MOLECULAR PHYLOGENY OF MUSACEAE IN INDIA

Thesis submitted to the
University of Calicut in partial fulfillment of the requirements for
the award of the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

by

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AUGUST-2024

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As the adjudicators have not mentioned any corrections in this thesis entitled **"Molecular Phylogeny of Musaceae in India"** it is certified that the thesis is being submitted without any corrections.

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CERTIFICATE

This is to certify that the thesis entitled **Molecular Phylogeny of Musaceae in India**, submitted to the University of Calicut by **Mr. Rajeesh E.P.**, in partial fulfillment of the award of the degree of Doctor of Philosophy in Botany is a bonafide record of the research work carried out by him under my supervision and guidance. No part of the present work has formed the basis for the award of any other degree or diploma previously.

Calicut University
29.08.2024

Prof. M. Sabu
(Supervising Teacher)

DECLARATION

I hereby declare that the work presented in the thesis entitled **Molecular Phylogeny of Musaceae in India** is based on the original work done by me under the guidance of **Prof. M. Sabu** and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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Date: 29-08-2024

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ACKNOWLEDGEMENTS

As I look back on this journey, I remember many faces who helped me directly or indirectly to achieve this milestone. It is time to extend my deepest appreciation and gratitude to all those who have helped me along the way to the completion of this thesis.

First and foremost, I express my deep sense of gratitude to my supervisor Prof. (Dr). M. Sabu, Emeritus Scientist, Malabar Botanical Garden and Institute for Plant Sciences, for his constant encouragement and valuable advice throughout this research work. His expertise, insightful feedback, and continuous motivation have been instrumental in shaping this thesis. I always cherished the experience of working with him- 'The Ginger man of India' whose exceptional enthusiasm and dedication have left a lasting impact on me. Thank you so much sir for your great support and for giving me the freedom to work and grow as a researcher.

I gratefully acknowledge KSCSTE, for providing financial support as research fellowship for doing this work.

I am very much indebted to Prof. (Dr.) Sailas Benjamin (Late) and Dr. A. K. Pradeep (Retd.) for being a source of inspiration towards research and their support had profoundly impacted my academic journey.

I express my sincere thanks to Sen. Prof. (Dr.) Santhosh Nampy for allowing me to carry out the molecular works and the compound microscope facilities in his lab.

I would like to thank Prof. (Dr.) C.C. Harilal, Head of the Department and Prof. (Dr.) Jos T. Puthur, Prof. (Dr.) V.V. Radhakrishnan, Sen. Prof. (Dr.) Santhosh Nampy, Prof. (Dr.) K. V. Mohanan, Prof. (Dr.) K. M. Jayaram, former Heads, Department of Botany, University of Calicut for facilities, and academic support that have facilitated the completion of this study.

I would like to express my sincere thanks to Dr. V.S. Hareesh, for being a back support during the entire span of my research. I am thankful to Dr. Alfred Joe and Ms. Smisha K.P. for their valuable suggestions and for providing me with some beautiful images of bananas.

I owe my deep sense of gratitude to Dr. P. Sunojkumar, Dr. Maju C. Nair, and Dr. C. Pramod, faculties of the Department of Botany, University of Calicut, for their valuable suggestions and friendly approach throughout the work. I extend my heartfelt thanks to all other teaching faculties, (Retd.) Prof. (Dr.) P. Manimohan, Prof. (Dr.) Jos T. Puthur, Sen. Prof. (Dr.) John E. Thoppil, Prof. (Dr.) A. Yusuf, Dr. M. Shamina, Dr. K.P. Deepna Latha, Dr. Resmi L. for their encouraging words. I would also like to express my sincere appreciation to all the non-teaching staff in the department of Botany for their assistance.

I would like to express my profound appreciation to Dr. Piyakaset Suksathan, Director, Queen Sirikit Botanical Garden and Dr. Sasivimon Swangpol, Mahidol University, Thailand, for their consideration, insightful advice and suggestions. Discussions with them have been extremely beneficial and have helped me to think differently.

I express my sincere thanks to Dr. Rithesh Kumar Choudhary, Scientist E, Agharkar Research Institute, Pune and his students Dr. Ashwini Darshetkar and Dr. Sathish Maurya for the valuable suggestions during the work.

I express my sincere thanks to Latheef Sir, Habeeba Miss, Dr. A. Usman, Dr. Priya T., Muhammed Ali Sir and JK Sir, for their support and motivation during my academic career.

I wish to express my sincere gratitude to the Chief Conservator of Forest of state Forest Department and officials of Mizoram, Arunachal Pradesh, Assam, Andaman & Nicobar, etc. for permission to conduct field studies in reserve forests and protected areas.

I extend my sincere thanks to Vision Scientific Services and Geene Spec, Cochin for DNA sequencing facility.

I express my sincere gratitude Mr. K.I. Reginold, Mr. Santhosh (Andaman), Nandan K.K., Joju Alappat IFS (Andaman) for their necessary and timely helps in the field trips.

A great word of thanks to Vasuettan and all other gardeners for planting and maintaining the materials in the Calicut University Botanical Garden (CUBG).

I express my sincere thanks to Dr. K.M. Prakasan and Mrs. Sony, Mrs. Sindhu K. (former Librarians, Department of Botany) and Mrs. Nusrath for their valuable help and moral supports. I am indebted to Mr. A.K. Vijayan for his lovely support and care during this research period.

I also extend my thanks to Mr. Rajesh and his co-workers, Bina Photostat, Villunniyal for type settings and printing.

I extend my sincere thanks to my seniors, Dr. V.P. Thomas, Dr. E. Sanoj Dr. K.M. Prabhukumar, Dr. Sreejith P.E., Dr. Mohammed Shameer Cheriyath, Mr. A.V. Prasanth, Dr. Aswani K., Mrs. Aswathi P. and Mrs. Linu for their valuable support during my work.

I am indebted to my friends and research colleagues Nikhil Krishna, Drisya V., Dr. Thoiba Kottekkatu, , Sreelakshmi, Sumitha K., Jeomol K.K., Aswathi Ganga, Ashna Toms, Alan Thomas, Krishnaraj T.P., Dr. Shinoj K., Sasi Kumar C., Jiji P., Dr. Geethika K., Akhil M.K., Harishma K.H., Krishna Priya M.P., Vaishnavi, Sreya, Aparna, Dr. Syam Radh, Dr. Reshmi S., Dr. Manudev K.M., Dr. Arun Kumar P.G., Dr. Janeesha, Dr. Prashob P., Sajitha, Dr. Mufeed, Shakira, Dr. Preethamol, Dr. Vimal K., Dr. Soumya P., Dr. Manu Philip, Jaise Mon, Binil E., and Vishnu Venugopal for their support, discussions, and encouragement throughout this endeavour.

A special word of thanks to Mr. Nikhil Krishna and Dr. Jayakrisnan T. for helping me to make my first trips to northeast India which inspired me to explore other parts of India.

I am immensely grateful for the unwavering support, encouragement, and companionship of my dear friends Dr. Amrutha A., Dr. Dani Francis, Dr. Vishnu Mohan, Sreekutty T.K., Sarath G. Nair, Irfan Mohammed, Dr. Litty R., Dr. Ramya Sree, Dr. Reshmi and Gokul. I remember all the fun we had together, and your friendship is a treasure I deeply value, and I look forward to creating many more beautiful memories together.

I want to take this moment to express my deepest gratitude to Dr. Amrutha for her support during field trips and at every stage of this research work.

I convey my deep sense of gratitude to Akhil, Shinojettan, JK, Sruthi, Vineesha, Harishma, Ambika, Haritha, Arunima, Sreya, Ashna, Vaishnavi and Aswathi Ganga for their immense helps during final stages of my thesis writing.

It is pleasure to thank my colleagues Dr. Musfir Mehaboob, Ms. Manasa and Mrs. Aysha Shirin for their encouragement and support.

Words are not adequate to thank my Achan, Amma, Sajeesh (Kuttan thattan), Hareesh (Vava), Athira, Eva mol, Hachi and all my family for their unconditional love, support and care throughout my life. I would like to express my sincere thanks to Ms. Krishna Priya (Kichu) for her unwavering support, patience and understanding.

Finally, I want to thank everyone who contributed to my thesis, whether directly or indirectly.

I would like to dedicate this thesis for all green thumbs.....

Rajeesh E.P.

ABSTRACT

The family of bananas, or "Musaceae", is does not require any special introduction to the human race. The present study is a marker based (nuclear ITS, chloroplast *trnL-F* and *rps*16) molecular phylogeny of wild bananas found in India. The classification of Indian Musaceae has been done earlier in terms of overall morphology, anatomy of leaves and micromorphological characters of seeds and pollen grains. However, molecular study is essential for the precise identity of some problematic taxa in India. Moreover, none of the molecular studies of Musaceae conducted globally have included Indian bananas. All these reasons validate the feasibility of the present study.

According to this study, a total of 34 taxa of wild bananas are present in India, including two species of *Ensete* and 32 taxa of the genus *Musa*. About 90% of India's wild bananas are distributed in the Northeast India. The rest are confined to the Western Ghats, Eastern ghats and the Andaman and Nicobar Islands. In contrast to the traditional classification by the morphological features of wild bananas, our molecular study found that two sections under the genus *Musa viz.*, *Eumusa and Rhodochlamys* should be merged into one section. This study helps to reveal the actual identity of *M. kattuvazhana* and reinstatement of *M. sabuana* and *M. balbisiana* var. *andamanica* from the synonymy of *M. balbisiana*. In addition, our study confirms the molecular identity of several Indian narrow endemic *Musa* taxa and reveals genetic distance between *M. acuminata* and *M. balbisiana* is greater than between it and ornamental bananas. Character evolution study reveals that bananas with small pseudostem, erect inflorescence and bright coloured bracts are evolved from bananas with large pseudostem, pendant inflorescence and dull coloured bracts. This study also discussed about the pollination switch happened in wild bananas during the course of evolution. Moreover, the study reveals that the diversification of Indian Musaceae, Indian *Ensete* and Indian *Musa* happened during c. 60 mya, 39 mya and 36.8 mya respectively. In addition, this study proposed several wild bananas with prominent agronomic traits for future breeding programmes.

Keywords: *Ensete*, Evolution, *Musa*, Phylogenetic tree, Wild banana

പ്രബന്ധ സംഗ്രഹം

മനുഷ്യ ഗണത്തിന് പ്രത്യേകം പരിചയപ്പെടുത്തലിന്റെ ആവശ്യമില്ലാത്തവയാണ് വാഴകളുടെ കുടുംബം അഥവാ " മൂസേസിയേ ". ഇന്ത്യയിൽ കാണപ്പെടുന്ന കാട്ടുവാഴകളുടെ ജനിതകതന്മാത്ര തലത്തിലുള്ള വർഗീകരണമാണ് പ്രസ്തുത പഠനം. ഇന്ത്യയിലെ കാട്ടുവാഴകളുടെ വർഗ്ഗീകരണം അവയുടെ ബാഹ്യ-ആന്തരിക ഘടന വെച്ചും, വിത്തുകളുടെയും പരാഗരേണുക്കളുടെയും സൂക്ഷ്മ ഘടന വെച്ചും ഇതിന് മുൻപ് നടത്തിയിട്ടുള്ളതാണ്. എന്നിരുന്നാലും ചില ഇനങ്ങളുടെ കൃത്യമായ ഐഡന്റിറ്റിയ്ക്ക് മോളികുലാർ പഠനം അനിവാര്യമാണ്. ഇതു കൂടാതെ ആഗോളതലത്തിൽ നടന്ന മൂസേസിയേയുടെ മോളികുലാർ പഠനങ്ങളിൽ ഒന്നും തന്നെ ഇന്ത്യൻ വാഴകളെ ഉൾപ്പെടുത്തിയിട്ടും ഇല്ല. ഈ കാരണങ്ങളെല്ലാം തന്നെ പ്രസ്തുത പഠനത്തിന്റെ സാധ്യതയെ സാധൂകരിക്കുന്നതാണ്.

ഈ പഠനപ്രകാരം ഇന്ത്യയിൽ ഇപ്പോൾ രണ്ട് ജനുസ്സുകളിലായി മൊത്തം 34 ഇനം വാഴകളാണ് ഉള്ളത്. ഇതിൽ രണ്ട് സ്പീഷീസ് 'എൻസിറ്റേ' എന്ന ജനുസ്സിലും ബാക്കിവരുന്ന 32 ഇനം 'മൂസ' എന്ന ജനുസ്സിലും ഉൾപ്പെട്ടിരിക്കുന്നു. ഇന്ത്യയിലെ കാട്ടുവാഴകളുടെ 90 ശതമാനവും കാണപ്പെടുന്നത് വടക്ക് കിഴക്കൻ സംസ്ഥാനങ്ങളിലെ കാടുകളിലാണ്. ബാക്കി വരുന്നവ പശ്ചിമഘട്ടങ്ങളിലും പൂർവ്വ ഘട്ടങ്ങളിലും ആൻഡമാൻ നിക്കോബാർ ദ്വീപുകളിലുമായി പരിമിതപ്പെട്ടു കിടക്കുന്നവയാണ്. കാട്ടുവാഴകളുടെ ബാഹ്യഘടനവെച്ചുള്ള തരംതിരിക്കലിന് വിപരീതമായി ഡി എൻ എ മാർക്കറുകൾ ഉപയോഗിച്ചുള്ള പഠനത്തിൽ ഇന്ത്യയിലെ ' മൂസ' ജനുസ്സിലെ രണ്ട് ഉപ ജനുസ്സുകളെ ഒന്നിപ്പിക്കേണ്ടതായി കാണുകയും, ഇന്ത്യൻ വാഴകളുടെ ബാഹ്യ ഘടനയുടെ പരിണാമവുമായി ബന്ധപ്പെട്ട അദ്ധ്യായത്തിൽ വലിയ ഇനം വാഴകളിൽ നിന്ന് പരിണമിച്ചാണ് അലങ്കാരത്തിന് ഉപയോഗിക്കുന്ന ചെറിയ ഇനം ഉണ്ടായതെന്ന് തെളിയുകയും ചെയ്തു. പ്രസ്തുത പഠനം ഇന്ത്യയിൽ മാത്രമായി കാണുന്ന ചിലയിനം വാഴകളുടെ ശരിയായ ഐഡന്റിറ്റി വെളിപ്പെടുത്താൻ സഹായകരമായിട്ടുണ്ട്. ഇതുകൂടാതെ ഇന്ത്യയിൽ വാഴ കുടുംബത്തിന്റെ ഉത്ഭവം 60 മില്യൺ വർഷങ്ങൾക്കു മുൻപാണെന്നും, ഇന്ത്യയിലെ 'എൻസിറ്റേ' ജനുസ്സുകൾ രൂപം കൊണ്ടത് 39 മില്യൺ വർഷങ്ങൾക്കു മുൻപും 'മൂസ' ജനുസ്സുകൾ ഉണ്ടായത് 36.8 മില്യൺ വർഷങ്ങൾക്ക് മുൻപാണെന്നും ഈ പഠനം വെളിപ്പെടുത്തുന്നു. ഇതിനുപുറമെ കാട്ടുവാഴകളിലെ ജനിതകപരമായ ഗുണങ്ങൾ ഭാവിയിൽ പുതിയ ഇനം നാട്ടുവാഴകളെ വികസിപ്പിക്കുന്നതിനായി ഉപയോഗിക്കാമെന്നും ഈ പഠനം ചൂണ്ടിക്കാണിക്കുന്നു.

സൂചകപദങ്ങൾ: കാട്ടുവാഴ, ജനിതകതന്മാത്ര, പരിണാമം, മൂസ, വർഗ്ഗീകരണം

CONTENTS

CHAPTER 1 INTRODUCTION

One general law on Earth which leads to the advancement of all living beings is: multiply, vary, and let the 'strongest survive and the weakest perish' (Darwin, 1964). Angiosperms are a prime example of this, having emerged as latecomers in the plant kingdom and quickly overtaking the gymnosperms. Darwin mentioned this rapid domination of Angiosperms as an 'abominable mystery' in the letter he drafted to Joseph Dalton Hooker in 1879. According to Lughadha *et al.* (2016), flowering plants comprise 369,434 accepted species and presently 95% of the plant kingdom is represented by these angiosperms (Judd *et al*., 2016). Among the flowering plants, monocots are the inevitable plant groups for us as they provide the primary food sources *viz*., rice, wheat, barley, corn, banana, oils from palm, coconut etc.

Zingiberales are the lately evolved clade aside with Commelinales in monocots (Tomlinson, 1962, 1969; Cronquist, 1981; Dahlgren *et al*., 1982; Kress, 1990, 1995; Rudall *et al*., 1999; Stevenson *et al*., 2000; Kress *et al*., 2001; Li *et al*., 2010; Liu *et al*., 2010; Janssens *et al*., 2016) and well known for its economic importance as spices, ornamentals and edible purposes. Bananas, birds of Paradise, Heliconias and Gingers are prime attractive members of the order Zingiberales (Kress *et al*., 2002). The notable midrib, relatively larger leaf blade and showy and colourful flowers or bracts distinguish this order from other monocots (Kress, 1990; Kress & Specht, 2005). The suitable sister group for Zingiberales is considered as 'Bromeliads' (Dahlgren & Rasmussen, 1983). The distribution pattern of order Zingiberales is mostly confined to old-world and new-world tropics (Kress, 1990).

In a broad sense the term "gingers" is used for the eight families in the order Zingiberales. In addition, two informal groupings are proposed in the order based on the number of viable pollen-bearing stamens such as 'Banana families' and 'ginger families'. The 'banana families' consists of four families *viz*., Musaceae (*Ensete* Bruce *ex* Horan., *Musa* L., & *Musella* (Franch.) H.W.Li.), Heliconiaceae (*Heliconia* L.), Strelitziaceae (*Strelitzia* Banks, *Ravenala* Adans., & *Phenakospermum* Endl.)

and Lowiaceae (*Orchidantha* N.E.Br.) (Petersen, 1889; Lane, 1955; Dahlgren *et al*., 1982; Kress & Specht, 2005; Liu *et al*., 2010; Li *et al*., 2010). These four families together constituted as basal lineage for order Zingiberales and large, oblong banana-like leaves with penni-parallel venation are seen as the synapomorphic character for these families. The remaining four families collectively formed the derived clades and together referred to as the 'ginger families', which consist of Zingiberaceae, Costaceae, Cannaceae and Marantaceae.

Musaceae are the basal lineage in the order Zingiberales and possess the sister group of Strelitziaceae, Lowiaceae and Heliconiaceae (Tomlinson, 1962; Kress, 1990, 1995; Kress *et al*., 2001). Musaceae comprises only three genera *viz*., *Musa* L., *Ensete* Bruce *ex* Horan. and *Musella* (Franch.) H.W.Li. *Musa ingens* N.W. Simmonds is one of the iconic members of Musaceae, which is titled the World's largest herbs (pseudostem reaches a height of up to 15 m). All economically important edible bananas come from the plant belonging to the genus *Musa.* The characteristic features of the family are large rhizome-bearing, perennial or monocarpic herbs. The inflorescence is terminal on the large stem or internal peduncle which is situated inside the folding of leaf sheaths. Flowers are zygomorphic (except of the actinomorphic flower in *Musa nanensis* Swangpol & Traiperm), epigynous, and unisexual (rarely bisexual). However, this family can be distinguished from other families in the order Zingiberales by the characters such as tall pseudostem, long inflorescence and large fruits (which are edible or not).

Musaceae shows the distribution range in Tropical Asia and Africa. In tropical Asia, it ranges from the Himalayas to northern Australia (Liu *et al*., 2002b; Chiu *et al*., 2011), even though the fossil data provide another interpretation that, this family may present in North America and perhaps in Europe during the past (Friis, 1988; Manchester & Kress, 1993; Burgos-Hernández *et al*., 2019). The origin of wild bananas is considered to be from Southeast Asia (Simmonds, 1962). The highest concentration of Musaceae members is confined to India, China, Malesia and Myanmar (Northern Indo-Burma) and this area is considered the cradle of the evolution of Musaceae (Janssens *et al*., 2016; Burgos-Hernández *et al*., 2019)

The banana is well-known as the most useful plant for mankind. It can be edible in various forms. Mostly, as sweet fruit that can be eaten raw. However, many varieties are fried, roasted, juiced, dried, or chipped to make nutritious and inexpensive snacks and street foods (IPGRI-INIBAP/CIRAD, 1996; Nayar, 2010). Bananas are well-known sources of potassium, carbohydrates, fat, protein, trace of Vitamin A, minerals and plenty of antioxidants. It is an excellent food for babies as well as athletes (Mohapatra *et al.*, 2010). The stem-extracted banana fibre (*viz*., 'Abaca fibre' from *Musa textilis* Née), leaf, rhizome, stem and flower bud are useful in many ways. Products made from the various plant parts are another important source of income. The stem-extracted banana fibre is used to make paper, ropes, clothing, wall hangings, baskets, and many creative arts. Aside from food, anything and everything is wrapped in banana leaves. So, the multifaceted uses of this plant satisfy the term 'Kalpatharu' (Sanskrit name means "plant of all value") (Joe & Sabu, 2019).

It is believed that the first hybrid origin of cultivated banana took place in Papua New Guinea Island and it migrated towards the north during the mid-Holocene (Kennedy, 2009; Denham & Donohue, 2009; Donohue & Denham, 2009). Furthermore, the cultivation of edible bananas as one of the oldest food crops was started 7000 years ago on the same Island as a part of early civilisation. The phytoliths from the Kuk area in Papua New Guinea Island during the period between 7000–4500 years can be put forward as evidence (Denham *et al*., 2003, 2004). The human shift from hunter-gatherers to settle humans lead to the cultivation of food crops (Wood, 1996). The banana cultivation is one of the examples of that shift.

The idea about the evolution of cultivated bananas was first proposed by Kurz (1867) and for a long time, this concept remained obscure. Later, Cheesman (1947a, 1948a,b,c) clarified the concept and Simmonds and Shepherd (1955) experimentally proved the bi-specific origin of cultivated bananas. They have crossed the two wild bananas, *M. acuminata* and *M. balbisiana* (the 'Adam' and 'Eve' of cultivars) with 'A'- genome and 'B'- genome respectively. Simmonds and Shepherd (*l.c*) compare the morphology and ploidy of resulting crosses with cultivated varieties and concluded that the majority of the edible bananas evolved from *M. acuminata* and *M. balbisiana* through major five stages (Fig. 1).

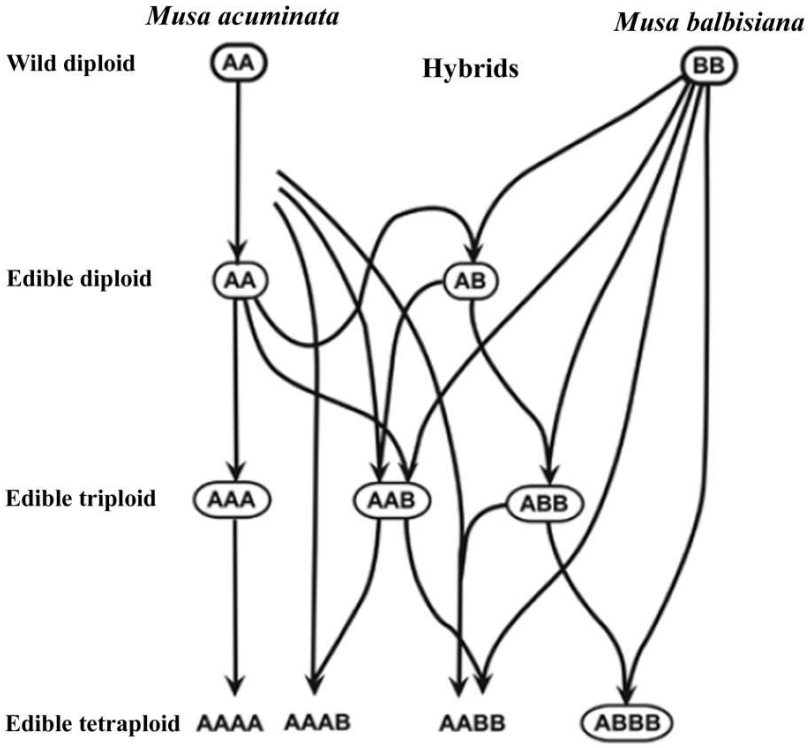


Fig. 1. Diagrammatic representation of the evolution of cultivated banana complex. **A.** *M. acuminata*; **B.** *M. balbisiana.* Genotypes known to occur naturally are encircled; those known only from experiments are not encircled. Crossing of tetraploid by diploid as a source of triploids has been neglected because natural tetraploids are very rare; many triploids have been made experimentally in this fashion [Adopted from Simmonds and Shepherd (1955)].

The genus *Musa* receives more attention in the family Musaceae for being the largest and economically most important genus, with a wide distribution range. The genus is discovered by Linnaeus, with *M. paradisiaca* L. as the type species. Later, Linnaeus (1759) added one more species, *M. sapientum* L. to the genus. Subsequent studies, however, indicated that the two Linnaean names are hybrids of two wild bananas (Cheesman, 1948c; Dodds & Simmonds, 1948; Simmonds & Shepherd, 1955), however, *M. paradisiaca* was chosen as a conserved type.

Ensete is another interesting genus that has good ornamental value and medicinal properties. It is only propagated through seeds and possesses a basic chromosome number nine. A recent study shows that this genus comprises eight species and one variety (Parmar *et al*., 2023) and possesses a disjunct distribution in Southeast Asia and tropical Africa (Simmonds, 1960). India is represented by only two *Ensete, viz*., *E. glaucum* (Roxb.) Cheesman and *E. superbum* (Roxb.) Cheesman. The monotypic genus *Musella* [*M. lasiocarpa* (Franch.) H.W.Li.] is

narrowly endemic to South-West China (Liu *et al*., 2002a; 2003). For a long time, the generic status of *Musella* has been under debate with *Ensete*, but now it is confirmed by the chloroplast sequence-based phylogeny (Fu *et al*., 2022).

Area of Study

India, (Fig. 2) is one among the 17 mega-biodiversity countries and the seventh largest country in the world harbours 7–8% of plants and animal species. It is located between 8°4′ to 37°6′ N latitude and 68°7′ to 97°25′ E longitude. Hence, India is located in the northeastern hemisphere. India is 2933 km in length from east to west and 3214 km in length from north to south with a coastline measuring 7516.66 km, it has a land boundary measuring 15200 km. In India, the south is bounded Indian Ocean, the Arabian Sea to the west, and the Bay of Bengal to the east. Northern frontiers of the country are borders with China, Bhutan and Nepal, north-west with Pakistan and the east with Bangladesh and Myanmar. India has been politically divided into 28 states and 8 union territories for administrative convenience.

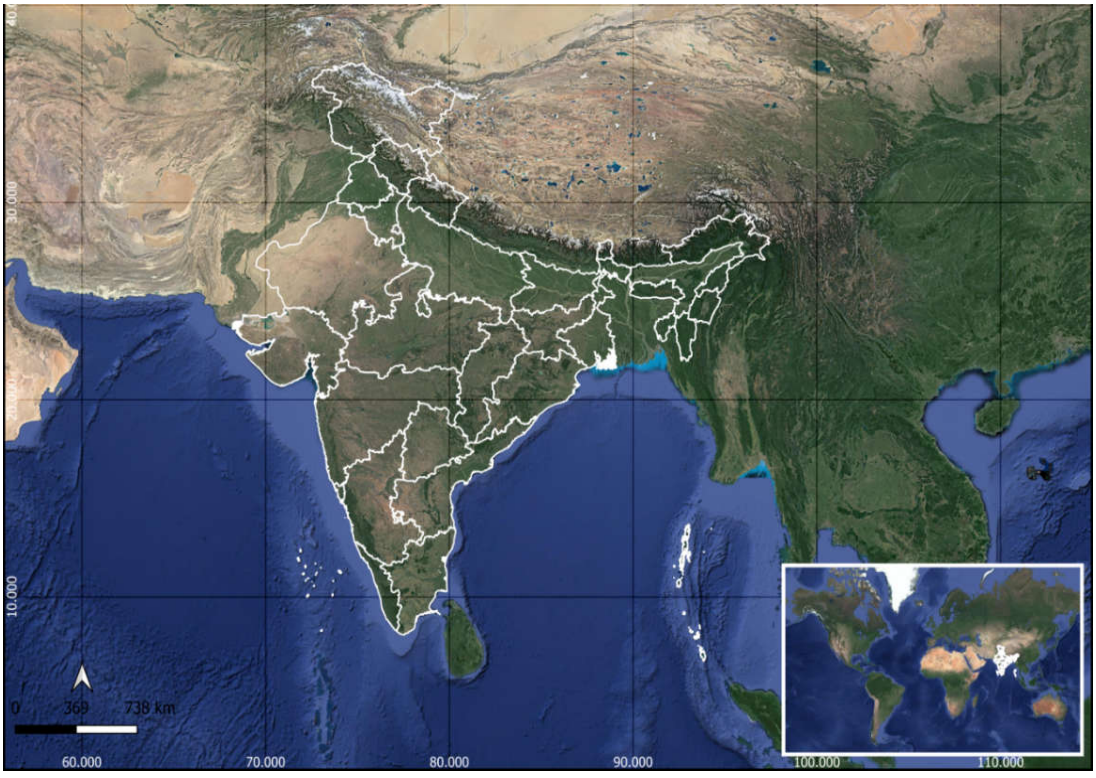


Fig. 2. Map of India, the study area (drawn using QGIS version 3.24.3).

Biogeographic Regions

Based on the biogeographical characteristics, there are 10 biogeographic regions or zones in India, (Fig. 3) which are further subdivided into 25 biogeographic provinces (Rodgers *et al*., 2002). The major biogeographical zones are mentioned below.

Trans-Himalayan Region

This zone includes the high-altitude areas of Ladakh, Jammu and Kashmir, North Sikkim and Lahul and Spiti areas of Himachal Pradesh. This region makes up 5.6 % of the total geographical area of India. Arid and cold conditions prevail in this region.

The Himalayas

There are wide altitudinal differences across this zone, which is made up of the entire Himalayan range. It is the youngest and most unstable mountain range in the world. It has a variety of forests and vegetation due to the diverse climatic conditions and complex topography. The cold-resistant, *M. sikkimensis* is the major *Musa* sp. grown in this zone.

Northeast India

It is located in the transition zone between Indian, Indo-Malayan and Indo-Chinese biogeographical regions. Moreover, this zone is a meeting point of the mountains of the Himalayas and Peninsular India. Forests in this region are extremely diverse in structure and composition, as well as serving as biogeographical gateways between Indian and Southeastern Asian plates. The major distributions of Indian Musaceae are confined to this area. Among the 34 taxa of Indian Musaceae, 30 taxa are reported in this zone, of which 15 taxa have shown endemism, which includes, *M. acuminata* var. *manipurensis*, *M. argentii*, *M. arunachalensis*, *M. balbisiana* var. *bheem-kola*, *M. balbisiana* var. *sepa-athiya*, *M. cheesmanii*, *M. cylindrica*, *M. flaviflora*, *M. mannii*, *M. markkui*, *M. ochracea*, *M. puspanjaliae*, *M. sanguinea*, *M. sikkimensis* var. *simmondsii* and *M. velutina* var. *variegata*.

Gangetic Plains

The largest plane constitutes around 10.8 % of the tropical geographical area. It is one of the most fertile regions of India, and the soil is formed by alluvial deposits from the Ganges. The vegetation is chiefly tropical moist and dry deciduous forests. *M. pradhanii* (endemic to West Bengal) and *M. thomsonii* are the *Musa* spp. representation to this zone.

Semi-Arid zone

This zone is represented by a transition zone between the Western Ghats and the desert. The habitat is often characterized by dry, rocky and sandy plains.

Deccan Plateau

This is the largest biogeographic region occupying about 42 % of the total geographic area. This zone is covered with different types of forests varying from semi-arid to moist deciduous or semi-evergreen type of forests. *Ensete superbum*, *M. balbisiana* and *M. ornata* are the common Indian Musaceae members seen in this zone.

Western Ghats

This is one of the eight "hottest hotspots" of biological diversity in the world. Western Ghat receives maximum rainfall from the southwest monsoon (Myers *et al*., 2000). There are a variety of vegetation types in this region, such as sholas, savannas, scrub jungles, humid deciduous forests, and tropical evergreen forests. *Ensete superbum*, *M. balbisiana* var. *elavazhai* and *M. kattuvazhana* are the only representations of this zone.

Coastal region

The Coastal region made up of about 2.5 per cent of India's land area, which is inhabited by marine or coastal Angiosperms. The mud flats, coral reefs, and sandy beaches are major land types. Backwater is another characteristic feature of the Coastal region.

Islands

Andaman and Nicobar Islands and Lakshadweep are the two major Islands in India. Lakshadweep, a Union territory is a group of Islands in the Arabian Sea, an archipelago comprising 36 islands that cover 32 square kilometres. Andaman and Nicobar archipelago consists of approximately 572 islands and islets, located in the Bay of Bengal (Kamble, 2020). The Andaman Island is separated from Nicobar group by a 150 km wide channel located at 10 degrees N latitude. The highest point is 2418 feet (737 metres) at Saddle Peak, North Andaman. There are 325 islands in the Andaman group and 247 in the Nicobar group. The forest of Andaman and Nicobar Islands is floristically rich by the influence of warm and ever-wet climatic conditions (Kamble, *l.c.*). The Andaman Islands harbours moist deciduous or wet evergreen forest type of vegetation. Central and Southern Islands of the Nicobar Islands are dominated by evergreen forests. *Musa sabuana* is endemic to the Andaman and Nicobar Islands and *M. balbisiana* var. *andamanica*, *M. kattuvazhana* are represented in the Andaman Islands.

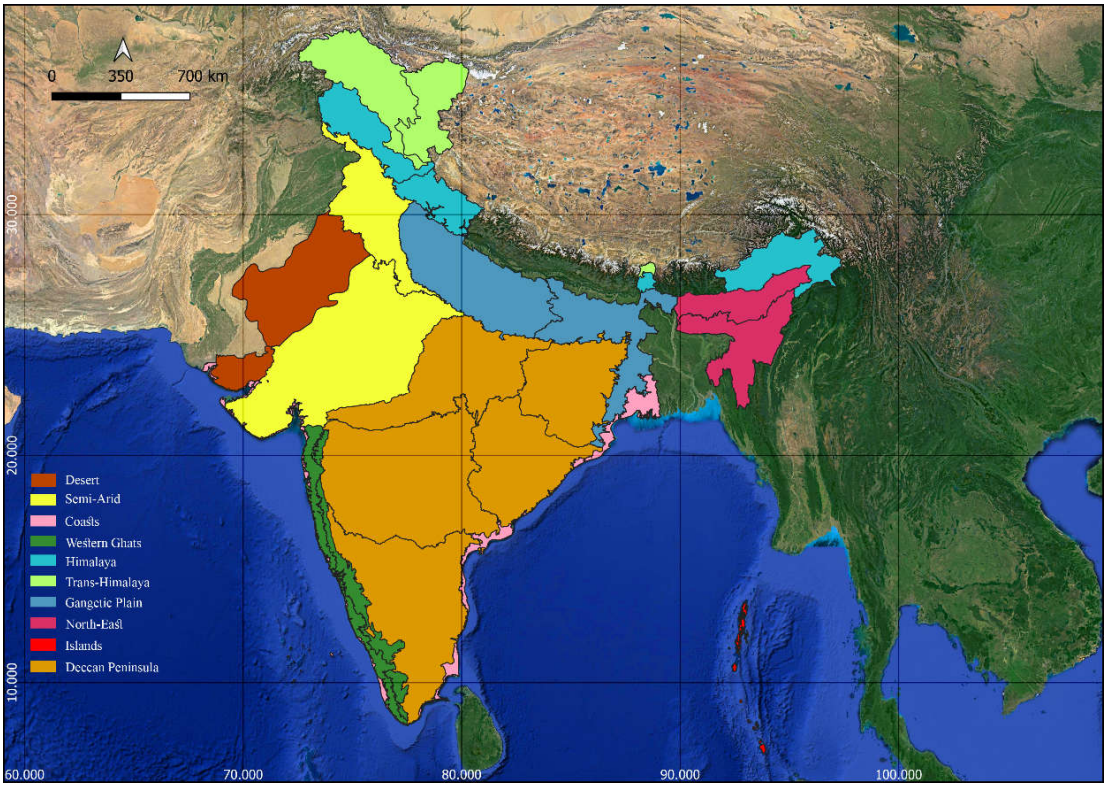


Fig. 3. Map showing Biogeographical zones of India (drawn using QGIS version 3.24.3).

Soil

A soil is an organic mixture of rock debris and organic materials that develops on the surface of earth. Mineral particles, humus, water, and air are the major constituents of the soil. Soil of India is classified into 8 different types based on formation, colour, composition and location. The types are Alluvial soils, Black soils, Laterite soils, Arid soils, Red and Yellow soils, Saline soils, Forest soils and Peaty soils. Alluvial soils are depositional soils and are widespread in the northern plains and the river valleys. Most of the Deccan Plateau is covered in black soil, which ranges in color from gray to deep black. In regions with low rainfall, particularly in the eastern and southern regions of the Deccan Plateau, red soil forms on crystalline volcanic rocks. The laterite soils develop in areas with high temperatures and high rainfall. Typically, this type of soil can be found in the hilly regions of Odisha and Assam, Karnataka, Kerala, Tamil Nadu, and Madhya Pradesh. Arid soils are characterized by red to brown hues, sandy nature and salty composition. These soils are characteristically developed in western Rajasthan. Saline soils are typically found in marshy and wet locations, as well as in arid and semi-arid regions. In forest regions with enough rainfall, forest soils normally form. A significant amount of decomposing organic matter builds up and enriches the humus and organic content that make up peaty soils in regions with high humidity and heavy rainfall.

Climate

Climate is the term used to refer to the average of the weather over a longer period. The climate in India varies depending on the wind, temperature and rainfall. The climate in India can be generally categorised as tropical monsoon type. The northeast monsoon and the southwest monsoon are two seasonal winds that affect the climate of India. In the country, the majority of the annual rainfall is brought about by the southwest monsoon. Officially four seasons are recognized by the Indian Meteorological Department (IMD), *viz*., 1. Winter, which starts in December and ends in early April; 2. Summer or pre-monsoon season (lasts from April to July

in north-western India); 3. Monsoon, also known as the wet season (June to September); and 4. post-monsoon, which lasts from October to December.

Rainfall

The precipitation in India is irregular over the course of the year. The annual rainfall is 118 cm according to the annual data from the meteorological department. Based on the amount of rainfall, India can be divided into four regions. The areas with over 300 cm of rain are categorized as regions of very heavy rainfall. This includes, southern slopes of the Eastern Himalayas, Assam, Bengal and the west coast region comprising the Konkan and Malabar Coast. The highest rainfall in India and the world is recognized as Mawsynram village in Meghalaya. The region of heavy rainfall constitutes an area with rainfall between 200 to 300 cm. The regions such as Middle Ganga valley, Western Ghats, Eastern Maharashtra, Madhya Pradesh and Odisha are included in this zone. Region of Moderate rainfall experiences 100 to 200 cm of rainfall. This includes the upper Ganga valley, Eastern Rajasthan and Punjab, and Southern Deccan comprising the plateau regions of Karnataka, Andra Pradesh and Tamil Nadu. The regions of scanty rainfall receive less than 50 cm. The northern parts of Kashmir, western Rajasthan, and southern Punjab come under this category.

Vegetation

The geological factors that regulate the growth of the vegetation in India are the temperature, rainfall, topology and soil. Based on this, natural vegetation can be categorized into six types. This includes tropical rainforests, tropical deciduous forests, Shrubs and Thorn forests, Desert vegetation, Mangrove forests, Mountain forests and grasslands. Areas with yearly precipitation over 200 centimetres are habitats to tropical rainforests. The forests are dense and comprised of tall trees. This forest type is mostly seen in the lower slopes of the Himalayas, the Andaman and Nicobar Islands, the western part of the Western Ghats, and certain areas of Assam and Odisha. The tropical deciduous forests are monsoon forests and usually occur in regions with an annual rainfall of between 70 and 200 cm. These forests are located in the northeastern states, on the eastern slopes of the Western Ghats,

Jharkhand, West Odisha, Chhattisgarh, and along the foothills of the Himalayan Mountains. The majority of shrubs and thorn forests can be found in regions with prolonged dry seasons and the usual annual rainfall is less than 75 cm. It is mainly found in semi-arid regions of Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Southwestern Punjab, and Western Haryana, the leeward side of Western Ghats, Maharashtra, Karnataka, Andra Pradesh and Tamil Nadu. Desert vegetation is found in the region where the annual rainfall is less than 25 cm. They occur in Rajasthan, Kutch and Saurashtra, southwestern Punjab and parts of the Deccan. Mangrove forests can be found in coastal regions that are frequently inundated by sea tides. They are abundant on the coastlines of the Andaman Islands and Sundarbans, as well as in the deltas of the Ganga, Mahanadhi, Godavari, Krishna, and Kaveri. Grasslands are regions in which vegetation is dominated by grasses, sedges and rushes families. In India, it spans across north India some regions of Gujarat and some regions of Ghats.

Relevance of the study

Bananas are inevitable plants for tropical farmers and mankind, but same time it is a troublesome group for taxonomists. The Indian people rely heavily on bananas for daily needs as well as religiously. India is one of the major centres of diversity for Musaceae and the Northeastern states of India harbour the lion's share of the wild *Musa* (Joe & Sabu, 2019). Musaceae are a difficult group due to the large size and succulent nature of the plants and similarities in the vegetative parts. So, conventional herbarium research is not amenable. Moreover, the collection and processing of plant specimens is another tedious task (Hareesh *et al*., 2017). Succinctly, the vegetative morphological resemblance and large pseudostem may create difficulties for beginners in identifying between the infra-generic and infra-specific taxa. In truth, the recognition during the vegetative phase is quite a task for most of the botanists.

The last decade has witnessed a steady increase in newly described taxa from Northeast India, South India and Andaman and Nicobar Islands. These include *M. acuminata* Colla var. *manipurensis* A.Joe & M.Sabu (Joe & Sabu, 2019), *M.*

argentii Gogoi & Borah (Gogoi & Borah, 2014a), *M. arunachalensis* Sreejith, A.Joe & M.Sabu (Sreejith *et al*., 2013), *M. balbisiana* var. *bheem-kola* A.Joe & M.Sabu (Joe & Sabu, 2019), *M. balbisiana* var. *elavazhai* A.Joe, Sreejith & M.Sabu (Joe *et al*., 2014c), *M. balbisiana* var. *sepa-athiya* Borborah, Borthakur & Tanti (Borborah *et al*., 2016), *M. cylindrica* A.Joe, Sreejith & M.Sabu (Joe *et al*., 2014b), *M. markkuana* (M.Sabu, A.Joe & Sreejith) Hareesh, A.Joe & M.Sabu (Hareesh *et al*., 2017), *M. markkui* Gogoi & Borah (Gogoi & Borah, 2013), *M. pradhanii* A.Joe & M.Sabu (Joe & Sabu, 2019), *M. puspanjaliae* Gogoi & Häkkinen (Gogoi & Häkkinen, 2013a), *M. sabuana* K.Prasad, A.Joe, Bheem. & B.R.P.Rao (Prasad *et al*., 2013), *M. sikkimensis* var. *simmondsii* A.Joe & M.Sabu (Joe *et al.*, 2016c) and *M. velutina* var. *variegata* A.Joe, M.Sabu & Sreejith (Joe *et al*., 2014a). Apart from these taxa, several other species of *Musa* were published from India, during this period. However, Joe and Sabu (2019) synonymised a total of 14 taxa based on critical taxonomic studies. Conclusively, now India harbours two species of *Ensete* and 32 taxa of *Musa* (including 25 species, one variety of *M. acuminata*, four varieties of *M. balbisiana*, one variety of *M. sikkimensis* Kurz. and one variety of *M. velutina* H.Wendl. & Drude.). Among these, 20 taxa are endemic to India. Joe and Sabu (2019) tentatively treated all these Indian ornamental bananas (sect. *Rhodochlamys*) and sect. *Musa* members under the section *Musa* (n=11) following Häkkinen's (2013) revised sectional classification based on molecular data. However, Indian endemic taxa are no longer represented in any recent molecular studies. The taxonomy of Indian Musaceae based on morphology, anatomy and palynology was well explored. However, molecular studies of this family in India have not been attempted to date. Considering the vast variety and variability of Indian *Musa*, this situation seems surprising. When we overlook on this topic, there is still some confusion about the actual identity of some taxa in Indian Musaceae, particularly concerning the status of 1) recently described taxa 2) Indian endemic taxa and 3) infraspecific taxa of *M. balbisiana* collected from various parts of the country.

AFLP-based (Wong *et al.*, 2002; Nwakanma, 2003b) and molecular marker-based (Li *et al*., 2010; Liu *et al*., 2010) phylogeny of Musaceae was already

attempted and reconstructed the sectional classification in the genus *Musa*. Based on these studies, the earlier five sections *viz.* sect. *Australimusa*, sect. *Callimusa*, sect. *Ingentimusa*, sect. *Eumusa* and sect. *Rhodochlamys* are amalgamated into two sections. Presently, the genus *Musa* represents only two sections *viz*., sect. *Musa* (n=11; sect. *Eumusa* and sect. *Rhodochlamys* merged into this section) and sect. *Callimusa* (n=7/9/10; sect. *Australimusa*, sect. *Ingentimusa* are merged into this section). However, the lack of Indian species in these studies is projected as a major shortcoming. Hence, a thorough molecular phylogenetic study of Musaceae included by Indian endemics may provide new insights. Subsequently, molecular dating of Musaceae was carried out in a world context (Christelova *et al*., 2011; Janssens *et al.*, 2016; Burgos-Hernández *et al*., 2019; Fu *et al.*, 2022). The results of these studies were rather contrary but suggest that Musaceae originated from the Northern Indo-Burma during the Cretaceous Palaeogene boundary, followed by Eocene-Oligocene diversification. Indian species, however, were not included in these studies. Inta *et al*. (2023) found the utility of the morphology of male flowers for the classification of Musaceae and studied the evolution of those flower characters on the phylogenetic tree. However, their study mainly focused on wild bananas in Thailand. Besides the flower characters, Musaceae possesses notable evolutionary significant characters, the study of the evolution pattern of those characters may provide further understanding to the history of Musaceae.

By examining these backgrounds, a thorough phylogenetic study of Indian Musaceae is necessary. Here we have chosen the appropriate nuclear and chloroplast markers based on the proven utility with the possible number of accessions from the Indian taxa. This will help to reveal the actual identity of Indian *Musa*, their taxonomic positions and relations with other *Musa* members in a global context. Moreover, elucidating evolutionary significant characteristics and conducting molecular dating studies of Indian Musaceae will enhance our understanding of the current evolutionary results of the banana family. Additionally, elucidating the phylogenetic relationships of *Musa* will provide valuable information for the utilization of genetic resources in future banana improvement.

Objectives

- Collection of specimens from different parts of India and add to the existing germplasm of CUBG.
- To prepare Herbarium specimens as per traditional techniques.
- To undertake the Molecular characterization of the specific and infra-specific taxa using suitable molecular markers. (nrITS, *trnL-F*, *rps*16).
- To test the morphology and infer the infra-generic phylogeny of *Musa* and correlate molecular phylogeny with traditional classifications.
- To construct a phylogram based on the molecular data and phenogram using morphological data inferred during the current study using suitable software.
- To elucidate the evolution of morphological characters and divergence time of Indian Musaceae.
- To establish a complete taxonomic account of Indian Musaceae using morphological and molecular data.

CHAPTER 2 REVIEW OF LITERATURE

Flowering plants or angiosperms are evolutionarily dominant and recently evolved plant groups on Earth. Pollen grains from the early Cretaceous period (140 million years ago) are the earliest fossil evidence of flowering plants. Therefore, angiosperm origin could have occurred 140 million years ago or even earlier. Once it emerged, it rapidly radiated, gradually replaced gymnosperms, and eventually took over as the dominant plant life form on Earth (Soltis *et al*., 2018).

Monocots are one of the prevailing and monophyletic groups in angiosperms, now placed between basal angiosperms and eudicots (APG, 1998; APG II, 2003; APG III, 2009; APG IV, 2016). There are eleven orders, 82 families and approximately 65,000 species represented in monocots, including the most useful plant families *viz*., Musaceae (bananas), Poaceae (true grasses), Orchidaceae (orchids), Zingiberaceae (gingers), Liliaceae (lilies), Arecaceae (palms), Iridaceae (irises) etc. (Givnish *et al*., 2010).

Musaceae, commonly known as banana family, is a recently evolved lineage in Monocots and basal to the order Zingiberales (Kress & Specht, 2006; Deng *et al.,* 2016) with about 128 taxa (Joe & Sabu, 2019; Hareesh & Sabu, 2023) and mainly distributed in tropical Asia, ranging from the eastern Himalayas to northern Australia and Africa (Liu *et al*., 2010). It represents three genera *viz*., *Musa* L., *Ensete* Bruce *ex* Horan. and *Musella* (Franch.) C.Y.Wu *ex* H.W.Li. The genus *Musa* is distributed in tropical Asia (Indian subcontinents and Malayan archipelago) and the exact number of taxa is still up for debate. In the revised sectional taxonomic studies of *Musa*, Häkkinen (2013) showcased only 70 species. In accordance with Govaerts & Häkkinen (2006), it comprises about 120 taxa, which include around 80 species, and the rest are infraspecific taxa (subspecies, varieties and hybrids). Väre and Häkkinen (2011) recognized eight species and one variety of *Ensete* (basic chromosome number n=x=9) in their checklist. Later, Joe *et al*. (2016b), reported the genus *Ensete* has seven species and one variety and is sparsely distributed in tropical Africa and South-East Asia. Recently, Parmar *et al*. (2023) added *E. nepalensis* to

this account and re-assigned the number to eight species and one variety. The monotypic genus *Musella* (*M. lasiocarpa*) is narrowly endemic to southwestern China (Wu & Kress, 2000). The systematics of the genus *Musa* is quite interesting due to its large representation when compared to the other two genera in this family and its socio-economic importance (Roux *et al*., 2008; Hareesh & Sabu, 2023).

Taxonomic History of the Banana family

The connection between bananas and the life of humans is remarkably intense, particularly the bonding of this plant with our country India is indispensable and has many facets *viz*., bananas are interwoven with India's epics, Hindu and Buddhist culture, rituals, history, civilizations and economy (Reynolds, 1951). The epics *Ramayana* and *Mahabharatha* had mention about this fruit plant. As a history and utility, bananas appeared in Kautilya's *Arthasastra* (300–400 B.C.), Theophrastus (371–287 BCE) works, *Susrutha samhita* (the first medical treatise in Sanskrit, 4th century), and Rheede's (1678–1693) '*Hortus Malabaricus*' (Reynolds, 1951).

The formation of the generic name *Musa* might be from the Sanskrit word '*Moca*' or from the Arabic word '*mauz*', '*mouz*' or '*mauwz*' which refers to banana. *Musa* is also a male name of Arabic origin. It means "saved from the water" and is based on the name of Moses. Another hypothesis that the genus name, *Musa*, is named in honor of Antonia Musa, who was a Roman physician in the 1st century B.C (Rumphius, 1747; De Candolle, 1886; Nayar, 2010).

Hendrik Andriaan van Rheede, the Governor of Dutch Malabar mentioned the Indian bananas for the first time with beautiful illustrations in his *Hortus Malabaricus* (Rheede, 1678–1693). It was a Latin botanical treatise, dealing with medicinal plants and uses from the Malabar coast of Kerala, India. In Malayalam bananas are called by the name 'Pazham' and the names related to bananas *viz*., '*bala*', '*vazha*' are mentioned in *Hortus Malabaricus*.

Rumphius (1747) included a few wild and cultivated bananas of Indonesia as '*Pissang*' in his *'Herbarium Amboinense'*. According to Merrill (1917), this classical work includes sixteen *Musa* forms along with illustrations for four taxa.

Colla's '*Memoria sul Genere Musa*' was the first monograph of the genus *Musa* (Colla, 1820). This work dealt with 12 species of *Musa* and mentioned about the anatomy, physiology, cultivation and uses of the genus altogether. In the same work, Colla (*l.c.*) described *M. acuminata* and *M. balbisiana*- the progenitors of the now a day's bananas by selecting the illustrations of Rumphius (1747)- '*M. simiarum pissang Jacki*' and '*Musa XI. Pissang Batu*' respectively as the types.

Dr. William Roxburgh, a Scottish Botanist and Physician, his contribution to Indian botany is irreplaceable to any other's work. In the second volume of '*Flora Indica*' (Roxburgh, 1824) he validated *M. ornata*, *M. glauca* and *M. superba* with elaborate descriptions. But, later *M. glauca* and *M. superba* were transferred into *Ensete.* A total of 6 species of *Musa* were described in the same work.

The distribution of *M. superba* was also reported by Wight (1853) during his exploration of South India. However, now this species is treated as *E. superbum*.

For the '*Flora Indiae Batavae*' or '*Flora van Nederlandsch Indie*', Miquel (1855) included 10 taxa of *Musa* with pre-Linnaean names and considered the genera *Ravenala* and *Heliconia* under the family Musaceae along with *Musa*.

Later, Horaninow (1862) recognized two tribes under the family as the tribe Heliconiae (including the genus *Heliconia*) and tribe Strelitziae (including the genera *Ensete*, *Musa*, *Ravenala*, and *Urania* DC.). This study also confirmed the generic status of *Ensete* by choosing *E. edule* Bruce *ex* Horan. as the type species and mentioned 25 species of *Musa* in detail.

Subsequently, Kurz (1867) discussed about 15 species of *Musa* and some edible varieties in his work based on plantains of the Indian archipelago. Several species in this study were later treated as synonyms and four species were transferred to *Ensete. Musa dasycarpa* Kurz (= *M. velutina*), an ornamental species

was described in this work. Later, Kurz (1878) described one more species, *M. sikkimensis* in his work 'The banana: a pomological contribution' from Sikkim.

Bentham and Hooker (1883) briefed on three species of *Musa* (*M. paradisiaca*, *M. coccinea,* and *M. rosacea*) in their botanical classic- '*Genera Plantarum*. In this work, they treated *Ravenala, Strelitzia,* and *Heliconia* under the tribe Museae along with the genus *Musa*.

A sensible classification of the genus *Musa* was introduced by Sagot (1887 a, b) by dividing it into three groups: giant bananas, fleshy bananas, and ornamental bananas (with erect inflorescence and bright-coloured bracts) with three, nine and four species respectively. In addition, he analysed the species *M. aurantiaca*, *M. ornata*, *M. sanguinea*, *M. superba* Roxb. and *M. nepalensis* Wall. in detail.

Later, Baker (1892) documented 6 species of *Musa* and several cultivated varieties of banana from the Indian subcontinent as part of the treatment of Scitaminae in Hooker's *Flora of British India*. Afterwards, Baker (1893), published another work- 'A synopsis of the genera and species of Museae' and documented 32 species from India. He validated *M. mannii* H.Wendl. *ex* Baker and *M. aurantiaca* G.Mann *ex* Baker from the manuscripts of Hermann Wendland and Gustav Mann respectively. By following the criteria of Sagot's (1887a) classification, Baker (*l.c.*) divided the genus *Musa* into three subgenera- *Physocaulis*, *Eumusa*, *Rhodochlamys*. Later, 7 species that he treated in the subgenus *Physocaulis* were transferred to *Ensete* by Cheesman (1947b).

In the early nineties, Prain (1903) addressed two species of *Musa* in his book 'Bengal Plants', even if it dealt with the ferns and fern allies. Later, Fischer (1928), represented two species of *Musa viz*., *M. superba* (= *E. superbum*) and *M. rosacea* (a name mistakenly given to *M. ornata*) for the *'Flora of Presidency of Madras'*.

Cowan and Cowan (1929) studied the plants of Bengal and comprehended *Musa* with two species and two varieties. He elevated the 'form' status of Baker's (1893) *M. sapientum* subsp. *seminifera* form *thomsonii* into *M. thomsonii* (King *ex* Baker) A.M.Cowan & Cowan. However, two taxa mentioned in the same work *viz*.,

M. hookerii (King *ex* Baker) A.M.Cowan & Cowan and *M. sapientum* var. *pruinosa* (King *ex* Schum.) A.M.Cowan & Cowan are now synonymized under *M. sikkimensis* and *M. balbisiana* respectively (Joe & Sabu, 2019). Karthikeyan *et al*., (1989) enumerated two species of *Ensete* and 18 species of *Musa* from India.

The first Musaceae diversity studies in Andaman and Nicobar Islands were carried out by Singh *et al.* (1998) and he described a variety *viz*., *M. balbisiana* var. *andamanica*. Later, Sinha (1999) reported *M. acuminata,* a wild *Musa* from the Great Nicobar Islands. The wild *Musa* diversity in the Islands was well explored recently by reporting three new species such as *M. sabuana* (Prasad *et al*., 2013), *M. indandamanensis* L.J.Singh (Singh, 2014) and *M. paramjitiana* L.J.Singh (Singh, 2017). In addition, Hareesh *et al*. (2017) reported *M. kattuvazhana* from the Andaman Islands.

After Baker's (1893) study, there is a large knowledge gap that can be seen regarding the Indian Musaceae until the revisionary study of Joe (2015). In the 20th century, only four true species were described from India *viz*., *M. nagensium* Prain (Prain, 1904), *M. ochracea* K.Sheph. (Shepherd, 1964), *M. cheesmanii* N.W.Simmonds (Simmonds, 1957) and *M. flaviflora* N.W.Simmonds (Simmonds, *l.c.*; by elevating 'Mariani form' of *M. acuminata*). The major floristics and revisionary studies of Indian Musaceae started after the beginning of 21st century. It resulted in a few numbers of new taxa, new reports and rediscoveries from the mainland of India, which include *M. shankarii* Subba Rao & Kumari (now stand as the synonymy of *M. balbisiana*) from Visakhapatnam District of Andhra Pradesh (Rao & Kumari, 2008). Rajib Gogoi and Souravjyoti Borah along with Markku Häkkinen published three species from Arunachal Pradesh *viz*., *M. puspanjaliae* (Gogoi & Hákkinen, 2013a), *M. markkui* (Gogoi & Borah, 2013) and *M. argentii* (Gogoi & Borah, 2014a). In the same period, the research team of M. Sabu including Alfred Joe and P.E. Sreejith described *M. arunachalensis* (Sreejith *et al*., 2013) from Arunachal Pradesh, and *M. cylindrica* (Joe *et al*., 2014b) from Meghalaya. In addition to this, they included several infra-specific taxa to the account of Indian *Musa*, such as *M. velutina* subsp. *markkuana* [Sabu *et al*., 2013a; now it is elevated

to the species status as *M. markkuana* by Hareesh *et al*. (2017)], *M. velutina* var. *variegata* (Joe *et al*., 2014a), *M. balbisiana* var. *elavazhai* (Joe *et al*., 2014c), *M. sikkimensis* var. *simmondsii* (Joe *et al.*, 2016c), *M. acuminata* var. *manipurensis* (Joe & Sabu, 2019) and *M. balbisiana* var. *bheem-kola* (Joe & Sabu, 2019). Sabu *et al.* (2013b) collected *M. chunii* Hákkinen from Arunachal Pradesh as a new record to India. Furthermore, the species *viz*., *M. cheesmanii*, *M. flaviflora*, *M. mannii*, *M. nagensium* and *M. thomsonii* are discovered from India after a gap of more than 50 years (Joe & Sabu, 2019).

The scientific naming of the genus *Musa* first appeared in Linnaeus's *Species Plantarum* (1753), unfortunately, it was a triploid interspecific hybrid, *M. paradisiaca* (AAB). The classification of the banana family is quite interesting and the path is mentioned here briefly. The primary classification of this genus was put forward by Sagot (1887a), with a division of three unnamed groupings. That are, (1) "Bananas with fleshy fruits, often edible" which includes *M. paradisiaca*, *M. sapientum* and *M. troglodytarum*; (2) "Ornamental bananas" with upright inflorescences and brightly coloured bracts comprising *M. ornata*, *M. sanguinea* and *M. coccinea*; and (3) "Giant bananas" like *M. ensete* J.F.Gmel. Subsequently, Baker (1893) defined three subgenera under the genus by adopting the previous Sagot's (1887a) grouping. which includes, (1) Subgenus '*Eumusa*' Baker, which possesses cylindrical stem; many flowers in a bract; green, brown or dull violet bracts; fruits usually edible (2) Subgenus *Rhodochlamys* Baker, which possesses cylindrical stem; few flowers in a bract; brightly coloured bracts; seeded and non-edible fruits (3) Subgenus *Physocaulis* Baker: stem bottle-shaped; flowers many in a bract; fruits inedible (the type is *M. ensete*, currently accepted in the genus *Ensete* as *E. edule* Bruce *ex* Horan.). Subsequently, after five decades, Cheesman (1947a) segregated the genus *Ensete* from *Musa* and proposed a stable sectional classification for the genus *Musa* based on the basic chromosome numbers, morphological characters like pseudostem aspect, the shape of inflorescences, the shape of fruits, colour of bracts and shape of seeds. This classification has been widely accepted for almost half a century without any modification. He proposed four sections in the genus *Musa viz*., sect. *Australimusa* (x=10), sect. *Callimusa* (x=10, except *M. beccarii* x=9), sect.

Eumusa (x=11) and sect. *Rhodochlamys* (x=11). Three decades later, Argent (1976) added sect. *Ingentimusa* (x =7), for a single species *M. ingens* N.W.Simmonds, confined in Papua New Guinea. Cheeseman's (1947) classification is the most accepted but failed to discuss the evolutionary relationships between these four sections. The occurrence of natural hybrids between these sections and the presence of morphologically intermediate species in the wild become another opposition. Simmonds (1954) found that *M. rubra* (= *M. laterita*), a species in sect. *Rhodochlamys* can cross more easily with the subspecies of *M. acuminata* (sect. *Eumusa*) than with species from its own section. Nwakanma *et al*. (2003b) mentioned the common occurrence of natural crosses between taxa belonging to the sect. *Musa* and sect. *Rhodochlamys*. These observations had questioned the Cheesman's (1947a) sectional classification and finally became one of the major reasons for the reassessment of this system. Additionally, the recently discovered species *viz*., *M. argentii*, *M. arunachalensis*, *M. markkui*, forms an intermediate position of sect. *Musa* and sect. *Rhodochlamys* (Medium sized plant, bright coloured bract, semi pendent or arched inflorescence) and these species stood up as physical evidence for inter-sectional hybridisation.

Phenetic and Genetic Marker based studies in Musaceae

The phylogenetic approach to the classification of the family Musaceae was started in the 1990s based on morphological characters, isozymes polymorphisms, molecular cytogenetics, intergenic spacers, random amplified polymorphic DNA markers (RAPDs), restricted fragment length polymorphisms (RFLPs), inter simple sequence repeats (ISSRs), variable number of tandem repeats (VNTRs), Inter retrotransposon amplified polymorphisms (IRAPs), sequence-related amplified polymorphisms (SRAPs), directed amplification of minisatellites DNA (DAMD) and microsatellites (Simmonds & Weatherup, 1990; Bhat *et al*., 1992a, b; Gawel & Jarret, 1991; Gawel *et al*., 1992; Bhat & Jarret, 1995; Crouch *et al*., 1999; Wong *et al*., 2001a, b; Youssef *et al*., 2010; Lamare & Rao, 2015; Saraswathi *et al*., 2020). However, Wong *et al*. (2002) paved the way for the present sectional classification system in the genus *Musa* by Amplified Fragment Length Polymorphism (AFLP).

Simmonds and Weatherup (1990) conducted a numerical taxonomic study based on morphological data in the wild *Musa* species. These data comprised the descriptions of 28 entities (including species and subspecies). In the result, five groupings were obtained instead of the four sections traditionally recognized. These include, I. *Australimusa*, II. *Callimusa*, III. *Rhodochlamys*, IV. *Eumusa* 1, and V. *Eumusa* 2. The fifth group (*Eumusa* 2) contains five taxa (*M. balbisiana*, *M. cheesmanii*, *M. nagensium*, *M. boman* and *M. ingens*) previously included in section *Musa*. *Eumusa* 1 grouping includes the taxa *viz*., *M. acuminata* and its subspecies *malaccensis*, *microcarpa*, *burmannica*, *siamea* and *banksii*, *M. flaviflora*, *M. itinerans*, *M. basjoo*, *M. schizocarpa* and *M. sikkimensis*. Moreover, this study placed *Musa ingens* into the *Eumusa* group and *Musa beccarii* into *Callimusa* group.

Isozymes (multiple forms of the same enzymes that catalyse the same chemical reaction) analysis is one of the techniques used to discriminate the genomic groups in the banana family. Bhat *et al*. (1992a, 1992b) studied the isozymes phenotype variability study in 44 Indian cultivar bananas belonging to the different genomic groups (AB, AAB, ABB, and AAA). The isozymes of esterase, acid phosphatase, catalase, peroxidase, superoxide dismutase, shikimate dehydrogenase, and malate dehydrogenases were investigated in these 44 banana groups. However, they do not find any genome-specific isozyme patterns in these cultivars studied indicating the common origin of the cultivar used for this study.

Gawel and Jarret (1991) used the application of chloroplast DNA (cpDNA) restriction fragment length polymorphism (RFLP) analysis to *Musa* taxonomy. Total Thirteen accessions (including all four sections) were used for this study and the phylogenetic tree was constructed using the software PAUP. Here in the tree, *M. balbisiana* shows similarity with *M. textilis* and subspecies of *M. acuminata* are clustered together. However, the placement of *M. beccarii* and *M. basjoo* did not follow morphology-based classification.

Gawel *et al*. (1992) carried out the morphology-based and RFLP-based phylogenetic analyses of 19 accessions of *Musa*. The result of both analyses was almost similar with some exceptions. This includes the placement of *M. boman* in

section *Australimusa* and the placement of *M. beccarii* as a sister to *M. acuminata*. In addition, they found that there was no support for separating section *Rhodochlamys* from section *Musa*.

Howell *et al*. (1994) used the random amplified polymorphic DNA (RAPD) technique to analyse the genetic variation in *Musa* germplasm by using 9 accessions (*M. acuminata* subsp. *burmannicoides*, *M. balbisiana* and edible hybrids). This work identified 116 amplification products by using nine primers. The study helps to identify RAPD markers that are specific to each of the nine genotypes of *Musa* representing AA, AAA, AAB, ABB, and BB genomes. As well, they observed the pattern of genomic variation was very similar to the pattern of variation defined using morphological characters. So, the result suggested that RAPD can used for *Musa* germplasm characterization, identification, and fingerprinting of varieties.

Bhat and Jarret (1995) carried out the RAPD analysis of 57 accessions of *Musa* from India including the wild taxa like *M. balbisiana*, *M. acuminata* subsp. *banksii*, *M. acuminata* subsp. *malaccensis*, *M. velutina* and cultivated clones of different genomic groups. A total of 60 random 10-mer primers were used for the study and selected 49 reproducible primers from it. Based on both the phenetic and the PCO analyses, previously unclassified cultivars were clubbed together with already classified cultivars in accordance with their genomic group and morphological characteristics.

Osuji *et al*. (1998) localized the physical sites of 18S-5.8S-25S and 5S rRNA genes and telomeric sequences in the *Musa* genome by fluorescent in situ hybridization (FISH). This study suggested that the variation in the number of copies of the 18S-5.8S-25S rRNA genes and 5S rDNA genes can be used for separating the different genomic groups in the cultivar bananas.

Crouch *et al*. (1999) conducted a study to compare different PCR-based marker systems (RAPD, VNTR, and AFLP) for the analysis of breeding populations generated from two diverse *Musa* breeding schemes. All three assays detected a high level of polymorphism between parental genotypes and within progeny populations.

Wong *et al*. (2001a) used the AFLP study in three subspecies of *M. acuminata* (subsp. *truncata*, subsp. *malaccensis*, and subsp. *microcarpa*) to elucidate their relationships. With the help of 8 primers, they found these three taxa are distinct and *M. acuminata* subsp. *truncata* is far distinct from the other two subspecies. This study has therefore provided the genetic basis for the proper taxonomic placement of the three taxa of *M. acuminata*

Wong *et al*. (2001b) attempted an AFLP study for the proper sectional placement of three *Musa* species *viz*. *M. beccarii*, *M. monticola*, and *M. suratii*. After the analyses of the phylogram, they came to know that *M. monticola* and *M. suratii* as distinct species, *M. beccarii* and *M. monticola* clustered in sect. *Australimusa*, and *M. suratii* falling between sect. *Callimusa* and sect. *Australimusa* suggests that the two sections can no longer be maintained as distinct.

Wong *et al*. (2002) paved the primary step of the present sectional classification system in the genus *Musa* by AFLP with 21 taxa of *Musa* representing 4 sections. This study merged the previous four sections into two groups *viz*., section *Musa* and section *Callimusa*. The basic chromosome number is n=x=11 and n=x=10/9/7 in sect. *Musa* and sect. *Callimusa* respectively and it can be an apomorphic character.

Ude *et al*. (2002) assessed the genetic diversity and relationships in 28 accessions of *M. acuminata* and *M. balbisiana* and some of their natural hybrids with the help of AFLP. The findings showed that there are three major subspecies within the *M. acuminata* complex (subsp. *burmannica*, subsp. *malaccensis*, and subsp. *microcarpa*), which indicating that more than one *M. acuminata* subspecies is involved in the origin of cultivated bananas. Moreover, this study indicated the close genetic relation between "Calcutta 4" (*M. acuminata* subsp. *burmannicoides*) and "Long Tavoy" (*M. acuminata* subsp. *burmannica*). This study also showed the occurrence of two groups in *M. balbisiana* complex.

Nwakanma *et al*. (2003a) attempted to identify molecular markers that will facilitate discrimination of A and B genome based on restriction-site variation in the ITS region (700 bp). For this study, seven *M. acuminata* and five *M. balbisiana*

accessions were used. An Rsa1 digest of the ITS fragment revealed a fragment of 530 bp that is specific to the 'A' genome and two fragments of 350 bp and 180 bp that are specific to 'B' genome, with interspecific hybrid cultivars containing all three fragments.

Nwakanma *et al*. (2003b) performed a phylogenetic analysis of the genus *Musa* using restriction site polymorphisms of the chloroplast and mitochondrial DNA (mtDNA). They included thirteen species representing the four sections of *Musa* and *Ensete ventricosum* as the outgroup for the study. The result shows the two lines of evolution in *Musa*. One lineage comprised species of the sections *Australimusa* and *Callimusa*, while most species of sections *Eumusa* and *Rhodochlamys* formed the other lineage. Phylogenetic analysis indicated that *M. balbisiana* occupied a basal position in the cladogram, indicating its primitive evolutionary history. *Musa acuminata* and *M. balbisiana*, were evolutionarily distinct from each other. Moreover, *M. laterita* and *M. acuminata* showed close phylogenetic relationships.

Uma *et al*. (2005) studied the origin and diversification of *M. balbisiana* by using 29 accessions from the mainland of India and Andaman & Nicobar Islands. This RAPD-based study clearly distinguished two clusters in Indian *M. balbisiana*, one containing accession from the mainland (Indian sub-continent), and the other cluster formed by the accessions from the Andaman and Nicobar Islands. From the results, they proposed that *M. balbisiana* originated from Northeast India, and identified three distinguishable areas of *M. balbisiana* diversity including the Andaman and Nicobar Islands.

Uma *et al*. (2006) select the RAPD markers to evaluate the variation and infraspecific relationships in the *M. balbisiana* population in India. They studied 16 accessions of *M. balbisiana* from different geographical regions in the country by both morphological as well as molecular viewpoints. For the molecular study, four primers were screened. Morpho-taxonomic characterization resulted seven clusters but only four clusters were formed in the RAPD dendrogram, so here they concluded that genotypic variation is much restricted than phenotypic variation.

Youssef *et al.* (2010) analyzed the genetic variations and relationships of 40 *Musa* accessions by the help of sequence-related amplified polymorphism (SRAP) technique (aimed for the amplification of open reading frames) and AFLP. Study shows that SRAP exhibited approximately threefold more specific and unique bands than AFLP and it can used to separate the taxa up to the subspecies level. In this study, the subspecies of *M. acuminata*, *M. balbisiana* and *M. schizocarpa* are effectively discriminated.

Padmesh *et al.* (2012) explored the genetic diversity of *M. acuminata* subsp. *burmannica* in Western Ghats, South India by using ISSR markers. The UPGMA clustering and PCA analysis resulted in three major groups in the 32 *M. acuminata* subsp. *burmannica* accessions. The grouping corresponded to the geographic gradient and high level of genetic diversity was observed within the population.

Choudhary *et al.* (2014) studied the molecular variability of 12 ecotypes of plantains in India by using ISSR and RAPD markers. In this study, the authors identified 16 RAPD and 14 ISSR primers for the molecular profiling of plantain ecotypes. Moreover, they were recognised that, the ISSR is better tool over RAPD for genetic diversity studies.

Dayarani *et al*. (2014) carried out a RAPD-based molecular study on the family Musaceae in India to find out the phylogenetic relationship of sect. *Rhodochlamys* members with the sect. *Eumusa* members. For this study, five species from the sect. *Rhodochlamys* and two representatives from the sect. *Eumusa* were considered. The dendrogram shows that *M. laterita* and *M. acuminata* ssp. *burmannicoides* are from different sections sharing a common homology. Genetic relations of these species show the close relationship of section *Rhodochlamys* and *M. acuminata* (sect. *Eumusa*). Based on this finding they hypothesized that it may be the rationale for the occurrence of more natural hybrids of *M. acuminata* and sect. *Rhodochlamys* in nature.

Lamare and Rao (2015) examine the extent of genetic variability in *M. acuminata* from 7 districts of Meghalaya, Northeast India. This study collectively used RAPD, ISSR and directed amplification of minisatellite DNA (DAMD)

markers along with 25 genotypes of wild accessions. The result shows RAPD marker system has the highest resolving power than other two markers.

Saraswathi *et al.* (2020) assessed the gene diversity of Musaceae germplasm conserved in ICAR-NRCB, Trichy, India by examining the polymorphism in retrotransposon elements. The IRAP marker-based study resulted dendrogram with two major clusters in Indian Musaceae, one was formed by *Ensete* and another cluster was formed by taxa belonging to sections *Eumusa* and *Rhodochlamys*. This result supported the genetic relatedness of the aforementioned sections within the genus *Musa.*

Phylogeny of Musaceae based on Nuclear and Chloroplast Markers

At the end of 20th century and the beginning of the 21st century, another milestone in plant systematics was started- "DNA barcoding". Why barcoding is essential?. The simple answer is barcoding not only helps you for faster identification but also phylogenetic conclusions. Obviously, quick identification of biological specimens has always been desirable but has rarely been possible, because one reason is the shortage of natural history specialists. DNA barcodes are short gene sequences taken from a standardized portion of the genome and used to identify species (Kress *et al*., 2015). These genetic markers help to address questions relating to evolution, species identification, and conservation. The authentic molecular works by using DNA markers were started very recently. In the beginning, the molecular study was carried out by only one marker but later the number of the markers was increased to elucidate more accurate results. According to the CBOL plant working group (2009), the 2-locus combination of *rbcL-matK* as the one plant barcode. CBOL suggested that these DNA regions will provide a universal framework for the common use of DNA sequence data to identify specimens and help for the authentic discovery of unnoticed species of plants. Along with these markers two other markers *viz*. *trnH-psbA*, and the internal transcribed spacers of nuclear ribosomal DNA (nrITS) were added as plant barcodes by Hollingsworth (2011). But sometimes these markers fail to discriminate the plant group up to the infraspecific level. In the case of the banana family, along with the

nuclear ITS region-specific chloroplast DNA regions were also used for the accurate phylogenetic results, which are *trnL-F* (trnL intron and *trnL-F* intergenic spacer) (Liu *et al*., 2010; Li *et al*., 2010; Janssens *et al*., 2016), *atpB-rbcL* and *rps*16 intron (Li *et al*., 2010; Janssens *et al*., 2016).

The nuclear ITS and chloroplast *trnL-F* regions-based phylogenetic analysis of the family Musaceae were carried out by Liu *et al*. (2010). This study comprises of 28 species with 39 accessions, which covers the representatives from the three genera of the family and four sections in the genus *Musa* (excluded the section *Ingetimusa*). Five outgroups were selected *viz*, *Heliconia* (Heliconiaceae); *Strelitzia*, *Ravenala* and *Phenakospermum* (Strelitziaceae); and *Orchidantha* (Lowiaceae). The ITS region was amplified by using either primer pair ITS4 and ITS5 of (White *et al.*, 1990) or ITS4 and ITS5a (Stanford *et al*., 2000) and the plastid *trnL-F* region (spanning *trnL* intron, the 3' *trnL* exon, and intergenic spacer region) was amplified with primers *trnC* and *trnF* (Taberlet *et al*., 1991). As per the result of both the Maximum parsimony (MP) and Bayesian (BI) tree, the family is strictly monophyletic and three main internal clades were well-supported within the family. Two of these clades are represented by the genus *Musa*, and the third clade is made up of *Musella* and *Ensete*. In this study, the four sections of Cheesman (1947a) are merged into two sections *viz*., sect. *Musa* (including sect. *Rhodochlamys*) and sect. *Callimusa* (including sect. *Australimusa*). The genus *Musella* has placed as a sister group with the African clade of *Ensete* suggests that it is not warranting generic status. The ITS sequences provide a more informative signal in reconstructing the phylogeny of the banana family with 190 parsimony-informative characters when compared to the *trnL-F* sequences.

Li *et al*. (2010) also carried out the multi-locus marker-based phylogeny and systematics of the banana family by using nrITS and plastid sequences *viz*., *atpB-rbcL*, *rps*16, and *trnL-F*. DNA sequences of 36 species (42 accessions of ingroups representing three genera) and 10 accessions retrieved from the GenBank database were used for the construction of a phylogenetic tree. This study mainly emphasizes the infrageneric classification of the genus *Musa*. Five sections in *Musa* were

included in the phylogeny by incorporating *M. ingens* (sect. *Ingentimusa*). The maximum parsimony and Bayesian tree support the monophyly of the family, *Musella* and *Ensete* may be congeneric or at least closely related; two infrageneric clades were identified within the *Musa viz*., Sect. *Musa* n=x=11 (merged sect. *Rhodochlamys* into it) and Sect. *Callimusa* n=x=7/9/10 (merged sect. *Australimusa* and sect. *Ingentimusa* into it). *M. ingens* clustered within *Callimusa* clade.

The gene codes for the 45S ribosomal RNA are in several thousand copies in the genome and it contains 18S, 5.8S and 26S rRNA genes and these are separated by internal transcribed spacers ITS1 and ITS2. Whereas the rRNA genes are evolutionary conserved, ITS shows a high level of polymorphism between species and has been used regularly in genetic diversity as well as phylogenetic studies. Based on these aspects, Hribova *et al*. (2011) worked on the structure and diversity of the ITS region in 87 representatives in the family Musaceae. In the case of *Musa*, ITS 1 has 216−223 base-pair length and ITS 2 has 205−227 base pairs. Phylogenetic reconstruction based on this ITS sequence revealed that the genus *Musa* is splits into two separate clades *viz*, *Australimusa*-*Callimusa* clade and *Eumusa*-*Rhodochlamys* clade. Within the *Musa* clade, representatives of 'B' genome (*M. balbisiana* varieties and *M. nagensium*) form a distinct subclade.

Bekele and Shigeta (2011) used the complete sequences of transcribed spacers and introns from the *trnT*-*trnF* region of chloroplast DNA of *Ensete* (3 species), *Musa* (13 species) to establish the phylogenetic relationships. Phylogram resulted that, the *Ensete* and *Musa* as clearly distinguished clades. Most of the *Rhodochlamys* species used in this study clustered together with strong support establishing their distinctiveness from the *Musa* species studied.

Novak *et al*. (2014) analyzed the repetitive genome fractions of six *Musa* species representing various phylogenetic groups *viz*, *M. acuminata*, *M. ornata*, *M. textilis*, *M. beccarii*, *M. balbisiana*, and *Ensete gilletii*. *Ty1*/copia long terminal repeat (LTR) retrotransposons and the chromovirus lineage of *Ty3*/gypsy elements (highly repetitive DNA in all species) were selected as the molecular markers for this study. The differences in these repetitive DNAs were prominent between

species from different sections of the Musaceae family, while in closely related species like *M. acuminata* and *M. ornata*, *M. beccarii* and *M. textilis*, this marker shared similar populations of repetitive elements.

Cizkova *et al*. (2015) examined the nuclear DNA content and genomic distribution of 45S and 5S rDNA in 21 diploid accessions of *Musa*, representing both sections of the genus *Musa viz*. sect. *Musa* and sect. *Callimusa*. 2C DNA content in the sect. *Musa* ranging from 1.217 to 1.315 pg and species included in sect. *Callimusa* had 2C DNA contents ranging from 1.390 to 1.772 pg. The negative correlation between the basic chromosome number (x) and nuclear genome size (1C) ("the less chromosomes, the larger genome" trend) was one of the major outcomes of this study. This study also found a high level of nucleotide polymorphism and the presence of more than two kinds of ITS sequences (by Fluorescence In situ Hybridization) in eight wild diploids pointed to their origin by hybridization of different genotypes *ie*, these may be interspecific hybrids or backcross progenies of other wild bananas.

Janssens *et al*. (2016) conducted a phylogenetic study of Musaceae based on four DNA markers (nuclear ITS, plastid *rps*16, *atpB-rbcL*, and *trnL-F*) and 70 accessions covering three genera in the family. The results show Musaceae form a well-supported clade that is split into two major lineages: *Musa* clade and *Ensete-Musella* clade. The genus *Musa* was again divided into two large clades. One clade is well resolved and consists only of taxa that belong to the sections *Australimusa*, *Callimusa* and *Ingentimusa*. The other *Musa* clade was less resolved and had the representatives of sections *Musa* and *Rhodochlamys*.

Lamare *et al*. (2017) did the phylogeny of the *Musa* by a simple and cost-effective method, they sequenced the maternally inherited chloroplast genome (*atpB-rbcL* spacer, *trnK-matK* intron, and *psbK-psbI* spacer) and ITS region of nuclear ribosomal DNA of three species of *Musa viz*., *M. balbisiana*, *M. acuminata*, and *M. ornata* (five accessions each) along with data available in the GenBank database including the sequence of outgroups (*Heliconia caribaea* and *Ravenala madagascariensis*). Here also the phylogram supports the presence of two main

clades in the genus *Musa*. Clade I accommodate, species of sections *Eumusa* and *Rhodochlamys*, except *Musa* maclayi (section *Australimusa*). Clade II is formed with representatives of sections *Australimusa* and *Callimusa* along with *Ingentimusa*. A phylogram of the combined cpDNA sequences has shown a close genetic relationship between *M. schizocarpa (*sect. *Eumusa*) and *M. maclayi* (sect. *Australimusa*) in the present study. *Musa acuminata* clustered closely with *M. rubra* indicating their close relationship.

Fu *et al*. (2022) carried out the phylogenetic studies of Musaceae by using plastome sequences of 48 accessions. This study also recovers the sectional taxonomic results of the genus *Musa* in previous studies ((Liu *et al*., 2010; Li *et al*., 2010; Janssens *et al*., 2016) with high clade supports. Here also, the genus *Musa* was divided into two prominent clades, which matched well to the basic number of n=x = 11 (section *Musa*) and n = x =10/9/7 (section *Callimusa*), respectively. In addition, four subclades were found within the genus *Musa*. *Musa coccinea* and *M. paracoccinea* were together formed as subclade I, subclade II formed by the remaining species section *Callimusa.* The subclade III and IV were formed within the sect. *Musa*. *Musa balbisiana* and allied species like *M. cheesmanii*, *M. nagensium*, *M. puspanjaliae*, *M. itinerans*, *M. basjoo* etc. together formed as subclade III. Subclade IV consists of *M. acuminata* and its subspecies (subsp. *burmannica*, subsp. *banksii*, subsp. *microcarpa*, subsp. *truncata* etc.) together with previous members in Sect. *Rhodochlamys* (*M. chunii*, *M. mannii*, *M. veluina*, *M. ornata*, *M. rubra* and *M. rosea* etc.). Moreover, *M. balbisiana* was formed as the basal species to the other taxa in section *Musa* and *Musa rubra*, *M. laterita*, *M. rosea*, and *M. siamensis* (= *M. rubra* var. *siamensis*) are separated from the subspecies of *M. acuminata.* Based on this study, the genus *Musella* is formed as a sister to *Ensete* and confirms its species status.

Molecular dating and Ancestral Area Reconstruction studies

Molecular dating is a recently developed technique to estimate the approximate age of evolutionary events. Since DNA and amino acid sequences change continuously in the genome over time, we can estimate the time since they

last shared an ancestor by comparing DNA sequences between lineages. Zuckerkandl and Pauling (1965) made the initial suggestion for calibrated sequence differences as a method for dating evolutionary divergences.

Kress *et al*. (2001) carried out a phylogenetic work in the order Zingiberales to find out the evolutionary history, by using 24 spp. representing all families (8 families) within this order. They incorporated three members from the family Musaceae *viz*., *M. acuminata*, *E. ventricosum* and *M. lasiocarpa* and used 36 morphological characters and DNA sequences of 18S rDNA, *atpB* and *rbcL* genes. The combined morphological and molecular data provides a well-supported estimate of phylogenetic relationships: ie, 1st primary lineage Heliconiaceae (Zingiberaceae, Costaceae) (Cannaceae, Marantaceae) and the remaining three families in the order make up a 2nd primary lineage Musaceae (Lowiaceae, Strelitziaceae). They also carried out biogeographic studies and the results show that Zingiberales originated in the early Cretaceous period, earlier than 100 million years ago (Mya). Based on these two combined results, they proposed a revised classification for the order Zingiberales.

Kress and Specht (2006) assessed the divergence time of Zingiberales from remaining commelinoid monocots. For this study, multiple gene loci (*rbcL*, *atpB*, and 18S rDNA) and multiple calibration points were used with the help of fossils and previous monocot-wide age estimates. This study proved that Musaceae is the first family diverged from the order Zingiberales about 110 Mya, followed by Lowiaceae-Strelitziaceae clade about 109 Mya. It indicates the almost simultaneous origin of these families. Moreover, this study suggested that Musaceae having an ancestral distribution of America and Southeast Asia at the time of its origin (110 Mya).

Christelova *et al*. (2011) studied the phylogenetic inter-relationships of members in Musaceae and estimate the times of divergence of the major Musaceae clades. They used thirteen species for this study and DNA sequences obtained from a set of 19 unlinked nuclear genes having approximately 16 kb sequence length. *Strelitzia nicolai* (Strelitziaceae) was chosen as outgroup. This is the first study to

calculate the estimates for the divergence times of the family as well as sections in the genus *Musa*. Results revealed the Musaceae crown age as the Cretaceous/Tertiary boundary, about 69 Mya. and the evolution of the genus *Musa* takes place about 50 Mya. The lineage of 'B' genome (*M. balbisiana* species) was diverged during 27.9 Mya, followed by the lineage of *M. mannii*, representing the *Rhodochlamys* section, at 20 Mya. The major speciation events within the A genome lineage (*M. acuminata* species) began 11.4 Mya. Also, the phylogram shows the close relationship between the species of sect. *Rhodochlamys* and *M. acuminata* (sect. *Eumusa*), the genetic distance between *M. acuminata* and *M. balbisiana* is greater than between it and *Rhodochlamys*.

Janssens *et al*. (2016) conducted a phylogenetic study and aimed to find a link between the diversification and biogeography of Musaceae and the geological history of the Southeast Asian subcontinent. Four DNA markers were used for the study (nuclear ITS, plastid *rps*16, *atpB-rbcL*, and *trnL-F*). The taxa sampling represents five *Ensete* species (6 accessions), 38 *Musa* species (63 accessions) one species of *Musella*. By reconstructing the ancestral area and analyzing diversification rates, evolutionary trends within the Musaceae family were deduced here. In order to correctly estimate the node ages of the three genera under Musaceae, the authors used 156 Zingiberales species and two outgroup taxa. For the robust node age estimation, an extended sampling of Zingiberales fossils was utilized. It includes (1). fossil seeds of *Ensete oregonense* were obtained from deposits in Oregon (USA) with an age of about 43 Mya (middle Eocene), (Manchester & Kress, 1993) and it used as the crown node of *Ensete* and *Musella* or stem node of *Ensete* (2). leaves of *Zingiberopsis attenuata* from the Paleocene (estimated age about 65 Mya) Paskapoo formation of Alberta (Canada) (Hickey and Peterson, 1978), considered as the crown node of the family Zingiberaceae and (3). *Spirematospermum chandlerae*- the oldest known fossil (estimated as an age of 83.5 Mya) of the Zingiberales (Friis, 1988). For the Ancestral area reconstruction, 12 geographical areas were used, which mainly included in the Southeast Asian subcontinent and Africa. The divergence time for Musaceae was estimated at 51.9 Mya, suggesting an early Eocene origin, also the splitting of sister genera *Ensete* and

Musella occurred in early Eocene at 44.7 Mya. The diversification of *Musa* started during the late Eocene (37.9 Mya). Within the genus *Musa*, the Clade I (*Ingentimusa*, *Australimusa*, and *Callimusa*) was diversified in the Oligocene at 26.3 Mya, whereas Clade II (represented by species of sections *Musa* and *Rhodochlamys*) started to radiate in the early Miocene at 20.9 Mya. The ancestral area analysis revealed that all the genera (*Musa*, *Ensete* and *Musella*) originated from Northern Indo-Burma. *Musa* species dispersed from the mainland of Southeast Asia to Malayan Archipelago with only a few back-dispersal events towards northern Indo-Burma.

Deng *et al*. (2016) carried out phylogenetic and ancestral area reconstruction (AAR) studies in the order Zingiberales with the help of plastid genome data (from 76 coding genes and 4 rDNA loci, comprised of 61,966 nucleotide characters). Seventeen species represent eight families under the Zingiberales and one outgroup (*Xiphidium caeruleum*, Haemodoraceae, Commelinales) was used in the study. Bayesian phylogram based on combined 80 plastid genes shows that Musaceae clade first diverged in the order Zingiberales and form the sister clade to the remaining 7 families. The ginger families (clade I) formed into two subclades, (Zingiberaceae, Costaceae) and (Marantaceae, Cannaceae), and the remaining families clustered into clade II. Within clade II, Heliconiaceae was sister to a subclade of Lowiaceae and Strelitziaceae. Here only the *M. textilis* and *M. acuminata* subsp. *malaccensis* was used as the representative of the banana family. The AAR study using the Bayesian Binary Markov Chain Monte Carlo (BMM) method shows that the major radiation of this order occurred in Africa (including Madagascar), Neotropical America, and Australia (including Melanesia) and the most likely ancestral area was Australia for Zingiberales.

Burgos-Hernandez *et al*. (2019) studied the biogeographic analysis of Musaceae, highlighting the significance of the fossil record in comprehending the early evolutionary history of the family. Informative loci like nrITS, *atpB-rbcL*, and *trnL-trnF* sequences were used to estimate divergence times and ancestral areas were reconstructed using two models: one including and one excluding the fossil distribution. *Phenakospermum guyannense* (Rich.) Miq. was included as an

outgroup. The previous phylogenetic and biogeographic studies considered the ancestral areas of Musaceae only based on current distributional status and underestimated or little has been explained about the ancient presence of the family in the Euroamerican boreotropics as fossil record (Liu *et al*., 2010; Janssens *et al*., 2016). *Spirematospermum chandlerae* represents the oldest known record of reproductive structures (more affinity with Musaceae) collected from Cretaceous sediments (Santonian) of North Carolina, with an estimated minimum age of 83.5 Mya (Rodríguez-de la Rosa and Cevallos Ferris, 1994; Fischer *et al*., 2009), so here it is used as the stem node of Musaceae. The fossil of *Ensete oregonense* was used to calibrate the minimum divergence time between *Ensete* and *Musella*. The results showed that Musaceae underwent a major split (*Musa* and *Ensete*/*Musella*) during the Palaeocene 61.0 Mya. The divergence between the allied genera *Ensete* and *Musella* happened in the Eocene at 46.0 Mya. Also, the results suggested that *Musa* began to diverge into its two known sections during the Early Eocene (52.0 Mya). The section *Musa* diversified in the Late Eocene at 33.7 Mya, whereas species of section *Callimusa* (37 Mya) radiated approximately 3 Mya earlier than section *Musa*. According to Model I (based on current distribution), Indo-Burma and southern China is the most likely ancestral area (MLAA) of Musaceae. However, Model II (based on current and fossil distribution) revealed North America, Indo-Burma, southern China and Europe as the MLAA for the family. Based on the present results and the elucidation of the fossil records, most of the Euroamerican lineages of Musaceae may undergo extinction between the Late Eocene and Early Oligocene, with surviving lineages limited mainly to Southeast Asia. Accordingly, North America and Europe may currently be considered the graveyard of ancient Musaceae lineages, while tropical Asia has been considered the cradle of the most recent lineages of Musaceae.

Fu *et al*. (2022) carried out the phylogeny of Musaceae by using plastome sequences of 48 accessions. This study sheds new insight into the Global Musaceae phylogeny and produced the following major outcomes: plastomes of the Musaceae having a length ranging from 166,782 bp to 172,514 bp. Four plastid regions *viz*., *ndhF-trnL*, *ndhF*, *matK-rps*16, and *accD* are identified as specific DNA barcodes for Musaceae. The molecular dating analysis showed that the divergence of the Musaceae family originated in the Palaeocene about 59.19 Mya and the genus *Musa*

diverged into two clades in the Eocene about 50.70 Mya and then started to diversify from the late Oligocene (29.92 Mya) to the late Miocene. The age comparisons of different clades in Musaceae from the aforementioned studies are depicted in Table 1.

Table 1. Comparison of molecular dating and divergence time studies of Musaceae

Publications	**Divergence time in Million years ago (Mya)**				
	Musaceae	***Ensete*/ *Musella***	***Musa***	**Sect. *Musa***	**Sect. *Callimusa***
Fu *et al*. (2022)	59.19 (46.26 −74.47)	44.77 (41.14−48.8)	50.70 (34.03−69.01)	29.92 (16.74−45.17) *M. balbisiana* clade- 27.32	30.16 (14.40−48.85)
Burgos-Hernandez *et al*. (2019)	61 (45.9−80.1) Palaeocene	46 (43.1− 51.6) Eocene (Split b/w *Ensete* and *Musella*)	52 (32−74) Early Eocene	33.7 Late Eocene	36.7
Janssens *et al*. (2016)	51.9 (45.6− 61.2) Early Eocene	44.7 (43.1−48.2) Early Eocene. Radiation of *Ensete* about 28.5 (16.9–42.1)	37.9 (24.5−50.5) Late Eocene.	20.4 (13.3−30.4) Early Miocene	26.3(16−38.9) Oligocene (Diversified)
Christelova *et al*. (2011)	69.1 (57.8−80.5)		50.7 (40.4−61.5)	27.9 (21.5−34.4)	28.7 (21.2−36.6)
Kress & Specht (2006)	96.5−110				

Literature survey

Information about the family Musaceae was gathered from a variety of sources, including institutional and university libraries, as well as through information retrieval systems such as New York Botanical Garden (http://www. biodiversityheritagelibrary.org), Missouri Botanical Garden Libraries (http://www.botanicus.org and http://www.tropicos.org), information from online libraries such as JSTOR (https://www.jstor.org), Internet archive (http://www.archives. com) etc. were also utilized.

Specimen collection and enrichment of germplasm

Materials for the current study were gathered through extensive field trips in north-eastern parts of India and Andaman and Nicobar Islands during 2018–2022. The list of collected taxa is given in Table 2. Primary information on the collected species was directly recorded in the field note. Attempts were made to study each species in its natural habitat and also under cultivation. Around 25 additional accessions were added to the existing germplasm of Musaceae in Calicut University Botanical Garden (CUBG) and the 'Ginger Valley' of Malabar Botanical Garden and Institute for Plant Sciences (MBGIPS).

Herbarium preparation

Collected specimens *viz*., leaves, male buds, bracts, flowers and fruits were treated with 20-30% formaldehyde solution and sealed in polythene bags. Herbaria were prepared following the wet method (De Vogel, 1987; Forman & Bridson, 1998). Specimens were dried using the oven and wet newspaper was changed with dry ones as frequently as possible. Dried specimens were mounted on handmade (24 × 42 cm) herbarium sheets and a label (14.5 × 11 cm) containing all the relevant information was affixed and all the sheets were deposited at Calicut University

Herbarium (CALI). The digitized images of all the herbarium sheets are available in online (https://CaliHerbarium.org).

Table 2. List of Indian Musaceae collected during the present study.

Sl. No.	Taxa	Herbarium accession No	Locality	Collector & Date of Collection
1	*Musa acuminata* Colla	159775 (CALI)	2 Km away from Namdang checkpost, Arunachal Pradesh	Rajeesh E.P 06-08-2018
		159777 (CALI)	4 km after Namdang checkpost, on the way to Changlang from Margherita, Assam	Rajeesh E.P 06-08-2018
2	*M. argentii* Gogoi & Borah	159766 (CALI)	Forest area between Namchik and Namphai, Arunachal Pradesh	Rajeesh E.P 05-08-2018
3	*M. arunachalensis* A.Joe, Sreejith & M.Sabu	159739 (CALI)	Kenibreed plants (cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
4	*M. aurantiaca* G.Mann *ex* Baker	159768 (CALI)	Namdapha Forest Area, Arunachal Pradesh	Rajeesh E.P 05-08-2018
5	*M. balbisiana* Colla var. *balbisiana* Colla	159713 (CALI)	Tashiding, on the way to Phamrong waterfall from Jorethang, Sikkim	Rajeesh E.P 29-07-2018
		159732 (CALI)	Kenibreed plants (cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
6	*M. balbisiana* var. *andamanica* D.B.Singh,Sreek., T.V.R.S.Sharma & A.K.Bandyop.	164030 (CALI)	Near kalimandir, krishnapuri, Diglipur, A&N Island	Rajeesh E.P & Amrutha. A 08-07-2019
7	*M. cheesmanii* N.W.Simmonds	159779 (CALI)	Demve, on the way to Tidding, Arunachal Pradesh	Rajeesh E.P 10-08-2018
		159729 (CALI)	Keinbreed plants (Cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
		159737 (CALI)	Keinbreed plants (Cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018

8	*M. chunii* Hakkinen	159788 (CALI)	Way to Tidding from Tezu, 12 km after Tohangam viewpoint, Arunachal Pradesh	Rajeesh E.P 10-08-2018
		159790 (CALI)	20 km before Tidding, on the way to Tidding from Tezu, Arunachal Pradesh	Rajeesh E.P 10-08-2018
9	*M. flaviflora* N.W.Simmonds	164712 (CALI)	Senki View, Itanagar, Arunachal Pradesh	Rajeesh E.P 30-05-2022
		164713 (CALI)	Near Military area, Senki View, Itanagar, Arunachal Pradesh	Rajeesh E.P 30-05-2022
10	*M. itinerans* Cheesman	159769 (CALI)	Namdapha National Park, Arunachal Pradesh	Rajeesh E.P 05-08-2018
		159734 (CALI)	Keinbreed plants (Cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
11	*M. kattuvazhana* K.C.Jacob	164050 (CALI)	8 km after kadamtala to Rangat, on the way to North Andaman from Portblair, Andaman & Nicobar Islands	Rajeesh E.P & Amrutha. A 06-07-2019
		164054 (CALI)	3 Km after Sabari junction, on the way to Portblair from Mayabander, Rangat, A&N Island	Rajeesh E.P & Amrutha. A 10-07-2019
12	*M. mannii* H.Wendl. *ex* Baker	159776 (CALI)	In between Namdang check post and Longran, Arunachal Pradesh	Rajeesh E.P 05-08-2018
		159735 (CALI)	Keinbreed plants (Cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
13	*M. markkuana* (M.Sabu, A.Joe & Sreejith) Hareesh, A.Joe & M.Sabu	159738 (CALI)	Keinbreed plants (Cultivated), L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
14	*M. markkui* Gogoi & Borah	159787 (CALI)	Way to Tidding from Tezu, 13 km after Tohangam viewpoint, Arunachal Pradesh	Rajeesh E.P 10-08-2018
		159789 (CALI)	23 Km after Tezu, On the way from Tezu to Tidding, Arunachal Pradesh	Rajeesh E.P 10-08-2018

15	*M. nagensium* Prain	159767 (CALI)	Namdapha Forest, Arunachal Pradesh	Rajeesh E.P 05-08-2018
		159736 (CALI)	Keinbreed plants (Cultivated) L.B Road, Kalimpong, West Bengal	Rajeesh E.P 30-07-2018
16	*M. ochracea* K.Sheph.	164759 (CALI)	Near Vairengten entry Checkpost, Mizoram	Rajeesh E.P 06-06-2022
17	*M. puspanjaliae* Gogoi & Hakkinen	159785 (CALI)	Way to Tidding from Tezu, 1 km after Tohangam viewpoint, Arunachal Pradesh	Rajeesh E.P 10-08-2018
18	*M. rubra* Wall. *ex* Kurz	159727 (CALI)	Jorethang town, Sikkim	Rajeesh E.P 28-07-2018
19	*M. sabuana* K.Prasad, A.Joe, Bheem. & B.R.P.Rao	164076 (CALI)	Galathea, Nicobar Island Galathea, Great Nicobar Island	Rajeesh E.P & Amrutha. A 17-07-2019
		164075 (CALI)	Vijaya Nagar, on the way to Galathea, Great Nicobar	Rajeesh E.P & Amrutha. A 17-07-2019
		164074 (CALI)	5 km after the Great Nicobar Biosphere Reserve check gate, Great Nicobar Biosphere Reserve	Rajeesh E.P & Amrutha. A 17-07-2019
20	*M. sikkimensis* Kurz	159750 (CALI)	Namahatta, On the way to Darjeeling, West Bengal	Rajeesh E.P 31-07-2018
		164721 (CALI)	Sessa Villege, Arunachal Pradesh	Rajeesh E.P 01.06.2022
		159712 (CALI)	Near Rajbhavan, Gangtok, Sikkim	Rajeesh E.P 29-07-2018
21	*M. thomsonii* (King *ex* Baker) A.M.Cowan & Cowan	164732 (CALI)	Foot hills of Tura Peak, Meghalaya	Rajeesh E.P 03.06.2022
22	*M. velutina* H.Wendl. & Drude	159765 (CALI)	On the way to Margherita to Mpen, Assam & Arunachal Pradesh Border	Rajeesh E.P 05-08-2018
23	*M. velutina* var. *variegata* A.Joe, M.Sabu & Sreejith	159764 (CALI)	Makum forest, Assam	Rajeesh E.P 05-08-2018

Preparation of Photo plates

Colour photo plates were prepared by using using Adobe Photoshop CS Version 8.0. Taxonomically significant characters and morphological variations are highlighted in the photo plates.

Morphological descriptions

This description part begins with correct name, reference to protologue and homotypic synonyms. Followed by a short description, distribution and notes of each taxon comes under Indian Musaceae.

1. *Ensete glaucum* (Roxb.) Cheesman, Kew Bull. 2(2): 101. 1947. **Fig. 4**

Musa glauca Roxb., Hort. Bengal. 19. 1814.

Musa agharkarii Chakravorti, J. Indian Bot. Soc. 27: 93. 1948.

Monocarpic, rhizomatous, non-stoloniferous, non-suckering herbs. Pseudostems 1–5 m tall, conical with swollen base, glaucous, pale green to green. Leaves arranged at the apex of pseudostem, apex acute, bases symmetric and both sides pointed; petioles glaucous, yellowish green to green. Inflorescence pendulous; female and male buds lanceolate, imbricate; bracts and flowers integral with each other and with axis; bracts green on both sides, persistent; flowers arranged in two rows. Infructescence compact with 5–14 hands; fingers perpendicular to the rachis; fruits straight, sessile, slightly ridged, acuminate or blunt at apex with floral relicts, faintly glaucous, green when young, yellow when ripen; seeds ovoid.

***Distribution*:** China, India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland & Tripura), Indonesia, Laos, Myanmar, Philippines, Papua New Guinea and Thailand.

***Notes*:** This species can be easily identified by its green conical pseudostem with leaves arranged at the apex and prominent pendulous inflorescence with persistent green bracts. Recently, Parmar *et al.* (2023) reinstated *Ensete nepalensis* from the synonymy of this species, based on the short nature of pseudostem (0.15–1.2 m), purplish colour of distal bracts (*vs.* green colour), revolute nature of distal bract apex (*vs.* non-revolute nature) and small size of seeds (4–8 × 3–8 mm) of *E. nepalensis*. They used two specimens, *viz*., *Gaurav Parmar* GP1 (KATH, 6 sheets), *Gaurav Parmar* GP2 (KATH, 3 sheets) from Bagmati, Lalitpur district of Nepal for their study.

Fig. 4. *Ensete glaucum*: A & B. Habit; **C.** Infructescence; **D & E.** Female bud.

2. *Ensete superbum* (Roxb.) Cheesman, Kew Bull. 2(2): 100. 1947. **Fig. 5**

Musa superba Roxb., Hort. Bengal. 19. 1814.

Monocarpic, rhizomatous, non-suckering, non-stoloniferous herbs. Pseudostems 1–4 m tall, conical with swollen base; leaves arranged from middle towards the apex of pseudostem, apex acute, bases symmetric and both sides pointed; petioles barely glaucous, green or yellow-green. Inflorescence pendulous to sub-horizontal; female and male bud lanceolate, imbricate, bracts and flowers integral with each other and with axis; bracts pink-purple to brown-purple on both sides, persistent or deciduous; flowers arranged in two rows. Infructescence compact with 3–14 hands; fingers perpendicular to the rachis; fruits pedicellate, straight, sparsely ridged, slightly pointed at apex with floral relicts, faintly glaucous, green when young, yellow when ripen; seeds ovoid.

***Distribution*:** India (Peninsular India), Myanmar, Thailand and Vietnam.

***Notes*:** This species can easily differentiate from *E. glaucum* by its dry appearance of pseudostem due to the persistent nature of old leaves and spatially arranged leaves and purplish-coloured bracts.

3. *Musa acuminata* Colla, Mem. Reale Accad. Sci. Torino 25: 394. 1820. **Fig. 6**

Musa simiarum '*Pissang Jacki*' Rumph., Herb. Amb. 5: 138. 1747.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1–3.2 m tall, cylindrical. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 30–65 cm long, moderate to profusely glaucous, green or yellowish-green. Inflorescence horizontal, bending downwards at maturity; female and male buds lanceolate, convolute; bracts and flowers attached separately on the rachis; bracts reflexed, revolute before falling, faintly glaucous, adaxially brownish-purple to pinkish-purple with or without yellow apex, abaxially red-purple to pale yellow; flowers arranged in two rows. Infructescence lax with 3–7 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight or curved, slightly ridged, pointed at apex without floral relicts, glabrous, green, greenish-yellow or pale yellow when young, yellow when ripen; seeds oblate.

Fig. 5. ***Ensete superbum*****: A & B.** Habit; **C–F.** Different stages of Inflorescence. (PC: **C-** K.P. Smisha).

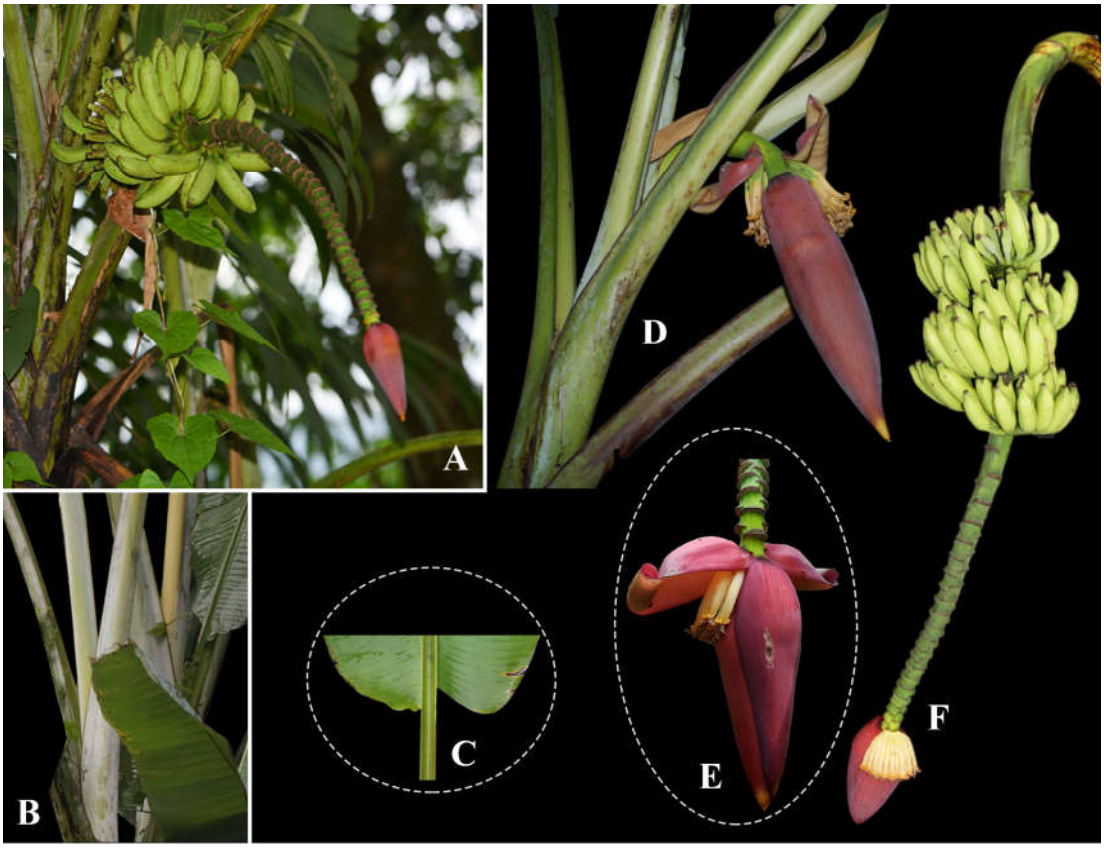


Fig. 6. *Musa acuminata*: A. Habit; **B.** Glaucous petiole; **C.** Leaf base; **D.** Female bud; **E.** Male bud; **F.** Infructescence. (PC: **D & F-**V.S. Hareesh).

***Distribution*:** China, India (Northeastern states), Java, Malaya, Myanmar and Thailand.

***Notes*:** This species is allied with *M. flaviflora* and *M. thomsonii* in its shape of male bud, but it has red-purple or brown-purple bracts.

4. *Musa acuminata* var. ***manipurensis*** A.Joe & M.Sabu A.Joe & M.Sabu, Revision of Indian Musaceae, 2019.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1.1–3 m tall, cylindrical. Leaves arranged terminally, bases symmetric, both sides rounded; petioles 35–65 cm long, moderate to profusely glaucous, green or yellow-green. Inflorescence horizontal, bending downwards at maturity; female and male bud lanceolate, convolute; bracts and flowers attached separately on the axis; bracts reflexed, revolute before falling, faintly glaucous, adaxially red to red-purple, abaxially cream; flowers arranged in two rows. Infructescence lax with 3–7

hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight or curved, slightly ridged, pointed at apex without floral relicts, glabrous, green, greenish-yellow or pale yellow when young yellow when ripen; pedicels 0.2–0.4 cm long; fruit apex 0.2–0.3 cm long; seeds oblate.

***Distribution*:** Endemic to Northeastern India (Manipur).

***Notes*:** This variety can be distinguished from typical *M. acuminata* by its fruit. The fruit apex is distinct at maturity, 0.2–0.3 cm long; fruit pedicel is 0.2–0.4 cm long.

5. *Musa argentii* Gogoi & Borah, Edinburgh J. Bot. 71(2): 182. 2014. **Fig. 7**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1.5–2.5 m tall, cylindrical. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 50–120 cm long, glabrous, green or dark pinkish-red. Inflorescence horizontal or pendulous; female and male bud lanceolate, convolute; bracts and flowers attached separately on the axis; bracts reflexed, revolute before falling, glabrous, adaxially pink or pink-purple, abaxially pinkish-red; flowers arranged in two rows. Infructescence compact with 5–11 hands; fingers perpendicular to the axis; fruits broadly oblong, pedicellate, straight or curved, slightly ridged, glabrous, acute to truncate at apex without floral relicts, glabrous, purplish-green or pinkish-green when young; seeds oblate.

***Distribution*:** Endemic to Arunachal Pradesh.

***Notes*:** Several clumps of the same population of *M. argentii* show horizontal, arched and pendulous inflorescence. Mostly the inflorescences are with partially erect or partially pendulous nature, representing the major characters of *M. velutina* and *M. itinerans*. We could find such plants in a mixed population of *M. velutina* and *M. itinerans* and therefore it is presumed that they may be hybrids of the latter two taxa.

Fig. 7. *Musa argentii*: A & B. Habit; **C.** Female bud; **D.** Infructescence; **E.** Split opened fruit. (PC: **A, B** and **C-** V.S. Hareesh).

6. *Musa arunachalensis* A.Joe, Sreejith & M.Sabu, Phytotaxa 134(1): 50. 2013.

Fig. 8

Musa kamengensis Gogoi & Hakkinen, Acta Phytotax. Geobot. 64(3): 149. 2013.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1–3 m tall, cylindrical. Leaves arranged terminally, bases asymmetric, one side rounded other side pointed; petioles 25–40 cm long, glabrous, green. Inflorescence arch-shaped; female and male buds lanceolate, convolute; bracts and flowers attached separately on the rachis; bracts reflexed, revolute before falling, glabrous, adaxially reddish-orange, abaxially yellow-orange; flowers arranged in one or two rows. Infructescence lax or compact with 2–6 hands; fingers pointed

upwards; fruits broadly oblong, pedicellate, straight, prominently ridged, pointed at apex, glabrous, green when young, yellow when ripen; seeds oblate.

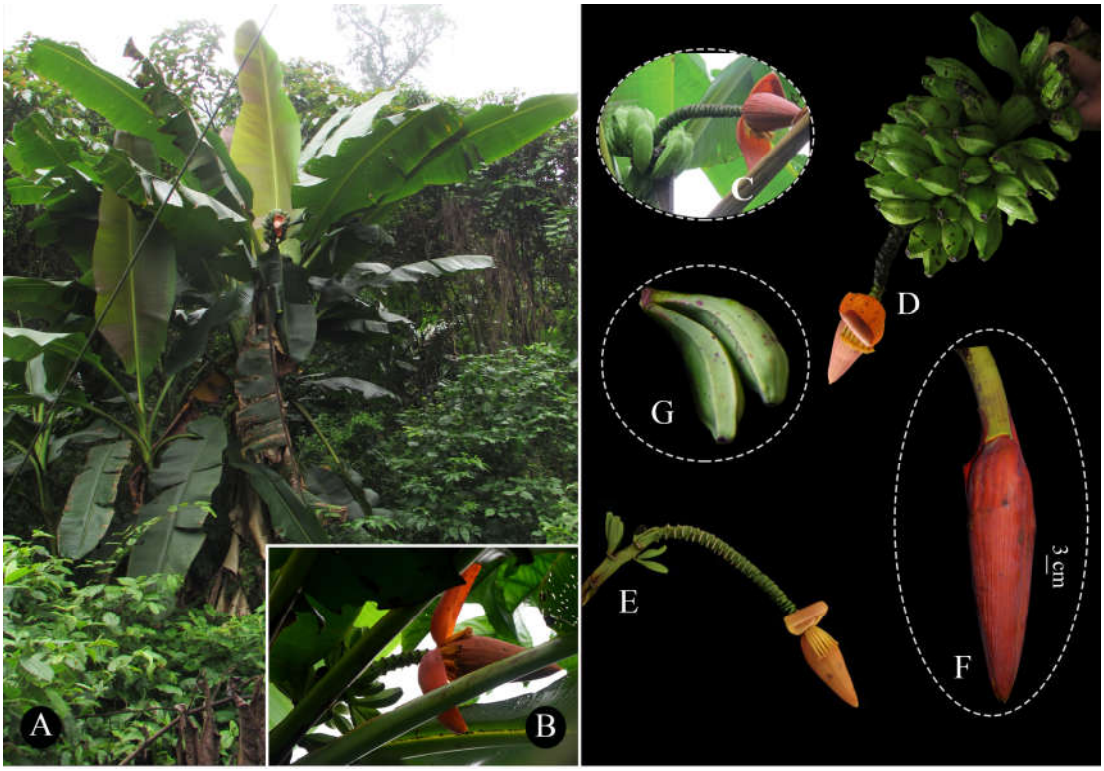


Fig. 8. ***Musa arunachalensis*****: A.** Habit; **B–E.** Different stages of Inflorescence; **F.** Female bud; **G.** Fruit. (PC: **A, D** and **F-** V.S. Hareesh; **C & E-** A. Joe).

Distribution**:** Endemic to Arunachal Pradesh

Notes**:** Inflorescence is completely arched, but rarely, the arch nature is restricted to the rachis. It begins by standing upright and then bends to one side before forming an arch. Bracts are reddish-orange with yellow tips adaxially and yellow-orange abaxially. Authors mentioned this species belongs to section *Rhodochlamys* based on the colour of bract and shape of inflorescence, but its pseudostem height and fruit nature are allied with the characteristics of section *Musa*. Therefore, it is considered an intermediate form between section *Musa* and section *Rhodochlamys*. This species is commonly found aside with *M. sikkimenis* and *M. markkuana*, sharing many morphological traits with these species. Consequently, there is a possibility that *M. arunachalensis* is a hybrid resulting from the combination of the above-mentioned species.

7. *Musa aurantiaca* G.Mann *ex* Baker, Ann. Bot. 7(26). 222. 1893. **Fig. 9**

Musa aurantiaca var. *homenborgohainiana* Gogoi, Nordic J. Bot. 32(6): 702. 2014.

Musa aurantiaca var. *jengingensis* Gogoi, Nordic J. Bot. 32(6): 702. 2014.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 0.7–2.5 m tall, cylindrical. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 40–80 cm long, glabrous, green or green with red tinge. Inflorescence erect; female and male buds lanceolate, convolute or imbricate at apex, bracts and flowers inserted separately on the axis; bracts reflexed, revolute or rarely non-revolute before falling, glabrous, bright orange on both sides; flowers arranged in one row. Infructescence compact with 0–13 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight, slightly pointed at apex, green when young, greenish-yellow or pale yellow when ripen; seeds oblate.

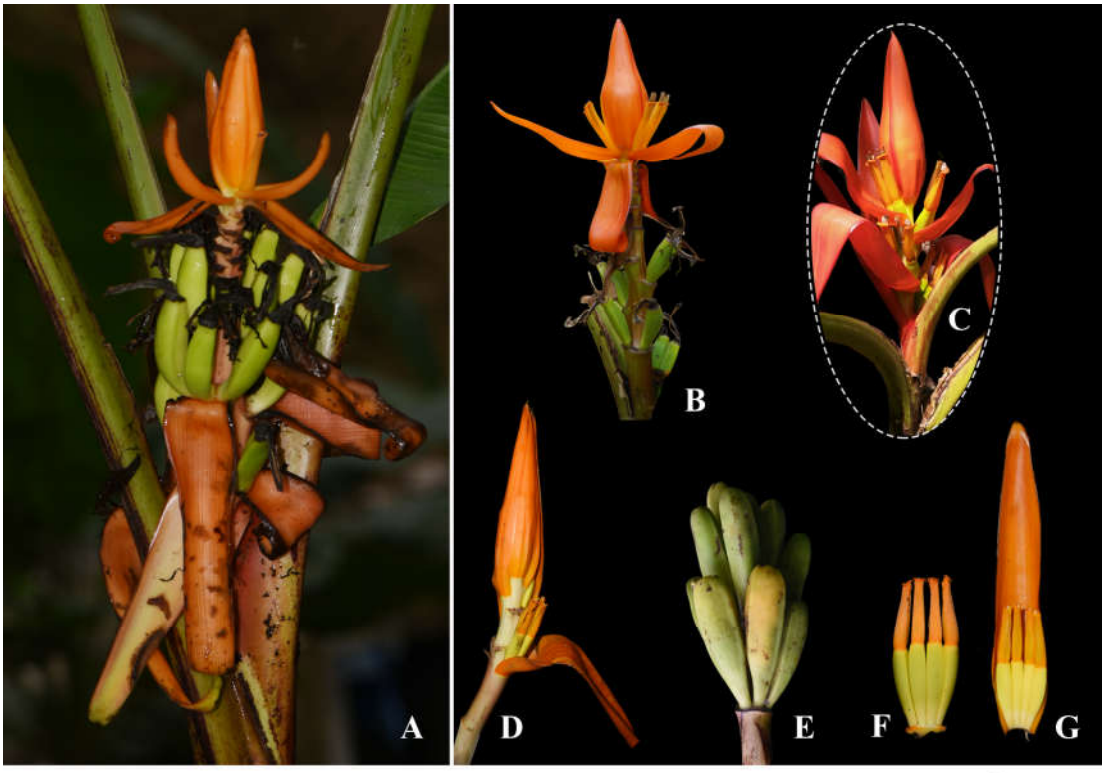


Fig. 9. *Musa aurantiaca*: A. Habit; **B & C.** Inflorescence; **D.** Female bud; **E.** Fruit bunch; **F & G.** Female flower.

***Distribution*:** India (Assam, Arunachal Pradesh and Nagaland) and Tibet.

***Notes*:** This species has an upright inflorescence with bright, orange-coloured bracts.

8. *Musa balbisiana* Colla, Mem. Reale Accad. Sci. Torino 25: 384. 1820. **Fig. 10**

Musa shankarii Subba Rao & Kumari, Fl. Visakhapatnam Distr. (Andhra Pradesh) 2: 266. 2008.

Musa nagalandiana S.Dey & Gogoi, Nordic J. Bot. 32(2): 584. 2014.

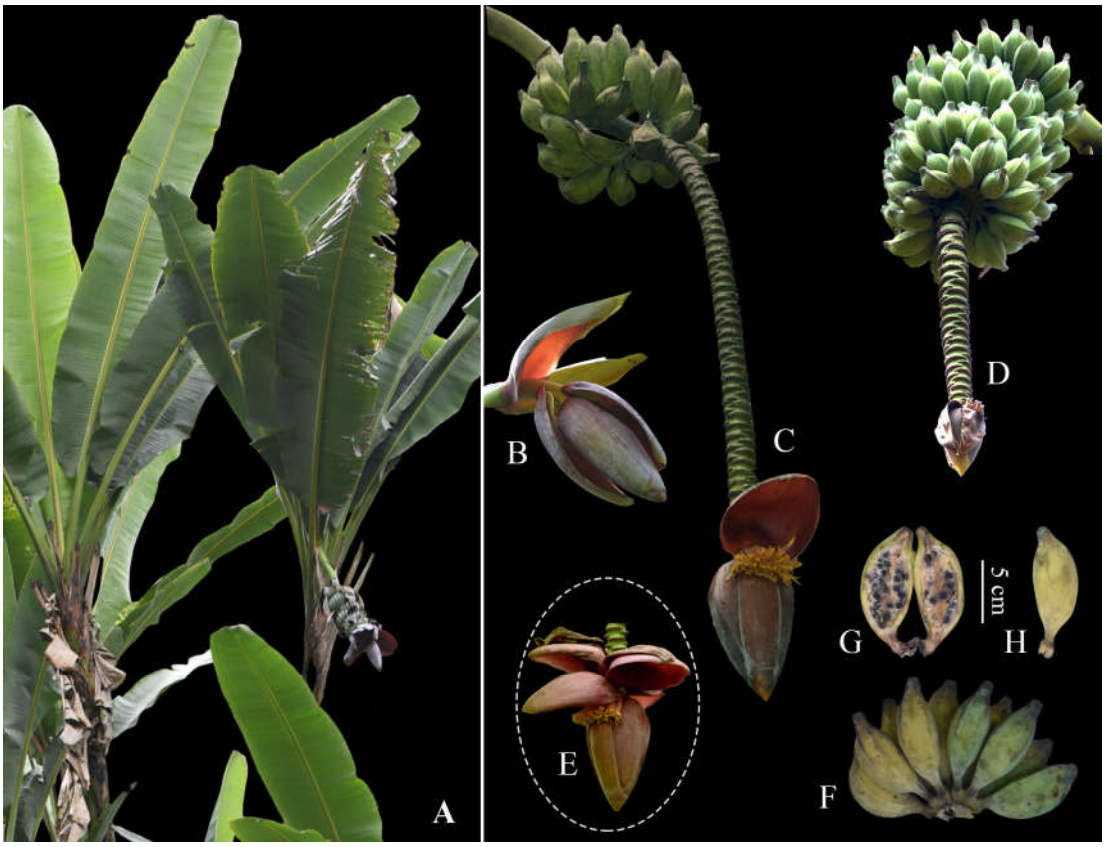


Fig. 10. *Musa balbisiana*: A. Habit; **B.** Female bud; **C & D.** Infructescence; **E.** Male bud; **F.** Fruit bunch; **G.** Fruit split opened; **H.** Fruit. (PC: **D-** V.S. Hareesh).

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 3–4.5 m tall, cylindrical. Leaves arranged terminally, bases asymmetric, both sides auriculated; petioles 30–65 cm long, slightly glaucous, green or yellow green to pale yellow. Inflorescence sub-horizontal; female and male bud lanceolate, convolute or rarely imbricate in male bud; bracts and flowers inserted separately on the rachis; bracts reflexed, non-revolute before falling, faintly glaucous, adaxially pink-purple, abaxially reddish-purple; flowers arranged in two rows. Infructescence compact with 3–10 hands; fingers pointed upwards; fruits broadly oblong, pedicellate, straight or curved, prominently ridged, 4-angled,

minutely pointed at apex without any floral relicts, slightly glaucous, green when young, yellow when ripen; seeds sub-globose.

***Distribution*:** Widely distributed in South and Southeast Asia. In India, this taxon is common in the Northeastern States and Eastern Ghats.

***Notes*:** It is the commonest and most widely distributed *Musa* species. This species shows closely appressed leaf bases and erect leaves with much shiny upper surface like a waxy coating.

9. *Musa balbisiana* Colla var. ***andamanica*** D.B.Singh, Sreek., T.V.R.S.Sharma & A.K.Bandyop, Malayan Nat. J. 52(3–4): 157. 1998. **Fig. 11**

Musa paramjitiana L.J.Singh, Nordic J. Bot. 35: 77. 2017.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 4–4.5 m tall, cylindrical, glaucous, dull green, greenish-black towards base and greenish towards apex. Leaves arranged terminally, bases asymmetric, both sides auriculated; petioles 40–65 cm long, slightly glaucous, yellowish green. Inflorescence sub-horizontal; female bud lanceolate, imbricate; male bud lanceolate or top-shaped in advanced blooming, convolute or slightly imbricate at apex; bracts and flowers inserted separately on the axis; bracts reflexed, non-revolute before falling, glabrous, adaxially pink-purple, faintly glaucous, abaxially red-purple; flowers arranged in two rows. Infructescence compact with 3–10 hands; fingers pointed upwards; fruits broadly oblong, short-pedicellate or almost sessile, straight, prominently ridged, blunt at apex without floral relicts, slightly glaucous, green when young, pale yellow when ripen; seeds sub-globose.

***Distribution*:** India (Andaman and Nicobar Islands, Tripura).

***Notes*:** This variety has fruits without distinct apex and pedicel (tapering to both ends, 0.4–0.6 cm long or almost sessile)

Fig. 11. ***Musa balbisiana*** var. ***andamanica*****: A.** Habit; **B.** Female bud; **C.** Bract with female flowers; **D.** Fruit bunch; **E.** Fruit; **F.** Fruit split-opened. (PC: **E & F-** A. Joe).

10. ***Musa balbisiana*** Colla var. ***bheem-kola*** A.Joe & M.Sabu, Revision of Indian Musaceae, 2019. **Fig. 12**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 4.5–8.5 m tall, cylindrical, olive green with black patches, faintly glaucous towards apex. Leaves arranged terminally, bases asymmetric, both sides auriculated; petioles 35–45 cm long, glaucous pale green. Inflorescence sub-horizontal, at least for the fruit-bearing part; female and male bud lanceolate to intermediate, imbricate at apex, male bud top-shaped to ovoid in advanced blooming; flowers inserted separately on the axis; bracts reflexed, non-revolute

before falling, glaucous, adaxially pinkish-purple, glabrous, abaxially reddish-purple; flowers arranged in two rows. Infructescence compact with 8–10 hands; fingers pointed upwards; fruits broadly oblong, pedicellate, straight or curved, prominently ridged, faintly glaucous, prominently pointed at apex without floral relicts, green when young, pale yellow with brownish dry patches when ripen; seeds sub-globose.

Fig. 12. ***Musa balbisiana*** var. ***bheem-kola*****: A.** Habit; **B.** Female bud; **C.** Bract with female flowers; **D.** Fruit bunch; **E.** Fruit; **F.** Fruit split-opened.

Distribution**:** Endemic to Assam.

Notes**:** This variety is differing from others by its large sized fruits (15–24 cm long, circumference 17–21 cm, pedicel 4–5 cm long and apex 2.7–3.5 cm long). Its pericarp shows dull yellow colour with brown markings or patches with a dry appearance at maturity.

11. *Musa balbisiana* Colla var. ***elavazhai*** A.Joe, Sreejith & M.Sabu, Phytotaxa 175(2): 113. 2014. **Fig. 13**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 5–7 m tall, cylindrical, glaucous, green. Leaves arranged terminally, bases asymmetric, both sides auriculated; petioles 50–65 cm long, glabrous, yellowish-green, pendulous, then horizontal up to fruit bearing part; female and male bud lanceolate, imbricate at apex; bracts and flowers inserted separately on the axis; bracts reflexed, non-revolute before falling, faintly glaucous, adaxially dark purple to purplish pink, abaxially purplish red, glabrous; flowers arranged in two rows. Infructescence lax with 4–7; fingers pointed upwards; fruits broadly oblong, pedicellate, curved, prominently ridged, extensively pointed at apex with or without floral relicts, slightly glaucous when young, green, yellow when ripen; seeds sub-globose.

Fig. 13. *Musa balbisiana* var. ***elavazhai*****: A.** Habit; **B.** Early stage of inflorescence**; C.** Mature infructescence; **D.** Male bud with rachis; **E & F.** Bracts.

Distribution**:** Endemic to South India (Kerala, Karnataka and Tamilnadu).

Notes**:** Male bud of this variety is lanceolate-shaped and pedicel 3.8–4 cm long.

12. *Musa balbisiana* Colla var. ***sepa-athiya*** Borborah**,** Borthakur & Tanti, Bangladesh J. Plant Taxon. 23(1): 75. 2016 **Fig. 14**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 3.5–5.25 m tall, cylindrical, slightly glaucous towards apex, yellow-green or green. Leaves arranged terminally, bases asymmetric, both sides auriculated; petiole slightly glaucous. Inflorescence sub-horizontal; female bud lanceolate to intermediate, imbricate at apex; male bud ovoid, imbricate at apex; bracts and flowers inserted separately on the axis; bracts glaucous, adaxially pink-purple with yellow apex, rarely yellowish-green, abaxially purplish-red; flowers arranged in two rows. Infructescence compact, with 7–16 hands; fingers pointed upwards; fruits broadly oblong, pedicellate, straight or curved, angled, faintly glaucous, apex broadly pointed, green when young, yellow when ripen; seeds sub-globose.

Fig. 14. *Musa balbisiana* var. ***sepa-athiya*****: A.** Habit; **B.** Female bud with green bracts; **C & D.** Infructescence; **E.** Male bud. (PC: **B-** A. Joe).

***Distribution*:** Endemic to Northeastern India (Assam and Meghalaya).

***Notes*:** Fruits of this variety show broadly pointed apex (pericarp broadly end to tip).

13. *Musa cheesmanii* N.W. Simmonds, Kew Bull. 11(3): 479. 1957. **Fig. 15**

Musa swarnaphalya Uma, Saraswathi & Durai, Indian J. Hort. 68(2): 146. 2011.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 3–6.5 m tall, cylindrical, glaucous, black, upper portion green. Leaves arranged terminally, bases asymmetric, one side rounded and other side auriculated; petiole 40–55 cm long, greenish yellow to purple. Inflorescence pendulous; female and male bud lanceolate, imbricate at apex; bracts and flowers inserted separately on the axis; bracts reflexed, non-revolute before falling, dark violet, adaxially sometimes pale yellowish green, faintly glaucous, abaxially red purple or pale orange; flowers arranged in two rows. Infructescence lax with 3–14 hands; fingers pointed upwards; fruits broadly oblong, pedicellate, curved, angled, minutely pointed at apex without any floral relicts, glaucous, dull green in mature; seeds sub-globose.

***Distribution*:** Endemic to Northeastern India (Arunachal Pradesh, Assam, Manipur and Nagaland).

***Notes*:** This species shows a unique black pseudostem.

14. *Musa chunii* Häkkinen, J. Syst. Evol. 47(1): 87. 2009. **Fig. 16**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1–2 m tall, cylindrical, pale green. Leaves arranged terminally, bases asymmetric, both sides pointed; petioles 30–45 cm long, glabrous, pale green. Inflorescence pendulous; female and male buds lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts pale lilac on both sides; flowers arranged in a single row. Infructescence lax with 3–6 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight or slightly curved, slightly ridged, blunt at apex with floral relicts, glabrous, pale green when young, yellow when mature; seeds oblate.

Fig. 15. ***Musa cheesmanii*****: A.** Habitat; **B.** Habit; **C.** Black coloured pseudostem; **D.** Mature infructescence; **E.** Female bud; **F.** Immature infructescence; **G.** Male bud with persistent bracts.

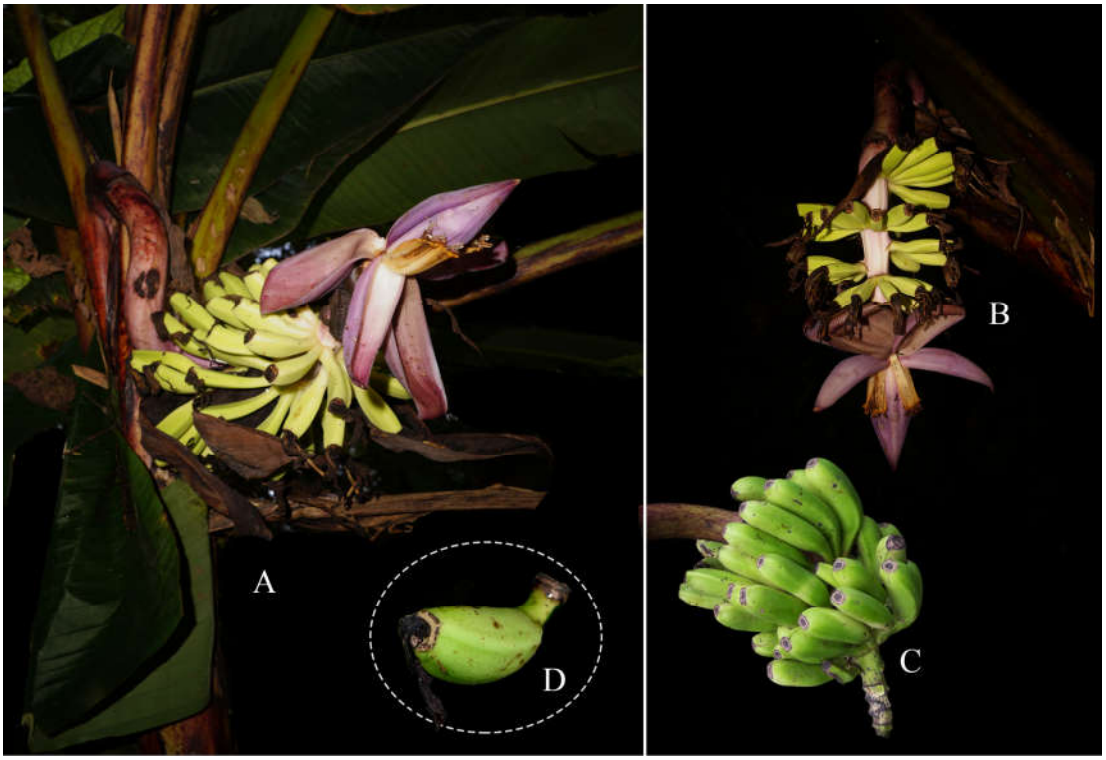


Fig. 16. ***Musa chunii*****: A.** Habit; **B. I**nfructescence; **C.** Fruit bunches; **D.** Single fruit. (PC: **A & B-** V.S. Hareesh).

Distribution**:** China, India (Arunachal Pradesh) and Myanmar.

Notes**:** Pendulous inflorescence, bracts pale lilac-coloured, creamy peduncle which turns brown at maturity.

15. ***Musa cylindrica*** A.Joe, Sreejith & M.Sabu, Phytotaxa 172(2): 138. 2014.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 2–3.3 m tall, cylindrical, slightly glaucous towards apex. Leaves arranged terminally, bases asymmetric, one side rounded and other side auriculated; petiole 70–82 cm long, faintly glaucous. Inflorescence first pendulous, then slightly horizontal; female bud cylindrical, male bud lanceolate, imbricate; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, adaxially faintly brown-purple, glaucous, abaxially cream; flowers arranged in two rows. Infructescence compact with 5–7 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight or slightly curved, minutely pointed at apex, green when young, yellow when ripen; seeds oblate.

***Distribution*:** Endemic to Meghalaya.

***Notes*:** This species possesses a cylindrical-shaped female bud.

16. *Musa flaviflora* N.W. Simmonds, Kew Bull. 11(3): 471. 1956. **Fig. 17**

Musa acuminata 'Mariani form' Cheesman, Kew Bull. 3(1): 28. 1948.

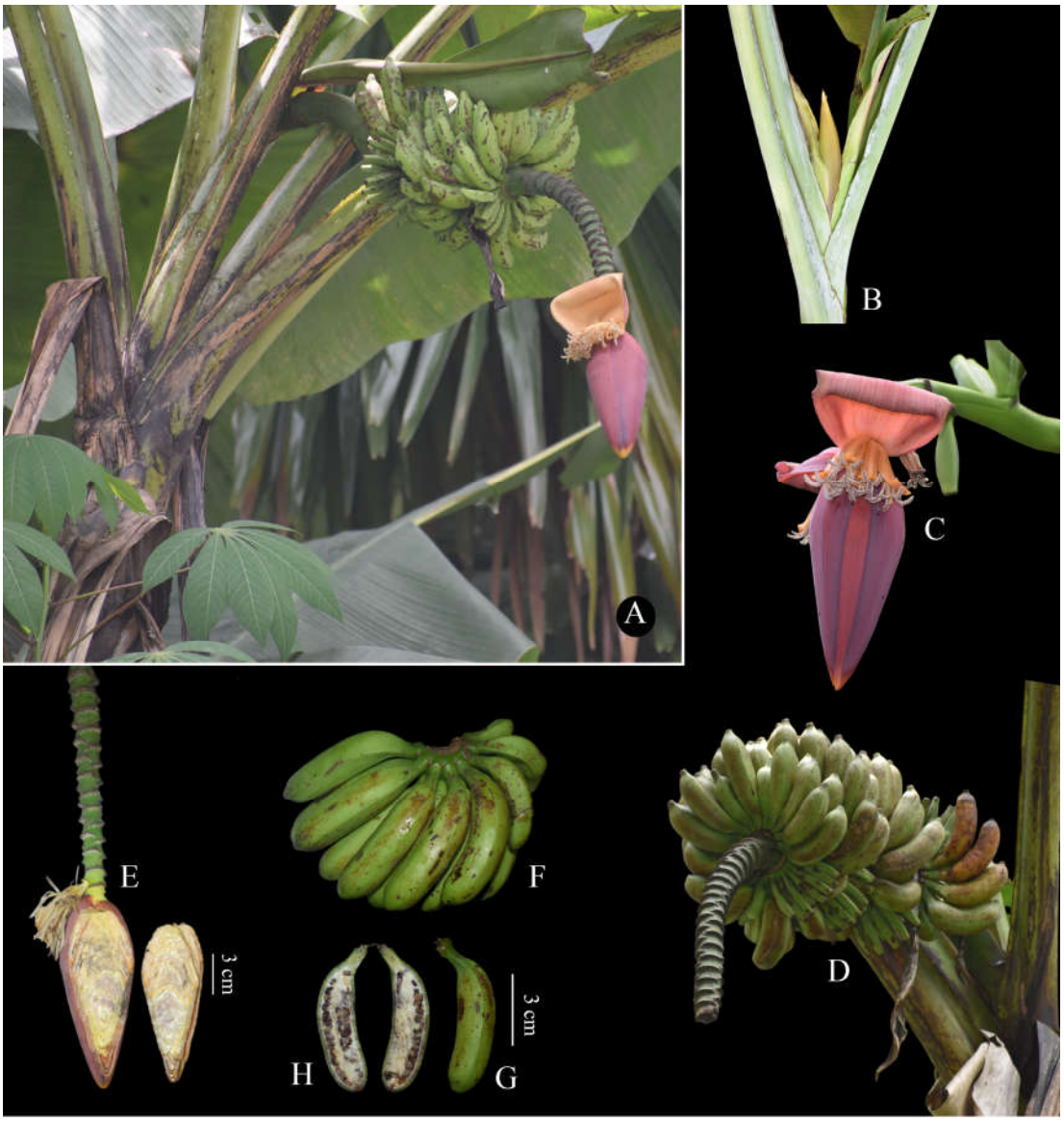


Fig. 17. ***Musa flaviflora*: A.** Habit; **B.** Emerging stage of inflorescence; **C.** Immature infructescence; **D.** Mature infructescence; **E.** Male bud; **F.** Fruit bunch; **G.** Fruit.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1.5–2.5 m tall, cylindrical, glaucous, green with black blotches. Leaves

arranged terminally, bases symmetric, both sides rounded; petioles 40–55 cm long, glaucous, green with black blotches. Inflorescence first erect, then horizontal; female buds lanceolate, convolute, minutely imbricate at apex; male buds top-shaped or convolute, imbricate at apex; bracts and flowers inserted separately on the axis; bracts reflexed and revolute before falling, adaxially reddish-pink, sparsely glaucous, abaxially cream with pink tinge; flowers arranged in two rows, yellow. Infructescence lax with 5–8 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight, slightly ridged, minutely pointed at apex without floral relicts, glabrous, green when young, yellow with brown blotches when mature; seeds oblate.

***Distribution*:** Endemic to Northeastern India (Assam, Meghalaya and Nagaland)

***Notes*:** This species is morphologically similar to *M. acuminata*, *M. thomsonii* but differs by its cream with pink tinge colour on the abaxial side of the bract and yellow flowers.

17. *Musa itinerans* Cheesman, Kew Bull. 4 (1): 23. 1949. **Fig. 18**

Perennial, rhizomatous, suckering, non-clump forming herbs, spreading by running rhizomes. Pseudostems 3–5.5 m tall, cylindrical, yellowish green or reddish brown. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 60–70 cm long, pale green or yellowish green. Inflorescence pendulous, then nearly horizontal; female and male bud lanceolate, convolute, male bud ovoid in advanced blooming; bracts and flowers inserted separately on the axis; bracts reflexed and revolute before falling, glabrous, adaxially dark maroon with yellow striations or yellow margins, abaxially cream to yellowish cream or yellow; flowers arranged in two rows. Infructescence lax with 3–6 hands; fingers pointed upwards, fruits narrowly oblong, pedicellate, straight, slightly ridged, minutely pointed at apex, glabrous, pale green when young, green when mature; seeds oblate.

Fig. 18. ***Musa itinerans*****: A.** Habitat; **B.** Apex of pseudostem; **C.** Habit; **D.** Female bud; **E & F.** Male bud; **G.** Fruit bunch.

***Distribution*:** China, India (Arunachal Pradesh, Manipur and Nagaland), Myanmar and Thailand.

***Notes*:** Running rhizomes, flower bracts dark maroon with yellow striations or yellow margins towards apex adaxially, cream to yellowish cream or yellow abaxially.

18. *Musa kattuvazhana* K.C.Jacob, Madras Bananas Monogr. 129. 1952. **Fig. 19**

Musa banksii F. Muell. var. *singampatti* T.G.Nayar, Indian J. Hort. 9(1): 14. 1952.

Musa acuminata subsp. *burmannica* N.W.Simmonds, Kew Bull. 11(3): 468. 1957.

Musa acuminata subsp. *burmannicoides* E.De Langhe, Bull. Jardin Bot. Etat Bruxelles 30: 377. 1960.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1.5–4 m tall, cylindrical, pale green or greenish-yellow or yellow with reddish-brown patches. Leaves arranged terminally, bases asymmetric, both sides pointed; petioles 20–40 cm long, pale yellow to yellowish green, glabrous. Inflorescence pendulous, then sub-horizontal; female and male buds lanceolate, prominently imbricate at apex; bracts reflexed, revolute before falling, adaxially dark purplish-violet, slightly glaucous, abaxially blood red. Infructescence compact with 3–10 hands; fingers pointed upwards; fruits narrowly oblong, straight or curved, slightly ridged, pointed at apex without floral relicts, glabrous, green when young, pale yellow when ripen; seeds oblate.

***Distribution*:** India (Karnataka, Kerala, North & South Andaman Islands and Tamil Nadu), Myanmar and Thailand.

***Notes*:** This species has Yellow-shaded and waxless pseudostem and petiole. Hairy peduncle and strongly imbricate bracts are prominent characteristics which help to differentiate this species from *M. acuminata*.

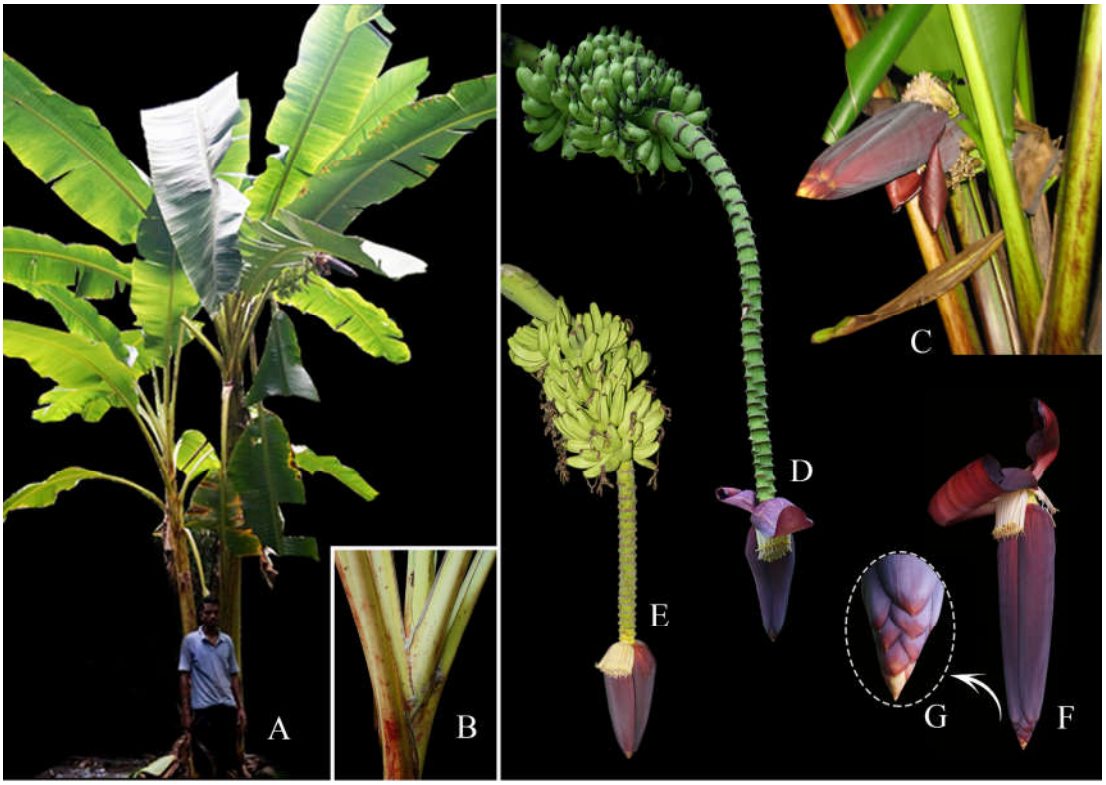


Fig. 19. ***Musa kattuvazhana*****: A.** Habit; **B.** Apex of pseudostem; **C.** Female bud**; D & E.** Mature infructescence; **F.** Male bud; **G.** Apex of male bud. (PC: **A, C, E, F & G-** V.S. Hareesh).

19. ***Musa mannii*** H.Wendl. *ex* Baker in Hook.f. (ed.), Fl. Brit. India 6: 263. 1892.

Fig. 20

Musa mannii var. *namdangensis* Gogoi & Borah, Taiwania 59(2): 94. 2014.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 0.8–1 m tall, cylindrical, pale green with reddish brown patches. Leaves arranged terminally, drooping, bases asymmetric, both sides rounded; petioles 40–45 cm long, pale green or with pale to deep pink patches, margin wrinkled. Inflorescence pendulous or horizontal; female and male bud lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute or rarely non-revolute before falling, pale pink on both sides, adaxially puberulent, abaxially glabrous; flowers arranged in a single row. Infructescence lax with 3 or 4 hands; fruits narrowly oblong, pedicellate, straight, faintly ridged, slightly pointed at apex without floral relicts, glabrous, pale green when young, yellowish-green when ripen; seeds oblate.

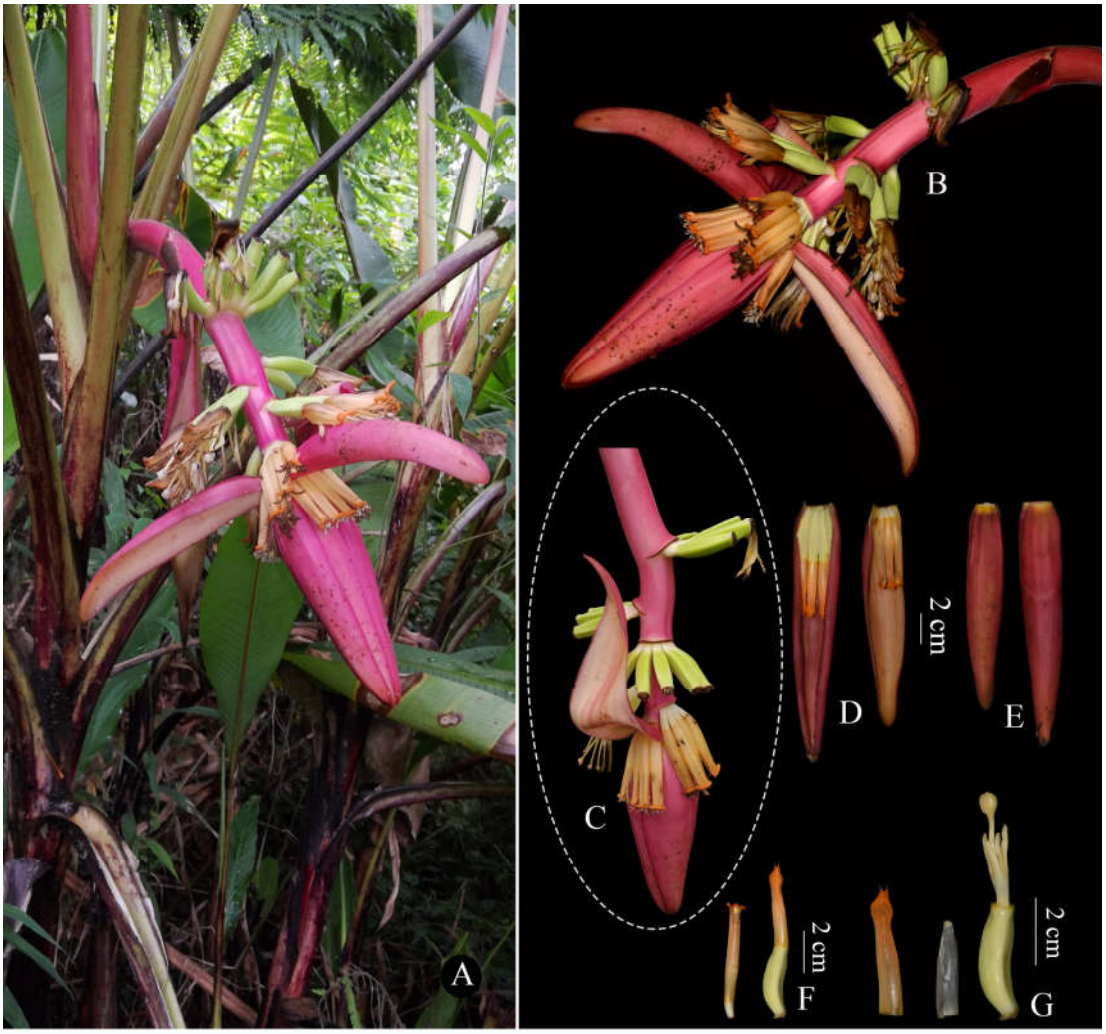


Fig. 20. ***Musa mannii*: A.** Habit; **B & C.** Inflorescence; **D.** Abaxial side of female bracts with female flowers; **E.** Adaxial side of female bracts; **F.** Male and Female flower; **G.** Floral parts of female flower.

***Distribution*:** Endemic to Northeastern India (Arunachal Pradesh and Nagaland).

***Notes*:** This species shows larger leaves in proportion to short pseudostem and which is highly drooping and its bract colour is pale crimson. Recently Tiatemsu *et al.* (2023) reported the occurrence of *M. mannii* from Nagaland.

20. ***Musa markkuana*** (M.Sabu, A.Joe & Sreejith) Hareesh, A.Joe & M.Sabu, Phytotaxa 303(3): 283. 2017. **Fig. 21**

Musa velutina H.Wendl. & Drude subsp. *markkuana* M.Sabu, A.Joe & Sreejith, Phytotaxa 92(2): 49. 2013.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems cylindrical, 0.7–1.7 m tall, slender, pale green or with pale purple patches. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 50–70 cm long, glabrous, pale brownish-pink, margins scarious. Inflorescence erect; female and male buds lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts reflexed and revolute before falling, pink to deep pink on both sides, minutely puberulent to glabrous adaxially, glabrous abaxially; flowers in a single row. Infructescence compact with 4–6 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight, prominently ridged, blunt at apex with or without floral relicts, glabrous, deep pink when young, pale pink when mature; seeds oblate.

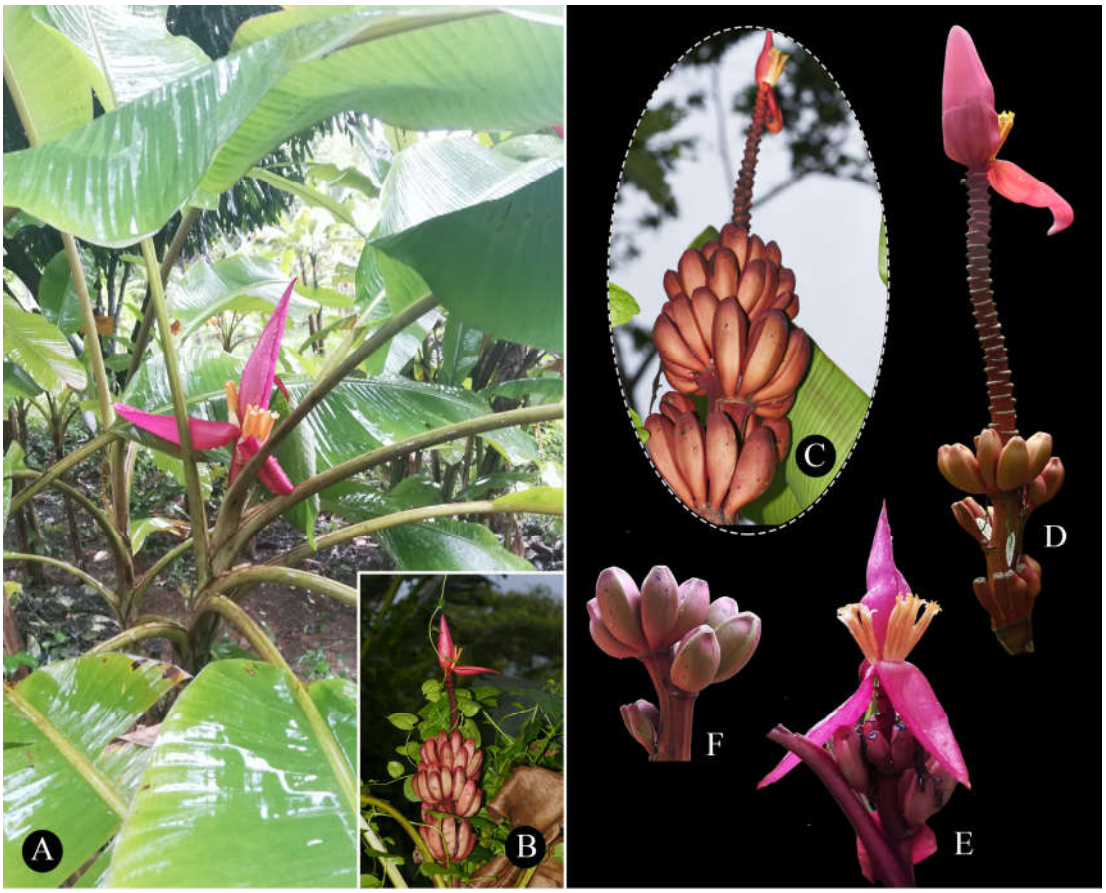


Fig. 21. ***Musa markkuana*****: A.** Habit; **B–E.** Different stages of infructescence; **F.** Fruit bunch. (PC: **D-** K.P. Smisha).

Distribution**:** India (Arunachal Pradesh and Nagaland) and Myanmar.

Notes**:** Glabrous fruits and peduncle help to differentiate this species from *M. velutina* and *M. velutina* var. *variegata*.

21. *Musa markkui* Gogoi & Borah, Gard. Bull. Singapore 65(1): 20. 2013. **Fig. 22**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1–3.5 m tall, cylindrical, pale-green with reddish-brown patches. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 40–80 cm long, pale green with pink colouration, wings wrinkled. Inflorescence erect or serpent or sub-horizontal or pendulous; female and male buds lanceolate, convolute; bracts and flowers arranged separately on the axis; bracts reflexed, revolute before falling, adaxially pale pink to pink or pale orange, abaxially pale pink or pale orange with pink striations; flowers arranged in a single row. Infructescence compact with 5–8 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight, ridged, glabrous, faintly pointed at apex without floral relicts, pale green to green when young, yellow with brownish-red small patches when ripen; seeds oblate.

Fig. 22. *Musa markkui*: A. Habit; **B.** Inflorescence; **C.** Female bud; **D & E.** Different type of infructescence. (PC: **A & C-** V.S. Hareesh).

***Distribution*:** Endemic to Northeastern India (Arunachal Pradesh and Nagaland)

***Notes*:** Usually, peduncle shows a bend.

22. *Musa nagensium* Prain, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 73(1): 21. 1904.

Fig. 23

M. nagensium var. *hongii* Hakkinen, Novon 18(3): 337. 2008; Gogoi, R. Taiwania 58(1): 50. 2013.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 3.5–7 m tall, cylindrical, glaucous, olive green to brownish-red or black. Leaves spatially arranged, drooping, bases asymmetric, one side rounded and other side auriculated; petioles 40–55 cm long, glaucous, yellowish-green. Inflorescence horizontal then pendulous; female and male buds lanceolate or cylindrical, prominently imbricate; bracts and flowers inserted separately on the axis; bracts reflexed, non-revolute before falling, glaucous, adaxially yellowish-orange with brick-red coloration and sometimes pale greenish-brown towards the apex, abaxially pale orange to orange; flowers arranged in two rows. Infructescence lax with 4–8 hands; fingers pointed downwards; fruits broadly oblong, pedicellate, straight, prominently angular, slightly pointed at apex, without floral relicts, glaucous, pale green to green when young, dark green when ripen; seeds oblate.

***Distribution*:** China, India (Arunachal Pradesh and Nagaland), Myanmar and Thailand.

***Notes*:** Leaves are spatially arranged almost half the length of the pseudostem. Pseudostem, peduncle and abaxial side of leaves are highly glaucous. Flower buds are prominently imbricated and orange shaded. Positively geotropic nature of fruit is another peculiar character of this species.

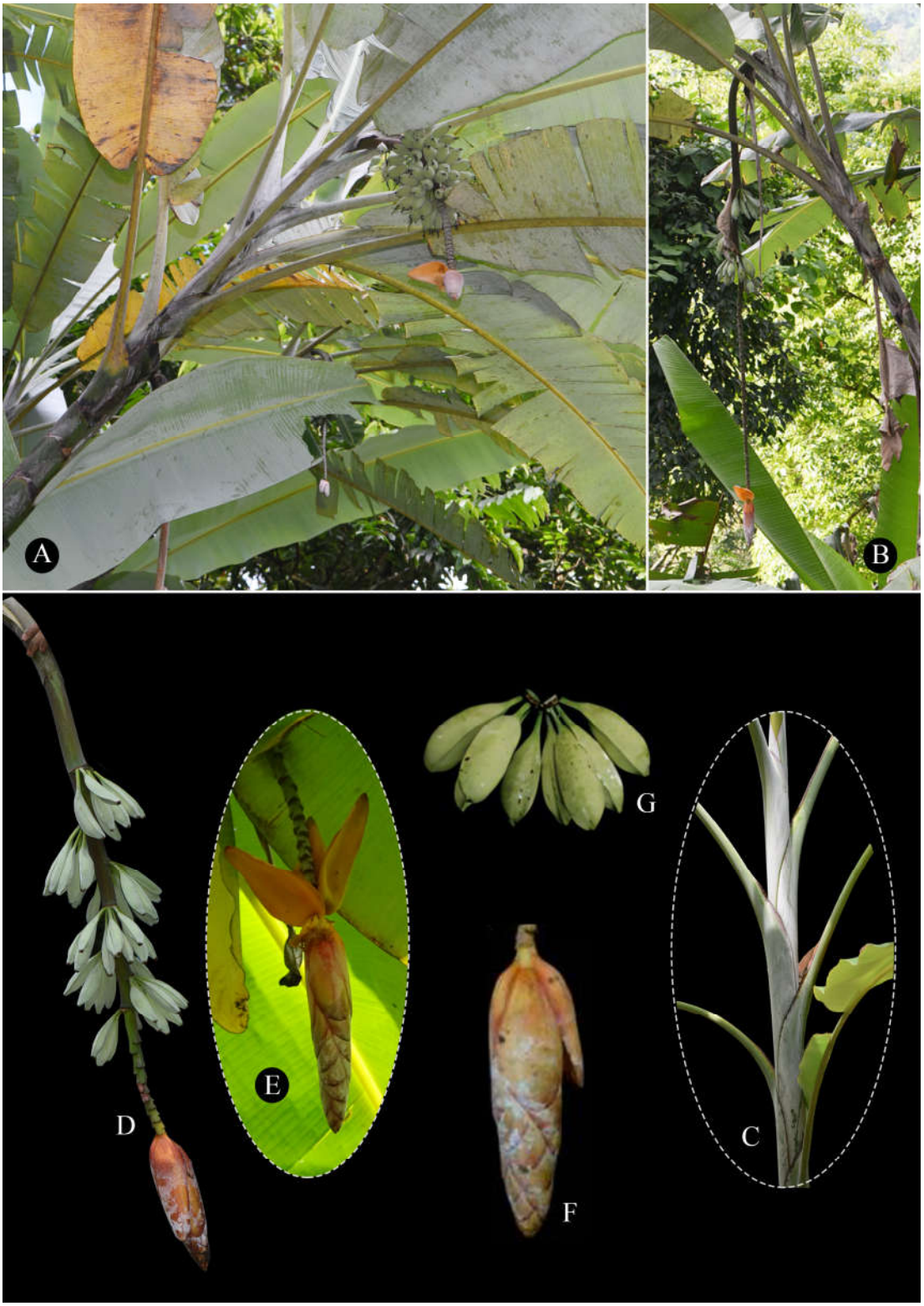


Fig. 23. ***Musa nagensium*: A & B.** Habit; **C.** Spatially arranged leaves with glaucous petiole base; **D.** Infructescence with positively geotropic fruits; **E & F.** Male buds; **G.** Fruit bunch. (PC: **E-** K.P. Smisha).

23. *Musa ochracea* K.Sheph., Kew Bull. 17(3): 461. 1964. **Fig. 24**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1.5–3m tall, cylindrical, ochreous yellow. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 60–85 cm long, greenish yellow to yellow, glabrous, wings margins prominent, dry-wrinkled.

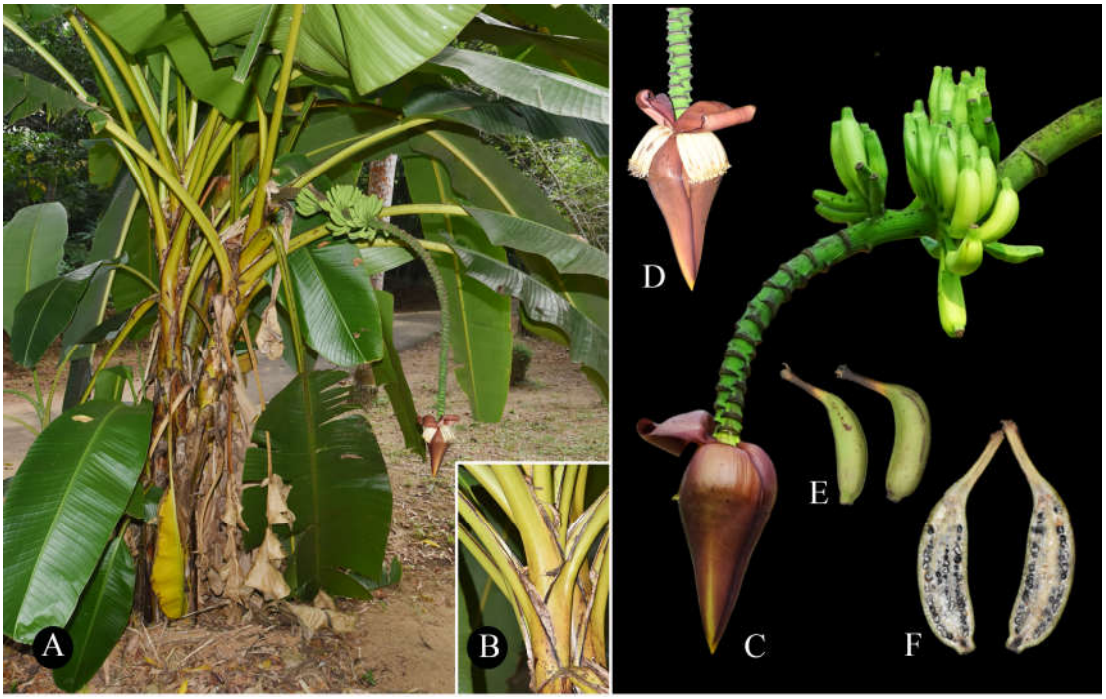


Fig. 24. *Musa ochracea*: A. Habit; **B.** Apex of pseudostem; **C.** Infructescence; **D.** Male bud; **E.** Fruit; **F.** Split opened fruit.

Inflorescence horizontal at least fruit-bearing part, peduncle densely puberulent; female bud lanceolate, convolute; male bud lanceolate to top-shaped, convolute; bracts and flowers inserted separately on the axis; bracts revolute before falling, adaxially dark brown-purple or with yellow lines towards the apex, abaxially cream to brown purple; flowers arranged in two rows. Infructescence compact with 5–7 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, curved, pointed at apex, glabrous except minutely puberulent base, green when young and maturity; seeds ellipsoid**.**

***Distribution*:** Endemic to Northeastern India (Manipur, Mizoram and Tripura).

***Notes*:** Ochreous yellow colour of pseudostem. Wrinkled petiole margins, a typical character that it shares with the *M. aurantiaca, M. mannii* and *M. sikkimensis*.

However, a broad and highly wrinkled leaf sheath is characteristic of *M. ochracea* and can identify this species in the vegetative stage by this character. This species also differs from other members of the genus by its very small seeds.

24. *Musa ornata* Roxb., Hort. Bengal. 19. 1814. **Fig. 25**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 1–2.5 m tall, glaucous, pale green. Leaves arranged terminally, erect to intermediate, bases asymmetric, one side rounded and other pointed or both sides rounded; petioles 24–55 cm long, glaucous. Inflorescence erect; peduncle glabrous; female and male bud lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, adaxially lilac with yellow apex, faintly glaucous, abaxially pale lilac with cream towards the base; flowers arranged in a single row. Infructescence compact with 3–6 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, slightly ridged, glabrous, pale green to green when young, greenish-yellow or pale yellow when ripen; seeds oblate.

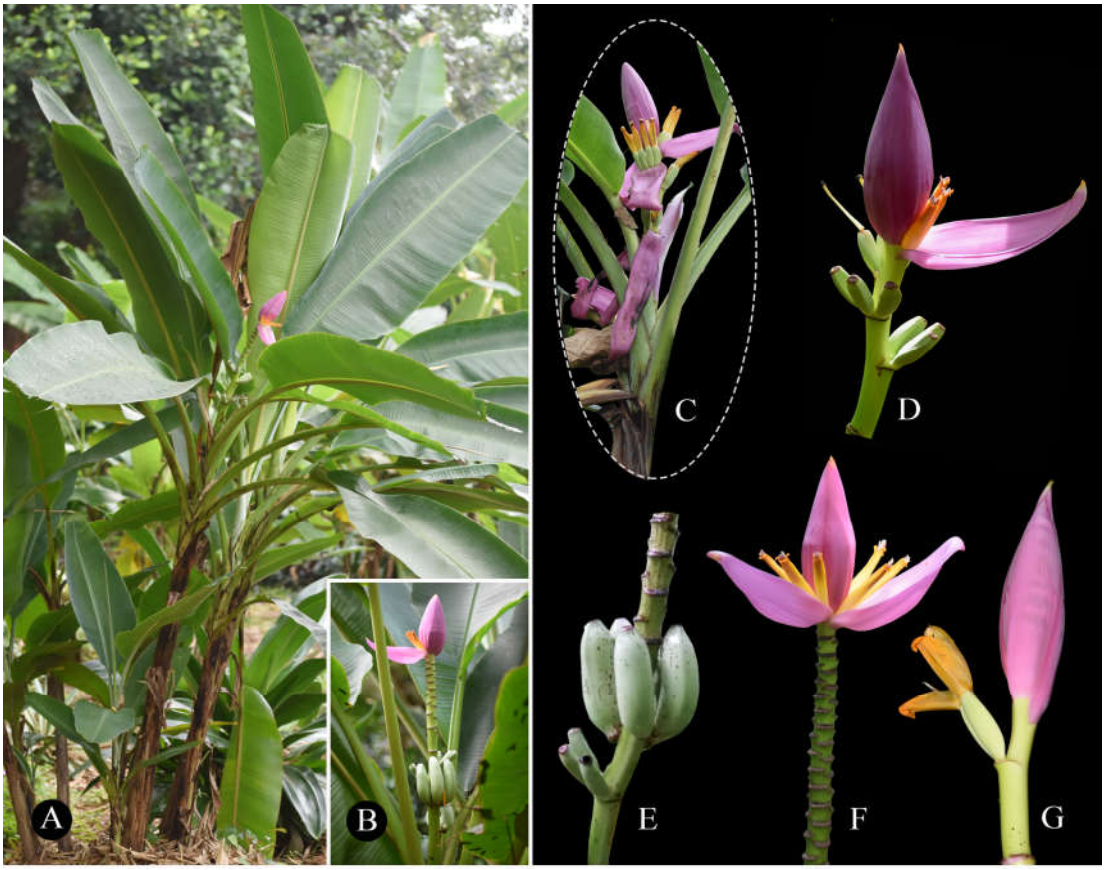


Fig. 25. *Musa ornata*: A. Habit; **B–D.** Different stages of inflorescence; **E.** Fruit bunch; **F.** Male bud; **G.** Female bud with female flower.

***Distribution*:** Bangladesh, India (Andhra Pradesh, Mizoram and Odisha), and Myanmar

***Notes*:** Lilac-coloured bracts distinguished this species from other ornamental bananas.

25. *Musa pradhanii* A.Joe & M.Sabu, Revision of Indian Musaceae, 2019.

Fig. 26

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 2–2.5 m tall, cylindrical, glaucous towards apex, green or greenish-yellow. Leaves arranged terminally; petioles 30–53 cm long, pale green or greenish-yellow. Inflorescence sub-horizontal; peduncle puberulent, green or green with purple colouration; female and male bud lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, faintly glaucous adaxially, pinkish-purple on both sides; flowers arranged in two rows. Infructescence lax with 4–6 hands, fingers pointed upwards; fruits narrowly oblong, pedicellate, straight or curved, prominently angled, glabrous, slightly pointed at apex, green when young and mature; seeds oblate.

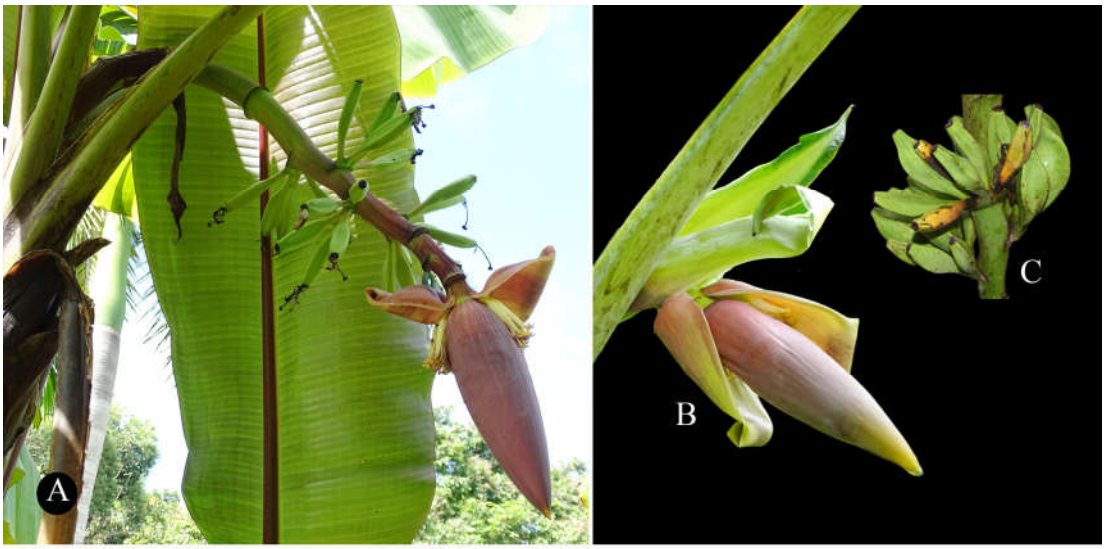


Fig. 26. ***Musa pradhanii*: A.** Habit; **B.** Female bud; **C.** Fruit bunch. (PC: **A & B-** K.P. Smisha).

***Distribution*:** Endemic to West Bengal.

***Notes*:** This species is similar to *M. sikkimensis*, but differs from it in having pseudostem colour as green or yellow-green (*vs.* green with dark reddish black blotches or completely dark reddish black), female and male buds lanceolate (*vs.* ovate-oblong), male bracts pink-purple with creamy yellow apex (*vs.* dark violet-purple with yellow apex), seeds small, 0.3–0.4 × 0.5–0.6 cm (*vs.* seeds large, 0.9–1.2 × 0.9–1.1 cm) and seed surface warty (*vs.* seed surface smooth).

26. *Musa puspanjaliae* Gogoi & Häkkinen, Nordic J. Bot. 31(4): 473. 2013. **Fig. 27**

Fig. 27. *Musa puspanjaliae*: A. Habit; **B–D;** Different types of infructescence. (PC: **A, B & C-** V.S. Hareesh).

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 7.5–9 m tall, glaucous, green to dark green with greenish-black blotches. Leaves arranged terminally, bases asymmetric, both sides rounded; petioles 30–45 cm long, faintly glaucous, green. Inflorescence pendulous; peduncle glabrous to pubescent; female bud lanceolate, imbricate; male bud ovoid, imbricate; bracts and flowers attached separately on the axis; bracts reflexed, non-revolute

before falling, glaucous, adaxially pale pink or pink-purple or pale yellowish green, abaxially pale-pink to purple; flowers arranged in two rows. Inflorescence compact with 5–10 hands; fruits broadly oblong, straight, prominently 3-angled, slightly pointed at apex without floral relicts, glaucous, pale green when young, green with powdery appearance when ripen; seeds oblate.

***Distribution*:** Endemic to Arunachal Pradesh.

***Notes*:** Fruits of this species are more allied to the species of *Ensete* than the species of *Musa*. It is perennial and takes more than one year to flower.

27. *Musa rubra* Wall. *ex* Kurz., J. Agric. Soc. Ind. 14: 301. 1867. **Fig. 28.**

Musa laterita Cheesman, Kew Bull. 4(3): 265. 1949.

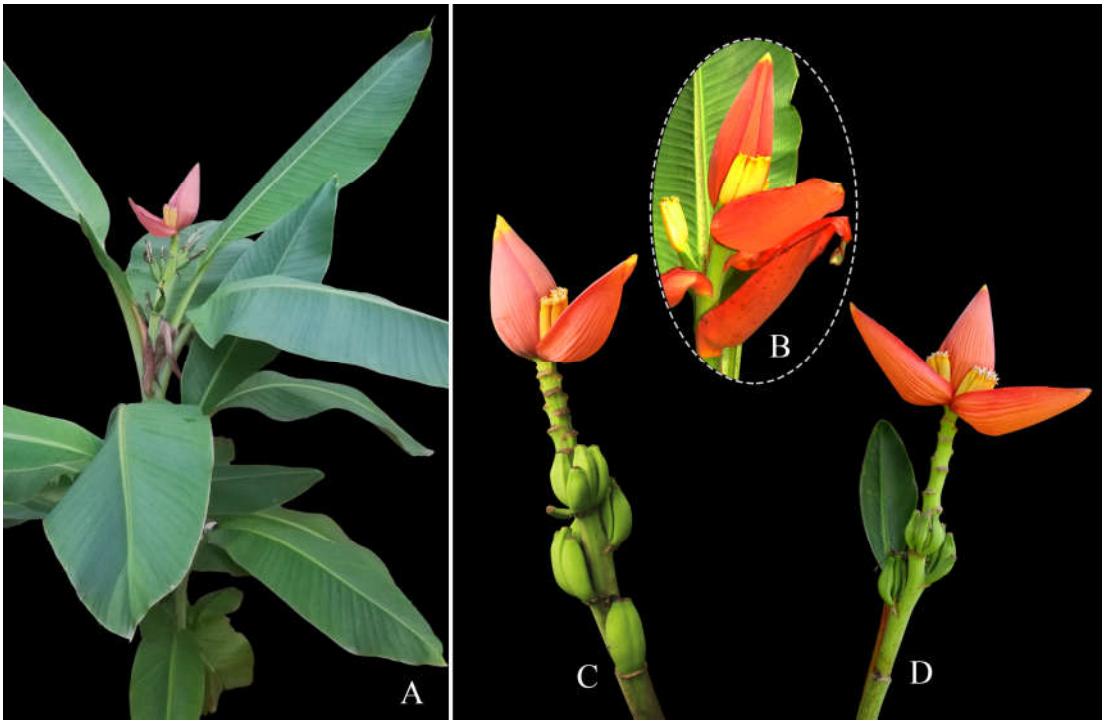


Fig. 28. *Musa rubra*: A. Habit; **B.** Female bud with female flowers; **C & D.** Inflorescence. (PC: **B & D-** K.P. Smisha).

Perennial, rhizomatous, suckering, not-clump-forming herbs, spreading by running rhizomes. Pseudostems 0.3–2.5 m tall, cylindrical, pale green with black or dark reddish-brown patches. Leaves arranged terminally, erect, bases symmetric, both sides pointed; petioles 20–40 cm long, margin scarious, blackish-brown.

Inflorescence erect; peduncle densely puberulent; female and male bud lanceolate, imbricate at apex, male bud top-shaped in an advanced stage; bracts and flowers inserted separately on the axis; bracts reflexed, non-revolute before falling, revolute in female bracts, red to brick red on both sides, faintly glaucous adaxially; flowers arranged in two rows. Infructescence compact with 4–6 hands; fingers pointed upward; fruits narrowly oblong, pedicellate, straight, prominently ridged, slightly pointed at apex without floral relicts, glabrous, pale green to green when young, yellow when ripen; seeds oblate.

***Distribution*:** India (Manipur and Mizoram), Myanmar and Thailand.

***Notes*:** Running rhizome, red-coloured bract, acute or obtuse-shaped leaf apex are the unique features of this ornamental species.

28. *Musa sabuana* K.Prasad, A.Joe, Bheem. & B.R.P.Rao, Indian J. Forest. 36(1): 151. 2013. **Fig. 29, 30**

Musa indandamanensis L.J.Singh, Taiwania 59(1): 27. 2014.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 5–9 m tall, cylindrical, pale to dark green or greenish-yellow with reddish brown patches. Leaves arranged terminally in a perfect spiral manner, bases asymmetric, one side pointed other side rounded; petioles 50–70 cm long, green, glaucous. Inflorescence sub-horizontal, peduncle glabrous, pale green to dark green; female bud cylindrical, imbricate at apex; male bud lanceolate; bracts and flowers inserted separately on the axis; bracts reflexed, non-revolute before falling, adaxially green to brown-purple (green or with brown-purple tinge or striations or patches, brown-purple or with green striation or tinge), glabrous, abaxially greenish-white, pale greenish-brown or purple; flowers arranged in two rows. Infructescence compact with 8–13 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, curved, glabrous, faintly ridged, bottle-necked at apex with or without floral relicts, pale green to dark green when young, yellow when ripen, pulp yellowish-orange; seeds oblate.

***Distribution*:** Endemic to Andaman and Nicobar Islands, India.

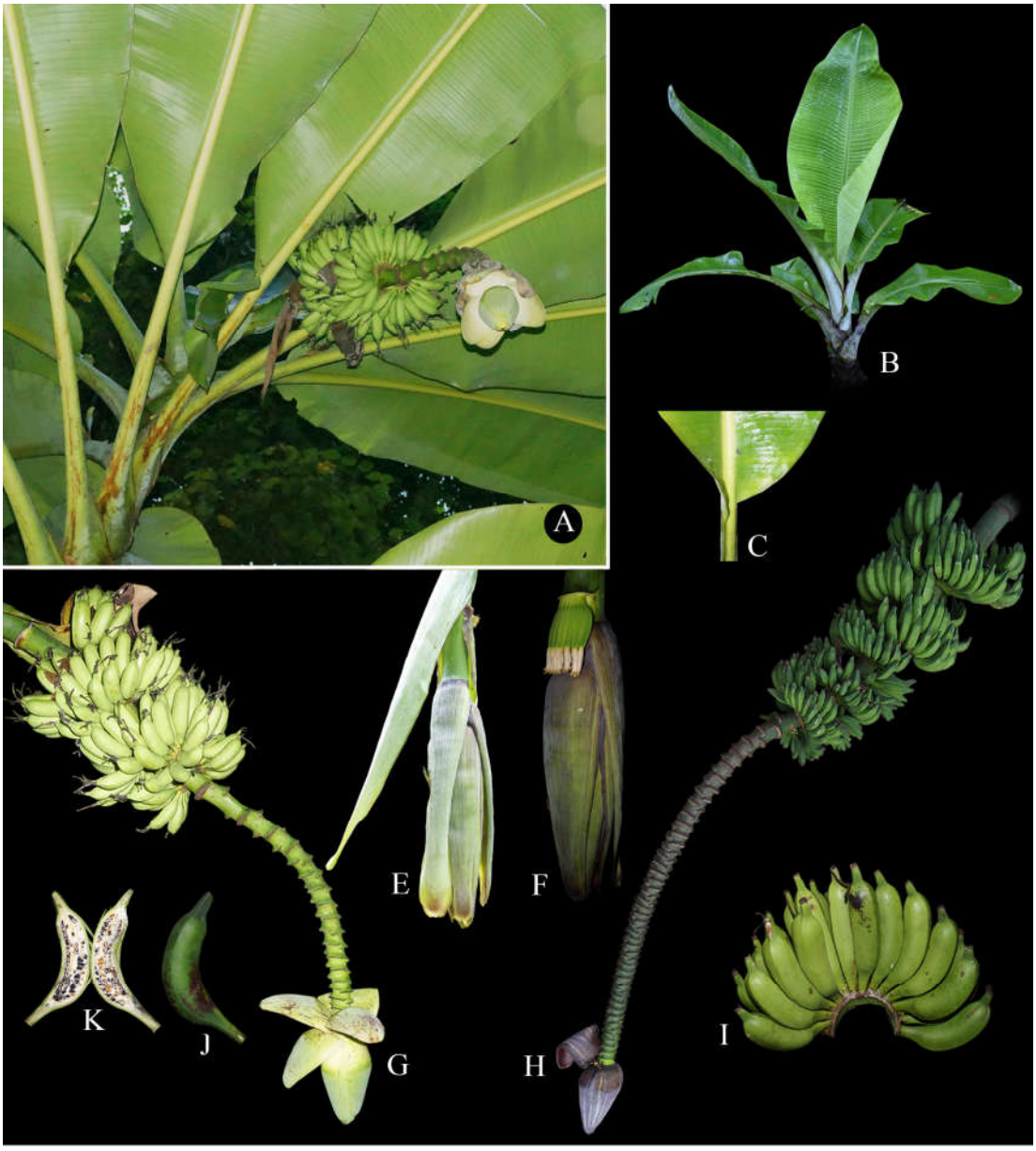


Fig. 29. ***Musa sabuana*****: A.** Habit with spiral arrangement of leaves; **B.** Young plant with glaucous petiole; **C.** Leaf base; **E & F.** Female bud; **G & H.** Infructescence with colour variation in bracts; **I.** Fruit bunch; **J.** Single fruit; **K.** Split opened fruit. (PC: **A, E, F, G, H & I-** V.S. Hareesh).

Notes**:** Protologue suggests that this species has unique features like "perfect spiral arrangement of leaves, fruits with bottle-necked apex (rarely absent) and brown-purple bracts with green striations". Based on the extensive study on the Musaceae of the Andaman and Nicobar Islands, Hareesh *et al*. (2017) reported the extended distribution of this species from the Great Nicobar Islands and gave a detailed account of the variation in bract colour from green to purple with intermediate shadings. Recently, Singh *et al.* (2020) treated *M. sabuana* as a synonym of a

morphologically distinct species *M. balbisiana* based on molecular study with only the ITS and *trnL-F* intergenic spacer as markers. However, while examining voucher specimens of their molecular materials, we found inconsistency in the collection locality.

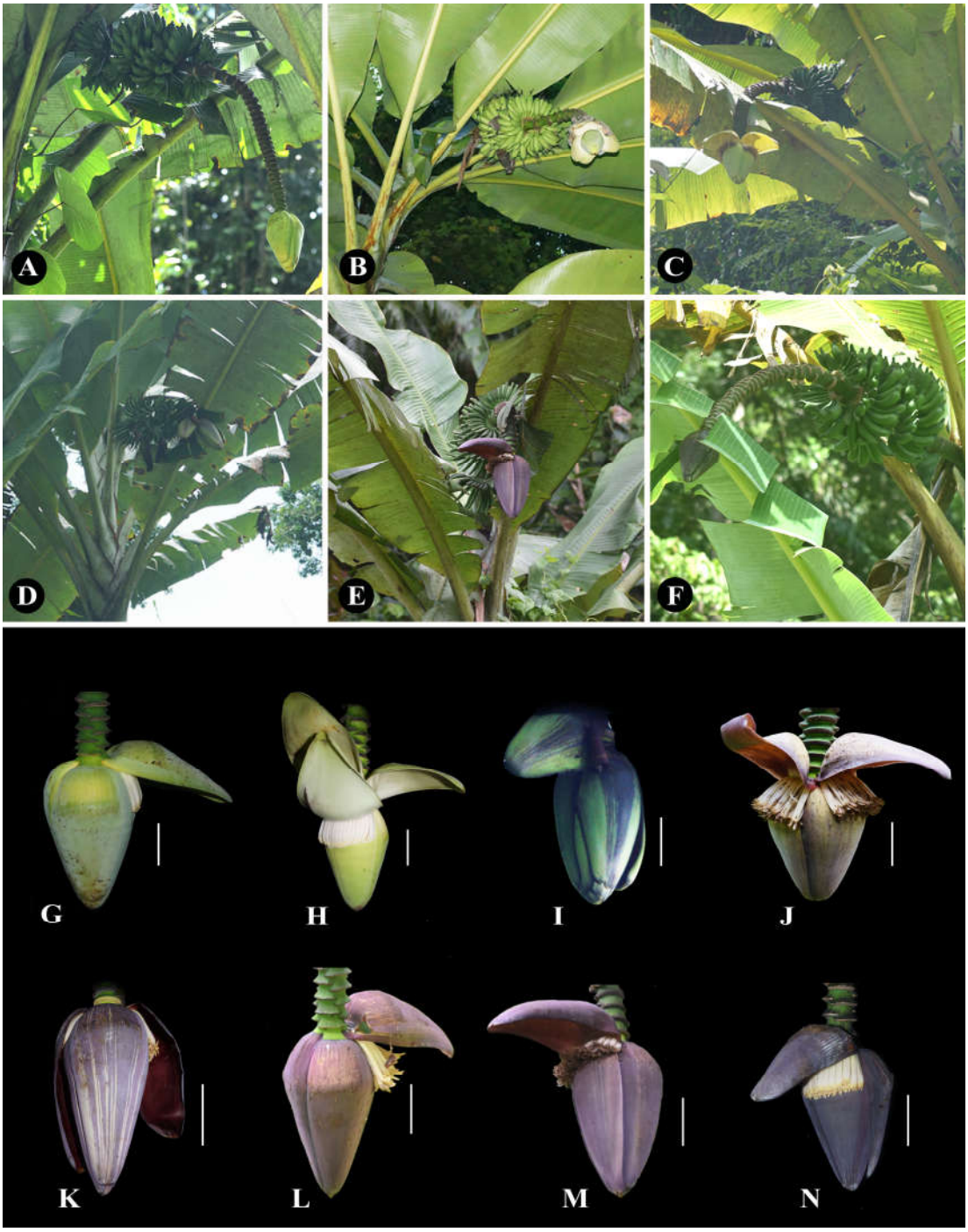


Fig. 30. Phyllotaxy and Bract colour variations in ***Musa sabuana*: A–F.** Habit with spiral arrangement of leaves; **G–N.** Male buds with bracts showing colour variation from green to brown-purple (Scale = 5 cm). (PC: **B, H, I, J & K-** V.S. Hareesh).

So, we carried out a three-marker (ITS, *trnL-F*, *rps*16) based phylogenetic study with three accessions of *M. sabuana* and two accessions of *M. balbisiana*. Based on this study we reinstate *M. sabuana* from the synonymy of *M. balbisiana* and establish the colour variation of bract in *M. sabuana*. Moreover, the present study supports the synonimization of 'green-bracted' species- *Musa indandamanensis* under *M. sabuana*. The spiral arrangement of leaves, bracts colour ranges from green to brown-purple, fruits with bottle-necked apex and yellowish-orange pulp are the unique character of this narrow endemic Island banana.

29. *Musa sanguinea* Hook.f., Bot. Mag. 98: t. 5975. 1872. **Fig. 31**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 0.9–1.2 m tall, cylindrical, green. Leaves arranged terminally, erect, bases symmetric, both sides rounded or cordate; petioles 30–60 cm long, margin smooth. Inflorescence erect; peduncle glabrous, bright red; female and male bud lanceolate, convolute at apex; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, blood red on both sides, faintly glaucous adaxially; Basal flower hermaphroditic; flowers arranged in single rows. Infructescence lax with 3–4 hands with 3 fruit per hand; fingers pointed upward; fruits narrowly oblong, pedicellate, straight, prominently ridged, slightly pointed at apex, glabrous, pale green to green when young, pale-yellow green when ripen; seeds oblate (Reproduce morphologically important characters from Hooker, 1872).

***Distribution*:** Endemic to India (Assam)

***Notes*:** Bright red coloured bracts that reflex and revolute before falling. This species shows similarities with *M. rubra* but differs by its revolute nature of bracts (*vs.* non-revolute) and clump-forming rhizomes (*vs.* running rhizomes). The recent studies of Joe and Sabu (2019) mentioned that this species is possibly extinct in the wild because the repeated efforts to collect this species from Assam and other parts of northeastern India were all in vain.

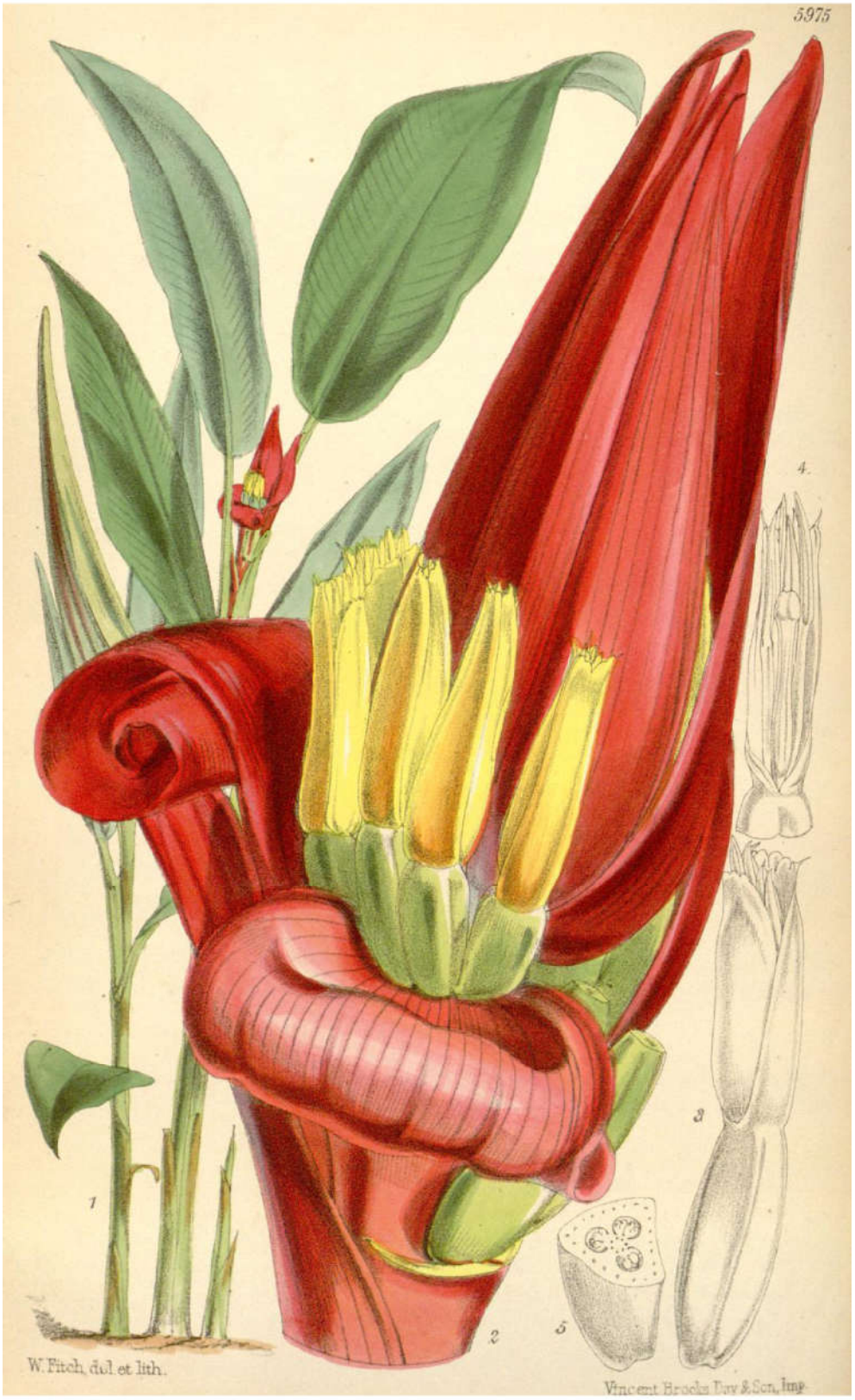


Fig. 31. Lectotype of ***Musa sanguinea*:** Hook.f., Icon in Bot. Mag. 98: t. 5975.

30. *Musa sikkimensis* Kurz., J. Agric. Hort. Soc. India 5: 164. 1878. **Fig. 32**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 5–8 m tall, cylindrical, dark reddish-black or green with dark reddish-black blotches. Leaves arranged terminally, bases symmetric, one side pointed other side rounded; petioles 35–60 cm long, glaucous, green, margins dry wrinkled, scarious. Inflorescence horizontal; peduncle green, puberulent; female and male bud ovate-oblong, imbricate; bracts and flowers inserted separately on the axis; bracts reflexed and revolute before falling, adaxially dark violet-purple with or without yellow apex, faintly glaucous, abaxially reddish-purple; flowers arranged in two rows. Infructescence lax with 4–7 hands; fingers pointed upwards; fruits broadly oblong, pedicellate, curved, prominently angled, slightly pointed or truncate at apex, glabrous, green when young and maturity; seeds oblate.

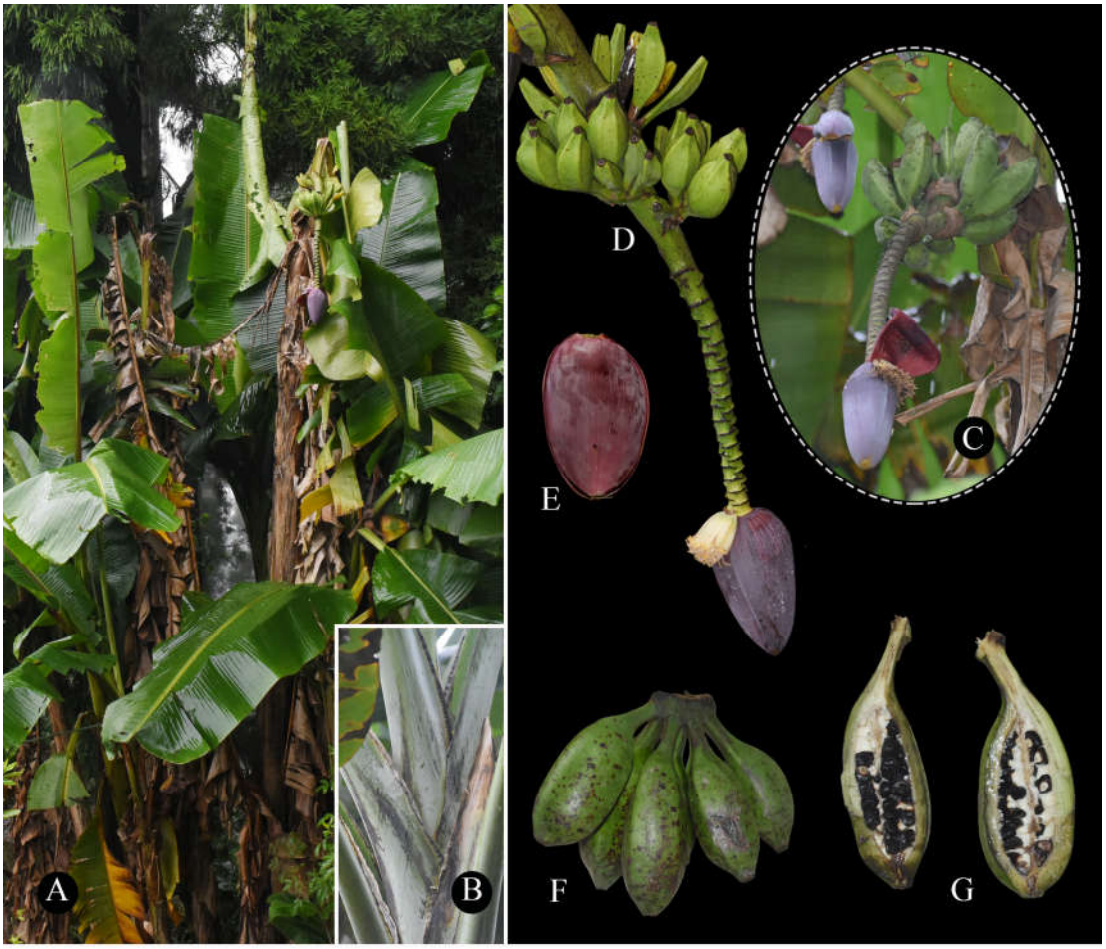


Fig. 32. *Musa sikkimensis*: A. Habit; **B.** Petiole bases with wrinkled margins; **D & E.** Infructescence; **E.** Bract; **F.** Fruit bunch; **G.** Split opened fruit.

***Distribution*:** Bhutan, India (Arunachal Pradesh, Manipur, Mizoram, Sikkim and West Bengal), Nepal

***Notes*:** It is a cold-resistant banana species. Usually occurring at an elevation from 150-2000 m but prefer high altitude and low-temperature conditions. The ovate-oblong shape of the male bud and dark violet-purple bract are unique features of this species.

31. *Musa sikkimensis* Kurz var. ***simmondsii*** A. Joe & M. Sabu, Webbia 71(1): 56. 2016. **Fig. 33**

Fig. 33. *Musa sikkimensis* var. ***simmondsii*****: A.** Habit; **B.** Mature infructescence.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 4–7 m tall, cylindrical, greenish-yellow with red pigmentation, faintly glaucous towards apex. Leaves arranged terminally, bases symmetric, one side pointed other side rounded; petioles 32–50 cm long, glaucous, green to pale greenish-yellow, margins wrinkled. Inflorescence pendulous, peduncle green, puberulous; male bud ovate-oblong, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, faintly glaucous, adaxially dark violet-purple with yellow apex, abaxially red-purple with yellow apex; flowers arranged in two rows. Infructescence compact with 2–3 hands; fruits broadly oblong, pedicellate, pedicel fused at base, straight, pointed upwards, nearly

rounded without ridges, slightly pointed at apex without floral relicts, glabrous, green when young and maturity; seeds oblate.

***Distribution*:** Endemic to Manipur.

***Notes*:** This variety differs from *M. sikkimensis* var. *sikkimensis* by the characters *viz.*, Male bracts closely arranged (*vs*. spatially arranged), bracts scar prominent (*vs*. scarcely prominent), fruit bunch compact (*vs*. lax), fruits almost spherical (*vs*. elongated), pedicel base fused (*vs*. distinct) and seeds sharply angled (*vs*. smooth).

32. *Musa thomsonii* (King *ex* Baker) A.M.Cowan & Cowan, Trees North Bengal 135. 1929. **Fig. 34**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 4–5 m tall, cylindrical, glaucous towards the apex, pale green with reddish-brown patches. Leaves arranged terminally, bases symmetric, both sides rounded; petioles 46–50 cm long, faintly glaucous, petiole margins blackish-brown, scarious. Inflorescence horizontal, peduncle, glabrous dark green; female buds lanceolate, imbricate; male bud lanceolate to ovate or intermediate to top-shaped at advanced blooming, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, adaxially purplish-brown with yellow streaks, faintly glaucous, abaxially cream to creamy yellow; flowers arranged in two rows. Infructescence lax with 8–12 hands; fingers pointed upwards; fruits narrowly oblong, pedicellate, straight or curved, prominently ridged, pointed at apex without any floral relicts, glabrous, pale green to green when young, yellow when ripen; seeds oblate.

***Distribution*:** Endemic to Northeastern India (Meghalaya, Mizoram and Sikkim).

***Notes*:** The adaxial surface of the bract with vertical streaks of yellow and purplish-brown, yellow abaxially are the major character of this species.

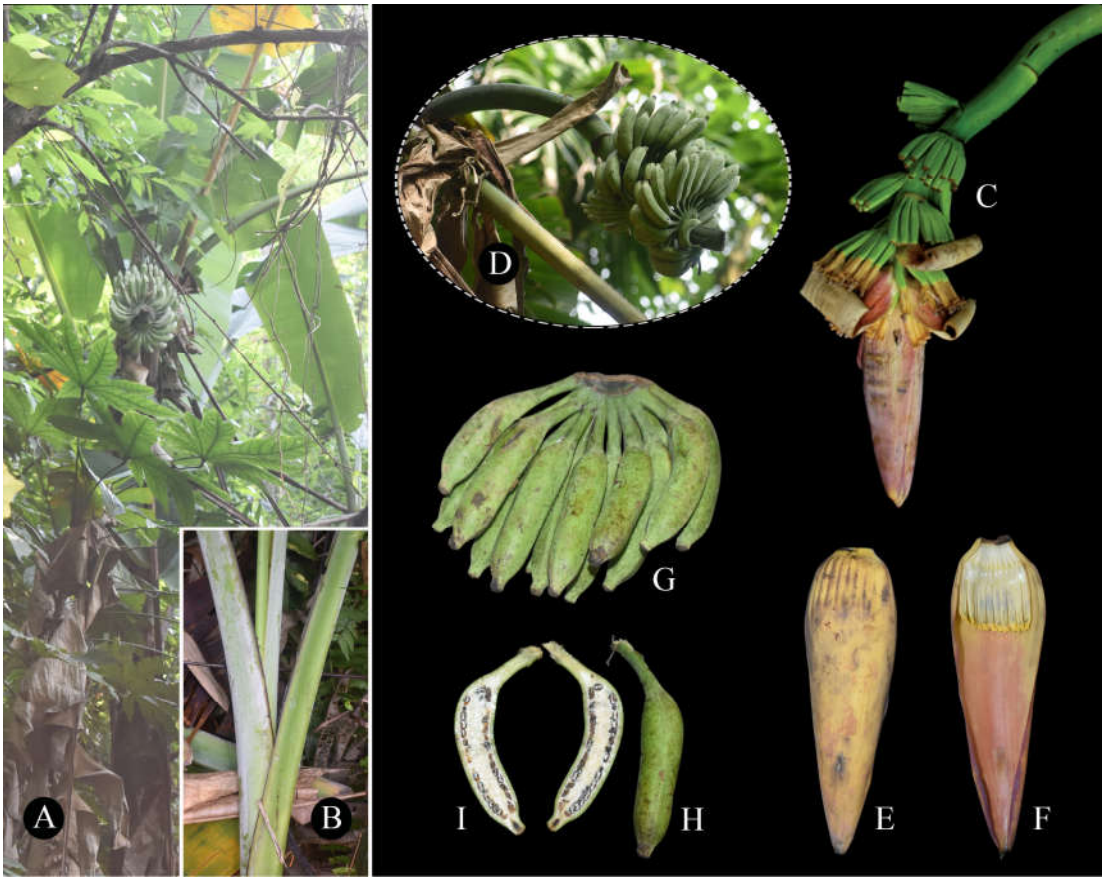


Fig. 34. ***Musa thomsonii*****: A.** Habit; **B.** Petiole base; **C.** Immature infructescence; **D.** Mature infructescence; **E & F.** Male bracts; **G.** Fruit bunch; **H.** Fruit; **I.** Split opened fruit. (PC: **C**-A. Joe).

33. ***Musa velutina*** H.Wendl. & Drude, Gartenflora 24: 65, t. 823. 1875. **Fig. 35**

Musa dasycarpa Kurz, J. Agri. Soc. India 14(1): 301. 1867.

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 0.45–1.8 m tall, cylindrical, pale green or creamy green to pale purplish, with brown or reddish-brown blotches. Leaves arranged terminally, intermediate to drooping, bases asymmetric, one side pointed other side rounded or both sides rounded; petioles 15–55 cm long, green, margins scarious with dry appearance. Inflorescence erect; peduncle pubescent, cream or creamy-pink to deep pink or red; female and male buds lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, adaxially pale pink, abaxially pink or reddish-pink; flowers arranged in a single row. Infructescence compact with 3–6 hands; fingers pointed upwards; fruits narrowly oblong, straight,

prominently ridged, blunt at apex with or without floral relicts, densely pubescent, pink or red when young, peel splits open when ripen, exposing pulp and seeds; seeds oblate.

Fig. 35. ***Musa velutina*****: A.** Habit; **B & C.** Immature infructescence; **D & E.** Mature infructescence with schizocarpic fruits.

Distribution**:** India (Arunachal Pradesh, Assam, Meghalaya and Nagaland) and Myanmar.

Notes**:** Fruits pinkish with densely covered hairs. Fruit wall splits open or shows self peeling nature (schizocarpous), which is a unique feature shared with *M. schizocarpa* from Papua New Guinea.

34. ***Musa velutina*** H.Wendl. & Drude var. ***variegata*** A.Joe, M.Sabu & Sreejith, Pl. Syst. Evol. 300(1): 13. 2014. **Fig. 36**

Perennial, rhizomatous, suckering, stoloniferous, clump-forming herbs. Pseudostems 0.6–0.7 cm tall, cylindrical, pale green. Leaves arranged terminally,

bases asymmetric, one side pointed other side rounded; petioles 18–24 cm long, pale pink. Inflorescence erect, peduncle whitish-pink, densely pubescent; female and male bud lanceolate, convolute; bracts and flowers inserted separately on the axis; bracts reflexed, revolute before falling, dark red with whitish-pink striations on both sides, densely pubescent with white hairs towards the apex of adaxial surface; flowers arranged in single row. Infructescence compact with 3–4 fruits per hand; fingers pointed upwards; fruits narrowly oblong, pedicellate, pubescent, angled or round, truncate at apex without floral relicts, pink to red when young, split opened when ripen; seeds oblate.

Fig. 36. ***Musa velutina*** var. ***variegata*****: A.** Habit; **B.** Inflorescence; **C.** Immature inflorescence showing female bud and female flower; **D.** Male bracts.

Distribution**:** Endemic to Assam.

Notes**:** Bracts abaxially variegated and red with cream or creamy pink striations.

CHAPTER 4 PHENETICS OF INDIAN MUSACEAE

Phenetics or taximetrics is the overall similarity-based system of classification. Here, the character states of each character are converted into numerical data, which is then analysed using computer algorithms and statistical techniques to generate phenograms that depict the degree of similarity between different taxa (Sneath & Sokal, 1973).

Materials and Methods

The present study included 33 taxa of Indian Musaceae (Operational Taxonomic Unit- OTU), including two species of *Ensete* and 31 taxa of *Musa*. A total of 64 morphological characters were selected and many of the morpho-taxonomic traits were adopted from the INIBAP *Musa* Descriptor List (IPGRI-INIBAP/CIRAD, 1996). The present morpho-taxonomic characters consisted of vegetative and reproductive phases, quantitative and qualitative characters as well as binary and multistate characters. For the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree building [Optimality criterion = distance; Branch swapping algorithm = Tree Bisection Reconnection (TBR)], PAUP* 4.0a 169 version software (Swofford, 2003) was used. All characters were given equal weights and directly converted to numeric codes with respect to the character states. Microsoft Excel was used to plot all 33 OTUs, 64 characters and their corresponding character states. This tabulated data was then used to create a phenogram (UPGMA tree). The morpho-taxonomic characters used in the present study are given in table 3.

Table 3. Morpho-taxonomic characters used in phenetic study

Sl. No.	Character	Character states	Remarks
i. Vegetative Characters **a. Underground stem**			
1.	Perennial/ monocarpic	0. Monocarpic 1. Perennial	

2.	Rhizome	0. Non-suckering 1. Suckering	
3.	Rhizome behaviour	0. Single 1. Clump forming 2. Running	
b. Pseudostem			
4.	Aspect	0. Slender 1. Stout	
5.	Shape	0. Cylindrical 1. Conical	
6.	Base shape	0. Almost similar to the width of entire pseudostem 1. Swollen 2. Distinctly swollen	
7.	Height (m)	0 <1.5 1. 1.5–3 2. 3.1–5 3. 5.1–7 4. >7	Recorded from the base of petiole to emergence point of peduncle
8.	Pseudostem base circumference (cm)	0. <10 1. 10–20 2. 21–49 3. 50–90 4. >90	
9.	Surface nature	0. Glaucous 1. Glabrous	Towards the apex of pseudostem
10.	Sap colour	0. Watery 1. Milky 2. Orange	
c. Leaf			
11.	Leaf habit	0. Erect 1. Horizontal to drooping (Intermediate) 2. Erect to intermediate or drooping	
12.	Position on pseudostem	0. Terminal tuft 1. Spatially arranged in the pseudostem	

13.	Arrangement on pseudostem	0. Irregular 1. Spiral	
14.	Shape of mature lamina	0. Oblong 1. Oblong or obovate 2. Oblanceolate	Recorded from the 3rd leaf. The 3rd leaf is counted from the last leaf that emerged just before the inflorescence bud emergence.
15.	Maximum lamina length (cm)	0. <150 1. 151– 200 2. >200	Measured from 3rd leaf
16.	Shape of lamina apex	0. Acute to obtuse 1. Truncate	Observed from 3rd leaf
17.	Appearance of the lower surface of leaf	0. Glaucous 1. Non-glaucous	
18.	Insertion point of lamina on petiole	0. Symmetric 1. Asymmetric	Observed from 3rd leaf
19.	Shape of lamina base	0. Both sides rounded 1. One side rounded and one pointed 2. Both sides pointed 3. Both sides auriculated 4. One side rounded and other auriculated	Observed from 3rd leaf
d. Petiole			
20.	Colour	0. Green 1. Yellow 2. Green with pink or purple patches	Observed from 3rd leaf
21.	Shape of petiole canal	0. Wide V-shaped 1. U-shaped 2. Margins curved inward	Observed from 3rd leaf. Cut the petiole halfway between the pseudostem and leaf blade and examine the cross-section.
22.	Petiole margins	0. Winged and not clasping the pseudostem	Observation should be made on the fusion point of

		1. Winged and clasping the pseudostem 2. Not winged and clasping the pseudostem	petiole and pseudostem.
23.	Petiole surface nature	0. Glabrous 1. Glaucous	Observed from 3rd leaf
24.	Nature of base margin	0. Wrinkled 1. Smooth	
ii. Reproductive characters			
a. Infructescence			
25.	Shape	0. Pendulous 1. Erect 2. Horizontal 3. Sub horizontal 4. Arch shaped 5. Horizontal to pendulous	
b. Peduncle			
26.	Maximum Peduncle length (cm)	0. < 20 1. 20–40 2. 41–60 3. >60	Measured from the point of emergence from the pseudostem to first hand in the mature infructescence.
27.	Predominant colour	0. Light green to green 1. Green to red 2. Green to yellow-green 3. Pale pink to maroon	
28.	Surface nature	0. Glabrous (hairless) 1. Pubescent or puberulent (hairy) 2. Glaucous (powdery appearance)	
c. Inflorescence (buds and bracts)			
29.	Male bud shape	0. Ovoid 1. Lanceolate	
30.	Arrangement of female bracts (female bud imbrication)	0. Convolute 1. Moderately imbricate 2. Highly imbricate	

31.	Longevity of bract	0. Deciduous 1. Deciduous or persistent 2. Persistent	
32.	Groove nature in bract	0. Few grooves or not grooved 1. Moderately grooved 2. Strongly grooved	
33.	Colour fading in bract	0. Colour discontinuing towards the apex 1. Colour homozygous	
34.	Striations on the external phase of bract	0. Absent 1. Present 2. Present or absent	
35.	Bract apex shape	0. Acute 1. Obtuse	
36.	Colour of the bract internal face (abaxial)	0. Cream to creamy yellow 1. Green 2. Pink-purple to red-purple 3. Creamy red to red 4. Orange 5. Lilac 6. Pale pink to pink 7. Light green to purple 8. Bright red	
37.	Colour of the bract external face (adaxial)	0. Cream to orange yellow 1. Green 2. Pink purple to dark brown purple 3. Pinkish red to dark red 4. Orange 5. Lilac 6. Maroon 7. Pink to pale pink 8. Green to purple 9. Bright red	
38.	Bract behaviour before falling	0. Revolute 1. Not revolute	Recorded as the bract lifted up to the horizontal.

	d. Flower		
39.	Number of rows of female flowers (or fruits) per bract	0. One 1. One or two 2. Two	
40.	Insertion point of flower	0. Base of the bract 1. Peduncle	
41.	Arrangements of ovule per locule	0. Two rowed 1. Four rowed	
42.	Compound tepal basic colour (female)	0. Creamy 1. Yellow to orange 2. Creamy with pink flush	
43.	Free tepal appearance	0. Smooth 1. Slightly corrugated 2. Highly corrugated	
44.	Compound tepal nature	0. Divided up to the base 1. Divided only at apex	
45.	Free tepal nature	0. Divided with two lateral sub-orbicular lobe and elongated central lobe 1. fused	
46.	Style basic colour	0. Cream 1. Creamy yellow to creamy orange	Noticed in fresh flower
47.	Stigma colour	0. Cream to yellow 1. Grey to brown	Noticed in fresh flower
48.	Ovary surface nature	0. Glaucous 1. Glabrous 2. Hairy	
49.	Ovary colour	0. Different shades of green 1. Cream 2. Yellow 3. Creamy pink 4. Maroon	Noticed in fresh flower
	e. Fruit bunch/ Fruit		
50.	Bunch position (arrangement) on peduncle	0. Erect 1. Horizontal 2. Hanging	
51.	Bunch appearance	0. Lax 1. Compact	

52.	Fruit general appearance	0. Slender 1. Stout	
53.	Fruit peel colour	0. Green 1. Pink to maroon	
54.	Maximum number of fruits on mid hand	0. <10 1. ≥10	
55.	Maximum fruit pedicel length (mm)	0. < 10 1. 10–30 2. >30	
56.	Fruit shape (longitudinal curvature)	0. Straight 1. Straight or curved 2. Curved (sharp curve)	
57.	Fruit length (cm)	0. <10 1. 10–15 2. >15	
58.	Fruit surface nature	0. Glabrous 1. Glaucous 2. Hairy	
59.	Geotropism of fruits	0. Negatively geotropic 1. Positively geotropic 2. Fruits almost perpendicular to axis	
60.	Cross-section of fruit	0. Pronouncedly ridged 1. Slightly ridged 2. Rounded	
61.	Shape of fruit apex	0. Pointed 1. Lengthily pointed 2. Blunt-tipped	
62.	Remains of floral relicts at fruit apex	0. Without any floral relicts 1. With persistent floral relicts 2. With or without floral relicts	
63.	Mature fruit peel colour	0. Yellow with black blotches 1. Yellow with a powdery or dry appearance. 2. Pink to maroon 3. Dull green to brownish green	
f. Seed			
64.	Seed size (cm)	0. < 0.8 × 0.8 1. ≥ 0.8 × 0.8	

Here attempted the phenetics or taximetrics or numerical taxonomy of Indian Musaceae based on 64 reliable and useful morpho-taxonomic characters. Almost all vegetative (24) and reproductive (40) phases are considered. The characters are selected irrespective of the phylogenetic relations. Quantitative (8) and qualitative (56) as well as binary (22) and multistate (42) characters are included in this analysis. Character states started from 2 and extended up to 10. The character types are given in the table 4.

Table 4. Character types used in the present phenetic study

Types	**Characters**	**Numbers**
Phases of plant	Vegetative	24
	Reproductive	40
Quantitative/qualitative	Quantitative	8
	Qualitative	56
Binary/ multistate	Binary	22
	Multistate	42
Total		64

Results and Discussion

On the basis of 64 morpho-taxonomic characters, the genus *Musa* in India has been classified into two groups (Cluster I and Cluster II). Cluster I is made up of the taxa that belong to Cheesman's (1947a) section *Eumusa* and Cluster II is formed by the members of the section *Rhodochlamys* (ornamental bananas) except *M. arunachalensis*, this ornamental species is clustered in Cluster I aside with other section *Eumusa* taxa. *Ensete glaucum* and *E. superbum* are formed as out-groups (Fig. 37).

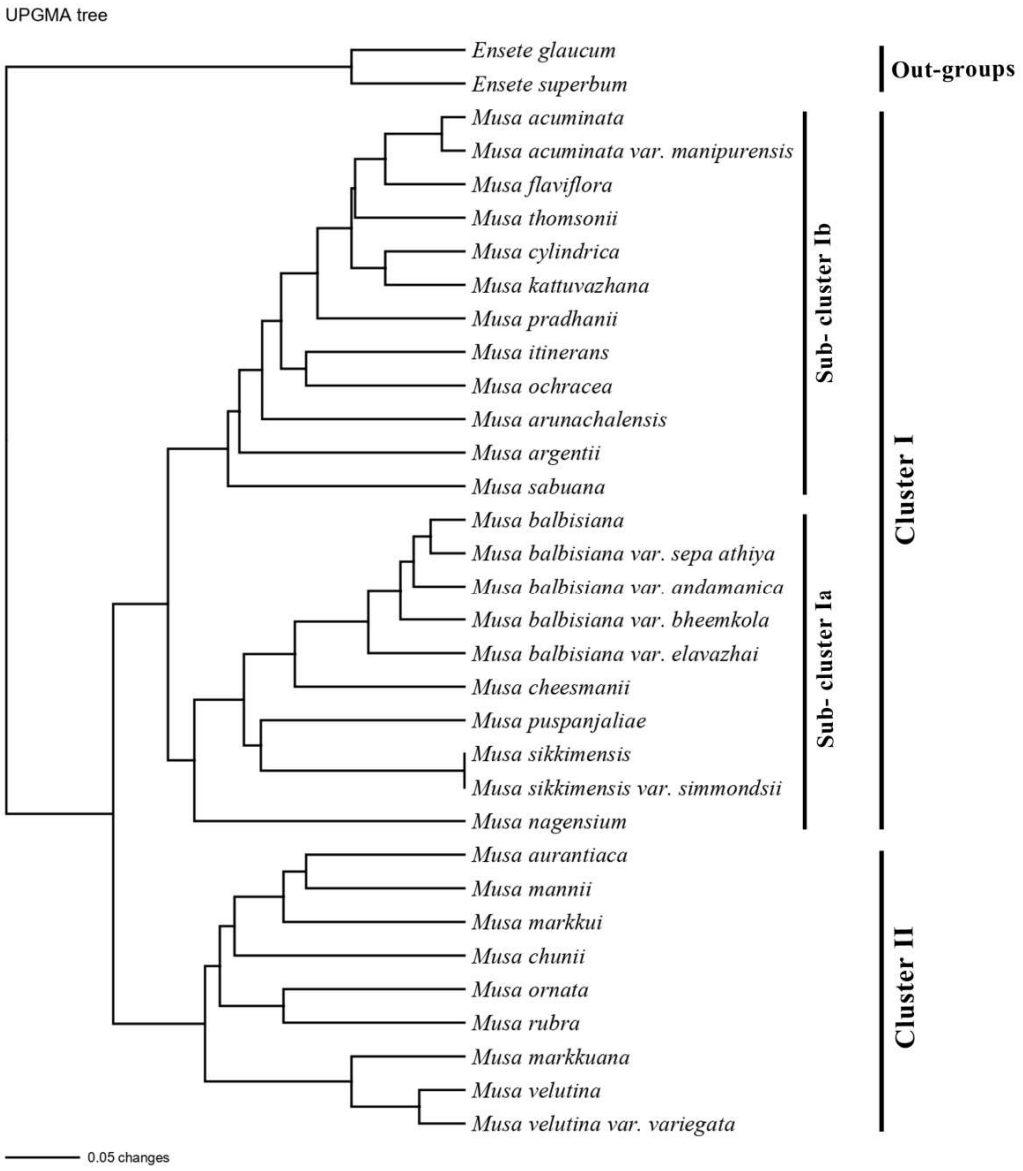


Fig. 37. UPGMA tree of Indian Musaceae based on 64 morpho-taxonomic characters.

Cluster I consisted of 22 taxa of section *Eumusa* and all are medium to large-sized (> 3m) plants having horizontal to pendulous type inflorescence with two-rowed flowers or fruits in a single bunch. This includes the most important progenitors of the cultivated bananas *viz*., *M. acuminata* and *M. balbisiana*. Cluster I is further divided into two sub-groups, namely sub-cluster Ia and sub-cluster Ib.

Sub-cluster Ia formed by 10 taxa, that are *M. balbisiana* and its varieties (var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. *sepa-athiya*) as well as the

morphologically related taxa *viz*., *M. nagensium*, *M. sikkimensis*, *M. sikkimensis* var. *simmondsii*, *M. puspanjaliae* and *M. cheesmanii*. They are characterized by large pseudostem, broadly oblong or stout fruits, non-revolute nature of bracts before falling and large seeds (≥ 0.8 cm). *Musa balbisiana*, one of the progenitors of cultivars and its 4 varieties (var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. *sepa-athiya*) are grouped together in this sub-cluster support their taxonomic resemblance. *Musa cheesmanii,* clustered along with *M. balbisiana* and its varieties, demonstrate the morphological similarity of these taxa. The high-altitude taxa, *M. sikkimensis* and its variety (*M. sikkimensis* var. *simmondsii*) did not show any variation and these taxa show phenetic similarity with *M. puspanjaliae*. The morphology of these taxa presented very unique traits, as they show similarity in fruit shapes and seed size. *Musa nagensium* is formed as a separate entity in this cluster; the possible reason for this placement may be due to its specialized morphological characters like the glaucous nature of pseudostem, petiole and leaf; spatially arranged leaves; highly imbricated flower bud apex and hanging or positively geotropic nature of fruit bunches. Considering the overall morphology of the taxa in sub-cluster Ia, this cluster can be referred to as the "*M. balbisiana* group", and it is possible that all taxa in this group may have the genome which is similar to the 'B'- genome.

The sub-cluster Ib is formed by 12 taxa. It includes the prime progenitor of cultivated bananas, *M. acuminata* along with its morphologically allied taxa (*M. acuminata* var. *manipurensis*, *M. flaviflora*, *M. thomsonii*, *M. cylindrica* and *M. kattuvazhana*), *M. itinerans* and some Indian endemic wild bananas like *M. pradhanii*, *M. ochracea*, *M. arunachalensis*, *M. argentii* and *M. sabuana.* The present study strictly supports the morphological synergy of *M. acuminata* and its allied taxa, *M. itinerans* in the sub-cluster Ib, by sharing the characters such as revolute nature of bract before falling, narrowly oblong or slender fruits and small seeds (< 0.8 cm). *Musa arunachalensis* and *M. argentii* are two species (both are recently described from Northeast India) included in this sub-cluster (sub-cluster Ib), and their placement in this UPGMA tree (Fig. 37) needs further clarification. While describing *M. arunachalensis*, the authors (Sreejith *et al*., 2013) considered it as a

representative of the section *Rhodochlamys*, but the overall morphological features like medium-sized pseudostem (1.4−3 m), arch-shaped inflorescence, showy orange-coloured bracts and one or two rows of flowers or fruits in a bunch reveals that it is an intermediate form of the sections *Eumusa* and *Rhodochlamys*. *Musa argentii* is also an intermediate form of these two sections, even though it is considered as a taxon under section *Eumusa* in the protologue by the authors (Gogoi & Borah, 2014a). Its overall morphology corresponds well with section *Musa*, however, some characters like bright-coloured bract, fruit colour *etc.* suited with section *Rhodochlamys*. In the current study, this species occupied in the section *Eumusa* cluster (Cluster I) and shows close affinity with *M. arunachalensis*. *Musa sabuana* is an endemic wild banana in Andaman and Nicobar Islands with the distinguishing features of its very large lamina, perfect spiral phyllotaxy, bract colour variation from green to brown-purple and bottle-neck apex of fruit. These unique characters may help to place this species as a separate entity in this sub-cluster. As a whole, all the taxa in this group have morphological features alike *M. acuminata* and we can assume that all the members carry the genomes corresponds to the 'A'- genome. Consequently, these 12 taxa can be used as an alternative for 'A genome' donors in future breeding programmes.

Cluster II exclusively includes the ornamental bananas with small pseudostem (<3 m), erect inflorescence, bright coloured bracts and one rowed flowers or fruits in a bunch; key morphological features of section *Rhodochlamys*. A total of 9 taxa are represented in this cluster, that are *M. velutina*, *M. velutina* var. *variegata*, *M. markkuana*, *M. rubra*, *M. ornata*, *M. chunii*, *M. markkui*, *M. mannii* and *M. aurantiaca*. The clustering of *M. velutina*, *M. velutina* var. *variegata* and *M. markkuana* indicates the relatedness of these taxa.

The results of the present phenetic study of Indian Musaceae reflected Cheesman's (1947a) sectional classification. By following Cheesman's (*l.c.*) classification, Joe (2015) reported that, India represents 2 sections under the genus *Musa*, which are section *Eumusa* and section *Rhodochlamys*. The present result highly corroborates with both the observations of Cheesman (*l.c.*) and Joe (*l.c.*).

Moreover, these results were congruent with the numerical taxonomical studies of the genus *Musa* by Simmonds and Weatherup (1990). According to Simmonds and Weatherup (1990), instead of 4 sections in the genus *Musa,* there are 5 informal groupings obtained such as *Australimusa*, *Callimusa*, *Rhodochlamys*, *Eumusa* (1) and *Eumusa* (2). The *Eumusa* (1) and *Eumusa* (2) groups were derived from the splitting of Cheesman's (1947a) traditional *Eumusa* section. In the phenetic tree of Simmonds and Weatherup (1990), the *Eumusa* (2) group includes species such as *M. balbisiana*, *M. cheesmanii*, *M. nagensium M. ingens*, and *M. boman*. The *Emusua* (1) group accommodates the taxa *viz*., *M. acuminata*, ssp. *malaccensis*, ssp. *microcarpa*, ssp. *burmannica*, ssp. *banksii*, *M. flaviflora*, *M. itinerans*, *M. basjoo, M. schizocarpa,* and *M. sikkimensis*. The 5th group, *Eumusa* (2) of Simmonds and Weatherup (*l.c.*) showed similarity with the sub-cluster Ia in the present phenogram and species like *M. balbisiana*, *M. cheesmanii* and *M. nagensium* are common in both 5th group of Simmonds and Weatherup (*l.c.*) and sub-clade Ia in our UPGMA tree. The other species, *M. ingens* and *M. boman* are not present in India so they are excluded from our study. Similarly, some taxa belonging to the group *Eumusa* (1) of Simmonds and Weatherup (*l.c.*) are similar to the taxa in sub-clade Ib of the present study (*M. flaviflora*, *M. acuminata*), except for the placement of *M. sikkimensis* and *M. itinerans.*

Potential candidates for the breeding programme from Indian wild bananas

Musa paradisiaca L. is now treated as a hybrid name '*Musa* x *paradisiaca*', this finding is experimentally proved by Simmonds and Shepherd (1955). According to their study, *Musa acuminata* ('A'- genome contributor) and *M. balbisiana* ('B' genome contributor) are two wild banana species from which nearly all the edible bananas originated. The natural existence of cultivated forms of *M. acuminata* in diploid ('Kadali', 'Karivazhai', 'Pisang linin' etc.) and triploid ('Dwarf cavendish', 'Grand naine', 'Red', 'Robusta' etc.) conditions supports the possibility of the above view. However, edible diploid forms ('BB' type) of *M. balbisiana* are not known till date (Simmonds & Shepherd, 1955; Sreejith & Sabu, 2017). Based on this hybrid origin, now we have different genome constitutions in cultivars such as AAA

(dessert bananas), AAB (plantains) and ABB (cooking bananas) (Silva *et al*., 2001; Venkataramana *et al*., 2015). The 'B' genome content in the hybrid increases the starch content and it also facilitates the seed set in the cultivated forms.

For the genome classification ('A' & 'B' genome), Simmonds and Shepherd (1955) used 15 characters as being most useful for diagnostic purposes. Which includes Pseudostem colour, characteristics of petiolar canal, appearance of peduncle, length of pedicels, arrangement of ovules, ratio of bract shoulder, behaviour of bract after opening, shape of bract, shape of bract apex, colour of bract, colour fading on bract, nature of bract scars, free tepal morphology of male flower, colour of male flower and colour of stigma. The character states for these 15 characters are assigned based on the corresponding morphology of the aforementioned two species- *M. acuminata* and *M. balbisiana* (Table 5).

Each character was scored from one (typical *M. acuminata*) to five (typical *M. balbisiana*) so the possible range of total scores was therefore 15 for typical *M. acuminata* character and 75 for typical *M. balbisiana* character. The scores in between these two values were given to the hybrid genome constitutions *viz.* 45 for AB, 35 for AAB, 55 for ABB *etc*.

The present phenetic study based on 64 characters includes most of the characters mentioned by Simmonds and Shepherd (1955; Table 5). Interestingly, the character states correspond to the 15 characters for *M. acuminata* and *M. balbisiana* are observed in other *Musa* members in India.

Table 5. Fifteen morphological characters and character states used by Simmonds and Shepherd (1955).

Character	*Musa acuminata*	*Musa balbisiana*
Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches very slight or absent
Petiolar canal	Margins erect or spreading with scarious wings below, not clasping	Margin inclosed, not winged below, clasping pseudostem
Peduncle	Usually downy or hairy	Glabrous
Pedicels	Short	Long
Ovules	Two regular rows in each locule	Four irregular rows in each locule
Bract shoulder (ratio of the vertical distance from base to broadest point and base to apex)	Usually high	Usually low
Bract curling	Bracts reflex and roll back after opening	Bracts do not reflex and roll back after opening
Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
Bract apex	Acute	Obtuse
Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
Colour fading	Inside bract colour usually fades to yellow towards the base	Inside bract colour usually continuous to base
Bract scars	Prominent	Scarcely prominent
Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
Male flower colour	Creamy white	Variably flushed with pink
Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink

The character states that correspond to *M. acuminata* are also observed in *M. kattuvazhana*, *M. flaviflora*, *M. thomsonii*, *M. ochracea*, *M. argentii*, *M. sabuana*

and character states correspond to *M. balbisiana* is found exactly in *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazahi*, var. *sepa-athiya*, *M. cheesmanii*, *M. puspanjaliae*. Apart from this, the taxa like *M. pradhanii*, and the wild ornamental bananas such as *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*, *M. aurantiaca*, *M. chunii*, *M. mannii*, *M. markkui*, *M. ornata* depicts the characters which are almost corroborate with *M. acuminata*. Also, some species reflect their morphological characters corresponding to *M. balbisiana* in some extent, which includes *M. nagensium*, *M. sikkimensis* and *M. itinerans*. So, based on these observations, we hypothesize that the morphological characteristics of some Indian wild bananas correspond well with *M. acuminata* and some others correspond with *M. balbisiana*. Therefore, the morphologically allied taxa of *M. acuminata* such as *M. kattuvazhana*, *M. flaviflora*, *M. thomsonii*, *M. ochracea*, *M. argentii* and *M. sabuana* may have a strong gene pool which corresponds to 'A'- genome and the taxa like *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazahi*, var. *sepa-athiya*, *M. cheesmanii*, *M. puspanjaliae* may carry the strong gene pool which corresponds to 'B'- genome. Thus, these candidates can be strongly recommended as genome donors for breeding experiments. Apart from this, the other wild bananas include *M. nagensium*, *M. sikkimensis* and *M. itinerans* (morphologically allied more with *M. balbisiana* than *M. acuminata*) and *M. pradhanii*, *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*, *M. aurantiaca*, *M. chunii*, *M. mannii*, *M. markkui*, *M. ornata* (Morphologically allied more with *M. acuminata* than *M. balbisiana*) can be tested by interspecific hybridization to analyse their vigour. Among the above-mentioned taxa, some taxa possess unique traits such as (1). *M. ochracea*- unique taste of fruit pulp (2). *M. sabuana*- yellow-orange coloured fruit pulp (3) *M. sikkimensis*, *M. pradanii*- cold resistance (4) *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazahi*, var. *sepa-athiya*- large plant height, drought resistance, disease resistance, high sucker formation *etc*. (5) *M. cheesmanii*, *M. puspanjaliae*- large size of fruit, high number of fruit bunch *etc.* These traits can be exploited in the breeding programme.

CHAPTER 5 MOLECULAR PHYLOGENY OF MUSACEAE

The evolution of higher organisms is a challenging and interesting event to study, however being difficult due to its slow pace and the physical evidence is obtained only through fossils and ancient DNA (Ronquist, 2004). Sequencing technology was increased at the starting point of the 21st century and now it has become one of the essential parts of taxonomy as supporting evidence for the actual identity of any taxon. Moreover, this DNA data helps to elucidate the relationship, hierarchy, evolution and position of a taxon in the Tree of Life (Judd *et al.*, 2016). India is one of the diversity-rich countries with a high level of endemism, however, the molecular systematic studies and evolutionary relationships of the plant groups are poorly understood (Pandey *et al*., 2020).

The Banana family (Musaceae) is a recently evolved lineage in Monocots and basal to the Zingiberales order. Zingiberales are one of the advanced orders among the eleven orders of monocots. The Musaceae represent three genera *viz*., *Musa* L., (around 120 taxa), *Ensete* Bruce *ex* Horan. (8 species) and monotypic *Musella* (Franch.) C.Y.Wu *ex* H.W.Li (Hareesh & Sabu, 2023). The systematics of the genus *Musa* is difficult due to its large representation when compared to the other two genera in this family (Hareesh *et al*., 2017). The sectional classification of the genus *Musa* by Cheesman (1947a) was widely accepted for almost half a century without any modification, but the evolutionary relationships among the four sections of the genus *Musa* cannot be discussed with this classification. Also, the common occurrence of natural crosses between taxa belongs to Sect. *Musa* and Sect. *Rhodochlamys* has questioned this classification (Nwakanma *et al*., 2003b). Simmonds (1954) found that *Musa rubra*, an ornamental species in sect. *Rhodochlamys* can cross more easily with *M. acuminata* (sect. *Eumusa*) subspecies than with species from its own section. Moreover, the recently discovered Indian endemic species *viz*., *M. argentii, M. arunachalensis, M. markkui*, are in fact

intermediate forms of these two sections (Medium-sized plant, bright coloured bract, Inflorescence semi-pendent or arched).

AFLP-based (Wong *et al*., 2002; Nwakanma, 2003b) and molecular marker-based (Li *et al*., 2010; Liu *et al*., 2010) phylogeny of Musaceae was already attempted and reconstructed the sectional classification in the genus *Musa*. Based on this study, now only two sections are present under the genus *Musa viz*., sect. *Musa* (n=11) and sect. *Callimusa* (n=7/9/10). However, the Indian species were not represented in these studies.

Recently, many attempts were conducted to document the diversity of wild Musaceae and several new taxa were described from Northeast India, South India and the Andaman and Nicobar Islands. That includes *M. acuminata* var. *manipurensis*, *M. argentii*, *M. arunachalensis*, *M. balbisiana* var. *bheem-kola*, *M. balbisiana* var. *elavazhai*, *M. balbisiana* var. *sepa-athiya*, *M. cylindrica*, *M. markkuana*, *M. markkui*, *M. pradhanii*, *M. puspanjaliae*, *M. sabuana*, *M. sikkimensis* var. *simmondsii* and *M. velutina* var. *variegata*. Apart from these taxa, several other taxa *viz*., *M. kamengensis*, *M. aurantiaca* var. *homenborgohainiana*, *M. aurantiaca* var. *jengingensis*, *M. shankarii*, *M. nagalandiana*, *M. paramjitiana*, *M. swarnaphalya*, *M. mannii* var. *namdangensis*, *M. nagensium* var. *hongii* and *M. indandamanensis* were published during this period (Rao & Kumari, 2008; Uma *et al*., 2011; Gogoi, 2013; Gogoi, 2014; Gogoi & Borah, 2014b; Singh, 2017). However, Joe and Sabu (2019) synonymized a total of 14 taxa based on critical taxonomic studies. Conclusively, now India harbours two species of *Ensete* and 32 taxa of *Musa* (including 25 species, one variety of *M. acuminata*, four varieties of *M. balbisiana*, one variety of *M. sikkimensis* Kurz. and one variety of *M. velutina* H. Wendl. & Drude.) under two sections *viz*., sect. *Musa* (n=11) and sect. *Rhodochlamys* (n=11). Among these, 20 taxa are endemic (65%) to India. The taxonomy of Indian Musaceae based on morphology, anatomy and palynology was well explored. However, a complete molecular study of this family in India has not been attempted to date. The only molecular attempt in Musaceae from India was done by Lamare *et al.* (2017) and Singh *et al.* (2021), but both studies are in a

narrow level based on the representation of Indian endemic taxa. There is still some confusion about the actual identity of some taxa in Indian Musaceae, particularly with regard to the status of 1) recently described taxa 2) Indian endemic taxa 3) infraspecific taxa of *M. balbisiana* collected from various parts of the country.

By trailing these backgrounds, a thorough phylogenetic study of Indian Musaceae is demanding. So, here we have chosen the appropriate nuclear and chloroplast markers based on the proven utility with the possible number of accessions from the Indian taxa. This will help to reveal the actual identity of the Indian taxa of *Musa*, their taxonomic positions and relations with other *Musa* members in the world. Moreover, clarifying the phylogenetic relationships of *Musa* will offer valuable insights for leveraging the wild gene pool in future efforts to enhance edible banana varieties.

Materials and methods

Compositions of Stock Solutions

1. CTAB (Cetyl Trimethyl Ammonium Bromide) extraction buffer (Sambrook *et al*., 1989) for 1L.

   100 mL of 1M Tris (pH 8)

   280 mL of 5M NaCl

   40 mL of 0.5 M EDTA (pH 8)

   20 gm of CTAB

   Make the final volume to 10 mL using ddH_2O

2. 1M Tris, (pH 8) for 1L

   121.1 gm of Tris base is dissolved in 800 mL of ddH_2O (about 80% of the final volume)

   Make final volume to 1L.

3. 0.5M EDTA (Ethylene Diamine Tetra Acetic Acid, pH 8) for 1L.

   186.12 gm of EDTA is dissolved in ddH_2O and make up into 1L. EDTA will not dissolve until solutions pH 8. NaOH pellets were added to adjust pH.

4. 5M NaCl

   To make up 1L solution, 292.2 gm of NaCl dissolved into 70% of the final volume of ddH_2O. Bring final volume after saturation.

5. 50X TAE (Tris- Acetate- EDTA) for 1L

   242 gm of Tris base

   57.1 mL Glacial acetic acid

   100 mL of 0.5 M EDTA (pH 8)

   Tris base was dissolved in 60% of the final volume of ddH_2O and EDTA and Acetic acid were added to the solution. Make final volume to 1L using ddH_2O

6. 10X TE (Tris EDTA) for 10 mL

   1 mL of 1 M TrisHCl (pH 8)

   0.2 mL 0.5 M EDTA (pH 8)

   Make the final volume 10 mL using ddH_2O

DNA Isolation

The total genomic DNA was isolated from the fresh/silica-dried cigar leaves using the CTAB method (Doyle & Doyle, 1990) with some modifications.

Procedure:

1. Preheat 1ml of 2% CTAB extraction buffer + 2 µl β- mercapto ethanol per sample at 65^0C in the water bath on the fume bench. (β- mercapto ethanol is toxic and should be used on the fume bench.)

2. Grind 100 mg fresh leaf sample or 20- 50 mg silica dried sample using mortar and pestle. Use required volume of liquid N_2 during grinding. Add 1 ml of preheated CTAB/ β- mercapto ethanol buffer and a pinch of PVPP (Polyvinyl poly pyrrolidone) to each ground sample. Gently shake the sample; the homogenate should be green with small tissue fragments at this stage. The material: buffer ratio should be such that the solution is thick green, but not so thick that it does not pour.
3. Incubate the sample for 30 min at 65^0C in the heated block (sample can be stored or left longer at this stage for up to 1 hr). Mix the sample twice during the incubation stage by inverting the tube.
4. Remove the tube from the heated block and allow it to cool to ambient temperature for 1-2 min.
5. Add 500 µl of chloroform: IAA (Iso amyl alcohol) (24:1) to each tube. Mix gently by shaking to obtain a momentary single phase. Transfer the tubes to the orbital shaker and shake on minimum speed for 10-20 min (samples can be left upto 1 hr at this stage)
6. Centrifuge for 10 min. at 13000 rpm.
7. Carefully remove the supernatant (upper layer) and transfer it into a clean 1.5 mL microcentrifuge tube and repeat the chloroform extraction (steps 5 & 6)
8. Precipitate the DNA by adding 600 µL of ice-cold isopropanol and mix by inverting the tube. DNA should be left in the freezer overnight or longer at this stage. (up to a few days or even weeks for herbarium specimens)
9. Precipitating the DNA by centrifuging for 10 min. at 13,000 rpm.
10. Remove the supernatant and add 500 µl of wash buffer (70% alcohol), vigorously agitate to release the pellet from the bottom of the tube and leave for at least 30 min. at room temperature. The DNA can be left in the refrigerator overnight at this stage
11. Centrifuge for 5 min. at 13000 rpm. Remove the supernatant and invert the tubes to allow the remaining wash buffer to drain away and allow to dry the pellet

12. Dissolve the pellet in 50-100 µl of TE buffer (more or less depending on the size of the pellet) and mix well.
13. Check the DNA quality and concentration by agarose gel electrophoresis
14. Store the DNA samples at -20^{0}C.

Electrophoresis

The isolated genomic DNA and PCR products were visualized using 0.8 % and 1.2 % agarose gels respectively and analyzed in agarose gel electrophoresis. Quality of the DNA/ PCR products was identified by the quality of the bands. The agarose gel was prepared using 1X TAE buffer. Ethidium bromide (0.025 µL/ mL) was used for visualization of the DNA bands under UV light, and it is directly added into the tank buffer. 1 µL of 6X gel loading dye (Himedia) was mixed with each sample and run the gel using an electrophoretic unit (Enduro horizontal electrophoresis system, Labnet International, Inc., Aplegen, USA) at 75V power supply with 1X TAE as an electrophoretic buffer for 1- 1.5 hr. until the dye front reaches almost bottom of the gel. The 100 bp DNA ladder (Invitrogen) 0.1 µg/ µL concentration was used to analyse the size of amplified DNA regions. Gels were visualized and documented using the Gel Documentation system (Enduro^{TM}GDS, Labnet International Inc., Aplegen, USA)

Primer dilution

The ITS forward lyophilized primer (ITS5P) of 23.6 n mol concentration was diluted with 236µL of milli-Q water. In the same way, 19.3 n mol of ITS reverse primer (ITS8P) added with 193 µL of milli-Q water, 22.4 n mol of *trnL-F* forward primer (*trnC*) added with 224 µL and 36.3 n mol of reverse primer (*trn F*) with 363 µL of milli-Q water,31.6 n mol of *rps*16 forward primer (*rpsF*) added with 316 µL and 29.8 n mol reverse primer (*rpsR2*) with 298 µL of milli-Q water. Minimum 12hr. incubation was needed for diluted primers. After completion of overnight incubation, take 1 µL of each primer from the stock primer and makeup to 10 µL using milli-Q water and used as working stock primer.

Polymerase Chain Reaction (PCR)

Three DNA loci were selected for the amplification, which include one nuclear (ITS1-5.8s-ITS2) and two chloroplast (*trnL-F & rps*16) regions. PCR was run by the primers used in previous studies (Table 6). The nuclear internal transcribed spacer (ITS) loci were amplified using published primers ITS5P and ITS8P (Möller & Cronk 1997). The *trnL-F* region consists of *trnL* intron and *trnL-trnF* intergenic spacer, these regions are amplified by the primer pair (*trnC* and *trnF*) of Taberlet *et al.* (1991). The *rps*16 is mainly constituted by an intron region which is situated aside of *rps*16 gene, this type II intron is amplified by using the primer pair *rps*F and *rps*R2 (Oxelman *et al.*, 1997). Mastermix composition and PCR conditions of three DNA loci were given in Tables 7 & 8.

Table 6. Details of primer pairs used for amplification

Loci	Primers	Primer sequence	Reference	Expected amplicon size (bp)
ITS	ITS5P	GGAAGGAGAAGTCTAACAAGG	Möller & Cronk (1997)	600
	ITS8P	CACGCTTCTCCAGACTACA		
trnL-F	*trnC*	CGAAATCGGTAGACGCTACG	Taberlet *et al.* (1991)	850
	trnF	ATTTGAACTGGTGACACGAG		
*rps*16	*rpsF*	GTGGTAGAAAGCAACGTGCGACTT	Oxelman *et al.* (1997)	800
	rpsR2	TCGGGATCGAACATCAATTGCAAC		

Table 7. Master Mix Composition of 20 µL PCR reaction mixture

Reagents	Volume
EmeraldAmp® GT PCR Master Mix (TaKaRa, India)	10 µL
Forward primer	0.75 µL
Reverse primer	0.75 µL
DMSO	0.4 µL
Nuclease free water or Milli-Q water	7.1 µL
Template DNA	1 µL

Table 8. PCR amplification conditions of ITS, *trnL-F* and *rps*16

	nrITS			*trnL-F*			*rps*16		
Initial denaturation	95 °C	5 min		95 °C	5 min		95 °C	5 min	
Denaturation	94 °C	30 sec	35 cycles	95 °C	30 sec	35 cycles	95 °C	30 sec	35 cycles
Annealing	56 °C	35 sec		56 °C	1 min		58 °C	1 min	
Elongation	72 °C	1 min		72 °C	1 min		72 °C	1 min	
Final extension	72 °C	5 min		72 °C	5 min		72 °C	5 min	
	4 °C	Infinity		4 °C	Infinity		4 °C	Infinity	

Sequence quality check and Multiple sequence alignment (MSA)

The contig sequences were generated from forward and reverse sequences by using Sequencher ver. 4.1.4 (Gene Codes) with manual checking of base calls in chromatograms by using AB1 files and editing was done accordingly. The final contig sequences were put in NCBI BLAST (Basic Local Alignment Search Tool) to find the sequence similarity indices. Multiple sequence alignment (MSA) was done by using MUSCLE incorporated in MEGA 7 (Kumar *et al*., 2016). All characters were given equal weight and gaps were treated as missing data and external gaps were represented by question marks. The end trimmed sequence file is used for further analyses and for NCBI GenBank deposit.

GenBank accession number generation

The properly aligned contig sequences are selected for NCBI GenBank upload. The ITS sequences were uploaded through the GenBank submission portal, while the sequences of *trnL-F* and *rps*16 were submitted through BankIt submission portal of NCBI. If the submission procedures are correct, we get the submission numbers and within 48 hours we can generate NCBI accession numbers for our sequences. There are options in the portal for delaying the sequence release date. Once we create a login ID in NCBI GenBank, we can submit our sequences at any time. If the sequences consist by only one region (eg:- ITS) then we can submit multiple sequences as a single file. While uploading the sequences, we must provide contact information, details of sequence author, name of organism, sequencing

technology, molecular type etc. Additionally, we can provide source modifiers like herbarium accession number, name of collector, date of collection, place of collection etc. It is better to submit the sequences in NCBI after getting the acceptance letter from the journal since we can furnish our sequences by adding the extra details such as the title of publication, journal name and publication status (in press or published) and it will help to increase the credibility of our sequences. We submitted 33 sequences (11 sequences each of ITS, *trnL-F*, and *rps*16) to NCBI, all of which were submitted after receiving acceptance from the journals.

Selection of substitution models

Sequence evolution model or the best-fit substitution models for three DNA regions (ITS, *trnL-F*, *rps*16) were calculated by jModeltest ver.2.1.3 (Darriba *et al*., 2012). Bayesian information criterion (BIC) was used as model selection strategies in the present study.

Steps

1. Open executable jar file of jModeltest
2. File→Load DNA alignment
3. Analysis→Compute likelihood scores → Compute Likelihods (with default settings)
4. Analysis→ Do AIC calculations, consecutively do BIC calculations and DT calculations
5. Results →show results table
6. The appropriate models for the sequence evolution were highlighted in red for AIC, BIC, and AICc. These models were selected for the tree construction in Bayesian analysis.

Maximum likelihood (ML) phylogenetic tree

Maximum Likelihood phylogenetic analysis was performed in IQ-TREE ver. 2.2.2.6 (Minh *et al*., 2021). The models for sequence evolution of different DNA loci were suggested by inbuilt ModelFinder (Kalyaanamoorthy *et al*., 2017) in this software. Ultra-fast bootstrap approximation was used to infer the Maximum Likelihood phylogenetic trees. To construct a ML tree the following steps were used.

1. Open the PowerShell window and drag iqtree2 application file into the window

2. Type the following commands, **–s filename.fas –spp filename.nex –bb 1000 –nt AUTO –m MFP+MERG -rcluster 10 –pre filename.merg**

3. Press Enter. The tree file (.tre) will automatically appear in the file after the run.

For a concatenated ML tree construction, a partition file in nexus format is needed. In the present study, the inbuilt Modelfinder recommended TrN +G4 model for ITS and TPM1uf +G4 model for *trnL-F* and *rps16* sequences. The strict consensus tree was generated and visualized in FigTree v 1.4.3 (Rambaut, 2016).

Partition homogeneity test or incongruence length difference (ILD) test

Combinability of the nuclear ITS and chloroplast *trnL-F*, *rps*16 were analyzed by Partition homogeneity test or incongruence length difference (ILD) test programmed in PAUP* 4.0a 169 version software. ILD test is a statistics-based test used to determine the differences in tree topology of individual dataset or partition. It will give a statistically significant result whether the partition is congruent or incongruent. Here, we initially test the combinability of chloroplast *trnL-F* and

*rps*16 sequences. We go for a congruence analysis for nuclear ITS and chloroplast *trnL-F* and *rps*16 dataset there after.

1. Open nexus file of the combined sequence in PAUP software.
2. Execute the command **charpartition genes = gene1:1-894, gene2:895-1750** for the combined *trnL-F* and *rps*16 sequences. For the combined nuclear ITS and chloroplast (*trnL-F*, *rps*16) region, the command used is **charpartition genes = gene1:1-637, gene2:638-2387**).
3. Execute command **hompart partition=genes**

Software begins the partition homogeneity test and the computation time depends on the number of taxa and genes. The P value > 0.5 indicates the congruence of the partition and < 0.5 represents the incongruence.

Bayesian Inference (BI) phylogenetic tree

It is a most useful, reliable and command-based Bayesian statistical framework, vastly used for phylogenetic works in nowadays. Bayesian analysis was performed by MrBayes ver. 3.2.6 (Ronquist *et al*., 2012). TIM3+G4 model was selected for ITS and HKY model was selected for *trnL-F* and *rps16* by jModeltest ver.2.1.4 (Darriba *et al*., 2012) for the Bayesian analysis. Two independent runs were performed, each run of Markov Monte Carlo (MCMC) with sample frequency 1000. Usually, the analysis starts by 10,000,00 (1 million) generations and increases until the average standard deviation of split frequency becomes less than 0.01. In the present study, analysis was performed for 10,000,000 to reach the standard deviation of split frequency of less than 0.01. The first 25% of the samples were discarded as burn-in. In the Bayesian tree, statistical branch/clade support was shown as posterior probability (PP) value. The generated tree file was visualized in FigTree v 1.4.3 (Rambaut, 2016). As mentioned in the ML analysis, here also a partition file (.nex) is required for the tree construction by concatenated sequences. The nst values (1, 2, 6 etc.) and rates (gamma or invgamma) are assigned in commands based on the models. Hence, each DNA markers have different rate of evolution. The sample command file is given in Fig. 38.

```
MrBayes > charset its=1-675;

   Defining charset called 'its'
   Expecting  command

MrBayes > charset trnl=676-2516;

   Defining charset called 'trnl'
   Expecting  command

MrBayes > partition names=2:its,trnl;

   Defining partition called 'names'
   Expecting  command

MrBayes > set partition=names

   Setting names as the partition, dividing characters into 2 parts.
   Setting model defaults
   Seed (for generating default start values) = 1800579388

MrBayes > lset applyto=(1) nst=6 rates=gamma

   Setting Nst to 6 for partition 1
   Setting Rates to Gamma for partition 1
   Successfully set likelihood model parameters to
   partition 1 (if applicable)

MrBayes > lset applyto=(2) nst=2 rates=invgamma

   Setting Nst to 2 for partition 2
   Setting Rates to Invgamma for partition 2
   Successfully set likelihood model parameters to
   partition 2 (if applicable)

MrBayes > mcmcp ngen=2000000 samplefreq=500 printfreq=500 diagnfreq=5000

   Setting number of generations to 2000000
   Setting sample frequency to 500
   Setting print frequency to 500
   Setting diagnosing frequency to 5000
   Successfully set chain parameters

MrBayes > mcmc
```

Fig. 38. Sample command lines in MrBayes.

Results

For the present phylogenetic study, we produced 138 new sequences, which include 46 sequences of each ITS locus, *trnL-F* region and *rps*16 region. The details of the amplification of each region were given in the methodology part. From the generated sequences, 33 sequences were deposited in NCBI database (Table 9). The length of ITS region varied from 615 to 695 bp while that of *trnL-F* varied from 886

to 1012 bp and *rps*16 varied from 844 to 953 bp. Only partial sequence of ITS could be generated for *M. balbisiana* var. *andamanica* two times and the third time it was amplified with 668 bp by some minor modification in template DNA and primer concentrations. In order to generate a robust phylogeny result, we retrieved 192 sequences (64 sequences of each ITS, *trnL-F* and *rps*16) or 64 accessions from the NCBI database which includes the taxa from the families, Musaceae, Strelitziaceae, Heliconiaceae and Lowiaceae. The outgroup consists of *Orchidantha chinensis* T.L.Wu, *O. fimbriata* Holttum, *O. siamensis* K.Larsen (Lowiaceae); *Ravenala madagascariensis* Sonn., *Strelitzia reginae* Banks (Strelitziaceae); *Heliconia psittacorum* L.f., *H. caribaea* Lam., *H. rostrata* Ruiz & Pav. (Heliconiaceae). For the world phylogeny of *Musa*, four species of *Ensete* and *Musella lasiocarpa* (Franch.) C.Y.Wu *ex* H.W.Li were considered as outgroup. Three taxa, namely *M. acuminata* var. *manipurensis*, *M. cylindrica*, *M. sikkimensis* var. *simmondsii* could not be included in the analysis due to the unavailability of materials. After the alignment, the total aligned length of ITS region was 637 bp while that of *trnL-F* region was 894 bp and *rps*16 was 856 bp. External gaps were coded with question marks. The total length of the combined dataset was 2387 bp. A total of 330 sequences (110 sequences of each ITS, *trnL-F* and *rps*16), 110 accessions (including both ingroups and outgropus) and 78 taxa (including both ingroups and outgropus) were incorporated in the present phylogenetic study.

Table 9. List of taxa sequenced in the present study. Asterisk (*) indicates the Indian endemic taxa. Dot (•) indicates the taxa sequenced first time for a phylogenetic study.

SL No	Taxa	Herbarium accession No	Locality	GenBank accession number		
				ITS	*trnL-F*	*rps*16
1	*Ensete superbum*	130896 (CALI)	CUBG, Kerala	Not submitted	Not submitted	Not submitted
2	*Musa acuminata*•	159775 (CALI)	Namdang check post, Arunachal Pradesh	PP125033	PP130489	PP130493
		116175 (CALI)	Khasi hills, Meghalaya	PP140666	PP130490	PP130494
3	*M. argentii**•	121885 (CALI)	Iduli, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
		159766 (CALI)	Namphai, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
4	*M. arunachalensis*•*	130837 (CALI)	Durga Mandir, Sessa Village, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
5	*M. aurantiaca*	159768 (CALI)	Namdapha Forest, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
6	*M. balbisiana* var. *balbisiana*	130742 (CALI)	Thumudibandha, Kandhamal Dist., Odisha	PQ154966	PQ181490	PQ181496
7	*M. balbisiana* var. *andamanica** •	116152 (CALI)	Durgapur, Tripura	PQ155079	PQ181491	PQ181497
		164030 (CALI)	Durgamandir, North Andaman	PQ155430	PQ181492	PQ181498

8	*M. balbisiana* var. *bheem-kola**•	130708 (CALI)	Tinsukia, Assam	Not submitted	Not submitted	Not submitted
		116111 (CALI)	SFRI Medicinal Garden, Chessa, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
9	*M. balbisiana* var. *elavazhai**•	123240 (CALI)	CUBG, Kerala	Not submitted	Not submitted	Not submitted
10	*M. balbisiana* var. *sepa-athiya**•	116172 (CALI)	Khasi Hills, Meghalaya	Not submitted	Not submitted	Not submitted
11	*M. balbisiana* var. 2*•	116158 (CALI)	Mandirghat, Agarthala, Tripura	Not submitted	Not submitted	Not submitted
12	*M. balbisiana* var. 3*•	116161 (CALI)	Hezamara, Tripura	Not submitted	Not submitted	Not submitted
13	*M. beccarii*	117239 (CALI)	CUBG, Calicut, Kerala	Not submitted	Not submitted	Not submitted
14	*M. cheesmanii**•	159779 (CALI)	Demve, on the way to Tidding, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
		159729 (CALI)	Kenibreed Nursery, West Bengal	Not submitted	Not submitted	Not submitted
15	*M. chunii*•	159788 (CALI)	Tidding, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
		159790 (CALI)	Tidding, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
16	*M. flaviflora**•	164712 (CALI)	Senki View, Itanagar, Arunachal Pradesh	Not submitted	Not submitted	Not submitted

17	*M. itinerans*	130715 (CALI)	Way to Changlang, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
		159734 (CALI)	Kenibreed Nursery, West Bengal	Not submitted	Not submitted	Not submitted
18	*M. kattuvazhana**•	164050 (CALI)	Kadamtala, Middle Andaman, Andaman Island	Not submitted	Not submitted	Not submitted
		164054 (CALI)	Rangat, Middle Andaman, Andaman Island	Not submitted	Not submitted	Not submitted
		148737 (CALI)	Munnar, Idukki, Kerala	Not submitted	Not submitted	Not submitted
19	*M. mannii*	159776 (CALI)	Namdang, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
		159735 (CALI)	Kenibreed Nursery, West Bengal	Not submitted	Not submitted	Not submitted
20	*M. markkuana**•	159738 (CALI)	Kenibreed Nursery, West Bengal	Not submitted	Not submitted	Not submitted
21	*M. markkui**•	159787 (CALI)	Tidding, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
22	*M. nagensium*	159767 (CALI)	Namdapha Forest, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
23	*M. ochracea**•	130815 (CALI)	Lengpui, Mizoram	Not submitted	Not submitted	Not submitted
		164759 (CALI)	Vairengten Checkpost, Mizoram	Not submitted	Not submitted	Not submitted

24	*M. ornata*	130818 (CALI)	Lengpui, Mizoram	Not submitted	Not submitted	Not submitted
		130758 (CALI)	Arakku valley, Andhra Pradesh	Not submitted	Not submitted	Not submitted
25	*M. pradhanii**•	130769 (CALI)	Darjeeling, West Bengal	Not submitted	Not submitted	Not submitted
26	*M. puspanjaliae**•	159785 (CALI)	Thohangam view point, Tidding, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
27	*M. rubra*	159727 (CALI)	Jorethang, Sikkim	Not submitted	Not submitted	Not submitted
28	*M. sabuana**•	164076 (CALI)	Galathea, Nicobar Island	PQ155431	PQ181493	PQ181499
		152776 (CALI)	Ramakrishnapur, Little Andaman	PQ155432	PQ181494	PQ181500
		164074 (CALI)	Great Nicobar Biosphere Reserve	PQ155433	PQ181495	PQ181501
29	*M. sikkimensis**•	159750 (CALI)	Namahatta, On the way to Darjeeling, West Bengal	Not submitted	Not submitted	Not submitted
		164721 (CALI)	Sessa villege, Arunachal Pradesh	Not submitted	Not submitted	Not submitted
30	*M. thomsonii**•	164732 (CALI)	Tura peak, Meghalaya	Not submitted	Not submitted	Not submitted
31	*M. velutina* var. *variegata*•	159764 (CALI)	Makum forest, Assam	Not submitted	Not submitted	Not submitted

Table 10. List of accessions with taxon name downloaded from NCBI-GenBank database.

Name of taxa	**Herbarium accession number**	**GenBank accession number**		
		ITS	***trnL- F***	***rps*16**
Ingroups (Musaceae)				
Ensete glaucum Roxb.	HBG: 2006–0705	FJ428103	FJ428154	FJ428124
E. superbum Roxb.	SS&JS-583	ON639613	ON453869	ON453859
E. ventricosum (Welw.) Cheesman	ITC1387	KU215100	KU215215	KU214976
Musa acuminata subsp. *banksii* (F.Muell.) N.W.Simmonds	ITC0623	FJ428097	FJ428161	FJ428138
M. acuminata subsp. *burmannica* N.W. Simmonds	ITC0283	FJ428083	FJ428169	FJ428135
M. acuminata subsp. *burmannicoides* De Langhe	ITC0249	FJ428085	FJ428170	FJ428133
M.acuminata subsp. *errans* (Blanco) R.V.Valmayor	ITC1028	FJ428094	FJ428160	FJ428126
M.acuminata subsp. *malaccensis* (Ridl.) N.W.Simmonds	ITC1511	KU215102	KU215205	KU214978
M. acuminata subsp. *malaccensis*	ITC0609	KU176107	KU176109	KU176108
M. acuminata subsp. *microcarpa* (Becc.) N.W.Simmonds	ITC0253	FJ428087	FJ428174	FJ428140
M. acuminata subsp. *microcarpa*	ITC0253	KU215076	KU215198	KU214952
M. acuminata subsp. *microcarpa*	ITC0308	KU215076	KU215198	KU214952
M. acuminata subsp. *siamea* N.W. Simmonds	ITC0660	FJ428084	FJ428175	FJ428137
M. acuminata subsp. *truncata* (Ridl.) Kiew	ITC0393	KU215124	KU215218	KU214999

M. acuminata var. *zebrina* (Van Houtte *ex* Planch.) Nasution	HBG:2003–0139	FJ428089	FJ428173	FJ428139
M. aurantiaca G.Mann *ex* Baker	HBG: 2007–0001	FJ428090	FJ428162	FJ428127
M. balbisiana Colla	HBG:2001–0390	FJ428102	FJ428159	FJ428145
M. barioensis Hakkinen	HBG: 2005–0738	FJ428067	FJ428185	FJ428152
M. basjoo Siebold *ex* Miq.	HBG: 2006–0701	FJ428100	FJ428188	FJ428147
M. basjoo	ITC0061	KU215073	KU215195	KU214949
M. beccarii var. *beccarii* N.W. Simmonds	HBG: 2001–0482	FJ428065	FJ428189	FJ428120
M. beccarii var. *hottana* Hakkinen	HBG: 2005–0826	FJ428066	FJ428190	FJ428115
M. campestris Becc.	HBG: 2001–0384	FJ428076	FJ428197	FJ428113
M. coccinea Andrews	HBG: 2001–0387	FJ428062	FJ428192	FJ428150
M. coccinea	ITC0287	KU215078	KU215200	KU214954
M. gracilis Holttum	HBG: 2001–0452	FJ428075	FJ428111	FJ428194
M. hirta Becc.	HBG: 2004–0366	FJ428074	FJ428199	FJ428117
M. ingens N.W. Simmonds	HBG: 2005–0375	FJ428077	FJ428184	FJ428118
M. itinerans Cheesman	HBG: 2005–0825	FJ428098	FJ428177	FJ428148
M. jackeyi W.Hill	ITC0588	KU215081	KU215203	KU214957
M. laterita Cheesman (=*M. rubra*)	HBG:2001-0448	FJ428082	FJ428157	FJ428136
M. lolodensis Cheesman	ITC0956	KU215094	KU215213	KU214970
M. maclayi F.Muell. *ex* Mikl.-Maclay	ITC0864	FJ428068	FJ428183	FJ428122
M. mannii H.Wendl. *ex* Baker	HBG: 2001–0454	FJ428091	FJ428166	FJ428129
M. mannii	ITC0543	KU215079	KU215201	KU214955
M. monticola M.Hotta *ex* Argent	HBG: 2004–0365	FJ428073	FJ428191	FJ428119
M. nagensium Prain	HBG: 2006–0700	FJ428101	FJ428158	FJ428144
M. nanensis Swangpol & Traiperm	SS & JS 612	ON639616	ON453871	ON453853

M. ornata Roxb.	HBG:2001–0398	FJ428096	FJ428164	FJ428130
M. peekeli Lauterb.	ITC0618	KU215084	KU215207	KU214960
M. rosea Baker	HBG:2001–0401	FJ428080	FJ428171	FJ428131
M. rubinea Hakkinen & C.H. Teo	HBG:2003–0768	FJ428093	FJ428163	FJ428128
M. rubra Wall. *ex* Kurz	HBG:2001–0402	FJ428081	FJ428172	FJ428132
M. salaccensis Zoll. *ex* Kurz	HBG: 2003–0784	FJ428072	FJ428196	FJ428112
M. schizocarpa N.W. Simmonds	ITC0846	FJ428088	FJ428176	FJ428142
M. schizocarpa	ITC0599	KU215082	KU215204	KU214958
M. serpentina Swangpol & Somana	SS & JS 353	ON639618	KT257594	ON453864
M. siamensis Hakkinen & Rich.H. Wallace (=*M. rubra* var. *siamensis* Hakkinen & Rich.H.Wallace)	HBG: 2002–0844	FJ428086	FJ428168	FJ428134
M. textilis Née	ITC0539	FJ428069	FJ428187	FJ428121
M. tonkinensis R.V. Valmayor, L.D. Danh & Hakkinen	HBG: 2001–0392	FJ428099	FJ428178	FJ428146
M. velutina H. Wendl. & Druce	HBG: 1998–0017	FJ428092	FJ428165	FJ428141
M. violascens Ridl.	HBG: 2003–0611	FJ428071	FJ428195	FJ428114
M. violascens	ITC1514	KU215123	KU215217	KU214998
M. yunnanensis Hakkinen & H. Wang	HBG: 2006–0702	FJ428095	FJ428163	FJ428143
Musella lasiocarpa (Franch.) C.Y.Wu *ex* H.W.Li	HBG:2005–0824	FJ428155	FJ428155	FJ428123
Out groups (Heliconiaceae, Lowiaceae and Strelitziaceae)				
Heliconia psittacorum L. f.	SCBG: 2005011	FJ428105	FJ428180	FJ428108
H. rostrata Ruiz & Pav.	19822412 (BR)	KU215042	KU215169	KU214907

Orchidantha chinensis T.L. Wu	SCBG: 2005012	FJ428104	FJ428181	FJ428153
O. fimbriata Holttum	L.B. Pedersen & B. Johansen 1071 (C)	AF434879	FJ621300	AF430098
O. siamensis K.Larsen	95KL47005	AF434887	AF431622	AF430106
Ravenala madagascariensis Adans	SCBG: 2005013	FJ428107	FJ428182	FJ428110
Strelitzia reginae Banks	Kress GH94-3783	FJ626403	FJ621298	JQ027166

Phylogenetic trees

For study the independent evolution of three DNA regions, separate trees of nuclear ITS locus, chloroplast *trnL-F* region and chloroplast *rps*16 intron region were constructed (Figs. 39, 40 & 41) for Indian Musaceae. The chloroplast region-based trees resulted in large unresolved polytomies and multiple low support branches, especially in *trnL-F* tree (Fig. 40). However, these trees helped to segregate the genera in Musaceae. Phylogenetic trees obtained from independent nuclear ITS and combined chloroplast *trnL-F- rps*16 analyses were congruent with minor differences (Figs. 39 & 42). Both analyses yielded two major clades in the genus *Musa*, which were similar to the tree generated by the combined analysis (Fig. 43). The incongruence in the placements of some species was observed and discussed in the following part in detail. Moreover, the phylogeny of world Musaceae resolved into three major clades in the family, which corresponds to *Ensete/Musella* clade, section *Callimusa* clade and section *Musa* clade. Detailed observations of all trees are provided in the following part.

ITS based phylogenetic analysis

ITS region (ITS1, 5.8s and ITS2) in nuclear DNA (nrDNA) is a potential marker for phylogenetic study (Alvarez & Wendel, 2003; Hrˇibova *et al*. 2011). This region is used here because of the proven utility in the banana family (Liu *et al*. 2010; Li *et al*. 2010). The dataset has 637 base pairs (bp) which includes 453 conserved sites (71.1 %) and 179 variable sites (28.1 %). The variable sites consist of 126 parsimony informative sites and 50 singleton sites. A total of 58 taxa were incorporated in the present study, which includes 45 newly generated sequences and 13 sequences from NCBI. Both Maximum Likelihood (ML) and Bayesian Inference (BI) tree display similar topology, hence we used BI tree to discuss the phylogenetic inferences (Fig. 39).

As per the present results, the Indian Musaceae are separated into two monophyletic clades corresponding to the two genera, *Ensete* and *Musa* with strong support (BS= 100, PP=1.00 in both clades). In the *Ensete* clade, two accessions of *E. superbum* are nested together and formed as a sister group to the *E. glaucum*. The Indian *Musa* clade represents 32 taxa with 55 accessions.

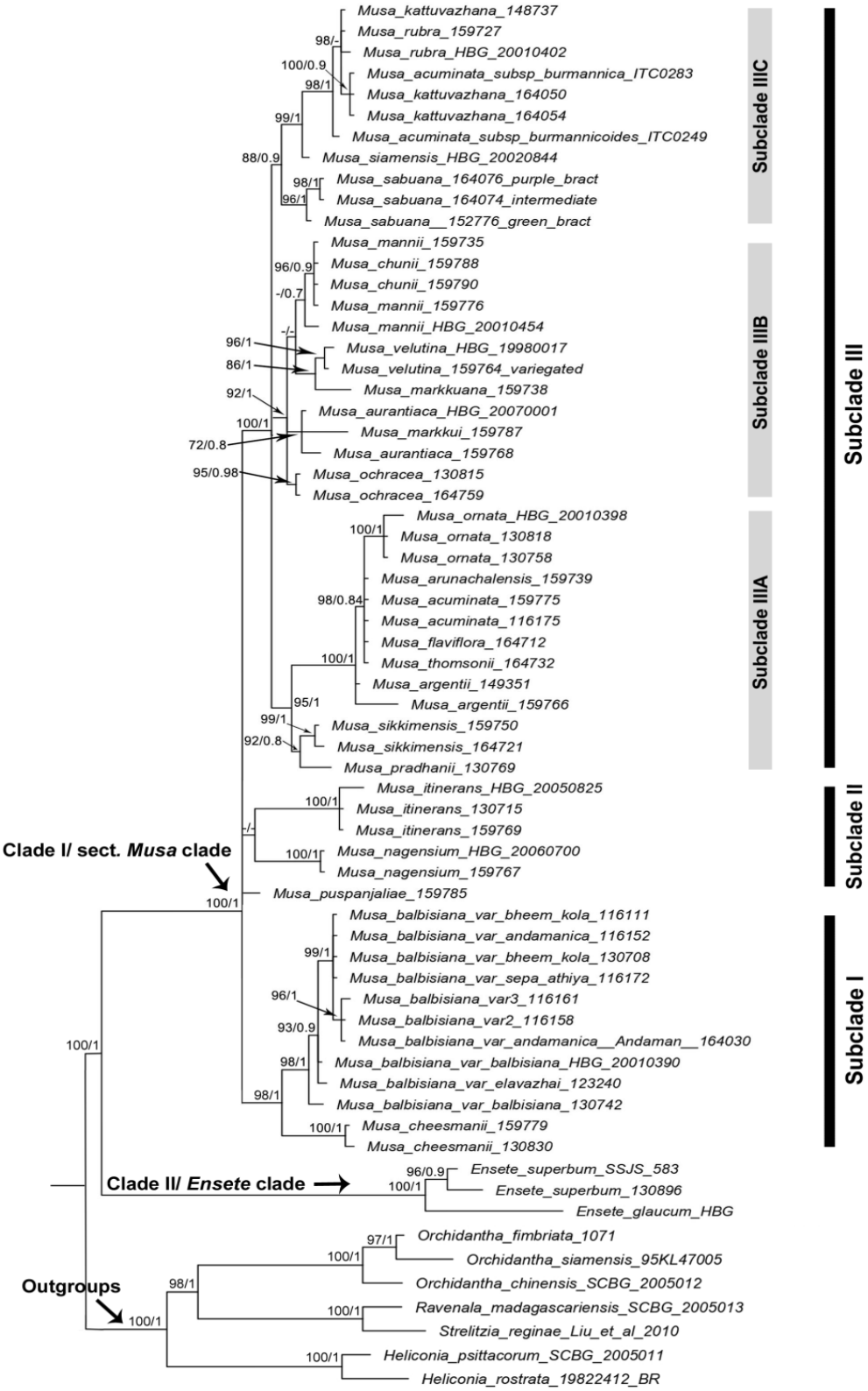


Fig. 39. Strict consensus tree of Indian Musaceae inferred from nuclear ITS sequences. Numbers above branches are Maximum Likelihood bootstrap support (BS) followed by Bayesian posterior probabilities (PP) when ≥ 70% (BS) or ≥0.70 (PP) (otherwise indicated as '-' when BS and PP are <70% or <0.70 respectively).

Previously, Joe (2015) treated Indian *Musa* into two sections following Cheesman's (1947a) sectional classification. This includes sect. *Eumusa* with large pseudostems (>3 m high) and pendant or semi-pendant type inflorescence, dull coloured bract and flowers arranged in two series in bract and sect. *Rhodoclamys* have ornamental bananas with small pseudo stems (< 3m high), erect or semi-pendant inflorescence, bright coloured bract and flowers arranged as single rows in bract. However, the present study showed that sect. *Rhodochlamys* is paraphyletic as it is nested in different subclades within the sect. *Musa*.

Musa puspanjaliae form as a basal monoclade. *Musa itinerans* and *M. nagensium* together formed as a clade with weak support (Subclade II; BS and PP < 70% and 0.7 respectively). *Musa cheesmanii*, *M. balbisiana* and its four varieties nested in another clade (Subclade I) and have received moderate support (BS=98, PP=1). However, *M. puspanjaliae* and subclade I (*M. cheesmanii–M. balbisiana* clade), subclade II (*M. itinerans – M. nagensium* clade) are formed as a basal polytomy in the Indian *Musa* clade.

The remaining taxa conclusively nested into a major clade (subclade III) and receive maximum clade support (BS=100, PP=1) and this subclade is further separated into three weakly supported clades (subclade IIIA, IIIB, IIIC). These three clades are not bifurcated, however, formed into a polytomy. The subclade IIIA (BS= 95, PP=1.00) consisted of *M. sikkimensis*, *M. pradhanii*, *M. argentii*, *M. ornata*, *M. acuminata*, *M. thomsonii*, *M. flaviflora* and *M. arunachalensis*. This particular subclade is bifurcated into two groups (*M. sikkimensis*-*M. pradhanii* clade, *M. acuminata* and its allied species clade). This clade incorporates two species from sect. *Rhodochlamys* (*M. ornata*, *M. arunachalensis*). The second clade (subclade IIIB; BS=92, PP=1) formed by the *M. ochracea* and 7 taxa of ornamental bananas *viz*., *M. markkui*, *M. aurantiaca*, *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*, *M. mannii* and *M. chunii*. The last clade (subclade IIIC; BS=88, PP=0.9) accommodates *M. sabuana*, *M. siamensis* (= *M. rubra* var. *siamensis*), *M. rubra*, *M. kattuvazhana*, *M. acuminata* subsp. *burmannica* (= *M. kattuvazhana*) and *M. acuminata* subsp. *burmannicoides* (= *M. kattuvazhana*). The placement of *Rhodochlamys* members *viz*., *M. rubra* and *M. rubra* var. *siamensis* in this clade is another example of paraphyly.

trnL-F based phylogenetic analysis

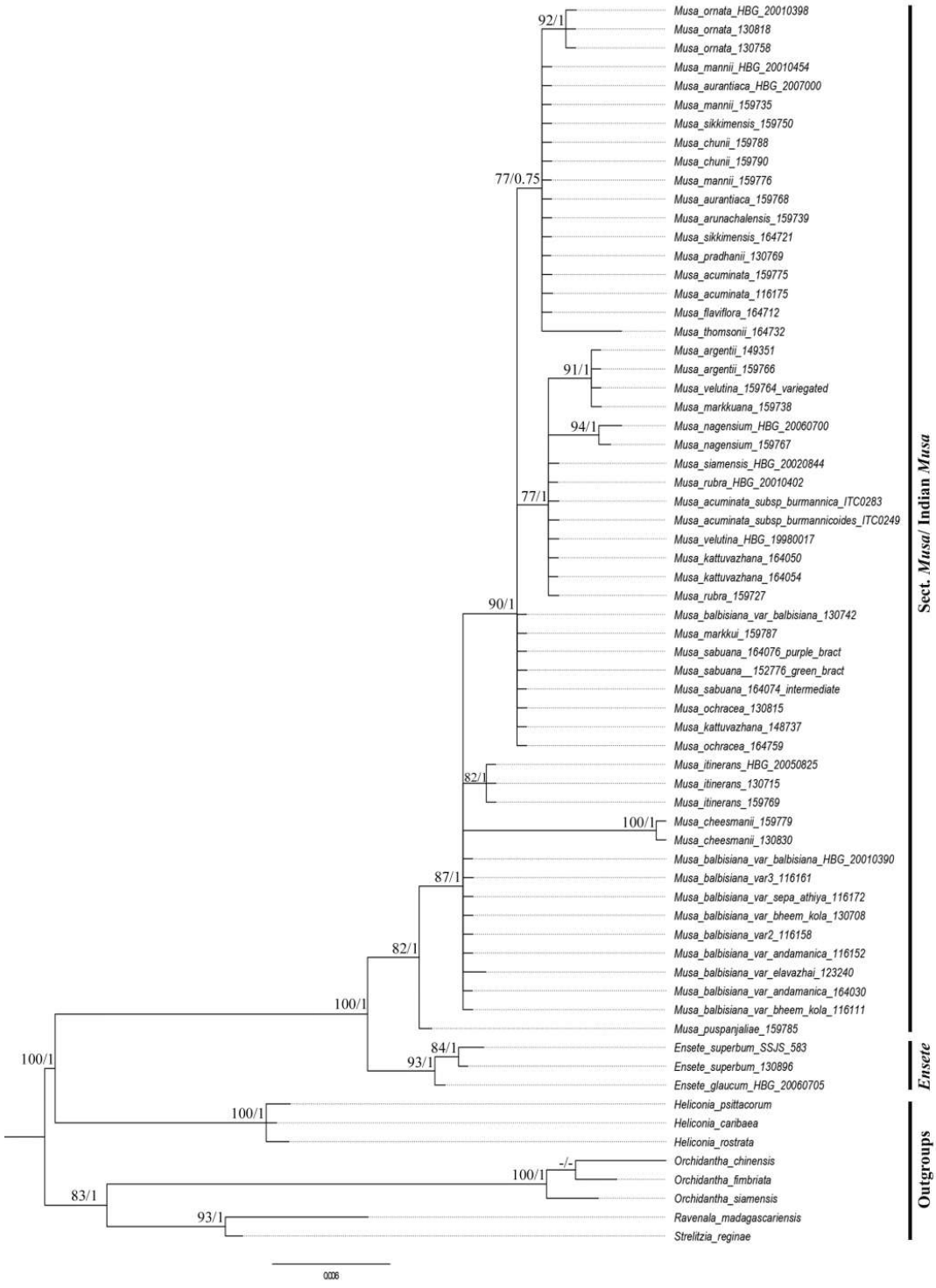


Fig. 40. Strict consensus tree of Indian Musaceae inferred from chloroplast *trnL-F* sequences. Numbers above branches are Maximum Likelihood Bootstrap Support (BS) followed by Bayesian Posterior Probabilities (PP) when ≥ 70% (BS) or ≥0.70 (PP) (otherwise indicated as '-' when BS and PP are <70% or <0.70 respectively).

This region is continuously used in all previous molecular studies of Musaceae based on its potentiality as a chloroplast marker (Liu *et al*., 2010; Li *et al*., 2010; Janssens *et al*., 2016). The total aligned length of this chloroplast DNA (cpDNA) region, including the *trnL* intron and *trnL-F* intergenic spacer, has 894 characters, of which 178 are variable sites and 117 sites are potentially parsimony informative (65.7 % of total variable sites). Both ML and BI analyses show congruent topology and here it is represented as a single tree (Fig. 40).

In the first look, this tree is analyzed as poorly resolved clades with plenty of polytomies and weakly supported branches. However, the species in outgroups are resolved well and placed in their corresponding family clades. Members in Lowiaceae (*Orchidantha chinensis*, *O. fimbriata*, *O. siamensis*) and Strelitziaceae (*Ravenala madagascariensis*, *Strelitzia reginae*) are placed as sister clades as well as Heliconiaceae (*Heliconia psittacorum*, *H. caribaea*, *H. rostrata*) situated as a separate clade. Within the Indian Musaceae (ingroup), *Ensete* (*E. glaucum*, *E. superbum*) and *Musa* are placed in separate clades. So, even this *trnL-F* tree is poorly resolved concerning the species, this region can be recommended for genus-wise delimitation, but not for infrageneric identification. Indian *Musa* (sect. *Musa*) is resolved as monophyletic (BS=100, PP=1) as same as in ITS tree and *rps*16 tree with only slight incongruence in topological results, however weak clade support evident here for each clade. The position of *M. puspanjaliae* observed as basal for section *Musa* (BS=82, PP=1). Compactly arranged fruits bunch, broadly oblong fruits, and large size of seeds of this species is a synapomorphic trait with *Ensete* and in contrast with all *Musa* sp. The placement of *M. puspanjaliae* may indicate that its maternal ancestor had a relation with the genus *Ensete.* The placement of *M. balbisiana* and its varieties supports their relatedness except for one accession (*M. balbisiana* var. *balbisiana*_130742). *Musa cheesmanii* (130830,159779; BS=100, PP=1) shows much variation with respect to other *Musa* sp., and its sequence data shows a notable number of insertions. Multiple accessions of *M. itinerans*, *M. nagensium*, *M. ornata* seem as resolved lineage when compared with other taxa. The remaining taxa from section *Musa* are formed as unresolved polytomies with low branch supports. The species from the ornamental banana section (*Rhodochlamys*) is

paraphyletically distributed throughout the section *Musa*. Interestingly, the polytomy formed by *M. argentii* (149351, 159766), *M. velutina* (variegated, 159764) and *M. markkuana* (159738) leads to the doubt that species may had some relations maternally. Even though the *M. argentii* belongs to section *Musa* and the other two taxa belong to section *Rhodochlamys*, the characters like pink-purple bracts, and pink shade of fruits are synapomorphy for these species.

_rps_16 based phylogenetic analysis

The utility of this intron region in cpDNA is proven in the previous molecular studies carried out in Musaceae (Li *et al*., 2010; Janssens *et al*., 2016). In comparison with *trnL-F* tree, this tree is much resolved for some species to some extent than the *trnL-F* region. The total aligned length of this intron has 856 bp, of which 125 are variable sites and 79 sites are potentially parsimony informative (63.2 % of total variable sites). Both ML and BI analyses show congruent topology and here it is represented as a single BI tree with BS and PP values (Fig. 41).

The phylogram obtained is similar to the *trnL-F* based tree in that outgroups are placed separately with strongly supported clades. *Ensete* clade and section *Musa* or Indian *Musa* clade are bifurcated with strong support (BS=100, PP=1.00). The ornamental bananas or sect. *Rhodochlamys* members are placed in different subclades of the Indian *Musa* clade or sect. *Musa* clade. In contrary with the *trnL-F* tree (Fig. 40), here *M. cheesmanii* formed as the primary lineage for section *Musa.* This tree has many similarities with *trnL-F* tree which consists (1). placement of *M. balbisiana* and its varieties as a clade (except one accession of *M. balbisiana* 130742) (2). *M. nagensium-M. itinerans* clade as sister to *M. balbisiana* clade. Apart from this, the formation of *M. sabuana-M. rubra-M. kattuvazhana* clade, corroborates with ITS tree. The remaining taxa are formed as unresolved branches or polytomies. A polytomy was found here as well between *M. velutina*, *M. markkuana*, and *M. argentii*. Besides that, multiple accessions of *M. itinerans*, *M. ochracea*, *M. kattuvazhana M. nagensium*, *M. ornata*, and *M. aurantiaca* were shown to be resolved lineages from other taxa, supporting the utility of the *rps*16 region as a cpDNA for phylogenetic analysis.

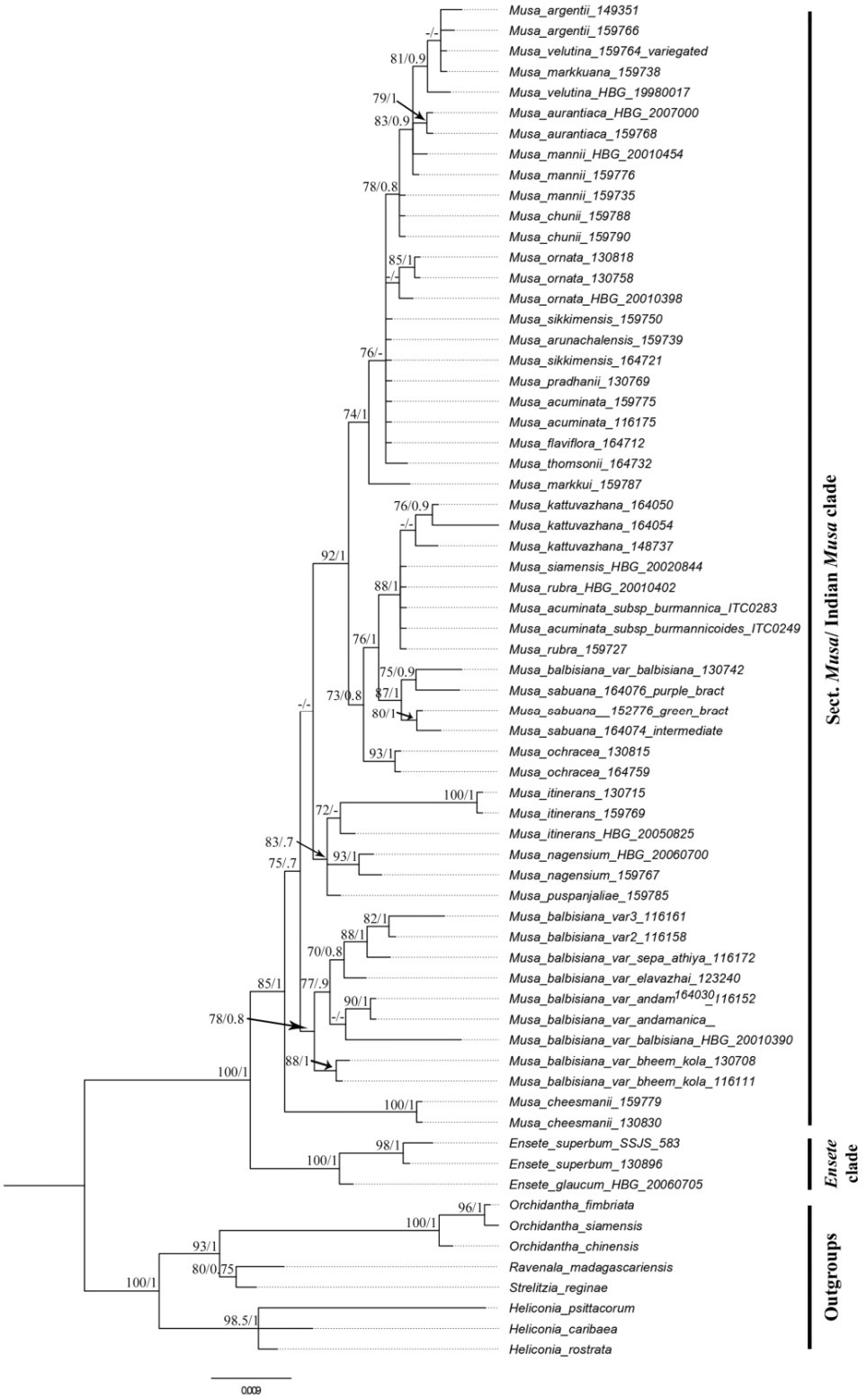


Fig. 41. Strict consensus tree of Indian Musaceae inferred from chloroplast *rps*16 intron sequences. Numbers above branches are Maximum Likelihood bootstrap support (BS) followed by Bayesian posterior probabilities (PP) when ≥ 70% (BS) or ≥0.70 (PP) (otherwise indicated as '-' when BS and PP are <70% or <0.70 respectively).

Chloroplast regions (*trnL-F* and *rps*16) based phylogenetic analysis

The partition homogeneity test supported the combinability of these cpDNA regions (p-value>0.05). The total length of the combined and aligned cpDNA regions have 1750 bp, of which 186 are variable sites and 120 sites are potentially parsimony informative (64.5% of variable sites). Both ML and BI analyses show congruent topology and here it is represented as a single tree (Fig. 42). The out groups are collapsed in the present phylogenetic tree. Within the Indian Musaceae, two major clades are displayed, clade I (BS=100, PP=1) and clade II (BS=100, PP=1) which corresponds to the section *Musa* and genus *Ensete* respectively.

The formation of clades and placement of taxa in each clade are quite similar with the independent *trnL-F* and *rps*16 tree (Figs. 40 & 41). However, this cpDNA tree majorly reflected the result of *rps*16 tree (Fig. 41) but the clade support is higher. Three main subclades are resolved in clade I (sect. *Musa* clade) *viz.*, subclades I, II and III. Moreover, two subclades are generated within subclade III, that are subclade IIIA and IIIB.

Here the basal lineages constituted by *M. cheesmanii* clade (BS=100, PP=1), *M. balbisiana* clade (subclade I; BS=72, PP<0.7) and *M. nagensium-M. itinerans-M. puspanjaliae* clade (subclade II; BS<70, PP<0.7). The subclade III is subdivided into two moderately supported subclades, subclade IIIA (BS=90, PP<0.7), subclade IIIB (BS=99, PP<0.96).

The subclade IIIA constituted by 15 taxa, of which majority of the taxa displays polytomy. However, three accessions of *M. ornata* (130818, 130758, HBG:2001-0398) and two accessions of *M. aurantiaca* (159768, HBG:2007-000) are clustered together and show their distinctiveness from other taxa. As mentioned in *trnL-F* tree and *rps*16 tree, here also *M. argentii* (sect. *Musa*), *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata* (all from sect. *Rhodochlamys*) shows very less sequence variation and displays a polytomy. Joe and Sabu (2019) mentioned the possibility of the hybrid origin of *M. argentii* from *M. velutina* and *M. itinerans*, based on their morphological similarity and the presence of a mixed population in the wild.

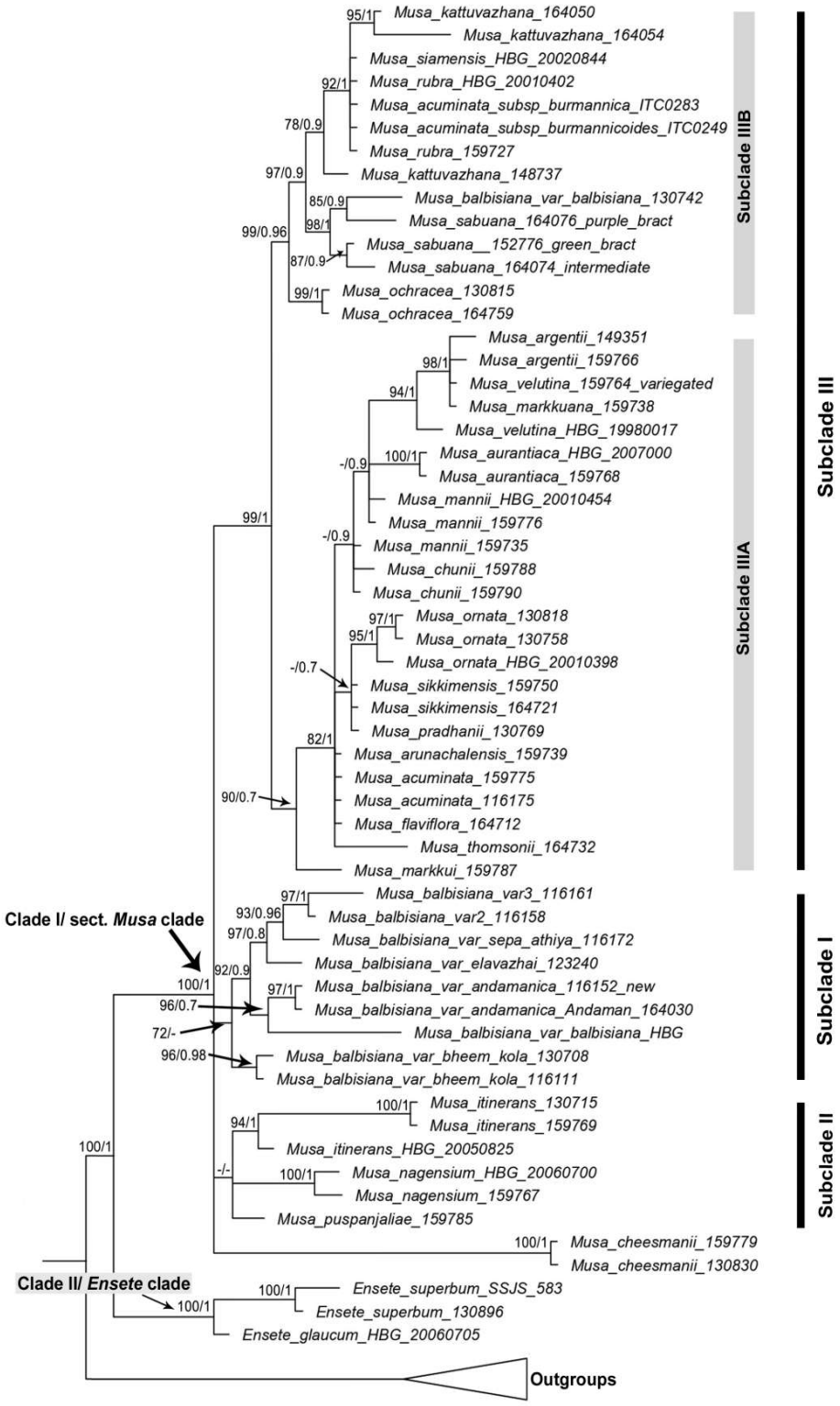


Fig. 42. Strict consensus tree of Indian Musaceae inferred from combined chloroplast (*trnL-F* and *rps*16) sequences. Numbers above branches are Maximum Likelihood bootstrap support (BS) followed by Bayesian posterior probabilities (PP) when ≥ 70% (BS) or ≥0.70 (PP) (otherwise indicated as '-' when BS and PP are <70% or <0.70 respectively).

Here, the chloroplast sequence (*trnL-F*, *rps*16) similarity between *M. velutina*, *M. velutina* var. *variegata*, *M. markkuana* (an allied species of *M. velutina*) and *M. argentii* strengthen the relatedness of these species, particularly from maternal side. Subclade IIIB is composed of 8 taxa, but the subspecies of *M. acuminata*, *viz.*, subsp. *burmannica* and subsp. *burmannicoides* are recently synomized with *M. kattuvazhana* (Joe *et al*. 2016a; Rajeesh *et al*., 2024) and this tree supported the synomization. The placement of accessions of *M. ochracea* (130815, 164759) and *M. sabuana* (164074, 152776, 164076) confirms their species status. *Musa rubra* and *M. siamensis* (=*M. rubra* var. *siamensis*) under section *Rhodochlamys* represented in this subclade and the remaining *Rhodochlamys* taxa are paraphyletically distributed in subclade IIIA, which supported the merging of *Rhodochlamys* and *Musa* sections.

Indian Musaceae trees by concatenated data (ITS, *trnL-F* and *rps*16)

Even though, the partition homogeneity test result is not enough (p-value is < 0.05) for combining the nrDNA (ITS) and cpDNA (*trnL-F*, *rps*16), we concatenated these sequences by visually inspecting the tree topology and based on the previous studies in Musaceae (Liu *et al*., 2010; Li *et al*., 2010; Janssens *et al*., 2016). The total length of the combined nuclear ITS and cpDNA (*trnL-F*, *rps*16) regions have 2387 bp, of which 363 are variable sites and 249 sites are potentially parsimony informative (68.6% of total variable sites). The topology of ML and BI trees are showing slight incongruence, so here represented as separately (Figs. 43 & 44). The main difference in two statistical approaches is the positioning of *M. puspanjaliae* as this species clustered along with *M. nagensium-M. itinerans* clade (subclade II) in ML tree (Fig. 43), whereas in BI tree (Fig. 44) it is separately placed as a basal monoclade.

The combined tree also supports the occurrence of two main clades in Indian Musaceae [*Ensete* clade (clade II) and sect. *Musa* clade (clade I); Figs. 43 & 44]. The clade I comprises the taxa from the previous sect. *Musa* and sect. *Rhodochlamys,* supporting the merging of two sections (Wong *et al*., 2002; Nwakanma *et al*., 2003b; Li *et al*., 2010; Liu *et al*., 2010; Hrˇibova´ *et al*., 2011; Janssens *et al*., 2016). That means the present phylogenetic tree reflects the results of previous studies.

All the representatives of Indian *Musa* are nested in clade I (Sect. *Musa*). The clade I consisted of three subclades (Subclade I, II, III) and subclade III further divides into three subclades, namely subclade IIIA, IIIB and IIIC.

Subclade I (*M. balbisiana-M. cheesmanii* clade; BS=99, PP=1) represents the *M. cheesmanii*, *M. balbisiana* and infraspecific varieties of *M. balbisiana*. Here, *M. balbisiana* var. *balbisiana* formed as the basal lineage of all other varieties of *M. balbisiana viz*., *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. s*epa-athiya*, var.2 and var.3. The present tree topology of *M. balbisiana* and its varieties moderately support the varietal status of *M. balbisiana* var. *andamanica*, *M. balbisiana* var. *bheem-kola*, *M. balbisiana* var. *elavazhai* and *M. balbisiana* var. s*epa-athiya.* However, further genetic marker and morphological phenetic studies are required for the confirmation of the identity of these infraspecific taxa. The tree depicted that, among all other varieties, *M. balbisiana* var. *elavazhai* is distinct as it shows good branch separation from other varieties of *M. balbisiana*. The characteristic morphological features of this variety including lanceolate male bud and fruit shapes supported its distinctiveness. Since this variety is only seen in South India, it may have facilitated the gene flow and caused their genetic differences.

Subclade II is represented by *M. nagensium* and *M. itinerans* in ML tree (Fig. 43) while in BI tree (Fig. 44), subclade II constituted by *M. puspanjaliae*, *M. nagensium* and *M. itinerans*. This subclade does not show much distinction from subclade I (*M. balbisiana-M. cheesmanii* clade), both clade shows polytomy in BI tree and with low clade support in ML tree. Large pseudostem (>4.5 m) and broadly oblong fruits are the synapomorphic traits of subclade II.

The majority of the Indian species is nested in the Subclade III (*M. acuminata*- sect. *Rhodochlamys* clade; BS=100, PP=1), constituted the members from Cheesman's section *Rhodochlamys*, *M. acuminata* and its sub species, Indian endemic *M. flaviflora*, *M. kattuvazhana*, *M. thomsonii*, *M. sikkimensis*, and recently published species from India, *viz*., *M. argentii*, *M. arunachalensis*, *M. markkuana*, *M. markkui*, *M. ochracea*, *M. pradhanii* and *M. sabuana.* The members of section *Rhodochlamys* (ornamental bananas) are nested in the subclade III, however paraphyletically distributed in subclades IIIA, IIIB and IIIC. This result suggested that the sect. *Rhodochlamys* is no longer warranting any taxonomic status.

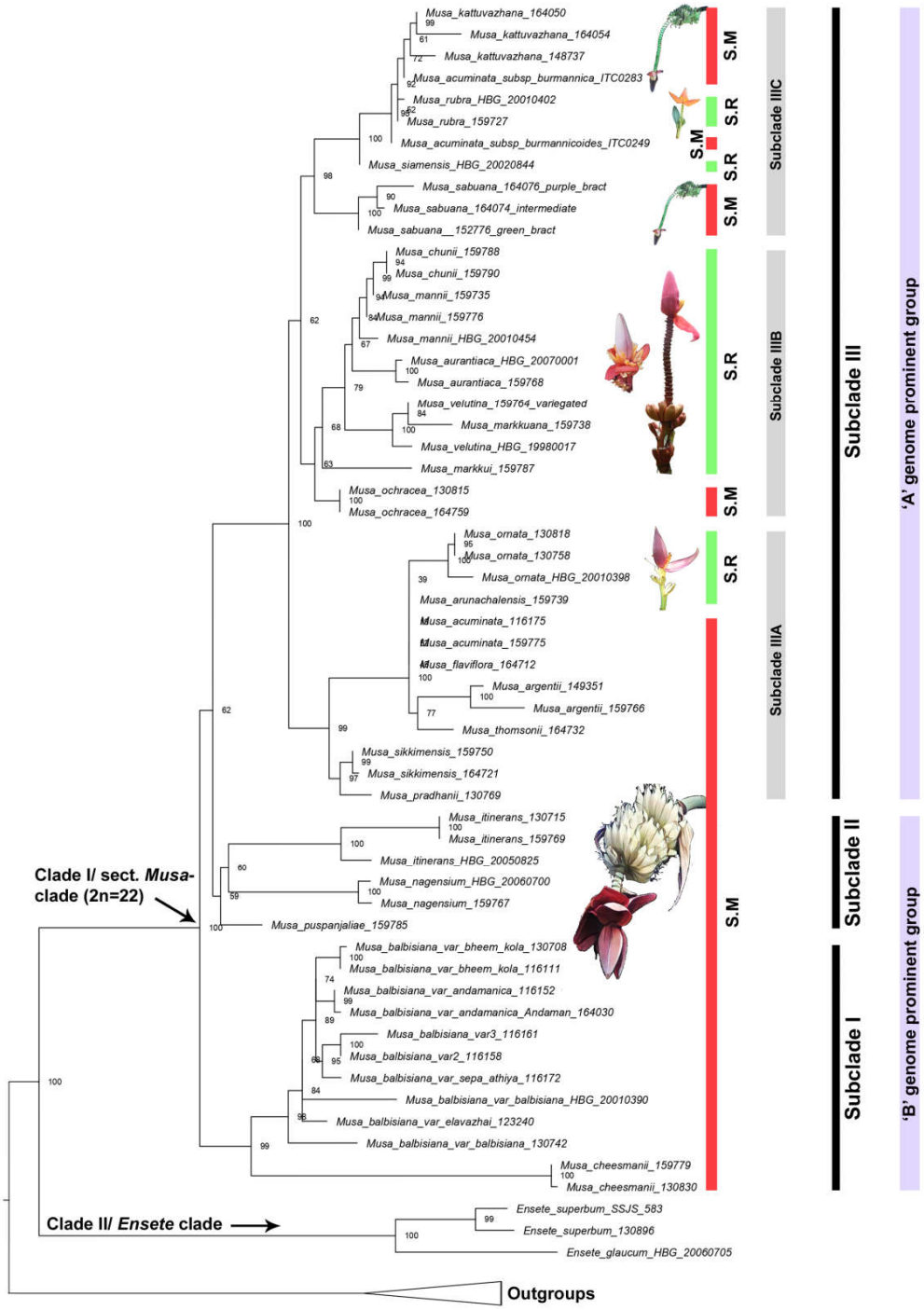


Fig. 43. Maximum Likelihood (ML) strict consensus tree of Indian Musaceae inferred from combined nuclear ITS and chloroplast (*trnL-F*, *rps*16 intron) dataset. Numbers in each node represent the Bootstrap support (BS). Red colour bar and S.M represent the section *Musa*, green bar and S.R represent section *Rhodochlamys* according to Cheesman's (1947a) sectional classification. Violet colour bar represents the 'A' and 'B' genome prominent groups within the section *Musa* clade.

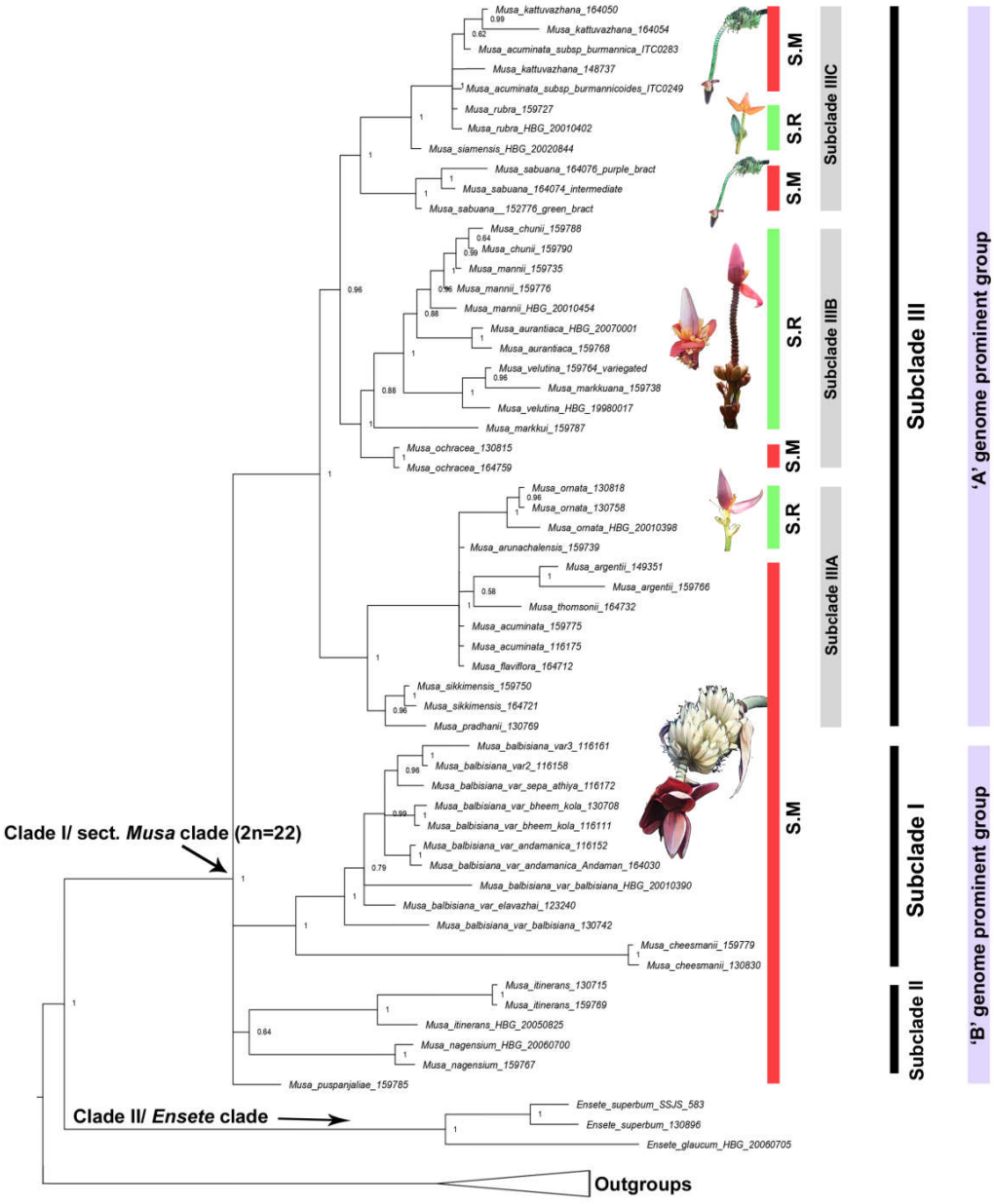


Fig. 44. Bayesian inference (BI) strict consensus tree of Indian Musaceae inferred from combined nuclear ITS and chloroplast (*trnL-F*, *rps*16 intron) dataset. Numbers in each node represent the Posterior probability (PP) value. Red coloured bar and S.M represent the section *Musa*, green bar and S.R represent section *Rhodochlamys* according to Cheesman's (1947a) sectional classification. Violet colour bar represents the 'A' and 'B' genome prominent groups within the section *Musa* clade.

Within the subclade III, the subclade IIIA (BS= 99, PP= 1) is represented by *M. sikkimensis*, *M. pradhanii*, *M. ornata* (sect. *Rhodochlamys*), typical *M. acuminata*, its allied species *M. flaviflora*, *M. thomsonii*, *M. argentii*, and *M. arunachalensis.* The concatenated tree shows polytomy for *M. acuminata*, *M. flaviflora* (allied species of *M. acuminata*) and *M. arunachalensis*. *Musa sikkimensis* and *M. pradhanii* are highly cold-resistant bananas, mainly seen at an elevation of 1500 m or above sea level and they are morphologically allied species. However, *M. sikkimensis* possesses the largest seeds among the wild bananas. Interestingly, *M. ornata*, with erect inflorescence and peculiar lilac-coloured bract is the only member of the sect. *Rhodochlamys* in this subclade (subclade IIIA). However, this species possesses glaucous nature in pseudostem and narrowly oblong fruits, corresponds characters of *M. acuminata* and these can be considered as synapomorphic characters for this subclade. It is still difficult for many taxonomists to differentiate between *M. thomsonii* and *M. flaviflora* and that was mentioned in some studies *(*Joe *et al*., 2013; Häkkinen *et al*., 2014; Joe & Sabu, 2019). However, these two species are distantly placed in this clade. The recently published *M. argentii* and *M. arunachalensis* are also clustered in this subclade A. While describing *M. arunachalensis*, authors treated these taxa under sect. *Rhodoclamys* due to its orange-red coloured bract. However, the characters like medium sized plants (1.4−3 m), arch-shaped inflorescence, highly grooved bract, one or two-rowed flowers and fruits shows the intermediate nature of this taxon between sections *Musa* and *Rhodochlamys*. Also, *M. arunachalensis* shows sympatric distribution with *M. sikkimensis*, *M. cheesmanii* (both belonging to sect. *Musa*) and *M. markkuana* (belongs to sect. *Rhodochlamys*). The vegetative character of *M. arunachalensis* shows similarity with *M. sikkimensis*. These all observation leads to a conclusion that *M. arunachalensis* may be a hybrid and the parents belong to sect. *Musa* and sect. *Rhodochlamys,* however, more studies are needed for the confirmation of its origin and identity of parents. The status of typical *M. acuminata* accessions is not fully resolved by our present markers (ITS, *trnL-F*, *rps*16) and formed a polytomy with *M. flaviflora* and *M. arunachalensis*. Morphologically *M. acuminata* and *M. flaviflora* shares notable similarities in pseudostem characters and inflorescence

characters. However, *M. arunachalensis* is a morphologically distinct species from *M. acuminata* and *M. flaviflora* (both belong to sect. *Musa*) and Sreejith *et al*., (2013) treated *M. arunachalensis* under sect. *Rhodochlamys* while describing the species. So, a multi accession study of this species is demanding to confirm its phylogenetic position.

The subclade IIIB (BS=63, PP=1) and subclade IIIC (BS=98, PP=1) are resolved with moderate support values. However, the subclade IIIB is mostly occupied by ornamental bananas (sect. *Rhodochlamys*) with small pseudostems and brightly coloured bracts. *Musa ochracea* (sect. *Musa*) is formed as the basal taxon to this clade and the present study helps to confirm its species status. It is a medium-sized plant (1.9–3 m) with an intermediate morphology of both sections *Musa* and *Rhodochlamys*. *Musa markkui* is a recently described species from Northeast India (Gogoi & Borah, 2013) and confirmed its identity. *Musa velutina*, *M. velutina* var. *variegata* and *M. markkuana* are clustered together in the present study and show their close affinity. The orange-bracted *M. aurantiaca* (159768) is clustered together with *M. aurantiaca* from Li *et al*.,'s (2010) accession. *Musa mannii* and *M. chunii* are taxonomically related wild ornamental bananas. Here the multiple accession study reveals their phylogenetic relationship.

Subclade IIIC is formed by *M. sabuana* (basal clade), *M. rubra*, *M. siamensis* (=*M. rubra* var. *siamensis*) and *M. kattuvazhana*. So, this subclade is also occupied by members from sections *Musa* (*M. sabuana*, *M. kattuvazhana*) and *Rhodochlamys* (*M. rubra*, *M. rubra* var. *siamensis*). Moreover, the present phylogenetic study supported the synonymization of *M. acuminata* subsp. *burmannica* N.W.Simmonds and *M. acuminata* subsp. *burmannicoides* De Langhe under *M. kattuvazhana.*

The members of the sect. *Rhodochlamys* are distantly distributed in subclade III which shows the lack of a Most Recent Common Ancestor (MRCA) for this section. It shows that the ornamental banana section (sect. *Rhodochlamys*) does not warrant any sectional validity. All members show paraphyletic placement in three subclades (subclades IIIA, IIIB and IIIC) within the subclade III. *Musa ornata* and

M. arunachalensis are housed in the subclade IIIA. The *Rhodochlamys* taxa *ie*., *M. markkui*, *M. velutina*, *M. velutina* var. *variegata*, *M. markkuana*, *M. aurantiaca*, *M. mannii* and *M. chunii* are clustered in subclade IIIB with highest representation. The remaining *M. rubra* and *M. rubra* var. *siamensis* (= *M. siamensis*) are nested in subclade IIIC, which shows the obvious paraphyly of the sect. *Rhodochlamys.*

Apart from these results, this phylogenetic tree reveals that the subclade I and subclade II representatives show symplesiomorphies, which include large pseudostems (>3 m), non-revolute bracts, broadly oblong fruits etc. These traits are common for *M. balbisiana* and we can expect that these phenotypic expressions are formed by the actions of specific genes within the 'B'-genome. So, we assume that the taxa from these subclades including *M. balbisiana* and its infraspecific varieties like *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. s*epa-athiya*, var.2, var.3, *M. cheesmanii*, *M. puspanjaliae*, *M. nagensium* and *M. itinerans* may carry the genome which is similar with 'B'- genome (*M. balbisiana* genome) and tentatively classify subclade I and II are 'B'- genome prominent group. Likewise, the members in subclade III have medium sized plants (<4 m; except *M. sikkimensis*, and *M. sabuana*), narrowly oblong-shaped fruits (except *M. sikkimensis*, *M. argentii* and *M. arunachalensis*), deciduos, revolute, bright or dull coloured bracts are shared characters for this clade. All these characters are corroborated with the characters of *M. acuminata.* Eventhough the sect. *Rhodochlamys* members are housed in this clade, we tentatively classified this subclade III as 'A'- genome prominent group. Because the aforementioned characters including plant height, narrowly-oblong fruit, deciduous and bright coloured bracts *etc.* are suited with sect. *Rhodochlamys*, moreover the findings of Simmonds (1954, 1962) as the close relationship of *M. rubra* (sect. *Rhodochlamys*) and *M. acuminata* (sect. *Musa*) supported this grouping. As a whole, the morphologically allied species of *M. acuminata* includes, *M. flaviflora*, *M. thomsonii*, *M. sabuana*, *M. kattuvazhana*, *M. ochracea*, and ornamental bananas (sect. *Rhodochlamys*) are the prime members in this 'A'- genome prominent group. The 'A' and 'B' genome prominent groups is does not meant that all members in these groups carry the pure 'A' or 'B' genome but they have the genome which is allied with the genome of their corresponding groups. Further studies want to confirm the actual genome status of each wild bananas.

Phylogenetic tree of Musaceae in world context

The concatenated dataset is used here for reconstructing the phylogenetic tree of Musaceae. The representation from the earlier five sections (*Eumusa*, *Rhodochlamys*, *Callimusa*, *Australimusa*, *Ingentimusa*) is used in this study. The ML tree and BI tree show some minor incongruence, particularly based on the position of *M. puspanjaliae*, hence both trees are shown separately (Figs. 45 & 46). The phylogenetic tree displayed three strongly supported clades, namely clade III (*Ensete/Musella* clade), clade II (sect. *Callimusa* clade) and clade I (sect. *Musa* clade). Within the genus *Musa*, two major clades resolved, that is clade I and clade II. The clade II consisted of the taxa from the earlier sect. *Australimusa*, sect. *Ingentimusa* and sect. *Callimusa,* which is represented by blue bar (S.A), yellow bar (S.I) and magenta bar (S.C) in the tree (Figs. 45 & 46) respectively. Based on the present result, these three sections are merged together in Clade II (Sect. *Callimusa*). Their chromosome number (2n=20/18/14) as observed as the synapomorphic character. Clade I is formed by the previous sections *viz*., *Rhodochlamys* (as green bar or S.R) and *Musa* (as red bar or S.M), so here it treated as sect. *Musa* (2n= 22) and which supported the previous studies (Wong *et al*., 2002; Li *et al*., 2010; Liu *et al*., 2010; Christelova *et al*., 2011; Janssens *et al*., 2016; Lamare *et al*., 2017; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022). This clade is mainly composed of Indian *Musa* and their relationships and placements are discussed in the concatenated tree of Indian Musaceae (Figs. 43 & 44). However, the non-Indian species like *M. basjoo* Siebold *ex* Miq., *M. tonkinensis* R.V.Valmayor, D.D.Lê & Häkkinen, *M. nanensis* Swangpol & Traiperm, *M. schizocarpa* N.W.Simmonds, *M. yunnanensis* Häkkinen & H.Wang, *M. rubinea* and infraspecific taxa of *M. acuminata viz.*, subsp. *errans*, subsp. *siamea*, var. *zebrina*, subsp. *microcarpa*, subsp. *banksii*, subsp. *truncata*, subsp. *malaccensis* are additionally incorporated in this tree.

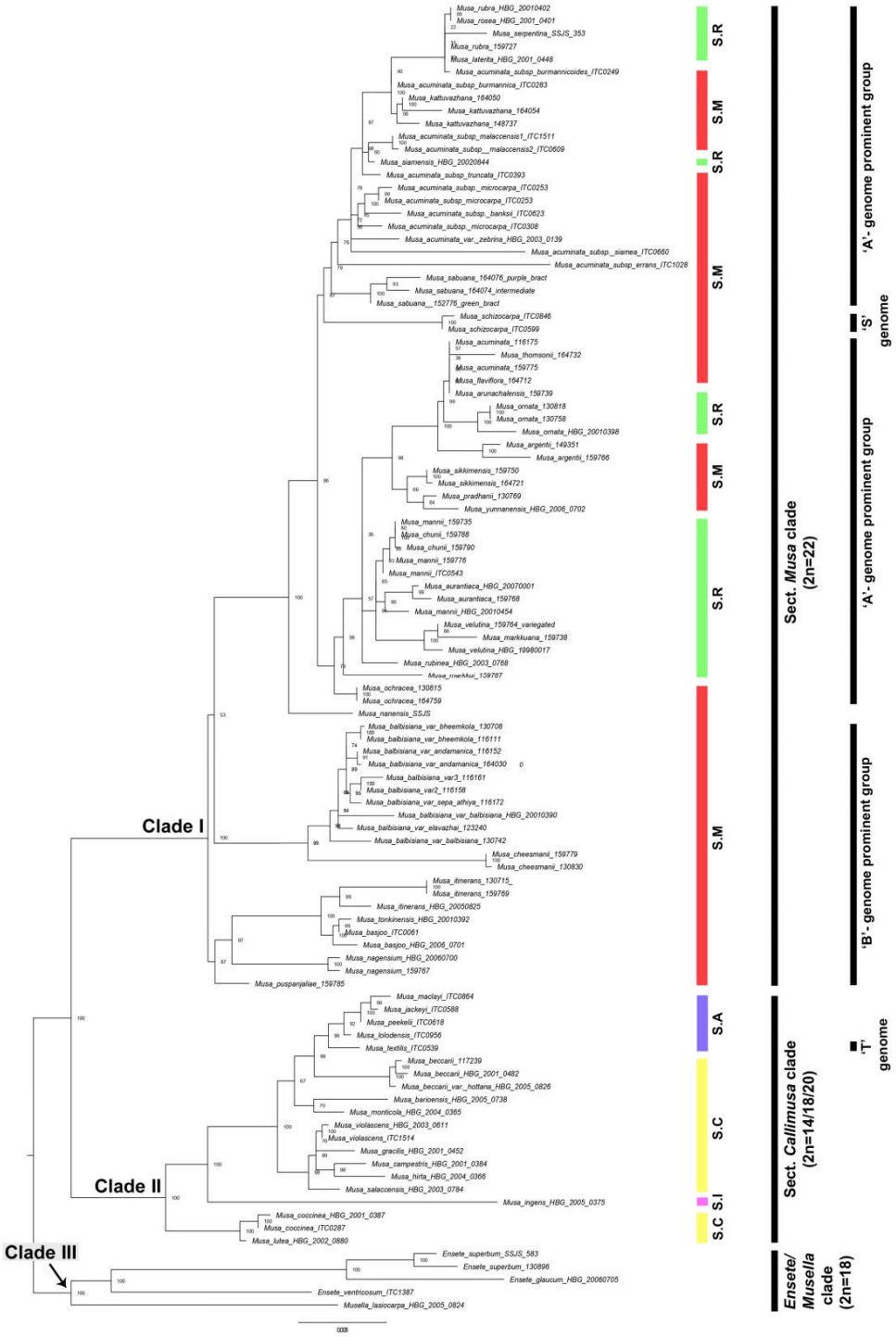


Fig. 45. Maximum Likelihood (ML) strict consensus tree of Musaceae inferred from combined nuclear ITS and chloroplast (*trnL-F*, *rps*16 intron) dataset. Numbers in each node represent the Bootstrap support (BS). Red colour bar, S.M represents the section *Musa*, green bar and S.R- section *Rhodochlamys*, blue bar and S.A- section *Australimusa*, yellow bar and S.C- section *Callimusa*, magenta bar and S.I- section *Ingentimusa* according to morphology and cytology based sectional classification by Cheesman (1947a) and Argent (1976).

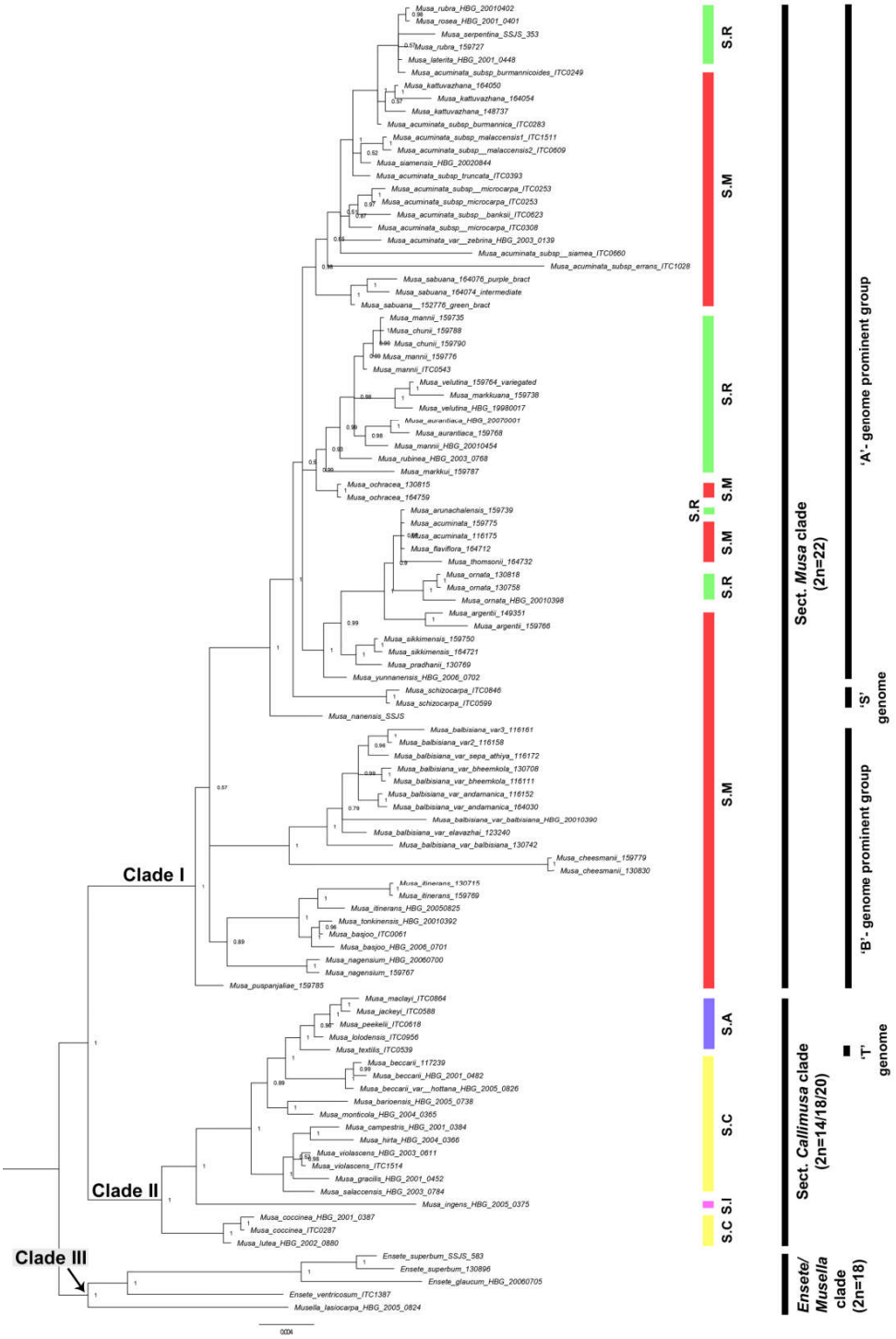


Fig. 46. Bayesian inference (BI) strict consensus tree of Musaceae inferred from combined nuclear ITS and chloroplast (*trnL-F*, *rps*16 intron) dataset. Numbers in each node represent the Posterior probability (PP) value. Red colour bar, S.M represents the section *Musa*, green bar and S.R- section *Rhodochlamys*, blue bar and S.A- section *Australimusa*, yellow bar and S.C- section *Callimusa*, magenta bar and S.I- section *Ingentimusa* according to morphology and cytology based sectional classification by Cheesman (1947a) and Argent (1976).

Musa basjoo and *M. tonkinensis* are nested along with *M. nagensium-M. itinerans* clade. The running rhizomes of *M. itinerans* and *M. tonkinensis* seem here as an autapomorphic character with respect to other *Musa* species. *Musa nanensis*, a remarkable species from Thailand (Swangpol *et al*., 2015; Inta *et al*., 2023) has radial symmetric or actinomorphic flowers with 6 united tepals. Here, it is positioned separately from other *Musa* sp. in the sect. *Musa* clade. The placement of *M. yunnanensis* is sister with *M. sikkimensis.* The morphology of *M. sikkimensis* and *M. yunnanensis* share many similarities *viz.*, Pseudostem characters, leaf nature, male bud nature, fruit shape, seed size and shape etc. Häkkinen and Wang (2008) reported four varieties of *M. yunnanensis* from china *viz*. *M. yunnanensis* var. *yunnanensis*, var. *caii*, var. *yongpingensis* and var. *jingdongensis*. The critical morphological study shows the similarity of *M. sikkimensis* with the varieties of *M. yunnanensis*. The validity of the findings will require further molecular confirmation. *Musa schizocarpa*, a unique wild banana with 'schizocarpic fruit' and 'S' genome contributor in some cultivated banana, is placed as sister taxa with *M. sabuana* in ML tree (Fig. 45) and sistered with *M. nanensis* in BI tree (Fig. 46). The self peeling nature of ripened fruit of this species and *M. velutina* is observed as a homoplasious trait, based on their placement in the present tree. The placing of seven infraspecific taxa of *M. acuminata* (subsp. *errans*, subsp. *siamea*, var. *zebrina*, subsp. *microcarpa*, subsp. *banksii*, subsp. *truncata* and subsp. *malaccensis*) are together and show sister relationship with *M. sabuana* and *M. kattuvazhana*. However, none of these infraspecific taxa placed in the clade of typical *M. acuminata* (subclade IIIA; Figs. 43 & 44). Here also, we tentatively separated clade I or section *Musa* into two major groups, which are 'A'- genome prominent group and 'B'- genome prominent group (alike in the concatenated tree of Indian Musaceae; Figs. 43 & 44). However, the 'S'- genome donor, *M. schizocarpa* is placed next to the 'A'- genome prominent group and 'T'- genome donor, *M. textilis* is nested as sister to *M. lolodensis* in the sect. *Callimusa* clade (Clade II).

Discussion

Musa balbisiana complex: problem of the status of different infraspecific taxa

Being one of the ancestors of cultivated banana and harbouring the major distribution range in India, *Musa balbisiana* drawn more attention among the wild bananas from India. As compared to *M. acuminata*, the species, *M. balbisiana* does not possess any distinct subspecies, and is mainly confined to the mainland area of southeast Asia *viz.*, northeastern states of India, Myanmar and southern China. These areas were considered as the major centre of origin of *M. balbisiana* (Daniells *et al*., 2001; Uma *et al*., 2006). This species is better adapted to drier climates and shows tolerance against major abiotic stresses like drought and temperature. So, heterogenomic banana hybrids evolved from *M. balbisiana* and *M. acuminata* display more drought and temperature tolerant than cultivars solely derived from *M. acuminata* (De Langhe, 2002). Moreover, the edible banana hybrids with higher *M. balbisiana* genomic content show the tendency of seed set.

So far, five varieties of *M. balbisiana* reported from india, *viz.*, *M. balbisiana* var. *balbisiana*, *M. balbisiana* var. *andamanica*, *M. balbisiana* var. *bheem-kola*, *M. balbisiana* var. *elavazhai* and *M. balbisiana* var. *sepa-athiya*. These varieties mainly show the differences in infructescence characters and fruit characters (Joe & Sabu, 2019). Apart from these varieties, several morphological variants were observed and collected from different parts of northeastern states in India, from 2011 to 2022. Altogether, this particular species stands as a species complex in the current circumstances. In the present molecular study, the ITS sequence-based tree (Fig. 39) and concatenated tree (cpDNA+ITS; Figs. 43 & 44) shows that, all the accessions of *M. balbisiana* and its varieties *viz.*, *andamanica*, *bheem-kola*, *elavazhai*, *sepa-athiya*, var. 2 and var. 3 are nested in a single clade (subclade I; Figs. 43, 44, 45 & 46). The var. 2 and 3 are the unidentified varieties collected from Tripura. This subclade I is formed as a basal position of sect. *Musa*. The previous phylogenetic studies of Musaceae (Li *et al*., 2010; Liu *et al*., 2010; Christelova *et al*., 2011; Janssens *et al*., 2016; Lamare *et al*., 2017; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022) did not include any varieties of *M. balbisiana*, showing this clade in their

studies seen as an unresolved basal lineage along with *M. cheesmanii*, *M. nagensium* and *M. itinerans*. The present study is carried out by including multiple accessions of *M. balbisiana* and its varieties helps to add more clarity for this clade. However, the varietal status of most of the varieties cannot be resolved by our three-marker based tree. The *M. balbisiana* var. *elavazhai* is seen as a distinct variety in the *M. balbisiana* clade, but the status of other varieties *viz.*, var. *andamanica*, var. *bheem-kola*, var. *sepa-athiya*, var. 2 and var.3 remains unresolved.

Liu *et al*. (2010) and Li *et al*. (2010) used two and one accession of *M. balbisiana* respectively. In both studies, *M. balbisiana* resolved as a basal lineage for the section *Musa* clade. Phylogenetic data from Liu *et al.* (*l.c.*) showed *M. balbisiana* forms a clade with *M. textilis* (clade G) and it is shown as an unresolved monoclade in the phylogenetic analysis by Li *et al.* (*l.c*.). According to Janssens *et al.* (2016) three accessions of *M. balbisiana* were formed as the first basal clade along with *M. cheesmanii*. Six accessions of *M. balbisiana* in the phylogenetic tree of Lamare *et al.* (2017) were found to be as a basal lineage for the Clade I (sect. *Musa*). Burgos-Hernández *et al*. (2019) study showed the same result as Liu *et al*. (*l.c.*), *ie*, three accessions of *M. balbisiana* formed a clade with *M. textilis.* The chloroplast genome-based phylogenetic study of Fu *et al*. (2022) depicts that, *M. balbisiana* and *M. cheesmanii* together formed as the basal lineage in section *Musa.* Even in the AFLP based UPGMA tree of Wong *et al*. (2002), the *M. balbisiana* was positioned as the primary lineage in sections *Musa- Rhodochlamys* group.

Positions of *Musa balbisiana* clade and *M. nagensium-M. itinerans* clade in different studies.

Both the *M. balbisiana* clade (subclade I) and *M. nagensium-M. itinerans* clade (subclade II) are formed as the primary lineage of section *Musa* in the present study as well as in all earlier studies (Liu *et al*., *l.c.*; Li *et al*., *l.c.*; Christelova *et al*., *l.c.*; Janssens *et al*., *l.c.*; Lamare *et al*., *l.c.*; Burgos-Hernández *et al*., *l.c.*; Fu *et al*., *l.c.*). In the present study, both clades are positioned differently in ML and BI tree. In the ML tree, *M. balbisiana* clade is shown as the basal clade, but interestingly, the BI tree resolved *M. puspanjaliae* as the basal clade. However, the statistical values

of this positioning in both ML and BI analyses are very poor. Most studies have remained uncertain about the placement of these two clades (Liu *et al*., *l.c.*; Li *et al*., *l.c.*), that because they are formed as a basal polytomy and do not have enough statistical evidence to support their position. *Musa balbisiana- M. cheesmanii* clade serves as the basal clade in Janssens *et al*. (*l.c.*), although the basal position is replaced by *M. nagensium* in Burgos-Hernández *et al*. (*l.c.*). So, the original basal lineage for the sect. *Musa* remains a controversial one. However, the most valuable phylogenetic work of the Musaceae family is the whole plastome sequence-based phylogeny by Fu *et al.* (*l.c.*), which indicates that basal lineage of sect. *Musa* is represented by *M. balbisiana-M. cheesmanii* clade.

***Musa sikkimensis- M. yunnanensis* clade**

In the phylogram of Indian Musaceae (Figs. 43 & 44), this clade is formed inside the subclade IIIA, which includes two high altitude froast tolerant Indian endemic species, *M. sikkimensis* ("winter hardy banana") and *M. pradhanii*. These two species are first time used for the phylogenetic study and their status is confirmed by this study. However, in world context phylogeny (Figs. 45 & 46) along with the abovementioned species, one Chinese species, *M. yunnanensis* Häkkinen & H.Wang is nested in their clade. This Chinese species also can tolerate seasonal cold stress (Häkkinen & Hong, 2007). In earlier molecular studies, this clade is not bifurcated, instead it forms a monoclade only by *M. yunnanensis* and seems as a sister clade with *M. schizocarpa* (Li *et al*., *l.c.*; Fu *et al*., *l.c.*) or sister with *M. aurantiaca-M. mannii-M. velutina* clade (Janssens *et al*., 2016). However, in the present study, we found that *M. yunnanensis* is placed along with its morphologically allied taxa (*M. sikkimensis*, *M. pradhanii*) and formed as a sister clade with *M. acuminata* clade and its allied taxa. The lack of material hindered us from including the sequences of *M. sikkimensis* var. *simmondsii* in this analysis, although this clade is well resolved.

Also, Häkkinen *et al*. (2008) described three additional varieties in *M. yunnanensis viz*., var. *caii*, var. *yongpingensis* and var. *jingdongensis*. Among these varieties, var. *caii* and var. *yongpingensis* shared some peculiar morphological and

ecological traits with *M. sikkimensis*, such as wrinkled petiole margins, bluish-purple or dark violet-purple bracts, broadly oblong fruits with pronounced ridges, large sized seeds, cold tolerant nature and can grow in an elevation upto 2000 m from sea level. Unfortunately, the sequences of *M. yunnanensis* varieties are not available. The actual identity of the *M. yunnanensis* and its varieties can be ascertained only by the critical study based on morphological and molecular aspects with the Indian endemic *M. sikkimensis* and *M. pradhanii*. However, we treated this clade as a cold-resistant banana group and the members in this clade such as *M. sikkimensis*, *M. pradhanii*, *M. yunnanensis* can be considered for the gene pool and cold-resistant edible banana development. Normally bananas are tropical crop plants, but through this process, we can introduce novel edible types into the new world temperate countries for cultivation.

Clade of typical *M. acuminata* and its allied species

The subclade IIIA is formed by both Indian endemic species *M. argentii*, *M. arunachalenis*, *M. flaviflora*, *M. thomsonii* and non-endemics, *M. acuminata* and *M. ornata*. Here, *M. ornata* is the only exceptional species which previously treated under sect. *Rhodochlamys,* all others are under sect. *Musa*. However, *M. ornata* shares some important morphological characters with *M. acuminata* and its allied species such as the glaucous nature of the pseudostem, lanceolate or top-shaped male bud, revolute nature of bracts, narrowly oblong fruits etc. Besides, this ornamental species is suggested as a potent parent for breeding programmes in some literature (Burgos-Hernández *et al*., 2013, 2014, 2017). *Musa argentii* is the Indian endemic species, which is an intermediate species of two previous sections, *viz.*, sect. *Musa*, and sect. *Rhodochlamys*, and the populations of this species are observed along with the populations of *M. itinerans* and *M. velutina.* Therefore, we may be skeptical that this species is a hybrid between *M. velutina* and *M. itinerans* as this species possesses the morphological traits of the previously mentioned species. Its bract colour and hairiness in the peduncle are allied with *M. velutina* and plant height, inflorescence nature is coined with *M. itinerans.* Similarly, *M. arunachalensis* is another intermediate taxon which shows the morphological traits

of both sections *Rhodochlamys* and *Musa*. This species is another Indian endemic reported from Arunachal Pradesh (Sreejith *et al*., 2013), shares its populations along with *M. sikkimensis* and its dry appearance of pseudostem resembles with *M. sikkimensis*. However, this species shares a peculiar orange-red bract, with *M. aurantiaca* and *M. rubra*. So, there is a possibility that *M. arunachalensis* may be an interspecific hybrid of above-mentioned species. Further studies demanding for the origin of this species. In the present phylogenetic tree (Figs. 43 & 44) we found that this subclade (subclade IIIA) is a newly resolved clade because all the taxa except *M. ornata* were used for the first time in phylogenetic analysis. Previous studies include only the accessions of *M. ornata* for this clade (Liu *et al*., 2010; Li *et al*., 2010; Christelova *et al*., 2011; Janssens *et al*., 2016; Lamare *et al*., 2017; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022). According to the results of Li *et al*. (*l.c.*) and Lamare *et al*. (*l.c.*) the *M. ornata* is placed as the sister taxa of *M. yunnanensis*. However, this species is placed along with *M. sanguinea* in the results of Liu *et al.* (*l.c.*) and Fu *et al.* (*l.c.*). The *M. sanguinea* is reported as 'extinct in the wild' in the Indian revision of Musaceae (Joe & Sabu, 2019), so the actual identity of this species mentioned in the study of Liu *et al.* (*l.c.*) and Fu *et al.* (*l.c.*) needs to be confirmed.

Subclade with the highest number of taxa in the section *Rhodochlamys*

Even if this clade (subclade IIIB; Figs. 43 & 44) is housed by the highest number of *Rhodochlamys* members (7 taxa), its basal lineage is formed by *M. ochracea*, a typical representative of the sect. *Musa*. Based on our observation, the fruit pulp of this species is unique and very sweet and it can be nominated as a parent for breeding studies. *Musa ochracea* is allied with *M. acuminata* by the nature of bud, flower architecture and fruit shape. The present molecular study ascertains the species status and position of *M. ochracea*. In the previous studies (Liu *et al*., 2010; Li *et al*., 2010; Christelova *et al*., 2011; Janssens *et al*., 2016; Lamare *et al*., 2017; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022), the species like *M. mannii*, *M. chunii*, *M. velutina* and *M. aurantiaca* were included but, *M. markkui, M. markkuana* and *M. velutina* var. *variegata* are considered for first time in the present study. Here, the molecular analyses supported the species status of *M.*

markkui and varietal status of *M. velutina* var. *variegata,* however, the data from this phylogram is not enough to segregate the *M. markkuana* from *M. velutina*. Previously, *M. markkuana* is considered as a subspecies of *M. velutina* and Hareesh *et al.*, (2017) elevated it into species rank with the support of morphological, anatomical and palynological evidence. The distinguishing features of *M. markkuana* from *M. velutina* are sterile bracts with glabrous adaxial surface (*vs*. hairy), glabrous ovary (*vs*. hairy) and glabrous fruits that do not split at maturity (*vs*. hairy schizocarpic fruits). Apart from these, the authors identified several differences in anatomy as well as in palynology. So, more DNA regions may be required for the segregation of these two species. Besides, multiple accessions of *M. mannii* (159735, 159776, HBG:2007-0001) and *M. chunii* (159788, 159790) are positioned as sister to each other in the present study in contrast with the results of Fu *et al*. (2022). In their study, *M. mannii* sistered with *M. aurantiaca* and *M. chunii* sistered with *M. ruiliensis* W.N.Chen, Häkkinen & X.J.Ge. *Musa ruiliensis*, a Chinese wild ornamental species (Chen *et al*., 2014), is allied with *M. chunii* but differs by its flower buds with abaxially pink-red and adaxially pale-yellow (*vs*. pale pink). In other molecular studies (Li *et al*., 2010; Liu *et al*., 2010; Christelova *et al*., 2011; Janssens *et al*., 2016; Lamare *et al*., 2017; Burgos-Hernández *et al*., 2019), *M. chunii* was not represented. The placement of *M. mannii* in Liu *et al.*'s (2010) study as sister taxa with *M. rosea*, whereas in the phylogram of Li *et al*. (*l.c.*), Janssens *et al*. (*l.c.*) and Lamare *et al*. (*l.c.*) it is placed along with *M. velutina*. However, the position of *M. mannii* in the molecular study of Burgos-Hernández *et al*. (*l.c.*) was aside with *M. laterita* (now a synonym of *M. rubra*), it looks like a strange positioning and more investigations are required for the identity of this *M. laterita* accession. Moreover, our accession of *M. aurantiaca* (159768) is placed as a sister to Li *et al*.'s (*l.c.*) accession of *M. aurantiaca*.

Reinstatement of *Musa sabuana* and *M. balbisiana* var. *andamanica* from the synonymy of *M. balbisiana*

Singh *et al*. (1998) described *M. balbisiana* var. *andamanica* based on a live plant grown on the campus of Central Agricultural Research Institute (CARI) (now CIARI; Central Islands Agricultural Research Institute) Port Blair, Andaman and Nicobar Islands. Later, Joe (2015) reported the occurrence of these taxa in Tripura,

India, which was a new record from the mainland. According to Hareesh *et al.* (2017)'s hypothesis, this taxon was brought onto the Andaman and Nicobar Islands from the mainland by Bengali settlers in the early 1950's. *Musa sabuana* is an endemic species, which was originally described based on collections from Middle Andaman and Little Andaman (Prasad *et al*., 2013). While describing this taxon, the authors studied the immature infructescence; hence the protologue lacks characters of ripened fruits and seeds. However, the protologue suggests that this species has unique features like "perfect spiral arrangement of leaves, fruits with bottle-necked apex (rarely absent) and brown-purple bracts with green striations". Subsequently, Hareesh *et al*. (2017) carried out an extensive study on the Musaceae of the Andaman and Nicobar Islands and reported the extended distribution of *M. sabuana* from the Great Nicobar Islands. They also gave a detailed account of the variation in bract colour from green to purple with intermediate shadings in *M. sabuana* with photographs. Moreover, during our field visit in 2019, we observed and studied several populations of *M. sabuana* with different bract colours (ranges from green to brown-purple). The colour of the flower bracts is green, green with brown-purple striations or patches, green with a brown-purple tinge, different gradations of brown-purple with a green tinge, brown-purple with cream striations, brown-purple with green striations and brown-purple. Whereas, recently Singh *et al*. (2020) treated *M. sabuana* and *M. balbisiana* var. *andamanica* as synonyms of a morphologically distinct *M. balbisiana* based on the molecular study. However, a close observation revealed that the voucher details in the herbarium specimen and NCBI data are rather contrary in this study. While examining the voucher specimens of *M. sabuana* used by Singh *et al.* (*l.c.*) for their molecular study, we noticed that the collection locality of *M. sabuana*- PBL29678 was from Panchawati area, Middle Andaman and *M. sabuana*- PBL29679 is from Ramakrishnapur, Little Andaman. But, based on the NCBI data, the collection localities of *M. sabuana*- PBL29678 is from Krishnapuri, North Andaman and *M. sabuana*- PBL29679 is from Kaushalya Nagar, Middle Andaman, where *M. balbisina var. andamanica* are widely distributed and not *M. sabuana*. So, the authenticity of the *M. sabuana* specimens used for the molecular study was controversial. North Andaman and Kaushalya nagar area in Middle

Andaman do not belong to the distribution range of *M. sabuana,* suggesting that the sequenced materials may be *M. balbisiana* var. *andamanica*, because these areas are mainly confined by this taxon. In the case of *M. balbisiana* var. *andamanica*-PBL29677 accession of Singh *et al.* (*l.c.*), its voucher details were also inconsistent in NCBI database. So, we have not included Singh *et al.* (*l.c.*) sequences in the present study.

The present phylogenetic tree with the multiple accessions of all these taxa shows that *M. sabuana* (three accessions of different bract colours; 152776, 164074 and 164076) formed a clade distinct from *M. balbisiana* clade with high support of BS =100 and PP =1.00. It strongly supports the distinctiveness of these two species and the bract colour variation (green to brown-purple) in *M. sabuana*. Two accessions of *M. balbisiana* var. *andamanica* (116152, 164030) and two accessions of *M. balbisiana* (130742, HBG: 2001-0390) are nested in the *M. balbisiana* clade along with other varieties of *M. balbisiana* and *M. cheesmanii*. Thirty-two variable sites are observed between *M. balbisiana* and two accessions of *M. balbisiana* var. *andamanica* in the concatenated nucleotide sequences and the tree supported the varietal status of *M. balbisiana* var. *andamanica* with high support (BS =98; PP = 0.99). Here, the *M. balbisiana* clade was recovered in the basal position of sect. *Musa* whereas, the *M. sabuana* clade was recovered as sister to the clade of *M. acuminata* subspecies, *M. rubra*, *M. kattuvazhana.* Based on these results, here we reinstate *M. sabuana* and *M. balbisiana* var. *andamanica* from the synonymy of *M. balbisiana.*

The 'green-bracted' *Musa indandamanensis* is conspecific with *M. sabauana*

Both *M. sabuana* and *M. indandamanensis* L.J.Singh were described from the Andaman Islands in the year 2013 and 2014 respectively. As mentioned earlier, while publishing *M. sabuana*, the authors gave only the details of immature infructescence; hence the protologue lacks characters of ripened fruit and seeds. However, this species has unique features like 'spiral arrangement of leaves, fruits with bottle-necked apex and brown-purple bracts with green striations'. Subsequently, Singh (2014) described another new species of *Musa*, *Musa indandamanensis* based on mature plant material with remarkable orange-purpled fruit and green bracts collected from areas adjacent to the paratype locality

(Ramakrishnapur, Little Andaman) of *M. sabuana.* Several aspects such as conservation, seed germination, storage study (Bohra *et al*., 2019, 2020) and whole plastid genome characterization (Maurya *et al*., 2023) were conducted. On the other hand, Hareesh *et al.* (2017) reported the bract colour variation in *M. sabuana* from green to purple and its extended distribution in the Great Nicobar Islands. Besides, the authors observed the overall morphological similarity of *M. sabuana* and *M. indandamanesis* and stated that the 'green-bracted' *M. indandamanensis* is a morphotype of former species and synonymized under it. Subsequently, Joe and Sabu (2019) provided detailed descriptions of *M. sabuana* (p. 230–236) including the ripened fruits and seeds characters, photographs showing bract colour variations, spiral phyllotaxy, bottle neck apex of fruit *etc.* in their revisionary study of Indian Musaceae. Recently, Singh *et al*. (2020) synonymised *M. sabuana* under *M. balbisiana* based on molecular study. It is surprising to note that both above-mentioned species show entirely different morphology in all aspects. Moreover, *M. sabuana* shows morphological similarity with *M. acuminata* and *M. kattuvazhana.* In order to reinstate *M. sabuana*, we used three accessions of *M. sabuana* with bract colour variation [152776 (green-bract), 164074 (green bract with purple tinge), 164076 (purple bract)]. These specimens were collected during our recent field visits in Andaman and Nicobar Islands. During this expedition, we found the *M. sabuana* with green-coloured bracts from Ramakrishnapur, Little Andaman and Vijayanagar, Great Nicobar Islands. Dark purple-coloured bracts from Galathea, Great Nicobar Islands and the intermediate bract colour shadings from all the way from Campbell Bay to Indira Point and Great Nicobar Biosphere Reserve. All the individuals studied showed uniform features like perfect spiral phyllotaxy and fruits with a bottle-neck apex (rarely absent). The prominent bottle-neck apex is seen in immature fruits and the bottle-necks gradually shorten when fruit becomes ripened in some populations. During the survey, the largest population of *M. sabuana* (with brown-purple bract) was observed in Galathea, Great Nicobar Islands (>70 individuals). All the plants in Galathea are directly exposed to sunlight and seem to be shorter and the dorsal side of the leaves are more glaucous when compared with the plants grown in shady areas. Moreover, whole leaves in the plants of these populations were torn and damaged by the continuous wind-blown from the seashore and these populations are at risk due to road widening.

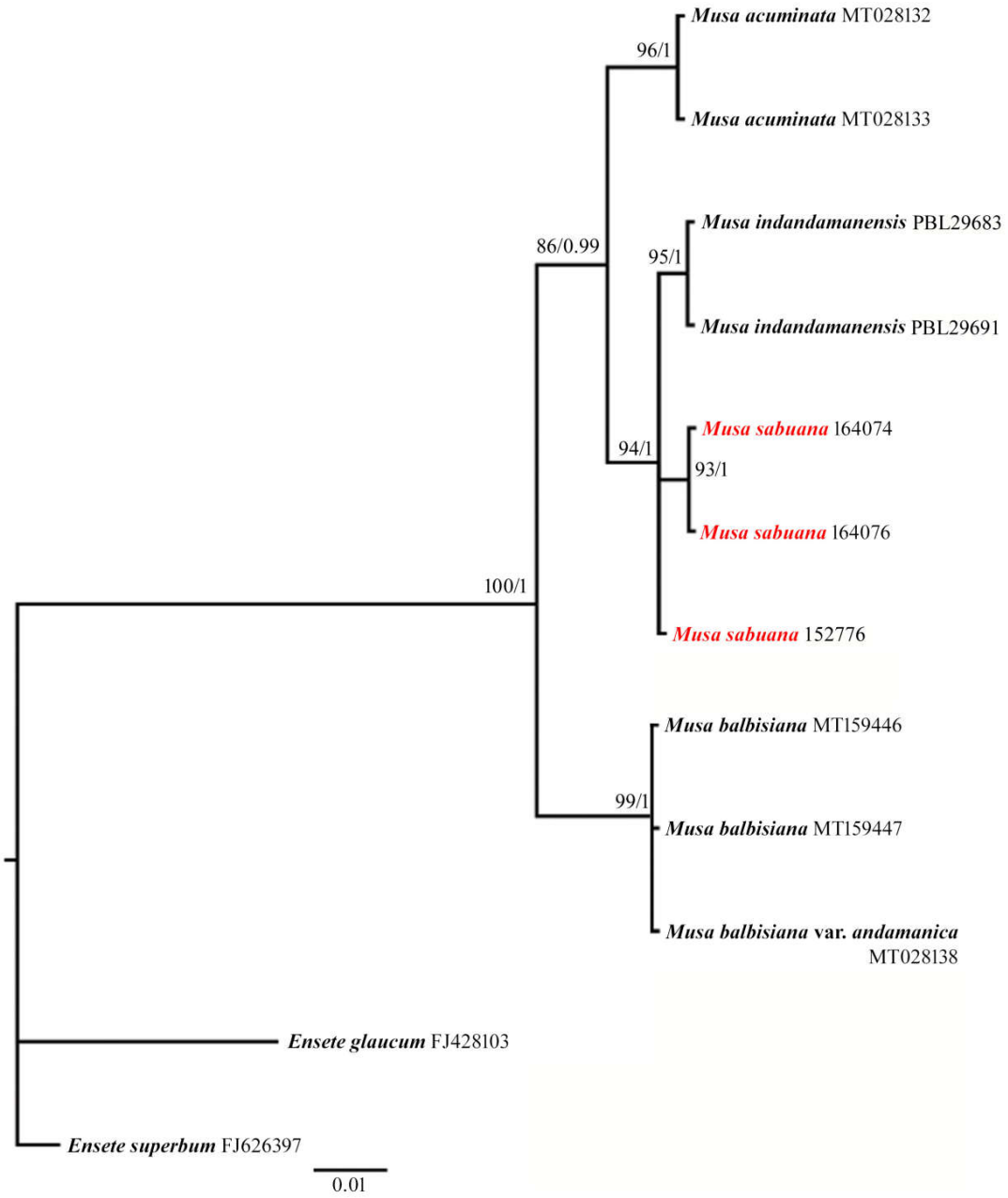


Fig. 47. Strictly consensus tree obtained from the ML and BI analyses of the combined ITS and *trnL-F* dataset. Bootstrap support value of ML and BI posterior probabilities are shown in each node.

Multiple accessions based on phylogenetic study of *M. sabuana* resulted that, all three accessions being nested together and formed as a clade sister to the clade of subspecies of *M. acuminata* (subclade IIIC; Figs. 43 & 44). The green bract form of *M. sabuana* (152776) was recovered a basal position to the other *M. sabuana* accessions (164074- green bract with purple tinge, 164076- purple bract) in the *M. sabuana* clade (Figs. 43 & 44). Moreover, the *M. indandamanensis* accessions

(PBL29683, PBL29691) of Singh *et al.* (*l.c.*) are nested along with our three accessions of *M. sabuana* (Fig. 47). So, in each aspect, our molecular result is in agreement with the findings of Hareesh *et al.* (2017) and we supported the synonymization of 'green-bracted' *M. indandamanensis* under the name, *M. sabuana*.

Molecular identity of *Musa kattuvazhana* and its taxonomic resurrection from *M. acuminata* and its subspecies

The identity of *M. kattuvazhana* is still in contention for some taxonomists. This species was originally published by Jacob (1952) from the Western Ghats of Madras Province, India. Based on the thorough literature study and live collections from South India, Joe *et al*. (2016a) and MosaChristas *et al.* (2021) treated *M. acuminata* subsp. *burmannica* and *M. banksii* var. *singampatti* as synonym of *M. kattuvzhana*. Also, the recent phylogenetic studies (Li *et al.*, 2010; Janssens *et al.*, 2016), show that of *M. acuminata* subsp. *burmannica* and subsp. *burmannicoides* phylogenetically show more similarity with the sect. *Rhodochlamys* species [*M. laterita* Cheesman (=*M. rubra*), *M. siamensis* Häkkinen & Rich.H.Wallace (=*M. rubra* var. *siamensis*), *M. rosea* Baker, and *M. rubra*] rather than other subspecies of *M. acuminata*. Besides, the recent plastome analysis of Musaceae revealed that *M. acuminata* subsp. *burmannica* is basal taxa to the *M. acumiata- M. rubra* clade (Fu *et al*., 2022). Thus, it is suggested that *M. acuminata* subsp. *burmannica* is genetically distinct from other subspecies of *M. acuminata*. However, still some studies (Sardos *et al*., 2016, Rouard *et al.*, 2018, Dupouy *et al.*, 2019, Singh *et al.*, 2020, Fu *et al.*, 2022) used the name *M. acuminata* subsp. *burmannica* without considering Joe *et al.* (2016a).

Moreover, based on a recent survey, Hareesh *et al*. (2017) reported the occurrence of *M. kattuavzhana* from the Andaman Islands. Later on, this species from Andaman was found to be nested along with *M. acuminata* subsp. *burmannica* in a phylogenetic study of Singh *et al*. (2020), which led them to conclude that the specimen found in the Andaman Islands was *M. acuminata* rather than *M. kattuvazhana*. However, we could not locate any single population of typical *M.*

acuminata during our repeated expeditions in the Islands. So, the actual identity of this particular Island *Musa* sp. remains unclear and needs to be proven.

By trailing these backgrounds, we include three accessions of *M. kattuvazhana* (Two accessions [164050, 164054] from middle Andaman, Andaman Islands and one accession [159727] from Munnar, Kerala, India), two accessions of typical *M. acuminata* from North-East India (116175, 159775) and accessions of *M. acuminata* subsp. *burmannica* (ITC0283) and supsp. *burmannicoides* (ITC0249), along with the other available sequences of *M. acuminata* subspecies from Li *et al.* (2010). In the phylogenetic tree (Figs. 43 & 44), *M. acuminata* (116175 and 159775; subclade IIIA), placed away from three accessions of *M. kattuvazhana* from Andaman (164050 and 164054) and Kerala (148737) in subclade IIIC with strong support (BS=98, PP=0.99). This indicates that *M. kattuvazhana* is phylogenetically distinct from *M. acuminata*. Moreover, three accessions of *M. kattuvazhana* is nested along with *M. acuminata* subsp. *burmannica*, subsp. *burmannicoides*, *M. rubra*, and *M. siamensis* (= *M. rubra* var. *siamensis*) in the phylogram of Indian Musaceae (Figs. 43 & 44). In the world phylogenetic tree (Figs. 45 & 46) of Musaceae, *M. kattuvazhana* accessions are nested along with the same taxa in Indian phylogram (Figs. 43 & 44) and apart from this, all other subspecies of *M. acuminata* and *M. sabuana* are placed in this subclade IIIC (BS =87, PP =0.98). However, the phylogenetic placement of *M. acuminata* subsp. *burmannica*, subsp. *burmannicoides* and *M. kattuvazhana* along with *M. rubra* and *M. rubra* var. *siamensis* supported the synonimization of *M. acuminata* subsp. *burmannica* and subsp. *burmannicoides* under *M. kattuvazhana* and its distinction with other subspecies of *M. acuminata.* Moreover, we analyzed Singh *et al*. (2020)'s two accessions (PBL29673, PBL29694) from Andaman (tree not shown) and concluded that, those species are *M. kattuvazhana*, not *M. acuminata*. Because the morphological characters of *M. kattuvazhana* include non-glaucous pseudostem (*vs*. glaucous), both sides pointed leaf base (*vs*. rounded), puberulent peduncle (*vs*. glaucous), strongly imbricate flower buds (*vs*. non-imbricate) and phylogenetic placement are entirely different from typical *M. acuminata.*

Bract colour variation- a common but unnoticed phenomenon in wild *Musa* spp.

The function of a bract is multifaceted, it may protect the young floral buds, fruits, seeds, attract pollinators, camouflage, seed dispersal, ameliorate the effect of abiotic stress on flowers, and photosynthesis (Song *et al*., 2024). The common bract colour of banana is different gradation of purple however, diversity and great variation can be observed in bract colour of wild bananas. The bright coloured-bract is the major attractive feature of ornamental banana species (sect. *Rhodochlamys*). Some examples are *M. aurantiaca* (orange), *M. arunachalensis* (red-orange), *M. chunii* (pale lilac), *M. mannii* (crimson red), *M. markkuana* (pink), *M. ornata* (lilac), *M. rubra* (brick red) and *M. velutina* (pink). Also, the members in sect. *Musa* shows unique colour in bracts *viz*., *M. argentii* (pink), *M. itinerans* (deep maroon with yellow margins), *M. ochracea* (dark brown-purple with yellow striation at apex). However, in Indian Musaceae, green-bracted species are very less, though the well-known example is *Ensete glaucum*. However, the bract colour variation from green to purple is rarely observed in some species. This colour variation is observed in the same population or in different populations inside the geographic boundary. One of the serious issues regarding this bract colour variation is that the bract colour is considered to be one of the main morphological traits for species delimitation and it is the eye-catching character in *Musa* species. So, researchers may be confused with the morphotype and publish it as a new taxon. Examples are, *M. swarnaphalya* Uma, Saraswathi & Durai (Uma *et al.*, 2011) a species with "greenish- yellow coloured male bud" and *M. paramjitiana* Singh (Singh, 2017) a "green bracted" species. But, after a detailed morphological variation study, Joe and Sabu (2019), treated the former species as conspecific with *M. cheesmanii* and Hareesh *et al*., (2017) treated the later one as the synonym of *M. balbisiana* var. *andamanica*. In the present molecular study also, the *M. cheesmanii* accession (159779), which was collected from the same clump of 'greenish-yellow bracted' *M. cheesmanii* from Itanagar, Arunachal Pradesh and violet-purple bracted *M. cheesmanii* (130830) from Tippi, Arunachal Pradesh are nested together in a clade along with varieties of *M. balbisiana*. Moreover, the 'green-bracted' accession of *M. balbisiana* var.

andamanica (164030) from middle Andaman, Andaman Island and 'pink-purple-bracted' *M. balbisiana* var. *andamanica* (116152) from Tripura, India formed a polytomy in *M. balbisiana* clade (subclade I; Figs 43 & 44). Therefore, the present molecular study is also in agreement with the observation of Hareesh *et al*., (2017) and Joe and Sabu (2019) regarding the synonymization of *M. swarnaphalya* with *M. cheesmanii* and *M. paramjitiana* with *M. balbisiana* var. *andamanica*. Moreover, wild bananas like *M. balbisiana* var. *elavazhai*, *M. balbisiana* var. *sepa-athiya* (Joe & Sabu, 2019: Page no. 145, Fig. 27B, O) show the colour variation in bracts from green to purple under cultivation in Calicut University Botanical Garden (CUBG), Kerala, India. The actual molecular mechanism in the colour variation of *Musa* sp. has not yet been studied. In addition, green bracts help to promote photosynthetic activity and provide photosynthates for developing fruits and seeds, especially late in a plant's life cycle, when leaves have started to senescence (Song *et al*., 2024). Based on the aforementioned facts, a thorough field study is necessary to determine the genuine source of polymorphism in the bract colour of *Musa* species.

Are there any unidentified wild ancestors for cultivated banana formation?

One of the major contradictory results of the present phylogenetic investigation is the positioning of two accessions of typical *M. acuminata* (116175, 159775). These accessions are placed in a different clade from that of subspecies of *M. acuminata* clade. So, the placement of our *M. acuminata* accessions questioned the previous phylogenetic results. In all the previous studies, they did not include the typical *M. acuminata* subsp. *acuminata* and we have used here it for the first time in a phylogenetic study. Being the 'A' genome contributor for the cultivated banana, this species gathered much attention. The placement of our *M. acuminata* subsp. *acuminata* in the phylogram is surprising and we have deeply investigated the contributions of the so-called ancestors to the edible bananas. Almost all scientific communities believe that the majority of edible bananas are formed by the result of inter-subspecific hybridisation of *M. acuminata* subspecies and hybridisation between *M. balbisiana* and subspecies of *M. acuminata* (Simmonds & Shepherd, 1955; Stover & Simmonds, 1987). Simmonds and Shepherd (1955) proved the

hybrid origin of cultivars from *M. acuminata* ('A' genome) and *M. balbisiana* ('B' genome) by experimental crosses between these species. The derived cultivars are classified into different genome groups based on their morphological similarity with the above-mentioned wild ancestors and their ploidy level. That is diploid 'AA', 'AB' and triploid 'AAA', 'AAB', 'ABB', however, edible tetraploids are very rare and no edible diploid forms of *M. balbisiana* are known (Simmonds & Shepherd, 1955; Baurens *et al*., 2019). Fifteen diagnostic morphological characters are used for this classification and the characters states similar with *M. balbisiana* will get maximum score '5' and that character states similar to *M. acuminata* will get a minimum score '1'. Hence, the possible range of total scores was therefore 15 (typical *M. acuminata* character) to 75 (typical *M. balbisiana* character). Apart from these 'A' and 'B' genome, a small genome contribution of *M. schizocarpa* N.W. Simmonds ('S' genome; Argent, 1976; Carreel *et al*., 1994) and *M. textilis* Nee ('T' genome; Brewbaker *et al*., 1956; Heslop-Harrison & Schwarzacher, 2007) were reported in cultivated bananas (Christelová *et al*., 2017; Němečková *et al*., 2018). However, most of the hybridisation studies were focused mainly on 'A' genome cultivar formation. The major contributor of banana domestication is, *M. acuminata* subsp. *banksii*, confined to the Papua New Guinea and this Island has been believed to be the 'cradle of banana domestication'. From this taxa, diploid form of 'cultiwild' (intermediate form of wild and cultivar banana) is formed and it diffused to the northern part, *ie,* into the whole South-East Asia. Subsequently, this form is hybridized with other local subspecies of *M. acuminata* and *M. balbisiana*. This process is enabled by the human movements and exchanges between Islands of South-East Asia (Perrier *et al*., 2009, 2011). All these hypotheses were made by trailing the results of Simmonds and Shepherd (1955). In general, the actual 'A' genome contributors are constituted by different subspecies of *M. acuminata* (Simmonds, 1956), however, not all subspecies of *M. acuminata* equally contributed for cultivar formation. In this process, the major contributors are *M. acuminata* subsp. *banksii* followed by subsp. *errans*, subsp. *malaccensis*, subsp. *zebrina* (Simmonds, 1956; Carreel *et al*., 2002; Denham *et al*., 2003; Perrier *et al*., 2009, 2011; Hippolyte *et al*., 2012; Sardos *et al*., 2016; Christelová *et al*., 2017;

Němečková *et al*., 2018; Martin *et al*., 2020a,b; Šimoníková *et al*., 2022). But some studies show that, the mainland subspecies, *M. acuminata* subsp. *burmannica*, now standing as the synonym of *M. kattuvazhana* by Joe *et al*. (2016a), is not much contributed for the cultivar formation (Li *et al*., 2013; Martin *et al*., 2020a,b). Moreover, some of the recent studies put forward doubts about the cryptic or unidentified wild contributor to cultivar formation (Martin *et al*., 2020b; Sardos *et al*., 2022), because these studies show higher genetic diversity in cultivars than their wild ancestors. Hence, we speculate that the history of edible banana formation is much complex than we expected and more study is required for a better understanding about this particular topic. The distant placing of *M. acuminata* (Sub clade III) and *M. balbisiana* (Sub clade I) supports their genetic difference and the morphologically allied wild bananas of these species can be exploited as wild gene pools (Li *et al*., 2010). As of the prime members in the 'A'-genome and 'B'-genome prominent groups (Figs. 43, 44, 45 & 46) we recommend the taxa like *M. balbisiana*, its varieties (var. *andamanica*, var. *bheemkola*, var. *elavazhai*, var. *sepa-athiya*) and its allied taxa (*M. cheesmanii*, *M. puspanjaliae*), also typical *M. acuminata* and its allied Indian endemics (*M. flaviflora*, *M. thomsonii*, *M. sabuana*, *M. ochracea etc*.) as genetic resources for breeding studies. No breeding studies have yet been carried out using Indian endemic wild bananas. This research sets the stage for future breeding programs utilizing promising candidates from the Indian endemic wild banana species.

CHAPTER 6 CHARACTER EVOLUTION AND ANCESTRAL STATE RECONSTRUCTION OF INDIAN MUSACEAE

The process of evolution cannot be directly experienced, but if we had a phylogenetic tree, we could trace the pattern of evolution by putting characters and character states onto the tree (Ronquist, 2004). Character state reconstruction is a strategy used to investigate the processes and patterns of character evolution (Cunningham, 1999; Maddison & Maddison, 1992; Ronquist, 2004).

Despite the importance of flower characters for the classification of angiosperms, they do not work well with Musaceae. The similarity in vegetative and floral parts among members in Musaceae, especially in the genus *Musa* makes this group taxonomically challenging (Hareesh *et al*., 2017; Joe & Sabu, 2019). The vegetative characters are shown a high level of polymorphism in these taxa. Furthermore, the herbarium-based study is not amenable due to fleshy and large-sized plant parts (Hareesh & Sabu, 2023). Cheesman (1947a) used cytological and morphometric characters including pseudostem characters, inflorescence characters, and seed characters for the sectional classification. Despite this, now revised classification of Häkkinen (2013), pointed only the chromosome numbers as a synapomorphic character for the prevailing two sections, such as section *Musa* (2n=2x=22) and section *Callimusa* (2n=2x=14/18/20). However, there are evolutionary important morphological traits seen in Musaceae, especially in the genus *Musa*. So, the mapping of those traits into a phylogenetic tree helps to comprehend the pattern of evolution. The current work aims to realize the evolution of phylogenetically important traits in Indian Musaceae.

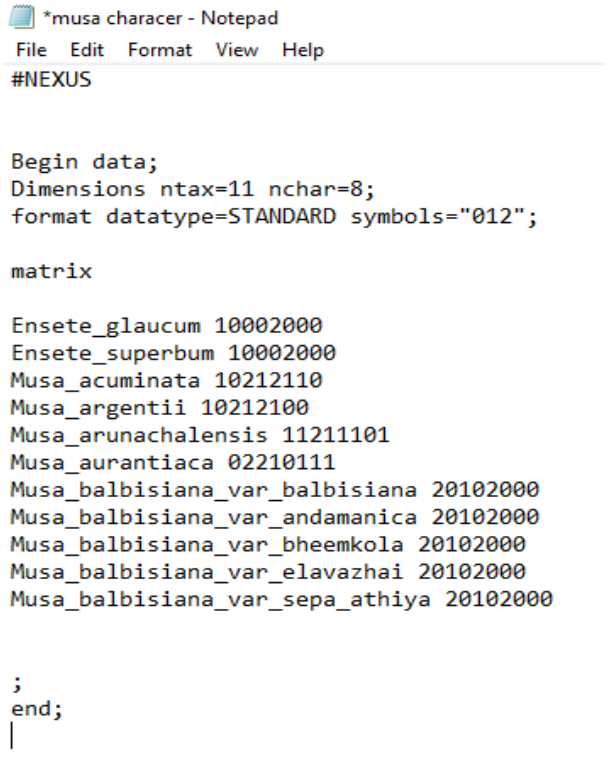
Materials and methods

In light of 64 morphometric characters analyzed in the morphological phenetic study (see Chapter 4), nine unique and evolutionary significant characters were selected to trace their evolutionary pattern in the Indian Musaceae. These

include the height of pseudostem, shape of inflorescence, longevity of the bract, behaviour of bract before falling, number of rows of fruit/flower per bract, shape of bract apex, colour of bract, fruit shape/appearance and seed shape. Each character and their corresponding character states (binary or multistate) are given in Table 11. The character states are coded/scored as whole numbers (0, 1, 2. etc.) and these codes were provided to each taxon corresponding to their character states (Table 12). After that, the data set was converted into nexus format. The analysis was performed in Mesquite 3.61 software (Maddison & Maddison, 2021) with 30 Indian taxa. The strict consensus Bayesian tree file as well as coded binary and multivariate characters was imported in Mesquite. The tree was plotted by using the ML algorithm. The final tree was visualized in Mesquite 3.61. The steps for the character evolution study are given below.

Steps

I. Characters and character states are coded in an Excel file. Import the file into nexus format. The sample file is given below.



II. Ancestral state trees are constructed in Mesquite software.

1. Open Mesquite software
2. File→Open nexus file of characters & character states
3. Characters→Make new matrix from Stored Matrices (rename the newly generated character matrix if needed.)
4. Taxa & Trees→Import file with trees→Link contents→open .tre file of selected BI phylogram (.tre file will appear on the left side of the tab) → click the trees icon→view trees
5. Analysis: Tree→Trace character history→ Parsimony character states →Ok

Table 11. Characters and their corresponding character states used in the study.

Sl No.	Characters	Character states	Score
1	Maximum pseudostem height (m)	Large (>4.5 m)	0
		Medium (2.2–4.5 m)	1
		Small (<2.2 m)	2
2	Shape of inflorescence	Pendulous	0
		Semi pendulous	1
		Erect	2
3	Longevity of bract	All bracts persistent throughout	0
		Persistent towards the male bud	1
		All bracts deciduous throughout	2
4	Bract behaviour before falling	Not- revolute	0
		Revolute	1
5	Number of rows of female flowers/ fruits per bract	Two	0
		One or two	1
		One	2
6	Bract apex shape	Obtuse	0
		Acute	1
7	Fruit shape/ appearance	Broadly oblong/ stout	0
		Narrowly oblong/ slender	1
8	Colour of bract	Dull	0
		Bright	1
9	Seed shape	Ovoid to sub-globose	0
		Oblate	1
		Ellipsoid	2

Table 12. Indian Musaceae with character score.

Taxa	Maximum pseudostem height (m)	Shape of inflorescence	Longevity of bract	Bract behaviour before falling	Number of rows of female flowers/ fruits per bract	Bract apex shape	Fruit shape/ appearance	Colour of bract	Seed shape
Ensete glaucum	1	0	0	0	0	0	0	0	0
E. superbum	1	0	1	0	0	0	0	0	0
Musa acuminata	1	0	2	1	0	1	1	0	1
M. argentii	1	0	2	1	0	1	0	0	1
M. arunachalensis	1	1	2	1	1	1	0	1	1
M. aurantiaca	2	2	2	1	2	1	1	1	1
M. balbisiana var. *balbisiana*	0	0	1	0	0	0	0	0	0
M. balbisiana var. *andamanica*	0	0	1	0	0	0	0	0	0
M. balbisiana var. *bheem kola*	0	0	1	0	0	0	0	0	0
M. balbisiana var. *elavazhai*	0	0	1	0	0	0	0	0	0
M. balbisiana var. *sepa-athiya*	0	0	1	0	0	0	0	0	0
M. cheesmanii	0	0	1	0	0	0	0	0	0
M. chunii	2	1	2	1	2	1	1	1	1
M. flaviflora	1	0	2	1	0	1	1	0	1
M. itinerans	0	0	2	0	0	0	1	0	1
M. kattuvazhana	1	0	2	1	0	1	1	0	1
M. mannii	2	1	2	1	2	1	1	1	1

M. markkuana	2	2	2	1	2	1	1	1	1
M. markkui	2	1	2	1	2	1	1	1	1
M. nagensium	0	0	2	0	0	0	0	0	1
M. ochracea	1	0	2	1	0	1	1	0	2
M. ornata	2	2	2	1	2	1	1	1	1
M. pradhanii	1	0	2	1	1	1	1	0	1
M. puspanjaliae	0	0	1	0	0	0	0	0	1
M. rubra	2	2	2	1	2	1	1	1	2
M. sabuana	0	0	2	0	0	0	1	0	1
M. sikkimensis	0	0	2	0	0	0	0	0	1
M. thomsonii	1	0	2	1	0	1	1	0	1
M. velutina	2	2	2	1	2	1	1	1	1
M. velutina var. *variegata*	2	2	2	1	2	1	1	1	1

Results and Discussion

The selected evolutionary significant characters and their corresponding evolution patterns along the phylogenetic tree of Indian Musaceae are discussed separately below. The ancestral state characteristics such as plesiomorphy or ancestral traits, symplesiomorphic conditions, apomorphy, synapomorphy and homoplasy were observed in the constructed character state trees.

1. Maximum pseudostem height

Even though the general vegetative characters are stable in the genus *Musa*, the height of plant (from the crown to the emerging point of petiole) shows variability and it ranges from 1−11.3 m. The pseudostem height is considered to be an important character by pioneers classifications (Colla, 1820; Spach, 1846; Sagot, 1887a, b; Baker, 1893) as well as modern Musaceae classifications (Häkkinen, 2013). In Cheesman's (1947a) sectional classification of the genus *Musa* as well as in the *Musa* description INIBAP *Musa* Descriptor List (IPGRI-INIBAP/CIRAD, 1996) the height of the pseudostem is taken as a key trait. Moreover, while describing a new species, height is always considered as a diagnostic character. Usually, in the traditional classification, pseudostem height <3 m is given for the section *Rhodochlamys* taxa and >3 m is assigned for the members in section *Musa*. However, our 64 morphological characters-based phenetic study shows that, sect. *Eumusa* (Fig. 37; Cluster I) is again diverged into two groups *viz.*, sub-clusters Ia and Ib. These sub-clusters show the differences in pseudostem height. So, following these observations here we divide this character into three-character states, which are large (>4.5 m), medium (2.2−4.5 m), small (<2.2 m) and coded as 0, 1 and 2 respectively. Based on the present results, the large pseudostem (>4.5 m) is observed as symplesiomorphy and small (<2.2 m) and medium (2.2−4.5 m) size of the pseudostem appear to be derived character states in the genus. However, derived traits such as small size and medium size of plants have been observed as evolved independently several times within the subclade III (*M. acuminata*- *Rhodochlamys* clade or 'A'- genome prominent group). The large-sized plants are mainly seen in an ancestral clade of Indian Musaceae, however, it reappears in *M. sabuana* and *M. sikkimensis* as homoplasy (Fig. 48).

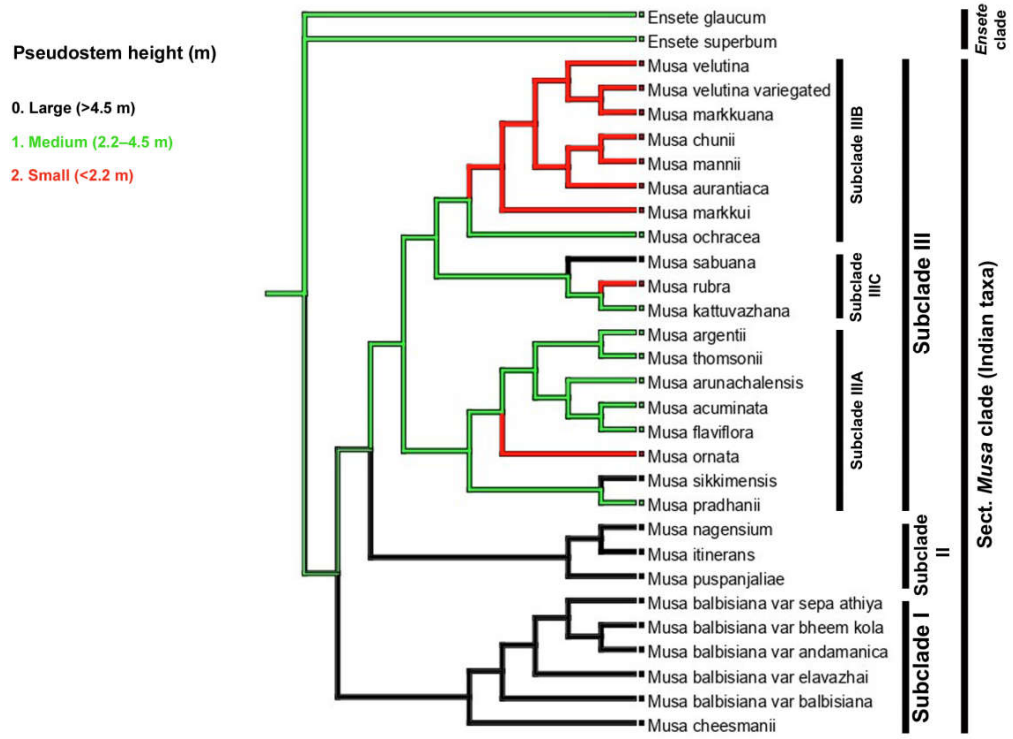


Fig. 48. Ancestral state reconstruction of the height of pseudostem in Indian Musaceae.

2. Shape of inflorescence

Inflorescence is the most useful morphological character to distinguish between infra-generic as well as sectional classifications in Musaceae in general and the genus *Musa* in particular. The importance of this character has been emphasized in many studies as a primary character (Colla, 1820; Spach, 1846; Sagot, 1887a; Baker, 1893; Cheesman, 1947a). According to Joe (2015), the genus *Musa* in India comprises members from sect. *Rhodochlamys* and sect. *Musa.* Both these sections show remarkable differences in inflorescence orientation or shape. In the prime concern, the ornamental bananas (sect. *Rhodochlamys*) are depicted with upright inflorescence and sect. *Musa* possess pendant inflorescence. However, more close observation reveals that Indian Musaceae possess pendulous, semi-pendulous and erect inflorescence, here it is taken as character states and given scores 0, 1 and 2 respectively. The character state tree suggests that pendulous inflorescence appears throughout the Indian Musaceae and it seems as the plesiomorphic trait for Indian *Musa*. The two species of *Ensete*, taxa in sub-clade I (*M. balbisiana* clade), taxa in

sub-clade II (*M. puspanjaliae-M. itinerans-M. nagensium* clade) are entirely housed by members with pendulous type of inflorescence (symplesiomorphy). Moreover, in sub-clade III, 9 species fall under this trait, that are *M. pradhanii*, *M. sikkimensis*, *M. flaviflora*, *M. acuminata*, *M. thomsonii*, *M. argentii*, *M. sabuana*, *M. kattuvazhana* and *M. ochracea*. These species are distributed throughout the subclade IIIA (clade of *M. acuminata* and its allied taxa), IIIB (*M. ochracea*-sect. *Rhodochlamys* clade) and IIIC (*M. sabuana-M. rubra-M. kattuvazhana* clade). The other forms of inflorescence (semi-pendulous, erect) are suggested as apomorphic characters and they evolved independently in the sub-clade IIIA, IIIB and IIIC. The erect inflorescence appeared in sub-clade IIIA as *M. ornata*, sub-clade IIIC as *M. rubra* and sub-clade IIIB as *M. aurantiaca*, *M. markkuana*, *M. velutina* and its variegated variety. Taxa with semi-pendulous inflorescences show discrete distribution in sub-clade III ('A' genome prominent group), as *M. arunachalensis* in subclade IIIA and as *M. markkui*, *M. chunii*, *M. mannii* in subclade IIIB (Fig. 49).

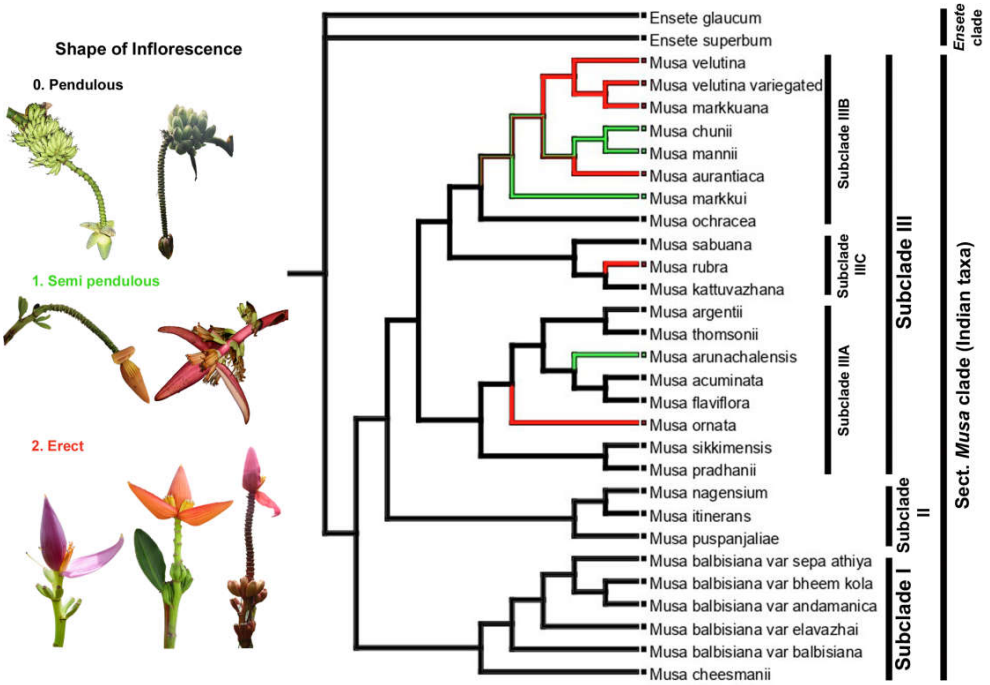


Fig. 49. Evolutionary pattern of the shape of inflorescence in Indian Musaceae.

3. Longevity of bract

In most of the flowering plants, the main function of the bract is providing protection for the flowers during the young stage and usually, it drops after the

maturity of the flower. In Musaceae bracts are the eye-catching part which protects one or two series of flowers. Usually, the bract in the genus *Musa* is fallen after the lifting. But, in *Ensete glaucum* all bracts are persistent throughout in the inflorescence and they are integral with the flowers. However, in some taxa of *Musa* and *E. superbum* show persistent bracts towards the male buds. So, here the character states assigned as all bracts persistent throughout, persistent towards the male bud, all bracts deciduous throughout and coded as 0, 1, 2 respectively. The results suggested that the persistent nature of the bract throughout in the inflorescence is observed as an ancestral phenotype in Indian Musaceae and it is observed only in *E. glaucum.* Whereas the persistent bract towards the male bud is seen as the symplesiomorphic trait for sub-clade I and it appears in *M. puspanjaliae.* All bracts deciduous throughout the inflorescence are observed as a synapomorphic character state for Indian *Musa*, this state has appeared in all remaining sub-clades in *Musa* (Fig. 50).

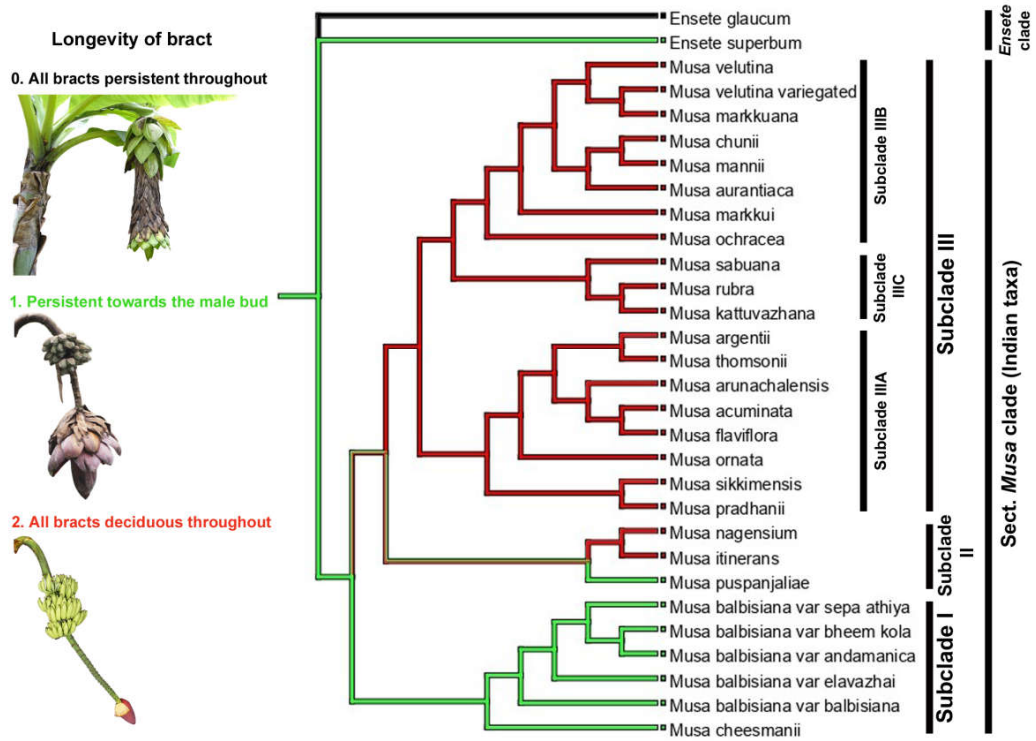


Fig. 50. Evolutionary pattern of the longevity of bracts in Indian Musaceae.

4. Bract behaviour before falling

The bracts are the protective covering of young flowers. In Musaceae, basal or first-formed flowers are mostly female (rarely bisexual) and male flowers are formed at the advanced blooming stage of inflorescence, so the chances of autogamy are rare (Nayar, 2010; Sreejith & Sabu, 2017). Generally, two types of bract behaviour are seen in Musaceae members before falling. These are revolute (curling of the tip of the bract into outer side) and non-revolute. These two types are considered here as character states and coded as '0' for non-revolute and '1' for the revolute nature of bract. The tree displays, non-revolute nature of bracts is the ancestral/plesiomorphic character and the revolute nature of bracts is formed as the derived character. The two species of *Ensete*, *M. cheesmanii*, *M. balbisiana* and its four varieties, *M. nagensium*, *M. puspanjaliae* and *M. sabuana* display the non-revolute, ancestral type of male bract. *Musa sabuana* rarely shows the revolute bract and the tree depicts that, the non- revolute nature of bract is independently acquired by this species. The rest of the Indian *Musa* taxa show the revolute type bracts. Which includes, *M. itinerans*, *M. sikkimensis*, *M. pradhanii*, *M. acuminata* and its relative species and ornamental bananas (previous sect. *Rhodochlamys*). The non-revolute bract is seen as a symplesiomorphic trait for ancestral sub-clade I and the revolute bract is depicted as a synapomorphic trait for the sub-clade III (except *M. sabuana*) (Fig. 51).

5. Number of rows of female flowers/ fruits per bract

Generally, the edible forms of bananas have always two rows of fruits within one hand and ornamental bananas have one row of fruits per hand (Sreejith & Sabu, 2017; Joe & Sabu, 2019). Here, the two rows of flower or fruit per bract are coded as 0 and the remaining character states such as two or one row and one row are coded as 1 and 2 respectively. The character state tree depicts two rows of fruit as ancestral phenotype, however, it reappears several times throughout the genus *Musa.* This character state is seen as symplesiomorphic for the *Ensete* clade, sub-clade I (*M. balbisiana* clade) and sub-clade II. One or two rows of flowers/ fruits are observed as apomorphic and it independently evolved for *M. pradhanii* and *M. arunachalensis*. However, under ideal conditions, both species produce two rows of flowers/ fruits. The one-rowed condition appeared as synapomorphic for sub-clade IIIB (except *M. ochracea*) and appeared in sub-clade IIIA and IIIC as *M. ornata* and *M. rubra* respectively (Fig. 52).

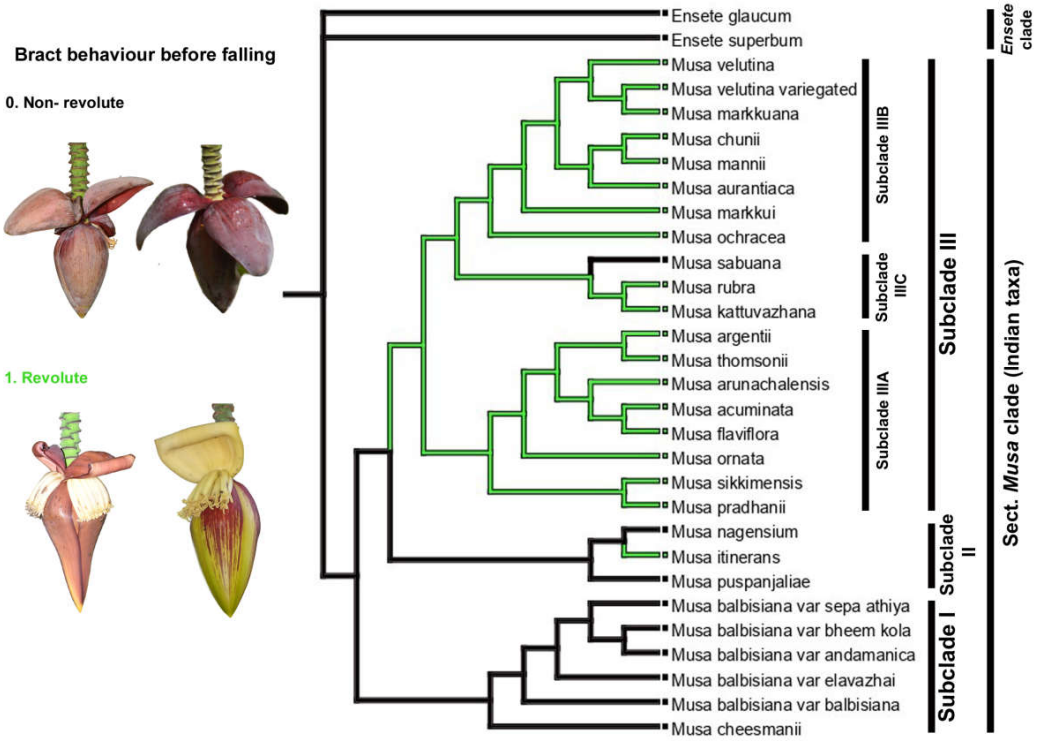


Fig. 51. Evolutionary pattern of bract behaviour before falling in Indian Musaceae.

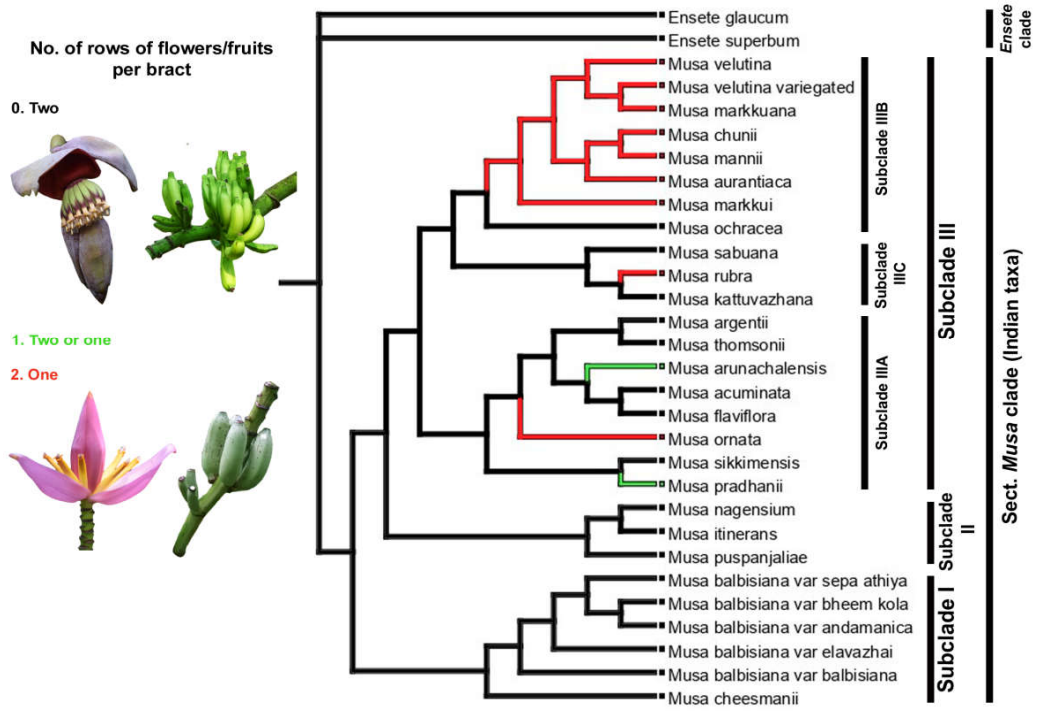


Fig. 52. Evolutionary pattern of the number of row of flowers/ fruits per bract in Indian Musaceae.

6. Shape of bract apex

The bract surface nature such as sulcate *vs*. plain, glaucous *vs*. non-glaucous and its behaviour before falling *ie.,* revolute *vs*. non-revolute, are mostly used for the section-wise discrimination within the genus *Musa* (Cheesman, 1947a; Simmonds & Shepherd, 1955). However, the bract apex shape has importance in the evolutionary perspective. The large-sized *Musa* species generally displays bracts with obtuse apex, while small-sized ornamental banana taxa possess bracts with acute apex. In this study, the obtuse and acute shapes of bract apex are coded as 0 and 1 respectively. The study suggested that obtuse shape of bract-apex is the ancestral trait for Indian *Musa* and it was also observed in the *Ensete* clade. Whereas bract-apex with acute shape is observed as a synapomorphic trait. The ancestral trait (obtuse bract-apex) is symplesiomorphic in sub-clade I and sub-clade II and homoplasious for *M. sikkimensis* and *M. sabuana* (both are in sub-clade III). The *Musa* species showing bract with acute apex is normally ornamental or allied with *M. acuminata*. Here, sub-clade III displays this synapomorphic (acute bract-apex) character except for *M. sikkimensis* and *M. sabuana* (Fig. 53).

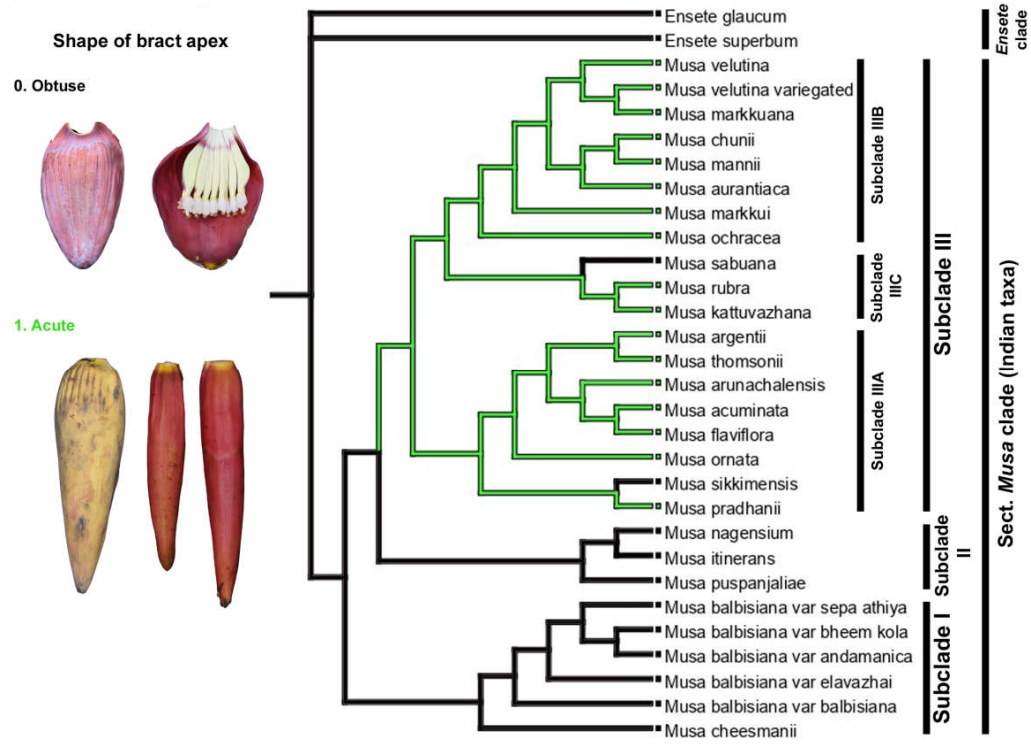


Fig. 53. Evolutionary pattern of the shape of bracts apex in Indian Musaceae.

7. Fruit shape/ appearance

The genus *Ensete* and *Musa* produces large fruits with respect to the other genera in the order Zingiberales. Fruit is the most useful part in both taxonomy as well as economic perspective (De Langhe *et al.*, 2009; Nayar, 2010; Sreejith & Sabu, 2017; Joe & Sabu, 2019). Here, for the ancestral state construction, the fruit shape is divided as broadly-oblong/stout and narrowly oblong/slender, which are coded as 0 and 1 respectively. The ancestral state tree suggested that the *Musa* spp. with broadly-oblong fruits is formed earlier than the taxa with narrowly oblong fruits. Hence, the plesiomorphic trait is broadly-oblong fruit and the apomorphic trait is narrowly-oblong fruit. The fruit with a broadly-oblong shape is most prevailing in Indian *Ensete* sp., infra-specific taxa of *M. balbisiana*, *M. puspanjaliae*, *M. sikkimensis* etc. The tree suggested that this ancestral character has appeared in the *Ensete* clade as well as sub-clade I (*M. balbisiana* clade) in sect. *Musa* as a symplesiomorphic character. Also, it independently re-appeared twice in sub-clade II (as *M. puspanjaliae*, *M. nagensium*) and three times in sub-clade III (as *M. sikkimensis*, *M. arunachalensis* and *M. argentii*). The narrowly oblong fruit evolved as a synapomorphy in sub-clade IIIB, IIIC. Among the Indian *Musa* having broadly oblong fruit, *M. balbisiana* ('B'-genome) shows greater genetic variability and distribution range and this character state can be considered as '*M. balbisiana* type'. In contrast with this, the narrowly oblong fruit is considered to be the characteristic feature of *M. acuminata* ('A'-genome), even though the ornamental bananas possess the same type of fruit shape (Fig. 54).

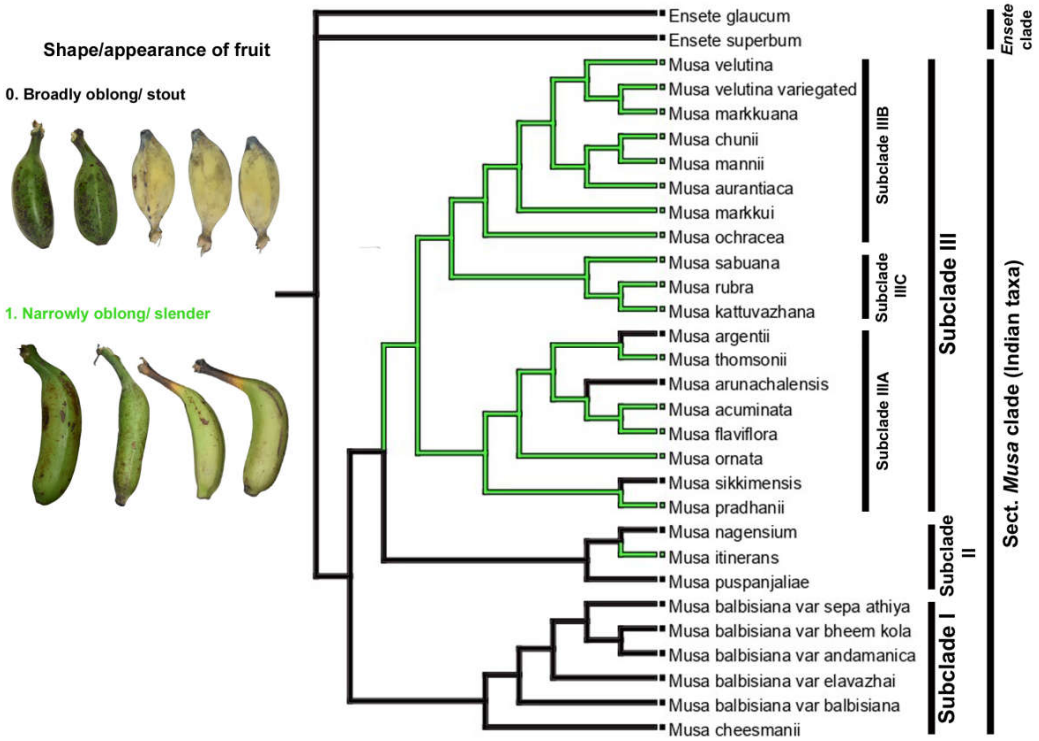


Fig. 54. Evolutionary pattern of the shape/appearance of fruits in Indian Musaceae.

8. Colour of bract

The colour of bract in Musaceae ranges from green, orange, lilac, pink, gradation of red and gradation of purple. However, this colour is highly polymorphic in some taxa of *Musa* such as *M. sabuana* (ranges from green to brown purple), *M. cheesmanii* (greenish-yellow to purple) etc. (Joe & Sabu, 2019). However, the bract colour can be majorly classified into two; dull-coloured and bright-coloured. Here these two are considered as character states for bract colour and coded as 0 and 1 respectively. Dull bract is distributed in entire sub-clades of the Indian *Musa*, however mainly confined as a symplesiomorphic trait for sub-clades I and II. The bright-coloured bract appeared as a derived trait, but it appears independently two times in sub-clade IIIA as *M. ornata* and *M. arunachalensis*, and also single time in sub-clade IIIC as *M. rubra*. In spite, its major appearance as a synapomorphy is found in sub-clade IIIB, this clade is confined by major ornamental bananas (Fig. 55).

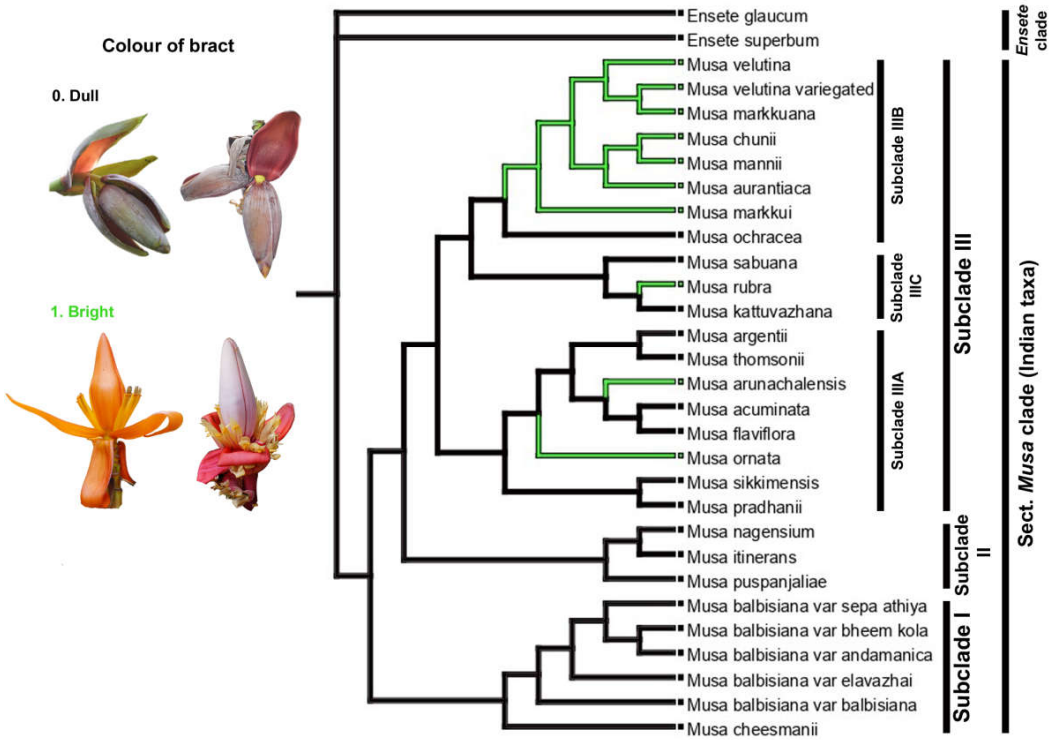


Fig. 55. Evolutionary pattern of the colour of bract in Indian Musaceae.

9. Shape of seed

The seed shape is a valuable character for the sectional classification, especially since this is the main character which discriminates the section *Australimusa* from section *Callimusa*. The former section possesses sub-globose or dorsi-ventrally compressed seeds, whereas the latter section possesses cylindrical or barrel-shaped or top-shaped seeds. However, the section *Musa* and section *Rhodochlamys* do not show much distinction in the seed shape (Cheesman, 1947a). Indian *Musa* generally shows round or angular seeds with warty or smooth surfaces (Joe, 2015; Joe & Sabu, 2019). The recent seed morphological and anatomical study of Indian Musaceae by Hareesh and Sabu (2023), clearly depicts that Indian species of *Ensete* have ovoid-shaped seeds and members in section *Musa* from India possess sub-globose, oblate and ellipsoid shapes in seeds. In the present character evolutionary analysis, the seed shape is divided into three-character states such as

ovoid to sub-globose (0), oblate (1) and ellipsoid (2). The ancestral state tree resulted in that ovoid to sub-globose shaped seed being an ancestral trait and appeared in the *Ensete* clade and sub-clade I in section *Musa.* Whereas the other two traits seem as derived character states. However, the ellipsoid seed is only seen in *M. ochracea* and *M. rubra*, so it appears only two times in sub-clade III. According to Hareesh & Sabu (2023), oblate seeds are the predominant seed shape for Indian *Musa.* Here, these oblate shapes of the seeds were observed as synapomorphic trait, which appeared entirely in sub-clade II and sub-clade III (except for *M. ochracea* and *M. rubra*) (Fig. 56).

Fig. 56. Evolutionary pattern of the seed shape in Indian Musaceae.

Character states correspond to 'A'- genome prominent and 'B'- genome prominent groups in section *Musa*

This is the first attempt to trace out the evolution of key morphological characters in Indian Musaceae based on a phylogenetic context. The current phylogenetic study results in two major grouping in section *Musa viz.*, 'A'- genome

prominent group (sub-clade III) and 'B'-genome prominent group (sub-clade I and II) (see chapter 5; Figs. 43 & 44). The basal lineage in this section is sub-clade I and II, which are constituted by *M. balbisiana* and its allied taxa such as *M. cheesmanii*, *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. *sepa-athiya*, *M. puspanjaliae*, *M. nagensium* and *M. itinerans.* A total of 19 taxa included in sub-clade III, which are members of the former section *Rhodochlamys* (*M. ornata*, *M. rubra*, *M. arunachalensis*, *M. markkui*, *M. aurantiaca*, *M. mannii*, *M. chunii*, *M. markkuana*, *M. velutina* and *M. velutina* var. *variegata*), *M. acuminata* and its allied taxa (*M. flaviflora*, *M. thomsonii*, *M. argentii*, *M. ochracea*, *M. kattuvazhana*, *M. sabuana*), *M. sikkimensis* and *M. pradhanii*. The 'A' and 'B' genomes come from the *M. acuminata* and *M. balbisiana* respectively (Simmonds & Shepherd, 1955). Here we provide the same name for the two groups as tentatively, based on the morphological similarity of taxa confined to the particular 'A'-genome prominent and 'B'-genome prominent groups.

The character evolution study of Indian Musaceae showed that the character states like large pseudostem (>4.5 m); pendent inflorescence; dull-coloured, non-revolute bracts which persistent towards male bud; obtuse apex of male bract; two rows of flower/fruits; broadly oblong or stout nature of fruits and ovoid to sub-globose seeds were ancestral/plesiomorphic traits. These ancestral characters are very well suited with *M. balbisiana* characters, and its allied taxa, so the corresponding ancestral clades (sub-clade I and II) in sect. *Musa* is considered tentatively here as the 'B'-genome prominent group. Also, the character states *viz.*, small to medium-sized pseudostem (≤4.5 m); erect or semi-pendent inflorescence; deciduous, revolute, bright or dull-coloured bracts; acute apex of male bract; narrowly-oblong or slender fruits and oblate or ellipsoid seeds were observed as derived/apomorphic characters. These characters are more related to the taxa in sub-clade III members *viz*., *M. acuminata,* its allied taxa (*M. flaviflora*, *M. thomsonii*, *M. ochracea*, *M. sabuana*, *M. kattuvazhana*), ornamental bananas (*M. ornata*, *M. aurantiaca*, *M. arunachalensis*, *M. markkui*, *M. markkuana*, *M. chunii*, *M. mannii*, *M. velutina*, *M. velutina* var. *variegata*, *M. rubra*) and *M. pradhanii*. Even though ornamental bananas are conclusively nested in this clade, it is represented here as

'A'-genome prominent group. Because the characters like curling or revolute nature of bract, acute bract apex, narrowly oblong fruit are observed as synapomorphies for the majority members in this subclade (subclade III). As an exception, two taxa in sub-clade III, *M. sikkimensis* and *M. sabuana* show ancestral characters such as large pseudostem, obtuse apex of bracts. Also, the non-revolute bracts in *M. sabuana* (rarely shows revolute bract) and broadly oblong fruits in *M. sikkimensis* are separately observed as a plesiomorphic trait.

Does the evolution of morphological character help to switch the pollination mechanism in bananas?

As mentioned earlier, the family Musaceae possess erect to pendulous type of inflorescence, revolute or non-revolute, dull-coloured or bright-coloured bracts, obtuse or acute-shaped bract apex. These characters have direct or indirect relations with the pollination mechanism. Since this family possess monoecious condition (rarely bisexual), insects, birds and bats are helping for the cross-pollination (Endress, 1994; Liu *et al*., 2002b; Kaushik *et al.*, 2012; Halder *et al*., 2019; Shawer *et al*., 2019; Waniale *et al*., 2021). Apart from these pollinators, several floral visitors are reported in bananas such as honeybees, hoverflies, butterflies, wasps, and stingless bees (Nilapaka *et al.*, 2019; Shawer *et al*., 2019; Sindhu *et al*., 2021). Insect pollination is mainly confined to the monotypic genus *Musella* (Liu *et al.*, 2002a), which indicates that Indian Musaceae members are mainly pollinated by bats and birds. Even the nectar-eating bats are considered as the primary pollinators of the normal banana, bird pollination (ornithophily) has also reported (Nur, 1976; Itino *et al*., 1991; Liu *et al*., 2002b; Pedrozo *et al*., 2018). Nur (1976) speculates that bat pollination occurs mostly in *Musa* spp. with pendant-type inflorescence, while bird pollination occurs in ornamental bananas with erect inflorescences. However, both chiropterophilic (bat pollination) and ornithophilic pollinations were observed in bananas with pendant type inflorescence *viz*., *M. itinerans* (Liu *et al*., 2002b), *M acuminata* subsp. *halabanensis* (Itino *et al*., 1991). The design of banana flowers and bracts are very much suited to bat pollination (Fleming *et al*., 2009; Alpízar *et al*., 2020). The flower characters include large size, usually pendent on a thick and

strong rachis, tubular corolla, off-white or cream colour and plenty of nectar having jelly-like or thick consistency, also the dull, purplish-coloured bract with unique odour helps to attract bats. Though, the ornamental bananas possess erect and large flowers with cream, orange or yellow coloured tubular corolla, copious amounts of nectar having relatively dilute consistency and bright coloured (red, orange, lilac, pink) bracts, which helps to attract birds for pollination (Itino *et al*., 1991; Liu *et al*., 2002b; Fleming *et al*., 2009; Murphy *et al*., 2016; Mărgăoan *et al*., 2019).

Bracts are closed during the young stages of flowers in the bananas and when the flowers are ready for pollination it opens and lifted up for display the flowers to pollinators. One to three bracts are lifted/reflexed at a time based on the species. But in some taxa, bracts do not revolute during their lifting time, somehow in other taxa revolute or curl their bracts into the adaxial side after opening (Nayar, 2010; Joe & Sabu, 2019). The present character evolution study depicts that the bracts of *Ensete* sp., *Musa balbisiana* and its allied taxa including *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. *sepa-athiya*, *M. cheesmanii* (subclade I), and members in subclade II such as *M. puspanjaliae*, *M. nagensium* (except *M. itinerans*) possess non-curling or non-revolute bracts, however the members from sub-clade III are showing curling or revolute nature (except *M. sabuana*) of bracts before it falls. So, the character mapped tree shows that the plesiomorphic non-reflexing nature of bract is gradually changed into reflexed nature and the curling or revolute nature of bract is observed as an acquired adaptation through evolution. Also, we speculate that the curling of bracts helps to display the flowers more than that of non-revolute bract and it helps to attract pollinators like bats as well as birds. The actual mechanism and purpose of bract curling is not studied yet. However, this could be due to the anatomical features and thickness of the bracts, as well as the success of pollination and selection of pollinators. The study of Murphy *et al*. (2016) shows that bats use the large concave bracts as acoustic beacons mean it would reflect the echoes to the ultrasounds created by the bats and in this way, the pollinator bats can eco-locate the flowers or nectar source. So, the presence of large bracts helps the pollinating bats to facilitate its quick approach to the flower and helps to increase the foraging efficiency. So, we

hypothesize that the ancestral taxa with non-curling or large concave shaped bract (members in subclade I and II; *Musa balbisiana*, *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. *sepa-athiya*, *M. cheesmanii*, *M. puspanjaliae*, *M. nagensium*) may have only pollinated by bats and the bracts of such plants help to ensure their pollination success. Another interesting fact is that, the study of Pedrozo *et al*. (2018) showed hovering type pollination (pollinators stay in the air by moving their wings quickly and pollinate the flower without landing on it) is a more evolved type in bat pollination, while comparing to the upside landing strategy (pollinators approach to the flower from the upper side after landing) and downside landing strategy (pollinators approach to the flower from the lower side after landing). The small body-sized bats more effectively do the hovering strategy than the large body-sized bats and so it also helps them to escape from the quick striking of potential predators (Murphy *et al*., 2016). Moreover, sunbirds and hummingbirds are usually following this type of hovering behaviour for pollination (Itino *et al*., 1991). We speculate that a hovering type of landing is more efficient in bananas with revolute-type bracts because the curling of the bract helps present the flower to pollinators. Also, both birds (hummingbirds in New World and sunbird from Old World) and small body-sized bats may pollinate the *Musa* spp. with revolute nature of bracts such as *M. itinerans*, *M. pradhanii*, *M. sikkimensis*, *M. flaviflora*, *M. acuminata*, *M. arunachalensis*, *M. thomsonii*, *M. argentii*, *M. kattuvazhana* and *M. ochracea*. The ornamental bananas with erect inflorescence (*M. ornata*, *M. rubra*, *M. markkui*, *M. aurantiaca*, *M. mannii*, *M. chunii*, *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*) do not have this problem because the bract itself serves as a landing platform for the pollinators and the showy bracts and flowers facilitate the bird pollination. Altogether, the nectar-eating bat with a large body size prefers the ancestral non-revolute bracted *Musa* sp. and small body-sized bat and birds choose the *Musa* sp. with revolute bract or erect inflorescence.

Moreover, the characters like large pseudostem (>4.5), pendent inflorescence, dull-coloured bracts are appeared as symplesiomorphic for the subclade I and II. In contrast with this, majority of the members in the derived clade (subclade III) possess small sized (≤ 2.2 m; *M. ornata*, *M. rubra*, *M. markkui*, *M.*

aurantiaca, *M. mannii*, *M. chunii*, *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*) or medium sized (2.2–4.5 m; *M. pradhanii*, *M. flaviflora*, *M. acuminata*, *M. arunachalensis*, *M. thomsonii*, *M. argentii*, *M. kattuvazhana*, *M. ochracea*) pseudostems, erect inflorescence (*M. ornata*, *M. rubra*, *M. aurantiaca*, *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*) or semi-pendent inflorescence (*M. arunachalensis*, *M. markkui*, *M. mannii*, *M. chunii*) and bright-coloured bract (*M. ornata*, *M. arunachalensis*, *M. rubra*, *M. markkui*, *M. aurantiaca*, *M. mannii*, *M. chunii*, *M. markkuana*, *M. velutina*, *M. velutina* var. *variegata*). The symplesiomorphic characters such as large pseudostem (>4.5), pendent inflorescence, dull-coloured bracts in subclade I and subclade II supported the bat pollination mechanism and synapomorphic characters such as small (≤ 2.2 m) to medium-sized (2.2–4.5 m) plants, erect or semi-pendent inflorescence and bright-coloured bract supported the bat as well as bird pollination mechanisms. The apomorphic characters include erect inflorescence, bright coloured bracts that enhance the ornithophilic/bird pollination. So, based on these observations we believe that the morphological character evolution supported the shift from bat pollination to bird pollination in the Indian *Musa*.

According to Fleming *et al.* (2009), bat pollination is an energy expense pollination mechanism and a derived trait throughout the angiosperm lineage, and it may be evolved from diurnal bird-pollinated species (hummingbird, sunbird) or diurnal or nocturnal pollinated taxa like bee or moth. This hypothesis is based on the observation of the entire angiosperm lineage. However, surprisingly our study shows the reverse of the above observation, which is in *Musa* lineage, bird pollination is derived from the bat pollination.

Chapter 7 MOLECULAR DATING OF INDIAN MUSACEAE

The fossil evidence helps to narrate the story about the age of organisms. The age of Angiosperm is calculated by using the pollen fossil and is estimated to be originated in the early Cretaceous and diversified in the late Cretaceous (De Bodt *et al*., 2005; Frohlich & Chase, 2007). However, this ageing calculation may vary based on the new fossil findings. The findings of Li *et al.* (2019) suggested that the origin of angiosperm is much earlier than the previous findings (De Bodt *et al*., 2005; Frohlich & Chase, 2007), based on Li *et al.*'s (*l.c.*) finding, the origin of flowering plants happens during the Triassic period and undergo diversification during the Jurassic period. The age of fossils is primarily determined by counting the radioactive atoms remaining in certain fossils or adjacent mineral layers. Radiocarbon dating is used for calculating the age of very recent plant and animal fossils having an age below 60,000 years because the half-life of Carbon-14 (C^{14}) is 5730 years. Radiometric dating by using Uranium-238 (U^{238}) and Uranium-235 (U^{235}) gives the approximate age of older fossils. These radioactive atoms have a greater half-life and are easily traced out from the mineral layers of the fossil strata. Uranium-Lead dating from the zircon minerals present in the ash layer is a commonly used dating technique (Scott, 2020).

The molecular dating of Musaceae was carried out in a world context (Christelova *et al*., 2011; Janssens *et al.*, 2016; Burgos-Hernández *et al*., 2019; Fu *et al.*, 2022). The results of these studies were rather contrary but suggest that Musaceae originated from the Northern Indo-Burma during the Cretaceous-Palaeogene boundary, followed by Eocene-Oligocene diversification. Indian species, however, were not included in that study. So, here we attempted the molecular dating study of Indian Musaceae, by incorporating more Indian endemic taxa and we hope that this study will add more understanding to the current evolutionary results of the banana family.

Materials and Methods

The divergence time estimation of Indian Musaceae was performed using the combined dataset of all three markers (ITS, *trnL-F* and *rps* 16) in BEAST 2.6.6 (Bouckaert *et al*., 2019). All the parameters were specified in BEAUti 2.6.6 (Bouckaert *et al*., 2019), with the independent nucleotide substitution models for the partitioned dataset of nuclear and chloroplast markers (following the models used in the Bayesian phylogenetic tree). The random local clock model was used to test the rate of evolution and rate parameters. Yule model was selected as prior and applied to test the single tree model with random tree sampling and constant birth rate. The input file (.xml file) was created in BEAUti 2.6.6 (Bouckaert *et al*., 2019) for BEAST analysis. We performed one run of four MCMC (Markov Chain Monte Carlo) chains, each with 10,000,000 generations and sampling was done for each 1000^{th} generation. The convergence and stationarity of final parameters were assessed using Tracer v1.5 (Drummond & Rambaut, 2007) to confirm the ESS (Effective Sampling Size) value was above 200. The MCC (Maximum Clade Credibility) tree with mean divergence time was visualized in FigTree v1.4.4 (Rambaut, 2016). Below mentioned steps are followed for the input file creation and tree building.

1. The input file for BEAST software is built from BEAUti software.
2. The fasta file of the aligned sequences was converted into a nexus file in MEGA.
3. Set the partition in the end portion of the nexus file as follows and save.

   begin assumptions;

   charset ITS = 1-678;

   charset *trnL-F* = 679-1695;

   charset *rps*16 = 1696-2652;

   end;

4. Open BEAUti 2 → File→Import alignment (fasta file is used for the analysis without partition or nexus file is used for the analysis with partition).

5. Site model →Select appropriate substitution models (here the same models were used for each marker that were used in phylogenetic tree construction), gamma category count, proportion invariant and substitution rate for each partition.

6. Clock model →Select optimized relaxed clock model/ random local clock.

7. Prior →Select Yule model or calibrated Yule model for each partition (otherwise, the prior can be selected from previous works).

The fossils of the order Zingiberales are well obtained and documented by comparing them to other groups of angiosperms and the age of Zingiberalean fossils ranges from Late Cretaceous to Eocene (Daghlian, 1981; Friis, 1988; Boyd, 1992; Manchester & Kress, 1993; Rodríguez-de la Rosa & Cevallos-Ferriz, 1994; Fischer *et al.*, 2009; Friis *et al.*, 2011). *Spirematospermum chandlerae* Friis is the oldest known fossil record of reproductive structures for the order Zingiberales, with an estimated minimum age of 83.5 mya. It is found in the Black Creek Formation of Cretaceous sediments (Santonian) of North Carolina, North America (Friis, 1988). The actual placement of this fossil made some controversy, some studies argued that it belongs to the family Zingiberaceae (Chandler, 1925; Koch & Friedrich, 1971) and major fossil conformation studies strongly recommended that *S. chandlerae* belongs to Musaceae (Manchester & Kress, 1993; Rodríguez-de la Rosa & Cevallos-Ferris, 1994). Moreover, recent dating studies of Musaceae considered this as a fossil of Musaceae (Janssens *et al*., 2016; Burgos-Hernández *et al*., 2019). The age of this fossil is selected here as the crown node age of Zingiberales or the stem node age of Musaceae. Another fossil related to the genus *Ensete*, was discovered from the Nut Beds of the Clarno formation in Oregon, North America with about an estimated age of 43.5 mya (Mid-Eocene). That is *Ensete oregonense* Manchester & Kress and was obtained as seeds and single fruit (Manchester & Kress, 1993; Manchester, 1994). For the current dating analysis, this fossil age is used as the crown node age of the *Ensete/Musella* clade or stem node age of *Ensete.* Eventhough the prime distribution range of Musaceae is confined to South-East Asia

in the current circumstances, the aforementioned fossils of this family were obtained from North America. This finding suggested that South-East Asia is considered the cradle of evolution of Musaceae and North America is suggested as a grave for this family (Burgos-Hernández *et al*., 2019). The present dating study is based on two calibration points or two fossil data as primary calibration and no secondary calibration data from previous studies were used.

For the analysis and result interpretation, we divided the years (million years ago or mya) depicted in the time tree into corresponding geological periods (Quaternary, Neogene, Palaeogene and Cretaceous) and epochs (Holocene, Pleistocene, Pliocene, Miocene, Oligocene, Eocene and Palaeocene). We follow the results of Burgos-Hernández *et al*. (2019) and Janssens *et al*. (2016) for allotting the geological periods and epochs for the time (mya). That is 0−10 mya for Pleistocene to Late Miocene, 10−20 mya for Late to Early Miocene, 20−30 mya for Early Miocene to Oligocene, 30−55 for Oligocene to Early Eocene and 55−70 Palaeogene-Cretaceous boundary (Fig.57).

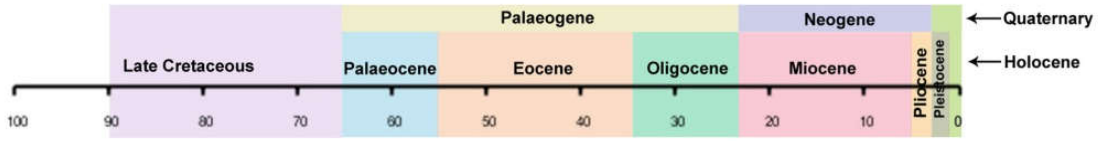


Fig. 57. Geological time scale representing the time period from Late Cretaceous.

Dating results of Indian Musaceae

The dated tree (Fig. 58) based on fossil data, *Spirematospermum chandlerae* with an estimated age of 83.5 mya (Zingiberales crown) and *Ensete oregonense* dated c. 43.5 mya (*Ensete/ Musella* crown) retrieved two major lineages in Indian Musaceae: *Ensete/ Musella* lineage and section *Musa* lineage. The divergence time of major clades in Musaceae is depicted here as a bubble diagram (Fig. 59). Indian Musaceae was diverted into *Musa* and *Ensete* branches in c. 60 mya [45−74; 95% Highest Posterior Density (HPD)] and underwent Palaeogene-Neogene diversification.

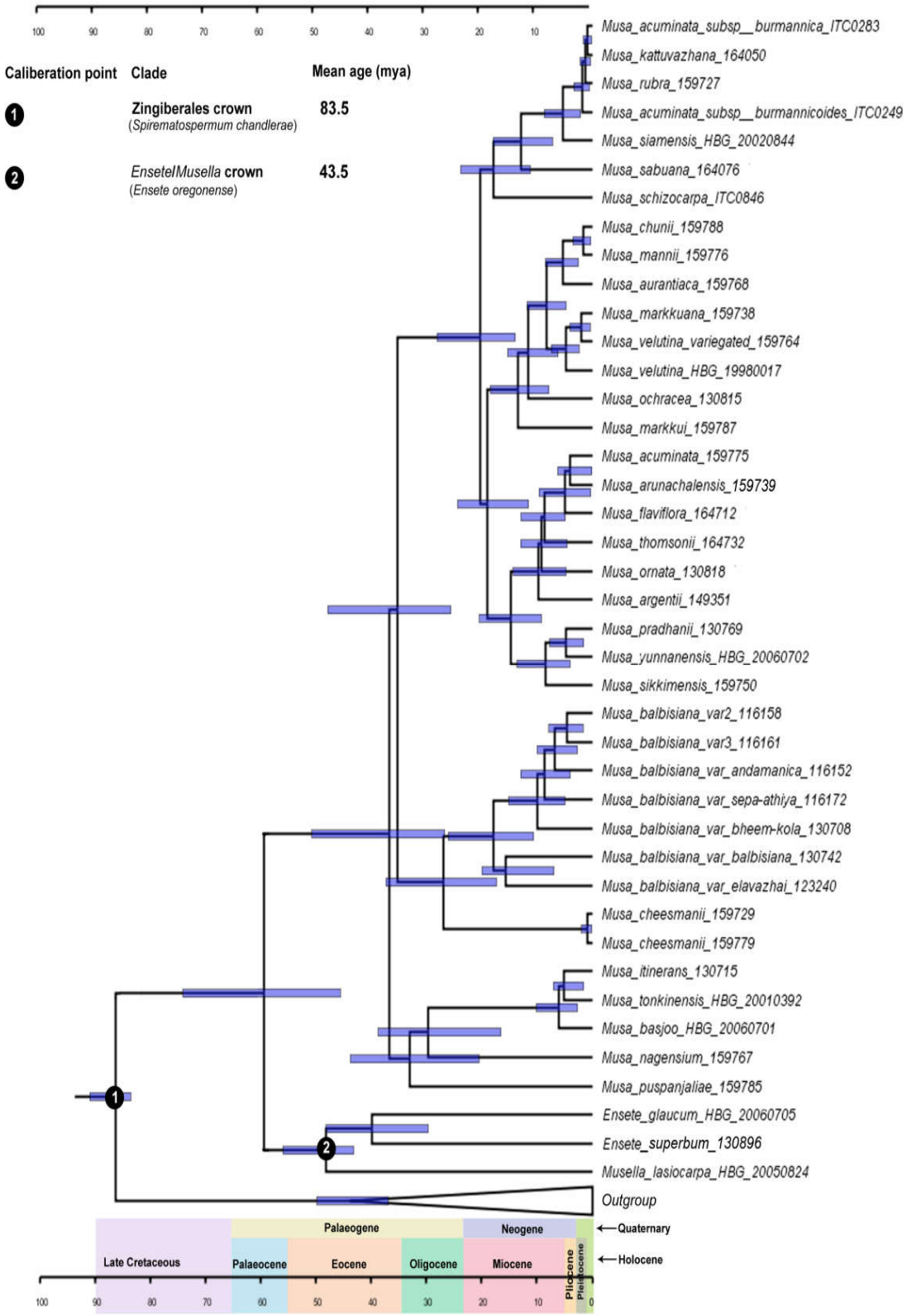


Fig. 58. Divergence time estimates of Indian Musaceae obtained from the BEAST analysis. Blue bar indicates the 95% HPD (Highest Posterior Density) intervals for the divergence date estimates. Time scale is given in million years (mya) with respective geological periods and epochs.

The *Ensete*/*Musella* clade originated from this evolutionary path during 48 mya (42.5–57; 95% HPD) and the Indian *Ensete viz.*, *E. glaucum* and *E. superbum* diverged from this lineage during the Eocene-Oligocene boundary and an estimated age of 39 mya (31–48; 95% HPD). After that, the genus *Musa* was formed from this evolutionary journey during Eocene-Oligocene boundary with an age of c. 36.8 mya (27–52; 95% HPD) and immediately 'B'-genome dominant group evolved during early Oligocene and age of c. 34 mya (25–48; 95% HPD). The divergence of 'A' genome dominant group was observed during the Early Miocene with an approximate age of 21 mya (14–27; 95% HPD). It observed that the 'B'-genome dominant group originated much earlier than the 'A'-genome dominant group. The *Rhodochlamys* or ornamental banana groups evolved during the Late Miocene; c.11.6 mya (7–15 mya; 95% HPD) and underwent rapid speciation or radiation. The major speciation events and divergence in Indian *Musa* occurred during the Pliocene/ Pleistocene period. A total of 22 taxa were diverged from its ancestors during this period.

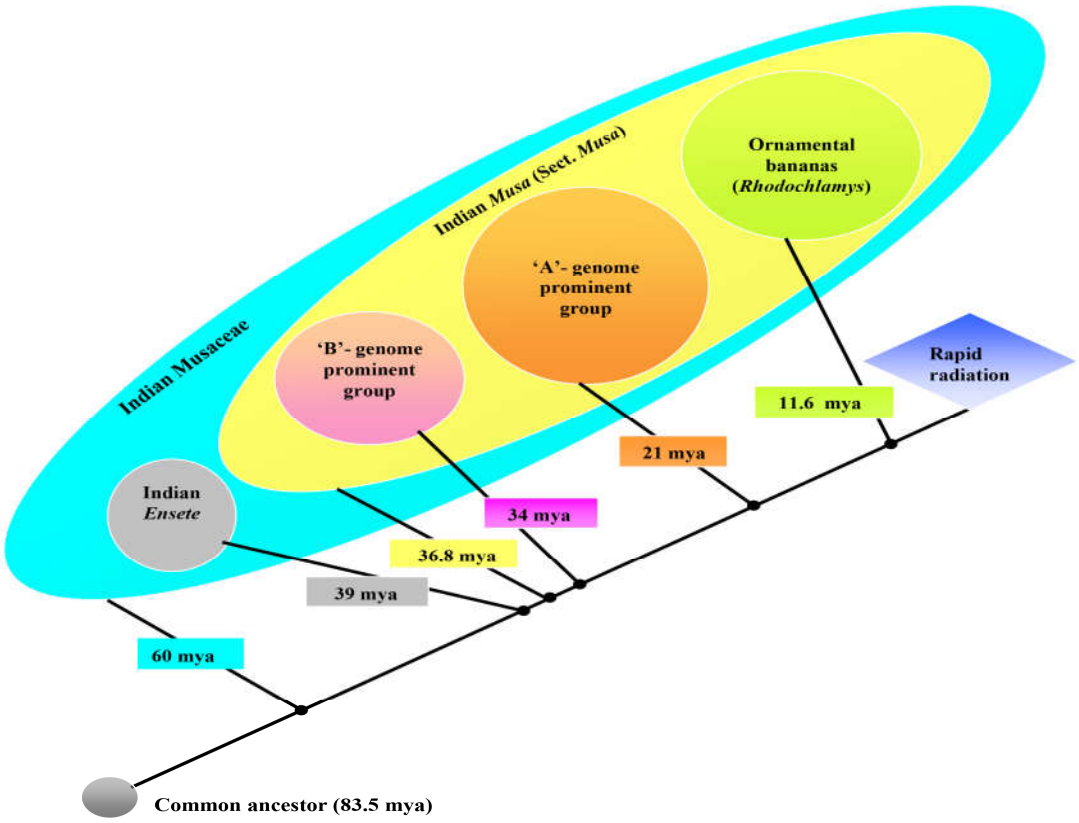


Fig. 59. Bubble diagram representing the divergence time of different lineages in Indian Musaceae.

Discussion

Accurate phylogeny based on the maximum representation of taxa and specific molecular markers and the precise divergence time estimation is important for the evolutionary history study. Here, we carried out the age estimation of Indian Musaceae with suitable markers (ITS, *trnL-F*, *rps* 16) and maximum representation of Indian taxa. The current dating study of Indian Musaceae is compared here with the previous dating studies in Musaceae (Christelova *et al*., 2011; Janssens *et al*., 2015; Magallón *et al*., 2015; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022) and this comparison is shown here as a bar diagram (Fig. 60). The age of Musaceae in the present study (c. 60 mya) is highly corroborated with the findings of Burgos-Hernández *et al*. (2019) and Fu *et al*. (2022). In their studies, Musaceae originated approximately during 60 mya (60±1 mya). However, according to Janssens *et al*. (2016), the age of Musaceae was c. 51.9 mya and it is younger than the present study. Also, the age of Musaceae was found to be older (c. 69 mya) in the result of Christelova *et al*. (2011). So, we hypothesize that the slight variations in the dating results of different studies may had a relation with the DNA regions assigned for the studies because specific sequences exhibit a specific rate of evolution. Our study is based on the DNA sequences of ITS, *trnL-F* and *rps* 16, however, Fu *et al*. (2022) used whole chloroplast sequences; Burgos-Hernández *et al*. (2019) used ITS, *trnL-F* and *atpB-rbcL* sequences; Janssens *et al.* (2016) analysed the age of Musaceae by the help of ITS, *trnL-F*, *atpB-rbcL* and *rps* 16 and Christelova *et al*. (2011) exploited 19 nuclear genes for their dating study. According to the initial dating study of Zingiberales based on *rbcL*, *atpB* and ribosomal 18S gene by Kress and Specht (2006), Musaceae originated in 110 mya and this was much earlier than the recent studies. This pioneer study suggested that the origin and diversification of the family Musaceae and genus *Musa* had a relation with the Gondwana land movement of the Indian plate towards Eurasia. However, our study and recent studies (Christelova *et al*., 2011; Janssens *et al.*, 2016; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022) do not agree with this hypothesis. The divergence of *Ensete/ Musella* clade happened c. 48 mya in the present study and this result is greatly matched with the results of Janssens *et al.* (2016), Burgos-Hernández *et al*. (2019) and Fu *et al*. (2022).

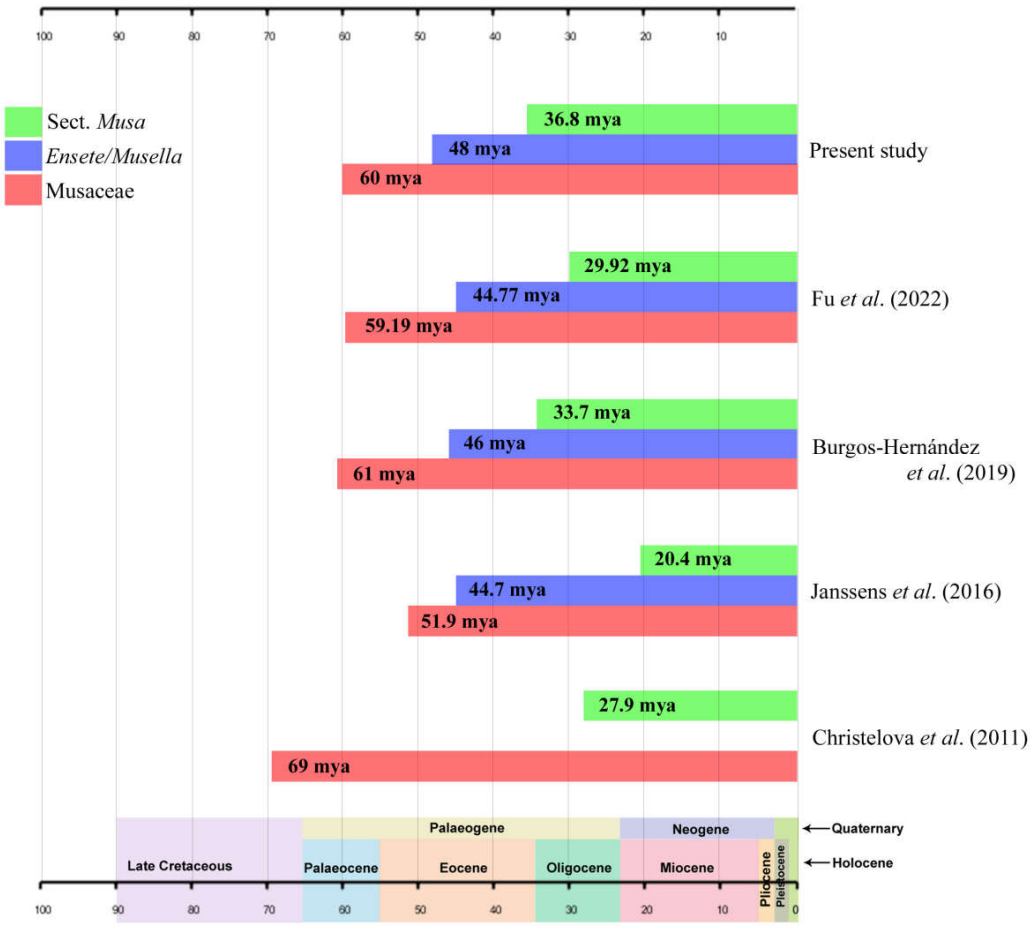


Fig. 60. Comparison of the divergence of different clades of Musaceae in recent dating studies.

The current age of *Ensete/ Musella* clade seems to be c. 2−3 mya elder than the aforementioned previous studies. Likewise, the age of section *Musa*/Indian *Musa* is observed as c. 37 mya and it is elder than the age mentioned in earlier studies (Christelova *et al*., 2011; Janssens *et al*., 2016; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022). However, the result is more corroborated with the findings of Burgos-Hernández *et al*. (2019), the difference is only 3 mya. Moreover, the timing of initial diversification of section *Musa* set at 37 mya in our result and is older than the previous studies (Christelova *et al*., 2011; Janssens *et al*., 2016; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022), somehow the result shows more similarity to the Burgos-Hernández *et al*. (2019) findings (33.7 mya). In addition, our study demonstrated that the 'B'-genome prominent group, 'A'-genome prominent group and *Rhodochlamys* members (ornamental bananas) are diverged during 37 mya, 21

mya and 11.6 mya respectively from the section *Musa* lineage in a successive manner. The numbers and accessions of taxa, calibration point allocation and selection of DNA markers play crucial role in the divergence-time calculation (Smith *et al*., 2018). Our study exploited more taxon, especially Indian endemics and the exact DNA marker helps to increase the accuracy in age calculation. Also, the most appropriate fossils are used here as the calibration point.

Along with the discussion of the origin and evolution of Musaceae in India, we must correlate it with major tectonics and climate fluctuation events in India and South-East Asia during the Cenozoic era. India was an Island during the Cretaceous period and was split from Madagascar (a part of Gondwana land) at some point in the late Cretaceous (Storey *et al*., 1995). Subsequently, the collision of the Indian plate with Asia occurred in the early Eocene (c. 50 mya) and it helped the diversification of South-East Asian flora and fauna during the Eocene epoch (Ali & Aitchinson, 2008, Klaus *et al*., 2016; Morely, 2018; Sen *et al*., 2019). The present study depicted that, during the movement of the Indian plate towards the Eurasian plate, no Musaceae members originated or evolved in the Indian plate even though the land possesses a rich tropical per humid climate. The lack of fossil evidence of Musaceae during the Cretaceous to Eocene period from India can be put forward as evidence for the above observation. It will strengthen the results of earlier studies (Janssens *et al*., 2016; Burgos-Hernández *et al*., 2019; Fu *et al*., 2022) as the origin of Musaceae was from South-East Asian part of Eurasia or specifically the northern Indo-Burma. However, the present study reinforces the Indian contribution in the origin of section *Musa*, because for the present study, we incorporate four varieties of *M. balbisiana viz.*, var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. *sepa-athiya* along with *M. balbisiana* var. *balbisiana* and *M. cheesmanii*. These taxa were nested in the ancestral clade (sub-clade I) of section *Musa* and another narrow Indian endemic species, *M. puspanjaliae* evolved as an ancestral species during the start of the Oligocene. The formation of these Indian endemic taxa as the ancestral radiation for section *Musa* will support the hypothesis as 'India served as a primary centre of ancestor radiation of section *Musa*'. Therefore, the contribution of the Indian sub-continent should be highlighted along with the existing results of

Janssens *et al*. (2016) and Burgos-Hernández *et al*. (2019). The present study also suggested that *M. balbisiana* lineage migrated from India to other South-East Asian regions like South China, the Philipines, and Thailand with some back dispersal into India. So, *M. balbisiana* clade obeyed the 'Into- and Out-of-India dispersal hypotheses' (Mani, 1974). The late Eocene (33.8–37 mya) connection between India and South-East Asia (Morely, 2018) favoured the distribution of 'B' genome dominant group (Sub-clades I & II) in both regions; the climatic conditions of both regions also supported this observation.

The continuation of the northward movement of India leads to another major event, the Himalayan uplift or orogeny. The uplifting started in the Oligocene, intensified during the Miocene and lasted for a Pleistocene period or the period between c. 30–4 mya (Zhao *et al*., 2015; Janssens *et al*., 2016; Pusok & Stegman, 2020; Ashokan *et al*., 2022). This orogenic event initiated the Asian monsoon (started in the late Oligocene) and this phenomenon strengthened in the Miocene period (Morely, 2012; Ding *et al*., 2017; Morely, 2018; Fu *et al*., 2022). The monsoon intensification and seasonal precipitation in South-East Asia will reflect in the North-Eastern region of India or the Eastern Himalayas, which helps for the rapid radiation in Indian Musaceae and trigger the formations of several narrow endemics *viz*., *M. cheesmanii*, *M. sabuana*, *M. kattuvazhana*, *M. ochracea*, *M. markkuana*, *M. velutina* var. *variegata*, *M. chunii*, *M. sikkimensis*, *M. pradhanii*, *M. argentii*, *M. thomsonii* and *M. flaviflora*. This taxa outbreak happened during the time lapse in mid-Miocene to the Pleistocene period.

The ancestors of Indian *Ensete* such as *E. glaucum* and *E. superum* originated in the northern Indo-Burma [North-East (NE) India, South-West (SW) China and Myanmar] during the Eocene- Oligocene boundary. *Ensete glaucum* was confined to the Indo-Burma regions without any natural radiations, in contrast, *E. superbum* migrated towards South India and colonization took place in the Western Ghats. The genus *Musa* is distributed in South-East (SE) Asia, starting from South and NE India, SW China, through Malaya, Borneo, New Guinea and up to North Australia. However, the major diversity centres are (1) NE India, SW China,

Myanmar and Thailand (2) Borneo, Indonesia (Sulawesi) and (3) New Guinea. The dispersion of sect. *Musa* started from Northern Indo-Burma (regions including NE India, SW China and Myanmar or mainland in SE Asia) and migrated towards the east by crossing the Wallace line. Most of the Islands in the East of the Wallace line (Indonesia, New Guinea etc.) formed by the gradual collision of the Australian plate (Sahul Shelf) and Eurasian plate (Sunda Shelf), it started in the Late Oligocene (Kooyman *et al*., 2019) and Islands are established during Late Miocene to Pliocene period (Janssens *et al*., 2016). Hence, the dispersion of *Musa* is depicted as it started from the mainland of SE Asia to the West side towards the Islands of SE Asia. However, some back dispersal occurs from the South to the mainland in the case of *M. ornata*, *M. rubra* etc.

CHAPTER 8 SUMMARY AND CONCLUSION

The present study is the first attempt to make a complete taxonomic account of Indian Musaceae by incorporating phylogenetic study into the already established taxonomy of Indian Musaceae by using morphology, anatomy, and palynology. Plant specimens for the study were collected during 2018-2022 through extensive field exploration from different parts of India including the Andaman and Nicobar Islands. Despite this, the Musaceae germplasm collection of CUBG and MBGIPS were also utilized for molecular analyses. We conducted a thorough examination of the morphological characteristics of each species and infra-specific taxa to explore their phylogenetic implications. To compare traditional morphology-based classification with molecular phylogenetic results, we constructed both a morphology-based phenogram (UPGMA tree) and phylogenetic trees. The significant outcomes of the present study are detailed below.

In this study, we constructed a UPGMA tree based on the morphological characters of Indian wild bananas for the first time. Previously, Sreejith & Sabu (2017) conducted a phenetic study of South Indian cultivated bananas. Our study, however, incorporated all available Indian wild taxa (33 OTUs) and utilized a comprehensive set of 64 morphological characters, including both vegetative and reproductive traits, as well as qualitative and quantitative, binary and multistate characters. The results of the present UPGMA tree are consistent with the earlier morphology and chromosome number based sectional classification by Cheesman (1947a). In our analysis, two species of *Ensete* formed the out-group while all Indian *Musa* taxa clustered into two distinct in-groups. These clusters- Cluster I and II correspond to the Indian *Musa* taxa of sections *Eumusa* and *Rhodochlamys,* respectively, as classified by Joe (2015). Cluster II, which comprises ornamental bananas with small pseudostems, and showy inflorescences contrasts with Cluster I, which includes taxa with normal morphology, and medium to large pseudostem with less ornamental values. Moreover, cluster I was further divided into sub-clusters Ia and Ib. Within sub-cluster Ia, all 10 taxa displayed characteristics of

Musa balbisiana, so they can be referred to as the "*M. balbisiana* group" or 'B'-genome prominent group. These taxa likely carry the abiotic stress-resistant genes similar to those found in the 'B- genome'. Similarly, the taxa in sub-cluster 1b exhibited characteristics of *M. acuminata*, hence they are termed the '*M. acuminata* group' or 'A'- genome prominent group. The 12 taxa in this cluster may carry valuable traits associated with the 'A- genome'.

The primary objective and major outcome of the present work was the construction of a comprehensive phylogenetic tree for Indian Musaceae. Here we constructed the phylogenetic tree of Indian Musaceae in multiple combinations by utilising the sequence data of nuclear ITS, chloroplast *trnL-F* and *rps*16. Which include a separate tree of individual ITS, *trnL-F* and *rps*16 sequence data, a combined tree of *trnL-F*+*rps*16 sequences and a concatenated tree of ITS+ *trnL-F*+*rps*16 sequences. In addition to these, we constructed a robust phylogenetic tree of Musaceae on a global scale by incorporating all available Musaceae taxa and selecting the most appropriate outgroups. During this study, we generated a total of 138 sequences and retrieved 192 authentic sequences from NCBI tree construction. The present phylogenetic trees were represented by a total of 330 sequences (110 sequences of each ITS, *trnL-F* and *rps*16), encompassing 110 accessions (including ingroups and outgroups) and 78 taxa. Of these sequences, 33 were deposited to NCBI. While the ITS sequence-based tree and cpDNA sequence-based tree (*trnL-F*, *rps16*) displayed similar topologies, the high informative signals of ITS provide more resolution to the ITS tree with high statistical support than the tree generated by cpDNA sequence. Our study suggests that the nuclear ITS region is a potential barcode for species delimitation in Musaceae, although it may not fully resolve certain data. According to the revisionary study of Indian Musaceae by Joe (2015), there were two sections in Indian *Musa* such as *Eumusa* and *Rhodochlamys*. However, recent phylogenetic studies of Musaceae (Li *et al*., 2010; Liu *et al*., 2010; Janssens *et al.*, 2016; Burgos-Hernández *et al*., 2019; Fu *et al.*, 2022) enforced the merging of these two sections. The present phylogenetic study corroborates these findings, leading us to merge the sections *Eumusa* and *Rhodochlamys* into a single section *Musa.* Henceforth, Indian *Musa* will be represented by a single section. All

phylogenetic trees in our study consistently resulted in two major clades within Indian Musaceae *viz.*, clade I (Indian *Musa* clade or sect. *Musa* clade) and clade II (Indian *Ensete* clade). In both the ITS tree and concatenated tree (ITS+ *trnL-F+rps*16 tree), the clade I is further divided into three subclades (subclades I, II and III). Subclade I consist of *M. balbisiana*, its varieties and *M. cheesmanii* forming the *M. balbisiana- M. cheesmanii* clade. In contrast to previous studies, this subclade is resolved more clearly in our analysis, likely due to the inclusion of all available infraspecific taxa of *M. balbisiana*. Subclade II was formed by *M. itinerans* and *M. nagensium* (*M. itinerans- M. nagensium* clade). In the ML tree *M. puspanjaliae* was also included in this subclade, however in the BI tree, this species separately formed as a monoclade. The subclade III was formed by the ornamental bananas (previous sect. *Rhodochlamys*) and allied taxa of *M. acuminata.* This subclade III (*M. acuminata- Rhodochlamys* clade) was further divided into subclades IIIA, IIIB and IIIC, and the ornamental bananas were paraphyletically distributed throughout all subclades in subclade III. Additionally, two major groupings were identified within sect. *Musa viz*., 'B'- genome prominent group (subclades I and II) and the 'A'- genome prominent group (subclade III). The B- genome prominent group includes *M. balbisiana* and its infraspecific varieties like *M. balbisiana* var. *andamanica*, var. *bheem-kola*, var. *elavazhai*, var. s*epa-athiya*, var.2, var.3, *M. cheesmanii*, *M. puspanjaliae*, *M. nagensium* and *M. itinerans.* The 'A'- genome prominent group, represented by subclade III comprises the remaining taxa, including ornamental bananas, *M. acuminata* and its allied taxa. The present molecular study plays a crucial role in substantiating the species identity of Indian narrow endemics, including *M. puspanjaliae*, *M. sikkimensis*, *M. pradhanii*, *M. thomsonii*, *M. argentii*, *M. ochracea*, *M. markkuii*, *M. sabuana* and *M. kattuvazhana*. Additionally, the study resolves the complex taxonomic issue of *M. flaviflora-M. thomsonii* complex. The typical *M. acuminata* is used first time ever for a phylogenetic study, and it is positioned entirely away from other infra-specific taxa of *M. acuminata*. The positioning of *M. acuminata*, the 'A'- genome contributor to cultivated bananas, reveals greater genetic differences than previously anticipated. Based on our findings, we suggest several wild candidates with desirable agronomic traits and a

strong gene pool. The potential candidates from the 'A'- genome prominent group, include *M. flaviflora*, *M. thomsonii*, *M. sabuana*, *M. ochracea*, *M. sabuana*, *M. kattuvazhana* and *M. sikkimensis*. From the 'B'- genome prominent group we recommend *M. cheesmanii*, *M. puspanjaliae*, *M. balbisiana* and its varieties like var. *andamanica*, var. *bheemkola*, var. *elavazhai*, var. *sepa-athiya*, var. 2 and var. 3.

In this character evolution study, we analysed the evolutionary patterns of nine key morphological traits: 1. Height of pseudostem, 2. Shape of inflorescence, 3. Longevity of bract, 4. Bract behaviour before falling, 5. Number of rows of fruit/flower per bract, 6. Shape of bract apex, 7. Colour of bract, 8. Shape/appearance of fruit and 9. Shape of seed. Our study showed that the following traits are ancestral (plesiomorphic): a large pseudostem (>4.5 m), pendent inflorescence, a male bud with obtuse apex, non-revolute and dull-coloured bracts, two rows of flower/fruits per bract, broadly oblong/stout nature of fruits and ovoid to sub-globose shaped seeds. The taxa belonging to 'B'- genome prominent group (subclade I and II) exhibited these ancestral characters. In contrast, derived (apomorphic) character states such as a small to medium-sized pseudostem (≤4.5 m); erect or semi-pendent inflorescence; deciduous and revolute, bright-coloured bracts, an acute apex of male bract; narrowly-oblong or slender fruits and oblate or ellipsoid seeds were observed. These characters are more related to *M. acuminata* and its allied taxa, which form the more advanced clade represented here as the 'A'- genome prominent group. The taxa within the 'A'- genome prominent group (subclade III) possessed these derived characters. This study also suggests that ancestral traits, such as large-sized plants, pendent inflorescence, dull coloured and non-curling bracts, are adapted to bat pollination. In contrast the derived characters *viz.*, small-sized plants, erect inflorescence, bright coloured and curling nature of bracts favour ornithophily (bird pollination). The evolution of these morphological traits, including the transition from large to small plants, pendent to erect inflorescences and dull-coloured to bright-coloured bracts, non-curling to curling nature of bracts helps to enhance the pollination success and supports a shift from bat to bird pollination.

The molecular dating study of Indian Musaceae aligns with the results of prior dating studies. According to the present study, the divergence of Indian Musaceae occurred around 60 mya (45−74; 95% HPD) in the Late Cretaceous-Palaeogene boundary. The estimated age of Indian *Ensete* is approximately 39 mya (31−48; 95% HPD) while Indian *Musa* is dated to around 36.8 mya (27−52; 95% HPD). Within Indian *Musa,* the ancestral clades or the 'B'- genome prominent group are estimated to have evolved around 34 mya (25−48; 95% HPD) and the 'A'-genome prominent group emerged around 21 mya (14−27; 95% HPD). The ornamental bananas are believed to have formed approximately c.11.6 mya (7−15 mya; 95% HPD) in the Late Miocene period. Earlier biogeographic studies suggest that the climatic condition during the Late Miocene-Pliocene-Pleistocene time period was highly favourable for speciation. In this context, our study observed that 22 taxa of *Musa* were formed during this time frame.

New synonyms identified

The molecular data confirmed the synonymization of *M. indandamanensis* with *M. sabuana* and *M. paramjitiana* with *M. balbisiana* var. *andamanica*. This molecular study also supported the synonymization of *M. acuminata* subsp. *burmannica* and subsp. *burmannicoides* with *M. kattuvazhana*

Reinstated taxa

Reinstated *Musa sabuana* and *M. balbisiana* var. *andamanica* from the synonymy of *M. balbisiana*.

Distributional record

This study confirms the occurrence of *M. kattuvazhana* in the Andaman and Nicobar Islands.

Sequence generation and NCBI deposit

A total of 138 new sequences were generated during this study, of which 33 sequences were deposited in NCBI.

CHAPTER 9 RECOMMENDATIONS AND FUTURE OUTLOOKS

The present study is the first phylogenetic attempt towards understanding Indian Musaceae (banana family) using molecular markers (nrITS, chloroplast *trnL-F* and *rps*16). This important plant group comprises only three genera *viz. Ensete*, *Musa* and *Musella* with about 128 taxa worldwide, distributed in Southeast Asia and Africa. India is considered a major center of evolution of *Musa*, and harbours 32 taxa along with two species of *Ensete.* The major distribution of these taxa is confined to North-East India, the Western Ghats, the Eastern Ghats and the Andaman and Nicobar Islands. Many wild bananas in Northeast India are at risk of extinction due to natural and anthropogenic activities, making immediate ex-situ conservation measures necessary. We are maintaining a Musaceae germplasm in CUBG and another at MBGIPS, Calicut.

Here we constructed the phylogenetic trees of Musaceae in Indian and World contexts to infer the phylogenetic relationships of taxa. Our study provides additional support for the merging of sections *Rhodochlamys* and *Eumusa* into a single section- 'sect. *Musa*'. Furthermore, this attempt has helped to solve the taxonomic identity of major narrow endemic taxa confined to India and helped to understand the relations of Indian endemic taxa with outside Indian taxa. However, our three marker-based phylogenetic tree is not enough for the complete resolution of infra-specific taxa problem in *M. balbisiana* complex. It must need more nuclear or chloroplast or genetic markers like AFLP, RFLP, RFLP or SRAP for a robust resurrection. The present study reveals the close relationship of *M. yunnanensis*, *M. sikkimensis* and *M. pradhanii*, so a further study should be carried out to analyze their actual status. As we have not included multiple accessions of *M. arunachalensis* in our study, there is uncertainty about the placement of this taxon in our phylogenetic tree and more accessions would be required to confirm its position. There is a possibility that some newly described taxa, such as *M. argentii* and M. *arunachalensis*, have been formed by natural hybridization, although further studies will be required to substantiate our hypothesis. In our study, we obtained two

groupings in sect. *Musa*, which is 'B'- and 'A'- genome prominent groups. From these groups, we propose some wild taxa as an alternative for *M. acuminata* and *M. balbisiana* for the future breeding programme. Considering its position as the leading producer of bananas and one of the major centres of origin of wild bananas, India has significant potential for research aimed at improving the quality of banana production. The current character evolution study is confined only to Indian Musaceae since we were not able to collect all the morphological characteristics of non-Indian Musaceae. However, the character evolution study of the whole Musaceae family definitely provides new insight into the concept of the evolution of bananas. Our molecular dating study particularly focuses on Indian Musaceae, as understanding the timing of evolution and divergence of this family in India is crucial. However, it would be beneficial to conduct a dating analysis of global Musaceae by incorporating the newly generated phylogenetic data from the present study. The future outlooks are outlined below.

- Future research should focus on conducting further phylogenetic analyses using multiple accessions of taxa and additional molecular markers or plastome sequencing of entire taxa to enhance the resolution of the global Musaceae phylogeny.

- equencing of entire taxa to enhance the resolution of the global Musaceae phylogeny.

- Collaborate with Zingiberales specialists to conduct molecular dating, ancestral area and ancestral state reconstruction studies within the order Zingiberales. Existing sequences along with newly generated sequences can be utilized for this proposed study.

- Karyomorphological studies of Musaceae have primarily focused on counting the chromosome numbers. However, being a key character in sectional classification, a detailed karyomorphological analysis of wild bananas is essential to understanding the chromosomal evolution.

- As mentioned earlier, the gene pool of potential wild banana candidates can be exploited to develop high-yielding or disease-resistant edible varieties.
- Develop appropriate agro techniques for the domestication and improvement of wild ornamental bananas with potential and introduce them into gardens.
- As many wild bananas are used as medicine by ethnic communities, the bioprospecting of these taxa needs to be thoroughly evaluated.

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APPENDIX

Publications

- **Rajeesh, E.P**., Hareesh, V.S. & M. Sabu 2024. Combining Morphological, Anatomical and Phylogenetic Evidence to Resolve the Identity of *Musa kattuvazhana* (Musaceae) in the Andaman Islands, India. *The Journal of Japanese Botany* 99(3): 146–158. DOI: https://doi.org/10.51033/jjapbot.ID0133
- Thomas, V. P., Sabu, M., Jayakrishnan, T. & **E.P. Rajeesh** 2020. A new species and a new combination of *Amomum* Roxb. (Zingiberaceae) from Sikkim Himalaya, India. *Phytotaxa* 430(1):46–50. https://doi.org/10.11646/phytotaxa.430.1.7

Paper presentations

- **Rajeesh E.P & M. Sabu 2023**. "Phylogeny and Molecular dating of Indian Musaceae". International symposium of IBS on "Synergy in Plant Science and Sustainable Future" held at Sant Gadge Baba Amravati University, Amravati, Maharashtra. 4-6 November 2023.

- **Rajeesh E.P & M. Sabu 2023**. "Molecular Phylogeny and character evolution of Indian Musaceae, with special reference to the genus *Musa*". International seminar on Gingers held at KSCSTE-MBGIPS, Kozhikode, Kerala,01-03 March 2023. (**KSCSTE-MBGIPS Overall Presenter Award**)

- **Rajeesh E.P & M. Sabu 2022**. "Combining morphological and molecular tools to understand the phylogeny of Indian Musaceae". National Conference of IAAT on "The Contribution of Angiosperm Diversity to Human Wellbeing and the Risks Associated with its Decline" held at Karnatak Science College, Dharwad, Karnataka, 11-13 November 2022. (**Fr. Antony Mukkath- K.S. Manilal Award for the best paper in modern techniques in Angiosperm Taxonomy**)

- **Rajeesh E.P & M. Sabu 2022**. "Investigation on the potential wild Indian bananas for breeding programmes". 34th Kerala Science Congress, Kerala, 10-12 February 2022.

- **Rajeesh E.P & M. Sabu 2022**. "Comparative morphological and ITS based phylogenetic studies of Indian Musaceae". International conference on "Sustainable utilisation of Bioresources" held at Dept. of Botany, University of Kerala, 10-15 January 2022.

- **Rajeesh E.P & M. Sabu 2019**. "Molecular phylogeny of *Musa balbisiana* complex in India using Nuclear and Chloroplast DNA sequences". National Symposium on "Modern Trends in Biosystematics of Angiosperms" held at Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Kerala, 11-13 November 2019.

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J. Jap. Bot. **99**(3): 146–158 (2024)
doi: https://doi.org/10.51033/jjapbot.ID0133

Combining Morphological, Anatomical and Phylogenetic Evidence to Resolve the Identity of *Musa kattuvazhana* (*Musaceae*) in the Andaman Islands, India

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(Accepted on 9 January 2024)

The validity of *Musa kattuvazhana* is still in contention for some taxonomists. The present study aimed to solve the correct identity of *M. kattuvazhana* through morphological comparison and phylogenetic analyses using nuclear ITS, chloroplast *trnL-trnL-trnF* and *rps16* intron. The distinctiveness of this species from *M. acuminata* is established here with morphological, anatomical, and phylogenetic evidence. In addition, the occurrence of *M. kattuvazhana* in Andaman was confirmed, contrary to the claims made by recent publications. Furthermore, the results supported that *M. acuminata* subsp. *burmannica* and subsp. *burmanicoides* should be treated as synonyms of *M. kattuvazhana*.

Key words: Andaman Islands, India, *Musa acuminata* subsp. *burmannica*, *Musa acuminata* subsp. *burmanicoides*, *Musa kattuvazhana*, Western Ghats, wild banana.

Musaceae (banana family) are the earliest diverging lineage within the order *Zingiberales* with three genera, *Musa* L., *Ensete* Bruce ex Horan. and *Musella* (Franch.) H.W.Li (Kress 1990, Kress et al. 2001, Li et al. 2010). Among the three genera, *Musa* is the largest and most economically important genus, which shows a wide range of distribution and occurs in tropical Asia from South India to Eastern Himalayas to Northern Australia (Häkkinen 2013). The genus *Musa* comprises about 70 species worldwide (Häkkinen 2013) and 32 taxa are represented in India with 20 endemics (Joe and Sabu 2019).

Musa kattuvazhana K.C.Jacob, which was originally published by Jacob (1952) from Western Ghats of Madras Province, India, is a taxonomically puzzling species. According to him, it is the only seeded banana growing wild in Madras Province. Parallel to Jacob's work, Nayar (1952) described *M. banksii* F.Muell. var. *singampatti* T.G.Nayar from Western Ghats, India. Subsequently, Simmonds (1957) described *M. acuminata* Colla subsp. *burmannica* N.W.Simmonds based on Cheesman's *M. acuminata* 'the Tavoy form' from Burma (Cheesman 1948). However, based on a critical survey of related literature and morphological study of living collections, Joe et al. (2016) and MosaChristas et al. (2021) treated *M. acuminata* subsp. *burmannica* and *M. banksii* var. *singampatti* as a synonym of *M. kattuvzhana*.

Musa acuminata was originally described

based on "*M. simiarum pissang Facki* Rumph. Amb. pag. 138 t. 61 fig. 1" by Colla (1820). So far, nine subspecies and one variety of *M. acuminata* have been recognized from various regions of South East Asia, viz. (1) subsp. *acuminata* from India, China and Malay Peninsula, (2) subsp. *banksii* (F.Muell.) N.W.Simmonds from New Guinea, (3) subsp. *malaccensis* (Ridl.) N.W.Simmonds and (4) subsp. *truncata* (Ridl.) Kiew from Malay Peninsula, (5) subsp. *burmannica* N.W.Simmonds, (6) subsp. *burmannicoides* De Langhe and (7) subsp. *siamea* N.W.Simmonds from North East India, Myanmar, southern China and Thailand, (8) var. *zebrina* (Van Houtte ex Planch.) Nasution (often erroneously treated as a subspecies) from Java, (9) subsp. *microcarpa* (Becc.) N.W.Simmonds from Indonesia, and (10) subsp. *errans* (Blanco) R.V.Valmayor from Philippines. Molecular analyses using restriction length fragment polymorphisms (RFLP) and simple sequence repeats (SSR) for seeded diploid forms of *M. acuminata* supported four unique clusters correspond to the subspecies and the variety: (1) subsp. *banksii* cluster from New Guinea, (2) subsp. *malaccensis* cluster from Malay Peninsula, (3) var. *zebrina* cluster from Java, and (4) subsp. *burmannica*, subsp. *burmannicoides*, subsp. *siamea* cluster from mainland of South East Asia (Perrier et al. 2009).

Li et al. (2010) and Janssens et al. (2016) described the phylogenetic relationship within the genus *Musa* based on nuclear ribosomal internal transcribed spacer (ITS) and chloroplast (*trnL* intron, *trnL-trnF* intergenic spacer, *rps16* intron, and *atpB-rbcL* intergenic spacer) sequences. The phylogenetic tree of Li et al (2010) depicted that *M. acuminata* was paraphyletic because four species of section *Rhodoclamys* (Baker) Cheesman (*M. laterita* Cheesman, *M. siamensis* Häkkinen & Rich.H.Wallace, *M. rosea* Baker, and *M. rubra* Wall. ex Kurz) are nested within the *M. acuminata* clade. In addition, *M. acuminata* subsp. *burmannica* and subsp. *burmannicoides* phylogenetically show more similarity with the four species rather than other subspecies of *M. acuminata*. However, the recent plastome analysis of *Musaceae* revealed that *M. acuminata* subsp. *burmannica* is sister to the clade including other subspecies of *M. acuminata* and four other *Musa* species (Fu et al. 2022). Thus, it is suggested that *M. acuminata* subsp. *burmannica* is genetically distinct from other subspecies of *M. acuminata*. However, still some studies (Sardos et al. 2016, Rouard et al. 2018, Dupouy et al. 2019, Singh et al. 2020, Fu et al. 2022) used the name *M. acuminata* subsp. *burmannica* without considering Joe et al. (2016).

During the recent survey, Hareesh et al. (2017) reported the occurrence of *Musa kattuvazhana* in the Andaman Islands. Later, two specimens of this species (as "*M. acuminata*") from Andaman were found to be closely related to *M. acuminata* subsp. *burmannica*, which was analyzed in Li et al. (2010), in the phylogenetic relationship based on the sequences of ITS and *trnL-trnF* intergenic spacer (Singh et al. 2020). Unfortunately, this phylogenetic analysis did not include other subspecies of *M. acuminata* and typical *M. kattuvazhana*. In addition, we could not find any populations of typical *M. acuminata* (*M. acuminata* subsp. *acuminata*) during our repeated expeditions in the Islands. Thus, the actual identity of this *Musa* species in the Islands remains unclear and needs to be confirmed.

The present study deals with the taxonomic identity and distribution of *Musa kattuvazhana* in the Andaman Islands and Western Ghats, India. To achieve that, here we (1) compared the morphological and anatomical differences between *M. acuminata* subsp. *acuminata* and *M. kattuvazhana*, and (2) examined the phylogenetic position of *M. kattuvazhana* from the Andaman Islands and from Western Ghats, India and *M. acuminata* subsp. *acuminata* from

North-East India based on the sequences of ITS, *trnL* intron, *trnL-trnF* intergenic spacer, and *rps16* intron.

Materials and Methods

Taxon sampling

For *Musa kattuvazhana*, two accessions were collected from two different areas of middle Andaman and one accession taken from Munnar, Idukki district of Kerala (Western Ghats), India (Fig. 1G–M, Appendix). Two accessions of *M. acuminata* subsp. *acuminata* were also collected from North-East India (Fig. 1A–F, Appendix). Fresh leaves and seeds were collected for anatomical observation. Cigar leaves were taken and stored in silica gel for DNA isolation. Fresh rhizome were taken to enrich the *Musa* germplasm collection of Calicut University Botanical Garden (CUBG) and Malabar Botanical Garden and Institute for Plant Sciences (MBGIPS), Kerala, India for further studies. The voucher specimens are deposited at Calicut University Herbarium (CALI), Kerala, India (Appendix).

Morphological and anatomical comparison

The morphological characters of *Musa kattuvazhana* and *M. acuminata* subsp. *acuminata* were described from the observation of living plants in the field and germplasm collections at CUBG and MBGIPS, according to the INIBAP *Musa* Descriptor List (IPGRI-INIBAP/CIRAD 1996).

For the anatomical study of these two species, the third leaf was selected from the top of the plant after the inflorescence appeared, and mature seeds were taken from the centre of the ripened fruit. The lamina sections were stained with safranin and mounted on a transparent glass slide with glycerin. Photomicrographs were taken using Axio Lab.A1 ZEISS microscope with AxioCam ERc 5s camera, ranging from 5X to 100X magnifications. The surface and longitudinal sections of seeds were subjected to scanning electron microscopy (SEM) to observe the detailed seed surface sculpturing and the structure of the seed coat. The seed coat thickness was measured between the chalazal chamber and the operculum (center of the seed) (Hareesh and Sabu 2023). SEM images were taken using a JEOL Model JSM-6390LV SEM and a Gemini SEM 300 at different magnifications.

DNA isolation, PCR amplification and sequencing

Total genomic DNA was extracted from fresh leaf samples taken from the germplasm collection and silica dried leaf samples by using modified 2x CTAB method (Doyle and Doyle 1990). Polymerase chain reactions (PCR) were conducted in a total volume of 20 µL containing 1 µL (10–25 ng) of DNA template, 10 µL EmeraldAmp® GT PCR Master Mix (TaKaRa Bio, India), 0.75 µL of each primer, 0.4 µL of DMSO. PCR conditions were 95 °C for 5 min; 35 cycles of 30 sec at 94 °C, 35 sec at 56 °C and 1 min at 72 °C; followed by a final extension of 5 min at 72 °C for ITS, 95 °C for 5 min; 35 cycles of 30 sec at 95 °C, 1 min at 56 °C and 1 min at 72 °C; followed by a final extension of 5 min at 72 °C for *trnL* intron and *trnL-trnF* intergenic spacer (*trnL-trnL-trnF*), and 95 °C for 5 min.; 35 cycles of 30 sec at 95 °C, 1 min at 58 °C and 1 min at 72 °C; followed by a final extension of 5 min at 72 °C for *rps16* intron. The published primers were used: ITS5P and ITS8P for ITS (Möller and Cronk 1997), c and f for *trnL-trnL-trnF* (Taberlet et al. 1991), and rpsF and rpsR2 for *rps16* intron (Oxelman et al. 1997). PCR amplicons were outsourced to Sci Genome Lab Pvt. Ltd., Cochin, Kerala for further purification and sequencing. Sequences were checked and edited by Sequencher ver. 4.1.4 (Gene Codes).

Phylogenetic analyses

Based on previous studies (Li et al. 2010, Janssens et al. 2016, Singh et al. 2020), sequences of ITS, *trnL-trnL-trnF* (*trnL* intron

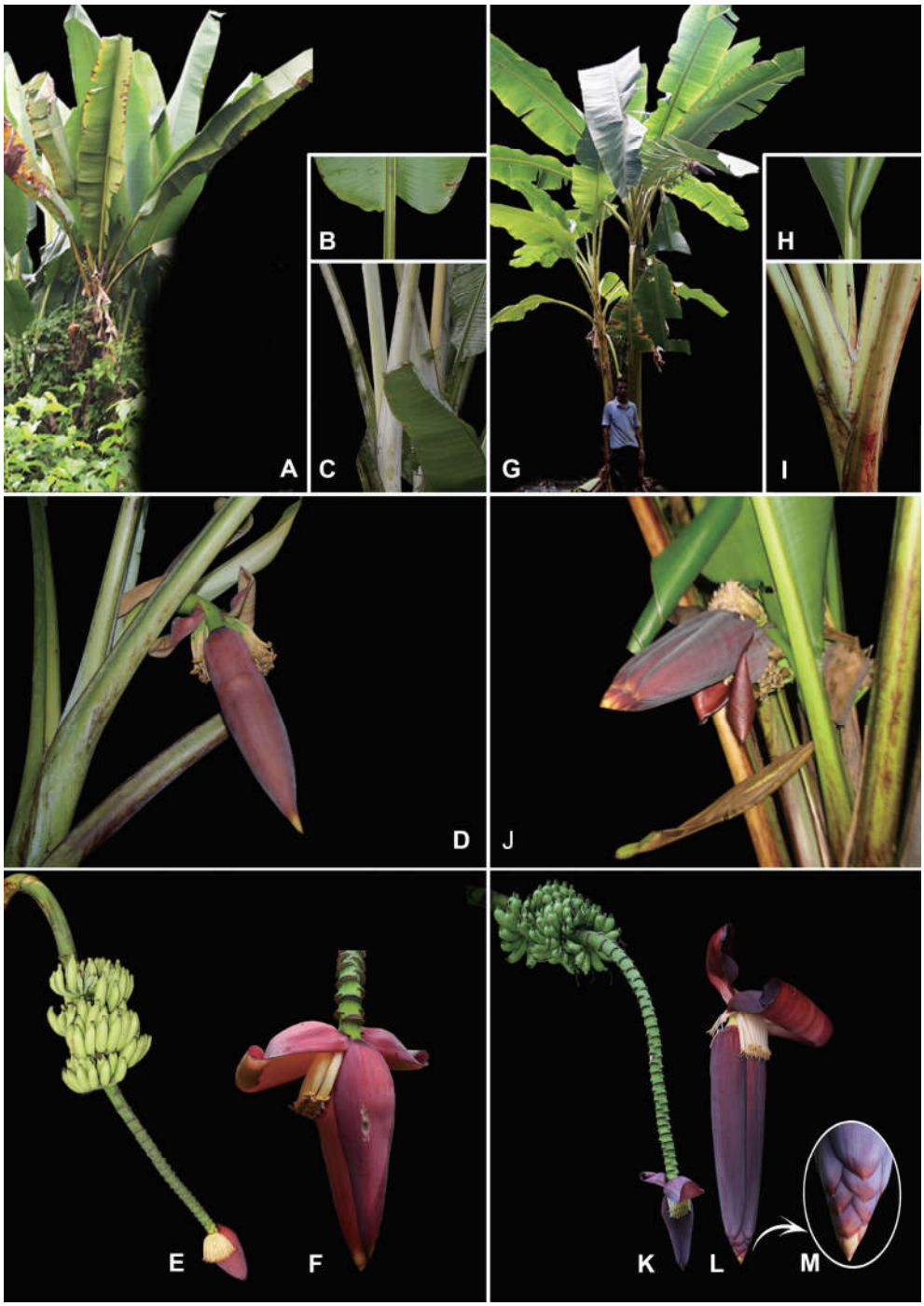

Fig. 1. Comparison of *Musa acuminata* subsp. *acuminata* (A–F) and *M. kattuvazhana* (G–M). A, G. Habit. B, H. Leaf base. C, I. Apex of pseudostem showing petiole. D, J. Initial stage of female bud. E, K. Mature Infructescence. F, L. Male bud. M. Imbricate apex of male bud. Photos: E.P.Rajeesh (F, K) and V.S.Hareesh (A–E, G–J, L, M).

and *trnL-trnF* intergenic spacer) and *rps16* intron of *Musa* and outgroups [*Ensete glaucum* (Roxb.) Cheesman and *Musella lasiocarpa* (Franch.) C.Y.Wu & H.W.Li] were retrieved from GenBank. However, *trnL* intron and *rps16* intron for two accessions of Singh et al (2020)'s *M. acuminata* were not available. First, the phylogenetic analyses were conducted based on the sequences of ITS and *trnL-trnF* intergenic spacer of 27 accessions. Subsequent analyses were based on the sequences of ITS, *trnL-trnL-trnF* and *rps16* intron of 25 accessions, excluding the two accessions from Singh et al. (2020). The sequences were aligned using MUSCLE embedded in MEGA7 (Kumar et al. 2016). For each dataset, phylogenetic trees were inferred using Maximum-likelihood (ML) and Bayesian approaches. ML analysis was performed using IQ-TREE ver. 2.1.2 (Minh et al. 2021) implementing the TIM3+G4 model for ITS and HKY model for *trnL-trnL-trnF* and *rps16* intron. Bayesian analysis was performed by MrBayes ver. 3.2.6 (Ronquist et al. 2012). TIM3+G4 model was selected for ITS and HKY model was selected for *trnL-trnL-trnF* and *rps16* intron by jModeltest ver.2.1.4 (Darriba et al. 2012) for the Bayesian analysis. Two independent runs were performed, each run of Markov Monte Carlo (MCMC) with sample frequency 1000. The analysis was performed for 10,000,000 generations until the standard deviation of split frequency was less than 0.01. The first 25% of the samples discarded as burn in. In the ML tree, branch/clade support was depicted by 1000 bootstrap (BS) replicates (Felsenstein 1985) with 100 random addition per replicates and the BS value above 80% are represented. In the Bayesian tree, statistical branch/clade support, posterior probability (PP) value, above 0.9 was shown. The strict consensus tree of both ML and Bayesian trees was generated and visualized in FigTree v 1.4.3 (Rambaut 2016).

Results and Discussion

Morphological and anatomical differences between M. acuminata *and* M. kattuvazhana

The identities of *Musa acuminata* and *M. kattuvazhana* have often been confused. However, they are distinct species with different distribution and morphological characters viz. pseudostem and petiole colour, shape of leaf base, nature of infructescence and fruit bunch, shape of male and female bud etc., which are the diagnostic characters used for the species delimitation of *Musa* (Fig. 1, Table 2).

Häkkinen and Väre (2008) designated the illustration of Rumphius (1747: t. 61 fig. 1) as the lectotype of *Musa acuminata* (Fig. 2A). This illustration shows the beaked apex of male bud. Our collections of *M. acuminata* from North-East India show the same type of male bud (Fig. 2B).

Jacob (1952) originally designated the specimen (K.C.Jacob, No. 88134) at the Madras Herbarium as the type of *Musa kattuvazhana*. However, as the specimens could not be verified, Häkkinen and Väre (2008) designated the photograph of Jacob (1952: fig. 72 'Kaattu Vazha') as the lectotype of *M. kattuvazhana* (not shown). In addition, they mentioned that "the lectotype is very closely related to *M. laterita* but cannot be identified with certainty." Nevertheless, Jacob (1952) had noted that "fig. 72" displayed an erect inflorescence "due to unsuccessful introduction of the taxa to the dry condition of Coimbatore." This may have led Häkkinen and Väre (2008) to mistakenly believe that *M. kattuvazhana* related to *M. laterita* with an erect inflorescence. After that, Häkkinen (2013) treated this species under section *Musa* with erect inflorescence adding significantly to the confusion. Therefore, Joe et al. (2016) designated the photograph of Jacob (1952: fig. 73 'Kaattu Vazha') as the epitype of the name (Fig. 2C) in order to avoid the uncertainty of the lectotype selected by Häkkinen and Väre (2008).

The Cheesman (1948) photographs of 'the

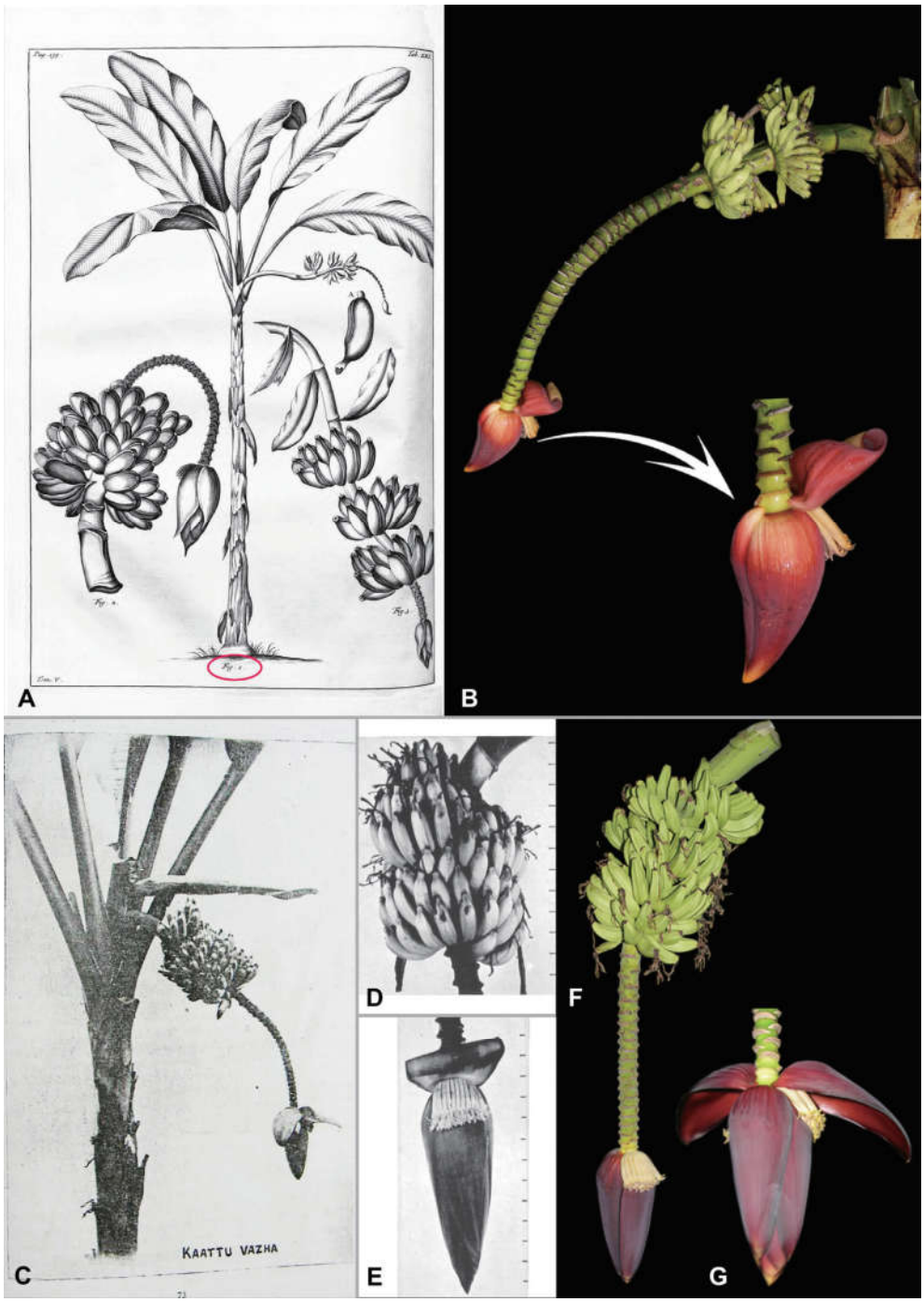

Fig. 2. *Musa acuminata* (A, B) and *M. kattuvazhana* (C–G). A. Lectotype of *M. acuminata* (Icon in Rumph., Herb. Amb. 5: t. 61, fig. 1. 1747). B. Infructescence showing beaked apex of male bud. C. Epitype of *M. kattuvazhana* (fig. 73 'Kaattu vazha' in Jacob, Madras Bananas Monogr. 1952). D, E. Photographs of *M. acuminata* subsp. *burmannica* 'the Tavoy form' [Cheesman pt. 4(2v) and pt. 3(4). 1948; Courtesy of Board of Trustees of Royal Botanic Garden Kew]. F. Infructescence. G. Male bud with imbricate apex. Photos: V.S.Hareesh (B, F, G).

Table 1. Morphological and anatomical comparison of *Musa acuminata* subsp. *acuminata* and *M. kattuvazhana*.

	M. acuminata subsp. *acuminata*	*M. kattuvazhana*
Morphology		
Pseudostem and Petiole	Glaucous, green with small blackish-brown blotches	Non-glaucous, yellow or greenish yellow with reddish brown patches
Leaf base	Both sides rounded	Both sides pointed
Peduncle	Glabrous	Puberulent
Floral bud apex	Imbrication absent, acuminate	Strongly imbricate, acute
Basal flowers	Female	Female or bisexual
Fruit bunch	Lax	Compact
Foliar Anatomy		
Lamina thickness above smaller vascular bundle (μm)	228–300	512–568
Lamina thickness above larger vascular bundle (μm)	292–327	570–596
Adaxial hypodermis	Two-layered	Three-layered
Adaxial hypodermis above larger vascular bundle	Single-layered	Two-layered
Seed micromorphology and Anatomy		
Inner periclinal wall structure	Warty	Smooth
Micropylar collar length (μm)	639–730	769–837
Inner endosperm (aleurone) layer thickness of seed coat(μm)	12.5–13.6	4.0–5.5

Tavoy form' [pt. 3(4) and pt. 4(2)], which were published as *Musa acuminata* subsp. *burmannica* (Simmonds 1957), are provided to show the fruit nature and imbricate nature of the apex of male bud (Fig. 2D, E). Our living collections of *M. kattuvazhana*, i.e., fruit nature (Fig. 2F) and imbricate nature of apex of male bud (Fig. 2G), are correspond to the typical morphological features of Cheesman (1948)'s 'the Tavoy form' as well as 'Kaattu Vazha' of Jacob (1952).

In the morphological comparison (Table 2, Fig. 1), *Musa kattuvazhana* shows that the pseudostem and petiole are non-glaucous, yellow or greenish yellow with reddish brown patches (Fig. 1I, J) [vs. glaucous, green with blackish brown blotches in *M. acuminata* subsp. *acuminata* (Fig. 1C, D)]. The leaf base pointed on both sides and continues as petiole margin in *M. kattuvazhana* (Fig. 1H), while *M. acuminata* subsp. *acuminata* possesses rounded leaf bases (Fig. 1B). Peduncle of *M. kattuvazhana* is puberulent (vs. glabrous in *M. acuminata* subsp. *acuminata*). In addition, a strongly imbricate and acute apex of floral bud is a characteristic feature of *M. kattuvazhana* (Fig. 1J, L, M), while *M. acuminata* subsp. *acuminata* possesses a floral bud with non-imbricate and acuminate apex (Fig. 1D, F).

Foliar anatomical comparison of *Musa acuminata* subsp. *acuminata* and *M. kattuvazhana* reveal the remarkable differences in the thickness of lamina and hypodermal layers (Table 2, Fig. 3). *Musa kattuvazhana* possesses greater lamina thickness above larger vascular bundles (570–596 μm; Fig. 3H) compared to *M. acuminata* (292–327 μm; Fig. 3A). In addition, the former can be easily differentiated from the latter (two-layered; Fig. 3A) in the presence of three-layered adaxial hypodermis (Fig. 3H). The adaxial hypodermal layer above the

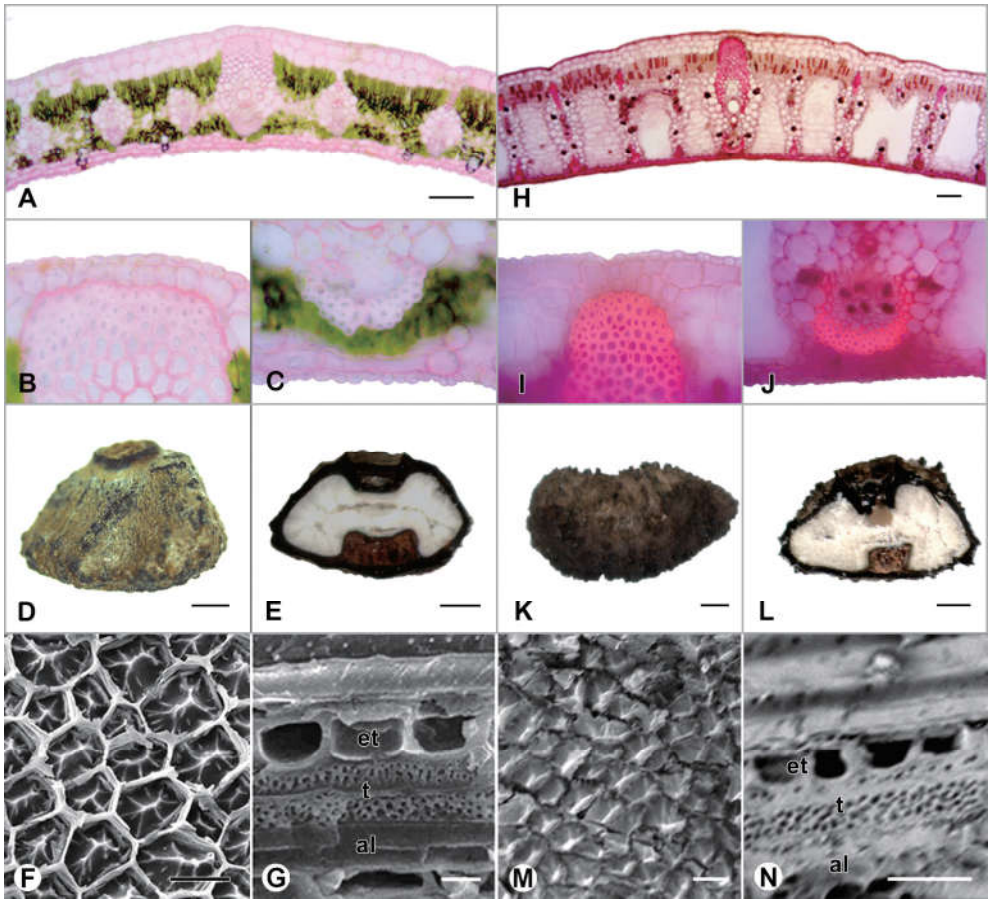

Fig. 3. Foliar anatomy and seed micro-morphology of *Musa acuminata* subsp. *acuminata* (A–G) and *M. kattuvazhana* (H–N). A, H. Cross section of lamina. B, I. Enlarged view of adaxial hypodermis above larger vascular bundle. C, J. Enlarged view of abaxial hypodermis below larger vascular bundle. D, K. Seeds. E, L. Longitudinal section of seeds. F, M. Inner surface sculpturing of seed coat. G, N. Transverse section of seed coat showing endo-testa (et), tegmen (t) and aleurone layer (al). Photos: V.S.Hareesh. Scale bars: 100 µm (A, H), 20 µm (F, M), 40 µm (G, N), 1 mm (D, E, K. L).

larger vascular bundle is single-layered in *M. acuminata* subsp. *acuminata* (Fig. 3B) whereas two-layered in *M. kattuvazhana* (Fig. 3I).

Scanning Electron Microscopic (SEM) studies on seeds of *Musa acuminata* subsp. *acuminata* and *M. kattuvazhana* shows significant differences in inner periclinal wall of inner surface sculpturing of seed coat (Hareesh and Sabu 2023). i.e., warty in the former (Fig. 3F) and smooth in the latter (Fig. 3M). Micropylar collar length in *Musa acuminata* subsp. *acuminata* is 639–730 µm (Fig. 3E), while *M. kattuvazhana* possesses 769–837 µm length (Fig. 3L). In addition, inner endosperm (aleurone) layer thickness of seed coats in *Musa acuminata* subsp. *acuminata* is 12.5–13.6 µm (Fig. 3N) and *M. kattuvazhana* is 4.0–5.5 µm (Fig. 3G).

Musa *identified as* M. acuminata *in the Andaman Islands*

The length of the *trnL-trnL-trnF* sequences analysed in this study are 975 to 980 bp. Those of *Musaceae* members from GenBank are 778 to 810 bp, except the sequences of two

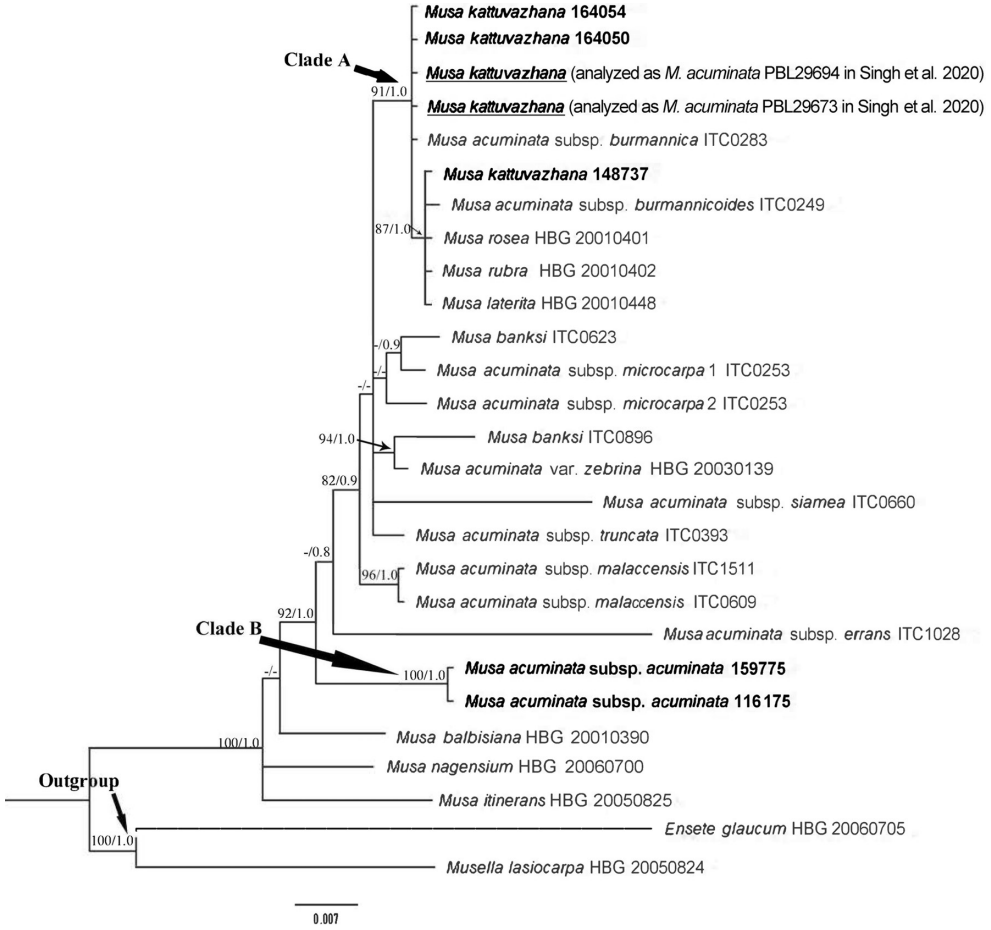

Fig. 4. Strict consensus tree of ML and Bayesian trees based on the combined sequences of ITS and *trnL-trnF* intergenic spacer. Number above the nodes are Maximum likelihood bootstrap support value (BS; ≥ 80%) followed by Bayesian posterior probabilities (PP; ≥ 0.9). Hyphens "-" indicate BS < 80% and PP < 0.9. Accessions in bold were analysed in the present study, those in bold and underlined were analysed in Singh et al. (2020), and standard ones are from GenBank (Appendix). The scale bar (0.007) represents substitution per site.

accessions of *M. acuminata* (PBL29673 and PBL29694 in Appendix) analysed in Singh et al. (2020), which are 350 bp of only *trnL-trnF* intergenic spacer without *trnL* intron. In the first phylogenetic analyses, the sequences of *trnL* intron were excluded from others in accordance with the two shorter sequences. The final aligned length of the combined sequences were 1047 bp (633 bp of ITS, 414 bp of *trnL-trnF*).

In the phylogenetic tree (Fig. 4), *M. acuminata* subsp. *acuminata* (116175, 159775), which was evidenced by morphological and anatomical characters (Clade B; BS = 100, PP = 1.0), was distinct from three accessions of *M. kattuvazhana* from Andaman (164050, 164054) and Kerala (148737) in Clade A (BS = 91, PP = 1.0). This indicates that *M. kattuvazhana* is phylogenetically distinct from *M. acuminata* subsp. *acuminata*.

On the other hand, two accessions analysed in Singh et al. (2020) as *M. acuminata* from Andaman (PBL29673, PBL29694) nested in Clade A along with three accessions of

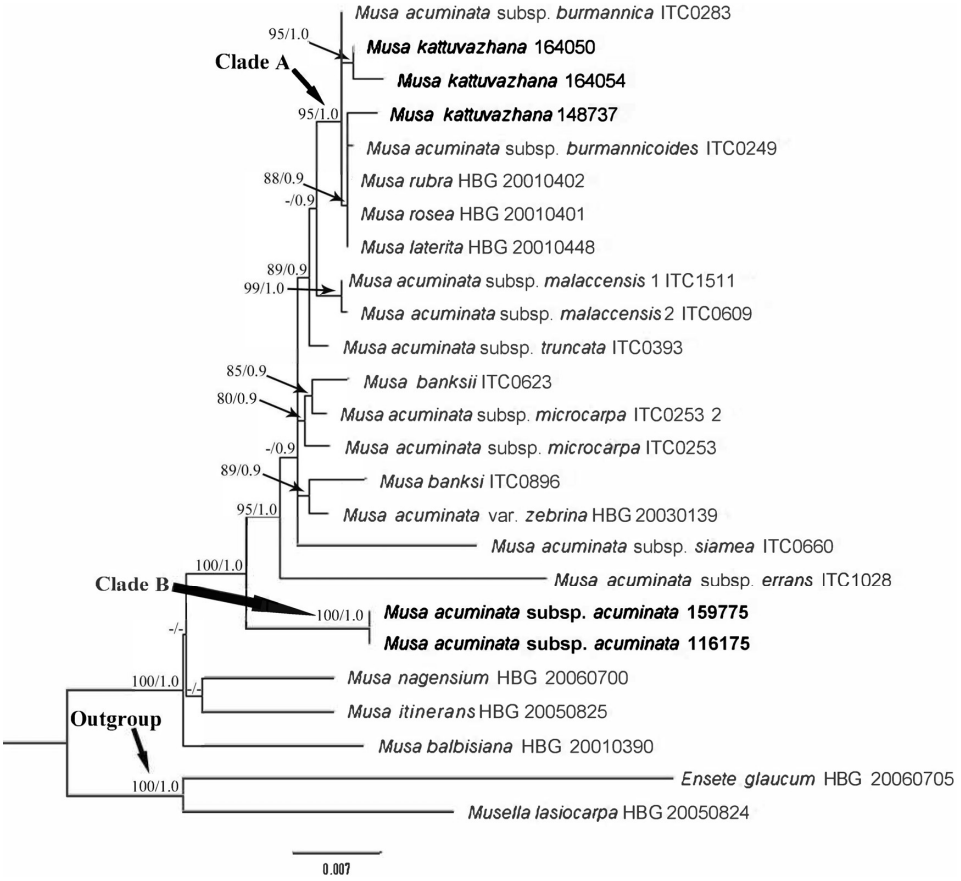

Fig. 5. Strict consensus tree based on the combined sequences of ITS, *trnL-trnL-trnF* and *rps16* intron. Number above the nodes are Maximum likelihood bootstrap support value (BS; ≥ 80%) followed by Bayesian posterior probabilities (PP; ≥ 0.9). Hyphens "-" indicate BS < 80% and PP < 0.9. Accessions in bold were analysed in the present study and standard ones are from GenBank (Appendix).

M. kattuvazhana, *M. acuminata* subsp. *burmannica*, subsp. *burmannicoides*, *M. rubra*, *M. laterita*, and *M. rosea*. For the identification of the two accessions from Andaman, Singh et al. (2020) did not consider the morphological distinction and the taxonomic history of *M. kattuvazhana*. Based on the photoplate of a plant identified as *M. acuminata* [fig. 7A in Singh et al. (2020)], the male bud has a highly imbricate apex, a characteristic feature of *M. kattuvaznana*. Thus, Singh et al. (2020)'s two accessions from Andaman are *M. kattuvazhana*, not true *M. acuminata*.

Phylogenetic position and taxonomic identity of Musa kattuvazhana

In the subsequent phylogenetic analyses, to resolve phylogenetic position of *Musa kattuvazhana*, the sequences of ITS, *trnL-trnL-trnF* (*trnL* intron and *trnL-trnF* intergenic spacer) and *rps16* intron were combined for 25 samples, excluding Singh et al. (2020)'s two accessions from Andaman. The aligned length of the combined sequences was 2267 bp (619 bp for ITS, 834 bp for *trnL-trnL-trnF*, and 814 bp for *rps16* intron). The phylogenetic tree (Fig. 5) mostly reflected the branching pattern of the tree in the first analyses (Fig. 4). Here also, the distinctiveness between *M. acuminata* subsp. *acuminata* (Clade B, BS = 100, PP = 1.0) and

M. kattuvazhana (nested in Clade A, BS = 95, PP = 1.0) is clearly evident (Fig. 5).

Three accessions of *Musa kattuvazhana* were not monophyletic, and were closely related to *M. acuminata* subsp. *burmannica* (ITC0283) from Myanmar, *M. acuminata* subsp. *burmannicoides* (ITC0249) from India and three other species of section *Rhodochlamys* in Clade A. However, *M. kattuvazhana*, *M. acuminata* subsp. *burmannica* and subsp. *burmannicoides* are characterized by a large pseudostem (> 3 m) with pendulous inflorescence, but *M. laterita*, *M. rosea*, and *M. rubra* are characterized by a small pseudostem (≤ 3 m) with erect inflorescence (Joe and Sabu 2019).

On the basis of our morphological, anatomical and phylogenetic evidence as well as taxonomic review, it is confirmed that *Musa kattuvazhana* is distinct from true *M. acuminata*. In addition, it is proved beyond doubt that the wild banana in the Andaman Islands is *M. kattuvazhana*, not *M. acuminata*. Furthermore, the morphological characteristics and phylogenetic results support the view that *M. acuminata* subsp. *burmannica* and subsp. *burmannicoides* should be treated as synonyms of *M. kattuvazhana*.

The authors are thankful to the Director, MBGIPS, Calicut and Head, Department of Botany, University of Calicut for providing necessary facilities to conduct this work. EPR acknowledged Kerala State Council for Science, Technology and Environment (KSCSTE), Ministry of Science and Technology, Kerala for the financial assistance as the research fellowship (No.26/FSHP/2016/KSCSTE, dated 24/03/2017). MS acknowledge the Emeritus Scientist programme of CSIR (No. 21(1103)/20/EMR-II Dated 21/09/2020). We express our sincere gratitude to the forests and wildlife Departments of Andaman and Nicobar Islands, Kerala and North-East India for providing necessary permissions for entry and logistic support during our field collection trips. The enormous helps from Dr. Alfred Joe, Asst. Professor, St Josephs' college, Irinjalakkuda, Kerala was greatly acknowledged, especially for his valuable collections of *Musa* spp. We thank Ms. Amrutha A., Research Scholar, Department of Botany, University of Calicut, Dr. Soumya P. and Mr. Nandan K.K. during the field trip and collection of specimens particularly in Andaman and Nicobar Islands.

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Appendix

List of samples used for the phylogenetic analyses and their GenBank accession numbers of ITS, *trnL-trnL-trnF* (*trnL* intron and *trnL-trnF* intergenic spacer) and *rps16* intron.

Name of taxa	Accession number	Country/ Locality of collection	GenBank accession number		
			ITS	*trnL-trnL-trnF*	*rps16* intron
Ensete glaucum	HBG20060705	–	FJ428103	FJ428154	FJ428124
Musella lasiocarpa	HBG20050824	China	FJ428079	FJ428155	FJ428123
Musa acuminata					
subsp. *acuminata**	116175 (CALI)	Meghalaya, India	PP140666	PP130490	PP130494
subsp. *acuminata**	159775 (CALI)	Arunachal Pradesh, India	PP125033	PP130489	PP130493
subsp. *burmannica*	ITC0283	Myanmar	FJ428083	FJ428169	FJ428135
subsp. *burmannicoides*	ITC0249	India	FJ428085	FJ428170	FJ428133
subsp. *errans*	ITC1028	–	FJ428094	FJ428160	FJ428126
subsp. *malaccensis*	ITC1511	–	KU215102	KU215205	KU214978
subsp. *malaccensis*	ITC0609	–	KU176107	KU176109	KU176108
subsp. *microcarpa*	ITC0253	Malaysia, S/E Borneo	KU215076	KU215198	KU214952
subsp. *microcarpa*	ITC0253	Malaysia, S/E Borneo	FJ428087	FJ428174	FJ428140
subsp. *truncata*	ITC0393	–	KU215124	KU215218	KU214999
subsp. *siamea*	ITC0660	Thailand	FJ428084	FJ428175	FJ428137
var. *zebrina*	HBG20030139	–	FJ428089	FJ428173	FJ428139
M. balbisiana	HBG20010390	–	FJ428102	FJ428159	FJ428145
M. banksii	ITC0623	Papua New Guinea	FJ428097	FJ428161	FJ428138
M. banksii	ITC0896	Papua New Guinea	KU215090	KU215211	KU214966
M. itinerans	HBG2005–0825	–	FJ428098	FJ428177	FJ428148
M. kattuvazhana (as *M. acuminata* in Singh et al. 2020)**	PBL29673	East Island Wildlife Sanctuary, North Andaman, India	MT028132	MT181062	–
M. kattuvazhana (as *M. acuminata* in Singh et al. 2020)**	PBL29694	Jhirkatanng, South Andaman, India	MT028133	MT181063	–
*M. kattuvazhana**	164050 (CALI)	Kadamtala, Middle Andaman, India	PP151596	PP114099	PP114098
*M. kattuvazhana**	164054(CALI)	Rangat, Middle Andaman, India	PP151595	PP130487	PP130491
*M. kattuvazhana**	148737 (CALI)	Munnar, Idukki, Kerala, India	PP151594	PP130488	PP130492
M. aterita	HBG20010448	–	FJ428082	FJ428157	FJ428136
M. nagensium	HBG2006–0700	–	FJ428101	FJ428158	FJ428144
M. rosea	HBG20010401	–	FJ428080	FJ428171	FJ428131
M. rubra	HBG20010402	–	FJ428081	FJ428172	FJ428132

Asterisk (*) indicates accessions sequenced in the present study.
Double asterisk (**) indicates the accessions taken from Singh et al. (2020) for which *trnL* intron and *rps16* intron sequence were unavailable.

E.P.Rajeesh[1], V.S.Hareesh[2], M.Sabu[2]：**形態学的，解剖学的，系統学的証拠によるインド，アンダマン諸島産*Musa kattuvazhana*（バショウ科）の分類学的位置付け**

インド，マドラス州の西ガーツ山脈から記載された*Musa kattuvazhana*（バショウ科）は，最近アンダマン諸島からも記録されたが，両者の分類学的な位置付けについては議論の余地があった．このことを明らかにするため，両産地の*M. kattuvazhana*および近縁種*M. acuminata*について，形態学的，解剖学的比較，および核DNA・葉緑体DNAに基づく系統解析を行った．その結果，*M. kattuvazhana*は*M. acuminata*とは独立した種であること，西ガーツ山脈とアンダマン諸島の*Musa*は*M. kattuvazhana*であること，*M. acuminata* subsp. *burmannica*と subsp. *burmanicoides*は，*M. kattuvazhana*の異名とすべきことが明らかになった．

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Author of Japanese title and summary: Masashi Nakata

Indian Association for Angiosperm Taxonomy

CERTIFICATE

This is to certify that Dr. / Mr. / ~~Ms.~~ Rajeesh. E. P.
University of Calicut has been awarded the Fr. Antony Mukkath - K.S. Manilal Award for the best paper in Modern Techniques in Taxonomy presented at the Annual Conference of the Indian Association for Angiosperm Taxonomy of the year 2022.. for his / ~~her~~ paper entitled Combining the Morphological and Molecular tools to understand the Phylogeny of Indian Musaceae.

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