

Asia-Pacific Biodiversity Observation Network (APBON)

22nd November 2022

Diversity, Taxonomy, and Conservation of Orchids in Malaysia

Speaker

DR. EDWARD ENTALAI BESI

*Department of Biology, Faculty of Science, UPM, Malaysia
Member of IUCN Species Survival Commission (SSC)*

Contributors

PROFESSOR DR. RUSEA GO

*Department of Biology, Faculty of Science, UPM, Malaysia
Member of IUCN Species Survival Commission (SSC)*

MS. RUNI SYLVESTER PUNGGA

International Affairs Division (IAD), Forest Department Sarawak, Malaysia

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WHY ORCHIDS?

ORCHIDACEAE Juss.

- **Most diverse & valuable group** of flowering plants
- Their survival depends on a delicate balance within healthy ecosystems
- Advanced in the floral variation
- Visually **stunning blooms** amongst the monocotyledon
- **Endangered, vulnerable** and **rare** species
- Orchids are mostly known for their importance as:



Corybas holttumii
J.Dransf. & G.Sm.



Dendrobium heterocarpum
Wall. ex Lindl.



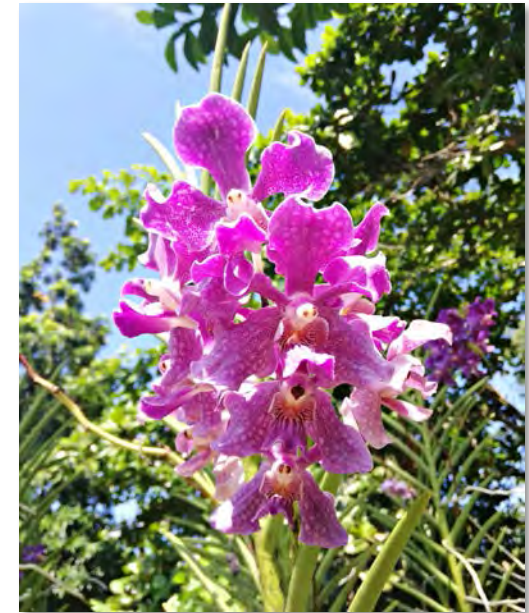
Paphiopedilum hookerae
(Rchb.f.) Stein

Medicinal Herbs



Vanilla flavour in our daily foods

Ornamental & Cut Flowers

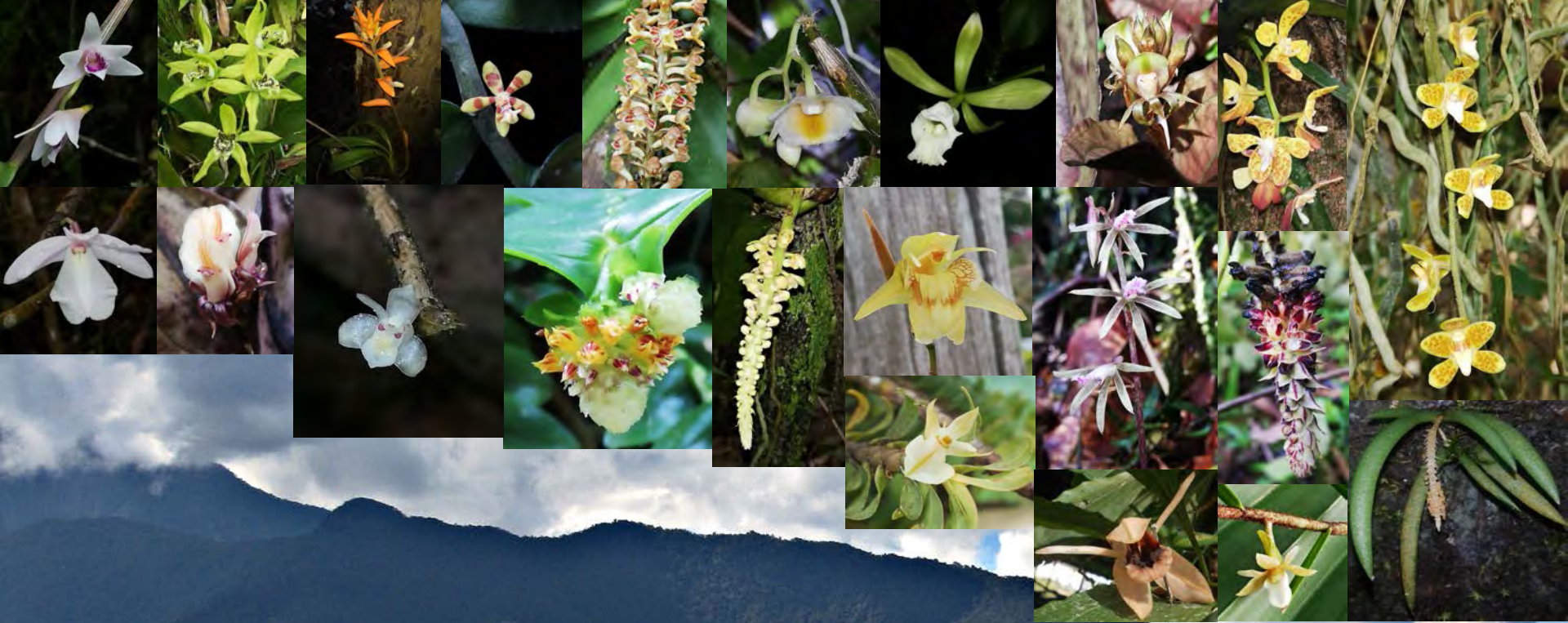


Hybrid orchids with beautiful flowers makes them popular for ornamental

Food Flavouring



Juice of pseudobulbs is applied in wound and boils



and more,
up to 5,000 species

Malaysian rainforests are considered
'hot spots' of orchid diversity

THREATS

Rescue mission



Ex-situ conservation

Salvaging orchids at-risk from the logged forests

- The issue of logging is rampant throughout Malaysia
- Orchids in logged forests experience extreme ecological conditions with higher ambient temperature and lower moisture level than in canopy-covered secondary forests
- Of concern to the orchid's survivability, a **joint rescue mission** was carried out in the disturbed forests of Malaysia, mainly in logging areas
- Through this effort, many **new records** and **new species** to science were discovered, described, and unveiled.



Dendrobium ainiae
R.Go et E.E. Besi



Dendrobium mizanii
R.Go et E.E. Besi



Dendrobium ruseae
E.E. Besi et Dome

New Species to science



RESEARCH FOCUS & CONSERVATION STRATEGY

Threatening processes

Clear-felling

Selective-logged

Plantation/human-managed

Mud-flood/secondary logged-over



Diversity & ecology studies:

Taxonomy, systematics, forest & habitat structure, microclimate, ecophysiology

In-situ conservation:
Habitat conservation, re-introduction programme & assisted migration



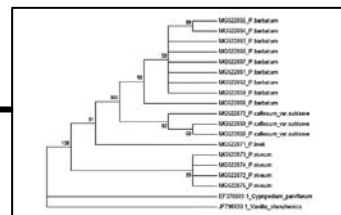
Pollinator interaction:
Limitations & reproductive success



TOWARDS SPECIES RECOVERY



Conservation genetics:
Genetic diversity & DNA barcoding for conservation priorities



Ex-situ conservation:
Species recovery, phenology study, seed banking, propagation



Mycorrhizal associations:
Endo- & ectophyte diversity, specificity & distribution

Developing Framework to reconcile Timber Extraction & Forest Management with Biodiversity Conservation for Rehabilitation

TECHNIQUES FOR CONSERVATION

(A) Diversity & Richness

- (1) Fieldworks & Sample Collections
- (2) Diversity & Ecological Study Design: **RCT**
- (3) Sample Processing & Identifications



(4) Diversity Analyses

- Shannon-Wiener Diversity Index (H)
- Simpson's Diversity Index (1-D)
- Dominance (D)
- Evenness (E) (PAST ver. 4)

(5) Statistical Analyses IBM SPSS version 24 (IBM Corp., Chicago, IL, USA)

(B) Ecology

- (1) *In-situ* Measurement of Ecological Data
temperature (degree Celsius), relative air humidity (1-100%), light intensity ($\mu\text{mol m}^{-2}\text{s}^{-1}$), bark moisture (0-100%)



- (2) Host Plants & Forest Structure Studies

Data Analyses

- ❖ Species richness (Individual-based Rarefaction) (EstimateS ver. 9.1.0) (Colwell, 2013)
 - ❖ Relative abundance (%Ao)
- ❖ Density of orchids within a plot (plants/m²)
- ❖ Canonical Correspondence Analysis (CCA) (Legendre & Legendre, 1998)
- ❖ Cluster Analysis UPGMA (PAST ver. 4)

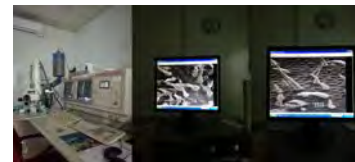
(C) Floral-surface micro-morphology taxonomy

- (1) Specimens' preparation for electron microscopy
(primary fixation with 4% Glutaraldehyde-washing with 0.1M Sodium Cacodylate Buffer-post fixation with 1% Osmium Tetraoxide-washing-dehydration with series of Acetone)

- (2) Critical Point Drying (CPD)

- (3) Sputter Coating

- (4) Micro-morphology examination via Scanning Electron Microscopy (SEM)



- (5) Enumeration & Comparative study of micro-morphology

(D) Phylogenetic & DNA Barcoding

- (1) DNA extraction & Polymerase Chain Reaction (PCR)

- (2) Bi-directional sequencing, sequence editing & data analyses (**BioEdit**)



(<https://www.allgenetics.eu/>)

- (3) Pairwise Genetic Distance & ML Phylogenetic analysis (**Mega X**), Barcoding Gaps Assessment

- (4) **GenBank & BOLD** Public data submissions

(E) Conservation Status Assessment

IUCN Red List Categories & Criteria version 14 (August 2019)



(<https://www.iucnredlist.org/>)



**Diversity of WILD ORCHIDS
in Forests of Different
Vegetation Types
in Malaysia**

Wild Orchids Of Logged Forests in Malaysia



Numerous orchids rescued from logged forests have been rehoused at an ex situ conservation site managed by conservationist and photographer Dome Nikong.

The fate of orchids in logged forests of Peninsular Malaysia

RUSEA GO and EDWARD ENTALAI BESI report on the alarming loss of orchids in Terengganu and the desperate attempts being made to save them

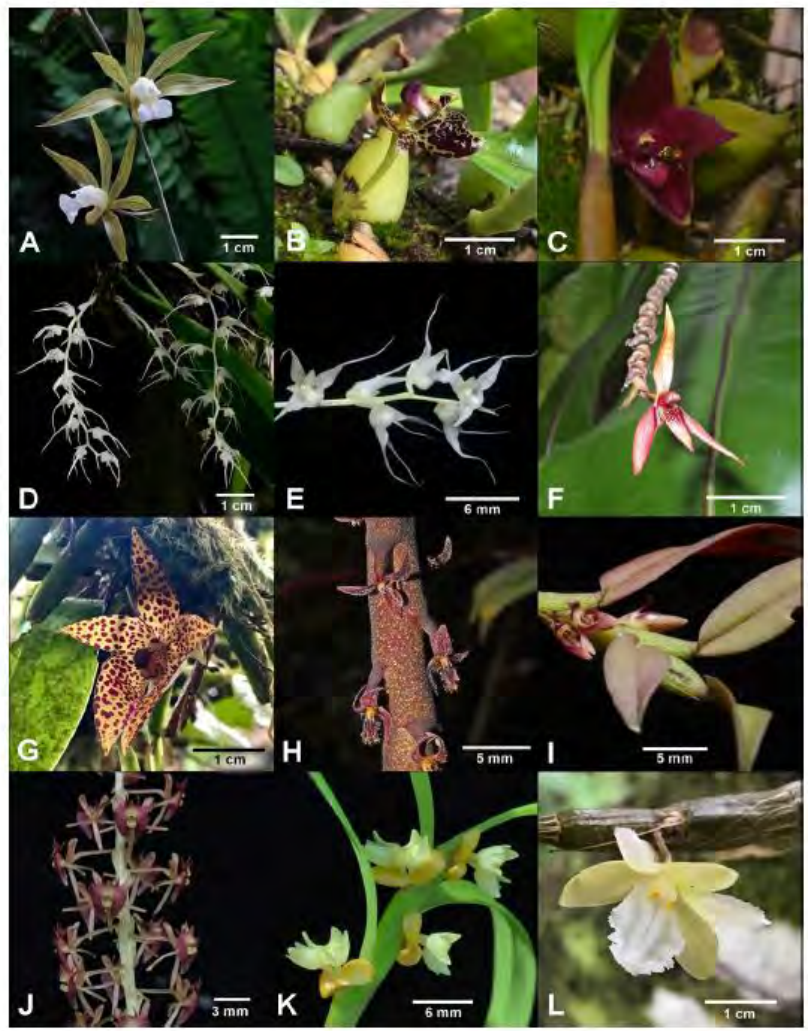


Figure 1 : Newly recorded orchid species found in the disturbed forests of Terengganu: A, *Ania penangiana*; B, *Bulbophyllum ecomutum* subsp. *ecomutum*; C, *Bulbophyllum elevatopunctatum*; D, *Bulbophyllum korthalsii*; E, *Bulbophyllum leptosepalum*; F, *Bulbophyllum lumbriciforme*; G, *Bulbophyllum sanguineomaculatum*; H, *Bulbophyllum setuliferum*; I, *Bulbophyllum tortuosum*; J, *Crepidium micranthum*; K, *Cylindrolobus biflorus*; L, *Dendrobium derryi*. Photos by DigitalDome.

Wild Orchids of Montane Forests in Peninsular Malaysia



Corybas holttumii



Corybas villosus



Corybas selangorensis



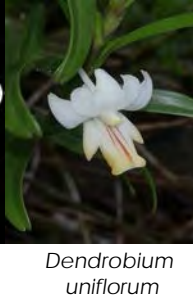
Dendrobium metrium



Anoectochilus reinwardtii



Dendrobium macropodum



Dendrobium uniflorum



Dendrobium hughii



Coelogyne roschussenii



Goodyera lanceolata



Nephelaphyllum tenuifolium



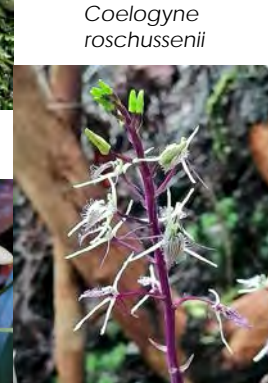
Zeuxine affinis



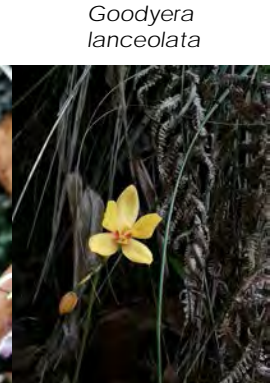
Trichoglottis scaphigera



Trichotosia microphylla



Liparis atropurpleoviridis



Spathoglottis aurea



Lepidogyne longifolia



Coelogyne xyrekes



Dendrobium macropodum

Wild Orchids of Montane Forests in BORNEO (SARAWAK & SABAH)

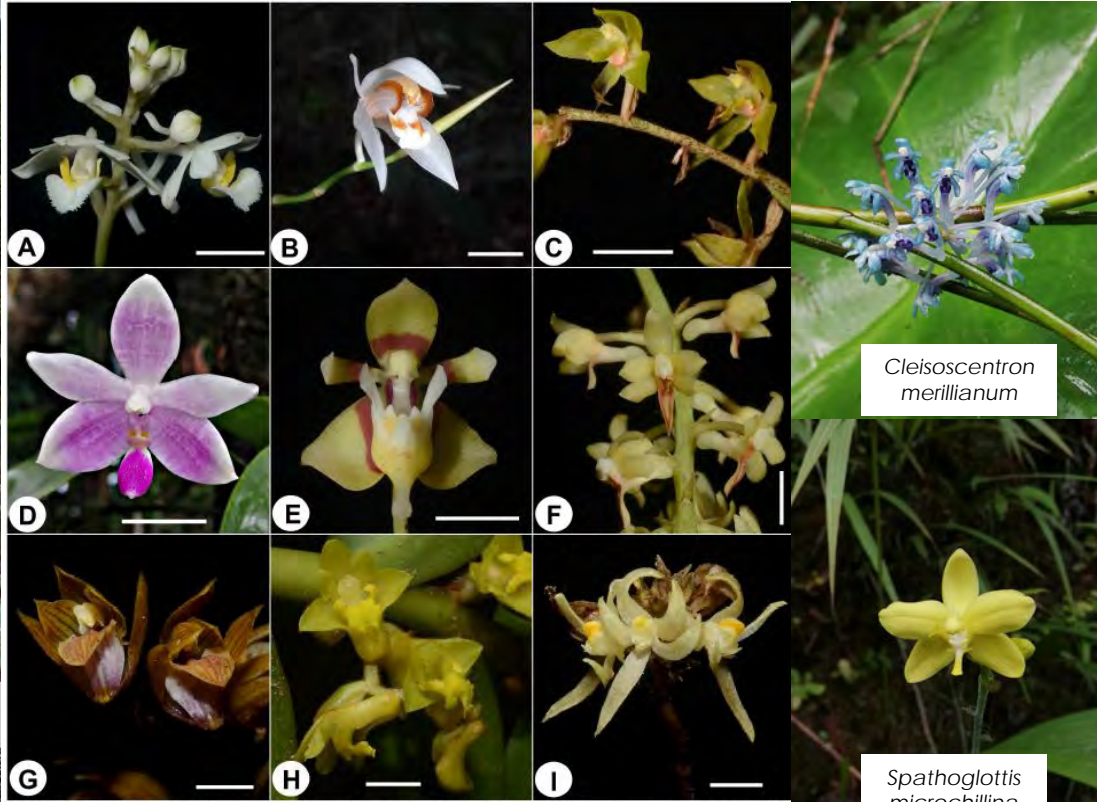
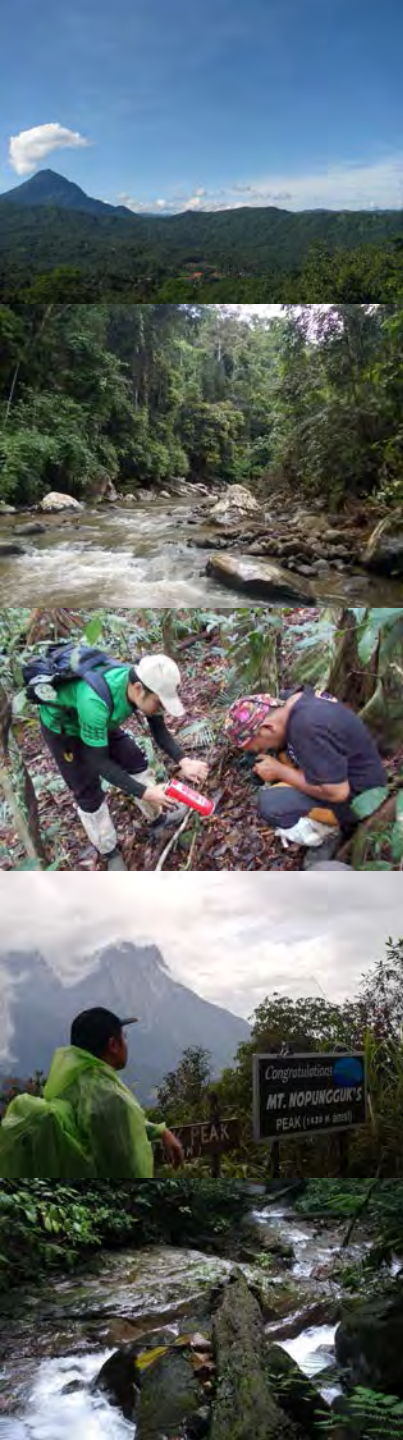


Fig. S2. Endemic species found in Tama Abu and Long Banga, Sarawak, Heart of Borneo. Designations: A) *Calanthe crenulata*; B) *Coelogyne endertii*; C) *Dendrochilum pubescens*; D) *Phalaenopsis modesta*; E) *Pteroceras fragrans*; F) *Robiquetia transversisaccata*; G) *Tainia scapigera*; H) *Trichostia brevipedunculata*; I) *Tropidia saprophytica*. Scale bars: A – 3 cm; B – 1 cm; C – 6 mm; D, E, F, H, I – 5 mm; G – 3 mm.



Taeniophyllum borneense



Jejewoodia jiewhoei



Cleisoscentron merillianum



Spathoglottis microchillina



Crepidium kinabaluense



Entomophobia kinabaluensis



Cystorchis variegata



Bulbophyllum pugilanthum

Wild Orchids of *kerangas padang* 'coastal heath' forest
in Peninsular Malaysia



Liparis elegans



Dendrobium villosulum



Bromheadia finlaysoniana



Spathoglottis aurea



Acriopsis densiflora var. *densiflora*



Peristylus lacertifer



Vanilla cf. *griffithii*

Wild Orchids of Limestone Forests in NORTHERN Peninsular Malaysia



*Paphiopedilum
niveum*



Oberonia calcarea



*Crepidium
inexpectatum*



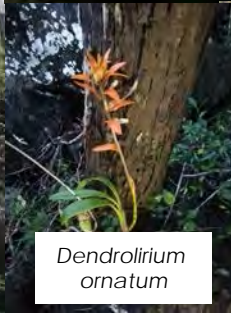
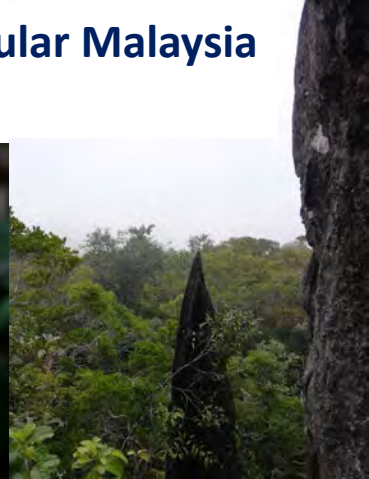
Pholidota gibbosa



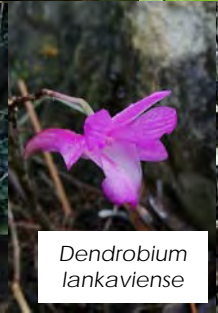
Porpax elwesii



Habenaria carnea



*Dendrolirium
ornatum*



*Dendrobium
lankaviense*



Crepidium sp.



Nervilia punctata



Taeniophyllum sp.

Wild Orchids of an Abandoned Mining Site in SABAH






ORCHID Flora of Mamut

EKSPEDISI SAINTIFIK MAMUT 2022, TAMAN KINABALU RANAU (19-30 SEPTEMBER 2022)
Edward Entalai Besi, *Rusea Go, Debbie Sandin, Christina Seok Yien Yong, Sebastian Wong
Department of Biology, Faculty of Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Hamelda Francisca Majit
Lembaga Pemegang Amanah Taman-Taman Sabah, Kota Kinabalu, Sabah, Malaysia
Tisun Gunggutau & Masius Gunggutau
Stesyen Mata Air Panas Poring, Taman-Taman Sabah, Ranau, Sabah, Malaysia
Evertius Enroe Soudi
Kg Kiulu Pahu, Tamparuli, Sabah, Malaysia
*Corresponding author

'BEAUTIFUL, ELEGANT, RARE, ENDEMIC, ENDANGERED!'

Abstract
The poster showcases the diversity of orchid species in Mamut, an abandoned copper mine, situated within the Kinabalu Parks area, covering a wide range of forest ecosystem ranging from the lowland to the mountain areas. The highland area lies between elevations of 1,300 to 1,600 metres above sea level. General collection of plants was carried out in two zones: Zone 1 (North and West) and Zone 2. In total, the study reported 80 orchid species, including not just the existing and enchanting common, rare and endemic species, but also the extraordinary new records to Kinabalu Parks. This preliminary compilation brings hope to add more solid information on the diversity of orchids in Mamut, including the threatened orchids community in the abandoned disturbed area, and shines better opportunities to study the climate changes and its effect towards orchids well-being, and their conservation priorities upon drafting their future conservation framework.



Agrostophyllum majus



Apostasia nuda



Appendicula congesta



Appendicula calcarata



Anoectochilus sp.



Arundina graminifolia subsp. *graminifolia*



Cymboglossum cymbidifolium



Bulbophyllum montense



Coelogyne monilirachis



Coelogyne pulverula



Crepidium kinabaluense



Crepidium lowii



Coelogyne vestita



Dendrochilum crassum



Dendrobium cymbulipes



Dendrobium lamelligerum



Obaronia kinabaluense



Dilochia sp. (cf. *cantleyi*)



Liparis elegans



Pinalia sp.



Pinalia sp.



Spathoglottis microcephala



Taeniophyllum sp.




Dendrochilum simile



Microsaccus griffithii



Odontochilus hydrocephalus



Discovery of rare
and new species

Vanilla yersiniana Guillaumin & Sigaldi

'A New Record For Peninsular Malaysia'

(Mat Esa et al., 2021)

Bryobium cordiferum subsp. *borneense*
(J.J.Wood) Schuit. [RE]

Crepidium lowii
(É.Morren) Szlach.
[NR]

Luisia brachystachys
(Lindl.) Blume [NR]

Rare Orchid Species
in Malaysia
**New Records [NR],
Recollections [RE]**

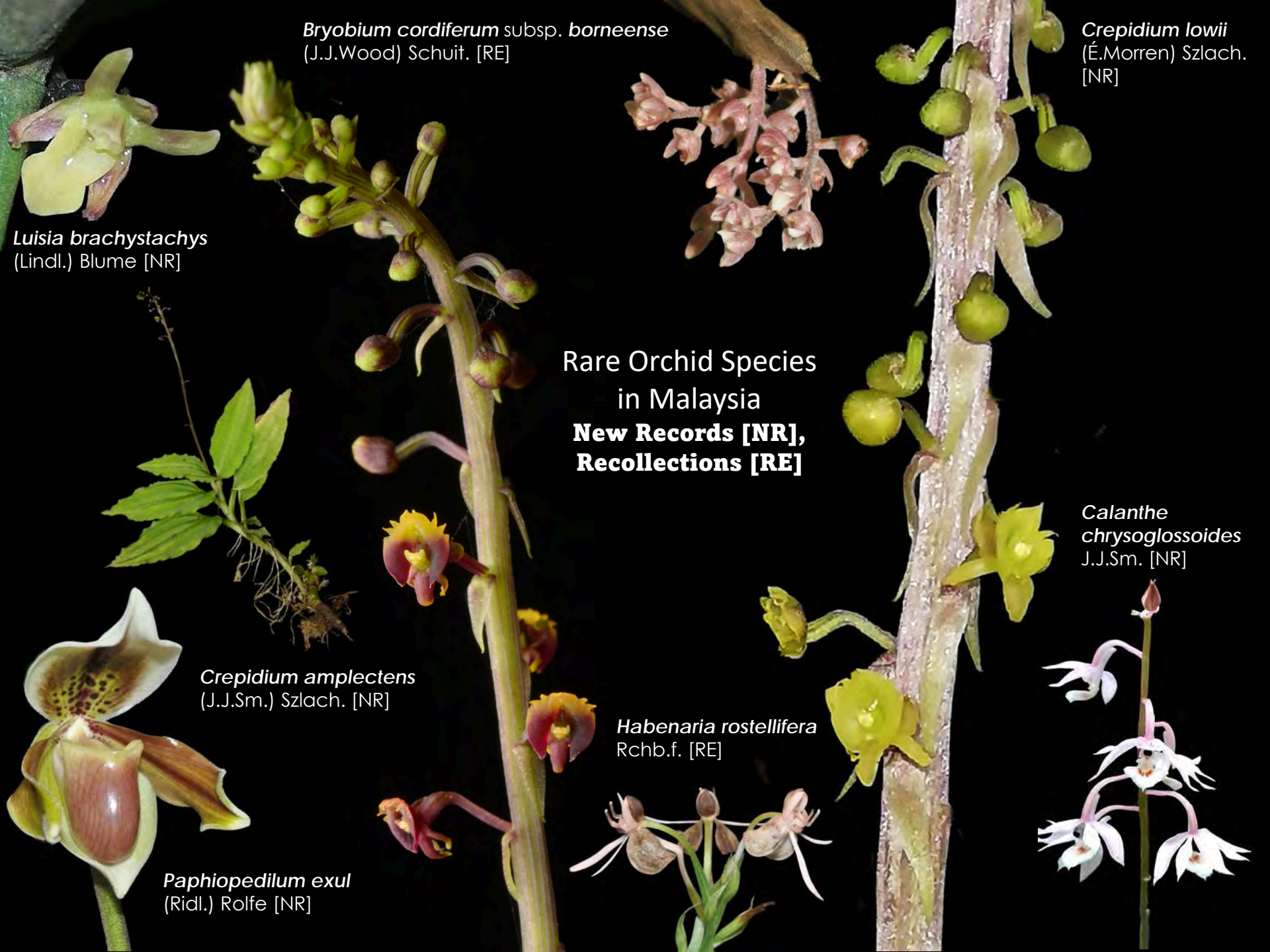


Crepidium amplexans
(J.J.Sm.) Szlach. [NR]

Habenaria rostelifera
Rchb.f. [RE]

Paphiopedilum exul
(Ridl.) Rolfe [NR]

Calanthe chrysoglossoides
J.J.Sm. [NR]





Dendrobium mizanii Go & Besi
(Besi et al., 2018)



Bulbophyllum abangjoei Go, Besi & Pungga
(Go et al., 2022)



Dendrobium ruseae Besi & Dome
(Besi et al., 2018)

New Species from Malaysia



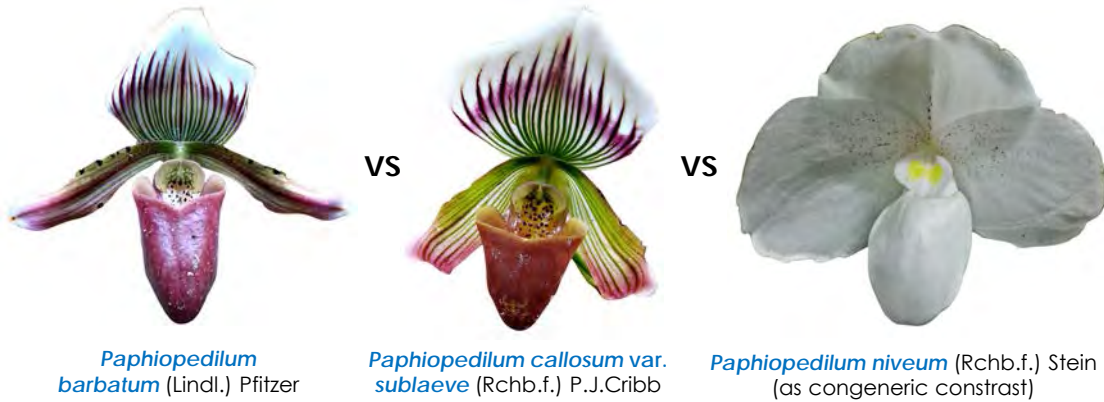
Bromheadia petuangensis Go & Besi
(Besi et al., 2020)



Dendrobium ainiae Go & Besi
(Besi et al., 2018)

**ROLES OF
TECHNOLOGY**
in Orchid Taxonomy &
Conservation Biology

Comparative Floral-surface Micromorphology Helps Discriminate between Species of *Paphiopedilum* (Orchidaceae: Cypripedioideae) from Peninsular Malaysia

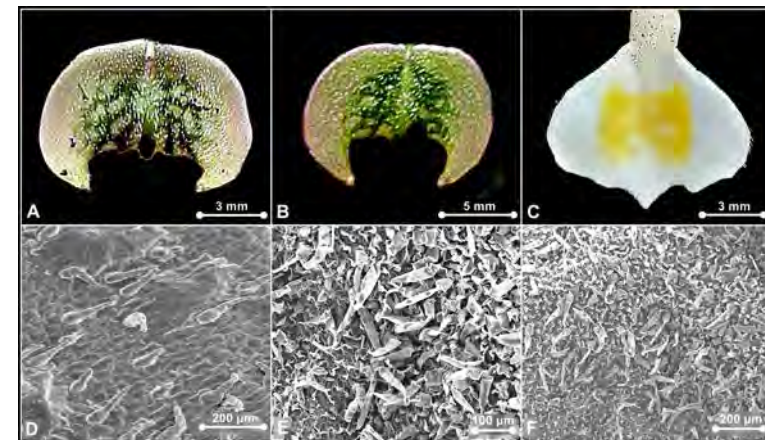


Features & distribution of floral-surface microstructures

Species	Floral Parts	Epicuticular ornamentation	Trichome type
<i>P. barbatum</i>	Dorsal Sepal	I, IV	III
	Synsepal	I, VII	III
	Lateral Petals	IV, VII	II, III
	Labellum	IV	II, III, V
	Staminode	VII	I, II, III
<i>P. callosum</i> var. <i>sublaeve</i>	Dorsal Sepal	II	I, III, V, VI
	Synsepal	II, VII	I, II, III, V
	Lateral Petals	II, III	II, V
	Labellum	IV	I, III, V
	Staminode	VI	V
<i>P. niveum</i>	Dorsal Sepal	III	I, IV, V
	Synsepal	III	I, IV, V
	Lateral Petals	II	II
	Labellum	II, V	II, III, V, VI
	Staminode	VI	V

- Species delimitation based on **general floral morphology & genetics** for the highly resemblant ones, for instance, *P. barbatum* & *P. callosum* var. *sublaeve* belong to subgenus *Paphiopedilum* is **DUBIOUS** (Seidenfeiden & Wood, 1992; Cribb, 1998; Leong, 2014)
- Given the above, we employed **scanning electron microscope (SEM) observations** to mainly evaluate surface microstructures' applicability in taxonomic delimitation & briefly on the physioecological functions
- The finding **supports the distinction** of *P. barbatum* from *P. callosum* var. *sublaeve*, which belong to subgenus *Paphiopedilum*, & from *P. niveum*, a species belonging to subgenus *Brachypetalum*, a separated monophyletic clade
- Multi-pattern **epicuticular ornamentation & trichomes** - offer a significant taxonomic value to discriminate the infrasubgeneric of *Paphiopedilum* species in Peninsular Malaysia.

Staminode & epicuticular surface



P. barbatum

P. callosum var. *sublaeve*

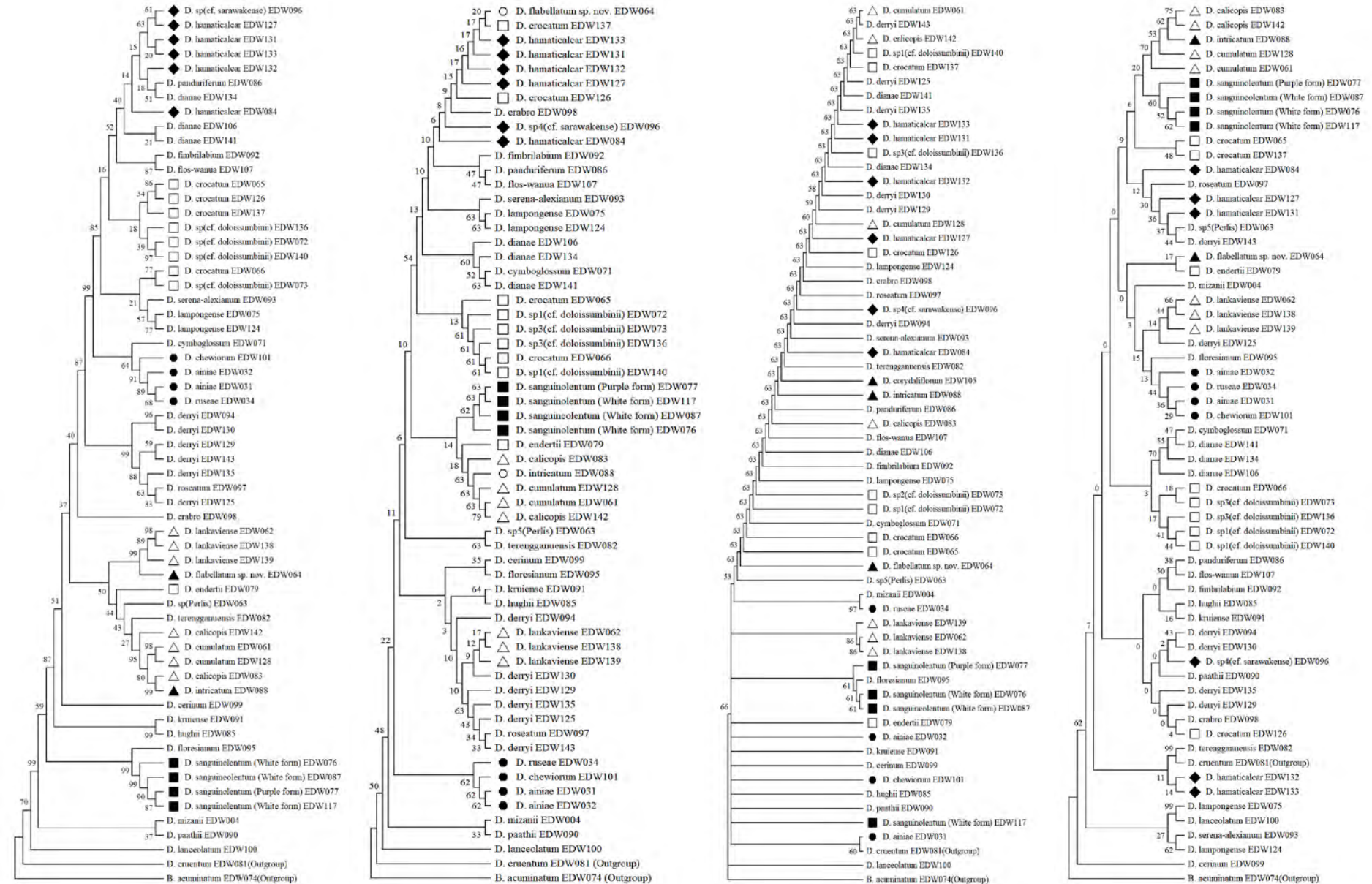
P. niveum

Profiling & resolving the uncertain & taxa using genetic-based evidence (Phylogenetic & DNA Barcoding)

- Efficacy of four DNA markers as barcode markers were tested across the several complexes and uncertain taxonomic groups
- 285 sequences of *nrITS*, *matK*, *rbcl*, and *psbA-trnH* loci were obtained, submitted to Barcoding of Life Data System (BOLD System) under a Project – *EBRG - DNA Barcoding of Selected Endangered and Cryptic Orchid Species (Orchidaceae)* and also GenBank.
- Our DNA barcoding work allows us to ascertain a correct identification and removes confusion when working with closely related species.
- The barcodes that we develop act as molecular tags that can be used to tackle the illegal trade of endangered orchid species in Malaysia.



PHYLOGENETIC TREES (Single & Combined Markers Analyses)



nrITS: The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model

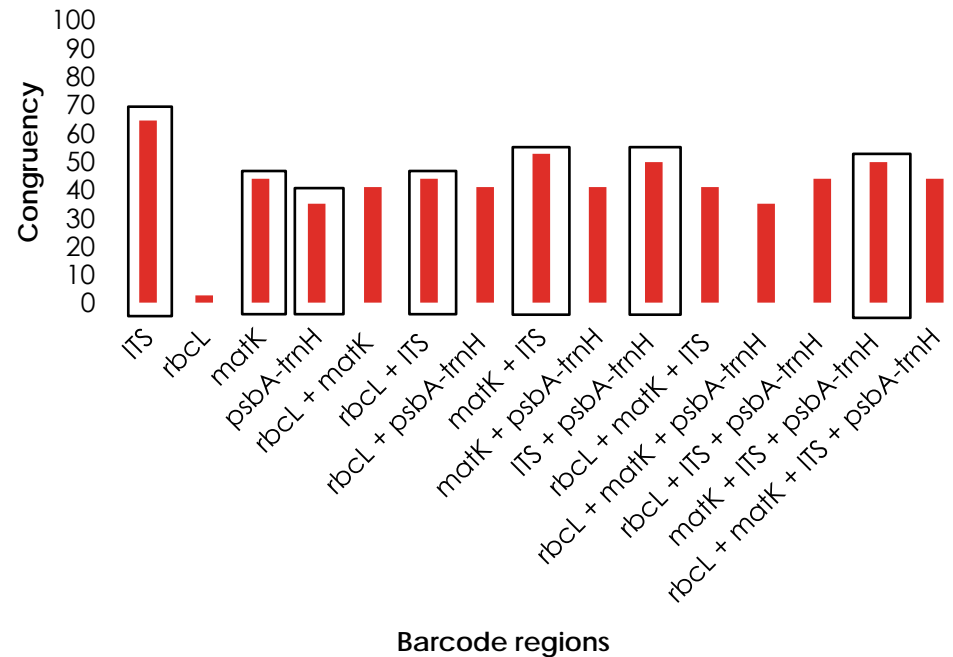
matK: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model

rbcl: The evolutionary history was inferred using Maximum Likelihood method and Jukes-Cantor model

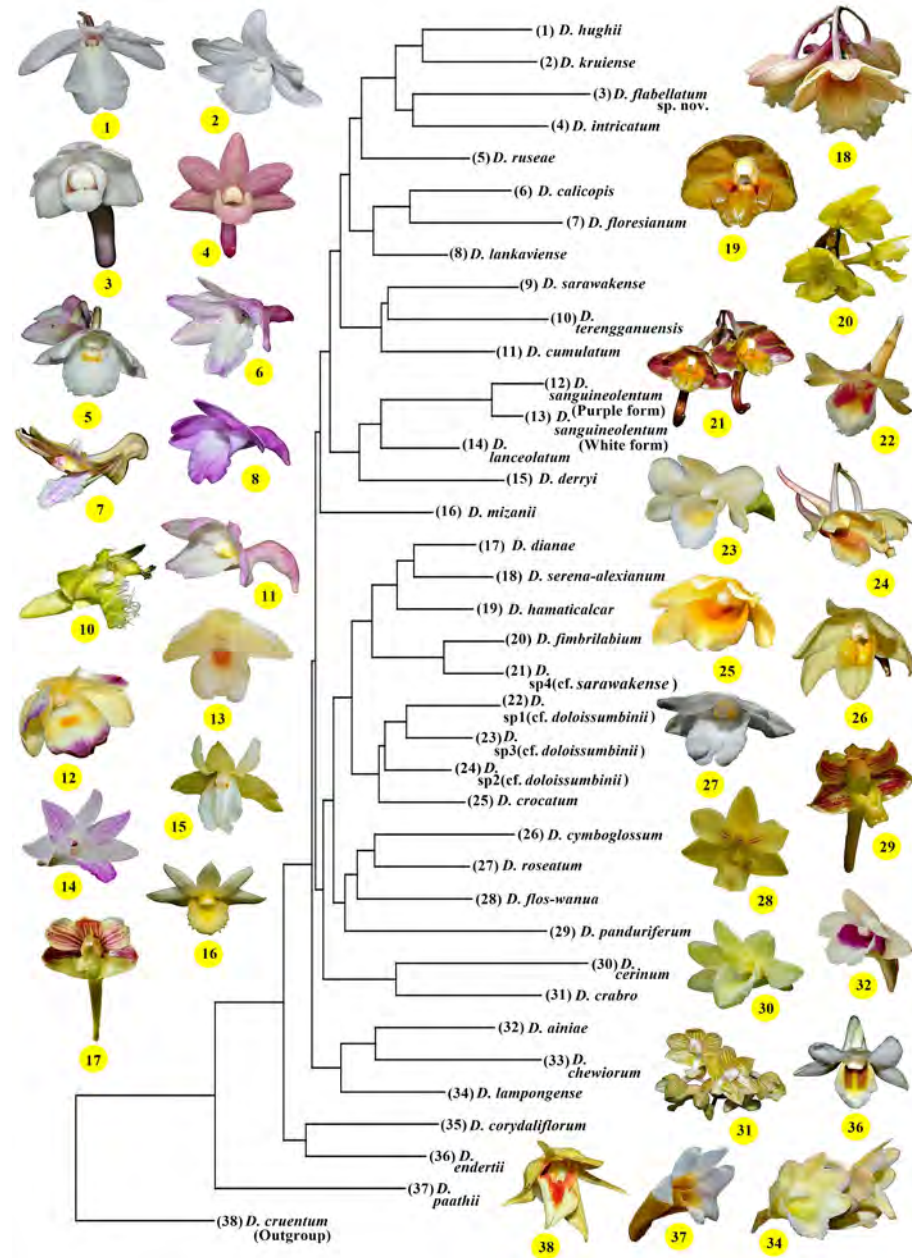
psbA-trnH: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model

Congruence between Molecular & Morphological-based Phenetic Analyses

- The ML phylogenetic trees constructed based on single *nrITS*, *matK*, & *psbA-trnH* are having highest congruency in topologies with the morphology-based phenetics analysis & each distinguishes the six complexes into separate cluster



Success rates of species identification for the four investigated regions and combinations of regions done by DNA barcoding based the coherency with the morphology-based NJ tree



Phenogram constructed according to Neighbor-Joining (NJ) cluster analysis (Similarity Index - Juke-Cantor) based on the morphological characters

Scientist-Community Collaboration

- Our effective strategy working with local communities is underpinned by frequent visits to privately managed gardens, nurseries and conservatories to survey and monitor rescued plants.
- Meeting with individual growers to understand their motivations, educate them on taxonomy and propagation, and explain our conservation framework.
- Engaging volunteer villagers to record biodiversity, detect, monitor, and survey species is a low-cost, inclusive approach.



Working with the local community on orchids ex-situ conservation in Peninsular Malaysia

Scientist-Forest Authorities Collaboration



A joint conservation work with the Silviculture and Forest Biodiversity Conservation Division of Forestry Department of Peninsular Malaysia

- A partnership with the **Silviculture and Forest Biodiversity Conservation Division** of Forestry Department of Peninsular Malaysia (FDPM), whom are also country representatives for CITES Plant Committee, was formed
- in highlighting orchid species currently threatened & protected by laws under **International Trade in Endangered Species Act 2008 (Act 686)**, **CITES Appendix 1 or 2**, & **National Forestry Act 1984 (Act 313)**
- **POSTERS** were designed & printed highlighting **30 endangered orchid species** including some of the newly discovered species.
- To raise awareness and prevent extinction of near-threatened and threatened species.



Posters on Endangered, Threatened and Rare Wild Orchids of Malaysia (Part 1 & 2)

Establishment of **Tengku Permaisuri Norashikin Conservatory** by UPM & Selangor Forestry Department in Fraser's Hills as Part of Conservation & Research Efforts



Scientist-Forest Management Unit (FMU) Collaboration

- A partnership with the Anap-Muput Forest Management Unit (FMU), through Forest Department Sarawak (FDS) spearheaded by Senior Assistant Director, Ms. Runi Sylvester Pungga.
- The high conservation and scientific value of the forest called for a thorough biological diversity assessment to ensure that the natural resources are sustainably managed for environmental and socio-economic benefit.
- Biodiversity, ecological and conservation studies have so far been largely confined to the protected and undisturbed zones.
- It is intended that the involvement of FDS and Universiti Putra Malaysia (UPM) in efforts to conserve biodiversity in Borneo will allow for the rescue of orchids from logging sites in Anap-Muput FMU.



Sites identification in logged forest in Anap-Muput FMU for rescue mission



In-situ data collection in logged forest in Anap-Muput FMU



Establishment of an ex-situ conservatory and rehabilitation program in Sarawak, by UPM and Forestry Department of Sarawak in collaboration with Anap-Muput FMU

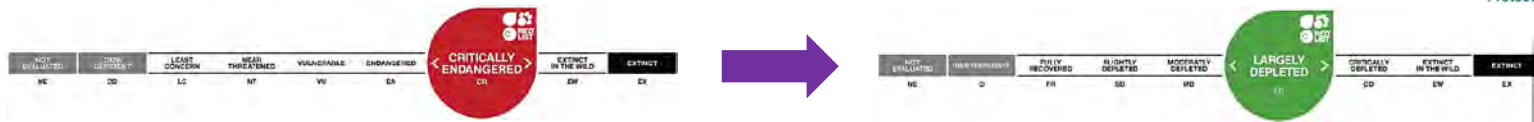
WHAT ARE THE NEXT STEPS?

OTHER THAN continuously monitoring the diversity and identifying the conservation sites for each species, we will be exploring...

- The ecological specificity of terrestrial orchids [especially on their relationship with the microbiome (including mycorrhizal)]
- Vertical stratification of epiphytic orchids assemblages & its association with the microclimatic gradients within vegetation
- Conservation values of the host suitable plants for epiphytic orchids diversification

IUCN Green Status of Species

(<https://iucngreenlist.org/>)



- Recognizing the imperative to evaluate species recovery and conservation impact, the IUCN called for the development of objective criteria for Green Lists of Species, Ecosystems & Protected Areas (IUCN & WCPA, 2017)
- A global standard for **measuring species recovery** and **assessing conservation impact** - prepared by the IUCN SSC Species Conservation Success Task Force (Version 2.0)
- A species moving to a lower category of extinction risk on the IUCN Red List due to conservation measures is a useful indicator of conservation impact (Butchart et al., 2006)
- However, many species may remain in a high threat category for long periods despite successful conservation efforts

I am giving thanks with grateful heart to God Almighty and to all of you..

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- Prof. Dr. Rusea Go (UPM)
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- Prof. Emeritus Dato' Dr. Abdul Latiff (UKM)
- Prof. Dr. Jean Yong (SLU)
- Mr. Rod Rice
- Dr. Norfaizal Ghazali (MARDI)
- Dr. Fitri (UKM)
- Ikhwanuddin Mat Esa (UPM)
- Dome Nikong (DigitalDome)
- Amin (MARDI)
- RGo Botany Team
- Mr. Alim Biun (Sabah Parks)
- Mr. Yabainus (Sabah Parks)
- Mr. Linus (Kipandi Park)
- Hamelda & her team (Sabah Parks)
- Andy Paul
- Department of Biology, FS
- Friends from IBTP, UMS

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- ✓ Skim Latihan Akademik IPTA (SLAI) (MOHE)
 - ✓ Tenaga Akademik Muda (TAM) (UPM)

- ✓ KRIBB
- ✓ Yayasan Sarawak

- Research Permits:**
- ✓ Jabatan Perhutanan Semenanjung Malaysia (JPMS)
 - ✓ Jabatan Perhutanan Sarawak (FDS)
 - ✓ Sabah Parks
 - ✓ SABc



Ministry of Higher Education (MOHE)



THANK
YOU

