MICROMORPHOLOGICAL AND MOLECULAR TAXONOMY OF GENUS OPHIOGLOSSUM L. IN KERALA, INDIA

Thesis Submitted to the University of Calicut
in partial fulfilment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY

in

BOTANY

Under the faculty of Science

by

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Under the guidance of

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KERALA, INDIA

February 2024

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CERTIFICATE

This is to certify that the thesis entitled **"MICROMORPHOLOGICAL AND MOLECULAR TAXONOMY OF GENUS OPHIOGLOSSUM L. IN KERALA, INDIA"** is an authentic record of research work carried out by **Mrs. Afsana Khan** under my supervision in fulfilment of the requirement for the degree of Doctor of Philosophy, in Botany of University of Calicut. The results embodied in this thesis have not been included in any other thesis submitted previously for the award of any degree or diploma of any other university or institution. Also certified that the contents of the thesis have been checked using anti-plagiarism database and no unacceptable similarity was found through the software check.

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02 February 2024

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This is to certify that the adjudicators of the Ph. D thesis of Ms. Afsana Khan, titled "MICROMORPHOLOGICAL AND MOLECULAR TAXONOMY OF GENUS OPHIOGLOSSUM L. IN KERALA, INDIA" have not given any directions for corrections or suggestions for change in their reports. The content of the CD is the same as in the hard copy.

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DECLARATION

I, **Afsana Khan**, hereby declare that the thesis entitled **"MICROMORPHOLOGICAL AND MOLECULAR TAXONOMY OF GENUS OPHIOGLOSSUM L. IN KERALA, INDIA"** submitted to the Universityof Calicut, for the award of the degree of Doctor of Philosophy in Botany is a bona fide record of the original research work carried out by me under the supervision and guidance of Dr. Ignatius Antony, Principal & Associate professor (Retd.), Department of Botany, St. Thomas College (autonomous), Thrissur and the Co-guidance of Dr. Anto PV, Assistant professor, Department of Botany, St. Thomas College (autonomous), Thrissur and that it has not been submitted earlier either in part or full for the award of any degree/diploma to any candidate of any University.

Thrissur
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ACKNOWLEDGEMENT

"With God, nothing shall be impossible." I owe my gratefulness to **"Almighty God"** for his showers of blessings throughout my studies.

I wish to express my sincere gratitude to my Research guide **Dr. Ignatius Antony**, Retd. Principal, St. Thomas College (autonomous), Thrissur and my Co-guide **Dr. Anto P V**, Assistant Professor, Department of Botany, St. Thomas college (autonomous), Thrissur, for his unceasing encouragement, strong support, excellent guidance, valuable suggestions, immense support as a result of which I could successfully complete this work.

I am grateful to the former Principals of St. Thomas College (Autonomous), **Dr. P O Jenson** and **Dr. Joy K L**, along with the current principal in charge, **Rev. Fr. Dr. Martin K A** and Bursar, **Rev. Fr. Biju Panengadan** who has provided me all the facilities required carrying out this research work. I wish to thank the entire college authorities for their warm support rendered to me for the successful completion of this research on time.

I express my sincere gratitude to **Dr. C. D. Varghese, Dr. Vimala Jose**, former Heads and **Dr. Geethu Elizabath Thomas**, Head of the Department of Botany, St. Thomas College (Autonomous), Thrissur, for all the facilities and help rendered for my research work.

I pay my deepest gratitude to and **Dr. Sreenivas V.K.**, Assistant Professor, Head of the Department of Botany, Sri Vyasa N.S.S College, Wadakkanchery, Thrissur, **Mr. Fraser-Jenkins C. R.,** for all the way they had prepared me to move forward in my studies.

I am grateful to **Dr. Suma Arun Dev**, Scientist, KFRI and Research Scholars of the Forest Genetics and Tree Breeding, KFRI, Peechi for providing lab facilities to carry out a part of my work and their valuable support and suggestions throughout my work.

I convey my sincere gratitude to **Sreehari S. Nair** for his immense support during my work.

I am thankful to **University Grants Commission (UGC),** New Delhi, India for grating Maulana Azad National Fellowship (MANF) to meet the financial assistance for the research.

I convey my heartfelt thanks to **Dr. Burrows J.E, Dr. Jomy Augustine, Dr. Stephen Sequeira, Dr. Prabhu Kumar K. M, Dr. Rajesh K. P, Dr. Raju Antony, Dr. Pradheep A. K, Dr. Lesly Augustine, Dr. Prakash P. S, Dr. Kishore Kumar, Dr. Pramod C., Goswami H. K, Dr. Salim Pichen, Dr. Pramod Kumar N., Alfred M.K, Renjima V.** for their immense support during my study.

I am extremely thankful to the teachers of the Department of Botany, St. Thomas' College, Thrissur, **Prof. Jacob Abraham Pulikal, Dr. Thomas M.T, Dr. Joby Paul, Dr. Sandhya Vincent** and to the teachers of my school days for their valuable advice and moral support.

I sincerely thank **Mr. Sanjo**, Librarian of St. Thomas College (Autonomous), Thrissur.

I extend my thanks to the lab assistants **Mr. Joy, Mr. Pauly, Mr. Paulson, Mr. Sharon, and Mr. Denny,** Botany Department, St. Thomas' College (Autonomous), Thrissur, for their co-operation and encouragement during this work.

I am deeply indebted to the **Chief Wildlife Warden** and staff of the Kerala Forest Department for permitting specimen collection

I express my sincere thanks to the **Curators** of **B, BM, CAL, K, KFRI, MH, MNHN, CALI, LINN, MH, STC, and TBGT** for kindly permitting me to consult their herbaria and also for sending the images of type specimens.

I also convey my gratitude to **STIC, Cochin University, SEM centre, NIT Calicut and Dr. Anu Yamuna Joseph** (Primordia Life Sciences) for providing lab facilities.

I express my sincere thanks to **Dr. Shibin Mohanan**, Assistant Professor, Department of Botany, Nirmala College, Muvattupuzha, **Mr. Bipin, Mr. Wilson** for the valuable suggestions in molecular phylogenetics.

I extend my deep sense of gratitude to my research colleagues **Mr**. **Vimal K. R., Mrs. Nimmi C. Domnigose, Mrs. Keerthana Nandakumar, Dr. Alina K Sebastian, Dr. Neenu A Santhosh and Dr. Seena K K, Mrs. Reshma Ashok, Ms. Reshma Rajan, Mrs. Lakshmi Devi P. R** and **Mrs. Dhanya Jose** for their immense help, assistance, constant motivation and suggestions during the entire period of my research.

My profound sense of gratitude is extended to the research colleagues in my lab, **Mrs. Smitha P. S., Mr. Jithin K. V., Mrs. Navya Patrik, Dr. Aiswariya K. S, Mrs. Hridhya M. V., Mrs. Rameena K Jamal, Mrs. Sreeshma P. S., Mrs. Aparna Joseph V., Mrs. Blessy Santhosh, Ms. Reshma Rajan, Mr. Shaibu Jacob and Mrs. Divya K Venugopal** for rendering their valuable suggestions, endless support and motivation.

I also thank all Teachers and Research Colleagues of various departments of St. Thomas College for their support and help.

Words cannot adequately express my deep sense of gratitude to my family, especially to my parents, especially my father **Moideen Khan**, mother **Isha Moideen**, Father-in-law **Habeeb**, mother-in-law **Sainaba**, Sisters **Ruksana Khan**, **Sabitha, Jisna**, Brother- in- laws **Kaja Husain**, **Riyas, Ansil, Ansif**, my husband **Siraj C. H,** and my lovely daughter, **Aliza Fathima**. My Grandmother Kadheeja is specially thanked for all her prayers and support during my work

Afsana Khan

Dedicated to my lovely family

ABSTRACT

Genus *Ophioglossum* is commonly called Adder's tongue or snake tongue fern owing to the shape of its tropophyll and fertile spike. It has 53 accepted species worldwide. The genus showed cosmopolitan distribution in all continents except in Antartica. The name was derived from combining two Greek words, ophios and glossa, meaning snake and tongue**.** *Ophioglossum* is a complex genus with bewildering morphological characters. The taxonomy of *Ophioglossum* was always confused from the past years and faced many nomenclatural issues.

The genus *Ophioglossum* is one of the least studied genera in Kerala. It can be easily distinguished from the other genera by its tropophyll, fertile spike and rhizome. This study aims to revise the genus *Ophioglossum* for the first time in Kerala, India. A combined morphological and molecular analysis was carried out to solve the nomenclatural problems within the genus and to trace the evolutionary relationship among them. Extensive field explorations were conducted during 2018 – 2022. Specimens were collected. The field observations, such as habit, habitat, rhizome, nature and colour of the tropophylls were noted in the field book. Morphological analysis includes both micromorphology and macro morphology. Micromorphology mainly focused on venation pattern, stomatal characters and spore morphology. Plant materials were dried and mounted on standard herbarium sheets. Herbarium sheets of each species, including type specimen, were deposited at St. Thomas College herbarium. Distribution maps of each species were prepared for future study. Molecular analysis was carried out using chloroplast genes (*rbc*L-F, *psb*A-*trn*H and *trn*L-F). The newly generated sequences were deposited into NCBI GenBank. Phylogenetic trees were constructed based on the three primer regions. The newly generated sequences and the sequence retrieved from GenBank were used for constructing the tree.

Previously there were 10 species reports from Kerala, the present study reports fourteen taxa in Kerala. Among them, *Ophioglossum* sp. nov. was described as a new species. *O. latifolium* was a new report for India and *O. indicum* was a new report for South India. The species status of *O. raphaelianum* and *O. indicum* was reinstated*, O. madhusoodananii* considered as a synonym of *O. costatum* and *O. trilokinathii* was considered as a synonym of *O. rubellum.* The occurance of *O. lusitanicum* and *O. vulgatum* in India was confirmed. Evaluation of the medicinal properties of genus *Ophioglossum* in Kerala and cytological studies on genus *Ophioglossum* in Kerala are the future aspects of this study.

സംഗ്രഹം

ജീനസ് ഒഫിയോഗ്ലോസം സാധാരണയായി 'അഡേഴ്സ് ടങ്' അല്ലെങ്കിൽ 'സ്നേക് ടങ്' പന്നൽ എന്നറിയപ്പെടുന്നു. ഇലയുടെയും റീപ്രൊഡക്റ്റീവ് സ്പൈക്കിന്റെയും ആകൃതിയാണ് ഈ ചെടികൾക്ക് ഈ പേര് നൽകുവാൻ കാരണം. ലോകമെമ്പാടും അംഗീകരിക്കപ്പെട്ട 53 സ്പീഷിസുകളാണ് ഈ ജീനസ്സിൽ ഉൾപ്പെടുന്നത്. ഇവ അന്റാർട്ടിക്ക ഒഴികെയുള്ള എല്ലാ വൻകരകളിലും വിന്യസിക്കപ്പെട്ടിരിക്കുന്നു. പാമ്പ് എന്നർത്ഥമുള്ള 'ഒഫിയോസ്', നാവ് എന്നർത്ഥമുള്ള 'ഗ്ലോസ' എന്നീ ഗ്രീക്ക് പദങ്ങൾ സംയോജിപ്പിച്ചാണ് ഈ പേര് നൽകിയത്. ആശയകുഴപ്പങ്ങൾ ഉണ്ടാക്കുന്ന രൂപശാസ്ത്രപരമായ പ്രതീകങ്ങളുള്ള ഒരു സങ്കീർണ്ണ ജീനസ്സാണ് ഒഫിയോഗ്ലോസം. ഒഫിയോഗ്ലോസത്തിന്റെ വർഗ്ഗീകരണം എല്ലായ്പ്പോഴും ആശയക്കുഴപ്പത്തിലായിരുന്നു. കൂടാതെ നിരവധി നാമകരണ പ്രശ്നങ്ങൾ അഭിമുഖീകരിക്കുകയും ചെയ്തു.

കേരളത്തിൽ ഏറ്റവും കുറവ് പഠനം നടത്തിയ ജീനസ്സുകളിൽ ഒന്നാണ് ഒഫിയോഗ്ലോസം. ഇല, റീപ്രൊഡക്റ്റീവ് സ്പൈക്ക്, റൈസോം എന്നിവയാൽ ഇതിനെ മറ്റ് ജീനസ്സുകളിൽ നിന്ന് എളുപ്പത്തിൽ വേർതിരിച്ചറിയുവാൻ കഴിയും. കേരളത്തിൽ ആദ്യമായി ഒഫിയോഗ്ലോസം ജീനസ്സിനെക്കുറിച്ച് പഠിച്ച് പരിഷ്ക്കരിക്കുവാനാണ് ഈ ഗവേഷണം ലക്ഷ്യമിടുന്നത്. ജീനസ്സിലെ നാമകരണ പ്രശ്നങ്ങൾ പരിഹരിക്കുന്നതിനും അവ തമ്മിലുള്ള പരിണാമപരമായ ബന്ധം കണ്ടെത്തുന്നതിനും ഒരു സംയോജിത രൂപഘടനയും, തന്മാത്രാ വിശകലനവും നടത്തി. 2018–2022 കാലയളവിൽ വിപുലമായ ഫീൽഡ് പര്യവേക്ഷണങ്ങൾ നടത്തി. ഓരോ ചെടിയും ശേഖരിച്ചു. ചെടിയുടെ സ്വഭാവം, ആവാസവ്യവസ്ഥ, ഭൂകാണ്ഡം, ഇലകളുടെ നിറം, സ്വഭാവം എന്നിവ ഫീൽഡ് ബുക്കിൽ രേഖപ്പെടുത്തി. മോർഫോളജിക്കൽ വിശകലനത്തിൽ മൈക്രോ മോർഫോളജിയും മാക്രോമോർഫോളജിയും ഉൾപ്പെടുന്നു. മൈക്രോ മോർഫോളജിയിൽ വീനേഷൻ പാറ്റേൺ, സ്റ്റൊമാറ്റൽ ക്യാരക്ടേർസ്, സ്പോർ മോർഫോളജി എന്നിവയ്ക്ക് കൂടുതൽ പ്രാധാന്യം നൽകി. ചെടിയുടെ ഭാഗങ്ങൾ ഉണക്കി ഹെർബേറിയം ഷീറ്റുകളിൽ ഒട്ടിച്ചു. ഓരോ ഇനത്തിന്റേയും ഹെർബേറിയം ഷീറ്റുകൾ, ടൈപ്പ് സ്പേസിമൻ എന്നിവ സെന്റ് തോമസ് കോളേജ് ഹെർബേറിയത്തിൽ നിക്ഷേപിച്ചു. ഭാവി പഠനത്തിനായി ഓരോ സസ്യത്തിന്റേയും ഡിസ്ട്രിബ്യൂഷൻ മാപ്പുകൾ തയ്യാറാക്കി. ക്ലോറോപ്ലാസ്റ്റ് ജീനുകൾ ഉപയോഗിച്ചാണ് തന്മാത്രാ വിശകലനം നടത്തിയത്. പുതുതായി സൃഷ്ടിച്ച സീകൻസുകൾ എൻ.സി.ബി.ഐ. ജെൻബാങ്കിൽ നിക്ഷേപിച്ചു. മൂന്ന് പ്രൈമറുകളെ അടിസ്ഥാനമാക്കിയാണ് ഒഫിയോഗ്ലോസം ജീനസ്സിലെ ഫൈലോജെനെറ്റിക് ട്രീ നിർമ്മിച്ചിരിക്കുന്നത്. പുതിയതായി സൃഷ്ടിച്ച സീകൻസുകളും

ജെൻബാങ്കിൽ നിന്നും വീണ്ടെടുത്ത സീക്വൻസുകളും ട്രീ നിർമ്മിക്കാൻ ഉപയോഗിച്ചു.

കേരളത്തിൽ നിന്ന് പത്ത് ടാക്സകളാണ് ഇതുവരെ റിപ്പോർട്ട് ചെയ്യപ്പെട്ടിട്ടുള്ളത്. എന്നാൽ പതിനാല് ടാക്സകളെയാണ് ഈ പഠനം റിപ്പോർട്ട് ചെയ്യുന്നത്. അവയിൽ ഒഫിയോഗ്ലോസം എസ്.പി. നോവ് പുതിയ ഇനമായി റിപ്പോർട്ട് ചെയ്തു. ഒഫിയോഗ്ലോസം ലാറ്റിഫോളിസം ഇന്ത്യയിൽ നിന്നും ഒഫിയോഗ്ലോസം ഇൻഡിക്കം ദക്ഷിണേന്ത്യയിൽ നിന്നും പുതിയ റിപ്പോർട്ടായിരുന്നു. ഒഫിയോഗ്ലോസം റാഫേലിയാനം, ഒഫിയോഗ്ലോസം ഇൻഡിക്കം എന്നിവയുടെ സ്പീഷീസ് സ്റ്റാറ്റസ് പുനഃസ്ഥാപിച്ചു. ഒഫിയോഗ്ലോസം മധുസൂദനാനിയെ ഒഫിയോഗ്ലോസം കോസ്റ്റേറ്റത്തിന്റെ സിനോണിമായും ഒഫിയോഗ്ലോസം ട്രൈലോക്കിനാത്തിയെ ഒഫിയോഗ്ലോസം റുബെല്ലത്തിന്റെ സിനോണിമായും പരിഗണിച്ചു, ഒഫിയോഗ്ലോസം ലുസിറ്റാനിക്കം, ഒഫിയോഗ്ലോസം വൾഗേറ്റം എന്നിവയുടെ സാന്നിധ്യം ഇന്ത്യയിൽ സ്ഥിരീകരിക്കുകയും ചെയ്തു.

ഒഫിയോഗ്ലോസത്തിന്റെ ഔഷധഗുണങ്ങളെക്കുറിച്ചുള്ള വിലയിരുത്തലുകളും കേരളത്തിലെ ഒഫിയോഗ്ലോസം ജീനസ്സിനെക്കുറിച്ചുള്ള സൈറ്റോളജിക്കൽ പഠനങ്ങളും ഈ പഠനത്തിന്റെ ഭാവി വശമാണ്.

Key words

1. ***Ophioglossum***
2. **Spike**
3. **Rhizome**
4. **Trilete**
5. **Bireticulation**

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ABBREVIATIONS

° C	Degree Celsius
µL	Microliter
µm	Micrometre
ml	Millilitre
mg	Milligram
M	Molar
g	Gram
cm	Centimetre
nm	Nanometre
%	Percentage
rpm	Revolutions per minute
V	Volt
var.	Variety
sp. nov.	New species
QGIS	Quantum Geographic Information System
LM	Light Microscopy
SEM	Scanning Electron Microscopy
viz.	Videlicet (Latin), namely
Dist.	District
Diam.	diameter

Chapter-1

Introduction

1.1. General Introduction

Pteridophytes are vascular plants with greater diversity and cosmopolitan distribution worldwide. Their origin was in the Silurian period of the Palaeozoic era and flourished during the Carboniferous to Jurassic periods. India is rich with 34 families, 130 genera, 1107 species and 50 subspecies of pteridophytes, of which 43 species are exotic and not part of the natural flora (Fraser-Jenkins *et al.,* 2017). Most of the plants thrive in moist shady areas, and the sporophytic plant body flourishes well during the rainy season. They are called vascular cryptograms due to the hidden reproduction in the presence of water. The life cycle of pteridophytes involves an alternation of generation between the two morphologically distinct and independent, haploid gametophytic generation and diploid sporophytic generation. The dominant, long living sporophytic plant body is well differentiated into roots, stems and leaves. The plants with microphylls are known as fern allies, and with megaphylls are known as ferns. Usually, sporangia are born on fertile leaves called sporophylls. The plants are either homosporous or heterosporous. Through spore germination, microspores give rise to the male gametophytic plant body, and megaspores give rise to the female gametophytic plant body. Pteridophytes reproduce both sexually and asexually. Sexual reproduction is by the fusion of male and female gametophytes, which gives rise to a diploid sporophyte, and asexual reproduction occurs by spore germination, which will result in the development of a haploid gametophytic plant body. The reproductive cycle repeats at regular intervals, which is called as the alternation of generations.

1.2. General account on Genus *Ophioglossum* L.

Genus Ophioglossum is commonly called Adder's tongue or snake tongue fern owing to the shape of its fertile spike. It is cosmopolitan with 53 accepted species worlds wide. The name was derived from combining two Greek words, ophios and glossa, meaning snake and tongue. The genus has gotten the attention of many taxonomists since its discovery by Bauhin (1620). The generic status of *Ophioglossum* was validated by Linnaeus (1753) in "Species Plantarum". Molecular clock approximations suggest the origin of *Ophioglossum* during the late carboniferous period of the cenozoic era (Pryer *et al.,* 2004).

The taxonomy of *Ophioglossum* was always confused from the past years. Clausen (1938) considered four subgenera under the genus *Ophioglossum* Viz. *Cheiroglossa* C. Presl, *Ophioderma* (Blume) Endl., *Euphioglossum* and *Rhizoglossum* C. Presl and identified 26 species for the genus. 23 species were placed under *Euphioglossum*, and three were treated under *Ophioderma*, one under *Cheiroglossa* and one species under *Rhizoglossum*. Later the subgenus *Cheiroglossa* was excluded by Pichi-Sermolli (1954) and recognized three subgenera viz. *Ophioderma* (Blume) Clausen, *Ophioglossum* and *Rhizoglossum* (Presl) Clausen. Burrows and Jhons (2001) dealt with 18 *Ophioglossum* species from east Africa. They considered subgenus *Ophioglossum* and *Ophioderma* under the genus *Ophioglossum.* Ophioderma included only the epiphytic species *Ophioderma pendulum* (L.) C. Presl.

The taxonomy of *Ophioglossum* genus is highly complex and controversial in India. Five species was identified by Balakrishnan *et al.*, (1960). Mahabale (1962) considered the four subgenus *Ophioderma, Euphioglossum, Cheiroglossa* and *Rhizoglossum* and identified ten species from India.The highest chromosome number found in *O. reticulatum* (Abraham *et al*., 1962) was a breakthrough discovery which increased the interest in the genus *Ophioglossum*. Goswami (2007) recognized two subgenera viz. *Ophioglossum* and *Ophioderma* with a total of 12 species and four varieties for the genus *Ophioglossum*. Roskov *et al.*, (2018), Balkrishna *et al.*, (2019), synonymised *Rhizoglossum* and *Ophioderma* under genus *Ophioglossum*. Balkrishna *et al.*, (2019) synonimised subgenus *Cheiroglossa* under the genus *ophioglossum.* Anto *et al.*, (2016) reported *O. raphaelianum* Anto, Afs.Khan, F. Francis & I. Antony from Kerala. Fraser-Jenkins *et al.*, (2017, 2021) doesn't considered the subgenus level classification and documented twelve *Ophioglossum* species from India, nine species from Kerala. "So totally, there were ten *Ophioglossum* species reports from Kerala viz. *O. costatum* R. Br., *O. gramineum* Willd., *O. gomezianum* Welw. Ex A. Braun, *O. lusoafricanum* Welw.ex. Prantl., *O. parvifolium* Grev. & Hook., *O. pendulum* L., *O. petiolatum* Hook., *O. reticulatum* L. and *O. rubellum* Welw. Ex A.Braun and *O. raphaelianum"* (Khan *et al.,* 2023). *Ophioglossum* is a beweldering genus and the plant body consists of a rhizome, tropophylls and a spike having two rows of sporangia. The variation in shape and the size of these plant parts is the readily available characters from the field for identification. Due to the confusing morphology of different species, many new names were added from India, without

considering the existance of morphotypes in this genus. So, Fraser-Jenkins *et al*., (2021) synonymised them under different species by considering the gross morphology and possibility for occurring different morphotypes of the same species.

1.3. Distribution and Habitat

The genus *Ophioglossum* is distributed worldwide and found in various habitats, including soil patches of loose rocks, in open grasslands, under the tree shades and in some cases, in the river banks. In Kerala, most species were found on the soil patches above exposed rock surfaces covered by mosses. The sporophytic plant body began to grow with the beginning of the rainy season and lasted until the end. After that, the plants will start yellowing and withering.

A wide distribution range is recorded for the *Ophioglossum* genus in all continents except for Antartica. (Fraser-Jenkins *et al.,* 2017). Africa: Throughout the mainland, Ascension Island Comoro islands, Madagascar, Mascarenes (Mauritius, La Réunion), Saint-Helena, Seychelles, Tanzania; America: South America, North America, Central America especially from Brazil, Caribbean Islands, Ecuador, Florida (naturalised), Galapagos Islands, Mexico, Pacific islands; Asia: Afghanistan, Bangladesh, Bahrain, Bhutan, China, India, Iran, Indonesia, Java, Japan, Korea, Kuwait, Malaysia, Myanmar, Maluku, Nepal, Oman, Pakistan, Russia, Saudi Arabia, Singapore, Siberia, Socotra, Sri Lanka, Sumatera Philippines, Thailand, Taiwan, Tibet, Yemen, Vietnam,; Australia: New Zealand, New Guinea, New Caledonia, Pacific islands and Europe: Azores, Macaronesi, South and West Europe, except for Antarctica, In India*, Ophioglossum* is well distributed in the northern and Southern states as well as in Andaman & Nicobar Islands. In north India, *Ophioglossum* is found in Arunachal Pradesh, Bihar, Gujarat, Goa, Himachal Pradesh, Jharkhand, Maharashtra, Madhya Pradesh, Odisha, Rajasthan, Punjab, Sikkim, Uttar Pradesh and Uttarakhand, West Bengal, Jammu and Kashmir and Chhattisgarh. In northeast states, *Ophioglossum* is distributed in Assam, Meghalaya, Mizoram, Nagaland, and Tripura. In south Indian states, *Ophioglossum* is found in Andhra Pradesh, Kerala, Karnataka, and Tamil Nadu.

1.4. Economic Importance

The *Ophioglossum* fronds shredded with coconut oil were used on the scalp to nourish the hair and promote the growth (Chakravarti, 1951). *O. reticulatum* is used as a

substitute for spinach in some villages of Uttar Pradesh in India (Sharma, 2000). *O. nudicaule* L. f. is consumed as a vegetable in Nepal (Manandhar, 2002). A study in Namibia suggested the use of *O. polyphyllum* A. Braun ex Seub. as a famine food (Larsen, 2001). *Ophioglossum* is a common vegetable used by some people of South-central Tibet, and it is rich in essential amino-acid that contribute to local diets (Lognay *et al.*, 2008). "The western corn rootworm in agricultural systems was controlled by the IPD079Ea protein encoded by the ipd079Ea gene from *O. pendulum"* (Boeckman *et al.,* 2022).

1.5. Relevance of the present study

Apart from a few records (Balakrishnan *et al.,* 1960; Augustine *et al.,* 1994, Nair and Ghosh, 1973; Nair *et al.,* 1992; Archana and Surabhi, 2021, Rajesh *et al.,* 2013, Madhusoodanan 2015, Anto *et al.*, 2016), the genus *Ophioglossum* is poorly explored in Kerala. The identification based on the simple morphology of the shape and size of rhizome, tropophylls, and spike seems confusing in this genus (Burrows & Edwards, 1995) and resulted in the misidentification and the accumulation of many species names in this genus. The size and shape of the plant tropophyll may vary depending upon the habitat and the availability of sunlight. So, a multidimensional approach by analysing the spore morphology and phylogenetic characters along with the morphology of vegetative plant parts is found to be more noteworthy and dependable in the case of the genus *Ophioglossum*. The spore characters are more consistent and not easily affected by environmental factors. So, the detailed examination of spore characters are noteworthy in this genus. The combined use of the coding region *rbcL*-F and the noncoding spacer region *psb*A-*trn*H as a global plant barcode for authentic species discrimination was recommended by Kress and Erickson (2007). Along with the *rbc*L-F and *psb*A-*trn*H regions, the *trn*L-F region also works well in the genus *Ophioglossum* for species identification.

So, the taxonomical studies of the genus *Ophioglossum* in Kerala by considering macromorphology, micromorphology and molecular characteristics will be helpful for the exact identification of different species by solving the existing nomenclatural problems in this genus. Moreover, documentation of several unreported species in Kerala will be new information to the society and will add many unnoticed *Ophioglossum* species to the pteridophyte flora of Kerala. The mapping of the

distribution of each species helps to get the correct location of all the species and will be helpful for future studies.

1.6. Objectives

- Systematic study of genus *Ophioglossum* from Kerala, India.
- Micromorphological studies on the stomata, venation and spores of each taxon.
- DNA isolation, amplification and sequencing of selected *Ophioglossum* plants.
- Molecular phylogenetic analysis and phylogenetic tree construction.
- Preparation of a distributional map for each species.

1.7. Area of the present study

"Kerala, the southwestern state of India, lies between 8°18'N and 12°48'E and eastern longitudes 74°52'N and 77°22'E" (Bhat, 1994). It lies between the West's Arabian Sea and the East's Western Ghats (Sahyadris) and covers about 38,863 km^2. 14 Districts are present in Kerala viz., Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam, Alappuzha, Idukki, Ernakulam, Thrissur, Palakkad, Malappuram, Kozhikode, Wayanad, Kannur and Kasaragod. Geographically the lands in Kerala are divided into high grounds in the East with mountains, central mid-lands and the lowlands in the western coastal plains. Thick evergreen forests are present in and near the areas of Western Ghats.

Rain

Kerala is nourished by two rainy seasons, The Southwest monsoon (Edavappathy) and the (Thulavarsham). The southwest monsoon begins in June and lasts up to September, and the annual rainfall decreases from the northern to the southern sides of Kerala. This is the most vital and dominant rain compared to the NorthEast monsoon. Western Ghats play a significant role in the rainfall by blocking the wind from passing through it. The North-East monsoon starts in October and ends in November. It increases from the northern parts to the southern parts of Kerala. North-East monsoon is weaker as compared to the Southwest monsoon. The annual rainfall varies from 360 – 180 cm from North to south.

Climate

Kerala experiences a tropical climate. A moderate climate is shared throughout the year with three main seasons viz., monsoon, winter and summer. From June-

November, Kerala is fed with the monsoon rain. The winter season begins from November-February and summer season from March-May.

Rivers

Kerala has mainly 44 rivers. Among these, 41 rivers originate from Westerghats, flowing westward into the Arabian Sea. Kasaragod, the northern district of Kerala, has more rivers compared to other districts of Kerala. The remaining three rivers, Kabani, Bhavani and Pambar, flow eastward to the Kavery River. Among these, Kabani originates from the Panamaram of Wayanad District and flows into Karnataka, Bhavani originates from Nilgiri hills of Western Ghats, and Pambar originates from Anamudi hills of Idukki District, and both the rivers flow into Tamilnadu.

Forest

Mainly there are seven forest groups in Kerala which are again divided into 16 forest types (Champion & Seth, 1968). They include Southern Hilltop Tropical Evergreen Forest, West Coast Tropical Evergreen Forest, Wet Bamboo Brakes, Pioneer Euphorbiaceous Scrub, West Coast Semi-Evergreen Forest, Very Moist Teak Forest, Moist Teak Forest, Slightly Moist Teak Forest, Southern Moist Mixed Deciduous Forest, Mangrove Forest, Myristica Swamp Forest, Southern Dry Mixed Deciduous Forest, Dry Grass Land, Southern Thorn Forest, Southern Montane Wet Temperate Forest and Southern Montane Wet Grassland.

Soil

As recorded by the "Department of soil survey and soil conservation of Kerala" (https://www.keralasoils.gov.in/ml/soils-kerala), the soils of Kerala are divided into coastal alluvium, mixed alluvium, acid saline, Kari, laterite, red, hill, black cotton and forest soils. Coastal alluvium is marine, sandy soils found in the coastal plains. Mixed alluvium is seen in banks of the rivers, basins, valleys and lowland plains such as the Kuttanad and Kole lands of the Thrissur District. Acid saline soils are seen in patches of the coastal area, mainly along the coastal side of Ernakulam, Kannur and Thrissur Districts. Laterite soil, if formed by weathering of rocks. Black soil is found in the plains of Chittur taluk in Palakkad District. Red soil is present in the Trivandrum district, mainly in the southern parts, with sandy clay to loamy clay structure. Hill soil is found in hilly areas, rocky cliffs, and narrow valleys. Forest soils are found in mountains and hills with steep slopes.

Chapter-2

Review of Literature

2.1. Taxonomical studies

The Eusporangiate fern family Ophioglossaceae was discovered by Bauhin (1620). The generic status of this snake tongue fern *Ophioglossum* was substantiated by Linnaeus (1753).

Presl (1845) documented 48 species in Ophioglossacea family, of which 25 species were included in the genus *Ophioglossum*.

Beddome (1883) recorded two species, *O. reticulatum* and *O. pendulum,* from the Ceylon and the Malay Peninsula. Subsequently, Beddome (1892) added *O. gramineum* to Ceylon. The morphology and illustrations of *O. pendulum*, *O. vulgatum* L., *O. reticulatum*, *O. fibrosum* Schum., and *O. nudicaule* were provided in detail.

Willis and Willis (1911) reported two species viz. *O. gramineum* and *O. pendulum* from Ceylon.

Clausen (1938a) discussed the status, taxonomical identity and relationship of *O. petiolatum* in detail and synonymised the names *O. moluccanum* Schlecht., *O. cordifolium* Roxb., *O. elongatum* A. Cunn., *O. cognatum* Presl., *O. cumingianum* Presl., *O. obovatum* and *O. timorense* Miquel., *O. vulgatum* var. *australasiaticum* Luerssen, *O. raciborskii* Alderw., *O. pedunculatum* Nakai., *O. littorale* Makino., *O. floridanum* E. P. St. John. under *O. petiolatum*. The comprehensive Monographic study of Clausen (1938b) recognized 26 species of *Ophioglossum* from a total of 52 species identified for the family. The author also recorded 23 species of *Botrychium*, One species of *Helminthostachys.* The genera *Rhizoglossum*, *Ophioderma* and *Cheiroglossa* were reduced to the rank of sub-genera under the genus *Ophioglossum*. According to him, the technical characteristics like venation and spore morphology are uncertain and impractical when identifying a species in *Ophioglossum.* Hence, he categorised *Ophioglossum* plants under four subgenera: *Cheiroglossa*, *Rhizoglossum*, *Ophioderma* and *Euphioglossum,* based on the gross morphology and correlation with geographical range. He also discussed and solved some nomenclatural confusion on *O. pedunculosum* Desv.

Chakravarty (1951) identified three species, *O. fibrosum*, *O. vulgatum*, and *O. pendulum* from Ceylon.

Pichi-Sermolli (1954) subdivided the genus *Ophioglossum* into three subgenera, *Ophioderma* (Blume) Clausen, *Ophioglossum* and *Rhizoglossum* (Presl) Clausen. He considered *O. costatum*, *O. fibrosum* identified by Clausen (1938) and *O. pedunculosum* by Weatherby (1939) are conspecific and hence, Pichi-Sermolli synonymised *O. fibrosum* and O. *pedunculosum* under *O. costatum*. He also concluded that the *O. capense* Sw. is a synonym of *O. nudicaule*. He rejected the name *O. regulare* (Schltdl.) C.Chr. *O. cuspidatum* Milde. is treated as synonyms of *O. polyphyllum*.

Horner (1958) discovered an unusual colony of *O. pendulum* ssp. *pendulum* from the forest reserve of Hawaii Island, apart from the previously known *O. pendulum* ssp. *falcatum* recorded by Clausen (1954). He recoerded mainly five characters to distinguish between the two species. *O. pendulum* ssp. *pendulum* was characterised by 105 cm long blades, 4 – 12 cm long fertile stalk, straight blade curvature, medium texture, and pointed blade apices, whereas the *O. pendulum* ssp. *falcatum* characterised by 10 – 45 cm blades, 0.5 – 3.0 cm long fertile stalk, falcate leaves, leathery texture, with a blunt apex.

India, a country with a mixture of wet and dry tropical climatic conditions, is more diverse for *Ophioglossum,* and the genus is distributed throughout the country. Therefore, extensive studies were carried out on this snake tongue fern from various parts, especially from the Northern sides of the country. Balakrishnan *et al.*, (1960) recorded five taxa of *Ophioglossum*, viz., *O. costatum*, *O. polyphyllum*, *O. grarmineum*, *O. nudicaule* var. *typicum* and *O. reticulatum*, from the localities coming under the southern circle of Botanical Survey of India (BSI). Morphotaxonomy, illustration, the key to the species and distributional map for the five collected taxa were provided.

The taxonomy of ten *Ophioglossum* species was discussed in India in detail by Mahabale (1962). He collected *O. lusitanicum*, *O. vulgatum*, *O. pedunculosum*, *O. nudicaule*, *O. fibrosum*, *O. japonicum* Prantl., *O. gramineum*, *O. aitchisoni* Nishida., *O. reticulatum* and *O. pendulum*. The author considered the leaf morphology, stipules, and epidermal and spore characters and prepared an artificial key based on the characters of each species. The probable phylogeny of the collected species was schematically represented. The subgenus *Euophioglossum* including *O. vulgatum* and

O. reticulatum, was shown as the main line of evolution, and then it was divided into subgenus *Ophioderma* and subgenus *Cheiroglossa*. The former includes *O. pendulum* and *O. aitchisoni,* whereas the latter comprises *O. palmatum* L. and *O. fibrosum*. Finally, the monotypic subgenus *Rhizoglossum* was placed distantly in the phylogeny tree. A key and a table comparing the morphological characters were given for the easy identification of the collected species. The author discussed the confusing taxonomy of *O. reticulam*, *O. vulgatum* and *O. aitchisoni* in this work.

Wieffering (1964) subsequently revised the *Ophioglossum* of the Indo-Pacific region and reported 14 species with some varieties and some forms. Disagreeing with Clausen's opinion, he observed the cytogenetic characters as the most reliable characters for species identification since the interspecific and intraspecific relationships cannot be depicted by considering the external morphology alone.

Thothathri *et al.*, (1969) collected the *O. pendulum* for the first time from Andaman Nicobar Island. Four species recorded by Linnaeus viz. *O. vulgatum*, *O. lusitanicum*, *O. reticulatum* and *O. palmatum* were retained in the *Ophioglossum* genus, and the other two were placed in the genus Lygodium by Abeywickrama and Fonseka (1973).

Khandelwal and Goswami (1983) described *O. eliminatum* Khand. & H.K. Goswami. Morphological characters were given by comparing with the characters of *O. costatum*, *O. nudicaule*, *O. lusitanicum*, *O. gramineum* and *O. thermale* Kom.

Dixit and Vohra (1984) reported *O. pendulum* from Andaman and Nicobar Islands.

Paul (1987) confirmed the status of *O. azoricum* C. Presl. as a separate species but not an ecotype of *O. vulgatum*. Derrick *et al.*, (1987) recorded four *Ophioglossum* species in the European flora.

Manickam and Irudayaraj (1992) reported five *Ophioglossum* species, *O. nudicaule*, *O. gramineum*, *O. reticulatum*, *O. petiolatum* and *O. vulgatum*, from South India. They provided the binomial, description, key to the species and illustrations for each species.

Burrows and Edwards (1993) discussed the nomenclatural confusions of the genus *Ophioglossum* in Africa. In this paper, the authors described *O. caroticaule* J.E. Burrows as a new species, *O. vulgatum.* subsp. *africanum* as a new subspecies, and a

status change for *O. latifolium* (basionym: *O. gomezianum* var. *latifolium*. Lectotypification of *O. gracillimum* Welw.; Bak., *O. lusoafricanum*, *O. latifolium*, and *O. rubellum*. were provided, and he discussed about the nomenclatural problems of *O. lancifolium* C. Presl. A new species, *O. gracile* Pocock ex J.E. Burrows. was discovered in South Africa by Burrows and Edwards (1995). According to them, the species concept of *Ophiglossum* is confusing due to the simple morphology of the genus.

Augustine *et al.*, (1994) discovered *O. pendulum* from the Periyar tiger reserve of Kerala. This discovery from the Idukki district along the Western Ghats sides of Kerala, South India, was a new report for the occurrence of *O. pendulum* in the mainland of India.

Assessment of the occurrence and habitat of *O. vulgatum* in European forests by Muller (2000) revealed that *O. vulgatum* is more abundant in undisturbed forest habitats, which were the primary habitat for this species. Still, it is also present in disturbed wet grassland ecosystems. He conducted his studies on several Lorraine forests. *O. vulgatum* was found in an oak-ash forest of the Carpinion alliance in all these forests. He also pointed out that, besides the more abundant young succession stages, it was present there in all phases of the forest cycle.

Burrows and Johns (2001) studies about 13 *Ophioglossum* species from East Africa. They provided detailed description, Artificial Key for the Identification and illustration of each species.

Kilian *et al.*, (2004) added *O. gomezianum* to the Arabian Peninsula.

Goswami (2007) documented about 12 *Ophioglossum* species from India. Their taxonomy, morphology and anatomical characters were discussed in detail.

Morphological description, spore morphology, and photographs of different species of the *Ophioglossum* genus were documented by Goswami *et al.*, (2008). This study coined the possibility of forming a new species, *O. eliminatum* (with the lowest chromosome number n=90 in the genus by natural hybridization and chromosomal elimination. This study considered all the possible morphological, molecular and biochemical techniques for knowing the evolutionary mechanisms. While assessing the rare and threatened Pteridophytes India.Chandra *et al.*, (2008) categorized *O.*

gramineum, *O. lusitanicum*, *O. pendulum* under the plants at risk category and *O. nudicaule*, *O. parvifolium*, *O. polyphyllum* under the rare category.

The Pachmarhi Biosphere Reserve (PBR) of Madhya Pradesh in India was explored for *Ophioglossum* plants by Singh *et al.*, (2009). They considered the morphology, taxonomy and distributional pattern of *Ophioglossum*. The diversity between and within the species over the Biosphere Reserve has been analysed and documented four *Ophioglossum* species, among these, *O. gramineum*, *O. nudicaule*, and *O. reticulatum* were broadly distributed within the Biosphere Reserve. This study reports *Ophioglossum* for the first time from PBR.

Yadav and Goswami (2010) discovered pink-brown *Ophioglossum*, *O. indicum* B. L. Yadav & H. K. Goswami. from the Rajasthan state of India based on the morphological characters and Scanning Electron Microscopic (SEM) characters of the spore. They distinguished *O. indicum* from other congeners by its pinkish tropophylls and petioles and its unique spore characters.

Later, Goswami (2011) collected and documented the population biology data of *O. eliminatum* from 1995 – 2009 from more than 12 sites in Central India. This study restated that the lowest chromosome number in the *Ophioglossum* genus is n = 86, not n = 30 as emphasised before, and the interspecific and intraspecific chromosome variations exist in many *Ophioglossum* species. Raju *et al.*, (2011) studied about the recognition and existence of *O. costatum* in Andhra Pradesh.

After the collection from the 19th century, *O. lusitanicum* L., which was considered extinct in the Croatian flora, was rediscovered in southern Istria of Croatia by Brana *et al.*, (2014). The authors agree with the opinions of Wagner (1990) in that *O. lusitanicum* was favoured by small disturbances like grazing and moderate trampling and measures to be taken for the conservation of this threatened taxa in Croatia.

Kholia and Jenkins (2014) rediscovered the *O. pendulum* after 125 years in Northern India. Their collection from Sikkim was significant for India as it is pointing to the possible westward distribution of this species. Previously it was reported from the eastern sides of the country including Assam and Arunachal Pradesh. Patil and Dongare (2014) recorded *O. parvifolium* and *O. polyphyllum* new to the Deccan Peninsula of India. The authors collected eight species from Maharashtra and the

paper discussed the taxonomy, phenology, and distribution and conservation status of the collected species. Patil and Lavate (2014) investigated the genus *Ophioglossum* in Western Ghats and reported eight species, viz. *O. gramineum*, *O. parvifolium*, *O. reticulatum*, *O. lusitanicum*, *O. petiolatum*, *O. polyphyllum, O. costatum* and *O. nudicaule*. Taxonomic characters, photographs, distribution, and IUCN conservation status for each species were provided along with a modified artificial key of Panigrahi and Dixit (1969) were also provided for easy identification. Amongst the eight species reported, the authors assigned the conservation status of *O. gramineum*, *O. parvifolium* and *O. reticulatum* as least concerned (LC), *O. lusitanicum*, *O. petiolatum* and *O. polyphyllum* as critically endangered (CE), *O. costatum* and *O. nudicaule* as vulnerable (V).

Kosenkov & Mardashova (2015) noted the occurrence of *O. vulgatum* near the "White sea biological station of Moscow State university at Kandalaksha gulf". He conducted a coastline registration survey to map its distribution. Peruzzi *et al.*, (2015) documented three new hybrids of *Ophioglossum.* Meza Torres *et al.*, (2015) reinstated the name *O. melipillense* Remy., from its doubtful names viz. *O. nudicaule*, *O. opacum* R.Br. *ex. Carmich.*, *O. vulgatum* and *O. crotalophoroides* Walter. According to them, *O. fernandezianum* was a doubtful species and the lectotype of *O. lusitanicum* was chosen and provided its synonyms.

Anto *et al.*, (2016) published *O. raphaelianum* from Kerala, the species was described based on its smaller size, bluish-green obovate tropophyll, subglobose-globose rhizhome. Patil *et al.*, (2016) documented *O. parvifolium* and *O. polyphyllum* for the first time from Maharashtra, they collected eight speciestaken during their study.

Fraser-Jenkins *et al*., (2017) discussed about 13 *Ophioglossum* species from India in their Annotated Checklist of Indian Pteridophytes. The study provided a detailed account of each species and provided detailed notes about the nomenclatural issues of each species.

Patel and Reddy (2018) described *O. malviae* M. Patel & M. N. Reddy. from the Western Ghats region of the Dang district of Gujarat. While evaluating the diversity and conservation status of pteridophytes in Gujarat, India. Patil *et al.*, (2018) described *O. gujaratens* S.M. Patil, R.N. Kachh., R.S. Patel & K.S. Rajput from the Gujarat state of India. Patel and Reddy (2018) reported *O. lusitanicum* and *O.*

thermale as new records from Gujarat state. Morphology, taxonomy and the ecological aspects of each species were documented. Patel *et al.*, (2018) reported a new species, *O. aletum* M. Patel, M.N. Reddy & H.K. Goswami., from Southern Gujarat. They distinguished the new species by possessing almost 90% alete spores. Kachhiyapatel *et al.*, (2018) revised the genus *Ophioglossum* for the Gujarat state and eight species were documented. *O. thermale* and *O. lusitanicum* were documented, first time for the Gujarat state.

Fraser Jenkins *et al.*, (2018) discussed some nomenclatural confusion that exists in some *Ophioglossum* species recorded from India. According to him, *O. lancifolium* was misidentified by some Indian authors as *O. thermale*, which needed further investigations to solve this problem. He criticized the report of *O. parvifolium* as *O. nudicaule* from India and a lectotype is designated.

Yadav and Meena (2019) collected *O. gujaratense* for the first time from Mainal, Chittorgarh district of Rajasthan and documented its morphological characters, and distribution along with its photographs. Patil *et al.*, (2019) considered the 1 – 4 branched sporangiophore of *O. thermale* collected from Gujarat state as an abnormal morphological character where the spores from each branch were found to be normal. *O. hitkishorei* M. Patel & M.N. Reddy., was described from Gujarat by Patel and Reddy (2019) based on morphological and Phylogenetic characteristics.

Goswami and Patel (2020) did an extensive study on *Ophioglossum* considering the morphology, palynology, chromosome studies and molecular characteristics. They coined that, preference should be given to spore characters and molecular data along with the morphological characters while differentiating species. The authors suggested that the unique morphological traits as the results of rare genetic combinations that arise due to autopolyploidy or allopolyploid conditions. So, a multidisciplinary approach including morphological, anatomical and biochemical characteristics was needed for identifying different *Ophioglossum* species. Goswami *et al.*, (2020) published a new species *O. chaloneri* H.K. Goswami, M. Patel & K.K. Nag., from India. Patil and Rajput (2020) investigated the *Ophioglossum* genus in Goa, and documented morpho-taxonomy, distribution, and conservation status along with the photo plates and illustrations of six species viz.*O. gramineum*, *O. lusitanicum*, *O. nudicaule*, *O. reticulatum*, *O. parvifolium*, and *O. costatum*. Out of

these six species, *O. parvifolium*, *O. reticulatum*, *O. lusitanicum*, and *O. nudicaule*, were reported for the first time from Goa state. Jash and De (2020) listed six *Ophioglossum* species in West Bengal. *O. petiolatum*, *O. parvifolium*, *O. gramineum*, *O. reticulatum*, *O. pendulum*, and *O. costatum*, with their distribution patterns. They found *O. pendulum* restricted in the northern side of West Bengal and *O. gramineum* restricted in the south parts. *O. costatum*, *O. parvifolium* and *O. reticulatum* showed their occurrence all over the state. They provided plant descriptions along with their Photographs. Patil *et al.*, (2020) described *O. jaykrishnae* S.M. Patil, S.K. Patel, Raole & K.S. Rajput from Gujarat state.

Fraser-Jenkins *et al*., (2021) discussed about the accumulation of many new names from India without considering the gross morphology and morphotypes of the same species. So, the authors synonymised them under existing species names. *O. chaloneri* was synonymised under *O. reticulatum, O. gujaratens* was considered as *O. gomezianum, O. hitkishorei* was synonymised under *O. costatum, O. malviae, O. indicum* and *O. raphaelianum* as *O. rubellum.*

2.2. Venation studies

Presl (1845) used the nature of venation to subdivide the genus *Ophioglossum*. He considered the reticulate venation in *Ophioglossum* as a key character to distinguish this genus from the related genera *Botrychium* and *Helminthostachys*.

Campbell (1904) had an opinion that even though the reticulate venation found in *Ophioglossum* is different from most other ferns, the genus *Kaulfussia* of the family Marattiacea shows some resemblance with the venation of Ophioglossaceae.

Mahabale (1962) illustrated and discussed the venation patterns of the *Ophioglossum* species in India. He noted the long areoles of *O. pendulum* without free endings and with a few free veins at the margins, *O. gramineum* with large vein meshes, *O. lusilanicum* having "enlarged mesh without free veins in the middle and a few free veins at the margin showing "Veins marginiaros", the embryonic leaf of *O. nudicaule* with Free vein-endings and older leaves with free endings from apex to the midrib. The venation pattern of *O. pedunculosum*, *O. japonicum* and *O. reticulatum* were described as "venatio anaxeti", and that of *O. aitchisoni* as large, elongated, swollen areole having "overlapping meshes of veins and free vein endings". The venation in

O. fibrosum is "regular hexagonal areolae with symmetrically bifurcated free vein endings on both the sides in the free areole" and *O. vulgatum* having irregularly shaped, elongated areole with free endings.

Wieffering (1964) discussed and illustrated the venation patterns of *O. costatum*, *O. nudicaule* var. *macrorrhizum* (Kunze) R.T. Clausen, *O. reticulatum* f. *dilatatum* (Miq.) Wieff., *O. gramineum* var. *majus* (Alderw.) Wieff., *O. intermedium* Hook. He provided a taxonomic key at the subgenus level and species level by treating venation patterns as important as morphology. The subgenus *Ophioglossum* was separated from *Ophioderma* by having secondary veinlets instead of the primary veins alone in *Ophioderma*. To separate different species in *Ophioglossum*, the venation is double is taken as the key character for *O. costatum* and venation is not double was taken for other species. “If the venation is not double, then the venation with very long stretched areoles in the basal part of the tropophyll is taken for *O. gramineum*, and venation without long-stretched areoles in the lower part is given as a character for identifying the *O. nudicaule* and *O. reticulatum”* (Wieffering, 1964). Then the two species were further separated based on other morphological characteristics.

Wagner (1979) suggested the reticulate venation in *Ophioglossum* as a product of convergent evolution. According to him, *Ophioglossum* possesses a Phyllodial type of reticulation in which the central veins were clustered along the tropophyll axis without forming a true midrib due to intercalary dilation. Based on this, he considered *O. bergianum* Schltdl., as the most primitive living form of that period, and it possesses reduced narrow linear leaves, which were then succeeded into wider blades in *O. reticulatum* and *O. vulgatum*. He also added about the two trends that happened during evolution, "towards greater complexity by formation from included veinlets of new areolets within the major areoles", and "towards decreasing complexity by enlargement of areoles and loss of included veinlets".

Wagner *et al.*, (1981) reinterpreted *O. dendroneuron* E. P. St. John as a form of *O. nudicaule*. The complex venation pattern of *O. dendroneuron* with small areoles inside large areoles was considered to distinguish the species from others. But later further observation of this species revealed a transition from small tropophylls with simple venation without included veinlets and large tropophylls with complex venation having included veinlets in which some of them form secondary veinlets.

Based on these observations the authors reinterpreted *O. dendroneuron* as a form of *O. nudicaule*.

Khandelwal and Goswami (1983) noted the venation of *O. eliminatum* as "venation not double and with long stretched areoles at the lower part of the sterile blade".

Wagner *et al.*, (1984) synonymised *O. ellipticum* Hook. under *O. nudicaule* by considering the venation pattern of the plants. The larger size of the plants along with complex venation having mini secondary areoles inside the large primary areoles, and the dorsal surface of the tropophyll having a pale central band were the main features that separate *O. ellipticum* from *O. nudicaule*. But later investigations in the forms of *O. nudicaule* showed small sized plants having simple veins without included areoles and large plants having complex veins with so many included areoles. This observation led them to synonymise *O. ellipticum* under *O. nudicaule*.

Kato (1987), considered the reticulated venation in *Ophioglossum* as a specialised character and the open venation in *Botrychium*, *Helminthostachys* and *Botrypus* as a primitive character. He used the venation pattern along with other morphological characters for the phylogenetic classification of Ophioglossacea.

Venation of *O. lusitanicum*. *O. azoricum*, and *O. vulgatum* were compared by Paul (1987) to prove the status of *O. azoricum* in the British Isles.

Singh *et al.*, (2009) considered venation patterns of *O. polyphyllum*, *O. nudicaule*, *O. gramineum* and *O. reticulatum* while identifying them from Pachmarhi Biosphere Reserve, Madhya Pradesh.

Yadav and Goswami (2010) considered the venation pattern of *O. indicum*, Anto *et al.*, (2016) discussed and illustrated the venation of *O. raphaelianum*.

Patel and Reddy (2018) noted the venation of *O. malviae*, Patel *et al.*, (2018) documented the venation of *O. aletum*, Patil *et al.*, (2018) noted the venation of *O. gujaratense*.

Patel and Reddy, M. (2019) commented on the venation of *O. hitkishoreii*, Goswami *et al.*, (2020) described the venation of *O. chaloneri*. Jash and De (2020) commented on the venation patterns of *O. costatum*, *O. gramineum*, *O. parvifolium*, *O. pendulum*, *O. petiolatum* and *O. reticulatum* present in West Bengal.

Yadav *et al.*, (2021) commented on the venation of *O. trilokinathii*.

2.3. Stomatal studies

Inamdar (1970) deals with the development of stomata in five species of O*phioglossum*. The author found hapIocheilic or perigenous development of stomata in vegetative as well as reproductive plant parts. Presence of superimposed twin stomata, persistent stomatal initial cells and aborted guard cells were observed. The study supported the foliar nature of the spike of genus *Ophioglossum*.

Mahabale (1962) documented the stomata in ten species of *Ophioglossum*, collected from India. He considedered the stomatal characters as a further supporting feature to distinguish different species. The study found that the stomata varies between differentt species. Unlike the other *Ophioglossum* species, the epiphytic *O. pendulum* has cyclic arrangement of guardcells.

Mital (1968) examined the stomatal and the epidermal features of *O. reticulatum, O. gramineum, O. nudicaule, O. costatum* and *O. petiolatum* from Rajasthan, India and according to him, these features did not constitute any major characters of diagnostic value.

The epidermal features of *O. palmatum* along with the statistical data on stomatal index were given in detal by Khandelwal and Goswami (1977). Association of potassium ions with stomatal movements in *O. engelmanni* Prantl. was reported by Dayanandan and Kaufman (1975).

Paul (1987) noted the status of *O. azoricum* in the British Isles. He checked the probability of *O. azoricum* for being a synonym of *O. vulgatum*. He compared between the stomatal and epidermal features of the two species and found that they didn't provide quality characteristics for differentiating *O. azoricum* from small specimens of *O. vulgatum*.

2.4. Spore studies

Mahabale (1962) discussed the spores of *O. vulgatum*, *O. reticulatum*. *O. japonicum*, O. *nudicaule*, *O. fibrosum*, *O. lusitanicum*, *O. aitchisoni*, *O. gramineum*, *O. pendulum* by analysing light microscopic view of the spores.

Goswami and Khandelwal (1973) talk about the dimorphic spores and their wall layers in *O. nudicaule*, *O. costatum* and *O. vulgatum* growing in the same habitat.

Whittier (1981) germinated *Ophioglossum* spores in axenic culture and studied young gametophyte development. The spore morphology of *O. vulgatum*, *O. azoricum*, and *O. lusitanicum* were compared in detail by Paul (1987) for confirming the species status of *O. azoricum.*

Spores of *O. caroticaule*, *O. lusoafricanum*, *O. gracillimum*, *O*. sp. aff. *lancifolium*, *O. vulgatum* subsp. *africanum*, *O. convexum* J.E. Burrows., compared in detail by Burrows and Edwards (1993) by SEM studies. The spore wall morphogenesis of *O. thermale* was studied by Uehara and Kurita (1989) using SEM analysis.

Yawen *et al.*, (1997) discussed the spore ornamentation of six species of *Ophioglossum* from China by SEM analysis viz. *O. nudicaule*, *O. pedunculosum*, *O. petiolatum*, *O. thermale*, *O. reticulatum*, *O. vulgatum* and *Ophioderma pendula*. They reasonably validated the separation of the *O. pendula* from the *Ophioglossum* genus based on the spore morphology.

Kilian (2004) examined the spore morphology of *O. gomezianum* in Yemen by SEM analysis.

Peruzzi *(*2015) considered the spore ornamentation for describing three new hybrids of *Ophioglossum* viz. O. × *pierinii, O.* × *giovanninii, O.* × *pseudoazoricum*. To solve the problems in species identification Meza Torres *et al.*, (2015), studied the ornamentation of spores of ten *Ophioglossum* species and correlated this data with morphology and suggested five spore types in *Ophioglossum* viz. *O. crotalophoroides* spore type consists of *O. crotalophoroides* and *O. crotalophoroides* var. *nanum*, *O. vulgatum* spore type having *O. coriaceum*, *O. nudicaule* var. *robustum*, *O. vulgatum*, and *O. vulgatum* var. *valdivianum*, *O. opacum* spore type including *O. crotalophoroides* var. *robustum* and *O. opacum*, *O. lusitanicum* spore type with *O. lusitanicum*, *O. melipillense* contain *O. lusitanicum* subsp. *coriaceum* spore type.

Anto *et al.*, (2016) analysed the spore ornamentation of *O. raphaelianum* to authenticate its new species identity.

Spore sculpturing of *O. vulgatum* was assessed by Olejnik *et al.*, (2018) to justify the taxonomic significance of spore morphology in Ophioglossaceae. Patel and Reddy (2018) considered and compared the spore characters of *O. malviae* to distinguish it from *O. parvifolium*. The alete nature of the spores was taken as a key character to justify *O. aletum* as a new species by Patel *et al.*, (2018). Patil *et al.*, (2018) used spore morphology as a taxonomic aid to substantiate *O. gujaratens* from other *Ophioglossum* species.

Patel and Reddy (2019) described *O. hitkishorei* from Gujarat, and they considered the unique pattern of exine ornamentation to state the species as a novel one.

Goswami *et al.*, (2020) considered the spore characters of *O. chaloneri* to substantiate it from other species of *Ophioglossum*. According to them, while dealing with species identification in *Ophioglossum* genus primary importance should be given to the exine ornamentation of its spore rather than giving importance to the plant's external morphology. They documented and compared the spore morphology of *O. gujaratense*, *O. petiolatum*, *O. costatum*, *O. vulgatum*, *O. polyphyllum*, *O. chaloneri*, *O. indicum*, *O. eliminatum*, *O. aletum*, *O. hitkishorei* and *O. malviae*.

Yadav *et al.*, (2021) analysed the exine ornamentation of *O. trilokinathii* and compared it with *O. costatum*, *O. gujaratense* and *O. parvifolium* to confirm its novelty.

2.5. Molecular studies

To check whether the ubiquitous expression in both reproductive and non-reproductive organs of Ceratopteris is unique to a highly derived lineage, Munster *et al.*, (2002) have characterised the MIKC-type MADS-box gene family of *Ophioglossum*. The isolated cDNAs from four different *Ophioglossum* MIKC-type genes disclosed the presence of more MADS-box genes in the *Ophioglossum*. They discussed the evolution of vascular plants in detail.

Chuang and Hu (2004) got a clear trnK5'-matK-trnK3' structure in *O. petiolatum*, *Lycopodiella cernua* and *Selaginella doederleinii*. They assessed the evolution of Chloroplast matK Genes in *O. petiolatum* and two Lycophytes and concluded that *matK* is not expressed consistently in lower-land plants.

Kress and Erickson (2007) recommended the use of a combination of the noncoding, spacer region *psb*A-*trn*H and a portion of the coding region *rbcL* as a global land plant barcode for authentic species discrimination.

Chung *et al.*, (2013) explored the allozyme variation of *O. vulgatum* in eight Korean populations to get an idea about its mating system and population establishment history. They detected homozygous genotypes owing to intra-gametic self fertilisation. By analysing Populations habor low within-population variation and high among-population differentiation. They concluded that the adverse effects of genetic drift and intragametophytic selfing reduced the size of *O. vulgatum* in South Korea. Grewe *et al.*, (2013) looked over the evolution of plastid genomes and demonstrated phylogenetic relationships among ferns by sequencing the plastid genomes of *O. californicum, Equisetum hyemale* and *Psilotum nudum*

Perruzi *et al.*, (2015) described three hybrids *O.* × *pierinii* as the hybrid between, *O.* × *giovanninii*, *O.* × *pseudoazoricum* by the genome size approximation and molecular taxonomy by using *trn*L-*trn*F IGS regions viz. *O.* × *pierinii* as a hybrid between O. *lusitanicum* and *O. azoricum*, *O.* × *pseudoazoricum* as the hybrid between *O. azoricum* and *O. vulgatum*, *O.* × *giovanninii* seen as the hybrid between *O. vulgatum,* O. × *pierinii*. Chernyshenko *et al.*, (2016) used SDS-PAGE for the assessment of the protein composition in *O. vulgatum*. To determine "the phosphorylation of studied proteins they applied electrophoresis using Phos-tag™ Acrylamide Gels.

Patel and Reddy (2018) analysed the three plastid DNA regions viz. *trn*L-F, *rbcL* and *psb*A-*trn*H to state *O. malviae* as a new species and provided a Phylogenetic tree of *Ophioglossum* species based on maximum likelihood analysis. Patel *et al.*, (2018) used the *rbcL* gene region to confirm the new species status of *O. aletum*. Patil *et al*., (2018) constructed a cladogram by using the Neighbor-Joining (NJ) method using the rbcL sequence of *O. gujaratense* and some other closely related species.

Patel and Reddy (2019) analysed *trn*L-F and *psb*A-*trn*H regions of *O. hitkishorei* for separating them from other species.

Goswami and Patel (2020), conclude that different phylogenetic analyses during recent years revealed the Indian representatives of *Ophioglossum* as offshoots of *O. vulgatum*, which were formed by the hybridisation of *O. vulgatum*, *O. costatum* and

O. reticulatum. In their study, they provided a maximum-likelihood phylogeny tree using the *rbc*L region. Goswami *et al.*, (2020) used *rbc*L, *trn*L-F, and *psb*A-*trn*H DNA sequences to prove *O. chaloneri* as a new species.

Yadav *et al.*, (2021) examined the phylogeny of *O. trilokinathii* to state it as a new species and constructed three phylogenetic trees based on *psb*A-*trn*H, *rbc*L, *trn*L-F regions. The chloroplast (cp) genome of *O. vulgatum* was completely sequenced by Hao *et al.*, (2021) and a phylogenetic tree was constructed based on Bayesian approach by using the complete chloroplast genome sequences of fifteen species. The study provided a molecular basic study for assessing the population variation in Ophioglossaceae.

Carlson *et al.*, (2022) assessed the Safety of the insecticidal protein IPD079Ea, a new protein derived from *O. pendulum*, which protects the plant from WCR in Genetically modified Maise. A weight-of-evidence approach was used for the analysis and the authors concluded that IPD079Ea is non-hazard to humans and other animals.

Chapter-3

Materials and Methods

3.1. Plant collection

Fresh *Ophioglossum* plants were collected from varoius localities of Kerala, during the year 2018 – 2022 (Fig.01). Herbarium preserved material and formalin preserved material was used for *O. pendulum*. Field trips were conducted from June-December of every year. The sporophytic plant body with fertile spikes could be found in the field during the monsoon season. Photographs of the plants were taken using Canon Eos R digital camera. The details of the plant including their habit, habitat, colour, number of tropophylls were recorded in the field note from the field itself. The plants were sealed in polythene bags to avoid cross contamination and then taken into the laboratory for further studies.

3.2. Descriptions and photoplates

Observations were done and photographs of each plant part were done under Leica stereo microscope. The photographs of the plant parts were taken using a Leica S8APO stereo microscope and using Canon Eos R digital camera. Morphological descriptions of each plant were prepared by detailed examinations of a wide range of specimens. Lellinger *et al.*, (2002), Simpson (2006) was followed for the terminology. Illustrations were prepared using 0.1-point micro tip pen.

3.3. Herbarium Preparation

Standard herbarium specimens were prepared by following De Vogel (1987) and Bridson & Forman (1998). The plant materials were dried using herbarium drying cabinet and pressed using herbarium press. After proper drying, the specimens were mounted on standard herbarium sheets (42 x 28 cm). The informations including scientific name, family, collection date, field notes, and the name of the collectors were labelled using standard herbarium labels. All specimens after fumigation, deposited in the St. Thomas College herbarium (STC).

3.4. Identification, nomenclature, and citations

Identification of the plants were done with the help of type specimens and protologues. Digital images of the type specimens were obtained from (B, BM, CAL, K, MH, and MNHN). The herbarium specimens from various herbaria including CALI, MH, KFRI, STC, were examined and digital images from LINN, BSID, CAL,

BM, AAU, B, E, TBGT, and NHM were also consulted. The examined type specimens were specified with exclamation mark (!). Acronyms of the herbaria were cited by following Theirs (2011). Brummitt and Powell (1992) was utilized for the citation of taxa. The list of herbaria was provided in appendix 1. The nomenclature of each species was provided based on the Shenzhen Code (Turland *et al.,* 2018). The plant names were updated by consulting the website of International Plant Names Index (http://www. ipni.org), The Plant List (http://www.theplantlist.org), Tropicos (http://www.tropicos.org).

3.5. Literature retrieval systems used

The database of Royal Botanic Garden Kew (http://apps.kew.org), Global Biodiversity Information Facility (GBIF, https://www.gbif.org) and World checklist of selected plant families, database of Missouri Botanic Garden (www. The plantlist.org) was also utilized. The literature retrieval systems of the Biodiversity Heritage Library (http://www.biodiversityheritagelibrary.org), literatures from Missouri Botanical garden library (http://www.botanicus.org) and New York Botanical garden and were also utilized. JSTOR Global Plants (https://plants.jstor.org), database was also utilized.

3.6. Species distribution map

The locations of each species were obtained during collection trips using GPS navigators (Garmin GPS etrex10). Other data were geo referenced from specimens preserved at various herbaria. A distributional map for each species was prepared using QGIS Desktop 3.22.12 software. (QGIS development team 2022).

3.7. Micromorphological analyses

3.7.1. Light microscopic analyses

Light microscopic views of the spore structure and stomata were observed under Binocular BIOMED Research Microscope (WESWOX DPT 1K, model: BXL Laboratory Microscope S. No.: 72056) and their photographs were taken in 40 X magnification. Venation pattern in the tropophylls of each species was examined using Leica S8APO stereo microscope.

3.7.2. Scanning Electron Microscopy analysis of the spore character

For SEM (Scanning Electron Microscope) analysis, the spores were directly dusted over a carbon tape coated stubs and sputter-coated with gold and examined using a Hitachi SU6600 VP Field Emission Scanning Electron Microscope of NIT, Calicut or JOEL, JSM-6390LV Scanning Electron Microscope of Sophisticated Test and Instrumentation Center (STIC), Cochin University of Science and Technology. Chemical treatments were not carried out since it was not recommended as a standard technique for the family Ophioglossaceae (Burrows 1997, Meza Torres *et al*., 2015) as it dissolves the fine exine ornamentation. The spore characters were analyzed, the Polar length (P) and the equatorial length (E) were measured and recorded from four spore samples of all the species, the P/E ratio was calculated, size of the spores was determined by following Erdtman (1986).

3.8. Molecular Characterization of selected samples

3.8.1. Genomic DNA extraction

The Genomic DNA was extracted from silica-dried leaf samples of the specimen using the QIAGEN DNeasy Plant Mini Kit following the manufacturer's protocol (Qiagen, Germany) as well as CTAB (Cetyl Trimethyl Ammonium Bromide) method of Doyle and Doyle (1987) with slight modifications.

Modified CTAB method

For the preparation of 2.5 ml of CTAB solution, 0.05 g of CTAB powder was weighed by using an electric weighing balance and then added to a clean 250 ml beaker. 350 µl of 0.7M (molar) Sodium chloride (NaCl) solution, 250 µl of 0.05M Ethylenediaminetetraacetic acid (EDTA) and 250µl of Tris Hydrochloric acid was added to the CTAB solution by using calibrated micropipettes.

For the DNA isolaton, the CTAB buffer was preheated at 65^0C prior to the grinded. 200 mg of silica-dried leaf samples were weighed and the leaf tissues were grinded using liquid nitrogen in chilled mortar and pestle to make a fine powder. The 1ml of preheated (65^0C) CTAB mix was added and mixed well. The homogenate was then transferred into a sterile centrifuge tube. 10 µl of RNase A solution was added, mixed by inverted shaking, incubated for 30 minutes at 37^0C with inverted shaking at every minute. The tube was then cooled at room temperature for 4 – 5 minutes. The aqueous

layer was collected from the solution and an equal amount of Chloroform: Isoamyl alcohol (24:1) was added and mixed by inversion, centrifuged at 5000 rpm for 10 minutes. After centrifugation, the aqueous layer was collected added an equal amount of Chloroform: Isoamyl alcohol again to it and mixed by inversion. After successive centrifugation the supernatant was collected and added double the volume of ice-cold Ethanol. After successive centrifugation steps at 5000 rpm for 10 minutes, the Ethanol was decanted and the tube was allowed to air-dry for about 10 minutes. After complete drying, 50 µl of autoclaved water was added to the tube to dissolve the DNA and then stored in a deep freezer

3.8.2. Agarose gel Electrophoresis

The extracted DNA was qualitatively analyzed using 1% agarose electrophoresis. For Preparing 1% agarose gel, 1.5 g of agarose was weighed, taken in a clean conical flask. 80 ml of 5x Tris- Borate- EDTA (TBE) buffer was added and mixed well. The solution was then heated using a microwave oven to completely dissolve all the particle and to get a clear solution. The conical flask was then allowed to cool at room temperature to reach a bearable heat. 1µl of Ethidium bromide (EtBr) solution was added to the agarose solution using a micropipette and mixed thoroughly and then poured into a previously cleaned gel casting tray with a comb placed on one side. Kept it undisturbed and the comb was removed after the gel was solidified. 5 µl of isolated DNA was then loaded into the wells and electrophoresis was carried out. The gel box was adjusted to 80V, 400A current and ran for 15 minutes.

The DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) at 260 nm.

3.8.3. Polymerase chain reaction

Polymerase chain reaction (PCR) was carried out to amplify *rbc*L-F (coding region) *trn*L-F and *psb*A-*trn*H, (intergenic spacer regions) regions of the plastid genome. The Primer sequences used for the study were given in (Table 1). Amplifications were done using the CBOL plant working group (2009) recommended universal primers for *rbc*L-F and *psb*A-*trn*H, *trn*L-F regions. A BIO-RAD T20 Thermal cycler was used for the consistent amplification of the target fragments. The annealing temperatures of *rbc*L-F region was at 50 °C, *psb*A-*trn*H and *trn*L-F was at 55 °C. The PCR products were documented using the Gel documentation system (BIO-RAD) after resolving on

2 % agarose gel. The products were then purified with a Nucleosieve Gel extraction and PCR purification Kit (Primordia Lifesciences Pvt. Ltd.). Cycle Big Dye Terminator cycle sequencing chemistry (Scigenom, Cochin) was used to carry out the cycle sequencing reactions.

Table 1: Sequences of the barcoding primers used in PCR reactions.

Sl no.	**Barcoding primers**	**Sequence (5 '–3 ')**
1.	*rbc*L 1F	ATGTCACCACAAACAGAAAC
	724 R	TCGCATGTACCTGCAGTAGC
2.	*trn*L	CGAAATCGGTAGACGCTAG
	*trn*F	ATTTGAACTGGTGACACGAG
3.	*trn*H	CGCGCATGGTGGATTCACAATCC
	*psb*A	GTWATGCAYG AACGTAATGCTC

3.8.4. Phylogenetic Analysis

The chromatogram of the barcode regions *rbc*L-F, *psb*A-*trn*H and *trn*L-F gave quality reads. BLAST analysis was carried out and the newly generated sequences were submitted to NCBI GenBank (http://www. ncbi.nlm.nih.gov/genbank). The accession numbers obtained against sequence submissions were given in Table 2.

Sequences for rest of the species included in this study were downloaded from NCBI (Table 3.) The sequence alignment was done using Clustal w with default parameters (Larkin *et al.*, 2007) in MEGA 11 software (Tamura *et al.*, 2021). The evolutionary history was analyzed using the Maximum Likelihood method using IQ-TREE software (Nguyen *et al.*, 2015). *Botrychium lunaria* of the same family (Ophioglossaceae) was taken as the out-group for the tree. The best fit model for each gene region was selected based on (Kalyaanamoorthy *et al.*, 2017). according to Bayesian Information score (BIC), the TNe+G4 was the best fit model for the *rbc*L tree, K3Pu+F+G4 was the best fit model for *psb*A- *trn*H tree and K2P was the best fit model for *trn*L-F tree.

Table 2: Accession numbers obtained against sequence submission in NCBI GenBank.

Sl. No.	Name of the taxa	*rbc*L-F Region	*psb*A-*trn*H Region	*trn*L-F Region
1.	*O. latifolium*	OQ696317 OQ696318	OR237822 OR237823	OR237814 OR237815
2.	*O. lusitanicum*	OR995597	OR237824 OR237825 OR237826	OR237816 OR237817 OR237818
3.	*O. lusoafricanum*	OQ714504	OR237827	OR237820
4.	*O. parvifolium*	OR995596	OR995594	OR995595
5.	*O. raphaelianum*	Not available	OR237819	Not available
6.	*O. rubellum*	OR995598	OR237821	OR237813
7.	*O.* sp.nov.	OP351703 OP351704 OP351705	OP484887 OP484888 OP484889	OP604552 OP604553 OP604554

Table 3: Nucleotide Sequences downloaded from NCBI GenBank.

Sl. No.	Name of the taxa	*rbc*L-F Region	*psb*A-*trn*H Region	*trn*L-F Region
1.	*O. costatum*	AY138418.1	MN524684.1	AY138453.1
2.	*O. gramineum*	AY138412.1	MW666172.1	AY138448.1
3.	*O. indicum*	MW666158.1	MW666178.1	MW666168.1
4.	*O. pendulum*	AY138420.1	Not available	Not available
5.	*O. petiolatum*	AB626647.1	OL519807.1	MW666159.1
6.	*O. reticulatum*	MN524783.1	MN524701.1	AY138446.1
7.	*O. vulgatum*	DQ026594.1	AB575344.1	DQ026627.1
8.	*O. gomezianum*	AY138419.1	Not available	AY138454.1
9.	*O. polyphyllum*	MT657859.1	Not available	Not available
10.	*B. lunaria*	KC482121.1	AB575330.1	MN524663.1

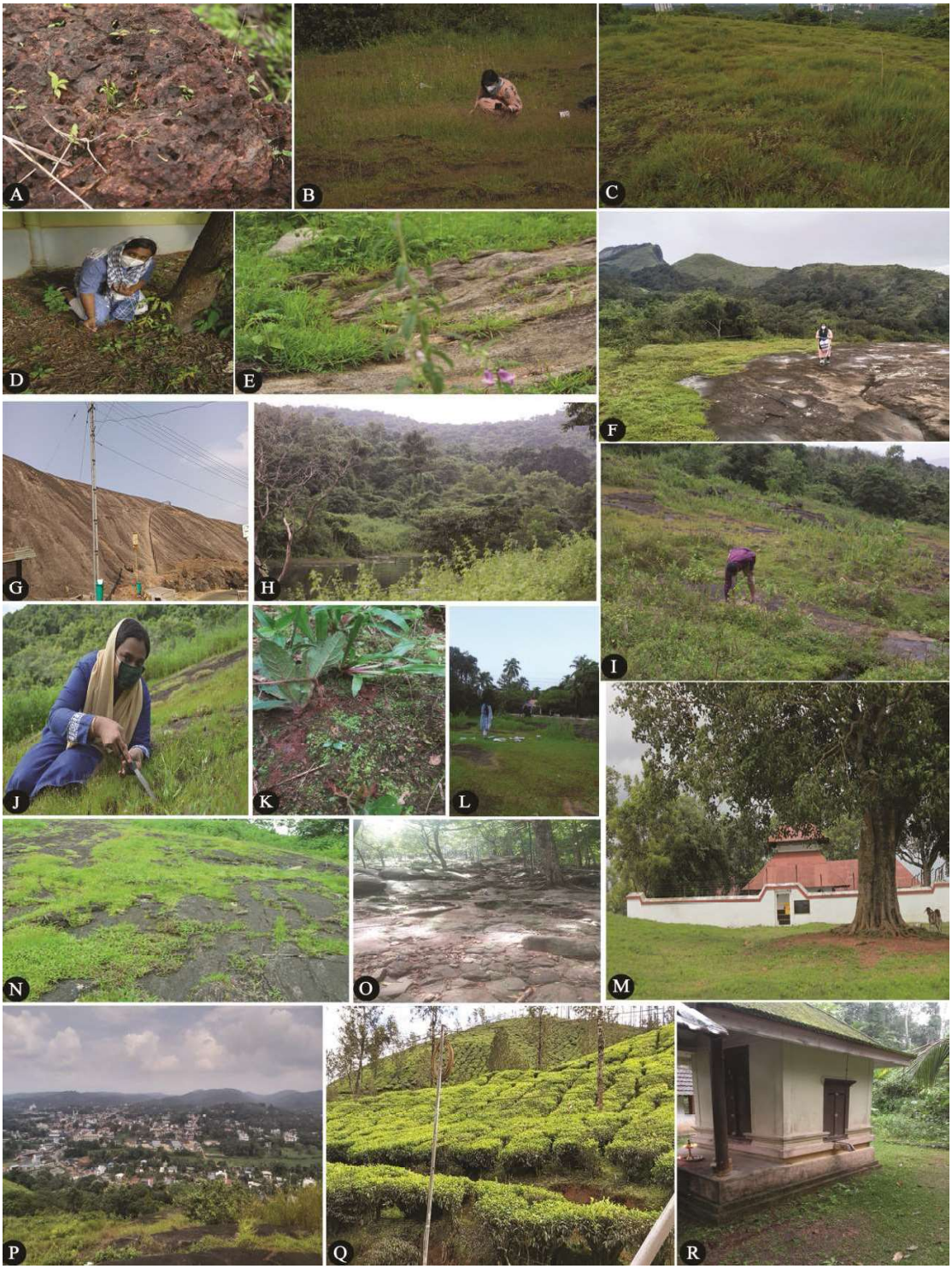

Fig. 01. Habitat diversity of Ophioglossum genus in Kerala: A. Posadi Gumpe, Kasaragod; B. Madayi Para, Kannur; C. Zamorins Guruvayoorappan College, Kozhikode; D. Farook College, Kozhikode; E. Kallekad, Palakkad; F. Kesavan Para, Palakkad; G. Cheppara, Thrissur; H. Cheruchakkichola,Thrissur; I. Kayampoovam, Thrissur; J. Killannur, Thrissur; K. mangadu, Thrissur; L. Pazhiyottumuri, Thrissur; M. Peruvanmala, Thrissur; N. Zeezan rock,Thrissur; O. Malayattoor, Ernakulam; P. Chutti Para, Pathanamthitta; Q. Munnar, Idukki; R. Elampally, Kottayam.

Chapter-4

Results

4.1. Systematic treatment

The systematic position of genus *Ophioglossum* based on the Pteridophyte Phylogeny Group (PPG I 2016) system of classification.

Kingdom: Plantae

Phylum: Tracheophyta

Class: Polypodiopsida

Subclass: Ophioglossidae

Order: Ophioglossales

Family: Ophiglossaceae

Genus: *Ophioglossum*

Genus: *Ophioglossum*

"It is a community-derived classification for extant lycophytes and ferns based on the phylogeny. The authors provided a modern, extensive classification for ferns and lycophytes, to the genus level, using a community-based approach" (PPG I 2016). The authors considered monophyly as the primary criteria for taxa identification and also aimed to maintain the existing taxa and the broadly accepted knowledge of the pteridophyte phylogeny. This classification treated "11916 species in 337 genera, 51 families, 14 orders, and two classes". "PPG I mainly recognized two classes viz., Lycopodiopsida (lycophytes) and Polypodiopsida (ferns)". Lycopodiopsida includes three orders viz., Lycopodiales, Isoetales, and Selaginellales. Order Lycopodiales have 16 genera belonginging to one family. Both the orders Isoetales and Selaginellales includes single monogeneric families. Order Polypodiopsida have four sub families viz., Equisetidae (horsetails), Ophioglossidae, Marattiidae, and Polypodiidae (leptosporangiates). Subclass Ophioglossidae posses two orders. Each having a single family, and altogether 12 genera. The order Ophioglossales contains family Ophioglossaceae and family psilotaceae. Family Ophioglossaceae includes genus *Ophioglossum*.

Ophioglossum L. Sp. Pl. 2: 1062. 1753.

Beddome, Handb. Suppl. Ferns Brit. India 108. 1892; Clausen, Mem: Torrey Bot. Club 19(2): 140. 1938; Balakrishnan *et al.*, Bull. Bot. Surv. India 2: 337. 1960; Mahabale, Nelumbo, 4(1 – 4), 71 – 84, 1962; Panigrahi and Dixit, Proc. Nat. Inst. Sci. India 35: 250. 1969; Nayar and Kaur, Comp. Beddome, Handb. 106. 1974; Dixit, Cens. Indian Pterid. 23. 1984; Manickam and Irudayaraj, Pterid. Fl. W. Ghats 48. t. 26. 1992; Nayar and Geevarghese, Fern.Fl. Malabar 78. 1993; Augustine *et al.*, JETB, 18(2): 445 – 447, 1994; Chandra, Ferns India, 7 – 8. 2000; Pullaiah *et al.*, Pterid. Andhra Pradesh 42. 2003; Goswami, Bionature, 1 – 73, 2007. Singh *et al.*, Taiwania, 54(4): 353 –364, 2009; Anto *et al.*, IJAR, 4:1268 – 73, 2016; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Khan *et al.*, PST. 186 – 195, 2023.

Lectotype: (J. Smith, Hist. Fil.: 367. 1875; Christensen, Index Fil.: lviii. 1906): *Ophioglossum vulgatum* L.

Cassiopteris H.Karst. in Linnaea 20: 437 (1847).

Cheiroglossa C.Presl in Suppl. Tent. Pterid.: 56 (1845).

Goswamia Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 23 (2022).

Haukia Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24 (2022).

Ophioderma (Blume) Endl. In Gen. Pl.: 66 (1836).

Rhizoglossum C.Presl in Suppl. Tent. Pterid.: 48 (1845).

Whittieria Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24 (2022).

Plants terrestrial herbs, except the epiphytic *O. pendulum*; rhizome fleshy, underground, discoid, globose, tuberous-elongated, fusiform, oblong, horizontal; roots fleshy, without root hairs, vegetatively propagating in some species; stipe slender, 0.1

– 11 cm long; tropophylls dorsiventral, 1 – 8 in number, glabrous, linear, lanceolate, elliptic, rhomboid, oblong, ovate, obovate, orbicular, linear-pendulous, cordate and spathulate (Lanceolate-narrowly ovate), margin entire, apex obtuse-acute-acicular-cuspidate-mucronate-rounded; venation anastomosing, reticulate or bireticulate; stomata generally anomocytic except for the cyclic stomata in *O. pendulum*; fertile spike arises from just below of the tropophyll lamina, from the centre of the tropophyll in *O. pendulum*; spike contain two rows of sporangia, either alternate or opposite; sporangia 4 – 80 in number; Spores yellow, globose, alete-trilete, ornamentation varies from reticulate-retate-foveolate-granulose-verrucate-rugate-patellate-lophate.

Phenology: June- January.

Habitat and Ecology: Various habitats, such as open grasslands, soil patches of loose rocks, river banks and under the tree shades.

Distribution: Cosmopolitian distribution in all continents except in Antartica.

Note: *Ophioglossum* is beweldering genus with greater taxonomic complixity. The simple morphology of the genus makes the species identification more difficult. The existence of several morphotypes for a single species is very common in genus *Ophioglossum*. Hence a combined approach by considering all the possible morphological characters along with the SEM analysis of spore characters and molecular characterisation sounds more authentic for identifying different species in this genus.

Key to the species of genus *Ophioglossum* in Kearala, India.

1a. Plants epiphytic, tropopyll pendulous……... *O. pendulum*

1b. Plants erect, terrestrial herbs, tropophylls not pendulous…….2

2a. Rhizome globose (Length shorter or equal to breadth) ……3

2b. Rhizome elongated (Length greater than breadth) 6

3a. Costa visible, bireticulate venation…… *O. costatum*

3b. Costa not visible, reticulate venation……...4

4a. Tropophyll away from the substratum, single, elliptic ……..…*O. latifolium*

4b. Tropophyll appressed or touching the substratum, one or more, ovate/ ovate-orbicular…… 5

5a. Tropophylls two-four, spores lophate, alete or trilete, like a rosette…. *O. rubellum*

5b. Tropophylls one-two, spores foveolate, trilete, not like a rosette..*O. raphaelianum*

6a. Tropophylls linear, apex acicular……*O. gramineum*

6b. Tropophylls not linear, apex not acicular……7

7a. Tropophylls cordate……*O. reticulatum*

7b. Tropophylls not cordate……8

8a. Number of tropophylls one……9

8b. Number of tropophylls more than one……11

9a. Venation bireticulate…….…… *O. vulgatum*

9b. Venation reticulate……10

10a. Plants below 10 cm high, spores verrucate……...*O. lusoafricanum*

10b. Plants above 10 cm high, spores rugate……*O. petiolatum*

11a. Tropophylls oblong-lanceolate, venation bireticulate…….*O.* sp. nov.

11b. Tropophylls not elliptic- lanceolate, venation reticulate……...12

12a. Tropophyll pinkish-green coloured…… *O. indicum*

12b. Tropophylls green coloured…… 13

13a. Subsidiary cells of the stomata are linear, cell walls not wavy…… *O. parvifolium*

13b. Subsidiary cells of the stomata are polygonal, cell walls wavy……*O. lusitanicum*

1. *Ophioglossum costatum* R. Br.Prod. Fl. Nov. Holl. 163.1810; Balakrishnan *et al.*, Bull. Bot. Survey India 2, 3 and 4: 33. 1960; Panigrahi & Dixit, Proc. Nat. Inst. Sci. India 35: 249. 1969; Burrows, and Johns, Fl. Trop. Ea. Afr.-Ophi., 2001; Patil & Dongare, Indian Fern Journal 31:17-24, 2014; Fraser-Jenkins *et al.*, An Ann. Check.

Ind. Pter. 1: 1 – 562, 2017; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Khan *et al.*, PST. 186 – 195, 2023.

Type: Australia, Arnheim Bay, R. Brown 118 (Lectotype, BM, digital image!).

Ophioglossum pedunculosum Desv. In Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 5:306. 1811.

Ophioglossum wightii Grev. and Hook. In Bot. Misc. 3: 218. 1833.

Ophioglossum brevipes Bedd. in Ferns. Southern India 23. t. 72. 1863.

Ophioglossum aphrodisiacum Welw. ex Hook. In Hooker and Baker, Syn. Fil.: 446. 1868.

Ophioglossum bulbosum Bedd. In Suppl. Ferns S. Ind.: 28. 1876.

Ophioglossum fibrosum Schumach. In Beskr. Guin. Pl.: 452. 1827; Bedd., Handb. 465. t. 289. 1883; Suppl. 109. 1892.

Ophioglossum vulgatum var. *pedunculosum* (Desv.) Domin in Biblioth. Bot. 20: 221. 1913.

Ophioglossum felixii Tardieu in Notul. Syst. (Paris) 13: 169 1948 publ. 1949.

Ophioglossum hitkishorei M.Patel and M.N.Reddy in Bot. Lett. 166: 426. 2019.

Ophioglossum madhusoodhananii Sojan, V.S.A. Kumar, Sindhu Arya, V. Suresh, L. Leeja & Alen Alex. syn. nov.

Fig. 02, 03, 04 & 05

Plants up to 6 – 28 cm high, erect, dark green; rhizome globose-tuberous elongated-discoid or cormlike like, 0.5 – 2 cm long, 1 – 5 cm in diam., unbranched; roots 1 – 7 cm long, fleshy; stipe 0.5 – 3 cm long, 0.2 – 0.5 cm in diam., forms a sheathing leaf base; tropophylls 3 – 8 in number, originating close to or away from the ground level, 2 – 8 cm long, 1 – 3 cm broad, tufted, dark green, simple, glabrous, elliptic-rhomboid-lanceolate, obtuse-acute-mucronate at apex, margine entire, midrib prominent, attenuate at base; fertile segment 4 – 23 cm long, stalk 3.5 – 16 cm long, 0.1 – 0.4 cm in diam., spike 0.5 – 7 cm long, 0.2 – 0.5 cm wide, occasionally bifurcated, sporangia

on either side, 11 – 75 in number, alternate-opposite or a mixture of both alternate and opposite arrangement.

Phenology: June-October.

Habitat and Ecology: Growing mainly in moist shallow soils on the exposed rock surfaces, as a group. Graminae members, *O. gramineum*, *O. lusitanicum* were also noted in the habitat.

Distribution: Africa, Australia, Asia: Bangladesh, **India** (Andhra Pradesh, Assam, Chhattisgarh, Gujarat, Himachal Pradesh, Jharkhand, Karnataka, **Kerala**, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal), Indonesia, Philippines, Sri Lanka, Thailand.

Note: *O. costatum* plants shows many morphotypes ranging from small sized to large sized plants. The size as well as shape of each plant parts varies among different population. The plants with globose or discoid rhizome and with elliptic or rhomboid or lanceolate tropophylls are common in *O. costatum.* The occurance of many morphotypes in this genus makes confusions among taxonomists and resulted in the accumulation many new names, which were synonymised by different authors. The micromorphology and the molecular characteristics are common in all the morphotypes, inspite of the small morphological changes in the vegetative parts.

Specimens examined: **India:** South India: **KERALA**: **Ernakulam Dist**.: Malayattor: (10° 12' 57.7152" N, 76° 30' 31.4856" E), *Afsana Khan & Anto P. V.* 20 (STC); **Idukki Dist.:** Pindimedu-Pooyamkutty, *P. Bharghavan 8740* (MH), Munnar (10° 4' 9.84" N, 77° 4' 3" E) *Stephen Sequeira, Afsana Khan & Anto P.V. 26* (STC); **Kannur Dist.:** Madayippara (12° 1' 35.3388" N, 75° 15' 50.0436" E), *Afsana Khan & Anto P.V. 22* (STC); **Kasaragod Dist.:** Posadigumpe (12° 40' 33.3768" N, 75° 1' 25.5504" E), *Afsana Khan & Anto P.V. 30* (STC); **Kozhikode Dist.:** Ramanattukara, *Jose 18358* (CALI). Palakkad Dist.: Dhoni Hills-Malampuzha, *Madhusoodhanan P.V 21270* (CALI); Kallekad (10º47'2"N, 76º36'2"E) *Afsana Khan & Anto P.V. 18* (STC), Kanjirapuzha, *Geevarghese 4105* (CALI); Malampuzha, *J. Joseph 17153* (MH);

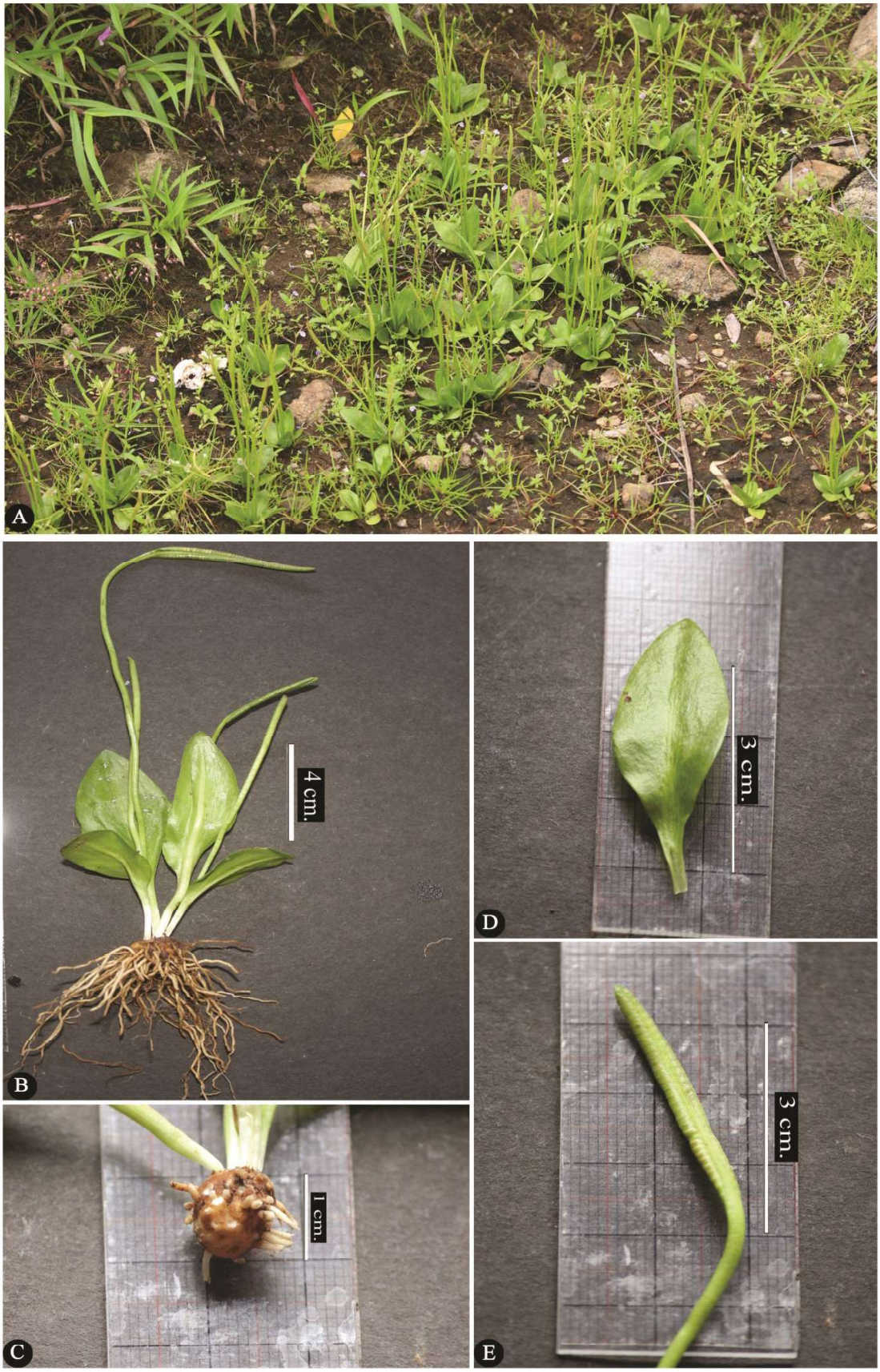

Fig. 02. *Ophioglossum costatum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

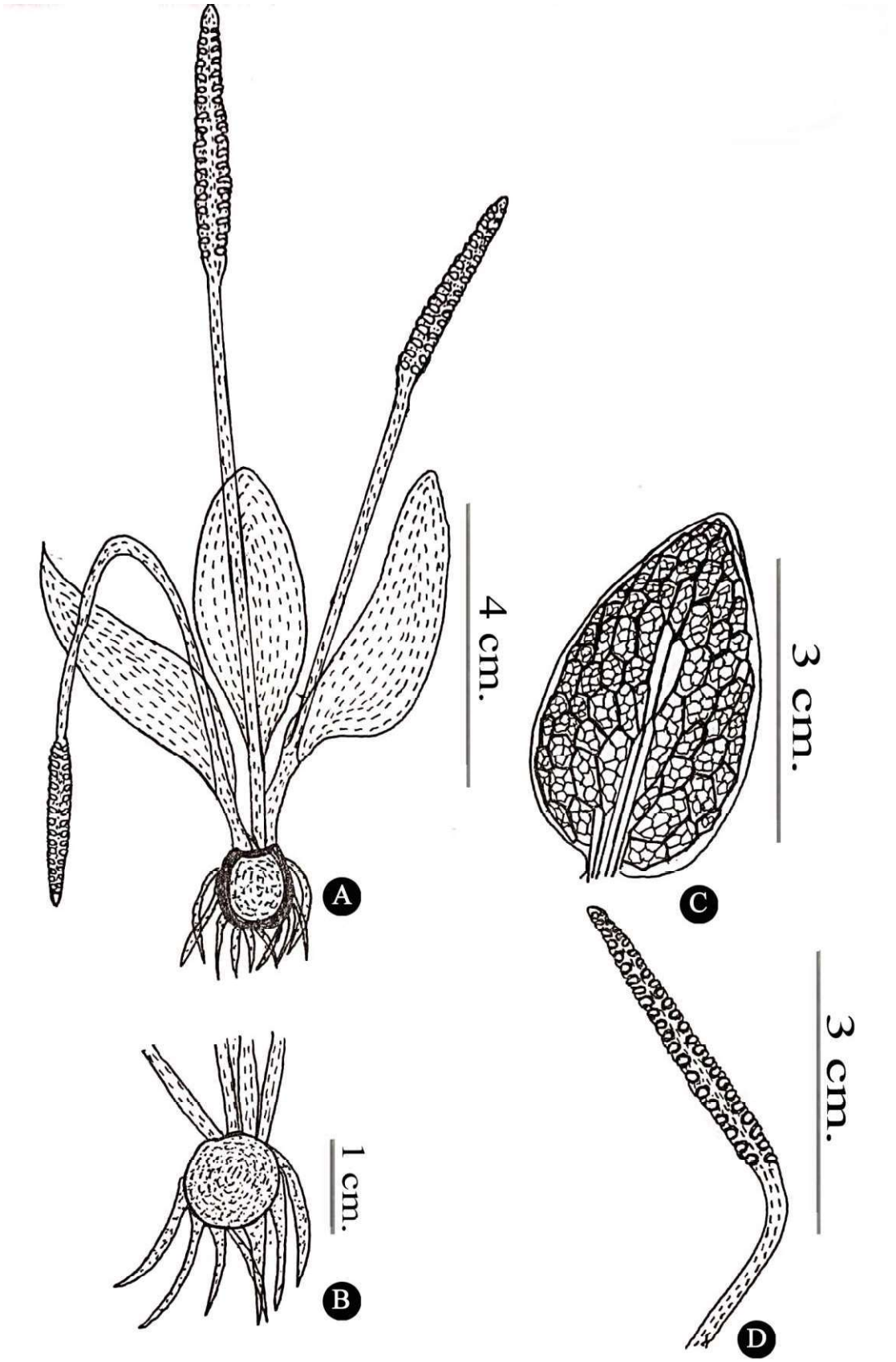

Fig. 03. Illustration of *Ophioglossum costatum*: A. Entire plant, B. Rhizome, C. Tropophyll, D. Spike.

Fig. 04. Lecto type of *Ophioglossum costatum* © The Board of Trustees of the Natural History Museum (BM), London.

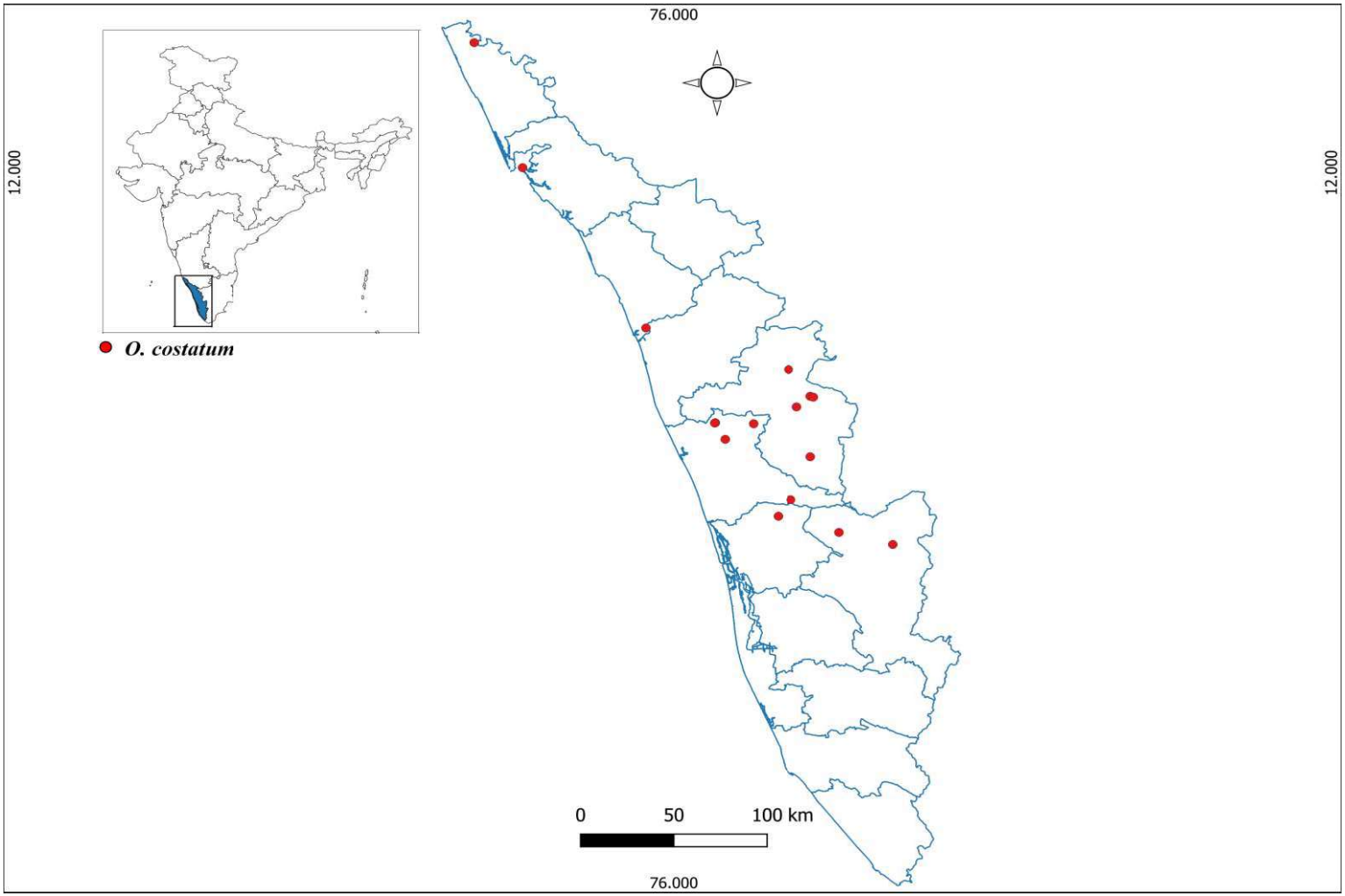

Fig. 05. Distribution map of *Ophioglossum costatum* in Kerala, India

Thrissur Dist.: Athirapilly, *V.V. Sivarajan 4841* (CALI); Cheruchakkichola (10º 42' 05.26"N, 76º11'59.07"E), *Afsana Khan & Anto P. V. 16* (STC); Kayampoovam (10º41'10.16"N, 76º23'07.50"E), *Afsana Khan & Anto P. V. 17* (STC); Killannur (10° 36' 56. 2968" N, 76° 14' 59.118" E) *Afsana Khan & Anto P.V. 4* (STC). **Madhya Pradhesh: Raipur Dist.:** Pachaida farm, Raipur, *K. Subramanyam 8573* (MH); **Maharashtra: Gondia Dist.:** Mahadev hills-Amagaon, *S. M. Bhuskute 344* (CALI);

Rajasthan: Baran Dist.: Atru, *C.B. Gena* (CALI). **Tamil nadu:** Coimbatore Dist.: Aliyar Sub mergible area, *K.M. Sebastine 14698* (MH); Dindigul Dist.: Kodaikanal, *T. Surendran CU 8612* (CALI); Pudukottai Dist.: *C. Arulappan, 243* (MH). Trichirapally Dist.: Narthamalai, *K. Ramamurthy 25950* (MH).

2. *Ophioglossum gramineum* Willd. Abhandl. Kurf. -Mainz. Akad. Wiss. Erf. 2(6): 18, t. 1, f. 1. 1802.; Beddome, Handb. Suppl. Ferns Brit. India 108. 1892; Balakrishnan *et al.*, Bull. Bot. Surv. India 2: 337. 1960; Mahabale, Nelumbo, 4(1 – 4), 71 – 84, 1962; Panigrahi & Dixit, Proc. Nat. Inst. Sci. India 35: 250. 1969; Nayar and Kaur, Comp. Beddome, Handb. 106. 1974; Dixit, Cens. Indian Pterid. 23. 1984; Manickam and Irudayaraj, Pterid. Fl. W. Ghats 48. t. 26. 1992; Singh *et al.*, Taiwania, 54(4): 353 –364, 2009; Patil & Dongare, Indian Fern Journal 31:17-24, 2014; Fraser-Jenkins *et al.*, Ann. Check. Ind. Pter 1: 1 – 562, 2017; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Khan *et al.*, PST. 186 – 195, 2023.

Type: West Africa, St. Thomae [São Tomé], De Friedland (lecto Type, B, digital image!).

Ophioglossum vulgatum var. gramineum (Willd.) Hook. f., in Fl. Nov. Zel., 2: 50. 1854.

Ophioglossum dietrichiae Prantl in Ber. Deutsch. Bot. Ges. 1:352. 1883.

Ophioglossum lanceolatum Prantl in Ber. Deutsch. Bot. Ges. 1: 352.1883.

Ophioglossum vulgatum var. *lanceolatum* Luerss. In J. Mus. Godeffroy 3: 247.1875.

Ophioglossum moluccanum f. *inconspicuum* Rac. In Natuurk. Tijdschr. Ned. -Indië 59. 237. 1900.

Ophioglossum prantlii C.Chr. in Index Filic.: 471. 1906.

Ophioglossum inconspicuum (Rac.) Alderw. in Bull. Dép. Agric. Indes Néerl. 21:9. 1908.

Ophioglossum inconspicuum f. *majus* Alderw. In Bull. Dép. Agric. Indes Néerl. 21: 9. 1908.

Ophioglossum gregarium Christ in Nova Guinea 8: 164.1909.

Ophioglossum vulgatum var. *prantlii* (C.Chr.) W.R.B. Oliv. In Trans. and Proc. New Zealand Inst. 49: 126.1917.

Ophioglossum gracile Pocock ex J.E.Burrows in Bothalia 25: 61. 1995.

Ophioglossum gramineum var. *majus* (Alderw.) Wieff. In Blumea 12: 324. 1964.

Fig. 06, 07, 08 & 09

Plants erect, 3 – 6 cm high, pale green; rhizome small, tuberous-elongated, light brown, 0.4 – 1 cm long, 0.6 – 1 cm in diam., unbranched; roots 0.5 – 1 cm long, 0.1 cm in diam.; stipe 1 – 2 cm, 0.1 – 0.2 cm in diam.; tropophylls one-two, minute, dark green, glabrous, 1 – 1.3 cm long, 0.1 – 0.3 cm wide, originating away from the ground level, narrowed, linear, acicular at apex, attenuate at base, margin entire; fertile segment 1.6 – 3 cm long, stalk dark green, 1 – 1.5 cm long, 0.1 – 0.2 cm in diam.; spike 0.6 – 1.5 cm long, 0.1 – 0.3 cm wide, with a short sterile pointed apex; sporangia 5 – 10 on either side, alternate.

Phenology: June-October.

Habitat and Ecology: Plants growing on grass lands and open rock surface with moist soil along with graminae members, *O. lusitanicum* and *O. indicum*.

Distribution: Africa, America, Australia, Asia: **India** (Andhra Pradesh, Chattisgarh, Gujarat, **Kerala**, Karnataka, Madhya Pradesh, Rajasthan, Maharashtra, Meghalaya, Rajasthan, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal), Indonesia, Jawa, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand.

Fig. 06. *Ophioglossum gramineum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

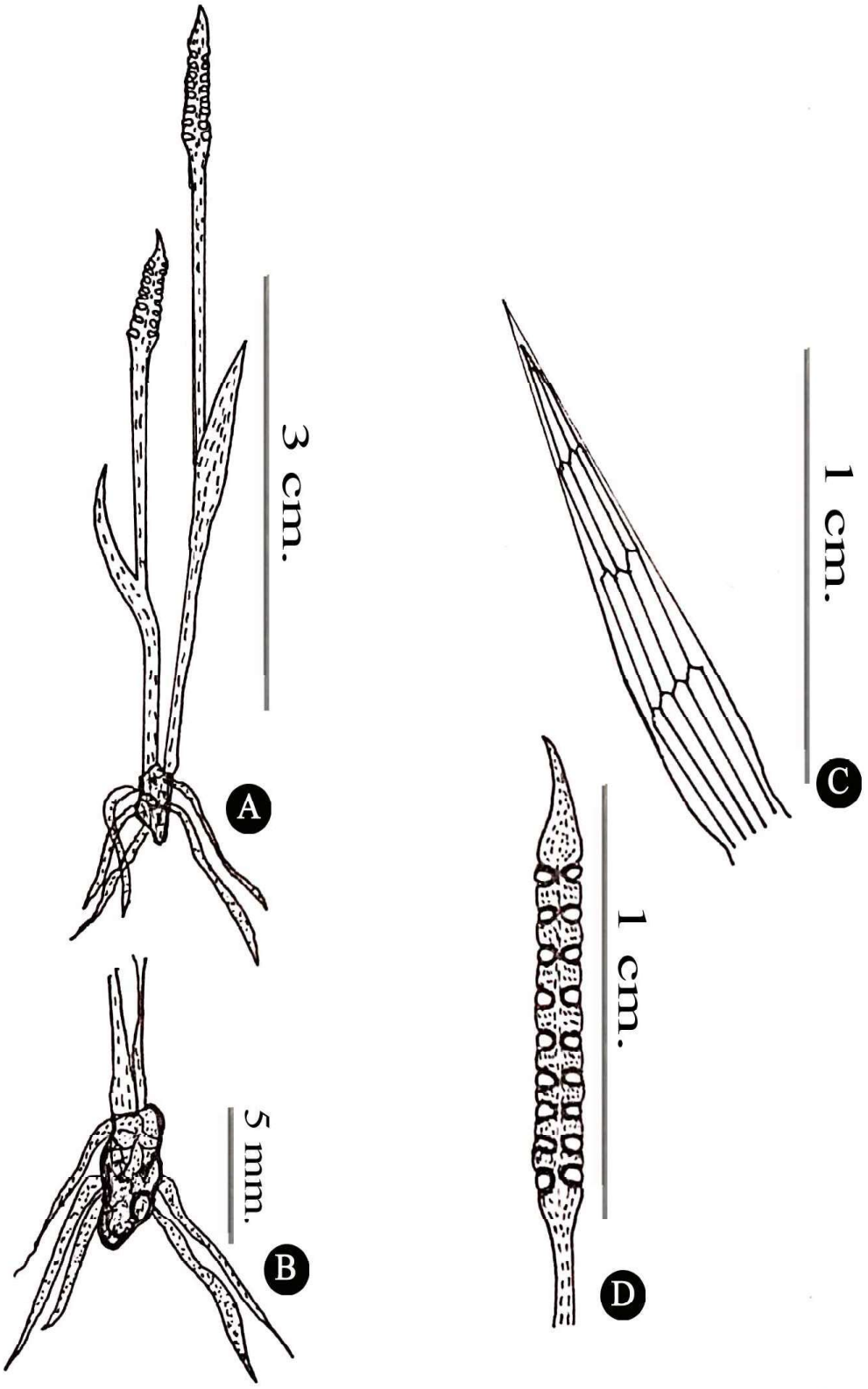

Fig. 07. Illustration of *Ophioglossum gramineum*: A. Entire plant, B. Rhizome, C. Tropophyll, D. Spike.

Fig. 08. Lectotype of *Ophioglossum gramineum*© The Board of Trustees of the herbarium of the Botanic Garden and Botanical Museum (B) Berlin-Dahlem.

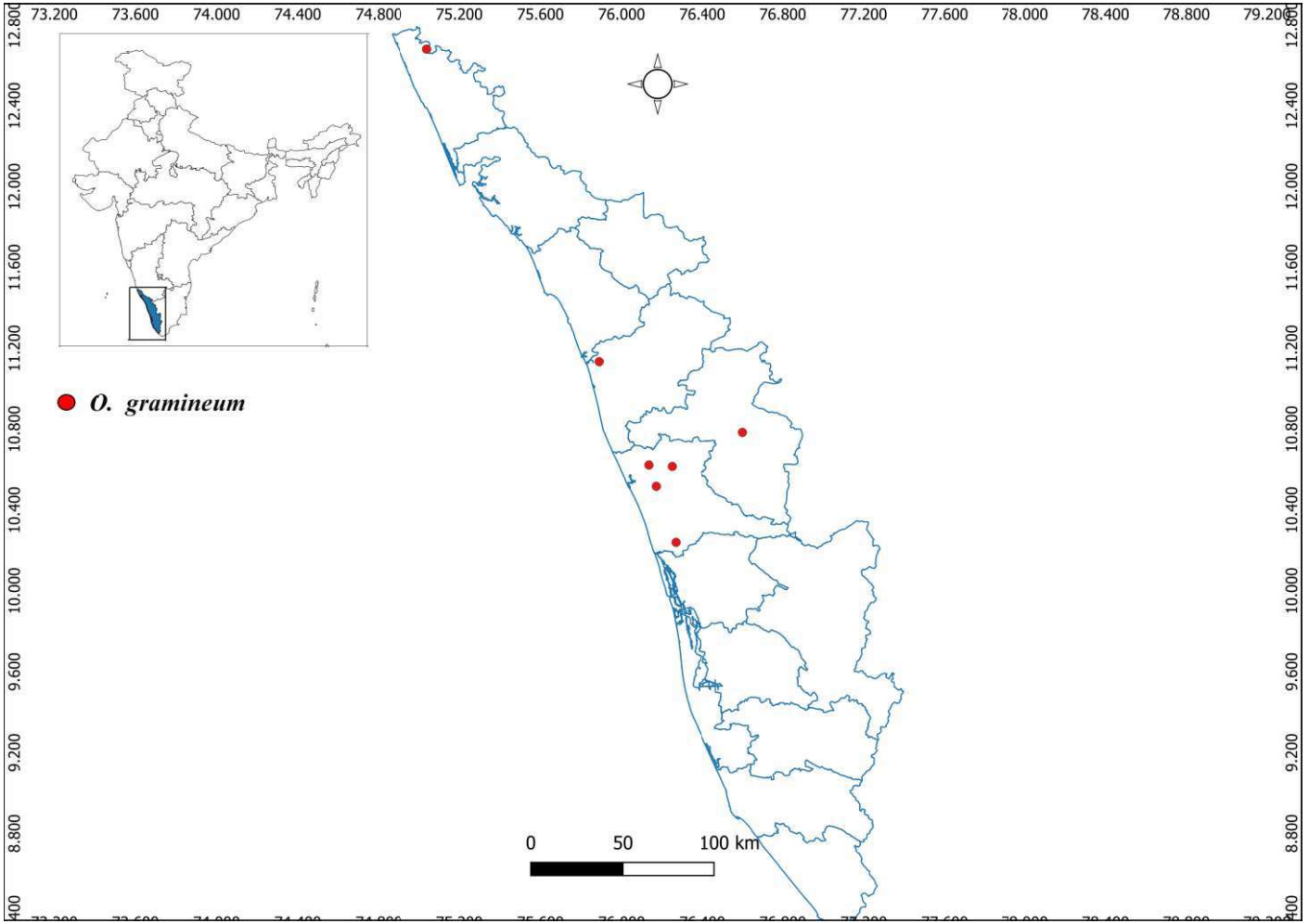

Fig. 09. Distribution map of *Ophioglossum gramineum* in Kerala, India.

Note: *O. gramineum* can be easily recognized from the field with its linear tropophylls. In Kerala, it is distributed mainly in central Kerala.

Specimens examined: **Kerala**: **Kasaragod Dist**.: Posadigumpe (12º40'33.55"N, 75º01'03.85"E), *Afsana Khan & Anto P. V. 32* (STC); **Malappuram Dist**.: Calicut University Campus, *Gheevarghese 1800* (CALI); Calicut University Campus, *Indira E. CU11697* (CALI); Calicut University Campus, *C. B. Gena CALI174948* (CALI); **Palakkad Dist**.: Kallekad (10°47'2"N, 76°36'2"E), *Afsana Khan & Anto P. V. 19* (STC); **Thrissur Dist**.: Killannur (10° 36' 56.2968" N, 76° 14' 59.118" E), *Afsana Khan & Anto P. V. 44* (STC); Mala (10° 14' 24"N 76° 16' 5.9982"E) *Afsana Khan & Anto P. V.* 38, Olari (10° 31' 08" N 76° 10' 24"E) *Afsana Khan & Anto P. V. 39*, Peruvanmala (10° 37' 20.4168" N, 76° 8' 4.8192" E), *Afsana Khan & Anto P. V. 1* (STC). **Madhya Pradesh**: **Hoshangabad Dist**.: Kesla-Banglapore, *J. Joseph 12838* (MH). **Tamil Nadu**: **Trichirapally Dist**.: Narathamalai, *K. Subramanyam 103787* (MH).

3. *Ophioglossum indicum* B. L. Yadav & H. K. Goswami. Bull. Natl. Mus. Nat. Sci., Tokyo, B. 36: 155. 2010. Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter.1: 1 – 562, 2017; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Zhang & Zhang in. Molec. Phylogen. Evol. 173 – 107512: 24 (2022). Khan *et al.*, PST. 186 – 195, 2023.

Type: Rajastan, B. L. Yadav 3011, (Isotype, CAL, digital image!)

Goswamia indica (B.L.Yadav & H.K.Goswami) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173 – 107512: 24 (2022).

Fig. 10, 11, 12 & 13

Plants erect, 3 – 6 cm high, pinkish green, glabrous; rhizome small, light brown, tuberous- elongated with a brown coating, 0.4 – 0.5 cm long, 0.4 – 1 cm in diam., unbranched; roots cylindrical, unbranched, arises from the entire surface of the rhizome, 0.5 – 2 cm long, 0.1 cm in diam.; stipe 0.6 – 1.5 cm long, 0.1 – 0.2 cm in diam.; trop- ophylls 1 – 4, originating close to the substratum, pinkish green, glabrous, 0.5 – 1 cm long, 0.4 – 0.6 cm wide, elliptic- lanceolate, acute at apex, obtuse at base; fertile segment 2 – 4 cm long, stalk pinkish green, 1 – 2.5 cm long, 0.1 – 0.2 cm in

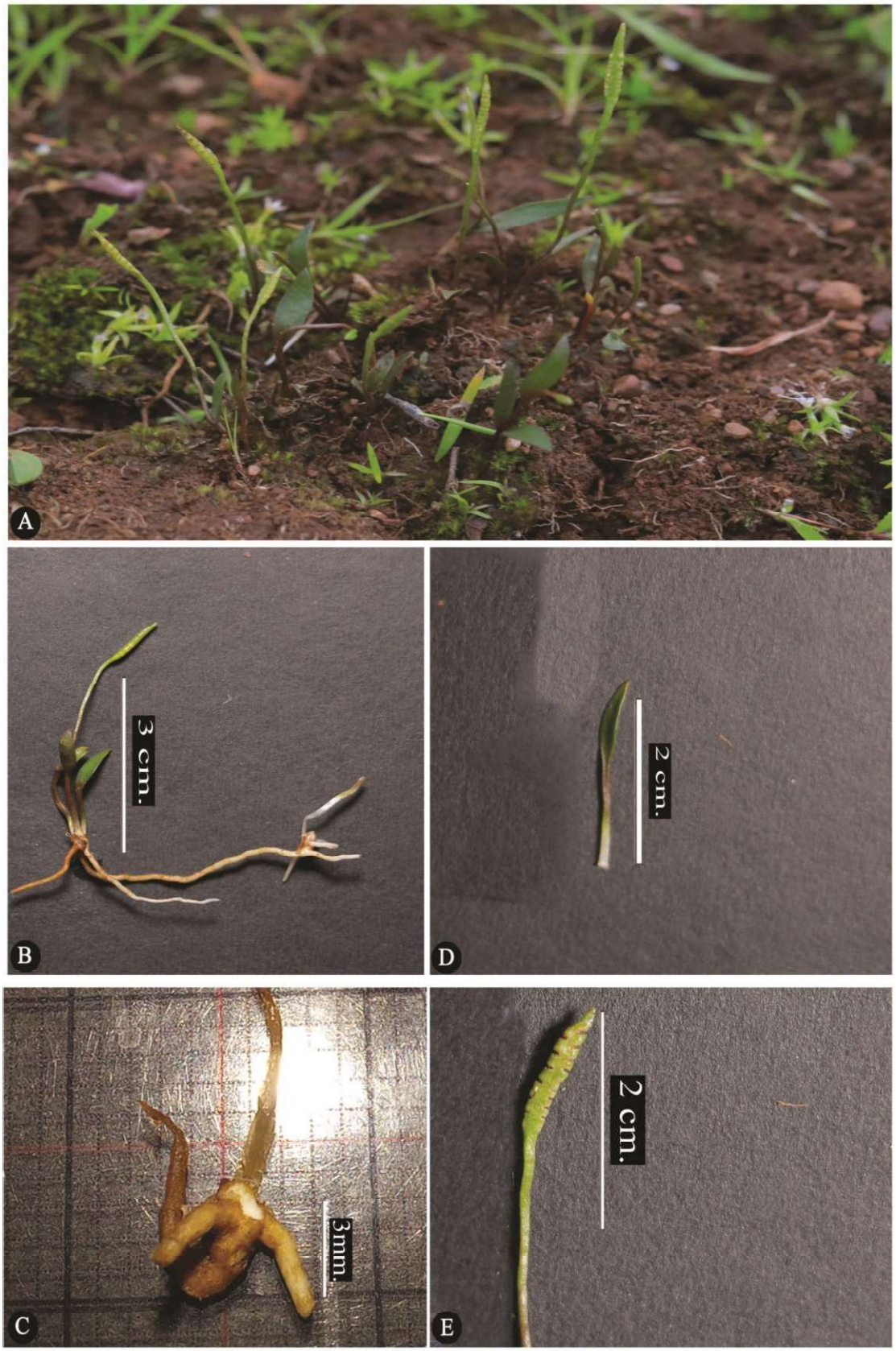

Fig. 10. *Ophioglossum indicum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

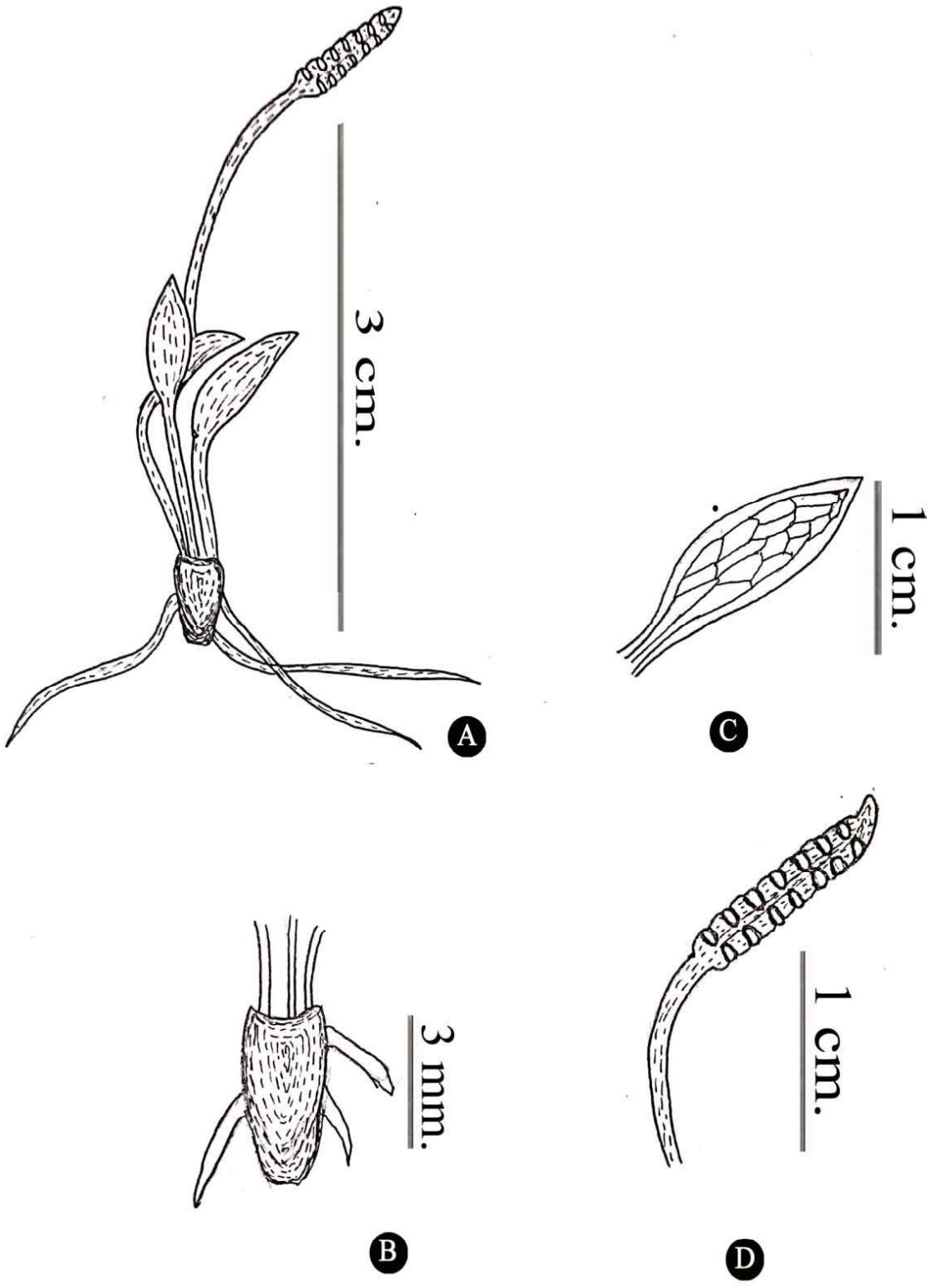

Fig. 11. Illustration of *Ophioglossum indicum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

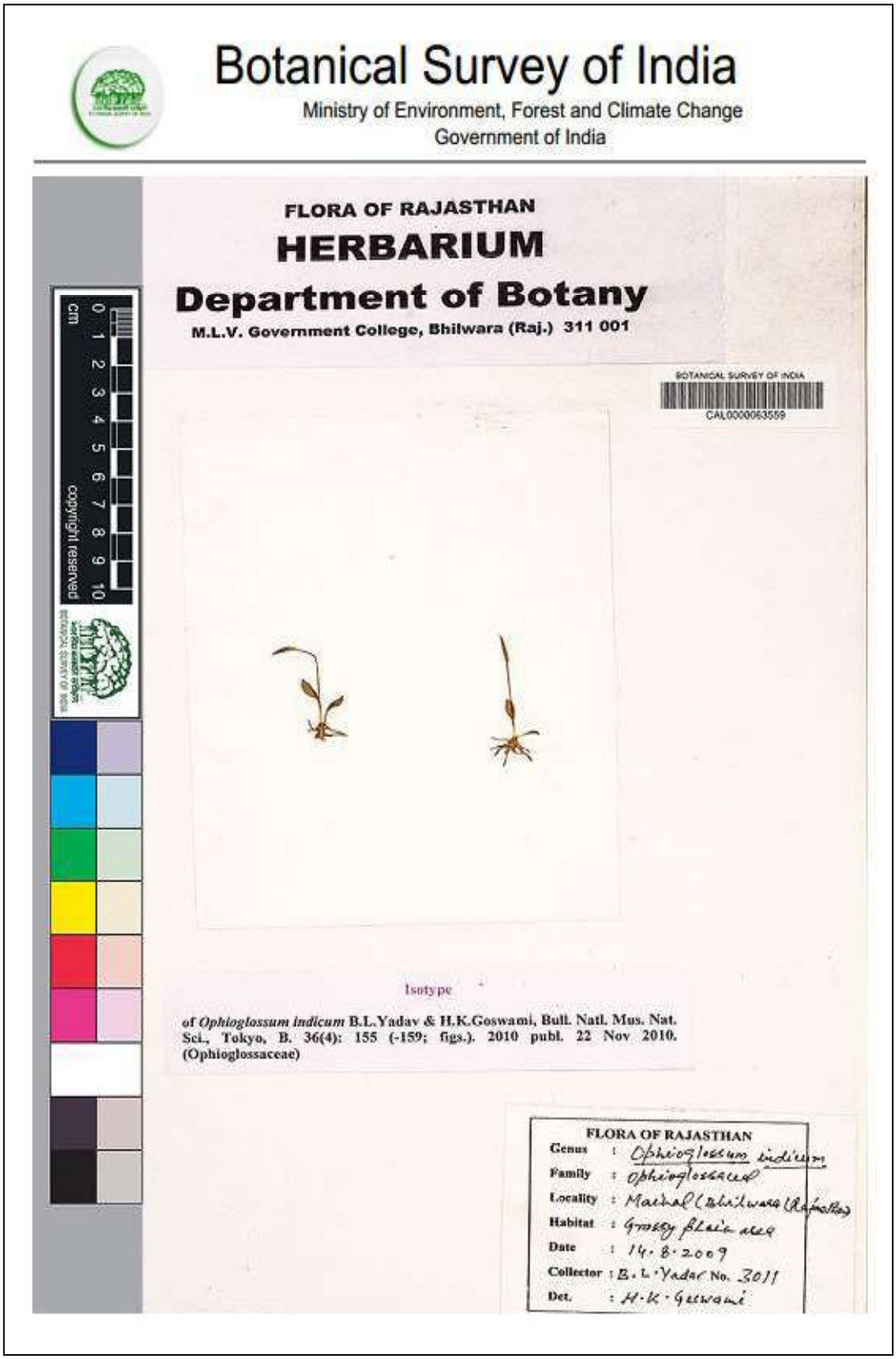

Fig. 12. Isotype of *Ophioglossum indicum*. © The Director, Botanical Survey of India, Kolkata.

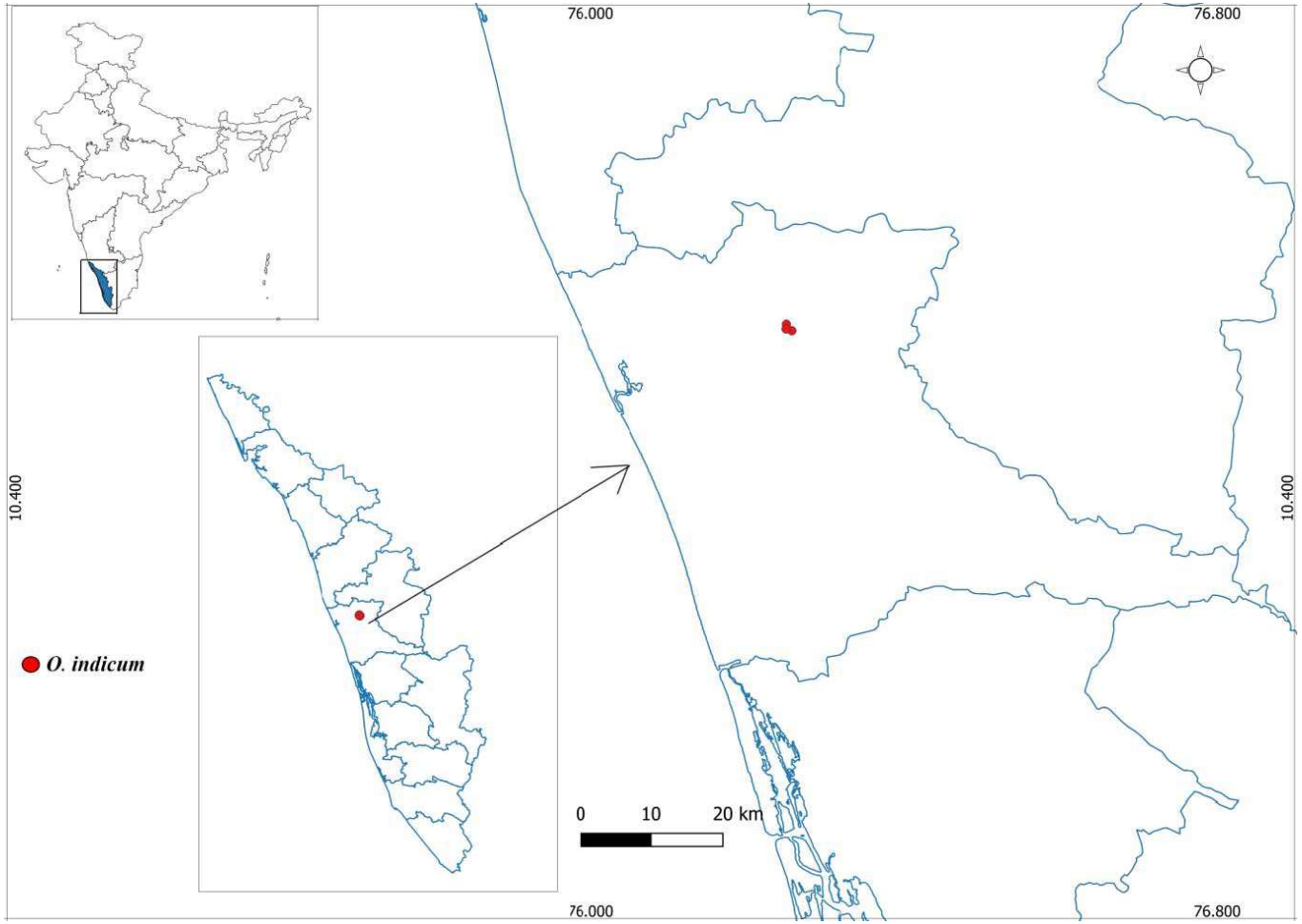

Fig. 13. Distribution map of *Ophioglossum indicum* in Kerala, India

diam.; spike 1 – 1.5 cm long, 0.1 – 0.3 cm wide, with a small sterile pointed apex; sporangia 6 – 7 on either side, opposite.

Phenology: June-October.

Habitat and Ecology: Exposed rock surfaces. *O. costatum*, *O. gramineum*, *O. lusitanicum* were also noted in the locality.

Distribution: India (Rajastan), **new record from Kerala.**

Note: Fraser-Jenkins *et al.*, (2021) Synonimised *O. indicum* as *O. rubellum.* But the present study collected seperate populations of *O. indicum* and *O. rubellum* from different locations of Kerala. By the detailed examination of macro and Micromorphological characters and comparing with the type specimens of both the species, this study recognized *O. indicum* and *O. rubellum* as two different species and hence the species status of *O. indicum* was reinstated in this study. Also *O. indicum* collected during this study is **a new distributional record for South India**.

Specimens examined: India: **Kerala**: **Thrissur Distr**., Cheppara (10° 37' 14.9988" N, 76° 15' 6.9984" E) *Afsana Khan & Anto P. V. 3* (STC), Killannur (10° 36' 56.0736" N, 76° 14' 59.2836" E) *Afsana Khan & Anto P. V. 36* (STC), Zezan rock (10° 36' 53" N, 76° 15' 40"E) *Afsana Khan & Anto P. V. 35* (STC).

4. *Ophioglossum latifolium* (Prantl) J.E. Burrows, Bothalia 23(2): 188 ,1993; Burrows and Johns, Fl. Trop. Ea. Afr.-Ophi., 2001; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018.

Type: Angola, Pungo Andongo, Welwitsch 32 (lectotype, K, digital image!).

Ophioglossum gomezianum var. *latifolium* Prantl in Jahrb. Königl. Bot. Gart. Berlin 3: 316. 1884.

Ophioglossum ellipticum Welw. ex Carruth. in Cat. Afr. Pl. 2(2): 279.1901., nom. illeg.

Fig. 14, 15, 16 & 17

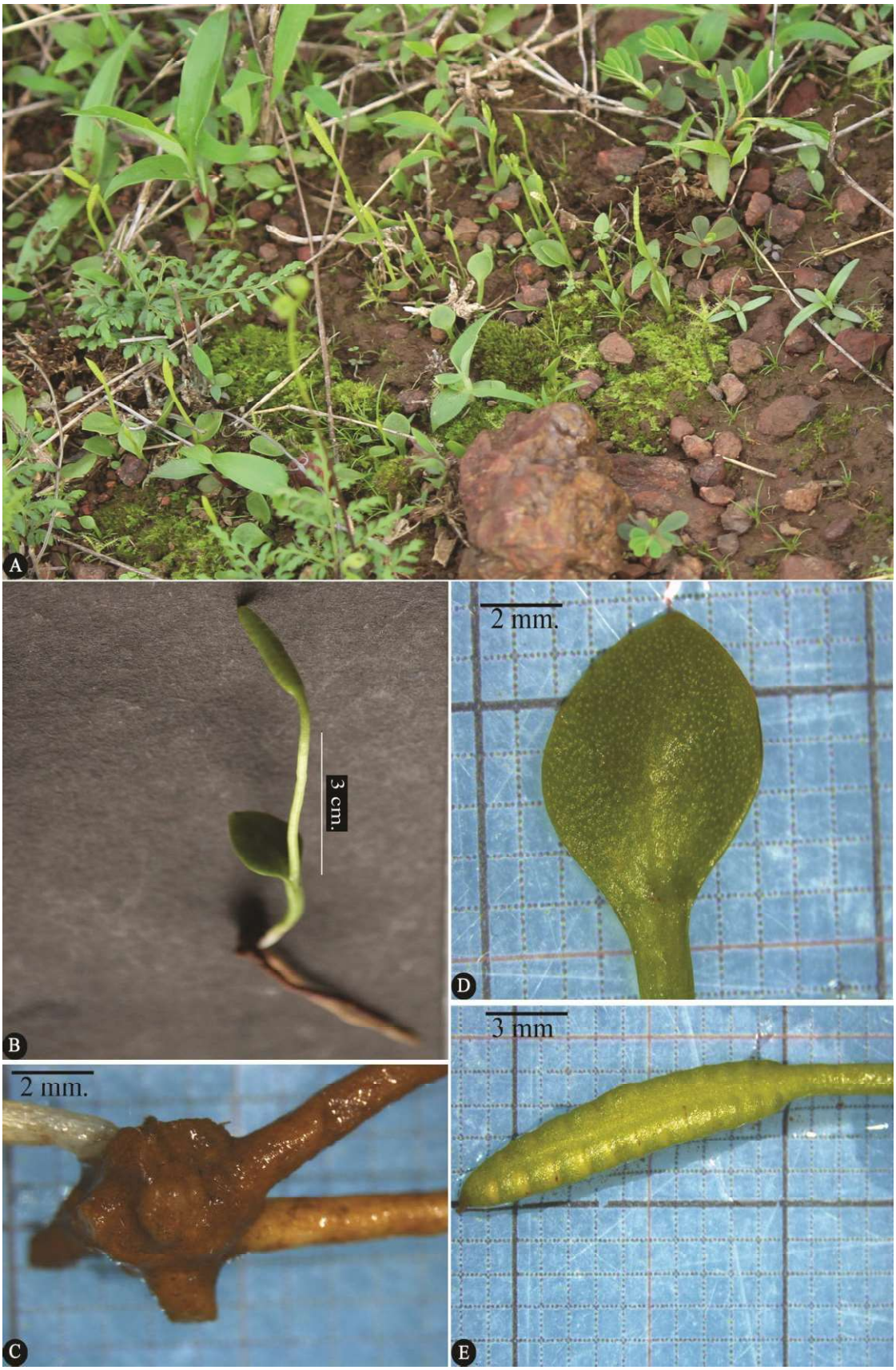

Fig. 14. *Ophioglossum latifolium*: A. Habitat, B. Entire plant, C. Rhizome, D Tropophyll, E. Spike.

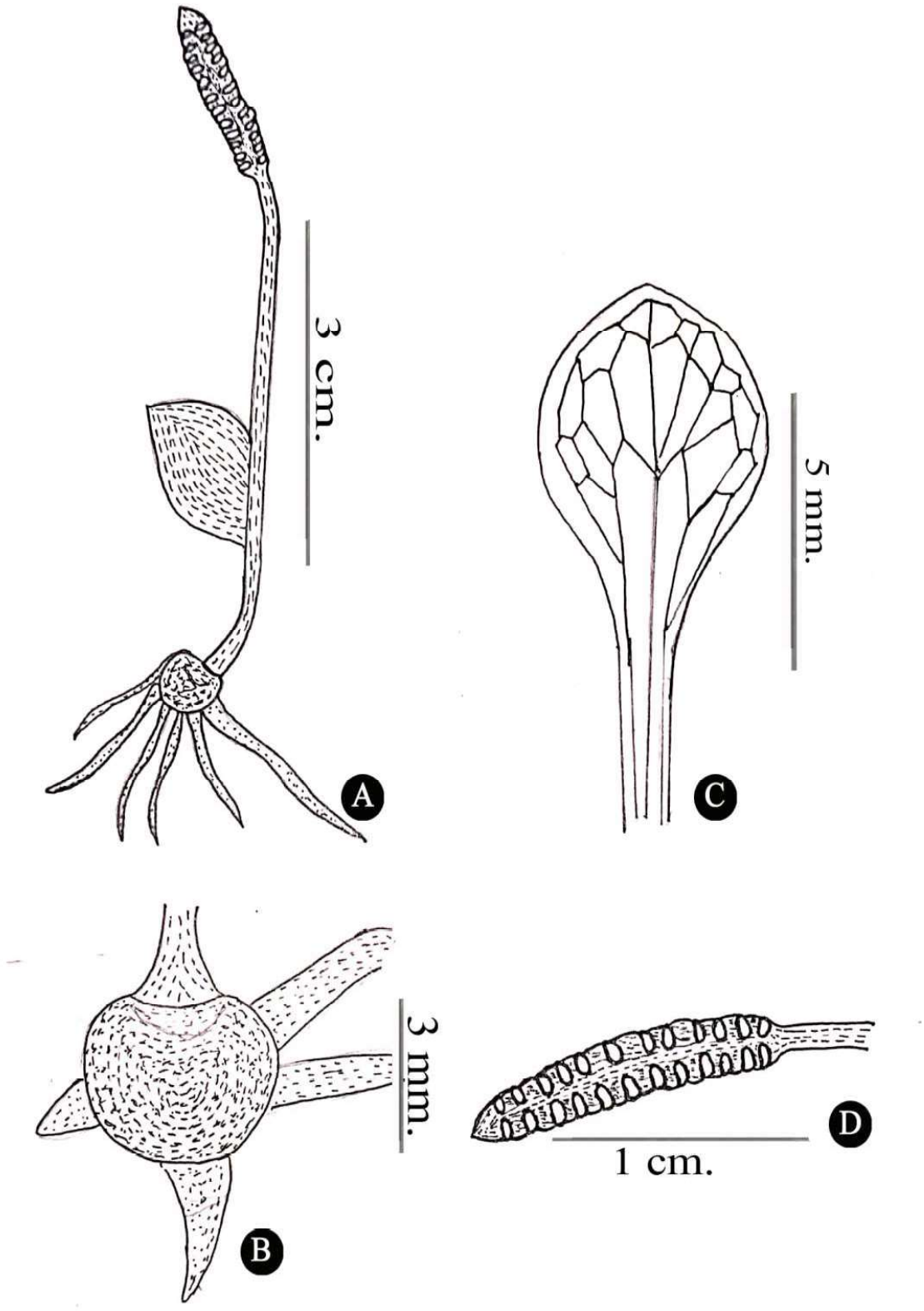

Fig. 15. Illustration of *Ophioglossum latifolium*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

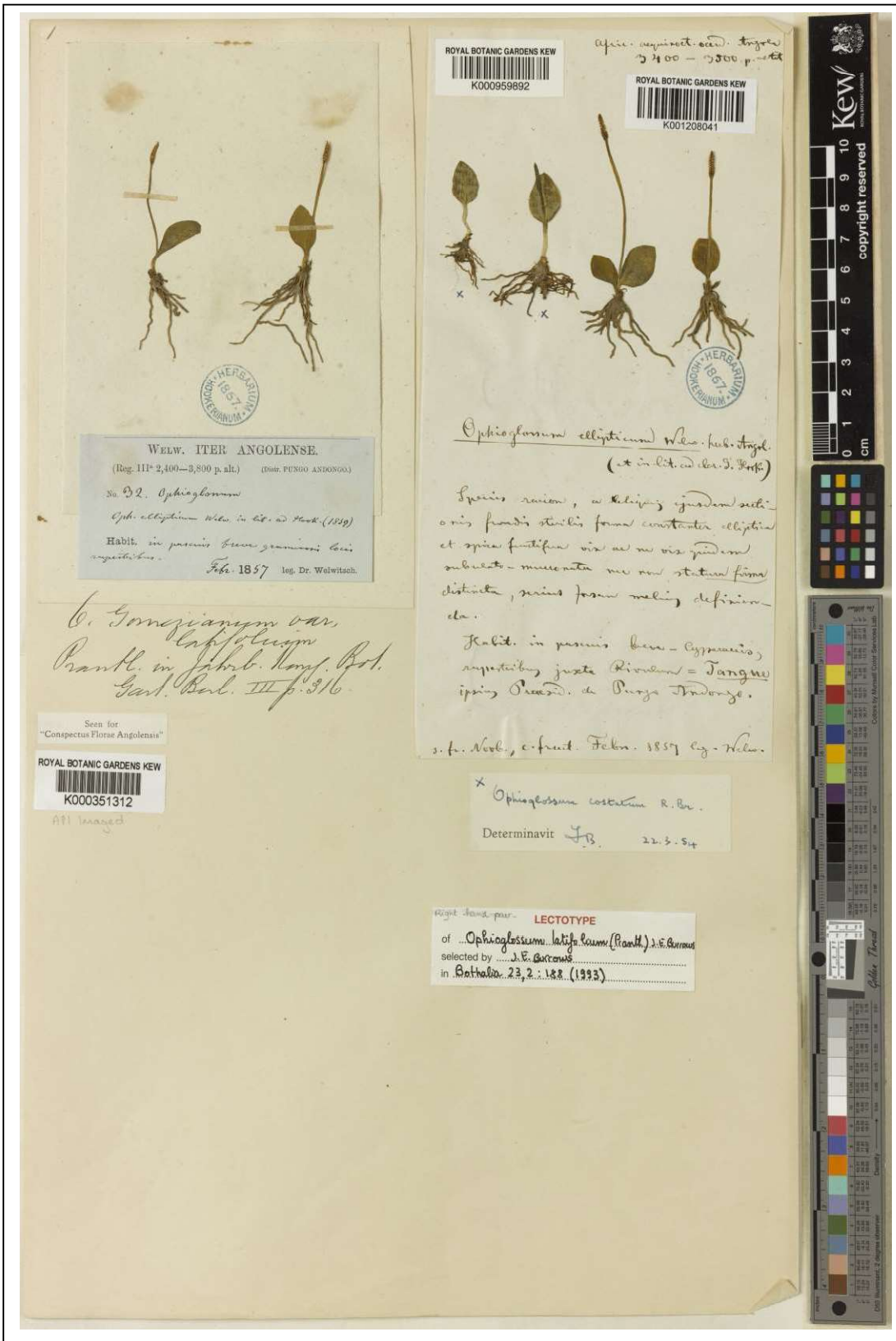

Fig. 16. Lectotype of *Ophioglossum latifolium*© The Board of Trustees of the RBG, Kew.

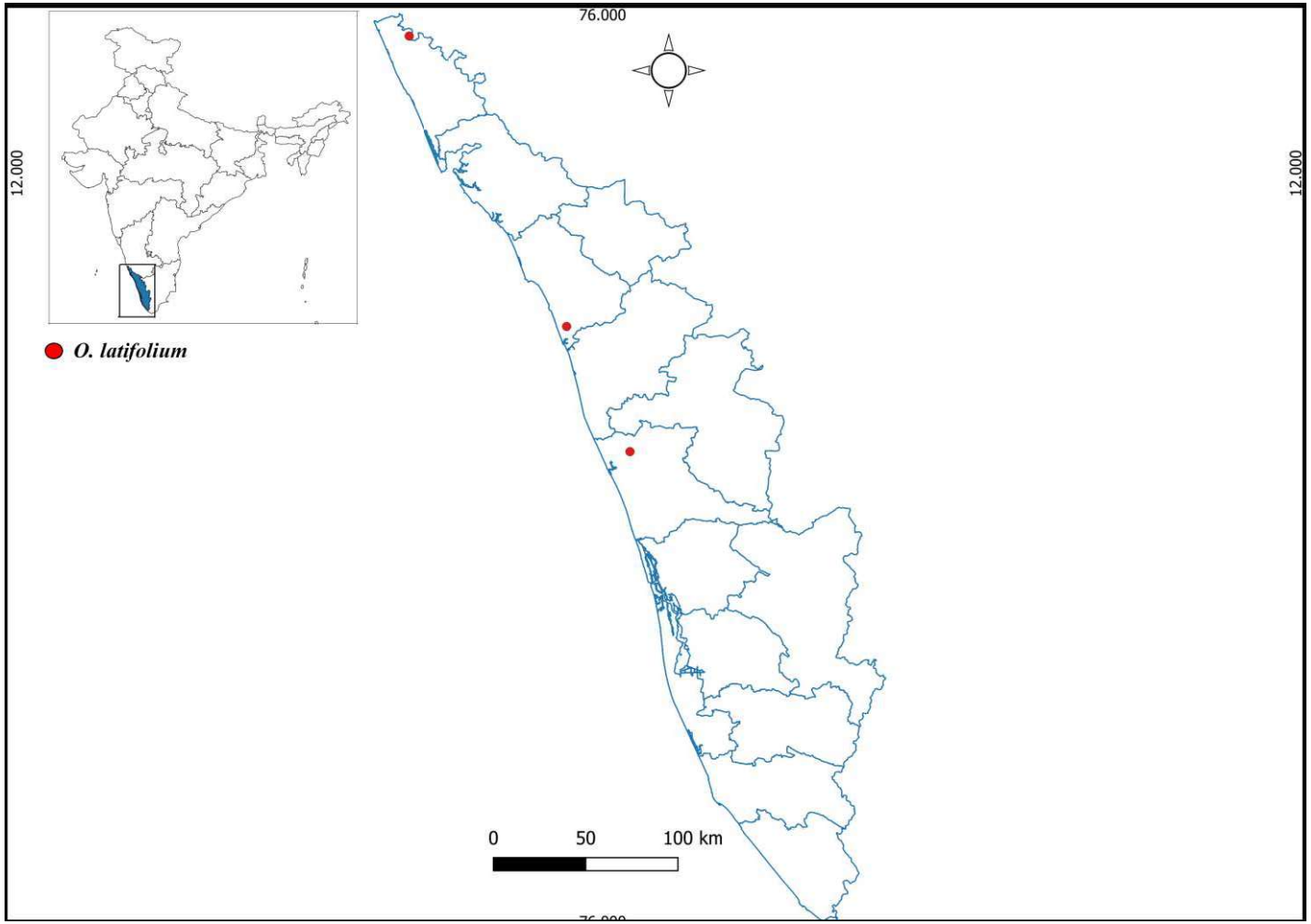

Fig. 17. Distribution map of *Ophioglossum latifolium* in Kerala, India.

Plants erect, 2 – 7 cm high, dark green, glabrous; rhizome small, light brown, globose, with a brown coating, 0.3 – 0.5 cm long, 0.8 – 1.5 cm in diam., unbranched; roots vegeratively propagating, cylindrical, unbranched, 0.2 – 1 cm long, 0.1 – 0.3 cm in diam.; stipe 0.2 – 1.5 cm long, 0.1 – 0.3 cm in diam.; tropophylls 1 – 2, originating very close to the substratum, green, glabrous, elliptic, obtuse at apex, cuneate at base, 0.4 – 1cm long, 0.4 – 0.9 cm wide; fertile segment 1.5 – 5 cm long, stalk 1 – 3.5 cm long, 0.1 – 0.3 cm in diam.; spike 0.5 – 1.5 cm long, 0.2 – 0.3 cm wide, with a small sterile pointed apex; sporangia 5 – 12 on either side, opposite.

Phenology: June-October.

Habitat and ecology: Growing on laterite rocks together with *O. gramineum*, *O. lusitanicum*, *O. parvifolium* and bryophytes. Mostly found in laterite soil.

Distribution: Africa, **New record from India (Kerala).**

Note: *O. latifolium* had restricted distribution in Kerala. It is found only in Kasaragod, Kozhikode, Thrissur Disrtricts. So, this taxon is mainly found in northern Kerala. The plants were distinguishable from other plants with their elliptic tropophyll and globose rhizome. The micromorphology and molecular characters are also different from other species. *O. latifolium* collected during this study is **a new report to India.**

Specimen examined: India: South India: **Kerala**: **Kasaragod Dist**.: Posadigumpe (12º40'15"N, 75º01'27"E), *Afsana Khan & Anto P. V. 29* (STC); **Kozhikode Dist**.: Zamorin's Guruvayoorappan College (11° 14' 31.416" N, 75° 49' 22.08" E), *AfsanaKhan & Anto P. V. 28* (STC); **Thrissur Dist.:** Peruvanmala (10° 37' 20.424" N, 76° 8' 4.812" E) *Afsana Khan & Anto P.V. 42* (STC).

5. *Ophioglossum lusitanicum* L., Sp. Pl. 2: 1063. 1753.; Clausen, Mem. Torry Bot. Club 19(2):159, 1938; Mahable, Bull. Bot. Surv. India 4:71, 1962; Panigrahi & Dixit, Proc. Nat. Inst. Sci. India 35: 251, 1969; Patil & Dongare, Indian Fern Journal 31:17-24, 2014; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Khan *et al.*, PST. 186 – 195, 2023.

Type: France: Brest, Deschamps 1806 (Epitpe, MNHN, digital image!).

Ophioglossum loureiroanum C.Presl in Suppl. Tent. Pterid.: 55. 1845.

Ophioglossum vulgatum var. *lusitanicum* (L.). Hook. & Arn. In Brit. Fl., ed. 8, éd. 8: 593.1855.

Ophioglossum vulgatum subsp. *Lusitanicum* (L.) Hook.f. in Student. Fl. Brit. Isl.: 469.1870.

Ophioglossum braunii Prantl in Ber. Deutsch. Bot. Ges. 1:351. 1883.

Ophioglossum vulgatum subsp. *Lusitanicum* Bonnier & Layens in Tabl. Syn. Pl. Vasc. France: 381. 1894.

Ophioglossum lusitanicum var. *latifolium* Rouy in G. Rouy& J. Foucaud, Fl. France. 14: 460. 1913.
Ophioglossum lusitanicum var. *longepedunculum* Rouy in G.Rouy & J.Foucaud, Fl. France 14: 460. 1913.
Ophioglossum vulgatum var. *macrophyllum* Rouy in G.Rouy & J.Foucaud, Fl. France 14: 458. 1913.

Fig. 18, 19, 20 & 21

Plants erect, up to 3 – 6 cm high, green, glabrous; rhizome small, light brown, tuberous- elongted, with a brown coating, 0.3 – 0.5 cm long, 0.5 – 1 cm in diam., un branched; roots cylindrical, unbranched, 0.5 – 1 cm long, 0.1 – 0.2 cm in diam.; stipe 0.6 – 1.5 cm long, 0.1 – 0.3 cm in diam.; tropophylls 1 – 2, originating close to the substratum, green, glabrous, 0.5 – 1 cm long,0.1 – 0.4 cm wide, lanceolate-elliptic, acute at apex, obtuse at base; fertile segment 2.1 – 4 cm long, stalk 1.4 – 3 cm long, 0.1 – 0.3 cm in diam.; spike 0.7 – 1 cm long, 0.1 – 0.3 cm wide, with a small sterile pointed apex; sporangia 4 – 9 on either side, opposite.

Phenology: June-October.

Habitat and Ecology: Growing in laterite soil. In Kerala, the distribution is restricted in Kasargode and Thrissur Districts.

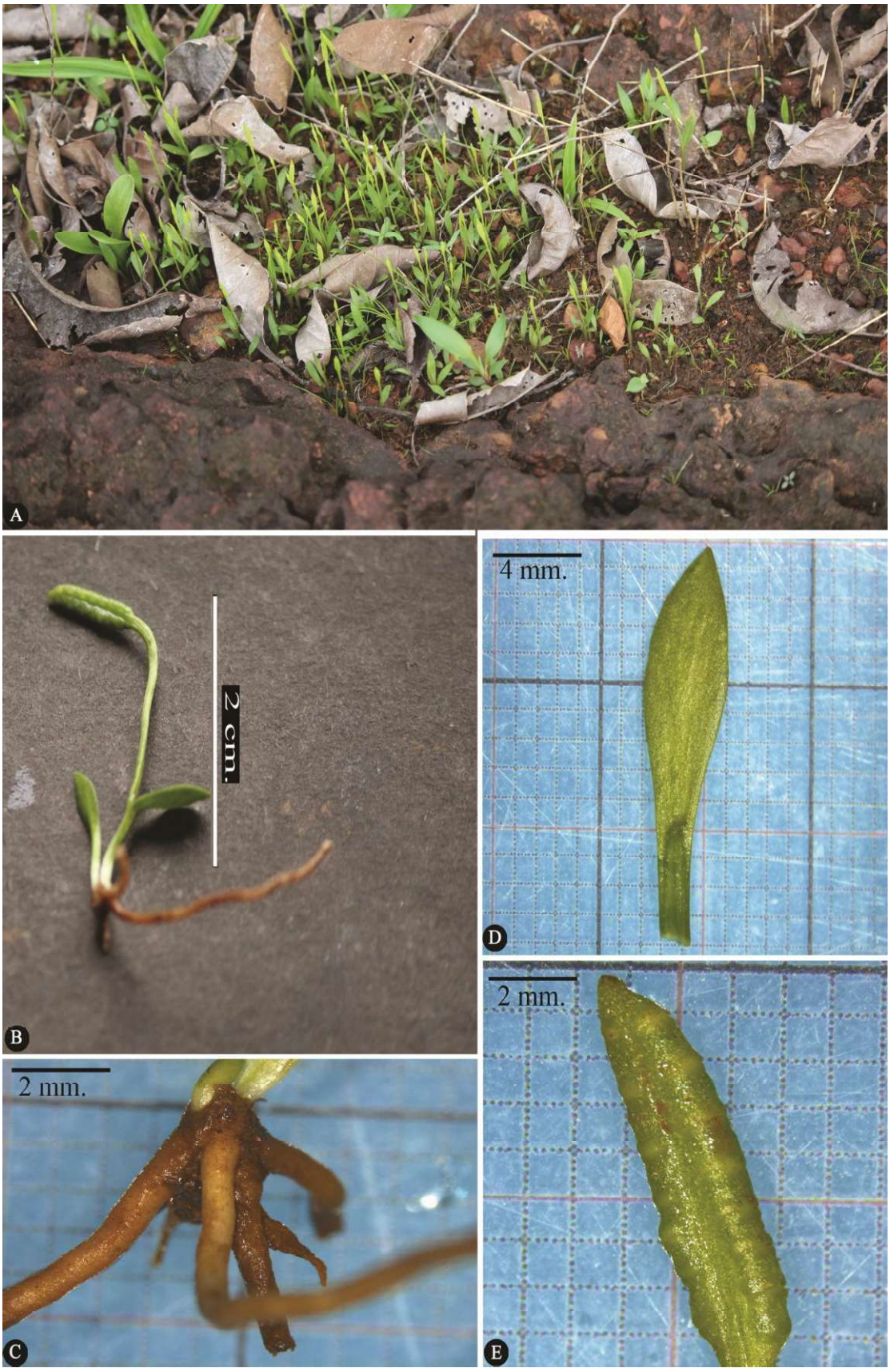

Fig. 18. *Ophioglossum lusitanicum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

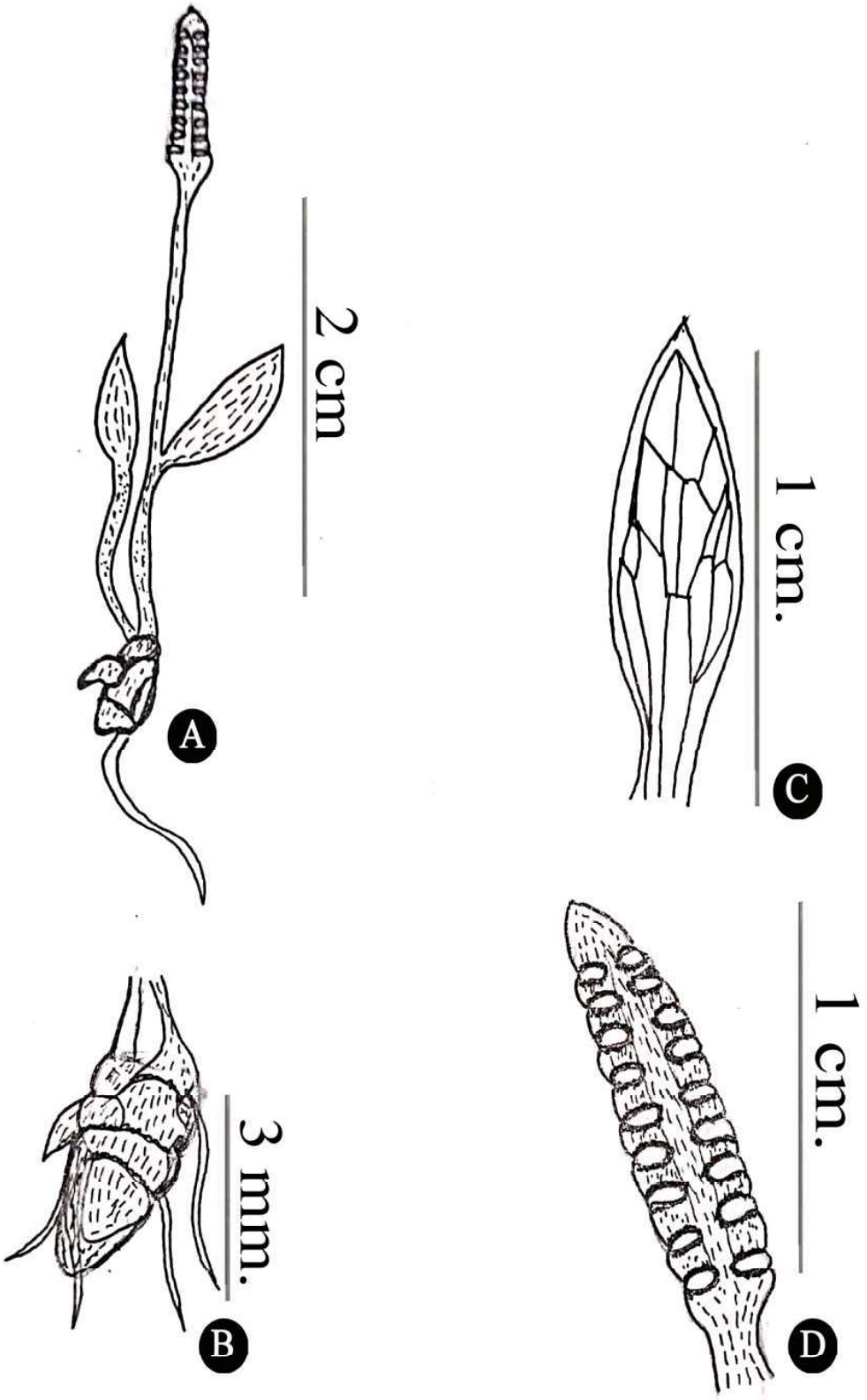

Fig. 19. Illustration of *Ophioglossum lusitanicum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

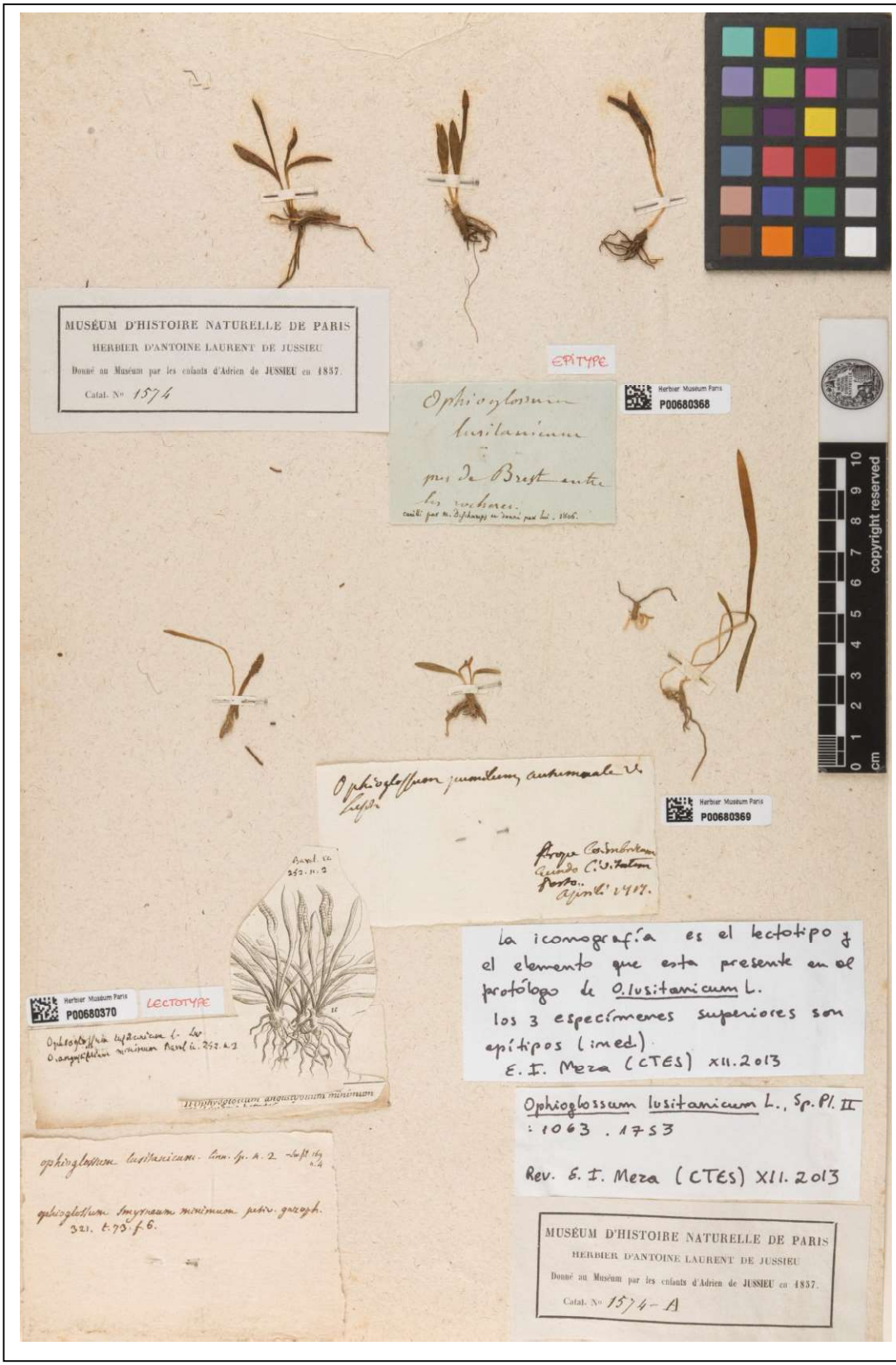

Fig. 20. Epitype of *Ophioglossum lusitanicum* © The Board of Trustees of the Herbarium of the Muséum national d'Histoire Naturelle (MNHN) Paris.

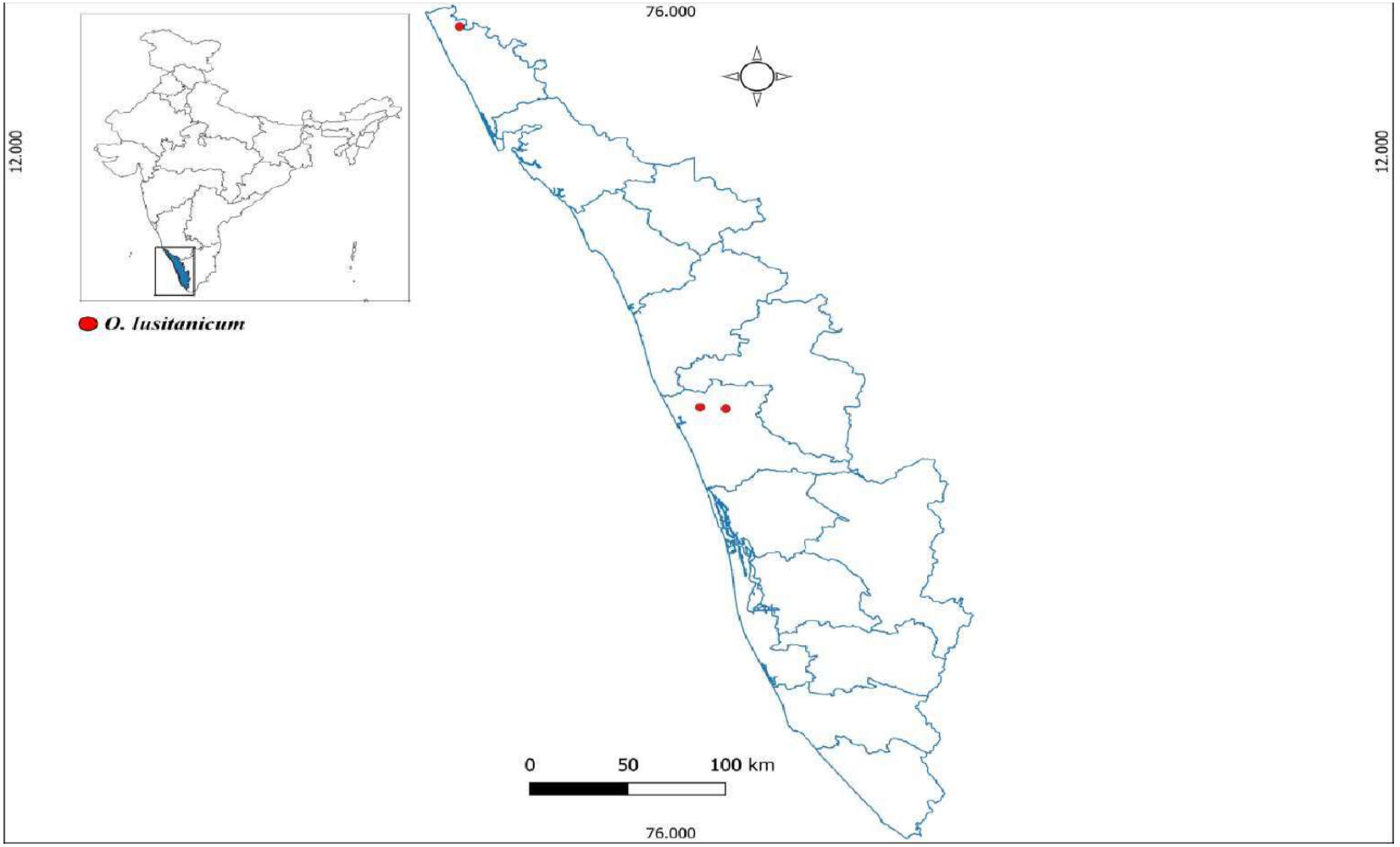

Fig. 21. Distribution map of *Ophioglossum lusitanicum* in Kerala, India.

Distribution: Africa, Europe, Asia: **India** (Karnataka, **Kerala**, Madhya Pradesh, Rajasthan, Tamil Nadu), Iran, Vietnam.

Note: Fraser Jenkins *et al*., (2021) had an opinion that the Indian *O. lusitanicum* corresponds to the African *O. lusoafricanum* (Fraser Jenkins (2021). But O. *lusitanicum* collected from different localities of Kerala during this study is different from *O. lusoafricanum. O. lusitanicum* are smaller than *O. lusoafricanum.* The tropophyll number is 1 – 2 in *O. lusitanicum*, where as *O. lusoafricanum* is characterized by single tropophyll. The fertile segment is 2.1 – 4 cm long in *O. lusitanicum* where as 3 – 8 cm long in *O. lusoafricanum*. By the detailed analysis of macro morphology, micro morphology, molecular characters and comparing with the type specimens, O. *lusitanicum* was found to be different from *O. lusoafricanum.* Hence the occurance of *O. lusitanicum* in India is confirmed.

Specimens examined: India: South India: **Kerala**: **Kasaragod Dist**.: Posadigumpe (12º40'33.55"N, 75º01'03.85"E), *Afsana Khan & Anto P. V. 31* (STC); **Thrissur Dist**.: Killannur (10° 36' 56.2968" N, 76° 14' 59.118" E) *Afsana Khan & Anto P. V. 7* (STC); Peruvanmala (10° 37' 20.4168" N, 76° 8' 4.8192" E), *Afsana Khan & Anto P. V.* 6 (STC).

6. *Ophioglossum lusoafricanum* Welw. ex. Prantl., Ber. Deutsch. Bot. Ges. 1: 351 (1883); Burrows, and Johns, Fl. Trop. Ea. Afr.-Ophi., 2001; Retief & Meyer, Plan. Of the fr. state inv. and idn. guide. 38: 1 – 1236, (2017); Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Khan *et al.*, PST. 186 – 195, 2023.

Type: Angola, apaungo Andongo, Welwitsch 34 (Isolectotype, K digital image!).

Fig. 22, 23, 24 & 25

Plants erect, up to 4 – 10 cm high, dark green; rhizome small, tuberous-elongated, light brown, 0.3 – 1 cm long, 0.5 – 1 cm in diam., unbranched; roots 0.6 – 1.3 cm long, 0.1 cm in diam.; stipe 0.7 – 1 cm, 0.1 – 0.3 cm in diam.; tropophylls one, originating away from the ground level, minute, dark green, glabrous, 0.5 – 1.5 cm

Fig. 22. *Ophioglossum lusoafricanum*: A. Habitat, B. Entire plant, C. Rhizome, D Tropophyll, E. Spike.

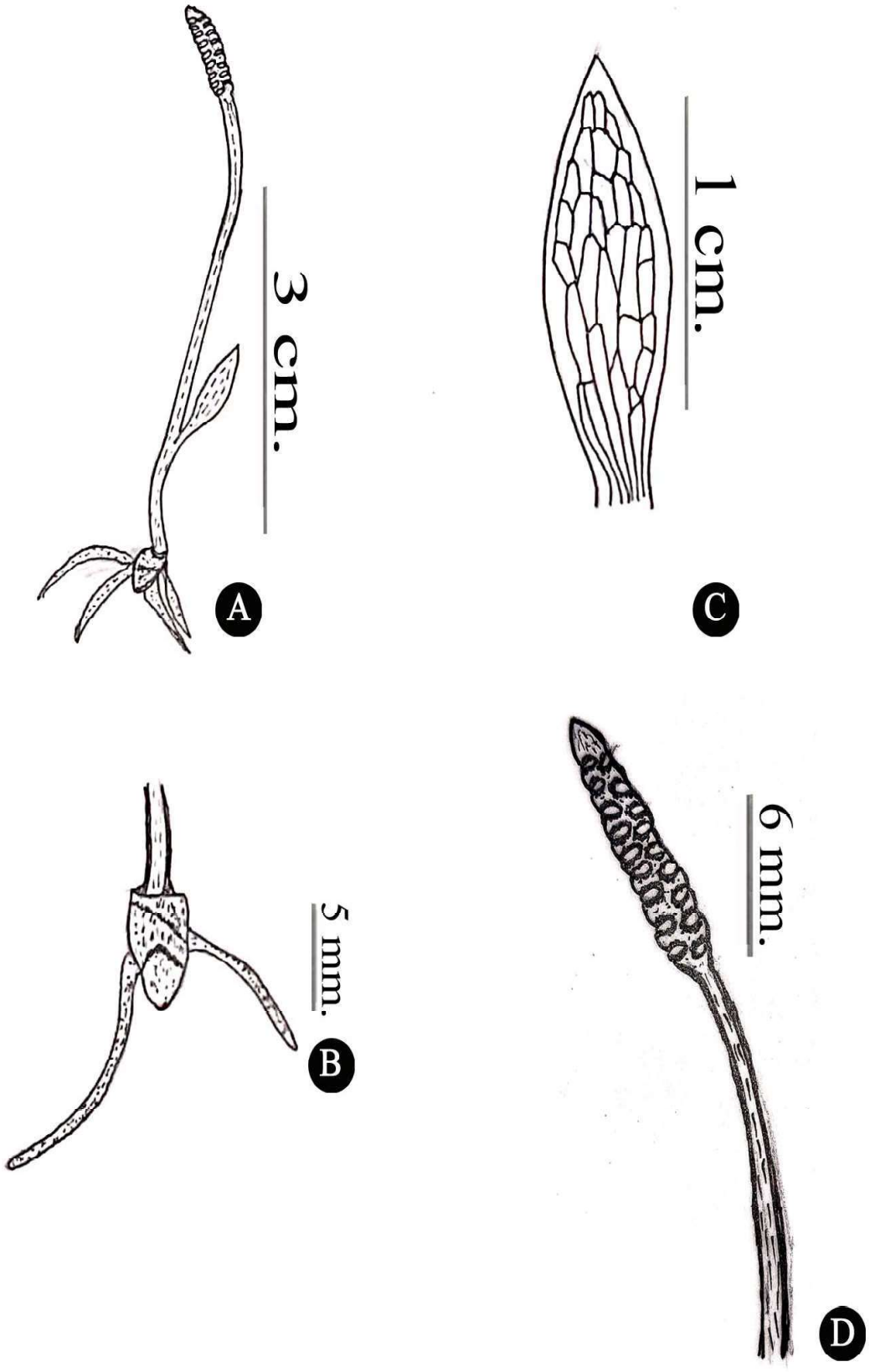

Fig. 23. Illustration of *Ophioglossum lusoafricanum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

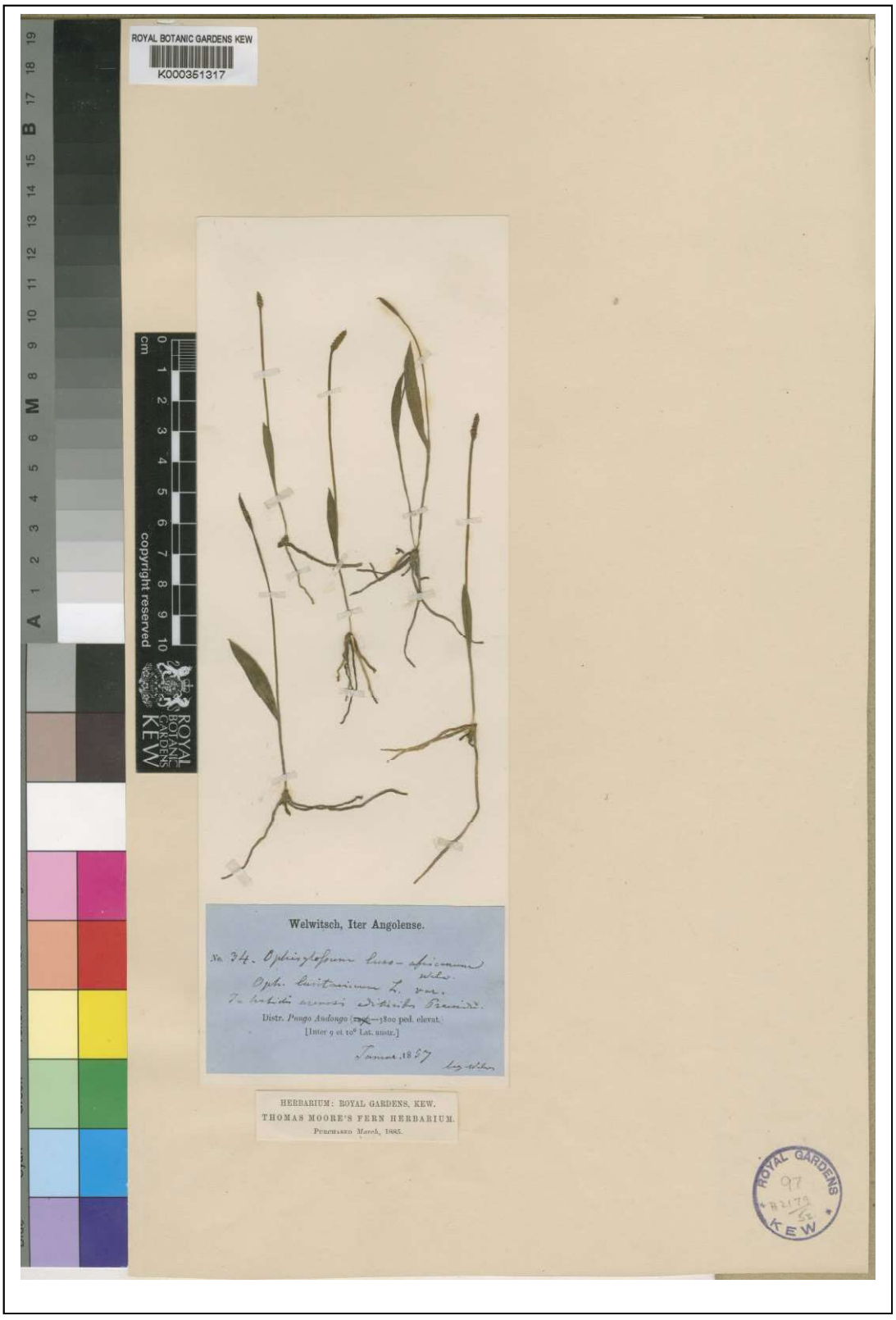

Fig. 24. Isolecto type of *Ophioglossum lusoafricanum*© The Board of Trustees of the RBG, Kew.

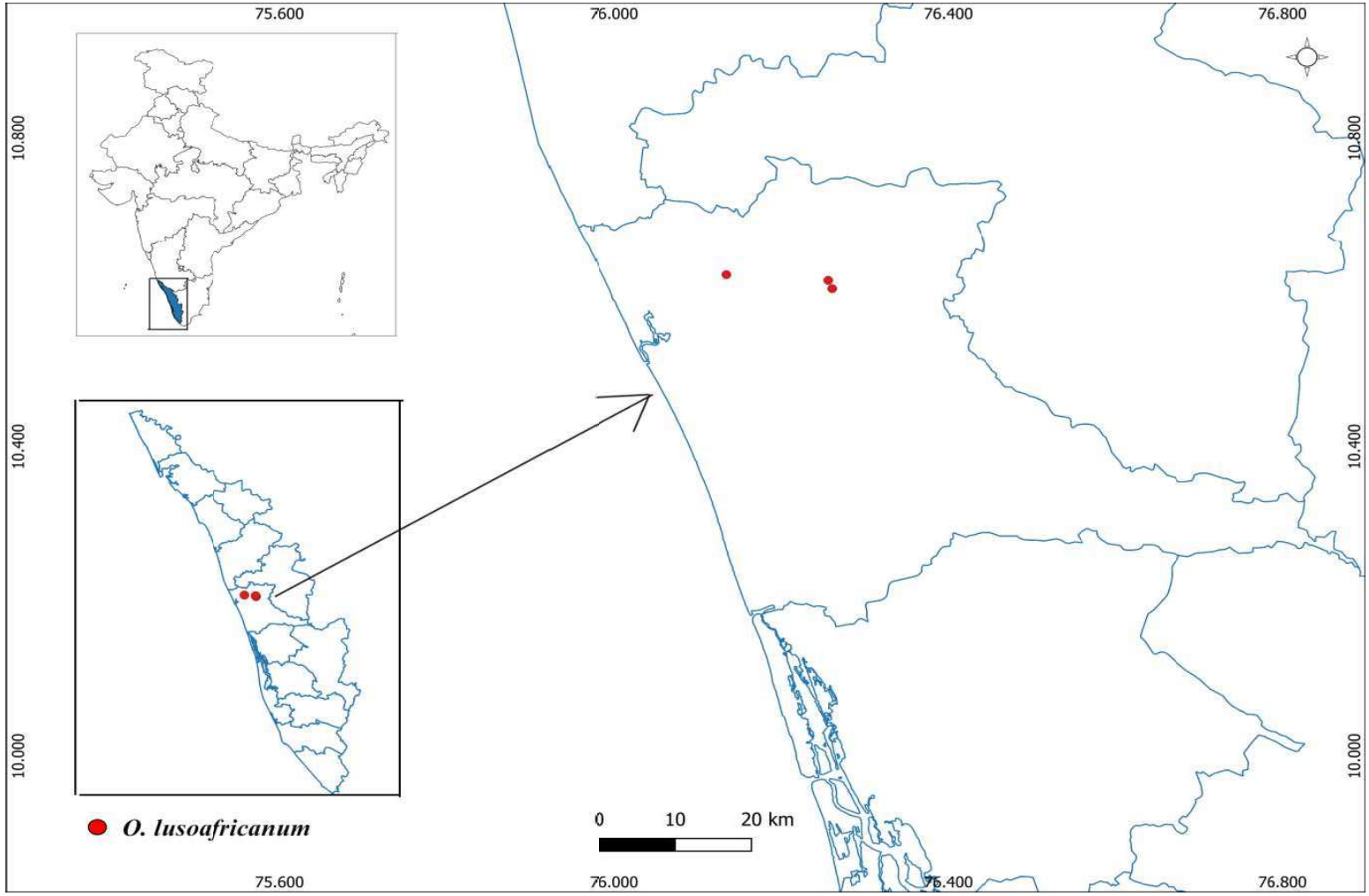

Fig. 25. Distribution map of *Ophioglossum lusoafricanum* in Kerala, India.

long, 0.2 – 0.4 cm wide, elliptic-lanceolate, acute at apex, attenuate at base; fertile segment 3 – 8 cm long, stalk dark green, 2.5 – 7 cm long, 0.1 – 0.2 cm in diam.; spike 0.5 – 1 cm long, 0.1 – 0.3 cm wide., with a short sterile pointed apex; sporangia 5 – 9 on either side, alternate.

Phenology: June-October.

Habitat and Ecology: Growing in open rock, intermingled with graminae members

Distribution: Africa, India.

Note: In Kerala, *O. lusoafricanum* showed a restricted distribution. It is distributed only in three adjascent localites of Thrissur District.*O. lusoafricanum* is morphologically comparable to *O. lusitanicum.* But *O. lusoafricanum* had single tropophyll whereas the *O. lusitanicum* had one-two tropophylls. *O. lusoafricanum* are large as compared to *O. lusitanicum*. The fertile segment is 3 – 8 cm long in *O. lusoafricanum* where as 2.1 – 4 cm long in *O. lusitanicum*. Notable differences are existing in the micromorphology and in molecular characteristics of *O. lusoafricanum* and *O. lusitanicum*.

Specimens examined: **India**: **Kerala**: **Thrissur Dist**: Cheppara (10° 36' 54"N 76° 15' 22.9998" E) *Afsana Khan & Anto P. V.* 47 (STC), Killannur (10° 37' 7.5828" N, 76° 15' 11.9628" E), *Afsana Khan & Anto P. V.*15 (STC), Peruvanmala (10° 37' 20.4162"N 76° 8' 4.8186" E) *Afsana Khan & Anto P. V. 46* (STC).

7. *Ophioglossum parvifolium* Grev. & Hook., Bot. Misc. 3: 218. 1833; Panigrahi and Dixit, Proc. Nat. Inst. Sci. India 35: 250. 1969; Patil and Dongare, I. F. J., 31:17 – 24. 2014; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 2: 1 – 573, 2018; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Fraser-Jenkins & Kandel, Fern. and Fern-Allies of Nep. 2: 1 – 446, 2019; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Chen *et al.*, Fern Gaz. 21: 292 – 419, 2022; Khan *et al.*, PST. 186 – 195, 2023.

Type: South India, Negapatam, Tamil Nadu, R. Wight, 1867 (Lectotype, K, digital image!).

Ophioglossum macrorrhizum Kunze in Analecta Pteridogr.: 2. 1837.

Ophioglossum schmidii Kunze in Linnaea 24: 246. 1851.

Ophioglossum vulgatum var. *macrorrhizum* (Kunze) Luerss. In J. Mus. Godeffroy 3: 242. 1875.

Ophioglossum luerssenii Prantl in Ber. Deutsch. Bot. Ges. 1: 352. 1883.

Ophioglossum tenerum Mett. ex Prantl in Ber. Deutsch. Bot. Ges. 1: 352. 1883.

Ophioglossum moluccanum f. *pumilum* Rac. In Natuurk. Tijdschr. Ned. -Indië 59. 237. 1900.

Ophioglossum pumilum (Rac.) Alderw. In Malayan Ferns: 774. 1909.

Ophioglossum schlechteri Brause in Bot. Jahrb. Syst. 49: 58. 1912.

Ophioglossum vulgatum var. *luerssenii* (Prantl) Domin in Biblioth. Bot. 20: 222. 1915.

Ophioglossum latifolium var. *macrorrhizum* (Kunze) R.T.Clausen in Mem. Torrey Bot. Club 19(2): 150. 1938.

Ophioglossum latifolium var. *tenerum* (Mett. ex Prantl) R.T. Clausen in Mem. Torrey Bot. Club 19(2): 146. 1938.

Fig. 26, 27, 28 & 29

Plants erect, minute, 2 – 6 cm high, green, glabrous; rhizome small, light brown, globose- elongated, with a brown coating, 0.2 – 0.5 cm long, 0.5 – 1 cm in diam., unbranched; roots vegetatively propagating, cylindrical, creeping, unbranched, 1 – 1.5 cm long, 0.1 – 0.2 cm in diam.; stipe 0.4 – 1 cm long, 0.1 – 0.3 cm in diam.; tropophylls one-two, originating very close to the substratum, green, glabrous, 0.2 – 1 cm long, 0.1 – 0.6 cm wide, elliptic-lanceolate-oblanceolate, obtuse-cuspidate-mucronate- rounded at apex, base attenuate; fertile segment 1.4 – 4.5 cm long, stalk 1 – 3.5 cm long, 0.1 – 0.3 cm in diam.; spike 0.4 – 1 cm long, 0.1 – 0.2 cm wide,.

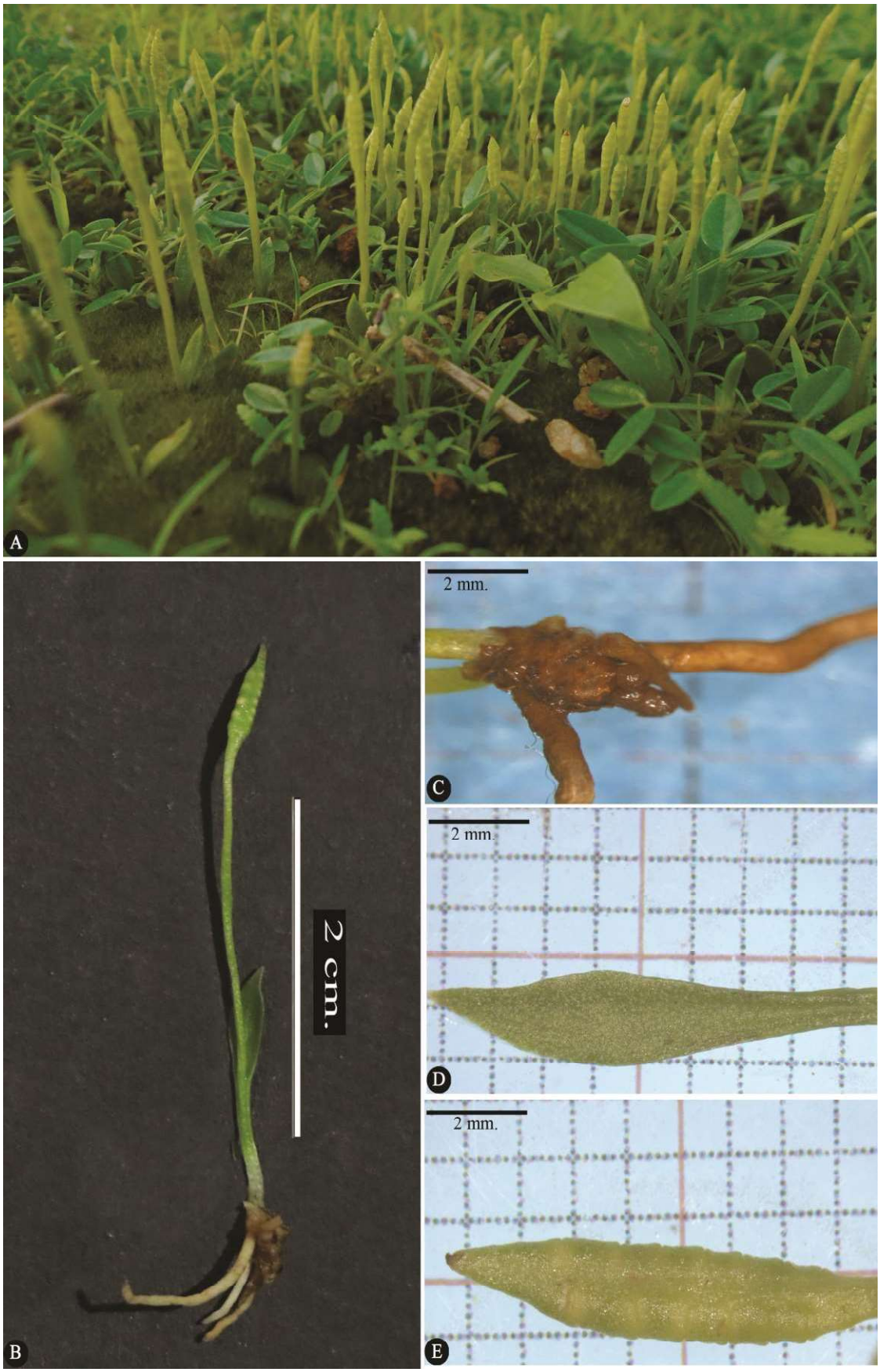

Fig. 26. *Ophioglossum parvifolium*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

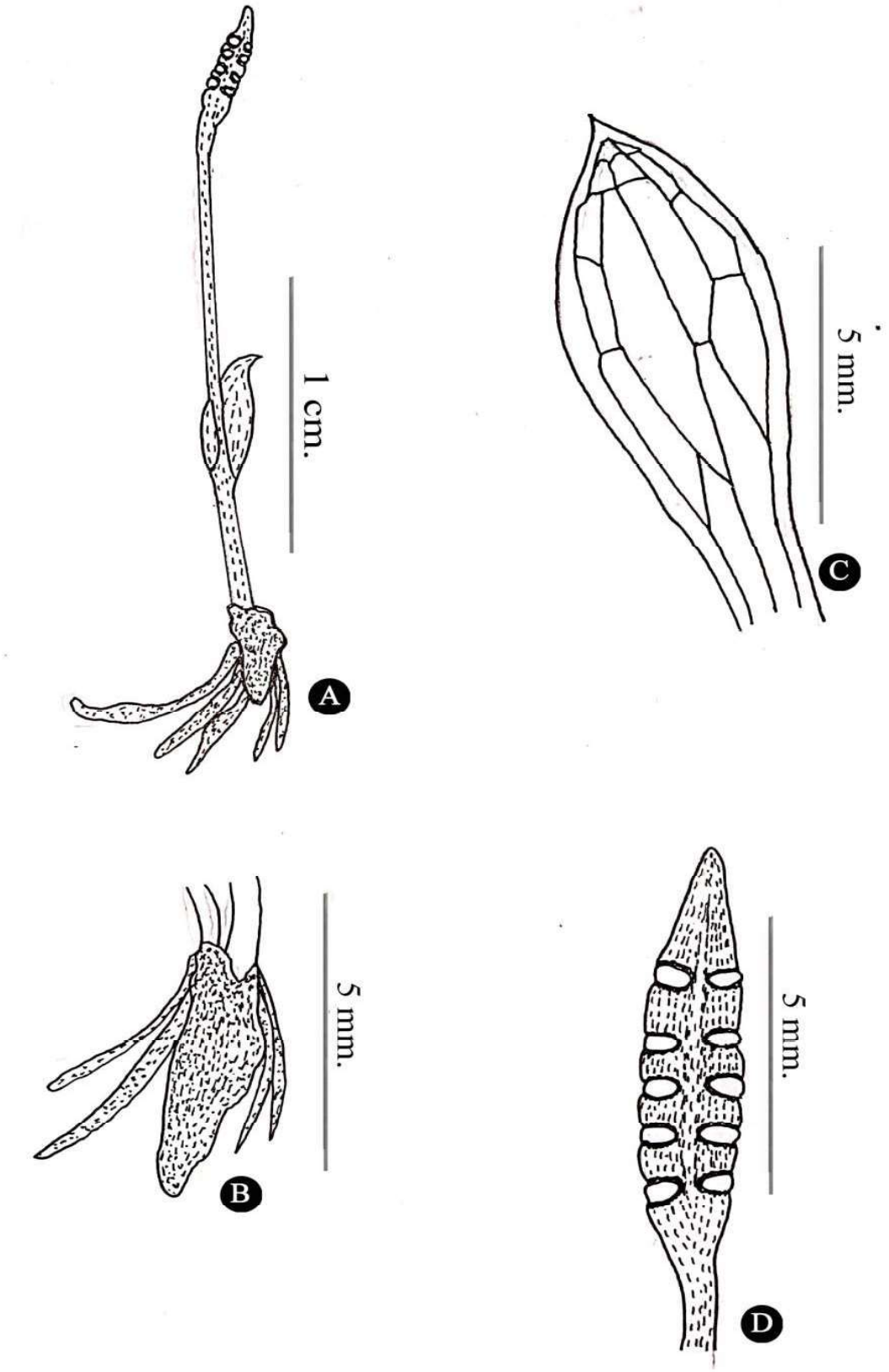

Fig. 27. Illustration of *Ophioglossum parvifolium*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, E. Spike.

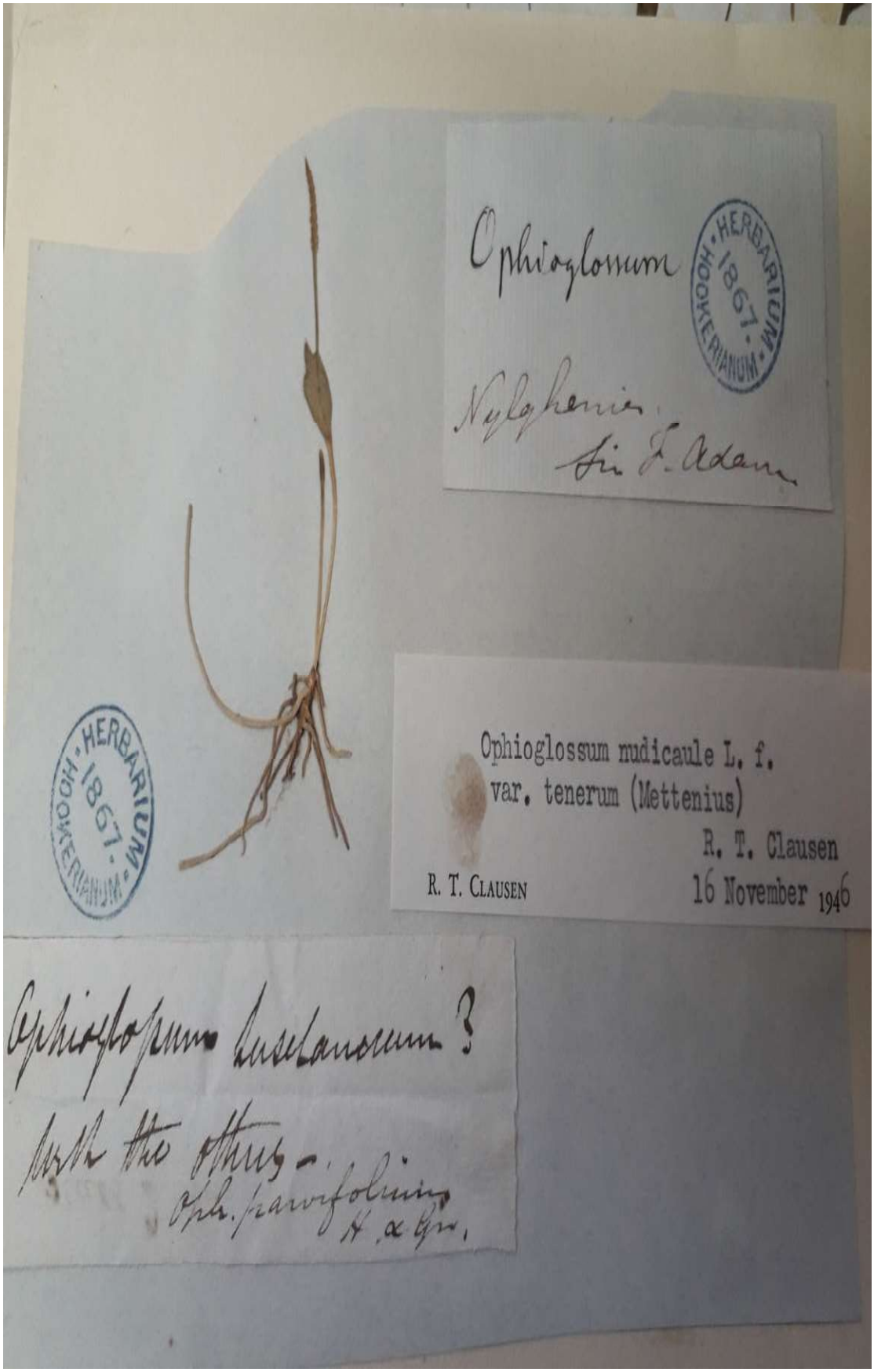

Fig. 28. Lecto type of *Ophioglossum parvifolium* © The Board of Trustees of the RBG, Kew.

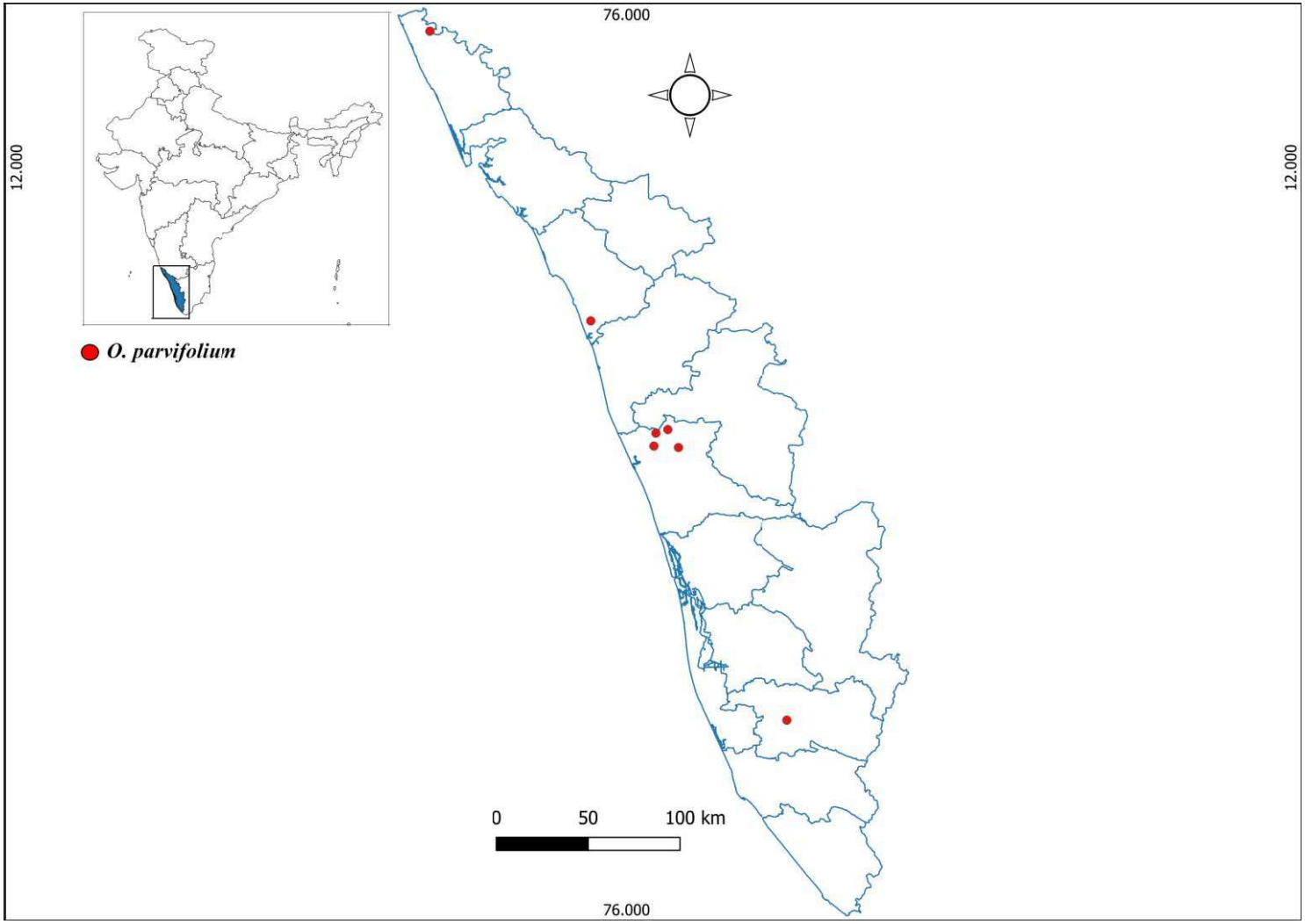

Fig. 29. Distribution map of *Ophioglossum parvifolium* in Kerala, India.

occasionally bifurcated, with a small sterile pointed apex; sporangia 4 – 6 on either side, opposite

Phenology: June-September.

Distribution: Africa, America, Australia, Asia: China, **India** (Andhra Pradesh, Arunachal Pradesh, Assam, Gujarat, Himachal Pradesh, Karnataka, **Kerala**, Madhya Pradesh, Maharashtra, Meghalaya, Rajasthan, Tamil Nadu, Uttarakhand, Uttar Pradesh, West Bengal) Indonesia, Japan, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Taiwan, Thailand.

Note: Many morphotypes of *O. parvifolium* was collected from different locations. They showed slight variation in the macro morphology. Rhizome varies from globose-elongated shape. In some population, the tropophyll arised very close to the substratum and in some other population tropophyll arised away from the substratum. This results in the variation in stipe length. The tropophylls also showed variation from elliptic-lanceolate-oblanceolate shape. Inspite of these small variations, all the morphotypes showed consistant micromorphologycal and molecular characters.

Specimens examined: India: South India: Kerala: **Kasargode Dist.:** Posadigumpe (12°40'33.55"N, 75°01'03.85"E), *Afsana Khan & Anto P. V.*, 33 (STC). **Kozhikode Dist.:** Zamorin' s Guruvayurappan College (11° 14' 31.4118"N 75° 49' 22.0902" E) *Afsana Khan & Anto P. V. 27* (STC), **Pathanamthitta Dist.:** Chuttippara (9° 15' 56.1414" N76° 47' 45.8946"E) 37 (STC), **Thrissur Dist.:** Cheppara (10° 36' 54" N76° 15'22.9998"E) *Afsana Khan & Anto P. V.* 48 (STC), Cheruchakkichola (10°42'13. 82"N, 76°12'12.39"E). *Afsana Khan & Anto P. V.* 8 (STC), Pazhiyottumuri (10° 41' 10.899" N 76° 8' 40.452"E), *Vimal K.R*, *Afsana Khan & Anto P. V.*, 40. (STC), Peruvanmala. (10° 37' 20.4168" N 76° 8' 4.8192" E), *Afsana Khan & Anto P. V.* 5 *(*STC).

8. *Ophioglossum pendulum* L. Sp. Pl., ed. 2. 2: 1518. 1763. Mahabale, Bull. Bot. Surv. India. 1 – 4, 1962; Thothathri *et al.*, Bull. Bot. 352 - Surv.India ll (3): 347 – 349.1969; Augustine *et al.*, 1. Econ. Tax. Bot. 18: 445. 1994. Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Khan *et al.*, PST. 186 – 195, 2023.

Lectotype: Indonesia, Maluku "Scolopendria major Rumphius, Herb. Amboin., 6: 84, t. 37, f. 3, 1750".

Ophioderma pendulum L. C.Presl in Suppl. Tent. Pterid.: 56. 1845.

Ophioglossum furcatum J.Sm. in Ferns Brit. For.: 272. 1866, nom. nud.

Ophioglossum moultonii Copel. in J. Straits Branch Roy. Asiat. Soc. 63: 72. 1912.

Fig. 30, 31, 32 & 33

Plants epiphytic, green, ribbon like, hanging downward from the branch of a host plant, 23 – 33 cm long; rhizome short, 0.7 – 2.4 cm long, creeping, horizontal-rounded; stipe 0.3 – 0.6 cm long, 0.2 – 0.3 cm in diam., tropophylls long, 3 – 4 in number, 22 – 30 cm long, 0.5 – 1.5 cm wide, linear-pendulous, ribbon like, green, fleshy, base tapering, unbranched or branched towards the apex, margin entire, base tapering, apex obtuse, fertile segment 13 – 19 cm long, situated nearly middle of the tropophyll, stalk 1 – 3 cm long, 0.2 – 0.5 cm in diam. spike solitary, unbranched, 12 – 16 cm long, 50 – 80 in number, alternate.

Phenology: June-September.

Habitat and Ecology: Epiphytic plants seen in associated with *Nephrolepis* species Inside the Periyar Tiger Reserve.

Distribution: Africa, America, Australia, Asia: Bangladesh, China, **India** (Arunachal Pradesh, Assam, **Kerala** (Periyar tiger reserve), Sikkim), Pacific islands.

Note: *O. pendulum* is the only one epiphytic species found in genus *Ophioglossum*. In Kerala, *O. pendulum* is present only inside the Periyar Tiger Reserve, Idukki District. The pendulous and ribbon like nature of the tropophylls makes it unique and distinguishable from other species of *Ophioglossum*.

Specimens examined: **India**: **South India**: **Kerala**: Idukki Dist., Periyar Tiger Reserve. *Jomy Augutine 12832*, (KFRI).

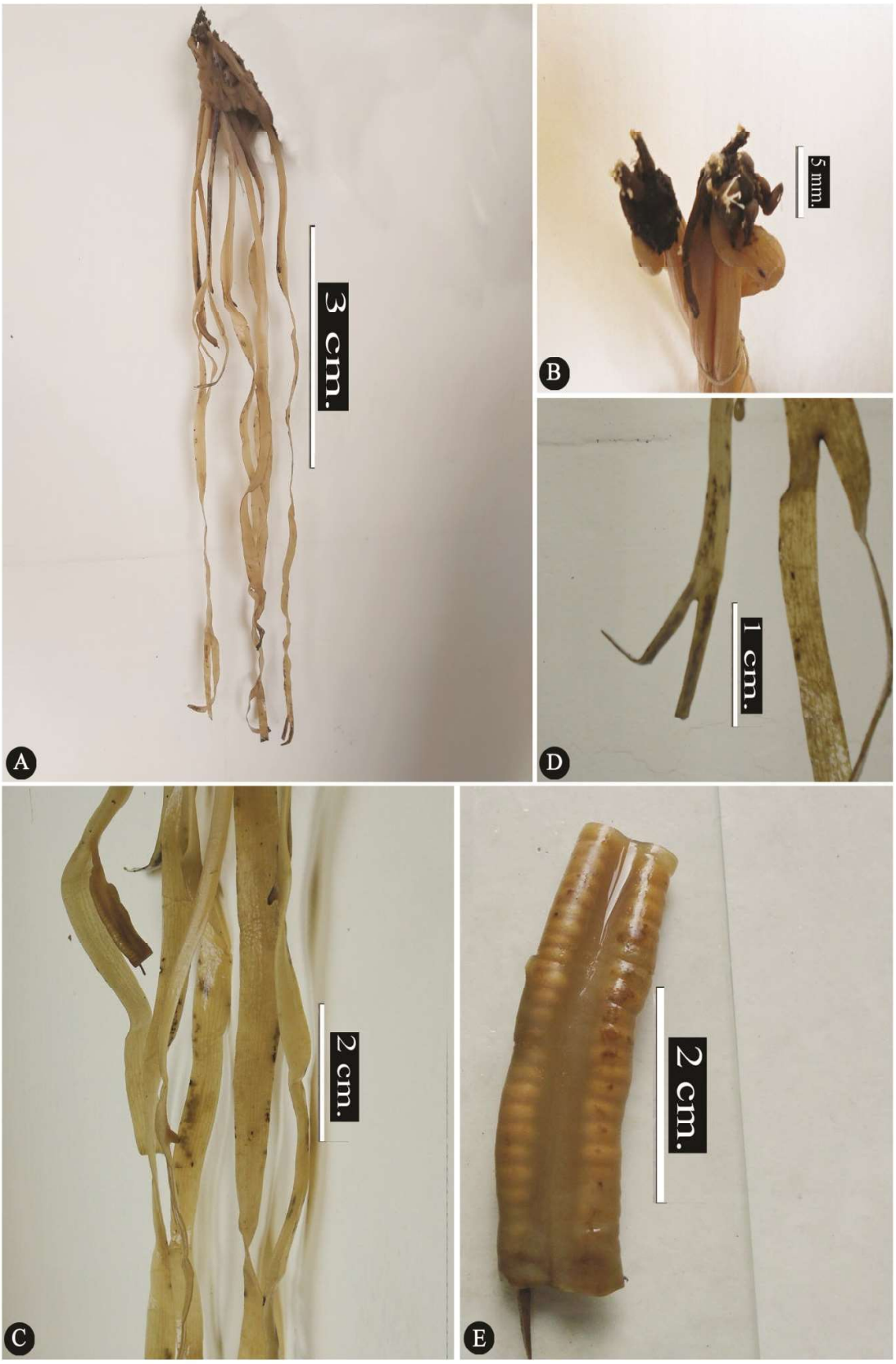

Fig. 30. *Ophioglossum pendulum*. A. Entire plant, B. Rhizome, C. Tropophyll, D. Bifurcation at tropophyll tip, E. A part of the Spike.

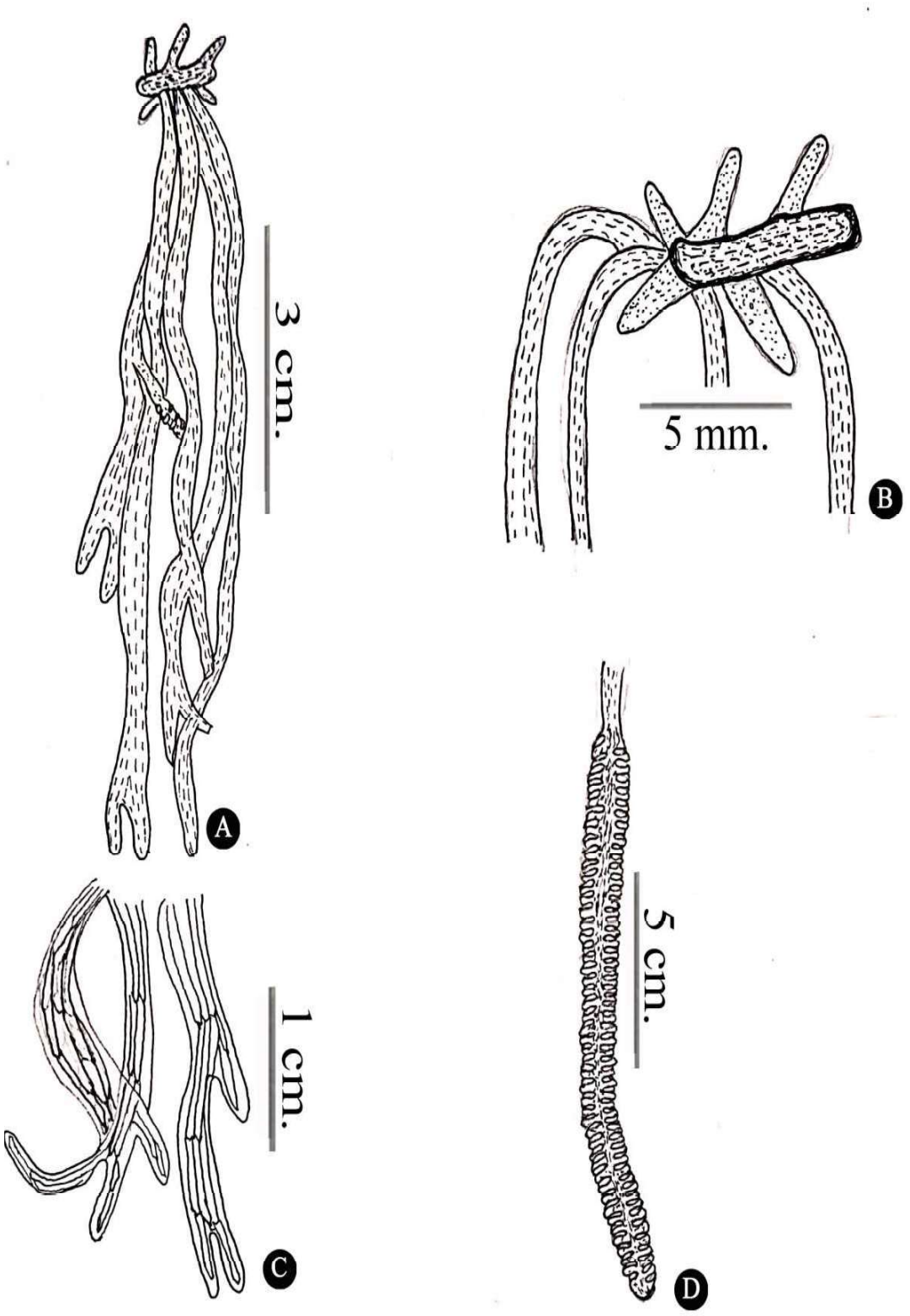

Fig. 31. Illustration of *Ophioglossum pendulum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

Fig. 32. Linnean specimen of *Ophioglossum pendulum*©The Board of Trustees of the Linnean society (LINN), London.

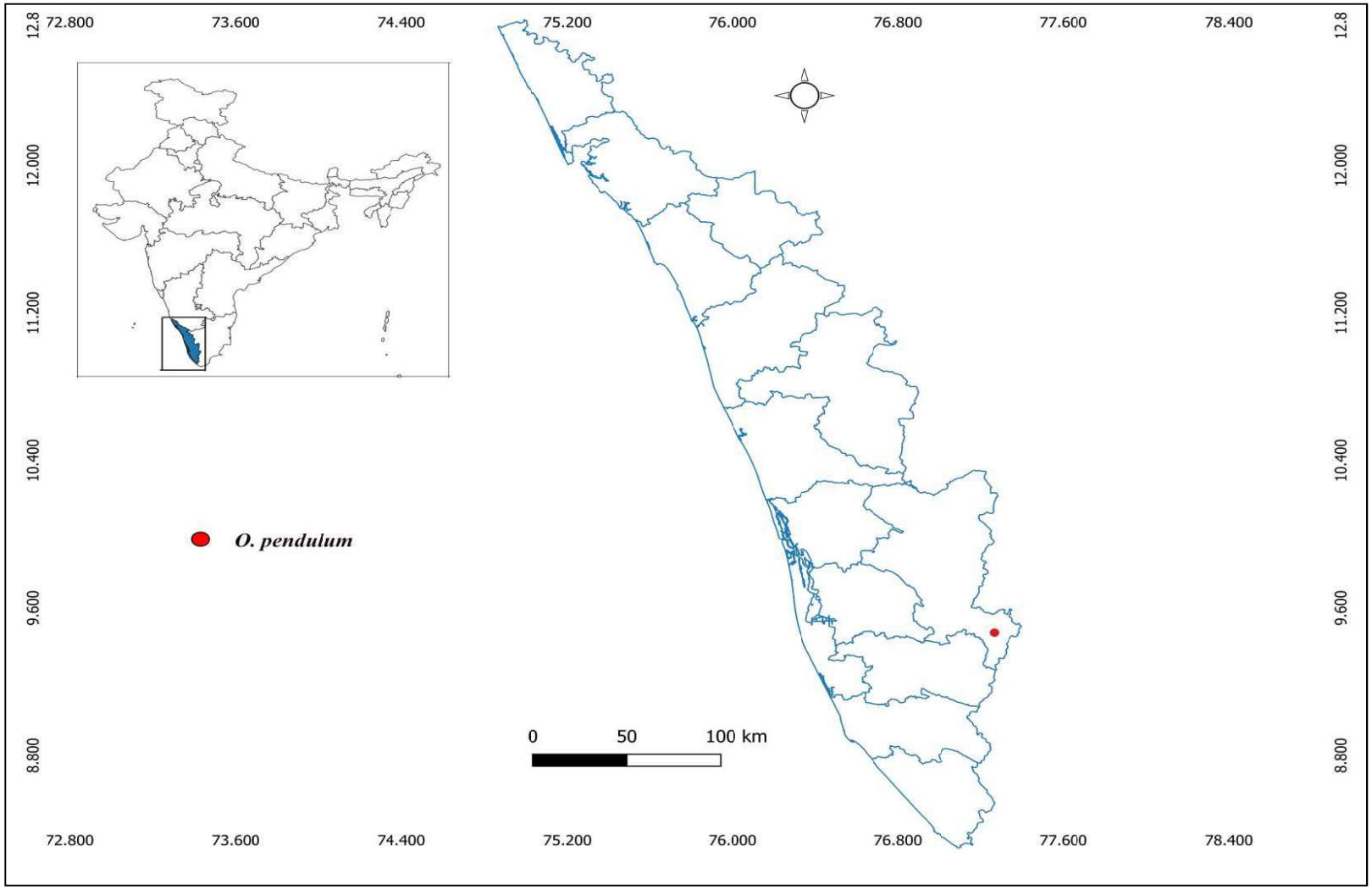

Fig. 33. Distribution map of *Ophioglossum pendulum* in Kerala, India

9. *Ophioglossum petiolatum* Hook.,Exot. Fl. 1(4): t.56. 1823; Panigrahi & Dixit, Proc. Natl. Inst.Sci. 35. 260-61 (1969); Manickam, Fern Fl. Palani Hills 14. 1986; Manickam & Irudayaraj Pterid. Fl. W. G. 52. t. 30 (1992); Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Khan *et al.* PST. 186 – 195, 2023.

TYPE: West Indies, Shepperd (K, digital image!).

Ophioglossum moluccanum Schltdl. in Adumbr. Pl.: 9.1825.

Ophioglossum elongatum A.Cunn. in Compan. Bot. Mag. 2: 361.1837.

Ophioglossum moluccanum f. *complicatum* Miq. in Ann. Mus. Bot. Lugduno-Batavi 4: 290. 1870.

Ophioglossum vulgatum var. *australasiaticum* Luerss. in J. Mus. Godeffroy 3: 246.1875.

Ophioglossum litorale Makino in J. Jap. Bot. 6: 27.1929.

Ophioglossum floridanum E.P.St.John in Amer. Fern J. 26: 53.1936.

Ophioglossum floridanum f. *favosum* E.P.St.John in Amer. Fern J. 26: 54.1936.

Ophioglossum floridanum f. *reticulosum* E.P.St.John in Amer. Fern J. 26: 55.1936.

Ophioglossum reticulatum f. *complicatum* (Miq.) Wieff. in Blumea 12: 330.1964.

Fig. 34, 35, 36 & 37

Plants erect, large, 8 – 13 cm high, green, glabrous; rhizome small, light brown, fusiform, with a brown coating, 0.5 – 1 cm long, 0.5 – 1 cm in diam., unbranched; roots, cylindrical, unbranched, 0.5 – 1 cm long, 0.1 – 0.3 cm in diam.; stipe large, 4.5 – 7 cm long, 0.1 – 0.2 cm in diam.; tropophyll one, green, glabrous, elliptic-rounded, obtuse-rounded at apex, cuneate at base, margin entire, 2 – 3 cm long, 1.5 – 2.5 cm wide, fertile segment 3 – 5 cm long, stalk 2 – 3 cm long, 0.1 – 0.3 cm in diam.; spike

Fig. 34. *Ophioglossum petiolatum:* A. Habitat, B. Entire plant, C. Rhizome, D. tropophyll, E. Spike.

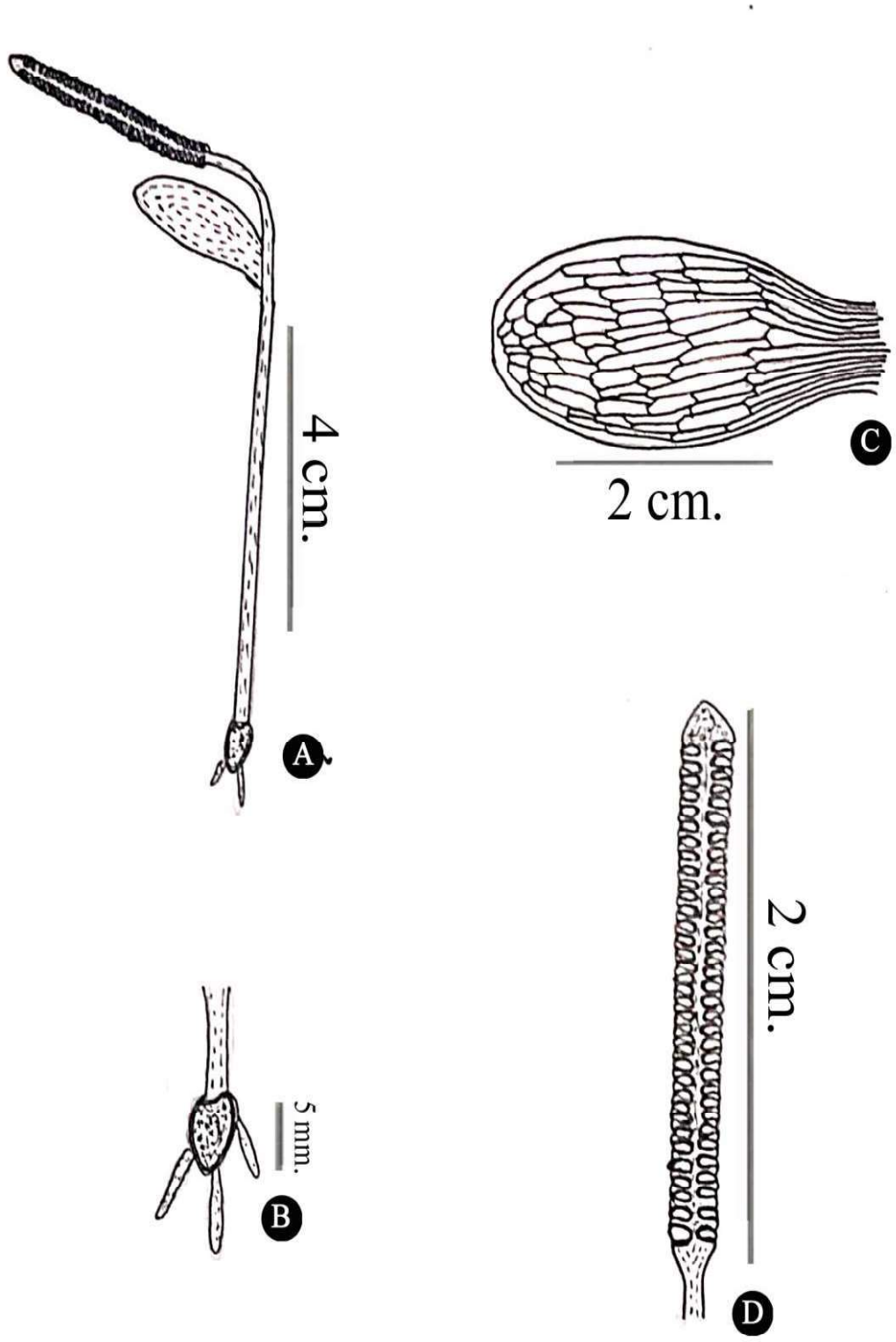

Fig. 35. Illustration of *Ophioglossum petiolatum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

Fig. 36. Type specimen of *Ophioglossum petiolatum*©The Board of Trustees of the RBG, Kew.

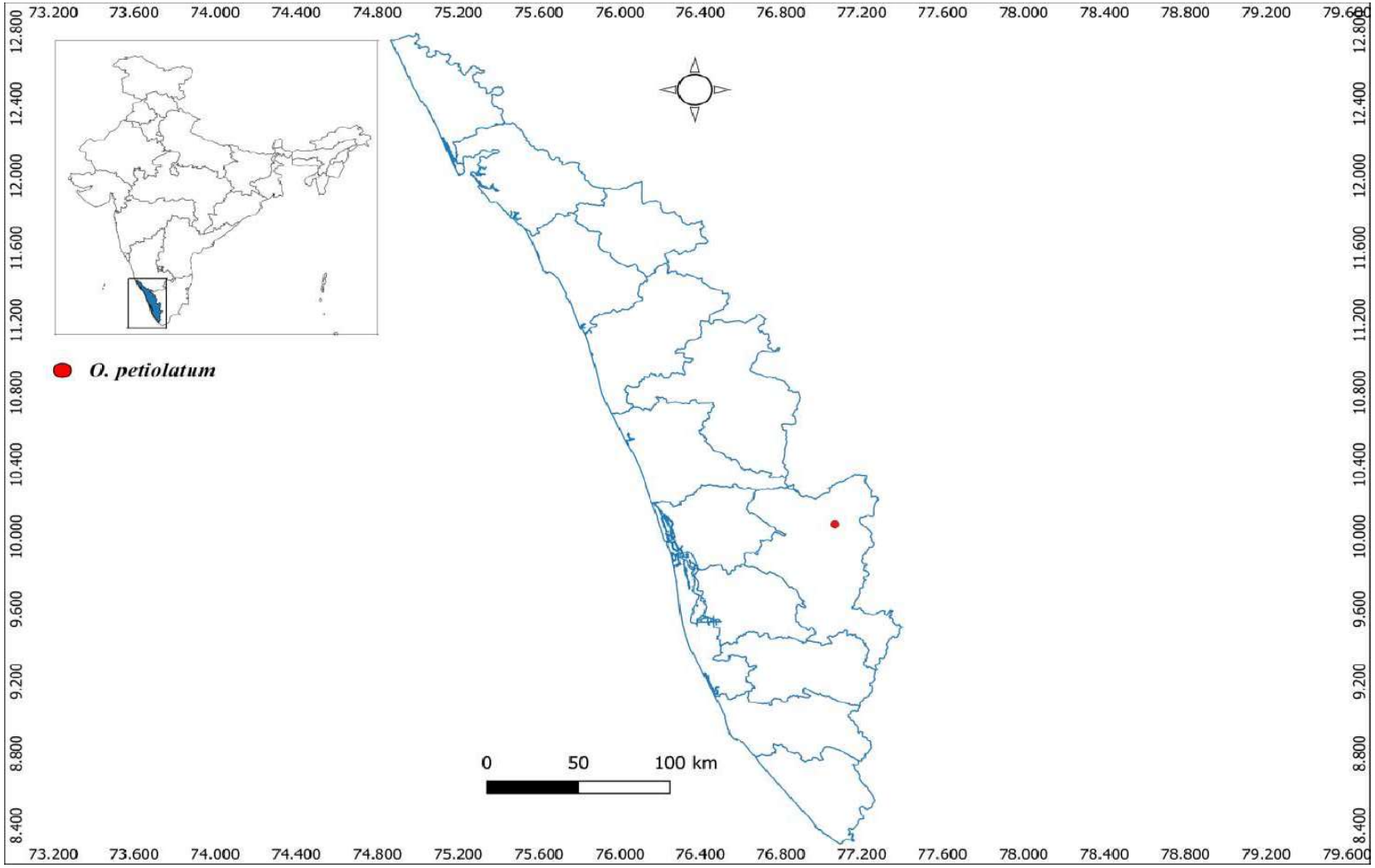

Fig. 37. Distribution map of *Ophioglossum petiolatum* in Kerala, India.

1 – 2 cm long, 0.1 – 0.2 cm wide, cylindrical, with a small sterile pointed apex; sporangia 25 – 28 on either side, alternate.

Phenology: June-December.

Habitat and Ecology: River Banks.

Distribution: America, Australia, Asia: Bhutan, China, **India** (Andhra Pradesh, Arunachal Pradesh, Assam, Chandigarh, Chhattisgarh, Himachal Pradesh, Jammu & Kashmir, **Kerala**, Madhya Pradesh, Meghalaya, Nagaland, Punjab, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal), Indonesia, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, Tibet.

Note: *O. petiolatum* is not common In Kerala. It could collect only from Munnar of the Idukki Dist rict. *O. petiolatum* is characterised by long petiole, single elliptic-rounded tropophyll. Rounded tropophylls are more common in *O. petiolatum* found in Kerala.

Specimens examined: **India: South India: Kerala: Idukki Dist.**: Munnar (10° 4' 5.988" N, 77° 4' 0.984" E) *Stephen Sequeira, Afsana Khan & Anto P. V.* 14 (STC). **Tamilnadu: Nilgiri Dist.** *D. B. Deb*, 31732 (MH).

10. *Ophioglossum raphaelianum* Anto, Afs.Khan, F.Francis & I.Antony, Int. J. Advanced Res. 4(5): 1269, f.1-2. 2016.; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Khan *et al.*, PST. 186 – 195, 2023.

Type: Kerala, East Mangad, Anto P. V. 112 (Isotype, MH!).

Ophioglossum malviae M. Patel & M. N. Reddy in Sci. Rep.8 (art. 5911): 1. 2018.

Goswamia malviae (M. Patel & M. N. Reddy) Li Bing Zhang & Liang Zhang in

Molec. Phylogen. Evol. 173-107512: 24. 2022.

Goswamia raphaeliana (Anto, Afs. Khan, F. Francis & I. Antony) Li Bing Zhang

& Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24. 2022.

Fig. 38, 39, 40 & 41

Plants erect, minute, 1.4 – 4 cm high, olivaceous or bluish gereen, glabrous; rhizome small, light brown, globose-ellipsoid, with a brown coating, 0.3 – 0.7 cm long, 0.9 – 1.2 cm in diam., unbranched; roots cylindrical, unbranched, 0.5 – 1 cm long, 0.1 – 0.2 cm in diam.; stipe 0.1 – 0.3 cm long, 0.2 – 0.4 cm in diam.; tropophylls one-two, subterranean, olivaceous or bluish green in colour, glabrous, 0.2 – 0.5 cm long, 0.3 – 0.4 cm wide, mostly widely ovate-orbicular, occasionally elliptic, obtuse at apex, base obtuse-rounded, either lying flat on the ground or slightly upward directed from the ground, margin entire; fertile segment 1 – 3 cm long, stalk 0.7 – 2 cm long, 0.1 – 0.2 cm in diam.; spike 0.3 – 1 cm long, 0.1 – 0.3 cm wide, with a small sterile pointed apex; sporangia 6 – 7 on either side, alternate.

Phenology: June-July.

Habitat and Ecology: Growing on laterite rocks. only a few *O. raphaelianum* plants could be found in the field. *O. gramineum*, *O. lusitanicum,* were also found in the habitat.

Distribution: **India**: Kerala.

Note: In agree with the opinion of Fraser-Jenkins *et al.*, (2021), *O. malviae* is conspecific with *O. raphaelianum.* The plant height is comparable for *O. malviae* (1 – 3 cm) and *O. raphaelianum* (1.4 – 4). The sub globose rhizome, ovate – obovate tropophylls, 4 – 8 pairs of sporangia, spore characters and molecular charcters of *O. malviae* is similar with the charactes of *O. raphaelianum.* Hence *O. malviae* is considered as a synonym of *O. raphaelianum* in this study.

Fraser-Jenkins *et al.*, (2021) synonimised *O. raphaelianum* as the synonym of *O. rubellum.* But the present study collected seperate populations of *O. raphaelianum* and *O. rubellum* from different locations of Kerala. By the detailed examination of macro, Micromorphological characters, molecular phylogenetic characters and comparing with the type specimens of both the species, this study recognized *O. raphaelianum* and *O. rubellum* as two distinct species and hence the species status of *O. raphaelianum* is reinstated.

Specimens examined: India: South India: Kerala: Thrissur Dist.: Mangadu, Kottappuram (10° 41' 21.876" N76° 11' 43.3788" E) *Afsana Khan & Anto P.V.* 2

Fig. 38. *Ophioglossum raphaelianum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

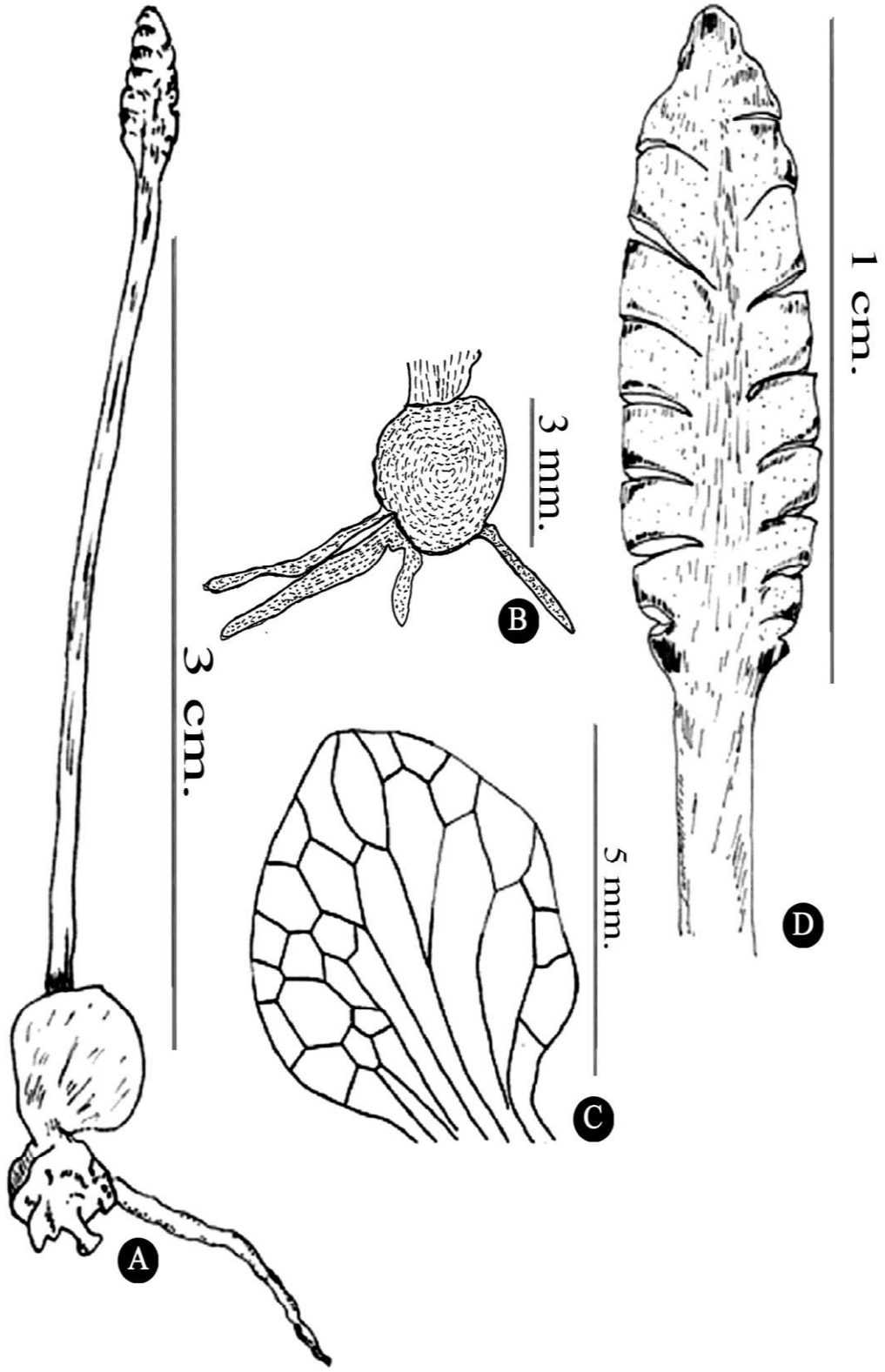

Fig. 39. Illustration of *Ophioglossum raphaelianum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

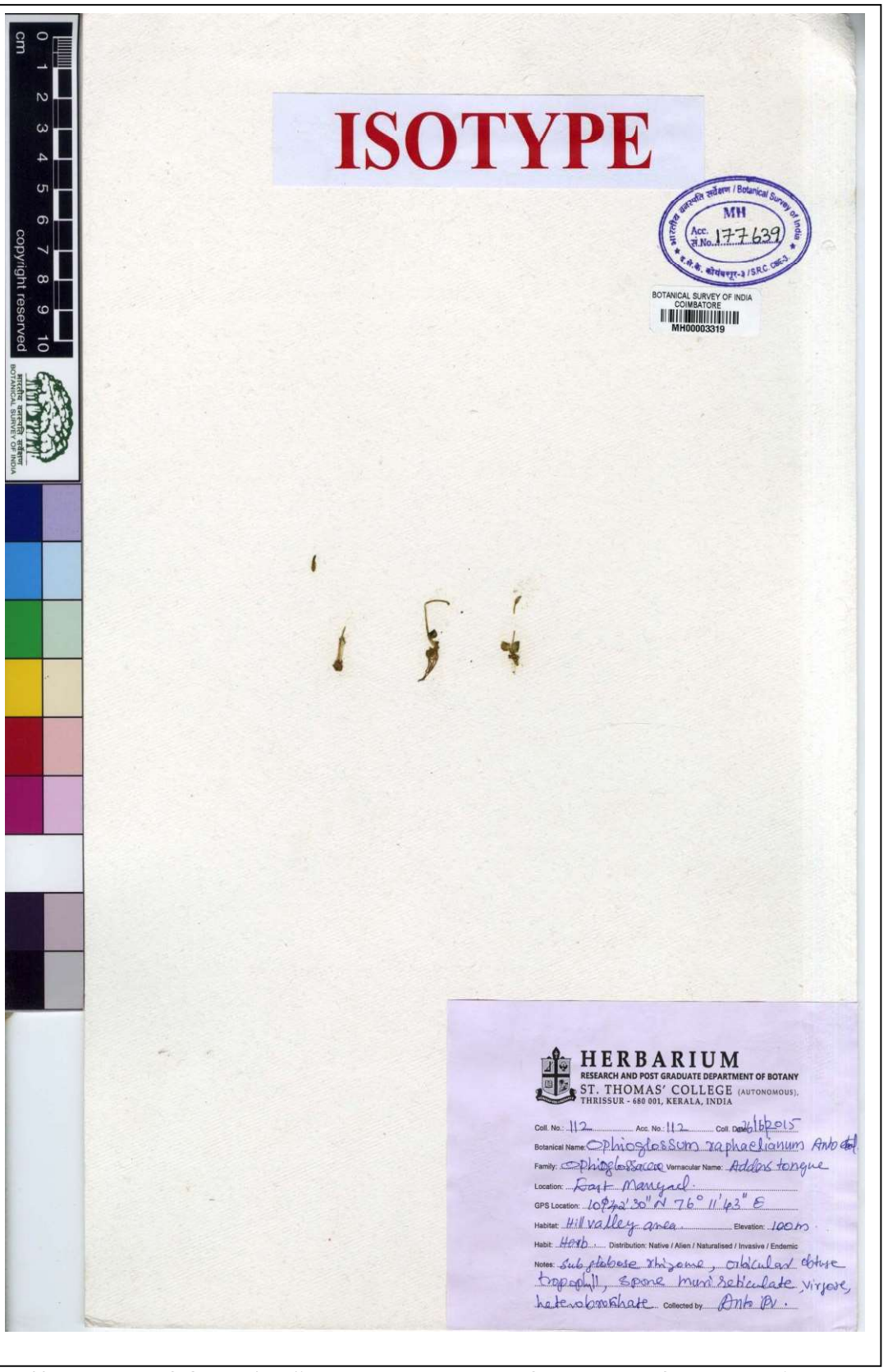

Fig. 40. Isotype of *O. raphaelianum*©The Board of Trustees of the Botanical Survey of India.

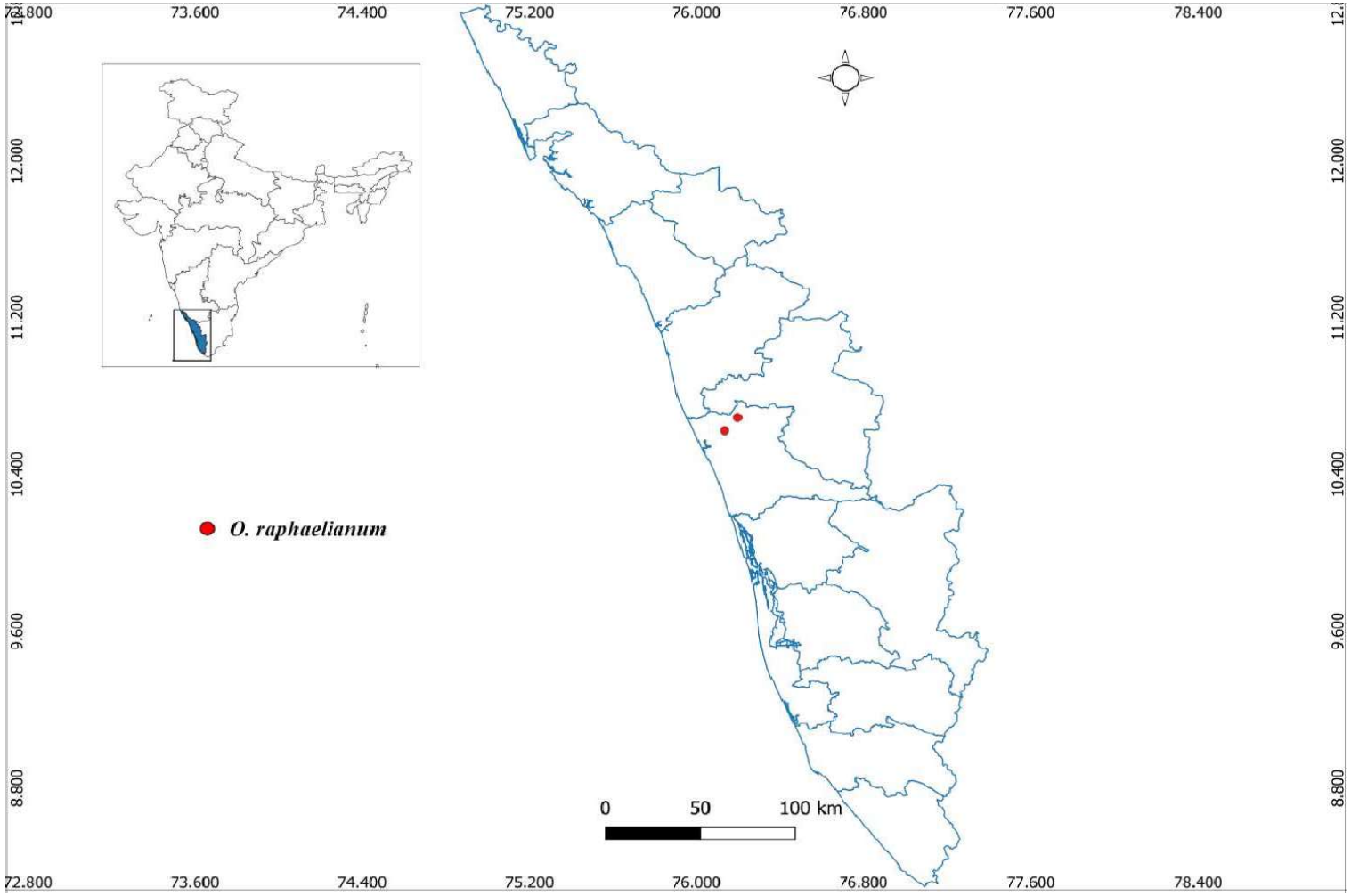

Fig. 41. Distribution map of *Opioglossum raphaelianum* in Kerala, India.

(STC), Peruvanmala (10° 37' 21.7128" N 76° 8' 4.6968" E) *Afsana Khan & Anto P.V.* 112 (CAL!).

11. *Ophioglossum reticulatum* L., Sp. Pl. 2: 1063 (1753). Beddome, Ferns. South. India 23. t. 70. 1863; Beddome, Handb. Ferns. Bri. India, 465. t. 290 (1883); Balakrishnan *et al.*, Bull. Bot. Surv. India 2: 337. 1960; Mahabale, Nelumbo, 4 (1 – 4), 71– 84, 1962; Panigrahi & Dixit, Proc. Nat. Inst. Sci. India 35. 257. 1969; Manickam & Irudayaraj, Pterid. Fl. West Ghats: 51. t. 29. 1992; Patil and Dongare, I. F. J. 31:17 – 24. 2014; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Khan *et al.*, PST. 186 – 195, 2023.

Type: Lectotype (Tardieu Blot, in Aubreville, Flor. Gabon 8: 30. 1964): from C. America

Ophioglossum ovatum Bory in Voy. iles Afrique 2: 206. 1804.

Ophioglossum cordifolium Roxb. In Numer. List: n. 47; Roxb. Calc. Journ. 1829.

Ophioglossum peruvianum C. Presl in Suppl. Tent. Pterid.: 52. 1845.

Ophioglossum cognatum C. Presl in Suppl. Tent. Pterid.: 53. 1845.

Ophioglossum cumingianum C. Presl in Suppl. Tent. Pterid.: 52. 1845.

Ophioglossum timorense Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 93. 1868.

Ophioglossum obovatum Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 93. 1868.

Ophioglossum moluccanum f. *dilatatum* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 92. 1868.

Ophioglossum vulgatum var. *minutum* F.M. Bailey in Bot. Bull. Dept. Agric. Queensland 5: 27. 1892.

Ophioglossum reticulatum var. *acutius* Christ in Denkschr. Kaiserl. Akad. Wiss., Wien. Math. -Naturwiss. Kl. 79(1): 56. 1908.

Ophioglossum reticulatum var. *polyangium* Christ in Denkschr. Kaiserl. Akad. Wiss., Wien. Math. -Naturwiss. Kl. 79(1): 56. 1908.

Ophioglossum usterianum Christ in A. Uster, Fl. Umgebung São Paulo: 137. 1911.

Ophioglossum raciborskii Alderw. in Bull. Jard. Bot. Buitenzorg, sér. 2, 28: 35. 1918.

Ophioglossum pedunculatum Desv. & Nakai in Bot. Mag. (Tokyo) 40: 373. 1926.

Ophioglossum ramosii Copel.in Philipp. J. Sci. 56: 97. 1935.

Ophioglossum austroasiaticum Nishida in J. Jap. Bot. 34: 46. 1959.

Ophioglossum reticulatum f. *dilatatum* (Miq.) Wieff. in Blumea 12: 329. 1964.

Ophioglossum holm-nielsenii B. ollg. in Fl. Ecuador 66: 16. 2001.

Ophioglossum aletum M. Patel, M.N. Reddy & H.K. Goswami in Indian Fern J. 35: 323. 2018.

Ophioglossum chaloneri H.K. Goswami, M. Patel & K.K. Nag in Phytotaxa 468: 103. 2020.

Fig. 42, 43, 44 & 45

Plants erect, large, 15 – 27 cm high, dark green, glabrous; rhizome small, light brown, fusiform, with a brown coating, 0.5 – 1 cm long, 0.6 – 1.5 cm in diam., unbranched; roots cylindrical, unbranched, 1 – 5 cm long, 0.1 – 0.2 cm in diam.; stipe 7– 11 cm long, 0.1 – 0.3 cm in diam.; tropophylls one-two, dark green, glabrous, 4 – 6 cm long, 2 – 4 cm wide, cordate, obtuse at apex, cordate at base; fertile segment 7.5 – 15 cm long, stalk 6.5 – 12 cm long, 0.1 – 0.3 cm in diam.; spike 1 – 3 cm long, 0.7 – 1 cm wide, with a small sterile pointed apex; sporangia 20 – 35 on either side, alternate.

Phenology: June-October.

Habitat and Ecology: Found in the slopes of tea estates in higher altitudes and under the tree shade.

Distribution: Africa, America**,** Australia**,** Asia: Bangladesh, Bhutan, China, **India** (Andhra Pradesh, Arunachal Pradesh, Assam State, Bihar, Chhattisgarh, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka, **Kerala**, Madhya Pradesh, Maharashtra, Meghalaya, Mizoram, Odisha, Rajasthan, Sikkim, Tamil Nadu, Tripura,

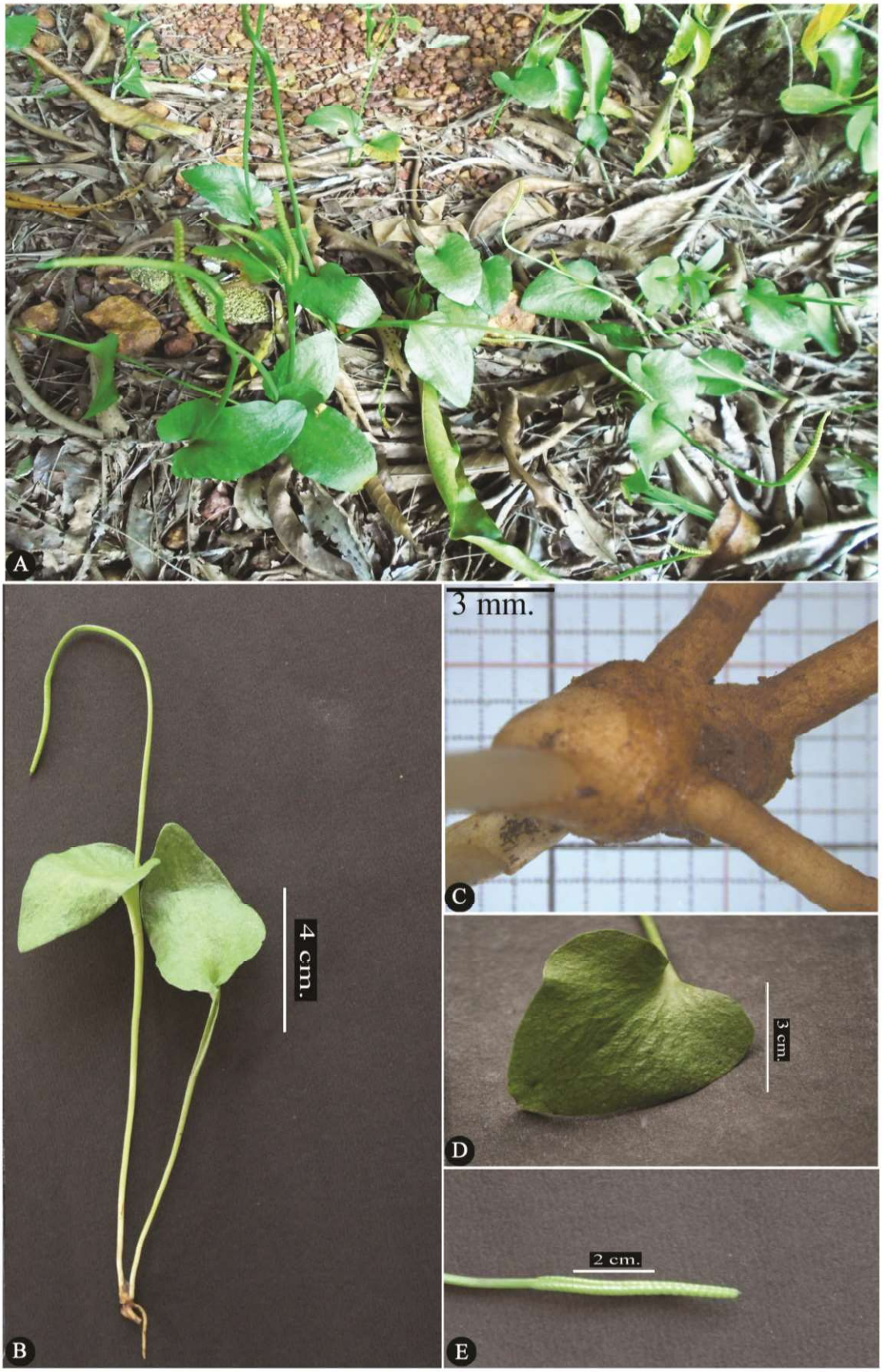

Fig. 42. *Ophioglossum reticulatum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

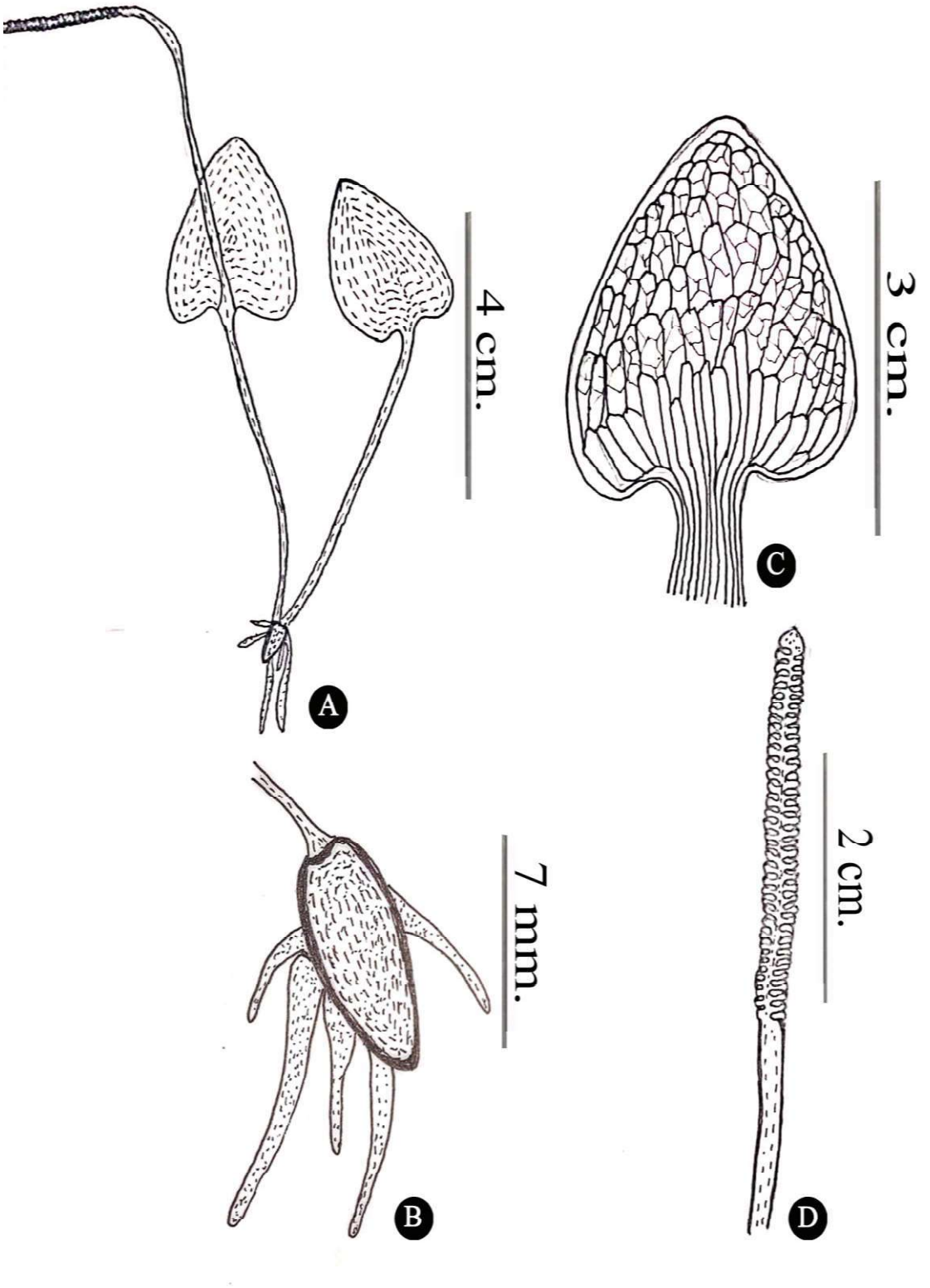

Fig. 43. Illustration of *Ophioglossum reticulatum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

Fig. 44. Linnean specimen of *Ophioglosssum reticulatum*©The Board of Trustees of the Linnean society (LINN), London.

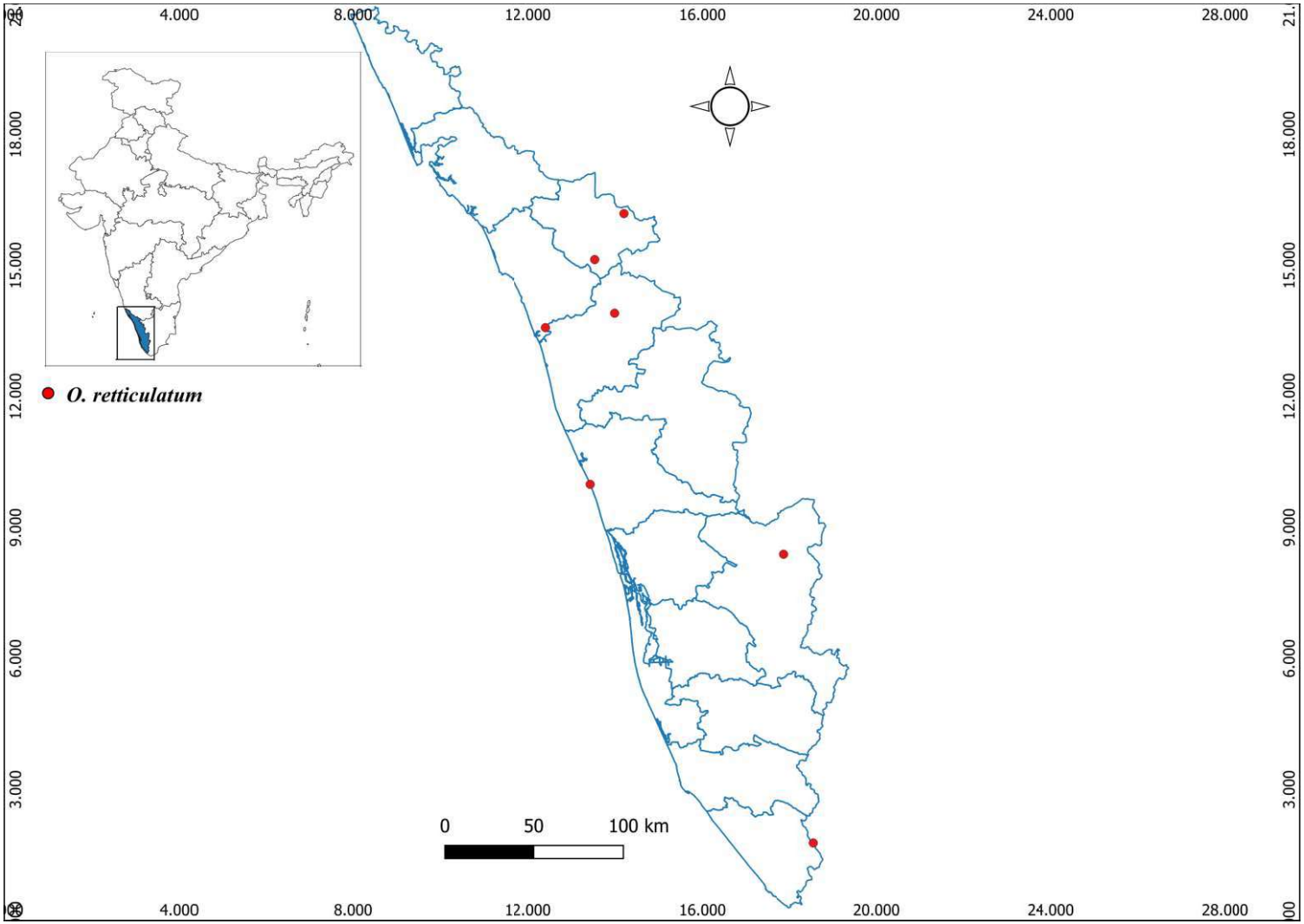

Fig. 45. Distribution map of *Ophioglossum reticulatum* in Kerala, India.

Uttarakhand, Uttar Pradesh, West Bengal), Indonesia, Japan, Korea, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Singapore, Socotra, Sri Lanka, Taiwan, Thailand, Tibet, Yemen.

Note: The cordate tropophylls of *O. reticulatum* makes it easily identifiable. In Kerala, *O. reticulatum* is not common and distributed mainly in the northern parts.

Specimens examined: **India**: **South India**: **Andra Pradesh**: **Kurnool Dist**. Gundlabrahmeswaram, *J. L. Ellis 16950* (MH). **Karnataka**: **Mysore Dist**.: *B. D. Naithani 21230* (MH); **Kerala**: **Idukki Dist**.: Munnar (10°04'07.10" N, 77°03'58.74" E) *Stephen Sequeira*, *Afsana Khan & Anto P. V. 21* (STC); **Kozhikode Dist**.: Farook College (11°11'57.44"N, 75°51'27.12"E), *Afsana Khan & Anto P.V. 23* (STC); **Malappuram Dist.:** Nilambur (11°16'13.61"N, 76°12'26.46"E), Afsana *Khan & Anto P. V. 12* (STC); **Thrissur Dist.:** Thrissur, *Varghese 29774* (CALI); **Thiruvanathapuram Dist.** Attayrar, *Raju Antony 69785* (TBGT); **Wayanad Dist.; Chembra Peak,** *B. K. Nayar* and *P. V. Madhusoodhanan 21302* (CALI); Chedaleth, *J. L. Ellis 19968* (MH); Chedaleth, *J. L. Ellis 24053* (MH); **Lakshadweep:** *P. Bhargavan 101906* (MH). **Madhya Pradhesh: Bastar Dist.;** Parawenala stream-Keskal, *K. Subramanyam 10377*(MH); Dharba, *K. Subramanyam 10379* (MH); **Rajastan**, **Sirohi Dist.:** Mount Abu, *T. N Bhardhwaju and party* (CALI); **Tamilnadu**: **Salem Dist.;** Balmadies Estate, yercaud, *K. Subramanyam 6564* (MH); **Trichirappalli Dist.**; Kondaiampatty, K. *Subramanyam 7780* (MH); Konkanpallam, Yercaud, *N. C. Nair 74250* (MH).

12. *Ophioglossum rubellum* Welw. Ex A. Braun., Filic. Afr. [Kuhn] 179. 1868. Burrows & Jhons Fl. of TrPl. East Afr.: Ophioglossaceae, 1 – 19 .2001; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 3: 318 – 327, 2021; Khan *et al.*, PST. 186 – 195, 2023.

Ophioglossum oleosum Khand. In Indian Fern J. 4: 102 (1987).
Ophioglossum trilokinathii B.L.Yadav, Meghvansi, Meena & Gena, Sci. Rep. 11(24396): 2 (2021).

Type: Africa; Angola, welwitsch 33 (Isotype, BM, digital image.,.

Fig. 46, 47, 48 & 49

Fig. 46. *Ophioglossum rubellum*: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

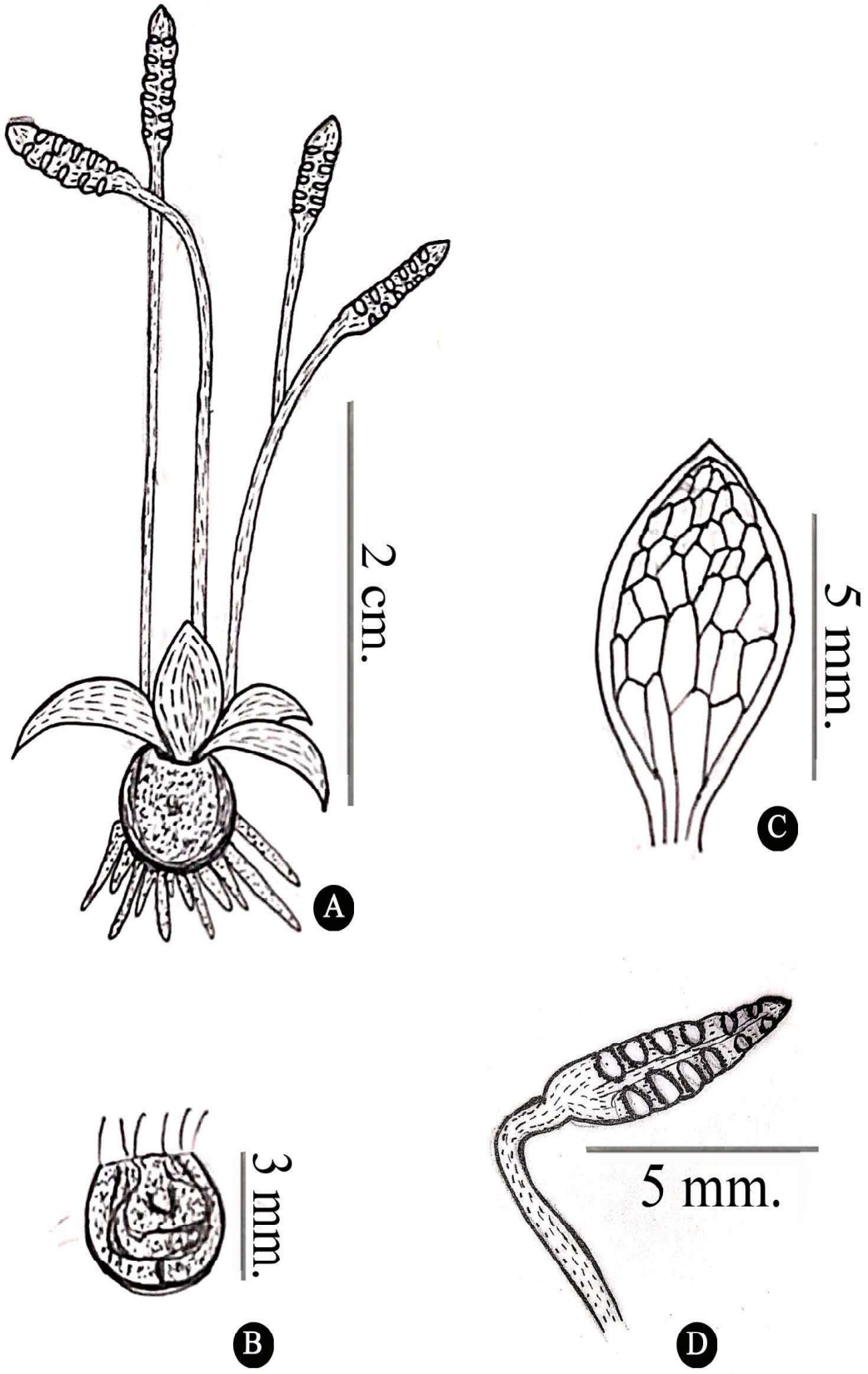

Fig. 47. Illustration of *Ophioglossum rubellum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

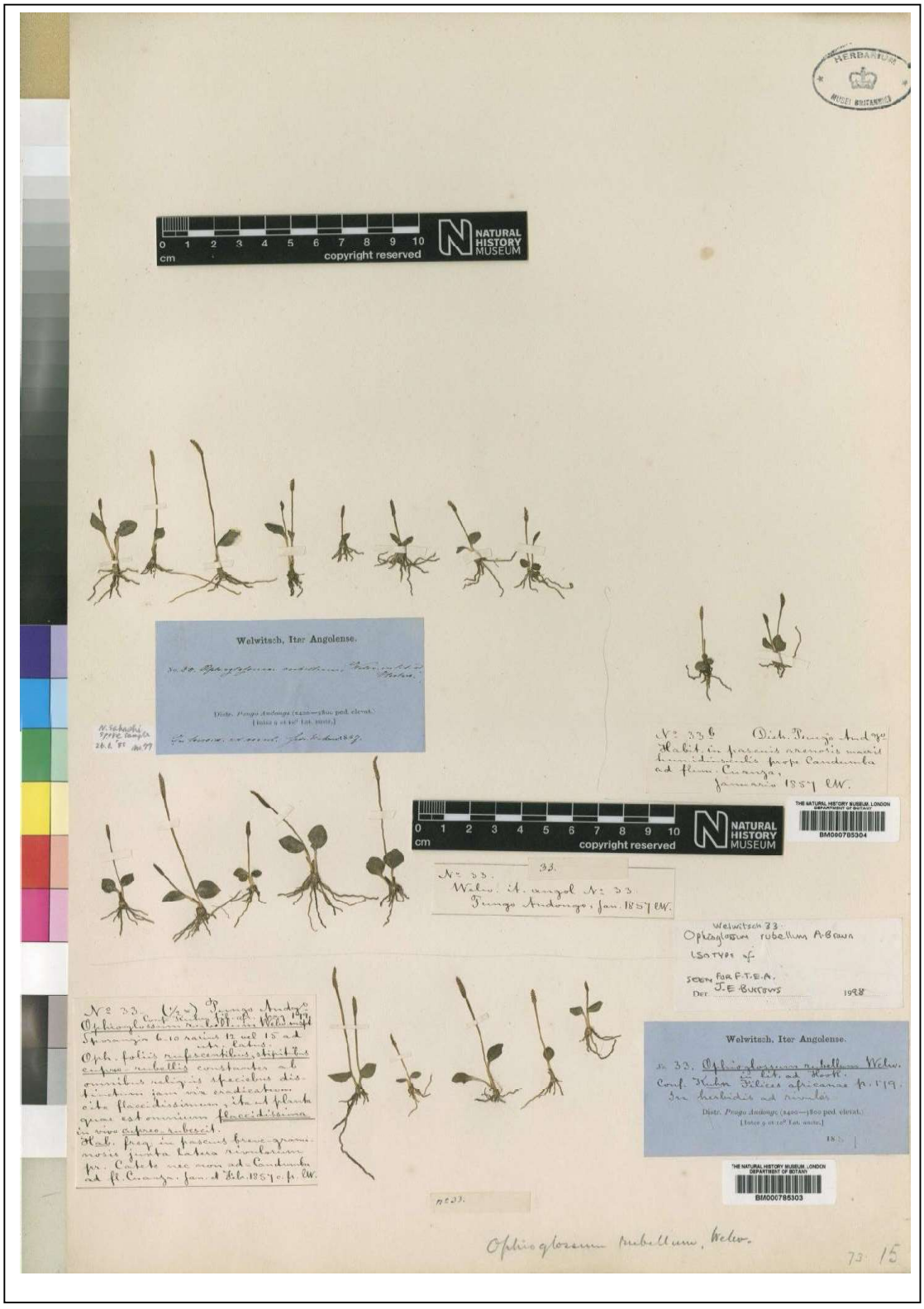

Fig. 48. Isotype of *Ophioglossum rubellum* ©The Board of Trustees of the Natural History Museum (BM), London.

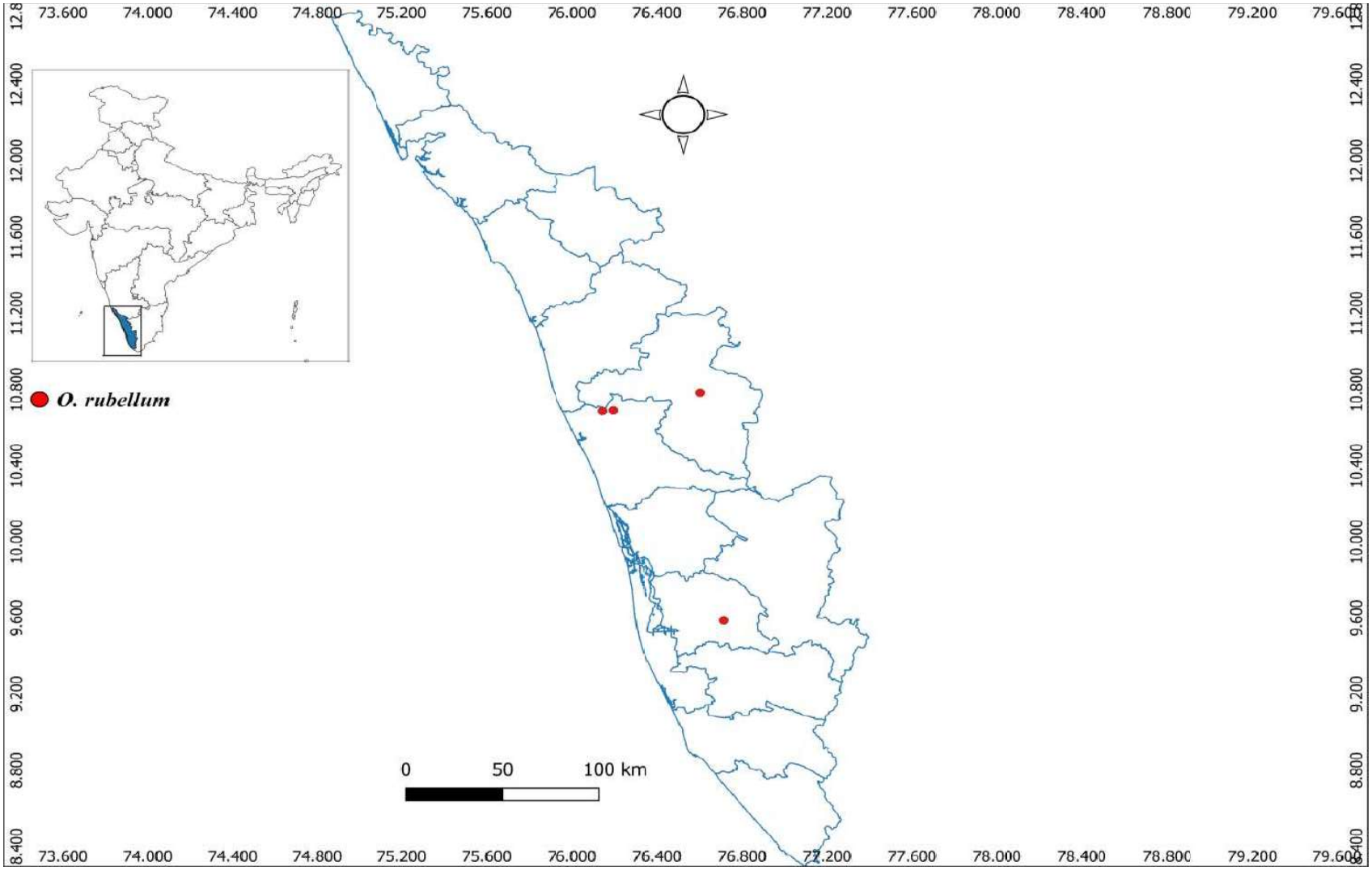

Fig. 49. Distribution map of *Ophioglossum rubellum* in Kerala, India.

Plants erect, up to 2.1 – 7.8 cm high, dark green; rhizome small, globose-fusiform, light brown, 0.5 – 0.6 cm long, 0.5 – 1.5 cm in diam., unbranched; roots vegetatively propagating by forming adventitious buds, unbranched, 0.5 – 1 cm long, 0.1 – 0.2 cm in diam.; stipe 0.1 – 0.2 cm long. 0.1 – 0.2 cm in diam; tropophylls two-four, minute, dark green, glabrous, 0.5 – 1.3 cm long, 0.2 – 0.8 cm wide, mostly ovate, occasionally elliptic or spathulate-obovate or sub orbicular, obtuse at apex, acute-cuneate at base, arranged like a rosette, sometimes touching the substratum; fertile segment 1.5 – 7 cm long, stalk dark green, 1 – 5 cm long, 0.1 – 0.2 cm in diam.; spikes 0.5 – 2 cm long, 0.1 – 0.3 cm wide, with a short sterile pointed apex; sporangia 6 – 10 on either side, opposite.

Phenology: June-October.

Habitat and Ecology: The plants growing in the undisturbed open grounds. Rooted in very thin loamy soil in moist and shady areas. It is usually found growing along with mosses and liverworts.

Distribution: Africa, Asia: **India** (Rajastan, **New record from Kerala).**

Note: *O. rubellum* is characterized by ovate-elliptic or spathulate-obovate or sub orbicular tropophylls originated very close to the substratum. Two-four tropophylls were present per plant. The populations of *O. rubellum*, collected from different localites of Kerala showed slight variations mainly in the plant height. The red colour is not prominent in all the plants.

Specimens examined: **India**: **South India**: **Kerala**: **Kottayam Distr**. Elampally (9°35' 13.902" N, 76° 42' 46.4688" E), *Afsana Khan & Anto P.V. 24* (STC); **Palakkad District:** Kallekad (10°46'56"N, 76°36'07"E) *Afsana Khan & Anto P.V. 10* (STC); **Thrissur Dist.:** Mangadu, Kottappuram (10° 41' 21.876" N, 76° 11' 43.368" E) *Afsana Khan & Anto P.V. 9* **(**STC**)**; Pazhiyottumuri (10° 41' 9.978" N, 76° 8' 40.776" E), *Vimal K.R*, *Afsana Khan & Anto P.V. 41* (STC).

13. *Ophioglossum vulgatum* L., Sp. Pl. 2: 1062 (1753). Clausen, Mem: Torrey Bot. Club 19(2): 140. 1938; Mahabale, Nelumbo, 4(1 – 4), 71 – 84, 1962; Manickam and Irudayaraj, Pterid. Fl. W. Ghats 48. t. 26. 1992; Burrows, and Johns, Fl. Trop. Ea.

Afr.-Ophi., 2001; Fraser-Jenkins *et al.*, An Ann. Check. Ind. Pter. 1: 1 – 562, 2017; Roskov *et al.*, World Ferns: Check. of Fer. and Lyco. of the World. 2018.

Type: Europe, herb. Linnaeus 1243.1, (Lectotype, LINN, digital image!)

Ophioglossum ovatum Salisb. in Prodr. Stirp. Chap. Allerton: 401.1796., nom. superfl.

Ophioglossum microstichum Ach. in Kongl. Vetensk. Acad. Nya Handl. 30: 59.1809.

Ophioglossum ovatum Sw. ex Opiz in Kratos 1(4): 12.1819., nom. superfl.

Ophioglossum ovatum var. *obtusum* Opiz in Kratos 1(4): 12.1819., nom. superfl.

Ophioglossum ovatum var. *mucronatum* Opiz in Kratos 1(4): 12.1819.

Ophioglossum vulgatum var. *microstichum* (Ach.) Hartm. In Handb. Skand. Fl.: 373.1820.

Ophioglossum vulgatum var. *typicum* Wherry in Bartonia 21: 13.1942, not validly publ.

Fig. 50, 51, 52 & 53

Plants erect, up to 7 – 11 cm high, green; rhizome small, light brown, elongated, 0.5 – 1 cm long, 0.7– 1.5 cm in diam., unbranched; roots unbranched, 0.3 – 0.5 cm long, 0.1– 2 cm in diam.; stipe 2 – 3 cm long, 0.1 – 0.2 cm in diam.; tropophyll one, dark green, glabrous, 3 – 4 cm long, 0.3 – 0.7 cm wide, Spathulate (Lanceolate-narrowly ovate), acute-obtuse at apex, obtuse at base; fertile segment 4.5 – 7 cm long, arising directly from the leaf base; stalk dark green, 2.5 – 4 cm long, 0.1 – 0.2 cm in diam.; spike 2 – 3 cm long, 0.4 – 0.6 cm wide, with a small sterile pointed apex; sporangia opposite, 25 – 35 on either side.

Phenology: June-December.

Habitat and Ecology: Growing mainly on on the river banks, soil patches on the open rocks. In Kerala, *O. vulgatum* found only in higher altitudes (Munnar and Nelliyampathy).

Distribution: America, Africa, Europe, Asia: China, **India**, Japan, Korea, Russia.

Fig. 50. *Ophioglossum vulgatum*: A Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

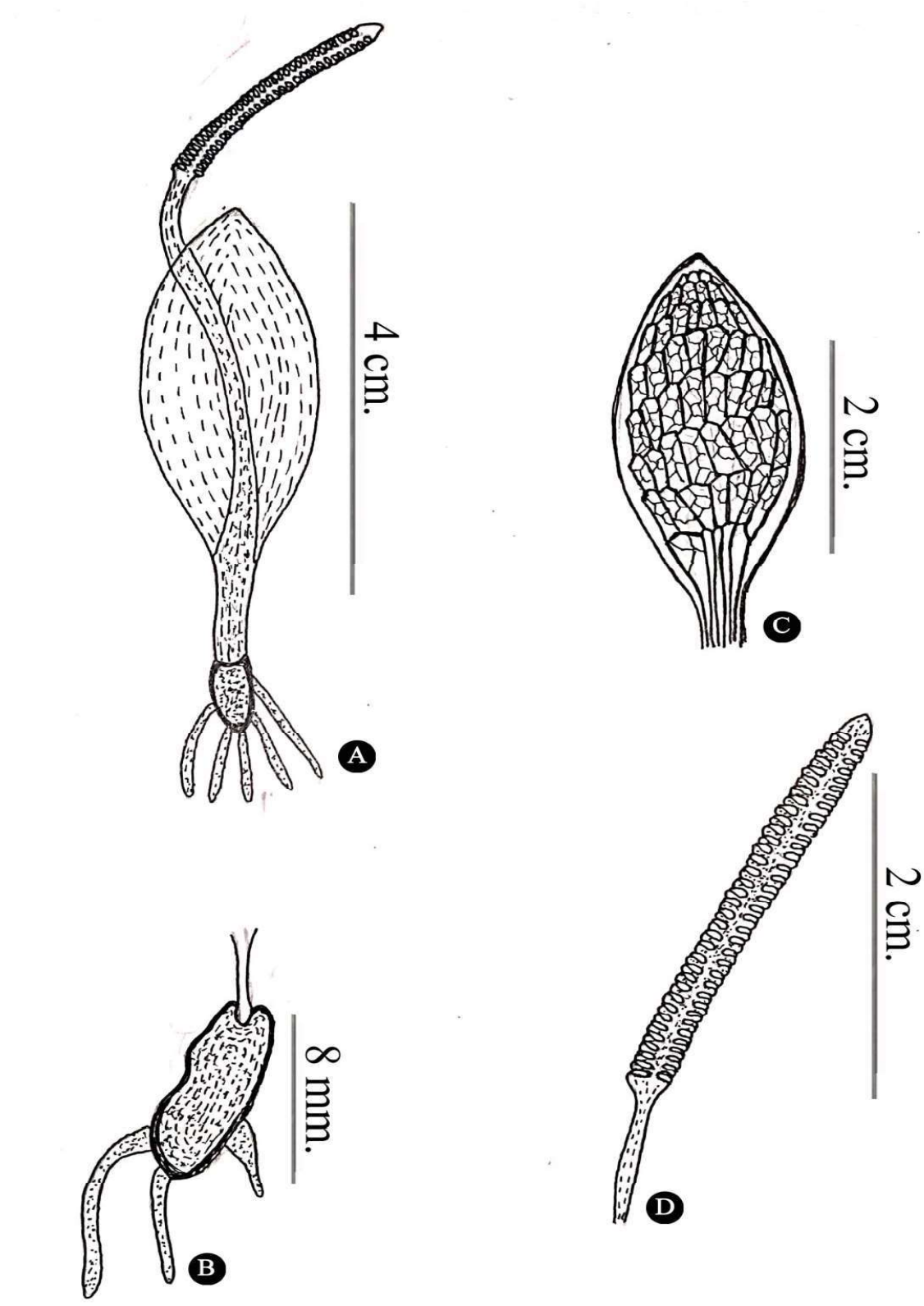

Fig. 51. Illustration of *Ophioglossum vulgatum*: A. Entire plant, B. Rhizome, C. Venation of tropophyll, D. Spike.

Fig. 52. Lectotype of *Ophioglossum vulgatum*©The Board of Trustees of the Linnean society (LINN), London.

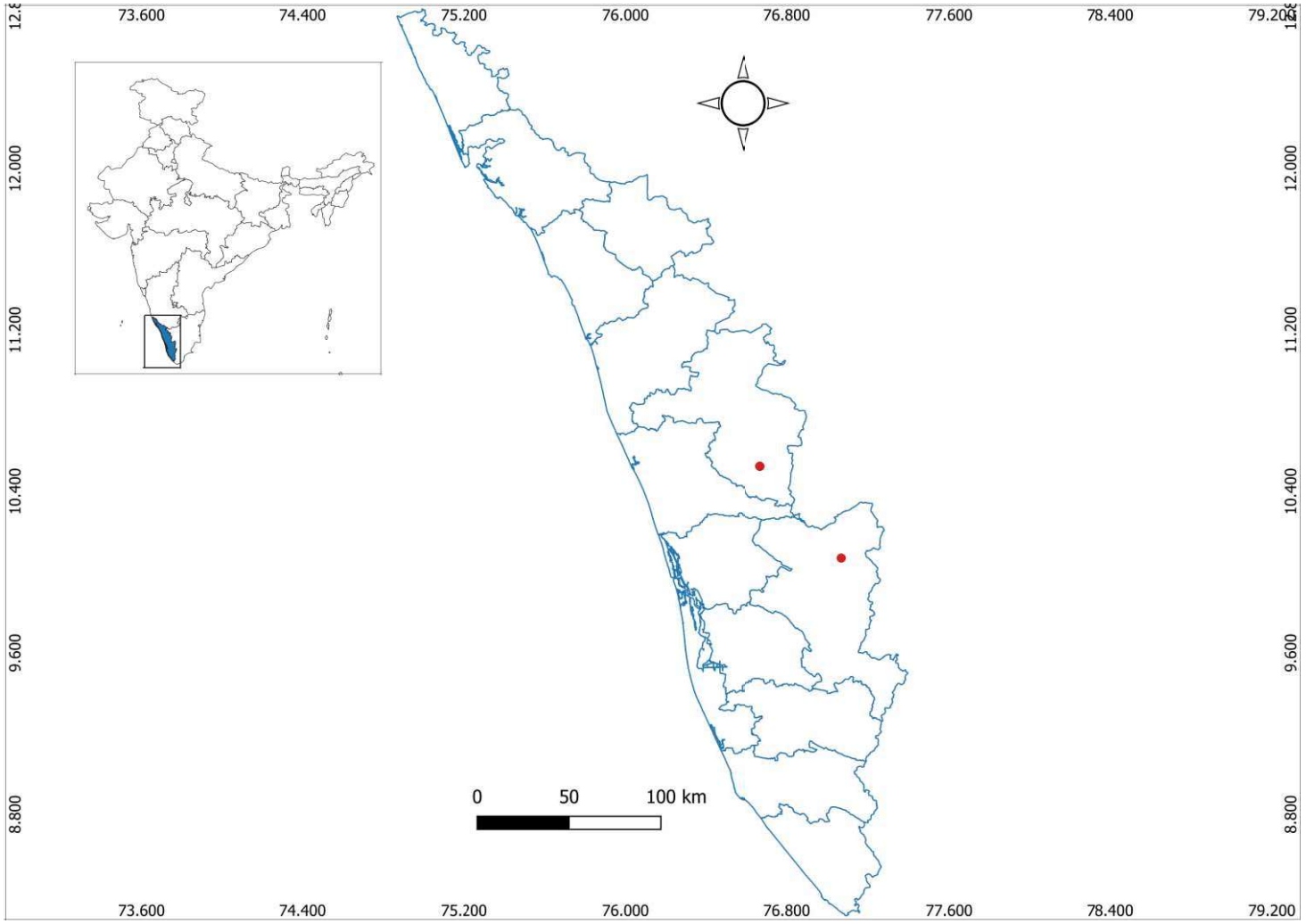

Fig. 53. Distribution map of *Ophioglossum vulgatum* in Kerala, India.

Note: *O. vulgatum* is not very common in Kerala. *O. vulgatum* plants possessed unique morphological characters different from all other *Ophioglossum* species collected. The elongated rhizome of *O. vulgatum* is different from globose- discoid-tuberous elongated rhizome of *O. costatum* and fusiform rhizome of *O. reticulatum*. The spathulate tropophylls of *O. vulgatum* is different from the elliptic-rhomboid-lanceolate tropophylls of *O. costatum* and cordate tropophylls of *O. reticulatum*. The venation, stomata, Spore characters and molecular characters were also different from all other *Ophioglossum* species found in Kerala. So, the presence of *O. vulgatum* in India was confirmed by the detailed morphological analysis and comparison with the type specimen.

Specimens examined: **Idukki Dist.:** Munnar (10° 4' 5.988" N, 77° 4' 0.984" E) *Stephen Sequeira, Afsana Khan & Anto P.V. 25* (STC). **Palakkad Dist.:** Nelliyampathy (10° 31' 28. 9992" N76° 40' 3" E), *Vimal K.R*, *Afsana Khan & Anto P.V. 43* (STC).

14. *Ophioglossum* sp. nov.

Type: India. Kerala: Palakkad Dist: Kallekad (10º46'55.56"N, 76º36'08.39"E) *Anto P.V. & Afsana Khan*. 11, 13 (Holotype-11, STC. Isotype-13, CALI.)

Fig. 54, 55, 56

Diagnostic features: Tuberous- oblong rhizome, oblong-lanceolate tropophylls, short stipe, 0.1 – 0.5 cm long, Rugate spores with longer and wider muri, smooth, flattened proximal face without a deep proximal cavity.

Plants erect, 5 – 12 cm high, dark green; rhizome small, tuberous- oblong, light brown, 0.5 – 1 cm long, 1 – 1.5 cm in diam., unbranched; roots vegetatively propagating by forming adventitious roots, horizontal, cylindrical, unbranched, 3 – 7 cm long, 0.1– 2 cm in diam.; stipe short, 0.1 – 0.5 cm long, 0.1 – 0.2 cm in diam.; tropophylls two-three, dark green, glabrous, 1.5 – 3.5 cm long, 0.5 – 2.5 cm wide, oblong-lanceolate, acute at apex, oblique at base; fertile segment 4.4 – 10.5 cm long, very close to the rhizome; stalk dark green, 3.4 – 8.5 cm long, 0.1 – 0.2 cm in diam.; spike 1 – 2 cm long, 0.1 – 0. 4 cm wide, occasionally bifurcated, with a sterile pointed apex, sporangia 6 – 11 pairs on either side, opposite.

Phenology: June-September.

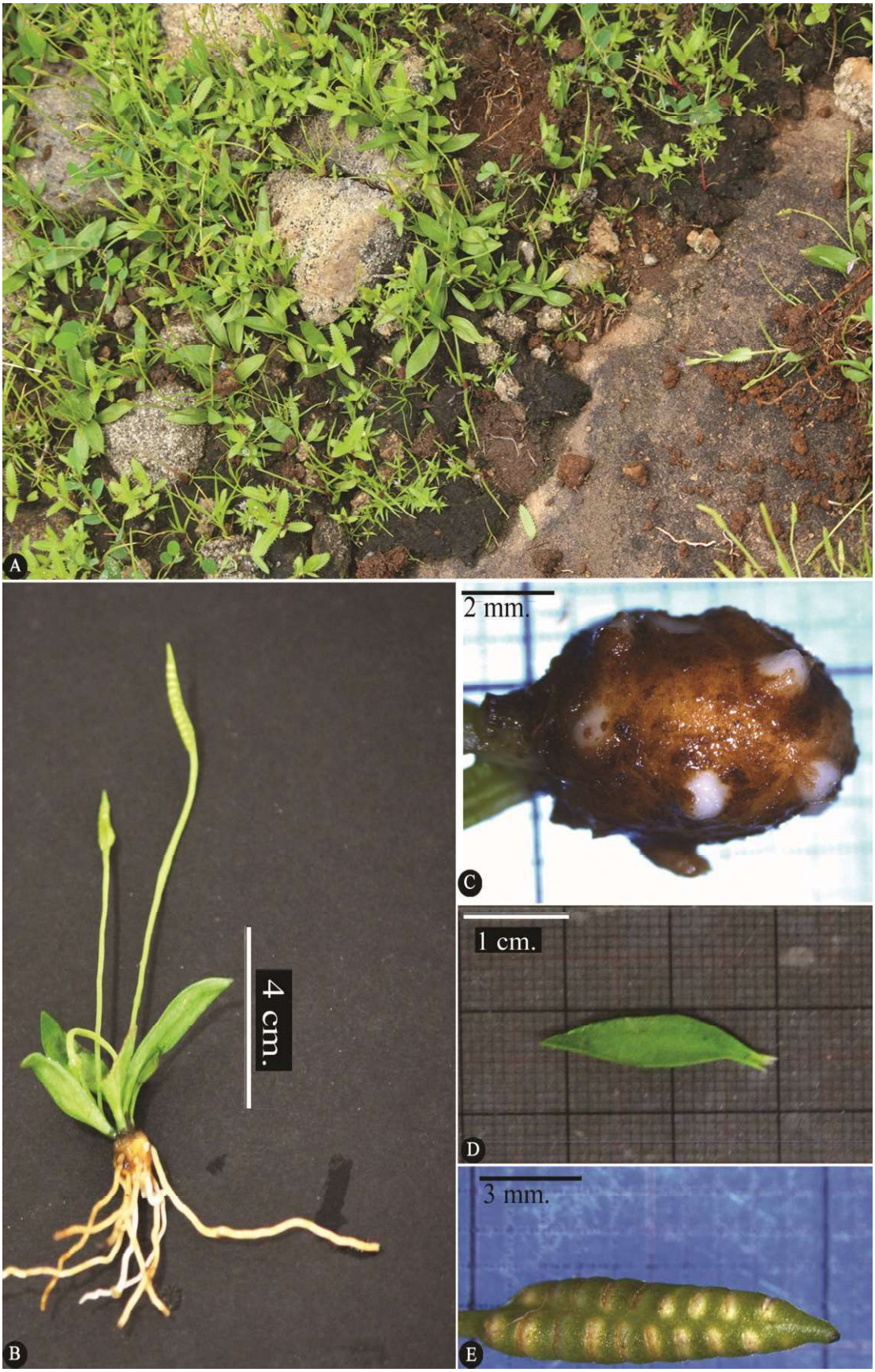

Fig. 54: *Ophioglossum* sp. nov.: A. Habitat, B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike.

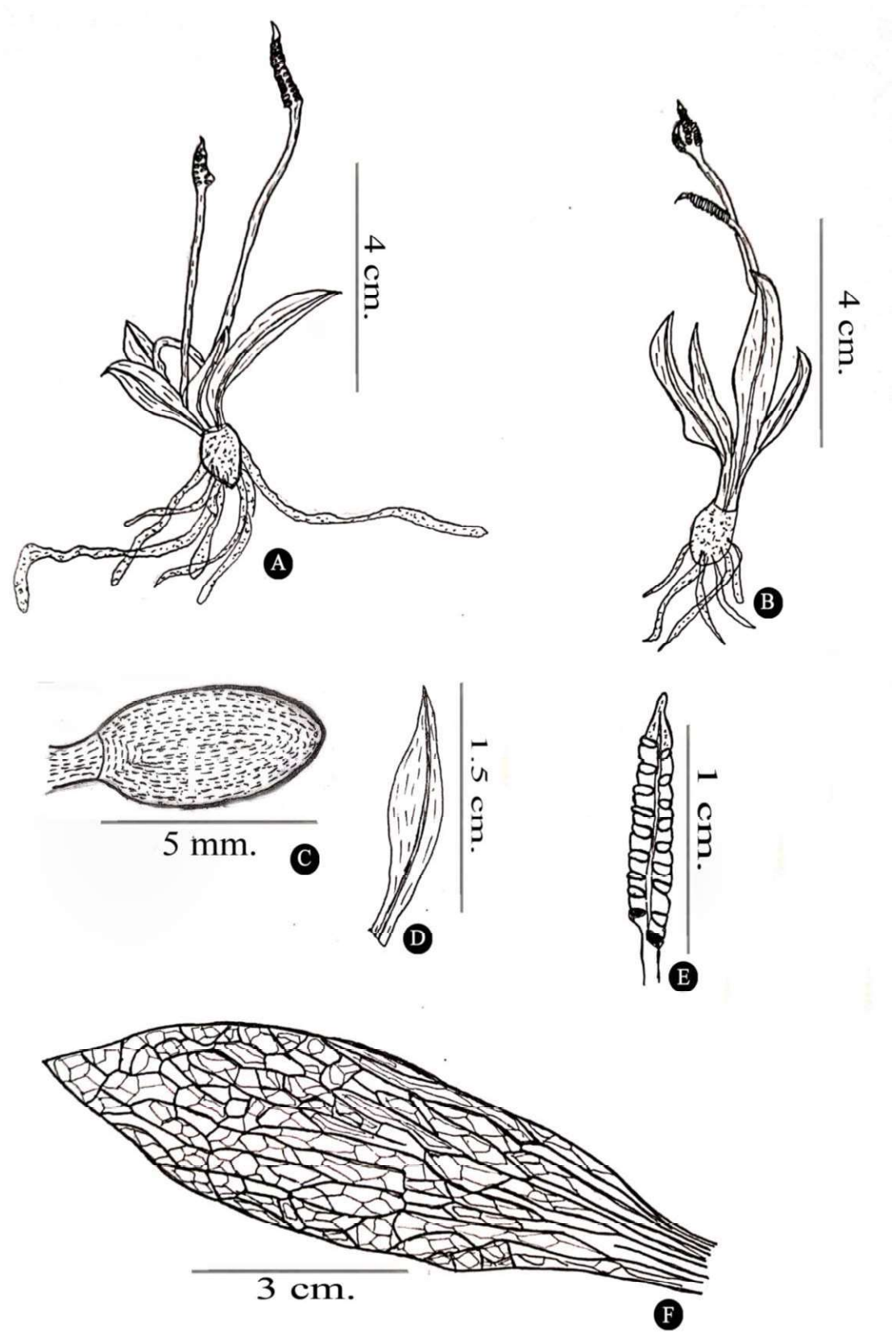

Fig. 55. Illustration of *Ophioglossum* sp. nov.: A & B. Entire plant, C. Rhizome, D. Tropophyll, E. Spike, F. Venation of tropophyll.

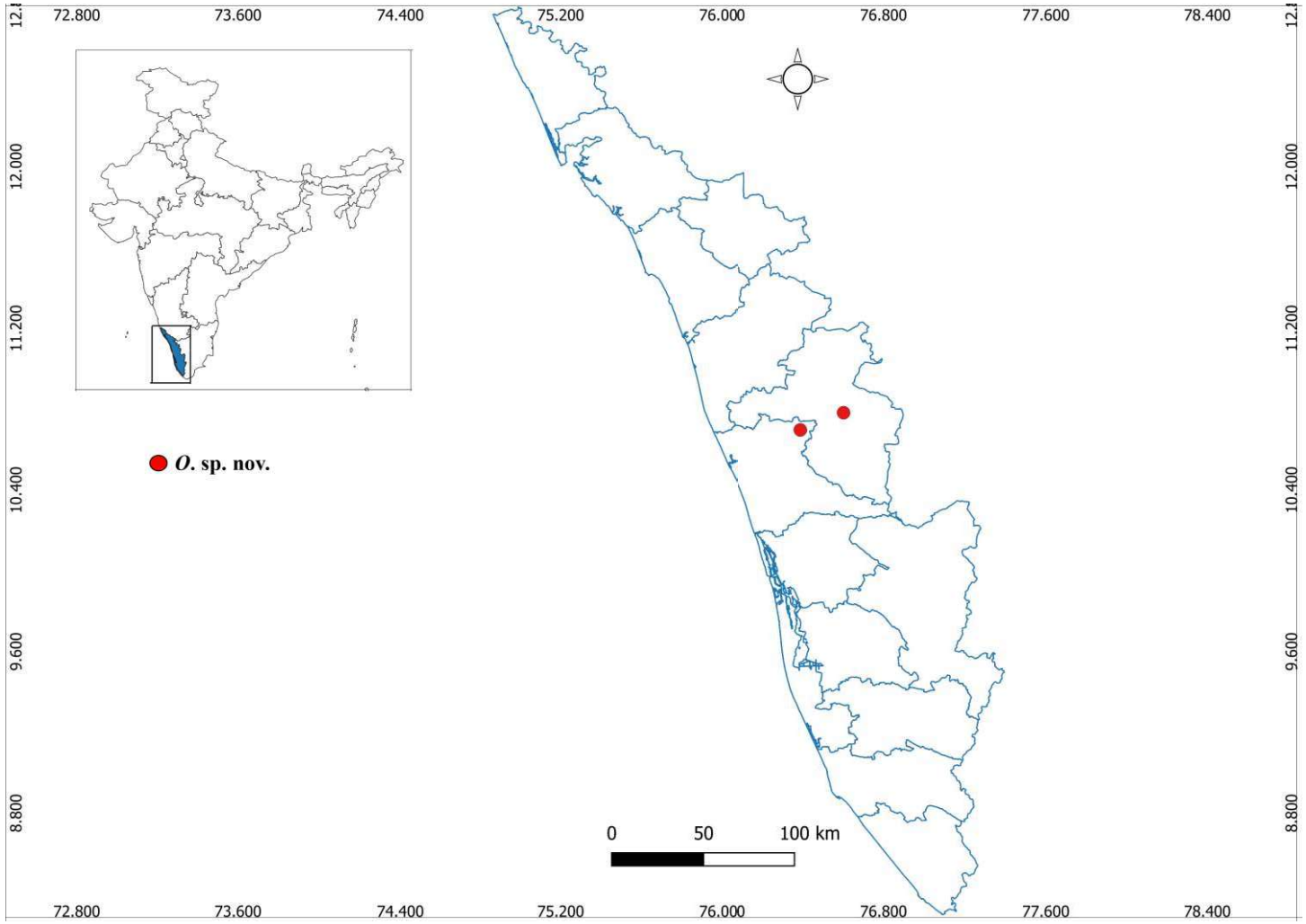

Fig. 56. Distribution map of *Ophioglossum* sp.nov. in Kerala, India.

Habitat and Ecology: The plants grow on weathering rock surfaces. vegetative propagation was observed.

Distribution: Open rocks.

Note: *Ophioglossum* sp. nov. has slight resemblence with *O. gomezianum*, *O. costatum* and *O. polyphyllum.* But the detailed analysis of macro morphology, micromorphology along with the molecular phylogenetic analysis revealed *Ophioglossum* sp. nov. as a distinct taxon.

Specimen examined: India, Kerala, **Palakkad dist.:** Kallekad (10°46'55.56"N, 76°36'08.39"E). **Thrissur Dist.:** Kayampoovam (10º41'09.54"N, 76º23'09.06"E) *Anto P.V. & Afsana Khan*. 11, 13, 34 (Holotype-11, STC.; Isotype-13, CALI.).

4.2. Micromorphological Characters

Micromorphology includes spore morphology, stomata and venation pattern

4.2.1. Venation Pattern (Fig. 57)

Venation is the pattern of arrangement of total veins of a frond (Lellinger *et al.*, 2002). Genus *Ophioglossum* has the most complex venation pattern among eusporangiate ferns, probably arised due to the amplification of true midrib (Wagner 1979). The veins in *Ophioglossum* fronds were joined together to form networks and enclose areolae (anastamosing). In general, two kinds of venation patterns were observed in *Ophioglosum* genus, the reticulated and bireticulated condition. The reticulated venation is simple primary veins and primary areoles. In bireticulate condition, the primary areoles enclose the secondary veinlets and secondary areoles. The areoles in the basal part were large and elongated whereas the size of the areoles decreased towards the apex and formed short hexagons. The peripheries of the tropophylls lack the venation. The closed network of the veins ends just before reaching at the peripheries. This kind of venation is dintinct in *Ophioglossum* genus.

1. *O. costatum*

The costa can be seen from the field itself in light yellow colour and hence the species got the name costatum. The venation is anastomosing, bireticulate. The anastomosed veins formed a network with five veins parellaly originating from the base. The median central vein is continuous and run towards the tip. The remaining four lateral

veins joins each other to form network which encloses elongated areoles. The basal central portion consists of only primary areoles formed by primary veins. Except this portion, all other portions of tropophyll were bireticulate. The secondary veins form the secondary, small aereoles within the primary areoles. The secondary veinlets were with free endings.

2. *O. gramineum*

Venation is anastomosing, reticulate, five veins arising from the base. One median vein is continous and run upto the tip of the tropophyll. The remaining four lateral veins joined to form a network. The two veins adjacent to the cental veins were exactly parallel to the median vein and hence the areoles adjascent to the median veins were long stretched and have a tube-like shape. The remaing two veins were not completely parallel, hence the areoles were just elongated and not like a tube. Bireticulation is absent. The veins lack free endings.

3. *O. indicum*

Veins are anastomosing, reticulate without included secondary areoles, four longitudinal-veins arises from the base. The median vein runs parallel towards the apex. The remaining four lateral veins anastomosed to form large areoles. The areoles are large, hexagonal in shape. Veins are without free endings.

4. *O. latifolium*

Veins are anastomosing, reticulate. Bireticulation is absent. Three veins arising from the base. The median vein is continuos up to the leaf tip. The remaining two lateral veins run towards the tip by joining together and forming primary areoles. The areoles are long up to the middle of the tropophyll starting from the base. Then the areoles became short hexogons-rounded in shape. Veins are without free endings.

5. *O. lusitanicum*

Veins are anastomosing, reticulate. Three veins arising from the base. The median vein continous to the tip. Two lateral veins anastomosed to form primary areoles. Areoles are elongated at the base, short and polygonal-rounded towards apex. Veins are without free endings.

6. *O. lusoafricanum*

Venation is Anastomosing, reticulate, five veins run parallel from the base and divided in to seven veins. The veins jointed to form several short, elongated areoles which arranged parallel to each other, veins are without free endings.

7. *O. parvifolium*

Veins are anastomosing, reticulate. Three veins starting from the base, running towards the apex by forming short hexagonal-elongated areoles. Secondary veinlets and secondary areoles are absent. Veins are without free endings.

8. *O. pendulum*

Veins are anastomosing, reticulate. Numerous veins run parallel to each other and joints to form large elongated, tubular areoles. Veins are without free endings.

9. *O. petiolatum*

Veins are anastomosing, reticulate. Numerous veins arising from the base. Areoles are elongated at the basal central regions and becomes shortens towards the tip. Veins are without free endings.

10. *O. raphaelianum*

Veins are anastomosing, reticulate. Six veins arising from the base. Mid vein continuos to the tip. Veins are anastomosed to form primary areoles. Areoles are elongated at the base, shorten and hexagonal towards the tip. Veins lack free endings.

11. *O. reticulatum*

Veins are anastomosing, bireticulate, numerous veins run from the base towards apex, forming many elongated, areoles. A prominent midvein is absent. Veins are reticulate at the basal and central portion of the tropophhyll, rarely bireticulated towards the apex. More elongated and lage areoles are present at the base. The size of the areoles decreased towards the apex and forms short hexagons. Veins lack free endings.

12. *O. rubellum*

Veins are anastomosing, reticulate. Seven veins arised from the base and run towards the tip. Median vein continous to the tropophyll tip. The six latersl veins anastomosed to form primary areoles. Areoles are elongated at the base and become short hexagons toward the apex. Veins lack free endings.

13. *O. vulgatum*

Veins are anastomosing, bireticulate. Six veins arising from the base. Median vein continous to the tip. Lateral veins form slightly elongated areoles at the base. The areoles shorten and become hexagonal toward tip. Secondary veinlets and secondary areoles were included by primary areoles. The veins are without free endings.

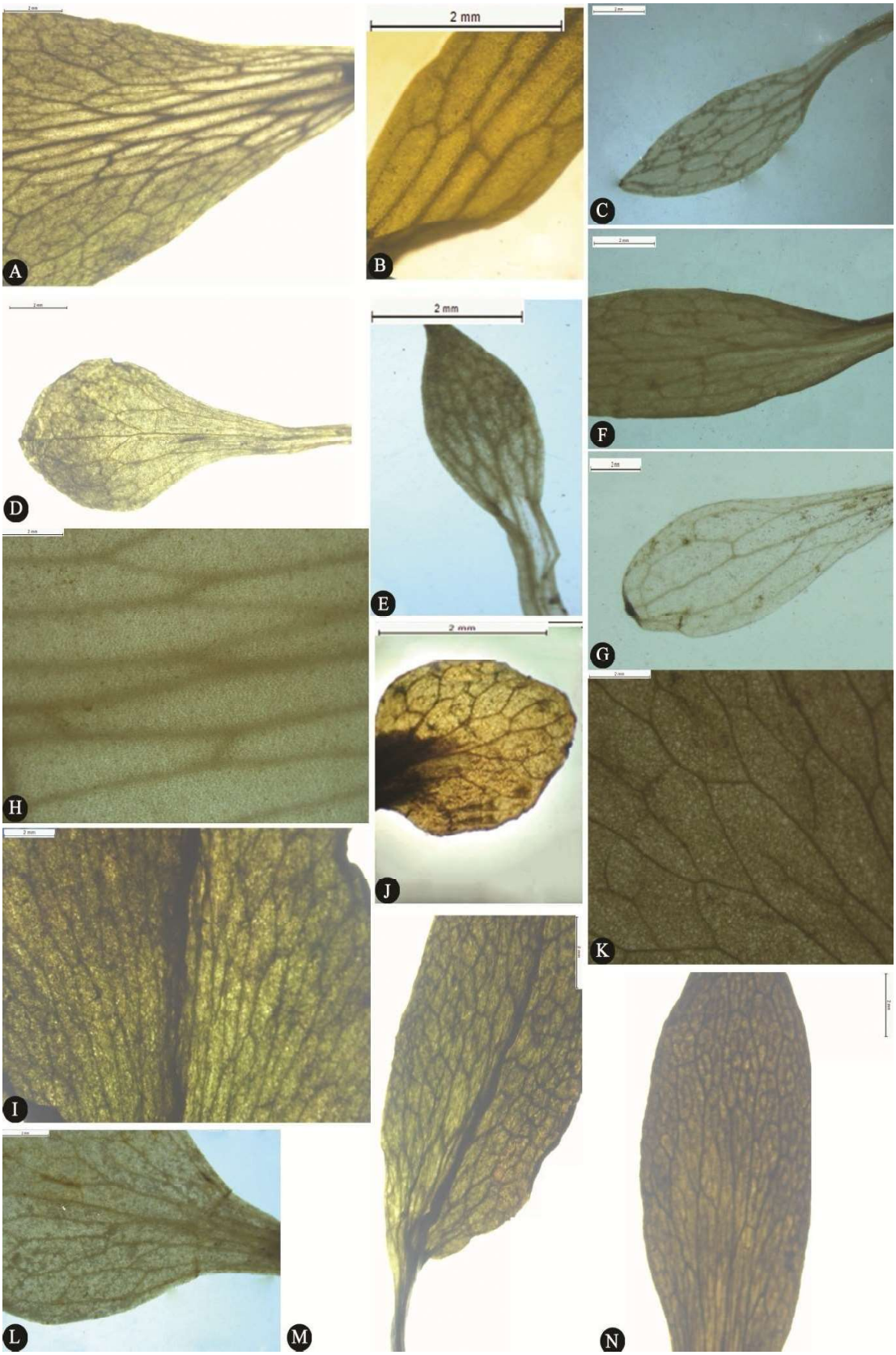

Fig. 57. Venation pattern of different *Ophioglossum* species: A. *O. costatum,* B. *O. gramineum,* C. *O. indicum,* D. *O. latifolium,* E. *O. lusitanicum,* F. *O. lusoafricanum,* G. *O. parvifolium,* H. *O. pendulum,* I. *O. petiolatum,* J. *O. raphaelianum,* K. *O. reticulatum,* L. *O. rubellum,* M. *O. vulgatum,* N. *O.* sp. nov.

14. *O.* sp. nov.

Veins are anastomosing, bireticulate, 5 veins start from the base and run toward the apex. The median veins continous up to the middle of the tropophylls and then anastomosed to form primary areoles. The lateral veins anastomosed to form primary areoles. Primary areoles include many secondary areoles. The secondary veinlets are branched, with and without free endings. The areoles are elongated at the base and shortens towards the apex.

4.2.2. Stomatal Characters (Fig. 58 & 59)

The stomata are anomocytic in *Ophioglossum*. There is no difference exists between the epidermal cells and the subsidiary cells. The epidermal cells are elongated in all the species. The cell walls are wavy. The mature stomata are surrounded by more than 3 subsidiary cells in each species. The number of subsidiary cells, their size varies in different species.

1. *O. costatum*

Stomata anomocytic, stomatal pore 5.67 – 10.72 µm long, 1.5 – 3 µm wide; guard cells 32 – 49 µm long, 12 – 14 µm wide, subsidiary cells 4 – 7 in number, 20 – 79 µm long, 13 – 30 µm wide, subsidiary cells are polygonal-rounded, anticlinal walls are wavy.

2. *O. gramineum*

Stomata anomocytic, stomatal pore 22 – 26 µm long, 4 – 5 µm wide, guard cells 48 – 50 µm long, 12 – 15 µm wide, subsidiary cells 5 – 6 in number, polygonal-elongated, 77 – 102 µm long, 30 – 36 µm wide, anticlinal walls are wavy.

3. *O. indicum*

Stomata anomocytic, stomatal pore 24.06 – 25.44 µm long, 2.33 – 3.93 µm wide, guard cells 63.66 – 73.54 µm long, 17.24 – 18.06 µm wide, subsidiary cells 4 – 5 in number, elongated, 88.66 – 140 µm long, 11.57 – 39.18 µm wide, anticlinal walls are wavy.

4. *O. latifolium*

Stomata anomocytic, stomatal pore 9 – 19.48 µm long, 1.19 – 2 µm wide, guard cells 60.95 – 68.19 µm long, 21 – 25.9 µm wide, subsidiary cells 5 – 7 in number,

polygonal-elongated, 53.55 – 101 µm long, 30 – 48 µm wide, anticlinal walls are wavy.

5. *O. lusitanicum*

Stomata anomocytic, stomatal pore 28 – 30.30 µm long, 2.5 – 4 µm wide, guard cells 57.47 – 65.12 µm long, 10.93 – 13.2 µm wide, subsidiary cells 5 in number, polygonal-elongated, 80 – 155 µm long, 30.35 – 62.99 µm wide, anticlinal walls are wavy.

6. *O. lusoafricanum*

Stomata anomocytic, stomatal pore 25.37 – 27.15 µm long, 1.60 – 4.5 µm wide, guard cells 69 – 73.63 µm long, 13 – 14 µm wide, subsidiary cells 4 – 6 in number, elongated, 109.34 – 176.29 µm long, 12.70 – 37.92 µm wide, anticlinal walls are wavy.

7. *O. parvifolium*

Stomata anomocytic, stomatal pore 10.17 –12.98 µm long, 1.15 – 1.36 µm wide, guard cells56.72 – 63.25 µm long, 14.69 – 16.04 µm wide, subsidiary cells 4 – 5 in number, linear, 102.74 – 187.35 µm long, 11.21 – 29.99 µm wide, anticlinal walls are wavy.

8. *O. pendulum*

Stomata cyclic, stomatal pore 16 – 18 µm long, 3 – 4 µm wide, guard cells 82 –89 µm long, 18 – 27 µm wide, subsidiary cells 6 in number, polygonal-rounded, 50 – 85 µm long, 27 – 46 µm wide, anticlinal walls are wavy.

9. *O. petiolatum*

Stomata anomocytic, stomatal pore 13.52 – 17.72 µm long, 0.5 – 0.77 µm wide, guard cells 40.76 – 51.07 µm long, 14.11 – 16.60 µm wide, subsidiary cells 6 in number, polygonal-rounded, 37 – 61.10 µm long, 28.37 – 45.33 µm wide, anticlinal walls are wavy.

10. *O. raphaelianum*

Stomata anomocytic, stomatal pore 22.49 – 27.32 µm long, 2.44 – 3.84 µm wide,

guard cells 54.45 – 62.72 µm long, 16.75 – 17.71 µm wide, subsidiary cells 5 – 6 in number, elongated, 103.68 – 120 µm long, 21.87 – 50.01 µm wide, anticlinal walls are wavy.

11. *O. reticulatum*

Stomata anomocytic, stomatal pore 10 – 15 µm long, 1.9 – 2.5 µm wide, guard cells 61.55 – 64.57 µm long, 21.83 – 22.13 µm wide, subsidiary cells 6 – 8 in number, polygonal-rounded, 34. 49 – 78 µm long, 39.30 – 77.09 µm wide, anticlinal walls are wavy.

12. *O. rubellum*

Stomata anomocytic, stomatal pore 11 – 12.5 µm long, 1.33 – 1.5 µm wide, guard cells 40.55 – 43.75 µm long, 11.35 – 13.45 µm wide, subsidiary cells 4 in number, polygonal- elongated, 42.47 – 98.41 µm long, 27.73 – 56.21 µm wide, anticlinal walls are wavy.

13. *O. vulgatum*

Stomata anomocytic, stomatal pore 6.78 – 7.50 µm long, 1.44 – 1.6 wide, guard cells 33.33 – 37.09 µm long, 31.49 – 38.23 µm wide, subsidiary cells 5 – 6 in number, short, polygonal- rounded, 31.44 – 43.44 µm long, 18.33 – 23.45 µm wide, anticlinal walls are wavy.

14. *O.* sp. nov.

Stomata anomocytic, stomatal pore 14.59 – 16.07 µm long, 1.14 – 2 µm wide, guard cells 55.04 – 58.27 µm long, 17.08 – 18.55 µm wide, subsidiary cells 5 in number, elongated cells, 59.39 – 98.18 µm long, 35.15 – 41.74 µm wide, anticlinal walls are wavy.

4.2.3. Spore morphology

The spore characters are more stable and are not easily affected by environmental factors. So, a detailed examination of the spore characters was carried out along with the examination of vegetative characters. The spore morphology it is found to be an effective tool for delimiting species in the genus *Ophioglossum*.

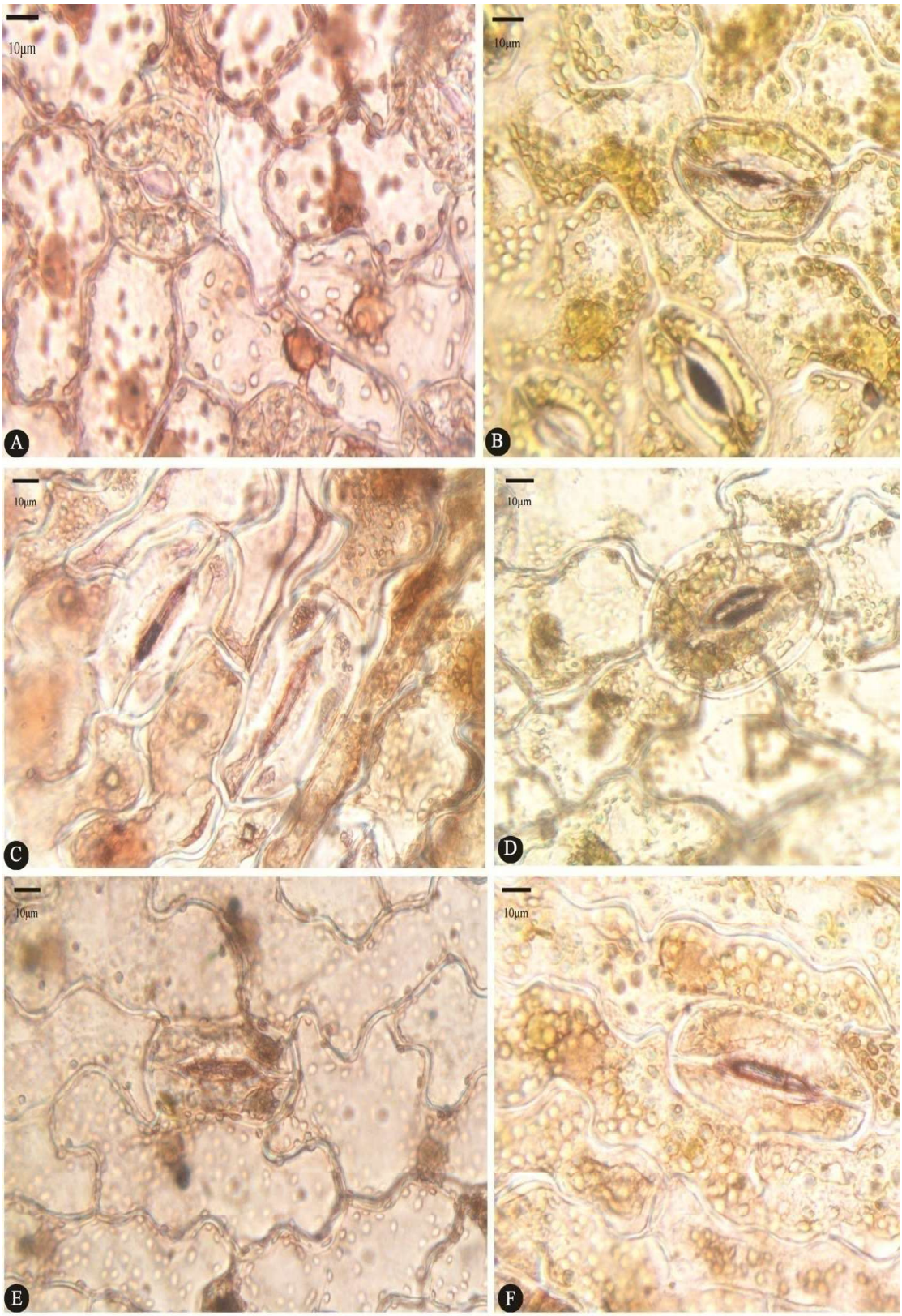

Fig. 58. Stomata of different *Ophioglossum* species: A. *O. costatum*, B. *O. gramineum*, C. *O. indicum*, D. *O. latifolium*, E. *O. lusitanicum*, F. *O. lusoafricanum.*

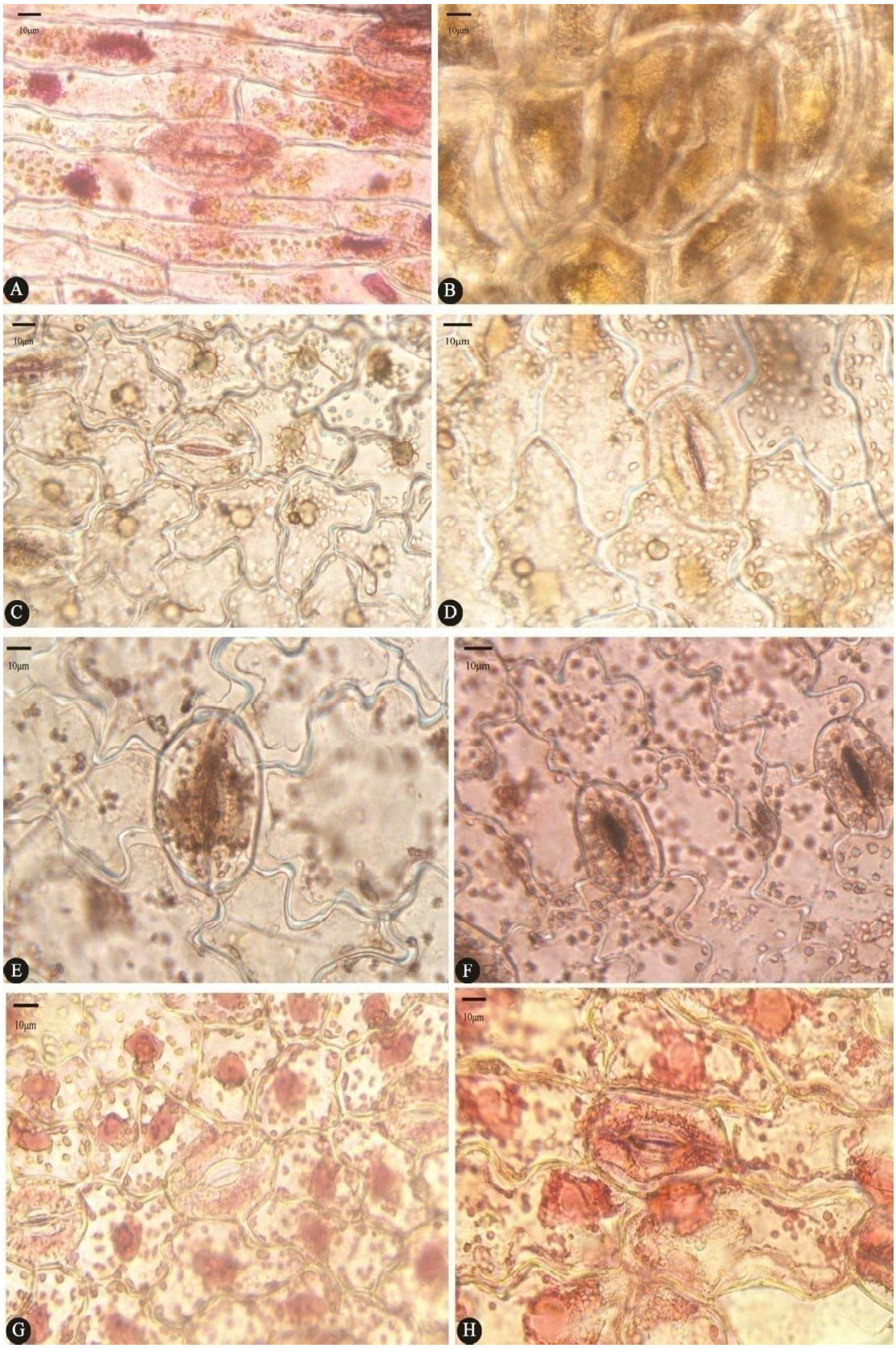

Fig. 59. Stomata of different *Ophioglossum* species: A. *O. parvifolium*, B. *O. pendulum*, C. *O. petiolatum*, D. *O. raphaelianum*, E. *O. reticulatum*, F. *O. rubellum*, G. *O. vulgatum*. H. *O.* sp. nov.

1. *O. costatum.* (Fig. 60)

"Spores globose and trilete, foveolate; distal face sizes up to 30.28 x 31.8 – 40.66 × 41.44 µm, foveolate, granulose, reticulate, muri anastomosing; lumina tetragonal-pentagonal-circular, narrow-wide; proximal face concave with a deep proximal cavity having shallow lumina, triradiate leasural arms unequal, one arm double the length of rest of the two arms, more or less straight, less wavy, extending up to the rim; polar axis 16.35 – 18.42 µm, equatorial axis (proximal face) 25.19 – 37.02 µm mean P/E ratio is 0.58 ± 0.04 µm, large spores" (Khan *et al.,* 2023).

2. *O. gramineum.* (Fig. 61)

"Spores globose and trilete, retate- reticulate; the distal face sizes up to 36.59 × 35.42 µm,granulose, retate- reticulate; lumina are deep, conical-circular, muri reticulate with pointed triangular edges; proximal cavity narrow, with shallow lumina on its face, triradiate leasaral arms are short, less wavy, restricted only to the half portion of the proximal cavity, only one among the three leasural arms is extended to the rim and jointed to the edge, rest of the two arms are ends within the cavity; polar axis 17.28 – 20 µm, equatorial axis (proximal face) 25.36 – 33.3µm, mean P/E ratio is 0.59 ± 0.03 µm, large spores" (Khan *et al.,* 2023).

3. *O. indicum.* (Fig. 62)

Spores globose, trilete, granulose; the distal face sizes up to 32.02 × 28.27 µm, muri reticulate, smooth, lumina shallow, more or less circular; proximal cavity deep, the triradiate leasaral arms almost equal, less wavy, jointed, ends inside proximal cavity. Abortive spores are also present. Polar axis 15.58 – 22.97 µm, equatorial axis (proximal face) 30.3 – 48.73 µm, mean P/E ratio is 0.51 ± 0.02 µm, large spores.

4. *O. latifolium.* (Fig. 63)

Spores globose, trilete, reticulate, patellate; the distal face sizes upto 32.02 × 28.27 µm; the tapetal residue is present; muri reticulate, narrow, ridges broad; lumina shallow, conical-square shaped; proximal cavity shallow, with embedded triradiations, leasural arms are short, less wavy, jointed and ends at the rim; polar axis 16.31 – 18.21 µm, equatorial axis (proximal face) 31.73 – 34.9 µm, mean P/E ratio 0.51 ± 0.00 µm, large spores.

5. O. lusitanicum. **(Fig. 64)**

"Spores globose-ellipsoid, trilete, patellate, dimorphic; distal face sizes up to 34.69 × 29.21µm; sporoderm is smooth due to the extensive deposition of perine layer over it, lumina shallow, irregular in shape; muri reticulate, smooth and flattened; the proximal face is dimorphic with and without a proximal cavity; the triradiate leasural arms almost equal, straighter, less wavy, extending to the rim and jointed to the edges. Polar axis 14.36 – 17.21µm, equatorial axis (proximal face) 22.33 – 30.36 µm, mean P/E ratio is 0.58 ± 0.03 µm, large-sized spores" (Khan *et al.,* 2023).

6. *O. lusoafricanum.* **(Fig. 65)**

"Spores globose, verrucate, dimorphic with alete and trilete spores, globose; distal face non astamosing, areoles are absent, proximal face alete or trilete; Trilete spores have arms extending up to rim, unequal, one arm double the size of rest of the two arms, the deep proximal cavity have small lumina. Polar axis 21.49 – 32.54 µm, equatorial axis (proximal face) 42.28 – 66.73 µm, mean P/E ratio is 0.52 ± 0.02 µm, large spores" (Khan *et al.,* 2023).

7. *O. parvifolium.* **(Fig. 66)**

"Spores globose and trilete; distal face sizes up to 33.67 × 30.69 µm; granulose, reticulate- patellate, perine layer is present; lumina are shallow, more or less circular, muri reticulate; triradiations less wavy, extending up to the rim; one leasural arm is double the length of rest of the two arms, Polar axis is 14.11 – 16.7µm, the equatorial axis (proximal face) 21 – 33.01µm, mean P/E ratio is 0.56 ± 0.05 µm, large spores" (Khan *et al.,* 2023).

8. *O. pendulum*. **(Fig. 67)**

"Spores are globose and trilete, reticulate, comparatively more enormous than the spores of other *Ophioglossum* species included in this study; the distal face sizes upto 51.83 × 52. 26 µm; perine layer deposition towards the centre; lumina narrow, more or less conical, muri reticulate, very thin and narrow without sharply pointing edges, somewhat wavy and smooth in appearance; the proximal cavity is shallow, triradiate leasural arms extending to the margins; polar axis 25 – 28.02 µm, equatorial axis (proximal face) 39.7 – 43.69 µm, mean P/E ratio is 0.62 ± 0.02 µm, large spores" (Khan *et al.,* 2023).

9. *O. petiolatum.* (Fig. 68)

"Spores trilete and globose, rugate, granulose; distal face sizes upto 30.68 × 33.75 µm; lumina as minute depressions, more or less rounded, muri as discontinuous ridges with thick and thin exine regions, thick ridges have granulose deposition over it, perine layer absent; proximal face trilete, leasural arms are almost equal, wavy, jointed, extending up to the rim of the deep proximal cavity, granulose. Polar axis 17.21 – 18.09 µm, equatorial axis (proximal face) 33.01 – 34.07 µm, mean P/E ratio is 0.52 ± 0.00 µm, large spores" (Khan *et al.,* 2023).

10. *O. raphaelianum.* (Fig. 69)

"Spores trilete and globose-ellipsoid, foveolate,; distal face sizes up to 26.24 × 20.44 µm; distal face foveolate, granulose; lumina shallowly depressed, more or less square shaped; muri reticulate, narrow without sharp pointed edges, perine layer present; proximal face trilete, leasural arms are more or less wavy, jointed, up to the centre of the proximal cavity and are pointing towards the wall in between trilobed proximal end; the distal face is granulose, polar axis 14.27 – 18.96 µm, equatorial axis (proximal face) 30.53 – 37.4 µm, mean P/E ratio is 0.53 ± 0.04 µm, large spores" (Khan *et al.,* 2023).

11. *O. reticulatum.* (Fig.70)

"Spores globose-ellipsoid, trilete, reticulate, granulose; distal face sizes upto 32.95 µm × 27.50 µm; lumina shallowly reticulate, perine layer is present, muri narrow, without pointed edges; proximal face trilete, leasural arms are wavy, more or less equal, jointed, up to the rim. Polar axis 15.55 –23.72 µm, equatorial axis (proximal face) 28.2 – 40.82 µm, mean P/E ratio 0.55 ± 0.01 µm, large spores" (Khan *et al.,* 2023).

12. *O. rubellum.* (Fig. 71)

"Spores globose, trilete or alete, lophate or smooth; distal facesizes up to 22 ×33 – 25.69 × 22.29 µm, lophate or smooth; muri are lophate with outer exospore wall raised to form ridges, muri surrounds the lumina, lumina are polygonal with three-five angles, some spores have very smooth and plain surface in the complete absence of

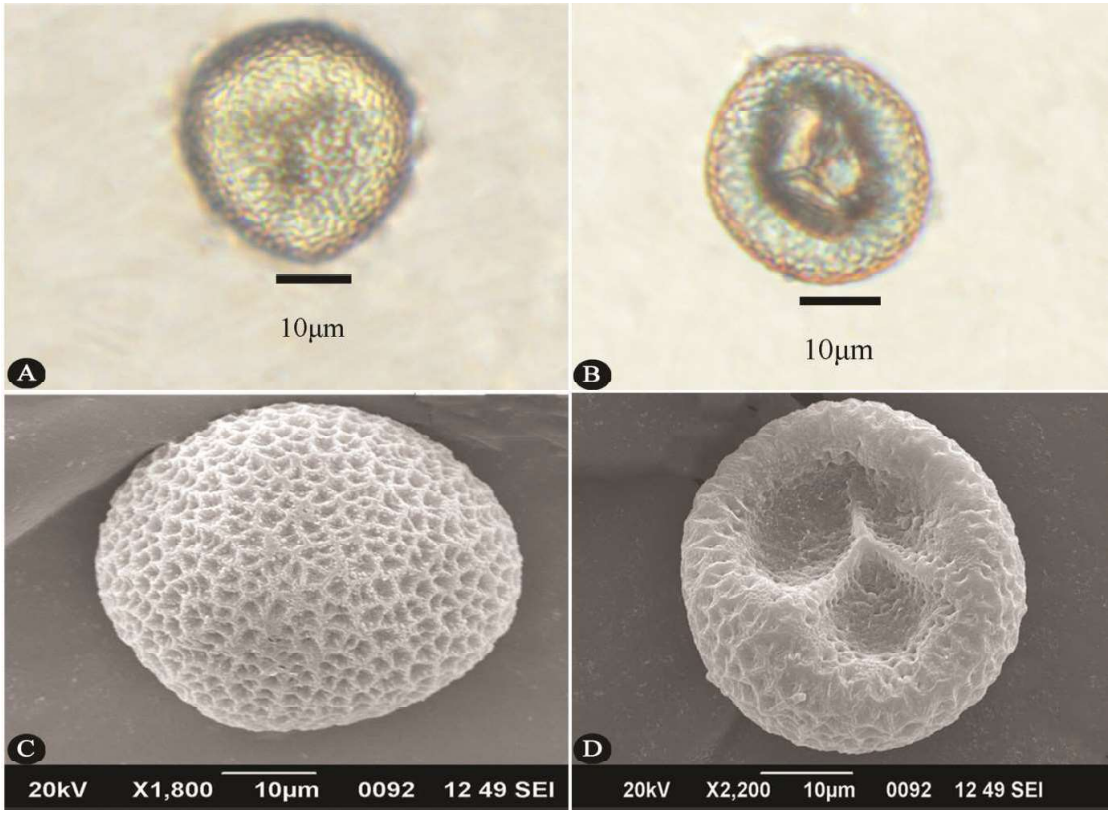

Fig. 60. Spores of *Ophioglossum costatum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

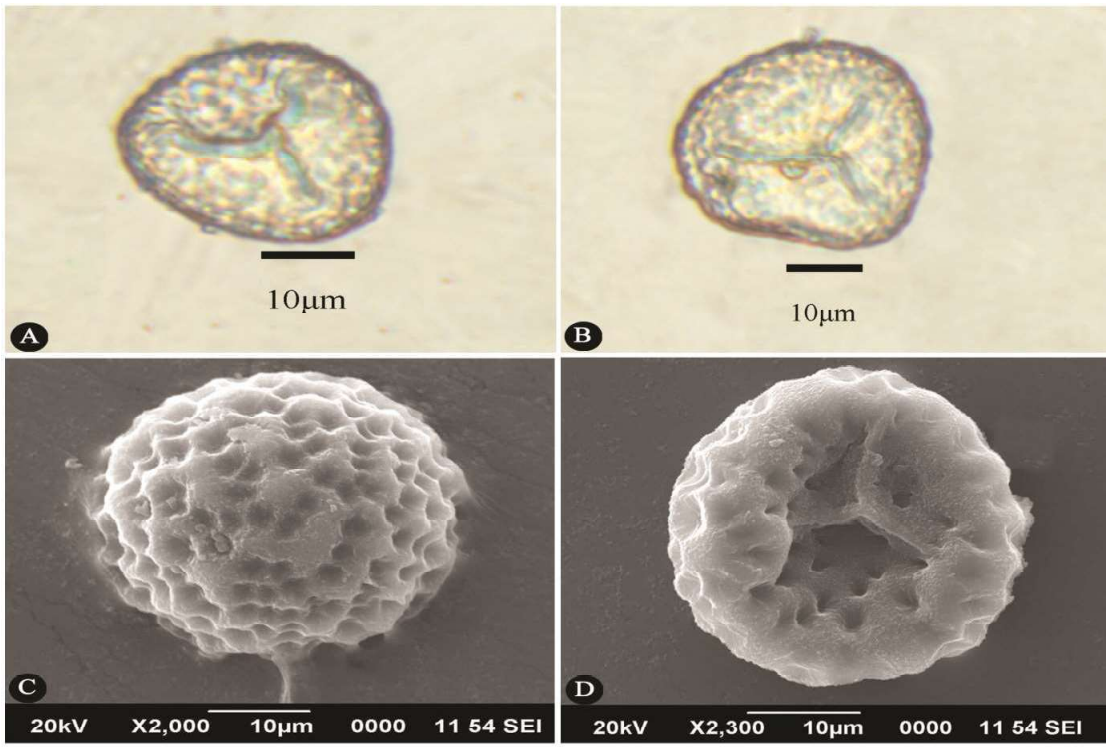

Fig. 61. Spores of *Ophioglossum gramineum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

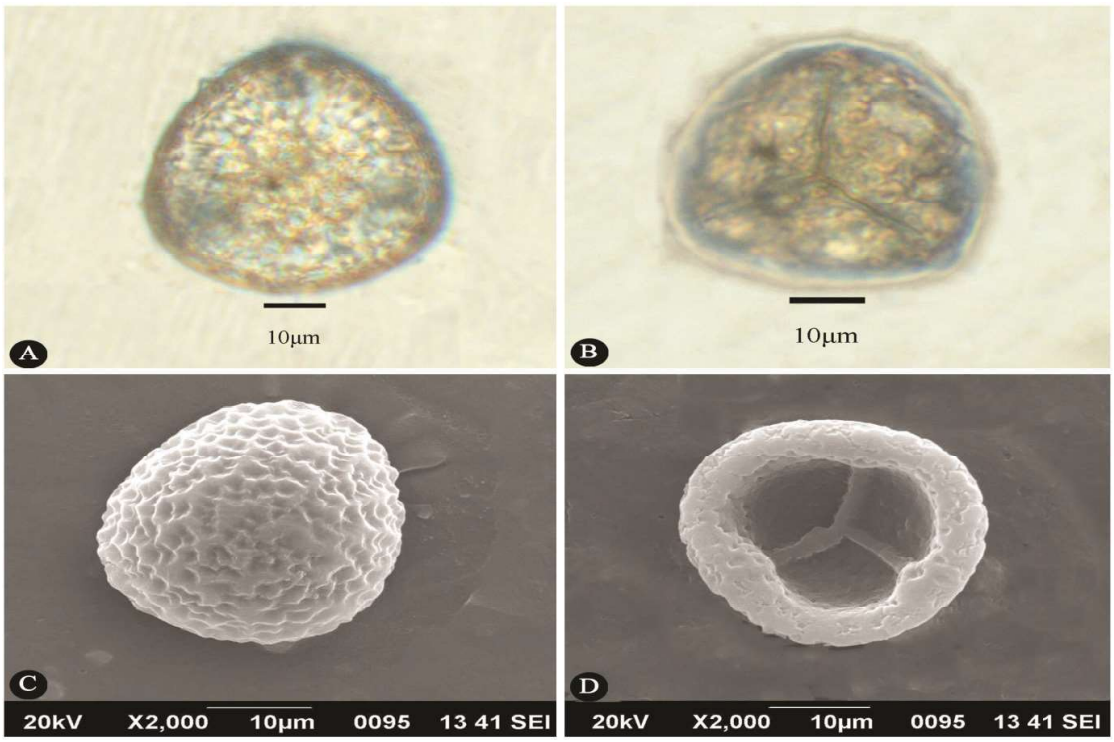

Fig. 62. Spores of *Ophioglossum indicum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

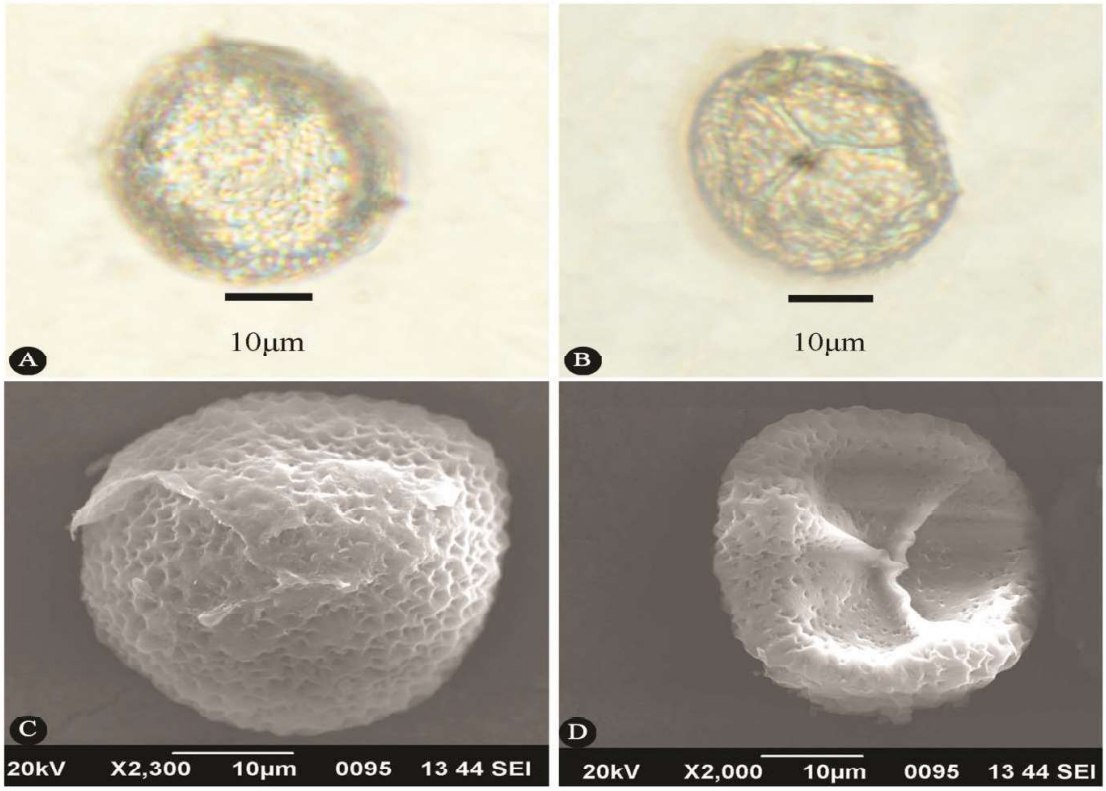

Fig. 63. Spores of *Ophioglossum latifolium*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

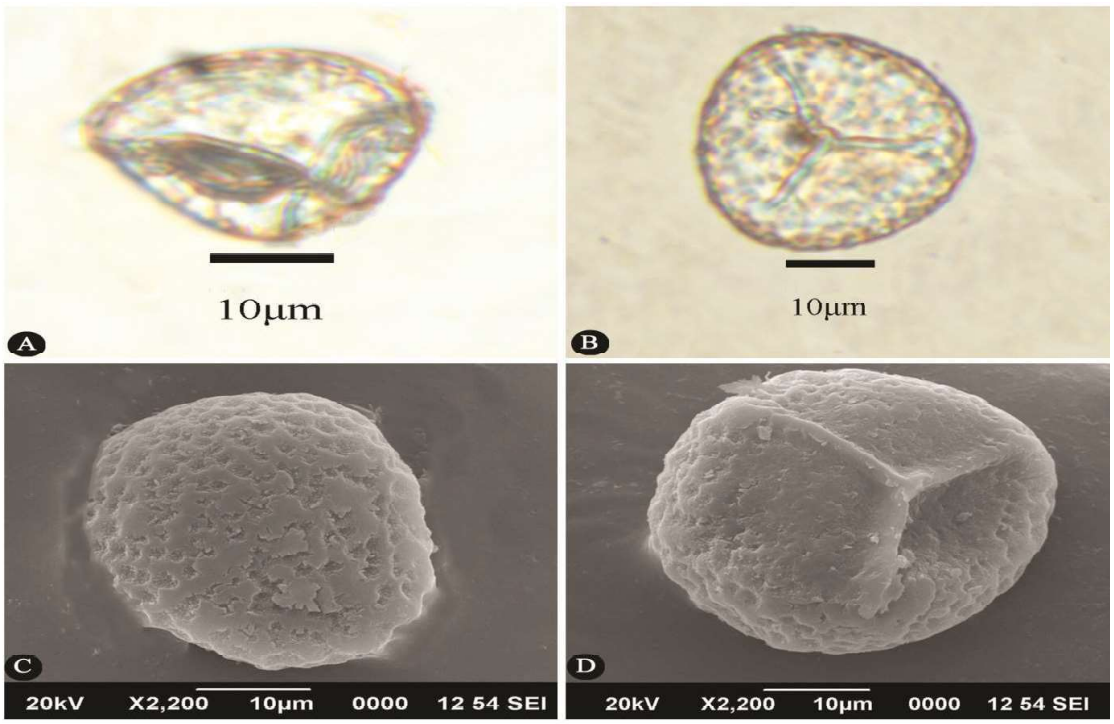

Fig. 64. Spores of *Ophioglossum lusitanicum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

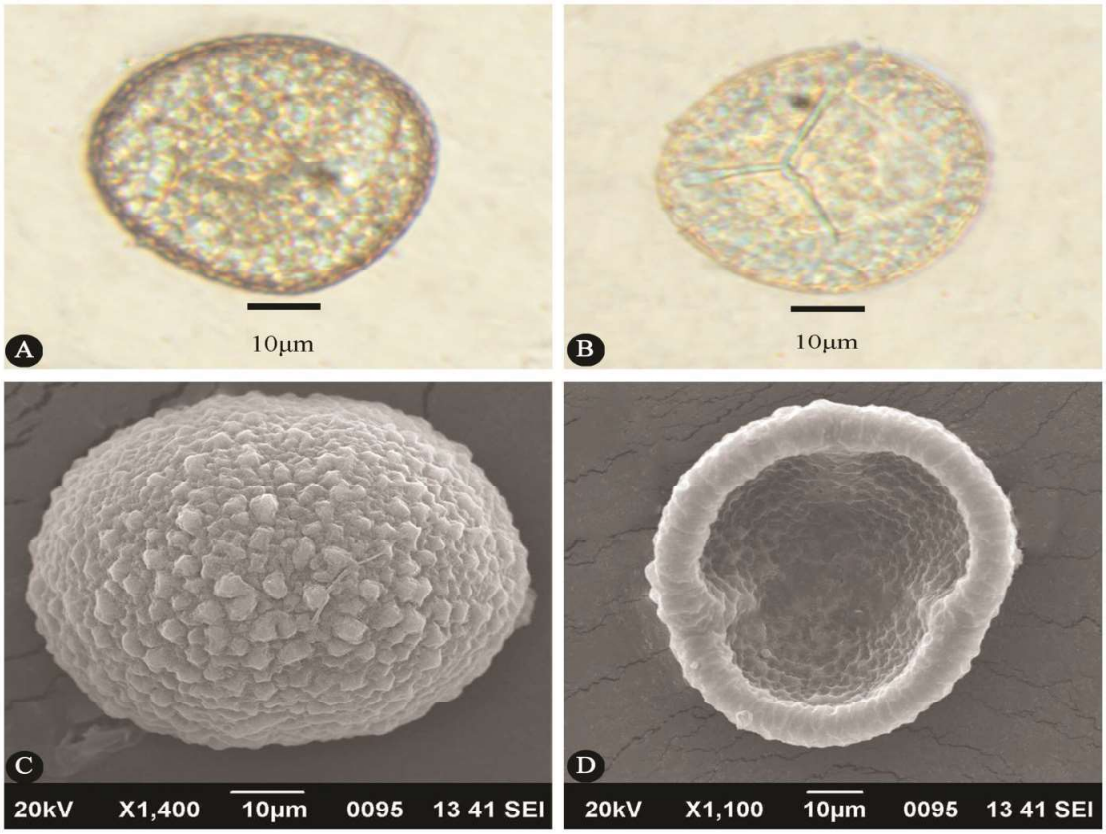

Fig. 65. Spores of *Ophioglossum lusoafricanum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

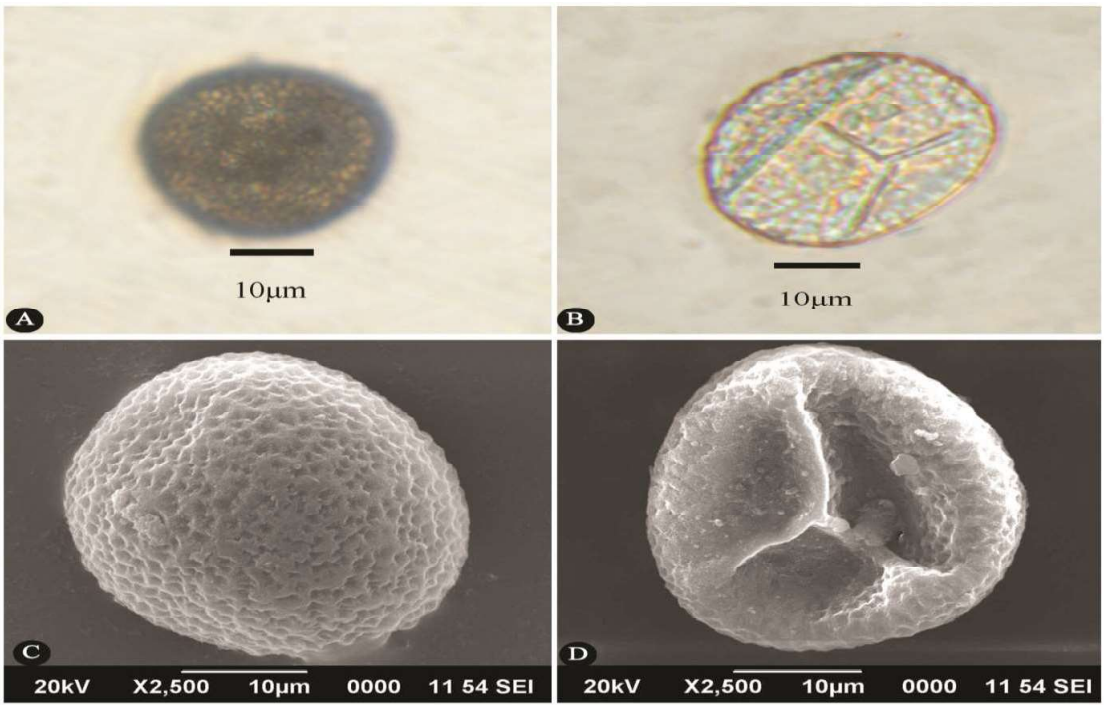

Fig. 66. Spores of *Ophioglossum parvifolium*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

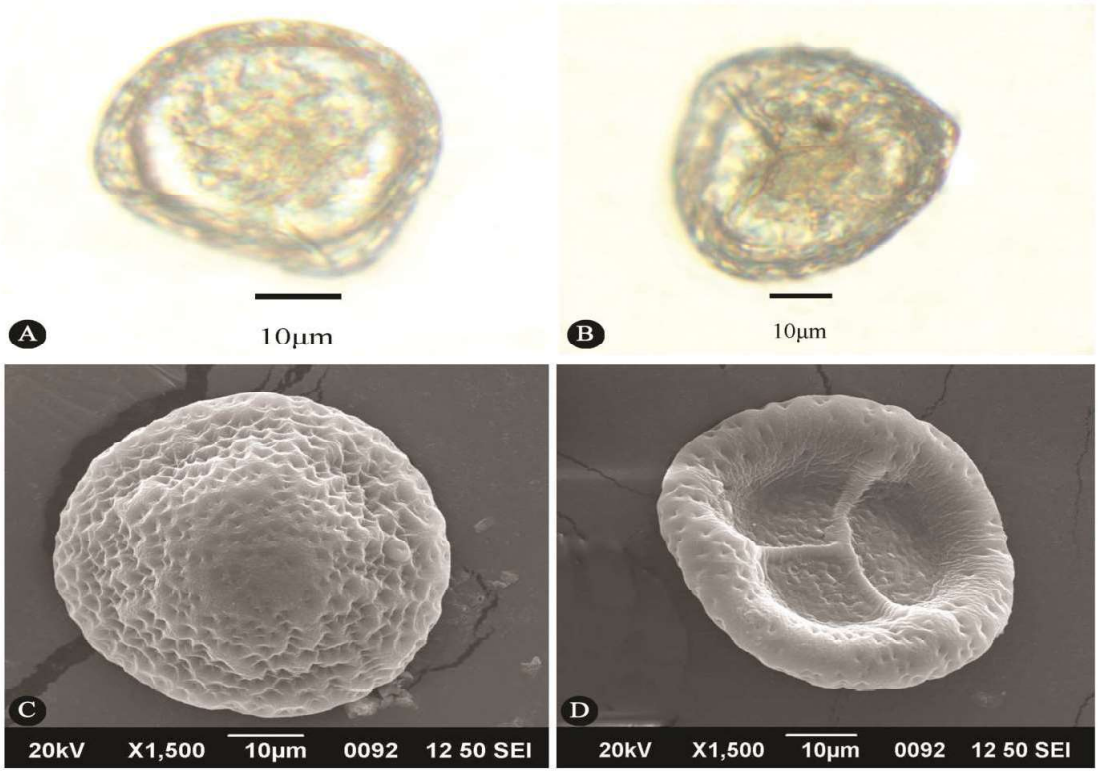

Fig. 67. Spores of *Ophioglossum pendulum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

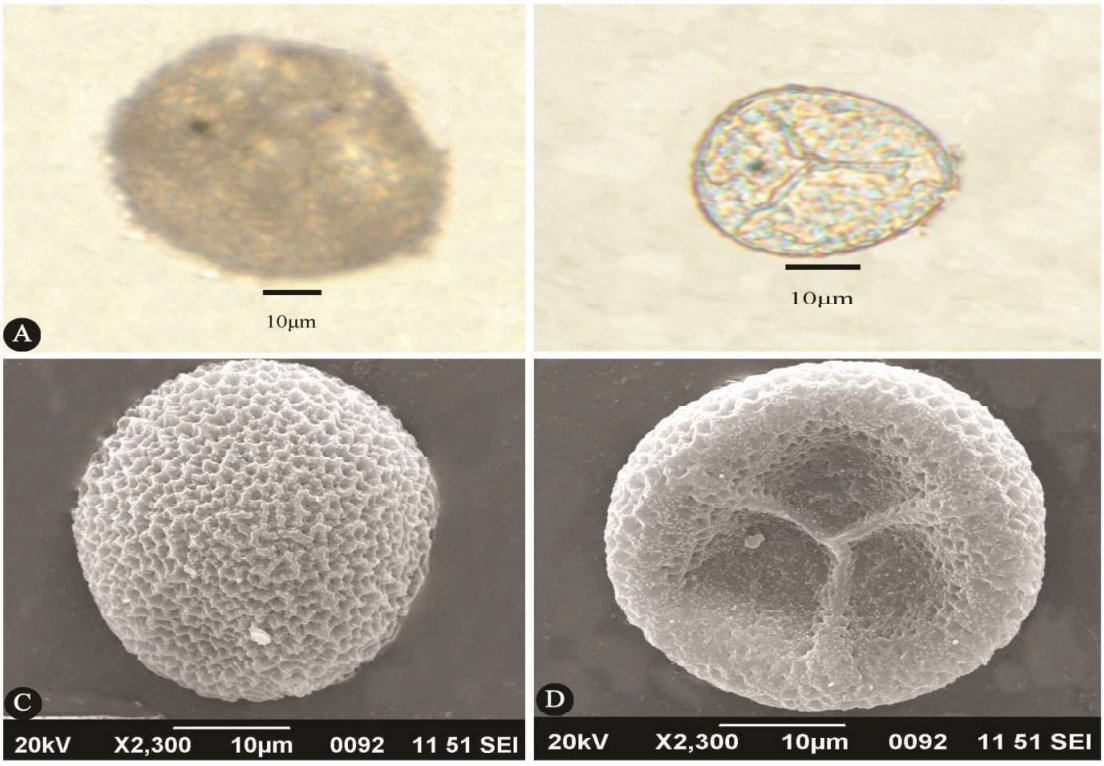

Fig. 68. Spores of *Ophioglossum petiolatum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

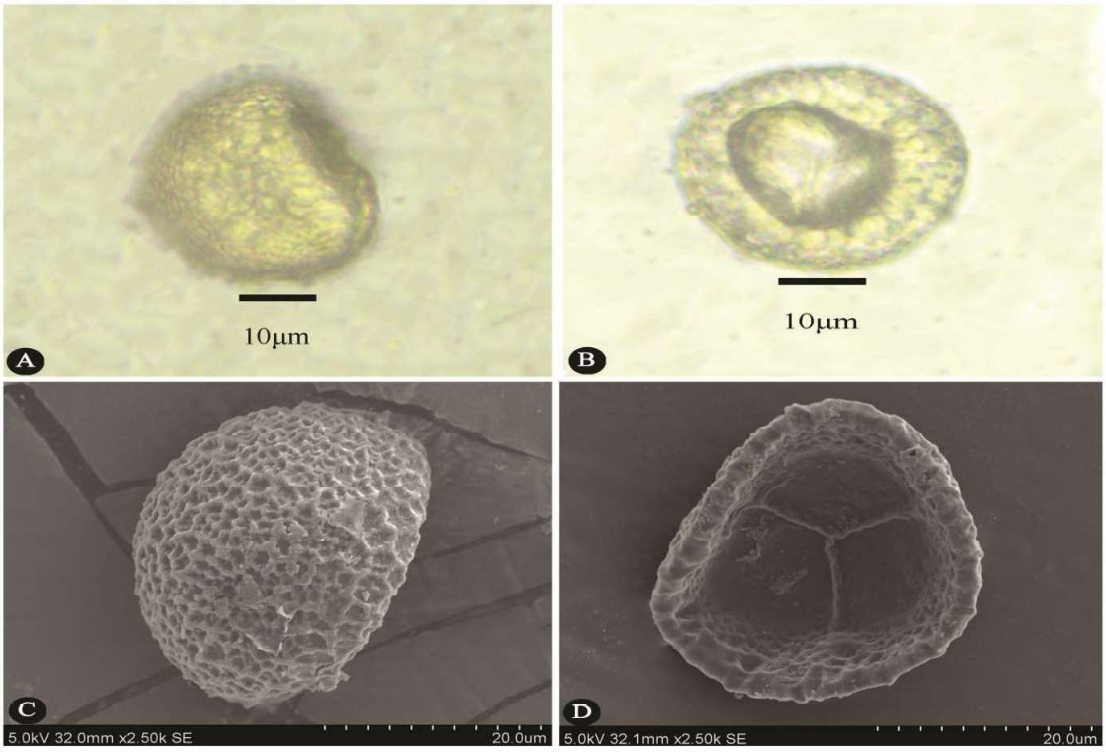

Fig. 69. Spores of *Ophioglossum raphaelianum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

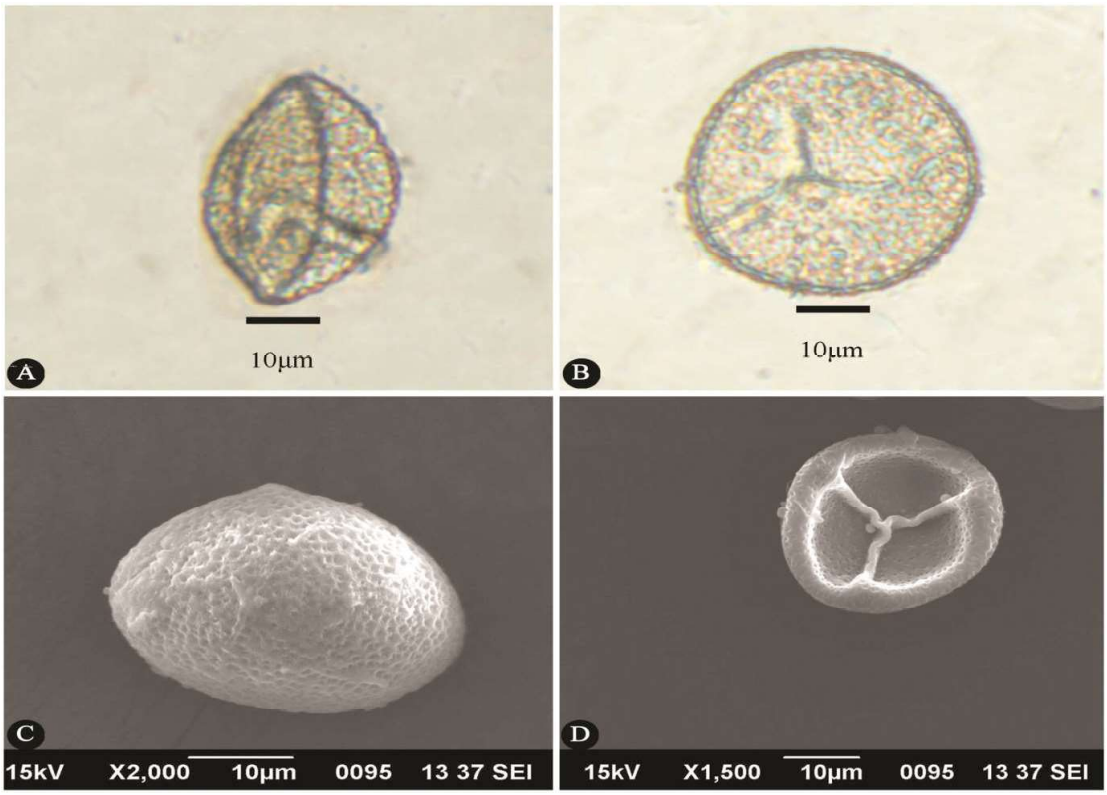

Fig.70. Spores of *Ophioglossum reticulatum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

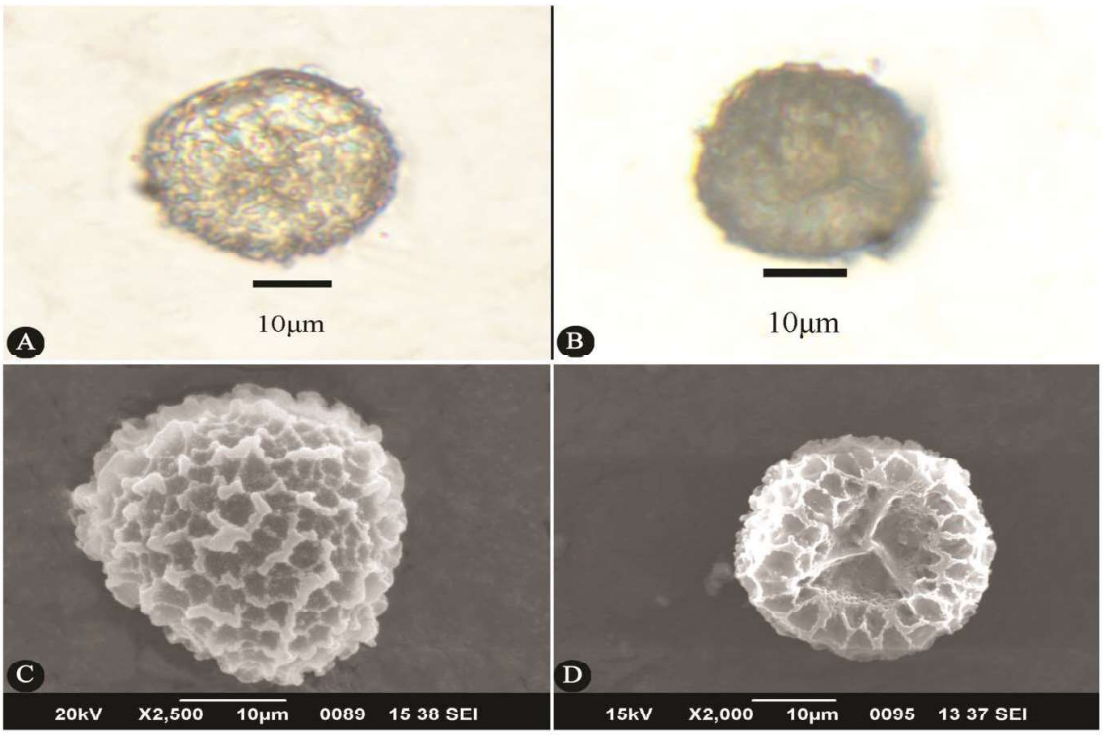

Fig. 71. Spores of *Ophioglossum rubellum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

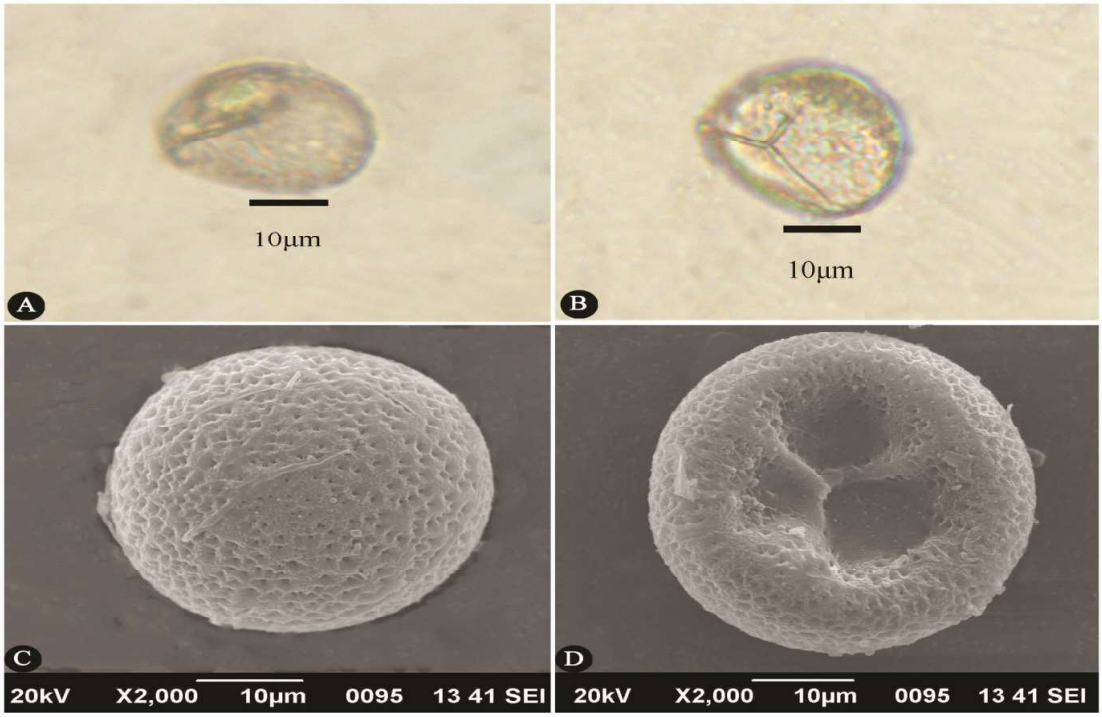

Fig. 72. Spores of *Ophioglossum vulgatum*: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

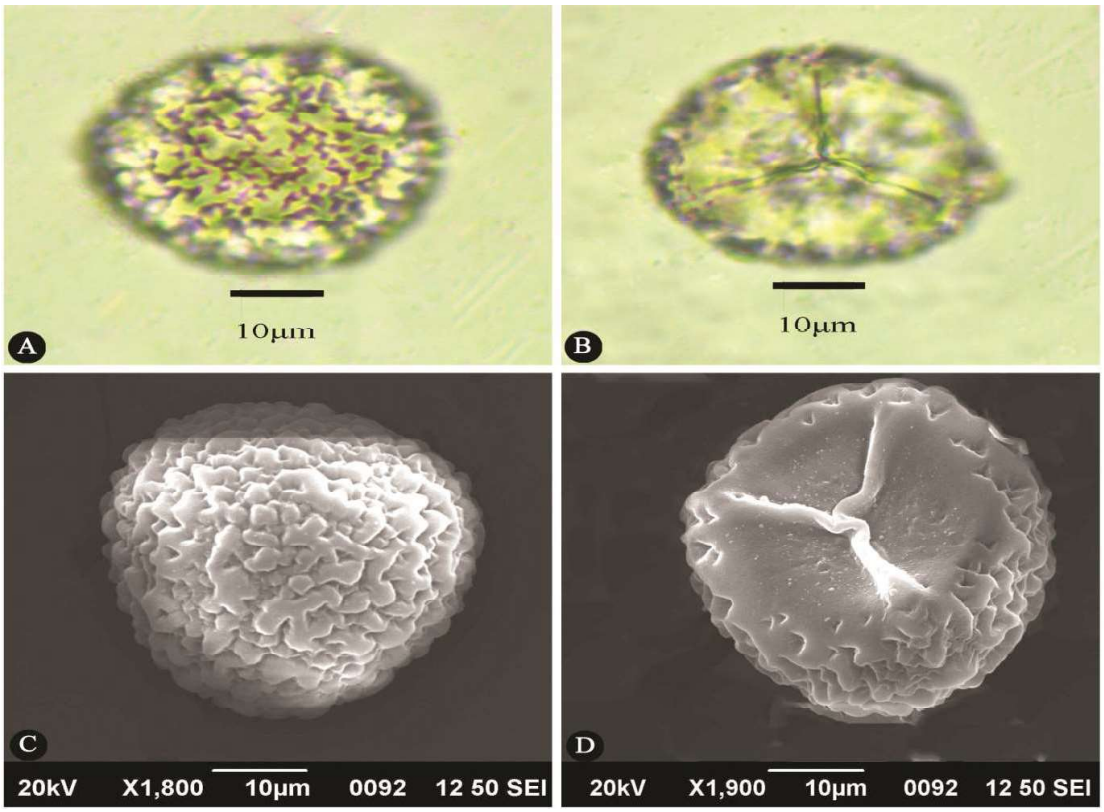

Fig. 73. Spores of *Ophioglossum* sp. nov.: A. Distal surface under LM., B. Proximal surface under LM., C. Distal surface under SEM., D. Proximal surface under SEM.

lumina and muri; in trilete spores, the leasaral arms more wavy and jointed, almost equal, ends at middle of the proximal cavity; polar axis 8.76 – 14.1µm, equatorial axis (proximal face) 22.15 – 28.89 µm, mean P/E ratio 0.46 ± 0.03 µm, medium sized spores" (Khan *et al.,* 2023).

13. *O. vulgatum.* (Fig. 72)

Spores are globose, trilete, granulose, verrucate; distal face sizes upto 36. 94 µm × 35.43 µm, granulose, verrucate, lumina as small deep pits, muri reticulate, with narrow and small cavity, with or without pointed ridges; proximal cavity shallow, triradiate leasural arms small, unequal, one arm is double the size of rest of the two arms; polar axis 16. 37 – 20.46 µm, equatorial axis (proximal face) 20.99 – 34.45 µm, mean P/E ratio 0.57 ± 0.02 µm, largesized spores.

14. *O.* sp. nov. (Fig. 73)

Spores globose, trilete rugate with longer and wider muri; distal face up to 39.14 × 39.99 µm; distal face gives a human brain-like appearance; exine plates form broad irregular muri, exine raised to form ridges and grooves, more or less flattened towards the centre; lumina triangular as continuous grooves, irregular in shape, less deep in the distal face whereas deeper towards the proximal end; proximal face is smooth, flattened, a deep proximal cavity is absent, unlike the distal face proximal face is plain and smooth in the absence of lumina; tri radiations more wavy and jointed,extending to the rim, projecting outside from the plane of the proximal cavity; polar axis 17.69 – 19.86 µm, equatorial axis (proximal face) 30.32 – 37.85 µm, mean P/E ratio is 0.55 ± 0.03, large sized spores.

Detailed examination of the spore characters revealed distinctspore characters for each species. The exine ornamentation is different for different species. So, the SEM studies of the spore characters along with the analysis of macromorphological characters found to be good and effective in identifying different species of the genus *Ophioglossum*.

4.3. Molecular Phylogenetic Analysis

Phylogeny of *Ophioglossum* species based on the analysis of *rbc*L-F region. (Fig. 74)

Fig.74. Phylogenetic tree of *Ophioglossum* species based on the analysis of *rbcL* region using Maximum likelihood method. ML bootstrap values are shown on branches.

The *rbc*L-F region of 16 sequences had an aligned length of 429 nucleotide sites. The final data set had 33 parsimony informative sites, 343 invariant (constant or ambiguous constant) sites. The best fit model according to Bayesian Information score (BIC) was TNe+G4. Bootstrap consensus tree from 1000 replicates was used and the ML bootstrap values were shown in the branches. The tree was supported by good bootstrap values. The genus *Ophioglossum* formed a distinct lineage diverged

from the out-group *B. lunaria.* Based on *rbc*L-F sequences, genus *Ophioglossum* formed two clades. Clade 1 was formed with 5 species and clade 2 was formed with 10 species included in the study. Clade 1 included *O. parvifolium*, *O. lusitanicum*, *O. latifolium*, *O. lusoafricanum* and *O. indicum* very closely aligned and originated from a common ancestor. The sequence divergence was less among the members of clade 1. Polytomy was also observed in clade 1, which may be aroused as result of simultaneous speciation. The species of clade 1are comparatively smaller and are morphologically relatable.

Clade 2 was formed by all the other species included in the study. Clade 2 was again divided in to 3 sub clades. In Sub clade 1, *O. costatum* and *O. gomezianum* are grouped together. Subclade 2 contains majority of the species included in the analysis (7 species). In sub clade 2, *O*. sp. nov., *O. rubellum* and *O. polyphyllum* formed distinct lineages nearest to each other. *O. reticulatum*, *O. vulgatum*, *O. petiolatum* and *O. gramineum* grouped together showing their origin from a common ancestor. The sequence divergence was less in this group and they also showed polytomy. The origin of epiphytic *O. pendulum* was more divergent from all other terrestrial taxa and formed a separate lineage as subclade 3.

Phylogeny of *Ophioglossum* species based on the analysis of *psb*A-*trn*H region. (Fig. 75)

The *psb*A-*trn*H region of 14 sequences had an aligned length of 279 nucleotide sites. The final data set has 178 parsimony informative sites, 61 invariants (constant or ambiguous con stant) sites. The best fit model according to BIC was K3Pu+F+G4. Bootstrap consensus tree from 1000 replicates was used and the ML bootstrap values were shown in the branches. The tree waswell supported by good boot strap values The genus *Ophioglossum* formed a seperate lineage divergerd from the out-group *B. lunaria.* Based on the *psb*A-*trn*H sequences, genus *Ophioglossum* was mainly divided in to two clades. Clade 1 have 8 species and clade 2 have 5 species included within it.

Clade 1 contains two subclades. Subclade1a and subclade 1b, more diverged from each other. Subclade 1a have *O*. sp. nov. and *O. rubellum* grouped together ., *O. lusitanicum* as seperete entity, *O. latifolium*, O. *parvifolium* and *O. lusoafricanum* as a separate group showing less sequence divergence and polytomy. Subclade 1b contains *O. costatum* and *O. indicum* grouped together. In clade 2 *O. raphaelianum* and *O.*

gramineum were formed as separate entities, *O. reticulatum*, *O. petiolatum* and *O. vulgatum* were closely aligned and formed a separate group with polytomy.

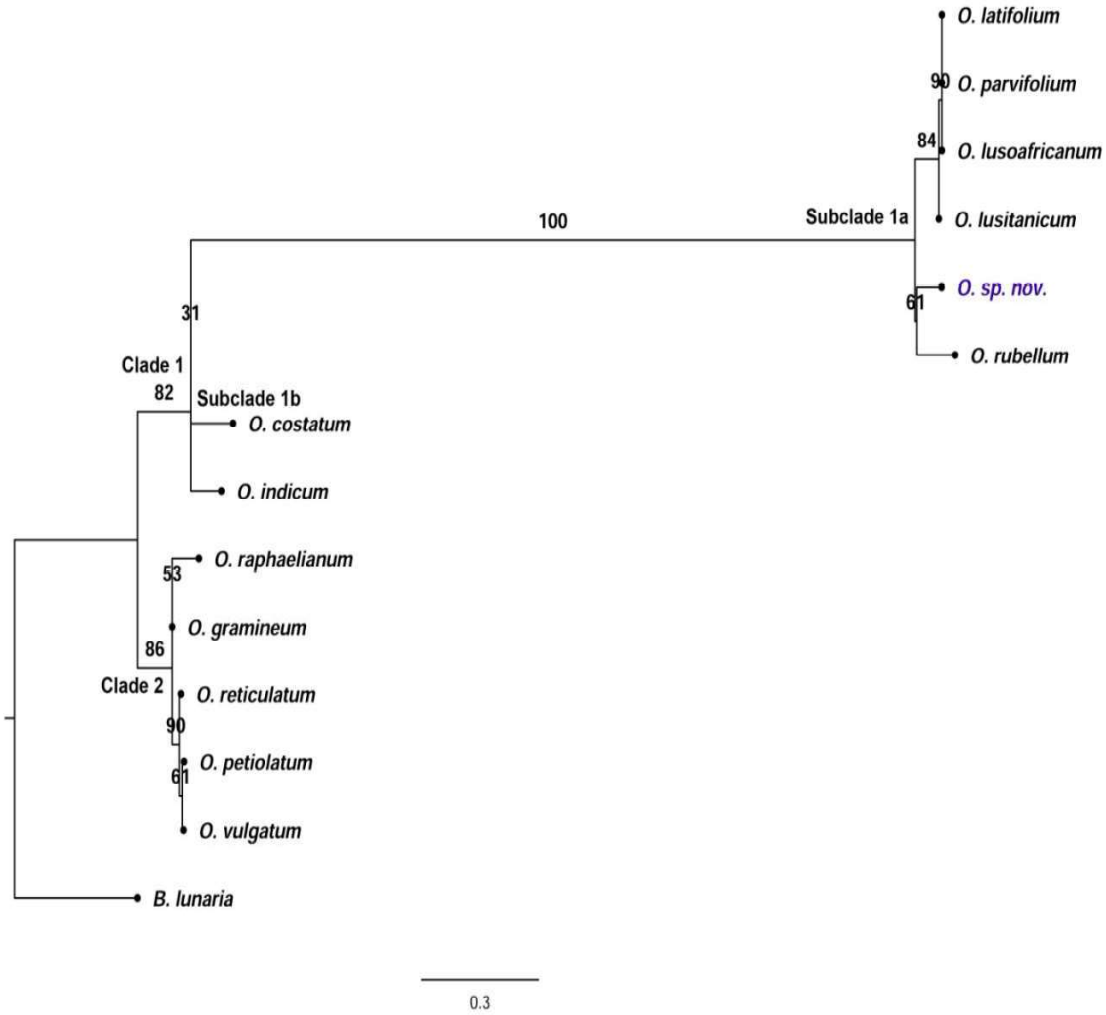

Fig. 75. Phylogenetic tree of *Ophioglossum* species based on the analysis of *psb*A-*trn*H region using Maximum likelihood method. ML bootstrap values are shown on branches.

Phylogeny of *Ophioglossum* species based on the analysis of *trn*L-F region. (Fig. 76)

The *trn*L-F region of 14 sequences had an aligned length of 252 nucleotide sites. The final data set has 57 parsimony informative sites, 130 invariant (constant or ambiguous constant) sites. The best fit model according to BIC was K2P. Bootstrap consensus tree from 1000 replicates was used and the ML bootstrap values were shown in the branches. The tree was supported by good bootstrap values. High sequence divergence was observed between the out-group *B. lunaria* and the clade containing genus *Ophioglossum*. According to the *trn*L-F sequences, the genus *Ophioglossum* was separated in to 3 clades. Clade 1 had a single species. Clade 2 had

4 species and clade 3 had 8 species included within it. Clade 1 was formed with *O. latifolium* distinct from all other species. Clade 2 consists of *O. lusitanicum*, *O. parvifolium*, *O. indicum* and *O. lusoafricanum* very closely aligned and showing polytomy and less sequence divergence.

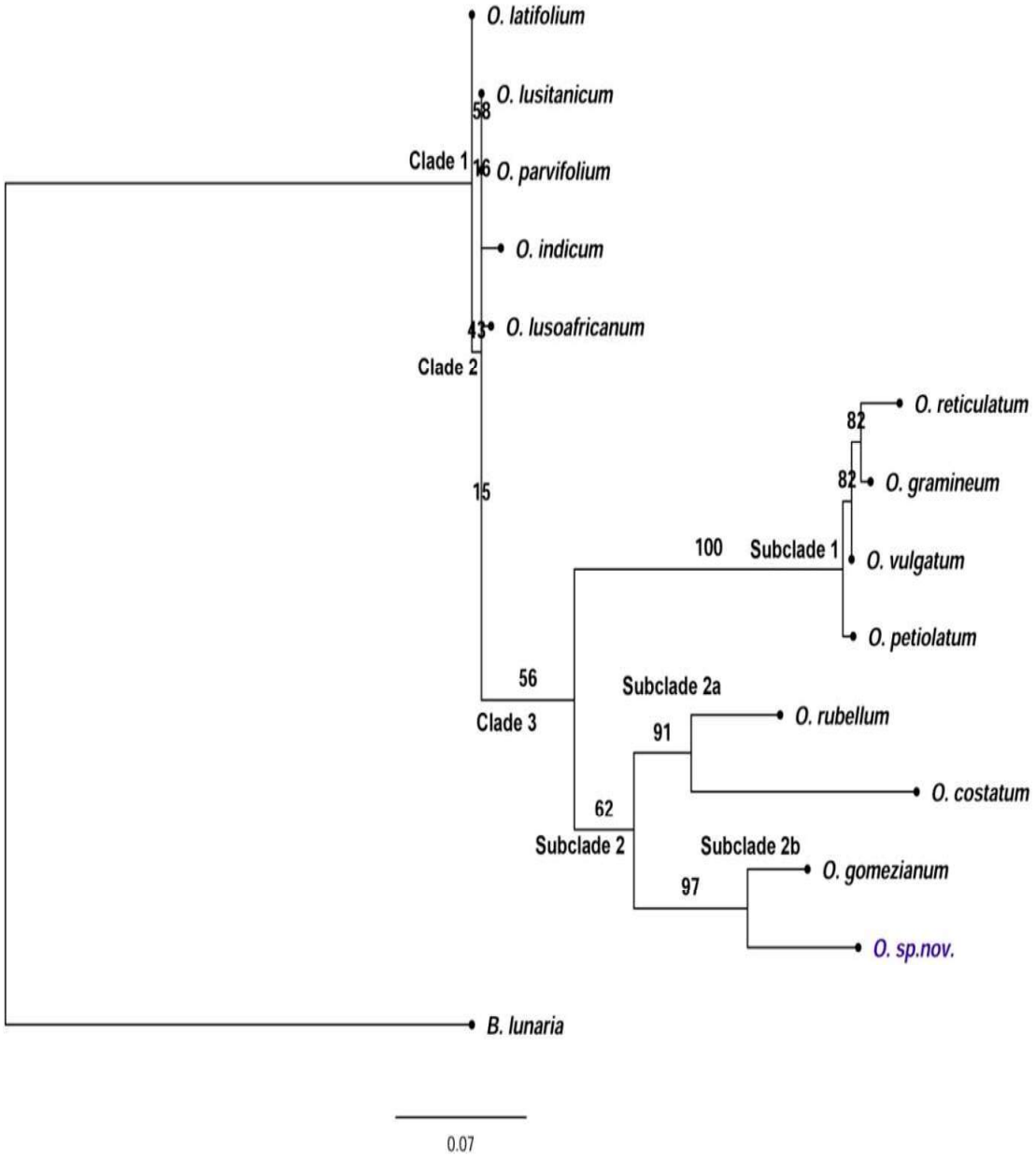

Fig. 76. Phylogenetic tree of *Ophioglossum* species based on the analysis of *trn*L-F region using Maximum likelihood method. ML bootstrap values are shown on branches.

The clade 3 was divided into 2 subclades. Subclade 1 contains *O. petiolatum* and *O. vulgatum* as separate entities, *O. reticulatum*, and *O. gramineum* grouped together. Subclade 2a have *O. rubellum* and *O. costatum* and Subclade 2b have O. *gomezianum* and *O*. sp. nov.

The new species status of *O.* sp. nov. has been well supported by good bootstrap values in the phylogenetic trees. In the *rbc*L-F Tree, *O.* sp. nov. formed a separate lineage with *O. rubellum* as the sister group and it was supported by of 36 % bootstrap value. In the *psb*A-*trn*H, *O.* sp. nov. formed as a separate entity and formed a clade with *O. rubellum* as the sister group. It was supported by 61% bootstrap value. In trnL-F tree, O. sp. nov. separated as distinct taxa and formed a clade with *O. gomazianum*. It was supported by 97 % bootstrap value. *O. rubellum* and *O. costatum* was formed a separate clade sister to the clade containing *O.* sp. nov. From the analysis of the three choloroplast regions, it was evident that *O.* sp. nov. is phylogenetically more related to *O. rubellum, O. gomazianum* and *O. costatum.*

4.4. Reinstating the species status of *O. indicum* and *O. raphaelianum* by differentiating from *O. rubellum.* (Fig. 77 & Table 4.)

4.4.1. Reinstating *O. indicum*

O. indicum was characterized by pinkish green colour where as *O. rubellum* was characterized by red or thin copper tinged colour. The tropophylls of *O. indicum* is elliptic-lanceolate but the tropophylls of *O. rubellum* are mostly ovate, occasionally elliptic or spathulate-obovate or sub orbicular in nature. The tuberous-elongated rhizome of *O. indicum* is different from the globose rhizome of *O. rubellum.* Fertile segment is 2 – 4 cm cm long in *O. indicum* and 1.5 – 7 cm long in *O. rubellum*. *O. indicum* had 6 – 7 sporangia whereas *O. rubellum* has 6 – 10 sporangia. In *O. indicum*, four veins are arising from the base but in *O. rubellum* seven veins are start from the base. The stomatal apparatus of *O. indicum* is larger, elongated and the walls were wavier than the stomatal apparatus of *O. rubellum.* The subsidiary cells were elongated in *O. indicum* where as polygonal-elongated in *O. rubellum*. Presence of twin stomata was observed in *O. indicum* but twin stomata was absent in *O. rubellum*. The spore characters were also distinct for the two species. Spores of *O. indicum* were trilete but that of *O. rubellum* were dimorphic with alete or trilete spores. The distal face of *O. indicum* is granulose where as the distal face of *O. rubellum* is lophate in nature. In the *rbc*L-F tree and trnL-F tree *O. indicum* and *O. rubellum* lies within separate clades, indicating more sequence divergence. Eventhough *O. indicum* lies within the subclade 1a and *O. rubellum* lies within the subclade 1b of Clade 1 in the *psb*A-*trn*H tree, the two subclades showed high sequence divergence. So, by the detailed examination of all the morphological characters along molecular characters

and comparing with the type specimens, *O. indicum* and *O. rubellum* collected during this study was found to be two distinct species and hence the species status of *O. indicum* was reinstated.

4.4.2. Reinstating *O. raphaelianum*

O. raphaelianum was distinguished by it's olivaceous or bluish green colour whereas *O. rubellum* was distinguished by its red or copper colour tinge. *O. raphaelianum* has mostly widely ovate-orbicular rarely elliptic tropophylls whereas *O. rubellum* has mostly mostly ovate, occasionally elliptic or spathulate-obovate or sub orbicular tropophylls. However, *O. raphaelianum* have 1 – 3 cm long fertile segment with 6 – 7 alternately arranged sporangia per spike which is different from 1.5 – 7 cm long fertile segment of *O. rubellum* having 6 – 10 oppositely arranged sporangia. Six veins arise from base of tropophyll in *O. raphaelianum* where as it is seven in *O. rubellum*. *O. raphaelianum* has 5 subsidiary cells whereas *O. rubellum* has 4 subsidiary cells. The spore ornamentation of *O. rapahealianum* and *O. rubellum* is distinct from each other. *O. raphaelianum* has trilete spores whereas *O. rubellum* is dimorphic with trilete or alete spores. The spores with shallow lumina in *O. raphaelianum* make it distinct from the lophate spores of *O. rubellum*. In the phylogenetic analysis, *O. raphaelianum* and *O. rubellum* formed separate clades showing high sequence divergence. By the detailed examination of all the morphological characters along with molecular characters and comparison with the type specimens, *O. raphaelianum* and *O. rubellum* collected during this study is found to be two distinct species and the species status of *O. raphaelianum* was reinstated (Khan *et al.,* 2023). The detailed comparison between the morphological characters of *O. rubellum, O. indicum* and *O. raphaelianum* is given in Table 4.

4.5. Synonymising *O. trilokinathii* in to *O. rubellum*

The macromorphological and spore characters of *O. trilokinathii* showed similarity with *O. rubellum*. The globose-subglobose-tuberous rhizome, 1–4 tropophylls, ovate or orbicular tropophylls, arranged as a rosette, appressed on the soil by horizontally touching the substratum, lophate spores are coincides with the characters of *O. rubellum*. The spore characters of *O. trilokinathii* including, 18 – 30 µm sized lophate

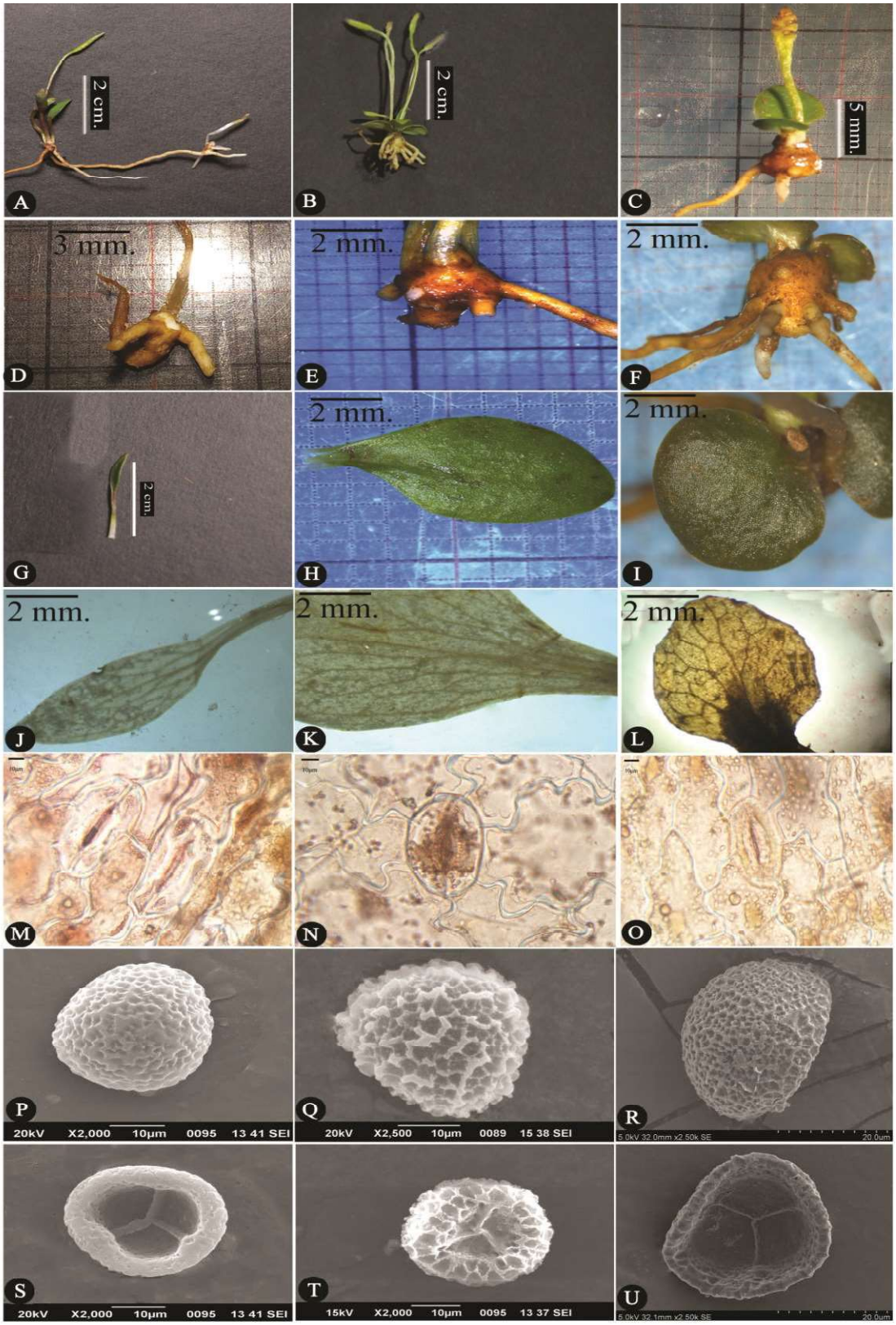

Fig. 77. Comparison between the characters of *O. indicum*, *O. rubellum* and *O. raphaelianum* A-C. Entire plants, D-F. rhizomes, G- I. tropophylls, J-L. venation of tropophylls, M-O. stomata, P-R. distal spore surfaces, S-U. proximal spore surfaces.

Table 4. Comparison between the morphological characters of *O. rubellum, O. indicum and O. raphaelianum.*

Character	*O. rubellum*	*O. indicum*	*O. raphaelianum*
Colour	Red or thin copper tinged	Pinkish green	Olivaceous or bluish green
Plant height	2.1 – 7.8 cm	3 – 6 cm	1.4 – 4 cm
Tropophylls	Mostly ovate occasionally elliptic or spathulate-obovate or sub orbicular	Elliptic-lanceolate	mosltly Widely ovate-orbicular occasionally elliptic
Rhizome	Globose-fusiform	Tuberous-elongated	Globose-ellipsoid
Fertile segment	1.5 – 7 cm	2 – 4 cm	1 – 3 cm
Sporangia Number	6– 10	6 – 7	6 – 7
Number of veins from base	Seven	Four	Six
The stomatal apparatus	Polygonal-elongated, less wavy	Elongated, wavier	elongated, less wavy
Twin stomata	Absent	Present	Absent
Spore	Lophate	Granulose	Foveolate

spores, having thick muri, forming large hexagonalas at distal and proximal surfaces, non-granulate proximal face, straight laesural arms are coincides with the characters of *O. rubellum*. Also, the *rbc*L region showed 100 % identity, *trn*L-F region showed 99.38 % similarity, *psb*A-*trn*H region showed 97.24 % identity between *O. rubellum* and *O. trilokiathii*. So, *O. trilokinathii* was recognized as a smaller form of *O. rubellum* and considered as a synonym of *O. rubellum* in this study.

4.6. Occurrence of different morphotypes of *O. costatum* (Fig. 78, Table 5.) and synonimising *O. madhusoodhananii* in to *O. costatum*

From the repeated field studies and observation on different population of *Ophioglossum* plants within the same locality and in different localities we could find that many morphotypes are present for a single species. Eight morphotypes for *O. costatum* was found in the study area. They showed slight variation in the plant size, size and shape of rhizome, tropophyll and fertile segment. But all of these morphotypes shared the same spore characteristics when observed under SEM. The size of the plants from different population changes from 6 – 28 cm. Morphotype 1 was the shortest, only up to 6 cm high, and morphotypes 6 was the tallest, with 28 cm hight. *O. costatum* showed more diverse rhizome owing to several morphotypes. Globose-tuberous elongated-discoid or cormlike rhizomes are found in different morphotypes. Globose rhizome is present in morphotypes 3 and 5, tuberous elongated rhizome is present in morphotypes 2 and 4 and discoid rhizomes are found in morphotypes 1, 6, 7 and 8. The number, size and shape of the tropophylls vary in different moorphotypes. Morphotypes 3 and 8 have have the fewest number of tropophylls (3) whereas morphotype 6 have highest number of tropophylls (8). Morphotypes 1, 3 have elliptic tropophylls, 2 has elliptic-rhomboid tropophyll and all the other morphotypes have lanceolate tropophylls. A gradual change from alternate to the opposite arrangement or a mix of both was observed in the sporangia of the different morphotypes.

Besides these minor variations, all the morphotypes of *O. costatum* produced the same kind of spores. The spores were globose, reticulate and foveolate in all the morphotypes. A slight variation was observed in the lumina width, lumina isnarrow in morphotypes 5, 1, 7 and gradually widen in morphotypes 8, 6 and 4 and highest in morphotype 3. Morphotypes 1, 2 and 5 have more granular deposition and morphotypes 8, 7 and 3 have less deposition. The detailed comparison between the characters of the eight morphotypes of *O. costatum* is given in Table.5.

The characteristic features of *O. madhusoodhananii* including, discoid rhizome, oppositely arranged sporangia, bifurcated spike, 35 – 60 pairs of sporangia, acute apex of the tropophylls, fusion of the fertile segment at the tropophyll base, spike with a sterile tip, 35–40 µm sized, foveolate spores with unequal leasural arms are shown

Table 5. Comparison between the characters of the eight morphotypes of *O. costatum*.

Character	M. 1	M.2	M.3	M.4	M.5	M.6	M.7	M.8
Height	5 – 8 cm	12 – 17 cm	14 – 18 cm	11 – 17 cm	24 – 26 cm	24 – 28 cm	25 – 27 cm	16 – 20 cm
Rhizome	Discoid	Tuberous elongated	Globose	Tuberous elongated	Globose	Discoid	Discoid	Discoid
Size	0.5 – 1 cm	1 – 2 cm	0.5 – 1 cm	1 – 2 cm	0.5 – 1 cm	1 – 2 cm	0.5 – 1 cm	0.5 – 1 cm
Tropophyll								
Number	5	5	3	4	4	6 – 8	6	3
Size	1 – 2 cm	1 – 4 cm	3 – 5 cm	4 – 6 cm	1.5 – 6 cm	4 – 8 cm	4 – 6 cm	3 – 5 cm
Shape	Elliptic	Elliptic-rhomboid	Elliptic	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate
Apex	Sub obtuse	Sub obtuse	Sub obtuse-acute	acute	Acute	Acute	Acute	Acute
Fertile segment								
Stalk	1 – 2 cm	9 – 12 cm	9 – 11cm	10 – 13 cm	2 – 24 cm	14 – 16 cm	13 – 14 cm	9 – 11 cm
Spike	1 – 2 cm	1.5 – 2.5 cm	1 – 3 cm	3 – 5 cm	3 – 5 cm	3 – 7 cm	2 – 4 cm	3 – 4 cm
Number of Sporangia	17 – 20	11 – 27	21 – 23	39 – 44	8 – 50	60 – 75	48 – 63	40 – 48
Spores	Foveolate	Foveolate	Foveolate	Foveolate	Foveolate	Foveolate	Foveolate	Foveolate

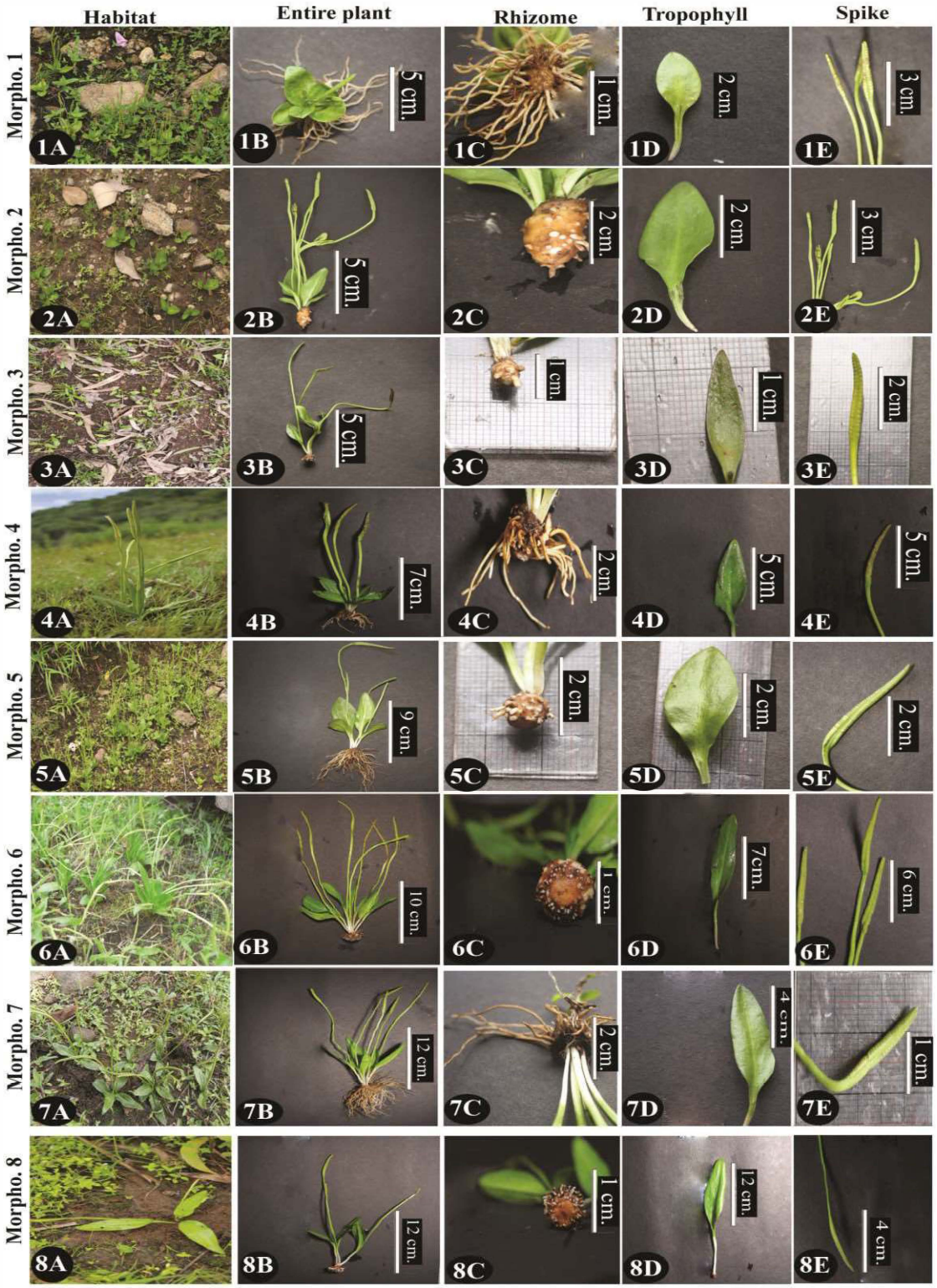

Fig. 78. Eight morphotypes of *Ophioglossum costatum*. A. Habitat, B. Habit, C. Rhizome, D. Tropophylls, E. Spike; 1A-1E. Morphotype 1, 2A-2E. Morphotype 2, 3A-3E. Morphotype 3, 4A-4E. Morphotype 4, 5A-5E. Morphotype 5, 6A-6E. Morphotype 6, 7A-7E. Morphotype 7, 8A-8E. Morphotype 8.

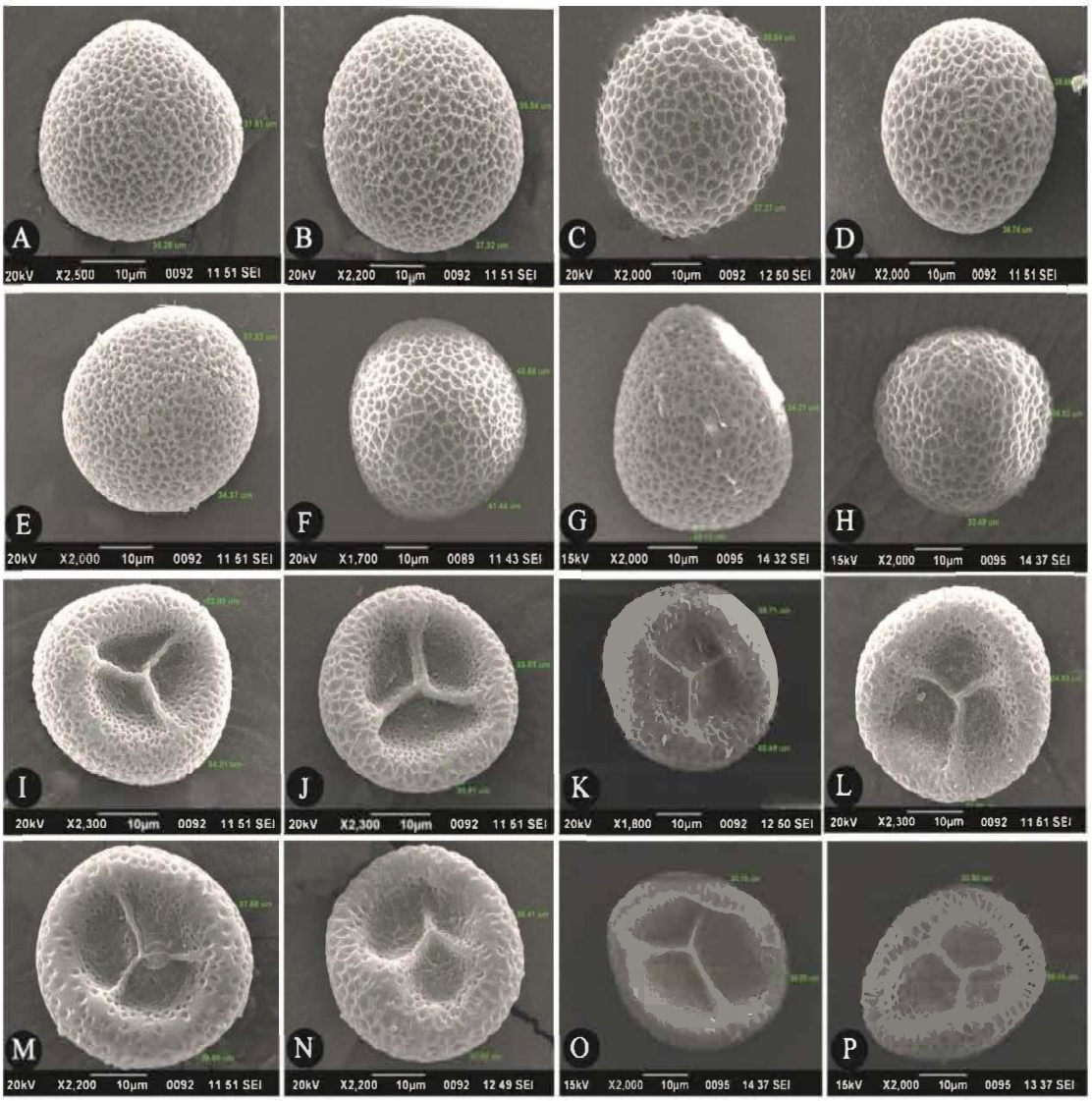

Fig. 79. Spore surfaces of eight morphotypes of *Ophioglossum costatum*: A, I. Morphotype 1, B, J. Morphotype 2, C, K. Morphotype 3, D, L. Morphotype 4, E, M. Morphotype 5, F, N. Morphotype 6, G, O Morphotype 7, H,P Morphotype 8.

by different morphotypes of *O. costatum.* Inspite of the small changes, in the vegetative morphology, the micromorphological and molecular characters are same for all the morphotypes. Only small variations were observed in the luminal width of the spore. So, by considering all these characters and occurance of several morphotypes of *O. costatum*, O. *madhusoodhananii* is considered as a morphotype of *O. costatum*.

Chapter-5

Discussion

The first and the detailed study of the genus *Ophioglossum* from Kerala, India, has been carried out. Fresh *Ophioglossum* plants were collected from different localities of Kerala. Herbarium preserved or formalin preserved material was also used in the case of *O. pendulum*. Due to the mere morphological characteristics of this genus, the species concept faced many issues from year to year (Mahabale1962, Burrows and Edwards 1995, Wieffering 1964, Fraser Jenkins *et al.*, 2018, Yadav and Goswami 2010, Rajesh *et al.*, 2013, Patel *et al.*, 2018, Patel and Reddy 2019, Patil *et al.*, 2020, Goswami *et al.*, 2020, Patil *et al.*, 2022). So, the complete analysis of rhizome morphology, tropophyll, spike, micromorphology of stomata, venation, SEM analysis of spore characters, and molecular phylogenetic analysis were discussed here.

5.1. Habitat

Ophioglossum is a cosmopolitan genus found in a variety of habitats. In Kerala, the *Ophioglossum* plants were mainly found in loose rocks, laterite rocks, river banks, tea estates, under tree shades and grasslands during the rainy season. *O. reticulatum* was collected from the decaying organic mesh under the tree shade. They grow in close association with bryophytes, graminae members, and insectivorous plants. They were not effortlessly eye-catching. Rhizome of all the species has Vesicular Arbescular Mycorrhizal association and hence the plants were habitat specific. Multi species communities as well as single species communities were present in several habitats. Presence of several morphotypes was common in all the species. So careful observations were needed to identify different species growing together in a small area and a wide range of specimens were needed for the exact identification.

5.2. Comparative morphology

5.2.1. Habit

Most of the *Ophioglossum* plants were small and fleshy terrestrial herbs, except epiphytic *O. pendulum* as discussed by Clausen (1938), Burrows and Jhons (2001). The plant body usually bears a discoid or globose or tuberous or elongated or fusiform or oblong or horizontal rhizome with fleshy roots. Rhizomes generally have a mycorrhizal association. Tropophylls are either solitary or many. The tropophylls continues to a fertile segment bearing a spike at its apex. Sporangia were arranged on the spike either alternately or oppositely. A mix of alternate and opposite sporangia

was found in some plants like *O. costatum.* The plant size may vary from 1.4 – 33 cm in different species. The plants like *O. gramineum, O. indicum*, *O. latifolium*, *O. lusitanicum*, *O. lusoafricanum*, *O. parvifolium, O. petiolatum, O. raphaelianum, O. rubellum, O. vulgatum* and *O.* sp nov. were attains a height below 15 cm. *O. gramineum, O. indicum*, *O. lusitanicum*, *O. lusoafricanum*, *O. parvifolium, O. latifolium, O. raphaelianum* and *O. rubellum* showed minute plants below 5 cm. Smaller forms of *O. costatum* with mature spike was also present in the field. But majority of *O. costatum* plants attain a height upto 28 cm. *O. costatum, O. reticulatum* and *O. pendulum* (33 cm) are comparatively larger plants attaining a height above 15 cm.

5.2.2. Rhizome

The rhizome shape in *Ophioglossum* is a character to be considered while identifying a species. The shape generally varies as discoid, globose, tuberous-elongated, fusiform, oblong, horizontal in different species. Among the 14 species studied, *O. costatum* showed globose-tuberous elongated-discoid or cormlike rhizome. Discoid rhizome was present only in morphotypes 1, 6, 7 and 8 of *O. costatum*. Globose rhizome was seen in morphotypes 3, 5 of *O. costatum, O. latifolium*. Globose-ellipsoid rhizome was seen in *O. raphaelianum* and globose-fusiform rhizome was seen *O. rubellum.* Both globose and elongated rhizome was observed within the population of *O. parvifolium*. *O. vulgatum* has elongated rhizome. morphotypes 2, 4 of *O. costatum, O. gramineum*, *O. indicum*, *O. lusitanicum*, *O. lusoafricanum* have tuberous-elongated rhizome. Fusiform rhizome was present in *O. petiolatum* and *O. reticulatum,* horizontal-rounded rhizome was present in *O. pendulum*, tuberous-oblong rhizome was seen in *O.* sp. nov. *O. costaum* and *O. parvifolium* showed variations in the rhizome morphology within the same population from the same locality. Morphotypes of *O. costaum* showed globose-tuberous elongated-discoid rhizome and Morphotypes of *O. parvifolium* showed either elongated or globose rhizome.

5.2.3. Tropophyll and the stipe

Tropophylls of *Ophioglossum* were simple, fleshy, green and glabrous. Erect and upright tropophylls were found in all the species except *O. pendulum*. The tropophyll shape in *Ophioglossum* genus varies as linear, lanceolate, elliptic, rhomboid, oblong,

ovate, obovate, orbicular, linear-pendulous, cordate and spathulate (Lanceolate-narrowly ovate). The pendulous tropophylls of *O. pendulum*, linear tropophylls of the *O. gramineum*, widely ovate-orbicular- elliptic tropophylls of *O. raphaelianum,* cordate tropophylls of *O. reticulatum,* and lanceolate-narrowly ovate (spathulate) tropophylls of *O. vulgatum* serves as a major taxonomic tool in identifying these plants from the field. Lanceolate tropophylls were present in morphotypes 4, 5, 6, 7and 8 of *O. costatum.* In the case of *O. lusitanicum*, lanceolate-elliptic tropophylls were present in which lanceolate tropophylls were shown by the majority. Elliptic tropophylls were present in morphotypes 1, 2 and 3 of *O. costatum* and in *O. latifolium.* Elliptic-rhomboid tropophyllls present in morphotype 2 of *O. costatum.* Elliptic-lanceolate tropophylls found in *O. indicum, O. lusoafricanum*. In *O. parvifolium*, elliptic-lanceolate-oblanceolate tropophylls were found in which elliptic tropophylls were shown by majority of the plants. lanceolate-oblong tropophylls found in *O*. sp.nov. This oblong nature was a unique character for *O*. sp. nov., which was dinstict from all other plants. *O. rubellum* was characterized by mostly ovate, rarely elliptic or spathulate-obovate or sub orbicular tropophylls. Elliptic-rounded tropophylls were found in *O. petiolatum*.

The number of tropophylls differs from 1 – 8 in different species. *O. lusoafricanum, O. petiolatum* and *O. vulgatum* have single tropophyll. 1 – 2 tropophylls were present in *O. gramineum, O. latifolium, O. lusitanicum, O. parvifolium, O. raphaelianum* and *O. reticulatum.* 1 – 4 tropophylls present in *O. indicum.* 2 – 3 tropophylls are seen in *O*. sp. nov., 2 – 4 in *O. rubellum. O. pendulum* has 3 – 4 tropophylls and *O. costatum* shows 3 – 8 tropophylls.

The tropophylls of *O. rubellum* and *O. raphaelianum* were found appressed on the soil. The tropophyll colour could be taken as a character for identification in *O. raphaelianum* and *O. indicum*. *O. raphaelianum* is characterized by its olivaceous or bluish-green colour of the tropophyll and *O. indicum* was distinguished by its pink-coloured tropophylls. *O. parvifolium*, *O. lusitanicum*, *O. raphaelianum*, and *O. rubellum* have minute tropophylls. The largest tropophylls were present in the epiphytic *O. pendulum*. In *O. pendulum,* the tropophylls were long (22 – 30 cm) and ribbon-like, usually showing bifurcation at the tip. Among terrestrial species, *O. costatum* has the largest tropophyll (8 cm). The shorter stipe was present in and in *O.*

rubellum (0.1 – 0.2 cm) and *O. raphaelianum* (0.1 – 0.2 cm) the longest stipe was present in *O. reticulatum* (11 cm).

5.2.4. Fertile segment

The fertile segment in the *Ophioglossum* genus always starts from the base of tropophyll lamina. Although the number of sporangia per spike and the arrangement of sporangia vary in different species. Fewer number of sporangia was present in *O. parvifolium* (4 – 6) and *O. lusitanicum* (4 – 9). The size of the fertile segment also varies in different species. The shortest segment was observed in *O. raphaelianum* (1 – 3 cm), and the longest fertile segment was observed in *O. costatum* (up to 23 cm). The arrangement of sporangia was either alternate or opposite or a mix of alternate and opposite arrangements in some species. Alternate sporangia were common in *O. lusoafricanum, O. gramineum, O. pendulum, O. petiolatum, O. raphaelianum, O. reticulatum,* and in morphotypes 1, 2, 4 and 8 of *O. costatum.* Opposite arrangement of sporangia was present in *O. indicum, O. lusitanicum, O. latifolium, O. parvifolium, O. rubellum, O. vulgatum, O.* sp. nov.*,* morphotypes 6 and 7 of *O. costatum.* A rare combination of alternate and opposite arrangements of sporangia were found in *O. costatum.* Occasionally bifurcated sporangia were observed in *O. costatum, O. parvifolium* and in *O.* sp. nov. The comparison between the vegetative characters of the 14 *Ophioglossum* species found in Kerala is given in Table 6.

5.3. Micromorphology

The venation pattern, stomata and spore characters are discussed here.

5.3.1. Venation

Many pteridologists had considered the venation pattern as a character of taxonomic importance for classifying species, genera and families (Wagner 1979). The members of genus *Ophioglossum* with closed veins were considered as advanced than the members of *Botrichium* having free veins, a more closely related genus of *Ophioglossum*. Two trends were occurred in the venation pattern of *Ophioglossum* genus in agreeing with Wagner's point of view.

One trend was the "formation of veins towards decreasing complexity by enlargement of areoles and loss of included veinlets". This reticulated condition was present

among the majority of the species (10 species) studied. The plants like *O. gramineum, O. indicum, O. latifolium, O. lusitanicum, O. lusoafricanum, O. parvifolium, O. petiolatum, O. raphaelianum, O. rubellum* and the epiphytic *O. pendulum* decreases their complexity in the absence of secondary veinlets and and secondary areoles.

Another trend was the formation of veins with "increased complexity by formation from included veinlets of new areolets within the major areoles". This bireticulated condition is more complex than the simple reticulated condition. Comparitively large terrestrial species like *O. costatum, O. reticulatum, O. vulgatum* and *O.* sp. nov. follows the bireticulated condition.

Among the 14 species studied, 10 species evolved with decreasing complexity and only 4 species had the tendency to evolve with increased complexity. Small plants had simple reticulated venation whereas the large plants have complex bireticulated venation. But there was an exception in the case of the epiphytic *O. pendulum. O. pendulum* is a large species but it had simple reticulated veins. The venation pattern in *O. costatum*, *O. vulgatum* and *O.* sp. nov. were comparable. But the two long stretched and prominent areoles present at the central basal part of the *O. costatum* make it distinct from *O. vulgatum* and *O.* sp. nov. The venation in *O. vulgatum* starts with six veins, *O.* sp. nov. starts with five veins. The areoles in *O. vulgatum* are short as compared to the elongated areoles of *O.* sp. nov. The number of secondary veins as well as secondary areolates were less in *O. vulgatum* as compared to *O. costatum* and *O.* sp. nov., *O. reticulatum* has numerous veins arising from the base. The double venation of *O. costatum,* Long-stretched areoles at the lower part of *O. gramineum* and the absence of long stretched areoles in the lower part of *O. reticulatum* agrees with the findings of Wieffering (1964).

The venation of *O. indicum*, *O. latifolium, O. lusitanicum, O. lusoafricanum, O. parvifolium, O. petiolatum, O. raphaelianum,* and *O. rubellum,* are comparable. The difference exists in the number of primary veins and correspondingly in the number of primary areoles. The number of primary veins in *O. indicum* was four, in *O. latifolium* was three, in *O. lusitanicum* was three, in *O. lusoafricanum* was five, in *O. parvifolium* was three, in *O. petiolatum* was14, in *O. raphaelianum* was six and in *O.*

Table 6. Comparison between the vegetative characters of O. *costatum, O. gramineum, O. indicum, O. latifolium , O. lusitanicum, O. lusoafricanum, O. parvifolium, O. pendulum , O. petiolatum , O. raphaelianum, O. reticulatum, O. rubellum, O. vulgatum* and *O.* sp. nov.

Name of the taxon	Height(cm)	Rhizome		Stipe (cm)	Tropophyll				Fertile segment			
		Shape	Size (cm)		No.	Size (cm)	shape	Apex	Stalk (cm)	Spike (cm)	Spor-angia (no.)	Arran-gement
O. costatum	6 – 28	globose-tuberous-discoid	0.5 – 2	0.5 – 3	3 – 8	2 – 8	elliptic-rhomboid-lanceolate	obtuse- acute-mucronate	3.5– 16	0.5 – 7	11 – 75	alternate-opposite
O. gramineum	3 – 6	tuberous-elongated	0.4 – 1	1 – 2	1 – 2	1 – 1.3	linear	acicular	1 – 1.5	0.6 – 1.5	5 – 10	alternate
O. indicum	3 – 6	tuberous-elongated	0.4 – 0.5	0.6 – 1.5	1 – 4	0.5 – 1	elliptic-lanceolate	acute	1 – 2.5	1– 1.5	6 – 7	opposite
O. latifolium	2 – 7	globose	0.3 – 0.5	0.2 – 1.5	1 – 2	0.4 – 1	elliptic	obtuse	1 – 3.5	0.5 – 1.5	5 – 12	opposite
O. lusitanicum	3 – 6	tuberous-elongated	0.3 – 0.5	0.6 – 1.5	1 – 2	0.5 – 1	lanceolate-elliptic	acute	1.4 – 3	0.7 – 1	4 – 9	opposite
O. lusoafricanum	4 – 10	tuberous-elongated	0.3 –1	0.7 – 1	1	0.5 – 1.5	elliptic-lanceolate	acute	2.5 – 7	0.5 – 1	5 – 9	alternate
O. parvifolium	2 – 6	globose-elongated	0.2 – 0.5	0.4 – 1	1 – 2	0.2 – 1	elliptic-lanceolate-oblanceolate	obtuse-cuspidate-mucronate-rounded	1 – 3.5	0.4 – 1	4 – 6	opposite
O. pendulum	23 – 33	horizontal-rounded	0.7 – 2.4	0.3 – 0.6	3 – 4	22 – 30	linear, pendulous	obtuse	1 – 3	12 – 16	50 – 80	alternate
O. petiolatum	8 – 13	fusifom	0.5 – 1	4.5 – 7	1	2 – 3	elliptic- rounded	obtuse-rounded	2– 3	1 – 2	25 – 28	alternate
O. raphaelianum	1.4 – 4	Globose-ellipsoid	0.3 – 0.7	0.1 – 0.3	1 – 2	0.2 – 0.5	Elliptic-widely ovate-orbicular	obtuse-rounded	0.7 – 2	0.3 – 1	6 – 7	alternate
O. reticulatum	15 – 27	fusiform	0.5 – 1	7– 11	1 – 2	4 – 6	cordate	obtuse	6.5– 12	1 –3	20 – 35	alternate
O. rubellum	2.1 – 7.8	globose-fusiform	0.5 – 0.6	0.1 – 0.2	2 – 4	0.5 – 1.3	ovate-elliptic or spathulate-obovate or suborbicular	obtuse	1– 5	0.5 – 2	6 – 10	opposite
O. vulgatum	7– 11	elongated	0.5 – 1	2– 3	1	3 – 4	spathulate	acute-obtuse	2.5 – 4	2– 3	25 – 35	opposite
O. sp nov.	5 – 12	tuberous-oblong	0.5 – 1	0.1 – 0.5	2 – 3	1.5 – 3.5	oblong-lanceolate	acute	3.4 – 8.5	1 – 2	6 – 11	opposite

rubellum it was seven. *O. gramineum* has more tubular areoles and five veins arising from the base.

5.3.2. Stomata

The size of stomal pore, guard cells, and subsidiary cells differ in each species. So, the stomatal characters could be considered along with other morphological characters while distinguishing species in *Ophioglossum*. All the studied species except *O. pendulum* had anomocytic stomata. *O. pendulum* had cyclic stomata in agree with the observation of Mahabale (1962). The stomata and its apparatus of *O. pendulum* was different from all the free-living land species. Unlike other species, the stomata of *O. pendulum* were cyclic in nature in which 6 subsidiary cells cyclically arranged outside the stomata. The subsidiary cells are smaller than the guard cells, not wavy like the other *Ophioglossum* species.

The length of the stomatal pore ranges from 5.67– 30.30 µm in dfferent species. Small pores were observed in *O. costatum* with (5.67 – 10.72 µm) and in *O. vulgatum* (6.78 – 7.50 µm) and longest pore were observed in *O. lusitanicum* with (28 – 30.30 µm). The width of the pore differs from 0.5 – 5 µm. *O. petiolatum* (0.5 – 0.77 µm) had the narrowest pore and O. *gramineum* (4 – 5 µm) had the widest pore. The smallest guard cells are present in *O. costatum* (32 – 49 µm) and *O. vulgatum* (33.33 –37.09 µm) and the longest and widest guardcells are present in *O. pendulum* (82 – 89 µm long, 18 – 27 µm wide). The number of subsidiary cells ranges from 4 –8 among different species. *O. indicum* (4 – 5) and *O. parvifolium* (4 – 5) had lesser number of subsidiary cells as compared to rest of the species. Highest number of subsidiary cells were found in *O. reticulatum* (6 – 8).

The length and breadth of the subsidiary cells were differed from one species to another. The subsidiary cells were polygonal rounded in *O. costatum, O. pendulum, O. petiolatum* and *O. reticulatum, O. vulgatum*. Polygonal-elongated subsidiary cells were found in *O. gramineum, O. latifolium, O. lusitanicum* and *O. rubellum.* Elongated subsidiary cells were found in *O. indicum, O. lusoafricanum, O. parvifolium, O. raphaelianum* and in *O.* sp nov. The shortest subsidiary cells are present in *O. costatum* (20 – 79 µm) and in *O. vulgatum* (31.44 – 43.44 µm) and the longest subsidiary cells are present in *O. lusitanicum* (80 – 155 µm). The subsidiary cells are narrowest in *O. parvifolium* (11.21 – 29.99 µm) and widest in *O. reticulatum*

(39.30 – 77.09 µm). In general, the large terrestrial species like *O. costatum* and *O. vulgatum* have the shortest stomatal apparatus as compared to all the smaller terrestrial species. *O. indicum* population shows twin stomata in tropophyll which was comparable to the superimposed twin stomata found in *O. nudicaule* reported by Inamdar (1970).

The cell wall natures of the subsidiary cells are also varying in different species. More rounded stomata, guard cells, subsidiary cells as well as the epidermal cells are present in *O. costatum.* The stomatal apparatus of *O. lusoafricanum* has more wavier cell walls and *O. parvifolium* has more linear cell walls.

5.3.3. Spore morphology (Fig. 80, 81 & Table 7.)

The spores of 14 *Ophioglossum* species considered in this study had unique and distinct spore characters. The plants were homosporous in agree with Goswami (2007). Generally, *Ophioglossum* genus had reticulated spores as mentioned by Peruzzi *et al.*, (2015). The spore characters were found to be a valid mark for identifying different species in this genus. "Foveolate granulose spores are present in *O. costatum*, retate-reticulate spores present in *O. gramineum*, patellate spores present in *O. lusitanicum*, verrucate spores in *O. lusoafricanum*, reticulate-patellate spores in *O. parvifolium*, rugate spores in *O. petiolatum,* reticulate spores found in *O. pendulum* and reticulate-granulose spores present in *O. reticulatum*, foveolate in *O. raphaelianum*, lophate spores present in *O. rubellum"* (Khan *et al.,* 2023). Patellate spores are common in *O. latifolium*, reticulate and granulose spores in *O. indicum*, and granulose-verrucate spores found in *O. vulgatum* and and *O.* sp. nov. The spores of *O. vulgatum* has narrow muri where as *O.* sp. nov. was characterized by wide zigzag exine plates on the spore surface. Dimorphic spores are present in *O. lusoafricanum, O. lusitanicum* and *O. rubellum* in agree with the observation of dimorphic spores within the same sporangium by Goswami (2007).

The coexistence of dimorphic spores in *O. reticulatum* (alete and monolete), *O. vulgatum* (monolete and trilete), *O. costatum* (alete, monolete and trilete), and *O. eliminatum* (alete, monolete, bilete and trilete) had already reported (Pant and Khare, 1971). The population of *O. lusoafricanum* collected during our study agree with this since it possesses both alete and monolete spores.

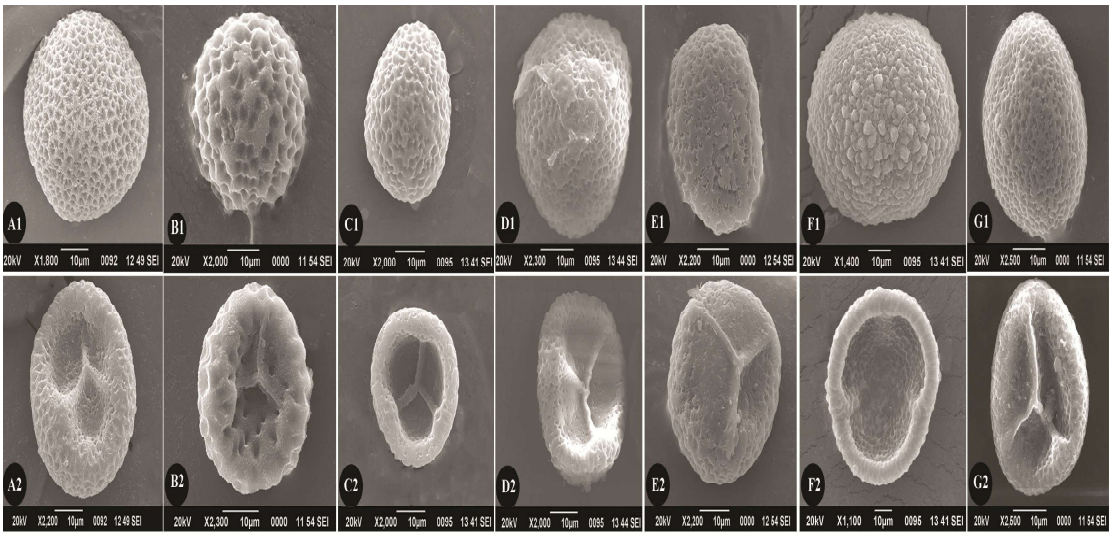

Fig. 80. Distal and proximal spore surfaces of different *Ophioglossum* species: A1, A2. *O. costatum,* B1, B2. *O. gramineum,* C1, C2. *indicum,* D1, D2. *O. latifolium,* E1, E2. *O. lusitanicum,* F1, F2. *O. lusoafricanum,* G1, G2. *O. parvifolium.*

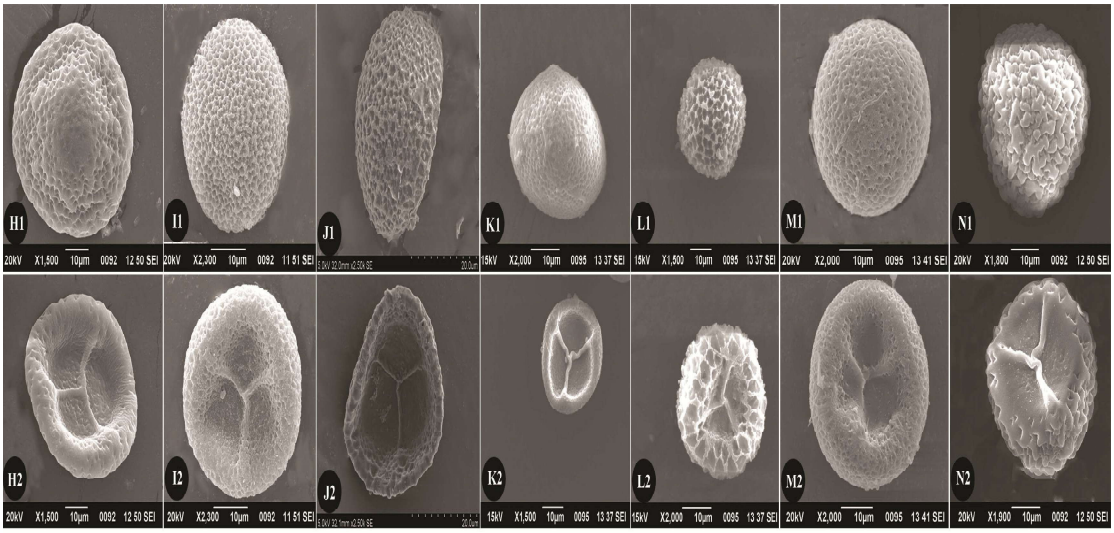

Fig. 81. Distal and proximal spore surfaces of different *Ophioglossum* species: H1, H2. *O. pendulum,* I1, I2. *O. petiolatum,* J1, J2. *O. raphaelianum,* K1, K2. *O. reticulatum,* L1, L2. *O. rubellum,* M1, M2. *O. vulgatum,* N1, N2. *O.* sp. nov.

Among the 14 species studied, spores of the *O. pendulum* were the largest. Even though *O. pendulum* is an epiphytic species, the spore characters are similar with the other terrestrial species, independent of their habitat (Khan *et al.,* 2023). *O. rubellum*

Table 7: Comparison between the Polar length, Equatorial Length, P/E ratio and size class of the spore of *O. costatum, O. gramineum, O. indicum, O. latifolium , O. lusitanicum, O. lusoafricanum, O. parvifolium, O. pendulum , O. petiolatum , O. raphaelianum, O. reticulatum, O. rubellum, O. vulgatum, O.* sp. nov.

Name of the taxa	Polar length (P) (µm)		Equatorial length (E)(µm)		P/E ratio (µm)		Size class
	Min.- Max.	Mean± SE.	Min.- Max.	Mean± SE.	Min.- Max.	Mean±SE.	
O. costatum	16.35 – 18.42	17.78±0.48	25.19 – 37.02	30.62±2.05	0.53 – 0.72	0.58±0.04	large
O. gramineum	17.28 – 20	18.27±0.60	25.36 – 33.3	31.01±1.8	0.53 – 0.68	0.59±0.03	large
O. indicum	15.58– 22.97	19.41±1.77	30.3 – 48.73	38.28±4.27	0.44 – 0.55	0.51± 0.02	large
O. latifolium	16.31 – 18.21	17.40±0.36	31.73 – 34.9	33.778±0.6	0.50 – 0.52	0.51±0.004	large
O. lusitanicum	14.36 – 17.21	15.57±0.70	22.33 – 30.36	27.1±1.79	0.49 – 0.65	0.58±0.03	large
O. lusoafricanum	21.49 – 32.54	26.03±2.39	42.28 – 66.73	50.19±5.58	0.48 – 0.58	0.52±0.02	large
O. parvifolium	14.11 – 16.7	15.51±0.46	21 – 33.01	28.11±2.63	0.47 – 0.67	0.56±0.05	large
O. pendulum	25 – 28.02	26.02±0.69	39.7 – 43.69	41.94±0.78	0.57 –0.67	0.62±0.02	large
O. petiolatum	17.21 – 18.09	17.55±0.21	33.01 – 34.07	33.60±0.25	0.50 – 0.54	0.52±0.00	large
O. raphaelianum	14.27 – 18.96	17.15±1.03	30.53 – 37.4	32.57±1.6	0.38 – 0.61	0.53±0.04	large
O. reticulatum	15.55 – 23.72	20.09±1.94	28.2 – 40.82	36.19±2.8	0.49 – 0.58	0.55±0.01	large
O. rubellum	8.76 – 14.1	11.46±1.09	22.15 – 28.89	24.5±1.5	0.36 – 0.51	0.46± 0.03	medium
O. vulgatum	16.37 – 20.46	17.19±1.84	20.99 –34.45	29.82±2.6	0.56 – 0.77	0.57±0.02	large
O. sp. nov.	17.69 – 19.86	19.15±0.50	30.32 – 37.85	35.03±1.64	0.48 – 0.63	0.55±0.03	large

has the smallest spores. They lie under medium sized spore class. The polar axis was shortest in *O. rubellum* with an average size of 8.76 – 14.1 µm, longest in *O. pendulum* with an average size of 25 –28.02 µm. Equatorial axis was longest in *O. lusoafricanum* with a mean value of 42.28 – 66.73 µm and shortest in *O. rubellum* with a mean value of 22.15 – 28.89 µm. The population of *O. lusoafricanum* shows greatest range of variation in the length of polar axis as well as in equatorial axis followed by *O. reticulatum* and *O. indicum* respectively. Abortive spores were found in *O. indicum* and *O. lusoafricanum* populations. Spores of *O. rubellum,* collected from Palakkad and Thrissur Districts possessed similar exine ornamentation. But the ornamentation showed slight variations in the specimens collected from Kottayam District. Small variations were also noted in the morphotypes of *O. costatum.* "The morphological variations in the vegetative characters and spore characters were common within the same species of genus *Ophioglossum"* (Fraser-Jenkins *et al.*, 2017, 2021). Comparison between the polar length, equatorial Length, P/E ratio and size class of the spore of 14 *Ophioglossum* species in Kerala is given in Table 7.

5.4. Molecular phylogeny

The phylogenetic analysis along with the analysis of external morphological characters and spore morphology were found to be good and effective in the genus *Ophioglossum* (Goswami and Khandelwal1973, Goswami 2007, Torres *et al.,* 2015, Goswami and Patel 2019, Patel and Reddy 2019). The Intergenic spacer barcode region, *psb*A-*trn*H has a high rate of nucleotide sequence variation viz. indels, transitions, transversions, and inversion and is very powerful in delineating even closely related taxa (Kress and Erickson 2007). The analysis of *rbc*L-F, *psb*A-*trn*H and *trn*L-F regions worked well in genus *Ophioglossum.* From the analysis of the *rbc*L-F, *psb*A-*trn*H and *trn*L-F regions it was convenient that the smaller species like *O. lusitanicum*, *O. parvifolium*, *O. indicum, O. latifolium* and *O. lusoafricanum* are genetically closer to each other. These species have almost similar morphology and can't easily distinguish from the field. The spore morphology was the main tool for the species identification since these plants have overlapping and confusing morphological characters. *O. reticulatum*, *O. gramineum*, *O. petiolatum*, *O. vulgatum* showed more sequence similarity and are grouped together. Except *O. gramineum,* rest of the three species have comparatively larger size and have a greater number of sporangia. *O.* sp. nov. is genetically more related to *O. costatum*, *O. rubellum* and *O.*

gomezianum. The possibility for the simultaneous speciation was indicated by the polytomy present in the branches.

The molecular analysis in *Ophioglossum* genus was not easy due to the unavailability of the plant material as well as a uniform dataset. Also, the three primers were not properly amplified in all the species included in the study. In *O. raphaelianum*, only the primer for the *psb*A- *trn*H could be amplified after repeated attempts. In the case of *O. gomezianum*, the sequence for *psb*A-*trn*H region was not available, for *O. pendulum* and *O. polyphyllum* only the *rbc*L-F sequences were available in the NCBI GenBank. Inspite of this limitation, the analysis of the *rbc*L-F, *psb*A-*trn*H and *trn*L-F gave almost similar tree topology with similar clade.

5.5. New species status of *O.* sp nov.

The species identification in the *Ophioglossum* genus was confusing and problematic since the plants have only limited morphological characters. Therefore, the species delimitation dependenting only on the external morphological characters unreliable in the case of this genus. Nevertheless, we found that the application of micromorphology, spore morphology and phylogenetic analysis along with the morphological characters were excellent in the genus *Ophioglossum.*

The characteristic features of *O.* sp nov. include height 5 – 12 cm, tuberous oblong rhizome, oblong-lanceolate tropophylls, occasionally bifurcated spikes in some plants, 6 – 11 pairs of oppositely arranged sporangia, rugate, wide plated exines of the dorsal spore surface with shallow lumina at the centre of the distal face whereas forming sharply pointed ridges and deep lumina towards the proximal face, flattened and smooth proximal surface with outwardly projected triradiations in the absence of an exact proximal cavity.

At first sight, *O.* sp nov. slightly resembles *O. gomezianum* in its size and appearance. But *O. gomezianum* differs from *O.* sp nov. by having the ellipsoid-fusiform rhizome, elliptic-lanceolate tropophylls, non-bifurcated spikes with alternate sporangia and tuberculated exine ornamentation.

There are other chances for misidentifying *O.* sp nov. as a smaller form of *O. costatum*. But the larger size range of *O. costatum* up to 28 cm, globose tuberous elongated-discoid rhizome, elliptic-rhomboid-lanceolate tropophylls with acute-

obtuse-mucronate apex, long fertile segment (4 – 23 cm), 11 – 75 pairs of alternate or opposite sporangia, and foveolate, granulose spores of *O. costatum* make it different from *O.* sp.nov.

O. sp nov. is phylogenetically more related to *O. rubellum.* But the globose-fusiform rhizome, ovate elliptic or spathulate obovate or sub orbicular tropophylls, 6 – 10 pairs of opposite sporangia, trilete or alete spores wih lophate ornamentation makes *O. rubellum* distinct from *O.* sp.nov.

O. sp nov. is also comparable to *O. polyphyllum.* unlike *O.* sp. nov., *O. polyphyllum* possessed a cylindrical rhizome, 2 – 6 elliptic-oblanceolate tropophylls, 14 – 36 pairs of sporangia, and a clear proximal cavity at the proximal surface of the spore with inserted triradiations. Even though the dorsal surface of the spores of these two taxa have some similarities, the exine plates form sharply pointed ridges and deep lumina towards the proximal face in *O.* sp nov. whereas the exine plates didn't formed sharply pointed ridges and deep lumina in *O. polyphyllum*.

Comparison of *trn*L-F, *rbc*L and *psb*A-*trn*H nucleotide sequence datasets yielded 97%, 36% and 61% ML bootstrap values respectively for *O.* sp. nov. with its closest specimens, *O. gomezianum* and *O. rubellum* confirming its novelty. Comparison between the morphological characters of *O.* sp. nov., *O. gomezianum, O. costatum, O. rubellum* and *O. polyphyllum* is given in Table 8.

5.6. Reinstating *O. indicum* and *O. raphaelianum* by differentiating from *O. rubellum*

O. indicum, O. raphaelianum and *O. rubellum* are three different species with taxonomically distinct characters. But some taxonomists treated *O. indicum* and *O. raphaelianum* as a synonym of *O. rubellum*. *O. indicum, O. raphaelianum* and *O. rubellum* were coloured *Ophioglossum* species. But the colour varies from species to species. The macromorphology, micromorphology was different among these three species and also the SEM analysis of the spore characters revealed distinct ornamentation, specific for each species. This confirms the species status of *O. indicum* and *O. raphaelianum* different from *O. rubellum* and thus the species status of *O. indicum* and *O. raphaelianum* were reinstated.

5.7. Synonimising *O. trilokinathii* in to *O. rubellum*

The macromorphological and micromorphological characters of *O. trilokinathii* was similar to the characters of *O. rubellum*. Also, the *rbc*L-F, *trn*L-F and *psb*A-*trn*H of *O. rubellum* and *O. trilokinathii* showed 100 %, 99.38 % and 97.24 % similarity respectively. So, this study considered *O. trilokinathii* as a synonym of *O. rubellum.*

5.8. Occurance of different morphotypes of *O. costatum* and synonimising *O. madhusoodananii* in to *O. costatum*

An increased number of morphotypes were observed even within the plants collected from the same population. The species of *Ophioglossum* were well known for the variability found within a species. Our observations from the various natural habitat confirmed that when the habitat was more diverse with more *Ophioglossum* species, the morphotype diversity also increased. Kallekad, Mangad and Keerankulangara were examples of such habitats. Kallekad was diverse with *O. costatum*, *O. gramineum, O. rubellum* and Mangad was diverse with *O. costatum,* and *O. parvifolium,* Keerankulangara was diverse with *O. costatum*, *O. gramineum*, *O. lusitanicum* and *O. indicum.* In those habitats, the morphotypes existed independently of their environmental factors. The possible genetic recombinations due to natural hybridisation between the different species of the same locality may brought variability within the intra-specific level. Due to the mere morphological characters of the genus *O. costatum* always showed a tendency to evolve continousily by changing its vegetative characters without changing the spore characters. This may be the reason for the similarity of majority of the newly described *Ophioglossum* species with *O. costatum*. The possibility for the occurance of different morphotypes and for the evolution of new species was increased with the increase in the number of species in the habitat. Since all these species are confined to pocketed and limited geographic areas, there was an increased chance for the cross fertilization between the gametes of different species. This may be the reason for the variability and the formation different morphotypes. The presence of different morphotypes of *O. costatum* found during our study agrees with Patil's point on the occurance of several biotypes in *Ophioglossum* genus (Patil et al., 2019).

Table 8. Comparison between the morphological characters of *O.* sp. nov., *O. gomezianum, O. costatum, O. rubellum* and *O. polyphyllum*

Character	***O.* sp. nov.**	***O. gomezianum***	***O. costatum***	***O. rubellum***	***O. polyphyllum***
Height	5 – 12 cm	5 – 12 cm	6 – 28 cm	2.1 – 7.8 cm	4 –13 cm
Rhizome	Tuberous-oblong	Ellipsoid-fusiform	Globose-tuberous-discoid	Globose-fusiform	Fusiform-linear
Tropophyll					
Number	2 – 3	2 – 4	2 – 8	2 – 4	2 – 6
Shape	oblong-lanceolate	elliptic - lanceolate	Elliptic-rhomboid-lanceolate-	ovate-elliptic or spathulate-obovate or sub orbicular	elliptic - oblanceolate
Fertile segment					
Stalk	3.4 – 8.5 cm	3.6–5.7cm	3.5 – 16 cm	1 – 5	0.7 – 1.2 cm
Spike	1 – 2 cm	1.7 – 3.1 cm	0.5 – 7 cm	0.5 – 2	1.7 – 8cm
Sporangia	6 – 11 pairs	8 – 10 pairs	11 – 75 pairs	6 – 10	14 – 36 pairs
Arrangement	Opposite	Alternate	Alternate-opposite	Opposite	Opposite
Spore characters	Rugate, with wide muri	Tuberculate exine with uneven tubercles	Foveolate	Lophate	sinous ridges, small gaps in the ridges and furrows

Even though the plants collected from different populations of *O. costatum* showed a little variation in shape and size of the rhizome, tropophyll, and fertile segment all these plants have the same spore morphology. Mahabale (1962) documented several specimens from India with cormatous rhizome, lanceolate-ovate tropophylls and with bifurcated spikes and identified them as "*O. fibrosum* Schumach". *O. fibrosum* was then synonymised by Roskov *et al.*, (2018) as *O. costatum* (Khan *et al.,* 2023). By considering the characters of *O. madhusoodhananii* and the occurance of several morphotypes of *O. costatum, O. madhusoodhananii* was recognized as a morphotype of *O. costatum* and hence it was considered as a synonym of *O. costatum.*

5.9. Occurance of *O. lusitanicum* in India

O. lusitanicum and *O. lusoafricanum* collected from various localities of Kerala was different in morphological characters of their vegetative parts, exine ornamentation and in molecular characters. *O. lusitanicum* plants (3 – 6 cm high) are smaller than *O. lusoafricanum* (4 – 10 cm high) plants. *O. lusitanicum* has one-two tropophylls whereas *O. lusoafricanum* has single tropophyll. The fertile segment is 2.1 – 4 cm long in *O. lusitanicum* where as 3 – 8 cm long in *O. lusoafricanum*. Venation is reticulate in both *O. lusitanicum* and *O. lusoafricanum* but the number of veins arising from the base is three in *O. lusitanicum* whereas five in *O. lusoafricanum.* The stomata and the subsidiary cells of *O. lusoafricanum* are elongated than that of *O. lusitanicum.* The Subsidiary cells of *O. lusoafricanum* are 109. 34 – 176.29 µm long and that of *O. lusitanicum* are 57. 45 – 65.12 µm long. The distal spore surface of *O. lusitanicum* is patellate whereas the distal spore surface of *O. lusoafricanum* is verrucate. The spores of *O. lusoafricanum* are alete or trilete with a proximal cavity whereas the spores of *O. lusitanicum* are trilete and dimorphic with or without a deep proximal cavity. By the detailed examination of macro morphology, micromorphology and comparing with the type specimen the occurance of *O. lusitanicum* in India was confirmed.

5.10. Occurance of *O. vulgatum* in India

O. vulgatum plants collected during this study showed distinct morphological characters. *O. vulgatum* can be distinguished from *O. costatum*, *O. reticulatum* and *O. petiolatum* in its macromorphology, micromorphology and molecular characteristics. The rhizome is elongated in *O. vulgatum* which can be distinguished from the globose-tuberous-discoid rhizome of *O. costatum* and fusiform rhizome of *O. reticulatum* and *O. petiolatum* The spathulate tropophylls of *O. vulgatum* is different from elliptic-rhomboid-lanceolate tropophylls of *O. costatum*, cordate tropophylls of *reticulatum* and elliptic-rounded tropophylls of *O. petiolatum.* Eventhough *O. vulgatum, O. costatum* and *O. reticulatum* have bireticulated venation, the number of veins arising from the base of *O. costatum* is five, *O. reticulatum* is numerous, *O. vulgatum* is six. *O. petiolaum* has reticulated venation. *O. vulgatum* has more polygonal-rounded and smaller stomatal apparatus than the other species. Granulose-verrucate spores of *O. vulgatum* are different from the foveolate-granulose spores of *O. costatum*, reticulate granulose spores of *O. reticulatum* and rugate spores of *O. petiolatum*. By the detailed examination of macro morphology, micromorphology and comparing with the type specimens the occurance of *O. vulgatum* in India was confirmed.

5.11. New reports to South India and Kerala

O. latifolium collected from Kasaragode, Kozhikode and Thrissur District is a new report for India and *O. indicum* Collected from Thrissur District is a new report for South India.

Chapter-6

Summary

The present study on the Micromorphlogy and Molecular taxonomy of genus *Ophioglossum* in Kerala identified 14 *Ophioglossum* species. They include *O. costatum*, *O. gramineum, O. indicum, O. latifolium, O. lusitanicum, O. lusoafricanum, O. parvifolium, O. pendulum, O. petiolatum, O. raphaelianum, O. reticulatum, O. rubellum, O. vulgatum* and *O.* sp. nov. One new species was described during the course of this study and one species was recorded as a new report to India and one species was recorded as a new report to South India. This treatment provides correct and updated nomenclature of each taxa with reference to the type materials and protolougue data. Fresh *Ophioglossum* plants collected from different localities of Kerala during repeated field trips conducted. *Ophioglossum* plants were distributed throughout Kerala in various habitat including grasslands, laterite rocks and and loose rocks, river banks and in tea estates during the rainyseason. *O. costatum* was the widely distributed plant with a greater number of individual plants and *O. raphaelianum* had restricted distribution, with a lesser number of individual plants. *O. costatum* plants also showed wide range of variability among different populations resulting in eight different morphotypes.

The morphological description for each species was provided along with an artificial key to the species for easy identification. Micromorphological characterization was done by examining the venation pattern, stomatal characters and spore characters of each species. Light Microscopy was used for the venation and stomatal studies and Scanning Electron Microscopy (SEM) analysis was used for the spore ornamentation studies. Molecular characterization was done using *rbc*L-F, *psb*A-*trn*H and *trn*L-F regions (Chloroplast regions) and phylogenetic trees were prepared. Colour photographs, illustrations and distributional map of each species were provided.

New species discovered

Ophioglossum sp. nov. was described as new species.

New reports

O. latifolium was a new report for India and *O. indicum* was a new report for South India.

Reinstatement of taxa

O. raphaelianum and *O. indicum* found to be different from *O. rubellum* and their species status were reinstated.

New synonyms recognized

O. madhusoodananii was recognized as a synonym of *O. costatum* and *O. trilokinathii* was recognized as a synonym of *O. rubellum*.

Species Occurance confirmed in India

Presence of *O. lusitanicum* and *O. vulgatum* were confirmed in India.

Recommendations

1. A detailed chromosomal analysis of the genus *Ophioglossum* will be useful for Cytogenetical studies.

2. An extensive biochemical characterization of *Ophioglossum* species in Kerala.

3. Ecological niche modelling of different *Ophioglossum* species by using QGIS technique.

4. Determining the conservation status of different *Ophioglossum* species in Kerala using IUCN strategies.

5. Studies on the spore viability and their developmental pattern.

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Annexure

Annexure 1. List of Herbaria Cited

1. Aarhus University, Denmark. Aarhus (AAU).
2. Botanical Survey of India, Deccan Regional Centre, India. Andhra Pradesh. Hyderabad. (BSID).
3. Botanical Survey of India, Southern Regional Centre, Coimbatore, India (MH)
4. Calicut University Herbarium, Department of Botany, University of Calicut (CALI)
5. Central National Herbarium, Kolkata, India (CAL)
6. Kerala Forest Research Institute Herbarium, Peechi, India (KFRI).
7. Linnean Society of London, U.K. England. London (LINN)
8. Museo Nacional de Historia Natural, Cuba. Habana (Havana) (MNHN)
9. Royal Botanic Gardens, U.K. England. Kew. (K)
10. Royal Botanic Garden Edinburgh, U.K. Scotland. Edinburgh (E)
11. St. Thomas' College (Autonomous) Thrissur, India. Kerala. Thrissur (STC)
12. The herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem (B)
13. The Natural History Museum, U.K. England. London. (BM)
14. Tropical Botanical Garden Herbarium, India.Kerala. Thiruvananthapuram (TBGT).
15. University of Nottingham U.K. England. Nottingham. (NHM)

Annexure II **Chromatogram of different *Ophioglossum* species**

1 *rbc*L-F region of *Ophioglossum latifolium*

2. *psb*A-*trn*H region of *Ophioglossum latifolium*

3. *trn*L-F region of *Ophioglossum latifolium*

4. *rbc*L-F region of *Ophioglossum lusitanicum*

5. *psb*A-*trn*H region of *Ophioglossum lusitanicum*

6. *trn*L-F region of *Ophioglossum lusitanicum*

7. *rbc*L-F region of *Ophioglossum lusoafricanum*

8. *psb*A-*trn*H region of *Ophioglossum lusoafricanum*

9. *trn*L-F region of *Ophioglossum lusoafricanum*

10. *rbc*L-F region of *Ophioglossum parvifolium*

11. *psb*A-*trn*H region of *Ophioglossum parvifolium*

12. *trn*L-F region of *Ophioglossum parvifolium*

13. *rbc*L-F region of *Ophioglossum rubellum*

14. *psb*A-*trn*H region of *Ophioglossum rubellum*

15. *trn*L-F region of *Ophioglossum rubellum*

16. *rbc*L-F region of *Ophioglossum* sp. nov.

17. *psb*A-*trn*H region of *Ophioglossum* sp. nov.

18. *trn*L-F region of *Ophioglossum* sp. nov.

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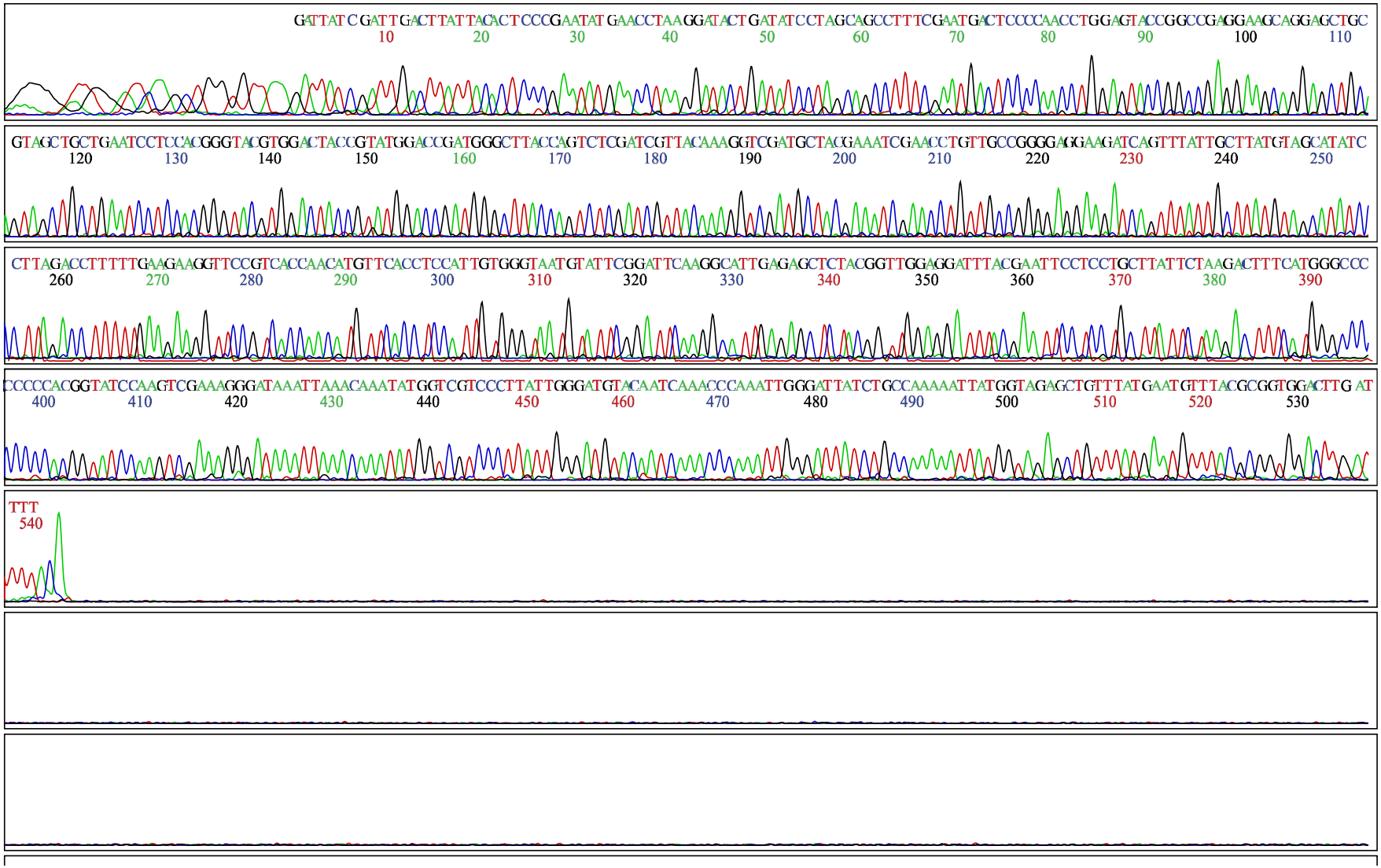

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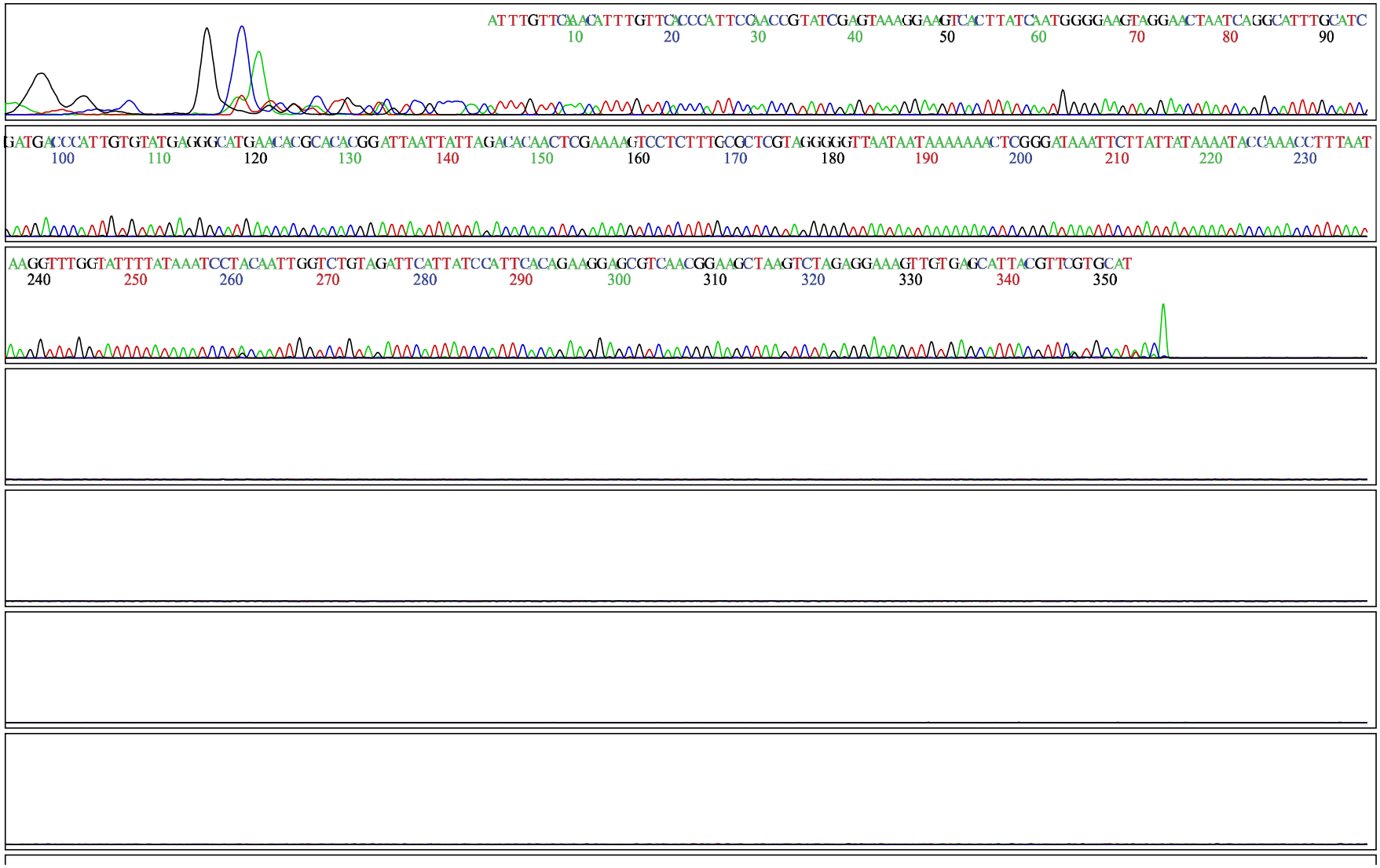

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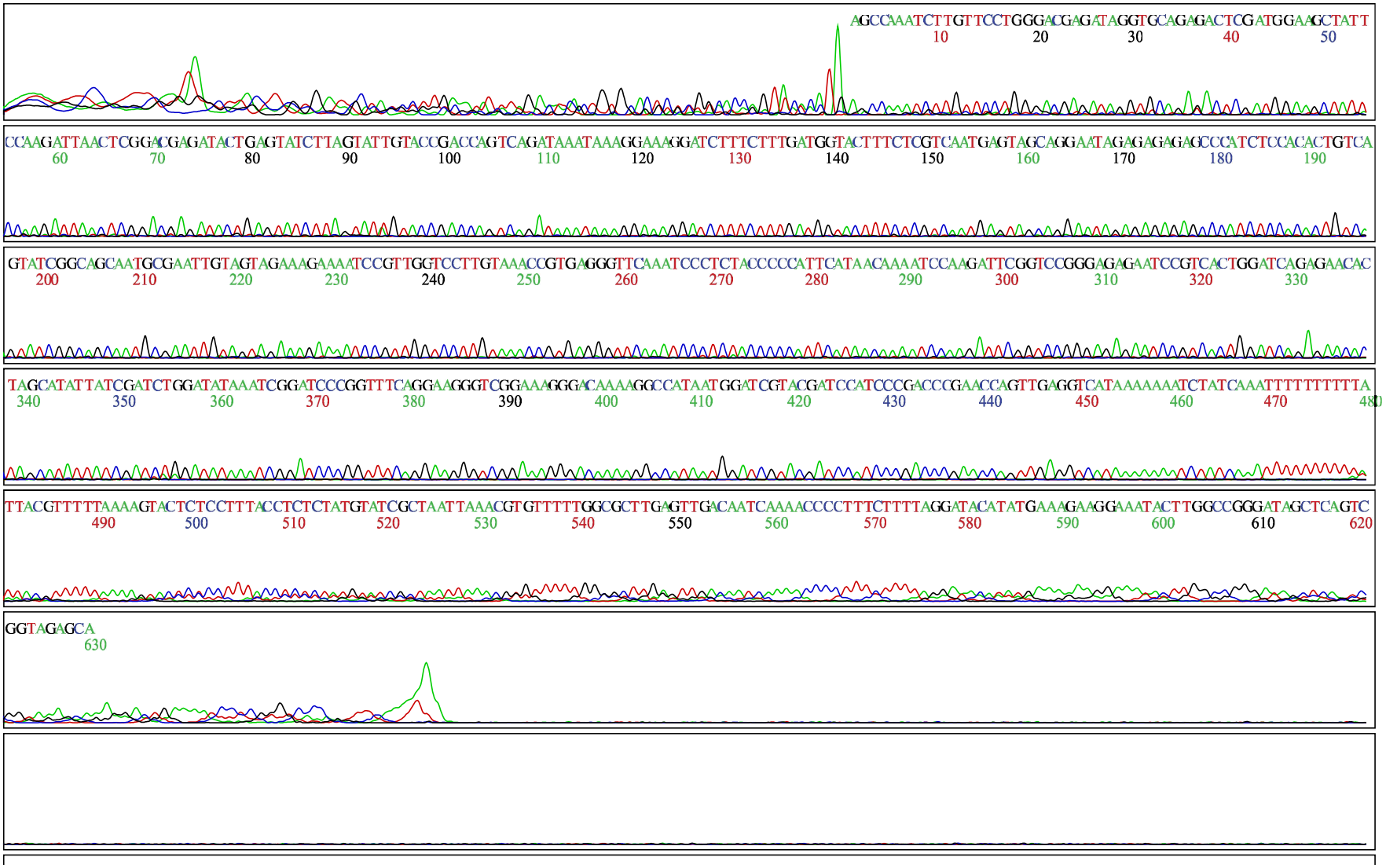

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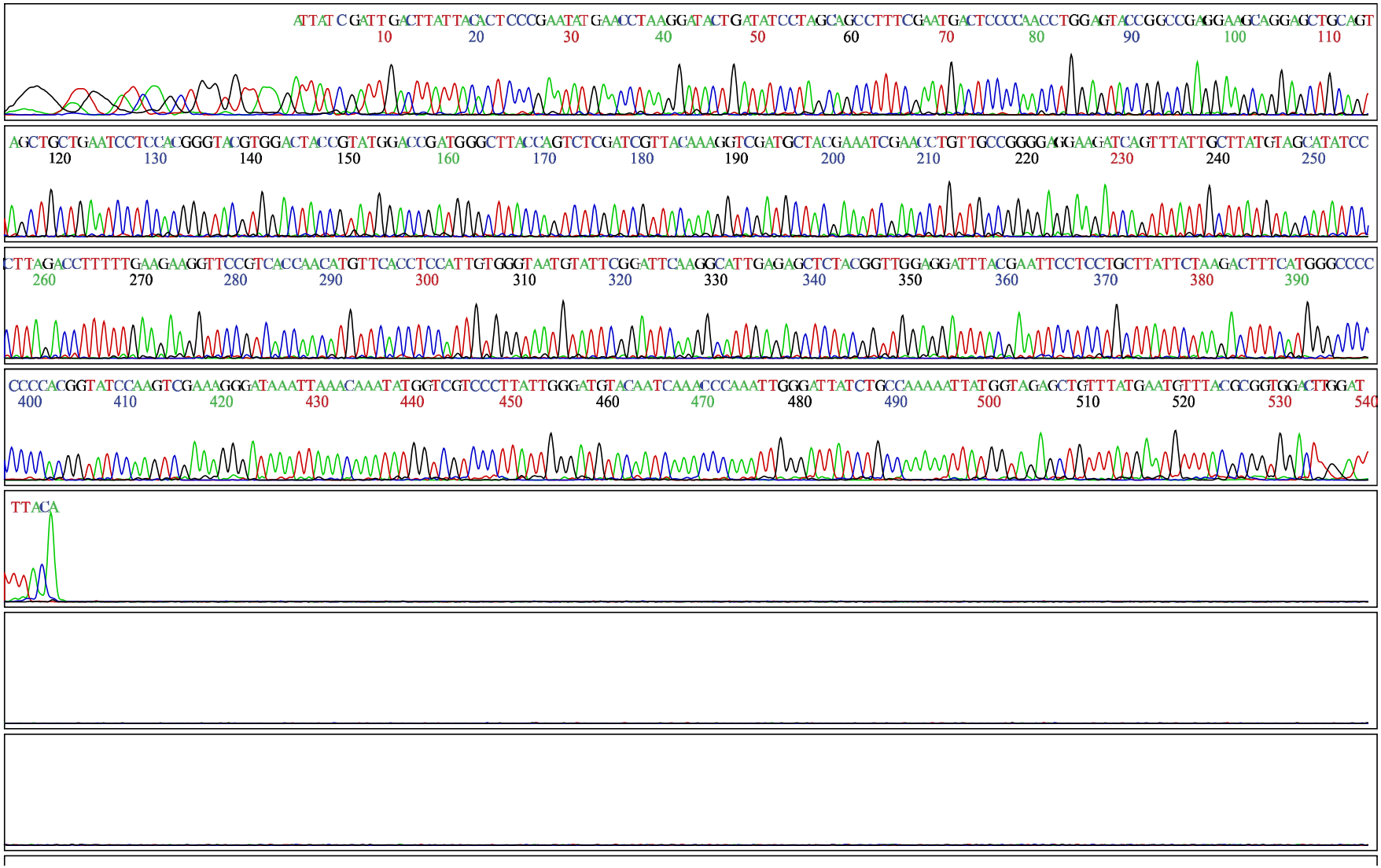

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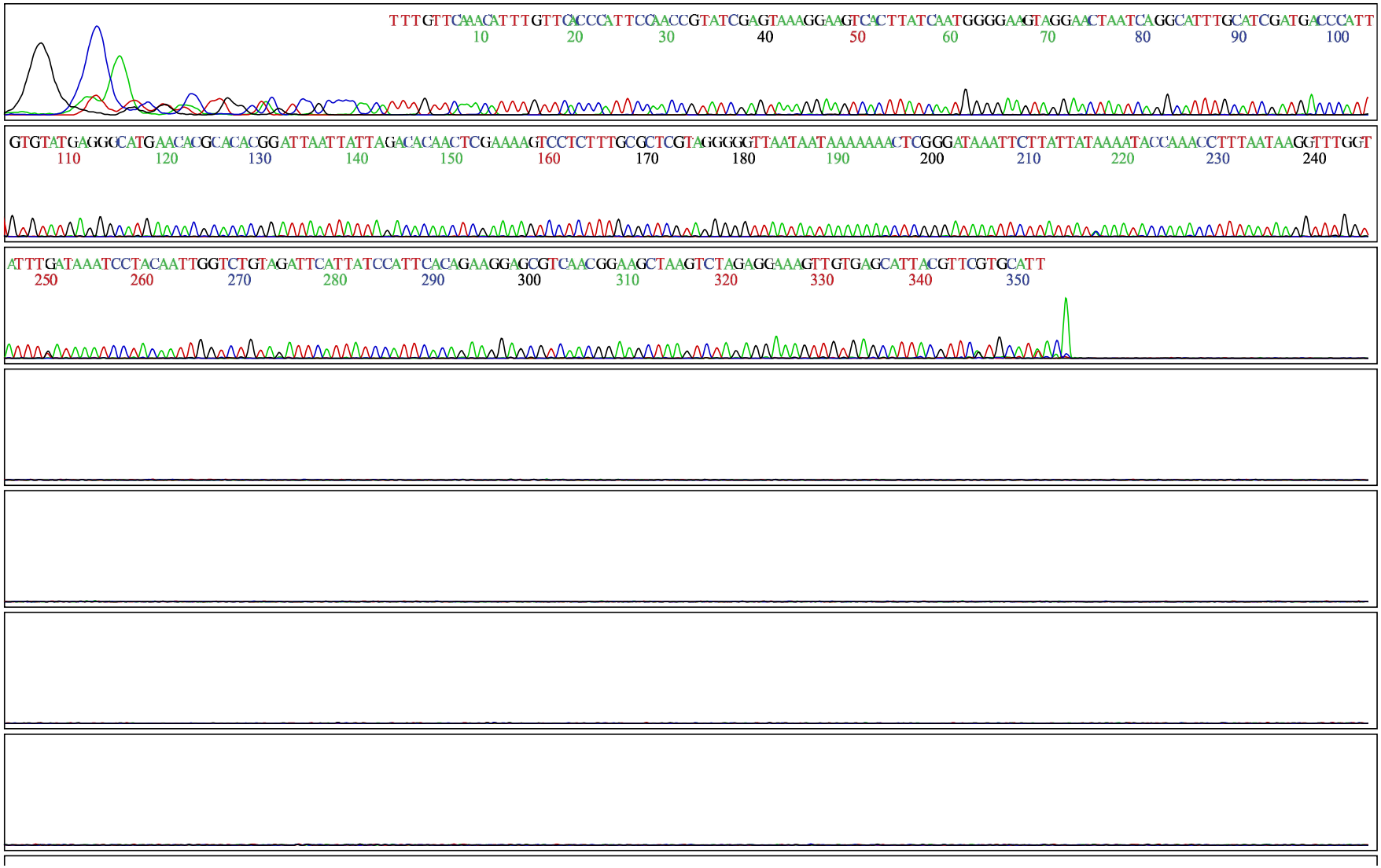

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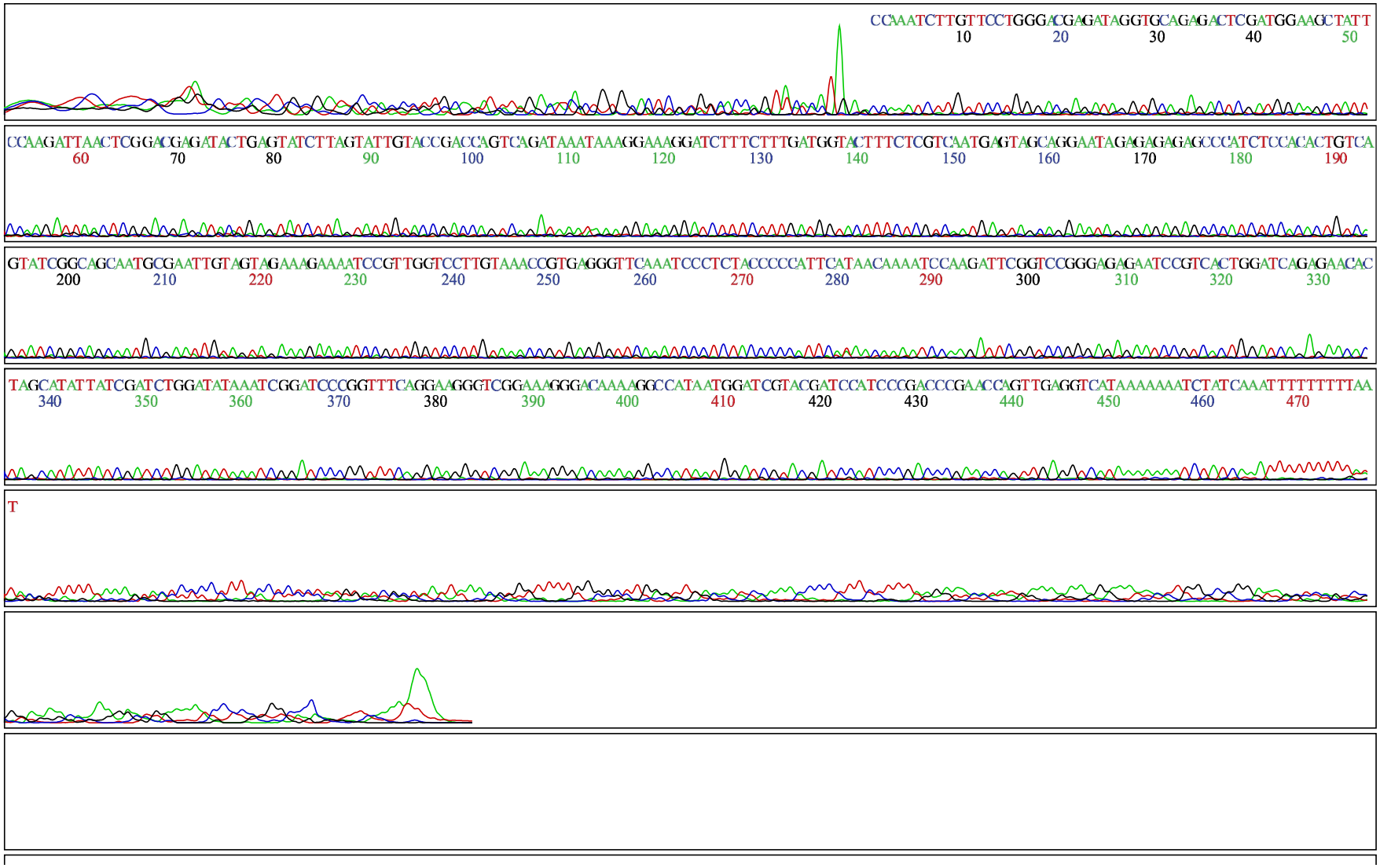

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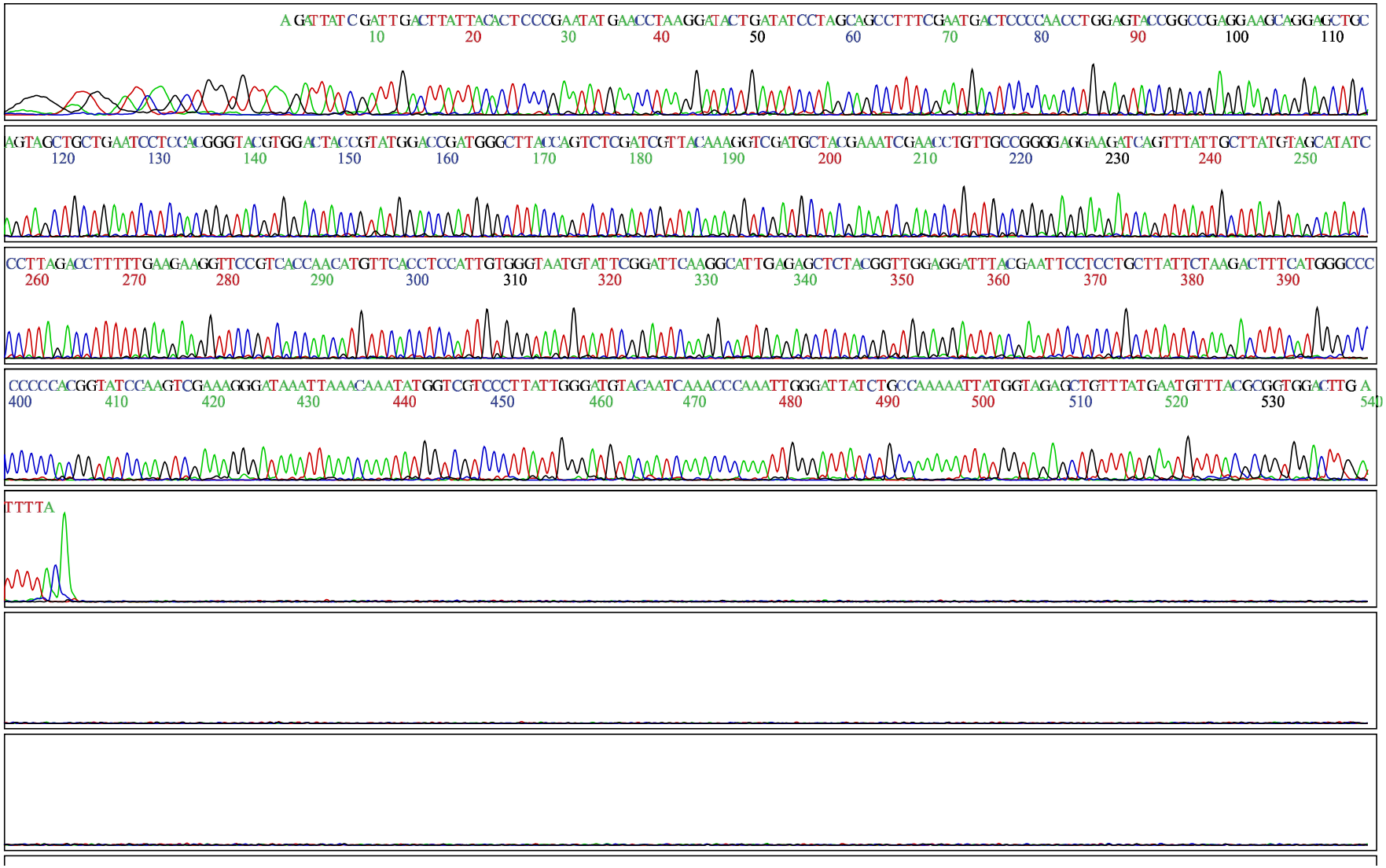

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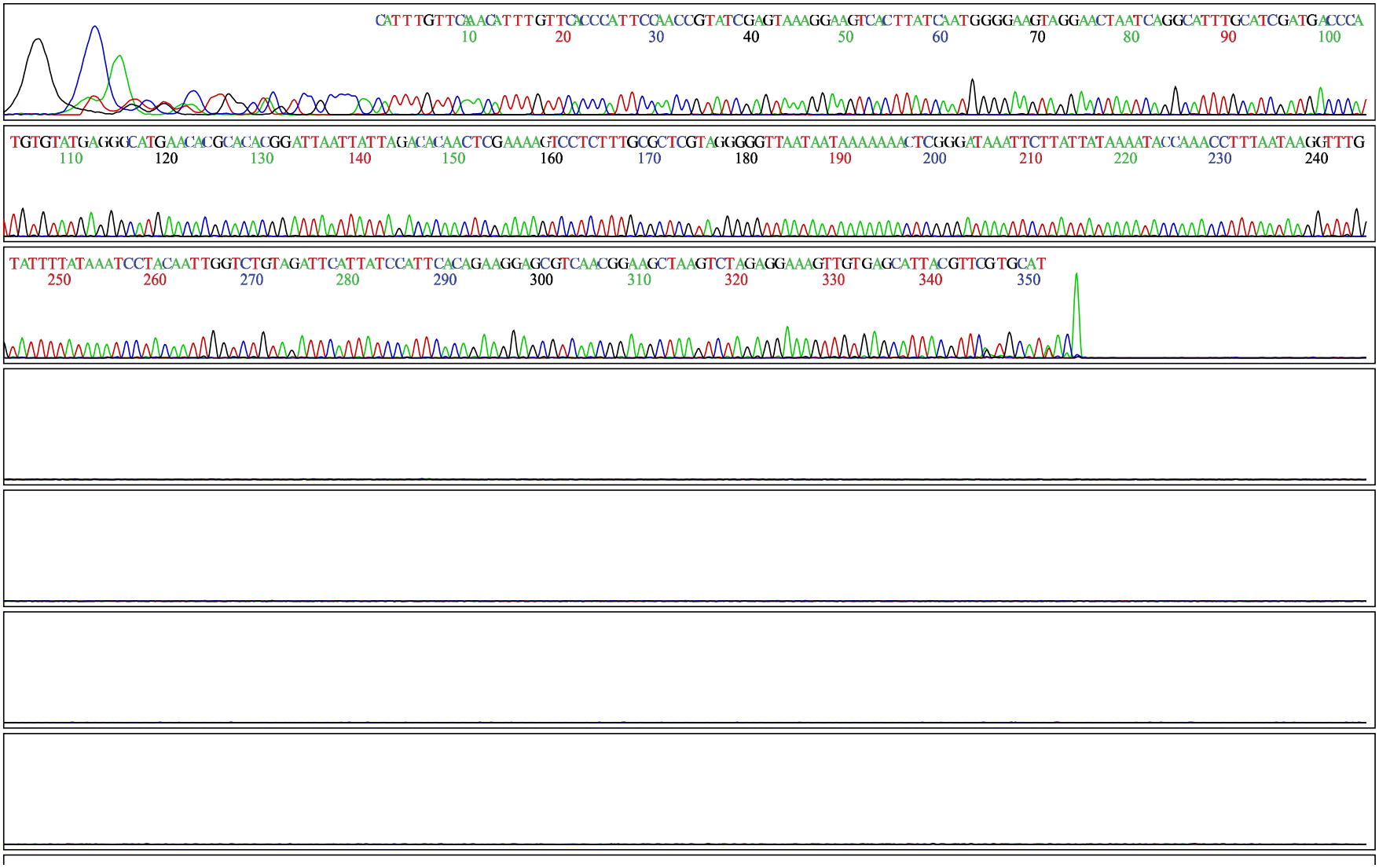

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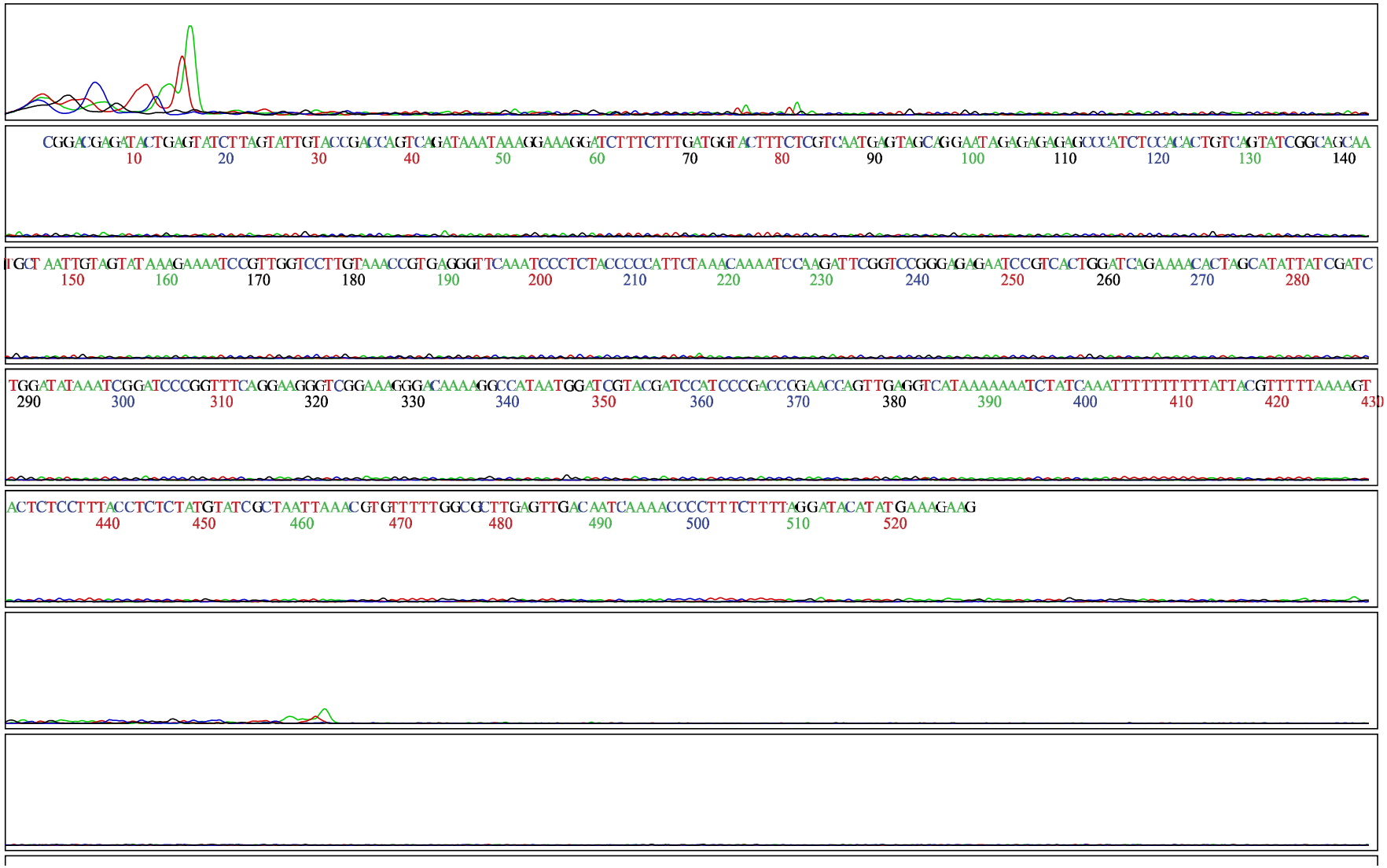

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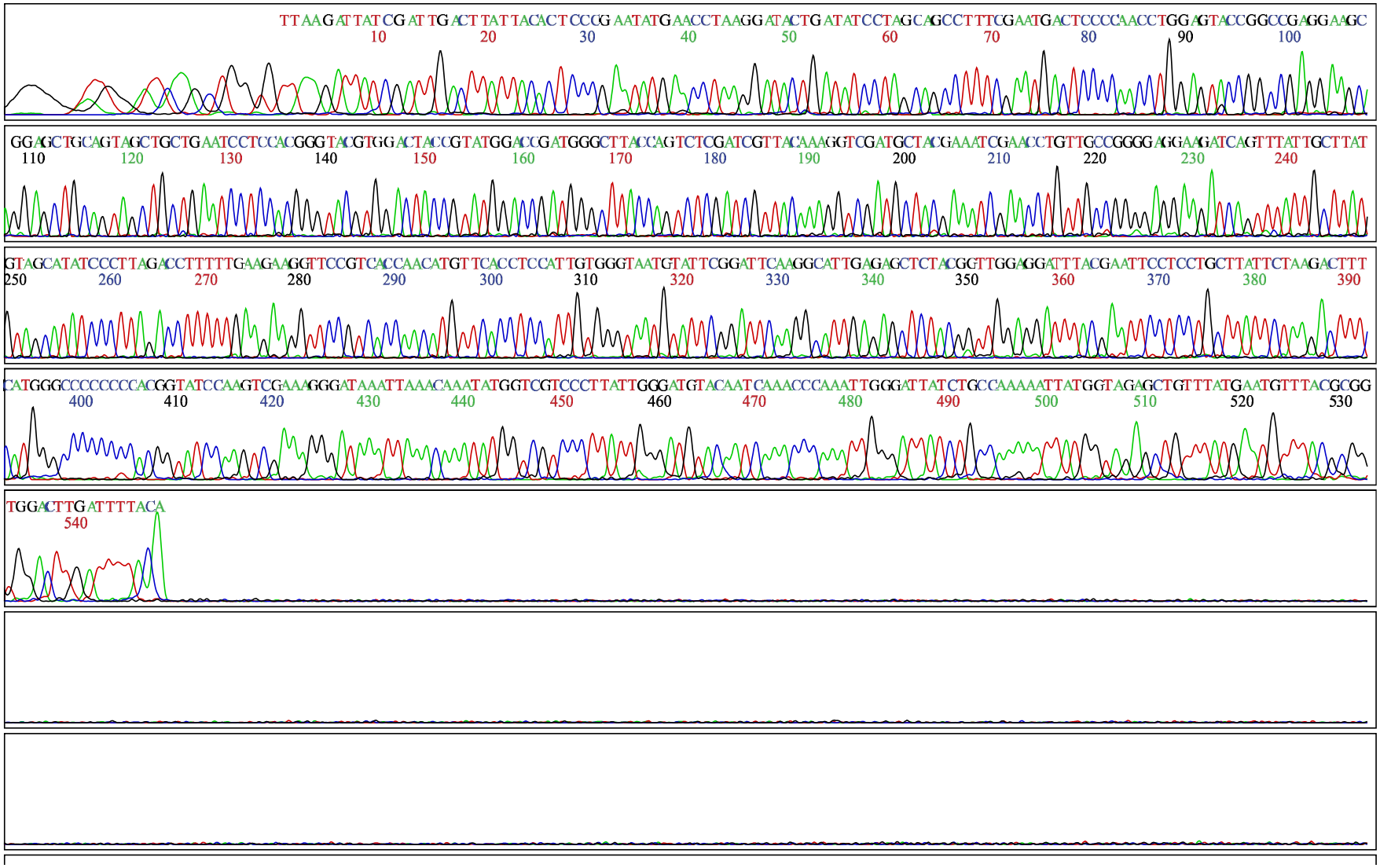

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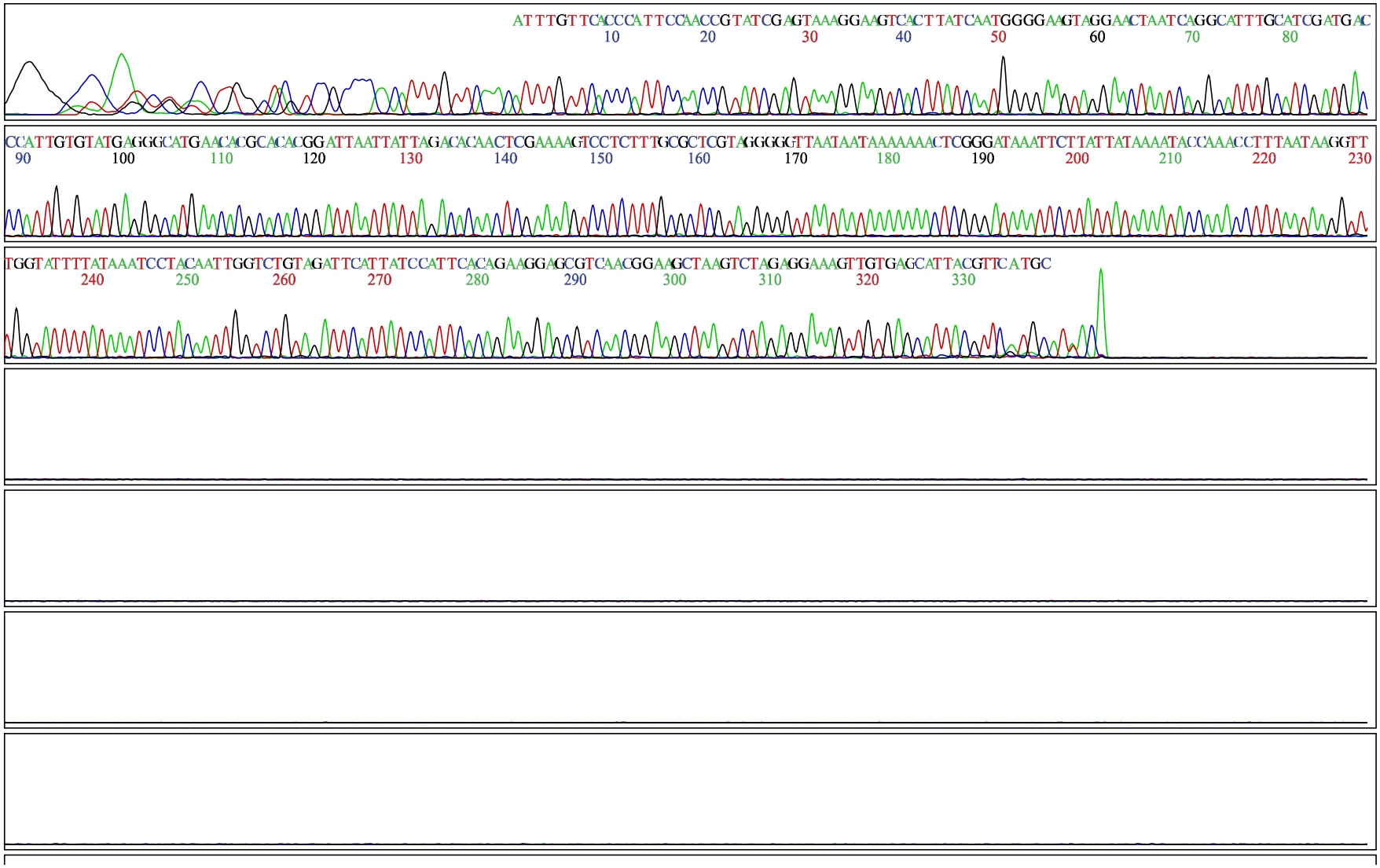

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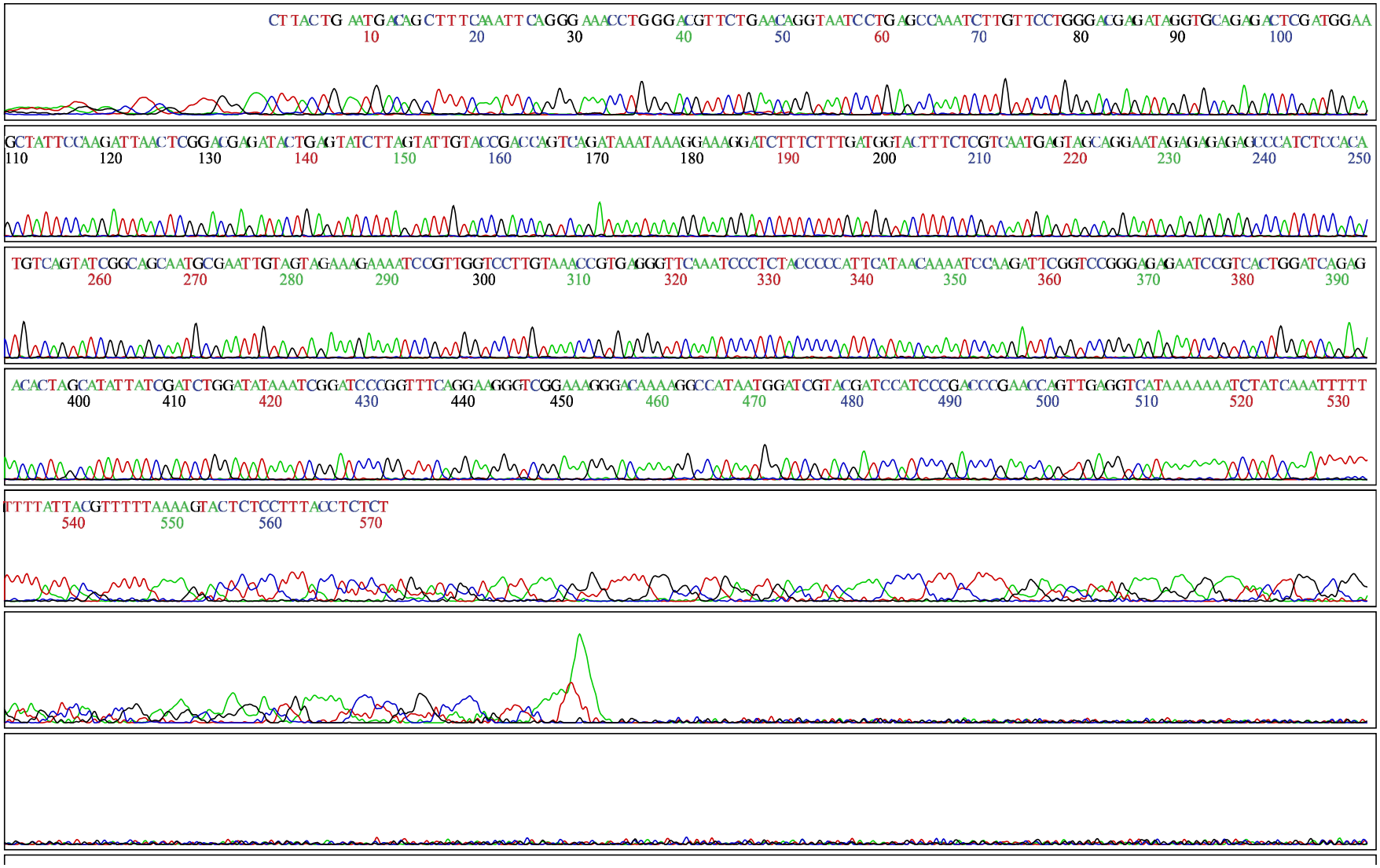

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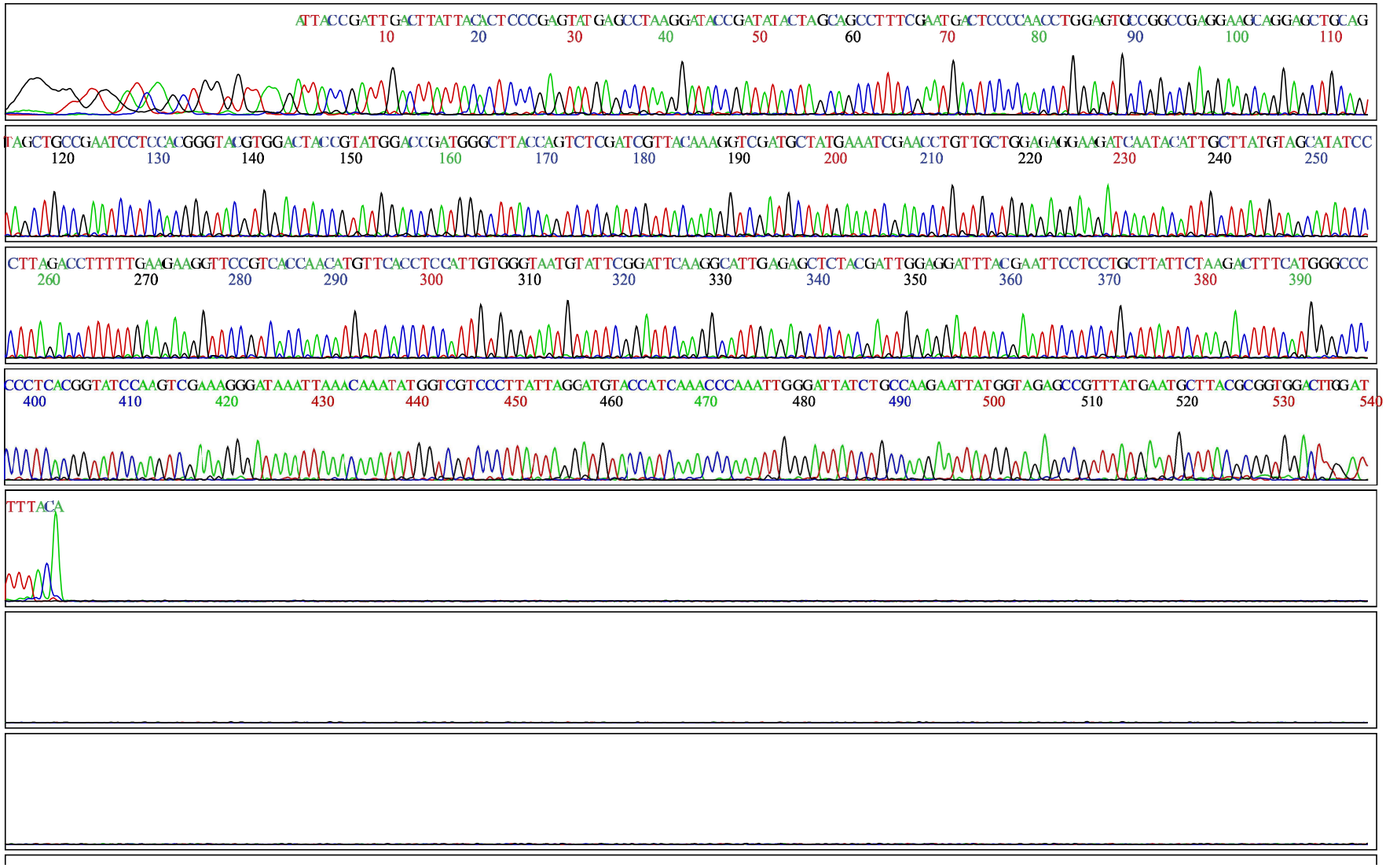

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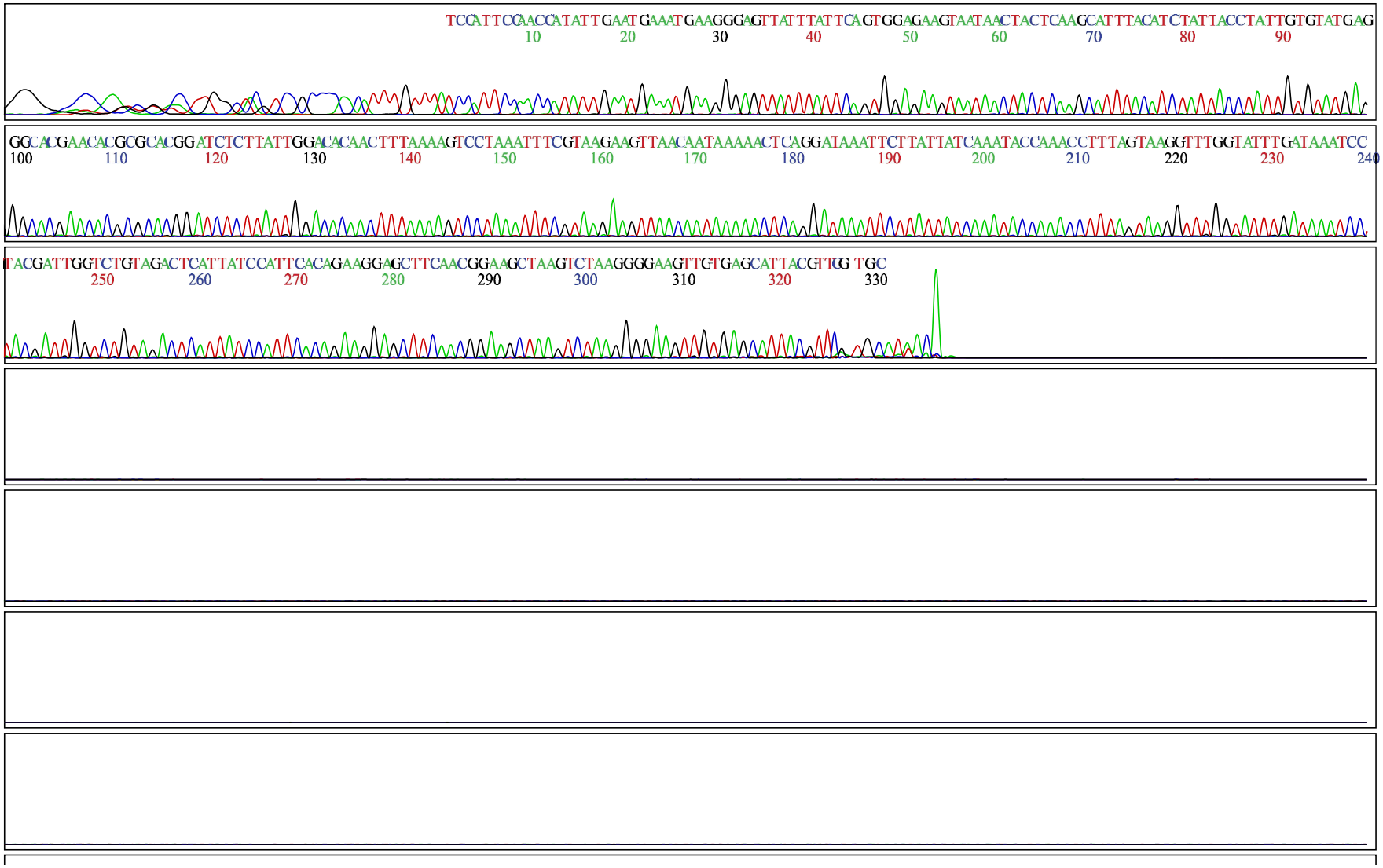

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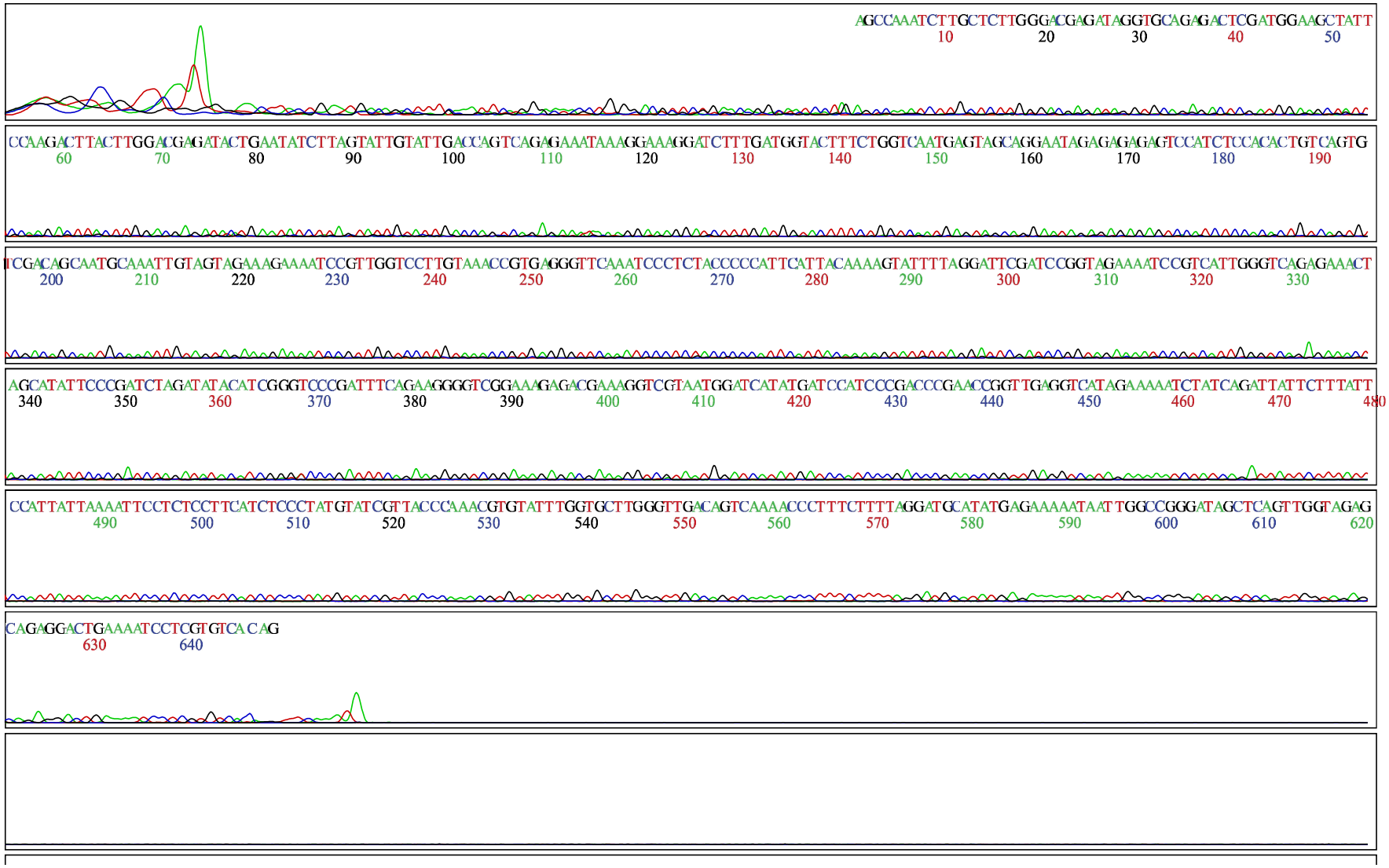

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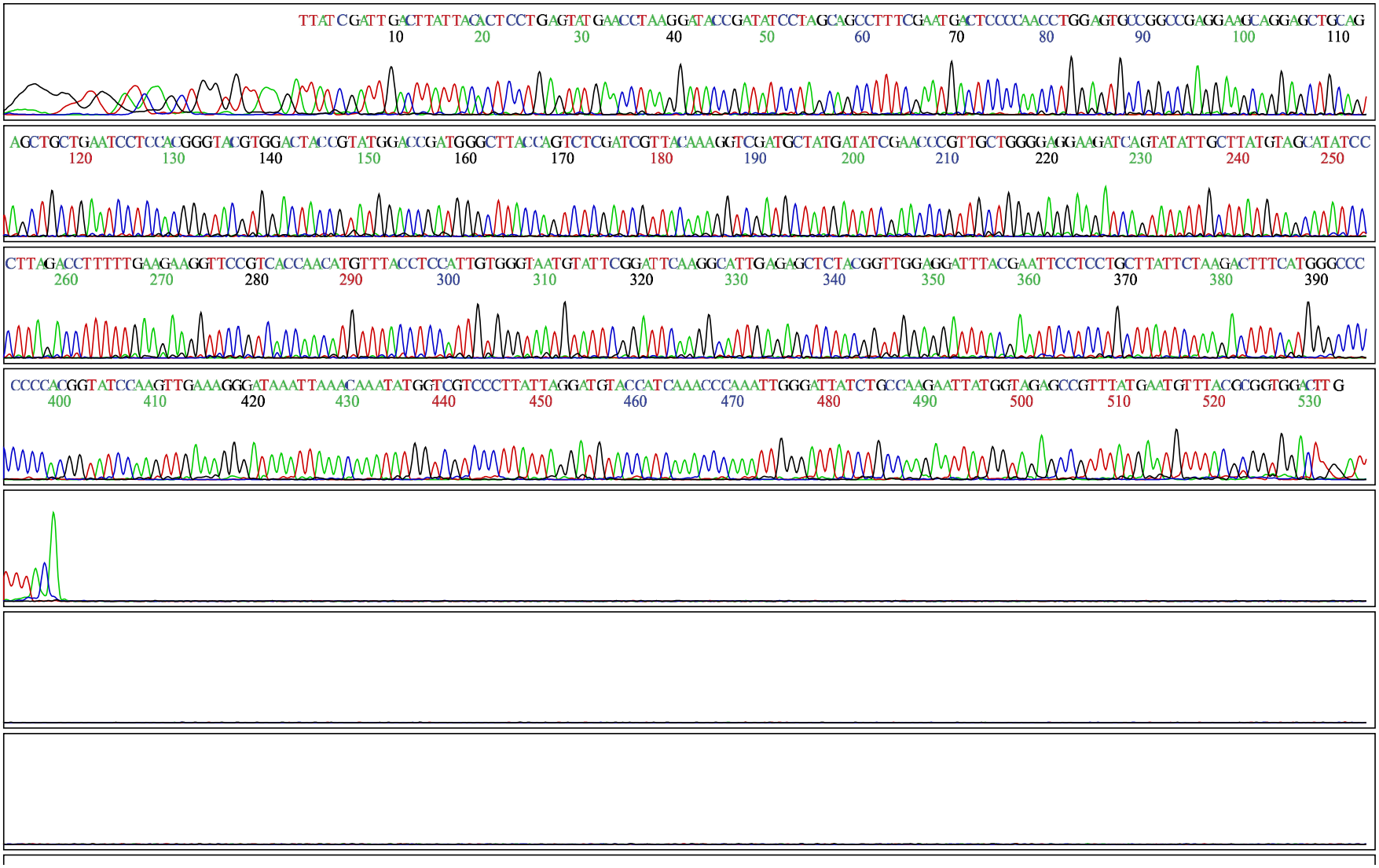

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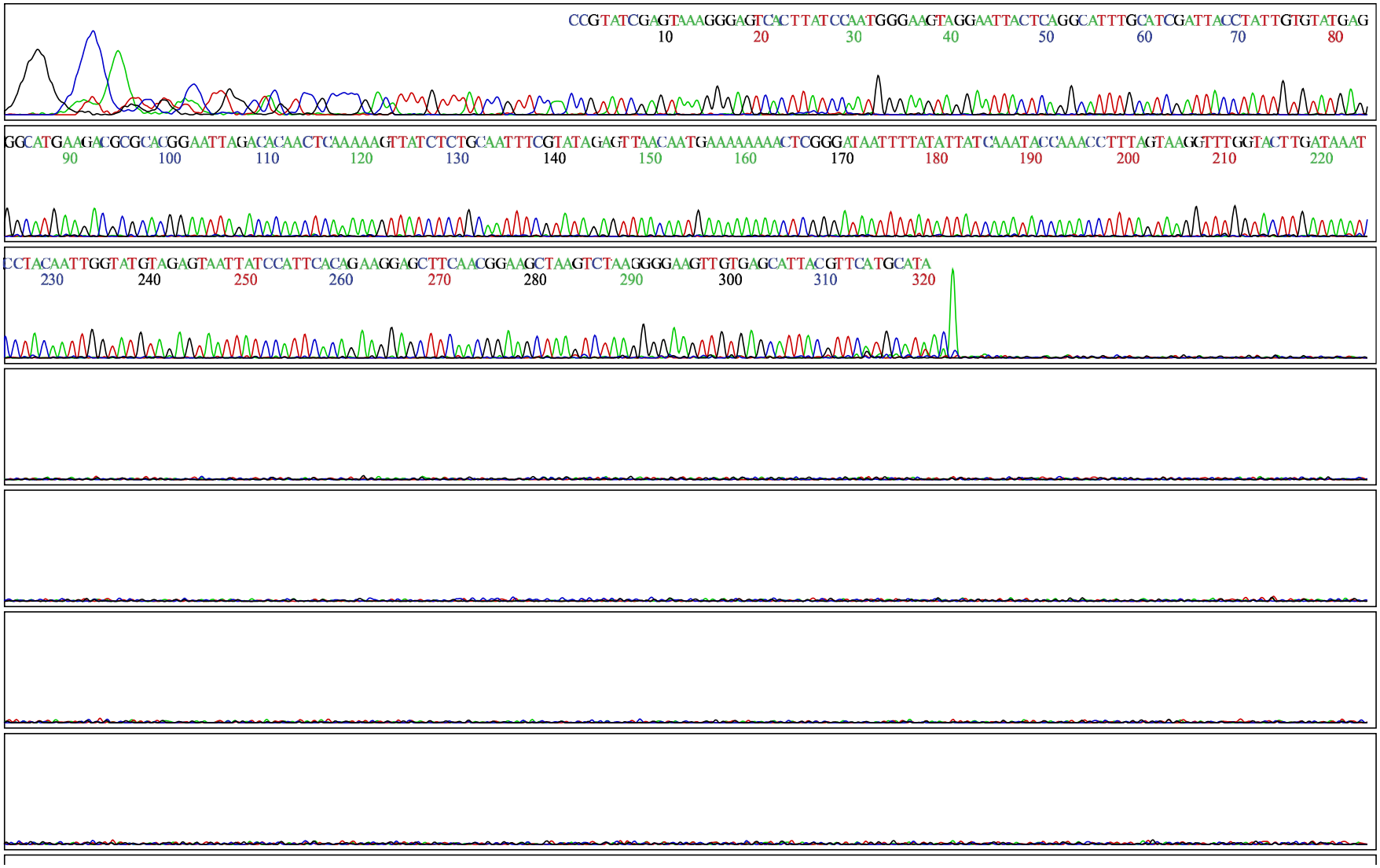

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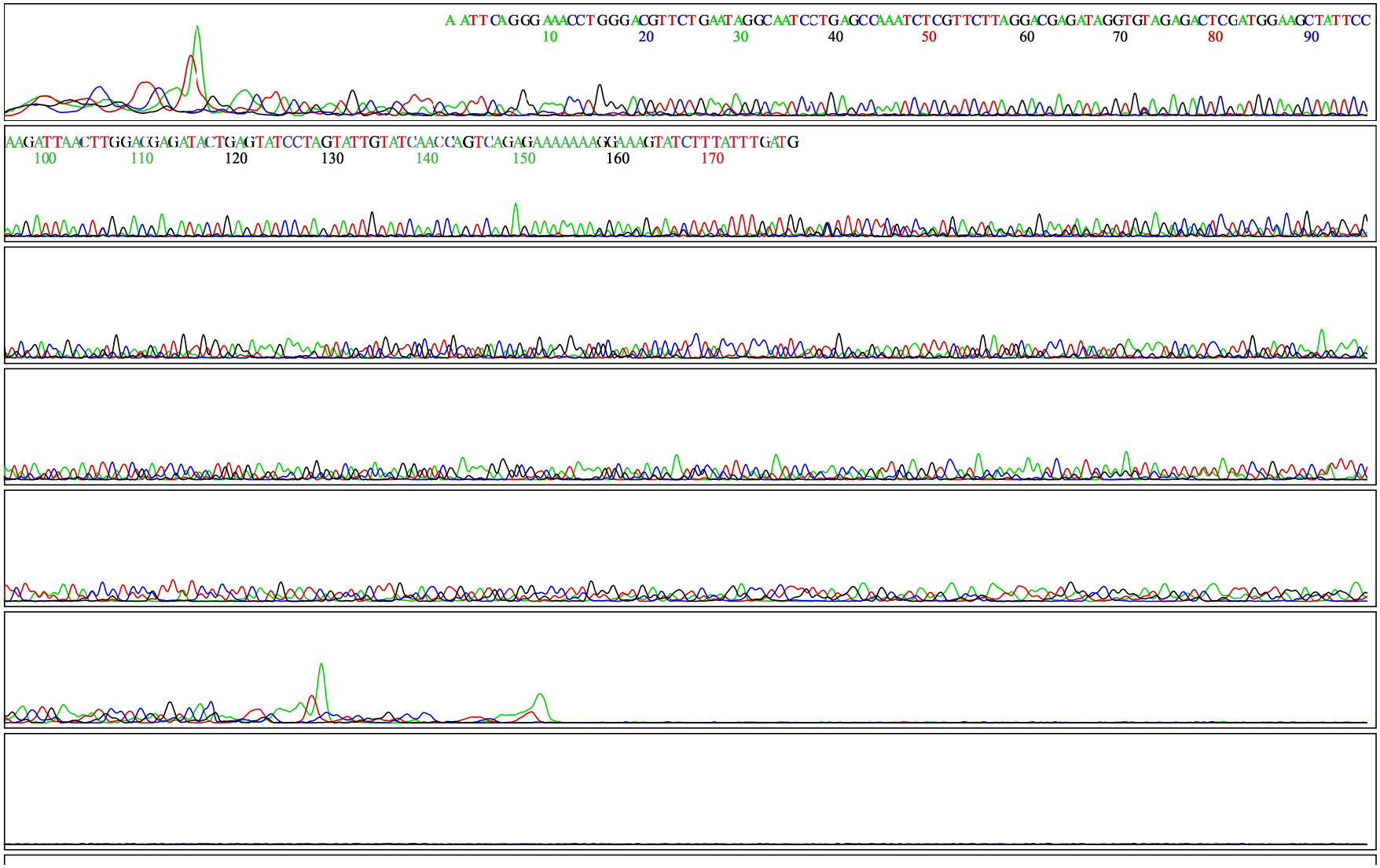

LIST OF PUBLICATIONS

- Afsana Khan, Anto P V & Ignatius Antony (2023). Comparative spore morphology of ten species of the genus *Ophioglossum* L. from Kerala, India. *Plant Science Today*. Vol 11(1): 186–195. https://doi.org/10.14719/pst.2497
- Khan, A., Kishore Kumar, K., & Anto, P. V. (2023). Reporting *Ophioglossum reticulatum* L. for the first time from the campus of Farook College (Autonomous), Kozhikode, Kerala.
- Afsana Khan, Anto P.V & Ignatius Antony (2022) Genus *Ophioglossum* in Thrissur District, Kerala; Proceedings of the National Seminar "Species the Passion 7", ISBN 978-93-91691-01-1.
- Afsana Khan, Anto P.V & Ignatius Antony (2022) A Review on the Taxonomical studies of Genus *Ophioglossum*. Perspective in Plant Science; 978-93-5627-274-3.

LIST OF PRESENTATIONS

- Taxonomic studies on the occurrence of different morphotypes of *Ophioglossum costatum* R. Br. In the National Seminar "Species the Passion 9" at St. Thomas' College (Autonomous), Thrissur, 15/ 12/ 2023.
- "Diversity and distribution of *Ophioglossum* genus in Central and Northern Kerala, India" in the International Seminar on Plant Science Research (ISPSR2022) organized by Post Graduate & Research Department of Botany, Mahatma Gandhi College, Thiruvananthapuram in association with Indian Science Congress Association Cochin Chapter on 3rd and 4th March 2022.
- "A Review on Taxonomical Studies on Genus *Ophioglossum* with special reference to India" In the National Seminar on "Emerging Trends in Genomic Research" at St. Thomas' College (Autonomous), Thrissur, 22/ 02/ 2019.

PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(1): 186–195
https://doi.org/10.14719/pst.2497

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RESEARCH ARTICLE

Comparative spore morphology of ten species of the genus *Ophioglossum* L. from Kerala, India

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OPEN ACCESS

ARTICLE HISTORY

Received: 11 March 2023
Accepted: 12 September 2023

Available online
Version 1.0 : 15 October 2023
Version 2.0 : 01 January 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Khan A, Anto P V, Antony I. Comparative spore morphology of ten species of the genus *Ophioglossum* L. from Kerala, India. Plant Science Today. 2024; 11(1): 186–195. https://doi.org/10.14719/pst.2497

Abstract

The eusporangiate fern *Ophioglossum* brings some uncertainty among taxonomists while identifying the species. Therefore, a combined approach by considering the spore morphology and other morphological characters is more authentic for delimiting species of this genus. This study documented and compared the spore morphology of *O. costatum*, *O. gramineum*, *O. lusitanicum*, *O. lusoafricanum*, *O. parvifolium*, *O. pendulum*, *O. petiolatum*, *O. raphaelianum*, *O. reticulatum*, and *O. rubellum* collected from Kerala, India by Scanning Electron Microscopy (SEM) analysis. SEM images of the spores for each species were provided, the P/E ratio was calculated, and the size classes of the spores were determined here. The occurance of *O. lusitanicum* in India was confirmed, and *O. madhusoodhananii* was synonymised under *O. costatum.*

Keywords

Ophioglossum; spore; trilete; SEM; fern; India

Introduction

The snake tongue or Adder's tongue fern, *Ophioglossum*, is a eusporangiate fern that belongs to the family Ophioglossaceae. Taxonomists have worked on *Ophioglossum* in India (1-11) since its discovery by Bouhin (12) and validation of its generic status by Linnaeus (13) in his "Species Plantarum". Previously, there were a total of ten *Ophioglossum* species reports in Kerala, of which nine *Ophioglossum* species, viz., *O. costatum* R. Br., *O. gramineum* Willd., *O. gomezianum* Welw. Ex A. Braun, *O. lusoafricanum* Welw. ex. Prantl., *O. parvifolium* Grev. & Hook., *O. pendulum* L., *O. petiolatum* Hook., *O. reticulatum* L. and *O. rubellum* Welw. Ex A.Braun was recorded by Fraser Jenkins *et al*. (14, 15), and *O. raphaelianum* Anto, Afs. Khan, F. Francis & I. Antony was reported by Anto *et al*. (16). However, morphological variations due to the polyploidy and highest chromosome number found in some species (17) and the simple morphology of the genus make species identification more problematic (18-22). *Ophioglossum* is a complex genus with bewildering morphological characters. Recently, many new species have been reported from India without considering the morphotypes of this genus. So Fraser-Jenkins *et al*. (15) synonymised different species of *Ophioglossum* in their work. So, considering the spore characters and the rhizome morphology, tropophylls and fertile segments are more reliable in the case of *Ophioglossum* (23, 24). Moreover, the number of taxonomists considering the spore characters for species identification has increased with time (25-32). In this study, the spore morphology of ten *Ophioglossum* species was compared

and plated using their light microscopy and scanning electron microscopy images, which will be helpful for the exact identification of each species.

Materials and Methods

Fresh *Ophioglossum* plants were collected from different areas of Kerala state. Field photographs were taken, and all the characters that could be documented from the field were noted in the field book. The plants were then taken into the laboratory for further examination. The sporangia were covered with an envelope from the field to preserve the spore morphology. The morphological characters of the external plant parts were recorded. The light microscopic structures of the spores were observed under a Binocular BIOMED Research Microscope using 40 X, and their photographs were taken. For SEM (Scanning Electron Microscope) analysis, the spores were directly dusted over carbon tape-coated stubs and sputter-coated with gold and examined using the JOEL, JSM-6390LV Scanning Electron Microscope. Chemical treatments were avoided since they dissolve the fine ornamentation and are hence not recommended as a standard technique for the family Ophioglossaceae (33, 34). The spore characters were recorded. Polar length (P) and equatorial length (E) were measured from four spore samples of each species, and the P/E ratio was calculated, and the size of the spores was determined accordingly (35).

Results

Ophioglossum costatum R. Br. Prod. Fl. Nov. Holl. 163.1810. (Fig. 1: 1A-1D)

Ophioglossum pedunculosum Desv. In Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 5:306. 1811, *Ophioglossum fibrosum* Schumach. In Beskr. Guin. Pl.: 452. 1827, *Ophioglossum wightii* Grev. & Hook. In Bot. Misc. 3: 218. 1833, *Ophioglossum vulgatum* var. *costatum* (R.B .) Hook.f. in Fl. Tasman. 2: 153. 1858, *Ophioglossum brevipes* Bedd., in Ferns. Southern India 23. t. 72. 1863, *Ophioglossum aphrodisiacum* Welw. ex Hook. In Hooker &Baker, Syn. Fil.: 446. 1868, *Ophioglossum bulbosum* Bedd. In Suppl. Ferns S. Ind.: 28. 1876, Bedd., Handb. 465. t. 289. 1883, Suppl. 109. 1892, *Ophioglossum vulgatum* var. *pedunculosum* (Desv.) Domin in Biblioth. Bot. 20: 221. 1913, *Ophioglossum felixii* Tardieu in Notul. Syst. (Paris) 13: 169 1948 publ. 1949, *Ophioglossum hitkishorei* M.Patel & M.N.Reddy in Bot. Lett. 166: 426. 2019. *Goswamia hitkishorei* (M.Patel & M.N.Reddy) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 23. 2022, *Goswamia costata* (R.Br.) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 23. 2022

Spores are globose and trilete; the distal face sizes up to 30.28 x 31.8-40.66 × 41.44µm; In micro ornamentation, the spores are foveolate, granulose, reticulate, muri anastomosing; lumina tetragonal-pentagonal-circular, narrow-wide; proximal face concave with a deep proximal cavity having shallow lumina; triradiate leasural arms unequal, one arm double the length of the rest of the two arms, more or less straight, less wavy, extending up to the rim; Polar axis 16.35-18.42 µm, Equatorial axis (proximal face) 25.19-37.02 µm mean P/E ratio is 0.58±0.04 µm, large spores (Fig 3: 1A-1D).

Specimens examined : India: South India: Kerala: Ernakulam Dist: Malayattoor: 10°21'60.32"N, 76°50'87.46"E. Kannur Dist: Madayippara 12°02'68.86"N, 75°26'30.12"E. Palakkad Dist.: Kallekad 10°47'2"N and 76°36'2"E, Thrissur Dist.: Cheruchakkichola 10°42'05.26"N, 76°11'59.07"E, Killannur. 10°61'56.38"N, 76°24'97.55"E, Kayampoovam 10° 41'10.16"N, 76°23'07.50"E: Afsana Khan, Anto P.V. 20, 22, 18, 16, 24, 17 (STC!)

Ophioglossum gramineum Willd. Abhandl. Kurf.-Mainz. Akad. Wiss. Erf. 2(6): 18, t. 1, f. 1. 1802. (Fig. 1: 2A-2D)

Ophioglossum vulgatum var. *gramineum* (Willd.) Hook.f. in Fl. Nov.-Zel. 2: 30.1854, *Ophioglossum dietrichiae* Prantl in Ber. D tsch. Bot. Ges. 1: 352. 1883, *Ophioglossum lanceolatum* Prantl in Ber. Deutsch. Bot. Ges. 1: 352. 1883, *Ophioglossum vulgatum* var. *lanceolatum* Luerss. In J. Mus. Godeffroy 3: 247. 1875, *Ophioglossum moluccanum* f. *inconspicuum* Rac. In Natuurk. Tijdschr. Ned.-Indië 59. 237. 1900, *Ophioglossum prantlii* C.Chr. In Index Filic.: 471. 1906, *Ophioglossum inconspicuum* (Rac.) Alderw. In Bull. Dép. Agric. Indes Néerl. 21: 9. 1908, *Ophioglossum inconspicuum* f. *majus* Alderw. In Bull. Dép. Agric. Indes Néerl. 21: 9.1908, *Ophioglossum gregarium* Christ in Nova Guinea 8: 164. 1909, *Ophioglossum vulgatum* var. *prantlii* (C.Chr.) W.R.B.Oliv. In Trans. & Proc. New Zealand Inst. 49: 126. 1917, *Ophioglossum gramineum* var. *majus* (Alderw.) Wieff. In Blumea 12: 324. 1964, *Ophioglossum gracile* Pocock ex J.E.Burrows in Bothalia 25: 61. 1995

Spores are globose and trilete; the distal face sizes up to 36.59 × 35.42 µm; granulose, retate-reticulate; lumina are deep, conical-circular; muri reticulate with pointed triangular edges; proximal cavity narrow, with shallow lumina on its face; triradiate leasaral arms are short, less wavy, restricted only to the half portion of the proximal cavity, only one among the three leasural arms is extended to the rim and jointed to the edge, rest of the two arms are ends within the cavity. Polar axis 17.28-20µm, Equatorial axis (proximal face) 25.36-33.3µm, mean P/E ratio is 0.59±0.03 µm, large spores (Fig. 3: 2A-2D)

Specimens examined : India: South India: Kerala: Kasaragod District: Posadigumpe: 12°40'33.55"N, 75°01'03.85"E., Palakkad District: Kallekad 10°47'2"N, 76°36'2"E, Thrissur District: Killannur. 10°61'56.38"N, 76°24'97.55"E. Peruvanmala 10°62'25.27"N, 76°13'46.49"E., Afsana Khan, Anto P.V. 23, 19, 20, (STC!)

Ophioglossum lusitanicum **L** , Sp. Pl. 2: 1063. 1753. (Fig. 1: 3A-3D)

Ophioglossum loureiroanum C.Presl in Suppl. Tent. Pterid.: 55. 1845, *Ophioglossum vulgatum var. lusitanicum (L.). Hook. & Arn.* In Brit. Fl., ed. 8, éd. 8: 593. 1855,*Ophioglossum vulgatum* subsp. *lusitanicum* (L.) Hook.f. in Student. Fl. Brit. Isl.: 469. 1870, *Ophioglossum braunii* Prantl in Ber. Deutsch. Bot. Ges. 1:351. 1883, *Ophioglossum vulgatum* subsp. *lusitanicum* Bonnier & Layens in Tabl. Syn. Pl. Vasc.

France: 381. 1894, *Ophioglossum lusitanicum* var. *latifolium* Rouy in G. Rouy & J. Foucaud, Fl. France. 14: 460. 1913, *Ophioglossum lusitanicum* var. *longepedunculum* Rouy in G.Rouy & J.Foucaud, Fl. France 14: 460. 1913, *Ophioglossum vulgatum* var. *macrophyllum* Rouy in G.Rouy & J.Foucaud, Fl. France 14: 458. 1913.

Spores globose-ellipsoid, trilete, dimorphic; distal face sizes up to 34.69 × 29.21µm; patellate, sporoderm is smooth due to the extensive deposition of perine layer over it, lumina shallow, irregular in shape; muri reticulate, smooth and flattened; the proximal face is dimorphic with and without a proximal cavity; the triradiate leasural arms almost equal, more straight, less wavy, extending to the rim and jointed to the edges. Polar axis 14.36-17.21µm, Equatorial axis (proximal face) 22.23-30.36µm, mean P/E ratio is 0.58±0.03 µm, large-sized spores (Fig. 3: 3A- 3D).

Specimens examined : India: South India: Kerala: Kasaragod District: Posadigumpe 12°40'33.55"N, 75°01'03.85"E., Thrissur District: Killannur. 10°61'56.38"N, 76°24'97.55"E, Peruvanmala. 10°62'25.27"N, 76°13'46.49"E., Afsana Khan, Anto P.V. 31, 7, 6 (STC!)

Note : There was an opinion that the Indian *O. lusitanicum* corresponds to the African *O. lusoafricanum* (Fraser Jenkins *et al*. 2021). But *O. lusitanicum* (Type: France: Brest, Deschamps, (Epitype, MNHN, digital image!)) and *O. lusoafricanum* (Type: Angola, apaungo Andongo, Welwitsch 34, K, digital image!) collected from different localities of Kerala during this study is different in the morphological characters of vegetative parts and in the exine ornamentation. The dorsal face of *O. lusitanicum* is smooth, whereas in *O. lusoafricanum,* spores are verrucate. The spores of *O. lusitanicum* are trilete and dimorphic with or without a clear deep proximal cavity, whereas *O. lusoafricanum* spores are alete or trilete with a proximal cavity.

Ophioglossum lusoafricanum Welw.ex Prantl., Ber. Deutsch. Bot. Ges. 1: 351.1883. (Fig. 1: 4A-4D)

Spores under Light microscope (LM) are large, verrucate, dimorphic with alete and trilete spores, globose; In SEM, the dorsal surface non astamosing, areoles are absent, ventral surface alete or trilete. Trilete spores have arms extending up to rim, unequal, one arm double the size of rest of the two arms, the deep proximal cavity have small lumina. Polar axis 21.49-32.54 µm, Equatorial axis (proximal face) 42.28-66.73 µm, mean P/E ratio is 0.52 ± 0.02 µm, large spores (Fig. 3: 4A-4D)

Specimens examined : Kerala, Thrissur Distr. Killannur, 10° 37' 7.5828'' N, 76° 15' 11.9628'' E., Afsana Khan, Anto P.V.15, (STC!)

Ophioglossum parvifolium Grev. & Hook., Bot. Misc. 3: 218. 1833. (Fig. 1:5A-5D)

Ophioglossum macrorrhizum Kunze in Analecta Pteridogr.: 2. 1837, *Ophioglossum schmidii* Kunze in Linnaea 24: 246. 1851, *Ophioglossum vulgatum* var. *macrorrhizum* (Kunze) Luerss. In J. Mus. Godeffroy 3: 242. 1875, *Ophioglossum luerssenii* Prantl in Ber. Deutsch. Bot. Ges. 1:352. 1883, *Ophioglossum tenerum* Mett. ex Prantl in Ber. Deutsch. Bot. Ges. 1:352. 1883, *Ophioglossum moluccanum* f. *pumilum* Rac. In Natuurk. Tijdschr. Ned.-Indië 59. 237. 1900, *Ophioglossum pumilum* (Rac.) Alderw. In Malayan Ferns: 774. 1909, *Ophioglossum schlechteri* Brause in Bot. Jahrb. Syst. 49: 58. 1912, *Ophioglossum vulgatum* var. *luerssenii* (Prantl) Domin in Biblioth. Bot. 20: 222. 1915, *Ophioglossum nudicaule* var. *macrorrhizum* (Kunze) R. T. Clausen in Mem. Torrey Bot. Club 19(2): 150. 1938, *Ophioglossum nudicaule* var. *tenerum* (Mett. ex Prantl) R. T. Clausen in Mem. Torrey Bot. Club 19(2): 146. 1938.

Spores globose and trilete; distal face sizes up to 33.67 × 30.69 µm; granulose, reticulate- patellate, perine layer is present; lumina are shallow, more or less circular, muri reticulate; triradiations less wavy, extending up to the rim; one leasural arm is double the length of rest of the two arms, Polar axis is 14.11-16.7µm, the Equatorial axis 21 -33.01µm, mean P/E ratio is 0.56±0.05 µm, large spores (Fig. 3: 5A-5D).

Specimens examined : India: South India: Kerala: Thrissur District: Cheruchakkichola 10°42'13.82"N, 76°12'12.39"E., Peruvanmala. 10°62'25.27"N, 76°13'46.49"E., Afsana Khan, Anto P.V. 8, 5 (STC!)

Ophioglossum pendulum L.Sp. Pl., ed. 2. 2: 1518. 1763. (Fig. 2: 1A-1D)

Ophioderma pendulum L. C. Presl in Suppl. Tent. Pterid.: 56. 1845, *Ophioglossum furcatum* J. Sm. In Ferns Brit. For.: 272. 1866, nom. nud., *Ophioglossum moultonii* Copel. In J. Straits Branch Roy. Asiat. Soc. 63: 72. 1912.

Spores are globose and trilete and comparatively more enormous than the spores of other *Ophioglossum* species included in this study; the distal face sizes up to 51.83 × 52. 26 µm; perine layer deposition towards the centre; lumina narrow, more or less conical, muri reticulate, very thin and narrow without sharply pointing edges, somewhat wavy and smooth in appearance; the proximal cavity is shallow, triradiate leasural arms extending to the margins. Polar axis 25-28.02 µm, Equatorial axis 39.7-43.69 µm, mean P/E ratio is 0.62±0.02 µm, large spores (Fig. 3: 6A - 6D).

Specimens examined : 12832 (KFRI!)

Ophioglossum petiolatum Hook., Exot. Fl. 1(4): t.56. 1823. (Fig. 2: 2A-2D)

Ophioglossum moluccanum Schltdl. In Adumbr. Pl.: 9. 1825, *Ophioglossum elongatum* A. Cunn. In Compan. Bot. Mag. 2: 361. 1837, *Ophioglossum moluccanum* f. *Complicatum* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 290. 1870, *Ophioglossum vulgatum* var. *Australasiaticum* Luerss. In J. Mus. Godeffroy 3: 246. 1875, *Ophioglossum litorale* Makino in J. Jap. Bot. 6: 27. 1929, *Ophioglossum floridanum* E.P.St.John in Amer. Fern J. 26: 53. 1936, *Ophioglossum floridanum* f. *Favosum* E.P.St.John in Amer. Fern J. 26: 54. 1936, *Ophioglossum floridanum* f. *Reticulosum* E.P.St.John in Amer. Fern J. 26: 55. 1936, *Ophioglossum reticulatum* f. *Complicatum* (Miq.) Wieff. In Blumea 12: 330. 1964.

Spores are trilete and globose, rugate, granulose; distal face sizes up to 30.68 × 33.75 µm in SEM.; lumina as minute depressions, more or less rounded; muri as discontinuous ridges with thick and thin exine regions, thick

ridges have granulose deposition over it, perine layer absent; the triradiated leasural arms are almost equal, wavy, jointed, up to the rim of the deep proximal cavity, granulose. Polar axis 17.21-18.09 µm, Equatorial axis 33.01-34.07 µm, mean P/E ratio is 0.52±0.00 µm, large spores (Fig. 3: 7A - 7D).

Specimens examined : India: South India: Kerala: Idukki District: Munnar 10° 4' 5.988'' N, 77° 4' 0.984'' E, Stephen Sequeira, Afsana Khan, Anto P.V.14 (STC!)

Ophioglossum raphaelianum Anto, Afs.Khan, F.Francis & I.Antony, Int. J. Advanced Res. 4(5): 1269, f.1-2. 2016. (Fig. 2: 3A-3D)

Ophioglossum malviae M. Patel & M. N. Reddy in Sci. Rep.8 (art. 5911): 1. 2018, *Goswamiana malviae* (M. Patel & M. N. Reddy) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24. 2022, *Goswamiana raphaeliana* (Anto, Afs. Khan, F. Francis & I. Antony) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24. 2022.

Spores are trilete and globose-ellipsoid, foveolate; distal face sizes up to 26.24 × 20.44 µm in SEM.; lumina shallowly depressed, more or less square shaped; muri reticulate, narrow without sharp pointed edges, perine layer present; the triradiated leasural arms are more or less wavy, jointed, up to the centre of the proximal cavity and are pointing towards the wall in between trilobed proximal end; the distal face is granulose, Polar axis 14.27-18.96 µm, Equatorial axis 30.53-37.4 µm, mean P/E ratio is 0.53±0.04 µm, large spores (Fig. 3: 8A-8D).

Specimens examined : India: South India: Kerala: Thrissur District: Mangadu, Kottappuram. 10°68'90.41"N, 76° 19'53.83"E. 2 (STC!), Peruvanmala. 10°62'25.27"N, 76° 13'46.49"E.Afsana Khan, Anto P.V 112 (CAL!)

Note : *O. raphaelianum* is distinguished by olivaceous or bluish green-coloured tropophylls, whereas *O. rubellum* is distinguished by its red or copper-coloured tinge. The globose-ellipsoid rhizome of *O. raphaelianum* is distinguishable from the orbicular-fusiform rhizome of *O. rubellum*. *O. raphaelianum* has elliptic-orbicular tropophylls, whereas *O. rubellum* has spathulate-obovate, ovate, or suborbicular tropophylls. However, *O. raphaelianum* has a 1-3 cm long fertile segment with 6-7 alternately arranged sporangia per spike, whereas *O. rubellum* has a 1.5-7 cm long fertile segment with 6-10 oppositely arranged sporangia. The spore characters are also distinct for the two species, and hence the species status of *O. raphaelianum* is reinstated here. The spores of *O. raphaelianum* are foveolate-granulose with more or less square-shaped shallow lumina and narrow muri, whereas *O. rubellum* has lophate spores with an outer exospore wall raised to form ridges. Detailed examination of the fresh specimens as well as the type specimens of *O. rubellum* (Type: Africa; Angola, Welwitsch 33 (Isotype, BM, digital image!)), and *O. raphaelianum* (Type: India; Kerala, Anto P.V.112 (holotype, CAL!) were carried out. Based on the morphological characters of vegetative parts and spore ornamentation of both species, the species status of *O. raphaelianum* is reinstated here.

***Ophioglossum reticulatum* L.**, Sp. Pl. 2: 1063. 1753. (Fig. 2: 4A-4D).

Ophioglossum ovatum Bory in Voy. iles Afrique 2: 206. 1804, *Ophioglossum cordifolium* Roxb. In Numer. List: n. 47; Roxb. Calc. Journ. 1829, *Ophioglossum peruvianum* C. Presl in Suppl. Tent. Pterid.: 52. 1845, *Ophioglossum cognatum* C. Presl in Suppl. Tent. Pterid.: 53. 1845. *Ophioglossum cumingianum* C. Presl in Suppl. Tent. Pterid.: 52. 1845. *Ophioglossum timorense* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 93. 1868, *Ophioglossum moluccanum* f. *Dilatatum* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 92. 1868, *Ophioglossum vulgatum* var. *Minutum* F.M.Bailey in Bot. Bull. Dept. Agric. Queensland 5: 27. 1892, *Ophioglossum reticulatum* var. *Acutius* Christ in Denkschr. Kaiserl. Akad. Wiss., Wien. Math. -Naturwiss. Kl. 79(1): 56. 1908, *Ophioglossum reticulatum* var. *Polyangium* Christ in Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 79(1): 56. 1908, *Ophioglossum usterianum* Christ in A.Uster, Fl. Umgebung São Paulo: 137. 1911, *Ophioglossum raciborskii* Alderw. In Bull. Jard. Bot. Buitenzorg, sér. 2, 28: 35. 1918, *Ophioglossum pedunculatum* Desv. & Nakai in Bot. Mag. (Tokyo) 40: 373. 1926, *Ophioglossum ramosii* Copel. In Philipp. J. Sci. 56: 97. 1935, *Ophioglossum austroasiaticum* Nishida in J. Jap. Bot. 34: 46. 1959, *Ophioglossum reticulatum* f. *Dilatatum* (Miq.) Wieff. In Blumea 12: 329. 1964, *Ophioglossum holm-nielsenii* B.ollg. In Fl. Ecuador 66: 16. 2001, *Ophioglossum aletum* M.Patel, M.N.Reddy & H.K.Goswami in Indian Fern J. 35: 323. 2018, *Ophioglossum chaloneri* H. K. Goswami, M. Patel & K. K. Nag in Phytotaxa 468: 103. 2020.

Spores are globose-ellipsoid, trilete, reticulate, distal face sizes upto 32.95 µm × 27.50 µm; lumina shallowly reticulate, perine layer is present, muri narrow, without pointed edges; triradiate leasural arms are wavy, more or less equal, jointed, up to the rim. Polar axis 15.55-23.72µm, Equatorial axis 28.2-40.82 µm, mean P/E ratio 0.55±0.01 µm, large spores (Fig. 3: 9A- 9D).

Specimens examined : India: South India: Kerala: Idukki District: Munnar 10°04'07.10" N, 77°03'58.74" E. Stephen Sequeira, Afsana Khan, Anto P.V, Kozhikode District: Farook College 11°11'57.44"N, 75°51'27.12"E., Malappuram District: Nilambur 11°16'13.61"N, 76°12'26.46"E., Afsana Khan, Anto P.V. 21, 23, 12 (STC!)

Ophioglossum rubellum Welw. ex A.Braun., Filic. Afr. [Kuhn] 179. 1868. (Fig. 2: 5A-5D).

O. oleosum Khand. In Indian Fern J. 4: 102. 1987.

Spores under Light microscope (LM) are trilete or alete and globose, lophate or smooth. In SEM, spore sizes up to 22 ×33-25.69 × 22.29 µm, the dorsal surface is convex in shape, muri are lophate with outer exospore wall raised to form ridges, muri surrounds the lumina; lumina are polygonal with three- five angles, some spores have very

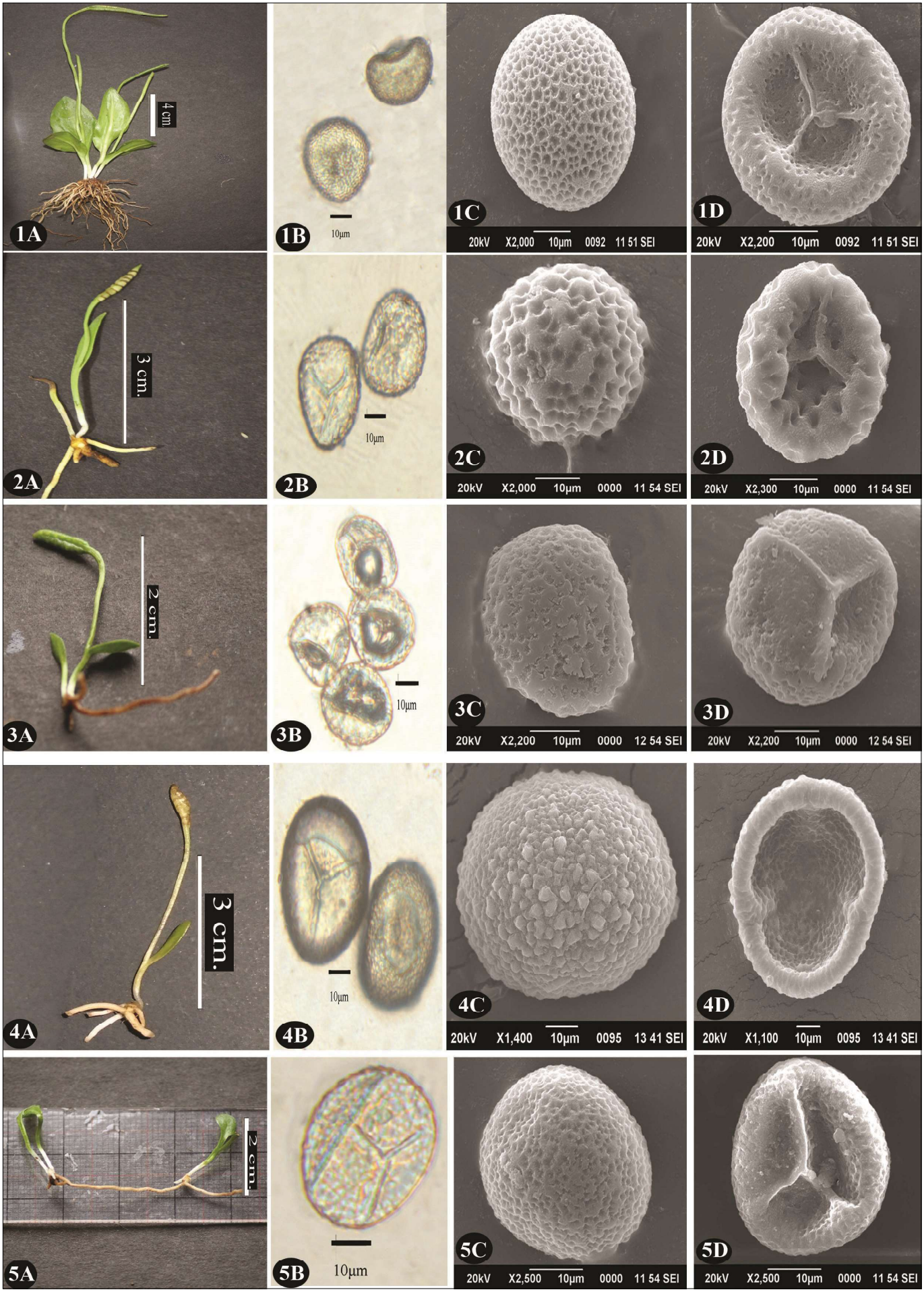

Fig1. Entire plant, Spore surface under LM., Distal spore surface under SEM, Proximal spore surface under SEM of different *Ophioglossum* species. **1A-1D**. *O. costatum*, **2A-2D**. *O. gramineum*, **3A-3D**. *O. lusitanicum,* **4A-4D**. *O. lusoafricanum*, **5A-5D**. *O. parvifolium*

smooth and plain surface in the complete absence of lumi-

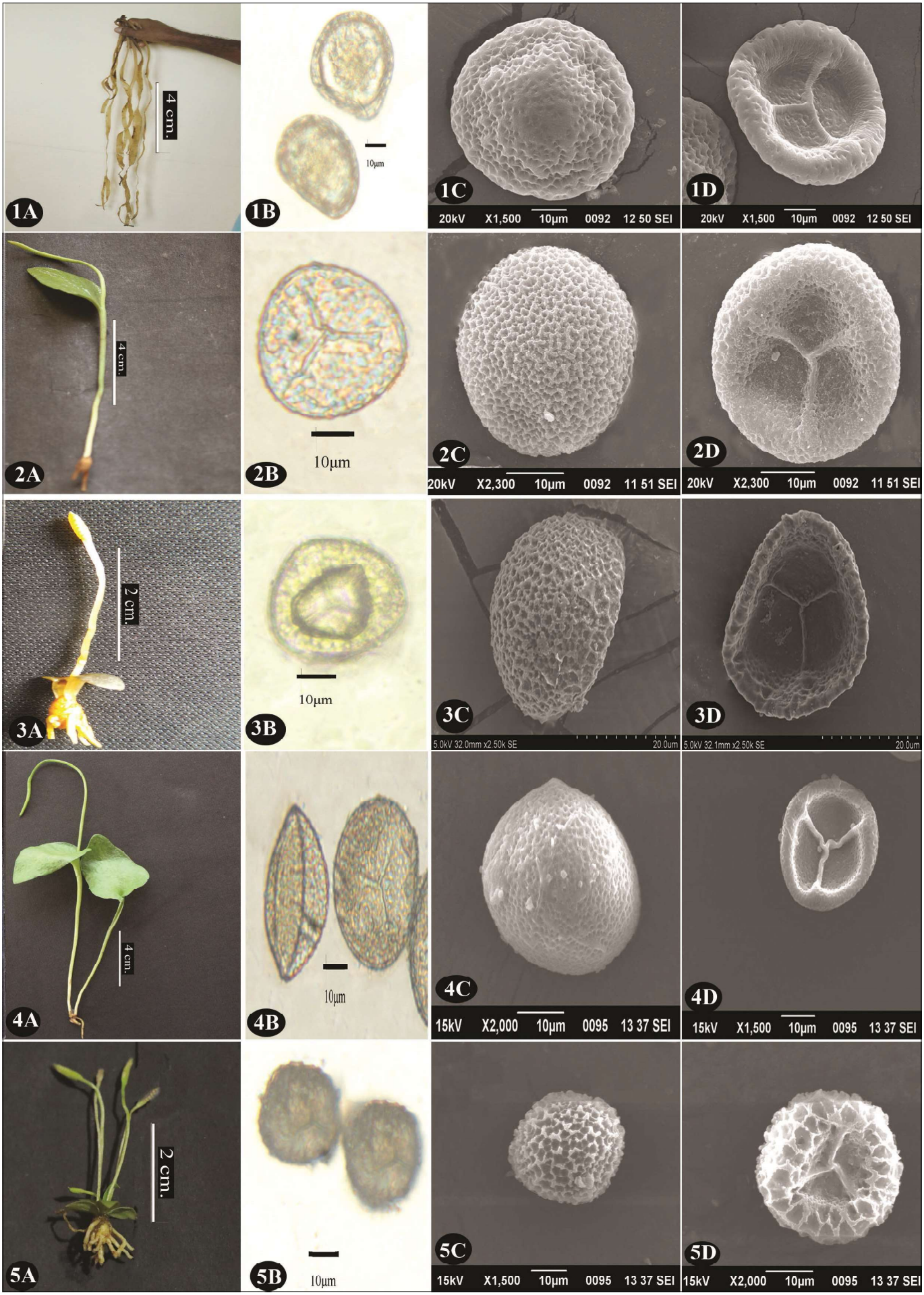

Fig 2. Entire plant, Spore surface under L.M., Distal spore surface under SEM, Proximal spore surface under SEM of different *Ophioglossum* species. **1A-1D**. *O. pendulum*, **2A-2D**. *O. petiolatum*, **3A-3D** *O. raphaelianum*, **4A-4D**. *O. reticulatum*, **5A-5D**. *O. rubellum*

na and muri; in trilete spores, the leasaral arms more wavy and jointed, almost equal, ends at middle of the proximal

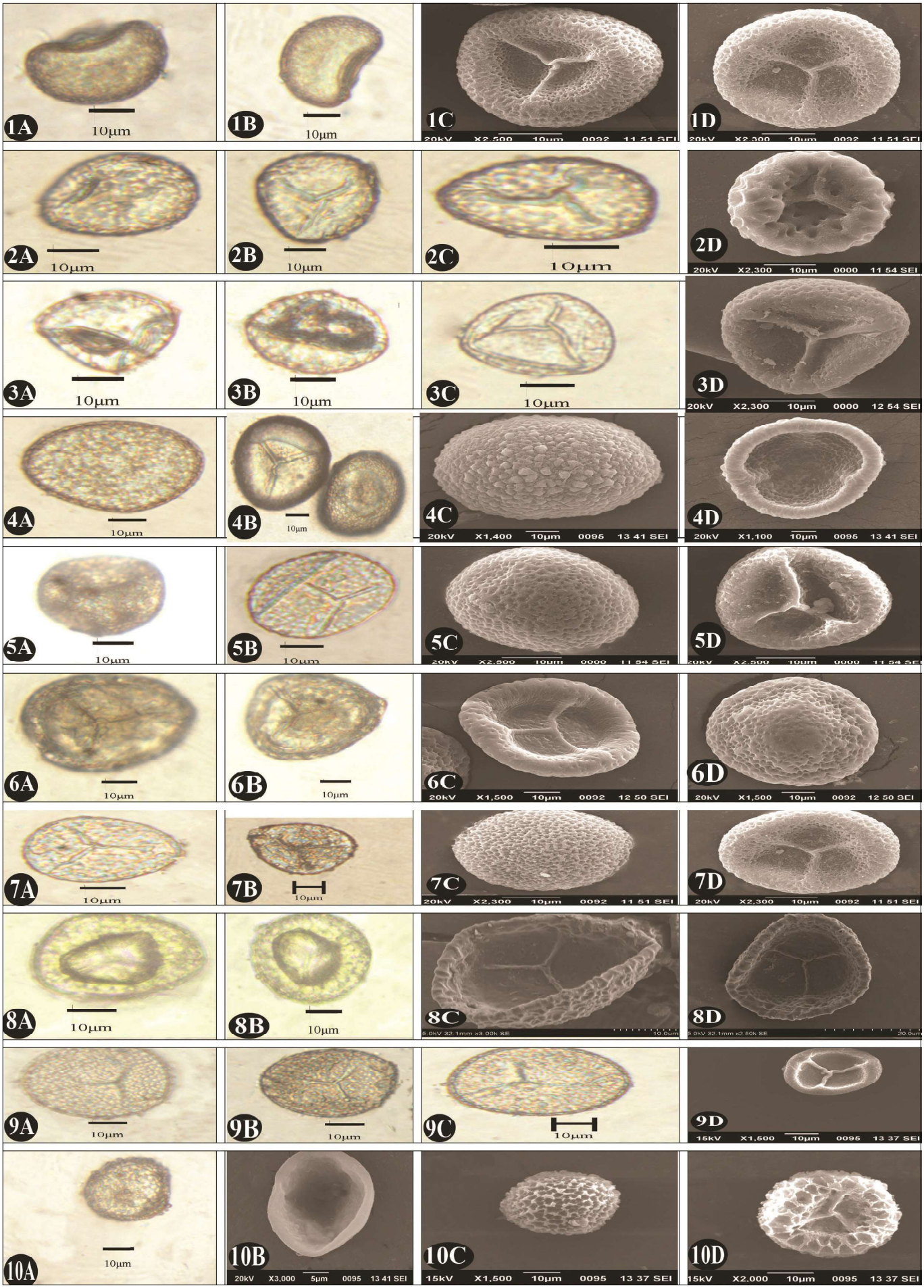

Fig 3. Spores used for P/E ratio calculation. **1A-1D**. *Ophioglossum costatum,* **2A-2D**. *O. gramineum*, **3A-3D**. *O. lusitanicum*, **4A-4D**. *O. lusoafricanum*, **5A-5D**. *O. parvifolium*, **6A-6D**. *O. pendulum*, **7A-7D**. *O. petiolatum*, **8A-8D**. *O. raphaelianum*, **9A-9D**. *O. reticulatum*, **10A-10D**. *O. rubellum*

cavity; ventral surface is concave shaped, and having an ornamentation similar to that of distal side, with de-

Table 1. Comparison of the spore characters of *Ophioglossum costatum*, *O. gramineum*, *O. lusitanicum*, *O. lusoafricanum*, *O. parvifolium*, *O. pendulum*, *O. raphaelianum*, *O. reticulatum* and *O. rubellum*

Name of the taxa	Polar length (P) (µm)		Equatorial length (E)(µm)		P/E ratio (µm)		Size class
	Min.- Max.	Mean± SE.	Min.- Max.	Mean± SE.	Min.- Max.	Mean±SE.	
O. costatum	16.35-18.42	17.78±0.48	25.19-37.02	30.62±2.05	0.53-0.72	0.58±0.04	large
O. gramineum	17.28-20	18.27±0.60	25.36-33.3	31.01±1.8	0.53-0.68	0.59±0.03	Large
O. lusitanicum	14.36-17.21	15.57±0.70	22.33-30.36-	27.1±1.79	0.49-0.65	0.58±0.03	Large
O. lusoafricanum	21.49-32.54	26.03±2.39	42.28-66.73	50.19±5.58	0.48-0.58	0.52±0.02	Large
O. parvifolium	14.11-16.7	15.51±0.46	21-33.01	28.11±2.63	0.47-0.67-	0.56±0.05	Large
O. pendulum	25-28.02	26.02±0.69	39.7-43.69	41.94±0.78	0.57-0.67	0.62±0.02	Large
O. petiolatum	17.21-18.09	17.55±0.21	33.01-34.07	33.60±0.25	0.50- 0.54	0.52±0.00	Large
O. raphaelianum	14.27-18.96	17.15±1.03	30.53-37.4	32.57±1.6	0.38-0.61	0.53±0.04	Large
O. reticulatum	15.55-23.72	20.09±1.94	28.2-40.82	36.19±2.8	0.49-0.58	0.55±0.01	Large
O. rubellum	8.76-14.1	11.46±1.09	22.15-28.89	24.5±1.5	0.36-0.51	0.46± 0.03	Medium

pressed lumina and lophate muri. Polar axis 8.76-14.1µm, Equatorial axis 22.15-28.89µm, mean P/E ratio 0.46±0.03µm, medium sized spores (Fig. 3: 10A- 10D).

Note: By the detailed examination of vegetative as well as spore morphology and comparison with the type specimens, *O. Indicum* B. L. Yadav & H. K. Goswami in Bull. Natl. Mus. Nat. Sci., Tokyo, B. 36: 155 (2010) (Type:Rajastan, B. L. Yadav 3011, (Isotype, CAL, digital image!))

Collected from Kerala was found to be distinct form *O. rubellum* (reinstatement in press) and hence the names *O. indicum* and *Goswamia indica* (B. L. Yadav & H. K. Goswami) Li Bing Zhang & Liang Zhang are not considered as the synonym of *O. rubellum* in this study.

Specimens examined : Kottayam Distr.**:** Elampally 9° 35' 13.902'' N, 76° 42' 46.4688'' E, Thrissur Dist.**:** Mangadu 10° 41' 21.876'' N, 76° 11' 43.368'' E, Afsana Khan, Anto P.V, 24, 9, (STC!), Pazhiyottumuri 10° 41' 9.978'' N, 76° 8' 40.776'' E, Vimal K. R, Afsana Khan, Anto P.V, 12, (STC!)

Discussion

Taxonomists used the exine patterns as the most significant features while differentiating species in the *Ophioglossum* genus (36-39). The spore characters were treated as valid for identifying some species (40). They are more consistent, less easily affected by environmental factors and are more reliable; and hence, a thorough examination of the wall layers is necessary, along with the morphological study of each plant part (41, 42, 43, 44, 45, 46). Common spores of the *Ophioglossum* genus have a reticulated pattern (47). The presence of dimorphic spores within the same sporangium was observed by (36, 48). In agreement with this, dimorphic spores are observed in *O. lusitanicum*, *O. lusoafricanum* and *O. rubellum*. Foveolate-granulose spores are found in *O. costatum*, reticulate-retate spores are present in *O. gramineum*, patellate spores are common in *O. lusitanicum*, verrucate spores in *O. lusoafricanum*, reticulate-patellate spores in *O. parvifolium*, reticulate and granulose spores in *O. pendulum* and *O. reticulatum*, rugate spores in *O. petiolatum*, foveolate in *O. raphaelianum* and lophate spores present in *O. rubellum*. The exine patterns of *O. parvifolium*, *O. costatum*, *O. lusitanicum* and *O. petiolatum* in this study correlate with (19, 32, 23). Except for *O. rubellum*, all the species examined have large spores; the spores of the epiphytic *O. pendulum* are the largest, with a P/E ratio of 0.0.62±0.02. Even though *O. pendulum* is an epiphyte, its spore characters are similar to those of other terrestrial species. Trilete spores are common among all the species, but alete spores are present in *O. lusoafricanum* and *O. rubellum*. The detailed comparison of spore characters between the ten collected species is given in Table 1.

The morphological variations in the vegetative characters and spore characters are common within the same species of genus *Ophioglossum* (14, 15). Spores of *O. rubellum* collected from Thrissur and Palakkad districts have similar spore ornamentation. But the ornamentation slightly varies in the specimens collected from Kottayam District. Variations are also observed in the cytotypes of *O. costatum.* The morphological characters of *O. madhusoodhananii* Sojan, V.S.A. Kumar, Sindhu Arya, V. Suresh, L. Leeja & Alen Alex (49) are similar to the characters of *O. costatum*. Mahabale (2) collected and described several specimens from India with cormatous rhizomes, bifurcated spikes, lanceolate-ovate tropophylls. He identified these specimens as *O. Fibrosum* Schumach. *O. fibrosum* was synonymised as *O. costatum* by Roskov *et al*. (50). The reticulate, granulose, and foveolate spores are common in all the cytotypes of *O. costatum*. The luminar width may vary within the spores of the same species. So, the present study considered *O. madhusoodhananii* as a cytotype of *O. costatum*.

Conclusion

The species identification by considering the morphology of the external plant parts along with the spore morphology sounds more authentic in the genus *Ophioglossum*. The spores of the ten *Ophioglossum* species collected from Kerala, Southern India, *viz*., *O. costatum*, *O. gramineum*, *O. lusitanicum*, *O. lusoafricanum*, *O. parvifolium*, *O. pendulum*, *O. petiolatum*, *O.raphaelianum*, *O. reticulatum*, and *O. rubellum* possess unique and distinct characters for each species. The presence of *O. lusitanicum* in India was con-

firmed, the species status of *O. raphaelianum* was reinstated and *O. madhusoo- dhananii* was synonymised with *O. costatum*.

Acknowledgements

The authors are grateful to DST-FIST for funding SIFC St. Thomas' College (Autonomous), Thrissur and The Sophisticated Analytical Instruments Facility (SAIF), Cochin University of Science and Technology for providing lab facilities to carry out this work, University Grants Commission (UGC), New Delhi, India for grating Maulana Azad National Fellowship (MANF) for the study. Fraser-Jenkins C. R, Burrows J.E, Sreenivas V. K, Vimal K.R, Stephen Sequeira, Prabhu Kumar K.M, Rajesh K. P, Raju Antony, Jomy Augustine, Pradheep A. K, Lesly Augustine, Prakash P.S, Kishore Kumar K., Pramod C., Goswami H. K, Alfred M.K, Salim Pichen, Pramod Kumar N., Renjima V. are also thanked for their support during this study.

Authors contributions

AK carried out the spore morphology studies, participated in the plant collection and identification and drafted the manuscript. APV participated in collection, identification and drafting of the manuscript. IA participated in the coordination of the work and correction of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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Devagiri Journal of Science 9(1),122-124

www.devagirijournals.com
ISSN 2454-2091

Reporting *Ophioglossum reticulatum* L. for the first time from the campus of Farook College (Autonomous), Kozhikode, Kerala

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Received: 12.07.2023

Revised and Accepted: 9.10.2023

Key Words: *Ophioglossum*, Farook College, Kozhikode District, Kerala

Abstract

The Snake-tongue fern *Ophiolossum* is a taxonomically bewildering genus due to its simple, but confusing morphological characters. The genus gets more noticed among taxonomists after the report of the highest chromosome number in *O. reticulatum*. The species was collected and documented for the first time from the campus of Farook College, Kozhikode district, Kerala state.

1. Introduction

The genus *Ophioglossum* which belongs to the family Ophioglossaceae, was discovered by Bauhin (1620) and the genus status was confirmed by Linnaeus (1753). *Ophioglossum* has 53 accepted species world-wide and has cosmopolitan distribution. The name *Ophioglossum* was formed by combining two Greek words, '*Ophios*' meaning snake and '*glossa*' meaning tongue. The report of highest chromosome number in *O. reticulatum* (*n* = c.630, 2*n* = c.1260), by Abraham *et al*. (1962) was a great discovery in the taxonomic history. In India, the genus is represented by 13 species, out of which 8 species are from the Western Ghats (Patil & Dongare, 2014; Fraser-Jenkins *et al*., 2017).This study reports *O. reticulatum* for the first time from the campus of Farook College, Kozhikode district of Kerala state.

2. Methodology

Fresh *O. reticulatum* plants were collected from the Farook College Campus during the monsoon period and photographs were taken using Canon EOS R digital camera. The morphological characters of the plants were studied under Leica stereo microscope. Descriptions was prepared and the plants were identified using relevant literatures.

3. Results

***Ophioglossum reticulatum* L.**, Sp. Pl. 2: 1063 (1753). (Fig. 1)

Ophioglossum reticulatum is a terrestrial herb and comparatively larger species attaining a height up to 27 cm. The plants are characterised by quadrate tropophylls, fusiform rhizome and long stipes. One-two tropophylls were present in a single plant, up to 6 cm long and 2 – 4 cm broad, fertile segment up to 15 cm long, with a stalk up to 12 cm long,

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spike up to 3 cm long, up to 35 pairs of sporangia, arranged alternatively on the spike. Spores are trilete, globose-ellipsoid in nature, granulose, reticulate, the triradiate leasural arms are wavy and jointed, "Polar axis 15.55 –23.72 µm, equatorial axis (proximal face) 28.2 – 40.82 µm, mean P/E ratio 0.55 ± 0.01 µm, large spores" (Khan *et al.,* 2023).

Due to the variations in the morphological characters and the existence of many morphotypes within the same species, many synonyms were present under the name *O. reticulatum* viz., *O. ovatum* Bory., O. *cordifolium* Roxb., *O. peruvianum* C. Presl., *O. cognatum* C. Presl., *O. cumingianum* C. Presl., *O. timorense* Miq., *O. obovatum* Miq., O. *moluccanum* f. *dilatatum* Miq., *O. vulgatum* var. *minutum* F.M. Bailey., *O. reticulatum* var. *acutius* Christ.,*O. reticulatum* var. *polyangium* Christ., *O. usterianum* Christ., *O. raciborskii* Alderw., *O. pedunculatum* Desv. & Nakai., *O. ramosii* Copel., *O. austroasiaticum* Nishida., *O. reticulatum*f. *dilatatum* (Miq.) Wieff., *O. holm-nielsenii* B. Ollg., *O. aletum* M. Patel, M.N. Reddy & H.K. Goswami and *O. chaloneri* H. K. Goswami, M. Patel & K.K.Nag.

Fig.1:*Ophioglossum reticulatum*: A. Habitat, B. Entire plant.

4.Conclusion

Previously *O. reticulatum* was recorded from Kozhikode, Thiruvananthapuram, Thrissur and Wayanad Districts of Kerala. During this study *O. reticulatum* was collected and documented for the first time from the campus of Farook College, Kozhikode District, Kerala.

5. References

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Genus *Ophioglossum* fromThrissur District, Kerala

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Abstract

The Adders toungue fern *Ophioglossum* is a Eusporangiate fern belonging to family Ophioglossacea. Thrissur district of Kerala was not well explored yet for *Ophioglossum* genus. So, a taxonomic investigation for *Ophioglossum* in Thrissur district results in the collection of four *Ophioglossum* species namely*, O. costatum O. gramineum, O. lusitanicum and O. raphaelianum.*Their morphological description and photographs are given in the paper.

Keywords: *Ophioglossum*, Thrissur, Kerala

Introduction

The family Ophioglossaceae was discovered by Bauhin (1620) and Linnaeus (1753) confirmed the generic status of Ophioglossum in his "Species plantarum". The *Ophioglossum* genus is not well studied in Kerala, especially in Thrissur District due to its smaller size, distribution in the pocketed areas and its occurance only during the rainy season. The plant body is small having a small underground rhizome, tropophyll and a fertile spike. During this study we could collect four *Ophioglossum* species namely*, O. costatum O. gramineum, O. lusitanicum and O. raphaelianum*.

Methodology

Fresh *Ophioglossum* plants were collected from different localities of Thrissur district (Fig.1). Photographs from the field were taken using Canon 01 camera and Canon ixus 115 H5 camera. Morphological characters of Rhizome, Trophophore, Trophophyll, Strobilus were studied and Photographs were taken using LABOMED CCD stereomicroscope and Leica S8AP0 microscope.

Results

***Ophioglossum costatum* R.Br.** Prod. Fl. Nov. Holl. 163 (1810). Panigrahi & Dixit, Proc. Nat. Inst. Sci. India 35: 249 (1969); Frazer Jenkins Taxo. Rev. Three Hund. Ind. Pterido. 19 (2007).

Ophioglossum brevipe Bedd., Ferns. Southern India 23. t. 72 (1863).

Ophioglossum bulbosum Bedd. Ferns. Brit. India Supl. t. 28, (1876). (Fig. 2)

Plants 23- 25cm high, rhizome hemi spherical, corm like, rhizoids 1 cm-5.5 cm long, tropophyll dark green, glabrous, 6-8 cm long, 1.5-2 cm wide, lanceolate-elliptic, base cuneate, margine entire, acute or obtuse apex, fertile strobilus 8-12 cm long, 32-39 sporangia, alternate.

Disttribution: Kallampara,1°00'0.0" N, 1°00'0.0"E , Cheruchakkichola, 10.7002° N, 76.1993°E.

***Ophioglossum gramineum* Willd.** Nov. Act. Acad. Erfurt. 2: 18. t. f. 1 (1802); Beddome, Handb. Suppl. Ferns Brit. India 108. 1892; Balakrishnan *et al.*, Bull. Bot. Surv. India 2: 337 (1960)

Ophioglossum clietrichiae Prantl, Ber. Deut. Bot. Ges. 1: 352 (1883).

Ophioglossum dietrichiae Prantl, Ber. Deut. Bot. Ges. 1: 352 (1883).

Ophioglossum gregarium Christ, Nova Guinea Bot. 8: 164 (1909). (Fig. 3)

Plants green, grass like, 3-6 cm high, growing together with gramminae members; rhizome cylindrical, tuberous, two-four tropophylls, narrowed, linear, acicular, glabrous, acute at the apex, peduncle 1.2–3.5 cm long, strobilus up to 1 cm long, 9-10 sporangia per spike, alternate.

Distribution: Kallampara**,**1° 00' 0.0" N, 1° 00' 0.0" E**,** Pazhiyottumuri10.6836° N, 76.1421° E

Ophioglossum lusitanicum Linn. sp. Pl., 2: 1063, 1753; Clausen, Mem. Torry Bot. Club, 19 (2): 159, 1938; Mahable, Bull. Bot. Surv. India, 4: 71, 1962.

O. vulgatum var. *lusitanicum* (L).Hook. f (Fig. 4)

Plants 2-3 cm high, dark green; rhizome small, brown, tuberous; one- two trophophyll; trophophylls small, green, glabrous, lanceolate- elliptic, apex acute; strobilus short, 5-6 sporangia with a small pointed tip, alternate.

Distribution: Kallampara,1° 00' 0.0" N, 1° 00' 0.0" E, Vattayi, 10.6011° N, 76.2647° E, Peruvanmala, 10°37′20.86″N,76°8′6.5″E

O. raphaelianum Anto**,** Afs.Khan, F. Francis & I. Antony (Fig. 5)

Plants 2-5 cm high; rhizome subglobose, 0.2-0.6 cm long; tropophylls olivaceous-bluish green, subterranean, orbicular, minute, strobilus small with a sterile pointed apex; sporangia 6-7, opposite.

Distribution: Peruvanmala10°37′20.86″N**,** 76°8′6.5″E

Discussion

During this study we could collect four *Ophioglossum* species, *O. costatum O. gramineum, O. lusitanicum and O. raphaelianum* from different localities of Thrissur District. Among these O. *costatum* is the larger species, which is up to 25 cm high, and could be easily identified from the field by its large elliptic-lanceolate tropophylls (leaves) with central yellowish margins (costa) and corm like rhizome. Rests of the three species are smaller and they sizes up to 6 cm. Among these *O.gramineum* is distinguished by its erect needle like tropophylls, *O. lusitanicum* is distinguished by lanceolate-ovate tropophylls and *O. raphaelianum* is distinguished by its subterranean, olivaceous or bluish green coloured orbicular tropophylls.

Conclusion

Thrissur district of Kerala was not explored well for *Ophioglossum* genus. During our study we could collect four species from different localities. They are *O. costatum O. gramineum, O. lusitanicum and O. raphaelianum*

Acknowledgements

The authors are grateful to DST-FIST funded SIFC St. Thomas' College (Autonomous), Thrissur for providing lab facilities to carry out this work. We would also like to thank Mr. Fraser-Jenkins for his valuable comments.

Funding -This study was funded by Maulana Azad National Fellowship (MANF)

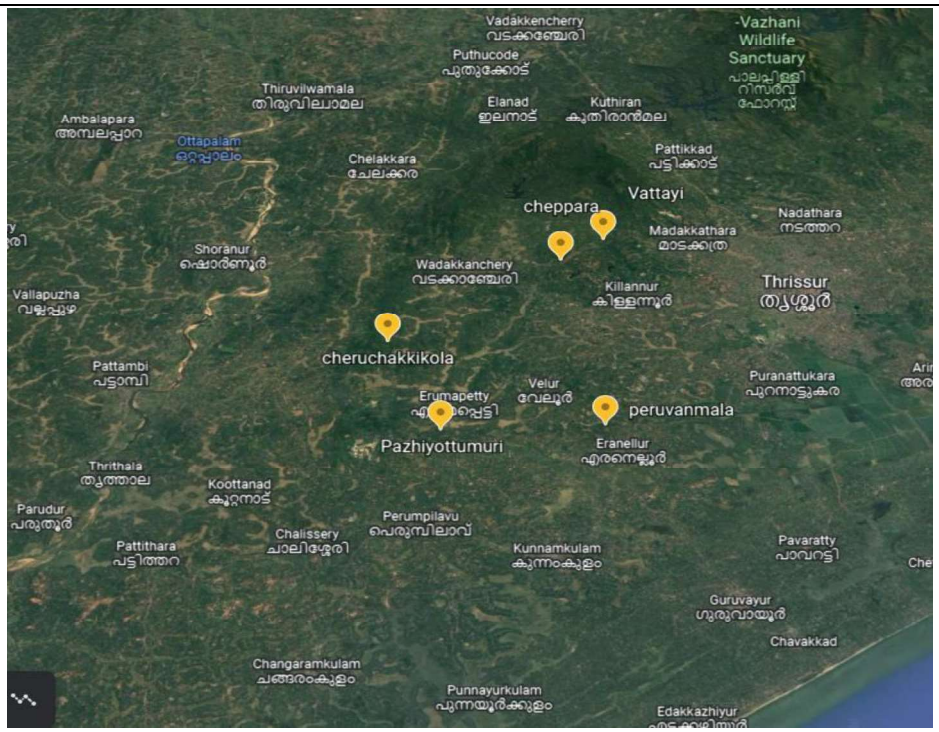

Fig.1. Geographical location of *Ophioglossum* plants in Thrissur District

Fig. 2. *O. costatum*

Fig. 3. *O. gramineum*

Fig. 4. *O. lusitanicum*

Fig. 5. *O. raphaelianum*

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A Review on the Taxonomical studies of Genus Ophioglossum

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Abstract

The family Ophioglossaceae was discovered by Bauhin (1620). The generic status of the snake toungue fern *Ophioglossum* was substantiated by Linnaeus (1753). Four species recorded by Linnaeus viz. *O. vulgatum, O. lusitanicum, O. reticulatum* and *O. Palmatum* were retained in *Ophioglossum* genus and the other two have been placed to the genus *Lygodium* by wickrama and Fonseka (1973).

The comprehensive Mongraphic study of Clausen (1938) recognized 28 species of *Ophioglossum* from the total of 52 species identified for the family. The author also recorded 23 species of *Botrychium*, One species of *Helminthostachys* and the genera *Rhizoglossum*, *Ophioderma* and *Cheiroglossa* were reduced to the rank of sub-genera under the genus *Ophioglossum*. The author think that the technical characters like venation and spore morphology are uncertain and not practical while identifying a species in *Ophioglossum* and hence he categorized *Ophioglossum* plants under four subgenera viz. *Cheiroglossa, Rhizoglossum, Ophioderma* and *Euphioglossum* based on the gross morphology and correlation with geographical range. 23 species were placed under *Euphioglossum,* three species were treated under *Ophioderma*, one species under *Cheiroglossa* and one species under *Rhizoglossum*. He also discussed and solved some nomenclatural confusions of *O. pedunculosum.*

Wieffering (1964) subsequently revised *Ophioglossum* of Indo-Pacific region and reported 14 species with some varieties and some forms. Disagreeing to Clausen's opinion he observed the cytogenetical characters as the most reliable characters for species identification since the interspecefic and

intraspecefic relationships cannot be depicted by considering the external morphology alone.

Horner (1958) discovered an unusual colony of *O. pendulum* ssp. *pendulum* from the forest reserve of the Hawaii Island, apart from the previously known *O. Pendulum* ssp. *falcatum* recorded by Clausen (1954). The former species differs from the later in five characters as follows. *O. pendulum ssp. pendulum* characterised by 105 cm long blades, 4-12cm long fertile stalk, straight blade curvature, medium texture, and the pointed blade apices, whereas the *O. Pendulum* ssp. *falcatum* characterised by 10-45 cm blades, 0.5-3.0 cm long fertile stalk, falcate leaves, leathery texture, with blunt apex.

Beddome (1883) records two species, *O. reticulatum* and *O. Pendulum* from the Ceylon and the Malay Peninsula. Subsequently he added two more species (1892), *O. gramineum* and *O. fibrosum* for Ceylon. Later, Chakravarty (1951) identified three species, *O. vulgatum*, *O. fibrosum* and *O. pendulum* L. from Ceylon. Willis and Willis (1911) repotred two species viz. *O. gramineum* and *O. pendulum* from Ceylon.

Burrows and Edwards (1993) discussed about the nomenclatural confusions of genus *Ophioglossum* in Africa. In this paper authors described *O. caroticaule* as a new species, *O. vulgatum*. subsp. *africanum* as a new subspecies, and a status chage for *O. latifolium* (basionym: *O. gomezianum var. latifolium*. Lectotypification of *O. gracillimum*, *O. lusoafricanum*, *O. latifolium*, and *O. rubellum*. were provided and he talked about the nomenclatural problems of *O. lancifolium*. A new species, *Ophioglossum gracile* was discoverd from South Africa by Burrows and Edwards (1995). According to them the species concept of *Ophiglossum* is confusing due to the simple morphology of the genus. Assessment of the occurrence and habitat of *Ophioglossum vulgatum* in European forests by Muller (2000) revealed that the *O. vulgatum* are more abundant in the undisturbed forest habitats, which are primary habitat for this species but it is also present in the disturbed wet grassland ecosystems. He

conducted his studies on several Lorraine forests. In all these forests, *O. vulgatum* was found in an oak-ash forest of the Carpinion alliance. He also pointed that, besides the more abundant young succession stages, it was present there in all stages of the forest cycle.

Taxonomical studies from India

India, being a country with a mixture of wet and dry tropical climatic conditions, it is more diverse for *Ophioglossum* and the genus is distributed throughout the country. Extensive studies were carried out in this snake toungue fern from various parts especially from the Northern sides of the country.

Balakrishnan *et al*. (1960) recorded five taxa of *Ophioglossum*, viz., *O. reticulaturn, O. polyphyllum, O. costatum, O. grarnineum* and *O. nudicaule* var. typicum from the localities coming under the southern circle of Botanical Survey of India (BSI). Morphotaxonomy, illustration, key to the species, distributional map for the five collected taxas were provided in the manuscript.

The taxonomy of ten species of *Ophioglossum* from India was discussed in detail by Mahabale (1962). The collected species were include *O. reticulatum* L., *O. Vulgatum*, *O. japonicum*, *O. peduwulosum*, *O. nudicuule*, *O. lusitanicum*, *O. fibrosum*, *O. gramineum*, *O. Aitchisoni* and *O. pendulum*.The author considered the leaf morphology, stipules, epidermal and spore characters and prepared an artificial key for based on the characters of each species. The probable phylogeny of the collected species was schematically represented. The subgenus Euophioglossum including *O. vulgatum*, *O. reticulatum* was showed as the main line of evolution and then it is divided into subgenus *Ophioderma* and subgenus *Cheiroglossa.* The former includes *O. pendulum* and *O. aitchisoni* whereas the later includes *O. palmatum* and *O. fibrosum*. The monotypic subgenus *Rhizoglossum* was placed distant in the phylogeny tree. A key and a table comparing the morphological characters were given for the easy identification of the collected species and the author discussed about the confused taxonomy of *O. reticulam*, *O. vulgatum* and *O. Aitchisoni* in this work.

Augustine *et a.l* (1969) discovered *O. Pendulum* from the Periyar tiger reserve of Kerala. Previously there were reports for *Ophioglossum* only from the Andaman and Nicobar islands (Dixit and Vohra, 1984). So this discovery from the Idukki district along the Westernghat sides of Kerala, South India was a new report for the occurance of *O. pendulum* in the mainland of India. Later Kholia and Jenkins (2014) rediscovered *O. pendulum* after 125 years from Northern India. Their collection from Sikkim was significant for India as it is pointining to the possible westward distribution of this species. Previously it was reported from the eastern sides of the country including Assam and Arunachal Pradhesh.

Khandelwal and Goswami (1984) described *O. eliminatum,* a new species collected in 1973 from Kanharjhir and at Chandpata. Morphological characters of the newly described species was given incorporating the characters of *O. costatum*, *O. nudicaule*, *O. lusitanicum*, *O. gramineum* and *O. thermal*e. The morphological description, some account of spore morphology, photographs of different species of *Ophioglossum* genus were documented by Goswami *et al*. (2008). This study coined the possibility of formation of a new species, *O. eliminatum* (with lowest chromosome number n=90 in the genus*)* by natural hybridization and chromosomal elimination. This study considered all the possible morphological, molecular and biochemical techniques for knowing the evolutionary mechanisms. Later, Goswami (2011) collected and documented the population biology data of *O. eliminatum* during 1995–2009 from more than 12 sites in Central India. This study restated that the lowest chromosome number in *Ophioglossum* genus is n = 86, not n = 30 as emphasized before, and the interspecific and the intraspecific chromosome variations are exists in many *Ophioglossum* species. Goswami and patel (2020) did an extensive study on *Ophioglossum* considering the morphology, palynology, chromosome studies and molecular characters. They coined that, preference should be given to spore characters and molecular data along with the morphological characters while differentiating species. The authors suggested that the unique morphological

traits are the results of rare genetic combinations arised due to autopolyploidy or allopolyploidy conditions. So a multidisciplinary approach including morphological, anatomical and biochemical characters is needed for identifying different *Ophioglossum* species.

Manickam and Irudayaraj (1992) reported five *Ophioglossum* species, *O. gramineum* Willd., *O. nudicaule* L., *O. vulgatum* L., *O. reticulatum* L., *O. petiolatum* Hook from South India. They provided the binomial, description, key to the species and illustrations for each species.

The Pachmarhi Biosphere Reserve (PBR) of Madhya Pradesh in India was explored for *Ophioglossum* plants by Singh *et al*. (2009). They took into account of the morphology, taxonomy and distributional pattern of *Ophioglossum* L. The diversity between and within the species over the Biosphere reserve have been analysed and documented four *Ophioglossum* species, among these *O. gramineum, O. nudicaule and O. reticulatum* were broadly distributed within the Biosphere Reserve. This study reports for the first time from PBR.

Yadav and Goswami (2010) discovered pink-brown *Ophioglossum, O. indicum* from Rajasthan state of India based on the morphological characters and Scanning Electron Microscopic (SEM) characters of the spore. They distinguished *O.indicum* from other congeners by its pinkish troppophylls and petioles, and also by its unique spore characters. Raju *et al*. (2011) looked over the recognition and existence of *O. costatum* R. Br., in Andhra Pradesh.

Anto *et al*. (2016) described a new bluish green colour *Ophioglossum*, *O. raphaelianum* from Kerala, of South India. The authors distinguished the new plant from other plants with its subglobose rhizome, orbicular, obtuse, bluish green and appressed tropophyll, sheathing trophophore, virugose, heterobrochate spore.

Patil and Dongare (2014) recorded *O. parvifolium* and *O. polyphyllum* new to the Deccan Peninsula of India. The authors collected eight species from

Maharashtra and the paper discussed about the taxonomy, phenology, and distribution and conservation status of the collected species. Patil and Lavate (2014) investigated the genus *Ophioglossum* in Westernghats and reported eight species, viz. *O. gramineum, O. parvifolium, O. reticulatum, O. lusitanicum, O. petiolatum, O. polyphyllum, O. costatum* and *O. nudicaule*. Taxonomic characters, photographs, distribution, and IUCN conservation status for each species were provided along with a modified artificial key of Panigrahi and Dixit (1969) were also provided for easy identification. Amongst the eight species reported, the authors assigned the conservation status of *O. gramineum, O. parvifolium* and *O. reticulatum* as least concerned (LC), *O. lusitanicum, O. petiolatum* and *O. polyphyllum* as critically endangered (CE), *O. costatum* and *O. nudicaule* as vulnerable (V). During the evaluation of diversity and conservation status of pteridophytes in Gujarat, India, Patel and Reddy (2018) reported *O. lusitanicum, O. thermale* as new records from Gujarat state. Morphology, taxonomy and the ecological aspects of each species were documented. Patel *et al*. (2018) reported a new species, O. aletum from the Southern Gujarat. They distinguished the new species by possessing almost 90% alete spores.

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