Establishment of Barcodes for Some Commercially Important Vandaceous Orchids of Nagaland

By

MR. JOYRISON KAMBA

THESIS SUBMITTED IN PARTIAL FULLFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY

DEPARTMENT OF BOTANY

NAGALAND UNIVERSITY, LUMAMI – 798627

NAGALAND, INDIA

2021

CONTENTS

Chapters	Particular	Page No.
	Declaration	1
	Acknowledgement	2
	Ph. D. Coursework Mark sheet and Certificate	3-4
	Abbreviations	5-6
	List of Tables	7
	List of Figures	8
	List of Photo Plates	9-10
1	**Introduction**	11-53
2	**Documentation of Vandaceous Orchids**	54-109
	INTRODUCTION	
	MATERIALS AND METHODS	
	RESULTS	
	DISCUSSION	
	SUMMARY AND CONCLUSION	
3	**Establishment of Barcode for Some Commercially Important Vandaceous Orchids**	110-163
	INTRODUCTION	
	MATERIALS AND METHODS	
	RESULTS	
	DISCUSSION	
	SUMMARY AND CONCLUSION	
4	**Summary and Conclusion**	164-168
	SUMMARY	
	CONCLUSION	
	References	169-187
	Appendix (I-III)	188-192
	I. GPS Coordinates from the collection sites	
	II. Chemicals, Enzymes & Reagents	
	III. PCR Cycle Conditions	
	List of Publications	193
	List of Seminar/Symposium, Conferences Attended and Paper Presented	194-195

Nagaland University
(A Central University Estd. By the Act of Parliament No. 35 of 1989)
Lumami 798 627, Nagaland, India
Mobile: +91-9436006808; E-mail: debchitta@rediffmail.com / debchitta@gmail.com
Prof. Chitta Ranjan Deb
Department of Botany

March 31, 2021

DECLARATION

I, Mr. Joyrison Kamba bearing Ph. D. Registration No. 722/2016 dated 06/08/2015 hereby declare that the subject matter of my Ph. D. thesis entitled 'Establishment of Barcodes for Some Commercially Important Vandaceous Orchids of Nagaland' is the record of original work done by me, and that the contents of this thesis did not form the basis for award of any degree to me or to anybody else to the best of my knowledge. This thesis has not been submitted by me for any Research Degree in any other University/Institute.

This is further certified that the Ph. D. thesis is submitted in compliance with the UGC Regulation 2016 dated May 05, 2016 (Minimum Standard and Procedure for Award of M. Phil. /Ph. D. Degree). It is certified that the content of the thesis is checked for 'Plagiarism' with licensed software 'Plagiarism Checker X' and satisfies with the norms of 'University Grants Commission, Govt. of India'. This thesis is being submitted to the Nagaland University for the degree of 'Doctor of Philosophy in Botany'.

Joyrison K 31/03/2021
(Joyrison Kamba)
Candidate

31/03/21
(Prof. Chitta Ranjan Deb)
Supervisor
Prof. Chitta Ranjan Deb
Department of Botany
Nagaland University
Lumami-798627. Nagaland, India

31/03/21
(Prof. Chitta Ranjan Deb)
Head, Department of Botany
Head
Department of Botany
Nagaland University
Lumami 798627. Nagaland

1

Acknowledgement

I would like to express my heartfelt gratitude to my Supervisor **Prof. Chitta Ranjan Deb** and Head of the Department, Department of Botany, Nagaland University for his continuous support, guidance, motivation, patience and providing all necessary facilities during the entire course of my research work.

My sincere thanks to Dr. A. Paul 'A man of action' for his insightful thought, suggestion, improvement and correction, all the faculty members of the Department of Botany, Nagaland University for their valuable support and guidance.

I would like to mention some eminent personalities who have help me in identification of some critical species like - Dr. Andre Schuiteman, Research Leader in Identification and Naming, Herbarium, Royal Botanic Gardens, Kew, Richmond, Survey, UK.

- Dr. Hubert Kurzweil, Orchid Specialist (Singapore Botanic Garden).
- Dr. Pankaj Kumar, IUCN-SSC Orchid Specialist Group Asia. Orchid Conservation Section KFGB Corporation, Hongkong S.A.R., China.
- Dr. Avishek Bhattacharjee, Scientist C (IUCN-SSC Orchid Specialist Group-Indian subcontinent) Central National Herbarium, Botanical Survey of India, Kolkata.
- Dr. Santanu Dey, former Research Scholar, Dept. of Botany, Nagaland University.
- Dr. Salam Pradeep Singh RA IBSD, Imphal, Manipur in submitting sequence to GenBank and Pradosh Mahadani RA CRRI, Cuttack, Orissa in sequence analysis and submission.

I am extremely thankful to the University Grant Commission (UGC), Govt. of India, New Delhi for the financial help through UGC-BSR Fellowship, and Institutional Biotech Hub Sponsored by DBT, New Delhi and UGC-SAP (DRS-III) and DST-FIST for all the facilities provided during my research work.

I express my gratitude to all the non-teaching staffs and research scholars especially my Molecular and Biotechnology Lab. members for which our memories will always be cherish and I shall always remain grateful to you all.

Last but not the least I express my sincere gratitude to my family and friends for their constant prayers and support.

Above all "TO THE ALMIGHTY GOD FOR HIS AMAZING GRACE".

Joyrikon K 31/07/2021
(Joyrikon Kamba)

Sl. No. : 14- 015919

NAGALAND UNIVERSITY

HEADQUARTERS LUMAMI

STATEMENT OF MARKS

Ph. D COURSE WORK EXAMINATION 2015

DEPARTMENT OF BOTANY

The following are the marks secured by Mr. **JOYRISON K**
Roll No. **Bot-Ph.D.-01/15** *of Ph.D Course Work Examination held in* **2015**

Subject(s)/Paper(s)	Max. Marks	Minimun Qualifying Marks	Marks Secured
Paper No. B.Ph.D. - I Research Methodology	100	55	**69**
Paper No. B.Ph.D. - II Elective Paper	100	55	**74**
Paper No. B.Ph.D. - III Review of Literature & Seminar	100	55	**88**
Total Aggregate Marks			**231**
Average Pass Mark – 55 %			

Result	Division	Percentage
Passed	**I Division**	**77 %**

09 DEC 2015

Marks compared by :

Asstt. Registrar/Controller of Examinations

NAGALAND UNIVERSITY

HEAD QUARTERS : LUMAMI

Ph. D COURSE WORK EXAMINATION

This is to certify that Mr/Ms. JOYRISON K

of Nagaland University bearing Roll No. Bot-Ph.D. - 01/15 is qualified in the Ph.D Course Work Examination

in the Department of BOTANY, Nagaland University held in the Year 20 15

10.12.15
Head of Department

Head
Department of Botany
Nagaland University
Hqs. Lumami.

15-12-2015
Dean

Dean
School of Sciences
Nagaland University
Hqs. Lumami Nagaland

School of Sciences

Abbreviations

Abbreviation	Expanded form
°C	Degree Celsius
%	Percentage
atpB	β-subunit of ATP synthase
B.C	Before Christ
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
Bp	Base pair
BSI	Botanical Survey of India
CBOL	Consortium for the Barcode of Life
CITES	Convention on the International Trade in Endangered Species of Wild Fauna and Flora
CO1	Cytochrome C Oxidase subunit 1
cpDNA	Chloroplast DNA
CTAB	Cetyl Trimethyl Ammonium Bromide
ddH_2O	Double-distilled water
DNA	Deoxyribo Nucleic Acid
dNTPs	Deoxynucleotide triphosphates
EtBr	Ethidium Bromide
EU	European Union
GPS	Global positioning system
$HgCl_2$	mercuric chloride
iBOL	International Barcode of Life
ICBN	International Code of Botanical Nomenclature
INR	Indian rupee
IR	Inverted Repeat
ISFR	India State of Forest Report
IUCN	International Union for Conservation of Nature and Natural Resources.
K2P	Kimura 2-Parameter
LSC	Large Single Copy
matK	*maturase K*

MEGA	Molecular Evolutionary Genetics Analysis
mg/ml	Milligram/ Millilitre
$MgCl_2$	MagnesiumChloride
mL	Millilitre
Mm	Millimetre
mM	Micrometer
N.E.	Northeast
NCBI	National Centre for Biotechnology Information
NEFA	North-East Frontier Agency
ng	Nanogram
NJ	Neighbour Joining
NMF	Natural Moistering Factor
NOR	Nucleolar Organizer Regions
nrITS	nuclear ribosomal Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
rbcL	*Rubisco Large Subunit*
rpm	Round Per Minute
rpoB	*RNA polymerase-β subunit*
rpoC1	*RNA polymerase-β' subunit*
rRNA	ribosomal RNA
Spp	Species
SSC	Small Single-Copy
SSC	Species Survival Commission
TAE	Tris-Acetate-EDTA
Taq polymerase	*Thermus aquaticus* polymerase
TBE	Tris Borate EDTA
TE	Tris-EDTA
tRNA	Transfer Ribonucleic Acid
US	United States
WCSP	World Checklist of Selected Plant
µL	Microlitre
µM	Micro meter

List of Tables

Table No.	Table Legend	Page No.
1.1	List of some primers used for amplification of potential candidates for DNA barcoding of plants.	43
2.1	List of orchid species collected and identified during the present study	107
3.1	PCR master mix composition 1	122
3.2	PCR master mix composition 2	122
3.3	Primers used for amplification of candidate DNA barcodes	123
3.4	Accession number obtained from GenBank	125
3.5	Amplification and sequencing success rates for three tested loci (80 individuals per loci)	128
3.6	Intra-specific K2P distance for three candidate loci	129
3.7	*matK* pairwise genetic distance	131
3.8	Average inter-specific K2P distance and percent species resolution for three investigated loci.	132
3.9	*rbcL* pairwise genetic distance	136
3.10	ITS pairwise genetic distance	141
3.11	Average K2P distances along with the number of species analyzed for each loci	154
3.12	Species Discrimination rates along with the number of species analyzed for each loci	155

List of Figures

Figure No.	Figure Legend	Page No.
1.1	Existing barcode candidates for the Plant Kingdom	41
1.2	Gene map of tobacco chloroplast DNA	42
1.3	ITS Regions of Nuclear Ribosomal Cistron	49
2.1	Shows the survey area for sample collection	57
3.1	Neighbour joining tree of 31 species (66 individuals) based on *matK* sequences. The species showing zero inter and intra specific divergence are shown bold, capital and both as well.	134
3.2	Neighbour joining tree of 68 species based on *rbcL* sequences. The species showing zero inter and intra specific divergence are shown bold, capital and both as well.	139
3.3	Neighbour joining tree of 67 species based on ITSsequences. The species showing zero inter and intra specific divergence are shown bold, capital and both as well.	143
3.4	*Acampe* species NJ trees (a) *matK* (b) *rbcL* (c) ITS	145
3.5	*Arachnis* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.	146
3.6	*Cleisostoma* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.	147
3.7	*Gastrochilus* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.	148
3.8	*Papilionanthe* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.	149
3.9	*Phalaenopsis* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.	150
3.10	*Vanda* species NJ trees (a) *matK* (b) *rbcL* (c) ITS	52-54

List of Plates

Plate No.	Plate Legend	Page No.
2.1	*Esmeralda clarkei* Rchb.f. (*Arachnis clarkei)* (A) Habitat (B) Flower.	64
	Arachnis labrosa (Lindl. & Paxton) Rchb.f. (C) Habitat (D) Flower.	63
2.2	*Acampe praemorsa* (Roxb.) Blatt. & McCann (A) Habitat (B) Flower.	60
	A. rigida (Buch.-Ham. ex Sm.) P.F.Hunt (C) Habitat (D) Flower	61
2.3	*Acampe ochracea* (Lindl.) Hochr. (A) Habitat (B) Flower.	59
	Gastrochilus inconspicuus (Hook.f.) Kuntze (C) Habitat (D) Flower.	70
2.4	*Cleisostoma simondii* (Gagnep.) Seidenf. (A) Habitat (B) Flower.	67
	C. williamsonii (Rchb.f.) Garay (C) Habitat (D) Flower.	68
2.5	*Cleisostoma paniculatum* (Ker-Gawl.) Garay (A) Habitat (B) Flower.	65
	C. racemiferum (Lindl.) Garay (C) Habitat (D) Flower.	66
2.6	*Gastrochilus acutifolius* (Lindl.) Kuntze (A) Habitat (B) Flower.	68
	G. pseudodistichus (King & Pantl.) Schltr. (C) Habitat (D) Flower.	72
2.7	*Gastrochilus obliquus* var. *obliquus* (Lindl.) Kuntze (A) Habitat (B) Flower.	71
	G. calceolaris (Buch.-Ham. ex Sm.) D.Don (C) Habitat (D) Flower.	69
2.8	*Papilionanthe teres* (Roxb.) Schltr.(A) Habitat (B) Flower.	73
	P. vandarum (Rchb.f.) Garay (C) Habitat (D) Flower.	74
2.9	*Phalaenopsis wilsonii* Rolfe (A) Habitat (B) Flower.	77
	P. braceana (Hook.f.) E.A. Christenson (C) Habitat (D) Flower.	75
2.10	*Renanthera imschootiana* Rolfe (A) Habitat (B) Flower.	78
	Smitinandia micrantha (Lindl.) Holttum (C) Habitat (D) Flower.	80
	Hygrochilus parishii (Veitch & Rchb.f.) Pfitzer (*Phalaenopsis*	

hygrochila) (E) Habitat (F) Flower. 76

2.11 *Stereochilus laxus* (Rchb.f.) Garay (A) Habitat (B) Flower. 81

Vandopsis undulata (Lindl.) J.J.Sm. (C) Habitat (D) Flower. 89

2.12 *Sarcoglyphis mirabilis* (Rchb.f.) Garay (A) Habitat (B) Flower. 79

Thrixspermumtsii W.H.Chen & Y.M.Shui (C) Habitat (D) Flower. 82

2.13 *Rhynchostylis retusa* (Linn.) Bl. (A) Habitat (B) Flower. 79

Aerides odorata Lour. (C) Habitat (D) Flower. 62

2.14 *Vanda ampullacea* (Roxb.) L.M. Gardiner (A) Habitat (B) Flower. 84

V. stangeana Rchb.f. (C) Habitat (D) Flower. 86

2.15 *Vanda coerulea* Griff. ex Lindl.(A) Habitat (B) Flower. 86

V. bicolor Griff. (C) Habitat (D) Flower. 85

2.16 *Vanda tastacea* (Lindl.) Rchb.f. (A) Habitat (B) Flower. 87

V. alpina (Lindl.) Lindl.(C) Habitat (D) Flower. 83

2.17 *Vanda pumila* Hook.f. (A) Habitat (B) Flower. 88

Luisia trichorrhiza (Hook.) Bl. (C) Habitat (D) Flower. 73

CHAPTER - 1

INTRODUCTION

Orchids belong to the family 'Orchidaceae', one of the two largest families after Asteraceae (Compositae) and highly specialized family of the flowering plants and biologically most complex. They are known for their strange fascinating shape, beautiful looks, longevity and, highly attractive colors besides their ornamental and medicinal importance and have become among the most studied flowering plant. They have outnumbered some other families of flowering plants by evolving higher levels of specialization in its vegetative and reproductive structures. Some of the major distinguishing features of orchid family are: the stamens are all on one side of the flower rather than being symmetrically arranged, stamen and the pistil are at least partly united, the seeds are tiny and numerous, flowers usually have a lip or labellum, flowers usually twists around in the course of development (resupination), part of stigma (rostellum) is usually involved in transferring pollen from one flower to another, pollen is usually bound together in a few large masses (pollinia) (Dressler, 1981). The world estimate of orchid species has been assessed between 17000 to 35000 (Dressler, 1993a). Some of the

other workers, however, suggest 19,128 species in 800 genera (Atwood, 1986), 30,000-35,000 (Garay and Sweet 1974; Gentry and Dodson, 1987), 17,500 species (Mabberley, 1990), 18,000 species in 750 genera (Haywood, 1993), 19,501 species from 803 genera (Dressler 1993b), 18,500 species in 778 genera (Mabberly, 1998), 20,000 species (Lawler and Rao, 2002). Recently well-documented estimation suggests more than 28,000 accepted species in 763 genera (Christenhusz and Byng, 2016) and approximately 26,567 species distributed worldwide (WCSP, 2020). *Pleurothallis*, which with an estimated 1120 species is the largest orchid genera in Orchidaceae. *Bulbophyllum* with about 1000 species constitutes the second largest genus and has a pantropical distribution with a large proportion of the species in tropical Asia (Vermeulen, 1991). *Dendrobium*, the third-largest genus is found in India and subtropical Asia (Bechtel *et al.*, 1992).

Orchidaceae shows great variation in their diversity and distribution pattern. Till date the smallest microscopic plants (*Platystele* species from Ecuador reported in 2009 measuring between 2-2.1 mm or genus *Oberonia* with 1.1-1.5 mm discover in mid-2010), *Bulbophyllum* to long vines in Vanilla and gigantic plants in*Cyrtopodium* and *Grammatophyllum speciosum* often referred to as the Sugar Cane Orchid, Giant Orchid, or Queen of the Orchids title the world largest orchid with flower spike measuring about 2.5 – 3 m (6-8 feet) in length (https://www.pumpkinbeth.com). On the other hand, there are thousands of manmade hybrid orchids produce each year and their number exceeds far than that of the natural species. For the first time, the first hybrid *Calanthi dominie* was flowered and named in 1856, and over 100,000 hybrids have been registered (Bechtel *et al.*, 1992). Orchidaceae is a rapidly evolving pollinator-oriented family (Dressler, 1981b; Benzing, 1987). The reason for the high diversity found in orchids could be due to large number of small seeds that favor the expression of genetic variability and high dispersal rates across geographical/ecological barriers, rapid life

cycles, high plasticity in floral architecture and fragrance, and preadaptation for epiphytes (Gentry and Dodson 1987; Burns-Balogh and Bernhardt 1988). Orchids are distributed all over the world in all terrestrial ecosystems except Antarctica, few isolated Islands inhabiting a variety of habitats except the aquatic system and hot deserts. The greatest diversity is found in the tropics (tropical and sub-tropical region) than any other ecosystem. Out of the total 19,501 species with 803 genera recognized (Dressler 1993b) for the entire family, 36 genera each with 100 or more species, comprising about 10,849 species (56% of the total) are found in the tropics (IUCN/SSC, 1996). They are adapted to various habitat *viz*. terrestrial, epiphytes, lithophytes, and subterranean life modes and are mainly concentrated in the tropical and sub-tropical regions where conditions are favorable (i.e., high humidity and thick vegetation) for their growth and development (Vij, 1995). Orchids are incredibly diverse and have a highly modified floral structure that continues to fascinate Botanists and enthusiasts alike (Janes, 2006).

HISTORY AND CLASSIFICATION OF FAMILY ORCHIDACEAE

The origin of orchids on earth can be dated back to about 120 millions year ago. However, they might have originated sometimes between 40 and 80 million years ago (Dressler, 1974). They are believed to have originated in Southeast Asia (Malaysia) (Garay, 1972) during the Cretaceous period and dispersed throughout the Tertiary period and becoming epiphytic in the Plio-Pleistocene (Schmid, 1977; Dressler, 1981; Janssen and Bremer, 2004). However, there is no concrete evidence of these events primarily due to no/poor fossil record (Schmid, 1977). It is generally believed and accepted that they have an ancient origin due to their close association with the families Liliaceae, Iridaceae, and Amaryllidaceae of the subclass Monocotyledonae (Dressler and Dodson, 1960; Schmid, 1977; Dockrill, 1992). The European were known to be the earliest to have kept the account of orchids which can be date back to the ancient Greeks and Romans, when

the classification was based on a plant's medicinal use. The Greeks and Romans believed that if a section of plants resembled the part of the human anatomy, then it could be used to heal that part of the body (Bedford, 1969; St. George, 1999; Rittershausen, 2002). Theophrastus, a Greek philosopher (370-285 B.C.) uses the term orchid for the first time in his book 'Enquiry into plants'. The name orchid is derived from the Greek word 'orchis' literally meaning 'testicles', because of the shape of the twin tubers in some species of orchis. He used the word orchis for a group of plants and observed that the roots of these plants were used in traditional pharmacopeias of Greece and neighboring Asia Minor as an antidepressant and as a stimulant. The term orchis is now used to describe the European genus and the name of the entire family - Orchidaceae, is also derived from it.

In 1521, a fleeting interest began in orchid when the Spanish discovered the Aztecs cultivating a species of Vanilla for their scented pods used to flavor chocolate (Rosengarten, 1969; Rittershausen, 2002). Throughout the 18^{th} and 19^{th} centuries, Europe has increased interest in Orchids, and the need for classification increased as more species were discovered. By 1753, Carl. Linnaeus has first developed systematic classification briefly described eight genera of orchids, of which 45 species were terrestrial (Bates, 1990). In 1789, De Jussieu recognized Orchidaceae as a separate family in his Genera Plantarum (De Jussieu, 1789). Olof Swartz recognized 25 genera in 1800 and predicted that many more new genera would be created in the future. He was the first person to bring out a critical review of orchid literature when he presented his classification and also reported the term Monandrous and Diandrous condition in orchids. By 1810, the first comprehensive guide to orchids was published by Robert Brown his work '*Prodromus Florae Novae Hollandiae et Insulare van Diemen*'. He described briefly many orchid species including 26 Australian genera containing 114 species (Bates, 1990; Dockrill,

1992). Louis Claude Richard introduced us in 1817 with descriptive terminology of orchids that we use today while working on European orchids (Richard, 1817). During 1830-1840, John Lindley published his book '*The Genera and Species of Orchidaceous' Plants* on which allmodern systems of orchid classification are founded. He is considered the father of orchidology. Lindley was the first to divide orchid families into distinct tribes based on anther number and type of pollinarium, recognizing seven with further divisions (Dressler and Dodson, 1960). In 1881, 'Bentham expanded/modified Swartz's classification of orchids and validated a total of 5 tribes and 27 subtribes in '*Genera Plantarum*' (Dressler and Dodson, 1960; Bedford, 1969). Ernst Pfitzer (1881) revised Bentham's system of classification and brought out a classification based on morphological features excluding characters of pollinia and proposed that there were 32 tribes, which became the basis for Rudolf Schlechter's 1926 treatment, *System der Orchidaceen*, recognizing four tribes and 80 subtribes (Dressler and Dodson, 1960). He divided the family into two subfamilies viz., Monandrae and Dinandrae. Schlechter's system is still used in some major herbaria. His tribes were based on anther and pollinarium characters while the subtribes were based on vegetative and floral characters. Dressler and Dodson (1960) reviewed the classification of Schlechter and made some changes to bring it by following the International Code of Botanical Nomenclature (ICBN). At about the same time i.e., 1960, Garay suggested the division of the family into 5 subfamilies; Apostasioideae, Cypripedioideae, Orchidoideae, Neottioideae and Epidendroideae. Vermeulen (1966), proposed 3 families, Apostasiaceae, Cypridiaceae, and Orchidaceae under the order Orchidales, and subdivided Orchidaceae into 2 subfamilies viz., the Orchidoideae and the Epidendroideae, the latter again being subdivided into two Neottianthae and Epidendranthae (Vermeulen, 1966). Dressler (1981) proposed 6 subfamilies (Apostasiodeae, Cypripedioideae, Spiranthoideae,

Orchidoideae, Epidendroideae, and Vandoideae), 21 tribes and 63 subtribes under the family Orchidaceae. Then in 1993 again, Dressler revised his classification based on morphological, palynological, anatomical, and cytological data and divided the family into 5 subfamilies (Apostasiodeae, Cypripedioideae, Spiranthoideae, Orchidoideae and Epidendroideae), 21 tribes and 69 subtribes. This system of classification is considered to be the most comprehensive presentation to be presented so far and has a considerable influence on later work (Dressler, 1993). Szlachetko (1995) accepted Vermeulen three family schemes and considered the most recent complete classification. From 1999 to 2014 over 15 years, 6 volumes was published in 'Genera Orchidacearum' and covers all the known orchids together with a description of each genus. During the 1990s, orchid taxonomy began to be influenced by molecular phylogenetics based on DNA sequences. In 1999, the first molecular phylogenetic study was published to include a substantial sample of orchids (Cameron *et al.*, 1999). Chase *et al.* (2003) published the first classification that was based on the cladistic analysis of DNA data, and update classification was again published in 2015. At present, there are 5 subfamilies broadly recognized orchids viz. Apostasiodeae, Cypripediodeae, Vanilloideae, Epidendroideae, and Orchidoideae.

ORCHID STUDIES IN INDIA AND NORTH-EAST INDIA

Due to its varied geographical conditions i.e., varied climatic conditions and diverse ecological habitats, ranging from temperate to tropical to arctic, India has a very rich resource of wild orchids. In India, the number of orchid species has been estimated between 127-184 genera and 810-1229 species by various workers (Pradhan, 1979; Bose and Bhattacherjee, 1980; Karthikeyan *et al.*, 1989; Kumar and Manilal, 1994; Karthikeyan, 2000). Misra (2007) reported 1,298 species, 5 subspecies, and 28 varieties under 186 genera from India. The diversity is not static and keeps on increasing from

time to time in the light of new additions by various authors and was being added every year. According to Verma and Lavania (2014) there are 1,378 species, 5 subspecies, 29 varieties, and 2 formae under 186 genera in the country. According to the Botanical Survey of India (BSI) latest publication 'Orchid of India: A Pictorial Guide' (2019) currently the total number stands at 1256 species under 155 genera and 307 which are endemic to India (Singh *et al.*, 2019).

In India, since the Vedic period orchids have been known to mankind. Around (1500 - 800 B.C.) two orchids (*Rasna – Vanda tessallata*; *Sanjeevani – Flickingeria macrai*) have been mentioned in the *Rig Veda* and the *Atharva Veda* as medicinally important. Accordingly, by Sushruta in *Sushruta Samhita* and Charak in *Charaka Samhita* listed a dozen of orchid plants used in Ayurveda. Van Rheede (1678-1693) published his monumental work "Hortus Malabaricus" in 12 volumes describing over 700 species of flowering plants from Malabar; it includes descriptions and notes on the medicinal properties of 16 Indian orchids from peninsular regions and thus laid the foundation of Indian orchid's fort the first time. Later on taxonomic works were carried out by Roxburgh (1832) recorded some 57 species of orchids in 8 genera in "Flora Indica" and proposed many new taxa which were later transferred to other genera. These collections were mainly from Sylhet district of Assam.Lindley (1830-1840) utilized the vast collections made by J. D. Hooker and G.M. Thomson and later on published "Genera and species of Orchidaceae plants". Lindley (1857), first described 71 species in 21 genera and later on dealt with the genera *Dendrobium, Crytochilus, Acanthephippium, Anthogonium* and documented 331 species belonging to 54 genera (Lindly,1858).Wight (1832) made extensive collections of the flora of Madras Presidency and he along with G. A. Walker - Arnott published their work. Later, Wight (1845-1853) subsequently published an illustrated account of Indian plants that contain invaluable information,

including a critical view on the affinities of orchid genera of India and its neighboring countries. The family Orchidaceae was studied at length by Griffith (1851) and gives a very good detailed account of their general morphology, methods of pollination, and affinities of each taxon. However, the treatment of all *Vanilla* spp. in a separate family Vanillaceae has invited many criticisms and consider invalid. His description was very simple and has provided a good number of beautiful illustrations both in Latin and English languages. Altogether he has described 147 species of orchids under 49 genera. Most of his species were recorded from the Khasi hills and its surrounding areas but some of his described species now belong to the adjacent Asian countries. Drury (1869) listed 275 species of orchids in 74 genera. He discovered Lady's slipper orchid from Augusteer Hill (1500-1800 m) in Travancore Hills and described as *Cypripedium druryi* Bedd. currently now known as *Paphiopedilum druryi* (Bedd.) Stein. Later, Beddome (1874) provided an excellent drawing and a very accurate description of this taxon. This endemic species was found in abundance by Beddome on the top of Calicut Hills which bloomed during the month of January. Atkinson (1882) listed as many as 55 species under 35 genera from the region comprising Kumaon, Nepal, and Tibet which include about 50 species of orchids under 33 genera from the Garhwal region alone. J. D. Hooker in his publication "Flora of British India" in Kew, London wherein his account of the family Orchidaceae in Vols. 5 & 6 where he included some 1250 species belonging to 117 genera collected from erstwhile British India. Hooker who has vast experience in the field had collected plants extensively from Bengal, Meghalaya, Nepal, and Sikkim had undoubtedly vast knowledge on the Indian orchids. Later on, he has also described with illustrations of hundred orchids "A century of Indian Orchids" in the Annals of the Royal Botanical Garden, Calcutta (1895). In (1854), his illustrations on Indian plants were published in the Himalayan Journal. King and Pantling (1898), published "Orchids of

Sikkim Himalayas" describing 448 species in 91 genera which is considered as the most extensive and pioneering work on family Orchidaceae from the North-Eastern region. Many regional treatments of orchids appeared in the year that followed. Collett (1902) described 38 species under 18 genera in his "Flora Simlensis" from Shimla and its neighboring areas, where key characters and distributional details are provided for each taxon but illustrations are given for some of the taxa only. Duthie (1903a, 1903b) studied on the orchids of North-Western Himalaya and published an illustrated account "Orchids of North-Western Himalayas", describing 173 species belonging to 45 genera and also provided a good number of illustrations. Cooke (1901-1908) published "The Flora of the Presidency of Bombay". Which provide an accurate diagnosis and described 74 species in 31 genera from the erstwhile Presidency of Bombay, which included Maharashtra, Gujrat, Sindh, and N. Kanara. The works of Hooker, King & Pantling, and Duthie have a considerable impact on Indian orchids

During the post-independence period, there has been an upsurge information on orchid embryology, occurrence of new species and new records, taxonomy and regional floristic works, cytology, breeding, habitat distribution, commercialization, etc. (Hedge, 1984; Manilal and Kumar, 1986). With the emergence of the Botanical Survey of India (BSI) after the post-independence ever since its establishment in 1956, orchids study has been mainly done by the scientists of the Botanical Survey of India. This has resulted in the publication of a number of booklets and papers both at National and International level (Santapau, 1953; 1957; Kataki, 1962; Hara, 1966; 1976; 1984a; Panigrahi andJoseph, 1966; Nair, 1966, 1978; Santapau and Kapadia, 1966; Arora, 1968; 1969a, b, c, d, 1972a, b; Rao and Verma, 1969 ; Rao and Hajra, 1974, 1984, 1987, 2001; Bhattacharjee, 1976; Das and Jain, 1978, 1979, 1980; Rao, 1979; Das and Jain, 1980; Rao andDeori, 1980 ; Joseph, 1982b; Hajra *etal.*,1983, 1984; Jain andMahrotra, 1984; Kataki

et al.,1984b; Kataki, 1986; Kataki and Hynniewta, 1986; De and Hajra 2001, 2004; Ansari, 1995; Chawngthantluage, 1996; Chowdhery and Pal, 1997; Chowdhery, 1998; Hynniewta *et al.*, 2000; Bhattacharjee and Chowdhery, 2018; Jalal *et al.*, 2018; Singh *et al.*, 2019).

Some of the major/important publications which have a considerable impact on the orchids of India mention may be made are Santapau and Kapadia (1966) published the book entitled 'Orchids of Bombay', Das and Jain (1980) published the book 'The Revision of the Orchidaceae'. Genus: *Coelogyne".* Jain andMahrotra (1984) published the preliminary "Inventory of Orchidaceae in India" listed 925 species under 144 genera of orchids with original citation, corrected nomenclature, and distribution in India along with the mention about their status (endemism). Kataki *et al.*,(1984b) published the plant bulletin conservation on the 'Distribution of Orchids of Sikkim &North - East India' and described 690 species belonging to 128 genera from Sikkim and N.E. India. Subsequently, Kataki (1986) wrote a book entitled 'Orchids of Meghalaya' which comprises of 280 species of orchids along with line drawings and identification keys for the genera and species. Meanwhile, Kataki and Hynniewta (1986) also reported 238 species and 4 varieties belonging to 59 genera from Nagaland. Chawngthantluage (1996) reported 253 species of orchids in his book entitled 'Orchids of Mizoram'. Chowdhery and Pal (1997) published the checklist of Arunachal Pradesh Orchidaceae. Consequently, Chowdhery (1998) published the book 'Orchid Flora of Arunachal Pradesh' reporting 545 species belonging to 123 genera of orchids known from Arunachal Pradesh. They have described identification keys to genera and species, along with the history of the genus and deviation of its name, distribution, flowering, and fruiting times. Hynniewta *et al.* (2000) reported 241 species and 4 varieties belonging to 63 genera in the work entitled 'Orchids of Nagaland'. Jalal and Jayanthi (2018) in 'Orchids of Maharashtra' has

described 106 taxa under 32 genera. Singh *et al.*, (2019) Orchids of India: A pictorial guide enumerated 1256 taxa belonging to 155 genera, out of which 307 are endemic to India.

HABIT AND HABITAT

Biologically orchids are highly specialized and able to grow on a variety of substratum. Based on their habitat they can be broadly classified into following groups:

(a) Epiphyte: (epi - upon; phyta - plants). Those found growing by perching on branches or on the tree trunk and sometimes even on moss covered boulder. Majority of the orchids belong to this group. They developed special kind of aerial roots which hang freely in the air or creep on the tree trunks but never draw nutrition from the host plants as parasite do. The roots are enclosed in a thick sheath of spongy tissue called "Velamen" which can absorb moisture from the atmosphere for the plant to survive. They draw their nutrition from the humus collected at the substratum by the normal roots and prepare their food with the help of green leaves through the process of photosynthesis. Example *Aerides, Dendrobium, Renanthera, Rhynchostylis* etc.

(b) Terrestrial: Those found growing on the soil (generally referred as ground orchids). These orchids store reserve food material in their rhizomes or tubers to survive during harsh environmental condition. During favorable condition young shoots are given out from the tubers or rhizomes and by the time the vegetative growth and flowering is complete sufficient reserve food is stored stocked in the new tuber for future use. These are mostly found in subtropical and temperate region of the world. Example. *Habernaria, Cymbidium, Calanthe, Phaius, Liparis, Malaxis*.

(c) Saprophyte: (sapros-rotten; phyta-plants). Orchids which are devoid of green leaves and grow in soil rich organic matter, decaying substances and derive their nutrition from

as they lack chlorophyll (green pigment). Example. *Epipogium* spp. *Galeola falconeri*, *Erythrorchis ochobiensis* etc.

(d)Lithophytes: Those that grow on rocks. The host plant substratum is replaced by rocks and boulder and found attached with it. Example *Bulbophyllum gymnopus, Coelogyn ovalis, Liparis* spp. and some species of *Paphiopedilum*, etc.

Orchids are further divided into two groups on the basis of vegetative structure:

1. Monopodial: Such orchids grow upright continuously from a single vegetative apex from season to season. Rhizomes and pseudobulbs are not present. The stem is swollen, erect or drooping, may be short or long. The leaves are borne along the entire length or at apex and bears aerial roots from parts of the stem. They sprout out new growth by developing axillary shoots which grow into new plants. Example. *Aerides, Gastrochilus, Rhynchostylis, Vanda, Vanilla* etc.

2. Sympodial: Such orchids have a number of growing apices situated at the base of the rhizome which is a modified stem, creeping or sometimes underground and in most cases produces more or less erect stem like structures. These stem like structures sometimes swell into reserve organs and are known as pseudobulbs. The leaves are borne at the base, apex or along the whole length of the pseudobulbs. These may be deciduous or persistent. Example. *Bulbophyllum, Dendrobium, Paphiopedilum*, etc

ECONOMIC USES

Orchids are known to mankind for the last several centuries for their beautiful attractive flowers and as medicinal plants to Indians from the Vedic period. They are unique groups of plants that exhibit an incredible range of diversity in size, shape, structure, color, and fragrance of the flower (Thomas and Michael, 2007). Several orchid species are cultivated for their various economic uses especially in floriculture. Orchids are grown primarily as ornamentals and are valued as cut flowers and potted plants

because of their exotic beauty and their long-lasting blooming period (Hew *et al.*, 1997). Though orchids are grown primarily as ornamentals, many are also used as herbal medicines, food, and other cultural value by many different cultures and tribes in the different parts of the world (Khasim and Rao, 1999; Kasulo *et al.*, 2009). The medicinal value of orchids is found recorded as early as 250-300 BC by Sushruta and Vagbhata respectively from ancient Sanskrit literature. The generic name 'Vanda' itself is a Sanskrit word in which the name orchid is mentioned as Vanda in Indian Vedic scriptures. The various uses of orchid are:

A. Orchids as ornamental/horticulture: Orchids have long been commercialized as ornamental plants in both horticultural and floricultural trade as a constant source of attraction and delight in one's eyes involving several distinct types of markets and consumers because of their beauty, variously colored, shaped, size, long-lasting, scented or unscented. More than tens of thousands of hybrids of a large number of orchids are now under cultivation for their flowers and today orchid growing is a multi-million dollar industry (Chowdhery, 1998). The vast majority of contemporary orchid trade involves artificially propagated plants and cut flowers are cultivated in commercial greenhouses/orchidarium. Reported Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) The trade of live artificially propagated plants is dominated by a small number of genera, with a large proportion of trade in hybrids e.g. *Cymbidium* Sw., *Dendrobium* Sw., *Phalaenopsis* Blume and *Vanda*W. Jones ex. R. Br. is reported by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Orchids had consistently ranked among the best sellers in the global potted plant trade (FloraHolland, 2015; USDA, 2016) and also comprise10% of all fresh cut flowers traded internationally (De, 2015). With exports of potted orchids, the Netherlands alone valued at almost €500 million in 2015 which

represents an economically significant importance of global trade (FloraHolland, 2015). The largest areas of production are in Thailand, Taiwan, The Netherlands, and Japan, with demand for both potted and cut flowers growing in economic value annually (Griesbach, 2002; Hanks, 2015). There is also considerable domestic and regional trade in cultivated orchids (Hinsley *et al.*, 2018).

B. Orchids as aphrodisiac: Several species of orchids have been used as medicinal herbs since time immemorial. According to the "Theory of the signatures" or "Doctrines of signatures" prevalent during that period, the shape of a plant indicates its medicinal usage. The Greeks and Romans believed that if a section of plants resembled the part of the human anatomy, then it could be used to heal that part of the body (Bedford, 1969; St. George, 1999; Rittershausen, 2002). The resemblance between the tubers of various species of *Orchis* and human testicles thus they established it as an aphrodisiac i.e. a drug that produces sexual desire/stimulant (the term aphrodisiac is derived from "Aphrodite" the Greek goddess of love). During the 18th century in Europe American *Vanilla*, the Oriental 'salep' and European *Satrium* were considered as an aphrodisiac as their uses became so common that men and women ate these tubers when they feel the need and 'salep' bars selling orchid beverage were opened. Thus Orchids gained widespread fame and publicity for its unique properties as an aphrodisiac and its use soon started in many parts of the globe (Chowdhery, 1998).

C. Orchids in cultural and religious beliefs: Orchid flowers have historically been and continue to be traded for their ornamental value in a wide range of cultural and religious ceremonies. For example, in Sri Lanka flowers of *Dendrobium maccarthiae* Thwaites are used as special temple offerings, and flowers, and pseudobulbs of species of *Laelia* Lindl. are used in Mexican Day of the Dead ceremonies (Duggal, 1971). *Rhynchostylis retusa,* the beautiful fox-tail orchid flower endearingly called as 'Kopou Phul' in Assam is worn

by ladies on their head as an ornament during 'Bihu' festival which symbolizes youthfulness during springtime, and similar species, such as *Bulbophyllum sukhakulii* Seidenf., which are often used to adorn women's hair (Goh, 2013). Orchid flowers are also used as national symbols, *Bulbophyllum auricomum* national flower of Myanmar,*Cattleya* Orchid (also known as *Cattleya labiata*) is the National Flower of Brazil, *Cattleya mossiae*-Venezuela's national flower, *Cattleya trianae*-Columbia's national flower, *Vanda miss joaquim*-Singapore's national flower and similar species, such as *B. sukhakulii* Seidenf., which are often used to adorn women's hair (Goh, 2013). There is also a religious belief where orchids have been considered to possess some magical properties too. The use of *Vanda roxburghii* and *Eria muscicola* for averting ill fortune, calamities and to bring prosperity and fortune was mention in the old Sanskrit writings. *Dendrobium acinaforme* plant was worn by the headhunting community of Suriname with a belief that it will provide the courage and good luck in their hunt. In Arunachal Pradesh, the flowers of *Cymbidium grandiflorum* is considered as important for holy worshipped while *Dendrobium hookerianum, D. nobile* and *D. gibsonii* symbolizes purity and sanctity. The orchids and Cyprus are believed to drive off evil spirits. For example, in Malaya Dendrobium crumenatum, Cymbedium finlaysonianum and Plocoglotis porphyrophylla are used to sprinkle water to prevent the ghosts from haunting the living (Tech Eng Soon, 1989).

D. Orchids as medicine: Orchids have been used to cure certain ailments and as a tonic in different parts of the world.

Some of the orchid species used for curing various ailments is listed below:

1. *Acampe papilliosa* (Lindl.) Lindl.: Root is used to treat rheumatism.
2. *Aerides multiflora* Roxb.: Leaf paste applied to treat cuts and wounds. Plant possesses antibacterial properties.

3. *Aerides odoratum* Lour.: Leaf paste is used to treat cuts and wounds, antibacterial properties.
4. *Anoectochilus roxburghii* (Wall.) Lindl.: Whole plant Consumed to treat tuberculosis.
5. *Arundina graminifolia* (D.Don) Hochr.: Root is used to relieve body aches.
6. *Brachycortis obcordata* (Lindl.) Summerh*:* Root is used in dysentery. Often taken with milk as a tonic, nutritious.
7. *Brachycortis obcordata* (Lindl.) Summerh.: Fresh pulp of pseudobulb is used in burns, leaves powder is used to cause abortion and recovery during childbirth.
8. *Bulbophyllum careyanum* (Hook.) Sprengel: Whole plant fresh pulp or juice is used in burns.
9. *Bulbophyllum odoratissimum* (Sm.) Lindl.: The whole plant is used to treat tuberculosis and fracture.
10. *Bulbophyllum umbellatum* Lindl.: The whole plant is used to enhance congenity.
11. *Calanthe plantaginea* Lindl.: Rhizome dry powder with milk is taken as a tonic and also as an aphrodisiac.
12. *Calanthe puberula* Lindl.: Rhizome dry powder with milk is taken as a tonic.
13. *Calanthe sylvatica* (Thou) Lindl.: Flower Juice is applied to stop nose bleeding.
14. *Calanthe tricarinata* Lindl.: Leaf paste applied on sores and eczema. Leaves and pseudobulbs are aphrodisiac.
15. *Cephalanthera longifolia* K. Fritsch: Rhizome is appetizer, tonic and it heals wound.
16. *Coelogyne corymbosa* Lindl.: Pseudobulbs juice is applied in the wound, the paste applied in the forehead to cure headaches.
17. *Coelogyne cristata* Lindl.: Pseudobulbs is given in constipation and also as an aphrodisiac. Juice of pseudobulbs is applied in wounds and boils. Gum from pseudobulb is used for sores.

18. *Coelogyne flaccida* Lindl.: Paste of pseudobulb is applied to the forehead to cure headache and fever, juice is taken for indigestion.
19. *Coelogyne fuscescens* Lindl.: Pseudobulbs paste and juice is used for abdominal pain and burns.
20. *Coelogyne nitida* (Wall. ex Lindl) D. Don.: Pseudobulbs paste and juice are applied in headache and fever and in burns.
21. *Coelogyne ovalis* Lindl.: Pseudobulbs is aphrodisiac.
22. *Coelogyne prolifera* Lindl.: Pseudobulbs paste is used to relieve from fever and headache and also applied in burns and also used in boils and backache.
23. *Coelogyne stricta* (D. Don) Schltr: Pseudobulbs paste is used to relieve headache and fever.
24. *Conchidium muscicola* (Lindl.) Lindl.: Whole plant is used in cardiac, respiratory, and nervous disorders.
25. *Crepidium acuminatum* (D. Don) Szlach: Root powder is used in burns, one of the ingredients of "Astavarga" of Ayurveda. Bulbs are used to treat bronchitis, fever, tuberculosis, and weakness and also given as a tonic.
26. *Cymbidium aloifolium* (L.) Sw.: Rhizome, root, bulbs paste is used for bone fracture and dislocated bones. Powder is used as a tonic.
27. *Cymbidium devonianium* Lindl. ex Paxton: Root paste is applied to treat boils, concentrated decoction is taken in cough and cold.
28. *Cymbidium elegans* Lindl.: Leaves, pseudobulbs and roots fresh juice is coagulating, applied in deep wound to stop bleeding.
29. *Cymbidium iridioides* D. Don: Leaves, pseudobulbs, and roots fresh juice is used to stop bleeding. Powder is used as tonic.
30. *Cypripedium cordigerum* D. Don: Roots is tonic, edible as a vegetable.

31. *Cypripedium elegans.* Reichenb .f. Nep: Roots nervine tonic in hysteria, spasm, madness, epilepsy, and rheumatism.
32. *Cypripedium himalaicum* (Rolfe) Kranzl: The whole plant is used to treat urine blocks treatment, Stone disease, heart disease, chest disorder, and cough.
33. *Dactylorhiza hatagirea* (D. Don) Soo: Tubers are tonic, wound healing, control bleeding, and burns. Also used as a farinaceous food. Used to treat fever and various other body disorders.
34. *Dendrobium amoenum* Wall. Ex Lindl.: Pseudobulbs fresh paste is applied to cure burnt skin and dislocated bones
35. *Dendrobium crepidatum* Griff.: Psudobulbs paste is used in fracture and dislocated bone.
36. *Dendrobium densiflorum* Lindl.: Pseudobulbs pulps of the pseudobulbs are used in boils and pimples and other skin eruptions.
37. *Dendrobium eriaeflorum* Griff.: Pseudobulbs paste is used to treat fractured and dislocated bones. Dried powder is used as a tonic.
38. *Dendrobium fimbriatum* Hook.: The whole plant is used in liver upset and nervous debility.
39. *Dendrobium heterocarpum* Wall.ex Lindl.: Pseudobulb paste is used to treat fractured and dislocated bones.
40. *Dendrobium longicornu* Lindl.: Whole plant juice is used to relieve fever and boiled roots are used to feed livestock suffering from cough.
41. *Dendrobium macaraei* (Lindl.) Seidenf.: Whole plant paste is used against snake bite, general stimulant, and demulcent, also used in Asthma, bronchitis, throat trouble, fever, and aphrodisiac.

42. *Dendrobium monticola* P.F. Hunt & Summerh.: Whole plant pulps of the pseudobulbs are used in boils and pimples and other skin eruptions.
43. *Dendrobium moschtum* Lindl.: Pseudobulb paste is used to treat fractured and dislocated bones.
44. *Dendrobium nobile*Lindl. Stem is tonic and useful in thirst and dryness of tongue. Also given in weakening and fever.
45. *Dendrobium transparens* Wall. ex Lindl.: Stem paste is used to treat fractured and dislocated bones.
46. *Dienia cylindrostycha* Lindl.: Pseudobulb power is used as a tonic.
47. *Epipactis helleborine* (L.) Crantz.: Tubers is used to treat insanity, gouts, headache, and stomachache.
48. *Eria spicata* (D. Don) Hand. Mazz.: Stem paste is taken internally to reduce stomachache and applied externally to reduce headache.
49. *Eulophia dabia* (D. Don) Hochr.: Rhizome is appetizer, tonic and aphrodisiac. Used in purulent cough and heart trouble. Tubers are given to infants in cough and cold.
50. *Eulophia nuda* Landl.: Tubers are appetizer, useful for tuberculosis glands in neck, tumors and bronchitis.
51. *Flickingeria fugax* (Rchb. f.) Seidenf.: Whole plant powder is used as a tonic general debility stimulant.
52. *Galeris strachaeyi* (Hook. f.) P. F. Hunt: Tubers used as a tonic and to cure headaches.
53. *Goodyera repens* (L.) R. Br.: Tuber plant paste is externally applied in syphilis and extract is taken as a blood purifier.
54. *Gymnadenia orchidis* Lindl.: Roots, pseudobulbs powdered are used to treat cuts and wounds. Also used for liver, urinary disorders and gastric.

55. *Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.: The whole plant is used as Salep in combination with dried tubers of various orchids, and also used as spices.
56. *Habenaria intermedia* D. Don.: Tubers is one of the ingredients of Astavarga of Ayurveda, which is used as a tonic. Tuber paste is used to cure various diseases such as hyperdipsia, fever cough, asthma, leprosy skin diseases.
57. *Habenaria marginata* Colebr.: Tubers thoroughly boiled plant extract taken in flatulence in wound and as tonic.
58. *Habenaria pectinata* (Sm.) D. Don: Leaf juice applied in snake bites and tuber is used against arthritis.
59. *Herminium lanceum* (Thunb. ex Sw.) Vuijk: Whole plant extract is given in suppressed urination.
60. *Herminium monorchis* (Linn.) R .Br.: Roots are used as tonic.
61. *Liparis nervosa* (Thunb) Lindl.: Tubers are used to treat stomachache, malignant ulcers.
62. *Luisia trichorhiza (*Hook.) Bl.: Tubers paste is applied externally to cure muscular pain.
63. *Luisia zeylanica* Lindl.: Leaves juice is used to treat chronic wounds, boils and burns.
64. *Malaxis muscifera* (Lindl.) Kuntze: Swollen stem base is useful in sterility, seminal weakness, dysentery, fever, and general debility as a tonic.
65. *Neottianthe calcicola* (W.W. Sm.) Soo.: Rhizome is tonic.
66. *Nervilia aragoana* Gaudich.: The whole plant is used in uropathy, hemoptysis cough asthma, vomiting, diarrhea, and mental instability.
67. *Oberonia caulescens* Lindl.: Tubers are used in liver ailments.
68. *Otochilus albus* Lindl.*:* Whole plant powder is used as a tonic.
69. *Otochilus lancifolius* Griff.: Pseudobulb is used to treat fractured and dislocated bones.
70. *Otochilus porrectus* Lindl.: The whole plant is used as a tonic and also in the treatment of sinusitis rheumatism.

71. *Papilionanthe teres* (Roxb.) Schltr.: Whole plant paste is applied to treat dislocated bones.
72. *Phaius tankervilliae* (Banks) Blume.: Tubers are tonic.
73. *Pholidota articulata* Lindl. : The whole plant is used as a tonic. Root powder is used to treat cancer, juice berries are used to treat skin ulcers and skin eruptions.
74. *Pholidota articulata* Lindl. var. *grifithii* Hook. f.: Pseudobulb Paste is applied to treat dislocated bones.
75. *Pholidota imbricata* (Roxb.) Lindl.: Bulbs, Pseudobulb juice is applied to relieve naval pain, abdominal pain, and rheumatic pain. Also used as a tonic.
76. *Pholidota pallida* Lindl.: Roots, pseudobulb juice is applied to relieve naval pain, abdominal pain, and rheumatic pain. Powder is used to induce sleep.
77. *Platanthera edgeworthii* (Hook. f. ex Collett) R. K. Gupta.: Root and leaves powder is used as a blood purifier.
78. *Platanthera sikkimensis* (Hook. f.) Kraenzlin.: Bulbs, pseudobulb juice is applied to relieve naval pain, abdominal pain, and rheumatic pain.
79. *Pleione humilis* (Sm.) D. Don: Pseudobulb dried powder is used as a tonic. Paste is used in cut and wounds.
80. *Pleione maculata* (Lindl.) Lindl.: Rhizome is used for liver and stomach ailments.
81. *Pleione praecox* (Sm.) D. Don: Pseudobulb dried powder is used as a tonic and also paste is used in cut and wounds.
82. *Rhynchostylis retusa* (L.). Bl.: Whole plant leaves are used to treat rheumatism. Root juice is applied to cuts and wounds.
83. *Satyrium nepalense* D. Don.: Tubers is used as a tonic and also used in diarrhea and malaria. Tubers edible juice is used externally in cut and wounds.

84. *Smitinandia micrantha* (Lindl.): Root powder is used as a tonic and stem has an antibacterial property.
85. *Spiranthes sinensis* (Pers.) Ames: Tuberdecoction of the plant is given in intermittent fever and tubers used as tonic. Paste of roots and stem is applied in sores.
86. *Thunia alba* (Lindl.) Rchb. F.: Whole plant paste is applied to treat dislocated bones.
87. *Trudelia cristata* (Lindl.) Senghas: Root paste is applied in cuts, wounds, boils, and dislocated bones.
88. *Vanda tessellata* (Roxb.) Rchb. f.: Roots and leaves are used in rheumatism and allied disorders. Paste of leaves is used for fever, rheumatism and allied disorders.
89. *Vanda testacea* (Lindl.) Rchb.f.: Leaves is used as antiviral and anticancer agent. Leaf drops are used for earache.
90. *Zeuxine strateumatica* (L.) Schltr.: Roots and tubers dry powder is used as a tonic.

(Source: Pant, 2013).

E. Orchids in Cosmetic Industry: For centuries the orchid flower has been used in Asia for its reparative and protective properties. They are well known in cosmetic industry for their moisturizing properties, fighting free radicals, increasing skin immunity and reducing the appearance of fine lines. For example, the pink orchid is ideal for all skin types and is rich in minerals which exist naturally in the skin, such as zinc, calcium, magnesium, iron and copper. Sugar and polysaccharides also help to seal in moisture while balancing the Natural Moistering Factor (NMF). In the United States (US), The European Union (EU), and the Japanese, the product Orchid Complex ^{TM} OS is used widely which is derived from the flower extract of *Cymbidium* species (*C. grandiflorum*). *Angraecum eburneum* which is another orchid species is also highly sought by the cosmetic industry for its fragrance in perfume making.

F. Orchids in Confectionary: The seed pods of *Vanilla* are used to flavor ice creams and cakes. Sometimes *Stanhopea tigrinum* is used to flavor tortillas.

G. Other uses of Orchids: The Santhal/Santal tribe ladies use fibers from *Vanda roxburghii* leaves for making anklets. The pseudobulb of *Coelogyne pandurata* is cut into half and used as a chalkboard eraser. *Anthogonium gracile* paste is in use for fixing broken glasses. The sticky substances from the corms of orchid *Aplectrum hyemale* give rise to its nickname "putty root" is use by pioneers to mend broken crockery. *Cymbidium canaliculatum*, sticky substance from the pseudobulbs is used as glue. In India *Phaius tankervilleae* is used for making fishing nets. The species *Bletilla hyacinthine* was used as insecticide in China and *Thecostele poilanei* is used for making rat poison in Vietnam. Yellow dye which is obtained from the flowers of *Dendrobium hookerianum* is used for dyeing yellow color to the yarn and cloth.

VANDACEOUS ORCHIDS

Vandaceousorchids are a group of orchid genera in the Subfamily HIGHER EPIDENDROIDEAE or formerly called/known as VANDIODEAE Endlicher Gen. pl. 196. 1837 (Tribe: CYMBIDIEAE, MAXILLARIEAE, POLYSTACHYEAE and VANDEAE) (Dressler, 1981). They are the second largest subfamily with about 300 genera and 5,000 species mostly being tropical epiphytes. The tribe VANDEAE Lindley with over 1700 species in more than 130 genera occurs in tropical Asia, Pacific Islands, tropical America, Australia, and Africa. The subtribe AERIDINEAE or formerly known as SARCANTHINAE Bentham with more than 1,000 species under 103 genera and also including about 200 hybrid species occurs mostly in Asia, with a few in Africa. According to Dressler (1981) this subfamily is characterized by stipes, dorsoventrally flattened pollinia, reduced anther partitions, and by operculate anthers that do not bend during their development. The vandoid orchids have been considered to be derived from

epidendroid ancestors. In the family Orchidaceae, the vandoid orchids are generally considered as the most highly evolved group. Parallelisms make the tribal classification of this group difficult. Besides their main stem growing in a single direction, many of the species developed pseudo bulbs (i.e. bulge at the base of the stem). The striking characteristic of the Vandoids are a cellular pollinium stalk/stipe, superposed pollinia and the unique development of the incumbent anther that bends early in the development (http://www.en.wikipedia.org).

The following features have been considered characteristic for vandoid group (Dressler, 1989)

1. **Viscidium:** With exception to some few autogamous species, the vandoid orchids have definite viscidia (or detachable viscidia in the terminology of Rasmussen, 1982). This feature, however, has evolved independently in many epidendroid orchids, as well as Orchidoideae and Spiranthoideae.
2. **Superposed pollinia:** Vandoid orchids have either two or four pollinia. When there are four pollinia, they are usually superposed or flattened parallel with the surface of the clinandrium, with one member of each pair above the other. Again, superposed pollinia are found in other groups, such as *Coelogyne* and *Sobralia.* Further, the pollinia of *Polystachya* may not be fu1ly superposed in the anther, though they spread upon removal, and then appear superposed. In some cases, pollinia may be ovoid or globose, rather than flattened and superposed especially in smaller flowers.
3. **Stipe:** This is the feature most often considered diagnostic of the vandoid orchids. In most cases, the pollinia are attached to the viscidium by a flat strap, orstipe. This is formed by the epidermis (or epidermis and adjacent cell layers) of the column, and is termed as tegula by Rasmussen (1982) as contrasted with a

hamulus, or stipe formed by the apex of the rostellum). Though a tegular stipe is present in most vandoid orchids, there is only a large viscidium in *Cymbidium* (Seidenfaden, 1983) and in some species of Maxillaria. Also, adefinite regular stipe has evolved independently in some Goodyerinae (Spiranthoideae). De Vogel (1986) reports a tegular stipe in *Geesinkorchis* of the *Coelogyninae*. Most other reports of stipes in epidendroid orchids appear to be based on misinterpretations of caudicles, as in *Epidanthus* (Dressler, 1983), (Mansfeld, 1934). It should be noted that hamular stipes occur in some species of *Bulbophyllum,* and similar structures occur in *Sunipia* (Rasmussen, 1986).

4. **Reduced anther partitions:** In most vandoid orchids, the internal walls of the anther are much reduced. This feature appears to be related to the superposed pollinia, and is also found in *Coelogyne*.
5. **Anther development:** In most epidendroid orchids, the anther shows an obvious change in orientation during ontogeny. During the early stages of development the anther is erect or parallel with the Column axis; then the anther bends downward until it forms an angle of about 90 degrees with the axis of the column. The anther may continue to bend back, forming an acut angle with the column axis. Such movement is not macroscopically evident in the development of most vandoid orchids, and this was taken by Dressler (198I a) as a key feature to distinguish the subfamilies Epidendroideae and Vandoideae. Dressler thus classified *Calypso* in the Epidendroideae, though it is vandoid in its other features. Hirmer (1920) suggested that the vandoid anther bent downward at a very early stage of ontogeny. Now Kurzweil (1987) shows clearly that the vandoid anther bentd ownward. The bending usually occurs much earlier than in the epidendroid orchids, but there is a good deal of variation within the vandoid

orchids. The development of the anther does not distinguish the vandoid orchids from the epidendroid orchids.

6. **Lateral inflorescence:** A lateral inflorescence is sometimes cited as a feature of the vandoid orchids. However, terminal inflorescence are found in most polystachyinea, in Acrolophia, Cyanaeorchis, Galeandra, and often in Ansellia. Further, lateral inflorescence occurs in many epidendroid orchids, such as the Bletiinae. The inflorescences of the Bletiinae and many vandoid orchids are basal. It appears basal in plants with very short stems, as in *Phalaenopsis,* but appears upper lateral in Vandeae.

DNA BARCODING

DNA barcoding is a technique projected for rapid and reliable identification of an unknown biological sample using short DNA sequences (Hebert *et al.*, 2003a). The short sequence of DNA from standardized locus/loci either from nuclear or cytoplasmic genome or both are called DNA barcodes. This technique can be used for rapid identification and detection of species and relies on DNA sequence variation that provides a unique recognition tag to a species (Hebert *et al.*, 2003a, b). This technique has several advantages than the morphotaxonomic method of identification:

I. The species can be identified even if a small amount of its tissue/DNA is available (Singh *et al.*, 2012).

II. The amplification and direct PCR sequencing of short barcode sequence can be carried out across taxonomically diverse species using universal primers.

III. Identification at any stage of life (juvenile or mature) is possible (Gonzalez *et al.*, 2009).

IV. It does not require cloning and sequencing of complete gene, making it less laborious and time consuming.

Therefore, DNA barcoding is an additional taxonomic tool with high potential of reviving modern taxonomy (Schindel and Miller, 2005). DNA barcoding started with the work of Prof. Paul Hebert at the University of Guelph in Ontario, Canada in 2003, who demonstrated that individual species from a collection of 200 closely allied species of lepidopteran using a short fragment (658 bp) could identified with 100% accuracy using mitochondrial gene Cytochrome c Oxidase subunit 1 (CO1) as a universal identification marker for species (Hebert *et al.,* 2003). The DNA sequence which was found to be applicable for barcoding animal species in their pioneering and subsequent studies was the "Folmer Region" at the 5' end of Cytochrome c Oxidase 1 (CO1), present in mitochondrial genome (Hebert *et al.,* 2003a, b, 2004a, b). Based on this initial success with animals, this region was projected as the locus that could provide unique recognition tags to all the organisms. Hebert *et al.* (2003b) argued that just 15 variable sites in COI could provide one billion combinations (4^{15}) of bases giving more than enough possible barcode 'patterns' for estimated 10 million eukaryotic species estimated to be presentin the world. Subsequently, the CO1 region was successfully tested in other animal groups (Barrett and Hebert, 2005; Cywinska *et al.,* 2006; Clare *et al.,* 2007). On exploring DNA barcoding issues, one of the first conference held was the DNA taxonomy workshop at Dentsche Staatssammlung in Munich during April 2002, funded by German Science Association with the participation of some 100 Scientists mainly from European countries (Teutz *et al.,* 2002) focusing mainly on the most useful marker for DNA taxonomy (i.e., universal DNA-base classification system to all organismal group) (Teutz *et al.,* 2003) and the implication for nomenclature (Minelli, 2003). Recognizing the potential approach of DNA barcoding, Alfred P. Sloan foundation funded two meeting at cold spring Harber

in March and September 2003. From these meeting came the idea that major natural museum should take the lead in connecting diagnostic DNA sequence both in specimen collection and existing taxonomic system i.e., Linnean system. In May 2004, the Sloan foundation decided to establish a secretariat for the "Barcode of Life" based at Smithsonians natural museum of natural history in Washington, DC, USA. Realizing the importance of this technology, an international "Consortium for the Barcode of Life" (CBOL) was established in 2004. It comprises of more than 170 member organizations from more than 45 countries (http://barcoding.si.edu). These CBOL's is an International initiative with a mission dedicated to supporting the development of DNA barcoding as a global standard for species identification, through rapid compilation of high quality DNA barcodes in a public library of DNA sequences (http://barcoding.si.edu) and later it was joined by many natural history museums and herbaria, research organization and private partners (www.barcoding.si.edu). Another organization, International Barcode of Life (iBOL), based at Guelph, Ontario in fact, is involved in barcoding of different group of plants and animals (http://ibol.org). Its aim is to generate DNA Barcodes for 5 million specimens and 500,000 estimated species existing on planet earth by 2015 (http://ibol.org). In 2005, the National Centre for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) sealed a partnership with CBOL where by barcode standard DNA sequence and relevant data can be archived in gene bank. Since then more than 200 organizations from more than 50 countries have joined CBOL and agreed to put their barcode data in a public database. In late 2005 CBOLs plant working group proposed *matK, rbcL* as the standard barcode region for land plants.

DNA barcodes besides helping Taxonomists in rapid identification of new/cryptic and polymorphic species (Lahaye *et al.*, 2008; Miwa *et al.*, 2009; Xiao *et al.*, 2010), it is also a powerful diagnostic tool in the hands of law enforcement agencies for checking

illegal trade of rare, endemic and endangered plants and animals species i.e., biopiracy (Eaton *et al.*, 2010; Jeanson *et al.*, 2011; Muellner *et al.*, 2011; Yesson *et al.*, 2011) and an excellent investigative tool for forensic specialists/experts (Coyle *et al.*, 2005; Ferri *et al.*, 2009). Moreover, it also helps in - (i) identifying invasive species right at quarantine stage (Bleeker *et al.*, 2008; van de Wiel *et al.*, 2009), (ii) authentication of herbal medicine and their adulterants (Yao *et al.*, 2009; Asahina *et al.*, 2010; Chen *et al.*, 2010; Srirama *et al.*, 2010), (iii) identifying complex food webs by studying species diversity in gut contents of animals (Soininen *et al.*, 2009), (iv) analyzing herbivore's diet components (Valentini *et al.*, 2009), (v) checking adulterations and substitutions in food products (Jaakola *et al.*, 2010) and (vi) determining the constituent plant species in different honey samples (Valentini *et al.*, 2010). Another breakthrough of DNA barcoding is that as opposed to the morphotaxonomic methods which require whole plant preferably in flowering/reproductive stage especially in plants for its authentic identification, DNA barcodes once standardized can identify the species even if a minute amount of tissue/fragment is available (Singh *et al.*, 2012). DNA barcodes could also act as genetic resource tags and in turn would of help in conservation of genetic diversity (Eaton *et al.*, 2010; Jeanson *et al.*, 2011; Muellner *et al.*, 2011; Yesson *et al.*, 2011). DNA barcoding could also hasten the process of biodiversity inventorization and analysis (Costion *et al.*, 2011) using still smaller fragments of DNA aptly called as minibarcodes (Meusnier *et al.*, 2008). DNA barcoding could also play a significant role in forest biosecurity and bio surveillance of habitats by identifying non-indigenous species from the native species (Armstrong and Ball, 2005; Humble and deWaard, 2010). The term 'Palaeobarcoding' coined for DNA barcoding is used for studying the effect of climate change on biotic diversity. To meet this objective, DNA in permafrost or sedimentary DNA (seda-DNA) is analyzed using metabarcoding (circumventing the need of cells or

tissues) technique and correlating it with the known temporal climatic changes that had taken place (Jørgensen *et al.*, 2011; Andersen *et al.*, 2011). Although, in animals, the mitochondrial gene region 'COI' with the requisite divergence and universality was found to be suitable for species distinction, However, in plants no such region of genome, cytoplasmic or nuclear, could be identified or found not suitable for most species, except a few of macro-algae. Rather, its search was compared with that for the "Holy Grail" (Rubinoff *et al.*, 2006). The plant mitochondrial genes with low nucleotide substitutions and low evolutionary rates were considered unsuitable for plants barcodes (Chase *et al.*, 2005; Kress *et al.*, 2005; Newmaster *et al.*, 2006). Therefore, nuclear and plastid genes have been the prime focus of research for the identification of the locus/loci which could become species level molecular tag/signatures for the plants.

The Potential Barcode Candidates

The loci tested for DNA Barcoding of plants from chloroplast genome include coding *Rubisco large subunit* (*rbcL),RNA polymerase-β subunit* (*rpoB*), *RNA polymerase-β' subunit* (*rpoC1*) and *maturase K* (*matK*) and non-coding *psbA-trnH* and nuclear ribosomal cistron Internal Transcribed Spacers (nrITS) from nuclear genome. The studies/investigation proved that the chloroplast genome showed high degree of variation and discrimination ability (Lahaye *et al.*, 2008). The slow rate of their substitution limits the ability to identify plants among the species (Baldwin *et al.*, 1995). The existing plant barcodes are shown in figure 1.1.

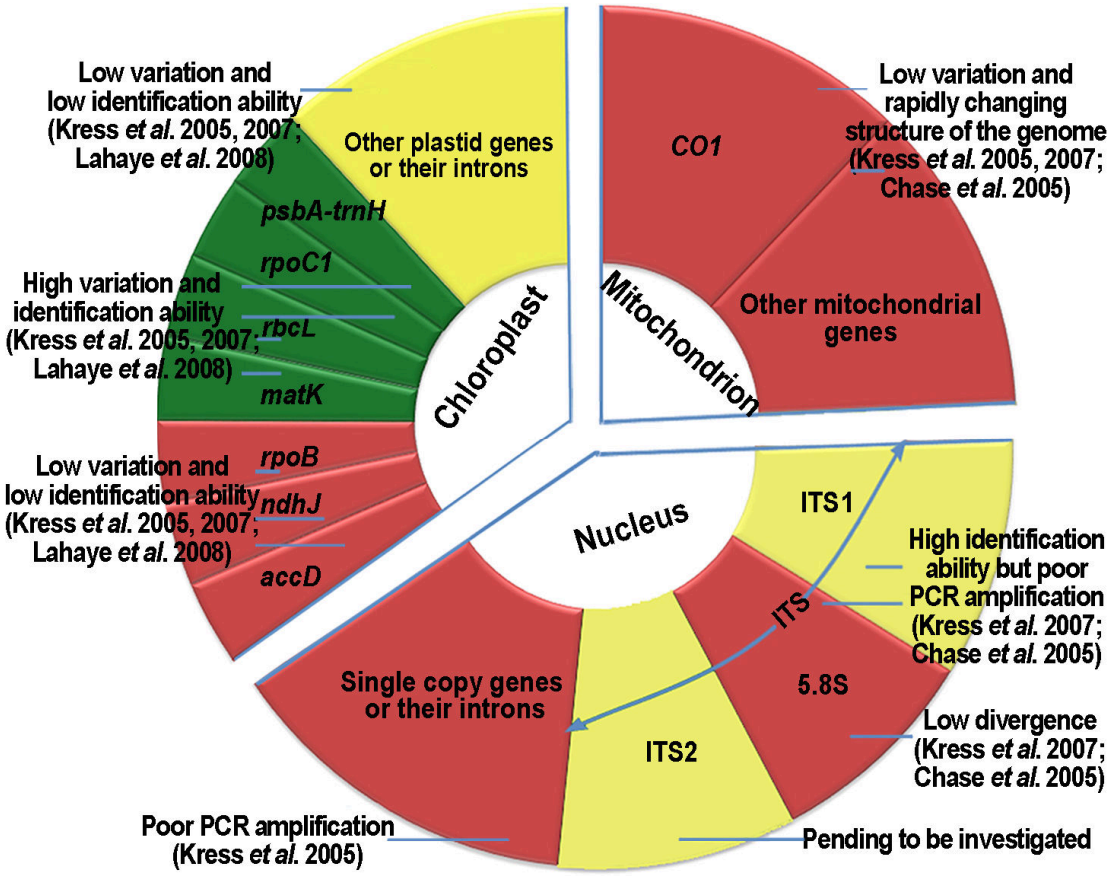


Figure 1.1: Existing barcode candidates for the Plant Kingdom (Source: Chen *et al.*, 2010)

Sequences from Chloroplast Genome

The chloroplast genome shares several attributes of mitochondrial genomes such as conserved gene order, high copy number per cell, amenability to PCR amplification and availability of universal primers. Hence, chloroplast genes could be considered as analogous to the mitochondrial gene that has been used for DNA barcoding in animals (Vijayan and Tsou, 2010). The chloroplastic genes have slower rate of evolution in plants as compared to mtDNA genes in animals, therefore finding suitable gene sequences with sufficient species discriminatory power is a great challenge. Moreover, due to the nature of uniparental inheritance, non recombination and structural stability in both the genic and intergenic regions of the chloroplast, many genes have been examined carefully for

their potentiality to test as barcodes in plants (Baldwin *et al.*, 1995; Goldman *et al.*, 1983). The chloroplast genome of higher plants has a circular structure which has a size of 120–160 k base pair (bp) (Figure 1.2).

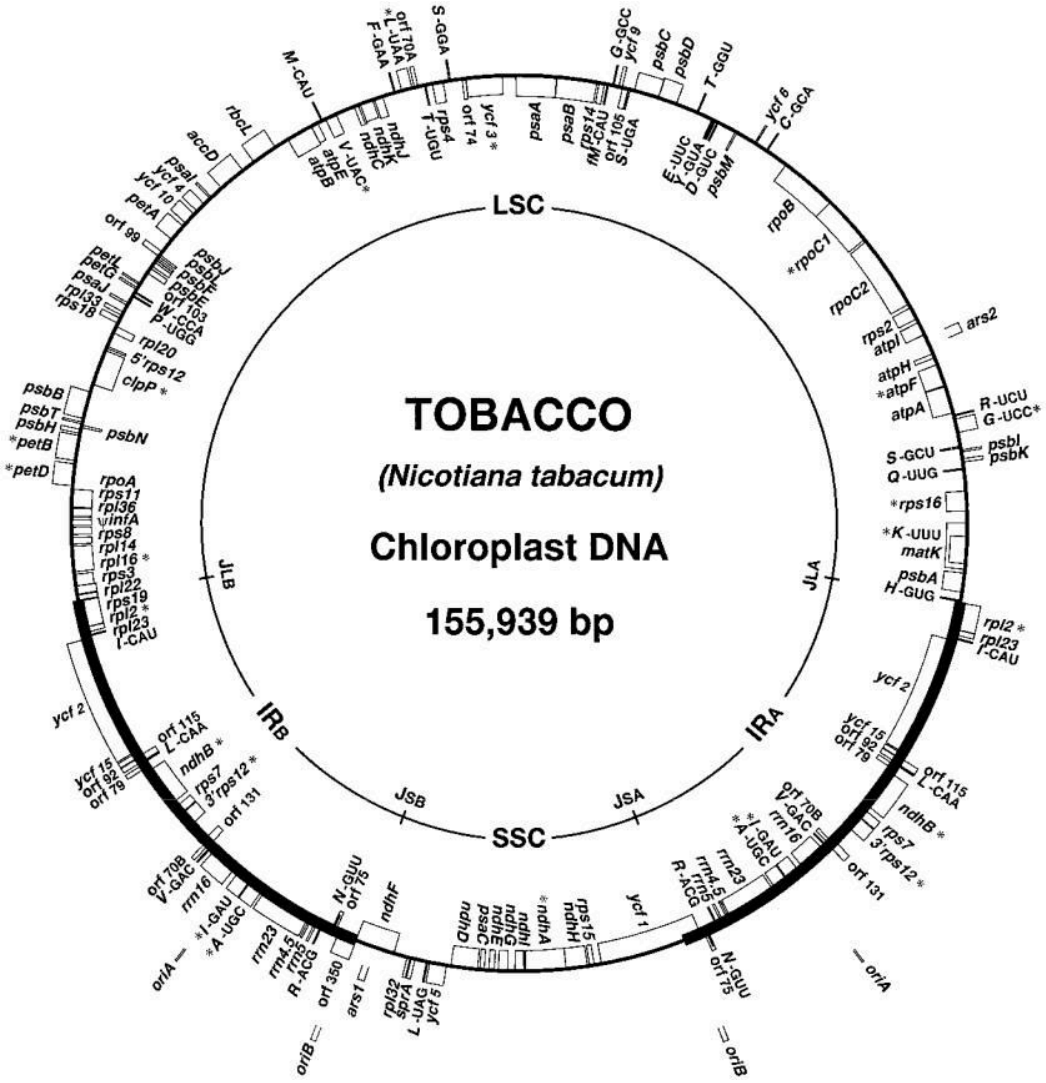


Figure 1.2: Gene map of tobacco chloroplast DNA (Source: Sugita *et al*., 1998)

It is represented by a large single copy (LSC) and a small single-copy (SSC) regions intervened by two copies of a large inverted repeat (IRa and IRb). The chloroplast genome contains all the rRNA genes (four genes in higher plants), tRNA genes (35 genes) and other genes for those proteins synthesized in the chloroplast (~100 genes) that are essential for its existence (Shinozaki *et al.*, 1986). On the basis of the considerable amount of information available from phylogenetic studies and recent testing with limited number of taxa, potentially useful genic and intergenic loci were initially selected as potential candidates for testing as barcodes for the land plants (Table 1.1). For testing the efficacy of these sequences as barcodes for plants, it has been examined individually or/and in combination with other loci on a large number of samples from a wide range of species covering all the major taxonomic lineages. The following loci were proposed by different investigating groups. Primers suitable for these genes are available in Table 1.1.

Table 1.1: List of some primers used for amplification of potential candidates for DNA barcoding of plants

Locus	Primer Name	Direction	Sequence 5′ → 3′	Reference
matK	2.1	Fwd→	CCTATCCATCTGGAAATCTTAG	(Kew website)
	2.1a	Fwd→	ATCCATCTGGAAATCTTAGTTC	(Kew website)
	5	Rev←	GTTCTAGCACAAGAAAGTCG	(Kew website)
	3.2	Rev←	CTTCCTCTGTAAAGAATTC	(Kew website)
	390F	Fwd→	CGATCTATTCATTCAATATTTC	Cuénoud et al. (2002)
	1326R	Rev←	TCTAGCACACGAAAGTCGAAGT	Cuénoud et al. (2002)
	matK_1	Fwd→	GAACTCGTCGGATGGAGTG	Wang et al. (1999)
	matK_1	Rev←	GAGAAATCTTTTTCATTACTACAGTG	Wang et al. (1999)
	matK_2	Fwd→	CGTACTTTTATGTTTACAGGCTAA	Wang et al. (1999)
	matK_2	Rev←	TAAACGATCCTCTCATTCACGA	Wang et al. (1999)
rbcL	1f	Fwd→	ATGTCACCACAAACAGAAAC	Lledó et al. (1998)
	724r	Rev←	TCGCATGTACCTGCAGTAGC	Lledó et al. (1998)

	a_f	Fwd→	ATGTCACCACAAACAGAGACTAAAGC	Kress & Erickson (2007)
	a_r	Rev←	CTTCTGCTACAAATAAGAATCGATCTC	Kress & Erickson (2007)
rpoB	rpoB 1	Fwd→	AAGTGCATTGTTGGAACTGG	(Kew website)
	rpoB 2	Fwd→	ATGCAACGTCAAGCAGTTCC	(Kew website)
	rpoB 3	Rev←	CCGTATGTGAAAAGAAGTATA	(Kew website)
	rpoB 4	Rev←	GATCCCAGCATCACAATTCC	(Kew website)
rpoC1	rpoC11	Fwd→	GTGGATACACTTCTTGATAATGG	(Kew website)
	rpoC12	Fwd→	GGCAAAGAGGGAAGATTTCG	(Kew website)
	rpoC13	Rev←	TGAGAAAACATAAGTAAACGGGC	(Kew website)
	rpoC14	Rev←	CCATAAGCATATCTTGAGTTGG	(Kew website)
accD	accD 1	Fwd→	AGTATGGGATCCGTAGTAGG	(Kew website)
	accD 2	Fwd→	GGRGCACGTATGCAAGAAGG	(Kew website)
	accD 3	Rev←	TTTAAAGGATTACGTGGTAC	(Kew website)
	accD 4	Rev←	TCTTTTACCCGCAAATGCAAT	(Kew website)
trnH-psbA	trnHf	Fwd→	CGCGCATGGTGGATTCACAATCC	Tate & Simpson (2003)
	psbA3f	Rev←	GTTATGCATGAACGTAATGCTC	Sang et al. (1997)
Ycf5	Ycf5 1	Fwd→	GGATTATTAGTCACTCGTTGG	(Kew website)
	Ycf5 2	Fwd→	ACTTTAGAGCATATATTAACTC	(Kew website)
	Ycf5 3	Rev←	ACTTACGTGCATCATTAACCA	(Kew website)
	Ycf5 4	Rev←	CCCAATACCATCATACTTAC	(Kew website)
ndhJ	ndhJ 1	Fwd→	CATAGATCTTTGGGCTTYGA	(Kew website)
	ndhJ 2	Fwd→	TTGGGCTTCGATTACCAAGG	(Kew website)
	ndhJ 3	Rev←	ATAATCCTTACGTAAGGGCC	(Kew website)
	ndhJ 4	Rev←	TCAATGAGCATCTTGTATTTC	(Kew website)
atpF-H	atpF	Fwd→	ACTCGCACACACTCCCTTTCC	Hollingsworth et al. (2009)
	atpH	Rev←	GCTTTTATGGAAGCTTTAACAAT	Hollingsworth et al. (2009)
psbK-I	psbK	Fwd→	TTAGCCTTTGTTTGGCAAG	Hollingsworth et al. (2009)
	psbI	Rev←	AGAGTTTGAGAGTAAGCAT	Hollingsworth et al. (2009)
trnL intron	trnL c	Fwd→	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)
	trnL d	Rev←	GGGGATAGAGGGACTTGAAC	Taberlet et al. (1991)

trnL-F	trnL c	Fwd→	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)
	trnL d	Rev←	GGGGATAGAGGGACTTGAAC	Taberlet et al. (1991)
	trnL e	Fwd→	GGTTCAAGTCCCTCTATCCC	Taberlet et al. (1991)
	trnL f	Rev←	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)
6 loop of nL intron	trnL g	Fwd→	GGGCAATCCTGAGCCAA	Taberlet et al. (1991)
	trnL h	Rev←	CCATTGAGTCTCTGCACCTATC	Taberlet et al. (1991)
ITS	ITS5a	Fwd→	CCTTATCATTTAGAGGAAGGAG	Stanford et al. (2007)
	ITS4	Rev←	TCCTCCGCTTATTGATATGC	White et al. (1990)
	IT1	Fwd→	TCGTAACAAGGTTTCCGTAGGT	Tsai et al. (2004)
	IT2	Rev←	GTAAGTTTCTTCTCCTCCGCT	Tsai et al. (2004)
ITS2	S2F	Fwd→	ATGCGATACTTGGTGTGAAT	Chen et al. (2010)
	S3R	Rev←	GACGCTTCTCCAGACTACAAT	Chen et al. (2010)

Maturase K (matK)

Among the chloroplast genes, *matK* is one of the most rapidly evolving genes and have been reported to have a high evolutionary rate (Yamane *et al.*, 2003). It is a type II intron present in the gene trnK that codes transfer RNA for lysine, having a length of about 1550 bp which encodes for an enzyme known as maturase. Since *matK* is embedded in the group II intron of the lysine gene *trnK* that flank matK on both sides (Jonson and Soltis, 1995)it can be easily PCR-amplified with a primer set designed from the conserved regions of the genes *trnK*, *rps16* and *psbA*. *matK* has been used as a marker to construct plant phylogenies because of its rapid evolution and the ubiquitous presence in plants (Hilu and Liang, 1997; Kelchner, 2000). However, failure of PCR amplification for *matK* in some taxonomic groups was also reported (Wolfe *et al.*, 1987). In order to overcome this problem, new sets of primers were developed, which work well in most of the major taxonomic groups. Most important and common primers developed are presented in Table 1.1). This primer set amplifies a DNA fragment of ~930 bp between positions 429 and 1313 of the *matK* sequence was developed (Schmitz-Linneweber *et al.*,

2001; Cuénoud *et al.*, 2002). Phylogenetically, the rate of evolution of *matK* was found suitable for resolving intergeneric as well as interspecies relationships in many angiosperms (Johnson and Soltis, 1995; Soltis and Soltis, 1998). Considering the high evolutionary rate of *matK*, it has been tested by several workers for suitability as a plant barcode and has been proposed either alone or in combination with other loci (Kress *et al.*, 2005; Chase *et al.*, 2007; Kress and Erickson, 2007; Sass *et al.*, 2007; Fazekas *et al.*, 2008; Lahaye *et al.*, 2008a; Newmaster *et al.*, 2008; CBOL Plant Working Group, 2009; Gonzalez *et al.*, 2009; Hollingsworth *et al.*, 2009; Kress *et al.*, 2009; Newmaster and Ragupathy, 2009; Parveen *et al.*, 2012). Chase *et al.*, (2007) include this locus and proposed three-locus combinations *viz*., *rpo*C1+*rpo*B+*matK* and *rpo*C1+*matK*+*psb*A-*trnH* for barcoding of plants. Fazekas *et al.* (2008) also suggested the inclusion of *matK* as multi-locus (*matK*+*atp*F-*atp*H+*psb*K-*psb*I) and observed that 69% species monopoly with the combination. Lahaye *et al.*, (2008) reported that *matK* alone or in combination with *trnH-psbA* could correctly identify >90% of the invested species while studying more than 1036 species of Mesoamerican orchids for checking the suitability of matK. Ford *et al.* (2009), after testing *matK* along with 12 other cpDNA loci in 98 land plant taxa, proposed a combination of *rpoC1*+*rpoB*+*matK* as the most promising combination for barcoding of land plants as in consensus with Chase *et al.* (2007). Recently, Starr *et al.* (2009), after testing *matK*, *rbcL*, *rpo*B, *rpo*C1and *trn*H–*psb*Aas barcodes in Cyperaceae, also advocated the use of *matK* alone as a universal barcode for land plants. Kelly *et al.* (2010) analyzed involving 23 individuals belonging to 11 species of the family Podostemaceae from Africa and recommended the use of *matK* as a core DNA barcode. The CBOL– Plant Working Group (2009) also tested *matK* in nearly 550 plant species and found that nearly 90% of the angiosperm samples were easily amplified and sequenced using a single primer pair, though the success rate was limited to 83% in

gymnosperms and much worse in cryptogams (10%). Because of this high universality and species discrimination, The CBOL–Plant Working Group recommended *matK* in combination with *rbcL* as the standard two-locus barcode for plants because of high universality and species discrimination.

Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RUBISCO, rbcL)

Among the plant genes, *rbc*L was the first characterized gene sequence. It encodes the large subunit of rubilose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), a critical photosynthetic enzyme. *rbcL*has been used so extensively in plant phylogenetic studies that more than 10,000 *rbcL* sequences are already available in GenBank (Newmaster *et al.*, 2006; Chase *et al.*, 2007). Various aspects of the molecular evolution of *rbcL*have also been studied in detail because of this wide utility (Albert *et al.*, 1994). Most of the phylogenetic studies suggest that *rbcL*is best suited to reconstruct the relationships down to the generic levels, but less effective at the species levels (Soltis and Soltis, 1998). Furthermore, in order to obtain enough species discrimination, the entire ~1430 bp needs to be sequenced, which acts as a limiting factor for its use as a barcoding sequence because an ideal DNA barcoding region should be short enough to amplify from degraded DNA and analysed via single-pass sequencing (Chase *et al.*, 2007). One probable solution for this was to amplify short sequences with enough variability. Accordingly for most of the taxa, primers for PCR amplification and sequencing for such short sequence within the *rbcL* gene have been developed (Fay *et al.*, 1997; Kress *et al.*, 2007). Owing to the ease in PCR amplification across a wide range of plant groups and the availability of sequence information in many plant groups, the CBOL Plant Working Group, 2009 has recently recognized *rbc*Las one of the most potential gene sequences for DNA barcoding in plants (Vijayan and Tsou, 2010). However, because of the low species discrimination, most of the investigating groups are of the opinion that *rbcL*should be

used in conjunction with other markers (CBOL–Plant Working Group, 2009; Chase *et al.*, 2007; Hollingsworth *et al.*, 2009; Soltis and Soltis, 1998). Therefore, the CBOL–Plant Working Group (2009) recommended a combination of *rbcL*and *matK*as the standard two-locus barcode for plants, because this combination of genes appears to be a pragmatic solution to a complex trade-off among universality, sequence quality discrimination and cost. This potential barcode candidate seems to stands next to the *matK* in the identification efficiency.

Nuclear Genome Sequence

Unlike chloroplast genome, till date internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) has been the only nuclear DNA that have been tested for suitability as barcodes in plants, although barcoding based on biparentally inherited nuclear DNA segment is expected to provide more information on species identity, including hybridization events, introgression and aneuploidy (Chase *et al.*, 2007; Kress *et al.*, 2007; Sass *et al.*, 2007). The possible major reasons why limited numbers of genes are being tested especially from degraded and low-quality DNA and the low species discriminatory power due to conservation of functional genes across large lineages could be due to difficulty in obtaining high universality of the PCR amplification of single or low-copy genes (Vijayan and Tsou, 2010).

Internal Transcribed Spacer Regions of Nuclear Ribosomal Cistron (nrITS)

The Internal Transcribed Spacer (ITS), a collective name for ITS1 and ITS2 of the Nuclear Ribosomal Cistron has been the most favorite locus to be sequenced by the plant molecular biologist for studying the phylogeny at species level (Alvarez and Wendel, 2003).

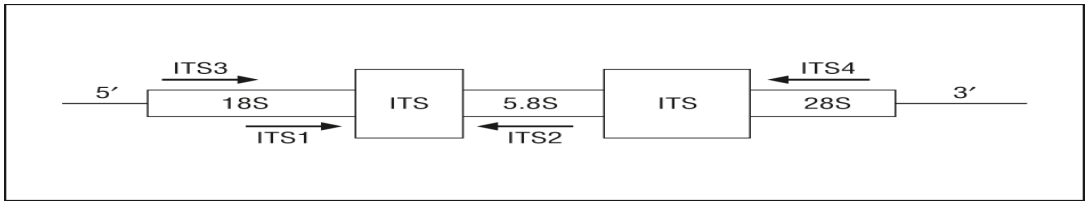


Figure 1.3:ITS Regions of Nuclear Ribosomal Cistron (nrITS) (Source: Yang *et al*., 2007)

The rDNA cistron is a multigene family encoding the nucleic acid core of the ribosome. The cistron contains genes for 18S rRNA and 26S rRNA, separated by two internal transcribed spacers (ITS1 and ITS2) having 5.8S rRNA gene in between (Figure 1.3). Within the cell, the rDNA is arranged as tandemly repeated units, reiterated thousands of times and are organized into large blocks in the chromosome called the nucleolar organizer regions (NOR) (Appels and Honeycutt, 1986; Hemleben *et al.*, 1988). One of the most remarkable features of the rDNA is that the individual unit of this multiple gene family does not evolve independently; instead all the units evolve in a concerted manner such that higher level of overall sequence homogeneity exists among copies of the rDNA within a species, but differs among different species (Hershkovitz and Zimmer, 1996; Brown *et al.*, 1972). This type of evolution is termed as horizontal or co-incidental or concerted evolution (Hershkovitz and Zimmer, 1996; Hood *et al.*, 1975), which involves unequal crossing over and gene conversions. Currently, *nr*ITS is considered as one of the most useful phylogenetic markers for both plants and animals, because of its ubiquitous nature, biparental inheritance, and comparatively higher evolutionary changes due to less functional constraints (Zimmer *et al.*, 1980; Baldwin *et al.*, 1995; Alvarez and Wendel, 2003; Rogers and Bendich, 1987). Likewise, species-level discrimination and technical ease have also contributed to its wider acceptability as a powerful phylogenetic marker. Another advantage is that the ITS1 and ITS2 regions can be PCR-amplified separately by anchoring primers in the conserved coding genes. This

facilitates easy amplification of ITS even from poor quality or degraded DNA (Kress *et al.*, 2005). However, some of the recent reports in tree plants and asexually propagated plants revealed the presence of some degree of intra-individual variations among the copiesof ITS1 and ITS2 sequences (Buckler *et al.*, 1997; Campbell *et al.*, 1997; Muir *et al.*, 2001; Bailey *et al.*, 2003; Feliner and Rosselló, 2007; Vijayan *et al.*, 2009). The reasons for such observed variations could be such as high mutation rate, recent hybridization, lineage sorting, recombination among copies, and pseudogene formation of cistrons (Zimmer *et al.*, 1980; Rogers and Bendich, 1987; Wendel *et al.*, 1995; Buckler *et al.*, 1997; Alvarez and Wendel, 2003). Nevertheless, nrITSis still considered to be a powerful phylogenetic tool at the species level (Vijayan *et al.*, 2009). Kress *et al.* (2005) when initially tested for its suitability as barcode in plants, ITSalong with nine other loci from the chloroplast genome, showed better universality (88%) and species discrimination than the chloroplast loci. Considering the availability of universal primers, presence of multiple copies in cells, easy retrieval of amplicons and high quality bidirectional sequences and a high resolution at species level and good species discriminatory power, ITSwas proposed as a potential candidate for barcoding in plants (Kress *et al.*, 2005; Sass *et al.*, 2007). Later, Hollingsworth *et al.* (2009) endorsed earlier view by suggesting that ITScan be considered for barcoding of species that have limited variations in the plastid genome. However, CBOL-Plant Working Group (2009) did not recognize ITS as an appropriate locus for barcoding species due to the problems arising from paralogous sequences (Alvarez and Wendel, 2003), pseudogenes (Bailey *et al.*, 2003) and intragenomic variability and difficulties in direct sequencing of PCR products, but as a supplementary barcode, where the loci from the chloroplast genome fail to resolve the species in question and the direct PCR product sequencing of ITS is possible. It is easier to recognize the amplicons and sequence it in both the directions as the length

of ITS2 is more conserved as compared to ITS1 among plants (Chen *et al.*, 2010). However, the number of informative sites available for identifications is reduced if ITS2 alone is used. Recently, China Plant Barcode of Life (China Plant BOL) group (2011) presented the inclusion of ITS in the core DNA barcode along with matK and rbcL, based on their study involving 6286 individuals belonging to 1757 species from 141 genera across 75 families of seed plants. They reported that ITS in combination with any one of the plastid DNA markers (*matK,rbcL* or *trnH-psbA*) could achieve 69.9-79.1% species resolution, which was higher than 49.7% resolution by the already proposed core DNA barcode for plants (*matK*+*rbcL*) (Babbar *et al.*, 2012).

The family Orchidaceae is one of the largest and highly evolved families of angiosperms (Chase, 2005). The estimated number of orchid species existing in India varies from 1,141 (Kumar and Manilal, 1994) to 1,600 (Medhi and Chakrabarti, 2009) to 1256 species in 155 genera of which 307 species are endemic (Singh *et al.*, 2019) which include commerciallyimportant species like *Arachnis*, *Cattleya*, *Phalaenopsis*, *Cymbidium*, *Dendrobium*, *Paphiopedilum*, *Vanda* and *Renanthera*. The orchids have taken a significant position in cut flower industry due to its attractiveness, diversity in forms, shape and colour, high productivity, right season of bloom, easy in packing and transportation. Indian orchid species having high ornamental values mention may be are *Aerides multiflorum, Aerides odoratum, Arundina graminifolia, Arachnis,Bulbophyllum, Calanthe masuca, Coelogyne elata, Coelogyne flavida, C. corymbosa, Cymbidium aloifolium, C.lowianum, C. devonianum, C. hookerianum, C. lancifolium, Dendrobium aphyllum, D. nobile, D. chrysanthum, D.farmeri, D. chrysanthum, D. farmeri, D. densiflorum, D. moschatum, D. fimbriatum, D. jenkinsii, Paphiopedilumvenustum, P. spicerianum, P. hirsutissimum, P. insigne, Phaius wallichii, Pleione praecox, Renanthera imschootiana, Rhyncostylis retusa, Thunia alba, Vanda cristata, Vanda caerulea* and

Vanda coerulescens (Lawler, 1984). The Himalayan region which is mainly a home to many orchids constitutes about 9% of the Indian flora which grow upto an elevation of 2000m from sea level and also scattered in Eastern and Western Ghats which harbour the small flowered orchids (http://www.orchidsasia.com). Orchids are generally considered promiscuous in nature as the reproductive isolation is mainly based on the specificity of the pollinator (Cameron, 2004). Because of this feature, it was envisaged that orchids would offer a stringent test to DNA barcoding concept in general and selected regions in particular. All orchid species with charismatic ornamental flowers and therapeutic properties are highly endangered due to their over exploitation and are thus listed in Appendix II of the Convention of International Trade in Endangered species of Fauna and Flora (CITES); some are even listed in Appendix I (http://www.cites.org; Sun *et al.*, 2011). This implies that trade of all orchids, could be undertaken only through export permits, while the trade of those listed under Appendix I is totally prohibited (http://www.cites.org). The export of orchids collected from wild is banned (http://www.cites.org). Despite these well regulated rules, orchids are still illegally traded using their parts or even fragments, which cannot be identified using traditional taxonomic methods. Orchidaceae species constituting a priceless genetic resource are common targets for conservation both *in situ* and *ex situ* in the national parks, germplasm banks and botanical gardens (http://www.sfri.org/orchidology.htm). In order to conserve these orchid species and to curb the illicit trade practices of biopiracy, special identification techniques are required which could distinguish these rare/endangered orchid species from other plants even if a part or fragment is used. DNA barcodes of such orchids once available could become powerful tools in the hands of the law enforcement agencies responsible for curbing such illegitimate practices (Parveen, 2012). Besides being used as identification tools, DNA barcodes of the endemic orchids could also serve

as genetic resource tags. Vandaceous orchids are the major ornamental crops as cut flower and potted plants as they have high demands in both National and International market (Lekawatana, 2010). They can be crossed within the same genus or with different genera leading to the production of various new hybrids having similar morphological traits. Thus, an alternative identification method for Vandaceous orchids is needed for variety and species certification and protection purposes (Peyachoknagul *et al.*, 2014). Therefore, the present investigation was initiated with the objectives:

1. Collection and documentation of wild Vandaceous orchids of Nagaland using conventional taxonomic tools and maintaining of these orchids in orchids house.
2. Use DNA sequence to identify species using different marker systems.
3. Explore and analyse the phylogenetics relationships between species.
4. To find common/universal barcodes for orchids.

CHAPTER - 2

DOCUMENTATION OF VANDACEOUS ORCHIDS

INTRODUCTION

The first historical account on the Orchidaceae of Nagaland can be traced back to 1889 during Charles Baron Clarke who was a British botanist wherein he reported to have collected 22 species of orchids under 18 genera. Before or until Independence of India, most of the plant collectors were Britishers. In 1837 William Griffith, a British doctor, naturalist, and botanist has made extensive collections on his way from Sadiya in Upper Assam to Upper Burma (Myanmar) through Assam Valley, Mishmi Hill (Arunachal Pradesh), Khasi Hills (Meghalaya) and Naga Hills (Nagaland). From 1885 onwards, C.B. Clark traveled the same route and collected about 1000 specimens and published an account in *J Linn Soc Lon* (Vol. 25, 1889) and for many years his collection remained the only source of information about the flora of Nagaland. Later J.D. Hooker in his publication *'Flora of British India'* Vols. 5 & 6 (1890), described about 507 species belonging to 85 genera of orchids from Eastern India and recorded 49 species under 24

genera of orchids including 1 (one) variety from Nagaland, after studying the collections of Collet, Griffith, Clarke including earlier/previous collections of Watt and Prain. King and Pantl (1898) reported 34 species under 18 genera, Burkill (1924) reported 10 species under 8 genera and Bor (1942) reported 8 species under 5 genera based on his own collection.

After Independence and with the reorganization of Botanical Survey of India (BSI) 1954 and establishment of Eastern Circle at Shillong on April 1956, several explorations were carried out in different parts of Nagaland by Dr. S.K. Kataki, Dr. C.L. Malhotra and C. Bahadur of the Botanical Survey of India, Shillong have made large collections and contributed towards the knowledge on the 'Orchidaceae of Nagaland'. Mitra (1958) reported 53 species under 26 genera and 1 variety, all of which were based on the collection of C.B. Clarke's, D. Prain's, G. Watt's, H. Collett's, W. Griffith's. Recent works on the Orchidaceae of Nagaland include those of Hynniewta (1984) recorded 59 genera, 238 species, and 4 varieties from Nagaland. Chankija *et al.* (1992) recorded 360 species under 85 genera, Deorani and Naithani (1995) recorded 238 species, Hynniewta *et al.* (2000) recorded 241 species under 63 genera including one endemic species i.e.,*Coelogyne hitendrae* Das and Jain, Deb and Imchen (2008) recorded 396 species of 92 genera and Jakha and Deb recorded 180 species belonging to 58 genera from the study of three districts of Nagaland *viz.*, Kiphre, Tuensang and Zunheboto bringing the total diversity of orchid taxa to about 429 species and 101 genera for the state. In Nagaland, the Vandaceous group of orchids has over 60 species under 22 genera (Deb and Imchen, 2008). Some of the commercially important Vandaceous orchid genera are *Vanda, Esmeralda, Cleisostoma, Papilionanthe, Phalaenopsis, Rhynchostylis, Arachnis, Aerides, Gastrochilus, Vandopsis* and *Renanthera*.

MATERIALS AND METHODS

Brief Account of the Survey Area

Nagaland with a total geographic area of 16,579 sq. km. is one of the 8th North-Eastern states of India which was formerly known as Naga Hills districts of Assam and Tuensang of 'North-East Frontier Agency' (NEFA). It was formed as the 16th state under the Union of India on 1st December 1963 and lies between 25˚60′N latitude and 93˚20′E and 95˚15′ E longitude. The state is located in the Indo-Burma hotspot belt and surrounded by Arunachal Pradesh and a part of Assam in the North, Myanmar (Burma) in the East, Manipur in the south and Assam in the west (Figure Map of Nagaland). The state comprises of 12 administered districts *viz*., Dimapur, Kipheri, Kohima, Longleng, Mokokchung, Mon, Noklak, Peren, Phek, Tuensang, Wokha and Zunhebhoto with its capital/headquarter at Kohima.

Nagaland has a typical monsoon climate. The seasons of the year can be divided into 4 (four) *viz*., winter (December-February), pre-monsoon (March-April), monsoon (May-September) and retreating monsoon (October-September). The average annual rainfall is recorded to be between 2000-2500 mm. The temperature of the state ranges from 16-37˚C during summer and drops as low as 4˚C during winter. As per Naithani (2011), the state forest and vegetation has broadly classified based on climatic and altitudes as Tropical, Sub-tropical, Temperate and Sub Alpine/Alpine vegetation.

1. Tropical vegetation – Moist evergreen forest, moist deciduous forest (up to 1000 m).

2. Sub- Tropical vegetation – Evergreen forest, Semi-evergreen forests, Degraded bamboo forests (1000-1800 m).

3. Temperate vegetation – Broad-leaved evergreen forests, Pine forests, Rhododendron forests (1800-3000 m).

4. Sub-Alpine/Alpine vegetation – Rhododendron forests (up to 3848 m).

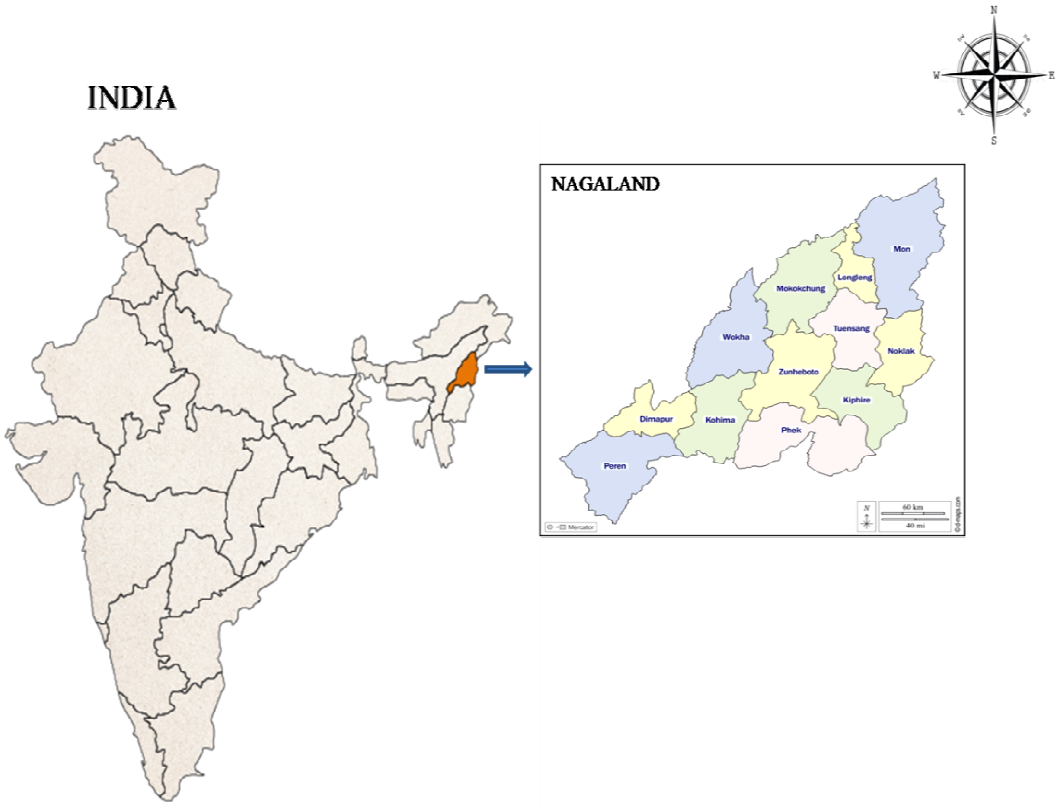


Figure 2.1: Shows the survey area for sample collection (Map not to scale)

Sample Collection

For documentation of Commercially Important Vandaceous Orchids of Nagaland regular field survey/exploration was carried out in different parts of the state in the forest areas of Dimapur, Kohima, Mokokchung, Peren, Phek, Tuensang, Wokha and Zunheboto districts from the year 2016–2019 covering all the seasons of the year i.e., spring, summer, autumn and winter to gather comprehensive information on the diversity of the orchids of the state of Nagaland. The sampled specimen were collected both in vegetative and flowering stage with global positioning system (GPS) location and photographs were taken. The collected specimens were identified after reviewing the available literature (taxonomic keys) and with the help of the experts. The specimens are brought under cultivation in the department orchidarium for further observation of flowering and other characteristics. All corresponding voucher samples were then deposited in the herbarium of the Department of Botany, Nagaland University, Lumami for future references.

Herbarium Preparation

Pressing and drying

The collected specimens were then treated/ soaked in 10% formaldehyde chemical and then pressed in wooden frames in between sheets of blotting paper and newspaper. The blotting paper/ newspaper were continuously changed until the specimens completely dried up. Fragile parts of the plants such as flowers were also preserved in 10% absolute alcohol for later dissection and observation.

Herbarium sheet preparation

The dried specimens were then poisoned by dipping in a saturated solution of mercuric chloride ($HgCl_2$) following (Jain and Rao, 1977) and then pressed in blotting paper. The dried specimens were then mounted on herbarium sheets of standard size 42 x 28 cm using adhesive (Fevicol) and then subsequently stitched. The standard herbarium

sheets were then labeled with details containing collection number, Date of collection, Family, Name of species, Locality of collection, Habitat, General description, Barcode and Name of collector. The herbarium sheets were then scanned with the department digital scanner and the digitized/scanned herbarium sheets were then kept it for future references. All the Herbarium sheets were then deposited in the herbarium of Department of Botany, Nagaland University, Lumami.

DOCUMENTATION

1. ***Acampe ochracea*** (Lindl.) Hochr. in Bull. New York Bot. Gard. 6:270. 1910; Hook. f., Fl. Brit. India 6:62. 1890; King & Pantl. in Ann. Ann. Roy. Gard. Calcutta 8:219, t. 292. 1898; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 39. 1998; Hynniewta, Kataki & Wadhwa. Orch Nagaland (BSI), 27. 2000; Sant. & kapad, Orch. Bombay, 234. 1966. *Saccolabium Ochraceum* Lindl. in Bot. Reg. Misc. 2. 1842. *Acampe dentate* Lindl., Fol. Orchid. *Acampe* 4:3, no. 8. 1853. *Acampe graffithi* Rchb. F., in Flora 55: 277. 1872.

Common English Name: The 'Ochre-Yellow Acampa'.

Stems upto 60-80 cm high, erect, stout. *Leaves* many, 10-20 x 1.5-2.5 cm, oblong, apex unequally and obtusely 2-lobed. *Inflorescence* 8-15 cm long, many-flowered, branched panicles. *Flowers* 1-1.2 cm across, pale yellow with irregular red streaks. *Dorsal sepals* obtuse, broadly oblanceolate; lateral sepals oblong, weakly falcate, obtuse. *Petals* spathulate, obtuse. *Lip* 3-lobed; lateral lobes erect, dentate below; mid-lobes fleshy, broadly oblong, obtuse, deflexed. *Spur* cylindric, curved, hirsute within.

Colour Plate 2.3: (A)&(B)

Flowering: January - February

Habitat and Ecology: Epiphytic and found growing on the tree trunk

Specimens examined: Lumami

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, West Bengal), Bhutan, Myanmar, Nepal, Vietnam.

Barcode: *matK* = MK160136, MW603187

rbcL = MN178485, MW603194

ITS = MW600257, MW600256, MN170563

2. ***Acampe praemorsa*** (Roxb.) Blatt. & McCann, J. Bombay Nat. Hist. Soc. 35: 495. 1932; Kumar et. Monilal, Cat. Ind. Orch. 63 (1994); Mishra, Orch. India. 280 (2007). *Acampe papillosa* (Lindl.) Lindl., Fol. Orchid. *Acampe* 4:2, nos.5. 1853; Pradhan, Indian Orchid 2:41; Hegde, Orchids of Arunachal Pradesh, 70. 1984; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh 524. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland 28. 2000. *Saccolabium papillosum* Lindl. in Bot. Reg. t. 1552. 1841; Hook. f., Fl. Brit. India 6: 63. 1890; King & Pantl., in Ann. Roy Bot. Gard. Calcutta. 8: 219. t. 290. 1898. *Gastrochilus papillosus* (Lindl.) O. Ktez., Rev. Gen. Pl. 2: 661. 1891.

Common English Name: The 'small Warty Acampe'.

Stems 20-30 cm long, stout, rigid. *Leaves* 7-15 x 1-1.2 cm, linear, conduplicate, apex truncate, sheathing at base. *Inflorescence* leaf opposite, 2-3 cm long, many flowered, sub-umbellate. *Flowers* 1.2 cm across, yellow, with brown stripes. *Dorsal sepals* oblong, apex spreading; lateral sepals oblong sub-acute apex, slightly falcate. *Petals* linear-oblong, sub-falcate, apex obtuse. *Lip* white with irregular transverse rose purple streaks, side lobes suppressed. *Spur* cylindrical, obtuse, hairy within.

Colour Plate 2.2: (A)&(B)

Flowering: October - December

Habitat and Ecology: Epiphytic and found growing on the main tree trunk.

Specimens examined: Jaluki

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, West Bengal), Bhutan, Myanmar, Nepal.

Barcode: *matK* = MK160137

rbcL = MK214919, MW603192

ITS = MN517126, MN170566

3. ***Acampe rigida*** (Buch.-Ham. ex Sm.) P.F.Hunt in kew Bull. 24:98. 1970; Hook. f., Fl. Brit. India 6:62. 1890; King Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 220, t. 292. 1898; Pradhan, Indian Orchid-II. 524. 1979; Kumar & Manilal, Cat. Ind. Orch. 63. 1994; Kataki, Orch. Meghalaya, 182. 1986; H.J. Chowdhery,Orch. Fl. Arunachal Pradesh 41. 1998; Hynniewta, Kataki & Wadha. Orch. Nagaland (BSI), 28. 2000. *Aerides rigida* Buch.-Ham. ex Sm. in A. Rees, Cycl. 39: 12. 1818. *Vanda multiflora*Lindl., Coll. Bot.: t. 38. 1826. *Vanda longifolia*Lindl., Gen. Sp. Orchid. Pl.: 215. 1833. *Acampe longifola* (Lindl.) Lindl., Fol. Orchid. 4: 1. 1853. *Acampe multiflora* (Lindl.) Lindl., Fol. Orchid. 4: 1. 1853. *Saccolobium longifolium* (Lindl.) Hook.f., Fl. Brit. India 6: 62. 1890.

Common English Name: The 'stiff Acampe'.

*Plants*50-90 cm long, stout, sheathed. *Leaves* 20-35 x 3-5 cm, oblong, apex unequally and obtusely 2-lobed. *Inflorescence* 10-15 cm long, many flowered, sub-corymbose, few branched. *Flowers* 1.5-1.8 cm across, slightly fragrant, yellow with purplish brown transverse stripes, lip creamish white, with purplish brown longitudinal stripes. *Dorsal sepals* obtuse, oblong; lateral sepals similar. *Petals* narrowly obovate, obtuse. *Lip* 0.5-1.0 x 0.3-0.4 cm, fleshy, 3-lobed; lateral lobes subquadrate; mid-lobe suberect, apex obtuse, slightly recurved. *Spur* conic, apex obtuse. *Column* 4 mm long, stout.

Colour Plate 2.2: (C)&(D)

Flowering: July - September

Habitat and Ecology: Epiphytic and found growing on tree branches.

Specimens examined: Jaluki

General distribution: India (Arunachal Pradesh, Assam, Meghalaya, Nagaland, Sikkim, West Bengal), Southern and East Africa, Sri Lanka, Burma, China, Thailand, Cambodia, Vietnam, Peninsular Malaysia.

Barcode: ***matK*** = MN523477, MW603189

rbcL = MG905912,MW603191

ITS = MN173056,MW617314

4. ***Aerides odorata*** Lour., Fl. Cochinch. 2: 525. 1790; Lindl. in Bot. Mag. t. 4139. 1845; Hook. f., Fl. Brit. India 6: 47. 1890; King & Pantl. in Ann. Roy. Bot Gard. Calcutta 8: 212. t. 282. 1898; Pradhan, Indian Orch. 2: 549. 1979; Hegde in Jour. Bombay Nat. History Soc. 82(2): 117. 1985; H.J. Chowdhery, Orchid Fl. Arunachal Pradesh 49. 1997; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI) 30. 2000. *Epidendrum odoratum* (Lour.) Poiret in Lamarck, Encycl. Suppl. 1:385. 1810. *Aerides cornutum*Roxb., Fl. Ind. 3: 472. 1832

Common English Name: The 'Fragrant Aerides'.

Plant erect to pendant, stem 10-30 cm long, branched. *Leaves* 20-30 x 2.2-3.0 cm, linear oblong, thickly leathery, fleshy, apex unequally and obtusely 2-lobed. *Inflorescence* 20-30 cm, many-flowered racemes, lax, drooping. *Flowers* 2.5 cm across, white, with pinkish or purple tinge, fragrant. *Dorsal sepals* oblong, obtuse; lateral sepals narrowly oblong, obtuse. *Lip* triangular, 3-lobed; mid lobe linear, erose at margins. *Spur* white, pale flushed, infundibuliform, incurved. *Column* white.

Colour Plate 2.13: (C)&(D)

Flowering: May - June

Habitat and Ecology: Epiphytic and found growing on the main tree trunks exposed to full sunlight.

Specimens examined: Lumami, Sema Settsu

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Orissa, Tamil Nadu, Uttar Pradesh, West Bengal, Bihar), Bangladesh, Bhutan, Borneo, Myanmar, Cambodia, Sri Lanka, Laos, Nepal, Vietnam.

Barcode: *matK* = MK064247, MW480552

rbcL = MN178484, MW480548

ITS = MG822846, MW599844

5. ***Arachnis labrosa*** (Lindl. & Paxton) Rchb.f., Bot. Centralbl. 28:343. 1886. ; Hook.f., Fl. Brit. India 6: 28. 1890; King & Pantl. In Ann. Roy. Bot. Gard. Calc. 8: 210 t. 280. 1898; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI). 47. 2000. *Arrhynchium labrosa* Lindl. & Paxton in Paxtons Fl. Gard.1.: 142. 1850. *Armadorum labrosa* (Lindl. & Paxt.) Schltr., in Fedde Repert. 10: 177. 1911. *Arachananthe bilinguis* Benth. in Benth. &Hook.f., Gen. Pl. 3: 573. 1883.

Common English Name: The 'Lip-like Arachnis'.

Plant 50-100 cm long, stem erect, cover by leaves sheaths, woody. *Leaves* 15-26 x 2-2.7 cm, linear-oblong, apex unequally and obtusely 2-lobed, sessile, bases overlapping. *Inflorescence* upto 40 cm long, axillary, arising from within leaf sheaths, 3-6 flowered. *Flowers* 3.5 cm across, pale-yellow with dark brown markings. *Dorsal sepal* oblong, subacute; lateral sepals oblong-lanceolate, acute. *Petals* oblong-spathulate, acute. *Lip* yellowish white, sagittate in general outline; side lobe short, round, mid lobe large, shortly clawed, apex rounded. *Spur* cylindric, obtuse, recurved. *Column* straight, whitish pink.

Colour Plate 2.1: (C)&(D)

Flowering: August - September

Habitat and Ecology: Epiphytic and found growing on the main tree trunk fully exposed to light.

Specimens examined: Dikhu, Doyang, Sema Settsu

General distribution: India (Arunachal Pradesh, Assam, Nagaland, Meghalaya, Sikkim), Bhutan, China, Vietnam.

Barcode: *matK* = MK064246, MW457584, MW603184

rbcL = MT227265, MW457587

ITS = MG820749, MW599843

6. **Esmeralda clarkei** Rchb.f. (***Arachnis clarkei*** (Rchb.f.) J.J.Sm.)in Gard. Chron. N.s. 26: 552. 1886. Type: Indian Himalayas, Clarke s.n., cult. Low (Holo. W! Herb. No. 3921, 3922); H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 387. 1998; Hook.f., Fl. Brit. India 6:28. 1890. *Vanda clarkei* (Rchb. F.) N.E. Brown in Bull. Misc. Inform. Kew 1888: 112. 1888. *Arachnanthe clarkei* (Rchb. f.) Rolfe in Gard. Chron. Ser.3, 4: 567. 1888. *Arachnis clarkei* (Rchb. f.) J.J. Smith in Natuurk. Tijdschr. Ned.- Indie. 72: 73. 1912.

Common English Name: The 'Clark's Arachnanthe'.

Plant upto 1m tall, stem erect, terete, cover with leaf sheaths. *Leaves* ligulate, unequally bilobed apically, 15-20 x1.5-3.5 cm, coriaceous. *Inflorescence* erect, 3-4 flowered, upto 30 cm long racemes. *Flowers* 5.5-7.5 cm across, yellow, transversely streaked with chestnut-brown, fleshy, fragrant. *Dorsal sepal* erect, oblong, obtuse; lateral sepal oblong, falcate. *Petals* falcate, oblong, obtuse. *Lip* free, pendent, 3-lobed; reniform midlobe and small erect side lobes, unspurred; disc with longitudinal keels and 2 raised calli in the centre of the lip. *Column* stout, clavate.

Colour Plate 2.1: (A)&(B)

Flowering: November - February

Habitat and Ecology: Epiphytic or lithophytic and found growing on tree trunk in shades.

Specimens examined: Tuensang, Meinkong

General distribution: India (Meghalaya, Nagaland, Sikkim, West Bengal), Nepal, Myanmar, China.

Barcode: *matK* = MN523478, MW457586

rbcL = MN220142, MW457590

ITS = MG820621, MW452979

7. ***Cleisostoma paniculatum*** (Ker-Gawl.) Garay in Bot. Mus. Left. Harv. 23 (4): 173. 1972; Seidenf. in Dansk Bot. Ark. 29 (3): 37. t. 16. 1975; Pradhan, Indian Orch. 2: 512. 1979; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 204. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 101. 2000. Aerides paniculata Ker-Gawl. In Bot. Reg. 3: Pl. 220. 1817. Vanda paniculata (Ker-Gawl.) R. Br. In Bot. Reg. 6. Sub, Pl. 516. 1820. Sacranthus paniculatus (Ker-Gawl.) Lindl. in Bauer, III. Orch. Pl. 9. 1832.

Common English Name: The 'Paniculate Inflorescence Cleisostoma'.

Stems 30-50 cm, erect, with rugose internodes, branching, many leaved. *Leaves* 15-25 x 1.5-2.0 cm, lanceolate, apex unequally and obtusely 2-lobed. *Inflorescence* 20-35 cm long, many flowered, longer than leaves, branched, lax. *Flowers* 1.0 cm across, opening widely, many; sepals and petals yellowish green, purplish brown adaxially, margins and midvein yellow. *Dorsal sepals* suboblong, concave, obtuse; lateral sepals obliquely oblong, base adnate to column foot. *Petals* smaller than sepals. Lip greenish yellow, side lobe oblong, obtuse, truncate; mid-lobe oblong, uncinate. *Spur* green, callus sub-equally 4-lobed. *Column* stout.

Colour Plate 2.5: (A)&(B)

Flowering: August - September

Distribution: Epiphytic and found growing on main tree trunk in open primary forest

Specimens examined: Mongchen, Lumami

Barcode : *matK* = MT974498, MW448188, MW448189

rbcL = MT974499, MW448192, MW448193, MW448196

ITS = MT422095, MW442838

8. ***Cleisostoma racemiferum*** (Lindl.) Garay in Bot. Mus. Leafl. Harv. 23(4): 173. 1972; *Saccolabium racemiferum* Lindl., Gen. Sp. Orchid. 224. 1833. *Sarcanthus pallidus* Lindl. in Bot. Reg. misc. 78. 1840; Hook. f., Fl. Brit. India 6: 68. 1890; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 204. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 101. 2000.

Common English Name: The spike shaped flower Raceme Cleisostoma.

Stems upto 15-20 cm long, stout, clothed with leaf sheaths. *Leaves* 20-25 x 3.5-4 cm, oblong, bilobed at apex. *Inflorescence* many flowered, much longer than the leaves, panicle many branched, branchlets long, sub-erect or drooping. *Flowers* 1-1.3 cm across, purplish yellow, floral bract short. *Sepals* and *petals* yellow with 2 dark purple median bands, obtuse. *Lip* white, longer than sepal, mid-lop large, ovate, glabrous. *Spur* cylindric, septate with 2 lobed calli.

Colour Plate 2.5: (C)&(D)

Flowering: July-August

Habitat and Ecology: Epiphytic and found growing on tree trunk in open forest

Specimens examined: Ungma

General distribution: India (Assam, Arunachal Pradesh, Meghalaya, Nagaland and Sikkim) Bhutan, Burma, China, Laos, Nepal, Thailand, Vietnam.

9. ***Cleisostoma simondii*** (Gagnep.) Seidenf.in Dansk Bot. Arkiv 29(3): 66. 1975; *Vanda simondii* Gagnep. in Bull. Mus. Paris 22(5): 628. 1950; *Vanda teretifolia* Lindl., Coll. Bot. t. 6. 1821. *Sarcanthus teretifolius* (Lindl.) Lindl., Gen. Sp. Orchid. 324. 1833; *Sarcanthus siamensis* Royle *ex* Dawnie in Kew Bull. 1925: 405. 1925; *Cleisostoma*

Seidenfadenii Garay in Bot. Mus. Leafl. Harvard Univ. 23(4): 175. 1972; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 208. 1998.

Common English Name: The Simond's Cleisostoma.

Stems upto 25-30 cm long, erect, terete. *Leaves* fleshy, terete. *Inflorescence* 15-20 cm, 8-14 flowered racemes, pendent. *Flowers* yellowish with brownish-purple markings. *Sepals* and *petals* subequal, spreading. *Lip* 3-lobed, mid lobe triangular, decurved, callus with short side horns at upper end. *Pollinia* stipe flate.

Colour Plate 2.4: (A)&(B)

Flowering: July - September

Habitat and Ecology: Epiphytic and found growing on tree trunk bark in tropical forest

Specimens examined: Khuzama

General distribution: India (Assam, Arunachal Pradesh, Nagaland), China, Laos, Thailand, Vietnam.

Barcode: *matK* = MK064249, MW448190, MW448191

rbcL = MN298853

ITS = MG822849, MW355894, MW362366

10. ***Cleisostoma williamsonii*** (Rchb.f.) Garay, Bot. Mus. Leafl. Harv. 23(4): 176. 1972. Seidenf. in Dansk Bot. Ark. 29 (3): 50. t. 21. 1975; Hook. f., Fl. Brit. India 6:67. 1890; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 208. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 103. 2000. *Sacranthus williamsonii*.f. in Gard, Chron. 674. 1865.

Common English Name: The 'Williamson's Cleisostom'.

Plants 30-60 cm long, slender, sheaths, branched, many leaved. *Leaves* 8-14 x 0.2-0.3 cm, terete, distichous, apex acute or obtuse. *Inflorescence* laxly many flowered, racemes, longer then the leaves. *Flowers* 3-5 mm across purplish pink. *Sepals* ovate-

oblong, obtuse. *Petals* linear-oblong, acute. *Lip* purplish pink, oblong, fleshy, thickly clawed. *Spur* globose, back wall callus 2-lobed with side horns.

Colour Plate 2.4: (C)&(D)

Flowering: May - June

Habitat and Ecology: Epiphytic, sometimes lithophytic and found growing on main tree trunk.

Specimens examined: Phek

General distribution: India (Arunachal Pradesh, Assam, Nagaland, Andaman Islands), Bhutan, Myanmar, Cambodia, China, Thailand, Vietnam.

Barcode: *matK* = MK160138

rbcL = MN298854, MW448194, MW448195

ITS = MN517118, MW442840

11. ***Gastrochilus acutifolius*** (Lindl.) Kuntze, Revis. Gen. Pl. 2: 661. 1891; Gastrochilus dentatus (Rchb.f.) Kuntze; Gastrochilus denticulatus (Paxton) Kuntze; Saccolabium acutifolium Lindl.; Saccolabium dentatum Rchb.f.; Saccolabium de nticulatum Paxton).

Common English Name: The 'Pointed-Leaf Gastrochilus'.

Stem elongated, upto 30 cm long, clothed with erose sheaths, rooting at nodes. *Leaves* 10-15 x 2-3 cm, fleshy, oblong-lanceolate, acute. *Inflorescence* umbel, 6-8 flowered, dense racemes. *Flowers* 2.0-2.5 cm across, spotted with dull brown colour. *Sepals and petals* pale-green or yellow, slightly reflexed, oblanceolate, blunt, subequal. *Lip* 3-lobed, white with yellow centre, saccate at base, side lobe narrow; mid lobe reniform, margin fimbriate, erose. *Column* short, thick.

Colour Plate 2.6: (A)&(B)

Flowering: November - December

Habitat and Ecology: Epiphytic and found growing in branches of trees in open forest.

Specimens examined: Mongchen

General distribution: India (Arunachal Pradesh, Nagaland,

Barcode: *matK* = MN239894, MW433889

rbcL = MN239898, MW433892

ITS = MT225573, MW475270

12. ***Gastrochilus calceolaris*** (Buch.-Ham. ex Sm.) D.Don, *Prodr.* Fl. Nepal. 32. 1825; Hook. f., Fl. Brit. India 6: 60. 1890; King & Pantl. in. Ann. Roy. Bot. Gard. Calc. 8:225. t. 300. 1898; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 413. 413. 1998; Buch- Ham. Ex J.E Smith in Rees cycl. (Addenda): 39. *Aerides*, no.11. 1819. *Saccolabium calceolare* (J. E. Sm.) Lindl. Gen. & Sp. Orch. 223. 1833. *Aerides calceolare* Buch.- Ham. ex J.E. Sm. in Rees Cycl. 39. 11. 1818.

Common English Name: The 'Shoe-Shaped Gastrochilus'.

Plants 15-20 cm tall, pendulose. *Leaves* 6-18 x 1.5-2.0 cm, oblong, apex unequally and obtusely 2-lobed. *Inflorescence* many flowered, dense, in corymbose raceme; peduncle stout. *Flowers* 1.5-2.0 cm across, green with scattered brown spots. *Sepals* sub-equal; dorsal sepal ovate-oblong, apex obtuse-rounded; lateral sepals oblong, falcate. *Petals* sub-similar to sepals, slightly smaller, apex obtuse-rounded. *Lip* with sac at base, brownish yellow with brownish red markings, mid lobe white, semicircular, hairy papillose. *Column* reddish, short.

Colour Plate 2.7: (C)&(D)

Flowering: March - May

Habitat and Ecology: Epiphytic and found growing in branches of trees in thick forest.

Specimens examined: Longkhum, Mongchen

General distribution: India (Arunachal Pradesh, Assam, Meghalaya, Nagaland, Sikkim, West Bengal), China, South-East Asia and Sumatra.

Barcode: *matK* = MN239897, MW480554

rbcL = MG925669, MW480549

ITS = MN517123, MW475266

13. ***Gastrochilus inconspicuous*** (Wall. ex Hook. f.) Kuntze, Rev. Gen. Pl. 2: 661. 1891; Seidenf. in Dansk Bot. Ark. 27(4): 94. 1971; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 416, 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 189, 2000; *Saccolobium inconspicum* Hook. Fl. Brit. India 6: 56 1890; *Luisia inconspicua* (Wall. ex Hook. f.) King & Pantl. in Ann. Roy. Bot. Gard. Calc. 8: 203. t. 272. 1898.

Common English Name: The inconspicuous Gastrochilus.

Plant upto 30 cm high, stem slender, terete with noded scars. *Leaves* 3.5-5.0 cm long, terete, weakly curved, sessile. *Inflorescence* lateral, racemose, 4-5 flowered. *Flowers* 0.5 cm across, extra axillary, yellowish green. *Dorsal sepal* ovate-lanceolate, sub-oblique, acute; lateral sepals similar. *Petals* oblong to ovate-elliptic, acute. *Lip* 3-lobed; hypochile saccate, lateral lobes rounded; mid-lobe flat, decurved, subreniform notched at the apex, minutely hairy, 5-7 vertical lines on the upper surface. *Column* purple, stout.

Colour Plate 2.3: (C)&(D)

Flowering: July - August

Habitat and Ecology: Epiphytic and found growing in branches of tree.

Specimens examined: Longkhum

General distribution: India (Arunachal Pradesh, Meghalaya, Nagaland, Sikkim), Bhutan, Nepal.

14. ***Gastrochilus obliquus***var. ***obliquus*** (Lindl.) Kuntze, in Gen. Pl. 2: 661 (1891); Z.H. Tsi, Guihaia 16: 141. 1996.

Common English Name: The 'Slanting Belly-Lip Orchid'.

Plants 16-24 cm tall, stem 1-2 cm, stout. *Leaves* 3-5, 8–20 × 1.7–6 cm, distichous, blade oblong to oblong-lanceolate, fleshy slightly or leathery.*Inflorescences* 1–4, from base of stem, subumbellate, often 5–8-flowered, peduncle straight, 1–2 cm, stout. *Flowers* 1-1.5 cm, fragrant, with yellow sepals and petals and white lip, with brownish purplish spots. *Sepals* similar, subelliptic, base contracted, apex obtuse. *Petals* spatulate, smaller than sepals, apex obtuse. *Lip* with an epichile and a saccate hypochile; epichile triangular, adaxially glabrous, with a central cushion, margin lacerate, apex obtuse and with a small wart abaxially; hypochile with yellow tip, with purplish red spots, nearly subglobose-cucullate, compressed laterally. *Column* short.

Colour Plate 2.7: (A)&(B)

Flowering: November - December

Habitat and Ecology: Epiphytic and found growing in branches of trees in thick forest.

Specimens examined: Jaluki

General distribution: India (Assam, Nagaland), Bhutan, Laos, Myanmar, Nepal, Thailand, Vietnam, China.

Barcode: *matK* = MN239895, MW433890, MW480553

rbcL = MN239899

ITS = MN240429

15. ***Gastrochilus pseudodistichus*** **(**King & Pantl.) Schltr. in Feddes Repert. Spec. Nov. Regni Veg. 12: 315. 1913; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 191. Fig. 63. 2000. *Saccolabium pseudodistichum*King & Pantl. in J. Aiat. Soc. Bengal, Pt. 2, Nat. Hist. 64(3): 341. 1895. *Gastrochilus hoyopse* (Rolfe ex Downie) Seidenfaden & Smitinand, Orch. Thail. 4(1): 623, t. 468. 1963.

Commom English Name: The 'distichous gastrochilus'.

Plant 15-35 cm, pendent, simple or branched, pendulous. *Leaves* 1.5-1.8 x 0.4-0.6 cm, distichous, many, fleshy, green with purplish red spot, ovate-lanceolate, apex acute. *Inflorescence* 4-5 flowered, subumbellate. *Flowers* 1 cm across, yellow, with purplish red marks or spots. *Dorsal Sepal* oblanceolate-oblong, apex obtuse; lateral sepals similar. *Petals* subobovate, slightly smaller than sepals, apex obtuse. *Lip* hypochile sub-hemispherically saccate, yellow; epichile broadly cordate, obtuse, orange. *Column* greenish purple.

Colour Plate 2.6: (C)&(D)

Flowering: September - October

Habitat and Ecology: Epiphytic and found growing in between two branches of tree.

Specimens examined: Meinkong

General distribution: India (Nagaland, Sikkim, West Bengal), China, Bhutan, Myanmar, Vietnam.

16. ***Luisia trichorrhiza*** (Hook.) Bl., Mus. Bot. Lugd. Bot. 1: 63. 1849; Hook. f., Fl. Brit. India 6: 21. 1890; Seidenf. in Dansk Bot. Ark. 27 (4): 66. t. 35. 1971; H. J. Chowdhery, Orch. Nagaland (BSI), 21. 2000. *Vanda trichorrhiza* Hook., Exot. Fl. 1: t. 1825. *Cymbidium triste sensu* Lindl., Gen. Sp. Orchid. Pl.: 167. 1833, non (Forster) Willdenow.

Commom English Name: The Hair-like root Luisia

Stems upto 20 cm long, erect, stout, usually branched (sometimes branched). *Leaves* 8-20 x 0.2-0.4 cm, terete, thick, jointed, narrowed at the apex. *Inflorescence* 3-5 flowered, axillary. *Flowers* 1.2 cm across, purplish green, with faint purple lines. *Dorsal sepal* oblong, obtuse; lateral sepals spreading, obliquely ovate to spathulate, acute, keeled. *Petals* oblong, obtuse, spreading. *Lip* 3-lobed; hypochile deeply concave with erect, rounded to triangular, subacute lateral lobes; epichile cordate, rigid, tapering to subtruncate, minutely emarginated apex. *Column* stout, purplish.

Colour Plate 2.17: (C)&(D)

Flowering: March - April

Habitat and Ecology: Epiphytic and found growing on tree trunk

Specimens examined: Longkhum

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim), Bhutan, Myanmar, Thailand.

17. ***Papilionanthe teres*** (Roxb.) Schltr. Orchis, 9. 78. 1951, Hook. f., Fl. Brit. India 6 :49. 1890; King & Pantl. in Ann. Roy. Bot. Gard. Calc. 8: 214. t. 285 1898; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 570. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 234. 2000. *Dendrobium teres* (Roxb.) Lindl., Gen. & Sp. Orch. 217. 1833.

Common English Name: The 'terete leaf Papilionanthe'.

Stem 40-100 cm long, slender, terete, branching, covered by leaf sheaths. *Leaves* 8-18 x 0.3-0.6 cm, terete, straight, suberect, curved, ridged. *Inflorescence* laxly 3-6 flowered racemes, arched. *Flowers* 8-10 cm across, large, whitish pink, lip darker pink with a yellow base, veined with pink. *Sepals* oblong or obovate, obtuse, laterals sub-falcate, spreading, margins undulate. *Petals* rounded, suborbicular, base twisted. *Lip* 3-lobed, pubescent; side lobe large, rounded; mid lobe obovate, deeply bifid. *Spur* funnel-shaped, compressed. *Column* pubescent in front.

Colour Plate 2.8: (A)&(B)

Flowering: April - May

Habitat and Ecology: Epiphytic and found growing on old growth tree near highway.

Specimens examined: Jaluki, Dikhu, Asukhomi

General distribution: India (Arunachal Pradesh, Assam, China, Meghalaya, Nagaland, Sikkim, West Bengal), Bangladesh, Bhutan, Cambodia, Myanmar, Thailand, Vietnam.

Barcode: *matK* = MK160135, MW448187

rbcL = MG925670, MW457591

ITS = MG821161, MW362367, MW362392

18. ***Papilionanthe vandarum*** (Rchb.f.) Garay in Bot. Mus. Leafl. 23(10). 372. 1974, Pearce & Cribb 2002. The Orch. of Bhutan Vol. 3(3). 539. 2000. *Aerides vandarum* Rchb. F. Gard. Chron. 1867:997. 1867. *Erides cylindricum* sensu Hook. in Bot. Mag. 83: t. 4982. 1857, non Lindl. 1832. *Vanda vandarum* (Rchb.f.) K.Karas.Orchid Atlas 8: 199. 1992.

Common English Name: The 'Vanda-Like Papilionanthe'.

Stems 50-60 cm or more, branched, terete, flexuous, sheaths. *Leaves* 8-25 x 0.3-0.5 cm, suberect, terete, grooved, jointed. *Inflorescence* leaf opposed, 1-4 flowered. *Flowers* 4-5 cm across, white with a purple-flushed base to lip and spur. *Dorsal sepal* obovate-oblong, obtuse, margins undulate; lateral sepals similar. *Petals* subrhombic, base twisted, margins undulate, apexobtuse. *Lip* 3-lobed; lateral lobes erect, unequally bifid, narrowly ovate, dentate on apical margin; mid lobe apex clawed, obovate, dilated and bilobulate at apex. *Spur* cylindric, base conic, straight. *Column* fleshy.

Colour Plate 2.8: (C)&(D)

Flowering: March - April

Habitat and Ecology: Epiphytic and found growing on tree trunk.

Specimens examined: Zunheboto

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim), Bhutan, South China, Myanmar.

Barcode: *matK* = MN523480, MW457585

rbcL = MN178486, MW457588, MW457589

ITS = MG821080, MW362394, MW362368

19. ***Phalaenopsis braceana*** (Hook.f.) E.A. Christenson in Selbyana 9: 169. 1986. *Doritis braceana* Hook. f., Fl. Brit. India 6(1): 196. 1890. *Kingidium braceanum* (Hook. f.) Seidenfaden in Opera Bot. 95: 187. 1988.

Common English Name: The 'Brace's Phalaenopsis'.

Plant 20-30 cm, stems very short, sheathed, brown, roots many, flattened, tortuous. *Leaves* 1 or 2, sometimes without, 5-8 x 1.2-2.5cm, deciduous, coriaceous, linear-oblong, obtuse to subacute. *Inflorescence* basal, erect, racemose, unbranched. *Flowers* 2.5-3.0 cm across, pendent, pinkish violet, lip darker in colour. *Sepals* obovate, acute, narrow at base. *Petals* obovate-oblong, obtuse. *Lip* 3-lobed, spurred; hypochile; lateral lobes ovate-lanceolate, subacute; dics with a forked callus; spathulate-obovate, rounded, boat shaped. *Spur* cylindric, straight. *Column* fleshy.

Colour Plate 2.9: (C)&(D)

Flowering: April - May

Habitat and Ecology: Epiphytic and found growing on tree trunk

Specimens examined: Longkhum

General distribution: India (Nagaland, Sikkim, West Bengal), China, Bhutan

Barcode: *matK* = MT974500

rbcL = MT974501

ITS = MT974319

20. ***Hygrochilus parishii*** (Veitch & Rchb.f.) Pfitzer in Engler, Nat. Pflanzenfam. Nachtr. 1: 112. 1897. *Vanda parishii* Veitch & Rchb.f. Xenia Orchid. 2: 138 1868; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 686. 1998. *Stauropsis parishii* (Veitch & Rchb.f.) Rolfe. Orchid Rev. 27: 97 1919. *Vandopsis parishii* (Veitch & Rchb.f.) Schltr. Repert. Nov. Regni Veg. 11: 47 1912. *Hygrochilus mariottiana* (Rchb.f.) Christenson. J. Orchideenfr. 12: 343 2005. *Phalaenopsis hygrochila* J.M.H.Shaw 2015.

Common English Name: The 'Moist Lip Phalaenopsis'.

Plants 10-30 cm tall, stems stout, sheathed. *Leaves* 17-25 x 4.0-6.0 cm, oblong or obovate-oblong, unequally bilobed. *Inflorescence* 1 or more, laxly 5-8 flowered. Flowers 4-5 cm across; sepals and petals yellow with deep purple spots, lip white, tinged with lilac on mid-lobe. *Sepals* broadly ovate, dorsally carinate, obtuse. *Petals* broadly ovate, obtuse. *Lip* 1-1.4 cm, fleshy, 3-lobed; lateral lobes suborbicular, small; mid-lobe cuneate-flabellate, with a central longitudinal keel and an erect appendage at the base near entrance of sac. *Column* winged, rostellum with ligulate.

Colour Plate 2.10: (E)&(F)

Flowering: April - June

Habitat and Ecology: Epiphytic and found growing on tree trunk and branches

Specimens examined: Jaluki

General distribution: India (Arunachal Pradesh, Assam, Nagaland), Bhutan, Chaina, Loas, Myanmar, Thailand, Vietnam.

Barcode: *matK* = MK160139, MW603188

rbcL = MN220143, MW603193

ITS = MN170567, MW599846, MW617318

21. ***Phalaenopsis wilsonii*** Rolfe**,** Bull. Misc. Inform. Kew 1909: 65 (1909).*Polychilos wilsonii* (Rolfe) Shim, Malayan Nat. J. 36: 27 (1982). *Kingidium wilsonii* (Rolfe) O.Gruss & Roellke, Orchidee (Hamburg) 47: 149 (1996). *Doritis wilsonii* (Rolfe) T.Yukawa & K.Kita, Acta Phytotax. Geobot. 56: 157 (2005).

Common English Name: The 'Wilson's Phalaenopsis'.

Stems very short 2-4 cm long. *Roots* well developed, densely verrucose, elongate, fasciculate, fleshy, flattened, greenish. *Leaves* usually 1-2, and sometimes leafless, 6-7 cm x 2.3 cm, purple reddish color, oblong-elliptic, acute. *Inflorescence* 9.4 cm, arcuate, few flowered in zigzag. *Flowers* widely opened, 4-5 cm, largely spaced, few 1-2, pink

sepals and petals, prominent at the base, mauve, lip magenta color, column white or pinkish. *Dorsal sepal* oblong-elliptic, 20 x 6 mm, acute or sub-obtuse. *Lateral sepals* oblique, 16 x 7 mm, acute. *Petals spatulate*, elliptic oblong-ovate, 18 x 8 mm, obtuse. *Lip* base clawed, lip 3-lobed; lateral lobes erect, sickle-shaped, 6 mm, adaxially incised-tipped keel; mid-lobe obcordate when spread, 9 x 5 mm, fleshy hump, convex, notched at apex, medium keel is raised at base. *Column* erect, 8 mm, stigmatic cavity. *Ovary* Pedicellate 3.4 cm. *Anther cap* with short beak, white-purplish on dorsal side. *Pollinia* 4, in 2 unequal pairs. *Capsule* cylindric.

Colour Plate 2.9: (A)&(B)

Flowering: April–June.

Habitat and Ecology: Epiphytic on mosses grown bark of a tree.

Specimens examined: Kithsakita

General distribution: India (Nagaland), China (Sichuan, Yunnan, and Hainan) Eastern Tibet and possibly even upto Myanmar and Vietnam.

Barcode: *matK* = MG958489

rbcL = MG958488

ITS = MG952632

22. ***Renanthera imschootiana*** Rolfe in Kew Bull. 1891: 200 (1891); H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 626. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 258. 2000.

Common English Name: The 'Red Vanda'.

Stems upto 1 m, cylindric, stout, erect. *Leaves* 8-11 x 1.5-2.0 cm, oblong-lanceolate, apex unequally, obtusely 2-lobed. *Inflorescence* 50 x 80 cm, 15-20 flowered, axillary, usually branched, paniculate racemes. *Flowers* 3.5-4.5 cm across, brownish red. *Dorsal sepal* subspatulate-oblanceolate, brownish red, obtuse, slightly keeled; lateral sepals bright red

with slightly brownish yellow, elliptic-ovate, margin undulate, apex obtuse. *Petals* brownish yellow, red spotted, linear spatulate. *Lip* small, bright red, 3-lobed; side lobes erect, triangular, exceeding column, base with 2 membranous lamellae, apex acute; mid lobe acute, strongly recurved, base with 3 fleeshy calli. *Column* terete, brownish red.

Colour Plate 2.10: (A)&(B)

Flowering: April - June

Habitat and Ecology: Epiphytic and found growing on tree trunk and branches

Specimens examined: Khuzama

General distribution: India (Arunachal Pradesh, Nagaland, Manipur, Sikkim), Cambodia, Nepal, Myanmar, Vietnam.

Barcode: *matK* = MN239896

rbcL = MG932128

ITS = MG820707, MW599845

23. ***Rhynchostylis retusa*** (Linn.) Bl.Bijdr. 286. t. 49. 1825; Hook. f., Fl. Brit. India 6: 32. 1890; King & Pantl. in Ann. Roy. Bot. Gar, Calc. 8: 213. 1898; Pradhan, Indian Orch. 2: 552. 1979; Kataki, Orch. Maghalaya 164. Pl. 61(1a, 1b, 1c) & N (iii). 1986; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 626. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 259. 2000. *Epidendrum retusum*Linn., Sp. Pl. 2: 953. *Aerides spicatum* D. Don, Prodr. Fl. Nepal.: 31. 1825

Common English Name: The 'Blunt Rhynchostylis/Foxtail'.

Plant 35-40 cm, stems stout, terete, covered by sheaths of leaves. *Leaves* 15-40 x 3-4 cm, linear-oblong, apex praemorse with a curved spine. *Inflorescence* upto 40 cm long, densely-flowered, drooping. *Flowers* 1-1.5 cm across, white with scattered pink blotches. *Dorsal sepals* oblong, acute; laterals gibbously orbicular-ovate, obtuse. *Petals* linear

oblong, spathulate, obtuse. *Lip* concave, basal lobe forming a sac; mid lobe variable in size, cuneiform, entire or emarginated at the apex, flat disc. *Spur* saccate.

Colour Plate 2.13: (A)&(B)

Flowering: April -May

Habitat and Ecology: Epiphytic and found growing on tree trunk and branches in open forest.

Specimens examined: Mongchen, Lumami

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim West Bengal), Bhutan, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Vietnam.

Barcode: *matK* = MK064250, MW480550, MW603185, MW603186

rbcL = MG867449, MW480544

ITS = MG822847, MW475272, MW475274, MW475276

24. ***Sarcoglyphis mirabilis*** (Rchb.f.) Garay in *Bot. Mus. Leafl. 23: 201. 1972*; Odyuo, Roy, Deori & Mao, J. Jpn. Bot. 94(1): 51–55. 2019.

Common English Name: The 'Wonderful Sarcoglyphis'.

Plant 10-15 cm, erect or sub-erect, stem 4–6 cm, base with persistent leaf sheaths. *Leaves* 8–12 x 1–2 cm, dark green, distichous, coriaceous, obliquely bi-lobed at apex. *Inflorescence* 4–6.0 cm long, racemose or paniculate, 10–12 flowered. *Flowers* 1-1.2 cm across, successively opening, sepals and petals yellowish green. *Dorsal sepals* 6.0 × 3.5 mm, oblong, apex retuse; lateral sepals 6.0 × 4.0 mm, oblong obtuse. *Petals* 5.0 × 2.0 mm, narrowly oblong, obtuse. *Lip* spurred, 3-lobed, purplish white, hypochile yellow, side lobes triangular, raised, tips pointed; mid-lobe triangular, acute, apex slightly curved upwards. *Spur* rounded. *Column* thick, purple.

Colour Plate 2.12: (A)&(B)

Flowering: April - May

Habitat and Ecology: Epiphytic and found growing in branches of tree.

Specimens examined: Punglwa

General distribution: India (Nagaland), China, Thailand, Vietnam.

Barcode: *matK* = MT419780, MW480551

rbcL = MT419781, MW480545, MW480546, MW480547

ITS = MT416451, MW475278

25. ***Smitinandia micrantha*** (Lindl.) Holttumin Gard. Bull. Singapore 25: 106. 1969; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 642. Fig 391. 1998. *Saccolabium micranthum*Lindl., Gen. Sp. Orchid. Pl. 220. 1833. *Cleisostoma micranthum* (Lindl.) King & Pantl., in Ann. Roy. Bot. Gard. 8:234, t. 312. 1898.

Common English Name: The 'Small flowered Smithinandia'.

Plant 10-25 cm tall, stems pendent. *Leaves* 8-10 x 1.5-2.0 cm, linear, obtusely bilobed at apex. *Inflorescence* 5-15 cm, densely flowered racemes. *Flowers* 0.4-0.6 cm across, opening widely, white to purple, tinged with purple, with triangular, acute. *Sepals* subequal; dorsal ovate, subacute; laterals broadly oblong-ovate, obtuse, slightly larger. *Petals* oblanceolate, obtuse, with erose margins, narrower than the sepals. *Lip* marked with violet, 3-lobed; side lobes erect; mid lobe oblong-ovate, blunt. *Spur* shortly conical, rounded apex.

Colour Plate 2.10: (C)&(D)

Flowering: April -May

Habitat and Ecology: Epiphytic and found growing on main tree trunk in open forest.

Specimens examined: Jaluki

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, West Bengal), Bhutan, Nepal, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia.

Barcode: *matK* = MN187220

rbcL = MN523482, MW603195

ITS = MN170568, MW617320

26. *Stereochilus laxus* (Rchb.f.) Garay in Bot. Mus. Leafl. 23: 205. 1972; *Sarcanthus laxus* Rchb.f., Bot. Zeitung (Berlin) 24: 378 (1866).

Common English Name: The 'Lax Flowered Stereochilus'.

Stems short, covered by leaves sheaths. *Roots* 4-6 cm. *Leaves* 4-5, thick, straight horizontal, fleshy, linear, oblong, 3-4 x 0.3- 0.5 cm, apex retuse. *Inflorescence* decurved, arising beneath leave sheaths, lax, glabrous, about 15-16cm, peduncle slender, somewhat zigzag, maroon. *Flower* glabrous, 1.5-2 cm across, raceme. *Sepals* and *petals* light pinkish-white and turn white over time, yellowish at maturity. *Dorsal sepal* ovate to oblong, spathulate, 1 x 0.3 cm. *Lateral sepals* ovate, obtuse, 0.8 x 0.3 cm. *Petals* spreading, ovate to sub-spatulate, 0.8-10 x 0.2-0.4 cm. Lip 3-lobed, adnate to column base, spurred, 0.5-0.7 cm, incurved at apex. *Column* greenish white, 0.5 cm. *Pedicel* and *ovary* 1.9 cm. *Anther* cap beaked. *Pollinia* 0.4 cm. *Seed capsule* fusiform, 3cm long.

Colour Plate 2.11: (A)&(B)

Flowering: September – October

Habitat and Ecology: Epiphytic in tropical forest.

Specimens examined: Kithsakita

General distribution: India (Nagaland), Myanmar and Thailand.

Barcode: *matK* = MT178331

rbcL = MT178332

ITS = MT178771

27. ***Thrixspermum tsii*** W.H.Chen & Y.M.Shui in *Brittonia 57: 55. 2005*.

Common English Name: The 'Tsi's Thrixspermum'.

Plant 20-30 cm long, 2-2.5 cm internodes, steam usually pendulous, stout, terete, slightly flattened, *Leaves* 6-13 x 1.5-2.2 cm, many, distichous, fleshy and thick, oblong, amplexicaul at base, unequally bilobed rounded apex. *Inflorescence* 1.5- 2.5 cm long, racemose, shorter then leaves, 1-3 flowers. *Flowers* 3.5-4 cm across, last only one day, large, white, fleshy, jasmine fragrant, lip golden yellow disk with a white tiped side lobes. *Sepals* veined, obtuse with a short tip. *Petals* oblong ovate, veined, acute. *Lip* slightly pouched, 1.1-1.2 cm, widely ellipsoid, pale-golden yellow, lateral lobes narrowed to acute apex, slightly curved forward, mid-lobe elongat into a broadly ligulate blade, fleshy, disc pale/golden-yellowish, disk with a single, narrow horizontal callus, with densely brownish irregular stripes or blotches. *Column* short.

Colour Plate 2.12: (C)&(D)

Flowering: May – June

Habitat and Ecology: Epiphytic and found growing in branches of tree**.**

Specimens examined: Jaluki

General distribution: India (Nagaland) and China (Yunnan)

Barcode: *matK* = MK160140

rbcL = MK214920

ITS = MN170569

28. ***Vanda alpina*** (Lindl.) Lindl., Fol. Orch. Vanda 10. 1853; Hook. f., Fl. Brit. India 6: 53. 1890: King & Pantl. in Ann. Roy. Bot. Gard, Calc. 8: 217. t. 289. 1898: H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 683. 1998. *Luisia alpina* Lindl. in Bot. Reg. 24: misc. 56, no. 101. 1838. *Trudelia alpina* (Lindl.) Garay in Orchid Digest 50(2): 76.

1986. *Stauropsis alpina* (Lindl.) T. Tang & F. T. Wang in Acta Phytotax. Sin. 1: 93. 1951.

Common English Name: The 'Montane Vanda'.

Plants 15-20 cm, stout, covered by leaf sheaths. *Leaves* broadly linear-oblong, arched, truncate, recurved, apex unequally 2-lobed. *Inflorescence* 2-3, 1-3 flowered, axillary, shorter then leaves. *Flowers* 2.5-3 cm across, greenish-yellow, pendent, thickly textured, not widely opening. *Sepals* almost equal; lateral sepals ovate-lanceolate, apex sub-acute. *Petals* narrowly oblong, lanceolate, apex obtuse-acute. *Lip* pale-yellow with purple ting, fleshy, base concave, spurless, 3-lobed; lateral lobes suberect, semi-circular, apex rounded; mid lobe, broadly lanceolate, subacute to obtuse, apex recurved.

Colour Plate 2.16: (C)&(D)

Flowering: July - August

Habitat and Ecology: Epiphytic and found growing on branches and tree trunks in an open forest.

Specimens examined: Asukhomi

General distribution: India (Arunachal Pradesh, Meghalaya, Nagaland, Sikkim), Bhutan, China, Myanmar, Malaysia, Nepal, Thailand.

Barcode: *matK* = MN187221, MW496851

rbcL = MN220141, MW405200

ITS = MN173057, MW362399, MW362401

29. ***Vanda ampullacea*** (Roxb.) L.M. Gardiner, Phytotaxa 61: 48. 2012, comb, nov. *Aerides ampullacea* Roxburgh, Fl. Ind., ed. 1832, 3: 476. 1832. *Saccolabium ampullaceum* (Roxb.) Lindl., Sert. Orchids. 4: t. 17. 1838; Hook. f., Fl. Brit. India 6 : 64. 1890; King & Pantl. In Ann. Roy. Bot. Gard. Calc. 8 : 220. t. 293. 1898. *Ascocentrum ampullaceum* (Roxb.) Schltr, Repert. Spec. Nov. Regni Veg. Beih. 1: 975. 1913; Seidenf.

& Smitnd. Orch. Thailand, 4(1):598. t. 449. 1963; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 74. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagaland (BSI), 48. 2000. *Gastrochilus ampullaceus* (Roxb.) Kuntze, Revis. Gen. Pl. 2: 661. 1891. *Ascocentrum ampullaceum* var. *aurantiacum* Pradhan, Indian Orch.: Guide Identif. 2: 561. 1979.

Common English Name: The **'**Vein-like Ascocentrum'.

Stems 2-4 cm, erect, stout, clustered. *Leaves* 3-5, 6-16 x 1-1.5 cm, narrowly oblong flat, abaxially tinged reddish with age, adaxially yellowish green with purplish red spots, thickly leathery, bifid at the apex. *Inflorescence* 2-3, 5-6 cm, axillary, erect, shorter then leaves, many flowered. *Flowers* red to bright orange, 1,5-2.2 cm across, opening widely. *Sepals* narrowly ovate, obtuse. *Petals* broadly ovate. *Lip* with cylindrical spur, 3-lobed; lateral lobes erect, subtriangular, very small, obtuse; mid-lobe narrowly oblong, apex obtuse to acute. *Spur* pale.

Colour Plate 2.14: (A)&(B)

Flowering: April-May

Habitat and Ecology: Epiphytic on tree trunk in an open forest**.**

Specimens examined: Khuzama, Meriema

General distribution: India (Arunachal Pradesh, Assam, Manipur, Nagaland, Sikkim, Andaman Island, West Bengal), Bangladesh, Bhutan, China, Laos, Myanmar, Nepal, Thailand, Vietnam.

Barcode: *matK* = MK064245, MW405193

rbcL = MK214918, MW433891

ITS = MN170562

30. ***Vanda bicolour*** Griff., Notul. Pl. 3: 354. 1851; Icon. Pl. Asiat.: t. 330.1851; Hook. f., Fl. Brit. India 6:52. 1890; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 683. 1998; Hynniewta, Kataki & Wadha, Orch. Nagaland (BSI), 274. 2000.

Common English Name: The ‘two-colored Vanda’

Plants 50-80 cm tall, stout stem, sheathed. *Leaves* 14-20 x 1.6 3 cm, ligulate, oblong, curved, trunate or bilobed at apex. *Inflorescence* arising from between leaf sheaths, laxly 3-6 flowered racemes. *Flowers* 4-5 cm across, yellowish-brown inside, violet externally, tessellated faintly. *Sepals* unequal, dorsal sepals small, dilated in the middle; lateral obovate, deflexed. *Petals* spathulate, clawed. *Lip* purple to violet, almost equaling the sepals; lateral lobes large, orbicular, yellow margin; mid lobe small, triangular, 2-lobed apex.

Colour Plate 2.15: (C)&(D)

Flowering: February - April

Habitat and Ecology: Epiphytic on branches of tree and trunk in an open forest.

Specimens examined: Longkhum, Lumami

General distribution: India (Arunachal Pradesh, Assam, Manipur, Nagaland), Bhutan, Myanmar.

Barcode: *matK* = MG925667, MW405194, MW405195, MW405196

rbcL = MG867451, MW405190, MW405191

ITS = MG822845, MW368614, MW365331

31. ***Vanda coerulea*** Griff. ex Lindl.in. Bot. Reg. 33: t. 30. 1847; Hook. f., Fl. Brit. India 6: 51. 1890; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 683. 1998; Hynniewta, Kataki & Wadhawa, Orch. Nagaland (BSI), 275. 2000.

Common English Name: The ‘Blue Vanda’.

Plants upto 40 cm tall, stem stout, erect, sheathed. *Leaves* 1-1.6 x 1.6-3.3 cm, thickly leathery, unequally bilobed at the apex. *Inflorescences* 1-3, 20-40 cm, sparsely 6-16 flowered. *Flowers* 6-8 cm across, pale-blue to deep-blue, widely opening, thinly textured, tessellated. *Sepal* spathulate, obovate, clawed; laterals larger than the dorsal one. *Petals*

obovate, short twisted claw. *Lip* dark blue, liner oblong, fleshy; mid lobe with 2 thick ridges. *Spur* conical.

Colour Plate 2.15: (A)&(B)

Flowering: September - November

Habitat and Ecology: Epiphytic on branches of tree and trunk in an open forest.

Specimens examined: Alichen, Lumami

General distribution: China, North East India, Myanmar, Nepal, Sri Lanka, Thailand, Java, Indonesia.

Barcode: *matK* = KY744816, MW496849, MW496850

rbcL = MG867450, MW537579

ITS = MG818987, MW362400, MW492905

32.***Vanda stangeana*** Rchb.f. in Bot. Zeit. 16: 351. 1858; Hook. f., Brit. India 6: 54. 1890; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 688. 1998.

Common English Name: The 'Stange's Vanda'.

Stems covered with old sheaths, stout, erect. *Leaves* distichous, unequally bilobed at apex, spreading-recurved. *Inflorescence* many flowered, erect to sub erect. *Flowers* golden green, tessellated with chestnut brown. *Dorsal sepal*s cuneate-obovate equaling the petals, lateral sepal larger in size. *Petals* obovate, with undulate margins, clawed. *Lip* white, violet in front with a streak of red dots on each side of the spur and a furrow under the column between auricle, blade gradually narrowed from a broad semi cordate, 2-lobed, auricles of lips semi ovate, divergent. *Column* short, white.

Flowering: April - June

Habitat and Ecology: Epiphytic on branches of tree and trunk in an open forest**.**

Specimens examined: Jaluki

General distribution: North East India (Arunachal Pradesh, Assam, Manipur, Nagaland),

Barcode: *matK* = MG925666, MW405199, MW405185, MW405186

rbcL = MG905911, MW433895, MW433896

ITS = MG822848, MW362402

33. ***Vanda testacea*** (Lindl.) Rchb.f.in Gard. Chron. 2: 166. 1877; Hook. f., Fl. Brit. India 6: 50. 1890; King & Pantl. In Ann. Roy. Bot. Gard. Calc. 8 : 215. t. 288. 1896; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 688. 1998; Hynniewta, Kataki & Wadhwa, Orch. Nagalang (BSI), 276. 2000. *Aerides testacea* Lindl., Gen. So. Orchid. Pl.:238. 1833. *Vanda parviflora*. Lindl. In Bot. Reg. 30 Misc. 45. 1835.

Common English Name: The 'Brick-Red Vanda'

Plants 10-30 cm tall, erect, stout, with thick, sheathed. *Leaves* linear, distichous, keeled and apex unequally 2-lobed. *Inflorescence* laxly 7-11 flowered, erect, equaling the leaves. *Flowers* 1.5-2 cm across, yellow to yellowish-brown, long peduncled. *Sepals* obovate-spathulate, obtusely rounded and incurved at the apex, spreading, incurved and twisted. *Petals* spathulate, concave, base narrow and twisted, spreading. *Lip* 0.5-0.8 x 0.2-0.4 cm, reflexed, spurred, 3-lobed; lateral lobes erect, oblong, obtuse; mid-lobe recurved, sub-quadrate to oblong, apex dilated; disc with 2 fleshy ridges. *Spur* conical, slender.

Colour Plate 2.16: (A)&(B)

Flowering: March - May

Habitat and Ecology: Epiphytic on branches and tree trunk in an open disturbed forest**.**

Specimens examined: Jaluki, Punglwa A

General distribution: India (Arunachal Pradesh, Assam, Nagaland, Sikkim), China, Myanmar, Thailand, Sri Lanka, Nepal, Bhutan.

Barcode: *matK* = MN187219, MW496852

rbcL = MK064251, MW433894

ITS = MN170573, MW425864

34. ***Vanda pumila*** Hook.f., Fl. Brit. India 6(1): 53. 1890; King & Pantl. in Ann. Roy. Bot. Gard. Calc. 8: 216. t. 288. 1898; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 688. 1998. *Trudelia pumila* (Hook. f) Senghas in Schltr., Orchideen ed.3, 1(19-20):1211. 1988.

Common English Name: The 'Dwarf Vanda'

Plants 10-20 cm tall, stout, stems covered with old sheaths. *Leaves* linear-oblong, unequally bilobed at the apex, dilated at the base. *Inflorescence* erect, laxly 2-3 flowered racemes, shorter then leaves. *Flowers* 4-5.5 cm across, cream coloured, red strecked, sweet scented. *Sepals* and *petals* subequal; lateral sepals oblong, obtuse, slightly larger in size, curved. *Lip* saccately spurred, 3-lobed; lateral lobes triangular; mid lobe broadly ovate, concave, ridged surface, 2 knob like lumps.

Colour Plate 2.17: (A)&(B)

Flowering: March - April

Habitat and Ecology: Epiphytic and found growing on the tree trunk in an open forest.

Specimens examined: Asukhomi

General distribution: India (Arunachal Pradesh, Meghalaya, Nagaland, Sikkim, West Bengal), China, Myanmar, Laose, Vietnam, Sumatra.

Barcode: *matK* = MN523479, MW405189

rbcL = MN523481, MW405192

ITS = MN517224, MW368597, MW493108

35. ***Vandopsis undulata*** (Lindl.) J.J.Sm. in Engler & Prantl, Nat. Pflanzenfam. 2(6): 210. 1889; King & Pantl. in Ann. Roy. Bot. Gard, Calc. 8: 205. t. 275. 1898; H.J. Chowdhery, Orch. Fl. Arunachal Pradesh, 692. 1998; Hynniewta, Kataki & Wadha, Orch. Nagaland

(BSI), 276. 2000. *Vanda undulata* Lindl. in Jour. Linn. Soc.3: 42. 1859. *Stauropsis undulates* (Lindl.) Benth ex Hook. f., Fl. Brit. India 6 : 27. 1890.

Common English Name: The 'Wavy Petaled Vandopsis'.

Plant 30-50 cm, sheaths warted, erect. *Leaves* 5-10 x 1.5-2.0 cm, distichous, linear-oblong, with unequally bilobed apex. *Inflorescence* 12-15 cm, leaf opposed, stout, rigid, laxly, 3-4 flowered. *Flowers* 3-4 cm across; sepals and petals white, lip yellow to white at apex, greenish yellow with purple streaks at base. *Dorsal sepals* oblong-spathulate, margins undulate; lateral sepals similar, spreading, yellow tinged at apex. *Petals* narrowly oblong-spathulate, margins undulate. *Lip* fleshy, adnate to the column, 3-lobed; lateral lobes erect, rounded; mid lobe spathulate, truncate, laterally compressed. *Column* whitish pink, stout.

Colour Plate 2.11: (C)&(D)

Flowering: March - April

Habitat and Ecology: Epiphytic on tree trunks in forests or lithophytic on rocks slopes.

Specimens examined: Zunheboto

General distribution: India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Sikkim, Nagaland, West Bengal), Bhutan, China, Nepal.

Barcode: *matK* = MN187222, MW603190

rbcL = MG925668, MW457592, MW457593

ITS = MG786550, MW452980

Plate 2.1: ***Esmeralda clarkei*** **Rchb.f. (*Arachnis clarkei* (Rchb.f.) J.J.Sm.) (A) Habitat (B) Flower.** ***Arachnis labrosa (Lindl. & Paxton)*** **Rchb.f. (C) Habitat (D) Flower.**

Plate 2.2: ***Acampe praemorsa*** **(Roxb.) Blatt. & McCann (A) Habitat (B) Flower.**

A. rigida **(Buch.-Ham. ex Sm.) P.F.Hunt (C) Habitat (D) Flower**

Plate 2.3: ***Acampe ochracea*** **(Lindl.) Hochr. (A) Habitat (B) Flower.**

Gastrochilus inconspicuus **(Hook.f.) Kuntze (C) Habitat (D) Flower.**

Plate 2.4: ***Cleisostoma simondii*** **(Gagnep.) Seidenf. (A) Habitat (B) Flower.**

C. williamsonii **(Rchb.f.) Garay (C) Habitat (D) Flower.**

Plate 2.5: ***Cleisostoma paniculatum*** **(Ker-Gawl.) Garay (A) Habitat (B) Flower.**

C. racemiferum **(Lindl.) Garay (C) Habitat (D) Flower.**

Plate 2.6: ***Gastrochilus acutifolius*** **(Lindl.) Kuntze (A) Habitat (B) Flower.**

G. pseudodistichus **(King & Pantl.) Schltr. (C) Habitat (D) Flower.**

Plate 2.7: *Gastrochilus obliquus* var. *obliquus* (Lindl.) Kuntze (A) Habitat (B) Flower. *G. calceolaris* (Buch.-Ham. ex Sm.) D.Don (C) Habitat (D) Flower.

Plate 2.8: ***Papilionanthe teres*** **(Roxb.) Schltr. (A) Habitat (B) Flower.**

P. vandarum **(Rchb.f.) Garay (C) Habitat (D) Flower.**

Plate 2.9: *Phalaenopsis wilsonii* Rolfe (A) Habitat (B) Flower.

***P. braceana* (Hook.f.) E.A. Christenson (A) Habitat (B) Flower.**

Plate 2.10: ***Renanthera imschootiana*** **Rolfe (A) Habitat (B) Flower.**
Smitinandia micrantha **(Lindl.) Holttum (C) Habitat (D) Flower.**
Hygrochilus parishii **(Veitch & Rchb.f.) Pfitzer /*Phalaenopsis hygrochila***
J.M.H.Shaw 2015 (C) Habitat (D) Flower.

Plate 2.11: ***Stereochilus laxus*** **(Rchb.f.) Garay (A) Habitat (B) Flower.**
Vandopsis undulata **(Lindl.) J.J.Sm. (C) Habitat (D) Flower.**

Plate 2.12: ***Sarcoglyphis mirabilis*** **(Rchb.f.) Garay (A) Habitat (B) Flower.**

Thrixspermumtsii **W.H.Chen & Y.M.Shui (C) Habitat (D) Flower.**

Plate 2.13: ***Rhynchostylis retusa*** **(Linn.) Bl. (A) Habitat (B) Flower.**

Aerides odorata **Lour. (C) Habitat (D) Flower.**

Plate 2.14: ***Vanda ampullacea*** **(Roxb.) L.M. Gardiner (A) Habitat (B) Flower.**

V. stangeana **Rchb.f. (C) Habitat (D) Flower.**

Plate 2.15: ***Vanda coerulea*** **Griff. ex Lindl.(A) Habitat (B) Flower.**

V. bicolor **Griff. (C) Habitat (D) Flower.**

Plate 2.16: ***Vandatestacea*** **(Lindl.) Rchb.f. (A) Habitat (B) Flower.**

V. alpina **(Lindl.) Lindl. (C) Habitat (D) Flower.**

Plate 2.17: ***Vanda pumila*** **Hook.f. (A) Habitat (B) Flower.**

Luisia trichorrhiza **(Hook.) Bl. (C) Habitat (D) Flower.**

RESULTS

While documenting the 'Vandaceous Orchid of Nagaland', a total of 35 species under 16 genera (Table 2.1) have been collected from different districts of Nagaland during the field survey (2016-2020). The genus *Vanda* recorded maximum at 7 (seven) species followed by *Gastrochilus* 5 (five) species and *Acampe, Cleisostoma, Phalaenopsis* at 3 (three) species each. All the Vandaceous orchid species which were collected were found to be epiphytic covering an altitudinal range from lowest 300 m ASL to highest 2400 m ASL. The 16 (sixteen) collected genera includes - *Acampe, Aerides, Arachnis, Cleisostoma, Gastrochilus, Luisia, Papilionanthe, Phalaenopsis, Renanthera, Rhynchostylis, Sarcoglyphis, Smitinandia, Stereochilus, Thrixspermum, Vanda* and *Vandopsis*.

Table 2.1: List of orchid species collected and identified during the present study

Sl. No.	Genus	Species Name	Accession No.	Flowering season
1	*Acampe*	1. *A. ochracea* (Lindl.) Hochr.	NUBOT-JK-AO -19	January - February
		2. *A. praemorsa* (Roxb.) Blatt. & McCann	NUBOT-JK-AP-21	October - December
		3. *A. rigida* (Buch.-Ham. ex Sm.) P.F.Hunt	NUBOT-JK-AR -17	July – September
2	*Aerides*	1. *A. odorata* Lour.	NUBOT-JK-AO-05	May – June
3	*Arachnis*	1. *Esmeralda clarkei* Rchb.f. (*Arachnis clarkei* (Rchb.f.) J.J.Sm.)	NUBOT-JK-EC-10	November - February
		2. *A. labrosa* (Lindl. & Paxton) Rchb.f.	NUBOT-JK-AL-06	August - September
4	*Cleisostoma*	1. *C. paniculatum* (Ker-Gawl.) Garay	NUBOT-JK-CP--15	August – September
		2. *C. racemiferum* (Lindl.) Garay	NUBOT-JK-CR--35	July-August
		3. *C. simondii* (Gagnep.) Seidenf.	NUBOT-JK-CS -14	July – September
		4. *C. williamsonii* (Rchb.f.)	NUBOT-JK-CW -20	May – June

		Garay		
5	*Gastrochilus*	1. *G. obliquus* var. *obliquus*(Lindl.) Kuntze	NUBOT-JK-GO-30	November – December
		2. *G. calceolaris* (Buch.-Ham. ex Sm.) D.Don	NUBOT-JK-GC-09	March – May
		3. *G. acutifolius* (Lindl.) Kuntze	NUBOT-JK-GA-22	November-December
		4. *G. inconspicuous* (Hook.f.) Kuntze	NUBOT-JK-GI-33	July – August
		5. *G. pseudodistichus* (King & Pantl.) Schltr.	NUBOT-JK-GP-32	September – October
6	*Luisia*	1. *L. trichorrhiza* (Hook.) Blume	NUBOT-JK-LT-34	March – April
7	*Papilionanthe*	1. *P. teres* (Roxb.) Schltr.	NUBOT-JK-PT-04	April – May
		2. *P. vandarum* (Rchb.f.) Garay	NUBOT-JK-PV-07	March – April
8	*Phalaenopsis*	1. *P. wilsonii* Rolfe	NUBOT-JK-PW-26	April–June.
		2. *P. braceana* (Hook.f.) E.A. Christenson	NUBOT-JK-PB-27	April – May
		3. *P. hygrochila* (Veitch & Rchb.f.) Pfitzer *(Hygrochilus parishii)*	NUBOT-JK-PH-24	April – June
9	*Renanthera*	1. *R. imschootiana* Rolfe	NUBOT-JK-RI -11	April – June
10	*Rhynchostylis*	1. *R. retusa* (Linn.) Bl.	NUBOT-JK-RR-03	April –May
11	*Sarcoglyphis*	1. *S. mirabilis* (Rchb.f.) Garay	NUBOT-JK-SM-23	April – May
12	*Smitinandia*	1. *S.micrantha* (Lindl.) Holttum	NUBOT-JK-SM-29	April –May
13	*Stereochilus*	1. *S. laxus* (Rchb.f.) Garay	NUBOT-JK-SL-25	September – October
14	*Thrixspermum*	1. *T.tsii* W.H.Chen & Y.M.Shui	NUBOT-JK-TT-28	May – June
15	*Vanda*	1. *V. coerulea* Griff. ex Lindl.	NUBOT-JK-VC-01	September – November
		2. *V. bicolor* Griff.	NUBOT-JK-VB-02	February – April
		3. *V. ampullacea* (Roxb.) L.M. Gardiner	NUBOT-JK-VA -12	April-May
		4. *V. stangeana* Rchb.f.	NUBOT-JK-VS -16	April – June
		5. *V. testacea* (Lindl.) Rchb.f.	NUBOT-JK-VT-13	March – May
		6. *V. alpina* (Lindl.) Lindl.	NUBOT-JK-VA -18	July – August
		7. *V. pumila* Hook.f.	NUBOT-JK-VP-31	March – April
16	*Vandopsis*	1. *V. undulata* (Lindl.) J.J.Sm.	NUBOT-JK-VU-08	March – April

DISCUSSION

The hilly state of Nagaland with few stretch of plain on the foot-hill in the western part of the state is blessed with wide altitudinal variation, topographical features coupled with favorable climatic condition (Tropical to alpine, humid forest with heavy rainfall) trigger the rich floristic biodiversity in the state. According to ISFR 2015 satellite interpretation, the state (Nagaland) forest cover was 12,489 sq km, which is 75.33% of the state geographical area. However, the state has reported a record forest area of 8,623 sq km (52.01%) of its geographical area. A net decrease of 450 sq km in forest cover area has been reported as per 2017 assessment from 2015-17 (ISFR, 2017) which could be attributed to an anthropogenic activities like unplanned developmental activities and shifting/jhum cultivation. Although, orchid species being distributed/cosmopolitan all throughout the state, care should be taken not to over exploit during collection and reduce anthropogenic activity.

SUMMARY AND CONCLUSIONS

Nagaland, the Northeastern part of India which is a part of Himalayas as well as Indo-Burma biodiversity hotspot provide rich reservoir of plant diversity. The Indian orchids are mostly confined to the Western Ghats (73 genera, 305 species) and the Northeastern state (125 genera, 714 species), out of which 73 species are endemic to the Northeastern region. Nagaland, one of the Northeastern states of India contributes significantly to the orchid flora of India with about 426 species belonging to 101 genera from the state. The present study to document the Vandaceous orchids of Nagaland reported 35 species belonging to 16 genera of which 3 species are new records for India bringing the total orchid diversity to about 429 species belonging to 101 genera for the state.

CHAPTER - 3

ESTABLISHMENT OF BARCODES FOR SOME COMMERCIALLY IMPORTANT VANDACEOUS ORCHIDS OF NAGALAND

INTRODUCTION

DNA barcoding is a technique which provides quick and reliable identification of species without involving the morphological characters. It uses a relatively small-standardized DNA fragment as a tag to define or discover a species. This technique can be used for rapid identification and detection of species and relies on DNA sequence variations that provide a unique recognition tag to a species (Hebert *et al*., 2003a). The short sequences of DNA can be from either standardized/agreed upon locus/loci of nuclear or cytoplasmic genome or from both the genome. The partial/whole genome is sequenced and compared to know their base-pair differences and then deposited in the barcode database, which is termed as DNA barcodes. These genetic codes/sequences

could be accessed through a digital library and used to identify the unknown species by any scientist around the World. The differences in their nucleotide sequence provide a unique molecular recognition tag/identity to a species. Therefore, DNA barcoding is an additional taxonomic or improvised tool with high potential of reviving modern taxonomy (Schindel and Miller, 2005).

The family Orchidaceae is one of the highly evolved and second largest family of angiosperm with approximately 25,000-35,000 species belonging to 750-850 genera distributed worldwide (Chase, 2005; Hossain, 2011), comprises some of the species that are difficult to identify and classify correctly even if available in flowering state (Dressler, 1993; van den Berg *et al*., 2000; Gravendeel *et al*., 2001; Cameron 2004). Moreover, the family contains several mega genera (over 1000 species) *viz*., *Bulbophyllum* Thouars, *Epidendrum* L., *Pleurothallis* R. Br. and *Dendrobium* Sw. in which explosive speciation has taken place due to adaptive radiations. The reasons for such explosive speciation possibly because of ecological adaptations, physiological/morphological innovations, or accelerated rates of morphological/ molecular change, are still not properly understood (Whitten *et al*., 2007). The genus *Holcoglossum* consists of both long-evolved and recently radiated species that adds to difficulty in the identification of species (Xiang *et al*., 2011). Events like hybridization play an important role in speciation in plants (Soltis and Soltis, 2009). A number of hybrids species came into existence as in the case of genus *Ophrys* that has a number of hybrid species (Soliva *et al*., 2001). The closely related species of *Paphiopedilum* form hybrids in nature as well as artificially. Due to the large number of hybrids and its difficulties to identify the hybrids from their parents especially in vegetative parts (Sun *et al*., 2011) it is difficult to classify the taxa into natural system (Soliva *et al*., 2001). The presence of cryptic species as in *Serapias* (Pellegrino *et al*., 2005) and sister species, like

Anacamptis morio and *A. longicornu* (Zitari *et al*., 2011), also make the morphological classification more difficult. For identification of these difficult to classify taxa, a rapid species identification technique like DNA barcoding is required (Schindel and Miller, 2005).

Orchids besides their beautiful, long-lasting, fragrant and attractive colorful flowers they are also known for their ornamental and therapeutic properties, for which the natural populations of orchids have been over exploited in the past, thus rendering these species threatened and endangered many important species. Since time immemorial orchids have also been used for curing various diseases and ailments (Jalal *et al*., 2008). India too being a home to the rich repository of medicinal herbs has a long history of utilizing orchid species in traditional folk medicine such as *Ayurveda*, *Siddha* and *Unani* (Jalal *et al*., 2008). Many orchid species have also been used in traditional system of medicine for curing various ailments like tuberculosis, paralysis, stomach disorders, chest pain, arthritis, syphilis, jaundice, cholera, acidity, eczema, tumour, piles, boils, inflammations, menstrual disorder, spermatorrhea, leucoderma, diahorrhea, muscular pain, blood dysentery, hepatitis, dyspepsia, bone fractures, rheumatism, asthma, malaria, earache, sexually transmitted diseases, wounds and sores (Bulpitt *et al*., 2007;Hossain, 2011;Pant, 2008). The therapeutic properties of different orchids are: aphrodisiac, rejuvenator, tonic, antibacterial, antioxidant and immune-modulating (Bulpitt *et al*., 2007). *Chyavanprash*, a popular Indian traditional polyherbal formulation that is widely used as tonic, rejuvenator, anabolic, immune-modulator and memory enhancer, consists of eight herbal components popularly known as '*Ashtavarga'* in '*Ayurveda'* (Singh and Duggal, 2009). Out of the eight components of *ashtavarga*, four are orchids. These are Riddhi (*Habenaria intermedia*), Vriddhi (*Habenaria edgeworthii*), Jivaka (*Malaxis muscifera*) and Rishabhaka (*Malaxis acuminata*) (Singh and Duggal, 2009). *Flickingeria*

macraei, known as '*Jeevanti'* in '*Ayurveda'* is used as astringent to the bowels, aphrodisiac and in asthma and bronchitis (Hossain, 2011). Other commonly used orchid drugs in the Ayurvedic system are salep (*Orchis latifolia* [accepted name is *Dactylorhiza incarnata*] and *Eulophia latifolia*), jivanti (*Dendrobium alpestre*), shwethuli and rasna (*Acampe praemorsa* and *Vanda tessellata*) (Hossain, 2011). In '*Sushruta Samhita'* it is mentioned that the underground tubers of *Orchis latifolia* is used in the drug 'munjatak' which pacifies cough (Hossain, 2011). The leaves of *Vanda roxburghii* are prescribed in the ancient Sanskrit literature for external application in rheumatism, ear infections, fractures and diseases of nervous system (Hossain, 2011). Most common compounds found in Orchidaceae are hydroxylbenzyl derivatives, fluorenones and stilbenoids (Yang *et al*., 2006) and flavones C-glycosides (Williams, 1979). Therefore, the Convention on International Trade of Endangered Species of Fauna and Flora (CITES) have listed all orchid species and their trade from the wild is banned (http://www.cites.org; Sun *et al*., 2011). In the absence of effective identification methods, rampant collection, trade and export of these endangered orchid species especially in vegetative form cannot be checked. The technique of DNA barcoding could provide a potent method for checking these illicit practices by offering a fool proof method for their detection in any form and stage, which could indirectly help in their conservation status.

The species of Orchidaceae are valued for cut flower production and as potted plants [e.g. *Dendrobiums, Paphiopedilums, Cypripediums, Phalaenopsis* etc.] (www.orchidsasia.com). They are prized for their incredible diversity in the size, shape and colour, attractiveness of their flowers and high keeping qualities even up to 10 weeks and enchantingly beautiful flowers which fetch a very high price at both national and international market (www.orchidsasia.com). Orchids have consistently ranked among the best sellers in the global potted plant trade (FloraHolland, 2015; USDA, 2016) and

also comprise 10% of all fresh cut flowers traded internationally (De, 2015). Among top ten cut flowers in the international market, orchids rank the sixth position and among orchids *Cymbidium* ranks the first position and in floricultural crops it accounts for 3% of the total cut flower production (De and Singh, 2016**)**. They are also harvested, grown and traded for a variety of purposes, including as ornamental plants, medicinal and food products. Thailand, Taiwan, The Netherlands and Japan are the largest areas of production, with demand for both potted and cut flowers growing in economic value annually (Griesbach, 2002; Hanks, 2015). The Netherlands alone valued at almost €500 million in 2015 with exports of potted orchids (FloraHolland, 2015). Thailand is the largest exporter of orchid cut flowers to India accounting up to 80.67% of total import followed by Netherlands 15.54%, New Zealand 2.29% and China 1.5%, respectively. Highest import of orchids was recorded in 2013-2014 (INR 3425.76 Lacs) followed by 2015-2016 (INR 2985.19 Lacs) and 2018-2019 (INR 2321.84 Lacs). Maximum export of orchids was found in 2016-2017 (INR 5.23 Lacs) followed by 2017-2018 (INR 4.89 Lacs) (De, 2020). However, despite this well-developed legal trade, orchids are also widely and illegally harvested from the wild for local, regional and international trade. There are growing concerns that trade, although largely unreported, is threatening wild orchid populations and species in many places.

The plant mitochondrial genes with low nucleotide substitutions and low evolutionary rates were considered unsuitable for barcodes of plants (Chase *et al*., 2005; Kress *et al*., 2005; Newmaster *et al*., 2006). Therefore, nuclear and plastid genes have been the prime focus of research for the identification of the locus/loci which could become species level molecular signatures for the plants. A number of plastid genes have been tested and proposed as probable plant barcodes by different groups. Kress *et al*. (2005) identified nine intergenic spacers (*trnK-rps16, trnH-psbA, rp136-rps8, atpB-rbcL,*

ycf6-psbM, trnV-atpE, trnC-ycf6, psbM-trnD and *trnL-F*), which were found to be the most variable regions and thus met the barcode criteria based on their comparison of the complete plastid genomes of *Nicotiana tabacum* and *Atropa belladonna* followed by determination of raw divergence levels across all genes, introns and intergenic spacers. From the nuclear genome they proposed the use of the internal transcribed spacer (ITS) and *trnH-psbA* spacer from the chloroplast genome as DNA barcodes for flowering plants (Kress *et al*., 2005). *rbcL* (Newmaster *et al*., 2006, Kress and Erickson, 2007), *rpoB, rpoC1* (Chase *et al*., 2005; Newmaster *et al*., 2006; Sass *et al*., 2007; Seberg and Petersen, 2009) and *matK* (Lahaye *et al*., 2008) are other loci which were tested for DNA barcoding of plants with some success. Beside the single locus barcodes, a two-locus barcode based on a combination of *trnH-psbA* spacer and *rbcL* was proposed (Kress and Erickson, 2007). Likewise, Chase *et al*. (2007) proposed two 3-locus barcode combinations comprising *matK+rpoB+rpoC1* and *matK+rpoC1+trnH-psbA*. Hollingsworth *et al*. (2009) proposed the combinations of *rbcL*, *matK, rpoC1* and *trnH-psbA* could provide a universal plant barcode for plants. Subsequently, the CBOL Plant Working Group (2009) proposed a combination of *matK* and *rbcL* as the core barcode for plants. Li *et al*. (2011a) too supported the two genes as core barcodes for ferns. Most of the single copy genes and their introns in the nuclear genome were not considered suitable for barcoding because of the lack of universal primers for their amplification (Kress *et al*., 2005; Chen *et al*., 2010). Kress *et al*. (2005) suggested the use of 5.8S ribosomal cistron from the nuclear genome along with the internal transcribed spacer (ITS) of the nuclear ribosomal DNA as the possible barcode for plants. Chen *et al*. (2010) validated the use of ITS2 region as an effective barcode for identifying medicinal plant species. Pang *et al*. (2010) also reported ITS2/ITS as the potential barcode for identification of members of the family Euphorbiaceae. Based on the analysis of ITS2

sequences of 50,790 plants and 12,221 animals downloaded from the GenBank, NCBI Yao *et al*. (2010) recommended ITS2 as the universal barcode for both animals and plants. Recently, the China Plant BOL Group (2011) has strongly advocated the inclusion of ITS in the core barcode for plants along with *matK*+*rbcL*.

A number of studies, mostly in the recent past, have evaluated the potential of various loci against a taxonomic backdrop by comparing the species discrimination ability within an order, family or genus. Some examples are Caryophyllales (Cuénoud *et al*., 2002) at the order level; Geraniaceae (Guisinger *et al*., 2008), Asteraceae (Gao *et al*., 2010b), Euphorbiaceae (Pang *et al*., 2010), Lemnaceae (Wang *et al*., 2010), Podostemaceae (Kelly *et al*., 2010), Caryoteae (Jeanson *et al*., 2011), Cycadaceae (Nicolalde-Morejón *et al*., 2011), Fabaceae (Gao *et al*., 2011), Grimmiaceae (Liu *et al*., 2011b), Meliaceae (Muellner *et al*., 2011), and Cacteaceae (Yesson *et al*., 2011) at the family level. At the generic level, different barcode loci have been tested for the discrimination of congeneric species of *Araucaria, Inga* (Hollingsworth *et al*., 2009), *Acacia* (Newmaster and Ragupathy, 2009), *Crocus* (Seberg and Petersen, 2009), *Carex* (Starr *et al*., 2009), *Solanum* (Spooner, 2009), *Dendrobium* (Yao *et al*., 2009, Asahina *et al*., 2010, Singh *et al*., 2012), *Alnus* (Ren *et al*., 2010), *Berberis*, *Ficus* and *Gossypium* (Roy *et al*., 2010), *Carex* and *Kobresia* (Le Clerc-Blain *et al*., 2010), *Agalinis* (Pettengill and Neel, 2010), *Picea* (Ran *et al*., 2010), *Taxus* (Liu *et al*., 2011a), *Quercus* (Piredda *et al*., 2011) and *Holcoglossum* (Xiang *et al*., 2011).

As per literatures the first DNA barcoding of orchid study was carried out by Lahaye *et al*. (2008) that sampled 1,036 Orchidaceae species from Mesoamerican biodiversity hotspot of southern Africa. They tested eight potential barcode loci and demonstrated that *matK* could identify almost all species. Lahaye *et al*. (2008) sampled 86 species of flowering plants along with 1,036 orchid species in two different data sets.

They observed that in the orchid data set, *matK* was amplified in all the sampled species, *ndhJ* and *ycf5* did not amplify efficiently, the alignment of *trnH-psbA* was difficult as it required addition of several gaps, and presence of *rps19* gene insertion in *trnH-psbA* and a less variable *rbcL* gene region.

The species identification success with *matK* alone was >90%. Their analysis pointed out that the 5′ end of *matK* exon was easy to amplify and align, and hence proposed *matK* as the preferred universal DNA barcode for flowering plants. However, Farrington *et al.* (2009) reported that *matK* region was not found to be suitable in discriminating species of Australian orchid genus *Cladenia.* They found that *matK* to be less informative at the subgeneric level and therefore, were not suitable for discriminating *Cladenia* species. On the other hand, they found *trnL* intron to be more useful for discrimination of *Cladenia* species. In another investigation, Yao *et al*. (2009) used *trnH-psbA* spacer, one of the candidate DNA barcode for testing the applicability of DNA barcoding technique in authentication/identification of 17 medicinally important *Dendrobium* species. Asahina *et al*. (2010) too investigated the species discriminating abilities of *matK* and *rbcL* barcode regions for five species of *Dendrobium*. The five medicinal species investigated were *Dendrobium fimbriatum, D. moniliforme, D. nobile, D. pulchellum* and *D. tosaense*. The phylogenetic tree analysis was carried out using whole gene sequences of *matK* and *rbcL, matK* could provide 100% species discrimination success while *rbcL* tree showed less discrimination power which was attributed to low variation in *rbcL* gene sequences (Asahina *et al*., 2010). These results based on only five *Dendrobium* species indicated that *matK* could provide 100% species identification in this genus. However, in another investigation, *matK* could resolve only 76.92% species among 36 species of *Dendrobium* analyzed (Singh *et al*., 2012), while ITS in the same study yielded 100% resolution. As opposed to the observation of Yao *et*

al. (2009), in this study, *trnH-psbA* spacer could not be tested for the discrimination of *Dendrobium* species because of the failure in obtaining its bidirectional sequences (Singh *et al*., 2012). Among the chloroplast genome, the loci *matK* + *rpoB* + *rpoC1* provided the best multi-locus combination. This three-locus barcode could resolve 92.31% (48 out of 52) species (Singh *et al*., 2012). Previously reported success combination of *matk*+*rbcL* in discriminating all five species of *Dendrobium* (Asahina *et al*., 2010) was ascribed to low sample size (Singh *et al*., 2012). The genus *Holcoglossum* of the subtribe Aeridinae with a complex taxonomy contains both long-evolved and recently radiated species, thus becoming an exceptional case to test DNA barcodes for Orchidaceae. Xiang *et al*. (2011) tested the potential of DNA barcoding for 12 species of this genus. The DNA regions tested from a subset of proposed barcode loci were *rbcL, matK, atpF-atpH, psbK-psbI, trnH-psbA* from the plastid genome and nuclear ITS. The amplification success was 100% for all loci in 52 samples except for *matK* in which amplification success rate was 92.3%. The sequencing success rate was 100% for *rbcL* and *trnH-psbA*, 84% in ITS and ~75% in *matK*. The sequencing of *atpF-atpH* and *psbK-psbI* loci was not successful because of the presence of mononucleotide repeats. The highest variability was found in *matK* and ITS regions. The six candidate barcode loci could not individually distinguish all 12 species of *Holcoglossum*, *matK* could resolved eight of the 12 species and thus, had the highest species discriminatory ability. Instead of *matK* alone, the combined sequences of *matK*+ITS afforded better species discrimination. This study concluded that *matK* was the best region to identify species of *Holcoglossum* but for identification of all species, other DNA regions would be required (Xiang *et al*., 2011). Parveen *et al*. (2012) tested the potential of five barcode loci in discriminating eight endangered *Paphiopedilum* species from India. In the analysis, nrITS could afford only 50% species resolution with 4.4% average inter-specific divergence value. On the other hand, *matK* yielded 100%

species resolution with 0.9% average inter-specific divergence value. Also, DNA barcodes of the three hybrids reflect their parentage. For identification of closely related endangered species of Indian *Paphiopedilums*, *matK* locus emerged as the signature sequence and also in elucidating the parentage of their inter-specific hybrids (Parveen *et al*., 2012).

Vandaceous orchids are the major ornamental crops as cut flower and potted plants as they have high demands in both National and International market (Lekawatana, 2010). They can be crossed within the same genus or with different genera leading to the production of various new hybrids having similar morphological traits. The export of orchids collected from the wild is banned (http://www.cites.org). Despite of this well regulated laws this medicinal and ornamental orchids are still illegally traded using their parts/even fragments, which cannot be identified using traditional taxonomic methods. Thus, an alternative identification method which can identify at any stages of life for Vandaceous orchids is needed for variety and species certification and protection purposes (Peyachoknagul *et al*., 2014). With this in mind, to conserve the orchid species and to curb the illicit trade practices of biopiracy, DNA barcoding technique was necessitated to establish the barcode for this important Vandaceous group of orchids which would become a powerful tool in the hands of the law enforcement agencies.

MATERIALS AND METHODS

Isolation of Total Genomic DNA

The total genomic DNA was isolated using CTAB methods/protocols depending on the type of tissue sampled. The protocols used are as follows:

CTAB Method

CTAB DNA extraction protocol (Doyle and Doyle, 1987)

1. Grind 0.5 g of plant tissue to a fine paste in liquid nitrogen using mortar and pestle. Add sample in pre-warmed 2% CTAB buffer (10 ml).

2. Incubate the CTAB/plant extract mixture for 1 hr at 60ºC in a water bath with timely mixing by inversion in between.
3. After incubation, the sample CTAB/plant extract mixture is spin at 10,000 rpm for 10 min.
4. Transfer the supernatant to clean centrifuge tubes.
5. To each tube add equal volume of Chloroform: Isoamyl Alcohol (24:1) and mix the solution by inversion for 5 min. After mixing, spin the tubes at 10,000 rpm for 8 min.
6. Transfer the upper aqueous phase only (contain the DNA) to clean centrifuge tubes.
7. DNA is precipitated using equal volumes of chilled isopropanol. Mix for 5 min. and incubate at -20ºC for 5-10 min.
8. Centrifuge at 14,000 rpm for 10 min. Supernatant is decanted slowly without disturbing the DNA pellet formation at the base of the centrifuge tube.
9. Wash pellet in 500-1000 µl of 70% chilled ethanol by inversion and centrifuged at 14,000 rpm for 5 min.
10. Supernatant/ethanol solution is decanted and air dried for 20-30 min. DNA should not be over dried or it will be hard to re-dissolve.
11. Add 50-200 µl of TE buffer to re-suspend the DNA pellet.
12. RNaseA (10 mg/ml) treatment can be done to remove the RNA from the sample by adding 6-10 µl of RNase and incubate at 37ºC in water bath for 45 min.
13. Finally, DNA sample is tested qualitatively in 0.8% agarose gel electrophoresis and quantified by Thermo Scientific Multiskan Go Spectrophotometer.

Modified CTAB method

1. Grind 200 mg of - 60°C stored plant leaf tissue to a fine paste in 2 ml CTAB buffer by using pre chilled mortar and pestle.
2. Transfer CTAB/plant extract mixture to a micro centrifuge tube and add 0.2% β-Mercaptoethanol (v/v) and incubate the mixture in a water bath for 1 h at 60˚C with frequent inversion.
3. After incubation, spin the CTAB/plant extract mixture at 12,000 rpm for 10 min at room temperature (20°C).
4. To each tube add equal volumes of Chloroform: Isoamyl (24:1) and mix the solution by inversion for 5 min and centrifuge at 12,000 rpm for 10 min.
5. Transfer the aqueous phase only to a clean micro centrifuge tube and repeat step 4 again (until solution is transparent).
6. The upper aqueous phase contains DNA. Add equal volume of chilled isoproponol to precipitate the DNA. Mixed the solution for 5 min by inversion and incubate at - 60˚C for 10-30 min (depending on the precipitation).
7. Centrifugation at 14,000 rpm for 15 min and the supernatant is discarded.
8. Wash pellet in 70% chilled ethanol by inversion and centrifuge at 12,000 rpm for 5 min (Repeat the same step 2-3 times).
9. Discard the supernatant and the pellet is air dried at room temperature (ethanol residue should be remove but it should not be over dried as it would be hard to re-suspend the DNA).
10. Add 100-200 µl of high salt TE buffer to re-suspend the DNA pellet.
11. RNase A (10 mg/ml) treatment can be done to remove RNA from the sample (3-5 µl RNase is added and maintain at 37°C for 45 min).

12. DNA concentration (quality and yield) was measured by running aliquots on 0.8% TAE agarose gel, stained with ethidium bromide and bands were observed in gel documentation system.

13. The DNA samples were stored at -20°C until further use.

PCR Amplification

PCR Protocol Used for Amplification of matK, rbcL and ITS

PCR master mix composition 1(One)

Table 3.1

Components	Initials Concentration	Vol. for 25 µl	Final Concentration
PCR buffer with 25 mM $MgCl_2$	10X	3.0 µl	1X
dNTPs	10 Mm	1.0 µl	0.2 mM
Forward primer	10 mM	0.5 µl	0.2mM
Reverse primer	10 mM	0.5 µl	0.2 mM
Template DNA	>1000 ng	3.0 µl	50-100 ng
Taq polymerase	3U	0.3 µl	≤1U
Nuclease free water		16.7 µl	

PCR Master Mix Composition 2(Two)

Pre-mix from company (Takara)

Table 3.2

Components	Vol. for 25 µl
PCR Pre-mix	**6.0**µl
Forward primer	**0.5** µl
Reverse primer	**0.5** µl
Template DNA	**3.0**µl
Nuclease free water	**15.0**µl

Bioinformatics Tools

The software used for analyzing the chromatograms of amplicons generated after sequencing were as follows:

a) BioEdit version 7.0.9.0 (Hall, 1999).

b) MEGA version 7.0 (Kumar *et al*., 2016).

Primers Used

Details of different primers used for the present study is given below in Table 3.3.

Table 3.3: Primers used for amplification of candidate DNA barcodes

Region	Primer Name	Sequence Direction	Bases
ITS	IT1 F	TCG TAA CAA GGT TTC CGT AGG T	22
	IT2 R	GTA AGT TTC TTC TCC TCC GCT	21
matK	*matK*-1 F	ATC CAT ATG GAA ATC TTG GTT C	22
	matK-1 R	GTT CTA GCA CAC GAA AGT CG	20
rbcL	*rbcL* F	ATG TCA CCA CAA ACA GAA ACT AAA CG	24
	*rbcL*R	CTT CGG CAC AAA ATA AGA AAC GAT CTC	27

Sequencing of the Amplicons and Sequence Analysis

The amplified amplicons were then sent for sequencing to 'Chromous Biotech Pvt. Ltd. Bangalore, India; 1st Base Laboratory, Singapore; Eurofins Genomics Pvt Ltd. Bangalore, India and Barcode Biosciences, Bangalore, India. Sequence quality check was performed by using BioEdit sequence alignment Editor Software and BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for search of the reference of each query sequence in NCBI GenBank. The targeted DNA sequences of *matK, rbcL,* ITS and those nucleotide sequences retrieved from the GenBank after blasting were subjected to multiple sequence alignment using ClustalW, a tool for multiple sequence alignment (Thompson *et al*., 1994) through MEGA-7 (Kumar *et al*., 2016) software. The nucleotide sequences of *matK, rbcL* and ITSregions for the candidate species were submitted to NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and accession numbers obtained. Phylogenetic trees were constructed with Neighbor Joining (NJ) method in MEGA and the evolutionary distances were computed using Kimura 2-parameter (K2P) distance as a model of substitution and running 1000 bootstrap replicates to assess the relative support for the branches for the

investigated species. The percentage of replicate tree in which the associated taxa clustered together in the bootstrap test is shown next to the branches. The inter-specific K2P distances were determined for each representative species sequence. The species identification success rate for each locus was determined using-

(i) K2P distances arrived at on the basis of analyses of the species utilizing MEGA 7.0,

(ii) Neighbour Joining trees constructed with the sequences of the selected loci and

(iii) BLAST method (Ross *et al*., 2008).

For the distance based method, species resolution of each locus was calculated according to the following formula:

$$\frac{\text{Total no. of species - No. of species with zero distance estimate}}{\text{Total no. of species}} \times 100$$

For tree based method, the species resolution was calculated according to the following formula:

$$\frac{\text{Total no. of species - No. of species which clustered with other species}}{\text{Total no. of species}} \times 100$$

In the BLAST method, the percent species discrimination was based on the BLAST search for homologous sequences in the Genbank with first hit maximum query coverage and identity with the query sequences.

RESULTS

The three potential barcode loci both from the chloroplast genome (*matK, rbcL*) and the nuclear genome (ITS) as DNA barcodes were tested for barcoding of Vandaceous orchid across 15 genus belonging to 31 species (80 individuals) of the family Orchidaceae. Altogether 201 barcode sequences were generated and submitted to NCBI GenBank (Table 3.4). Amplification and sequencing success rates for three selected barcode loci were evaluated and compared individually and their intra- and inter-specific divergence values were calculated using genetic distance method. Species resolutions for each locus were calculated based on genetic distances, phylogenetic tree method, and

through BLAST analysis. The intra-specific variations were estimated for only those species, which were represented by more than one individual. The inter-specific K2P distances and species resolution analysis was carried out at the generic level only. At the generic level, the inter-specific variations among the species of a genus were calculated using the distance matrix prepared by aligning the sequences of all the accessions belonging to different species of a genus. The K2P distances and tree building methods were used to discriminate the congeneric species of a genus.

Table 3.4: Accession number obtained from GenBank

Sl. no.	Genus	Species	*matK*	*rbcL*	ITS
1.	*Vanda*	1. *V. coerulea*	KY744816 MW496849 MW496850	MG867450 MW433893 MW537579	MG818987 MW362400 MW492905
		2. *V. bicolor*	MG925667 MW405194 MW405195 MW405196	MG867451 MW405190 MW405191 -	MG822845 MW368614 MW365331 -
		3. *V. ampullacea*	MK064245 MW405193	MK214918 MW433891	MN170562 -
		4. *V. stangeana*	MG925666 MW405199 MW405185 MW405186	MG905911 MW433895 MW433896 -	MG822848 MW362402 - -
		5. *V. testacea*	MN187219 MW496852	MK064251 MW433894	MN170573 MW425864
		6. *V. alpina*	MN187221 MW496851 -	MN220141 MW405200 -	MN173057 MW362399 MW362401
		7. *V. pumila*	MN523479 MW405189	MN523481 MW405192	MN517224 MW368597

			-	-	MW493108
2.	*Cleisostoma*	1. *C. paniculatum*	MT974498 MW448188 MW448189 -	MT974499 MW448192 MW448193 MW448196	MT422095 MW442838 - -
		2. *C. simondii*	MK064249 MW448190 MW448191	MN298853 MW645350 -	MG822849 MW355894 MW362366
		3. *C. williamsonii*	MK160138 MW645352 -	MN298854 MW448194 MW448195	MN517118 MW442840 -
3.	*Acampe*	1. *A. rigida*	MN523477 MW603189	MG905912 MW603191	MN173056 MW617314
		2. *A. praemorsa*	MK160137 -	MK214919 MW603192	MN517126 MN170566
		3. *A. ochracea*	MK160136 MW603187 -	MN178485 MW603194 -	MW600257 MW600256 MN170563
4.	*Gastrochilus*	1. *G. obliquus*	MN239895 MW433890 MW480553	MN239899 MW645351 -	MN240429 - -
		2. *G. calceolaris*	MN239897 MW480554	MG925669 MW480549	MN517123 MW475266
		3. *G. acutifolius*	MN239894 MW433889	MN239898 MW433892	MT225573 MW475270
5.	*Phalaenopsis*	1. *P. wilsonii*	MG958489	MG958488	MG952632
		2. *P. braceana*	MT974500	MT974501	MT974319
		3. *Hygrochilus parishii* *(P. hygrochila)*	MK160139 MW603188 -	MN220143 MW603193 -	MN170567 MW599846 MW617318
6.	*Papilionanthe*	1. *P. teres*	MK160135 MW448187 -	MG925670 MW457591 -	MG821161 MW362367 MW362392

		2. *P. vandarum*	MN523480 MW457585 -	MN178486 MW457588 MW457589	MG821080 MW362394 MW362368
7.	*Arachnis*	1. *E. clarkei (A. clarkei)*	MN523478 MW457586	MN220142 MW457590	MG820621 MW452979
		2. *A. labrosa*	MK064246 MW457584 MW603184	MT227265 MW457587 -	MG820749 MW599843 -
8.	*Stereochilus*	1. *S. laxus*	MT178331	MT178332	MT178771
9.	*Vandopsis*	1. *V. undulata*	MN187222 MW603190 -	MG925668 MW457592 MW457593	MG786550 MW452980 -
10.	*Renanthera*	1. *R. imschootiana*	MN239896 -	MG932128 MW645353	MG820707 MW599845
11.	*Aerides*	1. *A. odorata*	MK064247 MW480552	MN178484 MW480548	MG822846 MW599844
12.	*Rhynchostylis*	1. *R. retusa*	MK064250 MW480550 MW603185 MW603186	MG867449 MW480544 - -	MG822847 MW475272 MW475274 MW475276
13.	*Sarcoglyphis*	1. *S. mirabilis*	MT419780 MW480551 - -	MT419781 MW480545 MW480546 MW480547	MT416451 MW475278 - -
14.	*Smitinandia*	1. *S. micrantha*	MN187220 -	MN523482 MW603195	MN170568 MW617320
15.	*Thrixspermum*	1. *T. tsii*	MK160140	MK214920	MN170569

Amplification and Sequencing Success Rates

All together 80 orchidaceous individuals from 15 genus belonging to 31 species were tested for PCR amplification with different loci. The variable numbers of amplicons obtained for different loci were 74 for *matK*, 76 for *rbcL* and 73 for ITS. Thus, the

amplification success rates stands at 92.5% for *matK*, 95.00% for *rbcL* and 91.25% for ITS. Among the investigated individuals, some species belonging to *Acampe, Aerides* and *Arachnis* species showed multiple bands of amplicons with all the tested loci. The band having molecular weight nearest to that of the target locus was marked and gel extracted and sequenced/sometimes instructs the companies to elute while sending for sequencing. The sequencing success rates of *matK, rbcL* and ITS were 89.18%, 89.47% and 91.78%respectively. The total number of barcode sequences generated was 66 for *matK,* 68 for *rbcL* and 67 for ITS (Table 3.5).

Table 3.5: Amplification and sequencing success rates for three tested loci (80 individuals per loci)

Locus	Length of amplicons obtained (bp)	No. of amplicons obtained	Amplification success rates	No. of finished sequences generated	Sequencing success rates
matK	700-800bp	74	92.5%	66	89.18%
rbcL	600-700bp	76	95.00%	68	89.47%
ITS	600-700bp	73	91.25%	67	91.78%

Intra-specific Variation

The intra-specific distances were calculated for all the species that were represented by more than one accession per individuals for all the investigated loci. All the investigated loci showed intra-specific variations with variables range. The *matK* sequences of the multiple accession of *V. coerulea, V. alpina, A. clarkei (Esmeralda)* and *A. labrosa* had intra-specific variation from 0-0.002 with an average of 0.001. *V. bicolor, A. rigida* had intra-specific variation from 0-0.010, *V. pumila, P. vandarum* had 0-0.001, *C. paniculatum, G. obliquus* had 0-0.022, *A. ochracea, G. calceolaris, A. odorata* had 0-0.004 respectively. Some of the *rbcL* sequences of *V. bicolor, A. praemorsa* and *G. acutifolius* had average intra-specific variation of 0.001 ranges from 0-0.002. *V.*

ampullacea, P. vandarum had intra-specific variation from 0-0.010, *C. simondii, P. hygrochila (H. parishii)* had intra specific variation from 0-0.006, *A. rigida, R. retusa* and *S. mirabilis* had intra-specific variation from 0-0.003. Some of the ITS sequences of *V. coerulea, V. pumila* and *G. acutifolius* had intra-specific variation from 0-0.010 with an average 0.005. *C. paniculatum* and *A. odorata* had intra-specific variation from 0-0.008, *A. clarkei (Esmeralda)* and *S. mirabilis* had intra specific variation of 0-0.019 (Table 3.6).

Table 3.6: Intra-specific K2P distance for three candidate loci

Sl. No.	Species	Intra-specific K2P distances based on genetic distance method		
		matK	***rbcL***	**ITS**
1	*Vanda coerulea*	0-0.002	0-0.005	0-0.010
2	*Vanda bicolor*	0-0.010	0-0.002	0-0.089
3	*Vanda ampullacea*	-	0-0.010	-
4	*Vanda stangeana*	0-0.003	0-0.025	0-0.092
5	*Vanda testacea*	-	-	0-0.037
6	*Vanda alpine*	0-0.002	0-0.011	0-0.034
7	*Vanda pumila*	0-0.001	-	0-0.010
8	*Cleisostoma paniculatum*	0-0.022	0-0.009	0-0.008
9	*Cleisostoma simondii*	0-0.028	0-0.006	0-0.009
10	*Cleisostoma williamsonii*	0-0.005	0-0.008	0-0.001
11	*Acampe rigida*	0-0.010	0-0.003	0-0.012
12	*Acampe praemorsa*	-	0-0.002	0-0.050
13	*Acampe ochracea*	0-0.004	-	0-0.162
14	*Gastrochilus obliquus*	0-0.022	-	-
15	*Gastrochilus calceolaris*	0-0.004	-	0-0.007
16	*Gastrochilus acutifolius*	0-0.018	0-0.002	0-0.010
17	*Phalaenopsis wilsonii*	-	-	-
18	*Phalaenopsis braceana*	-	-	-
19	*Phalaenopsis hygrochila*	0-0.006	0-0.006	0-0.038
20	*Papilionanthe teres*	0-0.016	-	0-0.004

21	*Papilionanthe vandarum*	0-0.001	0-0.010	0-0.011
22	*Arachnis clarkei (Esmeralda)*	0-0.002	0-0.013	0-0.019
23	*Arachnis labrosa*	0-0.002	0-0.022	0-0.006
24	*Stereochilus laxus*	-	-	-
25	*Vandopsis undulata*	0-0.014	-	0-0.024
26	*Renanthera imschootiana*	-	-	0-0.002
27	*Aerides odorata*	0-0.004	-	0-0.008
28	*Rhynchostylis retusa*	0-0.023	0-0.003	0-0.034
29	*Sarcoglyphis mirabilis*	0-0.030	0-0.015	0-0.019
30	*Smitinandia micranta*	-	0-0.003	0-0.013
31	*Thrixspermum tsii*	-	-	-

Inter-Specific K2P Distance and Species Discrimination Rates for Three Loci

Inter-specific K2P distance and species discrimination rates for three locus were described separately under separate heads based on the three methods *viz*., Genetic distance, Phylogenetic tree and BLAST.

Using *matK*

Interspecific K2P Distances

Interspecific K2P distances were calculated for 66 *matK* sequences belonging to 31 species and revealed an average inter-specific K2P distance of 0.061 with a range of 0-1.099. Out of the 31 species (66 individuals) analyzed, 6 exhibited zero distance estimates with one or the other species. The maximum inter-specific K2P distance of 1.099 was between *Stereochilus laxus* and *Gastrochilus acutifolius*. (Table 3.7: *matK* pairwise genetic distance).

Species Resolution

The species discrimination rates were calculated on the basis of the following three methods:

Distance based method

The species resolution of aligned *matK* sequences from 66 species was calculated by K2P distance matrix method. The distance matrix revealed 9 species pair that had zero distance estimates. The formation of these species pairs involved 6 species; therefore the % species resolution stands at 90.90% (Table 3.8: Average inter-specific K2P distance). The species pairs formed were: *Vanda bicolor-V. bicolor*; *V. bicolor-V. bicolor*; *V. bicolor-V. bicolor*; *V. ampullacea-V. ampullacea*; *V. stangeana-V. stangeana*; *V. stangeana-V. stangeana*; *V. stangeana-V. stangeana*; *V. testacea-V. testacea*; *Vandopsis undulata-Papilionanthe vandarum*.

Table 3.8: Average inter-specific K2P distance and percent species resolution for three investigated loci

Locus	No. of Species analyzed	Average Inter-specific K2P distance (Range)	Species Discrimination Rates		
			Distance Based Method	Phylogenetic Tree Method	BLAST Method
matK	66	0.061 (0-1.099)	90.90%	95.45%	91.84%
rbcL	68	0.018 (0-0.066)	76.47%	92.64%	59.96%
ITS	67	0.150 (0-0.539)	95.52%	92.53%	79.40%

Phylogenetic Tree-Building

The aligned *matK* sequences analyzed from 31 species (66 individuals) revealed that out of 897 nucleotide sites compared, 440 were variable sites of which 154 were parsimony-informative sites (a site is parsimony-informative if it contains at least two types of nucleotide/amino acids, and at least two of them occured with a minimum frequency of two) and 286 were singleton sites (a singleton site contains at least two

types of nucleotides/amino acids with at the most one occurring multiple times). The Neighbour joining tree which is a distance based method, was constructed with 1000 bootstraps replicates, revealed five different clusters comprising 6 species and thus resulting in 90.90% species resolution. The species clusters form were (i) *Vanda bicolor, V. bicolor, V. bicolor, V. bicolor, V. bicolor,* (ii) *V. ampullacea, V. ampullacea,* (iii) *V. stangeana, V. stangeana, V. stangeana, V. stangeana, V. stangeana, V. stangeana,* (iv) *V. testacea, V. testacea*, (v) *Vandopsis undulata* and *Papilionanthe vandarum* (Figure 3.1: *matK* tree). The individuals of 4 species viz. *Vanda coerulea, V. stangeana, Arachnis labrosa* and *Cleisostoma paniculatum* that had intra-specific variations however formed a single cluster with all the accessions of each of these four clustering together. One each individual of *Vanda bicolor* and *Gastrochilus calceolaris* were clustered together with *V. stangeana* and *Cleisostoma simondii.*

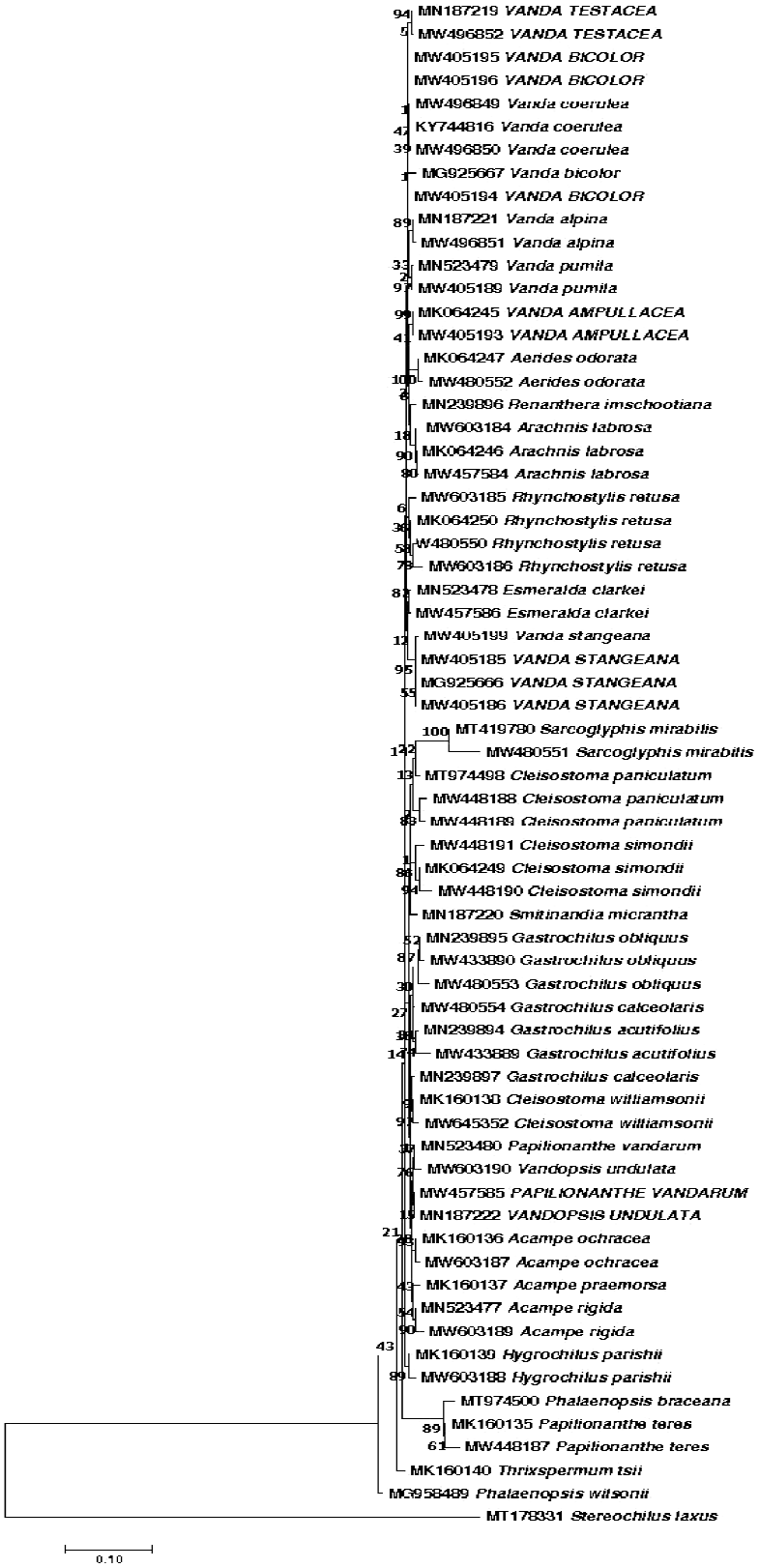


Figure 3.1: Neighbour joining tree of 31 species (66 individuals) based on *matK* sequences. The species showing zero inter and intra specific divergence are shown bold, capital and both as well.

Blast Analysis

In the BLAST analysis of 31 species (66 individuals) for *matK* sequences, 60 sequences correctly matched with the sequences of their own species. Out of the total 6 species that does not correctly matched/identified with their own species, 3 species are correctly identified up to genus level but not at the species level. Thus, the species resolution based on *matK* locus using BLAST method was 91.84% (Table 3.8: Average inter-specific K2P distance). The BLAST analysis was also carried out to determined that the query/amplified sequence is of the targeted locus and it is observed that all the 66 *matK* sequences generated in the present study were found to be only of the targeted locus and not contamination of fungi as they form symbiotic relationship with orchids/the host.

Using *rbcL*

Interspecific K2P Distances

Interspecific K2P distances were calculated for 68 *rbcL* sequences belonging to 31 species and revealed an average inter-specific K2P distance of 0.018 with a range of 0-0.066. Out of the 31 species (68 individuals) analyzed 16 exibited zero distance estimates with one or the other species. The maximum inter-specific K2P distance 0f 0.066 was between *Papilionanthe vandarum* and *Vanda stangeana*. (Table 3.9: *rbcL* pairwise genetic distance).

Species Resolution

Distance Based method

The species resolution of aligned *rbcL* sequences from 68 species was calculated by K2P distance matrix method. The distance matrix revealed 20 species pair that had zero distance estimates. Some of the species form zero distances with other species resulting in a number of species pairs with distance estimates as zero. The formation of these species pairs involved 16 species; therefore the % species resolution stands at 76.47% (Table 3.8: Average inter-specific K2P distance). The species pairs formed were: *Vanda coerulea-V. coerulea*; *V. bicolor-V. bicolor*; *V. stangeana-V. bicolor*; *V. stangeana-V. bicolor*; *V. testacea-V. testacea*; *V. pumila-V. pumila*; *Cleisostoma paniculatum-C. paniculatum*; *C. williamsonii-C. williamsonii*; *Acampe ochracea-A. ochracea*; *Gastrochilus obliquus-G. obliquus*; *G. calceolaris-G. calceolaris*; *Papilionanthe teres-P. teres*; *Vandopsis undulata-V. undulata*; *V. undulata-V. undulata*; *V. undulata-V. undulata*; *Renanthera imschootiana-R. imschootiana*; *Rhynchostylis retusa-R. imschootiana*; *R. retusa-R. imschootiana*; *Aerides odorata-A. odorata*; *Sarcoglyphis mirabilis-S. mirabilis*.

Phylogenetic Tree-Building

The aligned *rbcL* sequences analyzed from 31 species (68 individuals) revealed that out of 687 nucleotide sites compared, 159 were variable sites of which 88 were parsimony-informative sites and 71 were singleton sites. The Neighbour joining tree is constructed with 1000 bootstraps replicates, revealed 14 different clusters comprising 16 species and thus resulting in 76.47% species resolution. The species clusters form were (i) *Papilionanthe teres, P. teres* (ii) *Vanda coerulea, V. coerulea* (iii) *Acampe ochracea, A. ochracea* (iv) *Gastrochilus obliquus, G. obliquus* (v) *G. calceolaris, G. calceolaris* (vi) *Cleisostoma williamsonii, C. williamsonii* (vii) *Sarcoglyphis mirabilis, S. mirabilis* (vii)

Vandopsis undulata, V. undulata, V. undulata (viii) *Vanda stangeana, V. bicolor* (ix) *V. stangeana, V. bicolor* (x) *V.pumila, V. pumila* (xi) *V. testacea, V. testacea* (xii) *Cleisostoma paniculatum, C. paniculatum, C. paniculatum, C. paniculatum* (xiii) *Rhynchostylis retusa, Renanthera imschootiana* (xiv) *Aerides odorata, A. odorata*. (Figure 3.2: *rbcL* tree). The accession of 3 species *Cleisostoma paniculatum, Vandopsis undulata* and *Sarcoglyphis mirabilis* are all clustered with their own species.

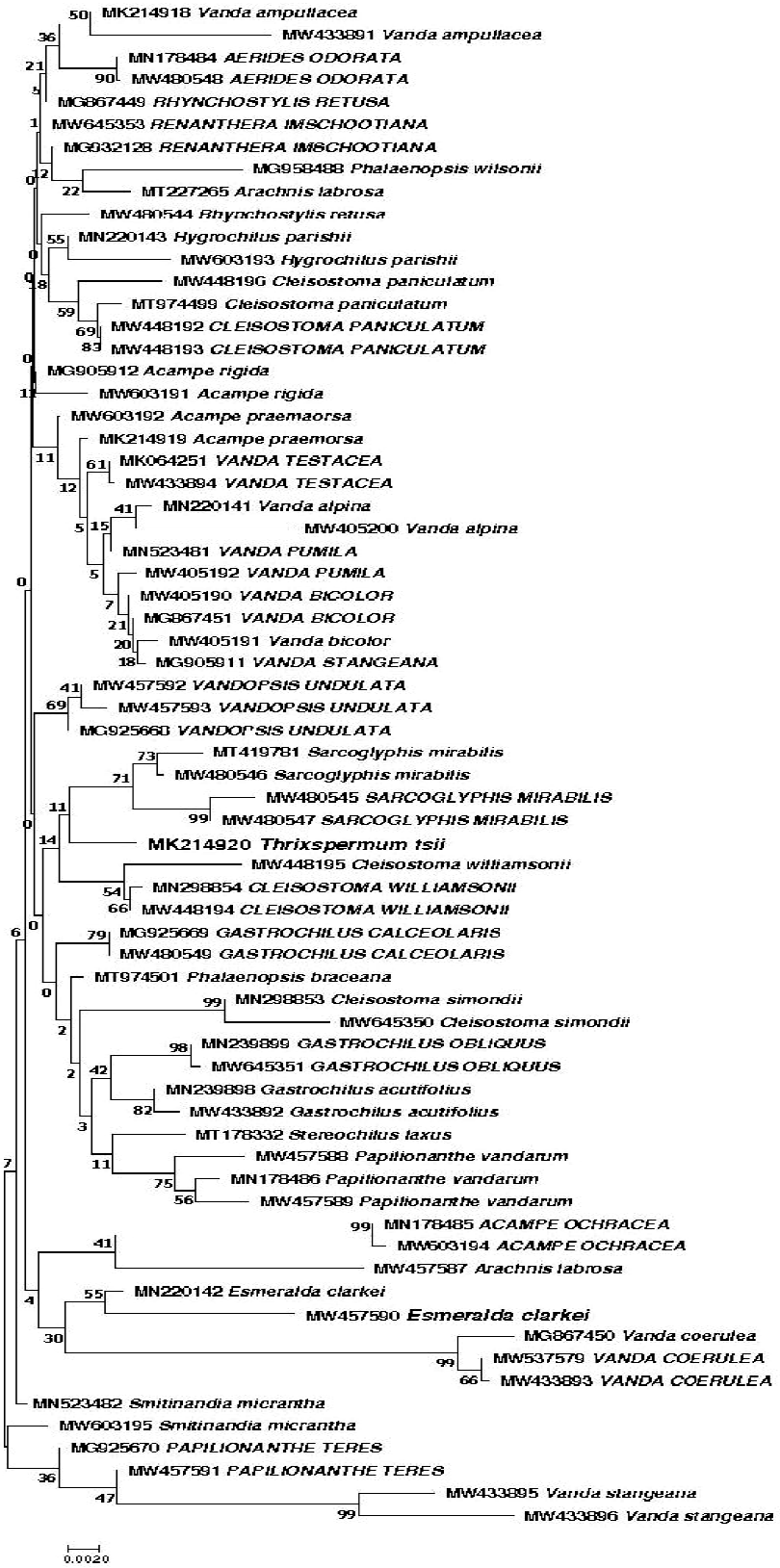


Figure 3.2: Neighbour joining tree of 68 species based on *rbcL* sequences. The species showing zero inter and intra specific divergence are shown bold, capital and both as well.

Blast Analysis

In the BLAST analysis of 31 species (68 individuals) for *rbcL* sequences, 41 sequences correctly matched with the sequences of their own species. Out of the total 27 species that does not correctly matched/identified with their own species, 26 species were correctly identified up to genus level but not at the species level. Thus, the species resolution based on ITS locus using BLAST method was 59.96% (Table 3.8: Average inter-specific K2P distance). The BLAST analysis was also carried out to determined that the query/amplified sequence is of the targeted locus and it was observed that all the 68 *rbcL* sequences generated in the present study were found to be only of the targeted locus and not contamination of fungi as they form symbiotic relationship with orchids/the host.

Using ITS

Interspecific K2P Distances

Interspecific K2P distances were calculated for 67 ITS sequences belonging to 31 species and revealed an average inter-specific K2P distance of 0.150 with a range of 0-0.539. Out of the 31 species (67 individuals) analyzed 3 exhibited zero distance estimates with one or the other species. The maximum inter-specific K2P distance 0f 0.539 was between *Acampe rigida* and *Vanda alpina*. (Table 3.10: ITS pairwise genetic distance).

Species Resolution

Distance Based method

The species resolution of aligned ITS sequences from 67 species was calculated by K2P distance matrix method. The distance matrix revealed 2 species pair that had zero distance estimates. The formation of these species pairs involved 3 species; therefore the % species resolution stands at 95.52% (Table 3.8: Average inter-specific K2P distance). The species pairs formed were: *Stereochilus laxus-Acampe praemorsa*; *Papilionanthe teres-P. teres*.

Phylogenetic Tree-Building

The aligned ITS sequences analyzed from 31 species (67 individuals) revealed that out of 848 nucleotide sites compared, 652 were variable sites of which 534 were parsimony-informative sites and 117 were singleton sites. The Neighbour joining tree was constructed with 1000 bootstraps replicates, revealed two different clusters comprising 3 species and thus resulting in 95.52% species resolution. The species clusters forms were (i) *Stereochilus laxus* and *Acampe praemorsa* (ii) *Papilionanthe* teres species. (Figure 3.3: ITS tree). The individuals of 4 species *viz*., *Vanda pumila, V. alpina, Rhynchostylis retusa* and *Cleisostoma simondii* that had intra-specific variations however formed a single cluster with all the accessions of each of these four clustering together. One each individual of *Vanda testacea* and *Acampe ochracea* were clustered together with *V. coerulea* and *Hygrochilus parishii.*

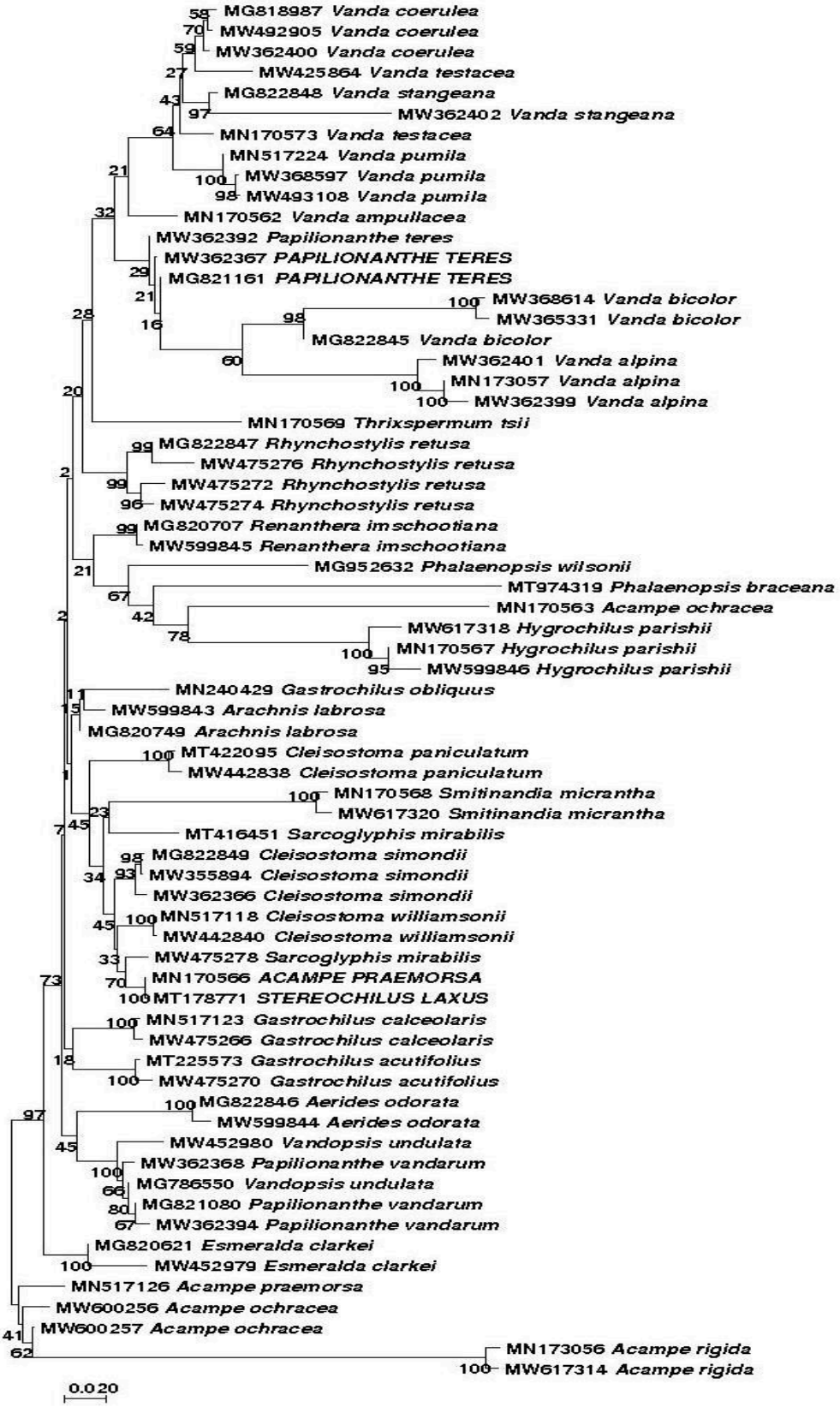


Figure 3.3: Neighbour joining tree of 67 species based on ITS sequences. The species showing zero inter and intra specific divergence are shown bold, capital and both as well.

Blast Analysis

In the BLAST analysis of 31 species (67 individuals) for ITS sequences, 55 sequences correctly matched with the sequences of their own species. Out of the total 12 species that does not correctly matched/identified with their own species, 9 species are correctly identified up to genus level but not at the species level. Thus, the species resolution based on ITS locus using BLAST method was 79.40% (Table 3.8: Average inter-specific K2P distance). The BLAST analysis was also carried out to determined that the query/amplified sequence is of the targeted locus and it is observed that all the 67 ITS sequences generated in the present study were found to be only of the targeted locus and not contamination of fungi as they form symbiotic relationship with orchids/the host.

Interspecific K2P Distances and Species Discrimination Rates for Three Loci at the Genus Level

The inter-specific variations and species discrimination rates among the congeneric species which are represented by more than one species were also calculated individually for all the 7 genera by three investigated loci. The species discrimination rates were calculated both by genetic distance and phylogenetic tree methods. The Neighbour joining tree methods were constructed with thousand bootstrap replicates for all the three tested loci.

Acampe

Three *Acampe* species were analyzed viz., *A. rigida* (2) individuals, *A. praemorsa* (2) individuals and *A. ochracea* (3) individuals. Only one individual from *matK* (*A. praemorsa*) could be compared as the sequence from these loci couldn't be obtained. The average inter-specific distances for *matK, rbcL* and ITS were 0.013, 0.016 and 0.187. The sequences from *matK*, *rbcL* and ITS could distinguished all the investigated *Acampe* species.

The Neighbour joining (NJ) tree constructed with all the accessions of the given genus for *matK*, *rbcL* and ITS (Figure 3.4: *Acampe* phylogeny a,b,c) distinguished all the three species correctly. The *matK* sequences with 841 nucleotides showed 22 variable sites of which only 5 were parsimony informative. The *rbcL* sequences had 644 nucleotides of which 18 were variable and 14 were parsimony informative sites. The number of variable and parsimony informative sites for ITS sequences were 231 and 145 out of 784 nucleotides.

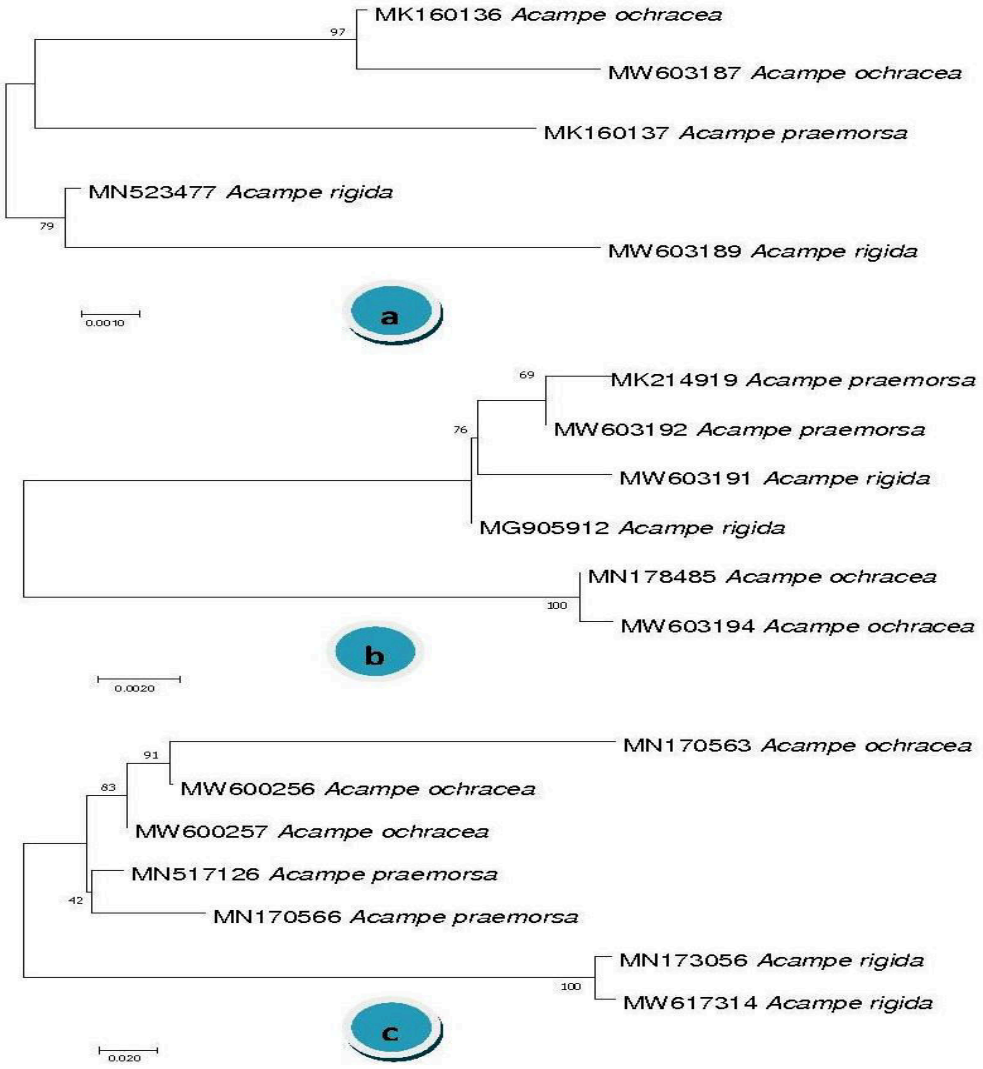


Figure 3.4: *Acampe* species NJ trees (a) *matK* (b) *rbcL* and (c) ITS.

Arachnis

Two *Arachnis* species were analyzed viz., *A. labrosa* (3) individuals and *A. clarkei (Esmeralda)* (2) individuals. The average inter-specific distances for *matK, rbcL* and ITS were 0.011, 0.023 and 0.032. The sequences from *matK*, *rbcL* and ITS could distinguished all the investigated *Acampe* species.

The Neighbour joining (NJ) tree constructed with all the accessions of the given genus for *matK*, *rbcL* and ITS (Figure 3.5: *Arachnis* phylogeny a,b,c) distinguished all the two species correctly. The *matK* sequences with 848 nucleotides showed 13 variable sites of which only 7 were parsimony informative. The *rbcL* sequences had 631 nucleotides of which 26 were variable and 5 were parsimony informative sites. The number of variable and parsimony informative sites for ITS sequences were 32 and 9 out of 716 nucleotides.

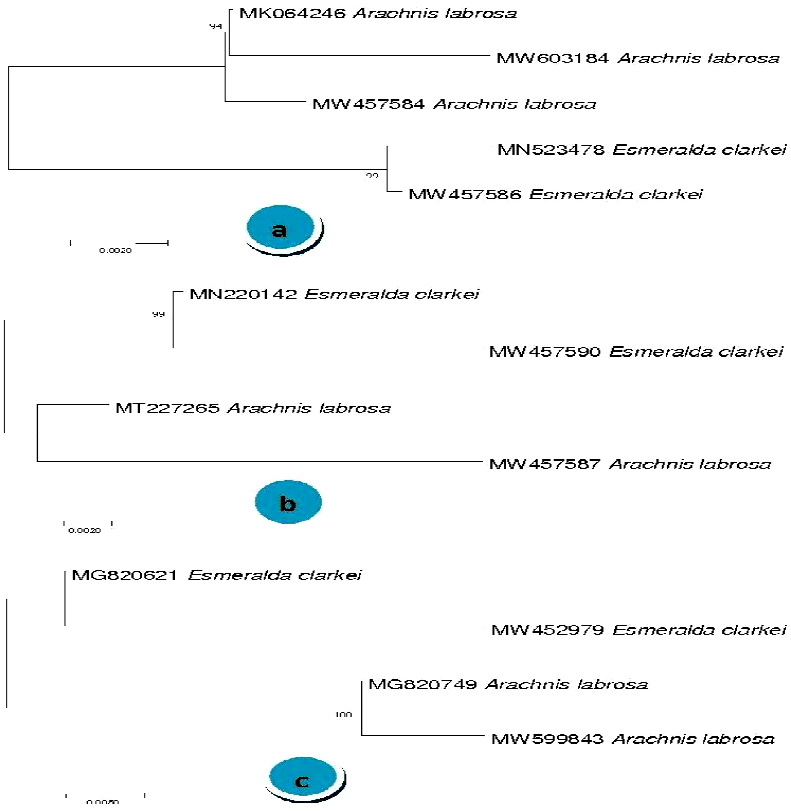


Figure 3.5: *Arachnis* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.

Cleisostoma

Three *Cleisostoma* species were analyzed *viz*., *C. paniculatum* (4) individuals, *C. simondii* (3) individuals and *C. williamsonii* (3) individuals. The average inter-specific distances for *matK, rbcL* and ITS were 0.024, 0.013 and 0.041. The sequences from *matK, rbcL* and ITS could distinguished all the investigated *Cleisostoma* species.

The Neighbour joining (NJ) tree constructed with all the accessions of the given genus for *matK*, *rbcL* and ITS (Figure 3.6: *Cleisostoma* phylogeny a,b,c) distinguished all the three species correctly. The *matK* sequences with 831 nucleotides showed 56 variable sites of which only 20 were parsimony informative. The *rbcL* sequences had 642 nucleotides of which 25 were variable and 14 were parsimony informative sites. The number of variable and parsimony informative sites for ITS sequences were 67 and 46 out of 744 nucleotides.

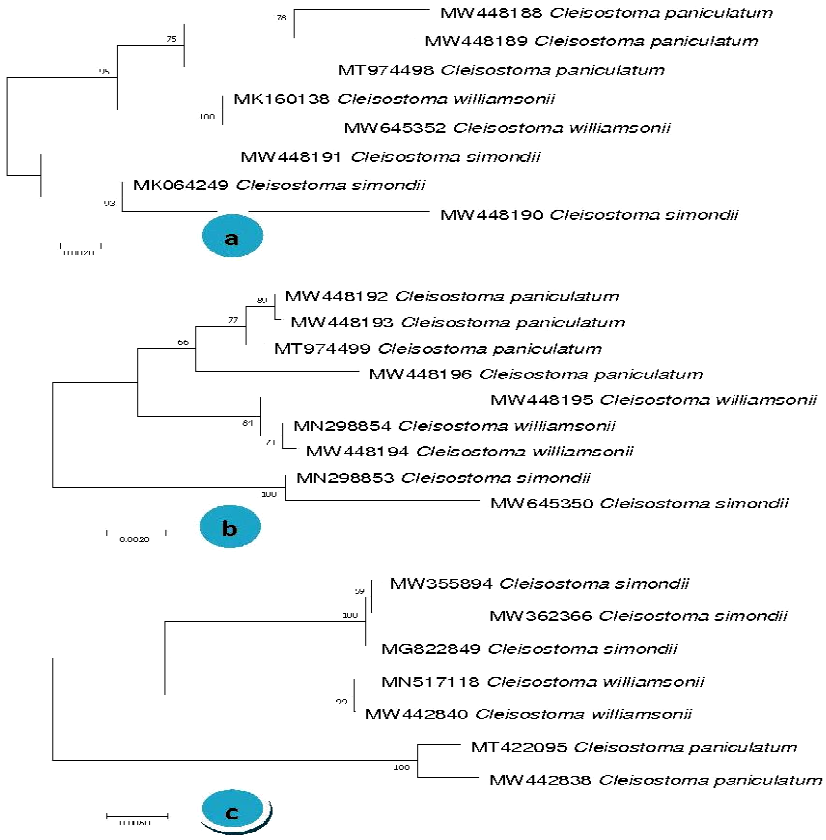


Figure 3.6: *Cleisostoma* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.

Gastrochilus

Three *Gastrochilus* species were analyzed viz., *G. obliquus* (3) individuals, *G. calceolaris* (2) individuals and G. acutifolius (2) individuals. The average inter-specific distances for *matK, rbcL* and ITS were 0.019, 0.007 and 0.054. The sequences from *matK*, *rbcL* and ITS could distinguish all the investigated *Gastrochilus* species.

The Neighbour joining (NJ) tree constructed with all the accessions of the given genus for *matK*, *rbcL* and ITS (Figure 3.7: *Gastrochilus* phylogeny a,b,c) distinguished all the three species correctly. The *matK* sequences with 809 nucleotides showed 47 variable sites of which only 11 were parsimony informative. The *rbcL* sequences had 637 nucleotides of which 10 were variable and 7 were parsimony informative sites. The number of variable and parsimony informative sites for ITS sequences were 71 and 26 out of 749 nucleotides.

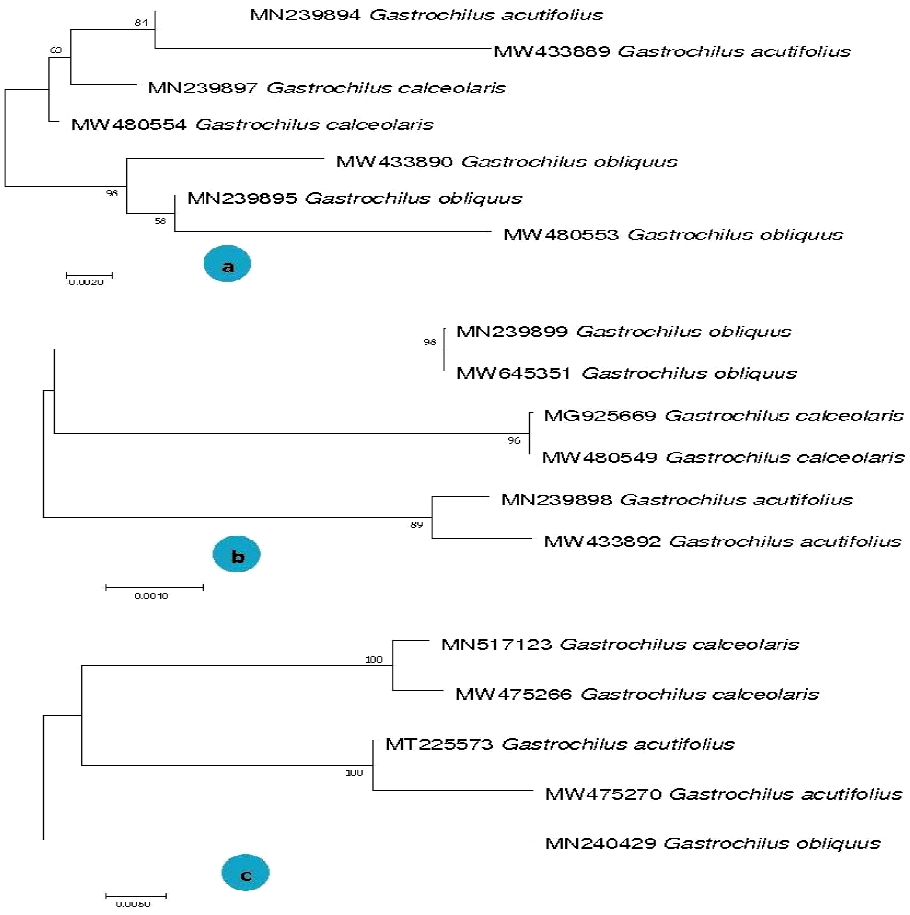


Figure 3.7: *Gastrochilus* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.

Papilionanthe

Two *Papilionanthe* species were analyzed *viz*., *P. teres* (3) individuals and *P. vandarum* (3) individuals. The average inter-specific distances for *matK, rbcL* and ITS were 0.049, 0.006 and 0.054. The sequences from *matK*, *rbcL* and ITS could distinguish all the investigated *Papilionanthe* species.

The Neighbour joining (NJ) tree constructed with all the accessions of the given genus for *matK*, *rbcL* and ITS (Figure 3.8: *Papilionanthe* phylogeny a,b,c) distinguished all the two species correctly. The *matK* sequences with 853 nucleotides showed 47 variable sites of which only 35 were parsimony informative. The *rbcL* sequences had 640 nucleotides of which 10 were variable and 3 were parsimony informative sites. The number of variable and parsimony informative sites for ITS sequences were 45 and 31 out of 778 nucleotides.

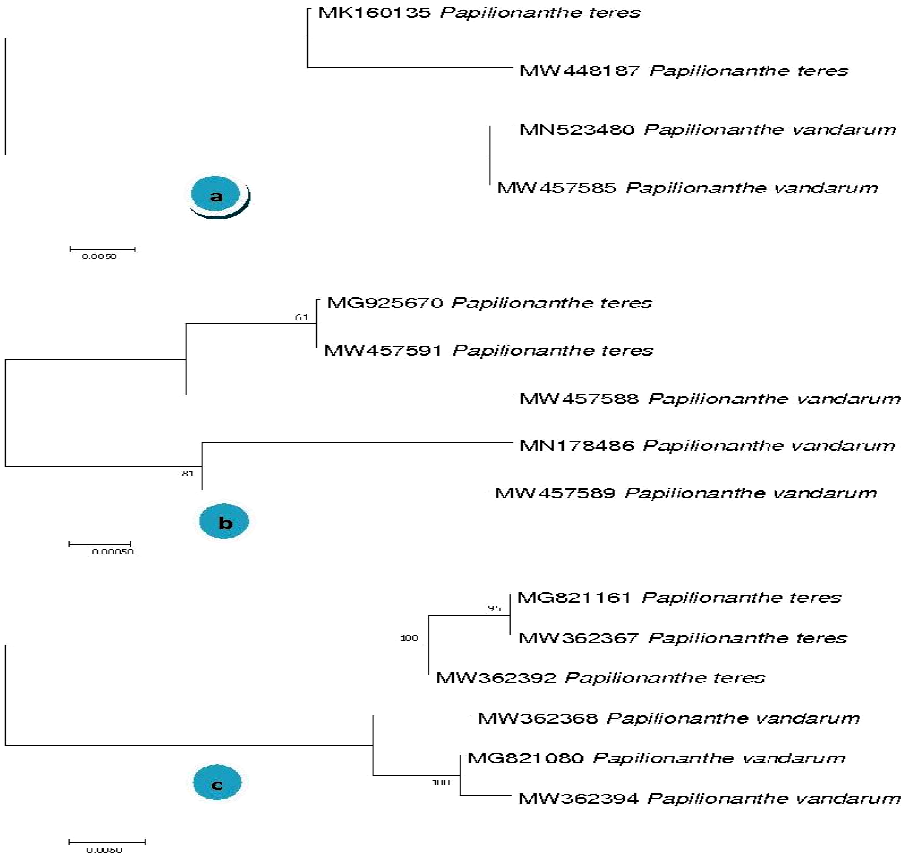


Figure 3.8: *Papilionanthe* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.

Phalaenopsis

Three *Phalaenopsis* species were analyzed *viz*., *P. wilsonii,P. braceana* one (1) each individual and *P. hygrochila (Hygrochila parishii)* (3) individuals. The average inter-specific distances for *matK, rbcL* and ITS were 0.056, 0.013 and 0.169. The sequences from *matK*, *rbcL* and ITS could distinguished all the investigated *Phalaenopsis* species.

The Neighbour joining (NJ) tree constructed with all the accessions of the given genus for *matK*, *rbcL* and ITS (Figure 3.9: *Phalaenopsis* phylogeny a,b,c) distinguished all the three species correctly. The *matK* sequences with 628 nucleotides showed 58 variable sites of which only 2 were parsimony informative. The *rbcL* sequences had 628 nucleotides of which 14 were variable and none of these was parsimony informative. The number of variable and parsimony informative sites for ITS sequences were 187 and 42 out of 680 nucleotides.

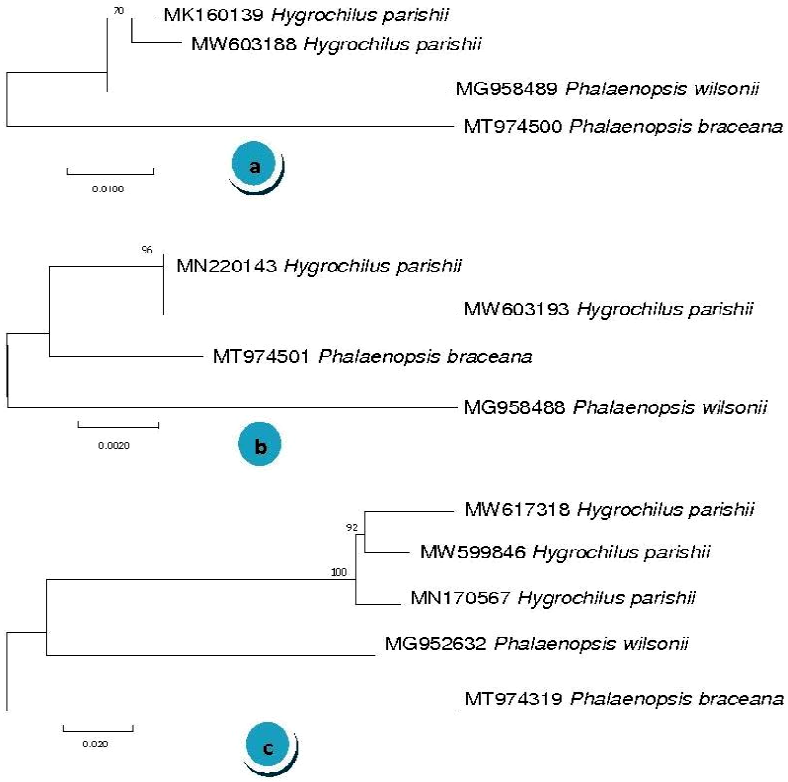
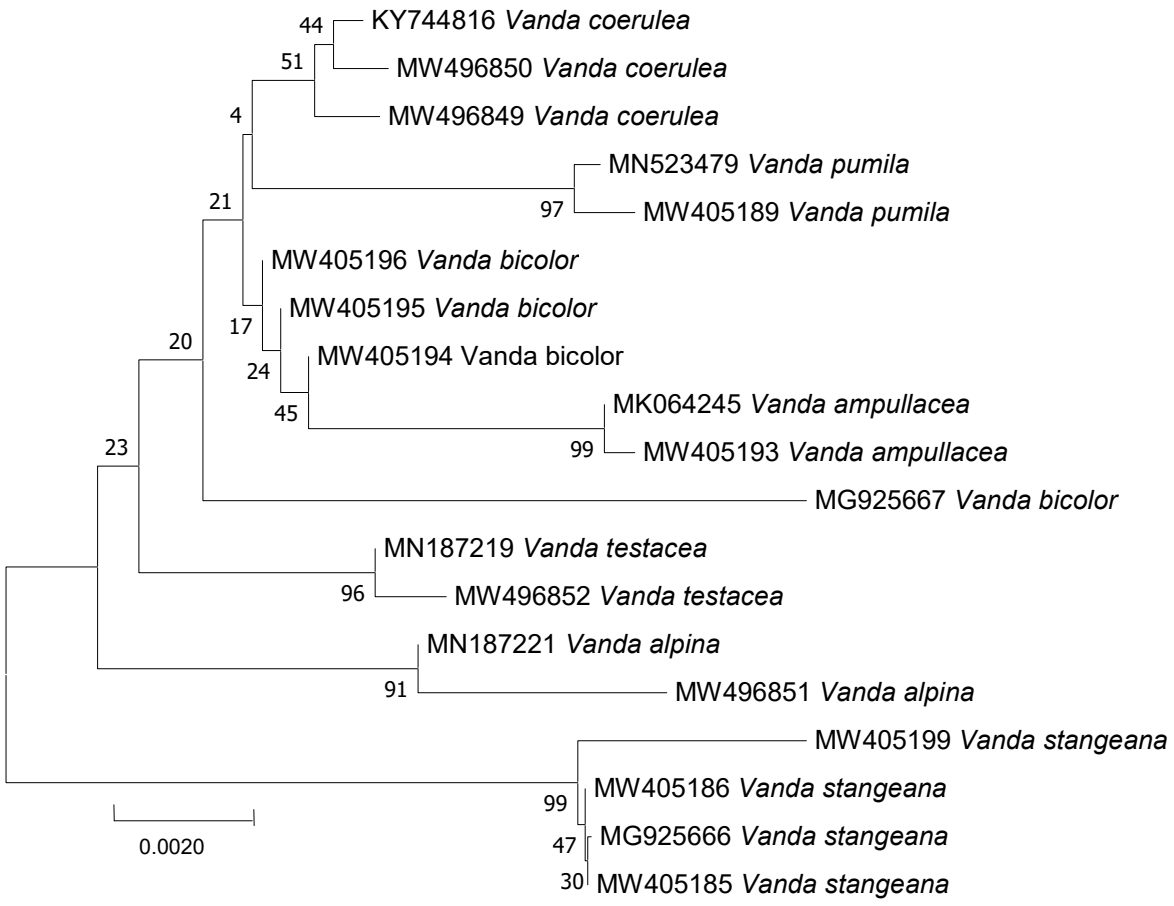


Figure 3.9: *Phalaenopsis* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.

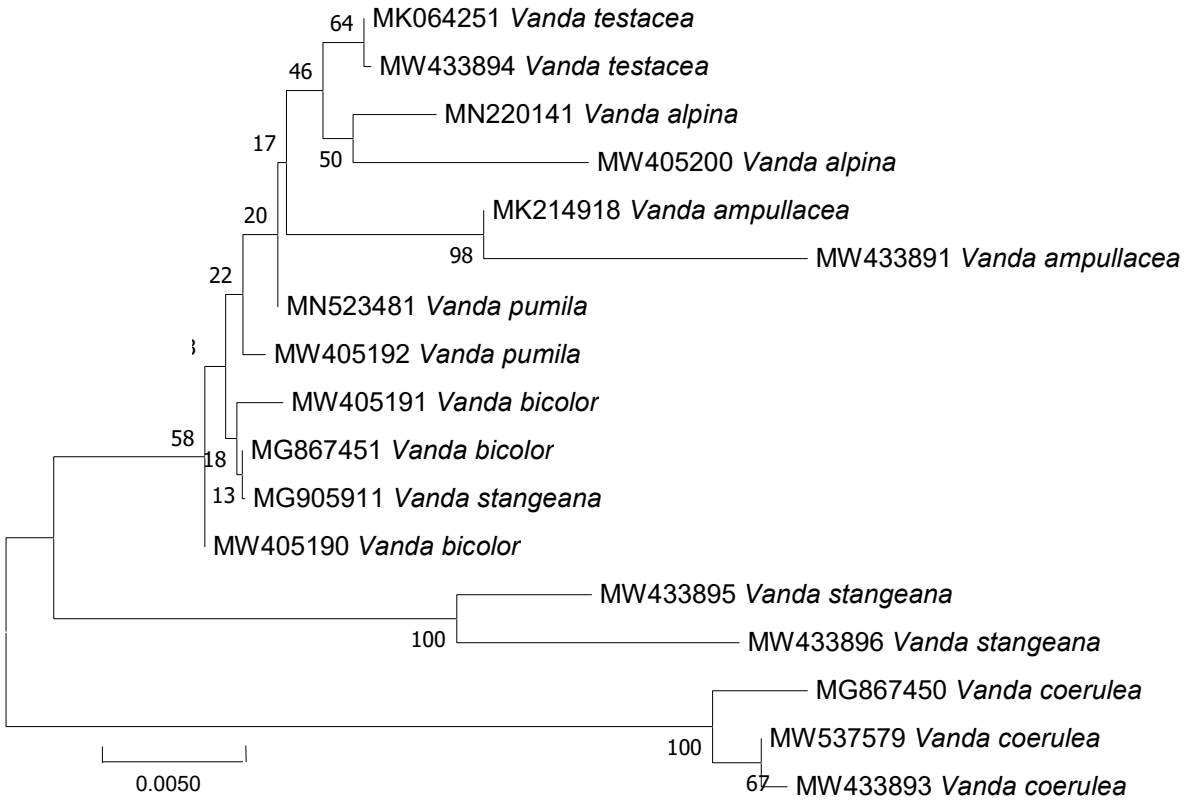
Vanda

Seven *Vanda* species were analyzed viz., *Vanda coerulea* (3) individuals, *V. bicolor* (3) individuals, *V. ampullacea* (2) individuals, *V. stangeana* (4) individuals, *V. testacea* (2) individuals, *V. alpina* (3) individuals and *V. pumila* (3) individuals. The average inter-specific distances for *matK, rbcL* and ITS were 0.010, 0.023 and 0.119. In *rbcL* matrix, one species pair of *Vanda stangeana* and *V. bicolor* showed zero distance estimates. The species discrimination success rates for *Vanda* species evaluated in the present studies for *matK* and ITS was 100% each and *rbcL* was 94.11% respectively.

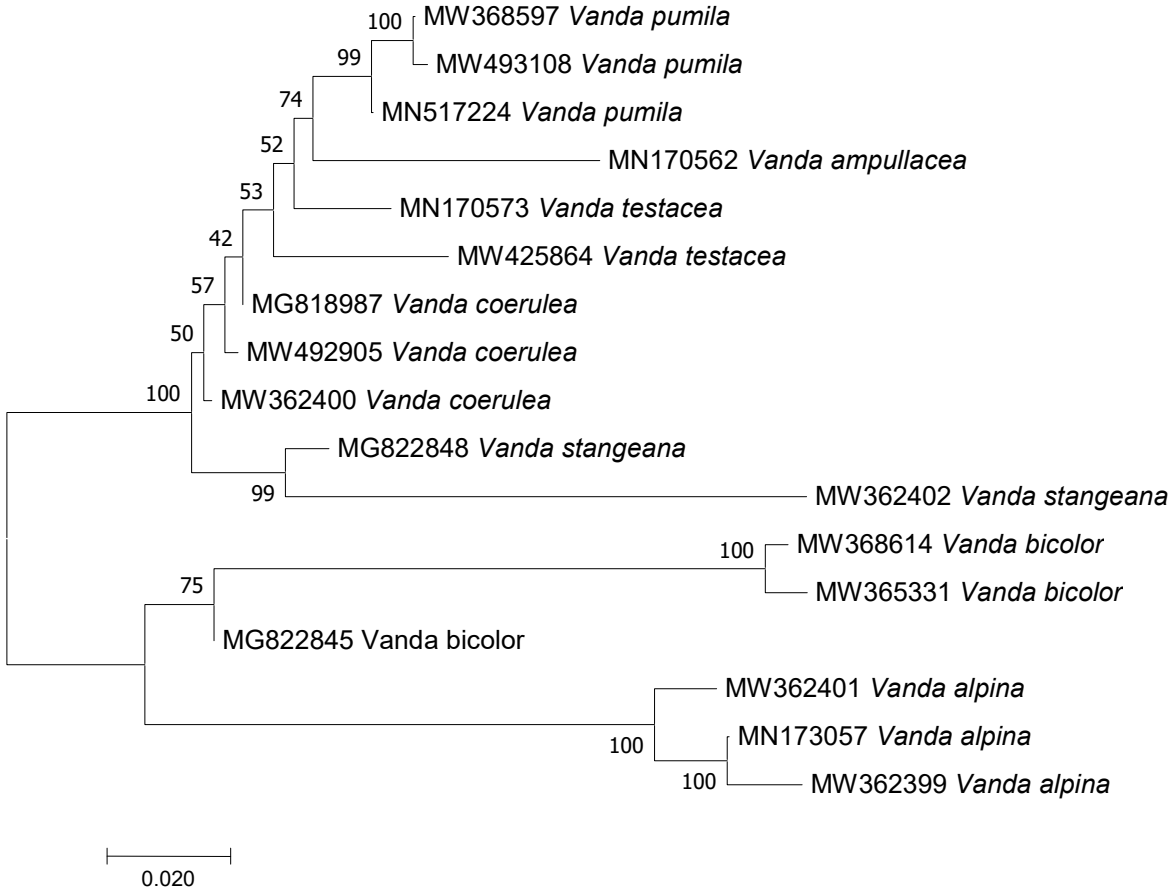
The Neighbour joining (NJ) tree constructed with all the accessions of *matK* sequences, it is observed that one individual of *Vanda bicolor* (MG925667) are cluster with other species (Figure 3.10: *Vanda* phylogeny a). The numbers of variable and parsimony informative sites among 875 nucleotides of *matK* are 46 and 22, respectively. For *rbcL,* one individual of *Vanda stangeana* (MG905911) are cluster with other species (Figure 3.10: *Vanda* phylogeny b). The numbers of variable and parsimony informative sites among 875 nucleotides of *rbcL* are 68 and 35, respectively. The numbers of variable and parsimony informative sites among 816 nucleotides of ITS were 320 and 218, respectively. The species discrimination rates stands at 94.73% for *matK,* 94.11% for *rbcL* and 100% for ITS. (Table 3.10 & 3.11).


KY744816 Vanda coerulea
MW496850 Vanda coerulea
MW496849 Vanda coerulea
MN523479 Vanda pumila
MW405189 Vanda pumila
MW405196 Vanda bicolor
MW405195 Vanda bicolor
MW405194 Vanda bicolor
MK064245 Vanda ampullacea
MW405193 Vanda ampullacea
MG925667 Vanda bicolor
MN187219 Vanda testacea
MW496852 Vanda testacea
MN187221 Vanda alpina
MW496851 Vanda alpina
MW405199 Vanda stangeana
MW405186 Vanda stangeana
MG925666 Vanda stangeana
MW405185 Vanda stangeana
44
51
4
97
21
17
20
24
45
99
23
96
91
99
47
30
0.0020

(a)


MK064251 Vanda testacea
MW433894 Vanda testacea
MN220141 Vanda alpina
MW405200 Vanda alpina
MK214918 Vanda ampullacea
MW433891 Vanda ampullacea
MN523481 Vanda pumila
MW405192 Vanda pumila
MW405191 Vanda bicolor
MG867451 Vanda bicolor
MG905911 Vanda stangeana
MW405190 Vanda bicolor
MW433895 Vanda stangeana
MW433896 Vanda stangeana
MG867450 Vanda coerulea
MW537579 Vanda coerulea
MW433893 Vanda coerulea
64
46
17
50
20
98
22
58
18
13
100
100
67
0.0050

(b)



(c)

Figure 3.10: *Vanda* species NJ trees (a) *matK* (b) *rbcL* (c) ITS.

Table 3.11: Average K2P distances along with the number of species analyzed for each loci

Sl. No.	Genus	No. of species	Average Inter-specific K2P Distance (Range)		
			matK	***rbcL***	**ITS**
1	*Acampe*	3	0.013 (0-0.021)	0.016 (0-0.029)	0.187 (0-0.431)
2	*Arachnis*	2	0.011 (0-0.020)	0.023 (0-0.040)	0.032 (0-0.063)
3	*Cleisostoma*	3	0.024 (0-0.042)	0.013 (0-0.028)	0.041 (0-0.070)
4	*Gastrochilus*	3	0.019 (0-0.041)	0.007 (0-0.011)	0.054 (0-0.078)
5	*Papilionanthe*	2	0.049 (0-0.078)	0.006 (0-0.010)	0.054 (0-0.067)
6	*Phalaenopsis*	3	0.056 (0-0.102)	0.013 (0-0.023)	0.169 (0-0.262)
7	*Vanda*	7	0.010 (0-0.024)	0.023 (0-0.064)	0.119 (0-0.269)

Table 3.12: Species Discrimination rates along with the number of species analyzed for each loci

Sl. No.	Genus	No. of species	Species Discrimination Rate		
			matK	***rbcL***	**ITS**
1.	*Acampe*	3	100%	100%	100%
2.	*Arachnis*	2	100%	100%	100%
3.	*Cleisostoma*	3	100%	100%	100%
4.	*Gastrochilus*	3	100%	100%	100%
5.	*Papilionanthe*	2	100%	100%	100%
6.	*Phalaenopsis*	3	100%	100%	100%
7.	*Vanda*	7	94.73%	94.11%	100%

DISCUSSION

DNA barcoding, a technique projected for rapid identification of unknown biological samples which uses short and agreed upon DNA sequences (Hebert *et al*., 2003 a, b). On the basis of its first initial success in more than 200 allied *Lepidopteran* species, it has been considered as a powerful tool for identification of all eukaryotes at the species level. The short DNA sequences which has been considered as the universal barcode region also known as the 'Folmer' region has 658 bp long, present at the 5′ end of the *CO1* mitochondrial genome (Hebert *et al*., 2003 a, b; 2004 a, b). Upon success in animals (Barrett and Hebert 2005; Cywinska *et al*., 2006; Clare *et al*., 2007), various programmed were also initiated to generate species specific molecular signature/tag to identify plants but it was found that *CO1* gene was not suitable for plants because as such there is no region of genome, cytoplasm or nuclear that could be identified. The plant mitochondrial genes with low nucleotide substitutions and low evolutionary rates were considered unsuitable for barcodes of plants (Chase *et al*., 2005; Kress *et al*., 2005; Newsmaster *et al*., 2006). Therefore, the present study was initiated to check the applicability of chloroplast (*matK, rbcL*) and nuclear genome (ITS) based on the earlier recommendation

made by Plant Working Group of consortium for the Barcode of Life (CBOL) and Barcode of Life Database (BOLD) standard guidelines (Ratnasingham and Hebert, 2007). The three loci were evaluated for three major criteria (i) Universality and robust amplification (ii) Sequence quality and coverage and (iii) Discrimination lay down by CBOL, Plant working group (2009). The details of the methodology followed and the results obtained are discussed below.

Sample Collection, Preservation and Isolation of DNA

The sampled specimen (as already mentioned in the documentation) was collected from different parts of Nagaland during the field survey. The investigated species assemblage represent Vandaceous orchids [subfamily: Highher Epidendroide (Formerly called Vandoideae), Tribe: Vandeae, Subtribe: Aeridinae (formerly Sarcanthinae)] comprising 15 genus and 31 species (80 individuals). The sampled specimen (individuals) collected during the field survey were brought to the Department laboratory and stored at -20°C in a deep freezer to minimize the degradation of DNA and to preserve them till DNA was extracted. For DNA isolation, CTAB method (Doyle and Doyle, 1987) protocol was followed for some species. However, some orchid species accumulate mucilage (*Acampe, Aerides, Arachnis*) to conserve water and as food reserve (Chowdhery 1998). The presence of high mucilage (polysaccharides and polyphenols) contents in such species was the major obstacle in DNA isolation and PCR amplification. Therefore, a modified CTAB method (Kamba and Deb, 2018) in concentration and a step-wise manner was modified for those species which has high mucilage content.

Amplification and Sequencing

A total of 31 species (80 individuals) was analyzed by comparing their amplification and sequencing success rates with all the three tested loci.

The *matK* (*maturase K*) gene from the chloroplast genome due to high variations is frequently used in the phylogenetic relationships study in the family Orchidaceae (Kores *et al*., 2001; Freudenstein *et al*., 2004; van den Berg *et al*., 2005). The PCR amplification success rate in the present study was 92.3% which is more/less equivalent to the findings of Xiang *et al*. (2011) for *Holcoglossum* species (Orchidaceae). Although, much higher amplification rates of 99% and 99.32% were reported by Lahaye *et al*. (2008) on Costa Rican orchid and Singh *et al*. (2012) on *Dendrobium* species. In 2009, Plant Working Group, CBOL had attained 90-98% successful amplification in angiosperms. In 2011, China Plant BOL Group reported 91% amplification success of *matK* from 6,286 individuals belonging to 1,757 species of seed plants. However, low PCR amplification rates were also reported by many workers like Sass *et al*. (2007), Kress and Erickson (2007), Fazekas *et al.* (2008), Hollingsworth *et al*. (2009), Kress *et al*. (2009) and Liu *et al*. (2010) from different groups of plants. The low amplification success and the difficulties encountered in sequencing could be attributed to higher variability of *matK* region especially in monocots (Chase *et al*., 2007; Fazekas *et al*., 2008). Some workers like Hollingsworth *et al*. (2011) and Parveen (2012) recommended for successful amplification and sequencing require not only family or genera specific primer but also species specific primer in some cases for cost effective and efficient plant barcoding.

The amplification rate stands at 95.00% for *rbcL* (RUBISCO large sub-unit) locus. The high amplification rate obtained in the present study is in congruent with most of the previous investigations reporting similar amplification of 95-100% (Kress *et al*., 2005; Kress and Erickson, 2007; Fazekas *et al*., 2008; Hollingsworth *et al*., 2009; Kress *et al*., 2009; CBOL Plant Working Group 2009; Ebihara *et al*., 2010; Parveen 2012). Also, Wang *et al*. (2010) observed 100% amplification success tested in 31 *Lemnacea*

species belonging to five genera. In 2011, China Plant BOL Group reported 94.5% amplification success among 6,286 individuals belonging to 1,757 species from 141 genera across 75 families of seed plants.

The amplification success rate for ITS was 91.25% in the present study, a relatively higher as compared to other workers (Kress *et al*., 2005; Kress and Erickson 2007; Parveen 2012). A higher amplification rate of 97% was reported by Roy *et al*. (2010) in the tested samples of 11 species of *Ficus* and 4 species of *Gossypium* and also Singh *et al*. (2012) while testing the congeneric species of *Dendrobium* with 98.97% amplification success rates.

Following amplification, the successful amplicons are packed, labelled and send it for sequencing after completing all the formalities/company instruction to various laboratories to sequence the desire size/targeted loci. The sequence success rate was 89.18% for *matK*, 89.47% for *rbcL* and 91.78% for ITS. The total number of barcode sequences generated for *matK, rbcL* and ITS was 66, 68 and 67 respectively.

Intra and Inter-Specific Variations

For correct identification and generation of DNA barcodes for species, the assessment of intra and inter-specific variations is important (Hebert *et al*., 2003a, b; Lahaye *et al*., 2008). The minimum inter-specific variation is greater than the maximum intra-specific variation and the difference between the two is referred as 'barcode gap' (Meyer and Paulay, 2005). The intra and inter-specific divergence were evaluated/expressed in terms of K2P distances as done by Chen *et al*. (2010) and also Parveen (2012). The intra-specific variations were evaluated for 27 species that were represented by more than one individual. Variable range of intra-specific distances was obtained in all the three loci in all different species. The minimum/lowest divergence among the investigated species was observed in *Vanda pumila (matK)*, *Cleisostoma*

williamsonii (ITS) and *Papilionanthe vandarum (matK)* while the maximum/highest divergence was observed in *Vanda stangeana* (ITS).

Species Discrimination Rates and Evaluation of DNA Barcodes

For evaluating species resolution and selecting DNA barcodes three methods were used viz., genetic distance, phylogenetic tree method and BLAST analysis. The genetic distance employs the assessment of intra and inter-specific divergence. The intra and inter-specific divergence should not overlap in an ideal barcode. The difference/gap between the two specific divergences provides a perfect barcode which is referred as barcode gap (Hebert *et al*., 2003a; Lahaye *et al*., 2008). The phylogenetic tree method is constructed using sequences from the targeted locus and the percent species resolution was determined by cluster analysis (Lahaye *et al*., 2008; China Plant BOL Group, 2011). The species for which all the individuals clustered together in a single clade are considered as unequivocally identified species and those which clustered with the individuals of the other species were treated as unresolved. During the present analysis, the species resolution of the investigated loci calculated using both these methods showed different results.

The last method used for evaluating species discrimination is the BLAST analysis (Ross *et al*., 2008). In this method, the unknown individual barcode sequence is search in the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for a very similar/identical sequence from the datebase, containing reference barcodes of correctly identified species.

Among the tested loci in the present investigation, *matK* provided maximum species resolution of 90.90% and 95.45% by distance based method and phylogenetic tree method. When BLAST hits identification was analyzed only upto genus level, the species resolution increases from 91.84-95.45%. The overall species discrimination rate for *matK* was 92.73%. Lahaye *et al*. (2008) reported >90% of the species correctly identified either

alone or in combination with *trnH-psbA* based on their study involving more than 1,036 species of Mesoamerican orchids. However, the high resolving power of *matK* tends to decrease if the sampling was restricted to sister species rather than natural geographic assemblages of species as reported by many workers like Lahaye *et al*. (2008), Parveen *et al*. (2012), Singh *et al*. (2012) and Parveen 2012, that's the reason why most of the investigated species in *matK* loci showed/exhibited zero distances in matrix (*Vand bicolor-V. bicolor, V. ampullacea-V. ampullacea, V. stangeana-V. stangeana, V. testacea-V. testacea*). The probability of getting results might change if the number of species analyzed in each genus is increased as also reported by Singh *et al*. (2012), Parveen 2012 that the decrease in the species resolution with increase in size sampling.

Although, *rbcL* exhibited high amplification and sequence success rates, the overall species discrimination rate was low with 76.35% only. When BLAST hits identification was analyzed only up to genus level, the species resolution increases from 59.96-98.52%. Out of 68 species individuals, 67 species could be identified upto genus level but not up to species level when BLASTED. The suitability of *rbcL* locus for reconstructing phylogenies only at the family and sub-family level has been reported by many workers (Cameron *et al*., 1999; Soltis and Soltis, 1998) as it had limited application at the species level. The following species pair showed showed/exhibited zero distances (*Vanda coerulea-Vanda coerulea, V. bicolor-V.bicolor, V. stangeana-V. bicolor, V. testacea-V. testacea, V. pumila-V.pumila, Cleisostoma paniculatum-C. paniculatum, C. williamsonii-C. williamsonii, Acampe ochracea-A. ochracea, Gastrochilus obliquus-G. obliquus, G. calceolaris-G. calceolaris, Papilionanthe teres-P. teres, Vandopsis undulata-V. undulata, Renanthera imschootiana-R. imschootiana, Aerides odorata-A. odorata* and *Sarcoglyphis mirabilis-S. mirabilis*).

In spite of low amplification rate, ITS showed more species discrimination rates of 90.90% and 95.45% by distance based method and phylogenetic tree method. At the genus level, ITS BLAST hits increases from 79.40-95.52%. The overall species discrimination rates are 89.15%. The high species discrimination ability of this region could be due to its high rate of evolution leading to genetic changes that allows differentiation of closely related congeneric species (Kress *et al*., 2005; Sass *et al*., 2007; Liu *et al*., 2011a; Singh *et al*., 2012).

SUMMARY AND CONCLUSIONS

Besides elegant look of orchids, they are also known for medicinal values. For these purposes they are collected and smuggle extensively from the wild resulting in rare, endangered and have threatened many important species, that's why all orchids have been included under Appendix II of CITES and some under Appendix I. Moreover, orchid species hybridize easily and due to hybridization a number of hybrids come in existence making them difficult to differentiate between parents and their hybrids. Thus, the need for establishing the barcodes of these important species is necessary to check at the molecular level when traditional taxonomical methods fail to identified/differentiate especially when they do not flowered or in parts /tissue. The loci *matK* and *rbcL* from the chloroplast genome and nrITS from the nuclear genome were selected for establishing the barcodes for Vandaceous orchid species. The investigation carried out on the topic "Establishment of Barcodes for Some Commercially Important Vandaceous Orchids of Nagaland" across 15 genus, 31 species (80) individuals collected from different parts of Nagaland during the field survey from 2016-2020.

Among the tested loci, *rbcL* with 95.00% exhibited the highest amplification success rate, followed by *matK* with 92.5% and ITS 91.25 % respectively. Likewise, the sequence success rate for *matK, rbcL* and ITS were 89.18%, 89.47 and 91.78%

respectively. A total of 201 barcode sequences were generated and submitted to NCBI, GenBank and accession numbers were obtained. Intra-specific distances were also computed for those species with multiple accessions and exhibited intra-specific variations of variable range for all the tested loci. For *matK*, the individuals of *Vanda pumila*, *Papilionanthe vandarum* exhibited minimum intra-specific variation in the range of 0-0.001 while *Sarcoglyphis mirabilis* exhibited maximum intra-specific variation in the range of 0-0.030. *Vanda bicolor, Acampe praemorsa, Gastrochilus acutifolius* exhibited minimum intra-specific variation for *rbcL* in the range of 0-0.002 while Vanda stangeana exhibited maximum intra-specific variation in the range of 0-0.025. For ITS, the minimum and maximum intra-specific variations is exhibited by *Cleisostoma williamsonii* (0-0.001) and *Aerides odorata* (0-0.162) respectively.

The ITS sequences with 0.150 average inter-specific K2P distance ranges from 0-0.539 obtained the maximum variations. While, minimum variations were exhibited by *rbcL* sequences with 0.018 with a range of 0-0.066. The *matK* sequences have average inter-specific K2P distances of 0.061 with a range of 0-1.099.

The species discrimination rates were calculated on the following three methods viz. genetic distance, phylogenetic tree method and BLAST analysis. The loci *matK*, from the chloroplast genome provided the best species resolution of 90.90% by genetic distance, 95.45% by phylogentic tree method and 91.84% by BLAST analysis, followed by ITS from the nuclear ribosomal region with 95.52% by genetic distance, 92.53% by phylogenetic tree method and 79.40% by BLAST analysis. Whereas the loci, *rbcL* from the chloroplast genome could afford only 76.47%, 92.64% and 59.96% by genetic distance, phylogenetic tree method and BLAST analysis. The BLAST search also revealed that all the sequences generated in the present study were of only the targeted

loci and not from the contamination of fungi or from the host tissue as they are mostly being epiphytic.

Among the three loci tested during the present investigation, it is observed that the loci ITS from the nuclear genome turns out to afford the overall highest/maximum species resolution followed by *matK* from the chloroplast genome while *rbcL* from the chloroplast genome turns out to afford the least/minimum species resolution which is in agreement with most of the previously reported investigators and apparently point towards suitability of the loci for orchid barcode. The loci *matK, rbcL* and ITS were tested individually/separately. However, no multi-locus combinations were tested during the present barcode establishment.

CHAPTER - 4

SUMMARY AND CONCLUSIONS

Orchids belong to the family 'Orchidaceae', one of the largest and highly advanced families of the flowering plants and biologically most complex. They are known for their striking features such as fascinating shape, beautiful looks, longevity and highly attractive colors besides their ornamental and medicinal importance and have become among the most studied flowering plant. They have outnumbered some other families of flowering plants by evolving higher levels of specialization in its vegetative and reproductive structures. India is a home to 1256 species of orchid in 155 genera of which 307 species are endemic and with three biodiversity hotspots *viz*., Indo-Burma, Himalayas, Western Ghats and Sri Lanka which make it one of the richest countries in terms of flora. Indian Orchids are mostly confined to the Northeastern region and the Western Ghats. The Northeastern states of India contribute significantly with about 750-850 species and Arunachal Pradesh being the highest number of orchid species reported. Nagaland, one of the Northeastern states of India with 426 species belonging to 101

genera has been reported for the state. With the addition of three more Vandaceous orchids during the present documentation, the total orchid diversity of the state stands at 429 species belonging to 101 genera.

The Vandaceous orchids are a group of orchid genera in the Subfamily higher Epidendroide or formerly known as Vandiodeae. They are the major ornamental crops as cut flower and potted plants with high demands in both National and International market. They can be crossed within the same genus or with different genera leading to the production of various new hybrids having similar morphological traits.

DNA barcoding, a technique which provides quick and reliable identification of speciesthat provides a unique recognition tag to a species using short DNA sequences. The mitochondrial CO1 with the requisite divergence and universality was found to be suitable for animal species identification but no such specific region of genome, cytoplasmic or nuclear in plants could be identified. The mitochondrial gene with low nucleotide substitution and low evolutionary rates were considered unsuitable for barcode of plants. The species of Orchidaceae are valued for cut flower production and as potted plantsbesides their ornamental and therapeutic properties resulting inrampant collection, trade and export of endangered orchid species. Orchid species are also difficult to identify correctly even if available in flowering state because of their similar morphological features. Moreover, hybridization also plays an important role in plant speciation. Therefore, it is difficult to identify the hybrids from their parents especially in vegetative parts. For identification and classification of these taxa, a rapid species identification technique like DNA barcoding have been undertaken by different groups utilizing DNA region from both the mitochondrial, plastid and nuclear genomes which can check illicit practices in any form and help indirectly in their conservation. Keeping these in mind, the present investigated on the topic “Establishment of Barcodes for Some Commercially

Important Vandaceous Orchids of Nagaland", was carried out to use DNA sequences to identify species using different marker (*matK, rbcL* from chloroplast genome and ITS from the nuclear ribosomal genome) and to find common/universal barcodes for orchids.

For establishment of barcodes for some commercially important Vandaceous orchids of Nagaland, regular field survey was conducted in different parts of the state.A total of 31 species (80 individuals) belonging to 15 genera were collected, identifiedand brought under cultivation in the Department orchidarium, Nagaland University, Lumami. Genomic DNA were isolated from the sampled specimen using standard CTAB protocol and stored in -20°C until further used. The isolated/extracted DNA was amplified with selected primers in Polymerase Chain Reaction. The amplicons were then sending it for sequencing to sequencing laboratory company. The amplification rates for three loci *matK, rbcL* and ITS are 92.5%, 95.00% and 91.25% respectively. The loci ITS (91.78%) has the highest sequencing success rate followed by *rbcL* (89.47%) and *matK* (89.18%) has the least/lowest sequence success rate. A total of 201 barcode sequences were generated and submitted to NCBI, GenBank. The identities of the sequences were confirmed by BLAST analysis on NCBI. The loci *matK* (91.84%) has the highest BLAST hits, followed by ITS (79.40%) whereas *rbcL* could afford only 59.96%. The BLAST results also confirmed that the sequences generated in the present were of only the targeted loci and not from the contamination of fungi especially in case of ITS or from the host tissue. Thereafter, the sequences of each loci were aligned in Clustal W and the aligned sequences were analyzed for genetic distance and phylogentic tree in MEGA 7. For constructing Neighbour Joining tree, 1000 bootstrap replicates were taken. Intra and inter-specific distances were calculated using K2P model. The percent species resolutions for each locus were calculated on the basis of K2P distances, phylogenetic tree and BLAST methods. The maximum average inter-specific K2P distance of 0.150 with a

range of 0-0.539 was obtained for ITS and the minimum average inter-specific K2P distance is observed in *rbcL* of 0.018 with a range of 0-0.066. The average inter-specific K2P distances for *matK* was 0.061 with a range of 0-1.099. Inter-specific variations and species discrimination rates among the congeneric species were also calculated individually for all three loci for 7 orchid genera (*Acampe, Arachnis, Cleisostoma, Gastrochilus, Papilionanthe, Phalaenopsis* and *Vanda*) which were represented by more than one species. Among the 7 orchid genera studied, all the investigated loci showed 100% species resolution except for genus *Vanda matK* and *rbcL* species resolution is 94.73% and 94.11% respectively. For *matK*, the individuals of *Vanda pumila*, *Papilionanthe vandarum* exhibited minimum intra-specific variation in the range of 0-0.001 while *Sarcoglyphis mirabilis* exhibited maximum intra-specific variation in the range of 0-0.030. *Vanda bicolor, Acampe praemorsa, Gastrochilus acutifolius* exhibited minimum intra-specific variation for *rbcL* in the range of 0-0.002 while *Vanda stangeana* exhibited maximum intra-specific variation in the range of 0-0.025. For ITS, the minimum and maximum intra-specific variations is exhibited by *Cleisostoma williamsonii* (0-0.001) and *Aerides odorata* (0-0.162) respectively.

Among the tested loci, ITS provided the highest species resolution of 95.52% based on genetic distance method. Whereas *matK* and *rbcL* could afford species resolution of 90.90% and 76.47% based on genetic distance. The maximum and minimum species resolution provided by phylogenetic tree based method was by *matK* (95.45%) and ITS (92.53%). The loci *rbcL* could afford 92.64% species resolution provided by phylogenetic tree based method.Thespecies resolution provided by BLAST method for *matK, rbcL* and ITS was 91.84%, 59.96% and 79.40% respectively. The overall maximum/highest species resolution was provided by *matK* (92.73%) from the

chloroplast genome, followed by ITS (89.15%) from the nuclear genome and *rbcL* (76.35%) from the chloroplast genome.

During the present investigation on the 'Vandaceous orchids of Nagaland', it has documented 35 species belonging to 16 genera, (tribe: Vandeae, Subfamily: Higher epidendroideae or formerly known as Vandiodeae) which are mostly epiphytes in habitat. While establishing the barcodes for some commercially important Vandaceous orchids of Nagaland, 31 species (80 individuals) belonging to 15 genus were selected and altogether 201 barcode sequences were generated. The loci selected were all tested individually and no multi-locus combination was tested here in the study. During the analysis, it is found that the *matK* from the chloroplast genome provided the best/highest species resolution, which was followed by ITS from the nuclear ribosomal genome and *rbcL* from the chloroplast genome, which apparently indicates the suitability of *matK* and ITS as the core barcodes for the land plants as suggested by many workers. However, the quest for the perfect universal barcode for plants providing 100% species resolution like as in the case of animal kingdom might not hold true in plant kingdom. Thus, the theoretical approach/projection that DNA barcoding would be able to identify any unknown samples upto species level whether in juvenile, fragmented/parts or available in any stages of life providing 100% perfection/accuracy might not hold true. The reason/outcome for the failure of DNA barcoding for not providing 100% accuracy in identifying the assign species should encourage both traditional/morphological and molecular taxonomist to re-think/re-consider or re-investigate the problem arising out and enhance it in the days to come.

References

Albert VA, Backlund A, Bremer K, Chase M.W, Manhart JR, Mishler BD, Nixon KC (1994) Functional constraints and *rbcL* evidence for land plant phylogeny. Ann Mo Bot Gard, 81: 534–567.

Alvarez I, Wendel JF (2003) Ribosomal ITS sequences and plant phylogenetic inference. Mol Phylogenet Evol, 29: 417–434.

Andersen K, Bird KL, Rasmussen M, Haile J, Breuning-Madsen H, Kjaer KH, Orlando L, Gilbert MTP, Willerslev E (2011) Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. Mol Ecol, DOI: 10.1111/j.1365-294X.2011.05261.x.

Appels R, Honeycutt RL (1986) rDNA: evolution over a billion years. In: Dutta SK (ed.) DNA Systematics Vol. II. Plants, CRC Press, Boca Raton 81–135.

Armstrong KF, Ball SL (2005) DNA barcodes for biosecurity: invasive species identification. Phil Trans R Soc B, 360:1813-1823.

Asahina H, Shinozaki J, Masuda K, Morimitsu Y, Satake M (2010) Identification of medicinal Dendrobium species by phylogenetic analyses using *matK* and *rbcL* sequences. J Nat Med, 64:133-138.

Atwood JT (1986) The size of the Orchidaceae and the systematic distribution of epiphytic orchids. Selbyana, 9(10): 171-186.

Babbar SB, Raghuvanshi S, Singh HK, Parven I, Malik S (2012) An overview of the DNA barcoding of plants. Phytomorphol, 62(1):69-99.

Bailey CD, Carr TG, Harris SA, Hughes CE (2003) Characterization of angiosperm nrDNA polymorphism, paralogy and pseudogenes. Mol Phylogenet Evol, 29: 435–455.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowsk MF, Campbell CS, Donoghue MJ (1995) The ITS regions of nuclear ribosomal DNA (1995) a valuable source of evidence on angiosperm phylogeny. Ann Mo Bot Gard, 82: 247–277.

Barrett RDH, Hebert PDN (2005) Identifying spiders through DNA barcodes. Can J Zool, 83:481-491.

Bates R, Weber JZ (1990) 'Orchids of South Australia.' (A.B. Caudell Government Printer: Adelaide).

Bechtel H, Cribb P, Launer E (1992) The manual of cultivated orchid species. Ed. 3. MIT Press, Cambridge, Massachusetts.

Beddome R. H. 1874. Icones lantarum Indiae Orientalis. Madras.

Bedford RB (1969) A Guide to Native Australian Orchids. (Angus and Robertson Ltd.: Melbourne).

Benzing DH (1987) Vascular epiphytism: taxonomic participation and adaptive diversity. Ann Missouri Bot Gard, 74: 183-204.

Bleeker W, Klausmeyer S, Peintinger M, Dienst M (2008) DNA sequences identify invasive alien Cardamine at Lake Constance. Biol Conserv, 141:692-698.

Bor NL (1942) Some remarks upon the Geology and the Flora of the Naga and Khasi Hills. Ann Roy Bot Gard Prt, I & II: 129-135, Calcutta.

Bose TK, Bhattacharjee SK (1980) Orchids of India. Naya Prokash, Calcutta.

Brown D, Wensink PC, Jordan E (1972) Comparison of the ribosomal DNA's of *Xenopus laecis* and *Xenopus muller*: the evolution of tandem genes. J Mol Biol, 63: 57–73.

Buckler ES, Ipplito SA, Holtsford TP (1997) The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. Genetics, 145: 111–125.

Bucklin A, Wiebe PH, Smolenack SB, Copley NJ, Beaudet JG, Bonner KG, Farber-Lorda J, Pierson JJ (2007). DNA barcodes for species identification of *Euphausiids* (Euphausiacea, Crustacea). J Plankton Res, 29(6): 483-493.

Bulpitt CJ, Li Y, Bulpitt PF, Wang J (2007). The use of orchids in Chinese medicine. JRSM, 10: 558-563.

Burkill IH (1924) Botany of the Abor expedition: the dispersal throughout the world of the spermatophyte collected in Abor land. Rec Bot Surv India, 10(2):74-141.

Burns-Balogh P, Bernhardt P (1988) Floral evolution and phylogeny in the tribe Thelymitreae (Orchidaceae: Neottioideae). Pl Syst Evol, 159: 19-47.

Cameron KM (2004) Utility of plastid psaB gene sequences for investigating intrafamilial relationships within Orchidaceae. Mol Phylogenet Evol, 31:1157-1180.

Cameron KM, Chase MW, Whitten WM, Kores PJ, Jarrell DC, Albert VA, Yukawa T, Hills HG, Goldman DH (1999) A phylogenetic analysis of the Orchidaceae: evidence from *rbcL* nucleotide sequences. Am J Bot, 86(2): 208-224.

Campbell CS, Wojciechowski MF, Baldwin BG, Alice LA, Donoghue MJ (1997) Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). Mol Biol Evol, 14: 81–90.

CBOL Plant Working Group (2009). A DNA barcode for land plants. Proc Natl Acad Sci USA, 106:12794-12797.

Chankija S, Kumar Y, Gurung PB (1992) Orchids of Nagaland. Forest Department, Nagaland.

Chase MW (2005) Classification of Orchidaceae in the age of DNA data. The Board of Trustees of the Royal Botanic Gardens, Kew 2-7.

Chase MW, Cameron KM, Barrett RL, Freudenstein JV (2003) DNA data and Orchidaceae systematics: a new phylogenetic classification. In: Dixon KW, KellSP, Barrett RLand Cribb PJ (eds.) Orchid Conservation. Natural History Publications, Kota Kinabalu, Sabah, Malaysia. Pp. 69-89.

Chase MW, Cowan RS, Hollingsworth PM, van den Berg C, Madrinan S, Petersen G, Seberg O, Jorgsensen T, Cameron KM, Carine M, Pedersen N, Hedderson TAJ, Conrad F, Salazar GA, Richardson JE, Hollingsworth ML, Barraclough TG, Kelly L, Wilkinson M (2007) A proposal for a standardized protocol to barcode all land plants. Taxon, 56:295-299.

Chase MW, Salamin N, Wilkinson M, Dunwell JM, Kesanakurthi RP, Haidar N, Savolainen V (2005) Land plants and DNA barcodes: short-term and long-term goals. Philos Trans R Soc Lond B Biol Sci, 360:1889-1895.

Chawngthantluanga (1996) Orchids of Mizoram Vol-1; Department of Environment & Forest, Govt. of Mizoram.

Chen S, Yao H, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X, Luo K, Li Y, Li X, Jia X, Lin Y, Leon C (2010) Validation of ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One, 5: e8613.

Chowdhery HJ (1998) Orchid Flora of Arunachal Pradesh. Bishen Singh Mahendra Pal Singh, Dehradun. India.

Chowdhury HJ, Pal GD (1997) Orchidaceae of Arunachal Pradesh. A checklist (Higher Plant of Indian Subcontinent). Vol.7. Bishen Singh Mahendra Pal Singh, Dehradun.

Christenhusz MJM, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa, 261: 201-217.

Clare EL, Kim BK, Engstrom MD, Eger JL, Hebert PDN (2007) DNA barcoding of Neotropical bats: species identification and discovery within Guyana. Mol Ecol Notes, 7:184-190.

Clark CB (1889) On the plants of Kohima and Muneypore. J Linn Soc, 25:71-74.

Collet H (1902) Flora Simlensis. Orchidaceae. 484-508. Thacker, Spink and Co., London.

Cooke T (1901-1908) The Flora of the Presidency of Bombay. London. Reprint1958. Calcutta.

Costion C, Ford A, Cross H, Crayn D, Harrington M, Lowe A (2011) Plant DNA barcodes can accurately estimate species richness in poorly known floras. PLoS One, 6:e26841.

Coyle HM, Lee CL, Lin WY, Lee HC, Palmbach TM (2005) Forensic botany: using plant evidence to aid in forensic death investigation. Croat Med J, 46:606-612.

Cuénoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ, Chase MW (2002) Molecular phylogenetics of caryophyllales based on nuclear 18S rDNA and plastid *rbcL, atpB,* and *matK* DNA sequences. Ame J Bot, 89: 132–144.

Cywinska A, Hunter FF, Hebert PDN (2006) Identifying Canadian mosquito species through DNA barcodes. Med Vet Entomol, 20:413-424.

Das S, Jain SK (1978a) New species of the genus *Coelogyne* Lindl. Proc Indian Acad Sci, 87B: 119-124.

Das S, Jain SK (1978b) New species of the genus *Coelogyne* Lindl. Orchid Rev 86: 195-196.

Das S, Jain SK (1980) Orchidaceae: genus *Coelogyne*. Fsc. Fl. India 5:1-33. BSI Howrah.

De Jussieu AL (1789) ORCHIDEAE. In: Genera Plantarum: Secundum Ordines Naturales Disposita.Pp. 64-66.

De LC (2015) Commercial Orchids. Berlin: De Gruyter Open. Available at: http://www.degruyter.com/view/product/456245.

De LC (2020) Export and import scenario of orchids in India.J Agric Forest Meteorol Res, 3(5): 402-404.

De LC, Singh DR (2016) Post –harvest management and value addition In Orchids. Int J Biol Sci, 3(1): 14-35.

Deb CR, Imchen T (2008) Orchid Diversity of Nagaland. SciChem Publishing House, Udaipur, Rajasthan, India.

Deorani SC, Naithani HB (1995) Orchids of Nagaland. Oriental Enterprises, Dehradun, India.

Dockrill AW (1992) Australian Indigenous Orchids. Surrey Beatty and Sons Pty Ltd: NSW.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull, 19:11-15.

Dressler RL (1974) Classification of the orchid family. In: Proc. 7thWorld orchid conf. (Ospina, M. ed.), Medellin, Colombia 259-279.

Dressler RL (1981) The Orchids: Natural History and Classification. Harvard University Press, Cambridge Massachusetts.

Dressler RL (1981b) The Orchids: Natural History and Classification. Harvard University Press, Cambridge, Massachusetts.

Dressler RL (1993a) Phylogeny and classification of the orchid family. Miss Bot Card, Cambridge Univ, 47: 25-67.

Dressler RL (1993b) Phylogeny and classification of the orchid family. Cambridge University Press, Cambridge.

Dressler RL, Dodson CH (1960) Classification and phylogeny in the Orchidaceae. Annals Missouri Bot Garden, 47: 25-68.

Drury H (1869) Hand Book of the Indian Flora. Vol. 3, Richardson and Co., London.

Duthie JF (1903a) The Orchids of the North-Western Himalaya. Annl Roy Bot Gard, 9:81-211.

Duthie JF (1903b) The Orchids of the North Western Himalaya. Bishen Singh Mahendra Pal Singh. Dehradun.

Eaton MJ, Meyers GL, Kolokotronis SO, Leslie MS, Martin AP, Amato G (2010) Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates. Cons Genet, 11: 1389-1404.

Ebihara A, Nitta JH, Ito M (2010) Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. PLoS One, 5:e15136.

Farrington L, Mac Gillivray P, Faast R, Austin A (2009) Investigating DNA barcoding options for the identification of *Caladenia* (Orchidaceae) species. Aus J Bot, 57:276-286.

Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). Kew Bull, 52: 111–120.

Fazekas AJ, Burgess KS, Kesanakurti PR, Graham SW, Newmaster SG, Husband BC, Percy DM, Hajibabaei M, Barrett SCH (2008) Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS One, 3:e2802.

Feliner GN, Rosselló JA (2007) Better the devil you know? Guidelines for insightful utilization of nrDNAITS in specieslevel evolutionary studies in plants. Mol Phylogenet Evol, 44: 911–919.

Ferri G, Alù M, Corradini B, Beduschi G (2009) Forensic botany: species identification of botanical trace evidence using a multigene barcoding approach. Int J Legal Med, 123:395-401.

FloraHolland (2015) Facts and Figures. FloraHolland Facts and Figures 2015. Available at:

https://www.royalfloraholland.com/media/5685262/RoyalFloraHolland_Annual Report_2015_ENG_facts_and_figures.pdf.

Ford CS, Ayres KL, Toomey N, Haider N, Stahl JVA, Kelly LJ, Wikstrom N, Hollingsworth PM, Duff RJ, Hoot SB, Cowan RS, Chase MW, Wilkinson MJ Soc (2009) Selection of candidate DNA barcoding regions for use on land plants. Bot J Linn, 159:1-11.

Freudenstein JV, Berg CV, Goldman DH, Kores PJ, Molvray M, Chase MW (2004) An expanded plastid DNA phylogeny of Orchidaceae and analysis of jackknife branch support strategy. Ame J Bot, 91:149-157.

Gao T, Sun Z, Yao H, Song J, Zhu Y, Ma X, Chen S (2011) Identification of Fabaceae plants using the DNA barcode matK. Planta Med, 77:92-94.

Gao T, Yao H, Song J, Zhu Y, Liu C, Chen S (2010b) Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family. BMC Evol Biol, 10:324-330.

Garay LA Sweet HR (1974) Orchudaceae. In: Howard RA (ed.) Flora of the Lesser Antilles. Arnold Arboretum, Harvard University, Jamaica Plains, Massachutsetts.

Garay, L. A. 1972. On the origin of the Orchidales II. J Arnold Arb, 53: 202-215.

Gentry AH, Dotson CH (1987) Diversity and biogeography of Neotropical vascular epiphytes. Ann Missouri Bot Gard, 74: 205-233.

Gentry AH, Dotson CH (1991) Diversity and biogeography of Neotropical vascular epiphytes. Ann Missouri Bot Gard, 78: 273-295.

Goldman WE, Goldberg G, Bowman LH, Steinmetz D & Schilessinger D (1983) Mouse rDNA: Sequences and evolutionary analysis of spacer and mature RNA regions.Mol Cell Biol, 3: 1488-1500.

Gonzalez MA, Baraloto C, Engel J, Mori SA, Pétronelli P, Riéra B, Roger A, Thébaud C, Chave J (2009) Identification of amazonian trees with DNA barcodes. PLoS One, 4:e7483.

Gravendeel B, Chase MW, De Vogel EDF, Roos MC, Mes THM, Bachmann K (2001) Molecular phylogeny of *Coelogyne* (epidendroideae; orchidaceae) based on plastid RFLPs, *matK*, and nuclear ribosomal ITS sequences: evidence for polyphyly. Ame J Bot, 88:1915-1927.

Griesbach RJ (2002) Development of *Phalaenopsis* orchids for the mass-market. In: Janick J, Whipkey A (eds.) Trends in New Crops and New Uses. Alexandria: ASHS Press, Pp. 458-465.

Griffith W (1851) Notulae ad Plantas Asiaticas. Vol. 3. W. Griffith, Calcutta.

Guisinger MM, Kuehl JV, Boore JL, Jansen RK (2008) Genome-wide analyses of Geraniaceae plastid DNA reveal unprecedented patterns of increased nucleotide substitutions. Proc Natl Acad Sci USA, 105:18424-18429.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser, 41:95-98.

Hanks F (2015) Orchid Cut Flower Industry. Report for the UK Agriculture and Horticulture Development Board and National Cut Flower Centre. Available at: https://horticulture. ahdb.org.uk/sites/default/files/u3089/A%20review%20of%20cutflower%20and% 20foliage% 20production%20statistics% 202015_0.pdf.

Haywood WH (1993) Flowering Plants of the World. B. T. Batsford Ltd., Oxford.

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003a) Biological identifications through DNA barcodes. Proc R Soc Biol Sci Ser B, 270:313-321.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004b) Ten species in one: DNA barcoding reveals cryptic species in neotropical skipper butterfly Astraptes fulgerator. Proc Natl Acad Sci USA, 101:14812-14817.

Hebert PDN, Ratnasingham S, de Waard JR (2003): Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc Biol Sci Ser B, 270:S96-S99.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004b) Identification of birds through DNA barcodes. PLoS Bio, 2:e312.

Hedge SN (1984) Orchids of Arunachal Pradesh. Forest Department. Itanagar.

Hemleben V, Ganal M, Gerstner J, Schiebel K, Torres RA (1988) Organization and length heterogeneity of plant ribosomal RNA genes. In: Kahl G (ed.) Architecture of Eukaryotic Genes VCH, Weinhein, Germany,Pp. 371–383.

Hershkovitz MA, Zimmer EA (1996) Conservation pattern in angiosperm rDNA–ITS2 sequences. Nucleic Acids Res, 24: 2857–2867.

Hilu KW, Liang H (1997) The *matK* gene: sequence variation and application in plant systematics. Ame J Bot, 84: 830– 839.

Hollingsworth ML, Clark A, Forrest LL, Richardson JR, Pennington RT, Long DG, Cowan R, Chase MW, Gaudeul M, Hollingsworth PM: Selecting barcoding loci for plants (2009) evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Mol Ecol Resour, 9:439-457.

Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. PLoS One, 6:e19254.

Hood L, Campbell JH, Elgin SCR (1975) The organization, expression and evolution of antibody genes and other multigene families. Annu Rev Genet, 9: 305–353.

Hooker JD (1890) The Flora of British India. Orchidaceae 5:667-856.1888; 6:1-198.1:1-101. Ashford, Kent. London.

Hossain MM (2011) Therapeutic orchids: traditional uses and recent advances - an overview. Fitoterapia, 82:102-140.

Humble ML, deWaard JR (2010) Application of DNA barcoding in forest biosecurity surveillance programs [abstract]. USDA Research Forum on Invasive Species GTR-NRS-P-75, 75:30.

Hynniewta T M, Kataki SK, Wadhwa BM (2000) Orchids of Nagaland. BSI, Calcutta.

Hynniewta TM (1984) Orchidaceae of Nagaland (A Contribution Towards a Detailed Knowledge of Orchid Flora). Ph.D. Thesis. Gauhati University, Guwahati. Assam, India.

IUCN/SSC Orchids Specialist Group (1996) Orchids – Status Survey and Conservation Action Plan. IUCN, Gland Switzerland and Cambridge, UK.

Jaakola L, Suokas M, Häggman H (2010) Novel approaches based on DNA barcoding and high-resolution melting of amplicons for authenticity analyses of berry species. Food Chem, 123:494-500.

Jain SK, Mehrotra A (1984) A Preliminary Inventory of Orchidaceae in India. Botanical Survey of India, Howrah, India.

Jain SK, Rao, RR (1977) A Handbook of field and Herbarium Methods. Today and tomarrow's Printers and Publishers, New Delhi.

Jakha HY (2018) Documentation of Orchid Resources of Kiphire, Tuensang and Zunheboto Districts of Nagaland and Micropropagation of Two Threatened Species. Ph.D. Thesis, Nagaland University, India.

Jalal JS, Jayanthi J (2018) An Updated Checklist of the Orchids of Maharashtra, India. Lankesteriana, 18(1): 23–62.

Jalal JS, Kumar P, Pangtey YPS (2008) Ethnomedicinal orchids of Uttarakhand, Western Himalaya. Ethnobot Leaflets, 12: 1227-1230.

Janes VK (2006) A Short Review on the History of Orchid Taxonomy.

Jeanson ML, Labat JN, Little DP (2011) DNA barcoding: a new tool for palm taxonomists? Ann Bot, 108:1445-1451.

Johnson LS, Soltis DE (1995) Phylogenetic inference in Saxifragaceae *sensu strict* and *Gilia* (Polemoniaceae) using *matK* sequences. Ann Mo Bot Gard, 82: 149–175.

Jørgensen T, Kjaer KH, Haile J, Rasmussen M, Boessenkool S, Andersen K, Coissac E, Taberlet P, Brochmann C, Orlando L, Gilbert MT, Willerslev E (2011) Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA Meta-barcoding. Mol Ecol, DOI: 10.1111/j.1365-294X.2011.05278.x.

Kamba J, Deb CR (2018) A new simple and efficient DNA extraction protocol for orchid without liquid nitrogen and phenol. Plant Cell Biotechnol Mol Biol, 19(3-4): 143-147.

Karthikeyan S (2000) A statistical analysis of flowering plants of India. In: Singh NP, Singh DK, Hajra PK, Sharma BD (eds.) Florae of India, Introductory Vol. III. Botanical Survey of India, Howrah, India.Pp.201-217.

Karthikeyana S, Jain SK, Nayar MP, Sanjappa M (1989) Florae Indicae Enumeratio: Monocotyledonae. Botanical Survey of India, Pune.

Kataki SK (1986) Orchids of Meghalaya. Forest Department, Shillong, Meghalaya.

Kelchner SA (2000) The evolution of noncoding chloroplast DNA and its application in plant systematics. Ann Mo Bot Gard, 87: 482–498.

Kelly LJ, Ameka GK, Chase MW (2010) DNA barcoding of African Podostemaceae (river-weeds): a test of proposed barcode regions. Taxon, 59:251- 260.

King G, Pantling R (1898) The orchids of Sikkim Himalaya. Ann Roy Bot Gard, Calcutta 8:1-342.

Kores PJ, Molvray M, Weston PH, Hopper SD, Brown AP, Cameron KM, Chase MW (2001) A Phylogenetic analysis of *Diurideae* (Orchidaceae) based on plastid DNA sequence. Ame J Bot, 88:1903-1914.

Kress JW, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. Proc Natl Acad Sci USA, 102: 8369–8374.

Kress WJ Erickson DL (2007) A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the noncoding *trnH–psbA* spacer region. PLoS ONE, 2: e508.

Kumar CS Manilal KS (1994) A Catalogue of Indian Orchids. Bishen Singh Mahendra Pal Singh, Dehradun.

Kumar S, Stecher G, Tamura K (2016) Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Mol Biol Evol, 33:1870-1874.

Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F, Gigot G, Maurin O, Duthoit S, Barraclough TG, Savolainen V (2008) DNA barcoding the floras of biodiversity hotspots. Proc Natl Acad Sci USA, 105:2923-2928.

Lawler L. 1984. Ethnobotany of the Orchidaceae. In: Arditti J (ed.) Orchid Biology, Reviews & Perspectives III. Ithaca: Cornell University, Pp. 27–149.

Lawler LJ, Rao AN (2002) Medicinal uses of orchids in some Asian countries. J Trop Med Pl, 3(1): 73-88.

Le Clerc-Blain J, Starr JR, Bull RD, Saarela JM (2010) A regional approach to plant DNA barcoding provides high species resolution of sedges (*Carex* and *Kobresia*, Cyperaceae) in the Canadian Arctic Archipelago. Mol Ecol Resour, 10:69-91.

Lekawatana S (2010) Thai Orchids: current situation.In: Taiwan International Orchids Symposium, Taiwan Orchids Plantation, Tainan, Taiwan, March 5.

Li FW, Kuo LY, Rothfels CJ, Ebihara A, Chiou WL, Windham MD, Pryer KM (2011)*rbcL* and *matK* earn two thumbs up as the core DNA barcode for ferns. PLoS One, 6: e26597.

Lindley J (1857) Contribution to the Orchidology of India. J Proc Linn Soc, 1: 170-190.

Lindly J (1830-1840) Genera and species of Orchidaceous Plants. London.

Liu JIE, Möller M, Gao L-M, Zhang D-Q, Li D-Z (2011a) DNA barcoding for the discrimination of Eurasian yews (*Taxus* L., Taxaceae) and the discovery of cryptic species. Mol Ecol Resour, 11:89-100.

Liu Y, Cao T, Ge XJ (2011b) A case study of DNA barcoding in Chinese Grimmiaceae and a moss recorded in China for the first time. Taxon, 60:185-193.

Mabberley DJ (1990) The Plant Book. Cambridge University Press, Cambridge.

Mabberley DJ (1998) The Plant Book (2^{nd} Edn.). Cambridge University Press, Cambridge. Madras.

Marutsuka T, Shimada S, Shiomori K, Hayashi N, Yagi Y, Yamane T, OgawaM (2003) Mechanisms of peritoneal metastasis after operation for non-serosa-invasive gastric carcinoma: an ultrarapid detection system for intraperitoneal free cancer cells and a prophylactic strategy for peritoneal metastasis**.** Clin Cancer Res, 9(2):678-85.

Meusnier I, Singer GAC, Landry JF, Hickey DA, Hebert PDN, Hajibabaei M (2008) A universal DNA mini-barcode for biodiversity analysis. BMC Genomics, 9:214.

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biol, 3:2229-2238.

Mishra S (2007) Orchids of India. Bishen Singh Mahendra Pal Singh, Dehradun.

Mitra JN (1958) Flowering plants of Eastern India. Orchidaceae, 1:261-357. Calcutta.

Miwa H, Odrzykoski IJ, Matsui A, Hasegawa M, Akiyama H, Jia Y, Sabirov R, Takahashi H, Boufford DE, Murakami N (2009) Adaptive evolution of rbcL in *Conocephalum* (Hepaticae, bryophytes). Gene, 441:169-175.

Muellner AN, Schaefer H, Lahaye R (2011) Evaluation of candidate DNA barcoding loci for economically important timber species of the mahogany family (Meliaceae). Mol Ecol Resour, 11:450-460.

Muir G, Fleming CC, Schlötterer C (2001) Three divergent rDNA clusters predate the species divergence in *Quercus petraea* (Matt.) Liebl. And *Quercus robur* L. Mol Biol Evol, 18: 112–119.

Nair NC (1978) Flora of Punjab plains. Vol. 21. Botanical Survey of India. Howrah.

Newmaster SG, Fazekas AJ, Ragupathy S (2006) DNA barcoding in land plants: evaluation of *rbcL* in a multigene tiered approach. Can J Bot, 84: 335–341.

Newmaster SG, Fazekas AJ, Steeves RAD, Janovec J (2008) Testing candidate plant barcode regions in the Myristicaceae. Mol Ecol Notes, 8:480-490.

Newmaster SG, Ragupathy S (2009) Testing plant barcoding in a sister species complex of pantropical Acacia (Mimosoideae, Fabaceae). Mol Ecol Resour, 9:172-180.

Nicolalde-Morejón F, Vergara-Silva F, González-Astorga J, Stevenson DW (2011) Character-based, population-level DNA barcoding in Mexican species of *Zamia* L. (Zamiaceae: Cycadales). Mitochondr DNA, 21:51-59.

Pang X, Song J, Zhu Y, Xie C, Chen S (2010) Using DNA barcoding to identify species within Euphorbiaceae. Planta Med, 76:1784-1786.

Panigrahi G, Joseph H (1966) A botanical tour to Tirap Frontier Division NEFA. Bull Bot Surv India, 8(2): 156-157.

Pant B (2008) Application of tissue culture for conservation of medicinal plants. In: PK. Jha et al (eds). Medicinal plants in Nepal: An anthology of contemporary research, Publisher, Ecological Society (ECOS) Kathmandu Nepal, Pp. 240-245.

Parveen I, Singh HK, Raghuvanshi S, Pradhan UC, Babbar SB (2012) DNA barcoding of endangered Indian *Paphiopedilum* species. Mol Ecol Resour, 12:82- 90.

Parveen I (2012) DNA Barcoding of Indian Orchids. Ph.D. Thesis, University of Delhi, India.

Pellegrino G, Musacchio A, Noce ME, Palermo AM, Widmer A (2005) Reproductive versus floral isolation among morphologically similar *Serapias* L. species (Orchidaceae). J Hered, 96:15-23.

Pettengill JB, Neel MC (2010) An Evaluation of candidate plant DNA barcode and assignment methods in diagnosing 29 species in the genus Agalinis (Orobanchaceae). Ame J Bot, 97:1391-1406.

Peyachoknagul S, Nettuwakul C, Phuekvilai, P, Wannapinpong S, Srikulnath K (2014) Development of microsatellite markers of Vandaceous Orchids for species and variety identification. Genet Mol Res, 13(3): 5441-5445.

Pfitzer EH (1887) Entwurf einer naturalichen Anordnung der orchiden. Carl winter, Heidelberg.

Piredda R, Simeone MC, Attimonelli M, Bellarosa R, Schirone B (2011) Prospects of barcoding the Italian wild dendroflora: oaks reveal severe limitations to tracking species identity. Mol Ecol Resour, 11:72-83.

Pradhan, U. C. 1979. Indian Orchids, Vol. I & II: Guide to Identification and Culture. U. C. Pradhan, Kalimpong.

Ran JH, Wang PP, Zhao HJ, Wang XQ (2010) A test of seven candidate barcode regions from the plastome in Picea (Pinaceae). J Integr Plant Biol, 52:1109-1126.

Ratnasingham S, Hebert PDN: BOLD (2007) The Barcode of Life Data System (http://barcodinglife.org). Mol Ecol Notes. 7:355-364.

Ren BQ, Xiang XG, Chen ZD (2010) Species identification of *Alnus* (Betulaceae) using nrDNA and cpDNA genetic markers. Mol Ecol Resour, 10:594-605.

Richard LC (1817) De Orchideis Europaeis annotationes. Parisiis, ex typographia A. Belin.

Rittershausen B Rittershausen W (2002) The Practical Encyclopedia of Orchids: A Complete Guide to Orchids and Their Cultivation. Annes Publication Limited, London.

Rogers SO, Bendich AJ (1987) Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol, 6: 339–345.

Rosengarten F (1969) The Book of Spices.Livingston Publishing Company, Wynnewood, PA.

Ross HA, Murugan S, Li WLS (2008) Testing the reliability of genetic methods of species identification via simulation. Syst Biol, 57:216-230.

Roxburgh W (1832) Flora Indica (Ed. W. Carey), 3: 450-488. Serampore.

Roy S, Tyagi A, Shukla V, Kumar A, Singh UM, Chaudhary BL, Datt B, Bag SK, Singh PK, Nair NK, Husain T, Tuli R (2010) Universal plant DNA barcode loci may not

work in complex groups: a case study with Indian *Berberis* species. PLoS One, 5:e13674.

Rubinoff D, Cameron S, Will K (2006) Are plant DNA barcodes a search for the Holy Grail? Trends Ecol Evol, 21:1-2.

Santapau H, Kapadia Z (1966) The Orchids of Bombay. New Delhi.

Sasaki T, Yukawa Y, Miyamoto T, Obokata J, Sugiura M (2003) Identification of RNA editing sites in chloroplast transcripts from the maternal and paternal progenitors of tobacco (*Nicotiana tabacum*): comparative analysis shows the involvement of distinct *trans*-factors for *ndhB* editing. Mol Biol Evol, 20(7): 1028-1035.

Sass C, Little DP, Stevenson DW, Specht CD (2007) DNA barcoding in the Cucadales: testing the potential of proposed barcoding markers for species indentification of cycades. PLoS ONE, 2: e1154.

Schindel DE, Miller SE (2005) DNA barcoding a useful tool for taxonomists. Nature, 435:17.

Schmid R, Schmid MJ (1977) Fossil History of the Orchidaceae. In: Arditti J (ed.) Orchid Biology Reviews and Perspectives. Cornell Uniersity Press: California.

Schmitz-Linneweber C, Maier RM, Alcaraz JP, Cottet A, Herrmann RG, Mache R (2001) The plastid chromosome of spinach (*Spinacia oleracea*): complete nucleotide sequence and gene organization. Plant Mol Biol, 45, 307–315.

Seberg O, Petersen G (2009) How many loci does it take to DNA barcode a Crocus? PLoS One, 4:e4598.

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J, 5(9): 2043-2049.

Singh A, Duggal S (2009). Medicinal Orchids: An Overview. Ethnobot Leaflets, 13:351-363.

Singh HK, Parveen I, Raghuvanshi S, Babbar SB (2012) Loci recommended as universal barcode for plants on the basis of floristic studies may not work with congeneric species as exemplified by DNA barcoding of Dendrobium species. BMC Res Notes, 5: 42.

Singh SK, Agrawala DK, Jalal JS, Dash SS, Mao AA, Singh P (2019) Orchids of India-A Pictorial Guide. BSI. Ministry of Environment, Forest & Climate Change, Kolkata.

Soininen EM, Valentini A, Coissac E, Miquel C, Gielly L, Brochmann C, Brysting AK, Sønstebø JH, Ims RA, Yoccoz NG, Taberlet P (2009) Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with highthroughput pyrosequencing for deciphering the composition of complex plant mixtures. Front Zool, 6:16-24.

Soliva M, Kocyan A, Widmer A (2001) Molecular phylogenetics of the sexually deceptive orchid genus *Ophrys* (Orchidaceae) based on nuclear and chloroplast DNA sequences. Mol Phylogenet Evol, 20:78-88.

Soltis DE, Soltis PS (1998) Choosing an approach and an appropriate gene for phylogenetic analysis. In: Soltis DE, Soltis PS, Doyle JJ (eds.)Molecular Systematicsof Plants II: DNA Sequencing.Kluwer, Dordrecht, Pp. 21–24.

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol, 60:561-588.

Spooner DM (2009) DNA Barcoding will frequently fail in complicated groups: an example in wild potatoes. Ame J Bot, 96:1177-1189.

Srirama R, Senthilkumar U, Sreejayan N, Ravikanth G, Gurumurthy BR, Shivanna MB, Sanjappa M, Ganeshaiah KN, Shaanker R U (2010) Assessing species admixtures in raw drug trade of *Phyllanthus*, a hepato-protective plant using molecular tools. J Ethnopharmacol, 130:208-215.

St. George I (1999) The Nature Guide to New Zealand Native Orchids. Random House New Zealand: Auchland.

Starr JR, Naczi RFC, Chouinard BN (2009) Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). Mol Ecol Resour, 9(1): 151–163.

Sun WY, Liao YJ, Hung SY, Chang JC, Sung MJ (2011) Development of ITS based SCAR markers for discrimination of *Paphiopedilum armeniacum*, *Paphiopedilum micranthum*, *Paphiopedilum delenatii* and their hybrids. Sci Hortic, 127:405-410.

Szlachetko DL (1995) Systema Orchidalium. Fragmenta Floristica et Geobotanica Supplementum, 3: 1-152.

Thomson JD, Higgins DG, Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple alignment through sequence weighting. Nucleic Acids Res, 22:4673-4680.

USDA (2016) Floriculture Crops 2015 Summary. United States Department of Agriculture, National Agricultural Statistics Service. Available at: http://usda.mannlib.cornell.edu/usda/current/FlorCrop/FlorCrop-04-26-2016.pdf.

Valentini A, Miquel C, Nawaz MA, Bellemain EVA, Coissac E, Pompanon F, Gielly L, Cruaud C, Nascetti G, Wincker P, Swenson JE, Taberlet P (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. Mol Ecol Resour, 9:51-60.

Valentini A, Miquel C, Taberlet P (2010) DNA barcoding for honey biodiversity. Diversity 2:610-617.

van de Wiel CCM, van der Schoot J, van Valkenburg JLCH, Duistermaat H, Smulders MJM (2009) DNA barcoding discriminates the noxious invasive plant species, floating pennywort (*Hydrocotyle ranunculoides* L.f.), from non-invasive relatives. Mol Ecol Resour, 9:1086-1091.

van den Berg C, Goldman DH, Freudenstein JV, Pridgeon AM, Cameron KM, Chase MW (2005) An overview of the phylogenetic relationships within Epidendroideae inferred from multiple DNA regions and recircumscription of Epidendreae and Arethuseae (Orchidaceae). Ame J Bot, 92:613-624.

Verma D, Lavania S (2014) Additions to the Orchids flora of Meghalaya, India. Richardiana, 15: 105-114.

Vermeulen JJ (1991) Orchids of Borneo. 2. Bulbophyllum. Royal Botanic Garden, Kew, and Sabah Society.

Vermeulen P (1966) The system of the Orchidales. Acta Bot Neerl, 15: 224-253.

Vij S P (1995) Genetic resources of Orchids. In: Chadha KL, Bhattacharjee SK (eds.) Advances in Horticulture, Vol. 12 Ornamental Plants. Malhotra Publishing, New Delhi, Pp. 153-181.

Vijayan K, Tsou CH (2010). DNA barcoding in plants: taxonomy in a new perspective. Curr Sci, 99:10.

Vijayan K, Zhang WJ, Tsou CH (2009) Molecular taxonomy of *Camellia* (Theaceae) as inferred from nrITS sequences.Ame J Bot, 96: 1348–1360.

Wakasugi T, Sugita M, Tsudzuki T, Sugiura M (1998) Updated Gene Map of Tobacco Chloroplast DNA. Pl Mol Biol Rep, 16: 231–241.

Wang W, Wu Y, Yan Y, Ermakova M, Kerstetter R, Messing J (2010) DNA barcoding of the Lemnaceae, a family of aquatic monocots. BMC Plant Biol, 10:205.

WCSP (2017) Kew Royal Botanic Gardens. World Checklist of Selected Plant Families. URL: http://apps.kew.org/wcsp/home.do (Accessed: October, 2020).

Wendel JF, Schnabel A, Seelanan T (1995) Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc Natl Acad Sci USA, 92: 280–284.

Whitten WM, Blanco MA, Williams NH, Koehler S, Carnevali G, Singer RB, Endara L, Neubig KM (2007) Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. Ame J Bot, 94:1860-1889.

Wight G (1832) Illustrations of Indian Botany. Government Press. Madras.

Wight R (1845-1853) Icones Plantarum Indiae Orientalis, Government Press, Wiley, New York.

Williams CA (1979) The leaf flavonoids of the Orchidaceae. Phytochem, 18:803-813.

Wolfe K, Li WH, Sharp PM (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc Natl Acad Sci USA, 84:9054-9058.

Xiang XG, Hu H, Wang W, Jin XH (2011) DNA barcoding of the recently evolved genus *Holcoglossum* (Orchidaceae: Aeridinae): a test of DNA barcode candidates. Mol Ecol Resour, 11:1012-1021.

Xiao JH, Wang NX, Li YW, Murphy RW, Wan DG, Niu LM, Hu HY, Fu YG, Huang DW (2010) Molecular approaches to identify cryptic species and polymorphic species within a complex community of fig wasps. PLoS One, 5(11): e15067.

Yang CY, Lin TL, Tzung TY, Cheng LC, Wang JT, Jee SH (2007) Direct identification of dermatophyte DNA from clinical specimens by a nested polymerase chain reaction assay. Arch Dermatol, 143(6): 799-800.

Yang L, Wang Z, Xu L (2006) Simultaneous determination of phenols (bibenzyl, phenanthrene, and fluorenone) in *Dendrobium* species by high-performance liquid chromatography with diode array detection. J Chromatogr A, 1104:230–237.

Yao H, Song J, Liu C, Luo K, Han J, Li Y, Pang X, Xu H, Zhu Y, Xiao P, Chen S (2010) Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One, 5:e13102.

Yao H, Song JY, Ma XY, Liu C, Li Y, Xu HX, Han JP, Duan LS, Chen SL (2009) Identification of *Dendrobium* species by a candidate DNA barcode sequence: the chloroplast psbA-trnH intergenic region. Planta Med, 75:667-669.

Yesson C, Bárcenas RT, Hernández HM, de la Luz Riz-Maqueda M, Prado A, Rodríguez VM, Hawkins JA (2011) DNA barcodes for Mexican Cactaceae, plants under pressure from wild collecting. Mol Ecol Resour, 11:775-783.

Zimmer EA, Martin SL, Beverley SM, Kan YW, Wilson AC (1980) Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. Proc Natl Acad Sci USA77: 2158–2162.

Zitari A, Scopece G, Helal AN, Widmar A, Cozzolino S (2011) Is floral divergence sufficient to maintain species boundaries upon secondary contact in Mediterranean food-deceptive orchids? Heredity, 108:219-228.

Appendix – I

GPS coordinates from the collection sites

LOCATION	LATITUDE (N)	LONGITUDE (E)	ELEVATION
Mokokchung	26°27′03.3″	094°44′56.9″	1196m
	26°15′21.0″	094°27′39.3″	873 m
	26°28′19.9″	94°46′35.8″	1200 m
	26°26′75.1″	94°40′49.4″	1131 m
	26°29′95.8″	094°27′99.3″	1026m
	26°29′95.9″	094°27′99.4″	1027m
	26°29′73.0″	094°27′57.0″	945.5m
	26°52′20.9″	094°67′21.6″	1276 m
	26°19′34.6″	094°31′07.8″	1226m
	26°37′86.1″	094°57′13.9″	950 m
	26°17′44.1″	094°29′53.2″	1249 m
Zunheboto	26°40′30.1″	094°32′22.5″	1648.1m
	26°12′57.2″	094°29′45.5″	722 m
	26°60′19.2″	094°32′14.0″	1529.2m
	26°20′72.4″	094°47′98.1″	491m
	26°20′53.4″	094°48′27.0″	876m
	26°20′46.5″	094°48′34.7″	823m
	26°12′38.5″	094°28′27.3″	1047m
	26°12′53.7″	094°28′46.3″	782 m

	26°12′61.0″	094°28′62.8″	790 m
	26°15′09.5″	094°29′40.9″	930 m
	26°15′31.3″	094°28′73.3″	1060 m
	26°04′03.5″	094°53′73.2″	1730m
	26°03′19.2″	094°32′05.0″	1757m
Wokha	26°11′27.6″	094°40′78.5″	545m
	26°11′34.7″	094°38′74.7″	411m
	26°11′50.3″	094°38′69.1″	343m
Peren	25°39′21.1″	093°39′39.2″	384m
	25°38′96.0″	093°39′61.8″	381m
	25°39′88.4″	093°50′99.5″	664m
	25°39′43.6″	093°51′47.9″	791m
	25°39′24.0″	093°51′47.9″	796m
	25°39′24.4″	093°51′65.0″	802m
Kohima	25°54′594″	094°12′37.2″	1605 m
	25°54′91.1″	094°13′44.0″	1637 m
	25°54′19.7″	094°13′11.8″	1672 m
	25°70′98.4″	094°13′81.4″	1407
	25°71′81.2″	094°13′57.2″	1348
Tuensang	26°13′38.5″	094°45′831″	1860 m
Phek	25°43′24.4″	094°28′07.3″	1248 m
	25°65′98.9″	094°36′79.8″	1253 m

Appendix – II

Chemicals& Enzymes

Absolute Ethanol	Isoamyl alcohol
Acetic acid (Glacial)	Isopropanol
Agarose	Na_2EDTA([2-[2-[bis(carboxy methyl)amino]ethyl-(carboxymethyl)amino] acetic acid]
Bromophenol blue sodium salt	NaCl (Sodium chloride)
Chloroform (C)	Ribonuclease A
CTAB (Hexadecyltrimethyl ammonium Bromide)	Sodium acetate
Deoxynucleotide set (dNTPs)	Sodium hydroxide pellets
DNA *Tag* polymerase	TAE Buffer
Ethanol	TBE Buffer
Ethidium bromide	TE Buffer
GeneRuler 100bp DNA Ladder	Tris-HCl
GeneRuler 1kb DNA Ladder	Tris-Phenol (P)
Glycerol	β-Mercaptoethanol
Hydrochloric acid	

Reagents

The following stock solutions were prepared for the isolation and purification of DNA (Following CTAB method, Doyle and Doyle, 1987) for 100 ml

a) 1.4 M NaCl – Dissolve 8.18 g/28 ml from 5M NaCl solution

b) 100 mM Tris-HCl – 5 ml from 2M Tris-HCl/pH 8.0

c) 20 mM Na_2EDTA – 4 ml from 0.5M Na_2EDTA/pH 8.0

d) 2% CTAB - 2.0 g CTAB to the buffer solution (to be added after autoclaving)

e) 0.2% β-Mercaptoethanol - 20µl for 10 ml CTAB solution (Add before adding sample)

f) 1 g PVP 40 (Polyvinyl pyrrolidone, Mw 40,000)

g) Chloroform : Isoamyl alcohol (24:1)

h) 70% Ethanol

i) 10 mg/ml RNase A

j) 5 mg/ml Ethidium bromide

k) 50X T.A.E. Buffer (Tris-HCl, Acetic acid, Na_2EDTA, pH 8.0)

l) Phenol equilibrated with Tris-HCl (pH 8.0)

m) 3 M Sodium acetate

All the reagents were prepared in Milli-Q water (MQ). The stock solutions, glassware and plasticware were sterilized by autoclaving at 103.4 KPa at 121°C for 20 min.

Appendix – III

PCR Cycle Conditions

(a) Chloroplast genome (*matK* and *rbcL*)

1.	Initial Denaturation	- 94°C, 5’	
2.	Final Denaturation	- 94°C, 45”	35 Cycles
3.	Primer Annealing	- 60°C,50”	
4.	Primer Extension	- 72°C, 01’	
5.	Final Extension	- 72°C, 10’	
6.	4°C	∞	

(b) Nuclear ribosomal ITS

1.	Initial Denaturation	- 94°C, 5’	
2.	Final Denaturation	- 94°C,45”	35 Cycles
3.	Primer Annealing	- 52°C,50”	
4.	Primer Extension	- 72°C, 01’	
5.	Final Extension	- 72°C, 10’	
6.	4°C	∞	

List of Publications

1. Deb CR, Kamba J, Longchar TB, Jakha H (2017) *Cymbedium bicolor* Lindl. (Orchidaceae): a new report for the orchid flora of Nagaland, India. Pleione 11(2): 498-500.
2. Kamba J, Deb CR (2018) A new simple and efficient DNA extraction protocol for orchid without liquid nitrogen and phenol. Plant Cell Biotechnol Mol Biol, 19(3-4): 143-147.
3. Chaturvedi SK, Kuotsu K, Kamba J (2020) Pollination and diversity of visitors and pollinators of *Alpinia blepharocalyx* K. Schum. (Zingiberaceae) in Nagaland (N-E India). IntJ Plant Repro Biol, 12(2): 1-5.

Research Paper Presented in Seminar/ Conference

1. Kamba, J. and Deb, C R. (2019) DNA Barcoding of Commercially Important *Vanda* species (Orchidaceae) of Nagaland (Best Poster Awarded). In: International Conference on 'Next Generation Plant Production and Bioresources Utilization Technologies', Department of Biosciences & Bioengineering and Centre for Rural Technology, IIT Guwahati India. February 11-13, 2019.

2. Kamba, J. and Deb, C R. (2020) DNA Barcoding: A Taxonomic Tool for Identification of Wild Orchids. In: National e-Conference on 'Bioresources and Sustainable Livelihood of Rural India', Department of Botany, Nagaland University, Lumami. September 28-29, 2020.

List of Seminar, Workshop and Conference attended

1. National Workshop on Computational Drug Designing-I, Bioinformatics Infrastructure Facility Centre, Nagaland University, Lumami on October 05-06, 2015.
2. National Seminar on 'Inventory, Sustainable Utilization and Conservation of Bioresoures', Dept. of Botany and Institutional Biotech Hub, Nagaland University, Lumami on February 26-27, 2016.
3. Hands on Training on Molecular Profiling and Genome Analysis, Bioinformatics Infrastructure Facility Centre, Nagaland University, Lumami on March 14-19, 2016.
4. National Training on Orchids Database Designing for Biologist, Bioinformatics Infrastructure Facility Centre, Nagaland University, Lumami on August 22-25, 2016.
5. National Seminar on Advances in Biological Science Research, Dept.of Botany, Nagaland university, Lumami on February 28-March 01, 2017.
6. International Conference on Natural Resources Management and Technology Trends (ICNRM-17), Center of Advance study, Department of Life Sciences, Manipur University, Imphal on 27-29 March, 2017.
7. Hands on Training on Functional Genomics, Institutional Biotech Hub and Dept. of Botany, Nagaland University, Lumami on November 14-21, 2017.
8. Short-Term Skill Development Training Programme in Biotechnology for students of North-East India on 'Orchids Propagation' organized by DBT Sponsored Institutional Biotech Hub & Department of Botany, Nagaland University, Lumami

jointly sponsored by Biotech Park, Lucknow, UP & Institutional Biotech Hub, Nagaland University, Lumami, November 16-December 15, 2017

9. Hands on Training on Genomics and Gene Expression Analysis, Department of Biotechnology, Govt. of India sponsored Advance level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami on July 18-23, 2018.
10. Workshop on Skill and Entrepreneurial Development of the Tribal Youth, with the theme 'Value-additions to Rich Bio-Resources with Special Reference to Medicinal and Aromatic Plants' jointly organized by Biotech Park, Lucknow & Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami on July 25-28, 2018.
11. International Conference on 'Next Generation Plant Production and Bioresources Utilization Technologies' Organized by Department of Biosciences & Bioengineering and Centre for Rural Technology, IIT Guwahati (IITG), India, In association with 'International Plant Propagator's Society' (IPPS) on February 11-13, 2019 and presented a poster on the topic "DNA Barcoding of Commercially Important *Vanda* species (Orchidaceae) of Nagaland".
12. National Conference of Stakeholders on 'Conservation, Cultivation, Resource Development and Sustainable Utilization of Medicinal Plants of North-Eastern India' jointly organized by Department of Botany (UGC-SAP DRS-III), DBT-Advance Level Institutional Biotech Hub, Nagaland University, Lumami & Society for Conservation and Resource Development of Medicinal Plants (SMP), New Delhi on March 6-7, 2019.
13. One-Day Workshop on "Importance of IPR in Academic Institutions" organized by IPR Cell, Nagaland University, held on 29th May, 2019.
14. Workshop on 'Research Ethics, Paper Writing & IPR' organized & Sponsored by UGC-SAP (DRS-III), Department of Botany & Department of Biotechnology, Govt. of India sponsored Advance Level Institutional Biotech Hub, Nagaland University, Lumami held on November 14-15, 2019.
15. National e-conference 'Bioresources and Sustainable Livelihood of Rural India' Organized by Department of Botany, Nagaland University, Lumami. Sponsored by Ministry of Enviroment, Forest and Climate Change supported NMHS Programme, UGC-SAP (DRS-III) Programme, Department of Botany & Nagaland University held on September 28-29, 2020 and presented a paper on the topic "DNA Barcoding: A Taxonomic Tool for Identification of Wild Orchids.

****