IAPT RESEARCH GRANT APPLICATION FORM

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Field of Specialisation : Plant systematics; Plant anatomy, Palynology

Employment status : Permanent

List of up to four relevant publications :

**Mohd Norfaizal, G.,** Amin Asyraf, T.Dome, N., A. Latiff, Muhammad Ikhwanuddin, M.E., Edward, E.B., Ahmad Zaki, Z. & Anuar Rasyidi, M.N. 2019. The systematic significance of leaf epidermal micromorphology of ten *Nepenthes* species (Nepenthaceae) from Peninsular Malaysia. *Reinwardtia* 18(2): 81-96 DOI 10.14203/reinwardtia.v18i2.3753.

**Amin Asyraf, T.,** Mohd Norfaizal, G., Dome, N., Muhamad Ikhwanudin M.E., Edward, E.B., A. Latiff, Anuar Rasyidi, M.N., & Mohamad Alias, S. 2020. *Nepenthes* × *setiuensis*, a new nothospecies of pitcher plant (Nepenthaceae) from montane cloud forest of Peninsular Malaysia. (in press). Malayan Nature Journal. Malaysian Nature Society Publication Press.

**Amin Asyraf, T.,** Mohd Norfaizal, G., Dome, N., Muhamad Ikhwanudin M.E., Edward, E.B., A. Latiff, Anuar Rasyidi, M.N., & Mohamad Alias, S. 2020. *Nepenthes malayensis* (Nepenthaceae), a new species of carnivorous pitcher plant from Peninsular Malaysia (in press). Kew Bulletin. Royal Botanic Gardens, Kew.

Names of people providing recommendation letters (2 letters are required):

1. Emeritus Prof. Dr. Abdul Latiff Mohamad
2. Assoc. Prof Dr. Noraini Talip

**Project Details**

Title of proposed project : Anatomical evolution and systematics of the

leaf characters in *Nepenthes* and natural hybrids collected in Peninsular Malaysia

**Description of the project:**

This research aims to study the structural changes occur in the leaf, stem, and pollen (if any) in *Nepenthes* natural hybrids, as compared with true *Nepenthes* species in Peninsular Malaysia. There are around 160-180 species predominantly in Malesian región of which 50 can be found in Malaysia (Clarke et al. 2012; Christenhusz et al. 2016; Murphy et al. 2019) . Compared to Malaysian Borneo, Peninsular Malaysia is home to accepted eleven (11) species (*N. alba* Ridl., *N. albomarginata* T. Lobb. ex Lindl., *N. gracillima* Ridl., *N. ampullaria* Jack, *N. benstonei* C. Clarke, *N. gracilis* Korth., *N. macfarlanei* Hemsl., *N. rafflesiana* Jack, *N. mirabilis* (Lour.), Druce, *N. ramispina* Ridl. and *N. sanguinea* Lindl.) and also a number of natural hybrids that thrive in their natural habitats (e.g*. Nepenthes ampullaria* x *N. gracilis; N. ampullaria* x *N. rafflesiana; N. benstonei* x *N. mirabilis; N. gracilis* x *N. rafflesiana, N. mirabilis* x *N. sanguinea*)*.* We believe that anatomy and palynology analysis could be developed as a tool as a method of identification and inheritance patterns from both parents of the true species. The information regarding this study will help us in understanding more on plant systematic and its functionality with implication towards the structures of plants cell and tissue and also pollen morphological characteristics. Hybrids taxonomic position could sometimes be difficult to resolve; hence, this knowledge is useful as screening method for species and natural hybrids identification and documentation for *Nepenthes* in Peninsular Malaysia.

Total budget request: USD2000.00

**Project Proposal**

**Project description**

**Introduction**

Pitcher plants of Peninsular Malaysia are considered as spectacular unique ornamental plants which include only a handful of eleven (11) species and they can be found thriving in less fertile soils from sea-level to over 2000m in elevations. Despite this, many systematic evidences regarding this genus are still scarce specifically on the cellular characters and how they influence systematic treatment of *Nepenthes*. Other than those eleven species thriving in disturbed heath forests, by the roadsides, near forest margins and on mountain peaks, another group of pitcher plants – known as natural hybrids or nothospecies – possesses a taxonomic significance and is quite interesting to study. It is crucial to document their inheritance patterns in terms of leaf anatomical characters and cell structures for the identification purpose; however, the data on such aspects are also lacking. Therefore, we see this information gap between true *Nepenthes* species and natural hybrids contains untapped, valuable information that can be utilised in adding significance data of systematics evaluation of pitcher plants species particularly in Peninsular Malaysia.

**Research questions**

1. How many *Nepenthes* species and its natural hybrids can be found in Peninsular Malaysia?
2. Can we differentiate the anatomical structures of *Nepenthes* species and its natural hybrids?
3. Are there any unique foliar (or pollen) structures of true *Nepenthes* species as compared to the natural hybrids, or vice versa?

**Objectives**

1. To elucidate the structural changes that occur in *Nepenthes* species and their natural hybrids.
2. To identify the "unique" structures occuring in *Nepenthes* species vs. natural hybrids.
3. To determine the diagnostic structure-based marker from the various part analysis of *Nepenthes* taxa with references on its anatomical, micromorphological and palynological (if any).

**Material and Methodology**

i. Field Study and Visits

The project will determine the distribution of *Nepenthes* and their natural hybrids in Peninsular Malaysia by conducting collection trips in targeted forest reserves, forested lands and non-forest habitats. The species will be characterised using their morphology, pollen grain characters and micromorphology. The collection mission and characterisation works will follow van Hintum et al. (2000). *Nepenthes* genetic resources will be conserved in the form of plant collection whenever possible, as the preliminary basal materials for taxonomic study. The collection trip will be carried out throughout Peninsular Malaysia with a special emphasis on habitats that has not been or less accessed by previous explorers. In order to meet the adequate study materials, 30 specimens of *Nepenthes* and their natural hybrids are targeted to be collected. Their passport data is based on morphological characters such as plant habit, leaf, inflorescence and pitchers as provided by Cheek & Jebb (2001), Clark 2001, and Jebb & Chek (1997). Morpho-systematics characterisation and evaluation will follow Anton et al. (2011) with some modifications. Ex situ conservation of the specimens will be conserved at MARDI Serdang's glasshouses and other designated facilities. Their voucher specimen of the species will be collected and kept at MDI herbarium for future references. Pollen grain analysis and micromorphological evaluation will follow the standard anatomy procedures with three replications for each accession (Sass 1950). Voucher specimens will be prepared for future references and will be kept at the MARDI Herbarium (MDI). Selected *Nepenthes* species/natural hybrids will be obtained from individual collections and also from the field, and these samples will undergo anatomy, micromorphology and palynology study (if any). Research and collection permit for samples taken from forest reserves will be obtained from related authorities such as the forestry department.

ii. Experimental Design

Three to five replications of *Nepenthes* samples will be provided in the selected study area. An inventory list of natural hybrids will be documented and analysed in this study for comparison with true species. Selected living parts from various species collected from the field and gained from individual’s collection/field will be preserved for future references.

iii. Anatomy Study

Leaf anatomy study will include such procedures: leaf clearing; leaf epidermal peeling methods; petiole, lamina, margin and midrib sectioning using sliding microtome; sections of petioles, lamina, midribs, leaf margin, cleared venation and scrapped abaxial and adaxial epidermis will be stained using Alcian green and Safranin, dehydrated, mounted on the slides using Euparal and Canada Balsam, dried in the oven and will be observed under light microscope; image will be captured using digital camera attached to a computer with Analysis Docu Software; illustration of petioles and midribs will be done under lucidar camera. Analyses will be done by precise description; comparative study and also phenetic analysis will be done using MVSP. Methods in leaf micromorphology study will include dehydration series methods, gold coating, and critical point drying before the samples get observed and captured under a scanning electron microscope (SEM). Micromorphology methods, observation and description will be made precisely and the data will be analysed together with leaf anatomical and micromorphological characteristics. In order to observe the anatomical structure of stem to differentiate among rice with a different disease, the middle part of the leaf tissue will be cut and fix in fixative solution Acetate: Alcohol 70% (1:3) for at least 24 hours.

The anatomical characteristics measurements will be measured and analysed by using a statistical software package attached to the microscope. The characters to be measured include a) the number of large vascular bundles (LBVB), b) the area of large vascular bundles (ALVB), c) the number of small vascular bundles (NSVB), d) the area of small vascular bundles (ASVB), e) the number of air chambers (NAC), f) the area of air chambers (AAC) and g) the thickness of mechanical tissue (TMT). Sections will then be photographed under the light microscope with a camera attached.

**Palynology study (depending on pollen availability)**

Methods will involve a) fixation of fresh specimens in the 4% of Glutaraldehyde solutions of the 70% alcohol solutions. Anther will be separated from the other parts of the flower on the watch glass. Anther will then be crushed using the scalpel under the dissecting microscope. Pollen will then be transferred into a vial, b) Slide preparation will be done for the observation under the light microscope where pollen will be dripped onto the glass slide. Glycerine jelly will then be used on the slide and closed with glass slip. The slide will be heated with temperature of 40-60°C. After the slide cooled down, slide will be labelled and ready to be viewed under the light microscope to acquire the reading for P/E ratio (polar length/equatorial diameter), C/P ratio (aperture length/polar length), d/D ratio (apocolpium length/diameter on polar view), c) Acetolysis method and observation under the scanning electron microscope to see the types, classes, and pollen exine ornamentation, while d) observation under the transmission electron microscope to observe the exine composition, e) Analysis and observation will be done using the Olympus BX43 microscope connected with digital video camera with assists of Cell^B software. A fenetic numerical analysis will be done using the MVSP 3.1 Kovach software to see the clumping of species. Pollen morphological observations under a light microscope, including dimensions of the pollen grain, pore and annulus diameter, and exine thickness were based on 50 fully expanded grains of each taxa. For ultrastructural studies, at least 20 grains were observed under SEM. SEM photomicrographs were taken of the center of the pollen grain in equatorial view at a uniform magnification of 620,000. The edges of the grains were also studied under SEM in order to classify and compare the fine exine surface excrescences.

**Literature review**

*Nepenthes* is the only genus of carnivorous plant classified in the dicotyledonous family Nepenthaceae (Adam, 1992; Shivas 1984). At present, a total of 11 species of *Nepenthes* have been recorded in Peninsular Malaysia that consist of lowland, highland intermediate and highland species (McPherson & Robinson, 2012). However, pitcher plant diversity inventory of Peninsular Malaysia is still lacking, taking into account the overall hills and mountains area of Peninsular Malaysia. Until now, at least 160 species were already discovered and documented all over the Old World from Madagascar, the Seychelles, Sri Lanka and India, towards China, the Phillipines, New Guinea, Australia and New Caledonia (Murphy et al.2019, Clarke et al. 2012; Philipps 2008). In spite of its importance and active explorations on new taxa by other scientists in other surrounding countries and regions, there has been virtually no effort and lack of work in improving knowledge on systematic study and species distribution of pitcher plants by local researchers in Peninsular Malaysia. One reason for this is due to the lack of local reference, or ‘in-home’ knowledge, on top of the difficulty in accessing their natural habitats especially those thriving near the rocky slopes and limestone areas which often rugged an in higher elevations. Existence of natural hybrids, intergrades and intra-specific variations of *Nepenthes* that are still improperly described in some remote areas becomes another factor that complicates morphological and systematic classification. Besides the previous works by Clarke (1997, 2001, 2002), Cheek & Jebb (2001) and McPherson & Alastair (2012), there is no other major taxonomic revisions revisited for this family. A single phylogenetic study on ten peninsular *Nepenthes* was conducted by Hamidun et al. (2017), but the population coverage was relatively limited and the study did not cover all 11 species and it excluded natural hybrids. In this project, we insist that a study needs be conducted in characterising anatomical structures that could serve and help in identification process of *Nepenthes* in a much greater depth. The data produced would be useful for future reference in discriminating species and hybrids, or even describing potential new species form unexplored terrains. As a part of the output, a complete collection (type specimens) of peninsular Malaysia’s *Nepenthes* species will be established for MDI Herbarium future references. With these additional informations for all species and natural hybrids, involved authorities and scientific personnel could optimise their diversity and systematics information in addition to increase awareness on our local biodiversity heritage and ornamental value appreciation of *Nepenthes.*

**Prospectives budget:**

**USD2000.00**

Selected References

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