

# Systematic Study of Genus *Nepenthes* L. (Nepenthaceae) in Western Sarawak, Borneo

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# Systematic Study of Genus Nepenthes L. (Nepenthaceae) in Western Sarawak, Borneo

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## DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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### ABSTRACT

Nepenthes is the sole genus of the monotypic family known as Nepenthaceae, under the order of Caryophyllales. This genus is native to Southeast Asia with Borneo and Sumatra being the centre of its diversity. *Nepenthes* are known as highly studied carnivorous plants for their unique morphological characteristics, ecology, carnivorous syndrome, ethnobotanical, phytochemical, and pharmacological properties. Hence, this study focused on revising and comparing the morphological characteristics of *Nepenthes* that are distributed particularly in western Sarawak as well as identifying the anatomical, micromorphological, and palynological characteristics of the Nepenthes studied. Distribution and ecology of each Nepenthes taxa were also studied. A total of eighteen taxa were recorded from western Sarawak (Samarahan, Kuching, Serian, and Samarahan). Most of the taxa were examined based on fresh samples collected from the field; Nepenthes albomarginata, N. ampullaria, N. gracilis, N. hirsuta, N. hispida, N. mirabilis, N. mirabilis var. echinostoma, N. nazreeana sp. nov. ined., N. rafflesiana and others. The remaining five taxa; N. bicalcarata, N. northiana, N. veitchii, N. tentaculata, and N. x bauensis were examined based on herbarium specimens deposited in SAR. The morphological examination revealed that the Nepenthes studied has interspecific and intraspecific variation among the taxa. The anatomical studies of 13 collected *Nepenthes* taxa under the light microscope were focused on two parts; the epidermal layers, and the midrib. The stomatal complex type of Nepenthes studied are all anomocytic either with straight, or sinuous type of anticlinal walls on their abaxial or adaxial surfaces. Additional data from Field Scanning Electron Microscopy revealed that the stomata have three types of formation; sunken, raised, and semi-raised. They are developed in various sizes and densities among the polygonal epidermal cells. The sessile glands also were noticed to appear mostly on the adaxial surfaces of leaves. Meanwhile, the midrib cross section

shows four types of outer shapes with different vascular bundle arrangements and have a complex closed vascular system with consistent closed, collateral vascular bundles. The micromorphological characteristics were observed mainly on the epidermal layers of the leaves, and the pitchers of Nepenthes under FESEM. Trichomes were present mostly on the abaxial surfaces of the leaves compared to the adaxial surfaces. More than five types of trichomes were identified and all of them were categorised into two which are glandular trichomes and non-glandular trichomes. The examination of the inner surface of the pitcher depicted a variety of features such as the lunate cells and the digestive glands. The lunate cells were found distributed abundantly on the upper part of the pitcher body which is known as the waxy zone. Ten taxa from the 13 Nepenthes studied own these features that are formed in a crescent-like shape which bends downwards and elongate horizontally in various lengths. Meanwhile, the digestive glands were observed present on the inner surface of the pitcher of all 13 Nepenthes studied. Most of them are developed in the basal half of the pitcher, known as the digestive zone. The digestive glands are conspicuously different in sizes and density among the 13 taxa studied. The palynological study focused on six Nepenthes taxa; N. ampullaria, N. gracilis, N. mirabilis, N. nazreeana sp. nov. ined., N. rafflesiana, and N. reinwardtiana are tetrahedral tetrad and inarpeturate with echinatemicroechinate ornamentation. The sizes of the pollen tetrads of the six Nepenthes studied are slightly varied between 17.02-19.91 µm in diameter, and all of them were identified as small size which falls between 10-25 µm. The pollen characteristics are homogenous among the six Nepenthes taxa studied. Therefore, they are not possessing any taxonomic value that can help in delimiting the taxa in the genus. But, they can be differentiated by their sizes, which can be influenced by the environment or the methods used to observe the pollen.

Keywords: Borneo, Carnivorous plants, Caryophyllales, Malaysia, taxonomy

#### Kajian Sistematik Genus Nepenthes L. (Nepenthaceae) di Barat Sarawak, Borneo

#### ABSTRAK

Nepenthes merupakan genus tunggal dalam keluarga monotip yang dikenali sebagai Nepenthaceae, di bawah order Caryophyllales. Genus ini berasal daripada Asia Tenggara, dengan Borneo dan Sumatera menjadi pusat kepelbagaiannya. Nepenthes merupakan tumbuhan karnivor yang banyak dikaji kerana ciri morfologinya yang unik, ekologi, sindrom karnivor, sifat-sifat etnobotani, fitokimia, dan farmakologi. Oleh itu, kajian ini tertumpu kepada penelitian dan perbandingan ciri-ciri morfologi Nepenthes yang terdapat di bahagian barat Sarawak khususnya, serta mengenal pasti ciri-ciri anatomi, mikromorfologi dan palinologi Nepenthes. Taburan dan ekologi setiap takson Nepenthes turut dikaji. Sebanyak 18 taksa telah direkodkan dari barat Sarawak (Samarahan, Kuching, Serian, dan Samarahan). Kebanyakan taksa telah diperiksa berdasarkan sampel segar yang dikumpul daripada lapangan; N. albomarginata, N. ampullaria, N. gracilis, N. hirsuta, N. hispida, N. mirabilis, N. mirabilis var. echinostoma, N. nazreeana sp. nov. ined., N. rafflesiana dan lainlain. Baki lima taksa; N. bicalcarata, N. northiana, N. tentaculata, N. veitchii dan N. x bauensis telah diperiksa berdasarkan spesimen herbarium SAR. Pemeriksaan morfologi mencirikan bahawa Nepenthes yang dikaji mempunyai variasi interspesifik dan intraspesifik dalam kalangan taksa. Kajian anatomi 13 taksa Nepenthes di bawah mikroskop cahaya tertumpu kepada dua bahagian; lapisan epidermis, dan pelepah. Semua Nepenthes yang dikaji mempunyai jenis kompleks anomositik sama ada dengan jenis dinding antiklinal lurus atau berliku pada permukaan abaksial atau adaksial. Data tambahan dari Mikroskop Elektron Pengimbasan Pelepasan Medan (FESEM) mecirikan stomata mempunyai tiga jenis pembentukan; tenggelam, timbul, dan separuh timbul dalam pelbagai saiz dan ketumpatan di kalangan sel epidermis poligon. Kelenjar sesil juga diperhatikan kebanyakannya muncul pada permukaan adaksial daun. Keratan rentas pelepah pula menunjukkan empat jenis bentuk luar dengan susunan berkas vaskular yang berbeza dan mempunyai sistem vaskular tertutup yang kompleks dengan berkas vaskular cagaran tertutup secara konsisten. Ciri-ciri mikromorfologi diperhatikan terutamanya pada lapisan epidermis daun, dan periuk Nepenthes di bawah FESEM. Kebanyakan trikom terdapat pada permukaan abaksial daun berbanding permukaan adaksial. Lebih daripada lima jenis trikom telah dikenalpasti iaitu trikom kelenjar dan trikom bukan kelenjar. Pemeriksaan permukaan dalam periuk menggambarkan pelbagai ciri seperti sel lunat dan kelenjar pencernaan. Sel lunat didapati tersebar banyak di bahagian atas badan periuk yang dikenali sebagai zon berlilin. Sepuluh daripada 13 taksa Nepenthes yang dikaji memiliki ciri-ciri ini, berbentuk seperti bulan sabit yang membongkok ke bawah dan memanjang secara mendatar dalam pelbagai panjang. Sementara itu, kelenjar pencernaan diperhatikan terdapat pada permukaan dalaman periuk kera bagi kesemua 13 Nepenthes yang dikaji. Kebanyakannya dibangunkan di separuh bawah periuk, yang dikenali sebagai zon pencernaan. Kelenjar pencernaan berbeza dengan ketara dalam saiz dan ketumpatan antara 13 taksa yang dikaji. Kajian palinologi tertumpu kepada enam taksa Nepenthes; N. ampullaria, N. gracilis, N. mirabilis, N. nazreeana sp. nov. ined., N. rafflesiana, dan N. reinwardtiana ialah tetrad tetrahedral dan tidak terapeturasi dengan hiasan echinate-microechinate. Saiz tetrad debunga enam Nepenthes yang dikaji sedikit berbeza antara diameter 17.02-19.91 µm, dan kesemuanya dikenal pasti sebagai saiz kecil, antara 10-25 µm. Ciri-ciri debunga enam takson Nepenthes yang dikaji ialah homogen. Oleh itu, tidak mempunyai sebarang nilai taksonomi yang boleh membantu dalam membatasi taksa dalam genus. Tetapi, boleh dibezakan mengikut saiznya, yang dipengaruhi oleh persekitaran atau kaedah yang digunakan untuk memerhatikan debunga.

Kata kunci: Borneo, Caryophyllales, Malaysia, taksonomi, tumbuhan karnivor.

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# LIST OF ABBREVIATIONS

| Abax    | Abaxial  |
|---------|--|
| Adax    | Adaxial  |
| C.      | Around or about                                    |
| CGS     | Centre for Graduate Studies                        |
| cm      | Centimeter   |
| DA      | Disturbed Areas                                    |
| DF      | Disturbed Forest                                   |
| FESEM   | Field Scanning Electron Microscopy                 |
| FOV     | Field of View                                      |
| FR      | Forest Reserve                                     |
| GPS     | Geographic Coordinate System                       |
| HUMS    | Herbarium of Universiti Malaysia Sarawak           |
| KF      | Kerangas Forest                                    |
| LDF     | Lowland Dipterocarp Forest                         |
| LF      | Limestone Forest                                   |
| m       | Meter  |
| m.a.s.l | Meter above the sea level                          |
| MDF     | Mixed Dipterocarp Forest                           |
| mm2     | Squared meter                                      |
| Mt.     | Mount  |
| N/A     | Not Available                                      |
| NH      | Prefix cord of herbarium specimen for Nabilah Huda |
| NP      | National Park                                      |

| pH     | Potential of Hydrogen                  |
|--------|--|
| PSF    | Peat Swamp Forest                      |
| SAR    | Herbarium of Sarawak Forest Department |
| SEM    | Scanning Electron Microscopy           |
| SF     | State Land Forest                      |
| SP     | Sandstone Plateau                      |
| UNIMAS | Universiti Malaysia Sarawak            |
| UP     | Upper pitcher plant                    |
| LP     | Lower pitcher plant                    |
| μm     | Micrometer                             |
| °C     | Degree celcius                         |
| %      | Percentage                             |

### **CHAPTER 1**

### **INTRODUCTION**

#### 1.1 Study Background

The diversity of *Nepenthes* can be found mostly scattered in Malesia Region (Cheek & Jebb, 2001). Many of them are endemic to Borneo island which include Malaysia (Sabah and Sarawak), Brunei as well as Indonesia (Sumatra). *Nepenthes* preferred to grow in soil that is alkaline, acidic and lacking in nutrients mainly Nitrogen which leads them to be carnivorous (Handayani, 2017). Amazingly, they adapted well to that condition by deriving nutrients using their unique morphological characteristic which is the modified leaf tip to attract, trap and digest the prey mainly insects (Adam, 1997; Mithofer, 2011).

*Nepenthes* L. was first described and formally published by Carl Linnaeus in 1753 which then *Nepenthes distillatoria* L., a species from Sri Lanka referred to as type specimens for the genus (Linnaeus, 1753). Phillipps et al. (2008) described that the *Nepenthes* can grow to more than one meter and carries a vase-like shape of a hollow flower or fruit in red or yellow colour with its lid. Also, they have modified leaves that are often known by various names such as jugs, little bags, pots, urns, jurns, tankards, flasks, beakers, mugs and stomachs (Phillipps et al., 2008). Their seeds are usually dispersed by wind and can travel not so far (Clarke, 1997).

Clarke and Lee (2004) stated that new species of *Nepenthes* L. are being described each year which contributes to the increasing number of species in this genus. Despite that, their population are declining due to overharvested of the plant and deterioration of their habitat. Now, many of the species are threatened with extinction. *Nepenthes* are economically important as they are collected for preparing foods and medicine as well as ornamentals in domestic collections (Rizqiani et al., 2018). These activities have caused too many problems which then various initiatives and actions are being taken to conserve it.

In taxonomy, the genus *Nepenthes* L. is problematic at the infrageneric level and it was taxonomically challenging to delineate the *Nepenthes* taxa due to their high level of intraspecific variability and polymorphisms, extreme heterophylly and a bias in the selection of herbarium material for extreme forms (Thorogood, 2010). Past monographic revisions of *Nepenthes* L. by Hooker (1873), Macfarlane (1908) and Danser (1928) have not fully resolved the family. The extreme heterophylly in the genus *Nepenthes* L. can be seen by the production of morphologically distinct lower and upper pitchers of the same species (Thorogood, 2010). Thus, the identification of *Nepenthes* species in the field must consider other characteristics including the morphology of the lid and peristome as well as the characteristics of the leaves and stems and not solely depend on the pitcher shape and colouration.

So, these present studies are aimed to revise the taxonomy of *Nepenthes* L. distributed in western Sarawak and identify the morphological, anatomical and palynological characteristics that could be useful data to differentiate those species. Other than that, this study also aimed to describe and documented its ecology and distribution in the selected region. This study will be focusing on the *Nepenthes* species that are distributed in western Sarawak which included several divisions such as Kuching, Samarahan, Serian and Sri Aman.

### **1.2 Problem Statement**

The relationship and differences between most of the taxa in the genus of *Nepenthes* are taxonomically challenging as they have a high level of intraspecific variability and polymorphism, an intense heterophylly and a bias in the selection of herbarium materials (Thorogood, 2010). Other than that, there are limited information and descriptions of the micromorphological, anatomical, and palynological characteristics of *Nepenthes*, especially in western Sarawak, Borneo, Malaysia. Therefore, it is very important to revise the taxonomy of *Nepenthes* species that are distributed in western Sarawak and identify other systematic characteristics through micromorphology, anatomy, and palynology to aid in delimiting the taxa in the genus. Along with that, it is also crucial to record the current distribution of *Nepenthes* and describe the ecological characteristics of the taxa that exist particularly in western Sarawak.

## 1.3 **Objectives**

The aims of this study were as follows:

- 1. To identify and revise the taxonomy of *Nepenthes* taxa and its characteristics that differentiate the taxa in western Sarawak.
- 2. To describe the distribution and ecology of *Nepenthes* taxa in western Sarawak.