex Macfarlane	KTKI	E05021003	AB675874
<i>Nepenthes danseri</i> Jebb & Cheek	KTKI	A14110901	AB675915
<i>Nepenthes densiflora</i> Danser	KTKI	C14110902	AB675875
<i>Nepenthes diatas</i> Jebb & Cheek	KTKI	D07110902	AB675876
<i>Nepenthes distillatoria</i> L.	KTKI	E05021004	AB675877
<i>Nepenthes ephippiata</i> Danser	KTKI	A14110902	AB675878
<i>Nepenthes faizaliana</i> J.H.Adam & Wilcock	KTKI	E05021005	AB675879
<i>Nepenthes fusca</i> Danser	KTKI	A14110903	AB675880
<i>Nepenthes glabrata</i> J.R.Turnbull & A.T.Middleton	KTKI	A11110902	AB675881
<i>Nepenthes gracilis</i> Korth.	BBG	KRB27100902	AB675882
<i>Nepenthes hirsuta</i> Hook.f.	KTKI	D07110903	AB675916
<i>Nepenthes khasiana</i> Hook.f.	KTKI	E05021006	AB675883
<i>Nepenthes lingulata</i> Chi.C.Lee, Hernawati & Akhriadi	KTKI	A11110903	AB675884
<i>Nepenthes longifolia</i> Nerz & Wistuba	KTKI	E05021007	AB675885
<i>Nepenthes macrovulgaris</i> J.R.Turnbull & A.T.Middleton	KTKI	B14110903	AB675886
<i>Nepenthes madagascariensis</i> Poir.	KBG	KBG02-0521	AB769064
<i>Nepenthes merrilliana</i> Macfarlane	KTKI	A11110904	AB675887
<i>Nepenthes mindanaoensis</i> Sh.Kurata	KTKI	A14110904	AB675888
<i>Nepenthes mirabilis</i> (Lour.) Druce (Bengkulu, Sumatra)	KTKI	E05021008	AB675889
<i>Nepenthes mirabilis</i> (Lour.) Druce (West Kalimantan)	KTKI	E05021009	AB675890
<i>Nepenthes naga</i> Akhriadi, Hernawati, Primaldhi & M.Hambali	KTKI	D07110904	AB675891
<i>Nepenthes neoguineensis</i> Macfarlane	KTKI	A14110905	AB675917
<i>Nepenthes ovata</i> Nerz & Wistuba	KTKI	B11110903	AB675892
<i>Nepenthes papuana</i> Danser	KTKI	B14110904	AB675918
<i>Nepenthes pervillei</i> Blume	KTKI	E05021010	AB675893
<i>Nepenthes platychlora</i> Chi.C.Lee	KTKI	E05021011	AB675894
<i>Nepenthes rajah</i> Hook.f.	KTKI	D07110905	AB675895
<i>Nepenthes reinwardtiana</i> Miq.	BBG	KRB27100903	AB675896
<i>Nepenthes rhombicaulis</i> Sh.Kurata	KTKI	B14110905	AB675897
<i>Nepenthes rowanae</i> F.M.Bailey	KTKI	A14110906	AB675919
<i>Nepenthes sanguinea</i> Lindl.	KTKI	B14110906	AB675898
<i>Nepenthes smilesii</i> Hemsl.	KTKI	C14110903	AB675899
<i>Nepenthes spathulata</i> Danser	KTKI	D07110906	AB675900
<i>Nepenthes spectabilis</i> Danser	KTKI	A14110907	AB675901
<i>Nepenthes</i> sp. Misool	KTKI	E05021012	AB675902
<i>Nepenthes stenophylla</i> Mast.	KTKI	A14110908	AB675903
<i>Nepenthes sumatrana</i> (Miq.) Beck	KTKI	A14110909	AB675904
<i>Nepenthes talangensis</i> Nerz & Wistuba	KTKI	D07110907	AB675905
<i>Nepenthes tentaculata</i> Hook.f.	KTKI	C14110904	AB675920
<i>Nepenthes thai</i> Cheek	KTKI	E05021013	AB675906
<i>Nepenthes tobaica</i> Danser	KTKI	B11110904	AB675907
<i>Nepenthes truncata</i> Macfarlane	KTKI	E05021014	AB675908
<i>Nepenthes veitchii</i> Hook.f.	KTKI	A14110910	AB675909
<i>Nepenthes ventricosa</i> Blanco	KTKI	A11110905	AB675910
<i>Nepenthes vieillardii</i> Hook.f.	KBG	KBG02-0522	AB769065
<i>Nepenthes villosa</i> Hook.f.	KTKI	B14110907	AB675911
<i>Nepenthes vogelii</i> Schuit. & de Vogel	KTKI	B11110905	AB675912
<i>Dionaëa muscipula</i> J.Ellis ex L.	KTKI	E05021015	AB675913

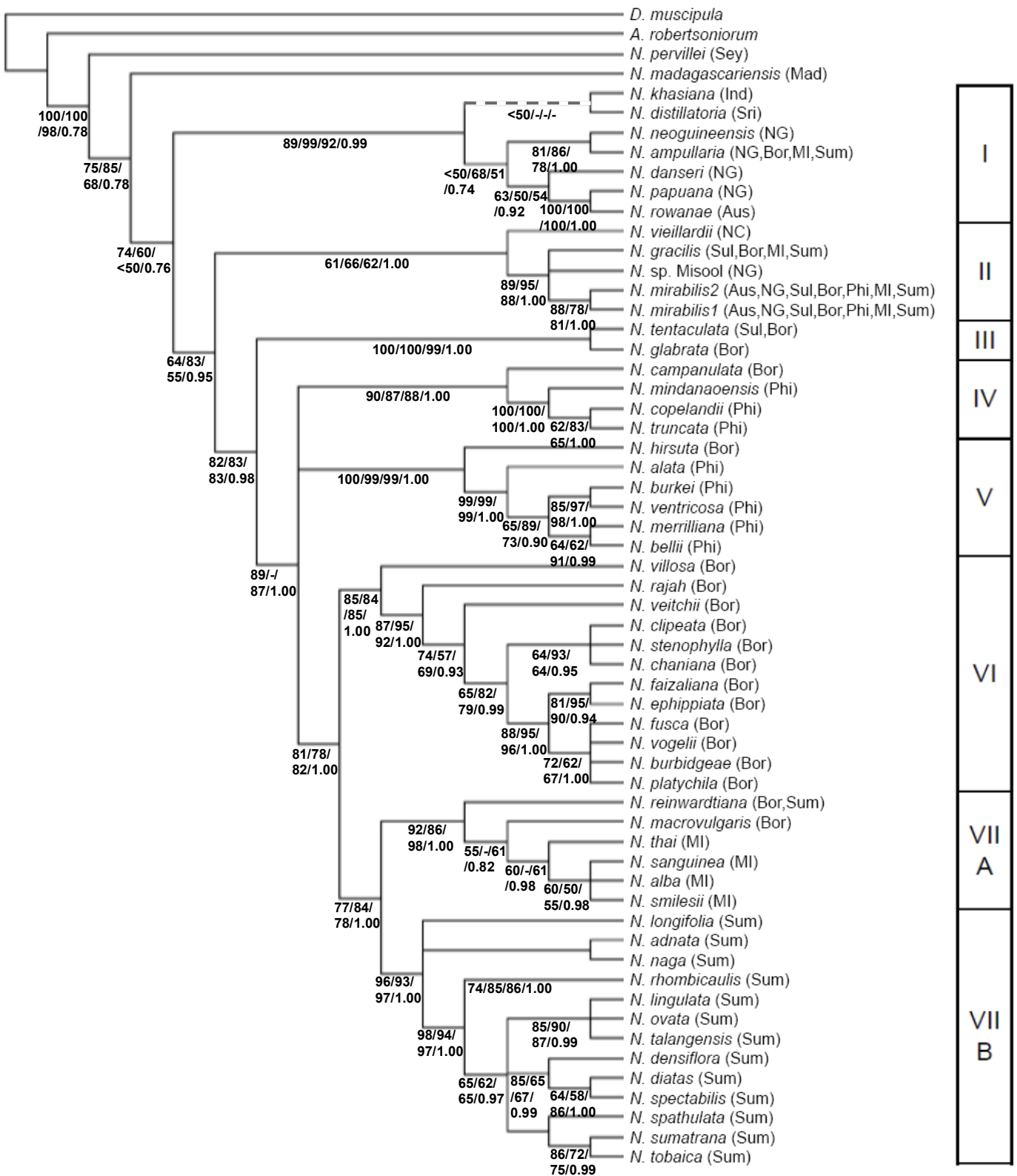


Figure II-1. Strict consensus tree derived from maximum parsimony analysis of ITS sequences for *Nepenthes* and outgroup taxa. Statistical support for each branch is shown below each branch with successive values of MP/NJ/ML/Bayesian. The minus signs (-) on the successive statistical values indicate different topologies or polytomies. Additional information of the distribution area of a species is given as an abbreviation next to the taxon name.

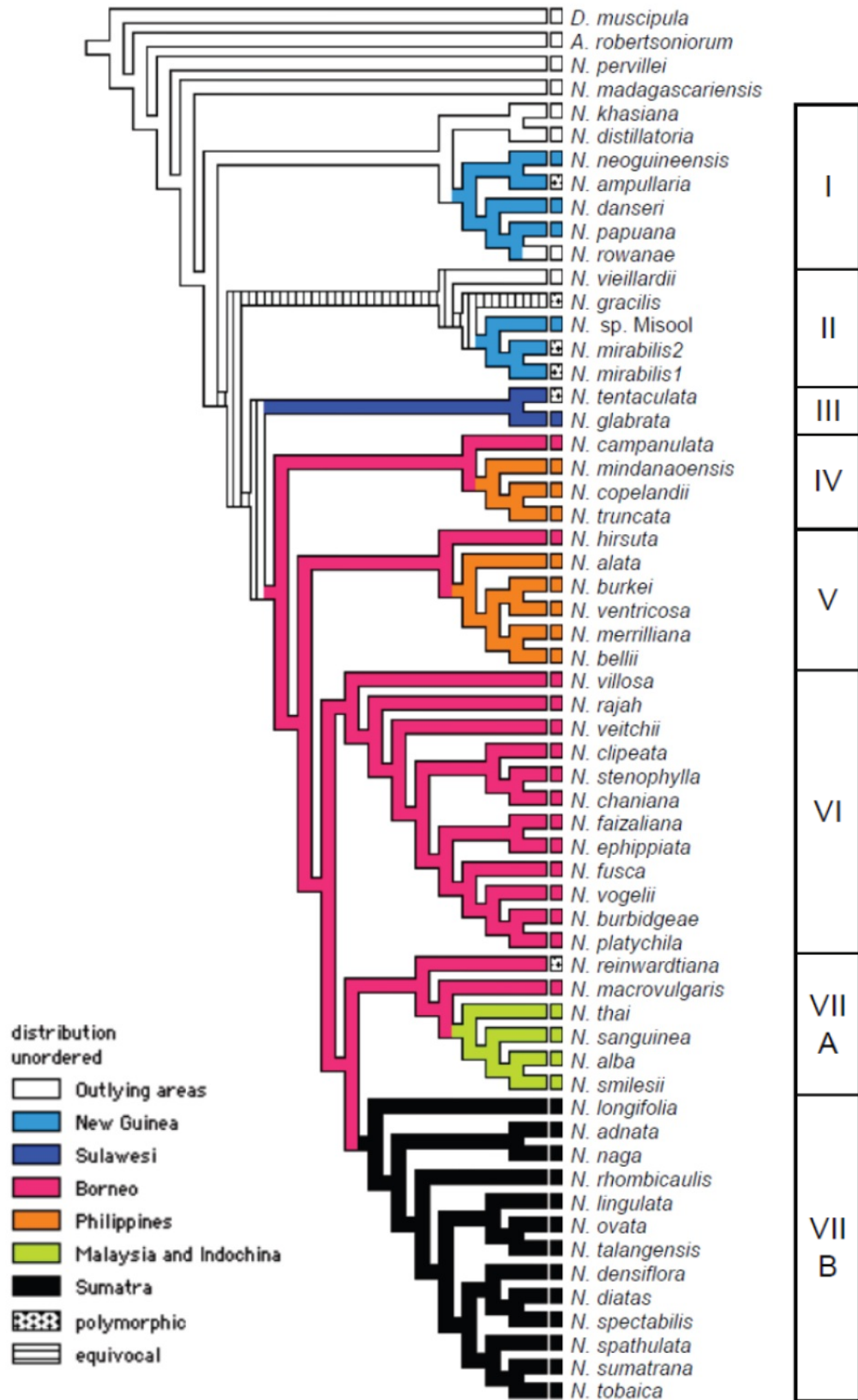


Figure II-2. Character state reconstruction of *Nepenthes* for distribution areas based on 1 of 108 MPTs using MacClade ver. 4.06 with ACCTRAN optimization.

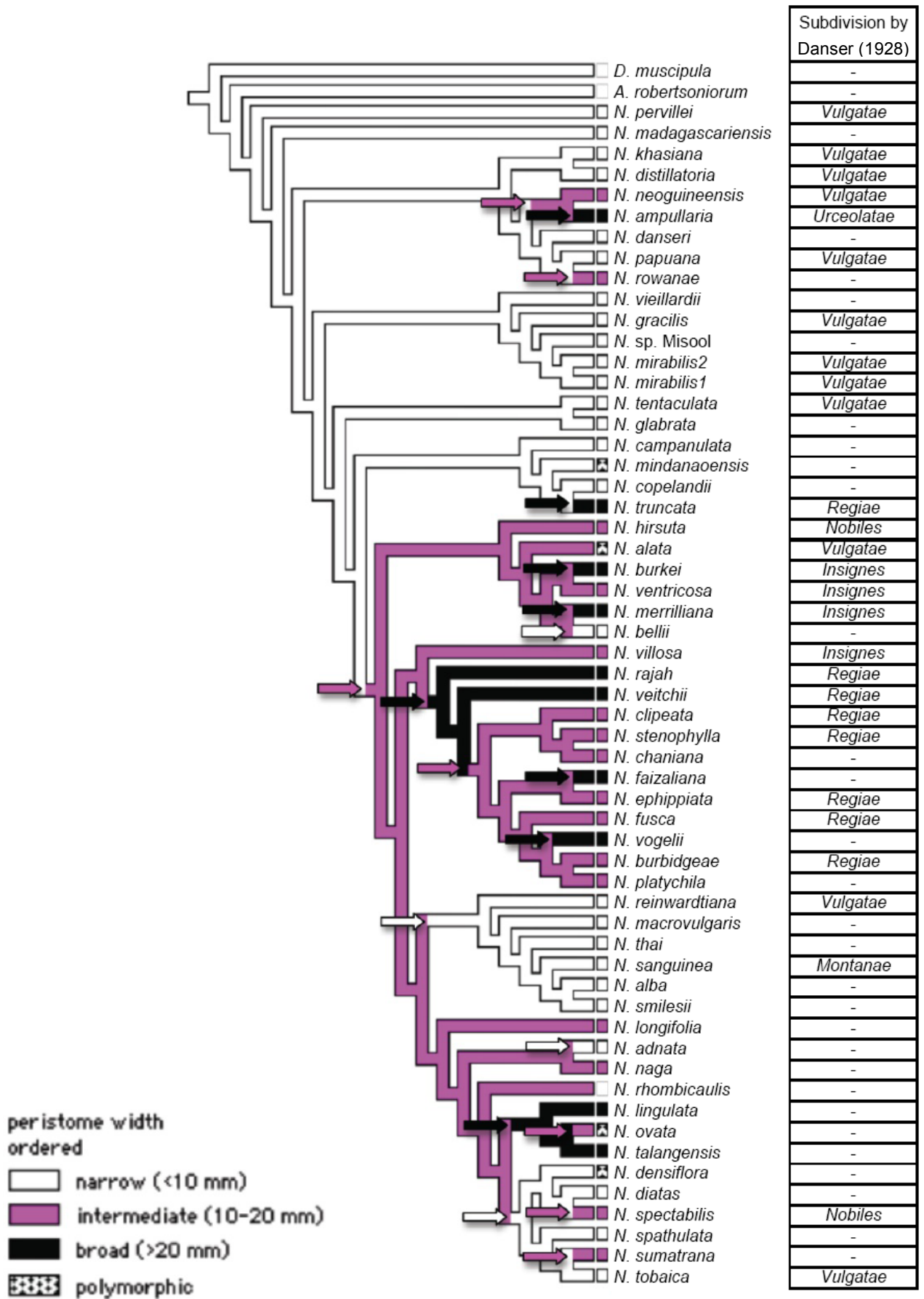


Figure II-3. Character state reconstruction of *Nepenthes* for peristomes based on 1 of 108 MPTs using MacClade v4.06 with ACCTRAN optimization with comparison to the classification system of the genus by Danser (1928). The *arrows* indicate the repeated evolution of narrow, intermediate, and broad peristomes. The characters included in the classification are provided in Appendix 3.

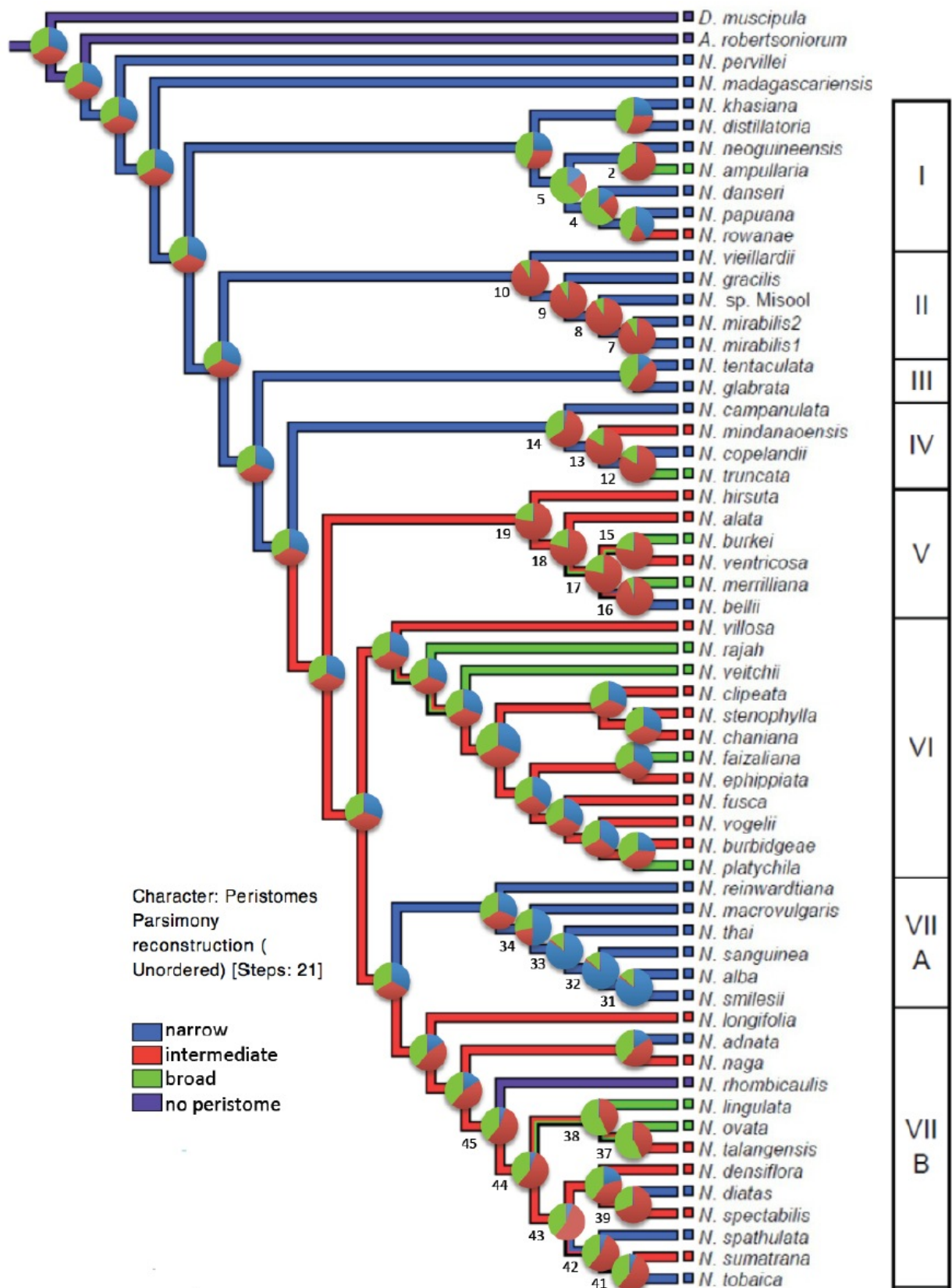


Figure II-4. Ancestral character reconstruction for the evolution of peristomes of genus *Nepenthes* based on 1 of 108 MPTs using Mesquite v2.75 and Bayes Traits v1.0. Pie graphs represent the likelihood of the four peristomes states at each node. Colored boxes represent present-day peristome characteristics of individual *Nepenthes*. Branch colors indicate inferred ancestral peristome characteristics under MP. Numbers represent the node's number.

## Chapter III

### Molecular and Adaptive Evolution of The *Nep2* Gene from the Carnivorous Plant *Nepenthes*

#### 3-1. Introduction

##### *Nepenthesin as the key enzyme for nitrogen uptake*

Carnivorous pitcher plants *Nepenthes* specifically evolved to inhabit marginal environments with nutrient deficiency, particularly nitrogen, phosphorus and potassium (Ellison, 2006; Osunkoya *et al.*, 2007; Schultze *et al.*, 1997), and to exploit niches where non-carnivorous plant species are less equipped to compete successfully. They augment their nutrient uptake by carnivory with their pitchers (McPherson, 2009). Morphological adaptations, including wetness-dependent peristome and slippery wax crystals, encourage the prey to fall into the pitcher (Bauer & Federle, 2009; Bauer *et al.*, 2011; Bohn & Federle, 2004; Gaume & Di Giusto, 2009). Then the body of the prey is drowned by the pitcher fluid (Di Giusto *et al.*, 2008; Gaume & Forterre, 2007) and subsequently digested by enzymes within the pitcher fluid (McPherson, 2009).

*Nepenthes* secrete acid proteinases to digest the protein of their prey that trapped and drowned in their pitcher fluid (Tokes *et al.*, 1974), and absorb the digestion product as a nitrogen source (Moran *et al.*, 2001; Schultze *et al.*, 1997). The acid proteinases inside the pitcher fluid were identified as nepenthesins, which were distinguished into nepenthesin I and II (Athauda *et al.*, 2004). Both enzymes have optimal activity at acidic pH and are most stable at pH 3 (Athauda *et al.*, 2004; Hatano & Hamada, 2008). Moreover, Athauda *et al.* (2004) reported that nepenthesin I and II from *N. distillatoria*

were quite different from each other in properties as they have different molecular masses. They also reported that the activity and stability of both enzymes were different at certain temperature and pH. In addition, they reported that nepenthesin I and II from *N. gracilis* had only 66.6% identity of their amino acid sequences. Despite their differences, these 2 acid proteinases are the only enzymes known to be specialized in prey digestion in the pitcher fluid of *Nepenthes* (Hatano & Hamada, 2008).

### ***Molecular evolution of plant aspartic proteinase (AP)***

The nepenthesin is an aspartic proteinase (AP), making a family of protease enzymes that use an aspartate (Asp) residue for catalysis of their peptide substrates (Athauda *et al.*, 2004). Aspartic proteinases are found widely in plants (Kervinen *et al.*, 1999; Simoes & Faro, 2004) and other living organisms including animals, fungi, bacteria and viruses (Davies, 1990; Rawlings & Barret, 1995). In plants, APs are distributed in seeds, leaves and flowers (Mutlu & Gal, 1999), as well as in the digestive fluid of carnivorous plants and pitcher fluid of *Nepenthes* (An *et al.*, 2002; Athauda *et al.*, 2004; Hatano & Hamada, 2008).

Some plant APs have been purified and well characterized, such as from barley called phytepsin (Roos *et al.*, 1991; Kervinen *et al.*, 1999), and from rice called oryzasin (Asakura *et al.*, 1995). These 2 plant APs were identified as intracellular vacuolar enzymes and shared a plant-specific insertion sequence in the middle of their DNA sequences. In addition, An *et al.* (2002) reported the cloning of AP homologs from the pitcher tissue of *Nepenthes alata* that also belong to the vacuolar aspartic proteinase. These APs contain a so-called plant-specific insertion. In contrast, nepenthesins from *N. gracilis* do not have any plant-specific insertion sequences. Instead, they have a specific insertion found only in



the nepenthesin aspartic proteinase (NAP). Therefore, APs from the pitcher fluid of *Nepenthes* plants are clearly belong to a novel subfamily of APs (Athauda *et al.*, 2004).

The plant AP gene has undergone both the gain and loss of introns during molecular evolution. Rice AP oryzasin 1 gene comprises 14 exons and 13 introns (Asakura *et al.*, 1995). Other plant AP gene from *Fagopyrum esculentum* (Genbank: AM422870), which is in the same order Caryophyllales with *Nepenthes*, also comprises the same number of exons and introns as oryzasin 1 gene. Non-plant AP genes have different composition of intron and exons, for instance, human cathepsin D (Redecker *et al.*, 1991), rat renin (Fukamizu *et al.*, 1988) and bovine chymosin (Hidaka *et al.*, 1986) are all composed of 9 exons and 8 introns. Otherwise, yeast proteinase A gene has no introns (Woolford *et al.*, 1986). Therefore, the molecular evolution of APs as well as their structure-function relationships and physiological roles have become an interested field of study.

In the present study, I have tried to isolate the nepenthesin-encoded genes for the first time to study their molecular evolution and structure-function relationships, and to detect positive selection operates on the gene. For these purposes, I designed universal primers for the amplification of both nepenthesin I and II based on the alignment of sequences of the genes and their homologs available in the Genbank. However, I only succeeded to amplify the nepenthesin II encoded-gene, named *Nep2*. The structure of the *Nep2* gene has affected its protein product as an extracellular proteinase with high stability and rapid secretion, which revealed its molecular evolution. Moreover, the *Nep2* gene has been the target of positive selection during the evolution of *Nepenthes* species growing in the lowest altitude habitats in Sumatra. The study of nepenthesin I-encoded gene (*Nep1*) of *Nepenthes* will be conducted in the near future.

## **3-2. Materials and Methods**

### ***Plant Materials***

I analyzed 29 *Nepenthes* species in total (Table III-1), representing most of geographical areas, including Peninsular Malaysia and Thailand (3 species), Sumatera (12 species), Borneo (7 species), Philippines (3 species), Sulawesi (2 species with species *N. tentaculata* also distributed in Borneo), New Guinea (1 species), and outlying areas (1 species endemic to India) and habitat types.

### ***Amplification and sequencing of Nep2 gene***

Total DNA was extracted from silica-dried leaf samples with a QIAGEN DNeasy Mini Plant Kit (Qiagen) following the manufacturer's protocol. Amplification was performed using 2 pairs of degenerated primers (Table III-2). Ex-Taq buffer and Ex-Taq DNA polymerase (Takara Bio) were used for the amplification of the *Nep2* gene. The polymerase chain reaction (PCR) protocol consisted of an initial 90-s pre-denaturation at 96°C; 40 cycles of 45-s at 96°C (denaturation), 80-s at 58.5°C (annealing), and 70-s at 72°C (extension); and a final 7-min extension at 72°C.

The PCR products were cleaned using Wizard SV Gel and PCR Clean Up System (Promega) and were used for autocycle sequencing reaction following the manufacturer's (Beckman Coulter) instructions. Autocycle sequencing products were cleaned by ethanol precipitation. Both forward and reverse sequences were analyzed with a CEQ8000 automated sequencer (Beckman Coulter), using the same primers as for PCR. Six internal primers (Table III-2) were designed to get better sequences of the *Nep2* gene. The DNA genomic sequence of the *Nep2* gene from each sample was used for phylogenetic analysis, and all sequences were deposited in DDBJ/EMBL/Genbank (Table III-1).

### ***Identification of the Nep2 gene and characterization of nepenthesin II***

To identify the *Nep2* gene, homology searching was conducted using BLASTn program on NCBI server. To characterize the nepenthesins II, DNA sequences of the *Nep2* genes were translated into amino acid sequences using ExPASy translate tool and followed by computation of isoelectric point (pI) of each enzyme on the ExPASy server. The pI is the pH at which a particular molecule or surface carries no net electrical charge (Sakakibara & Yanagisawa, 2007). Other characterization of nepenthesin II including determination of prepro-nepenthesin II form, along with their active sites, cystein residues, N-glycosylation sites, acid and basic residues, and the NAP-specific insertion, that conducted by comparison with nepenthesin II from *N. gracilis* (Athauda *et al.*, 2004).

### ***Phylogenetic analysis***

DNA sequences of nepenthesin-encoded genes were aligned with ClustalX. This multiple sequence alignment consisted of 29 isolated *Nep2* genes, 2 *Nep2* cDNAs of *N. gracilis* (Genbank: AB114915) and *N. mirabilis* (Genbank: JX494402); 3 *Nep1* cDNAs of *N. gracilis* (Genbank: AB114914), *N. mirabilis* (Genbank: JX494401) and *N. alata* (Genbank: AB266803); and 2 *Nep1* cDNAs of *Vitis vinifera* (Genbank: XM\_002272766) and *Ricinus communis* (Genbank: XM\_002522868) as outgroups. The multiple alignment was saved in phylip format. Phylogenetic analysis was conducted using the neighbor joining (NJ) method (Saitou & Nei, 1987) by using maximum composite likelihood model (Tamura *et al.*, 2004) with MEGA version 5.05 (Tamura *et al.*, 2011). Bootstrap values were calculated with 1000 replicates.

### ***Test of positive selection on Nep2 gene***

Testing for positive selection on *Nep2* gene by computing the average number of synonymous and nonsynonymous substitutions were performed with MEGA 5 (Tamura *et al.*, 2011) by using codon-based Z-test of selection based on Nei-Gojobori method (Nei & Gojobori, 1986) for sequence pairs, which involved 29 nucleotide sequences of the *Nep2* genes. All ambiguous positions were removed from each sequence pair.

The probability computed must be less than 0.05 for null hypothesis (strict neutrality) rejection at 5% level, where the number of synonymous substitutions per synonymous site ( $d_S$ ) and the number of nonsynonymous substitutions per nonsynonymous site ( $d_N$ ) are not the same, and the alternative hypothesis is  $d_N > d_S$ , indicates positive selection. The difference in synonymous and non-synonymous substitutions should be significant at the 5% level. For estimating the variance of the difference between  $d_N$  and  $d_S$ , the bootstrap method was conducted with 1000 replications.

## **3-3. Results**

### ***Nep2 genomic DNA sequences***

In the present study, I have succeeded to amplify for the first time the genomic DNA sequences of APs *Nep2* gene for all species examined. All of the amplified DNA sequences are most similar to the aspartic proteinase *Nep2* cDNA of *N. gracilis* and *N. mirabilis* from the Genbank, with identities between 94%–98% and 93%–98%, respectively. The *Nep2* genes of 29 *Nepenthes* species varied in length between 1314–1317 bps, but mostly 1317 bp. Some *Nep2* genes with 1314 bp in length have a deletion in their propeptide (*N. khasiana*) or in enzyme (*N. stenophylla*, *N. tentaculata*, and *N. rajah*). Based on the alignment of 29 *Nep2* gene sequences together with the *Nep2* cDNA

sequences from *N. gracilis* and *N. mirabilis*, all of the DNA sequences of the *Nep2* genes show no introns (Appendix 1).

### ***Nepenthesin II characterization***

The prepro-form of nepenthesin II from 29 *Nepenthes* species, most composed of 438 amino acids, including 24 residues putative signal sequence, 55 residues putative propeptide, and 359 residues enzyme (Fig. III-1). All nepenthesin II contain 12 cysteine residues per molecule protein, 2 active site sequence motifs: aspartic acid-threonine-glycine (D-T-G) and aspartic acid-serine-glycine (D-S-G); as well as the so-called flap tyrosine residue, assigned to residue 96 (Appendix 2). All nepenthesin II appear to have 22 residues of NAP-specific insertion, assigned to residue 70-91, except for *N. stenophylla* that has a deletion within its NAP-specific insertion sequence (Appendix 2). Each nepenthesin II contains 1 or 2 potential N-glycosylation sites (Table III-3). Each nepenthesin II also contains different number of acidic (aspartic acid and glutamic acid) and basic (histidine and arginine) residues. The number of acidic and basic residues are between 28–35 residues and 1–4 residues, respectively (Table III-3).

### ***Evolutionary relationships***

The alignment of 36 nucleotide sequences of *Nep1s* and *Nep2s* provided a final dataset with 1350 positions. The tree inferred from NJ method describes the evolutionary history of *Nep1* and *Nep2* (Fig. III-2). Corresponding to the tree topology, the *Nep1* and *Nep2* of *Nepenthes* species were clearly diverged into different clades. Within the *Nep2* clade, *Nep2* of *N. khasiana* and *N. papuana* were at basal positions with bootstrap supports of 100% and 97%, respectively. The subsequent bootstrap supports of internal branches

within *Nep2* clade were low. The base substitutions per site of internal branches within the *Nep2* clade were also low.

### ***Synonymous and nonsynonymous substitutions***

There were a total of 438 positions in the final dataset of *Nep2*. Table III-4 shows the relative abundance of synonymous and nonsynonymous substitutions that have occurred in DNA sequences of the *Nep2* genes of all sequence pairs by using codon-based Z-test of selection. It shows that the probability (P) of rejecting the null hypothesis of strict-neutrality ( $d_N = d_S$ ) in favor of the alternative hypothesis of positive selection ( $d_N > d_S$ ) with values of P less than 0.05, correspond to several of all sequence pairs involving *N. sumatrana*. This result suggests that the evolution of the *Nep2* gene of *N. sumatrana* has been under positive selection.

In contrast, the Z values calculated from the  $d_N - d_S$  of some sequence pairs involving *N. chaniana*, *N. naga*, *N. spathulata*, *N. stenophylla*, *N. glabrata*, *N. densiflora*, *N. lingulata*, *N. platyphila*, *N. diatas*, and *N. ventricosa*, were positive, indicating positive selection. However, the corresponding P values were above 0.05. This result suggests that the evolution of the *Nep2* genes of that 10 *Nepenthes* species have been under strong purifying selection.

## **3-4. Discussions**

### ***Structural and functional relationships of the Nep2 gene***

In the present study, it is revealed that the *Nep2* genes of 29 *Nepenthes* species have no introns. This is the first report presenting structural feature of nepenthesin-encoded gene from *Nepenthes* species. This structural feature may has relationship with the function of

the gene. Jeffares *et al.* (2008) reported that genes with rapidly changing expression levels in response to environmental stress have significantly lower intron densities in some eukaryotes. They proposed that introns could slow down the regulatory responses and were selected in genes whose transcripts need rapid adjustment for survival from environmental stress.

In some cases, transcription occurs at 1200-1500 nucleotides per minute (Izban & Luse, 1992) with the half-lives for splicing reactions are less than 1 minute for the first intron, but 2–8 minutes for the subsequent introns (Audibert *et al.*, 2002). Consequently, splicing of 2 or more introns need more longer time than the transcription itself (Jeffares *et al.*, 2008). Therefore, intronless *Nep2* gene will produce its protein product rapidly for digesting the trapped prey. This rapid production of nepenthesin II enzyme may help to avoid putrefaction of trapped prey, which resulted in an accumulation of ammonium that may harm the pitcher to die (McPherson, 2009). This result is concordant to the result of immuno-histochemical staining of *Nepenthes* pitcher tissue, which indicated that nepenthesins were directly secreted into the pitcher fluid and functioned without accumulation in the pitcher tissue (Athauda *et al.*, 2004). The rapid production of nepenthesin II enzyme is also corroborated by the small quantity of fluid contained in newly opened pitchers, which usually less than 1/6 of the total volume of the mature one (McPherson, 2009). Thus, *Nep2* gene is supposed to have adapted specifically to produce extracellular nepenthesin II digestive enzymes rapidly by removing its introns during the course of molecular evolution.

As an extracellular proteinase, nepenthesin II of the genus *Nepenthes* is synthesized in the endoplasmic reticulum (ER), travel to Golgi apparatus and then to plasma membrane for secretion (Brooker *et al.*, 2008). This route is known as the secretory pathway (Brooker

*et al.*, 2008). The signal sequence of nepenthesin II is recognized by specific cellular components that facilitate the proper routing of that protein. As synthesized in the ER and secreted via Golgi apparatus and plasma membrane, the signal sequence of nepenthesin II is included in the ER signal type, which is usually located near the amino terminus (Brooker *et al.*, 2008). As an ER signal type, the signal sequence of nepenthesin II is composed of mostly (67%) nonpolar amino acids: methionine (M), alanine (A), valine (V), glycine (G), leucine (L), isoleucine (I), and proline (P) (Figure III-3).

Based on the alignment of 24 amino acid residues of nepenthesin II signal sequences from 29 *Nepenthes* species, there are some substitutions within the signal sequences, including valine (V) to leucine (L) and alanine (A) to glycine (G) (*N. khasiana*), valine (V) to alanine (A) (*N. longifolia*), leucine (L) to glycine (G) (*N. tobaica*), leucine (L) to valine (V) (*N. papuana*), and glycine (G) to alanine (A) (*N. sanguinea*) (Fig. III-3). However, those substitutions of amino acids were expected to have no change of the protein properties, since the substituted amino acids are also nonpolar.

All of the enzyme of nepenthesin II examined contain 12 cysteine residues which would form 6 disulphide bonds expected to contribute greatly to the stability of the enzyme (Athauda *et al.*, 2004). Moreover, the stabilized structure with 6 disulphide bonds allows the protein to be resistant to protease degradation (Selitrennikoff, 2001). These structures suggest that nepenthesin II enzyme can remain in the pitcher fluid without digestion (Hatano & Hamada, 2008), which is indicated by the 85% of the original activity after 30 days at pH 3 (Athauda *et al.*, 2004).

All nepenthesin II of 29 *Nepenthes* species contain an approximately 22 residues of NAP-specific insertion, preceding the flap tyrosine residue (Appendix 2). This insertion contains 4 cysteine residues as well as 4 acidic residues, except for *N. sanguinea*, *N.*



*khasiana*, *N. campanulata*, *N. faizaliana*, and *N. spathulata* that have only 3 acidic residues (Appendix 2). In addition, *N. copelandii* and *N. bellii* have only 2 acidic residues within their NAP-specific insertion (Appendix 2). Overall, the differences of acidic and basic residues number would determine the pI of the enzymes which vary among nepenthesins II, with the highest point reach pH 3.45 (*N. khasiana*) and the lowest one at pH 2.95 (*N. thai*) (Table III-3).

The sequences of NAP-specific insertion of nepenthesins II are not conserved, since some substitutions appeared within the sequence insertions of some *Nepenthes* species (Appendix 2). In contrast, the flap tyrosine residues following the NAP-specific insertions, as well as the 2 active site motifs: aspartic acid-threonine-glycine (D-T-G) and aspartic acid-serine-glycine (D-S-G), are conserved among the 29 nepenthesin II enzymes (Appendix 2).

During the synthesis of prepro-nepenthesin II in the ER and following the travel to Golgi apparatus, the enzyme is attached with carbohydrate in the process of glycosylation (Brooker *et al.*, 2008). Since nepenthesin II has the N-glycosylation motif site(s), the carbohydrate which is oligosaccharide chains, is attached to a nitrogen of asparagine (N) side chains, in the sequence motif of asparagine-leucine-serine (N-L-S) and asparagine-valine-serine (N-V-S) (Blom *et al.*, 2004; Brooker *et al.*, 2008), within the nepenthesin II sequence of most *Nepenthes* species (Appendix 2). In addition, the sequence motif of asparagine-alanine-serine (N-A-S) (Blom *et al.*, 2004; Brooker *et al.*, 2008), which resulted from the substitution of valine (V) to alanine (A), could act as an N-glycosylation site within the nepenthesin II sequence from *N. tentaculata* (Appendix 2). A more detailed study of structure and functional relationships of *Nep2* gene will be conducted in the near future by examining the 3D-structure of the nepenthesin II.

### ***Adaptive evolution of nepenthesin II enzyme***

The present study is the first study that concern to the adaptive evolution on *Nep2* gene by using statistical analysis based on the relative abundance of synonymous and nonsynonymous substitutions. Adaptive evolution after gene duplication has been reported in several gene families (Hughes, 2002). In this study, *Nep2* gene, a member of a gene family of aspartic proteinase, suggestively experienced significant positive selection on *N. sumatrana* (Table III-4), a species inhabits the lowest altitude habitats (0-800 m) amongst Sumatran endemic species, which most of them are highland species (McPherson, 2009). Adaptation to lowland habitats in Sumatra would be influenced by multiple physiological factors. For instance, at the physiological level, an obvious adaptive phenotype is the development of unusual 2 types of lower pitchers of *N. sumatrana*. Lower pitchers of the first type are beared from seedlings and juvenile plants with wholly or partially ovate form. While, lower pitchers of the second type are produced by a basal offshoots developing from the rootstock, and have much more squat lower pitcher form than the first type (McPherson, 2009).

The function of both types of lower pitchers are to trap creeping insects (McPherson, 2009). Most of the nitrogen sources of some *Nepenthes* species inhabit lowland habitats were provided by ants (Adam, 1997; Bazile *et al.*, 2012; Moran & Moran, 1998; Moran *et al.*, 2001). Therefore, the 2 distinct types of lower pitchers of *N. sumatrana* are mirrored by the strategy employed to obtain nutrients from the trapped prey, and the abundance of nutrient uptake should be correlated to the fitness of the species in the lowland habitats. The 2 types of lower pitchers of *N. sumatrana* are not found among other lowland *Nepenthes* species as well as the highland species (McPherson, 2009). These 2

distinct types of lower pitchers reveal that *N. sumatrana* has developed specific adaptation in response to nutrient stress that characterize the habitat where it grows. Thus, they may demonstrate that *N. sumatrana* is under selective pressure of prey and environment. Three-dimensional structure of nepenthesin II will be conducted in the near future to examine the difference between *Nep2* of *N. sumatrana* and *Nep2* of other *Nepenthes* species.

Table III-1. Plant materials examined in the study of *Nep2* gene. The materials were collected from the Indonesian Carnivorous Plant Society (*Komunitas Tanaman Karnivora Indonesia / KTKI*).

Species	Source	Genbank accession no. of <i>Nep2</i>
<i>Nepenthes adnata</i> Tamin & M. Hotta ex Schlauer	West Sumatra, cult. KTKI	AB769066
<i>Nepenthes alba</i> Ridl.	cult. KTKI	AB769067
<i>Nepenthes bellii</i> K. Kondo	cult. KTKI	AB769068
<i>Nepenthes campanulata</i> Sh. Kurata	cult. KTKI	AB769069
<i>Nepenthes chaniana</i> C. Clarke, Chi. C. Lee & S. McPherson	cult. KTKI	AB769070
<i>Nepenthes copelandii</i> Merr. ex Macfarlane	cult. KTKI	AB769071
<i>Nepenthes densiflora</i> Danser	cult. KTKI	AB769072
<i>Nepenthes diatas</i> Jebb & Cheek	cult. KTKI	AB769073
<i>Nepenthes ehippiata</i> Danser	cult. KTKI	AB769074
<i>Nepenthes faizaliana</i> J.H. Adam & Wilcock	cult. KTKI	AB769075
<i>Nepenthes glabrata</i> J.R. Turnbull & A.T. Middleton	cult. KTKI	AB769076
<i>Nepenthes khasiana</i> Hook. f.	cult. KTKI	AB769077
<i>Nepenthes lingulata</i> Chi. C. Lee, Hernawati & Akhriadi	North Sumatra, cult. KTKI	AB769078
<i>Nepenthes longifolia</i> J. Nerz & Wistuba	cult. KTKI	AB769094
<i>Nepenthes naga</i> Akhriadi, Hernawati, Primaldhi & M. Hambali	North Sumatra, cult. KTKI	AB769079
<i>Nepenthes ovata</i> J. Nerz & Wistuba	North Sumatra, cult. KTKI	AB769080
<i>Nepenthes papuana</i> Danser	Papua, New Guinea, cult. KTKI	AB769081
<i>Nepenthes platyphila</i> Chi. C. Lee	cult. KTKI	AB769082
<i>Nepenthes rajah</i> Hook. f.	Sabah, Borneo, cult. KTKI	AB769093
<i>Nepenthes sanguinea</i> Lindl.	cult. KTKI	AB769083
<i>Nepenthes spatulata</i> Danser	Lampung, Sumatra, cult. KTKI	AB769084
<i>Nepenthes spectabilis</i> Danser	North Sumatra, cult. KTKI	AB769085
<i>Nepenthes stenophylla</i> Mast.	cult. KTKI	AB769086
<i>Nepenthes sumatrana</i> (Miq.) Beck	North Sumatra, cult. KTKI	AB769087
<i>Nepenthes talangensis</i> J. Nerz & Wistuba	cult. KTKI	AB769088
<i>Nepenthes tentaculata</i> Hook. f.	cult. KTKI	AB769089
<i>Nepenthes thai</i> Cheek	cult. KTKI	AB769090
<i>Nepenthes tobaica</i> Danser	North Sumatra, cult. KTKI	AB769091
<i>Nepenthes ventricosa</i> Blanco	cult. KTKI	AB769092

Table III-2. Primers used for amplification and sequencing of *Nep2* gene.

Primer name	Sequence (5'→ 3')
NepF1	GGYAGYGATCTYATYTGGAC
NepR2	ACYWGC RWKTYTTGCTGCTG
NepF5	ATGGCCTCRYCRCTR TAYTC
NepR6	YTACGACGCACCACAYTGAG
<i>Internal primers</i>	
NepF3	TRTCGCTTCCTTCTCAACTCG
NepR4	TGGATKAGGGTYGTAYTMGG
NepF7	CAAGCGTTCAC T GACCAGA
NepR8	CTTGGCAATACTGGCTCTCGCA
NepF9	TTGATCTCTCCAGCTGAAGG
NepF10	GAGGAGCTCTGCAACATAGCA



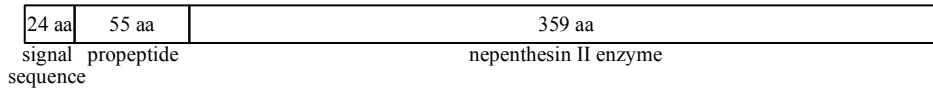


Figure III-1. Illustration of the prepro-form of nepenthesin II, composed of 438 amino acids, including 24 residues putative signal sequence, 55 residues putative propeptide, and 359 residues enzyme.

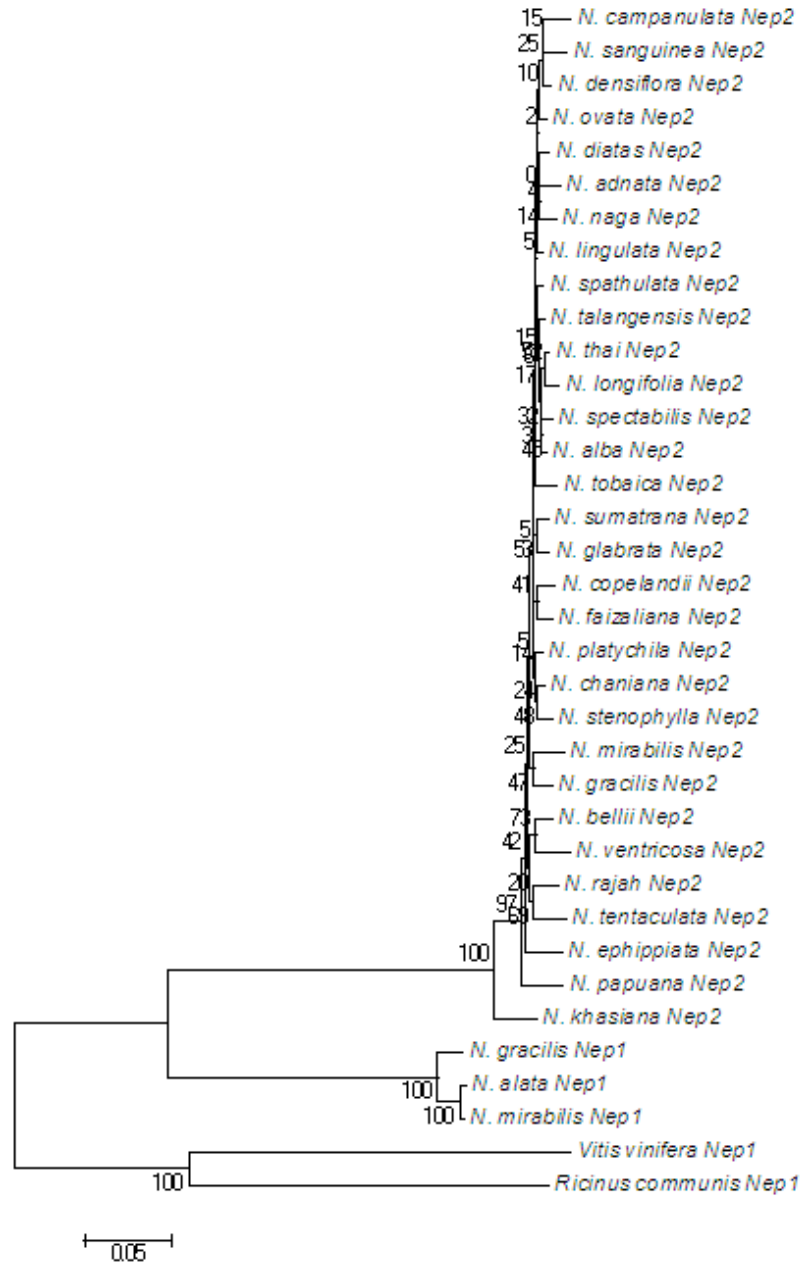


Figure III-2. Phylogenetic tree of nepenthesins-encoded genes obtained by the NJ method. Numbers above the branches indicate bootstrap support (%) with 1000 replicates. Scale indicates the base substitution per site.

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N.adnata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.bellii : MASSLYSVVLGLAIVSAIVAPTCS : 24
N.platychila : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.khasiana : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.ovata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.densiflora : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.diatas : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.lingulata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.talangensis : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.sumatrana : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.longifolia : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.campanulata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.tentaculata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.naga : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.spectabilis : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.tobaica : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.ephippiata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.glabrata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.copelandii : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.thai : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.faizaliana : MASSLYTVVLGLAIVSAIVAPTSS : 24
N.papuana : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.chaniana : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.alba : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.spathulata : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.stenophylla : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.sanguinea : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.rajah : MASSLYSVVLGLAIVSAIVAPTSS : 24
N.ventricosa : MASSLYSVVLGLAIVSAIVAPTCS : 24
MASSLY VVLG AI6SAIVAPTSS

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Figure III-3. Alignment of nepenthesin II signal sequences of 29 *Nepenthes* species with nonpolar amino acids and amino acid substitutions highlighted in grey and black, respectively.

## **Chapter IV**

### **General Discussion**

In this thesis, I have studied the genus *Nepenthes* for molecular phylogeny and phytogeography in Chapter II, and molecular evolution of nepenthesin II enzyme in Chapter III. In this Chapter, I would like to discuss their evolution and adaptation by integrating these results.

#### ***Correlation between nepenthesin II pI values and the number of habitat types***

The present study is the first one that examine the correlation between physical properties of nepenthesin II (i.e., pI) and the number of habitat types that occupied by *Nepenthes* species by using statistical-based comparative analysis. I distinguished *Nepenthes* species into generalist and specialist based on the number of habitat types they occupied. Generalists refer to species inhabit more than 1 habitat, which consists of 12 *Nepenthes* species, whereas the specialists refer to species grow in only 1 specific habitat, which consists of 17 *Nepenthes* species (Table IV-1).

I performed comparative analysis for examining the correlation between pI and the number of habitat types of *Nepenthes* species by using phylogenetically independent contrasts (PIC) (Felsenstein, 1985) with the program Mesquite (Maddison & Maddison, 2006), using its PDAP module (Midford *et al.*, 2005). A phylogenetic tree of *Nepenthes* based on the internal transcribed spacer (ITS) sequences was entered to the program, as a phylogenetic information incorporated into statistical analysis of the correlated evolution of pI and the number of habitat types. The data of habitat types number of *Nepenthes*



species examined in this study were taken from McPherson (2009).

Before calculating the phylogenetically independent contrasts, I performed diagnostic check of four assumptions which relate to the Brownian motion model of evolution that underlies independent contrasts, by using the first 4 entries in the PDAP:Chart menu (they contain numbers 1-8 at the end). The first assumption of “Abs. Contrast vs. Standard Dev. (1,2)” is probably the most important assumption to check whether the branch lengths of phylogenetic tree adequately fit the tip data (Diaz-Uriarte & Garland, 1996). A violation of this assumption would indicate that the character did not evolve by Brownian motion given the branch lengths that are used (Garland *et al.*, 1992). I performed 3 additional diagnostic checks which have associated numbers (3,4), (5,6), and (7,8). These diagnostics may be useful for various purposes, but have not been studied as much as the (1,2) (Midford *et al.*, 2005).

A phylogenetic regression analysis was performed to test whether each pair of traits (pI and the number of habitat types) show evidence of correlated evolution by clicking on “Y Contrasts vs. X Contrasts (positivized) (9)”. Positivized contrasts simply mean that the direction of subtraction for the contrasts is set so that the X variable is positive (Garland *et al.*, 1992). I chose pI values for the X-axis and the number of habitat types for the Y-axis, as pI values is the independent variable and number of habitat types are the dependent variable.

Based on the result of comparative analysis (Table IV-2), the 2-tailed p-values of the first 3 assumptions were not significant, i.e., above 0.05, indicating that the characters evolution (pI and the number of habitat types) did not violate the Brownian motion. In contrast, the last assumption “Est. Node Value vs. Node Height (7,8)” had 2-tailed p-value below 0.05. However, since most of the assumptions were fulfilled, phylogenetic

regression analysis of the 2 traits could be conducted that resulting significant result with 2-tailed p-value below 0.05, indicating that increases in pI of nepenthesin II enzyme are associated with the increases in the number of habitat types occupied by *Nepenthes* species. Therefore, the ability of the generalist to occupy more than 1 habitat are correlated to the higher pI of nepenthesin II enzymes than those of the specialist. A higher pI of nepenthesin II is supposed to contribute to render nepenthesin II much more stable, since the charge repulsion among the dissociating carboxylate groups should be less pronounced when the pH increased (Athauda *et al.*, 2004). At a pH above its pI, nepenthesin II carries a net negative charge. This net negative charge resulting charge repulsion among carboxylate groups (RCOO<sup>-</sup>) contain negative charge, that may lead to denaturation (Athauda *et al.*, 2004). This is corroborated by the results of Dice and Goldberg (1975) that showed proteins with lower isoelectric points tend to be degraded more rapidly than those with higher isoelectric points. They assumed that the degradative rates and isoelectric points of proteins seem to be related with a highly significant correlation (Dice and Goldberg, 1975).

These higher pI values may encourage the generalist to inhabit different habitats with different biogeographic characteristics, such as temperature and water, which are included as basic factors for growing requirements of *Nepenthes* (McPherson, 2009). Changes in temperature and water content in a solution will be reflected by a subsequent change in pH (Brooker *et al.*, 2008). Thus, the increase of temperature and the decrease of water content in pitcher fluid may be reflected by a subsequent increase in pH of pitcher fluid. Therefore, nepenthesin II from the generalist appear to be more stable in a wider range of pH, indicating an evolutionary adaptation of the enzyme to different habitats. This evolutionary adaptation permits the generalist to enlarge its ecological niche to a broader range of habitats and should contribute to its ecological success. The little differences of pI

among nepenthesin II enzymes probably attributed to the strong purifying selection operates on *Nep2* gene that suggests this enzyme plays an important role in *Nepenthes*. In contrast, the inclusion of *N. sumatrana* as the generalist with higher pI value may add the evidence of adaptive evolution of its *Nep2* gene.

### ***Adaptations of Nepenthes to nutrient and environmental stress***

The results of Chapter II demonstrate the distribution pattern of *Nepenthes* in Southeast Asia from the eastern region to the western region that may be influenced by the different climates between these 2 regions. The results of Chapter II also demonstrate the evolutionary trends of peristomes of upper pitchers. Most of upper pitchers of *Nepenthes* species distributed in the eastern region (Sulawesi and New Guinea) possessed narrow peristomes, whereas upper pitchers of species distributed in the western region (Borneo and Sumatra) possessed intermediate and broad peristomes.

The different characteristics of peristomes of upper pitchers facilitated different trapping strategies (Bauer *et al.*, 2011; Moran *et al.*, 1999), which may influenced by the different climates where *Nepenthes* species live. The intermediate and broad peristomes of upper pitchers facilitate better slippery surface trapping than the narrow peristome, since they serve larger surface for water droplets from rainfall or dew formation (Bauer *et al.*, 2008; Bauer & Federle, 2009; Di Giusto *et al.*, 2008). These characteristics of intermediate and broad peristomes of upper pitchers are corroborated by the wetter climate of western region of Southeast Asia, whereas the characteristics of narrow peristomes of upper pitchers are corroborated by the drier climate of eastern region as well as the Indochina of Southeast Asia mainland (Heaney, 1991; McPherson, 2009). Therefore, this different characteristics of peristome of upper pitchers reveals anatomical adaptations of *Nepenthes*

species to nutrient and environmental stress where they live. In addition, the results of Chapter III also demonstrate anatomical adaptations of *N. sumatrana* to nutrient and environmental stress, which is the development of unusual 2 types of lower pitchers to trap creeping insects in abundance in the lowland habitats.

Beside the anatomical adaptations, *Nepenthes* develop another mechanism of adaptations. The results of Chapter III show the evolution of *Nep2* gene producing extracellular nepenthesin II digestive enzyme with rapid secretion and high stability in the pitcher fluid. The rapid secretion of nepenthesin II enzyme will digest the trapped prey in the pitcher fluid subsequently after the prey fall down into the pitcher, to avoid putrefaction of the trapped prey that may harm the pitcher to die. In addition, the high stability of nepenthesin II enzyme protects the enzyme from digestion by protease in the pitcher fluid. Therefore, the production of nepenthesin II enzyme reveals molecular adaptations of *Nepenthes* species to nutrient and environmental stress.

The anatomical and the molecular adaptations of *Nepenthes* species are mirrored by the strategy employed to obtain nutrients under selective pressure of the prey and environment. The abundance of nutrient uptake should be correlated to the fitness of the *Nepenthes* species. The study by Kato *et al.* (1993) suggested that *Nepenthes* increase the rate of nutrient uptake by developing 3 strategies: (1) maximizing the rate of nutrient input into the pitchers, (2) maximizing the rate of decomposition and digestion of prey, and (3) minimizing loss of nutrient. These strategies are corroborated by the results of my study. The anatomical adaptations of the different peristome characteristics of upper pitchers and the development of 2 types of lower pitchers of *N. sumatrana* are included into the first strategy. The molecular adaptations of *Nep2* gene resulted in the rapid secretion of nepenthesin II digestive enzyme and the high stability of the enzyme in the pitcher fluid

are included into the second strategy.

### ***Adaptations of Nepenthes to avoid interspecific competition***

The results of Chapter II show some Sumatran species that have different characteristics of peristomes of upper pitchers and are distributed in the same region, i.e., Lake Toba of north Sumatra, and naturally hybridises (*N. ovata*, *N. tobaica*, and *N. spectabilis*) (Clarke, 2007; McPherson, 2009). This difference of peristome characteristics of upper pitchers from closely distributed species may demonstrates prey partition (Moran *et al.*, 1999; Kato *et al.*, 1993). Moran *et al.* (1999) studied 2 *Nepenthes* species, *N. rafflesiana* and *N. gracilis* which often grow together in the lowlands of northwest Borneo and potentially compete for the same prey resources, and show different size of peristome, where *N. rafflesiana* has larger pitchers with broader peristomes than those of *N. gracilis*. *N. rafflesiana* was more successful at catching flower-visiting insects; bees, moths, and flies. In contrast, *N. gracilis*, which possesses narrow peristomes, captured more beetles, hemipteran bugs, ants and total flightless preys. They suggested that the difference of peristome width revealed prey partition between those two closely distributed *Nepenthes* species. In addition, the study by Kato *et al.* (1993) also showed prey partition among 3 *Nepenthes* species grow together in montane forests at Gunung Gadut in west Sumatra, namely *N. spathulata*, *N. bongso*, and *N. sp. B* (undescribed species). They suggested that the difference between prey assemblages was due to different prey trapping strategies. *N. spathulata* bears narrow peristomes in upper pitchers (Chapter II), whereas *N. bongso* bears intermediate peristomes in upper pitchers (Clarke, 2007; McPherson, 2009). This difference of peristome width in upper pitchers should play a role in resulting prey partition among the Sumatran species.

Similarly, New Guinean species distributed in western region of New Guinea (*N. neoguineensis*, *N. ampullaria*, and *N. papuana*), and Philippine species distributed in Dinagat and northern Mindanao (*N. alata*, *N. mindanaoensis*, *N. truncata*, *N. merrilliana*, *N. bellii*, and *N. mirabilis*) also have different peristome characteristics of upper pitchers, that also may reveal prey partition of closely distributed *Nepenthes* species.

The results of Chapter III show *N. sumatrana* develops 2 types of lower pitchers to trap creeping insects in abundance in the lowland habitats. In addition, *N. sumatrana* has intermediate peristomes of upper pitchers, and is closely distributed and naturally hybridises with two widely distributed species *N. gracilis* and *N. mirabilis* (McPherson, 2009), both of which have narrow peristomes of upper pitchers; and with *N. longifolia*, a Sumatran species which has intermediate peristomes of upper pitchers. The different peristome characteristics of upper pitchers among these 4 closely distributed species may also reveal prey partition. Moreover, 2 types of lower pitchers developed by *N. sumatrana* may specialized to avoid interspecific competition with *N. longifolia*. From these results, I can assume that prey partition helps to avoid interspecific competition among closely distributed species, thus, the different species can co-exist together in the same habitats.

## Concluding remarks

1. Phylogenetic analysis of the genus *Nepenthes* based on ITS nrDNA sequences revealed monophyletic origin of this genus. Positional differences of species between the present *Nepenthes* phylogeny and previous studies were found, which may be caused by chloroplast capture phenomenon, concerted evolution, and intergenomic introgression of nrDNA genes.
2. The phytogeography of *Nepenthes* in Southeast Asia based on the tree topology was described by the distribution pattern of this genus from the eastern region to the western region of Southeast Asia, with Borneo as a secondary center of diversification for *Nepenthes*, allowing *Nepenthes* species to radiate within the Sunda Shelf of Southeast Asia.
3. The evolutionary trends of the peristome as a taxonomically important characteristics of the genus based on the tree topology revealed repeated evolution within the 3 character states and limitedness of Danser's system of classification for *Nepenthes*.
4. The molecular evolution study of the *Nep2* gene demonstrated that the structure of the *Nep2* gene influenced its protein product (nepenthesin II) as an extracellular proteinase with high stability and rapid secretion.
5. The *Nep2* gene has been the target of positive selection during the evolution of *Nepenthes* species growing in the lowest altitude habitats of Sumatra (*N. sumatrana*), which developed unusual 2 types of lower pitchers.
6. *Nepenthes* are adaptable plants to poor soils environment and actively respond to the environmental conditions and availability of the prey in their habitats, by changes in phenotypic and genotypic characteristics.

7. Changes in phenotypic characteristics which is demonstrated by the difference of the peristome characteristics among *Nepenthes* species and the development of 2 types of lower pitchers of *N. sumatrana* reveal nutrient sequestration strategies and prey partition among closely distributed species.
8. Changes in genotypic characteristics demonstrated by molecular evolution of the *Nep2* gene reveal the nutrient sequestration strategy in the pitcher fluid.
9. The strategies employed by *Nepenthes* to maximize nutrient uptake are correlated to the fitness of the plants and are supposed under strong selective pressure of nutrient and environment that characterize the habitats of *Nepenthes*.



Table IV-1. The value of isoelectric point of 29 *Nepenthes* species and the number of their habitat types, as raw data for comparative analysis using PIC.

No.	Species	Isoelectric point (pI)	Number of habitat types
1	<i>N. khasiana</i>	3.5	2
2	<i>N. papuana</i>	3.4	4
3	<i>N. adnata</i>	3.4	2
4	<i>N. bellii</i>	3.3	5
5	<i>N. sumatrana</i>	3.3	2
6	<i>N. sanguinea</i>	3.3	3
7	<i>N. stenophylla</i>	3.3	3
8	<i>N. tentaculata</i>	3.2	3
9	<i>N. ventricosa</i>	3.2	3
10	<i>N. glabrata</i>	3.2	3
11	<i>N. tobaica</i>	3.2	3
12	<i>N. longifolia</i>	3.2	3
13	<i>N. rajah</i>	3.0	1
14	<i>N. densiflora</i>	3.0	1
15	<i>N. naga</i>	3.0	1
16	<i>N. spathulata</i>	3.0	1
17	<i>N. faizaliana</i>	3.0	1
18	<i>N. spectabilis</i>	3.0	1
19	<i>N. ovata</i>	3.0	1
20	<i>N. lingulata</i>	3.0	1
21	<i>N. platyphila</i>	3.0	1
22	<i>N. campanulata</i>	3.0	1
23	<i>N. copelandii</i>	3.0	1
24	<i>N. chaniana</i>	3.0	1
25	<i>N. thai</i>	3.0	1
26	<i>N. alba</i>	3.0	1
27	<i>N. talangensis</i>	3.0	1
28	<i>N. diatas</i>	3.0	1
29	<i>N. ehippiata</i>	2.9	1

Table IV-2. 2-tailed p-values obtained from the statistical calculation of independent contrasts toward 2 characters evolution (pI and the number of habitat types). 2-tailed p-value of assumption (1,2) is significant if  $p < 0.05$ , whereas 2-tailed p-value for the contrasts (9) is significant if  $p < 0.05$

Assumption (1,2) and Contrasts (9)	2-tailed p-values	
	Isoelectric point (pI)	Number of habitat types
Abs. Contrast vs. Standard deviation (1,2)	0.096	0.303
Abs. Contrast vs. Est. Node Value (3,4)	6.710	4.134
Abs. Contrast vs. Node Height (5,6)	0.705	0.054
Est. Node Value vs. Node Height (7,8)	0.007	0.001
Y Contrasts vs. X Contrasts (positivized) (9)	0.036	

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Appendix 1. Alignment comparison between *Nep2* genes of 29 *Nepenthes* species and *Nep2* cDNAs of *N. gracilis* (AB114915) and *N. mirabilis* (JX494402) with red font color.

		*	20	*	40	*	
<i>N. bellii</i>	:	ATGGCCTCGTCGCTG	TACTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. ventricosa</i>	:	ATGGCCTCGTCGCTA	TACTCTGTGGTACTT	GGCTTAGCCATAGTTT	CTGC	:	50
<i>N. tentaculata</i>	:	ATGGCCTCGTCGCTG	TACTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. rajah</i>	:	ATGGCCTCGTCGCTG	TACTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. ephippiata</i>	:	ATGGCCTTGTGCTG	TACTCTGTGGTACTT	GGCTTAGCCATAGTTT	CTGC	:	50
<i>N. gracilis</i>	:	ATGGCCTCACC	ACTATACTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. mirabilis</i>	:	ATGGCCTCACC	ACTATACTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. chanihana</i>	:	ATGGCCTCGTCGCG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. stenophylla</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. platyphila</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. copelandii</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. faizaliana</i>	:	ATGGCCTCGTCGCTG	TATACTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. adnata</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. diatas</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. naga</i>	:	ATGGCCTCGTCGCTG	TACTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. campanulata</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. sanguinea</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. densiflora</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. ovata</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. alba</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. spectabilis</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. thai</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. longifolia</i>	:	ATGGCCTCGTCGCTG	TACTATGCGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. talangensis</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. spathulata</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. lingulata</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. glabrata</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. sumatrana</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCAATAGTTT	CTGC	:	50
<i>N. tobaica</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACT	AGGCGAGCAATAGTTT	CTGC	:	50
<i>N. papuana</i>	:	ATGGCCTCGTCGCTG	TACTCTGTGGTACTT	GGCGTAGCCATAGTTT	CTGC	:	50
<i>N. khasiana</i>	:	ATGGCCTCGTCGCTG	TATTCTGTGGTACTT	GGCTTAGCCATACTTT	CTGC	:	50
		ATGGCCTcgtCgCtgtA tctGtGGTACTtGgCttAGCaATAgTTTCTGC					
		60	*	80	*	100	
<i>N. bellii</i>	:	CATTGTTGCACCAAC	ATGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. ventricosa</i>	:	CATTGTTGCACCAAC	ATGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. tentaculata</i>	:	CATTGTTGGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. rajah</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. ephippiata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. gracilis</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. mirabilis</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. chanihana</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. stenophylla</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. platyphila</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. copelandii</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. faizaliana</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. adnata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. diatas</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. naga</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. campanulata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. sanguinea</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. densiflora</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. ovata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. alba</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. spectabilis</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. thai</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. longifolia</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. talangensis</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. spathulata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. lingulata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. glabrata</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. sumatrana</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. tobaica</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCATCTTC	ATCATG	:	100
<i>N. papuana</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCCCTTCTTC	ATCATG	:	100
<i>N. khasiana</i>	:	CATTGTTGCACCAAC	AAGCTCCACCTCAAG	AGGAACCC---TTC	ATCATG	:	97
		CATTGTTGcACCAACAaGCTCCACCTCAAGAGgAACCC tctTCATCATG					



	*	120	*	140	*	
<i>N. bellii</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. ventricosa</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. tentaculata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGGGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. rajah</i>	:	GTCAGAAAAGGCCACAACCCGGCCTCCGTGTCGTTCTCGAGCAGGTCGAT	:	150		
<i>N. ehippiata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. gracilis</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGATCTCGAGCAGGTCGAT	:	150		
<i>N. mirabilis</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. chaniana</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. stenophylla</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. platyphila</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. copelandii</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. faizaliana</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. adnata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. diatas</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. naga</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. campanulata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. sanguinea</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. densiflora</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. ovata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. alba</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. spectabilis</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. thai</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. longifolia</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. talangensis</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. spathulata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. lingulata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. glabrata</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. sumatrana</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. tobaica</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. papuana</i>	:	GTCAGAAATAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	150		
<i>N. khasiana</i>	:	GTCAGAAAAGGCCACAACCCGGCCTTCGTGTTGTTCTCGAGCAGGTCGAT	:	147		

	160	*	180	*	200	
<i>N. bellii</i>	:	TCGGGCATGGATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. ventricosa</i>	:	TCGGGCATGGATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. tentaculata</i>	:	TCGGGCATGGATTTGACCAAATACGAGATCATCAAACGTGCTATCATGCG	:	200		
<i>N. rajah</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCCATCAAGCG	:	200		
<i>N. ehippiata</i>	:	TCGGGCAGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. gracilis</i>	:	TCGGGCAGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. mirabilis</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. chaniana</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. stenophylla</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. platyphila</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. copelandii</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. faizaliana</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. adnata</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. diatas</i>	:	TCGGGCATGGATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. naga</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. campanulata</i>	:	TCGGGCATGGATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. sanguinea</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. densiflora</i>	:	TCGGGCATGGATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. ovata</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. alba</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. spectabilis</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. thai</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. longifolia</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. talangensis</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. spathulata</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. lingulata</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. glabrata</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. sumatrana</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. tobaica</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. papuana</i>	:	TCGGGCATGAATTTGACCAAATACGAGCTCATCAAACGTGCTATCAAGCG	:	200		
<i>N. khasiana</i>	:	TCGGGCATGAATTTGACCAAATACGAGATCATCAAACGTGCTATCAAGCG	:	197		

	*	220	*	240	*	
N.bellii	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.ventricosa	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATATTTGCAGAGCTCCTCCG	:	250		
N.tentaculata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.rajah	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.ehippiata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.gracilis	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.mirabilis	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.chaniana	:	TGGGGAGAAGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.stenophylla	:	TGGGGAGAAGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.platychila	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.copelandii	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.faizaliana	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.adnata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.diatas	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.naga	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.campanulata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.sanguinea	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.densiflora	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.ovata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.alba	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.spectabilis	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.thai	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.longifolia	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.talangensis	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.spathulata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.lingulata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.glabrata	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.sumatrana	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.tobaica	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.papuana	:	TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	250		
N.khasiana	:	TGGGGAGAAGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG	:	247		
		TGGGGAGAGGAGGATGCGAAGCATTAAATGCTATGTTGCAGAGCTCCTCCG				

	260	*	280	*	300	
N.bellii	:	GTATTGAAACTCCTGTTTATGCGGGACACGGTGAATATCTAATGAACGTA	:	300		
N.ventricosa	:	GTATTGAAACTCCTGTTTATGCGGGACACGGTGAATATCTAATGAACGTA	:	300		
N.tentaculata	:	GTATTCAAACTCCTGTTTATGCGGGAAACGGTGAATATCTAATGAACGCA	:	300		
N.rajah	:	GTATTGAAACTCCTGTTTATGCGGGAGGCGGTGAATATCTAATGAACGTA	:	300		
N.ehippiata	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.gracilis	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.mirabilis	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.chaniana	:	GTATTGAAACTCCTGTTTATGCGGGAAACGGTGAATATCTAATGAACGTA	:	300		
N.stenophylla	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.platychila	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.copelandii	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.faizaliana	:	GTATTGAAACTCCTGTTTATGCGGGAAACGGTGAATATCTAATGAACGTA	:	300		
N.adnata	:	GTATTGAAACTCCTGTTTATGCGGGTGACGGTGAATATCTAATGCACGTA	:	300		
N.diatas	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.naga	:	GTATTGAAACTCCTGTTTATGCGGGAGGCGGTGAATATCTAATGAACGTA	:	300		
N.campanulata	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.sanguinea	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGCATATCTAATGAACGTA	:	300		
N.densiflora	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGCATATCTAATGAACGTA	:	300		
N.ovata	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	300		
N.alba	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.spectabilis	:	GTATTGAAACTCCTGTTTATGCGGGAAACGGTGAATATCTAATGAACGTA	:	300		
N.thai	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.longifolia	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.talangensis	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.spathulata	:	GTATTGAAACTCCTGTTTATGCGGGAAACGGTGAATATCTAATGAACGTA	:	300		
N.lingulata	:	GTATTGAAACTCCTGTTTATGCGGGAAACGGTGAATATCTAATGAACGTA	:	300		
N.glabrata	:	GTATTCAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.sumatrana	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGCATATCTAATGAACGTA	:	300		
N.tobaica	:	GTATTGAAACTCCTGTTTATGCGGGAGGCGGTGAATATCTAATGAACGTA	:	300		
N.papuana	:	GTATTGAAACTCCTGTTTATGCGGGAAAGCGGTGAATATCTAATGAACGTA	:	300		
N.khasiana	:	GTATTGAAACTCCTGTTTATGCGGGAGACGGTGAATATCTAATGAACGTA	:	297		
		GTATTGAAACTCCTGTTTATGCGGGa CGGTGaATATCTAATGaACGtA				

	*	320	*	340	*	
N.bellii	:	TCGATTGGTACTCCGGCTAATTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.ventricosa	:	TCGATTGGTACTCCGGCTAATTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.tentaculata	:	TCAATTGGTACTCCGGCGAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.rajah	:	TCGATTGGTACTCCGGCAAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.ehippiata	:	TCGATTGGCACTCCGGATAGTTCTTTCTGGGCCATTATGGATAACCGGCAG	:	350		
N.gracilis	:	GCAATTGGTACTCCGGATAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.mirabilis	:	GCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.chaniana	:	TCGATTGGTACTCCGGCTATTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.stenophylla	:	TCGATTGGTACTCCGGCTAGTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.platychila	:	TCGATTGGTACTCCGGCTAGTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.copelandii	:	GCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.faizaliana	:	TCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.adnata	:	TCGATTGGTACTCCGGCCATTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.diatas	:	TCGATTGGTACTCCGGCCAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.naga	:	GCGATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.campanulata	:	TCGATTGGTACTCCGGCGAATTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.sanguinea	:	TCGATTGGTACTCCGGTTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.densiflora	:	TCGATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.ovata	:	TCGATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.alba	:	TCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.spectabilis	:	TCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.thai	:	GCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.longifolia	:	TCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.talangensis	:	GCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.spathulata	:	TCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.lingulata	:	TCGATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.glabrata	:	TCGATTGGTACTCCGGCCAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.sumatrana	:	GCGATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.tobaica	:	TCAATTGGTACTCCGGCTAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.papuana	:	GCAATTGGTACTCCGGATAGTTCTTTCTCGGCCATTATGGATAACCGGCAG	:	350		
N.khasiana	:	GCAATTGGTACTCCGACTACTCTTTCTCGGCCATTATGGATAACCGGCAG	:	347		
		C ATTGGtACTCCGgctAgTtCTTTcTcGGCCATTATGGATAACCGGCAG				

	360	*	380	*	400	
N.bellii	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.ventricosa	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.tentaculata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.rajah	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.ehippiata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.gracilis	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.mirabilis	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.chaniana	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.stenophylla	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.platychila	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.copelandii	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.faizaliana	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.adnata	:	TGATCTCATTGGACACCATGCCGGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.diatas	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.naga	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.campanulata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.sanguinea	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.densiflora	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.ovata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.alba	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.spectabilis	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.thai	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.longifolia	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.talangensis	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.spathulata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.lingulata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.glabrata	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.sumatrana	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.tobaica	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.papuana	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCAGTCAAC	:	400		
N.khasiana	:	TGATCTCATTGGACGCAATGCCAGCCATGTACGCAGTGCTTCCTCAAC	:	397		
		TGATCTCATTGGACgCaATGCgaGCCATGTACGCAGTGCTTCagTCAAC				

	*	420	*	440	*	
N.bellii	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.ventricosa	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.tentaculata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.rajah	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.ephippiata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.gracilis	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.mirabilis	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.chaniana	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.stenophylla	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.platychila	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.copelandii	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.faizaliana	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.adnata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.diatas	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.naga	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.campanulata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.sanguinea	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.densiflora	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.ovata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.alba	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.spectabilis	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.thai	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.longifolia	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.talangensis	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.spathulata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.lingulata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.glabrata	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.sumatrana	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.tobaica	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.papuana	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	450		
N.khasiana	:	CTACGCCCATTTTCAACCCACAGGACTCGTCTTCCTTCTCTACCCTTCCT	:	447		

	460	*	480	*	500	
N.bellii	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.ventricosa	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.tentaculata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.rajah	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.ephippiata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.gracilis	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.mirabilis	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAAT--	:	498		
N.chaniana	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.stenophylla	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAAT--	:	498		
N.platychila	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.copelandii	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.faizaliana	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.adnata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.diatas	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.naga	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.campanulata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.sanguinea	:	TGCGATAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.densiflora	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.ovata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.alba	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.spectabilis	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.thai	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.longifolia	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.talangensis	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.spathulata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.lingulata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.glabrata	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.sumatrana	:	TGCGGGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.tobaica	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	500		
N.papuana	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCAATAATAA	:	500		
N.khasiana	:	TGCGAGAGCCAGTATTGCCAAGATCTTCCGAGCGAAACCTGCTATAATAA	:	497		

	*	520	*	540	*	
<i>N. bellii</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. ventricosa</i>	:	TGAATGCCAATACACATACGGATACGGGGGCGTTCC	CAACCCAAGGTT	:	550	
<i>N. tentaculata</i>	:	TGAATGCCAATACACCTACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. rajah</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. ehippiata</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. gracilis</i>	:	TGAATGCCAATACATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. mirabilis</i>	:	-GACTGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	547	
<i>N. chaniana</i>	:	TGACTGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGAT	:	550	
<i>N. stenophylla</i>	:	-GACTGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	547	
<i>N. platyphila</i>	:	TGACTGCCAATACACCTACGGATACGGAGGCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. copelandii</i>	:	TGACTGCCAATACACCTACGGGTACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. faizaliana</i>	:	TTACTGCCAATACACCTACGGGTATGGGGACGGTCC	CACAACCCAAGGAT	:	550	
<i>N. adnata</i>	:	TGAATGCCAATACACATACGGATACGGAGACGGTCC	CACAACCCAAGGTT	:	550	
<i>N. diatas</i>	:	TGAATGCCAATACACATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. naga</i>	:	TGAATGCCAATACGCATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. campanulata</i>	:	TGAATGCCAATACACATACGGATACGGAGGCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. sanguinea</i>	:	TGAATGCCAATACACATACGGATACGGGGGCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. densiflora</i>	:	TGAATGCCAATACACATACGGATACGGAGGCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. ovata</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. alba</i>	:	TGAATGCCAATACACATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. spectabilis</i>	:	TGAATGCCAATACACATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. thai</i>	:	TGAATGCCAATACACATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. longifolia</i>	:	TGAATGCCAATACACATACGGATACGGAGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. talangensis</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. spathulata</i>	:	TGGATGCCAATACACATACGGATACGGAGGCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. lingulata</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CACAACCCAAGGTT	:	550	
<i>N. glabrata</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. sumatrana</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CAACCCAAGGTT	:	550	
<i>N. tobaica</i>	:	TGAATGCCAATACACATACGGATACGGGGACCGTTCC	CACAACCCAAGGAT	:	550	
<i>N. papuana</i>	:	TGAATGCCAATACACCTACGGATACGGGGACCGTTCC	CAACCCAAGGAT	:	550	
<i>N. khasiana</i>	:	TGAATGCCAATACACATATGGATATGGGGACGGTCC	CACAACCCAAGGAT	:	547	
		tgaaTGCCAATaCaCaTAcGGaTAcGG	GaCgGtTCC		CAACCCAAGGtT	

		560	*	580	*	600	
<i>N. bellii</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. ventricosa</i>	:	ATATGGCCACCGACACCTTCACTTTTCGAGACGGGCTCC	GTGCCGAATATC	:	600		
<i>N. tentaculata</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGGGCTCC	GTGCCGAATATC	:	600		
<i>N. rajah</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAAACGGGCTCC	GTGCCGAATATC	:	600		
<i>N. ehippiata</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAAACGGGCTCC	GTGCCGAATATC	:	600		
<i>N. gracilis</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. mirabilis</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	597		
<i>N. chaniana</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. stenophylla</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	597		
<i>N. platyphila</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. copelandii</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAAACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. faizaliana</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAAACGAGCTCC	ATGCCGAATATC	:	600		
<i>N. adnata</i>	:	ATATGGCCACCGACACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. diatas</i>	:	ATATGGCCACCGACACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. naga</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. campanulata</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAAACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. sanguinea</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAAACGGGCTCC	GTGCCGAATATC	:	600		
<i>N. densiflora</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. ovata</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAGACAAGCTCC	GTGCCGAATATC	:	600		
<i>N. alba</i>	:	ATATGGCAACCGACACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. spectabilis</i>	:	ATATGGCAACCGACACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. thai</i>	:	ATATGGCCACCGACACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. longifolia</i>	:	ATATGGCCACCGACACCTTCACTTTTCGAAACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. talangensis</i>	:	ATATGGCCACCGACACCTTCACTTTTCGAAACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. spathulata</i>	:	ATATGGCAACCGACACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. lingulata</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. glabrata</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. sumatrana</i>	:	ATATGGCAACCGAGACCTTCACTTTTCGAGACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. tobaica</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAAACGAGCTCC	ATGCCGAATATC	:	600		
<i>N. papuana</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAAACGAGCTCC	GTGCCGAATATC	:	600		
<i>N. khasiana</i>	:	ATATGGCCACCGAGACCTTCACTTTTCGAGTGGGCTCC	ATGCCGAATATC	:	597		
		ATATGGC	ACCGA	ACCTTCACTTTTCGA	aCgaGCTCCgTgCC	AAATATC	

	*	620	*	640	*																											
N.bellii	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.ventricosa	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.tentaculata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.rajah	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.ehippiata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.gracilis	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.mirabilis	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	647											
N.chaniana	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.stenophylla	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	647											
N.platychila	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.copelandii	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.faizaliana	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.adnata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.diatas	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.naga	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.campanulata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.sanguinea	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.densiflora	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.ovata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.alba	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.spectabilis	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.thai	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.longifolia	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.talangensis	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.spathulata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.lingulata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.glabrata	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.sumatrana	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.tobaica	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.papuana	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	650											
N.khasiana	:	GC	TT	CG	GT	TG	CG	GG	GA	AG	CA	AC	CA	AG	GC	AA	CG	GG	GC	:	647											
		GC	g	TT	CG	GT	TG	CG	GG	G	A	g	CA	AA	c	CA	g	GG	AT	TC	GG	G	c	a	AG	GC	AA	CG	GG	GC	:	

		660	*	680	*	700																									
N.bellii	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.ventricosa	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.tentaculata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.rajah	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.ehippiata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.gracilis	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.mirabilis	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	697	
N.chaniana	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.stenophylla	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	697	
N.platychila	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.copelandii	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.faizaliana	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.adnata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.diatas	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.naga	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.campanulata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.sanguinea	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.densiflora	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.ovata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.alba	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.spectabilis	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.thai	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.longifolia	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.talangensis	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.spathulata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.lingulata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.glabrata	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.sumatrana	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.tobaica	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.papuana	:	TG	GC	CT	GA	TC	GG	GA	TG	GG	TT	TG	GG	GC	CT	T	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	700	
N.khasiana	:	AG	GC	CT	GG	T	CG	GG	AT	GG	TT	TG	GG	GC	CT	T	C	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:	697
		t	GC	CT	G	a	T	CG	GG	AT	GG	TT	TG	GG	GC	CT	T	AT	CG	CT	T	C	T	T	CT	CA	ACT	CG	:		

	*	720	*	740	*	
N.bellii	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.ventricosa	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.tentaculata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.rajah	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.ehippiata	:	GCGTGGCC	CAGTTCTCTTACTGCATGACCTCCTATGGTAGCTCCTCACCC	:	750	
N.gracilis	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.mirabilis	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	747	
N.chaniana	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.stenophylla	:	GCGTGGGT	CAGTTCTCTTACTGCATGGCCCTCCTATGGAAGCTCCTCACCC	:	747	
N.platychila	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.copelandii	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.faizaliana	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.adnata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.diatas	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.naga	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCACCTCTGGAAGCTCATCACCC	:	750	
N.campanulata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.sanguinea	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.densiflora	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.ovata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.alba	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCATCACCC	:	750	
N.spectabilis	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCATCACCC	:	750	
N.thai	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCATCACCC	:	750	
N.longifolia	:	ACGTGGGT	CAGTTCTCTTACTGCATGACCTCCTCTGGAAGCTCATCACCC	:	750	
N.talangensis	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.spathulata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.lingulata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.glabrata	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.sumatrana	:	GCGTGGGT	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.tobaica	:	GCGTGAGC	CAGTTCTCTTACTGCATGACCTCCTATGGAAGCTCCTCACCC	:	750	
N.papuana	:	GCGTGGGC	AATTCTCTTACTGCATGACCTCCATGGAAGCTCCTCCCC	:	750	
N.khasiana	:	ACGTGACT	CAATTCTCTTACTGCATGACCCCTATGGTAGCTCCTCACCC	:	747	
		gCGTGGgt	CAgTTCTCTTACTGCATGAcctCCtaTGGaAGCTCctCaCCC			

	760	*	780	*	800	
N.bellii	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.ventricosa	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.tentaculata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.rajah	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.ehippiata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.gracilis	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.mirabilis	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGGGTGCCTGAAGGCTCCCC	:	797		
N.chaniana	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.stenophylla	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	797		
N.platychila	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.copelandii	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.faizaliana	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.adnata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.diatas	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.naga	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.campanulata	:	AGCACTCTCGCATTGGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.sanguinea	:	AGCACTCTCGCACTTGGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.densiflora	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.ovata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.alba	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.spectabilis	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.thai	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.longifolia	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.talangensis	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.spathulata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.lingulata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.glabrata	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.sumatrana	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.tobaica	:	AGCACTCTCGCACTTGGATCCGCAGCCAGTGGAGTGCCTGAAGGCTCCCC	:	800		
N.papuana	:	AGCACTCTCGCACTGGGATCCGCAGCCAGTGGTGTGCCCCGAAGGCTCCCC	:	800		
N.khasiana	:	AGCACTCTCGCACTGGGATCCGCAGCCAGTGGAGTGCCTGACGGCACTCC	:	797		
		AGCACTCTcGCACtTtGGATC	GCaGCCAGtGGaGTGCctGAaGGctCccc			

	*	820	*	840	*	
N.bellii	:	TAGTACGACCCTCATCCATAGTTCTTTGAATCCA	ACTTACTATTATATTA	:	850	
N.ventricosa	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.tentaculata	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.rajah	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.ephippiata	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.gracilis	:	GAGTACGACCCTCATCCATAGTTCTTTGAATCCA	ACGTACTATTATATTA	:	850	
N.mirabilis	:	GAGTACGACCCTCATCCATAGTTCTTTGAATCCA	ACTTACTATTATATTA	:	847	
N.chaniana	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.stenophylla	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	847	
N.platyphila	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.copelandii	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.faizaliana	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.adnata	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.diatas	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTCTATTATATTA	:	850	
N.naga	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTCTATTATATTA	:	850	
N.campanulata	:	GAGTACGACCCTCATCCATAGTTCTTTGAATCCA	ACTTCTATTATATTA	:	850	
N.sanguinea	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.densiflora	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTCTATTATATTA	:	850	
N.ovata	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTCTATTATATTA	:	850	
N.alba	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.spectabilis	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.thai	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.longifolia	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.talangensis	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.spathulata	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.lingulata	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTCTATTATATTA	:	850	
N.glabrata	:	GAGTACGACCCTCATCCATAGTTCTTTGAATCCA	ACTTACTATTATATTA	:	850	
N.sumatrana	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTCTATTATATTA	:	850	
N.tobaica	:	GAGTACGACCCTCATCCATAGTTCTTCGAATCCA	ACTTACTATTATATTA	:	850	
N.papuana	:	GAGTACGACCCTCATCCATAGTTCTTCCATCCA	ACTTCTATTATATTA	:	850	
N.khasiana	:	TAGTACAACCCTCATCCATAGCTCTCCAATTCCA	ACTTACTATTATATTA	:	847	
		gAGTACgACCCTCaTCCATAGtTcttcgaaTCCAACtT	CTATTATATTA			

		860	*	880	*	900	
N.bellii	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.ventricosa	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.tentaculata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.rajah	:	CGCTCCAAGGGATTATGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.ephippiata	:	CGCTCCAAGGGATGACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.gracilis	:	CGCTCCAAGGTATAACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.mirabilis	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	897		
N.chaniana	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.stenophylla	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	897		
N.platyphila	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.copelandii	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.faizaliana	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.adnata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.diatas	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTGCATCGAGT	:	900		
N.naga	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.campanulata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.sanguinea	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.densiflora	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.ovata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.alba	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.spectabilis	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTGCATCGAGT	:	900		
N.thai	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.longifolia	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.talangensis	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.spathulata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.lingulata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.glabrata	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.sumatrana	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.tobaica	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.papuana	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCATCGAGT	:	900		
N.khasiana	:	CGCTCCAAGGGATTACGGTTGGTGGCGATAA	TTTGGGTATTCCACCGAGT	:	897		
		CGCTCCAAGGgATtAcgGTTGGTGGCGATAa	TTTGGGTATTcCatCGAGT				



	*	920	*	940	*	
N.bellii	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.ventricosa	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.tentaculata	:	ACTTTTCAACTTCAAGGCGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.rajah	:	ACTTTTCAACTTCAAGGCGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.ephippiata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATTATTGACTCCGG	:	950	
N.gracilis	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.mirabilis	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGAATGATAATTGACTCCGG	:	947	
N.chaniana	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.stenophylla	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	947	
N.platychila	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.copelandii	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.faizaliana	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.adnata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.diatas	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.naga	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.campanulata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATAATAATTGACTCCGG	:	950	
N.sanguinea	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.densiflora	:	ACTTTTCAACTTCAAGACGATGGAAC	AGGCGGGATGATAATTGACTCCGG	:	950	
N.ovata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.alba	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.spectabilis	:	ACTTTTCAACTTCAAGACGACGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.thai	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.longifolia	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.talangensis	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.spathulata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.lingulata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.glabrata	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.sumatrana	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.tobaica	:	ACTTTTCAACTTCAAGACGATGGAAC	TGGCGGGATGATAATTGACTCCGG	:	950	
N.papuana	:	ACTTTTCAACTTCAAGACGATGGTAC	TGGCGGGATGATTATTGACTCCGG	:	950	
N.khasiana	:	ACTTTTCAACTTCAAAATGATGGAAC	TGGCGGGAGGATAATTGACTCCGG	:	947	
		ACTTTTCAACTTCAAGacGatGGaAc	TGGCGGgAtgATaATTGACTCCGG			

	960	*	980	*	1000	
N.bellii	:	GGCAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.ventricosa	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.tentaculata	:	CACGACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.rajah	:	CACAACGCTCACTTATCTTCCACAAGACGCATACAATGCGGTAGCACAAG	:	1000		
N.ephippiata	:	CACAACGCTCACTTATCTTCCACAGGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.gracilis	:	GACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.mirabilis	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	997		
N.chaniana	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.stenophylla	:	CACAACGCTCACTTATCTTCCACAGGACGCTTACAATGCGGTAGCACAAG	:	997		
N.platychila	:	GACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.copelandii	:	CACAACGCTCACTTATCTTCCACAGGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.faizaliana	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.adnata	:	CACGACGCTCACTTATCTTCCACAGACGCTTACTATGCGGTAGCACAAG	:	1000		
N.diatas	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.naga	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACTATGCGGTAGCACAAG	:	1000		
N.campanulata	:	CACCACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.sanguinea	:	GACGACGCTCACTTATCTTCCACAAGACGCTTACTATGCGGTAGCACAAG	:	1000		
N.densiflora	:	GACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.ovata	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.alba	:	CACAACGCTCACTTATCTTCCACAAGATGCTTACAATGCGGTAGCACAAG	:	1000		
N.spectabilis	:	CACAACGCTCACTTATCTTCCACAAGATGCTTACAATGCGGTAGCACAAG	:	1000		
N.thai	:	CACGACGCTCACTTATCTTCCACAAGATGCTTACAATGCGGTAGCACAAG	:	1000		
N.longifolia	:	CACGACGCTCACTTATCTTCCACAAGATGCTTACTATGCGGTAGCACAAG	:	1000		
N.talangensis	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.spathulata	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.lingulata	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTATCACAAG	:	1000		
N.glabrata	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.sumatrana	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.tobaica	:	CACCACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAAG	:	1000		
N.papuana	:	GACAACGCTCACTTATCTTCCACAAGACGCATACAATGCGGTAGCACAAG	:	1000		
N.khasiana	:	CACAACGCTCACTTATCTTCCACAAGACGCTTACAATGCGGTAGCACAGG	:	997		
		caC ACGcTCACTTATCTTCCaCaaGAcGcTtTACaATGCGgTAgCaCaAg				

	*	1020	*	1040	*
N.bellii	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.ventricosa	:	CGTTCACTGACCAGATAAAATCTCCCCACCGTCGAGGAATCCTCGAGCGGC	:	1050	
N.tentaculata	:	CGTTCACTGACCAGATACATCTCTCCACCGTCGA---ATCCTCGAGCGGC	:	1047	
N.rajah	:	CGTTCACTGACCAGATAGATCTCTCCACCGTTGA---ATCCTCGAGCGGC	:	1047	
N.ephippiata	:	CGTTCACTGACCAGATAGATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.gracilis	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.mirabilis	:	CGTTCACTGACCAGATAAAATCTCTCCCCCGTCGATGAATCCTCGAGCGGC	:	1047	
N.chaniana	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.stenophylla	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1047	
N.platychila	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.copelandii	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGA	:	1050	
N.faizaliana	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.adnata	:	CGTTCACTGACCAGATAGATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.diatas	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.naga	:	CGTTCACTGACCAGATAGATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.campanulata	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCCAACGGC	:	1050	
N.sanguinea	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.densiflora	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.ovata	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.alba	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.spectabilis	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.thai	:	CGTTCACTGACCAGATAGATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.longifolia	:	CGTTCACTGACCAGATAGATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.talangensis	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.spathulata	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.lingulata	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.glabrata	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.sumatrana	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.tobaica	:	CGTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.papuana	:	CGTTCACTTACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1050	
N.khasiana	:	CTTTCACTGACCAGATAAAATCTCTCCACCGTCGATGAATCCTCGAGCGGC	:	1047	
		CgTTCACTgACCAGATA ATCTC CcACCGTcGAtgaATCCTCgAgCGGC			

	1060	*	1080	*	1100
N.bellii	:	CTCAGTACGTGCTTCCAGCTACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.ventricosa	:	CTCAGTACGTGCTTCCAGCTACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.tentaculata	:	CTCAATACATGCTTCCAGCAACCGTCTGACGGATCATCCGTACAAGTTCC	:	1097	
N.rajah	:	CTCAGTACATGCTTCCAGCAACCGTCCGACGGATCATCCGTGCAAGTTCC	:	1097	
N.ephippiata	:	CTCAGCACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.gracilis	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.mirabilis	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1097	
N.chaniana	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.stenophylla	:	CTCAGCACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1097	
N.platychila	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.copelandii	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.faizaliana	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.adnata	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.diatas	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.naga	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.campanulata	:	CTCAATACGTGCTTCCAGTACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.sanguinea	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.densiflora	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.ovata	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.alba	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.spectabilis	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.thai	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.longifolia	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.talangensis	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.spathulata	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.lingulata	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.glabrata	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.sumatrana	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.tobaica	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.papuana	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1100	
N.khasiana	:	CTCAGTACGTGCTTCCAGCAACCGTCCGACGGATCAACCGTGCAAGTTCC	:	1097	
		CTCAGtACgTGcTTCCAGcaACCgTCCgACGGATCAaCCGTgCAAGTTCC			



	*	1220	*	1240	*
N.bellii	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.ventricosa	:	TCATCGCAGCAGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.tentaculata	:	TCATCGCAGCAGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1247	
N.rajah	:	TCATCGCAGCAGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1247	
N.ephippiata	:	TCATCGCAGCAGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.gracilis	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.mirabilis	:	TCATCGCAGCAGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1247	
N.chaniana	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATTTCCAGCAGCAAGAAAC	:	1250	
N.stenophylla	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1247	
N.platychila	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.copelandii	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.faizaliana	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.adnata	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.diatas	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.naga	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.campanulata	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.sanguinea	:	TCATCGCAGCTGGGAATTTCCATTTTTGGTAATATCCAGCAGCAAGAAAC	:	1250	
N.densiflora	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.ovata	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.alba	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.spectabilis	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.thai	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.longifolia	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.talangensis	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.spathulata	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.lingulata	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.glabrata	:	TCATCGCAGCTCGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.sumatrana	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.tobaica	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.papuana	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1250	
N.khasiana	:	TCATCGCAGCTGGGAATTTCCATTTTTGGGAATATCCAGCAGCAAGAAAC	:	1247	
		TCATCGCAGCTgGGAATTTcCaTTTTtGGgAATaTCCAGCAGCAAGAAAC			

	1260	*	1280	*	1300
N.bellii	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.ventricosa	:	GCAGGTGCTCTATGACCTTCAGAATATGGCCCGTTCGTTGTATTCTATGT	:	1300	
N.tentaculata	:	GCTGGTCTCTATGACCTTCAGAATGTGGCCGTGTCGTTTCGTTTCTACTC	:	1297	
N.rajah	:	GCAGGTGCTCTATGACCTTCAGAATTCGGCCGTGTCGTTTCGTTTCTACTC	:	1297	
N.ephippiata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.gracilis	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.mirabilis	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1297	
N.chaniana	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.stenophylla	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1297	
N.platychila	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.copelandii	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.faizaliana	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.adnata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.diatas	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.naga	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.campanulata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCATTTCGTTTCTACTC	:	1300	
N.sanguinea	:	GCAGGTGCTCTATGACCTTCAGAATTCGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.densiflora	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.ovata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.alba	:	GCAGGTGCTCTATGACCTTCAGAATTCGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.spectabilis	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.thai	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.longifolia	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.talangensis	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.spathulata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.lingulata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.glabrata	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1300	
N.sumatrana	:	GCAGGTGCTCTATGACCTTCAGAATGTGGCCGTGTCGTTGGTTTCTGCTC	:	1300	
N.tobaica	:	GCAGGTGCTCTATGACCTTCAGAATGTGGCCGTGTCGTTTCGTTTCTACTC	:	1300	
N.papuana	:	GCAGGTGCTCTATGACCTTCAGAATATGGCCTTGTTCGTTTCGTTTCTACGC	:	1300	
N.khasiana	:	GCAGGTGCTCTATGACCTTCAGAATTTGGCCGTGTCGTTTCGTTTCTGCTC	:	1297	
		GCAGGTGCTCTATGACCTTCAGAATtGGCCgtGTCgTTCgtTcCT ctc			

\*

N.bellii : AATGTGGTGCGTCGTAA : 1317  
N.ventricosa : GGTGCGTTGCGTCGTAA : 1317  
N.tentaculata : AGTGTGGTGCGTCGTAA : 1314  
N.rajah : AATGTGGTGCGTCGTAA : 1314  
N.ehippiata : AGTGTGGTGCGTCGTAA : 1317  
N.gracilis : AGTGTGGTGCGTCGTAG : 1317  
N.mirabilis : AGTGTGGTGCGTCGTAG : 1314  
N.chaniana : AGTGTGGTGCGTCGTAA : 1317  
N.stenophylla : GGTGTGGTGCGTCGTAA : 1314  
N.platychila : AGTGTGGTGCGTCGTAA : 1317  
N.copelandii : AGTGTGGTGCGTCGTAA : 1317  
N.faizaliana : AATGTGGTGCGTCGTAA : 1317  
N.adnata : GGTGTGGTGCGTCGTAA : 1317  
N.diatas : AGTGTGGTGCGTCGTAA : 1317  
N.naga : AATGTGGTGCGTCGTAA : 1317  
N.campanulata : AATGTGGTGCGTCGTAA : 1317  
N.sanguinea : AATGTGGTGCGTCGTAA : 1317  
N.densiflora : AATGTGGTGCGTCGTAA : 1317  
N.ovata : AATGTGGTGCGTCGTAA : 1317  
N.alba : AGTGTGGTGCGTCGTAA : 1317  
N.spectabilis : AATGTGGTGCGTCGTAA : 1317  
N.thai : AATGTGGTGCGTCGTAA : 1317  
N.longifolia : AATGTGGTGCGTCGTAA : 1317  
N.talangensis : AATGTGGTGCGTCGTAA : 1317  
N.spathulata : AGTGTGGTGCGTCGTAA : 1317  
N.lingulata : AGTGTGGTGCGTCGTAA : 1317  
N.glabrata : GGTGTGGTGCGTCGTAA : 1317  
N.sumatrana : GGTGTGGTGCGTCGTAA : 1317  
N.tobaica : GGTGTGGTGCGTCGTAA : 1317  
N.papuana : GGTGTGGTGCGTCGTAA : 1317  
N.khasiana : GGTGTGGTGCGTCGTAA : 1314  
TGTGgTGCGTCGTAA

Appendix 2. Alignment of nepenthesin II enzymes of 29 *Nepenthes* species with 12 cysteine (C) residues, 2 active sites (DTG and DSG), and a flap tyrosine (Y) residue highlighting in yellow, green, and pink, respectively; the 22 residues of NAP-specific insertion are marked with blue line, the acidic (D and E) and basic (H and R) residues with red and green font color, respectively; and the N-glycosylation site(s) are underlined.

	*	20	*	40	*	
<i>N. copelandii</i>	:	QSSSGIETPVYAGSGEYLMNVAIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. sanguinea</i>	:	QSSSGIETPVYAGDGAYLMNVSIGTPVSSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. bellii</i>	:	QSSSGIETPVYAGHGEYLMNVSIGTPANSFSAIM	<u>DTGS</u>	DLIWTQCQPCTQ	: 50	
<i>N. stenophylla</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. campanulata</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPANSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. naga</i>	:	QSSSGIETPVYAGSGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. adnata</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPAIPFSAIM	<u>DTGS</u>	DLMWTPCGPCTQ	: 50	
<i>N. tobaica</i>	:	QSSSGIETPVYAGGGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. glabrata</i>	:	QSSSGIQTPVYAGSGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. diatas</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. ovata</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. chania</i>	:	QSSSGIETPVYAGNGEYLMNVSIGTPAIPFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. spectabilis</i>	:	QSSSGIETPVYAGNGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. spathulata</i>	:	QSSSGIETPVYAGNGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. khasiana</i>	:	QSSSGIETPVYAGDGEYLMNVAIGTPPTPFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. tentaculata</i>	:	QSSSGIQTPVYAGNGEYLMNVAIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. longifolia</i>	:	QSSSGIETPVYAGSGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. thai</i>	:	QSSSGIETPVYAGSGEYLMNVAIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. densiflora</i>	:	QSSSGIETPVYAGDGAYLMNVSIGTPVSSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. rajah</i>	:	QSSSGIETPVYAGGGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. platychlila</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. ventricosa</i>	:	QSSSGIETPVYAGHGEYLMNVSIGTPANSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. talangensis</i>	:	QSSSGIETPVYAGSGEYLMNVAIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. papuana</i>	:	QSSSGIETPVYAGSGEYLMNVAIGTPDSSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. lingulata</i>	:	QSSSGIETPVYAGNGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. sumatrana</i>	:	QSSSGIETPVYAGSGAYLMNVAIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. alba</i>	:	QSSSGIETPVYAGSGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. faizaliana</i>	:	QSSSGIETPVYAGNGEYLMNVSIGTPASSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
<i>N. ehippiata</i>	:	QSSSGIETPVYAGDGEYLMNVSIGTPDSSFSAIM	<u>DTGS</u>	DLIWTQCEPCTQ	: 50	
		QSSSGI2TPVYAG GEYLMNVSIGTPa sFSAIMD3GsDL6WTqC PCTQ				
		60	*	80	*	100
<i>N. copelandii</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	YNNDCOYTYGYGDGS	: 100	
<i>N. sanguinea</i>	:	CFSQPTPIFNPQDSSSFSTLPCDSQY	<u>CODLPSETC</u>	CNNNECOYTYGYGGGS	: 100	
<i>N. bellii</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDRS	: 100	
<i>N. stenophylla</i>	:	CFSQPTPIFNPQHDPDSSSFSTLPCESQY	<u>CODLPSETC</u>	Y-NDCOYTYGYGDGS	: 99	
<i>N. campanulata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGGGS	: 100	
<i>N. naga</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYAYGYGGGS	: 100	
<i>N. adnata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. tobaica</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. glabrata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. diatas</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. ovata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. chania</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	YNNDCOYTYGYGDGS	: 100	
<i>N. spectabilis</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. spathulata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGGGS	: 100	
<i>N. khasiana</i>	:	CFPQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. tentaculata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. longifolia</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. thai</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. densiflora</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGGGS	: 100	
<i>N. rajah</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. platychlila</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGGGS	: 100	
<i>N. ventricosa</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGGGS	: 100	
<i>N. talangensis</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. papuana</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. lingulata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. sumatrana</i>	:	CFSQPTPIFNPQDSSSFSTLPCGSQY	<u>CODLPNDTC</u>	CNNNECOYTYGYGDRS	: 100	
<i>N. alba</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
<i>N. faizaliana</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	YNNYCOYTYGYGDGS	: 100	
<i>N. ehippiata</i>	:	CFSQPTPIFNPQDSSSFSTLPCESQY	<u>CODLPSETC</u>	CNNNECOYTYGYGDGS	: 100	
		CfsQPTPIFNPQDSSSFSTLPCESQYCODLPs 3C NNECOYtYGYG gs				

	*	120	*	140	*	
<i>N. copelandii</i>	:	STQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. sanguinea</i>	:	TTQGYMATE	TFTTF	ETGSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. bellii</i>	:	STQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. stenophylla</i>	:	STQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 149
<i>N. campanulata</i>	:	TTQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. naga</i>	:	TTQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. adnata</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. tobaica</i>	:	TTQGYMATE	TLTF	ETSSMPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. glabrata</i>	:	STQGYMATE	TFTTF	ETSSVPNIAFGCG	QDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. diatas</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLVGMWGPLSL	: 150
<i>N. ovata</i>	:	TTQGYMATE	TFTTF	ETSSVPNIAFGCG	ENNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. chania</i>	:	STQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. spectabilis</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLVGMWGPLSL	: 150
<i>N. spathulata</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. khasiana</i>	:	TTQGYMATE	TFTTF	ESGSIPNIAFGCG	ENNQFGQNGAGLVGMWGPLSL	: 150
<i>N. tentaculata</i>	:	STQGYMATE	TFTTF	ETGSVPNIAFGCG	QDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. longifolia</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. thai</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. densiflora</i>	:	TTQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. rajah</i>	:	STQGYMATE	TFTTF	ETGSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. platychila</i>	:	TTQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGPNGAGLIGMGWGPLSL	: 150
<i>N. ventricosa</i>	:	STQGYMATD	TFTTF	ETGSVPNIAFGCG	EDNQFGQNGAGLVGMWGPLSL	: 150
<i>N. talangensis</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. papuana</i>	:	STQGYMATE	TLTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. lingulata</i>	:	TTQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. sumatrana</i>	:	STQGYMATE	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. alba</i>	:	TTQGYMATD	TFTTF	ETSSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
<i>N. faizaliana</i>	:	TTQGYMATE	TFTTF	ETSSMPNIAFGCG	EDNQFGPNGAGLIGMGWGPLSL	: 150
<i>N. ehippiata</i>	:	STQGYMATE	TFTTF	ETGSVPNIAFGCG	EDNQFGQNGAGLIGMGWGPLSL	: 150
		3TQGYMAT	TtTFE3sS6PNIAFGCG	1NQFGqGNGAGL6GMGwGPLSL		

		160	*	180	*	200
<i>N. copelandii</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. sanguinea</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSIPT	: 200
<i>N. bellii</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSLNPT	: 200
<i>N. stenophylla</i>	:	PSQLGVGQFSYC	M	ASYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 199
<i>N. campanulata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLVHSSLNPT	: 200
<i>N. naga</i>	:	PSQLGVGQFSYC	M	TSGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. adnata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. tobaica</i>	:	PSQLGVSQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. glabrata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSLNPT	: 200
<i>N. diatas</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. ovata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. chania</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. spectabilis</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. spathulata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. khasiana</i>	:	PSQFDVTQFSYC	M	TPYGSSSPSTLALGSAASGVP	DGTPSTTLIHSSPIPT	: 200
<i>N. tentaculata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSPNPT	: 200
<i>N. longifolia</i>	:	PSQLDVGQFSYC	M	TSSGSSSPSTLALGSAASGVP	EGSPSTTLIHSSPNPT	: 200
<i>N. thai</i>	:	PSQLGVGQFSYC	M	TSSGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. densiflora</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. rajah</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. platychila</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. ventricosa</i>	:	PSQLGVGQFSYC	M	TSSGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. talangensis</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. papuana</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSHPT	: 200
<i>N. lingulata</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. sumatrana</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. alba</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. faizaliana</i>	:	PSQLGVGQFSYC	M	TSYGSSSPSTLALGSAASGVP	EGSPSTTLIHSSSNPT	: 200
<i>N. ehippiata</i>	:	PSQLgVgQFSYC	M	tYSSSPSTLALGSAASGVP	EGSPSTTLIHSSQNPT	: 200
		PSQLgVgQFSYC	M	tYSSSPSTLALGSAASGVP	EG3PSTTL6HSS nPT	

	*	220	*	240	*
<i>N. copelandii</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. sanguinea</i>	:	YYYITLQGITVGGDNLGIPSSSTFRLQDDGTGGMIIDSGTTLTYLPQDAYY	:	250	
<i>N. bellii</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGATLTYLPQDAYN	:	250	
<i>N. stenophylla</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	249	
<i>N. campanulata</i>	:	FYYITLQGITVGGDYLGISSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. naga</i>	:	FYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. adnata</i>	:	YYYITLQGITVGGDNLGIPSSSTFRLQDDGTGGMIIDSGTTLTYLPQDAYY	:	250	
<i>N. tobaica</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. glabrata</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. diatas</i>	:	YYYITLQGITVGGDNLGIASSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. ovata</i>	:	FYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. chaniana</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. spectabilis</i>	:	YYYITLQGITVGGDNLGIASSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. spathulata</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. khasiana</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGRIIDSGTTLTYLPQDAYN	:	250	
<i>N. tentaculata</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. longifolia</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYY	:	250	
<i>N. thai</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. densiflora</i>	:	FYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. rajah</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. platyphila</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. ventricosa</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. talangensis</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. papuana</i>	:	FYYITLQGITVGGDSLGISSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. lingulata</i>	:	FYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. sumatrana</i>	:	FYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. alba</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. faizaliana</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	
<i>N. ehippiata</i>	:	YYYITLQGITVGGDNLGIPSSSTFQLODDGTGGMIIDSGTTLTYLPQDAYN	:	250	

5YYITLQG6tVGGDnLGIPsSTFqLQ DGTGG IIDSGtTLTYLPqDAYn

	260	*	280	*	300
<i>N. copelandii</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. sanguinea</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. bellii</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQLPSDGSTVQVPEISMQFDGGMLN	:	300	
<i>N. stenophylla</i>	:	AVAQAFTDQINLPTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	299	
<i>N. campanulata</i>	:	AVAQAFTDQINLSTVDESSNGLNTCFQIPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. naga</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. adnata</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. tobaica</i>	:	AVAQAFTDQINLPTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. glabrata</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISLQYDGGVLN	:	300	
<i>N. diatas</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. ovata</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. chaniana</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. spectabilis</i>	:	AVAQAFTDQINLPTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. spathulata</i>	:	AVAQAFTDQINLPTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. khasiana</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. tentaculata</i>	:	AVAQAFTDQIHLSSTVE-SSSGLNTCFQQPSDGSSVQVPEISMQFDGGVLN	:	299	
<i>N. longifolia</i>	:	AVAQAFTDQIDLPTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. thai</i>	:	AVAQAFTDQIDLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. densiflora</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. rajah</i>	:	ALAQAFTDQIDLPTVE-SSSGLSTCFQQPSDGSSVQVPEISMQFDGGVLN	:	299	
<i>N. platyphila</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. ventricosa</i>	:	AVAQAFTDQINLPTVDESSSGLSTCFQLPSDGSTVQVPEISMQFDGGMLN	:	300	
<i>N. talangensis</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. papuana</i>	:	AVAQAFTYQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. lingulata</i>	:	AVSQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. sumatrana</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. alba</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. faizaliana</i>	:	AVAQAFTDQINLSTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	
<i>N. ehippiata</i>	:	AVAQAFTDQIDLPTVDESSSGLSTCFQQPSDGSTVQVPEISMQFDGGVLN	:	300	

A6aQAFTDQI L TVDESSsGLsTCFQqPSDGS3VQVP IS6Q5DGG6LN



	*	320	*	340	*
<i>N. copelandii</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISILGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. sanguinea</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNSAVS	:	350
<i>N. bellii</i>	:	LGEQNILISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. stenophylla</i>	:	LGEENILISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNWAVS	:	349
<i>N. campanulata</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. naga</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. adnata</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. tobaica</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNVAVS	:	350
<i>N. glabrata</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. diatas</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. ovata</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. chaniana</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNFQQQ	ETQVLYDLQNLAVS	:	350
<i>N. spectabilis</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. spathulata</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. khasiana</i>	:	LGEENILISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. tentaculata</i>	:	LGEENILISPAEGVICLAMGSSSSQGGISIFGNIQQQ	ETLVLYDLQNVAVS	:	349
<i>N. longifolia</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. thai</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. densiflora</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. rajah</i>	:	LGEQNILISPAEGVICLAMGSSSSQGGISIFGNIQQQ	ETQVLYDLQNSAVS	:	349
<i>N. platychila</i>	:	LGEENILISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. ventricosa</i>	:	LGEQNILISPAEGVICLAMGSSSSQGGISIFGNIQQQ	ETQVLYDLQNMMAAS	:	350
<i>N. talangensis</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. papuana</i>	:	LGEENILISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNMALS	:	350
<i>N. lingulata</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. sumatrana</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNVAVS	:	350
<i>N. alba</i>	:	LGEENVLISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNSAVS	:	350
<i>N. faizaliana</i>	:	LGEENILISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS	:	350
<i>N. ehippiata</i>	:	LGEENILISPAEGVICLAMGSSSSQGGIPFLGNIQQQ	ETQVLYDLQNLAVW	:	350
		LGE N6LISPAEGVICLAMGSSSSQLGISIFGNIQQQ	ETQVLYDLQNLAVS		

<i>N. copelandii</i>	:	FVPAQCGAS	:	359
<i>N. sanguinea</i>	:	FVPAQCGAS	:	359
<i>N. bellii</i>	:	FVPTQCGAS	:	359
<i>N. stenophylla</i>	:	FVPARCGAS	:	358
<i>N. campanulata</i>	:	FVPTQCGAS	:	359
<i>N. naga</i>	:	FVPTQCGAS	:	359
<i>N. adnata</i>	:	FVPARCGAS	:	359
<i>N. tobaica</i>	:	FVPTRCGAS	:	359
<i>N. glabrata</i>	:	FVPARCGAS	:	359
<i>N. diatas</i>	:	FVPAQCGAS	:	359
<i>N. ovata</i>	:	FVPTQCGAS	:	359
<i>N. chaniana</i>	:	FVPAQCGAS	:	359
<i>N. spectabilis</i>	:	FVPTQCGAS	:	359
<i>N. spathulata</i>	:	FVPAQCGAS	:	359
<i>N. khasiana</i>	:	FVPARCGAS	:	359
<i>N. tentaculata</i>	:	FVPTQCGAS	:	358
<i>N. longifolia</i>	:	FVPTQCGAS	:	359
<i>N. thai</i>	:	FVPAQCGAS	:	359
<i>N. densiflora</i>	:	FVPTQCGAS	:	359
<i>N. rajah</i>	:	FVPTQCGAS	:	358
<i>N. platychila</i>	:	FVPAQCGAS	:	359
<i>N. ventricosa</i>	:	LYSMWCVAS	:	359
<i>N. talangensis</i>	:	FVPAQCGAS	:	359
<i>N. papuana</i>	:	LFPTRCVAS	:	359
<i>N. lingulata</i>	:	FVPAQCGAS	:	359
<i>N. sumatrana</i>	:	LVPARCGAS	:	359
<i>N. alba</i>	:	FVPAQCGAS	:	359
<i>N. faizaliana</i>	:	FVPTQCGAS	:	359
<i>N. ehippiata</i>	:	FVTTQCGAS	:	359
		fvp CgAS		

Appendix 3. The characters included in the classification of the genus *Nepenthes* by Danser (1928)

Groups	General characteristics	Stem and leaves	Phyllotaxis	Indumentum	Pitcher form	Peristome	Appendage on underside of lid	Inflorescence	Distribution
<i>Vulgatae</i>	Simple forms.	Have no coarse stem, no large nor broad. Mostly lanceolate and a few are sessile.	Almost always 2/5.	No hirsute reddish nor brown indumentum.	Not large and rarely infundibuliform.	Narrow (<10 mm).	Mostly no appendage, very rarely 1 appendage.	Paniculate or racemose.	All over the generic area.
<i>Montanae</i>	The colour of herbarium specimens are rarely blackish.	Always sessile.	Always 2/5.	Striking short hairs on lower side of lid in some species.	Often infundibuliform and often larger than <i>Vulgatae</i> .	Generally broader than <i>Vulgatae</i> .	Same characteristics as <i>Vulgatae</i> .	Racemosa.	Found in the mountains of the Malay peninsula, Sumatra, Java and perhaps in those of southern Borneo.
<i>Nobiles</i>	The colour of herbarium specimens are yellowish.	Intermediate between <i>Vulgatae</i> - <i>Montanae</i> .	Intermediate between <i>Vulgatae</i> - <i>Montanae</i> .	Red-brown indumentum.	Intermediate between <i>Vulgatae</i> - <i>Montanae</i> .	Not broad.	Never appendages.	Racemosa.	Found in Sumatra, Palawan and Borneo.
<i>Regiae</i>	Most beautiful and largest species. Most remarkable forms. Large, coarse plant. The colour of herbarium specimens are mostly yellowish or reddish brown.	Large petiolate.	Often ½ in the elongated stems.	A coarse red-brown indumentum.	Infundibuliform upper pitchers.	Mostly flattened or expanded.	Appendage on the lower surface of the lid near the apex.	Racemosa.	Occur in the first place in Borneo, but in few species spread as far as Mindanao and New Guinea.
<i>Insignes</i>	Like that of <i>Regiae</i> , also comprises very beautiful, but in general less aberrant forms.	Large petiolate. Often sessile. Coarse stem with large leaves.	-	More sparse or even wholly absent.	Large pitchers. Campanulate-infundibuliform upper pitchers.	flattened or expanded.	Never appendages.	Racemosa.	occur in the first place in Borneo, but in few species spread as far as Mindanao and New Guinea.
<i>Urceolatae</i>	Not showing a strong likeness.	-	2/5.	-	Urceolate and glandular lower pitchers. Infundibuliform upper pitchers.	Flattened at the inner side and very delicately ribbed.	-	Paniculate.	Sunda shelf, New Guinea and north-western Borneo.