



# **Molecular Phylogeny of Some Moss Taxa and Its Implications for Identification**

**Thesis**

**Submitted In Fulfillment of the Requirements for the  
Degree of Doctor of Philosophy of Science in Botany**

**(Bryology)**

**By**

*Sahar Ibrahim Ali El-Sakaty*

**M.Sc. in Botany (Bryology)**

**(2009)**

**Ain Shams University  
Faculty of Science  
Botany Department  
2014**



# **Molecular Phylogeny of Some Moss Taxa and Its Implications for Identification**

A thesis

Submitted to the Faculty of Science - Ain shams University

In Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy of Science in Botany (Bryology)

by

***Sahar Ibrahim Ali El-Sakaty***

MSc 2009

***Supervised by***

**Prof. Dr. Wagieh El-Sayed El-Saadawi**

Botany Department

Faculty of Science

Ain Shams University

**Prof. Dr. Magdi Mahmoud Mohamed**

Biochemistry Department

Faculty of Science

Ain Shams University

**Dr. Usama Yahia Abou-Salama**

Assistant Prof. in Botany Department

Faculty of Science

Ain Shams University

**2014**



**Student Name:** Sahar Ibrahim Ali El-Sakaty

**Thesis Title:** Molecular Phylogeny of Some Moss Taxa and Its Implications for Identification.

**Degree:** Doctor of Philosophy of Science in Botany (Bryology)

**This thesis has been supervised by:**

**Prof. Dr. *Wagieh El-Sayed El-Saadawi***

Ex-head of Botany Department  
Faculty of Science,  
Ain Shams University

**Prof. Dr. *Magdi Mahmoud Mohamed***

Prof. of Biochemistry,  
Faculty of Science,  
Ain Shams University

**Dr. *Usama Yahia Abou-Salama***

Assistant Prof. of Bryology  
Faculty of Science,  
Ain Shams University

## Approval Sheet

*Title of Thesis:*

Molecular Phylogeny of Some Moss Taxa and Its Implications for Identification

*Name of Student:*

Sahar Ibrahim Ali El-Sakaty

*Degree:* Doctor of Philosophy of Science in Botany (Bryology)

**This thesis has been approved by:**

**Dr. Mohamed Abd-El-Salam Rashed**

Professor of Molecular genetics

Molecular genetics Department, Faculty of Agriculture

Ain Shams University

**Dr. Nahed El-Hoseiny**

Professor of Taxonomy of flowering plants

Faculty of Science

Cairo University

**Dr. Wegieh El-Sayed Al-Saadawi**

Professor of Botany and ex-head of Botany Department

Faculty of science

Ain Shams University

**Dr. Magdi Mahmoud Mohamed**

Professor of Biochemistry

Faculty of science

Ain Shams University

**Prof. Dr. Maher Mohamed Shehata**

Head of Botany Department

Faculty of Science

Ain Shams University



## 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.



# Acknowledgements

*First of all, thanks to **Allah** who gave 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, and effort to facilitate its production.*

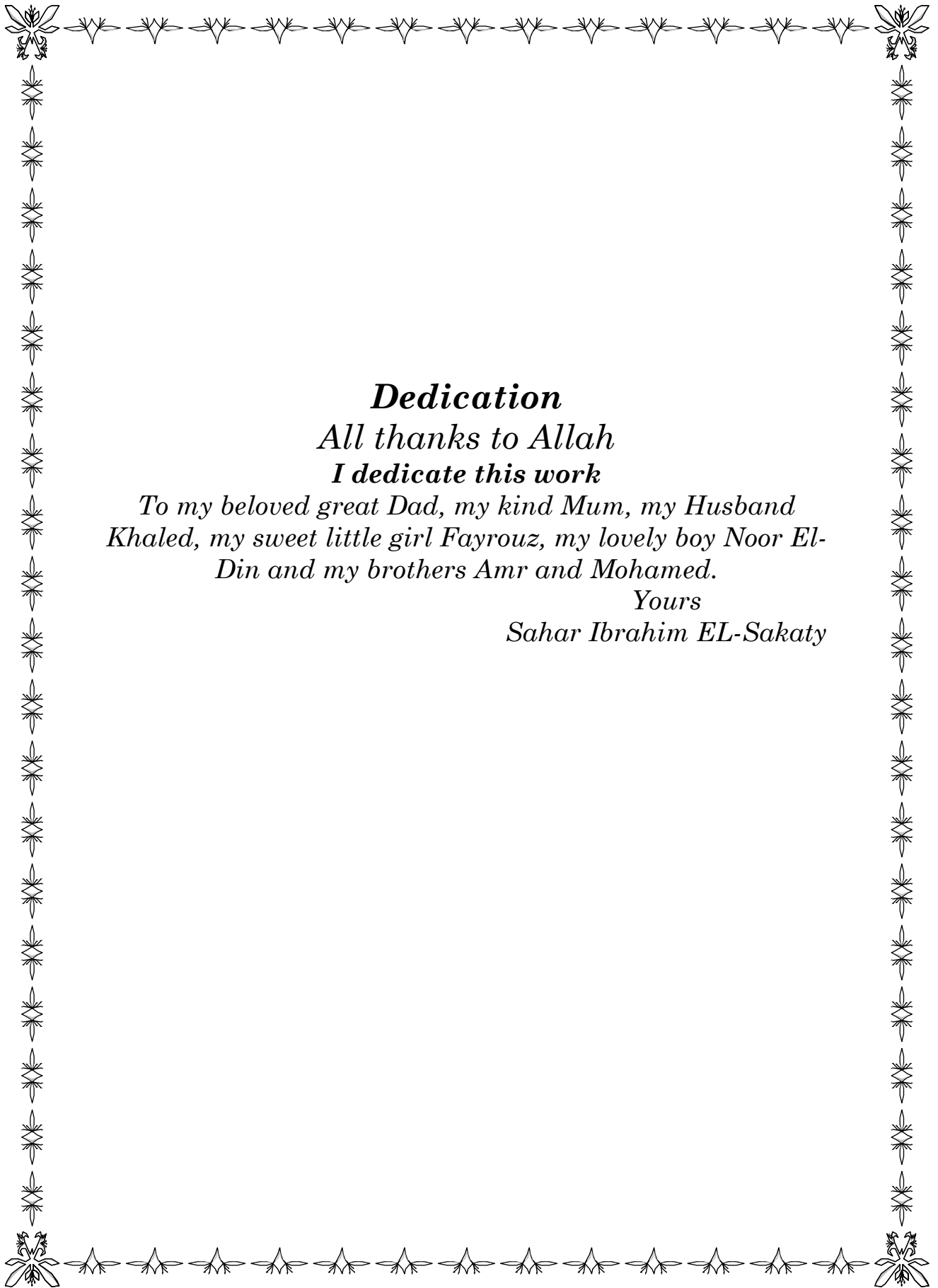
*I am deeply grateful to **Prof. Dr. Magdi Mahmoud and Dr. Usama Abou-Salama** who devoted their time, effort, and experience to facilitate the production of this work.*

*My appreciation is for **Dr. Hala El-Atroush**, for standing beside me, continuous guiding, and encouraging me.*

*My deepest thanks and appreciation to **Dr. Mahmoud Magdy El-Mosallami** who devoted his effort, time and experience to help me in this work.*

*Thanks are also to **Prof. Dr. Mohamed Tantawy**, Prof. of Taxonomy and Head of Botany Department, Faculty of Science, Ain Shams University, gratitude to **Prof. Dr. Amira A. Hassanein**, **Prof. Dr. Aza Elshafaii** and **Prof. Dr. Raiefa A. Hassanein** ex-head of the Botany Department for their support and continuous help.*

*Thanks to all members of Bryology unit (Dr. Hanaa, Dr. Mahmoud, Manal, Mai and Mohamed). All members of Botany Department for their support and help.*



***Dedication***

***All thanks to Allah***

***I dedicate this work***

*To my beloved great Dad, my kind Mum, my Husband  
Khaled, my sweet little girl Fayrouz, my lovely boy Noor El-  
Din and my brothers Amr and Mohamed.*

*Yours*

*Sahar Ibrahim EL-Sakaty*

بسم الله الرحمن الرحيم

﴿وما أوتيتم من العلم إلا قليلا﴾

صدق الله العظيم

/



# Contents

Subject	Page
➤ List of figures	I
➤ List of tables	III
➤ List of abbreviations	IV
➤ Abstract	V
➤ I. Introduction	1
➤ Aim of work	2
➤ II. Review	3
➤ III. Material and Methods	25
• Material	25
• Methods	
▪ Morphological study	25
▪ Molecular study	29
▪ Data Analysis	37
➤ IV. Results	45
• Morphological reinvestigation	45
• DNA extraction	51
• PCR reactions	51
• Data analysis	58
➤ V. Discussion	76
➤ VI. Summary	93
➤ VII. References	95
➤ VIII. Glossary	121
➤ IX. Appendix	123
➤ X. Arabic summary	٢
➤ XI. Arabic Abstract	١

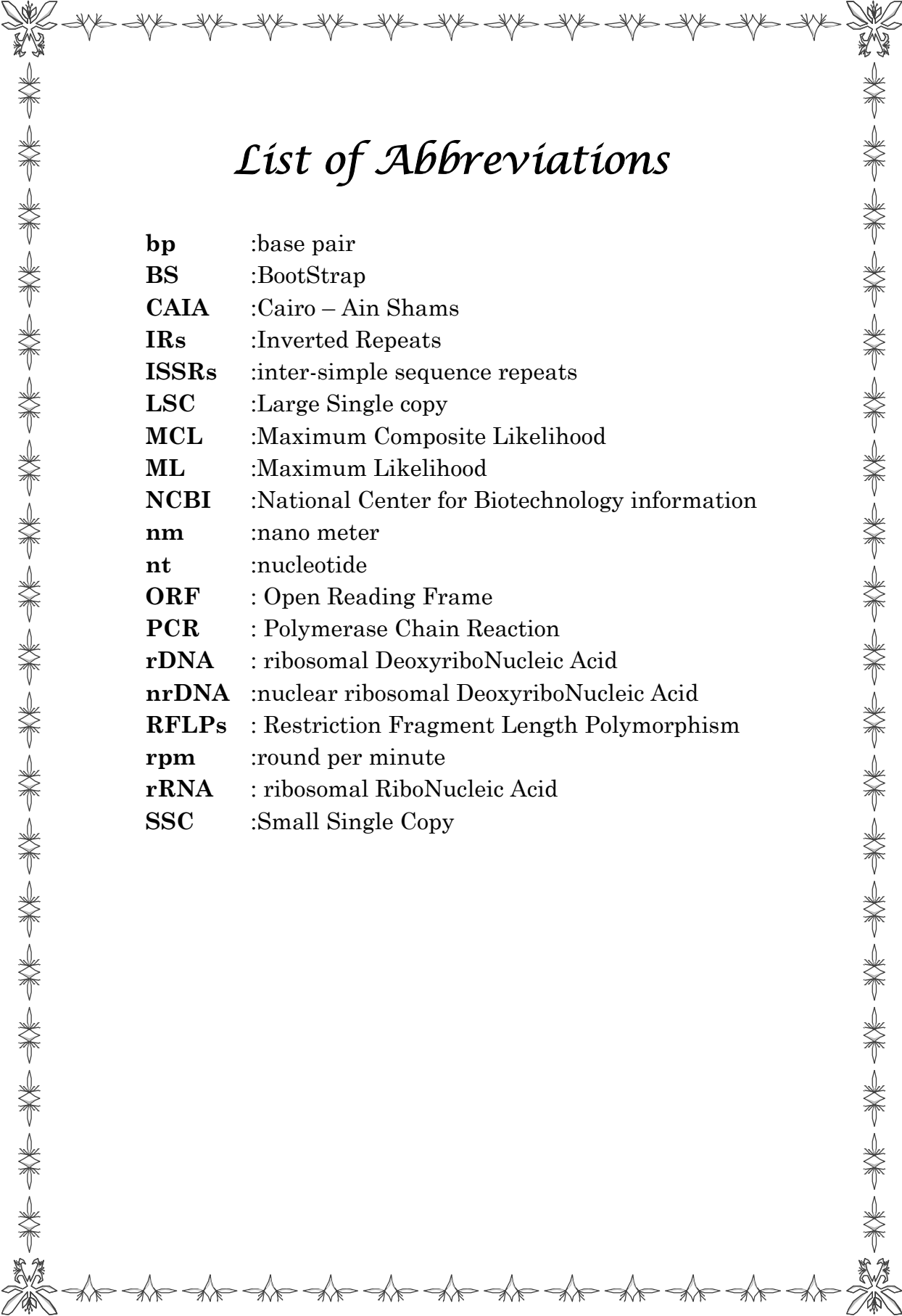
## *List of figures*

Figure no.	Figure caption	Page no.
1	Overview of the cpDNA indicating the positions of coding and non-coding regions which have so far been used in bryophyte phylogenetics.	18
2	Schematic representation of the universal structure of the rDNA region in plants.	23
3	Bootstrap analysis described in three steps.	43
4	Agarose gel electrophoresis using <i>rbcL</i> primers at 55°C annealing temp. and 60°C for ITS primers.	51
5	Agarose gel electrophoresis showing result of some samples of <i>rbcL</i> PCR reaction.	52
6	Agarose gel electrophoresis for some purified PCR products of <i>rbcL</i> region.	52
7	Agarose gel electrophoresis of ITS region using universal primers.	53
8	Agarose gel electrophoresis for ITS PCR reaction using primers designed for bryophytes.	54
9	Agarose gel electrophoresis for both ITS1 and ITS2.	54
10	Circular tree of all tested samples by Maximum likelihood method based on sequences of <i>rbcL</i> region.	60
11	The Strongly supported clade (A) of Bryaceae (BS=100).	61
12	The Moderately supported clade (B) of Pottiaceae (BS=80).	62
13	Unrooted phylogenetic tree of Bryaceae taxa by ML method.	65
14	Unrooted phylogenetic tree by ML method of <i>Barbula</i> taxa.	67
15	Unrooted phylogenetic tree by ML method of <i>Didymodon</i> taxa.	68
16	Unrooted phylogenetic tree by ML method of <i>Tortella</i> , <i>Trichostomum</i> and <i>Weissia</i> .	71

<b>17</b>	Part of chromatogram chart showing very good ( <i>rbcL</i> sequence	78
<b>18</b>	Part of chromatogram chart showing bad <i>rbcL</i> sequence	79
<b>19</b>	Part of taxon 15 ITS chromatogram, showing the noisy background giving unreliable sequence.	81
<b>20</b>	Different leaf shapes of taxon 5	84
<b>21</b>	Different leaf shapes of taxon 8	84
<b>22</b>	Leaf shapes of taxon 6	84
<b>23</b>	Leaf shape of taxon 7	84
<b>24</b>	Different leaf shapes of <i>Didymodon</i> tested taxa.	86
<b>25</b>	Different leaf shapes of taxa 16, 17, 18, 19, 20 and 22.	87

## *List of Tables*

<b>Table no.</b>	<b>Table caption</b>	<b>Page no.</b>
<b>1</b>	Herbarium data of samples used in the current study.	<b>26</b>
<b>2</b>	The primers used in DNA amplification and sequencing.	<b>31</b>
<b>3</b>	PCR reaction conditions.	<b>32</b>
<b>4</b>	Cycle program of PCR reaction.	<b>32</b>
<b>5</b>	GenBank data used in the Phylogenetic analysis with accession numbers of different regions.	<b>37</b>
<b>6</b>	The blast result for each locus of tested taxa.	<b>56</b>
<b>7</b>	The GenBank accession numbers of submitted regions.	<b>57</b>
<b>8</b>	Codes used for tested samples in the phylogenetic analysis.	<b>58</b>
<b>9</b>	Identification of different taxa by different methods.	<b>74</b>



## *List of Abbreviations*

<b>bp</b>	:base pair
<b>BS</b>	:BootStrap
<b>CAIA</b>	:Cairo – Ain Shams
<b>IRs</b>	:Inverted Repeats
<b>ISSRs</b>	:inter-simple sequence repeats
<b>LSC</b>	:Large Single copy
<b>MCL</b>	:Maximum Composite Likelihood
<b>ML</b>	:Maximum Likelihood
<b>NCBI</b>	:National Center for Biotechnology information
<b>nm</b>	:nano meter
<b>nt</b>	:nucleotide
<b>ORF</b>	: Open Reading Frame
<b>PCR</b>	: Polymerase Chain Reaction
<b>rDNA</b>	: ribosomal DeoxyriboNucleic Acid
<b>nrDNA</b>	:nuclear ribosomal DeoxyriboNucleic Acid
<b>RFLPs</b>	: Restriction Fragment Length Polymorphism
<b>rpm</b>	:round per minute
<b>rRNA</b>	: ribosomal RiboNucleic Acid
<b>SSC</b>	:Small Single Copy



# ABSTRACT



# Abstract

In this study, Forty six herbarium samples and one freshly collected sample were selected for morphological reinvestigation to confirm their identification. DNA was extracted and purified for PCR reaction; 17 samples were positive for both *rbcL* and ITS regions in addition to eight samples were positive for *rbcL* region only. DNA sequencing analysis shows that 20 samples gave high quality for *rbcL* region, 13 for partial ITS and three for ITS1 regions with high quality sequences which were used in the Phylogenetic analysis. All 20 sequences of *rbcL* and three out of 16 ITS sequences were submitted to GenBank as a new record for mosses and the others 13 sequences of ITS regions were already identified on GenBank. Phylogenetic analysis was performed using these two loci by Maximum Likelihood method using the obtained model of evolution. Two samples were omitted from final phylogenetic analysis because of conflicting data. The identification of *Tortella nitida* and *Didymodon vinealis* was proved by Phylogenetic analysis. The identification of 14 mosses was confirmed on the generic level but disapproved and therefore corrected specific level. Names of five samples were changed on both generic and specific level. Phylogenetic analysis revealed that our concept of morphological variation inside the same species must be wider.