

بسم الله الرحمن الرحيم

 $\infty\infty\infty$

تم رفع هذه الرسالة بواسطة / مني مغربي أحمد

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

AIN SHAMS UNIVERSITY

1992

1992

ملاحظات: لا يوجد





Botany Department

Mosses as Bioindicators of Spatial and Temporal Environmental Variations

A Thesis submitted for the degree of Doctor of Philosophy in Science in Botany

By
Mohamed Farag Abu Elhamd Ali

To
Department: Botany
Faculty of Science -Ain Shams University

Supervised by Dr. Wagieh El-Sayed El-Saadawi

Emeritus Professor of Botany Faculty of Science Ain Shams University

Dr. Hanaa Mostafa Shabbara

Dr. Mahmoud Magdy Abdallah

Emeritus Professor of Bryology Faculty of Science Ain Shams University Associate Professor of Genetics Faculty of Agriculture Ain Shams University

Dr. Olaf Franziskus Werner

Researcher of Molecular Systematics Faculty of Biology Murcia University-Spain

(2022)





Ph.D. Degree Supervisor's Signatures

Mosses as Bioindicators of Spatial and Temporal Environmental Variations

A Thesis submitted for the degree of Doctor of Philosophy in Science in Botany

By

Mohamed Farag Abu Elhamd Ali

To

Department: Botany Faculty of Science -Ain Shams University

Supervised by Dr. Wagieh El-Sayed El-Saadawi

Emeritus Professor of Botany Faculty of Science Ain Shams University

Dr. Hanaa Mostafa Shabbara

Dr. Mahmoud Magdy Abdallah

Emeritus Professor of Bryology Faculty of Science Ain Shams University Associate Professor of Genetics Faculty of Agriculture Ain Shams University

Dr. Olaf Franziskus Werner

Researcher of Molecular Systematics
Faculty of Biology
Murcia University-Spain

(2022)





Validity of Ph.D. of Science Thesis in Botany

- Student Name: Mohamed Farag Abu Elhamd Ali
- **Title of Thesis:** Mosses as Bioindicators of Spatial and Temporal **Environmental Variations**
- **Degree:** Ph.D. of Science (Botany)
- **Supervisory Authority**
- 1. Prof. Dr. Wagieh El-Sayed El-Saadawi
- 2. Prof. Dr. Hanaa Mostafa Shabbara
- 3. Dr. Mahmoud Magdy Abdallah
- 4. Dr. Olaf Franziskus Werner

•	Members of the Judging Committee
1.	
2.	
3.	
4.	
Da	ta for the administration of postgraduate studies

• Date of defense of the thesis:	/	/	
 Department Council approval date: 		/	/
• Date of approval of the Faculty Board:		/	/
 Date of approval of the University Council 	l:	/	/

Employee Signature

Director of Studies Department Signature

Faculty Secretary

Declaration

This thesis has not been previously submitted for any degree at this or any other university. The references in the text will show specifically the extent to which I have availed myself of the work of other authors.

This work was a result of co-operation between three laboratories:

- A. Archegoniatae Lab- Botany Department- Faculty of Science-Ain Shams University-Egypt.
- B. Molecular Genetics Lab- Genetics Department- Faculty of Agriculture- Ain Shams University-Egypt.
- C. Bryoflora, Biogeographic and Molecular Systematics Lab-Botany Department-Faculty of Biology- Murcia University-Spain.

This work was funded by culture affairs and scholar sector- High Education and Scientific Research Ministry as joint-supervision scholarship (2018-2020) between Ain Shams University and Murcia University.

Mohamed Farag

Acknowledgements

First of all, the ultimate praise gratitude and thanks be to Allah for granting me the ability to accomplish this work.

I would like to express my deep thanks and gratitude to

Prof.Dr. Wagieh El-Saadawi for supervising this work, effort to facilitate its production and critical revision of the manuscript.

I am deeply grateful to Prof.Dr. Hanaa Shabbara, for supervising this work, valuable suggestions, encouragement during this work, her kind help and patience.

All the words of thanks to Dr. Mahmoud Magdy. He is an advisor, supervisor, friend, and brother. All credit goes to him to put my foot on this track of molecular analysis, and he has taught me all that I have now.

My appreciation and thanks are to Dr. Olaf Werner, for his guidance and supervision of the practical work in his laboratory for two years. His advice was amazingly effective in solving technical problems during the analysis of data

It was an honour for me to deal with Prof. Dr. Rosa Maria Ros, who is considered for me as the fifth supervisor on this thesis. I have learnt a lot from her experience. I am very appreciative of her huge effort with me in all paper works when I was under her supervision in Spain.

"I will always be in debt for their great help and support"

Thanks, and gratitude are to ex-Heads of Botany Department, Faculty of Science, Ain Shams University and Prof. Dr. Amal Ahmed Morsi - Head of Botany Department- for their support and continuous help during the period of my Ph.D.

Special thanks are offered to my colleagues Dr. Manal Ibrahim, Dr. Sahar Ibrahim and Dr. Mai Taha, Mr. Ahmed Sherif in the faculty of Science, Ms. Hageer Hafny and Mr. Mostafa Nafe3 in the lab of genetics and Dr. Elena de la Cruz-Martínez in the lab at Murcia University. They shared with me the stages of this work, and always provided me with their useful assistance and continuous guidance throughout this work.

I wish to express my gratitude to the following people who generously contributed relevant literature and for their open discussions and help in this work: Dr. Abdulla Samy and Miss. Aliaa Mohamed (Faculty of Science, Ain Shams University) for their help in the revising of the part of species distribution modelling. I am very grateful to Dr. Yasmin Ibrahim (Lecturer-Faculty of Science- Suez Canal University) and Ms. Haidy Issac (Teaching assistant- Faculty of Education- Ain Shams University) for providing me with samples of Funaria hygrometrica from Ismailia and Beny Suef, respectively. Thanks continued to Eng. Ahmed Khalid (GIS solving problem engineer-ESRI) for teaching me the basics of GIS and maps editing. Thanks to all members of the Botany Department for their support, help and encouragement.

All my respectful gratitude is to my big family; my mother, father, brother, and sisters for their support through my life steps.

There are no words to express my thanks for my small family my lovely wife Randa and my daughters Habiba and Hanin and I apologize for leaving them two years during my scholarship.



List of Contents

List of Tables	i-v
List of Figures	vi-x
List of Abbreviations	xi-xiv
Abstract	1-2
Preface	3-7
Aim of the Work	8
Chapter 1: Environmental Diversity and the Distribution Modelling of <i>Funaria hygrometrica</i> Hedwig.	9-38
Chapter 2: Molecular Variation of Nuclear Ribosomal DNA Repeats of <i>Funaria hygrometrica</i> Hedwig	39-59
Chapter 3: Extranuclear Inheritance of Chloroplast and Mitochondrial Genomes Reveal the Complexity of the Moss <i>Funaria hygrometrica</i> Hedwig	60-90
Chapter 4: Whole Genome Resequencing of <i>Funaria hygrometrica</i> Hedwig, Uncovering Molecular Pathways of Ecological Adaptation	91-137
General Discussion	138-145
Conclusion and Recommendations	146
Summary	147-152
References	153-175
الملخص	3-7
المستخلص	1-2

List of Tables

Chapter 1: Environmental Diversity and the Distribution Modelling of <i>Funaria hygrometrica</i> Hedwig	9 - 38
Table 1. Data (Herbarium number, location, habitat, date of collection, Longitude, latitude and phytogeographic territory (Ph.T.)) of the 25 relatively recently collected <i>Funaria hygrometrica</i> samples	13-14
Table 2. Data of old <i>Funaria hygrometrica</i> samples kept at CAIA herbarium	15
Table 3. Descriptive analysis of 19 Bioclimatic data (BC1-19) of Global and Egyptian points. Minimum (Min.), maximum (Max.), mean, and standard deviation of means (SD) are given	21
Table 4. The five class intervals of <i>Funaria hygrometrica</i> ` occurrences, based on each bioclimatic variable (BC 1-19). Intervals arranged according to the number of occurrences per interval from high to low as 1 is the highest and 5 is the lowest, and the range of each interval are all shown	22
Table 5. Correlation matrix (Spearman (n)) of bioclimatic variables of <i>Funaria hygrometrica</i> ` samples, recently collected from Egypt.	27
Table 6. Analysis of bioclimatic variables (BC1-19) contributions in the two distribution modelling (SDM) of <i>Funaria hygrometrica</i> at the global and Egyptian levels. The data of BCs are the contribution percentage / permutation importance.	31
Chapter 2: Molecular Variation of Nuclear Ribosomal DNA Repeats of <i>Funaria hygrometrica</i> Hedwig	39-59

Table 1. Data (Herbarium number, date of collection, GPS data as well as temperature and precipitation) of <i>Funaria hygrometrica</i> sampling locations.	43
Table 2. Data of accessions at NCBI database, used for building phylogenetic trees.	47-48
Table 3. The summary of raw NGS data of the 17 samples of <i>Funaria hygrometrica</i> (F1-F17).	49
Table 4. Variations of nuclear ribosomal repeats (nrDNA) of the 17 samples of <i>Funaria hygrometrica</i> (F1-F17). The length of <i>de Novo</i> assembles and mapped nrDNA consensus are shown and the number of ambiguity (A) is mentioned. In addition, the length of internal transcript spacers 1 and 2 (ITS1 & ITS2) and intergenic spacers 1 and 2 (IGS 1 & IGS 2), and copies number are given.	51
Chapter 3: Extranuclear Inheritance of Chloroplast and Mitochondrial Genomes Reveal the Complexity of the Moss <i>Funaria hygrometrica</i> Hedwig	60-90
Table 1. Summary of data of chloroplast genome (cp-g) and mitogenome (mt-g) of 17 samples of <i>Funaria hygrometrica</i> (F1-F17). The number of mapped reads and their percentages (MR (%)) and produced cp-g and mt-g size are given. The length of large single-copy, inverted repeat, and small single-copy (LSC/IR/SSC) of cp-g are presented.	71
Table 2. SNP Data of twenty genes of chloroplast with the highest SNPs between the samples of <i>Funaria hygrometrica</i> .	75
Table 3. Summary of 162 SNP at 36 genes mitogenome coding regions, which had variants between <i>Funaria hygrometrica</i> and <i>Physcomitrium patens</i> .	76

Table 4. Data of DNA polymorphism between the 17 samples of <i>Funaria hygrometrica</i> and at the level of the three lenght groups of chloroplast genomes (C-GLn) and mitogenome (M-GLn).	77
Table 5. Simple sequence repeats (SSR) of chloroplast genome (cp-g) of 17 samples of <i>Funaria hygrometrica</i> (F1-F17). Size of the tripartite structure of cp-g and SSR length in each, number of SSR and corresponding types are given. As well as the number of six categories of SSR i.e., monomeric, dimeric, trimeric repeats (MoR, DiR and TriR) and their three compound forms (C) are presented.	84
Table 6. Types of SSR of three length groups of chloroplast genome (cp-g). Types and the number differences of SSR types among groups are presented.	85
Table 7. Description of simple sequence repeats (SSR) of mitogenome of <i>Funaria hygrometrica</i> . The length of SSR per each sample, number of SSR, monomeric repeats (MoR), microsatellite repeats (MSt), and mixed repeat-regions (MoR/Dimeric repeats (Di-R), DiM/MoR) are all given.	86
Chapter 4: Whole Genome Resequencing of <i>Funaria</i> hygrometrica Hedwig, Uncovering Molecular Pathways of Ecological Adaptation	
Table 1. Description of obtained single nucleotide polymorphism (SNP) data.	103
Table 2. Data of SNP-G1, which resulted from comparing <i>Funaria hygrometrica</i> with 27 chromosomes (Ch1-Ch27) of <i>Physcomitrium patens</i> as a reference. Total number of SNPs, SNP transition (Ts), SNP transversion (Tv), SNPs at noncoding and coding regions, number of genes, synonymous	105

SNPs (S), and non-synonymous(N) SNPs per chromosome are all given.

- **Table 3.** Summary of SNP-G3 data, which resulted from 108 comparing 17 samples of *Funaria hygrometrica* at coding regions, using 27 chromosomes (Ch1-Ch27) of *Physcomitrium patens* as a reference. Show total number of SNPs, SNPs according to types (Ts: SNP transition, Tv: SNP transversion); SNPs according to protein effect (S: synonymous, N: non-synonymous), types of SNPs in regard to protein effect and number of affected genes.
- **Table 4.** List of 20 genes, which had the highest number of SNP. Chromosome of gene, gene name and length, number of SNPs, SNP transition (Ts), SNP transversion (Tv), synonymous SNPs (S), non-synonymous SNP (N), % of number of SNP per gene length (GL) and per total number of SNP (T.SNP) are all given.
- **Table 5.** List of 20 genes, which had the highest percentage of number of SNP per gene. Chromosome of gene, gene name and length (GL), number of SNPs, SNP transition (Ts), SNP transversion (Tv), synonymous SNPs (S), non-synonymous SNP (N), length and per total number of SNP (T.SNP) are all presented.
- **Table 6.** Data of SNP were presented in both genetic groups (GGA, GGB). The total number of SNP and their number per group are all given as well as the types of SNP either transition (Ts) or transversion (Tv), number of genes, and the SNP effect either synonymous (S) or non-synonymous (N).
- **Table 7.** The four groups of the genes, which are based on 116 SNPs case in the genetic groups. The number of genes per each case probability is given.
- **Table 8.** Descriptive analysis of bioclimatic data (BC) of 17 124 samples of *Funaria hygrometrica*. Minimum (min.),

maximum (max.), mean and standard deviation of means (SD) are presented.

Table 9. The Partial Mantel test results between the sets of samples against climatic and bioclimatic variables. Bold typed figures represent significant data.

List of Figures

Chapter 1: Environmental Diversity and the Distribution Modelling of <i>Funaria hygrometrica</i> Hedwig	9-38
Fig. 1. Histogram of the major interval of Funaria hygrometrica occurrences based on each bioclimatic variable (BC 1-19) and the range of the interval being shown.	20
Fig. 2. Dendrogram of 25 classes of <i>Funaria hygrometrica</i> based on k-means and agglomerative hierarchical clustering of bioclimatic data of points, showing their clustering in 4 groups.	23
Fig. 3. A. World map of Köppen climate classification for 1901-2010. B. World map showing the distribution of four global ecological groups of <i>Funaria hygrometrica</i> based on bioclimatic data with a zoom into part of North America at left and Mediterranean basin at right.	24
Fig. 4. The biplot PCA of the four centroids of the four ecological groups regarding bioclimatic variables.	26
Fig. 5. The agglomerative hierarchical clustering for the samples of <i>Funaria hygrometrica</i> in five phytogeographic territories based on six bioclimatic variables (BC1, BC3, BC6, BC8, BC9 and BC11).	28
Fig. 6. The map of the second global species distribution modelling of <i>Funaria</i> . <i>hygrometrica</i> .	32
Fig. 7. The map of the SDM of <i>Funaria hygrometrica</i> in Egypt. A & B: The extracted maps from the two global SDM, respectively. C & D: the two regional SDM based on the relatively recently collected samples.	33
Chapter 2: Molecular Variation of Nuclear Ribosomal DNA Repeats of <i>Funaria hygrometrica</i> Hedwig	39-59
Fig. 1 . a. spin column DNA extraction method based on manual of E.Z.N.A.®SP Plant DNA kit; b. 1% gel electrophoresis of the	45

hygrometrica.

seventeen samples of <i>Funaria hygrometrica</i> (F1-F17). c. The major steps for obtaining raw data. d. The graph annotation of nuclear ribosomal DNA repeat.	
Fig. 2 . Phylogenetic analysis based on the concatenated sequence of four spacers (ITS1, ITS2, IGS1 and IGS2) of 17 samples of <i>Funaria hygrometrica</i> (F1-F17) with three accessions from the NCBI database.	54
Fig. 3. Phylogenetic analysis based on the concatenated sequence of internal transcript spacers (ITS1 and ITS2) of 17 samples of <i>Funaria hygrometrica</i> (F1-F17) with 14 accessions from the NCBI database.	56
Fig. 4. Phylogenetic analysis based on internal transcript spacer 1 (ITS1) of 17 samples of <i>Funaria hygrometrica</i> (F1-F17) with 35 accessions from the NCBI database.	57
Fig. 5. Phylogenetic analysis based on internal transcript spacer 2 (ITS2) of 17 samples of <i>Funaria hygrometrica</i> (F1-F17) with 21 accessions from the NCBI database.	58
Chapter 3: Extranuclear Inheritance of Chloroplast and Mitochondrial Genomes Reveal the Complexity of the Moss <i>Funaria hygrometrica</i> Hedwig	60-90
Fig. 1 . I.A, II.A. Map of the chloroplast and mitochondrial genomes of the <i>Funaria hygrometrica</i> , respectively. Gene colour is based on their belonging functional groups. The ratio of GC, AT content of the genomes are illustrated internally as dark and light grey circles, respectively. I.B, II.B. Histogram of different lengths of cp-g and mt-g mentions the three groups of the 17 samples of both genomes (Orange= GL1, Yellow= GL2; Green- GL3).	72
Fig. 2. Clustered bar of SNPs cases at tripartite parts i.e., large single-copy, inverted repeat, and small single-copy (LSC/IR/SSC) between the chloroplast genomes of 17 samples of <i>Funaria</i>	74