
**POLLINARIA DIVERSITY OF THE FAMILY ORCHIDACEAE:
A SYSTEMATIC APPROACH**

*Thesis Submitted to Midnapore City College for the Partial Fulfillment
of the Degree of Master of Science (Botany)*

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Declaration

We do hereby declare that the present Master thesis entitled '*Pollinaria Diversity of the Family Orchidaceae: A Systematic Approach*' embodies the original research work carried out by us in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. Soumitra Pal, Assistant Professor, Department of Biological Sciences, Midnapore City College and Kuturaia, Bhadutala, Paschim Medinipur. No part thereof has been submitted for any degree or diploma in any University.

Date:

Place: Midnapore City College, Paschim Medinipur

**Arundhuti Singha
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Approval Sheet

This project report entitled **POLLINARIA DIVERSITY OF THE FAMILY ORCHIDACEAE: A SYSTEMATIC APPROACH** by Arundhuti Singha, Debanjan Paria, Tanmay Santra is approved for the degree of M.Sc. in Botany.

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Place: _____

**Dedicated to my Parents, supervisor with
Soulful respect and love.**

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Arundhuti Singha

Debanjan Paria

Tanmay Santra

Abstract

Orchidaceae is one of the most widespread families of flowering plants. Almost 1300 species of orchids are reported in India, which represent about 5.98% of the world orchid flora. The unique flower structure attracted the human being. It has three sepals, three petals (one is modified and form the labellum), and a central column. Column bears the pollinia. The pollinaria are solid masses of pollen grains which represent the male reproductive unit. In present study, we focused on orchid pollinaria. So, we collected different orchid samples (total 15 species) from different places in South Bengal and North Bengal. Then work out those collected specimens under simple microscope and SEM. We observed various micro morphological characters of those pollinaria. A similarity matrix is prepared using 9 binary state characters and 4 multistate characters by correlation of the coefficient method, with the help of this similarity matrix, dendrogram was constructed by UPGMA method. The result shows that, two major clades are recognized in this treatment. The first clade represents the subfamily Orchidoideae and the second clade represent another subfamily Epidendroideae. All the species of Epidendroideae came from a monophyletic group.

Keywords: Orchidaceae, Pollinaria, pollinia, micro morphological characters, numerical taxonomy, clade, dendrogeam.

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Chapter 1: Introduction

1. Introduction:

In India, orchids are known to humans from the Vedic period. In Rig- Veda and Atharva Veda two orchid species were mentioned i.e., *Vanda tessellata* and *Flickingeria macraci* as medicinally important plants (Ninawe and Swapna, 2017). China was the country that first cultivate orchids as medicinal plants (Almost 4000 years ago). Around 300 B. C, Theophrastus was named for the first time Orkhis. Carl Linnaeus first used the word Orchidaceae in 1737. This name was fully established by Antoine Laurent de Jussieu in 1789 (Anghelescu *et al.*, 2020). Orchids are one of the most widespread families of flowering plants. It is the second largest family in the Liliopsida, with about 25,000-35,000 species with approximately 900 genera that are distributed all over the world (Cribb *et al.*, 2003). Orchids account for 8% of Angiosperm species diversity. This family is further divided into five subfamilies, they are Apostasioideae, Cyripedioideae, Epidendroideae, Orchidoideae, and Vanilloideae. Among them, Epidendroideae is larger. They are mostly perennial or annual herbs. Orchids are either epiphytes, lithophytic, or terrestrial (Yonzone *et al.*, 2012). Almost 90% of the orchids are epiphytes and the rest of them grow in terrestrial and lithophytic habitats.

Orchids have attracted people for their beautiful ornamented flowers. Orchids are not needed only for their attractive flowers but also for their great number of uses in therapeutic purposes. (Handa, 1986). It also has cultural and religious importance in the world (Rao *et al.*, 2003). *Rhynchostylis retusa* commonly known as fox tail has great cultural significance in India. The pinkish-colored flowers of 'fox tail' orchids attracted people and these orchids are adorned by lady dancers in the Bihu festival as a tradition. *Dendrobium hookerianum* is used by the Buddhists to decorate Gompas (pagodas) to worship Lord Buddha (Ninawe and Swapna, 2017). Besides them, *Vanda*, *Cymbidium*, *Calanthe*, *Dendrobium*, *Paphiopedilum*, *Ascocentrum*, etc. are in high demand in the International market (De, 2020). But nowadays

with extreme use of these orchids for various purposes, they are in danger. In 2017, IUCN global red list included 948 orchid species of which 56.5 percent are reported to be threatened (De, 2020).

About 1300 species of orchids belonging to 140 genera are reported in India (Bhunia *et al.*, 2021) which represent about 5.98% of the world orchid flora and 6.83% of the Angiosperms in India (De, 2020). It is the largest family among higher plants in India. The Eastern and North Eastern Himalayas, North Western Himalayas; Peninsular India, and Andaman and Nicobar Islands are the major orchid-dense region of India (De, 2020). In West Bengal, orchid-rich areas are almost overall North Bengal and some areas of South Bengal like Purulia, Midnapore, Birbhum, etc. (Mitra *et al.*, 2020).

Orchids have a remarkable note for a number of reasons, being monocots, they have a very interesting flower structure, having 3 sepals, 3 petals, and a central Column. The column is the reproductive organ of the orchids where the male and female reproductive parts are fused and form a finger-like structure. The anther is located at the distal end of the column and the stigma is located near the distal end and just on the underside of the column, just below the column, there is the labellum or lip. The labellum is a modified petal that acts as a landing area for pollinators (Phillips and Peakall, 2018). Orchidaceae have many characteristic features, (like the presence of labellum or lip, Aerial root, column, and Pollinaria) presence of Pollinaria is one of them. The Pollinaria of an orchid contains its male reproductive units (the Pollinaria) and structures associated with their transport during pollination. Pollinaria is covered by the anther cup for protection. The pollinaria are solid masses of pollen grains. Each pollinium is attached to a caudicle -an elongate, stalk-like structure derived from anther tissue. The caudicles are embedded in the sticky, noncellular material known as Viscidium, a structure derived from the stigma (Blackman and Yeung, 1983). Although an important part of the median stigmatic lobe generally became sterile, which produces secretions that are involved in

the pollination system. This modified stigmatic lobe is known as rostellum (Dressler,1981). The pollen characters are very important for species-level identification that has accepted APG-IV (2016). Thus, we study the pollinaria morphology of this orchid species using Scanning Electron Microscope (SEM). So, the present study was designed to prepare a dendrogram based on Numerical Taxonomy and determine the relationship among the different orchid species in North Bengal and South Bengal, India.

Chapter 2: Literature Review

2. Literature Review:

Orchidaceae members are mostly epiphytic to derive nutrition from the host plant body. Dressler (2005), reported more than 25,000 orchid species from 900 genera of the Orchidaceae family. Blackman and Yeung, in 1983 studied the Pollinaria using Transmission Microscope (TEM) and Fluorescence Microscope. They show the close similarity in development and structure between caudicles and pollinia and finally conclude that the caudicles evolved from the Pollinaria. Pridgeon (1982), collected different anatomical and floral data from 202 species of 34 genera of the Pleurothallidinae (Orchidaceae). A data matrix was compiled from scorings of 35 vegetative characters and 10 floral characters. Different cluster and factor analyses were performed with this data matrix in order to identify phenetically similar taxa and at the same time reveal differences in results owing to different statistical procedures. Regardless of the clustering method applied, several genera or complexes are measurably distinct from each other. DuPuy *et al.* (1985), worked on “A numerical taxonomic analysis of *Cymbidium*, section *Iridorchis* (Orchidaceae)”. Michael and Zavada (1990), studied the ultrastructure of Pollen grains of 30 orchid species from the Spiranthoideae and Epidendroideae (including the Vandaeae). The orchid pollen thus far studied exhibits a wide range of variation in pollen unit, aperture type, and wall ultrastructure. The least specialized Apostasioideae and some Cyripedioideae shed pollen in monads and have monosulcate grains with a tectate-columellate and perforate exine structure. These are features observed in most primitive monocotyledons. In the more specialized orchid subfamilies, pollen occurs in mealy or tightly packed, waxy pollinia, and the grains have a porate-ulcerate aperture or are inaperturate, and the sporopollenin wall is present only on most peripheral grains in the pollinium (a few species lack exines). The presence of the exine on only the most peripheral grains in the pollinium is accompanied by a loss of the foot layer in many taxa and elaboration of the intine. As in other monocots, endexine has not been unequivocally demonstrated in orchids. Freudenstein *et al.*

(1996), studied pollinium development and number in the Orchidaceae. They studied the development of anthers in three sub-families (Orchidoideae, Spiranthoideae, and Epidendroideae) anatomically to examine homology hypotheses of pollinium number characters and to produce a model of pollinium development for the family and showed a single meristematic region, which would later give rise to Pollen, per theca at the earliest stage; septation of these regions resulted in four or eight pollinia per anther, while lack of septation in some members of the Epidendroideae gave two pollinia. Freudenstein *et al.* (2002), studied the evolution of anther morphology in orchids: incumbent anthers, superposed pollinia, and the vandroid complex. They examined variation in pollinium and anther orientation in Epidendroideae and Vanilloideae using anatomical sections of columns at successive ontogenetic stages and related the observed changes to the final anther morphology to re-examine our homology hypotheses for these characters. Anther bending in vanilloids is achieved primarily by the massive expansion of the connective. In nonvandroid epidendroids, it results from column elongation and tipping of the mature anther, while in vandroids, it is due to a redirection of growth in very early ontogenetic stages.

Dressler (2005), in “How many orchid species?”, said that large-flowered species are relatively few, rather the vast majority of species are small-flowered if not microscopic. Ephemeral flowers, such as *Sobralia*, *Palmorchis*, and the *Thiphoreae*, have special problems associated with their identification, for which most field-collected herbarium specimens are nearly useless. In practice, most large-flowered orchids are poorly represented by herbarium specimens, and even many small-flowered species are scarce in the herbaria. Only abundant and widespread species, such as *Epidendrum radicans* or *Habenaria monorrhila*, are really well represented in the herbaria. According to Hidayat *et al.* (2006), Pollinarium is one of the most distinct synapomorphies of Orchidaceae. They examined the phylogenetic relationships among genera of subtribe Aeridinae by using characters derived from the pollinarium. Cladistic

analysis of their work showed that (1) Subtribe Aeridinae is a monophyletic group. (2) Five of six groups constructed in the analysis are consistent with the groups recognized in previous molecular phylogenetic analyses. (3) The genera *Cleisostoma* and *Phalaenopsis* are non-monophyletic groups. (4) Pollinarium morphology endorses monophyly *Trichoglottis* and *Phalaenopsis* alliances. (5) Although the transformation of the stipe and viscidium shapes in the subtribe is subjected to parallelism, the results showed that these characters are much more useful in determining relationships in the subtribe than those of pollinium.

Singer *et al.* (2008), discussed the structural variation of pollinia and pollinaria in Orchidaceae. Pollinia and pollinaria are restricted to two (of the five) orchid subfamilies: Orchidoideae and Epidendroideae. The attributes of pollinia and pollinaria of these subfamilies are commented on and discussed. Pollinia and pollinaria also occur in the plant family Apocynaceae, in the subfamilies Asclepiadoideae and Periplocoideae, but these are structurally different from those found in the family Orchidaceae. A number of morphological features of orchid pollinaria are informative taxonomically and ecologically. These features are briefly discussed and examples are given. The recent description of the first unequivocal fossil orchid (*Meliorchis caribea*; Orchidoideae: Goodyerinae) from a pollinarium attached to an insect pollinator is briefly discussed. This example is used to illustrate the use of informative morphological and palynological characters. They also discussed a fascinating new perspective, the possibility of species identification using DNA sequencing from pollinia or pollinaria attached to pollinators. Nieto and Damon (2008), studied the Morphology of the Pollinia and Pollinaria of Orchids from Southeast Mexico. This report shows that the authors are mainly focusing on the morphological characteristics of pollinia and Pollinaria. They pretend in the future the data and the images will use for the identification of Pollinaria carried by pollinators. Ezekiel *et al.* (2009), showed that reproductive characteristics are much better than the vegetative characters earlier used by Kraenzlin (1926) in the distribution of

Polystachya species into their sections. With the use of reproductive characters, more clusters have been reported which also indicates the occurrence of many more sections in *Polystachya* than those earlier reported. Damon and Nieto (2013), described the proper guideline of Pollinaria for the identification of orchids. The complete work was only focused on the biological corridor tacana´-boquero´n in Southeast Mexico. The authors were done the present work by using the morphology and micromorphological characteristics of Pollinaria like the number of Pollinaria, size of Pollinaria, length and width ratio, etc. Finally, the authors provide the taxonomical characters up to the subtribe level.

Mosquera-Mosquera, *et al.* (2019), studied on epidendroideae. They give us useful information to understand the evolution of the reproductive organs of Epidendroideae. L.C. De (2020), studied the morphological diversity in orchids. His investigation was conducted using all vegetatively propagated species and hybrids of commercially grown orchid genera. Amongst different morphological descriptors of commercially grown orchid genera, diversity in pseudobulb shape, leaf shape, inflorescence variation, floral characteristics, and their diversity were studied in detail. Li *et al.* (2020), studied the development of pollinia of a newly recorded orchid species *Pennilabium yunnanense*.

A recent study of “Pollinia Morphology of 14 Species in Dendrobium (Orchidaceae) and Taxonomic Significance” by Wang *et al.* (2021), observed 5 kinds of exine sculptures (psilate; psilate with threads; psilate-uneven, coarse reticulate, and rugulate) under a scanning electron microscope, as well as two types of crystals observed on the surface of pollinia in *Dendrobium wislsonii* Rolfe and *Dendrobium thyrsoiflorum* Rchb. The morphology and exine sculpture of pollinia were distinguished in different species of Dendrobium, which is of some significance to its taxonomic clarification. In the latest study by Fan *et al.* (2023), they carried out this study on the pollinium morphologies of subtribe Aeridinae with emphases on the aperture types and the pollinium sculpture to find out their systematic signification in light of molecular

phylogeny. The results indicate that most species have a cleft type and few with porate type or none. All samples in this study have a complete tectum exine and most with psilate surface implying an advanced position of Aeridinae in Orchidaceae. Exine sculpture characters are consistent in each clade except *Cleisostoma* clade and *Phalaenopsis* clade. It has a somewhat clear changing tendency in consist with the molecular phylogeny with few exceptions. *Gastrochilus* and *Trichoglottis* clades are supported by its non - flabellate pollinium, psilate bulgy sculpture, and irregular shape of individual units of pollinium. However, no distinguishing pollinium characteristics are found between them. Conclusionarily, exine sculpture characters are not systematic but useful characters in determining major systematic groups in subtribe Aeridinae.

Chapter 3: Aims and Objective

3. Aims and Objective:

3.1. Aims:

The present work was aim to study the Pollinaria diversity of the family Orchidaceae.

3.2. Objectives:

Therefore, the present observation's main objectives are-

- (i) To study macro morphology, and micromorphology of the collected specimen.
- (ii) To find out the host plants of the Orchidaceae members.
- (iii) To correlate the relationship of observed plant species through the implementation of numerical taxonomy.

Chapter 4: Study Area

4. Study Area:

Our study area is mainly in the different places of the state West Bengal, which is located in the Eastern part of the Indian subcontinent and situated between 21°38' and 27°10' North Latitude and between 85°30' and 89°53' East longitude. The state has a total area of 88,75m. West Bengals share three international boundaries: the first one with Bangladesh in the South-East, the second one with Bhutan in the North the third one with Nepal in North-West. The state shares the largest international boundary of approx. 2272 km with Bangladesh and the smallest with Nepal. The state also shares interstate boundaries with Odisha in the South, Bihar and Jharkhand in the West, Sikkim in the North, and Assam in the Northeast. The state shares the longest interstate boundary with Jharkhand and the Smallest with Sikkim. The width of the state from the North-South direction is 673 km and from the East-west direction is 320 km. The West Bengal provinces can be divided into 3 divisions: (i) The Northern Mountain region, which is situated in the North-Western part of West Bengal and Belongs to the Eastern Himalayan region includes the entire Darjeeling, Kalimpong districts and some portion of Jalpaiguri districts. Rivers Teesta, Jaldhaka, and Raidhak flow through this region. (ii) The Western plateau, which uplands comprise the entire Purulia district, the western part of Birbhum, Bankura, Bardhaman, and West Midnapore district. However, the Western Plateau is part of the Chotonagpur Plateau, and it consists of small hills, like - Ajodhya, Sushunia & Baghmundi hills, etc. This region is highly elevated between the Kansai & Subarnarekha rivers. (iii) The plains of the North & South Region, which extends from Siliguri to the coast of the Bay of Bengal. It is divided into two parts- the Plains of North Bengal & plains of South Bengal. The Ganges is the most important river in this region. The climate of West Bengal varies according to the location. It varies from moist tropical in the South-east to dry tropical in the south-west and from subtropical to temperate in the North. The climate of the northern mountains is cooler than Southern Plains. The temperature range is from well

below 0°C in the hills during winter, whereas about 45°C in South Bengal during summer. Snowfall is limited to the Himalayan regions. The state has an annual precipitation of 1625 mm or 64 inches.

The Forests of three distinct areas exist in the state. These are the forests of the North, which include the mountain temperate forests and the tropical forest of Dooars, the deciduous forest of the Plateau fringe, and the mangrove forests of Sundarbans. The total forest land in the state is 11879 km², of which 7054 km² is Reserved Forest, 3,722 km² is protected forest and 1053 km² is unclassed State forests, thus constituting 13.38% of the geographical area of the state.

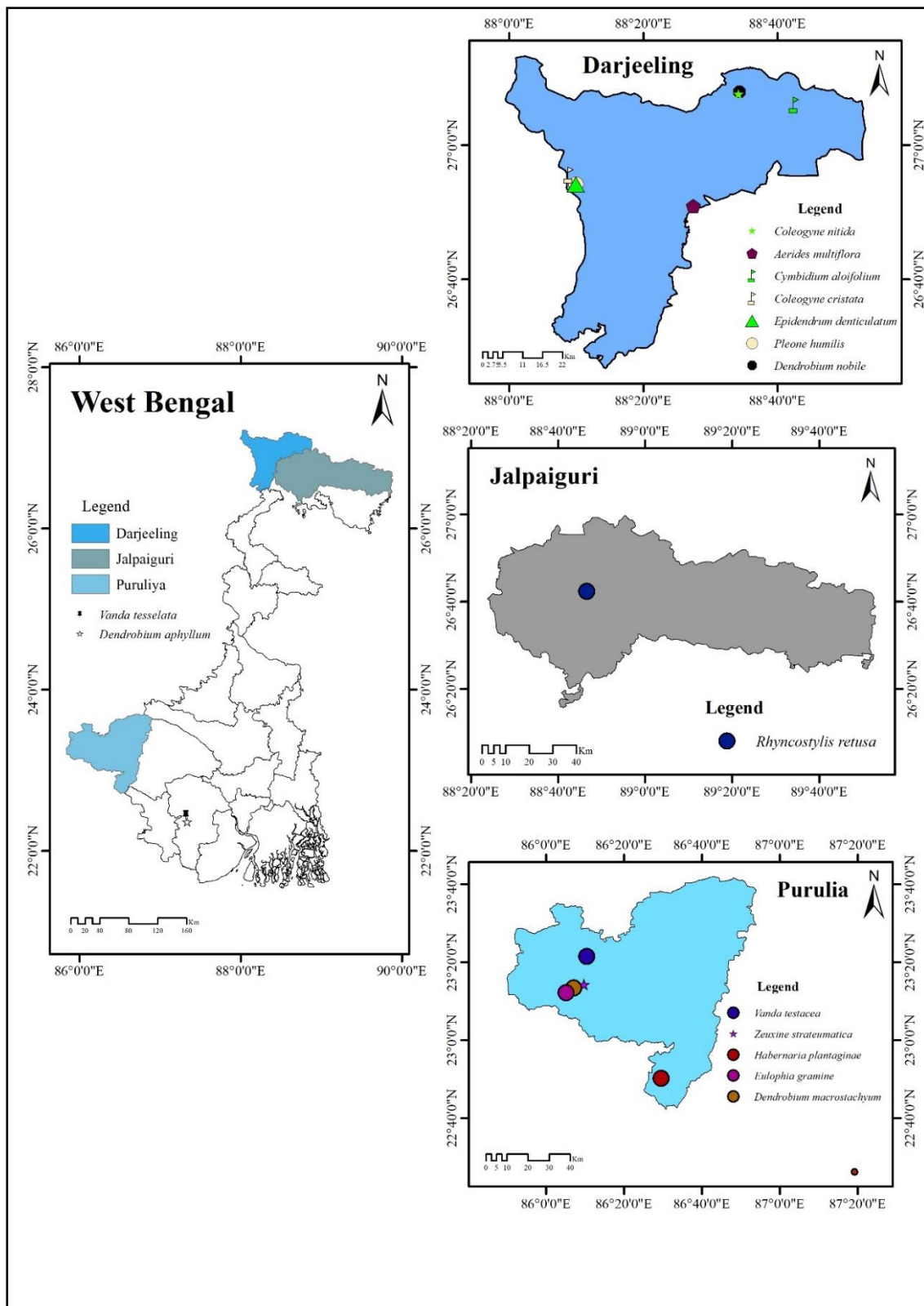


Figure 1: Distribution of orchid species in different part of West Bengal.

Chapter 5: Materials and Methods

5. Materials and Methods:

5.1. Collection of specimens:

In this study different field survey was undertaken between October 2022 to May 2023 in our selected study area, different parts of West Bengal, India. It was a big challenge for us to find the sample with the reproductive stage (flower only) for the diverse flowering seasons and distribution pattern of each plant specimen.

Fresh specimens are collected from different areas, put into the zipper bags, and then brought to the laboratory for further taxonomic workout and herbarium preparation. Many flowers and mature pollinia were collected into liquid preservatives (viz. FAA solution 30-40% formalin) for SEM analysis. The natural habitat, host plants, and pictures of samples were captured by a Nikon D3400 camera and smartphone. All geo-tag data was recorded by GPS.

5.2. Taxonomic observation:

Dissection of the flower was done and observed under a simple microscope. Photographs were taken with the help of a camera (Nikon D3400).

5.3. Preparation of herbarium:

For preparing the herbarium we followed the standard herbarium method (Jain and Rao, 1977).

5.4. Preparation of distributional map:

GPS coordinators were collected by GPS and prepared a simple collection map by GIS technology with the help of ArcGIS software (V. 10.5).

5.5. Microscopic data:

A. Simple microscope study:

Micro morphology of flowers as well as Pollinaria was studied under a simple microscope.

B. Stereo zoom microscope study:

Micro morphology, flower color, reproductive structures, Pollinaria structure, etc. were studied under a zoom stereo microscope for capturing images.

C. SEM study:

For the SEM study, mature Pollinaria samples were dehydrated into DMP and afterward, critical point Dried (Halbritter, 1998). Fresh Pollinaria were taken into stubs unfixed to obtain a dry state. The sample was coated with gold by a ‘Gold coater machine’. The SEM study was conducted at the CRF (Central Research Facility), IIT, Kharagpur. The images are shown in the result section.

5.6. Identification of collected specimens:

The collected specimen was identified by using obtainable monographs, and different flora books, keys of different research papers such as Bengal Plants by David Prain(1903), VOL- I and II, Flora of Bankura, West Bengal Flora VOL-2, Flora of India, British Flora of India by J.D Hooker (1872-1897), Flora of India, etc., and the species nomenclature were updated by using online browsers like Plants of the World Flora(<https://powo.science.kew.org>), International Plant names Index(<https://www.ipni.org>) etc. The review literature survey was performed exhaustively online using the Google search engine, PubMed and Google Scholar.

5.7. Character weighting and coding:

A practical definition of characters espoused by numerical taxonomists (Michener and Sokal, 1975) defines characters as a feature, which varies from one organism to another. For numerical taxonomy, we selected some unite characters (taxonomic characters of two or more states, which within the study at hand can't be subdivided logically, except for the subdivision bought about by the method of coding) from reproductive structures. Our selected all characters are equal-weighted. After that characters are converted to binary (only two forms) or multi-states (more than two forms) for computer handling. The coding of character states is very important. It is done by assigning a non-negative integral value. Binary characters are conveniently assigned 0 and 1. Plesiomorphic characters (primitive or ancestor characters) or absent were assigned 0 and apomorphic characters (advanced or descendant characters) or present were assigned 1. Where the characters were not distinguished as apomorphic or plesiomorphic, we assigned 0 for usual or common characters and 1 for unusual or rare or uncommon characters. In the case of multi states characters, assigned 1, 2, and 3 for plesiomorphic or common characters to apomorphic or uncommon characters respectively. In certain cases, information may be available for a particular character for most or some OTUs (Operational Taxonomic Units), but not for all. Such characters are used in analysis but for the taxa for which information is not available or is irrelevant, we gave NC code (Not comparable) in the data matrix.

5.8. Numerical analysis:

Fifteen numbers of samples are use under 11 numbers of genera for this analysis. To see the relationship among all the species, total 14 numbers of unit characters are considered here on pollinial morphology. The characters are both qualitative (9) and quantitative (5) in nature under which 9 are binary characters and 5 are multistate characters

allow for this analysis. A similarity matrix is prepared on the basis of Correlation coefficient method. Cluster analysis, Phenogram development are performed on average taxonomic distance between species and Unweighted pair-group methods with arithmetic averages (UPGMA) is implemented in 'PAST' software version 4.0.3 (Hammer, 2001).

Table No. 1: Collected specimens with their host plants.

| Sl. No. | Species | Subfamily | Habitat | Host plants |
|---------|---|------------------------|-------------|---|
| 1. | <i>Aerides multiflora</i> Roxb. | Epidendroideae Kostel. | Epiphytic | <i>Bombax ceiba</i> L., <i>Ficus benghalensis</i> L., <i>Mangifera indica</i> L., <i>Schleichera oleosa</i> (Lour.) Oken |
| 2. | <i>Coelogyne corymbosa</i> Lindl. | Epidendroideae Kostel. | Epiphytic | <i>Cryptomeria japonica</i> (Thunb. ex L.f.) D. Don |
| 3. | <i>Coelogyne cristata</i> Lindl. | Epidendroideae Kostel. | Epiphytic | <i>Schima wallichii</i> (DC.) Korth. |
| 4. | <i>Cymbidium aloifolium</i> (L.) Sw. | Epidendroideae Kostel. | Epiphytic | <i>Ficus religiosa</i> L. |
| 5. | <i>Dendrobium aphyllum</i> (Roxb.) C.E.C.Fisch. | Epidendroideae Kostel. | Epiphytic | <i>Ficus benghalensis</i> L., <i>Azadirachta indica</i> A. Juss. |
| 6. | <i>Dendrobium macrostachyum</i> Lindl. | Epidendroideae Kostel. | Epiphytic | <i>Diospyros melanoxylon</i> Roxb. <i>Shorea robusta</i> Roth. |
| 7. | <i>Dendrobium nobile</i> Lindl. | Epidendroideae Kostel. | Epiphytic | <i>Cyathea spinulosa</i> Wall. |
| 8. | <i>Epidendrum denticulatum</i> Barb.Rodr. | Epidendroideae Kostel. | Epiphytic | <i>Schima wallichii</i> (DC.) Korth. |
| 9. | <i>Eulophia graminea</i> Lindl. | Epidendroideae Kostel. | Terrestrial | Terrestrial |
| 10. | <i>Habenaria plantaginea</i> Lindl. | Orchidoideae Eaton. | Terrestrial | Terrestrial |
| 11. | <i>Pleione humilis</i> (Sm.) D. Don. | Epidendroideae Kostel. | Epiphytic | <i>Cryptomeria japonica</i> (Thunb. ex L.f.) D. Don , <i>Rhododendron</i> L. |

| | | | | |
|-----|--|------------------------|-------------|--|
| 12. | <i>Rhyncostylis retusa</i> (L.) Blume | Epidendroideae Kostel. | Epiphytic | <i>Mangifera indica</i> L., <i>Bombax ceiba</i> L.; <i>Diospyros melanoxylon</i> Roxb. |
| 13. | <i>Vanda tessellata</i> (Roxb.) Hook. ex G.Don | Epidendroideae Kostel. | Epiphytic | <i>Azadirachta indica</i> A. Juss., <i>Acacia catechu</i> (L.F.) Willd., <i>Diospyros melanoxylon</i> Roxb., <i>Ficus benghalensis</i> L., <i>Ficus religiosa</i> L., <i>Barringtonia acutangulata</i> (L.) Gaertn., <i>Mangifera indica</i> L., <i>Shorea robusta</i> Roth., <i>Madhuca longifolia</i> var. <i>latifolia</i> (Roxb.) A. Chev. |
| 14. | <i>Vanda testacea</i> (Lindl.) Rchb. f. | Epidendroideae Kostel. | Epiphytic | <i>Acacia catechu</i> (L.F.) Willd., <i>Diospyros melanoxylon</i> Roxb., <i>Ficus benghalensis</i> L., <i>Madhuca longifolia</i> var. <i>latifolia</i> (Roxb.) A. Chev. |
| 15. | <i>Zeuxine strateumatica</i> (L.) Schltr. | Orchidoideae Eaton. | Terrestrial | Terrestrial |

Chapter 6: Results

6. Results:

6.1. Collected plants:

In our intensive field work we found 15 orchid species that belong to 11 genera viz, *Aerides* (1 species), *Coelogyne* (2 species), *Cymbidium* (1 species), *Dendrobium* (3 species), *Epidendrum* (1 species), *Eulophia* (1 species), *Habenaria* (1 species), *Pleione* (1 species), *Rhyncostylis* (1 species), *Vanda* (2 species), and *Zeuxine* (1 species) from the family Orchidaceae. Below the photographs shows orchid plants species:



Figure 2: Collected plant samples. **A:** *Dendrobium nobile*, **B:** *Coelogyne corymbosa*, **C:** *Aerides multiflora*, **D:** *Coelogyne cristata*, **E:** *Dendrobium macrostachyum*, **F:** *Cymbidium aloifolium*.



Figure 3: Collected plant samples. **A:** *Pleione humilis*, **B:** *Vanda Testacea*, **C:** *Eulophia graminea*, **D:** *Vanda tessellata*, **E:** *Habenaria plantaginea*, **F:** *Zeuxine strateumatica*, **G:** *Epidendrum denticulatum*, **H:** *Rhynchosyilis retusa*, **I:** *Dendrobium aphyllum*.

In our study, we recorded that the most abundant orchid genera is *Vanda*. Among the 2 *Vanda* species, *V. tessellata* is found in many places of South Bengal. But *V. testacea* grew only in some areas of the Purulia District.



Figure 4: A-I Field photographs during sample collection.

Table No. 2: List and distribution of collected plants in the study area:

| Sl. No. | Specimen Name | Collection Place | Collection Date | Latitude & Longitude |
|---------|---|---|-----------------|---|
| 1. | <i>Aerides multiflora</i> Roxb. | Sevoke Forest, West Bengal 734007 | 14.05.2023 | Lat- N26°50'53.5" Long- E88°27'27.1" |
| 2. | <i>Coelogyne corymbosa</i> Lindl. | Icha Forest, West Bengal 734317 | 12.05.2023 | Lat-N27°07'36.5" Long- E 88°34'14.5" |
| 3. | <i>Coelogyne cristata</i> Lindl. | Gopal Dhara Tea Garden, West Bengal 734214 | 21.02.2023 | Lat- N26°55'33.0" Long- E88°08'54.2" |
| 4. | <i>Cymbidium aloifolium</i> (L.) Sw. | Raset forest, West Bengal 734319 | 12.05.2023 | Lat- N27°06.004' Long- E88°42.488' |
| 5. | <i>Dendrobium aphyllum</i> (Roxb.) C.E.C.Fisch. | Inda, Kharagpur, West Bengal 721301 | 13.04.2023 | Lat- N22°21.220' Long- E87°20.121' |
| 6. | <i>Dendrobium macrostachyum</i> Lindl. | Ajodhya Hills, Ajodhya, West Bengal 723152 | 24.05.2023 | Lat- N23°13'22.7" Long- E86°07'06.9" |
| 7. | <i>Dendrobium nobile</i> Lindl. | Icha Forest, West Bengal 734317 | 12.05.2023 | Lat- N27°07'55.3" Long- E88°34'24.3" |

Table No. 2: Continued.....

| Sl. No. | Specimen Name | Collection Place | Collection Date | Latitude & Longitude |
|---------|---|---|-----------------|---|
| 8. | <i>Epidendrum denticulatum</i> Barb.Rodr. | Bukim Tea Garden, West Bengal 734214 | 19.02.2023 | Lat- N26°54'09.1" Long- E88°09'56.4" |
| 9. | <i>Eulophia graminea</i> Lindl. | Ajodhya Hills, Ajodhya, West Bengal 723152 | 24.05.2023 | Lat- N23°12.16' Long- E86°5.17' |
| 10. | <i>Habenaria plantaginea</i> Lindl. | Ajodhya Hills, Ajodhya, West Bengal 723152 | 20.09.2022 | Lat- N23°14'15.6" Long- E86°09'48.9" |
| 11. | <i>Pleione humilis</i> (Sm.) D.Don. | Sonada Forest, West Bengal 734209 | 18.02.2023 | Lat- N26°57'42.6" Long- E88°17'55.2" |
| 12. | <i>Rhynchostylis retusa</i> (L.) Blume | Nimna Tandu Forest, West Bengal 73524 | 13.05.2023 | Lat- N26°42'21.0" Long-E88°46'37.1" |
| 13. | <i>Vanda tessellata</i> (Roxb.) Hook. ex G.Don | Midnapore, West Bengal 721102 | 20.05.2023 | Lat- N22°26.240' Long- E87°19.221' |
| 14. | <i>Vanda testacea</i> (Lindl.) Rchb. f. | Torang, West Bengal 723201 | 24.05.2023 | Lat- N23°21.465' Long- E86°10.439' |
| 15. | <i>Zeuxine strateumatica</i> (L.) Schltr. | Ghatihuli, West Bengal 723129 | 11.02.2023 | Lat- N22°50'14.70" Long- E86°29'35.02" |

6.2: Taxonomic Observation:

6.2.1. *Aerides multiflora* Roxb.

Synonym: *Aerides affinis* Wall. Ex J.Lindl. , *Epidendrum geniculatum* Hook.f.

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial, densely clustered, velamen well developed.

Stem: Long, slender, stout stem with many alternate leaves, sheathed at the base, green.

Leaves: Simple; sessile; linear to lanceolate; margin entire; apex obliquely bifid; coriaceous, glossy; glabrous; alternate; parallel venation; size- 10-18cm.

Inflorescence: Terminal racemose; taller than leaves, many-flowered (Figure 5: A&B).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; pinkish white with irregular purple patches; no fragrance, size- 1.4 cm (Figure 5: D).

Calyx: Sepals 3; polysepalous; sub-equal, oblong; pinkish white with irregular purple patches; size- 0.8 cm (Figure 5: C)

Corolla: Petals 3, one is modified into a labellum; polypetalous; sub-equal, oblong; larger than sepals; pinkish white with irregular purple patches; size- 0.8 cm labellum 3 lobed, pointing forward, margin denticulate; size- 1.4 cm (Figure 5: C).

Gynostemium: Stigma located at the base; anther cup covered the Pollinaria (Figure 5: F); pollinia-2 (Figure 5: H&G), globular, yellowish; size- 0.8 cm (Figure 5: E)

Fruit: Capsule; dry.

Seed: small, dust-like.

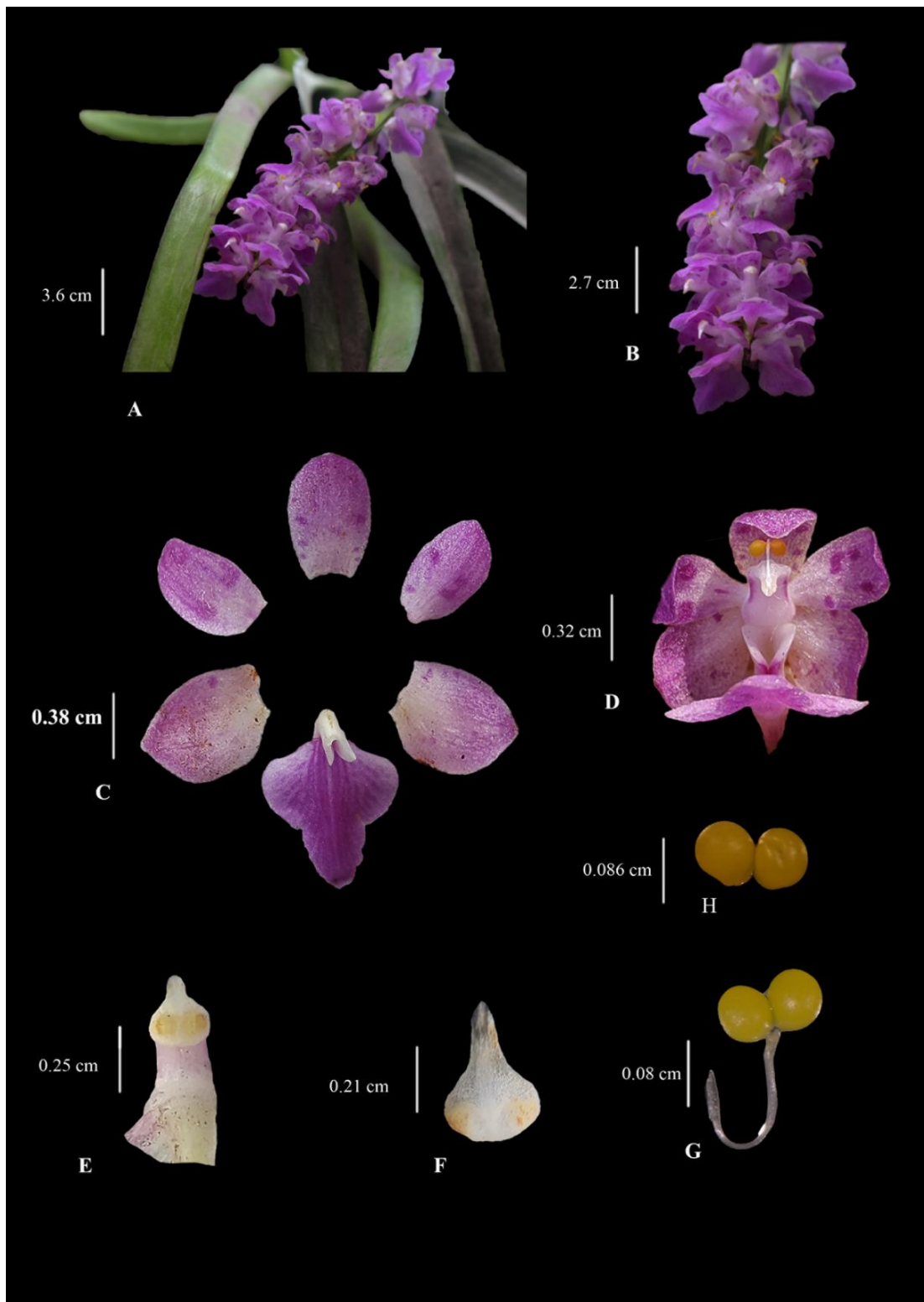


Figure 5: *Aerides multiflora* Roxb.: **A.** Habit; **B.** Inflorescence; **C.** Petals & Sepals; **D.** Flower; **E.** Column; **F.** Anther cap; **G & H.** Pollinia.

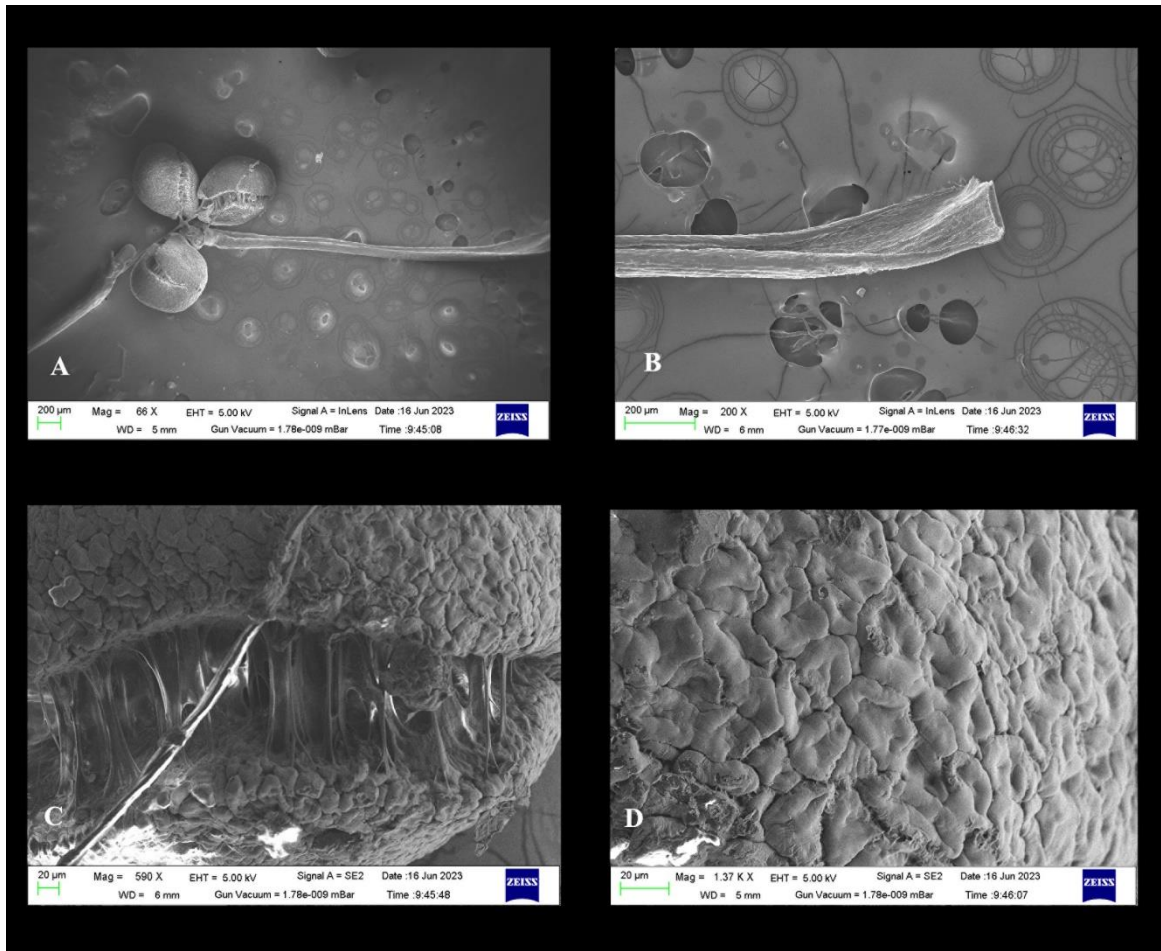


Figure 6: *Aerides multiflora* Roxb.: **A-D.** SEM photographs of Pollinia.

6.2.2. *Coelogyne corymbosa* Lindl.

Synonym: *Pleione corymbosa* (Lindl.) Kuntze

Habitat: Epiphytic.

Habit: Herb (Figure 7: A).

Root: Aerial, velamen well developed.

Stem: Pseudobulbs; farrow with ages; erect, stout, cylindrical; green.

Leaves: Simple; sessile; long; lanceolate; apex acute; margin entire; coriaceous, glossy; glabrous; alternate; parallel venation; dark green; size- 15-30cm.

Inflorescence: Racemose, flower arranged corymbose manner; shorter than leaves, multi flowered.

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; white; fragrance, size- 2.3 cm (Figure 7: B, C&D)

Calyx: Sepals 3; polysepalous; oval; apex acute; margin entire; white; size- 2.4 cm. (Figure 7: E)

Corolla: Petals 3, one is modified into labellum; polypetalous; oval, apex acute, margin entire, white, narrower than sepals, size- 2.2 cm; labellum lobed, fringed, white with yellow spot, size- 1.9 cm (Figure 7: E).

Gynostemium: column long, elongated, slender, white; anther cup covered the Pollinaria (Figure 7: G&H); stigma located beneath the anther cup; pollinia-4 (Figure 7: I&J), caudicle link the pollinia; yellowish; size- 1.4 cm (Figure 7: F)

Fruit: Capsule; dry; **Seed:** Small, dust-like.

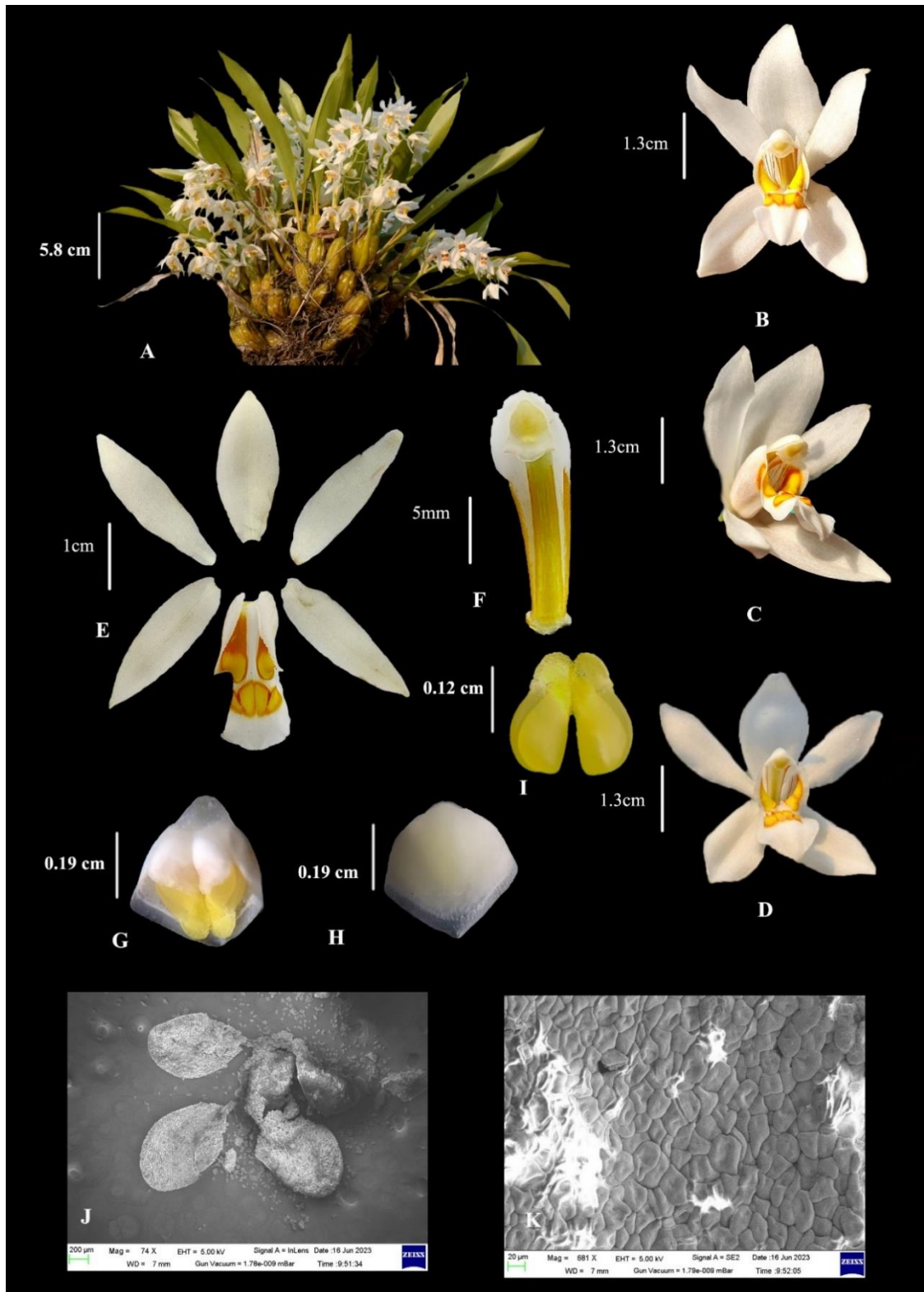


Figure 7: *Coelogyne corymbosa* Lindl.: A. Habit; B, C & D. Flower; E. Petals & Sepals; F. Column; G & H. Anther cap; I. Pollinia; J & K. SEM photographs of Pollinia.

6.2.3. *Coelogyne cristata* Lindl.

Synonym: *Cymbidium speciosissimum* D.Don , *Pleione speciosissima* (D.Don) Kuntze

Habitat: Epiphytic or lithophyte.

Habit: Herb (Figure 8: A).

Root: Aerial, velamen well developed.

Stem: Pseudobulbs; farrow with ages; erect, stout, cylindrical; sheathed at the base; green.

Leaves: Simple; sessile; long; linear to oblong; apex acute; margin entire; coriaceous, glossy; glabrous; alternate; parallel venation; dark green; size- 8-12 cm.

Inflorescence: Raceme, pendulous; 5-12 flowered.

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; white; fragrance, size- 4.1 cm (Figure 8: B, C&D).

Calyx: Sepals 3; polysepalous; elongated; lanceolate to oblong; apex acute; white; size- 4.1 cm (Figure 8: E).

Corolla: Petals 3, one is modified into a labellum; polypetalous; lanceolate, apex acute, white, petals are narrower than sepals, size- 3.8 cm; labellum lobed, boat-shaped, crested, the most attractive part of this flower, margin fringed, yellow at the center and white at the margin, size- 3.6 cm (Figure 8: E).

Gynostemium: Column long, elongated, white; anther cup covered the Pollinaria, stigma located beneath the anther cup; pollinia-2 (Figure 8: G&I), caudicles link the pollinia; yellowish; size- 2.4 cm (Figure 8: F).

Fruit: Capsule; dry.

Seed: Small, dust-like.

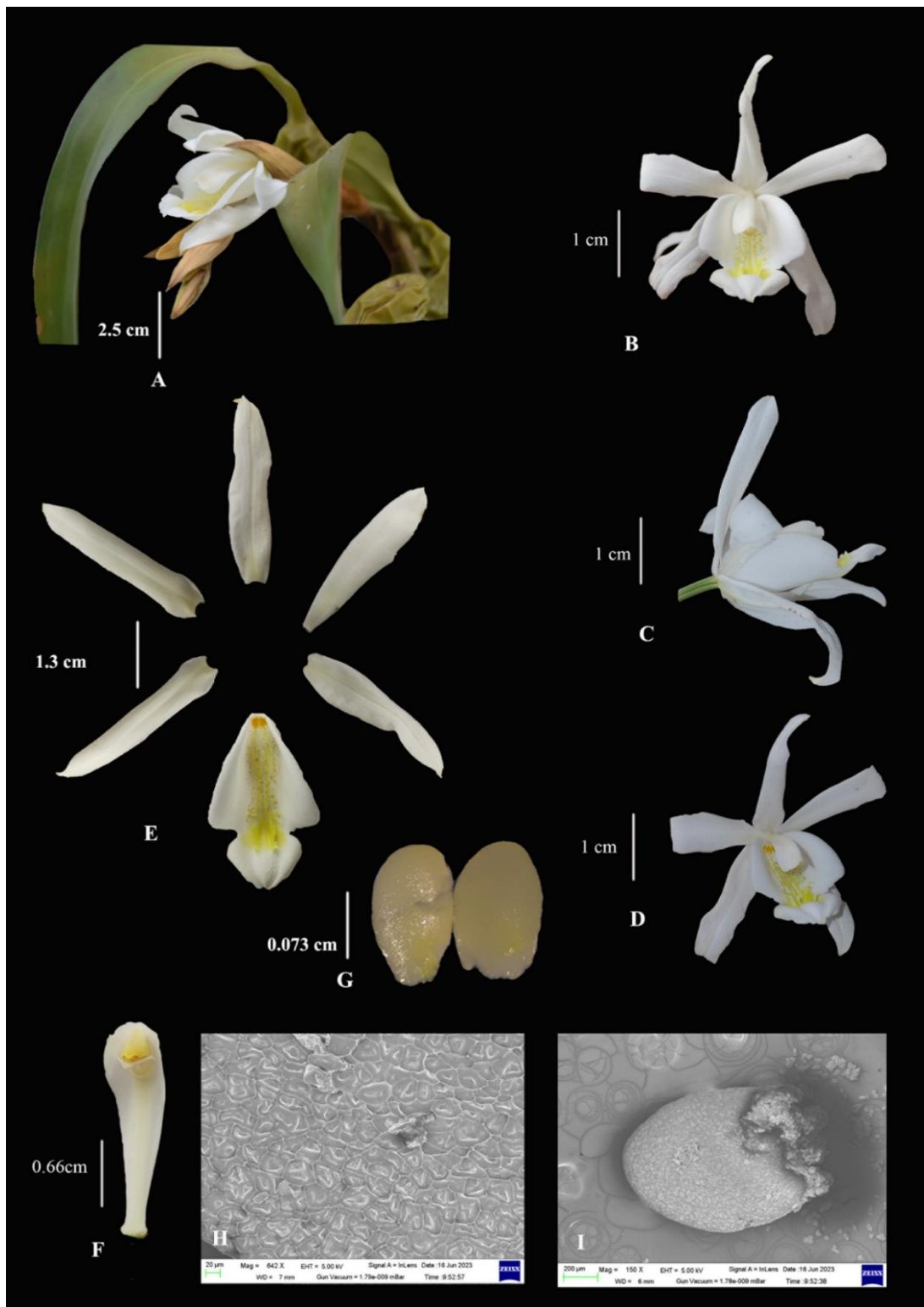


Figure 8: *Coelogyne cristata* Lindl.: A. Habit; B, C & D. Flower; E. Petals & Sepals; F. Column; G. Pollinia; H & I. SEM photographs of Pollinia.

6.2.4. *Cymbidium aloifolium* (L.) Sw.

Synonym: *Epidendrum aloifolium* L., *Limodorum aloifolium* (L.) Roxb.

Habitat: Epiphytic.

Habit: Herb.

Root: Numerous, aerial, densely cover base.

Stem: Erect, long, stout, slender; bearing many leaves; green;

Leaves: Simple; sessile; linear to oblong; narrow; margin entire; slightly notched at apex; coriaceous; glabrous; parallel venation; size- 35-50cm.

Inflorescence: Pendulous; raceme; 20-35 flowered (Figure 9: A).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; cream yellow with maroon; no fragrance, size- 2.2 cm (Figure 9: B&C).

Calyx: Sepals 3; polysepalous; apex obtuse; margin entire; longer than petals; cream yellow with maroon strips; size- 2 cm (Figure 9: D).

Corolla: Petals 3, one is modified into labellum; polypetalous; elongated and slightly oval; apex obtuse; margin entire; cream yellow with maroon strips; size-1.8 cm; labellum usually large; showy; 3 lobed; margin entire; maroon strip alternates with cream strip; size- 1.3 cm (Figure 9: D).

Gynostemium: Column elongated, long, maroon in colour (Figure 9: E).; anther cup yellow; anther cup covered the Pollinaria (Figure 9: G&F).; pollinia-2 (Figure 9: H&I)., caudicles link the pollinia; yellowish.

Fruit: Capsule; dry.

Seed: Small, dust-like.

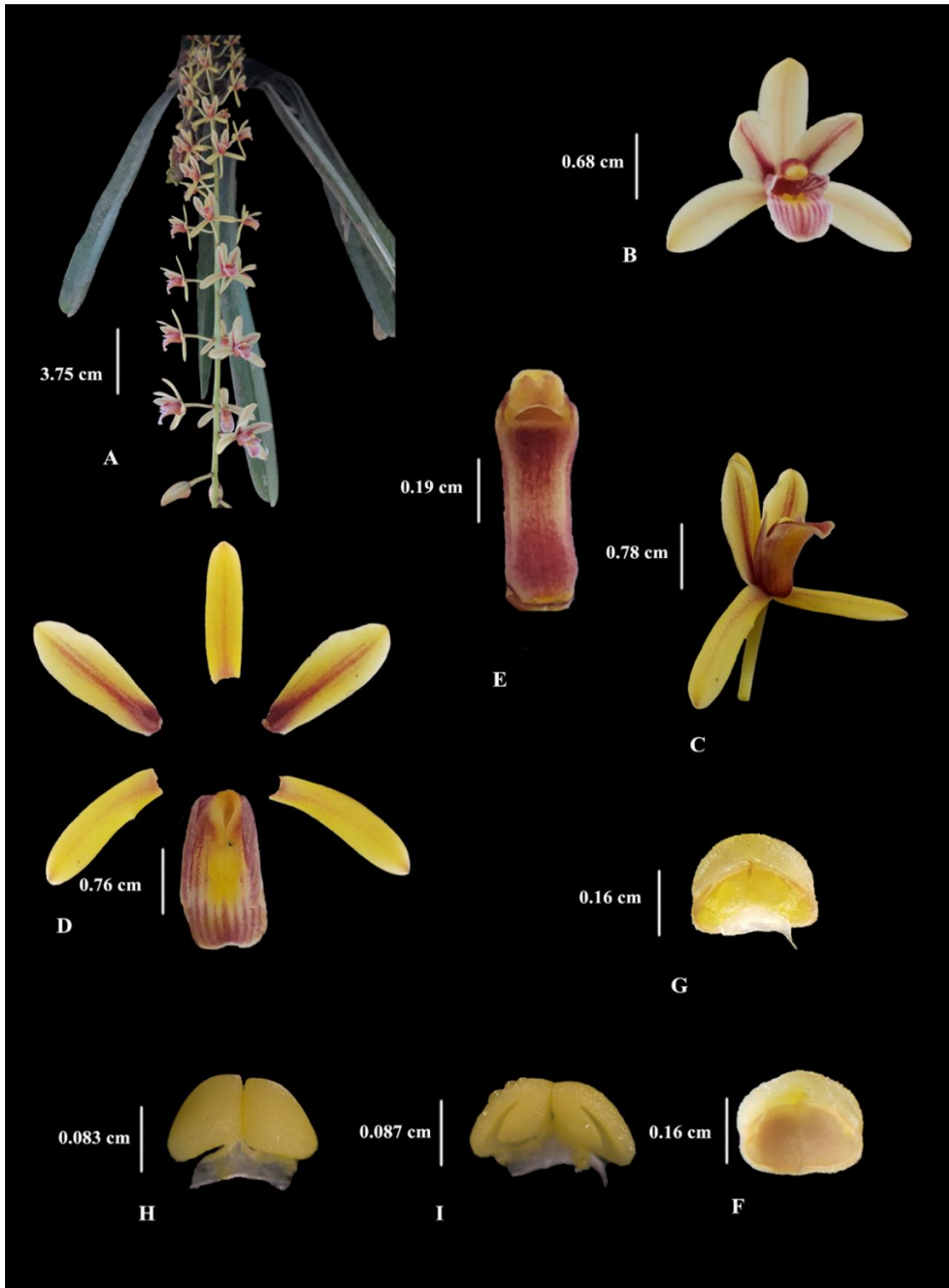


Figure 9: *Cymbidium aloifolium* (L.) Sw.: A. Habit; B & C. Flower; D. Petals & Sepals; E. Column; F & G. Anther cap; H & I. Pollinia.

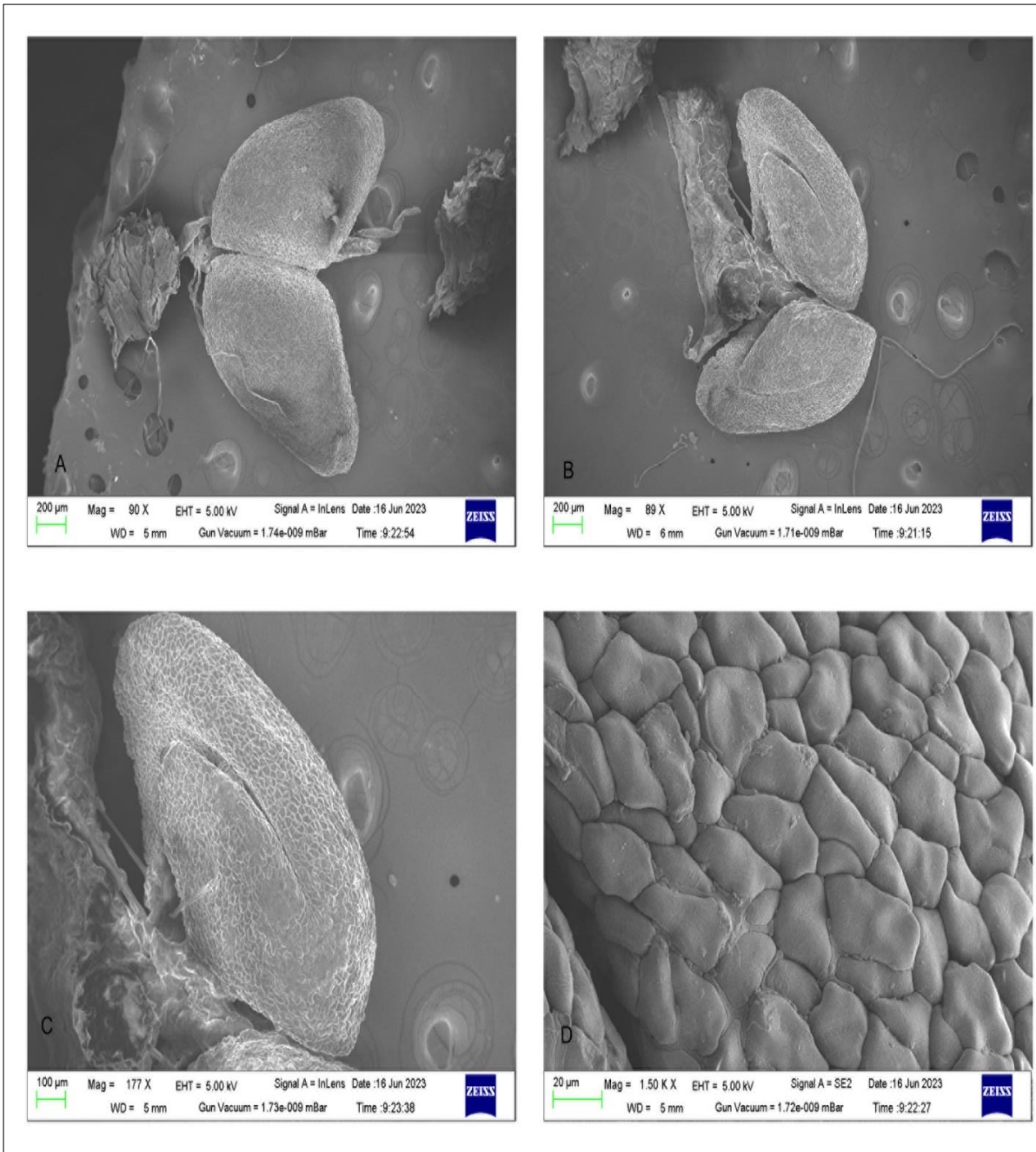


Figure 10: *Cymbidium aloifolium* (L.) Sw.: **A-D.** SEM photographs of pollinia.

6.2.5. *Dendrobium macrostachyum* Lindl.

Synonym: *Callista macrostachya* (Lindl.) Kuntze

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial, velamen well developed.

Stem: Erect, cane-like; stout, cylindrical; present nodes and internodes; green.

Leaves: Simple; sessile; long; lanceolate to oblong; apex acute; margin entire; coriaceous, glossy; glabrous; alternate; parallel venation; dark green; size-15-30cm.

Inflorescence: Raceme, multi flowered (Figure 11: A).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; small to medium; greenish yellow with brown vein like strip at lip; no fragrance, size- 2-4 cm (Figure 11: B).

Calyx: Sepals 3; polysepalous; lanceolate to oblong; apex acute; margin entire; greenish yellow with slightly reddish apex; dorsal sepal size- 1.4 cm, lateral sepal size- 1.9 cm (Figure 11: C).

Corolla: Petals 3, one is modified into labellum; polypetalous; lanceolate, apex acute, margin entire, greenish yellow with slightly reddish apex, size- 1.4 cm; labellum fringed, with velvety surface; greenish yellow with brown vein like strips; larger than other petals; size- 2 cm (Figure 11: C).

Gynostemium: Column long, elongated, slender, light yellow, size- 0.4 cm (Figure 11: D); anther cup covered the Pollinaria (Figure 11: E&F)., stigma located beneath the anther cup; pollinia-4, yellowish (Figure 11: H).

Fruit: Capsule; dry; **Seed:** Small, Dust-like.

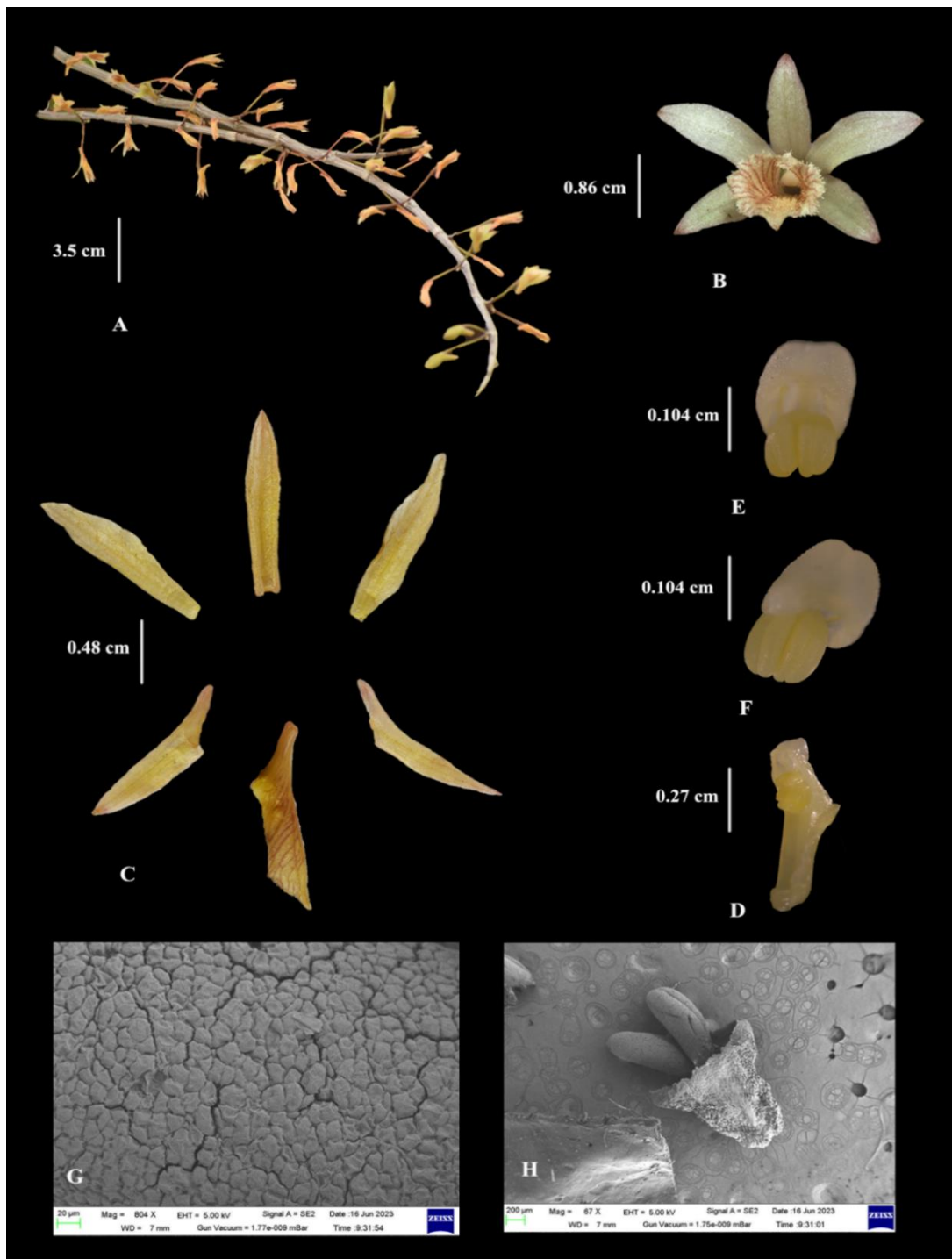


Figure 11: *Dendrobium macrostachyum* Lindl.: **A.** Inflorescence, **B.** Flower, **C.** Petals & Sepals, **D.** Coloumn, **E&F.** Pollinia with Anther cap, **G&H.** SEM photographs pf Pollinia.

6.2.6. *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch.

Synonym: *Callista aphylla* (Roxb.) Kuntze

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial, velamen well developed.

Stem: Clustered, cane-like; pendulous; stout, cylindrical; elongated; many noded; produces keikis; green.

Leaves: Simple; sessile; long; ovate to linear-lanceolate; textured; deciduous; apex acuminate; margin entire; coriaceous, glabrous; alternate; parallel venation; green; size-length 6 cm, breadth 2 cm.

Inflorescence: Racemose, borne on nodes of the old stem; many flowered; arising laterally; many numbers of inflorescence per plant (Figure 12: A).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; pinkish white; no fragrance, size- 3-4 cm (Figure 12: B&C).

Calyx: Sepals 3; polysepalous; oblong to lanceolate; apex acute; margin entire; narrower than petals; white; dorsal sepal size- 2.9 cm, lateral sepal size- 3.3 cm (Figure 12: D).

Corolla: Petals 3, one is modified into labellum; polypetalous; elliptic to oblong; apex acute, margin entire, pinkish white, size- 3.1 cm; labellum obscurely 3 lobed; trumpet-shaped; margin crenulate; off-white with purple strip at the base; covered with tiny hairs on the outer surface and along the border; larger than other petals; size- 3.9 cm (Figure 12: D).

Gynostemium: Column long, elongated, slender, white; anther cup covered the Pollinaria (Figure 12: F). stigma located beneath the anther cup; pollinia-4, yellowish (Figure 12: G&I).

Fruit: Capsule; dry.

Seed: Small, Dust-like.

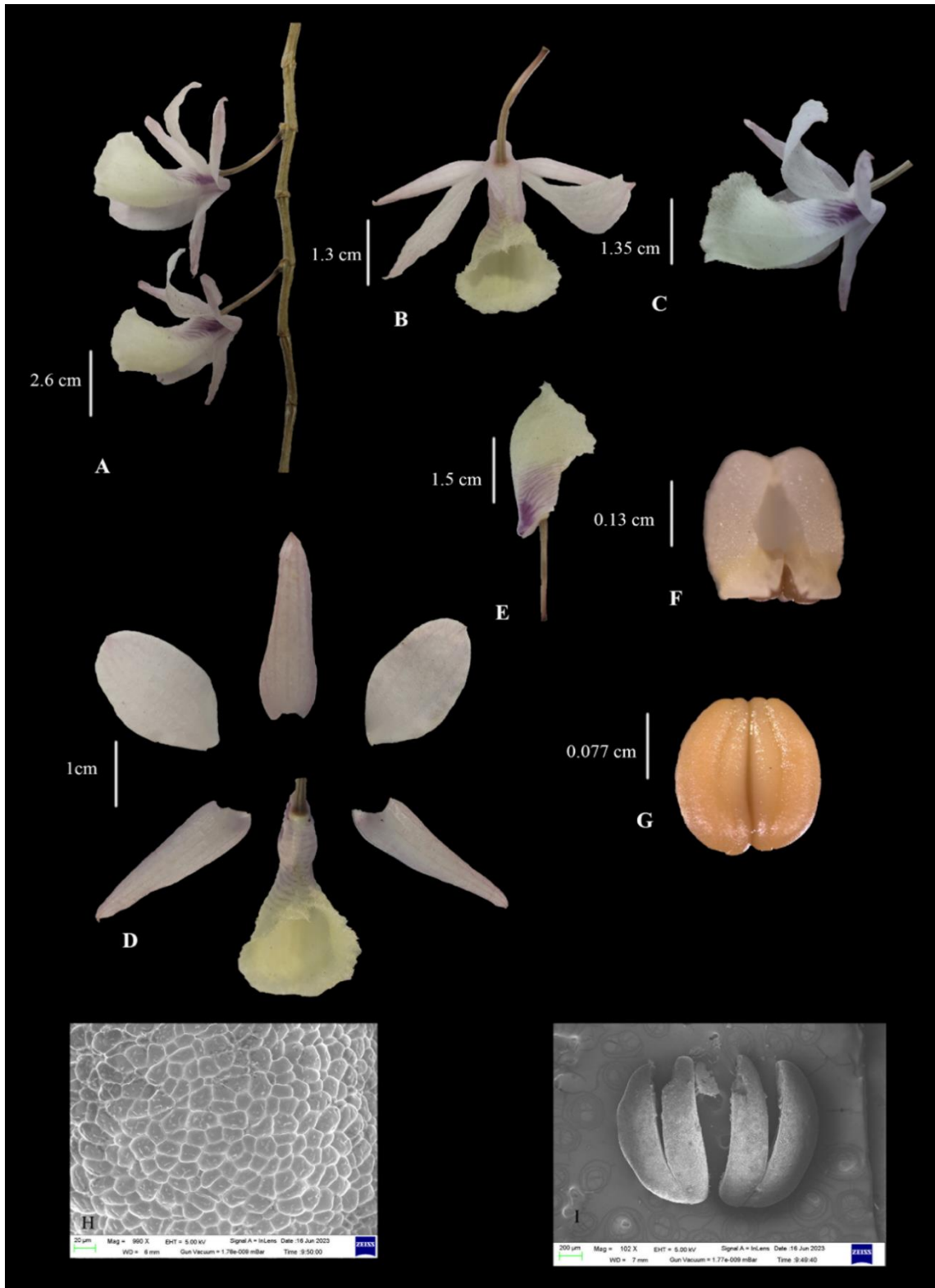


Figure 12: *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch.: **A.** Inflorescence; **B & C.** Flower; **D.** Petals & Sepals; **E.** Lip; **F.** Anther cap; **G.** Pollinia; **H & I.** SEM photograph of Pollinia.

6.2.7. *Dendrobium nobile* Lindl.

Synonym: *Callista nobilis* (Lindl.) Kuntze

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial, velamen present; cylindrical.

Stem: pseudobulbs erect, long, stout, slender, furrowed with age; bearing many leaves; green.

Leaves: Simple; sessile; oblong; alternate; margin entire; apex sub obtuse and notched; coriaceous; glabrous; parallel venation; size- 7-8cm.

Inflorescence: Short raceme; 3-5 flowered (Figure 13: A).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; pinkish white; no fragrance, size- 3.9 cm (Figure 13: B).

Calyx: Sepals 3; polysepalous; narrower than petals; oblong; apex obtuse; margin entire; colour purple turning white at the base; dorsal sepal size- 3.8 cm, lateral sepal size- 4.1 cm (Figure 13: C).

Corolla: Petals 3, one is modified into a labellum; broader than sepals; polypetalous; elliptic; apex acute; margin entire; colour purple turning white at the base; size- 3.9 cm; labellum showy; hairy; ovate to oblong; margin entire; deep purple inside and white outside; size- 3.9 cm (Figure 13: C).

Gynostemium: Column elongated, long; maroon in colour (Figure 13: D).; anther cup yellow; anther cup covered the Pollinaria (Figure 13: E&F).; pollinia-4, (Figure 13: G&H), Yellowish.

Fruit: Capsule. **Seed:** Small, Dust-like.

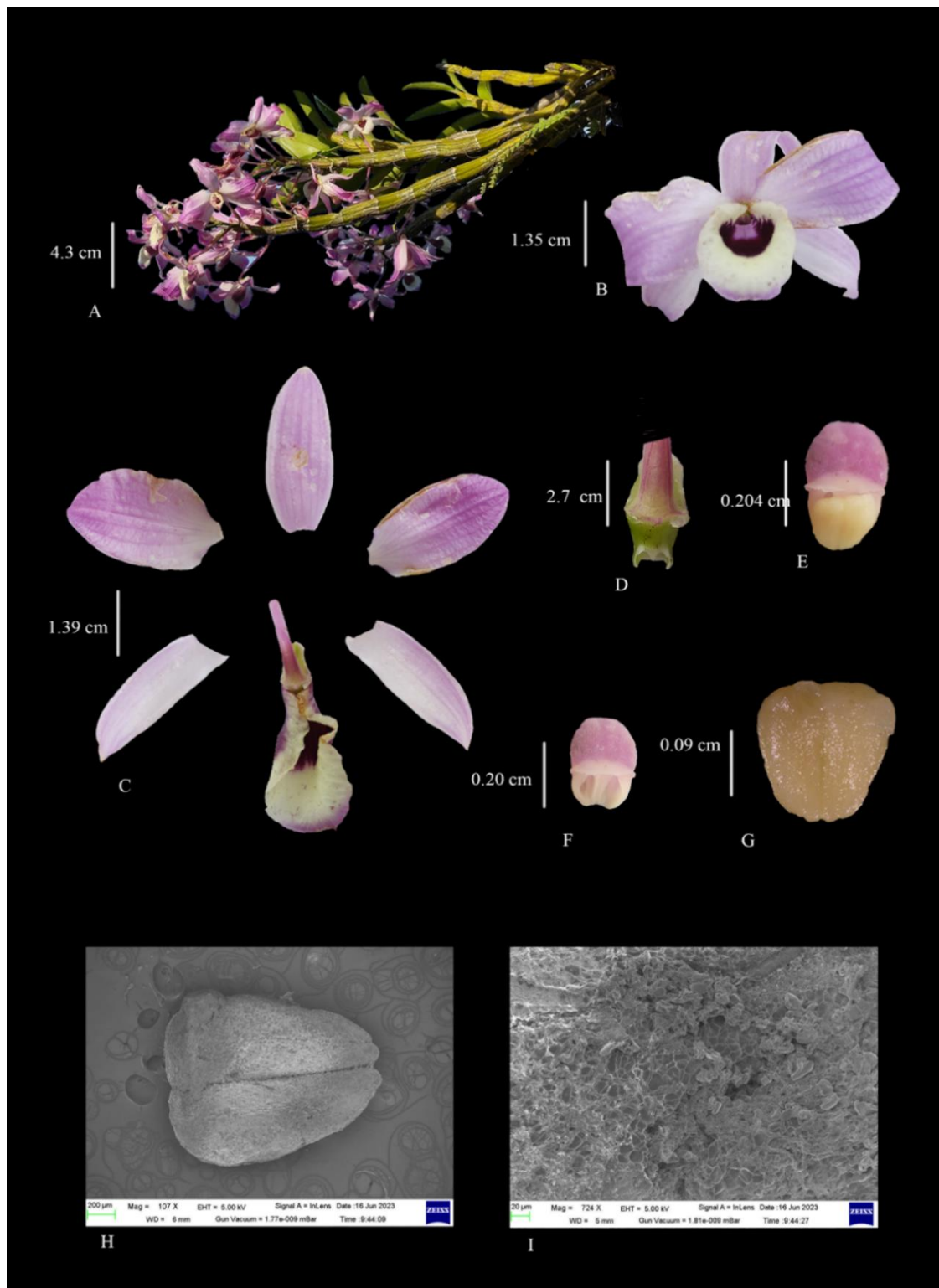


Figure 13: *Dendrobium nobile* Lindl.: **A.** Habit; **B.** Flower; **C.** Petals & Sepals; **D.** Column; **E & F.** Anther cap; **G.** Pollinia; **H & I.** SEM photograph of Pollinia.

6.2.8. *Epidendrum denticulatum* Barb. Rodr.

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial, long, thin, spongy velamen well developed.

Stem: Slender, erect, stout, distinct nodes and internodes, green.

Leaves: Simple; sessile; lanceolate to elliptic; margin entire; apex rounded; coriaceous, glossy; glabrous; alternate; parallel venation; size- 5-10cm.

Inflorescence: Terminal racemose; taller than leaves, 3-7 flowered (Figure 14: A).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; pink; no fragrance, size- 3.3cm (Figure 14: B).

Calyx: Sepals 3; polysepalous; oblong to obovate; apex acute; margin entire; pink; each of the calyx separated from the next by angle close to 60°; size- 1.1 cm (Figure 14: C).

Corolla: Petals 3, one is modified into labellum; polypetalous; obovate to elliptic, apex acute, margin entire, pink, narrower than sepals, size- 1.2 cm; labellum 3 lobed, margin serrate to fimbriate, pink, shorter than other petals; each of the corolla separated from the next by angle close to 60°; size- 0.7 cm (Figure 14: C).

Gynostemium: Column long, elongated, slender; anther cup covered the Pollinaria; pollinia-4 (Figure 14: F), yellowish.

Fruit: Capsule; dry.

Seed: Small, Dust-like.

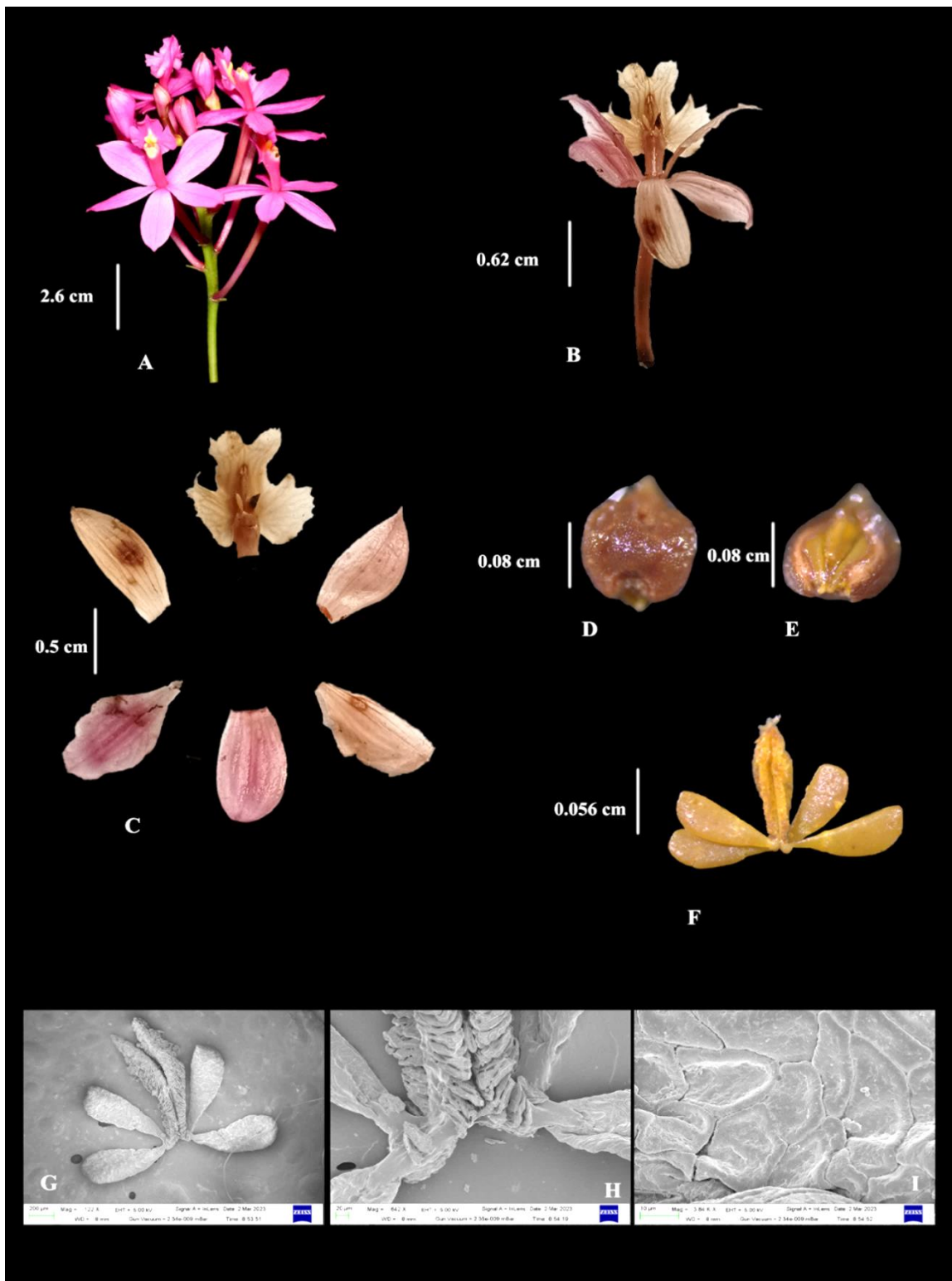


Figure 14: *Epidendrum denticulatum* Barb. Rodr.: **A.** Inflorescence; **B.** Flower; **C.** Petals & Sepals; **D.** Anther cap; **E.** Anther cap with Pollinia; **F.** Pollinia; **G, H & I.** SEM photograph of Pollinia.

6.2.9. *Eulophia graminea* Lindl.

Synonym: *Graphorkis graminea* (Lindl.) Kuntze

Habitat: Terrestrial

Habit: Pseudobulbous herb.

Root: Tuberos; Pseudobulbs; borne above ground or partially underground, erect, ovoid, conical, oblong, several noded, producing erect aerial stem; whitish brown in colour.

Stem: Becomes leafless at the flowering time, the leafy stem bearing 5 leaves.

Leaves: Simple; sessile; linear; acute; margin entire; glossy; glabrous; parallel venation; the upper and lower leaves being smaller; dark green; Size- 11-15 cm.

Inflorescence: Scapose, slender, erect, branched; racemose or sometimes paniculate; bearing many flowers that are about 1.5-3 cm apart from each other on a grey rachis; floral bracts linear-lanceolate, apex acuminate. Size- 60 cm (Figure 15: A).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; Sepals and petals olive green coloured with dark purple venation; lip white purplish red lamellae; Size- 1.5 cm (Figure 15: B).

Calyx: Sepals 3; Dorsal sepal narrowly oblong to oblanceolate; olive green coloured with purple venation; apex acuminate; lateral sepals similar to dorsal sepal, usually slightly longer and slightly oblique. Size- 1.3cm (Figure 15: C).

Corolla: Petals 3, one is modified into labellum; polypetalous; narrowly ovate, apex short acuminate, margin entire, olive green colored with middle dark brown venation, narrower than sepals, size- 1.1 cm; labellum 3 lobed, side lobes pale green, smaller than the mid lobe, mid lobe white at the margin and pinkish at the inner side,

subrounded, with undulate margin, densely fimbriate on mid lobe; larger than other petals; size- 1.4 cm; spur cylindric (Figure 15: C).

Gynostemium: Column long, Straight, slender, with a column foot; ovary glabrous, with equally long, hardly twisted pedicel; white; anther cup covered the Pollinaria, stigma located beneath the anther cup; pollinia-2 (Figure 15: F), yellowish. Size- 10 mm.

Fruit: Capsule; pendulous; dry; ellipsoid.

Seed: Small, dust-like.

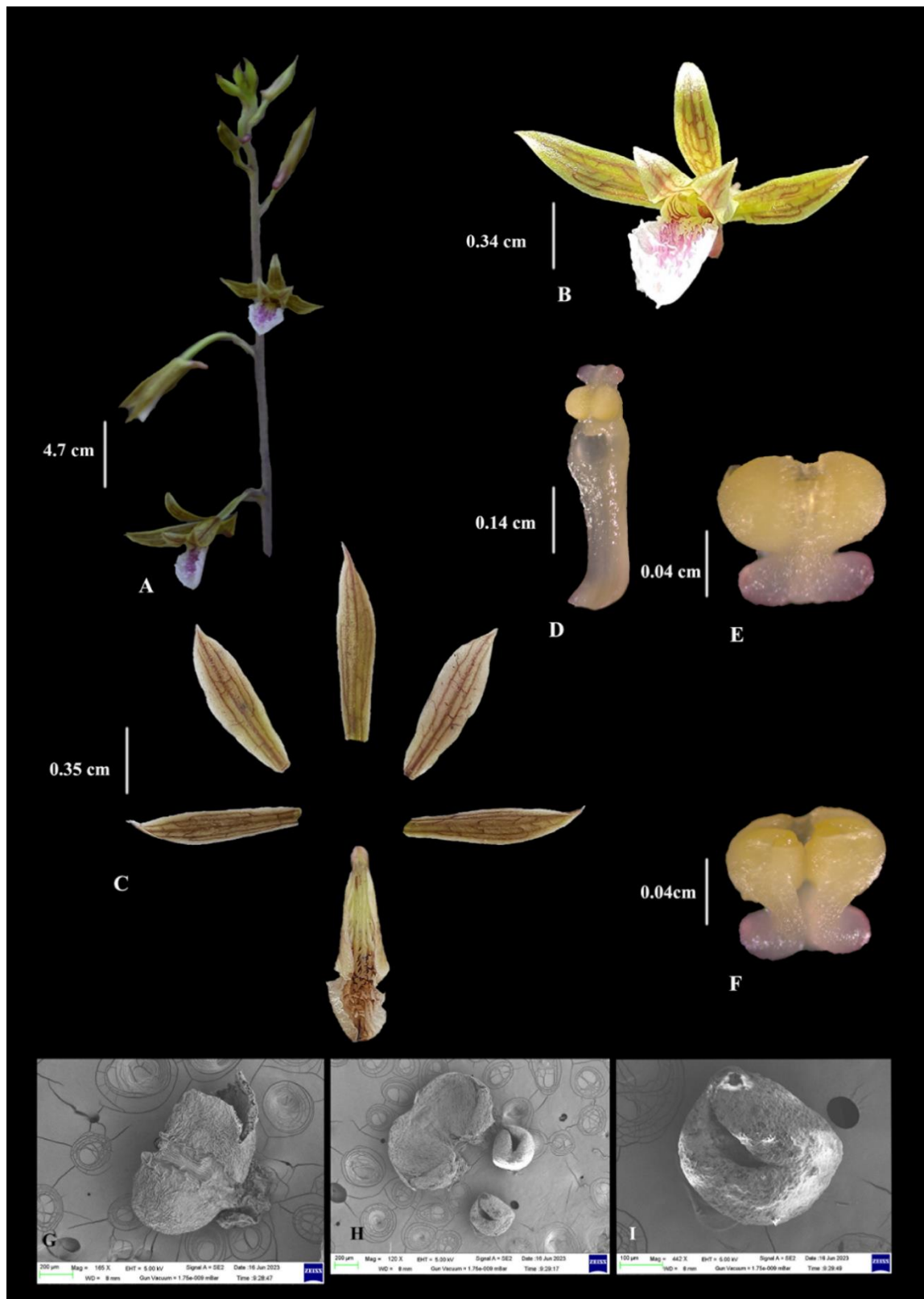


Figure 15: *Eulophia graminea* Lindl.: **A.** Inflorescence; **B.** Flower; **C.** Petals & Sepals; **D.** Column; **E&F.** Pollinia; **G.** SEM photograph of anther cup **H&I.** SEM photograph of Pollinia.

6.2.10. *Habenaria plantaginea* Lindl.

Synonym: *Plantaginorchis plantaginea* (Lindl.) Szlach.

Habitat: Terrestrial.

Habit: Annual herb; Erect; Size 40-45 cm in length.

Root: The presence of adventitious root, penetrate the soil where they anchor the plant to the forest floor. Some adventitious roots are modified to form ovoid or globose bulbs.

Stem: Aerial; Erect; Cylindrical; Glabrescent; Soft herbaceous.

Leaf: Exstipulate; Simple pinnate; Short petiole or sessile; Sheathing leaf base; Lamina shape elliptic to oblong, lamina base obtuse, Apex acute; Succulent, soft in texture; glabrous; phyllotaxy-alternate; Venation-parallel; Size- 10×3 cm.

Inflorescence: Raceme (Figure 16: A).

Flower: Resupinate; Scaly bracteate; Pedicilate; Cyclic; Hypogynous; Complete; Perfect; Bisexual; Zygomorphic; White in color; Size-15mm (Figure 16: C).

Calyx: Sepals 3; Anterior side (near to mother axis) sepal is fuse, but lateral side 2 petals are free spreading and reflexed; Irregular; Hypogynous; The anterior sepals covering the petals forming hood which further cover the column (Figure 16: D).

Corolla: Petals 5; Zygomorphic; Hypogynous; Bilabiate, upper 3 long lips are fuse but deeply lobed about 13 mm long known as labellum, two side lobes are much larger

than middle lob; Entire; Distinct venation. The base of the upper petals is modified into long cylindrical spurred about 35-37mm long. Lower lip 2 lobed free, 1.5-2mm long, narrow or short than the sepals, closely equal to sepal, elliptic, apex acute, broad in width, 1-2 nerved, hyaline in color. Both the lip is adnate at the base of the column (Figure 16: C).

Gynostemium: Formation of Gynostemium, column short, anther cells adnate to the front of the column, discrete or rarely touching, their bases often prolonged into tubes or a sack-like structure containing translator, caudicles short, glands exposed, flat and discoid or elliptic or elongate with truncate end, pollinia 2 (Figure 16: I), clavate or pyriform, massulae present (Figure 17: D); Carpel 3; Stigma 2; Placentation parietal (Figure 16: H).

Fruit: Dry dehiscent, persistent bract, 1.5 cm long, green in colour (Figure 16: G).

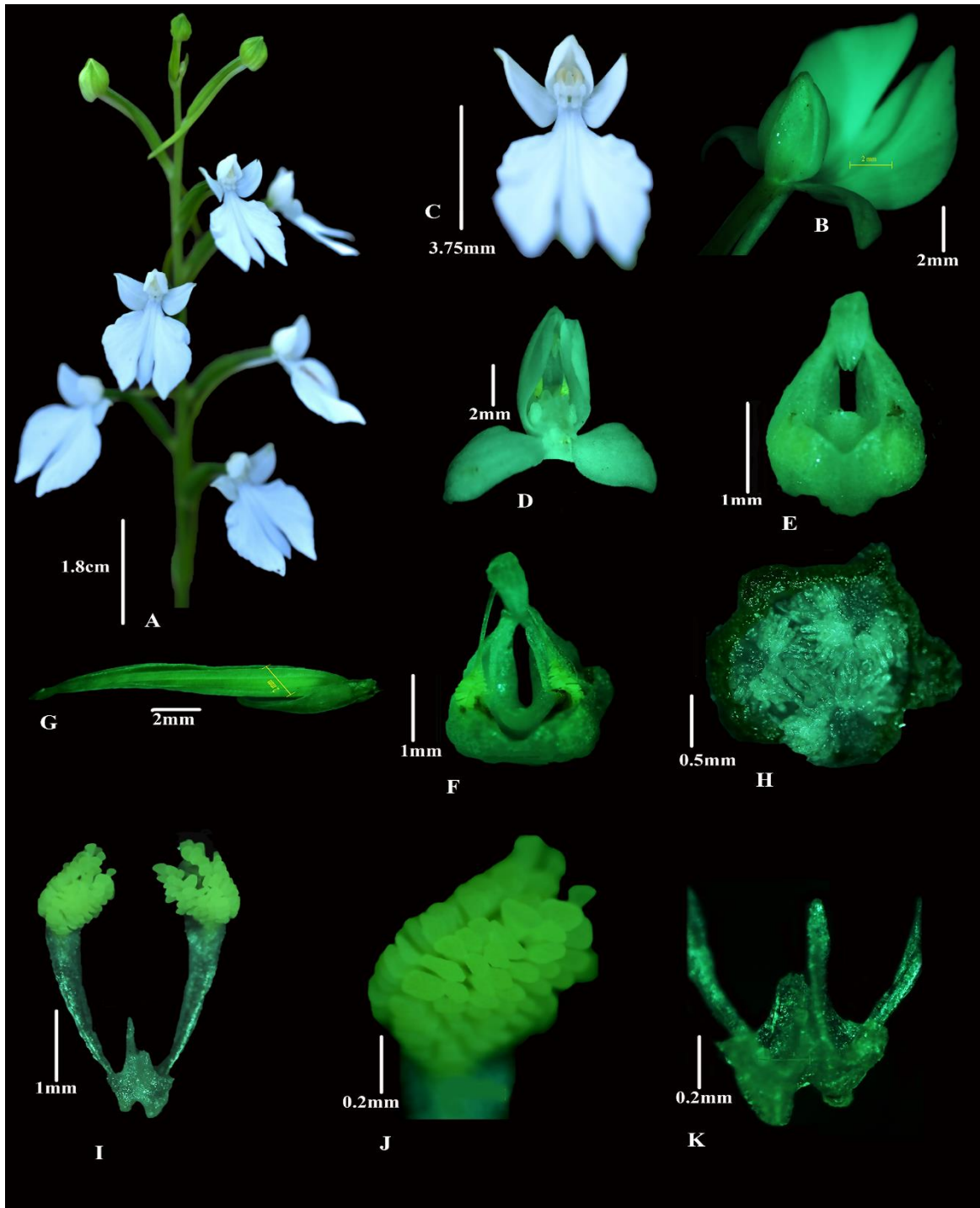


Figure 16: *Habeneria plantaginea* Lindl: **A.** Inflorescence of the plant; **B.** lateral view of flower; **C.** Front view of the flower; **D.** Flower with pollinia; **E-F.** Column; **G.** Fruit with bract; **H.** T.S. of the ovary; **I-K.** Pollinia

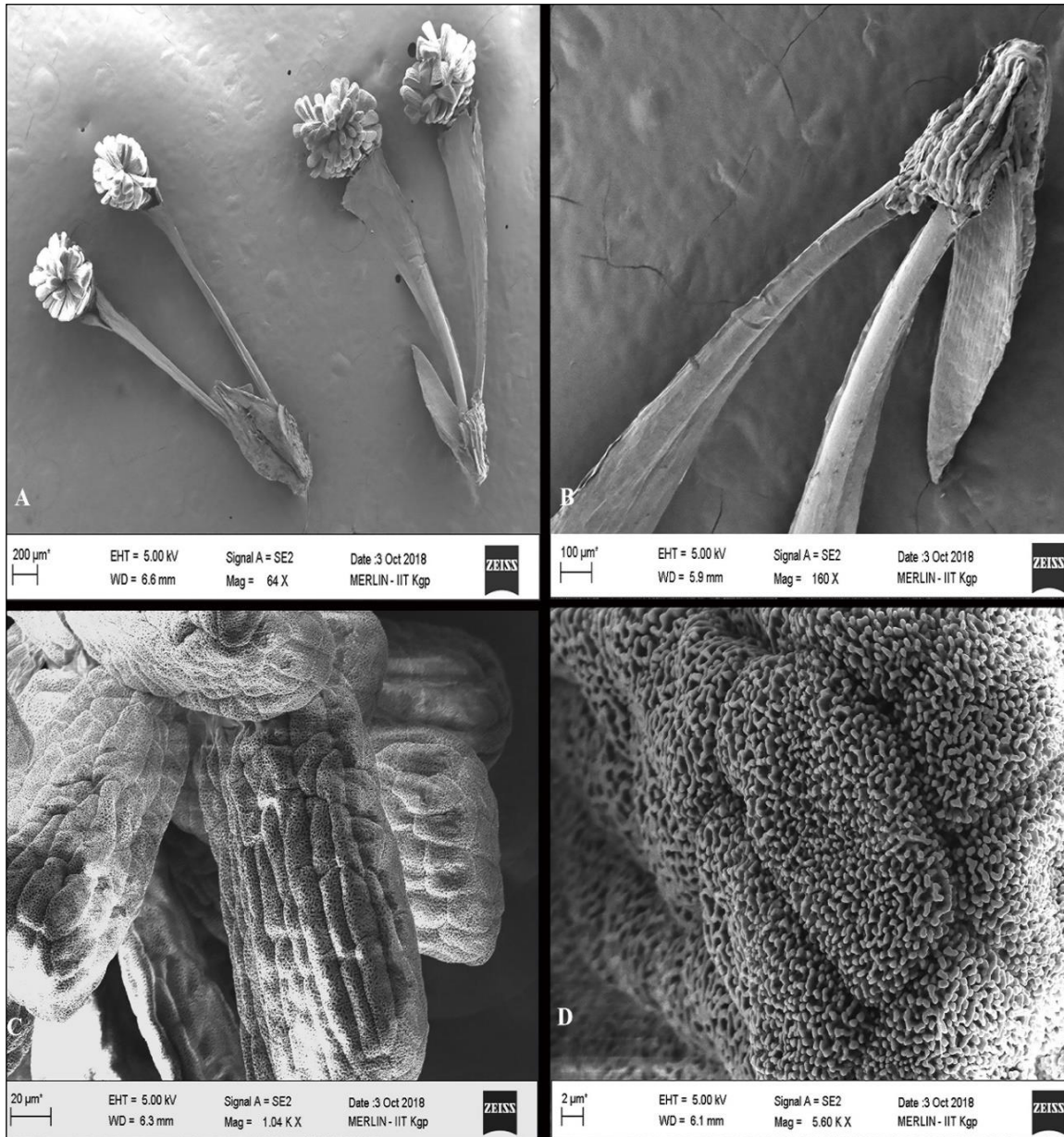


Figure 17: *Habenaria plantaginea* Lindl: A-D. SEM photographs of Pollinia.

6.2.11. *Pleione humilis* (Sm.) D. Don.

Synonym: *Coelogyne humilis* (Sm.) Lindl.

Habitat: Epiphytic or sometimes lithophyte (Figure 18: A).

Habit: Herb.

Root: Adventitious; aerial.

Stem: Leafy pseudobulbs; short; flask-shaped; erect; green.

Leaves: Simple; sessile; oblanceolate to elliptic; coriaceous; glossy; apex acute; membranous; tapering at the base; margin entire; glabrous; alternate; parallel venation; light green.

Inflorescence: Solitary; peduncle from the base of pseudobulb, erect, 1 flowered (Figure 18: B).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; white with brown strip; no fragrance, size- 4.5 cm (Figure 18: C).

Calyx: Sepals 3; polysepalous; sub-equal; apex sub-acute; margin entire; white (Figure 18: D).; size- 3.2 cm

Corolla: Petals 3, one is modified into labellum; polypetalous; obtuse, margin entire, white, narrower than sepals; labellum large; expanding into wide mouth; apex emarginate; margin lacerate; white with brown strip; larger than other petals; size- 3 cm.

Gynostemium: column long, elongated, slender, white with brown dotted; anther cup covered the Pollinaria, stigma located beneath the anther cup; pollinia-4 (Figure 18: H), caudicle link the pollinia; yellowish;

Fruit: Capsule; dry; **Seed:** Small, dust-like.

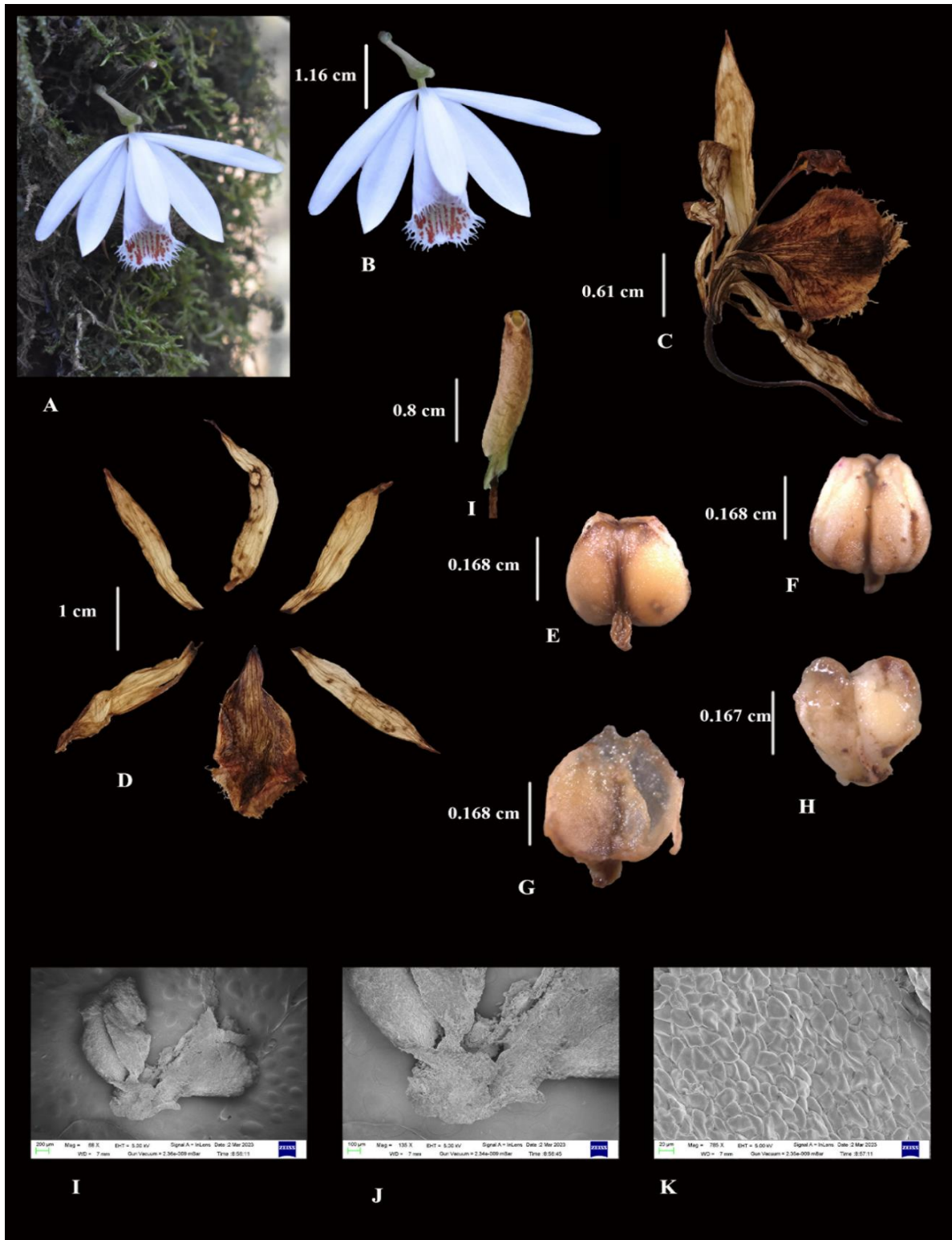


Figure 18: *Pleione humilis* (Sm.) D. Don: **A.** Habit; **B&C.** Flower; **D.** Petals & Sepals; **E&F.** Pollinia with anther cap; **G.** Anther cap; **H.** Pollinia; **I, J & K.** SEM photographs of Pollinia.

6.2.12. *Rhyncostylis retusa* (L.) Blume

Synonym: *Aerides retusa* (L.) Sw., *Epidendrum retusum* L.

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial; clustered; velamen well developed.

Stem: Terete; sheathed with a persistent sheathing base of fallen leaves; stout stem with many alternate leaves, green.

Leaves: Simple; sessile; linear to oblong; margin entire; apex slightly bilobed; coriaceous, glossy; glabrous; alternate; parallel venation; size-15-25cm.

Inflorescence: Racemose; pendulous; flower densely arranged; taller than leaves, many-flowered (Figure 19: A).

Flower: Bracteate, ovate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; white with pink spotted; no fragrance (Figure 19: B), size- 1.5 cm.

Calyx: Sepals 3; polysepalous; ovate; apex subacute; margin entire; white with irregular pink patches (Figure 19: C); size- 0.7 cm.

Corolla: Petals 3, one is modified into labellum; polypetalous; oblong; apex obtuse, narrower than sepals; margin entire; white with irregular pink patches; size- 0.7 cm; labellum 3 lobed, pointing forward; white at the base and pink at apex (Figure 19: C); size-1.5 cm.

Gynostemium: Column short; stout; Stigma located at the base; anther cup covered the Pollinaria; pollinia-2 (Figure 19: G), globular, with long caudicles, yellowish.

Fruit: Capsule; dry.

Seed: Small, Dust-like.

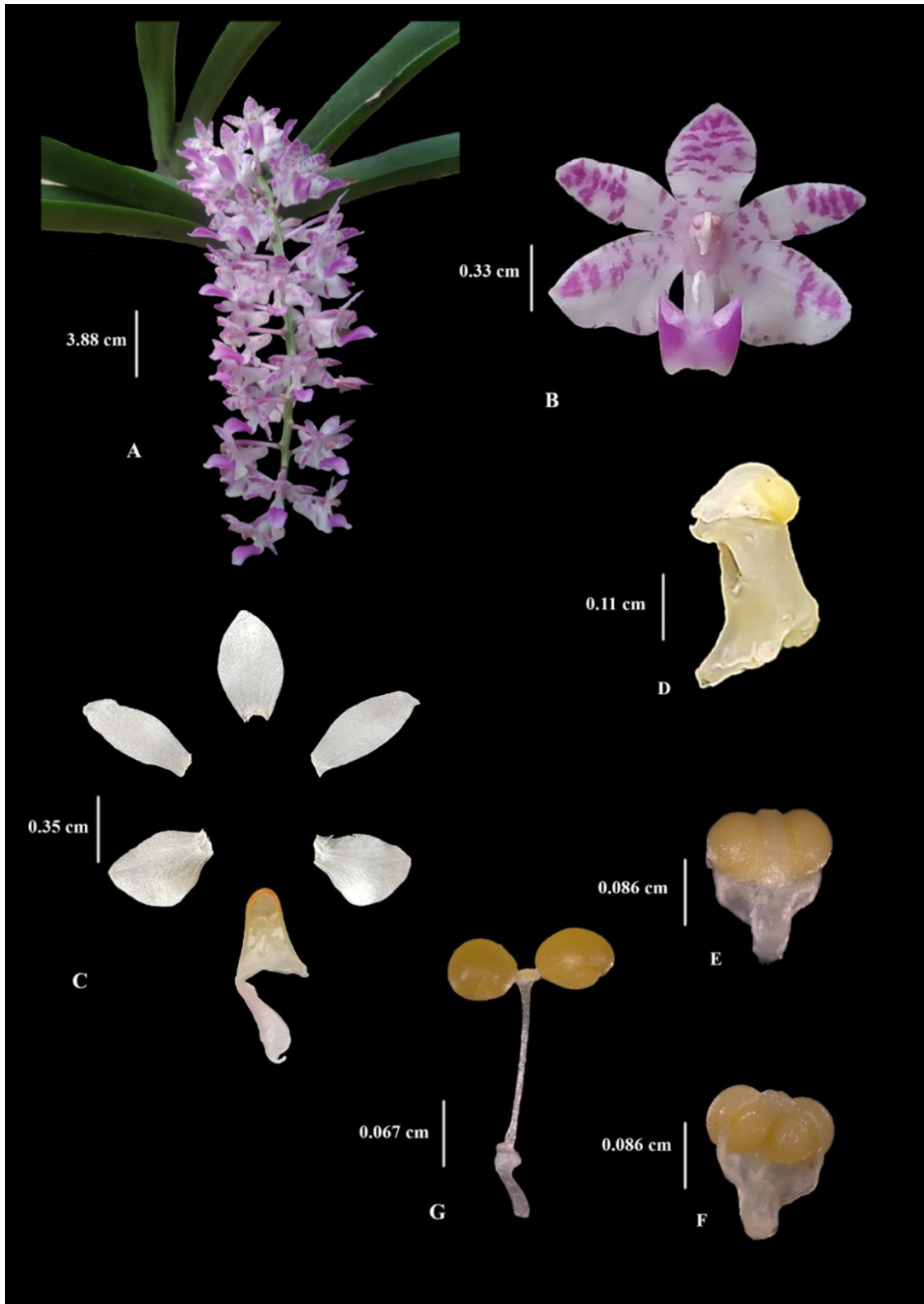


Figure 19: *Rhyncostylis retusa* (L.) Blume: **A.** Habit; **B.** Flower; **C.** Petals & Sepals; **D.** Anther cap; **E&F.** Pollinia with anther cap; **G.** Pollinia.

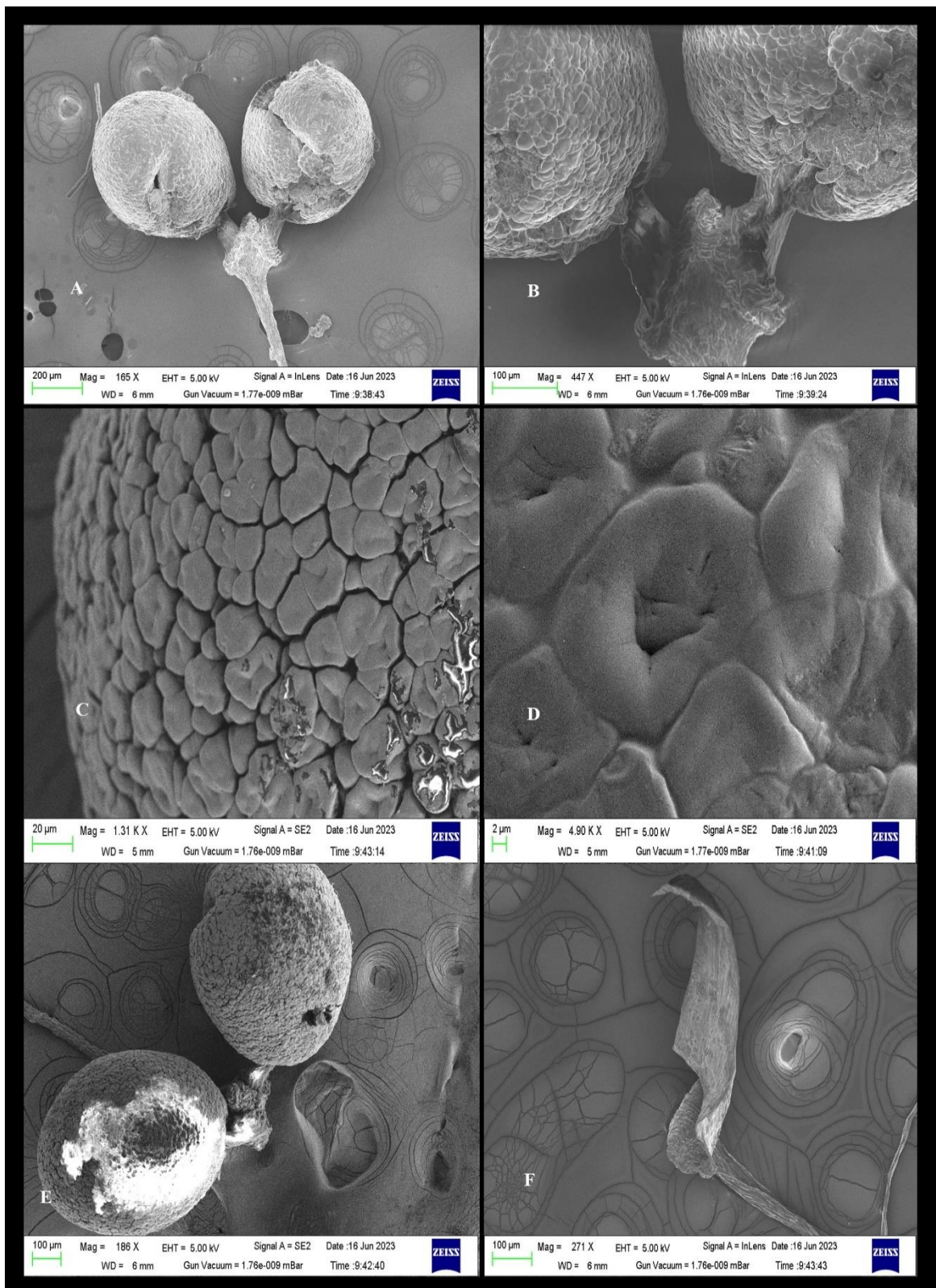


Figure 20: *Rhyncostylis retusa* (L.) Blume: A-F. SEM photographs of Pollinia.

6.2.13. *Vanda tessellata* (Roxb.) Hook. Ex G.Don.

Synonym: *Aerides tessellata* (Roxb.) Wight ex Lindl.

Habitat: Epiphytic.

Habit: Herb.

Root: Aerial, rhizomatous, fibrous; velamen well developed.

Stem: Long, aerial; monopodial; stout, cylindrical; scandent; green.

Leaves: Simple; sessile; long; linear to oblong; narrow; apex 3 toothed; margin entire; coriaceous, glabrous; alternate; parallel venation; green; size-10-18 cm.

Inflorescence: Racemose, erect; 6-12 flowered; size- 20-25 cm.

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; large; greenish violet; nectar gland present; no fragrance (Figure 21: B&C), size- 3- 3.5 cm.

Calyx: Sepals 3; polysepalous; narrower at the base; apex round; margin entire with waves; broader than petals; greenish yellow tessellated with brown spots (Figure 21: D); dorsal sepal size- 2.2 cm, lateral sepal size- 2.5 cm.

Corolla: Petals 3, one is modified into labellum; polypetalous; oblong; apex obtuse, margin entire with waves, greenish-yellow tessellated with brown spots, size-2.2 cm; labellum obscurely 3 lobed; boat-shaped; apex purple and white at the base (Figure 21: D); size-2 cm.

Gynostemium: column short, slender, slightly curved; white; size- 0.9 cm; anther cup covered the Pollinaria, stigma located beneath the viscidium; pollinia-2, caudicles link the pollinia (Figure 21: H&I); yellow.

Fruit: Capsule; dry.

Seed: Small, Numerous; Dust-like.

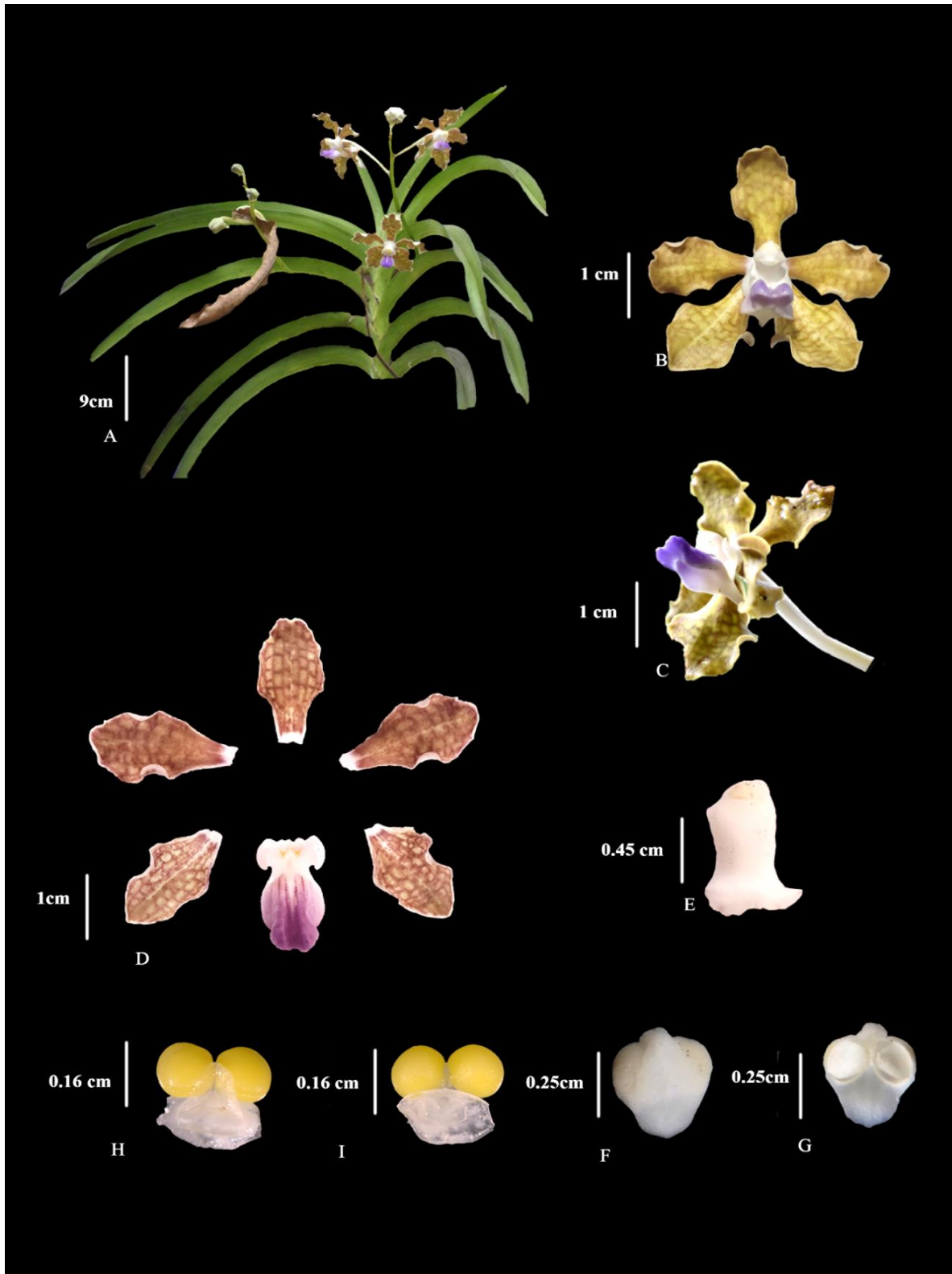


Figure 21: *Vanda tessellata* (Ex G.Don Roxb.) Hook.: **A.** Habit; **B & C.** Flower; **D.** Petals & Sepals; **E.** Coloumn; **F & G.** Anther cap; **H & I.** Pollinia.

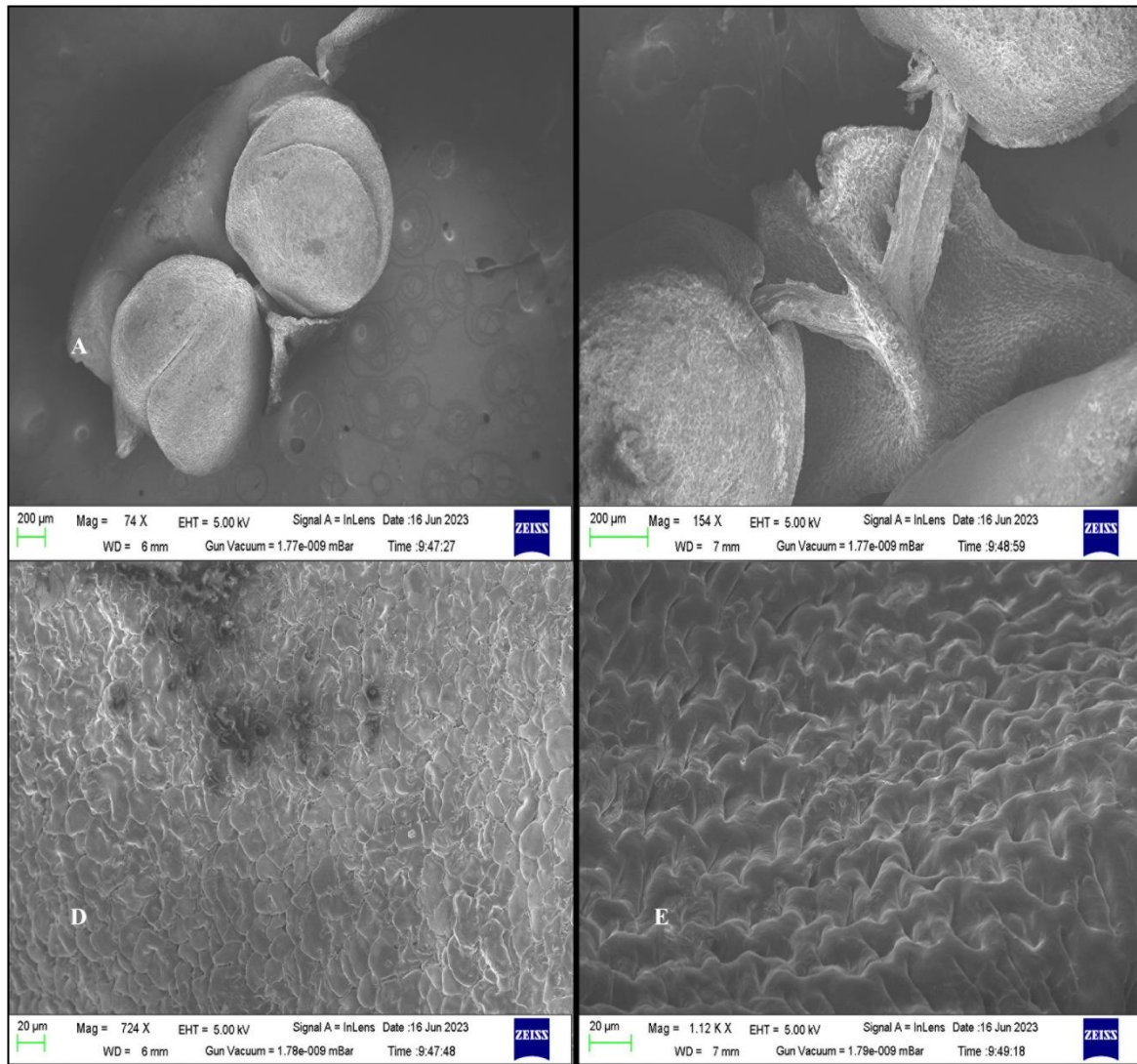


Figure 22: *Vanda tessellata* (Ex G.Don Roxb.) Hook.: **A-E.** SEM photograph of Pollinia.

6.2.14. *Vanda testacea* (Lindl.) Rchb.f.

Synonym: *Aerides testacea* Lindl.

Habitat: Epiphytic.

Habit: Herb (Figure 23: A).

Root: Aerial, rhizomatous, fibrous, velamen well developed.

Stem: Erect, short, stout, slender; bearing many leaves; sheathed; green.

Leaves: Simple; sessile; lanceolate to oblong; margin entire; apex 2-3 toothed; coriaceous; glabrous; alternate; parallel venation; size- 5-10cm.

Inflorescence: Racemose; shorter than the stem, 3-7 flowered (Figure 23: B).

Flower: Bracteate; pedicellate; trimerous; complete; perfect; bisexual; zygomorphic; small in size; light yellow; no fragrance, size-1.5-2 cm.

Calyx: Sepals 3; polysepalous; margin entire; dorsal sepal incurvate apex, side sepals obovate-spathulate; light yellow (Figure 23: E); dorsal sepal size- 0.9 cm, lateral sepal size- 0.7 cm.

Corolla: Petals 3, one is modified into labellum; polypetalous; apex acute, narrow at the base; twisted; margin entire, light yellow; size- 0.8 cm; labellum 3 lobed, side lobed erect; apical lobe recurved; margin crenulate; yellow with violet flash (Figure 23: E); size- 0.6 cm.

Gynostemium: Column truncate cylindrical, short; anther cup yellow; sticky stigmatic surface present below anther cup; anther cup covered the Pollinaria; pollinia-2 (Figure 23: H), caudicles link the pollinia; yellowish.

Fruit: Capsule; dry; **Seed:** Small, Dust-like.

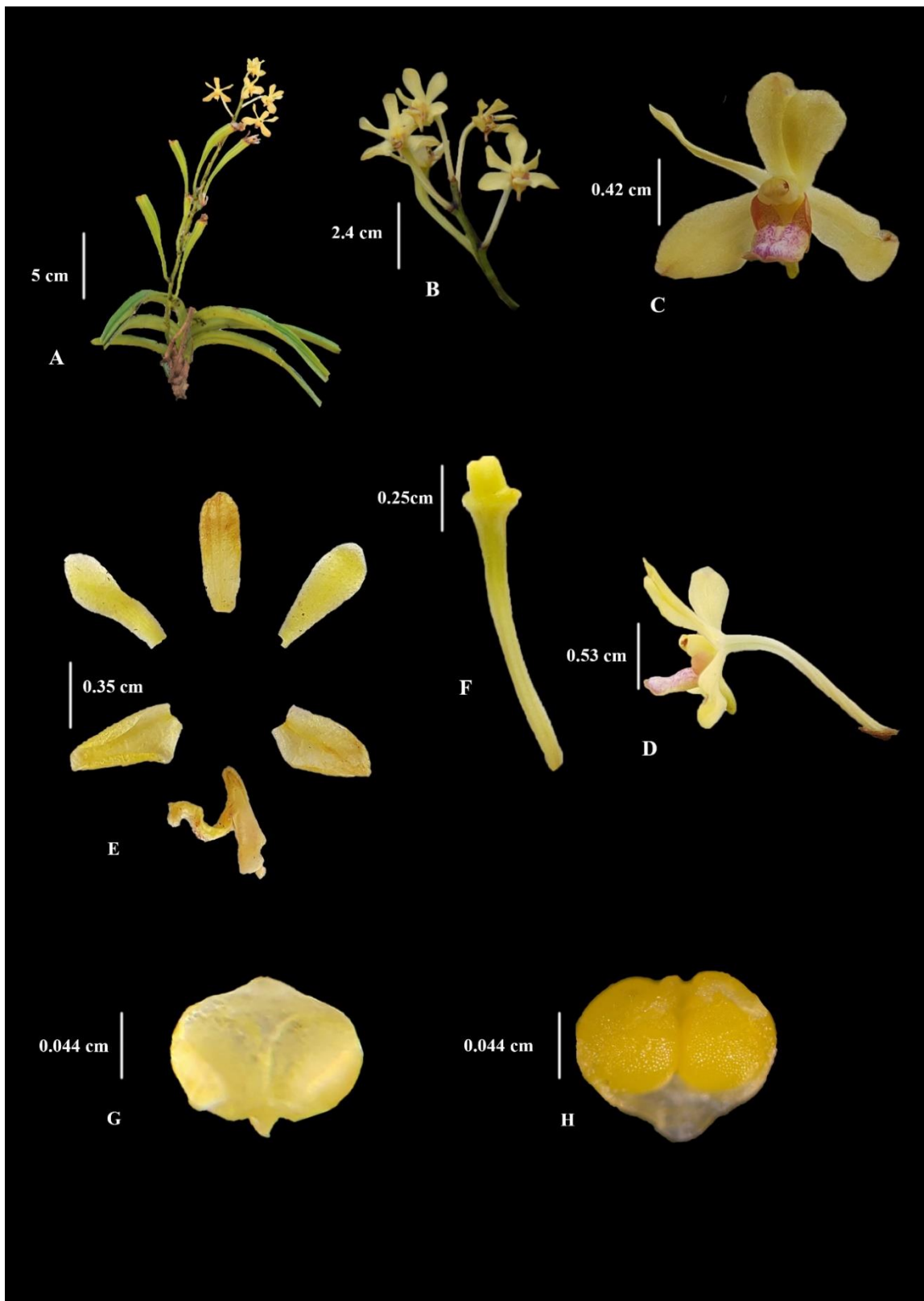


Figure 23: *Vanda testacea* (Lindl.) Rehb.f.: **A.** Habit; **B.** Inflorescence; **C&D.** Flower; **E.** Petals & Sepals; **F.** Column; **G.** Anther cap; **H.** Pollinia.

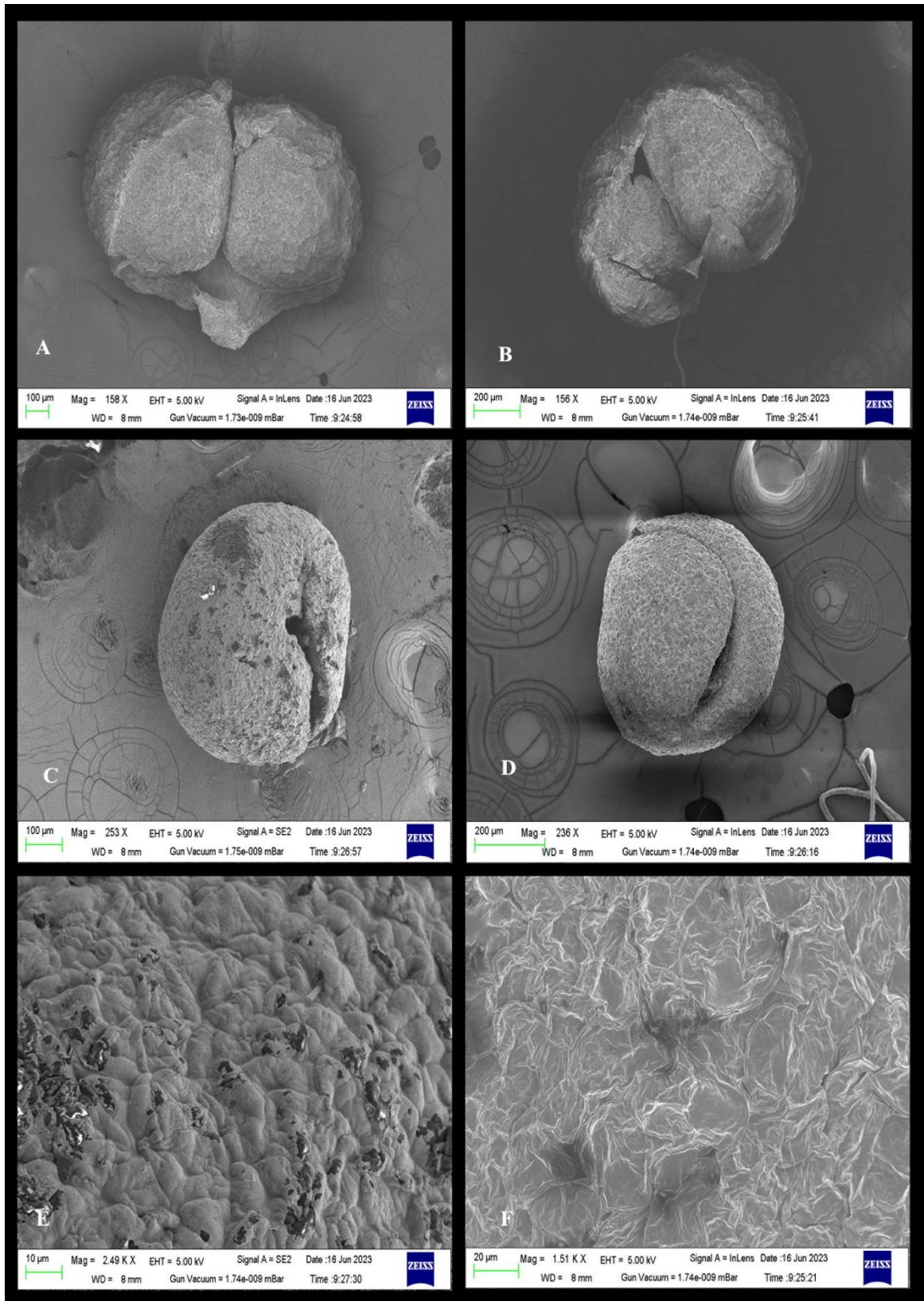


Figure 24: *Vanda testacea* (Lindl.) Rchb.f.: **A-F.** SEM photographs of Pollinia.

6.2.15. *Zeuxine strateumatica* (L.) Schltr.

Synonym: *Adenostylis strateumatica* (L.) Ames

Habitat: Terrestrial but these taxa born on the wet soil intermingled with the grass.

Habit: Erect, annual, herb, size 10-15cm.

Root: Fleshy tap root with distinct node and internodes, branching of the root occur from each node.

Stems: Aerial; Cylindrical; Terete; Glabrescent; Soft herbaceous; Stem totally covered with leaf base, stem white or to some extent green in colour and at the base of the stem reddish in colour, 10-13 cm long.

Leaves: Exstipulate; Simple; Sessile; Shape linear, apex acuminate, sheathing leaf base, margin entire. Leaves thick one nerved at the middle, green in colour but when mature its colour transfer into reddish brown like grasses (it helps for camouflaging). Surface glabrous; Phyllotaxy Spirally arranged, parallel venation, Size 30×4 mm.

Inflorescence: Spike racemose, flower densely arranged on the rachis (Figure 25: A).

Flower: Bracteate, Bract leafy, size 7 mm, ovate, 1-1.3×0.5cm, totally covering the ovary, pale green or pinkish in colour, acuminate apex, margin entire having 1-3 veins. Resupinate; Sessile; Cyclic; Hypogynous; Complete; Perfect; Bisexual; Zygomorphic; white in colour (Figure 25: B); Size-1-1.5cm.

Calyx: Sepals 3, free; Anterior 2 sepals, but posterior sepal forming hood-like structure which covers the inner lateral 2 petals; Irregular; Hypogynous (Figure 25: E); Size 3.3mm.

Corolla: Petals 3; Zygomorphic; Hypogynous; upper 2 lateral petals ovate, white in colour, size- 4 mm, Entire. Another 1 anterior petal is thicker than other petals, yellow in colour, 3 lobed known as a labellum, base of the labellum white in colour and it is sticky in nature (Figure 25: D), size-3-4mm.

Gynostemium: Formation of Gynostemium, pollinarium present, 1.5-2mm long, anther cells bilobed, adnate to the front of the column, 1.3-1.5×1-1.3 mm size, caudicles short, glands exposed, flat and discoid or elliptic or elongate with truncate end, pollinia 2 (Figure 25: N), clavate or pyriform, massulae present;Carpel 3; Stigma 2; Placentation parietal (Figure 25: O); ovary elliptic to fusiform, 4-5 × 1-3 mm, pale green, twisted, glabrous.

Fruit: Dry dehiscent, persistent bract, 1.5 mm long, brown in colour (Figure 25: G).

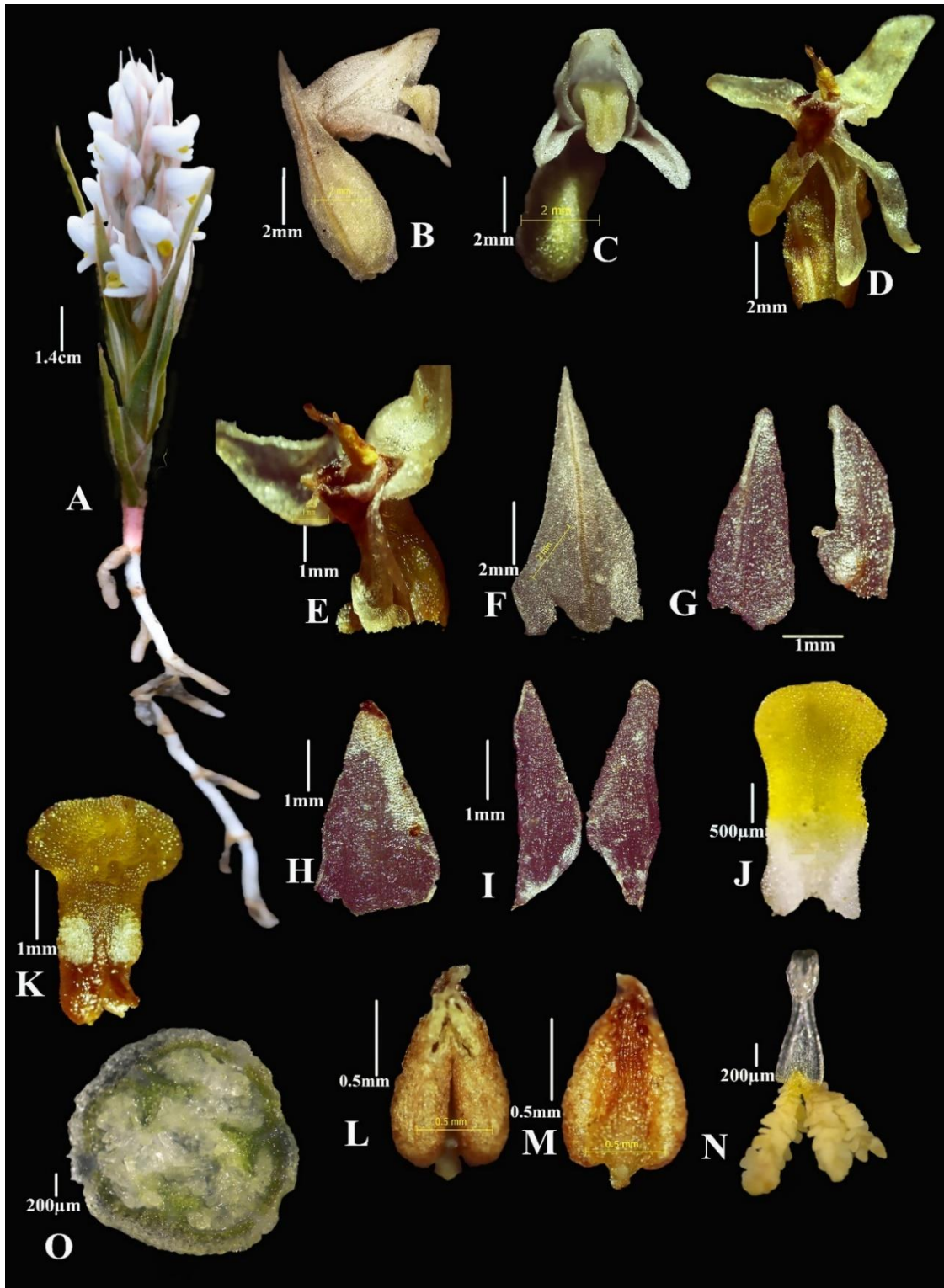


Figure 25: *Zeuxine strateumatica* (L.) Schltr.: **A.** Habit; **B.** Lateral view of Flower; **C.** Front View; **D & E.** Flower liberating Pollinia; **F.** Bract; **G.** Lateral Sepal; **H.** Dorsal Sepal; **I.** Petal; **J & K.** Anterior & Posterior side of the Labellum; **L & M.** Anterior & Posterior of anther; **N.** Pollinia; **O.** Parital Placentation.

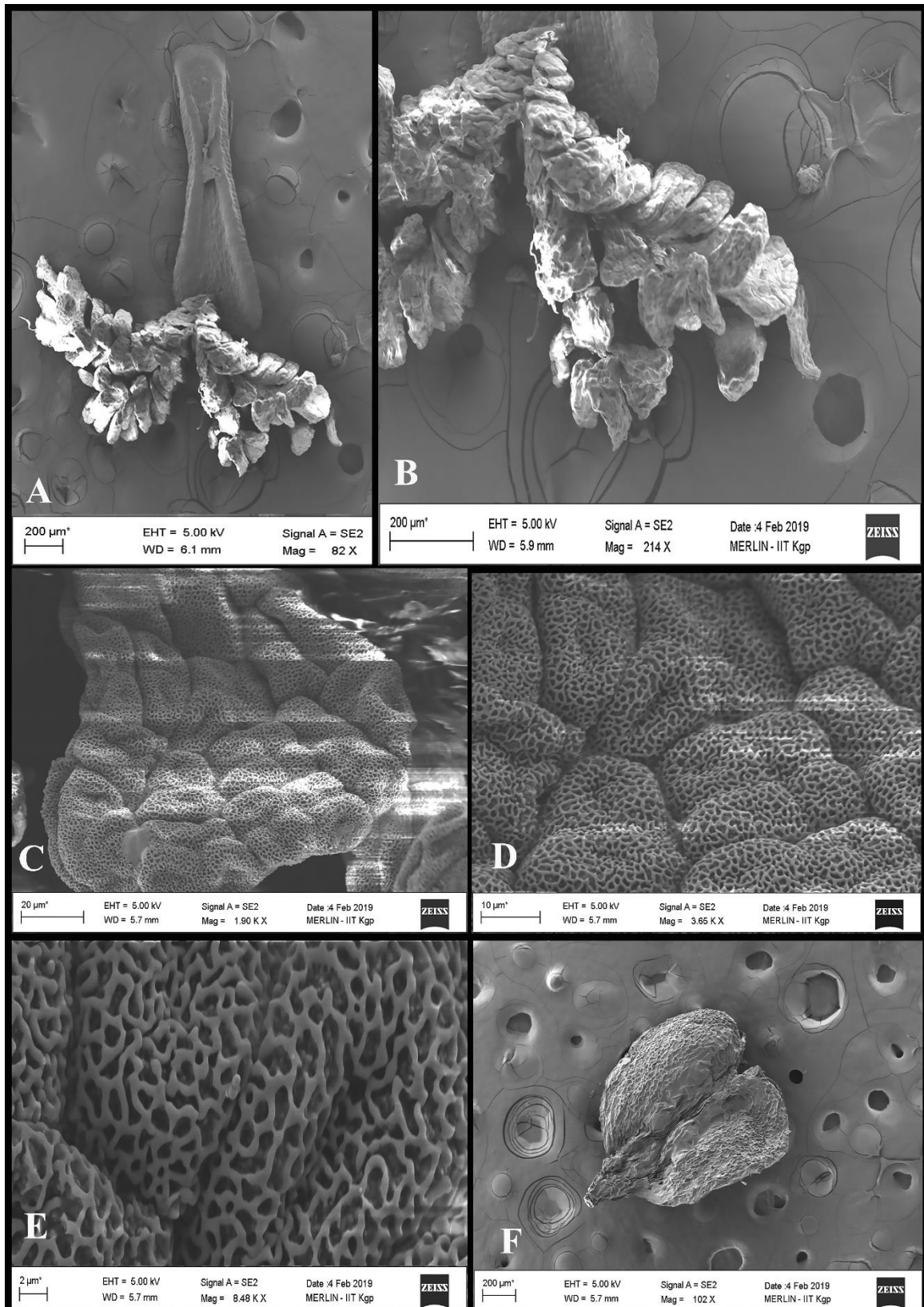


Figure 26: *Zeuxine strateumatica* (L.) Schltr.: **A-F.** SEM photographs of Pollinia.

6.3. Ultra-structure of Pollinia:

Pollinia is a characteristic feature in orchidaceae. Actually, it is a modified male reproductive structure, which consist of mass of pollen grain in a sac like structure. The pollinial micro morphology studied under simple microscope and SEM. From this study we found different characters. These are given below:

***Aerides multiflora* Roxb.:** It has 2 pollinia, which is round in shape. The orientation of pollinia is side by side. Aperture present. It has a transparent caudicle. The texture of pollinia is granular. The pollinia surface is rough (Figure 6. A-D).

***Coelogyne corymbosa* Lindl.:** It has 4 pollinia. The orientation of pollinia is superposed. Apex round and base is acute. Aperture absent. Having a short caudicle. The texture of pollinia is granular. The pollinia surface is rough (Figure 7. J-K).

***Coelogyne cristata* Lindl.:** It has 2 pollinia. The orientation of pollinia is superposed. The apex is round and base is acute. Aperture absent. Having a short caudicle. The texture of pollinia is granular. The pollinia surface is rough (Figure 8. H-I).

***Cymbidium aloifolium* (L.) Sw:** It has 2 pollinia. The orientation of pollinia is side by side. The apex is acute and base is round. Aperture present. It has a short caudicle. The texture of pollinia is granular. The pollinial surface is smooth (Figure 10. A-D).

***Dendrobium macrostachyum* Lindl.:** It has 4 pollinia. The orientation of pollinia is side by side. Aperture absent. The apex is acute and base is round. Caudicle absent. The texture of pollinia is granular. The pollinial surface is smooth (Figure 11. G-H).

***Dendrobium aphyllum* (Roxb.) C.E.C. Fisch.:** It has 4 pollinia. The orientation of pollinia is side by side. Aperture absent. The apex is acute and base is round. Caudicle absent. The texture of pollinia is granular. The pollinial surface is smooth (Figure 12. G-I).

***Dendrobium nobile* Lindl.:** It has 4 pollinia. The orientation of pollinia is side by side. Aperture absent. The apex is broad and base is acute. Caudicle absent. The texture of pollinia is granular. The pollinial surface is smooth (Figure 13. G-I).

***Epidendrum denticulatum* Barb. Rodr.:** It has 4 pollinia. The orientation of pollinia is side by side. Aperture absent. The apex is round and base is acute. It has double caudicle. The texture of pollinia is granular. The pollinia surface is rough (Figure 14. F-I).

***Eulophia graminea* Lindl.:** It has 2 pollinia, which is round in shape. The orientation of pollinia is side by side. Aperture present. Caudicle absent. The texture of pollinia is granular. The pollinial surface is rough (Figure 15. H-I).

***Habenaria plantaginea* Lindl.:** It has 2 pollinia. The shape of pollinia is unformed. The orientation of pollinia is side by side. Aperture absent. It has 2 transparent caudicle. Massulae present. The massulae surface is reticulated (Figure 17. A-D).

***Pleione humilis* (Sm.) D. Don.:** It has 4 pollinia, which is superposed. The apex is round and base is acute. Having a short caudicle. The texture of pollinia is granular. The pollinia surface is smooth (Figure 18. H-K).

***Rhyncostylis retusa* (L.) Blume:** It has 2 pollinia, which is round in shape and attached side by side. Aperture present. It has double caudicle. The texture of pollinia is granular. The pollinial surface is smooth (Figure 20. A-F).

***Vanda tessellata* (Roxb.) Hook. Ex G.Don.:** It has 2 pollinia, which is round in shape and attached side by side. Aperture present. It has a short caudicle. The texture of pollinia is granular. The pollinial surface is rough (Figure 22. A-E).

***Vanda testacea* (Lindl.) Rchb.f.:** It has 2 pollinia, which is round in shape and attached side by side. Aperture present. It has a short caudicle. The texture of pollinia is granular. The pollinial surface is rough (Figure 24. A-F).

***Zeuxine strateumatica* (L.) Schltr.:** It has 2 pollinia. The shape of pollinia is unformed. The orientation of pollinia is side by side. Aperture absent. It has 2 transparent caudicle. Massulae present. The massulae surface is rod-like (Figure 26. A-F).

6.4. Numerical Analysis:

To create a similarity matrix of Orchid species we selected 14-unit characters from pollinial morphology, all are equally weighted. Where 9 characters are binary states and 5 are multi-states. Most of the selected characters are qualitative (9), and few are quantitative (5).

Table No. 3: Selected characters for numerical analysis. Presumed binary characters (0/1) and multi state characters (1/2/3).

| Sl. No. | Characters | State |
|---------|--------------------------|---|
| 1. | Caudicle | Absent-0, Short-1, Long-2 |
| 2. | Number of pollinia | Two-0, Four-1 |
| 3. | Pollinium orientation | Side by side-0, Superposed-1 |
| 4. | Pollinium texture | Granular-0, Massulae-1 |
| 5. | Shape of pollinia | Round-0, Acute apex base round-1, round apex acute base-2, unformed-3 |
| 6. | Aperture of pollinia | Absent-0, Present-1 |
| 7. | Length of pollinium | <1 mm-0, 1-2 mm-1, 2 mm< -2 |
| 8. | Width of pollinium | <1 mm-0, 1 mm<-1 |
| 9. | Length of caudicle | <1 mm-0, 1-2 mm-1, 2 mm< -2 |
| 10. | Number of caudicle | Single-0, Double-1 |
| 11. | Pollinium fusion | Free-0, Fused-1 |
| 12. | Sculpture of massulae | Reticulate-0, Rod like-1 |
| 13. | Sculpture of the granule | Absent-0, Smooth-1, Rough-2 |
| 14. | Texture of pollinia | Soft-0, Hard-1 |

6.5. Binary characters:

Table No.4: 14 pollinial characters-state values for each taxon used in numerical analysis.

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---------------------------------|---|---|---|---|---|---|---|---|-----|-----|----|-----|----|----|
| <i>Aerides multiflora</i> | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | ... | 2 | 1 |
| <i>Coelogyne corymbosa</i> | 1 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | ... | 2 | 0 |
| <i>Coelogyne cristata</i> | 1 | 0 | 1 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 0 | ... | 2 | 0 |
| <i>Cymbidium aloifolium</i> | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | ... | 1 | 1 |
| <i>Dendrobium aphyllum</i> | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | ... | ... | 1 | ... | 1 | 0 |
| <i>Dendrobium macrostachyum</i> | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | ... | ... | 1 | ... | 1 | 0 |
| <i>Dendrobium nobile</i> | 0 | 1 | 0 | 0 | 2 | 0 | 2 | 0 | ... | ... | 1 | ... | 1 | 0 |
| <i>Epidendrum denticulatum</i> | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | ... | 2 | 1 |
| <i>Eulophia graminea</i> | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | ... | ... | 0 | ... | 2 | 1 |
| <i>Habenaria plantaginea</i> | 2 | 0 | 0 | 1 | 3 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 |
| <i>Pleione humilis</i> | 1 | 1 | 1 | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 1 | ... | 1 | 0 |
| <i>Rhynchostylis retusa</i> | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | ... | 1 | 1 |
| <i>Vanda tessellata</i> | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | ... | 2 | 1 |
| <i>Vanda testacea</i> | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | ... | 2 | 1 |
| <i>Zeuxine strateumatica</i> | 2 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |

6.6. Similarity matrix table:

Table no. 5: Similarity matrix for each taxon.

| | A. multiflor | C. corymbo | C. cristata | C. aloifoliur | D. aphyllur | D. macrostz | D. nobile | E. denticula | E. graminez | H. plantagi | P. humilis | R. retusa | V. tessellata | V. testacea | Z. strateum |
|---------------|--------------|-------------|-------------|---------------|-------------|-------------|------------|--------------|-------------|-------------|-------------|-------------|---------------|-------------|-------------|
| A. multiflor | 1 | 0.89745698 | 0.88505322 | 0.93075286 | 0.53686834 | 0.54498956 | 0.5289021 | 0.94722766 | 0.59422058 | 0.09523407 | 0.86324057 | 0.98645972 | 0.97063353 | 0.95272319 | -0.13604654 |
| C. corymbo | 0.89745698 | 1 | 0.986179 | 0.95038414 | 0.4113476 | 0.41197749 | 0.41996544 | 0.95123478 | 0.40070844 | 0.14238162 | 0.97915965 | 0.88184388 | 0.93907707 | 0.94590888 | -0.12669642 |
| C. cristata | 0.88505322 | 0.986179 | 1 | 0.95141895 | 0.41456405 | 0.40628355 | 0.42310902 | 0.92420486 | 0.39517025 | 0.17681733 | 0.97970874 | 0.86965592 | 0.92609808 | 0.9466699 | -0.12494535 |
| C. aloifoliur | 0.93075286 | 0.95038414 | 0.95141895 | 1 | 0.42208424 | 0.42205674 | 0.42229738 | 0.93059419 | 0.4287584 | 0.09932572 | 0.95848174 | 0.92930955 | 0.97015534 | 0.97937363 | -0.17800955 |
| D. aphyllur | 0.53686834 | 0.4113476 | 0.41456405 | 0.42208424 | 1 | 0.99734335 | 0.99731909 | 0.53315205 | 0.96883434 | 0.14334385 | 0.41293905 | 0.61569655 | 0.44163379 | 0.4242631 | -0.00486200 |
| D. macrostz | 0.54498956 | 0.41197749 | 0.40628355 | 0.42205674 | 0.99734335 | 1 | 0.99509828 | 0.54280341 | 0.97727007 | 0.1376335 | 0.40515107 | 0.62359275 | 0.44834531 | 0.42354106 | 0.00967822 |
| D. nobile | 0.5289021 | 0.41996544 | 0.42310902 | 0.42229738 | 0.99731909 | 0.99509828 | 1 | 0.54173989 | 0.96074017 | 0.19663842 | 0.42098587 | 0.60799218 | 0.43504944 | 0.41615972 | 0.04887264 |
| E. denticula | 0.94722766 | 0.95123478 | 0.92420486 | 0.93059419 | 0.53315205 | 0.54280341 | 0.54173989 | 1 | 0.55754665 | 0.2603776 | 0.91464007 | 0.94629864 | 0.95049463 | 0.92754191 | 0.02780323 |
| E. graminez | 0.59422058 | 0.40070844 | 0.39517025 | 0.4287584 | 0.96883434 | 0.97727007 | 0.96074017 | 0.55754665 | 1 | 0.11348791 | 0.36670018 | 0.66425632 | 0.48950921 | 0.45665721 | 0.00962334 |
| H. plantagi | 0.09523407 | 0.14238162 | 0.17681733 | 0.09932572 | 0.14334385 | 0.1376335 | 0.19663842 | 0.2603776 | 0.11348791 | 1 | 0.15206489 | 0.1330704 | 0.03612136 | 0.01572535 | 0.92125737 |
| P. humilis | 0.86324057 | 0.97915965 | 0.97970874 | 0.95848174 | 0.41293905 | 0.40515107 | 0.42098587 | 0.91464007 | 0.36670018 | 0.15206489 | 1 | 0.8609589 | 0.90823791 | 0.92665367 | -0.15126564 |
| R. retusa | 0.98645972 | 0.88184388 | 0.86965592 | 0.92930955 | 0.61569655 | 0.62359275 | 0.60799218 | 0.94629864 | 0.66425632 | 0.1330704 | 0.8609589 | 1 | 0.95662445 | 0.93803611 | -0.10280857 |
| V. tessellata | 0.97063353 | 0.93907707 | 0.92609808 | 0.97015534 | 0.44163379 | 0.44834531 | 0.43504944 | 0.95049463 | 0.48950921 | 0.03612136 | 0.90823791 | 0.95662445 | 1 | 0.9883569 | -0.20828097 |
| V. testacea | 0.95272319 | 0.94590888 | 0.9466699 | 0.97937363 | 0.4242631 | 0.42354106 | 0.41615972 | 0.92754191 | 0.45665721 | 0.01572535 | 0.92665367 | 0.93803611 | 0.9883569 | 1 | -0.25691147 |
| Z. strateum | -0.13604654 | -0.12669642 | -0.12494535 | -0.17800955 | -0.00486200 | 0.00967822 | 0.04887264 | 0.02780323 | 0.00962334 | 0.92125737 | -0.15126564 | -0.10280857 | -0.20828097 | -0.25691147 | 1 |

6.7. Dendrogram:

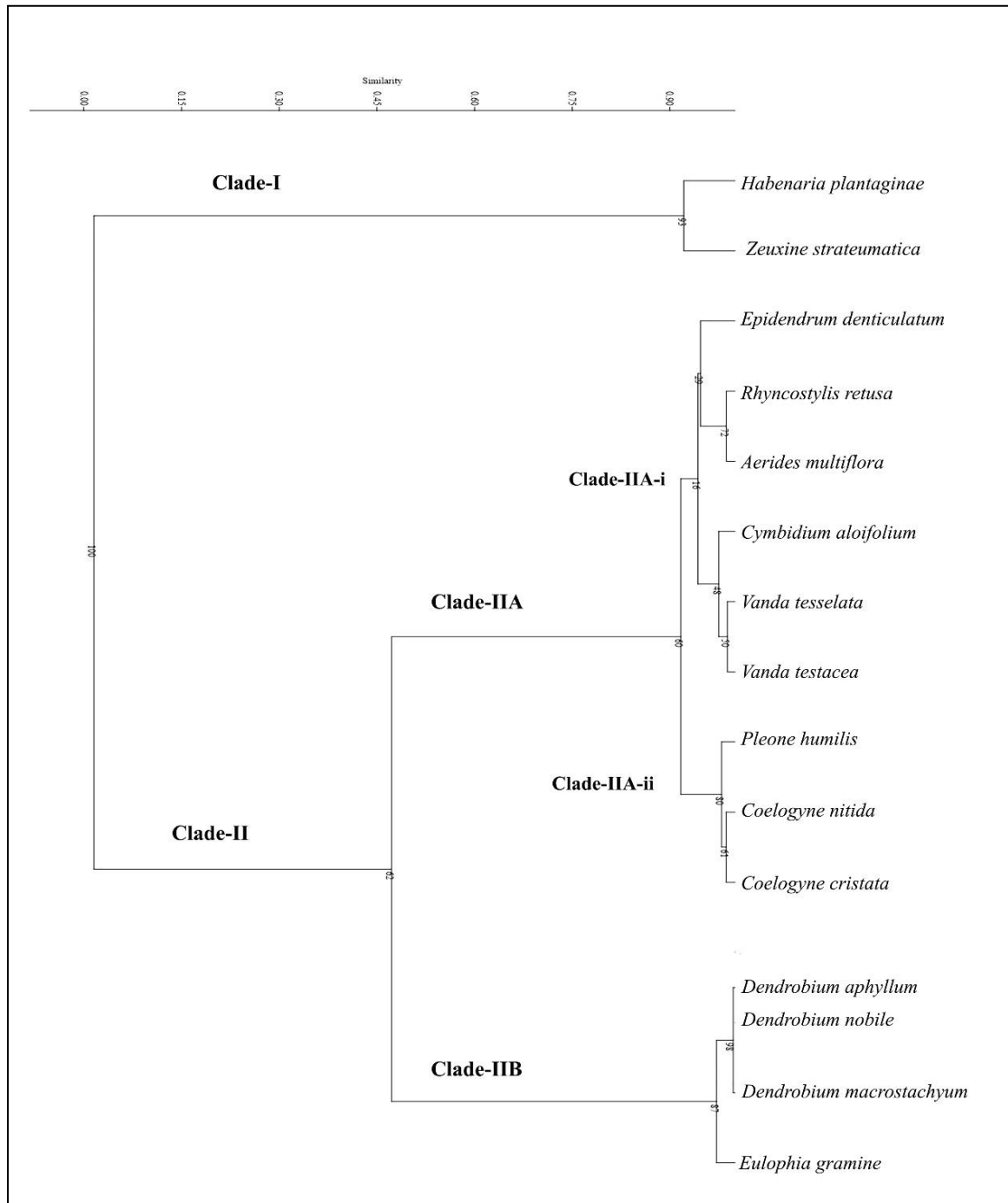


Figure.27: Dendrogram illustrating the hierarchical classification of 15 taxa based on 14 pollinial characters.

Chapter 7: Discussion

7. Discussion:

According to APG-IV (2016) classification, the family Orchidaceae was divided into five sub-families viz. Apostasioideae, Cyripedioideae, Epidendroideae, Orchidoideae, and Vanilloideae where Epidendroideae (Singer *et al.*, 2008) was further divided into 16 tribes, Orchidoideae divided into 7 tribes, and Vanilloideae divided into 2 tribes (APG-IV, 2016). From our six months of work, we successfully collected 15 species of which two species belongs to subtribe Orchidoideae and the remainings are belongs to subtribe Epidendroideae. Depending on 14 characters of pollinium, we prepare a similarity matrix by Correlation co-efficient method. The UPGMA method is implemented here for cluster analysis and dendrogram preparation.

In the present study, two clades are found in the dendrogram. The clade-I is separated from clade-II at the very beginning. That's why clade-I is treated here as an out-group which composed of *Zeuxine strateumatica* and *Habenaria plantaginae*. In the APG IV system of classification, these two taxa belongs to the sub-family Orchidoideae and the other 13 taxa are belonging to Epidendroideae sub-family, which develops clade-II. However, at that point, the present result supports the latest classification of the Orchidaceae family. Approximately 92% of similarities are found in the study between *Habenaria plantaginae* and *Zeuxine strateumatica*. These two species came in a sister clade with bootstrap value 93 for many similar pollinial morphology but have few differences. The main difference is having different Massulae sculptures *i.e.* reticular in *Habenaria plantaginae* and rod-like in *Zeuxine strateumatica*. The major difference between Epidendroideae and Orchidoideae members is the type of pollinial texture. Epidendroideae

members have a granular texture, whereas Orchidoideae members have Massulae type of texture.

Clade-II:

Clade-II represents 13 taxa of the sub-family Epidendroideae and it is further divided into Clade-IIA and Clade-IIB.

Clade-II-A:

Clade-II-A represent 9 taxa viz. *Epidendrum denticulatum*, *Rhynchostylis retusa*, *Aerides multiflora*, *Cymbidium aloifolium*, *Vanda tessellata*, *Vanda testacea*, *pleione humilis*, *Coelogyne corymbosa*, *Coelogyne cristata*. This subclade is separated from clade-IIB at 46% similarity.

Clade-IIA is now further divided into 2 sub-clades viz. clade-IIA-i & clade-IIA-ii at 90% similarity. The first sub-clade (clade-IIA-i) divided into 2 monophyletic groups at 93% similarity with 29 and 48 bootstrap value respectively. The 1st group is composed of *Epidendrum denticulatum*, *Rhynchostylis retusa*, and *Aerides multiflora* with 98% similarity (Hidayat *et al.*, 2006). The 2nd group is composed of *Cymbidium aloifolium* and two species of *Vanda* sp. (*Vanda testacea* and *Vanda tessellata*) in a sister clade with 98% similarity and 50 bootstrap value.

The clade-IIA-ii is a monophyletic group with 95% similarity and 80 bootstrap value that composed of *Pleione humilis* and two *Coelogyne* sp. (*Coelogyne corymbosa* and *Coelogyne cristata*) in a sister clade with 98% of similarity and 61 bootstrap value. This strongly supports the APG IV system of classification.

Clade-II-B:

This clade is also a monophyletic clade and has a common ancestor and it's composed of *Dendrobium* sp. and *Eulophia* sp. In the present work, *Dendrobium* has three species viz. *Dendrobium aphyllum*, *Dendrobium macrostachyum*, and *Dendrobium nobile* in a sister clade with 99% similarity and 87 bootstrap values which is a significant outcome of this work.

According to the APG IV system of classification *Eulophia graminea* and *Cymbidium aloifolium* belongs to the tribe Cymbidicae and sub-tribe Eulophiinae and Cymbidiinae respectively (Chase *et al.*, 2016). But it comes so far from each other in the present result. So APG-IV is denied here. To resolve that problem many more morphological characters are considered for that analysis instead of the selected 14 pollinial characters which is our further aim of this study.

Chapter 8: Conclusions

8. Conclusions:

After the overall conclusion, the present study reveals the 15 species under 11 genera belongs to 2 sub-family of the family Orchidaceae. Among these 15 species, 3 species (*i.e. H. plantaginae, E. graminea, and Z. strateumatica*) are terrestrial and the rest of 12 species (*i.e. Epidendrum denticulatum, Rhynchostylis retusa, Aerides multiflora, Cymbidium aloifolium, Vanda tessellata, Vanda testacea, Pleione humilis, Coelogyne corymbosa, Coelogyne cristata., Dendrobium aphyllum, Dendrobium nobile, and Dendrobium macrostachyum,*) are epiphytic in nature. High species diversity of orchids are found in our study area. But they grow at different times of the year. So, we collected a few species from the study area. *Vanda tessellata* shows the highest density of distribution. Along with altitude, precipitation, temperature, and other climatic factors are also a great influencing factors for the growth of orchid plants. For epiphytic orchid species, the dominant host plants are *Ficus benghalensis, Diospyros melanoxylon, Cryptomeria japonica, Azadiracta indica*, etc. On the basis of pollinial morphology the two subfamilies mainly differ from each other by their pollinial structure, Orchidoideae having massulae whereas Epidendroideae does not have massulae.

Therefore, the two subfamilies are placed in separated clades in the cluster analysis. All the thirteen species (*Epidendrum denticulatum, Rhynchostylis retusa, Aerides multiflora, Cymbidium aloifolium, Vanda tessellata, Vanda testacea, Pleione humilis, Coelogyne corymbosa, Coelogyne cristata., Dendrobium aphyllum, Dendrobium nobile, Dendrobium macrostachyum, Eulophia gramine*) which belong to the subfamily Epidendroideae, arise from a monophyletic clade because they all

have many similar characters. The other two genera (*Habenaria plantaginea*, and *Zeuxine strateumatica*) which belongs to the subfamily Orchidoideae, arise from the different clade. The two *Vanda* spp., two *Coeleogyne* spp., and 3 *Dendrobium* spp. are closely related. So, they develop sister clades respectively. In our present study, pollinial morphology takes a great role for identification at species level. But according to the APG IV system of classification, the species *Cymbidium aloifolium* and *Eulophia graminea* should be closely related. Whereas in our dendrogram their positions are far away from each other. So, our present study concludes that pollinial characters as well as other morphological characters play a major role in preparation of a proper phylogenetic tree.

Chapter 9: Future Scope

9. Future Scope:

1. By this systematic study identification of the different orchid species of West Bengal can be done (Epidendroideae and Orchidoideae).
2. This study is very helpful for those people who are dealing with orchids for commercial and research purposes.
3. The detailed taxonomical characters with supported clear photographs will be useful to future researchers for proper identification of the specimen.
4. The present study emphasizes on the orchid Pollinaria, which will be helpful to study the pollination and reproductive biology of Orchidaceae.
5. On the basis of pollinarial characters it can find 2 subfamilies (Epidendroideae and Orchidoideae) under our work. So, pollinial characters are a significant tool for the systematic study of Orchidaceae in the near future.

Chapter 10: References

10. References:

- Anghelescu, N., Bygrave, A., Georgescu, M., Petra, S., Toma, F., 2020. A History of Orchids A History of Discovery, Lust and Wealth. *Scientific Papers -B, Horticulture 2020*, 64(1), pp.519-530.
- Bhunja, A., Naskar, A., Tripathi, S., Dinda, S., Khatun, M., Dwari, S., Islam, S., Mondal, A., 2021. *Habenaria plantaginea* Lindl. (Orchidaceae): A new record for Eastern part of the Chhotonagpur Plateau, West Bengal, *India Plant Science Research*, 41(1&2), pp.48-54.
- Blackman, S.J., Yeung, E.C., 1983. Comparative Anatomy of Pollinia and Caudicles of an Orchid (*Epidendrum*). *Botanical Gazette*, 144(3), pp.331–337.
- Cribb, P., Kell, S., Dixon, K., Barrett, R., 2003. Orchid conservation: a global perspective, in: *Orchid Conservation. Natural History Publications, Kota Kinabalu*. pp.1–24.
- Damon, A., Nieto L., G., 2013. A Guide to the Morphology of the Pollinia and Pollinaria of Orchids from the Biological Corridor Tacaná-Boquerón in Southeast Mexico. *Selbyana*, 31(1), pp.5–39.
- De Beaugrande, R., Dressler, W., 1981. Introduction to text linguistics, *Scientific Research Publishing*, pp.17-32.
- De, L., 2020. Morphological diversity in orchids. *International Journal of Botany Studies*, 5(5), pp.229-238.
- Dressler, R., 2005. How Many Orchid Species? *Selbyana*, 26, pp.155–158.
- DuPuy, D.J., Ford-Lloyd, B.V., Cribb, P.J., 1985. A Numerical Taxonomic Analysis of *Cymbidium*, Section *Iridorchis* (Orchidaceae). *Kew Bulletin*, 40(2), pp.421.
- Falorunso, A.E., Jayeola, A.A., 2009. Application of Numerical Taxonomy to Lip Morphology in the Genus *Polystachya* Hook (Orchidaceae) in Nigeria. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 37(1), pp.45–47.

- Fan, J., Li, C.-C., Jin, X., 2023. Understanding the systematic implications of Pollinium morphology of Aeridinae (Orchidaceae). *Research Square*, pp.1-18.
- Freudenstein, J., Rasmussen, F., 1996. Pollinium development and number in the Orchidaceae. *American Journal of Botany*, 83, pp.813–824.
- Freudenstein, J.V., Harris, E.M., Rasmussen, F.N., 2002. The evolution of anther morphology in orchids: incumbent anthers, superposed pollinia, and the vandoid complex. *American Journal of Botany*, 89, pp.1747–1755.
- Halbritter, H., 1998. Preparing living pollen material for scanning electron microscopy using 2,2-dimethoxy propane (DMP) and critical-point drying. *Biotech Histochem*, 73(3), pp.137–143.
- Hammer, Q., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), pp. 9.
- Handa, S.S., 1986. Orchids for drugs and chemicals. In: biology, conservation and culture of orchids (ed. S.P. Vij). pp.89-100.
- Hidayat, T., Ito, M., 2006. Evolutionary analysis of pollinaria morphology of subtribe Aeridinae (Orchidaceae). *Reinwardtia*, 12.
- Hooker, J.D. (1890). Flora of British India–Vol. 6. L. Reeve, London, pp.184.
- Jain, S.K., and Rao, R.r., 1977. A handbook of field and herbarium technique. *Today and Tomorrow Publishers*, New Delhi, India.
- Kraenzlin, F. (1926). A monograph of the genus *Polystachya*. *Feddes Repertorium; Beih.* 39, pp.663-671.
- Li, L., Zhang, J., LI, C.-R., Wang, Y.-P., Tan, Q.-Q., Luo, Y., 2020. The pollinia development of *Pennilabium yunnanensis* (Orchidaceae) 40(4), pp.161–171.
- Michener, D. and Sokal, R.R., 1957. A quantitative approach to a problem of classification. *Evolution*, 11(2), pp.130-162.

Mitra, S., Bandyopadhyay, S. and Mukherjee, S. (2020) 'TAXONOMIC CENSUS OF THE ORCHIDS OF WEST BENGAL, INDIA', *Plant Archives*, 20, pp. 3951–3980.

Mosquera-Mosquera, H.R., Valencia-Barrera, R.M., Acedo, C., 2019. Variation and evolutionary transformation of some characters of the pollinarium and pistil in Epidendroideae (Orchidaceae). *Plant Systematics and Evolution*, 305(5), pp.353–374.

Nieto L., G., Damon, A., 2008. Morphology of the Pollinia and Pollinaria of Orchids from Southeast Mexico. *Selbyana*, 29, pp.20–68.

Ninawe, A.S., Swapna, T.S., 2017. Orchid Diversity of Northeast India – Traditional Knowledge and Strategic Plan for Conservation, pp.16.

Phillips, R.D., Peakall, R., 2018. An experimental evaluation of traits that influence the sexual behavior of pollinators in sexually deceptive orchids. *Journal of Evolutionary Biology*, 31(11), pp.1732–1742.

Prain, D. (1903). Bengal Plants, Govt. of India, *Central Publication Branch*, Calcutta. Vol. 1 and 2.

Pridgeon, A.M., 1982. Numerical analyses in the classification of the Pleurothallidinae (Orchidaceae). *Botanical Journal of the Linnean Society*, 85(2), pp.103–131.

Rao, C.K., B.L. Geetha & G. Suresh, 2003. Red List of Threatened Vascular Plant Species in India. *Botanical Survey of India*, Calcutta, pp.144.

Singer, R.B., Gravendeel, B., Cross, H., Ramirez, S.R., 2008. The Use of Orchid Pollinia or Pollinaria For Taxonomic Identification. *Selbyana*, 29, pp.6–19

Byng, James W; Chase, M; Christenhusz, M; Stevens, P 2016. Update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV; *Botanical Journal of the Linnean Society*, 181(1), pp.1–20.

Wang, Y.-P., Li, L., Yang, C.-X., Yan, L., Li, Q.-Q., 2020. Pollinia morphology of 14 species in *Dendrobium* Sw. (Orchidaceae) and taxonomic significance, *Bulletin of Botanical Research*, 41(1), pp.12-25.

Yonzone, R., Lama, D., Bhujel, R.B., Rai, S., 2012. Orchid species diversity of Darjeeling Himalaya of India. *Life Science*, 3(3). pp.3.

Zavada, M., 1990. A Contribution to the Study of Pollen Wall Ultrastructure of Orchid Pollinia. *Annals of the Missouri Botanical Garden*, 77, pp.785.