วงศ์วานวิวัฒนาการและสารทุติยภูมิของราที่ก่อให้เกิดไลเคนวงศ์ทริพิทิเลียซิอีในประเทศไทย



บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR) เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR) are the thesis authors' files submitted through the University Graduate School.

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต สาขาวิชาเทคโนโลยีชีวภาพ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2558 ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

## PHYLOGENY AND SECONDARY METABOLITES OF LICHEN-FORMING FUNGI IN

TRYPETHELIACEAE IN THAILAND

Mr. Theerapat Luangsuphabool



จุฬาลงกรณมหาวทยาลย Chulalongkorn University

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy Program in Biotechnology Faculty of Science Chulalongkorn University Academic Year 2015 Copyright of Chulalongkorn University

Thesis Title	PHYLOGENY AND SECONDARY METABOLI		ΓES			
	OF	LICH	IEN-F	ORMING	FUNGI	IN
	TRYPETHELIACEAE IN THAILAND					
Ву	Mr. Thee	erapat	Luan	gsuphabool		
Field of Study	Biotechn	nology				
Thesis Advisor	Assistant Professor Jittra Piapukiew, Ph.D.					
Thesis Co-Advisor	Assistant	it Profe	essor l	Ek Sangvichie	n, Ph.D.	

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

\_\_\_\_\_Dean of the Faculty of Science

(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

Chairman

(Assistant Professor Tosak Seelanan, Ph.D.) \_\_\_\_\_\_Thesis Advisor

(Assistant Professor Jittra Piapukiew, Ph.D.)

\_\_\_\_\_Thesis Co-Advisor

(Assistant Professor Ek Sangvichien, Ph.D.)

Examiner

(Assistant Professor Sanit Piyapattanakorn, Ph.D.)

Examiner

(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.....External Examiner

(Associate Professor Kansri Boonpragob, Ph.D.)

้ ธีรภัทร เหลืองศุภบูลย์ : วงศ์วานวิวัฒนาการและสารทุติยภูมิของราที่ก่อให้เกิดไลเคนวงศ์ทริพิทิเลียซิอีใน ประเทศไทย (PHYLOGENY AND SECONDARY METABOLITES OF LICHEN-FORMING FUNGI IN TRYPETHELIACEAE IN THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. จิตรตรา เพียภูเขียว, อ.ที่ ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. เอก แสงวิเซียร, 270 หน้า.

้ไลเคนวงศ์ทริพิทิเลียซิอีเป็นไลเคนชนิดครัสโตสพบได้ทั่วไปในเขตร้อน จัดอยู่ในอันดับ Trypetheliales (Dothideomycetes) จากการเก็บตัวอย่างไลเคนจากสถานที่ต่างๆ ในประเทศไทย จำนวน 28 แหล่ง ใน 24 จังหวัด 965 ้ตัวอย่าง พบไลเคนวงศ์นี้ได้ทุกระบบนิเวศ จากการแยกราที่ก่อให้เกิดไลเคนด้วยวิธีการปลดปล่อยแอสโคสปอร์สามารถ แยกได้ทั้งหมด 313 ไอโซเลต การใช้ลักษณะทางสัณฐานวิทยาในการจัดจำแนก พบไลเคนวงศ์นี้ในประเทศไทยจำนวน 8 สกุล ได้แก่ Astrothelium, Bathelium, Campylothelium, Laurera, Marcelaria, Polymeridium, Pseudopyrenula และ Trypethelium จากการวิเคราะห์วงศ์วานวิวัฒนาการจากลำดับนิวคลีโอไทด์ของแต่ละสกุล ได้แก่ Astrothelium, Laurera, Marcelaria และ Trypethelium ช่วยเปิดความสัมพันธ์ทางวิวัฒนาการและยืนยันไลเคนชนิดใหม่ในสกล Astrothelium จำนวน 5 ชนิด ส่วนสกุล Laurera มีความใกล้ชิดกันมากกับสกุล Marcelaria และ M. benguelensis และ M. cumingii เป็นไลเคนชนิดเดียวกัน ส่วนสกุล Trypethelium นั้นมีลักษณะทางสัณฐานวิทยาไม่สอดคล้องกับวงศ์ วานวิวัฒนาการและยังพบความหลากหลายทางพันธุกรรมของไลเคนในกลุ่ม T. eluteriae โดยสามารถจัดจำแนกไลเคน ในกลุ่มนี้เป็น 3 ชนิด ได้แก่ T. eluteriae, T. platystomum และ T. subeluteriae ซึ่งสารทุติยภูมิจากแทลลัสไลเคนมี ความสอดคล้องกับความสัมพันธ์วิวัฒนาการมากกว่าลักษณะทางสัณฐานวิทยา จากการศึกษาวงศ์วานวิวัฒนาการ ของไลเคนวงศ์ทริพิทิเลียซิอีที่ตำแหน่ง ITS, nuLSU, mtSSU rDNA และ RPB1 พบว่า ไลเคนวงศ์นี้มีความสัมพันธ์ทาง วิวัฒนาการที่แตกต่างหลากหลาย ซึ่งไม่สอดคล้องกันระหว่างวงศ์วานวิวัฒนาการกับลักษณะทางสัณฐานวิทยาและ ลักษณะสารเคมี ทั้งในระดับสกุลและชนิด โดยไลเคนในวงศ์ที่ส่วนใหญ่จัดเป็นกลุ่ม polyphyletic ได้แก่ Astrothelium, Bathelium, Laurera, Polymeridium และ Trypethelium ในขณะที่สกุล Campylothelium, Marcelaria และ Pseudopyrenula จัดเป็นกลุ่ม monophyletic ถึงแม้ว่าลักษณะทางสัณฐานวิทยาส่วนใหญ่จะไม่สอดคล้องกับของมูล ทางพันธุกรรม แต่สารเคมีที่ผลิตขึ้นจากราที่ก่อให้เกิดไลเคนกลับมีความสอดคล้องกับวงศ์วานวิวัฒนาการ ดังนั้นจึงควรมี การจัดจำแนกไลเคนวงศ์นี้ใหม่ โดยข้อมูลวิวัฒนาการชาติพันธุ์ร่วมกับลักษณะสารทุติยภูมิจากราที่ก่อให้เกิดไลเคน จาก การศึกษานี้สามารถจัดจำแนกไลเคนวงศ์นี้ในประเทศไทยทั้งหมด 62 ชนิด เป็นชนิดที่พบครั้งแรก 18 ชนิดและไลเคนชนิด ใหม่ของโลก 5 ชนิด จากการศึกษาสารทุติยภูมิทางสร้างขึ้นจากราที่ก่อให้เกิดวงศ์ทริพิทิเลียซิอี พบว่าสารทุติยภูมิส่วน ใหญ่เป็นสารที่มีขั้ว เมื่อนำมาทดสอบฤทธิ์ทางชีวภาพ พบว่า สารสกัดจากราที่ก่อให้เกิดไลเคน 11 ชนิด ได้แก่ A. neglectum, L. varia, M. cumingii, T. andamanicum, T. eluteriae, T. platystomum, T. subeluteriae, T. ubianense, Trypethelium sp.2, Trypethelium sp.7 และ Trypethelium sp.8 สามารถออกฤทธิ์ได้หลากหลายทั้งยับยั้ง การเจริญของ Staphylococcus aureus, Candida albicans และฤทธิ์ต้านอนุมูลอิสระ DPPH และไม่พบสารสกัดจาก ราที่ก่อให้เกิดไลเคนชนิดใดสามารถยับยั้งการเจริญของ Escherichia coli ได้

สาขาวิชา เทคโนโลยีชีวภาพ ปีการศึกษา 2558

ลายมือชื่อนิสิต
ลายมือชื่อ อ.ที่ปรึกษาหลัก
ลายมือชื่อ อ.ที่ปรึกษาร่วม

#### # # 5472882823 : MAJOR BIOTECHNOLOGY

KEYWORDS: TROPICAL LICHENS / MOLECULAR PHYLOGENY / TAXONOMY / SECONDARY METABOLITES / LICHENIZED FUNGI / TRYPETHELIALES

> THEERAPAT LUANGSUPHABOOL: PHYLOGENY AND SECONDARY METABOLITES OF LICHEN-FORMING FUNGI IN TRYPETHELIACEAE IN THAILAND. ADVISOR: ASST. PROF. JITTRA PIAPUKIEW, Ph.D., CO-ADVISOR: ASST. PROF. EK SANGVICHIEN, Ph.D., 270 pp.

Trypetheliaceae is a family of tropical crustose lichenized fungi belonging to the order Trypetheliales (Dothideomycetes). Nine hundred and sixty-five lichen specimens were collected from various localities in Thailand at 28 study sites in 24 provinces, in which species of this family were found in different habitats. Mycobionts were isolated by the ascopore discharge technique and 313 isolates were successfully isolated and cultivated in axenic cultures. In this study the following eight genera have been found in Thailand: Astrothelium, Bathelium, Campylothelium, Laurera, Marcelaria, Polymeridium, Pseudopyrenula and Trypethelium. Phylogenetic analysis of the genera Astrothelium, Laurera, Marcelaria and Trypethelium revealed evolutionary relationships among these lichenized fungi and supported previously unrecognized species, including five new species in the genus Astrothelium. The genus Laurera was found to be closely related to Marcelaria and the two currently accepted species M. benguelensis and M. cumingii were found to be conspecific. Morphological characters and phylogenetic relationships were incongruent within Trypethelium and within the T. eluteriae group a remarkable diversity was found with three species occurring in Thailand, viz. T. eluteriae, T. platystomum and T. subeluteriae. In contrast to morphological characters, secondary metabolites showed better correlation with phylogenetic relationships. Molecular phylogenetic studies of the family Trypetheliaceae based on ITS, nuLSU, mtSSU rDNA and RPB1 showed various genetic relationships, which demonstrated conflict in phylogeny, morphology and chemistry. Most genera in this family were found to be polyphyletic, including Astrothelium, Bathelium, Laurera, Polymeridium and Trypethelium, whereas Campylothelium, Marcelaria and Pseudopyrenula formed monophyletic groups. Although, most morphological characters did not correlate with molecular data, the metabolites produced in mycobiont cultures showed correlation with the phylogeny. Thus, the family requires a taxonomic revision based on molecular phylogeny in combination with the mycobiont substances. In this study, 62 species were recorded of Trypetheliaceae in Thailand, including 18 new records and 5 new species. According to the chemical study, secondary metabolites produced from mycobiont cultures are polar compounds. Crude extracts from eleven species A. neglectum, L. varia, M. cumingii, T. andamanicum, T. eluteriae, T. platystomum, T. subeluteriae, T. ubianense, Trypethelium sp.2, Trypethelium sp.7 and Trypethelium sp.8 showed effective inhibition of Staphylococcus aureus and Candida albicans and reacted to free radical DPPH, while all extracts from these lichens were ineffective against Escherichia coli.

Field of Study: Biotechnology Academic Year: 2015

Student's Signature
Advisor's Signature
Co-Advisor's Signature

#### ACKNOWLEDGEMENTS

I would like to express my greatest appreciation to my thesis advisor, Assistant Professor Jittra Piapukiew, Ph.D. and my thesis co-advisor, Assistant Professor Ek Sangvichien, Ph.D. for their valuable advice and encouragements throughout this study.

My appreciation is also expressed to Assistant Professor Tosak Seelanan, Ph.D., Assistant Professor Sanit Piyapattanakorn, Ph.D., Assistant Professor Warinthorn Chavasiri, Ph.D. and Associate Professor Kansri Boonpragob, Ph.D. for their kindness and helpful suggesting for the complements of this study and serving as thesis committee.

I would like to extend my thanks to Professor Anthony J. S. Whalley, Ph.D. from School of Biomolecular Sciences, Liverpool John Moores University (UK) and H. Thorsten Lumbsch, Ph.D. from Science & Education, The Field Museum (USA) for their valuable suggestions and comments to improve this thesis.

I also wish to express my appreciation to the Program in Biotechnology and Department of Botany, Faculty of Science, Chulalongkorn University and Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University for providing facilities during my study. The financial supports from CU. Graduate School Thesis Grant and scholarship of the Human Resource Development in Science Project (Science Achievement Scholarship of Thailand). I also thank members of Room 212, Department of Botany and Room SCO 327, Department of Biology, Ramkhamhaeng University for their friendship, help and kindness.

Finally, the greatest gratitude is expressed to my parents for their true loves and continuing support throughout this study.

### CONTENTS

Page	ì
THAI ABSTRACT iv	
ENGLISH ABSTRACTv	
ACKNOWLEDGEMENTS vi	
CONTENTSvii	
LIST OF TABLESx	
LIST OF FIGURES xi	
CHAPTER I INTRODUCTION	
CHAPTER II LITERATURE REVIEW4	
2.1 Lichen	
2.2 The fungal partner	
2.3 Lichen identification	
2.4 Molecular study of lichens	
2.5 Lichen metabolites	
2.6 Family Trypetheliaceae	
CHAPTER III MATERIALS AND METHODS	
3.1 Instruments used in this study	
3.2 Chemicals used in this study	
3.3 Taxon sampling and specimens preparation	
3.4 Mycobiont isolation and cultivation	
3.5 Taxonomic study and lichen identification	
3.6 Molecular study	
3.6.1 DNA extraction	

## Page

	3.6.2 Polymerase chain reaction (PCR), amplification and DNA sequencing3	37
	3.6.3 Basic Local Alignment Search Tool (Blast) and nucleotide submission	37
	3.6.4 Nucleotide sequence alignments	39
	3.6.5 Phylogenetic analysis of lichen-forming fungi Trypetheliaceae	39
3.7	Chemical study4	10
	3.7.1 Mycobiont extraction4	10
	3.7.2 Secondary metabolites analysis4	10
3.8	Antimicrobial activity determination4	11
	3.8.1 Microbial preparation	11
	3.8.2 Bioautography examination	11
3.9	Antioxidant activity detection	12
CHAP	TER IV RESULTS4	13
CHAP 4.1	TER IV RESULTS	13 13
CHAP 4.1 4.2	TER IV RESULTS	13 13 15
CHAP 4.1 4.2 4.3	TER IV RESULTS	13 13 15 19
CHAP 4.1 4.2 4.3	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4	13 13 15 19
CHAP 4.1 4.2 4.3	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4         4.3.2 Lichen identification       5	13 13 15 19 19
CHAP 4.1 4.2 4.3	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4         4.3.2 Lichen identification       5         Molecular study of family Trypetheliaceae       9	13 13 15 19 19 55 98
CHAP 4.1 4.2 4.3 4.4	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4         4.3.2 Lichen identification       5         Molecular study of family Trypetheliaceae       9         4.4.1 Molecular phylogeny of genus Astrothelium       9	13 13 15 19 19 55 98 98
CHAP 4.1 4.2 4.3 4.4	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4         4.3.2 Lichen identification       5         Molecular study of family Trypetheliaceae       9         4.4.1 Molecular phylogeny of genus Astrothelium       9         4.4.2 Phylogeny of genera Laurera and Marcelaria       10	13 13 15 19 19 55 98 98
CHAP 4.1 4.2 4.3 4.4	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4         4.3.2 Lichen identification       5         Molecular study of family Trypetheliaceae       9         4.4.1 Molecular phylogeny of genus Astrothelium       9         4.4.2 Phylogeny of genera Laurera and Marcelaria       10         4.4.3 Phylogeny of genus Trypethelium       10	<ul> <li>13</li> <li>13</li> <li>15</li> <li>19</li> <li>19</li> <li>55</li> <li>98</li> <li>98</li> <li>92</li> <li>96</li> </ul>
CHAP 4.1 4.2 4.3 4.4	TER IV RESULTS       4         Taxon sampling       4         Mycobiont isolation and cultivation       4         Taxonomic study and identification       4         4.3.1 Lichen taxonomy       4         4.3.2 Lichen identification       6         Molecular study of family Trypetheliaceae       9         4.4.1 Molecular phylogeny of genus Astrothelium       9         4.4.2 Phylogeny of genera Laurera and Marcelaria       10         4.4.3 Phylogeny of genus Trypethelium       10         4.4.4 Phylogeny and diversity of Trypethelium eluteriae group in Thailand       11	13 13 15 19 19 55 98 98 98 98 98 92 98

# Page

4.4.5 Phylogenetic relationships of lichen-forming fungi of Trypetheliaceae in	
Thailand	116
4.5 Chemical study	124
4.5.1 Mycobionts extraction and secondary metabolites study	133
4.6 Antimicrobial activity	136
4.7 Antioxidant activity	140
CHAPTER V Discussion	143
CHAPTER V Conclusion	153
REFERENCES	156
APPENDICES	182
APPENDIX A	183
APPENDIX B	185
APPENDIX C	188
APPENDIX D	200
APPENDIX E	263
APPENDIX F	267
VITA	270

## LIST OF TABLES

Table 1 Lichen genera commonly used in traditional medicine.         19
Table 2 Three main of secondary metabolites.   21
Table 3 The secondary metabolites of lichens and their biological activities
Table 4 Primers for nucleotide amplification in this study
Table 5 The information of study sites, number of lichen samples, number of isolatesand the number of mycobiont isolates.45
Table 6 Comparison of the major characteristics for genus delimitation within
Trypetheliaceae54
Table 7 List of lichen species in family Trypetheliaceae in Thailand based on
morphological characters and number of isolated of each species95
Table 8 Nucleotide sequences of genus Astrothelium were downloaded from
GenBank
Table 9 The nucleotide sequences of genera Laurera, Marcelaria and outgroup
were downloaded from GenBank103
Table 10 Nucleotide sequences of genus Trypethelium and outgroup were
downloaded from GenBank107
Table 11 Morphological characters of T. eluteriae, T. platystomum and T.
subeluteriae115
Table 12 Total amount of mycobiont colonies and crude extracts of lichen-forming
fungi family Trypetheliaceae133
Table 13 The Rf values of antimicrobial activity of lichen family Trypetheliaceae
Table 14 Antioxidant activity and Rf values from different solvent extraction of lichen-
forming family Trypetheliaceae

## LIST OF FIGURES

Figure 1 Lichen thallus structure
Figure 2 The thallus character of lichens7
Figure 3 Sexual and asexual reproductive structure of lichen9
Figure 4 The lichen tissue culture methods isolated from thallus fragments
Figure 5 Ascospore discharge from apothecia and transfer ascospores to culture
medium
Figure 6 The macroscopic and microscopic morphology for lichen identification
Figure 7 Schemes mapping of ribosomal DNA and protein-coding gene with primers
position
Figure 8 Biosynthetic pathways of secondary metabolites of lichens
Figure 9 The chemical structure of anthraquinone and xanthone group
Figure 10 The map of lichen collection sites in Thailand
Figure 11 Development of ascospore and formation of mycobiont colony on MYA
medium
Figure 12 Taxonomic characters of each genus within Trypetheliaceae
Figure 13 Morphological characters of thallus and ascospores of A. aenascens (A-
B), A. flavocoronatum (C-D), A. macrocarpum (E-F), and A.
macrostiolatum (G-H)58
Figure 14 Morphological characters of thallus and ascospores of A. neglectum (A-
B), A. neovariolosum (C-D), A. siamense (E-F), and B. albidoporum (G-H)62
Figure 15 Morphological characters of thallus and ascospores of B. madreporiforme
(A-B), <i>B. tuberculosum</i> (C-D), <i>Bathelium</i> sp.1 (E-F), and <i>C. nitidum</i> (G-H)65

Figure 16 Morphological characters of thallus and ascospores of L. alboverruca (A-
B), L. cf. aurantiaca (C-D), L. cf. columellata (E-F), and L. keralensis (G-
Н)67
Figure 17 Morphological characters of thallus and ascospores of <i>L. megasperma</i>
(A-B), <i>L. meristospora</i> (C-D), <i>L. meristosporoides</i> (E-F), and <i>L.</i>
phaeomelodes (G-H)
Figure 18 Morphological characters of thallus and ascospores of <i>L. phaeomelodes</i>
(A-B) / subdiscreta (C-D) / subphaerioides (E-E) and / varia (G-H) 71
Figure 19 Morphological characters of thallus and ascospores of <i>L</i> .
verrucoaggregata (A-B), L. vezdae (C-D), M. benguelensis (E-F), and M.
cumingii (G-H)73
Figure 20 Morphological characters of thallus and ascospores of <i>P. albidum</i> (A-B),
P. albocinereum (C-D), P. catapastum (E-F), and P. quinqueseptatum (G-
Н)75
Figure 21 Morphological characters of thallus and ascospores of Polymeridium sp.1
(A-B), Polymeridium sp.2 (C-D), Pseudopyrenula diluta var. degenerans
(E-F), and <i>P. subnudata</i> (G-H)77
Figure 22 Morphological characters of thallus and ascospores of T. cf. aeneum (A-
B), T. albopruinosum (C-D), T. andamanicum (E-F), and T.
cinereorosellum (G-H)79
Figure 23 Morphological characters of thallus and ascospores of <i>T. eluteriae</i> (A-B),
T. microstomum (C-D), T. neogabeinum (E-F), and T. nitidusculum (G-H)81
Figure 24 Morphological characters of thallus and ascospores of T. ochroleucum
var. subdissocians (A-B), T. aff. papulosum (C-D), T. pseudoplatystomum
(E-F), and <i>T. tropicum</i> (G-H)85

Figure 25 Morphological characters of thallus and ascospores of T. ubianense (A-
B), T. virens (C-D), Trypethelium sp.1 (E-F), and Trypethelium sp.2 (G-H)87
Figure 26 Morphological characters of thallus and ascospores of Trypethelium sp.3
(A-B), Trypethelium sp.4 (C-D), Trypethelium sp.5 (E-F), and Trypethelium
sp.6 (G-H)
Figure 27 Morphological characters of thallus and ascospores of Trypethelium sp.7
(A-B), Trypethelium sp.8 (C-D), Trypethelium sp.9 (E-F), and Trypethelium
sp.10 (G-H)92
Figure 28 Morphological characters of thallus and ascospore of <i>Trypethelium</i> sp.1194
Figure 29 Phylogenetic relationships of the genus Astrothelium based on maximum
likelihood and Bayesian inference analyses using four loci (ITS, nuLSU,
mtSSU and RPB1)101
Figure 30 Phylogenetic relationships of genera Laurera and Marcelaria in Thailand
based on two loci (nuLSU and mtSSU)
Figure 31 A maximum likelihood tree of genus <i>Trypethelium</i> based on nuLSU and
mtSSU regions
Figure 32 Phylogeny of the Trypethelium eluteriae group based on partial ITS and
mtSSU rDNA sequences113
Figure 33 Morphology of thallus and ascospores in the <i>T. eluteriae</i> group114
Figure 34 TLC plates of <i>T. eluteriae</i> group with anthraquinone pigment
Figure 35 Phylogenetic tree lichen-formin fungi family Trypetheliaceae in Thailand
based on four loci (ITS, nuLSU, mtSSU rDNA and RPB1)
Figure 36 Overall of phylogenetic relationships of genera within family
Trypetheliaceae based on four loci (ITS, nuLSU, mtSSU and RPB1)
Figure 37 TLC plates of chemical substances from mycobiont cultures

# CHAPTER I

Lichens are symbiotic organisms between fungal (mycobiont or lichen-forming fungi) and algae (photobiont) (Ahmadjian, 1993; Purvis, 2000; Nash III, 2008). Photosynthetic partners have been estimated to belong to nearly 40 genera including green algae (25 genera) and cyanobacteria (15 genera), and therefore the majority group associated with lichens are green algae (Büdel, 1992; Ahmadjian, 1993; Kirk et al., 2008). The photobiont has played a major role on synthesis and transfer of organic nutrients from CO<sub>2</sub> as sugar alcohols or glucose to the mycobiont. However in cyanobacteria can produces organic nitrogen compound, are produced by nitrogen fixation (Hale, 1983; Feige and Jensen, 1992; Nash III, 1996; Purvis, 2000). In contrast, the fungal partner absorbs water vapor from the air and protects the partnership from stress condition, ultraviolet radiation and insect pests (Ahmadjian, 1993; Emmerichet et al., 1993; Fröberg et al., 1993; Gauslaa and Solhaug, 2001). Stages of lichens symbiosis are very different in character depending on the with origin of fungal and algae partners (Purvis, 2000). Lichens grow and occur in most ecosystems of the earth: from polar, tundra, alpine, desert, mangrove forest and both temperate and tropical rain forest (Hale, 1983; Nash III, 1996; Purvis, 2000; Galloway, 2007; Kirk et al., 2008). Lichenforming fungi are poorly studied especially in tropical regions, and have been estimated to be betweent about 17,500 and 28,000 species in the world (2,720 genera, 37 order), almost all of them belong to Ascomycota and a few to the Basidiomycota (Hawksworth, 1991; Kirk et al., 2008; Boonpragob et al., 2013).

Trypetheliaceae is crustose lichen, with worldwide distribution in tropical and subtropical regions, with approximately 13 genera and 192 species being recorded (Harris, 1984; Del Prado *et al.*, 2006; Kirk *et al.*, 2008). This family has been only reported in Thailand classified into 6 genera and 33 species belonging to *Astrothelium, Campylothelium, Laurera, Polymeridium, Pseudopyrenula* and *Trypethelium* (Vongshewarat, 2000). Morphological characters are important for identification, and

ascospore characters are especially important for delimitation of genera within the Trypetheliaceae (Harris, 1995). Trypetheliaceae and Pyrenulaceae have only ascospore color and hamathecium characters to assign them the family, This has caused problems because of their lack of critical morphological characters (Aptroot, 2009a). In addition, some genera of Trypetheliaceae they cannot be separated based on ascospore characters in for example between Bathelium and Polymeridium, which both produce two ascospore characters as muriform and transversely septate (Harris, 1995). At present, morphology is the major method in lichen identification but there are problems due to lack of experts and lack the type specimen of tropical lichens for confirmation. Previously, Astrothelium and Trypethelium were reported as a non-monophyletic group based on DNA analysis (Del Prado et al., 2006; Nelsen et al., 2009; Nelsen et al., 2014). Accordingly because of conflict of morphological characters, molecular techniques are alternatively tools to help in lichen identification and to understand phylogenetic relationships within Trypetheliaceae. Internal transcribed spacer (ITS), nuclear large subunit ribosomal DNA (nuLSU), mitochondrial small subunit ribosomal DNA (mtSSU rDNA) and RNA polymerase II (RPB1) are conserved regions and are variable (Zoller et al., 1999; Martin and Rygiewicz, 2005; Ruibal et al., 2009), which provides the potential for the explanation of the relationships of lichen-forming fungi within genus and species level (Kasalicky et al., 2000; Tehler et al., 2000; Del Prado et al., 2006).

Lichen substances are an importance source of secondary metabolites mainly produced from the fungal partner, and which depends on the lichen species, nutrients and environment conditions (Stocker-Wörgötter *et al.*, 2004). Secondary metabolites of lichens and lichen-forming fungi have been estimated at about 1,050 substances (Molnar and Farkas, 2010), of which 50-60 substances were similar to higher plants and other fungi (Elix and Stocker-Wörgötter, 2008). Lichen-forming fungi in laboratory are produces substances both are similar and different from lichen thallus (Stocker-Wörgötter and Brunauer, 2005; Fazio *et al.*, 2009). Thus, the cultivation of the mycobiont is important for secondary metabolite studies. In addition, secondary metabolites of lichens have been using to folk medicine for expectorants and diuretics, dye coloring

agent, cosmetics and also in the perfume industry (González-Tejero *et al.*, 1995; Romagni and Dayan, 2002). In fact, lichens in nature produced substances to protect the thallus from UV light, insects and parasites (Emmerichet *et al.*, 1993; Fahselt, 1994), and are known to exhibite bioactivity such as antimicrobial, antiviral and enzyme inhibitor (Huneck, 1999; Heng *et al.*, 2013).

Trypetheliaceae produce the major groups of xanthones and anthraquinones such as lichexanthone, parietin, draculone, secalonic acid and haematommone (Harris, 1984; Mathey *et al.*, 2002; Manojlovic *et al.*, 2010a). Xanthone and anthraquinone groups have been reported for their antibacterial, antifungal, anticancer, antioxidant and anti-inflammatory properties (Mathey, 1979; Manojlovic *et al.*, 2002; Vasiljevic *et al.*, 2009; Manojlovic *et al.*, 2010b). Accordingly in this lichen family there is potential for various applications since very few from the tropics have been studied. Thus, Trypetheliaceae is not only important to study for its molecular phylogeny and for lichen identification but also to help understanding the relationships within this family. In addition studies on secondary metabolites produced from mycobiont cultivation can have other applications.

Therefore, the objectives of this study were to investigate the phylogenetic relationships of lichen-froming fungi within the family Trypetheliaceae and to study the secondary metabolites of Trypetheliaceae in Thailand for their bioactivity.

## CHAPTER II LITERATURE REVIEW

#### 2.1 Lichen

Lichen is symbiotic associations formed between fungal partner (mycobiont or lichen-forming fungi) and algae (green algae/cyanobacteria) partner (photobiont). Some lichen groups contain three organisms or more partners (Hawrksworth and Hill, 1984; Ahmadjian, 1993; Purvis, 2000; Nash III, 2008). The fungus forms the main structure of lichen thallus, whilst inside is the house of photobionts (Ahmadjian, 1993; Purvis, 2000; Gilbert, 2004). The lichen thallus in general has three different layers as cortex layer, algal layer and medulla layer, which photosynthetic partner cell are enveloped by fungal tissue (Figure 1). Lichen-forming fungi are heterotrophic organisms and do not contain chlorophyll; hence, they cannot produce their own nutrition as carbohydrates (Purvis, 2000). All nutrient is transfered from the autotrophic photobiont to the heterotrophic mycobiont, which is the main benefit of fungus to symbioses with photobionts as made up the specific of lichen pattern (Ahmadjian, 1993; Purvis, 2000). Photobiont was estimated about 7% of all lichen thallus (Collins and Farrar, 1978), mostly are eukaryotic algae (90%) such as genus Trebouxia or Trentepohlia, while the rest is cyanobacterium (10%) such as Nostoc (Ahmadjian, 1967; Purvis, 2000; Rankovic and Kosanic, 2015). The algae partner can be able to synthesis the carbohydrates from sunlight and  $CO_2$ uptake, which the types of carbon source depends on type of algae partner. The lichens are associates with green algae as sugar alcohols, while cyanobacteria produce glucose and also support the nitrogen compound to lichen fungus by fix nitrogen from the atmosphere (Feige and Jensen, 1992; Purvis, 2000). For mycobiont partner, they have the role to protect the photobiont from strong UV and stress the environments, and also absorb water and mineral nutrients from atmosphere and contaminate on thallus surface, respectively (Hale, 1983; Nash III, 1996; Purvis, 2000; Nash III, 2008). The Ascomycetes is the major group of fungus that forms lichen symbiotic, a few number are the Basidiomycetes and Deuteromycetes (Hawrksworth and Hill, 1984; Nash III, 2008).



## Figure 1 Lichen thallus structure. (Buaruang et al., 2009)

The main of lichens thalli are divided into three types such as crustose, foliose and fruitcose (Ahmadjian, 1993; Büdel and Scheidegger, 2008).

#### 2.1.1 Crustose lichens

The thallus seems to be powdery, very thinly and closely attached to the substrate surface by fungal hyphae at the lower cortex that cannot be removed from substrate. In some crustose species are lack the lower cortex. This lichen groups are grow on the wood bark or bare exposure rock (Figure 2, A-B), which highly tolerate extremes environments that occurred in various habitat as desert, tropical rain forest, highest attitude mountain in the Himalayas (7,400 m) and ice area in Antarctica. Crustose lichens were estimated approximately 15,000 species or 75% of all lichens (Hertel, 1988; Ahmadjian, 1993; Büdel and Scheidegger, 2008).

#### 2.1.2 Foliose lichens

Lichen thallus have circular, leaf-like, flat and dorsiventral lobes that more or less closely adhere to the substrate such as wood bark or rock, which attached by rhizine or holdfast (Figure 2, C-D). In general, foliose thallus consists of the medulla, and the upper and lower cortex, it is the great range of thallus size developed and their diversity. In addition, foliose lichen can be divided into two subtypes as Laciniate lichens and Umbilicate lichens, which both thalli are different on lower cortex in contact with substratum by rhizine hyphae or the margin of the lobes free and attached to substrate at the central of thallus by holdfast, respectively (Jahns, 1973; Jahns, 1988; Büdel and Scheidegger, 2008).

#### 2.1.3 Fruticose lichens

This lichen type is free for branching of thallus lobes, it looks likehairy, bushy or strap-shaped and the thallus may be cylindrical or flat shape. The fruticose thalli are attached at base to the substrate by the holdfast, which grow on the trees, rocks and soil (Figure 2, E-F). The pattern of lichen thalli are various size and characters, which depend on genera or species group. Some fruticose thalli can grow several meters long, hanging from trees as have upright stalks on the ground. Fruticose lichens are distributed in various ecosystems ranging from the desert to tropical rain forest (Jahns, 1973; Jahns, 1988; Büdel and Scheidegger, 2008).



Figure 2 The thallus character of lichens. A-B. crustose, C-D. foliose, and E-F. fruitcose.

Reproductive structures of lichens are produced from the fungal component that consists of sexual and asexual life cycle, which usually are teleomorph. Sexual reproductive structures have various characters, size and color, which contains hymennium tissue, ascus and ascospore. In general, there are two major types of sexual reproductive structures (Büdel and Scheidegger, 2008).

#### 2.1.4 Apothecia

The structure of the apothecia is cup or disk shaped that develops on the thallus (Figure 3, A-B). The inside consists of hymenium tissues, ascus and ascospore. Apothecia have exposed hymenium of ascospore maturity and released them (Hawrksworth and Hill, 1984; Büdel and Scheidegger, 2008).

#### 2.1.5 Perithecia

The perithecia are a globular to flask-shaped that rise on the thallus (Figure 3, C-D). This ascomata are usually solitary, but some genus aggregates into pseudostomata tissue. Ascospores are developed in the locule of perithecia hymenium, which also includes the ascus and paraphyses. The exciple is carbonized at the surrounded perithecia or only nearly ostiole in some genera. Perithecia are open at the small ostiole tube that is used for ascospore discharg (Hawrksworth and Hill, 1984; Büdel and Scheidegger, 2008).

Lichens have various asexual reproductive structures. The two most importance basic characters are the isidia (Figure 3E) and soredia (Figure 3F). The isidia are cylindrical, branches or finger-shape that develops on upper surface of the thallus, while soredia are dry, powdery and diffusely at similar to isidia ontogeny. Both of isidia and soredia includes photobiont cells that are enveloped by fungal hyphae, which their structure are brake to fragments and can be develops to new lichen thallus (Hawrksworth and Hill, 1984; Büdel and Scheidegger, 2008).



Figure 3 Sexual and asexual reproductive structure of lichen. A-B. apothecia, C-D. perithecia , E. soredia, and F. isidia.

#### 2.2 The fungal partner

The lichen-forming fungi or mycobionts are heterotrophic organism that associates with photobionts as the lichen is formed. Early twenty-one century (C.E.), lichens were described as plants, because they have developed thallus characters as plant-like structures. In fact, lichens are the fungus, which the name of the lichens refers to the fungal species (Honegger, 2008). Fungal partner is around 20% of all fungi and 40% of all Ascomycota as lichen symbiosis (Purvis, 2000; Kirk *et al.*, 2008). Lichenforming fungi are estimated approximately 17,500 – 28,000 species (2,720 genera, 37 order and 16 as only lichen mycobiont), which mostly about 98% of lichens belong to Ascomycota and a few species as Basidiomycota and anamorphic fungi (Hawksworth, 2001; Sipman and Aptroot, 2001; Honegger, 2008; Boonpragob *et al.*, 2013). The lichen-forming fungi form various lichen thalli characters, mainly more than 55% form crustose thalli, while 20% form squamulose or placodioid thalli and 25% form foliose or fruticose thalli (Honegger, 2008).

Isolation and cultivation of lichen-forming fungi have been studied more than 100 years ago with many lichens species (Ahmadjian, 1993). The early studies on these were done by Töbler (1909) and Thomas (1939), which they were primary interesting to resynthesis lichens from both symbiont partners (Turbin, 1996). A few studies on lichen formation have been successful, which *Endocarpon pusillum* and *Staurothelse clopima* are the first lichen groups that successes to synthesis in laboratory (Ahmajian and Heikkilä, 1970). Lichen-forming fungi have been isolated from temperate, Antarctic and Antarctic lichens species, which *Xanthoria parietina* was the lichen study model for ascospore isolation and culture condition (Chrismas, 1980; Oliver *et al.*, 1989; Crittenden *et al.*, 1995; Molina *et al.*, 1997; Molina and Crespo, 2000; Molina *et al.*, 2015). Although almost mycobiont isolation by ascospore discharge technique and axenic culture (Yoshimura *et al.*, 2002; Sangvichien *et al.*, 2011; McDonald *et al.*, 2013).

In general, lichen-forming fungi can be isolated from various parts such as thallus fragments, isidia and soridia by using lichen tissue culture method (Figure 4) (Yamamoto *et al.*, 1985; Yamamoto *et al.*, 2002), which might be get the wrong fungus, bacteria and yeast contaminates (Petrini *et al.*, 1990; Ahmadjian, 1993). Fungal partner grow more slowly than contaminate organisms; hence, lichen tissue culture from thallus fragments is a high risk of contamination when isolates on nutrient rich media (Ahmadjian, 1993; Yamamoto *et al.*, 2002). Ascospores discharge is the first choice and the best method for lichen mycobiont isolation (Figure 5), which might be difficult to ascospores germination (Ostrofsky and Denison, 1980; Ahmadjian, 1993). Crustose lichens have more percentage of ascospore germination than the foliose and fruticose lichens (Kofler, 1970; Sangvichien *et al.*, 2011). Aposymbiotic of pure fungal partner is different from the phenotype characters to the symbiotic phenotype, which mycobionts culture produce the balloon hyphae, compact and raise up colony (Lawrey, 1984; Ahmadjian, 1993; Honegger, 2008).



Figure 4 The lichen tissue culture methods isolated from thallus fragments. (Yamamoto *et al.*, 2002)



Figure 5 Ascospore discharge from apothecia and transfer ascospores to culture medium.

(Yamamoto et al., 2002)

#### 2.3 Lichen identification

Identification of lichen has been determined based on the traditional characters as morphological and chemical characters, which can be divided into three main parts (Harris, 1984; Awasthi, 1991; Makhija and Patwardhan, 1993; Vongshewarat, 2000; Aptroot *et al.*, 2008; Aptroot, 2009b).

#### 2.3.1 Macroscopic morphology

Lichens are observed by lichen thallus characters, growth formed, sexual and asexual reproductive structures, visible light color and color under ultraviolet light (Figure 6, A-B).

#### 2.3.2 Microscopic morphology

Lichen thallus and sexual reproductive structures are crossed section by razor blade and investigated under microscopes. The characters of thallus structures such as photobiont layer and cortex formation were observed. In addition, ascocarp characters as ascomata types, color of ascocarp, ascospore size, shape, color and also ascospore septation investigated, which ascospore character was the majority role to lichen identification (Figure 6).

#### 2.3.3 Chemical characters and spot test

Spot test or color test is a basic for characterized lichen substances that related to lichen classification, which based on chemical reaction to the surface of thallus cortex, medulla and ascocarp with K as 10% Potassium hydroxide (KOH), Sodium hypochlorite (C) and Paraphenyldiamine (Pd). The positive spot tests are investigated by the color changed to red, purple, brown or yellow, if the negative results as colorless. In addition, lichen chemotaxonomies are not only determined by spot test but also thin layer chromatography (TLC) with the standardized methods with solvent systems as solvent A: toluene/dioxane/acetic acid (180:45:5), solvent C: toluene/acetic acid (170:30) and solvent G: toluene/ethyl acetate/formic acid (139:83:8) (Culberson, 1972; Lumbsch, 2002; Elix and Stocker-Wörgötter, 2008).



**Figure 6** The macroscopic and microscopic morphology for lichen identification. A-B, lichen thallus characters, C. pseudostrama and perithecia, D. ascospores and asci, E. transverse septate ascospore, and F. muriform ascospore.

#### 2.4 Molecular study of lichens

In general, morphological and chemical characters are important to lichen classification (Culberson, 1969; Brodo, 1978; Lumbsch, 1998; Lumbsch, 2002). However, these characters are problems to lichen identification because some phenotypes are very complex or similar and also personal error. Thus, molecular techniques play an important role to lichen studies as the term of population genetics, systematic, especially to solve the taxonomic problems of lichen identification and also phylogenetic relationships between genus or species levels (Gargas et al., 1995; Lutzoni and Vilgalys, 1995; Bridge and Hawksworth, 1998; Lumbsch et al., 2007; Weerakoon et al., 2012; Kraichak et al., 2014). Molecular phylogeny of lichen has been analyzed on nucleotide sequences to conserved regions with specific primers (Gargas and Taylor, 1992; Gardes and Bruns, 1993; Crespo et al., 1997; Zhao et al., 2015), which various DNA loci have been used to evolutionally study and lichens identification such as internal transcribed spacer ribosomal DNA (ITS rDNA), nuclear large subunit ribosomal DNA (nuLSU rDNA), mitochondrial small subunit ribosomal DNA (mtSSU rDNA) and the largest subunit of RNA polymerase II (RPB1) protein-coding gene (Figure 7) (Zhenga et al., 2007; Nelsen et al., 2011; Fernández-Brime et al., 2013; Kraichak et al., 2014; Zhao et al., 2015). Ribosomal RNA genes have been commonly studied to fungal systematic in the term of single and multiple loci (Lutzoni et al., 2004); moreover, the protein-coding gene RPB1 was the best effective phylogenetic marker for the Ascomycetes and the lichen-forming fungi (Diezmann et al., 2004; Hofstetter et al., 2007a). These conserved regions are more advantage for molecular phylogeny as multi copy genes, not larger size, easy to amplification and high genetic variation among genus and species level, which refer to species delimitation (White et al., 1990; Gardes et al., 1991; Lee and Taylor, 1992; Sheen et al., 1993; Zoller et al., 1999).

Papong *et al.* (2012) reported that phylogenetic relationships of tropical lichen genus *Lecanora* are based on two loci of ribosomal DNA (ITS and mtSSU). The phylogeny of *Lecanora* species demonstrated that non monophyletic within group of species, with presence of usnic acid and dark hypothecium. This result indicated that these phenotypes may be evolved several times independently within the group, which adapted for tropical species. More molecular data and species are suggested for species delimitation and understanding the relationships within *Lecanora*.

Kraichak *et al.* (2014) showed the phylogenetic placement of *Chapsa lamellifera*, *C. megalophthalma* and *Diploschistes ocellatus* within Graphidaceae. Five genetic markers (mtSSU, nuLSU, RPB1, RPB2 and ITS) solved the problem based on morphology and chemistry conflictes on generic concept. Two *Chapsa* species and *D. ocellatus* were separated into two new genera as *Gintarasia* and *Xalocoa*, respectively, which confirmed by molecular evidence.

Gueidan *et al.* (2016) studied on molecular phylogeny of tropical custose lichen in family Pyrenulaceae using three ribosomal genes (nuLSU, mtSSU and ITS). Pyrenulaceae was divided into two major groups that correlate with the presence or absence of pseudocyphellae, while other taxonomic characters conflicted with phylogeny. In addition, the ribosomal DNA demonstrated many problems that showed *Pyrenula* form polyphyletic genus, which some species was synonym or cryptic species.









A, Internal transcribed spacer ribosomal DNA (ITS rDNA); B, Nuclear large subunit ribosomal DNA (nuLSU rDNA); C, Mitochondrial small subunit ribosomal DNA (mtSSU rDNA); D,The largest subunit of RNA polymerase II (RPB1) (Rehner and Samuels, 1994; Larena *et al.*, 1999; Zhou and Stanosz, 2001; Matheny *et al.*, 2002).

#### 2.5 Lichen metabolites

Lichen produces various substances that depend on specific conditions, which provide to support lichen living against herbivores, parasitic fungi, and the environmental stress (Culberson et al., 1977; Huneck, 1999; Solhaug and Gauslaa, 2004; Deduke et al., 2012; Delmail et al., 2013). Normally, lichens synthesized two metabolic groups as primary and secondary metabolites (Lawrey, 1986). The primary metabolites found in lichens which include protein, carotenoids, amino acids, vitamins and polysaccharides, which can be soluble in water and extract by hot water (Olafsdottir and Ingólfsdottir, 2001; Stocker-Wörgötter, 2008). These metabolites may occur in other fungi, algae and plants that are not specific in lichens (Huneck, 1999; Elix and Stocker-Wörgötter, 2008; Rankovic and Kosanic, 2015). The main metabolites synthesized in lichens as organic secondary compounds that originate from fungal partner, which stimulates fungal hyphae to protect the thallus and algae partner from UV sun screen, parasites and insects (Emmerichet et al., 1993; Fahselt, 1994; Romagni and Dayan, 2002). Most of secondary metabolites are specific only in lichens and a small substances can be found in free-living fungi and higher plants. These metabolites can be isolates by organic solvent; because, they are poorly soluble in water (Elix and Stocker-Wörgötter, 2008; Backorová et al., 2012).

The lichens are source of important natural products that have been a potential for agriculture, perfumes, medicine and pharmaceutical industries (Culberson and Armaleo, 1992; Huneck, 1999; Oksanen, 2006). In ancient times, lichens were recorded for medicine about the fourth and third century B.E. in the Greek era (Lebail, 1853). Some species groups have been used to the folk or traditional medicine, which their properties are different from lichen species and part of the world as the list in Table 1. The America Indians and European used the lichen for folk medicine (Turner, 1977; Crawford, 2015), some lichens species were used to expectorant in India and China (Saklani and Upreti, 1992; Elix, 1996). For modern medicine and chemical study, lichen secondary metabolites have been focused on bioactivity, chemical identification and characterization (Sun *et al.*, 1990; Li *et al.*, 1991). In 1860s, Nylander reported the first

study on lichens substances and tested by color test with lichen thallus surface (Nylander, 1866). After that, Asahina and Shibata (1954) reported the first analysis for chemical structure and identified lichen substances, based on biosynthetic pathways that can be divided into three main groups as shown in Figure 8 and Table 2 (Elix and Stocker-Wörgötter, 2008).

Table 1 Lichen genera commonly used in traditional medicine.

(Crawford, 2015)

Lichen genera	Main area of use
Usnea	🦢 Worldwide (except
	Australia)
Evernia and Pseudevernia	Europe and North Africa
Letharia	China
Lethariella	Europe
Cetraria	India
Parmotrema and Everniastrum	North America and Africa
Xanthoparmelia	North America, Europe,
	Asia
Cladonia and Cladina	Asia
Thamnolia	North America, Europe,
	Asia
Lobaria and Peltigera	North America, Europe,
	Asia
Umbilicaria	North America and Asia

#### HULALONGKORN UNIVERSITY

Many secondary metabolites from lichen exhibit bioactivity and other application (Table 3). For examples, usnic acid shows antimicrobial activity that can inhibit Grampositive bacteria such as *Streptococcus mutans*, which was added for shower gel in Europe. Moreover, this substance was antihistamine and antiviral agent (Elix, 1996). Some lichen substance groups of depisides, depsidones, ursolic acid and triterpene derivatives were presents as anti-HIV, anti-HSV and anti-RSV activity (Neamati *et al.*, 1997; Kashiwada *et al.*, 2000; Esimone *et al.*, 2009). In addition, leukotriene and prostaglandin inhibit inflammatory, while anthraquinones, depsides, depsidones and xanthones exhibit antioxidant activity (Hidalgo *et al.*, 1994; Choi *et al.*, 2000; Marx, 2001; Manojlovic *et al.*, 2010a; Oettl *et al.*, 2013). Anticancer was reported in various lichen substance groups as anthraquinones (chrysophanol, emodin and parietin)

(Cohen and Towers, 1995; Choi *et al.*, 1997; Backorová *et al.*, 2012; Basile *et al.*, 2015), naphthoquinones (naphthazarin) (Babula *et al.*, 2009) and xanthones such as lichexanthone (Brandão *et al.*, 2013). In addition, the other application were used to dyes color, perfumes and cosmetic industrials (Sanchez *et al.*, 1997), which two lichen species as *Evernia prunastri* (oak moss) and *Pseudevernia furfuracea* (tree moss) were used in perfumery in France and Monaco (Moxham, 1986; Romagni and Dayan, 2002) and also hair color treatment (Bachmann and Portmann, 1981).



Figure 8 Biosynthetic pathways of secondary metabolites of lichens. (Elix and Stocker-Wörgötter, 2008)

The *in vitro* cultures of mycobionts produce compounds both similar and different from lichens symbiosis (Stocker-Wörgötter, 2001), which depended on stages of interaction between mycobionts and photobionts such as normal lichens symbiosis, resynthesized lichens and only lichen-forming fungal cultivation (Ahmadjian, 1993). For example, aposymbiotic culture of some lichens, produced and unregulated of secondary metabilotes such as anthraquinone derivatives by a stress as lack of photobionts and source of culture medium, which the carbon source effected to activate polyketide production and quantification (Brunauer *et al.*, 2007).

 Table 2 Three main of secondary metabolites.

(Elix and Stocker-Wörgötter, 2008; Rankovic and Kosanic, 2015)

#### 1. Acetyl-polymalonyl pathway

- 1.1 Secondary aliphatic acids, esters and related derivatives
- 1.2 Polyketide derived aromatic compounds
  - 1.2.1 Mononuclear phenolic compounds
  - 1.2.2 Di-and tri-aryl derivatives of simple phenolic units
    - 1.2.2a Depsides, tridepsides and benzyl esters
    - 1.2.2b Depsidones and diphenyl ethers
    - 1.2.2c Depsones
    - 1.2.2d Dibenzofurans, usnic acid and derivatives
  - 1.2.3 Anthraquinones and biogenetically retated xanthones
  - 1.2.4 Chromones
  - 1.2.5 Naphthoquinones
  - 1.2.6 Xanthones

2. Mevalonic acid pathway

- 2.1 Di-,sester- and triterpenes
- 2.2 Steroids
- 3. Shikimic acid pathway
  - 3.1 Terphenylquinones
  - 3.2 Pulvinic acids derivative

Lichen compounds	Lichen species	Bioactivity	References
Acremonidin E	Graphis tetralocularis	Antitubercular	(Pittayakhajonwut
		Anticancer	<i>et al.</i> , 2009)
Alectoronic acid	Ochrolechia parella	Anticancer	(Millot <i>et al.</i> , 2007)
Atranorin	Parmotrema	Antimicrobial,	(Türk <i>et al.</i> , 2006;
	austrosinense,	Antioxidant,	Melo <i>et al.</i> , 2011)
	Cladonia foliacea,	Anti-inflammatory,	(Proksa <i>et al.</i> ,
	Stereocaulon	Anticancer,	1994; Ingólfsdóttir
	alpinum,	Probiotic activity,	<i>et al.</i> , 1998;
	Pseudevernia	Trypsin inhibition	Yilmaz <i>et al.</i> ,
	furfuracea,		2004; Gaikwad <i>et</i>
	Hypogymnia		<i>al.</i> , 2014;
	physodes,		Rankovic <i>et al.</i> ,
	Cladina kalbii		2014)
Baeomycesic acid	Thamnolia	Anti-lipoxygenase	(Ingólfsdóttir <i>et</i>
	subuliformis		<i>al.</i> , 1997)
Barbatic acid	Arthothelium	Antioxidant,	(Verma <i>et al.</i> ,
	awasthii,	Antimicrobial,	2008a; Verma <i>et</i>
	Cladia aggregata	Antityrosinase	<i>al.</i> , 2008b; Martins
			<i>et al.</i> , 2010)
Benzoic acid	Ramalina roesleri	Antioxidant	(Sisodia <i>et al.</i> ,
			2013)
Chloroatranorin	Pseudovernia	Antibacterial,	(Türk <i>et al.</i> , 2006)
	furfuracea	Antifungal	

Table 3 The secondary metabolites of lichens and their biological activities.
Lichen compounds	Lichen species	Bioactivity	References
Diffractaic acid	Parmelia nepalensis,	Analgetic,	(Okuyama <i>et al.</i> ,
	P. tinctorum	Antiproliferative,	1995; Kumar and
	Usnea diffracta	Antipyretic,	Müller, 1999; Honda
	U. subcavata	Antibacterial	<i>et al.</i> , 2010)
Divaricatic acid	Protusnea malacea,	Antioxidant,	(Hidalgo <i>et al.</i> ,
	Lecanora frustulosa	Antibacterial,	1994; Kosanic <i>et</i>
		Antifungal	<i>al.</i> , 2010)
Emodin	Caloplaca schaereri	Antibacterial,	(Manojlovic <i>et al.</i> ,
		Antifungal	2002)
Ergosterol peroxide	Ochrolechia parella	Anticancer	(Millot <i>et al.</i> , 2007)
Evernic acid	Evernia prunastri	Antifungal,	(Halama and Van
		Antioxidant,	Haluwin, 2004;
		Anticancer	Kosanic <i>et al.</i> ,
			2013)
Fallacinal	Caloplaca schaereri	Antibacterial,	(Manojlovic <i>et al.</i> ,
		Antifungal	2002)
Teloschistin	C. schaereri	Antibacterial,	(Manojlovic <i>et al.</i> ,
(fallacinol)		Antifungal	2002)
Fumarprotocetraric	Cladonia rangiferina	Antibacterial,	(Yilmaz <i>et al.</i> , 2004;
acid	C. furcate	Antifungal,	Rankovic and Mišic,
	C. foliacea	Antioxidant,	2008; Kosanic <i>et</i>
		Anticancer	<i>al.</i> , 2014)

Lichen compounds	Lichen species	Bioactivity	References	
Gyrophoric acid	Lobaria pulmonaria,	Light screening	(Kumar and Müller,	
	Lassalia pustulata	pigments,	1999; Candan <i>et al.</i> ,	
	Parmelia nepalensis,	Cytotoxicity	2006; McEvoy <i>et al.</i> ,	
	P. tinctorum,	activity	2007; Burlando <i>et</i>	
	Xanthoparmelia,	Anticancer,	<i>al.</i> , 2009)	
	pokornyi	Antibacterial,		
		Antifungal		
Homosekikaic acid	Ramalina roesleri	Antioxidant,	(Sisodia <i>et al.</i> , 2013)	
		Antibacterial		
Hypostatic	Parmotrema	Antibacterial	(Honda <i>et al.</i> , 2010)	
	sphaerospora			
Imbricaric acid	Cetrelia	Anti-inflammatory	(Lopes <i>et al.</i> , 2008;	
	monachorum		Oettl <i>et al.</i> , 2013)	
Lecanolic acid	P. tinctorum,	Antitumour,	(Rankovic and Mišic,	
	Ochrolechia	Antioxidant,	2008; Bogo <i>et al.</i> ,	
	androgyna	Antibacterial,	2010; Honda <i>et al.</i> ,	
		Antifungal	2010)	
Lichexanthone	Pyxine consocians	Larvicidal	(Kathirgamanathara	
		activity, Muman	<i>et al.</i> , 2006)	
		sperm motility		
		activity		
Lobaric acid	Stereocaulon	Antibacterial,	(Ingólfsdóttir <i>et al.</i> ,	
	alpinum	Anticancer	1998; Bucar <i>et al.</i> ,	
			2004)	
Melanin	Lobaria pulmonaria	Light screening	(McEvoy <i>et al.</i> ,	
		pigments	2007)	

Lichen compounds	Lichen species	Bioactivity	References
Napthoquinones	Astrothelium sp.	Antibacterial	(Sun <i>et al.</i> , 2010)
	(mycobiont)		
Naphthazarin	Lecanora	Cytotoxic activity	(Ernst-Russell <i>et al.</i> ,
	hybocarpa		1999)
Norstictic acid	<i>Ramalina</i> sp.	Antibacterial,	(Tay <i>et al.</i> , 2004;
	R. furinacea	Antifungal	Honda <i>et al.</i> , 2010)
Olivetoric acid	Pseudevernai	Antibacterial,	(Türk <i>et al.</i> , 2006)
	furfuracea	Antifungal	
Orcinol	Umbilicaria	Anti-	(Kim <i>et al.</i> , 1996;
	esculenta,	inflammatory,	Lopes <i>et al.</i> , 2008)
	Parmotrema	Antioxidant	
	tinctorum		
Orsellinic acid	P. tinctorum	Antioxidant	(Lopes <i>et al.</i> , 2008)
Pannarin	Erioderma chielense	Antioxidant,	(Hidalgo <i>et al.</i> ,
	Sphaerophorus	Anticancer	1994; Russo <i>et al.</i> ,
	globosus		2008)
Parietin	Laurera	Antibacterial,	(Fazio <i>et al.</i> , 2007;
	benguelensis,	Antifungal,	Vasiljevic <i>et al.</i> ,
	Caloplaca schaereri	Antiviral,	2009; Manojlovic <i>et</i>
	Xanthoria parietina	Anticancer	<i>al.</i> , 2010b; Basile <i>et</i>
	Teloschistes		<i>al.</i> , 2015)
	chrysophthalmus		
	(mycobiont)		
Parietinic acid	Caloplaca schaereri	Antibacterial,	(Manojlovic <i>et al.</i> ,
		Antifungal	2002)
Perlatolic acid	C. monachorum	Anti-inflammatory	(Oettl <i>et al.</i> , 2013)

Lichen compounds	Lichen species	Bioactivity	References	
Physodic acid	Pseudoevernia	Antibacterial,	(Türk <i>et al.</i> , 2006;	
	furfuraceae	Antifungal,	Kosanic <i>et al.</i> ,	
	Hypogymnia	Antioxidant,	2013; Rankovic <i>et</i>	
	physodes	Anticancer	<i>al.</i> , 2014)	
Protocetraric acid	Parmelia caperata	Antibacterial,	(Tay <i>et al.</i> , 2004;	
	Parmotrema	Antifungal,	Rankovic and Mišic,	
	dilatatum	Antioxidant,	2008; Honda <i>et al.</i> ,	
	Ramalina farinacea	Anticancer	2010; Manojlovic <i>et</i>	
			<i>al.</i> , 2012)	
Protolichesterinic	Cetraria islandica	Antibacterial,	(Ingólfsdóttir <i>et al.</i> ,	
acid	C. aculeata	Anticancer	1998; Türk <i>et al.</i> ,	
			2003; Bucar <i>et al.</i> ,	
			2004)	
Ramalin	Ramalina terebrata	Antibacterial	(Paudel <i>et al.</i> ,	
			2010)	
Resorcinol	Parmotrema	Antioxidant	(Lopes <i>et al.</i> , 2008)	
	tinctorum			
Salazinic acid	Bulbothrix	Antibacterial,	(Ingólfsdóttir <i>et al.</i> ,	
	Setschwanensis,	Antifungal,	1998; Behera and	
	Parmelia saxatilis	Antioxidant,	Makhija, 2002;	
		Antityrosinase,	Manojlovic <i>et al.</i> ,	
		Anti-xanthine	2012)	
		oxidase		
		Anticancer		
Secalonic acid	Pseudoparmelia	Antibacterial,	(Honda <i>et al.</i> , 2010)	
	sphaerospora	Antifungal		

Lichen compounds	Lichen species	Bioactivity	References
Sekikaic acid	Ramalina roesleri	Antioxidant,	(Sisodia <i>et al.</i> ,
		Antibacterial	2013)
Sphaerophorin	Sphaerophorus	Anticancer	(Russo <i>et al.</i> , 2008)
	globosus		
Stenosporic Acid	Xanthoparmelia	Antifungal,	(Candan <i>et al.</i> ,
	pokornyi	Antibacterial	2006)
Stictic acid	Usnea articulata	Antioxidant	(Lohézic-Le
			Dévéhat <i>et al.</i> ,
			2007)
Tenuiorin	Lobaria linita	Anti-	(Ingólfsdóttir <i>et al.</i> ,
		lipoxygenase	2002)
Umbilicaric acid	<i>Umbilicaria</i> sp.	Antioxidant,	(Buçukoglu <i>et al.</i> ,
		Antimicrobial	2013)
Usnic acid	Usnea diffracta	Antiviral,	(Okuyama <i>et al.</i> ,
	Parmelia saxatilis	Antibacterial,	1995; Pramyothin <i>et</i>
	Ramalina farinacea	Antifungal,	<i>al.</i> , 2004; Tay <i>et al.</i> ,
	R. nervulosa	Antipyretic,	2004; Fazio <i>et al.</i> ,
	R. pacifica	Analgetic,	2007; Rankovic and
	R. celastri	Anti-	Mišic, 2008;
	(mycobiont)	inflammatory,	Burlando <i>et al.</i> ,
	Hypogymnia	Aepatotoxic,	2009; Honda <i>et al.</i> ,
	physodes	Glucosidase	2010; Verma <i>et al.</i> ,
	Xanthoparmelia	inhibitor	2012; Huang <i>et al.</i> ,
	somloensis		2014; Rankovic <i>et</i>
			<i>al.</i> , 2014)

Lichen compounds	Lichen species	Bioactivity	References	
Variolaric acid	Ochrolechia parella	Anticancer	(Millot <i>et al.</i> , 2007)	
Vicanicin	Psoroma pallidum,	Anticancer	(Brisdelli <i>et al.</i> ,	
	P. pulchrum		2013)	
Vulpinic acid	Alectoria	Antifungal	(Lauterwein <i>et al.</i> ,	
	ochroleuca,	activity,	1995; Burlando <i>et</i>	
	Letharia vulpina	Anticancer	<i>al.</i> , 2009)	
Zeorin	Parmeliopsis	Antioxidant,	(Kosanic <i>et al.</i> ,	
	hyperopta	Antibacterial,	2010)	
		Antifungal		



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

## 2.6 Family Trypetheliaceae

Trypetheliaceae is the oldest lichen family (Goebel and Kunze, 1827), classified into class Dothideomycetes, order Trypetheliales, which a pyrenocarpous crustose and epiphytic lichen, occurring worldwide distribution in tropical habitats as grown on bark or rarely on bryophytes over soil. This family was characterized by thallus crustose, ecorticate to corticate, white to yellow-brown to olive-green color, sometime bright yellow, and orange or red of anthraquinone pigment on the thallus surfaces. Photobiont is Trentepohlia. Ascomata as perithecia formed inside pseudostrama tissues or neck and totally black-cabornized, monocarpic to polycarpic aggregate or solitary, single or fused ostiole. Hamathecium consists of prosoplectenchymatous hyphae, hyaline paraphysis, branched and anastomosing, sometimes inspersion with oil hyaline or yellow. Asci: bitunicate, obclavate to cylindrical, non-amyloid, 1-8 ascospores per ascus. Ascospore: fusiform-ellipsoid to oblong, hyaline to dark brown, transversally septate to muriform, septate locule usually round and sometime rectangular to diamondshaped lumina. Chemistry: thallus surface contained lichexanthone (1,8-dihydroxy-3,6dietnoxyxanthone) or anthraquinone such as parietic and perylenequinone in medulla layer (Harris, 1984; Harris, 1995; Del Prado et al., 2006; Aptroot et al., 2008; Hyde et al., 2013). In currently, Trypetheliaceae is recorded approximately 192 species and including 13 genera are accepted as follow; Aptrootia, Arcthitrypethelium, Ascocratera, Astrothelium, Bathelium, Campylothelium, Cryptothelium, Exiliseptum, Laurera, Marcelaria, Polymeridium, Pseudopyrenula and Trypethelium (Harris, 1984; Del Prado et al., 2006; Kirk et al., 2008; Aptroot et al., 2013). Few taxonomic studies and diversity of this family have been reported in Asia. Five genera and 45 species were reported in India, Nepal and Sri Lanka (Awasthi, 1991), whereas 6 genera and 33 species were found in Thailand (Vongshewarat, 2000). Recently, Trypetheliaceae was studied on phylogeny with small specimens in South America that showed monophyletic family within Dothideomycetes, which some genera form polyphyletic within the family (Del Prado et al., 2006; Nelsen et al., 2014).

For secondary metabolites, Trypetheliaceae produces the major groups of substance as anthraquinone and xanthone (Figure 9), which exhibit bioactivities for antibacterial, antifungal, anticancer, antioxidant, anti-inflammatory and enzyme inhibition properties (Mathey, 1979; Manojlovic et al., 2002; Vasiljevic et al., 2009; Manojlovic et al., 2010a; Verma et al., 2012). Lichexanthone is one of common xanthone that occured on thallus of this lichen family such as M. benguelensis and Astrothelium species (Aptroot et al., 2008; Manojlovic et al., 2010a). Anthraquinone group is found on thallus and pseudostroma such as parietin (yellow pigment), secalonic acid and haematommone (Harris, 1984; Mathey et al., 2002; Manojlovic et al., 2010a), which presents in common lichens M. benguelensis and T. eluteriae (Mathey, 1979; Makhija and Patwardhan, 1993; Vasiljevic et al., 2009) and perylenequinone group (isohypocrelline) was found in L. sunguinaria (Mathey et al., 1994). Naphthoquinones and dirivertives were found from mycobiont culture of Astrothelium sp. and T. eluteriae, while phenalenone dirivertives produced from Trypethelium sp. culture on malt-yeast extract medium (Mathey et al., 1980; Sun et al., 2010; Takenaka et al., 2013). The dirivertives of naphthoquinone from Astrothelium sp. show the antibacterial activity with Gram positive bacteria (Sun et al., 2010).

Although, the Trypehteliaceae is common lichen in tropical areas, a few reports have been studied in Southeast Asia (Vongshewarat, 2000; Aptroot *et al.*, 2007). This family was mostly investigated in South America with representative species and main focus on taxonomy (Harris, 1984; Harris, 1995; Aptroot *et al.*, 2008), while less reported in Asia not only taxonomy and molecular phylogeny but also bioactivities, especially in Thailand (Vongshewarat *et al.*, 1999; Vongshewarat, 2000; Aptroot *et al.*, 2007).



Figure 9 The chemical structure of anthraquinone and xanthone group.A. emodin, B. lichexanthone, C. parietin, and D. secalonic acid D. (Manojlovic *et al.*, 2010a)

## CHAPTER III

## MATERIALS AND METHODS

## 3.1 Instruments used in this study.

- Rotary evaporator (Model R, BÜCHI, Switzerland)
- Stainless steel beads (2.3 mm, BioSpec Products, Inc.)
- Compound microscope (BX41, Olympus optical Co., Ltd. Japan)
- Stereo microscope (SZ11, Olympus Co., Ltd. Japan)
- Differential interference contrast (DIC) microscopy (Olympus U-DICT)
- Camera (Canon EOS 650)
- Gel Documentation system (Model ECX-26.MX, Vilber Lourmat, France)
- 2-Digit and 4-Digit precision weighting balance (Model AG204, Mettler Toledo, Switzerland)
- Vortex mixer (VX-100, Labnet Internation, Inc.)
- Minispin microcentrifuge (Eppendorf)
- Micropipette P2-P1000 (Eppendorf)
- Autoclave (Model SS-325, Tomy Seiko Co., Ltd. Japan)
- Hot air oven (Model D06063, Memmert)
- Lamina flow (Model H1, Lab Service, Ltd. Thailand)
- 4 °C and -20 Refrigerators
- Incubator shaker
- pH meter
- DNA thermo cycle (Model TP600, TaKaRa Bio Inc., Otsu, Shiga, Japan)
- Parafilm (Lab M)
- Filter papers Whatman No.1 (GE Healthcare Life Sciences, Inc., Uk)
- Microtubes (0.2 and 1.5 ml) (Axygen Scientific, Inc. USA)
- Electrophoresis chamber set (Mupid-ex, Bruker BioSpin Inc., Switzerland)
- Thin Layer Chromatography (TLC) plate (Merck Millipore, Inc. USA)

## 3.2 Chemicals used in this study.

- Tris (hydroxymethyl) aminomethane
- Boric acid  $(H_3BO_3)$
- Ethylenediamine tetraacetic acid (EDTA) (Scharlau)
- Cetyltrimethylammonum bromide (CTAB) (Serva)
- Hydrochloric acid (HCI) (Merck, Germany)
- Isopropanol alcohol (Merck, Germany)
- Isoamyl alcohol (Carlo Erba)
- Chloroform
- Malt extract (Difco)
- Yeast extract (Difco)
- Polyvinylpyrrolidone
- Pfu DNA polymerase (Thermo)
- DNA Stain G (Serva)
- 2,2-diphenyl-1-picrylhydrazyl (DPPH)
- Butylated hydroxyanisole (BHA)
- Ethanol
- Methanol
- n-Hexane
- Dichloromethane

## 3.3 Taxon sampling and specimens preparation

Total lichen specimens in family Trypetheliaceae were collected on bark from various locations in Thailand. Before colleting, each lichen thallus was simply observed under 10x-40x hand magnifying lens for checking the fruiting bodies, after that the thallus was cut down into pieces containing perithecia approximately 3-5 cm per piece and depth 0.2-0.3 cm from thallus surface. Each specimen was wrapped by the tissue paper and recorded for their detail of study site. All lichen specimens were air dried at room temperature for 24 hours, recorded for code and details, and then enveloped in paper bag and store at 4 °C.

## 3.4 Mycobiont isolation and cultivation

Mycobionts were isolated from lichen thalli using ascospore discharge techniques (Sangvichien *et al.*, 2011). A small piece of each lichen sample that contain perithecia (0.5 x 0.5 cm) was attached with petroleum jelly on upper cover of petri dish, after that upside down the petri dish of water agar medium (WA) (Appendix A) and then incubated at room temperature for about 24 hours. Ascospores discharged on WA medium, which were observed and selected by stereo microscope and transferred to Malt-Yeast Extract medium (MYA) (Appendix A). The ascospores were incubated at room temperature for 9-12 weeks, until ascospore geminated and mycobiont colony developed. Their mycobiont colonies were prepared for DNA isolation and secondary metabolites extraction.

## 3.5 Taxonomic study and lichen identification

Lichen taxonomic was studied based on morphological and chemical characters. Macroscopic morphology was investigated on thallus character, color of thallus, sexual reproductive structures (perithecia), pattern of perithecia and ostiole under stereo microscope (Olympus SZ11). For microscopic examination, lichen thallus and perithecia were cross section by razor blade to observe thallus layer, perithecia ostiole and ascospore characters such as number per ascus, color, septate, size and shape of ascospores, which their importance to delimited genera (Aptroot, 2009b). The ascospore pictures were recorded by digital camera (Canon EOS650), which connected to the Olympus BX41 compound microscope with differential interference contrast (DIC) (Olympus U-DICT). Chemical character was determined the reaction by spot test (Hale, 1979) using 10% Potassium hydroxide (KOH) solution with thallus and pseudostroma, and TLC with solvent system A and C (Lumbsch, 2002). All of taxonomic characters were used to compare with classical keys for delimited lichen species.

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

## 3.6 Molecular study

## 3.6.1 DNA extraction

The mycobionts colony and lichen thalli of representative of each species were extracted total genomic DNA by using CTAB precipitation protocol (Cubero and Crespo, 2002). Firstly, prepare 20-50 mg of the sample in plastic tube 2.0 ml with 10 stainless steel beads (1 mm) and then dipped it in liquid nitrogen for 1 min after that 2 min grinded by using Mixer MM 400 at 30 hertz. Next, the homogenized sample was added 400 µl of CTAB extraction buffer (Appendix B) and 100 µl of 5% (w/v) PVPP (Polyvinylpolypyrrolidone) incubated at 70 °C for 30 mins and then added 500 µl of choloform / isoamyl alcohol (24:1) (Appendix B), mixed by vigorous hand shaking and centrifuge at 10000 G for 5 min at room temperature. After centrifuged, transfer the aqueous phase to new 1.5 ml plastic tube and 3 fold-diluted with CTAB precipitation buffer (Appendix B), centrifuged at 10000 G for 5 mins at room temperature after that eliminate the aqueous phase. The pellet was dissolved by 25 µl of 1.2 M NaCl, 3 µl of 10x RNAase buffer and 2 µl RNAase A (10mg/ml), vortexed and incubated at 37 °C for 30 mins, then add 370 µl of 1.2 M NaCl. The end of this process, plastic tube was repeated chloroform purification step by adding 500 µl of choloform / isoamyl alcohol (24:1), vortexed and centrifuge at 10000 G for 5 min at room temperature. The supernatant was transferred to a new 1.5 ml plastic tube. The DNA was alcohol precipitated by 0.6 times of isopropanol (300 µl if you have recovered 500 µl of supernatant) and centrifuged at 13000 G for 15 min at 4 °C, then discard the aqueous phase. The pellet was washed by adding 500 µl of 70% ethanol and centrifuged at 13000 G for 3 min at 4 °C, after that eliminate the supernatant. Finally, dry DNA pellet at room temperature for 30-60 min, then dissove in 50 µl of TE buffer and stored at -20 °C until use.

## 3.6.2 Polymerase chain reaction (PCR), amplification and DNA sequencing

Genomic DNA was amplified in four loci: ITS, nuLSU, mtSSU and RPB1, using primer pairs and sequences of each primer as shown in Table 4. The PCR amplifications were performed in 50  $\mu$ l containing a reaction mixture of 5  $\mu$ l 10x *Pfu* Buffer with MgSO<sub>4</sub>, 2 mM of dNTP mix, 20  $\mu$ M of each primer, 1.25 U of *Pfu* DNA Polymerase (Thermo Fisher Scientific Inc.), and 5  $\mu$ l of DNA solution (10 fold-dilution). The reactions were carried out in a thermal cycler TP600 (Takara Shuzo Co., Tokyo) and performed using the following program: initial denaturation for 1 min at 94 °C and 38 cycles of 94 °C for 1 min, 51 °C for 1 min (ITS1F/ITS4), 52 °C for 45 sec (LR0R/LR3), 53 °C for 45 sec (mrSSU1/MSU7) and 52 °C for 1.30 min (RPB1-Af/RPB1-Cr), followed by extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were checked by 1% agarose gel electrophoreses with 1x TBE buffer and 1  $\mu$ l of DNA stain clear G per 100  $\mu$ l agarose gel for 45 min. The size of DNA products were compared to DNA standard 100 bp DNA ladder by Gel Documentation at 312 nm. The products were cleaned by Gel/PCR DNA Fragments Extraction Kit (Genaid, Taiwan) according to the manufacturer's instructions. PCR products were DNA sequenced services (1st BASE Laboratories, Malaysia).

## 3.6.3 Basic Local Alignment Search Tool (Blast) and nucleotide submission

Total nucleotide sequences were compared to similarity with GenBank databases by Blast program in NCBI (www.ncbi.nlm.nih.gov), which setting for nucleotide collection database with other and somewhat similar sequences (Blastn). Each of DNA sequence was recorded for total score and percent identity blast from the highest value. Then, nucleotide sequences were submitted in DDBJ (www.ddbj.nig.ac.jp).

 Table 4 Primers for nucleotide amplification in this study.

D Io	NA oci	Primer name	Types	Sequences (5'->3')	References
ľ	TS	ITS1F	forward	CTTGGTCATTTAGAGGAAGTAA	(Gardes and
					Bruns, 1993)
		ITS4	reverse	TCCTCCGCTTATTGATATGC	(White <i>et al.</i> ,
					1990)
nu	LSU	LR0R	forward	ACCCGCTGAACTTAAGC	(Vilgalys and
					Hester, 1990)
		LR3	reverse	GGTCCGTGTTTCAAGAC	(Vilgalys and
					Hester, 1990)
mt	SSU	mrSSU1	forward	AGCAGTGAGGAATATTGGTC	(Zoller <i>et al.</i> ,
					1999)
		MSU7	reverse	GTCGAGTTACAGACTACAATCC	(Zhou and
					Stanosz, 2001)
RF	PB1	RPB1-Af	forward	GARTGYCCDGGDCAYTTYGG	(Matheny <i>et al.</i> ,
					2002)
		RPB1-Cr	reverse	CCNGCDATNTCRTTRTCCATRTA	(Matheny <i>et al.</i> ,
			CHULALO	DNGKORN UNIVERSITY	2002)

#### 3.6.4 Nucleotide sequence alignments

The sequences data sets were aligned separately each single genes and combines four loci (ITS, nuLSU, mtSSU and RPB1) using Clustal W (Thompson *et al.*, 1994), using outgroups from GenBank as *Capnodium coffeae* (DQ491515, FJ190609, DQ471162, KF902173), *Dothidea insculpta* (AF027764, DQ247802, FJ190602, DQ471154) and *Pyrgillus javanicus* (KT820171,KT808612, KT808549, DQ842010). All outgroups were selected from a member of class Dothideomycetes and Chaetothyriomycetes, which related to Trypetheliaceae (Trypetheliales) (Del Prado *et al.*, 2006; Nelsen *et al.*, 2009; Nelsen *et al.*, 2014). The alignments were manually improved using MEGA v.6 software (Tamura *et al.*, 2013).

## 3.6.5 Phylogenetic analysis of lichen-forming fungi Trypetheliaceae

Total DNA data sets (ITS, nuLSU, mtSSU and RPB1) were calculated for nucleotide substitution models. The model was chosen by using jModelTest v.2.1.4 (Darriba et al., 2012) with the Akaike Information Criterion (AIC). The best-fit model was set for phylogeny program analysis. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). Before analysis, Nucleotide sequences alignment data were converted to PHYLIP and NEXUS format for ML and BI analysis, respectively. The ML analysis was performed using the program RAxML v.8 (Stamatakis, 2006; Stamatakis et al., 2008; Stamatakis, 2014), bootstrap values were calculated using 1,000 pseudoreplicates and specified setting for outgroups. The BI tree and posterior probabilities were calculated using MrBayes v.3.2.1 (Ronquist and Huelsenbeck, 2003). Four independent runs were performed Markov chain Monte Carlo (MCMC) algorithms with 10,000,000 generations and discarded 0.25 burn-in first period. The nucleotide substitution model was same as in the ML analysis. The options were set as stoprule and aborting the analyses at the average standard deviation of split frequencies of 0.01. Every one hundred tree was saved into a file. Both of phylogenetic trees were viewed using FigTree v.1.3.1 (http:// tree.bio.ed.ac.uk/software/figtree/).

## 3.7 Chemical study

## 3.7.1 Mycobiont extraction

The colonies of representative species were prepared by making them into small pieces. These samples were extracted by three solvents from non-polar to polarity as n-hexane, dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) and methanol (CH<sub>3</sub>OH). The sample was dissolved in solvent volume as ratio 1:1 and incubated at room temperature for 24 hours, after extraction samples were changed to more polarity. Each solvent extraction was filtrated through-filter paper (Whatman No.1) and evaporated by rotary evaporator at 40 °C until solvent dried. The crude extracts were recorded for the dry weight and kept at -20 °C until use.

## 3.7.2 Secondary metabolites analysis

Crude extracts were dissolved by one milliliter of each solvent as dichloromethane and methanol. The samples were dropped 20  $\mu$ l on thin layer chromatography (TLC) plate, which crude dichloromethane extract and methanol extract were developed by solvent system as dichloromethane : ethyl acetate (7 : 5) and dichloromethane : methanol (100 : 4), respectively. The TLC plate was detected under UV light at 254 and 356 nm, then recorded the secondary metabolite profiles and calculated for retention factor value (Rf). The negative control was using to the solvent for dissolve crude extracts.

#### 3.8 Antimicrobial activity determination

#### 3.8.1 Microbial preparation

Three microorganisms were selected for bioautography test to Gram negative bacteria used *Escherichia* coli (ATCC25922), Gram positive bacteria used *Staphylococcus aureus* (ATCC25923) and yeast used *Candida albicans* (ATCC10231). Bacteria was prepared by steak plate on Nutrient Agar (NA) (Appendix A) and incubated at 37 °C for 24 hour. After that, single colony on NA plate was inoculated 50 ml of Nutrient Broth (NB) (Appendix A), then incubated in shaker at 37 °C for 24 hour. *Candida albicans* was prepared similar to previously bacteria preparation (24 hour), which different for cultured on Malt-Yeast Extract Agar (MYA) (Appendix A).

#### 3.8.2 Bioautography examination

Antimicrobial activities (bacteria and yeast) were tested from TLC direct bioautography method as described by (Zitouni *et al.*, 2005). The crude extracts were loaded and separated on TLC plate, until the plate is dry and recorded Rf values under ultraviolet light (253 and 365 nm). After that, prepare the Petri dish of Mueller-Hinton Agar and MYA medium (Appendix A) for testing bacteria and yeast, respectively. The TLC plates were placed down on their culture medium, then covered the top of TLC plate by warm semi solid medium (42-45 °C), which mixed with each of test microorganisms until have equal to 0.5 McFarland standard. The Petri dish sets were incubated at 37 °C for 18-24 hour, then was stainned the medium surface by lactophenol trypan blue. The activity was determined by comparison to clear zone and Rf values of secondary metabolites profile.

## 3.9 Antioxidant activity detection

Secondary metabolites of lichen compounds were determined for inhibition of oxidation property by TLC direct bioautography method (Bhattarai *et al.*, 2008). Crude extract of each lichen species were loaded and developed on TLC plate, with similar to solvent system as previously described in the step of secondary metabolites studied. The TLC plate was observed for Rf values under ultraviolet light (253 and 365 nm) and kept until the plate is dry. After that, the TLC plate was sprayed on the surface by 0.05% of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution and incubated at room temperature for 10 min. The standard of antioxidant used as 0.5 % Butylated hydroxyanisole (BHA) and blank TLC plate as negative control. The positive action was detected by the color of DPPH changes to yellow spot, and compared Rf values of chemical profile on TLC spot.

Chulalongkorn University

# CHAPTER IV RESULTS

## 4.1 Taxon sampling

The lichen thallus was observed on simple macroscopic morphology by magnifying glass in the field trip. Nine hundred and sixty-five lichen thalli were collected from various localities in Thailand consisting of twenty-eight study sites in twenty-four provinces (Figure 10) that included different types of forests such as tropical rain forest, hill evergreen forest, dry evergreen forest, dry dipterocarp forest, mixed deciduous forest and mangrove forest.



Figure 10 The map of lichen collection sites in Thailand.

Figure 10 (continued).

- 1. Doi Chiang Dao Wildlife Research Station (Chiang Mai)
- 2. Doi Suthep-Doi Phi National Park (Chiang Mai)
- 3. Doi Khun Tan National Park (Lamphun)
- 4. Wiang Sa district (Nan)
- 5. Saritsena camp (Phitsanulok)
- 6. Phu Hin Rong Kla National Park (Phitsanulok)
- 7. Thung Salaeng Luang National Park (Phitsanulok)
- 8. Phetchabun Rajabhat University (Phetchabun)
- 9. Phra That Si Song Rak temple (Loei)
- 10. Phu Luang Wildlife Sanctuary (Loei)
- 11. Pa Hin Ngam National Park (Chaiyaphum)
- 12. Nam Pung dam (Sakon Nakhon)
- 13. Phu Chong Nayoi National Park (Ubon Ratchathani)
- 14. Umphang district (Tak)
- 15. Si Sawat district (Kanchanaburi)
- 16. Sai Noi district (Nonthaburi)
- 17. Khao Yai National Park (Nakhon Ratchasima)
- 18. Thap Lan National Park (Prachinburi)
- 19. Khao Soi Dao Wildlife Santuary (Chanthaburi)
- 20. Koh Chang island (Trat)
- 21. Koh Samae San island (Chonburi)
- 22. Suan Phueng district (Ratchaburi)
- 23. Cha-Am district (Phetchaburi)
- 24. Pala-U waterfall (Prachuap Khiri Khan)
- 25. Mueang Ranong district (Ranong)
- 26. Si Phang-nga National Park (Phang Nga)
- 27. Chawang district (Nakhon Si Thammarat)
- 28. Khaopra-Bangkhram Wildlife Sanctuary (Krabi)

## 4.2 Mycobiont isolation and cultivation

The lichen mycobionts of Trypetheliaceae were isolated using lichen ascospore discharge (Sangvichien *et al.*, 2011). The multiple ascospores were geminated and cultivated on MYA medium. Three hundred and thirteen mycobionts were successful for isolation and colony development (Table 5). Ascospore germination and colony development of isolated mycobiont growth on MYA medium were shown in Figure 11.

 Table 5 The information of study sites, number of lichen samples, number of isolates

 and the number of mycobiont isolates.

Collection sites	Code	Number of	Number of	Mycobiont isolates	
	- Januar	samples	isolates		
				CP1, 5, 48, 54, 69, 70,	
Chaiyaphum:				72, 73, 74, 78, 79, 81,	
Pa Hin Ngam National	CP	101	21	86, 89, 98, 100, 111,	
Park				112 113 110 123	
Chanthaburi:				112, 113, 113, 123	
Khao Soi Dao Wildlife	CBR	4	4	CBR12, 13, 16, 51	
Santuary					
Chiang Mai:					
Doi Suthep-Doi Phi	СМ	32	6	CM156, 159, 161, 168,	
National Park				190, 192	
Chiang Mai:					
Doi Chiang Dao Wildlife	DCD	22	11	DCD2, 3, 4, 5, 7, 11,	
Research Station				12, 19, 20, 94, 95	
Chonburi:	0140	0	F		
Koh Samae San island	5IVI5	9	5	SM57, 17, 72, 73, 74	
Kanchanaburi:					
Khao Nam Phu Wildlife					
Conservation and	KJB	33	6	кјв1, 2, 62, 70, 72, 74	
Development Center					

Collection sites	Code	Number of samples	Number of isolates	Mycobiont isolates
				KRB36, 42, 58, 59, 72,
				74, 75, 76, 78, 79, 80, 81,
Krabi:				82, 83, 84, 87, 91, 99,
Khaopra-Bangkhram	KRB	106	34	100, 105, 106, 107, 118,
Wildlife Sanctuary				125, 128, 139, 155, 158,
				172, 176, 177, 179, 183,
				203
				DKT30, 35, 36, 42, 45,
Lamphun:				48, 54, 58, 66, 67, 71, 73,
Doi Khun Tan National	DKT	36	25	82, 87, 92, 94, 95, 98,
Park.				104, 105, 108, 109, 110,
				115, 116
Loei:				
Phra That Si Song Rak	จุหกลง	6 4 6	Man 3	L45, 48, 52
temple				
Loei:				
Phu Luang Wildlife	PHL	77	11	PHL4, 7, 20, 53, 61, 62,
Sanctuary				89, 119, 128, 146, 191
				KY11, 17, 52, 76, 354,
				418, 472, 517, 655, 710,
				716, 743, 759, 777, 780,
Nakhon Katchasima:	KY	77	31	781, 783, 784, 803, 808,
Mao rai Nalional Park				811, 812, 814, 832, 835,
				838, 839, 842, 845, 848,
				853, 857

**Table 5** (continued). The information of study sites, number of lichen samples, numberof isolates and the number of mycobiont isolates.

Collection sites	Codo	Number of	Number of	Mycobiont isolates	
	Code	samples	isolates	Wycobiont isolates	
Nakhon Si Thammarat:	NCD	0	7	NSR6, 14, 16, 17, 34,	
Chawang district	NON	9	I	54, 57	
				NAN5, 9, 16, 18, 23,	
New				25, 39, 50, 59, 71, 72,	
Nan:		07	00	76, 86, 90, 93, 79, 95,	
Lai-Ivan Sub-district,	NAN	97	28	104, 118, 119, 124,	
Wiang Sa district				126, 127, 129, 130,	
				131, 143, 146	
Nonthaburi:					
Sai Noi district	NBR	1	1		
Phang Nga:					
Si Phang-nga National	PNG	13	5	PNG1, 2, 3, 29, 61	
Park					
Phetchabun:					
Phetchabun Rajabhat 🕞	PB	GKOP11 UNIN	ERST4	PB20, 24, 25, 45	
University					
Phetchaburi:					
Huai Ta Paet reservoir,	PBR	25	8	PBRZ, 3, 4, 5, 27, 28,	
Cha-Am district				24, 31	
Phitsanulok:					
Phu Hin Rong Kla	HRK	3	2	HRK42, 93, 98	
National Park					
Phitsanulok:					
Saritsena camp	PL	18	3	PL35, 45, 99	

**Table 5** (continued). The information of study sites, number of lichen samples, numberof isolates and the number of mycobiont isolates.

Collection sites	Number of Num Code samples isc		Number of isolates	Mycobiont isolates	
<b>Prachinburi:</b> Thap Lan National Park	TLN	3	2	TLN3, 19	
Prachuap Khiri Khan: Pala-U waterfall	PJK	25	10	PJK8, 9, 14, 15, 16, 17, 18, 20, 21, 24	
Ranong: Mueang Ranong district	RN	7///	3	RN26, 55, 104	
Ratchaburi: Suan Phueng district	SP	10	5	SP46, 118, 119, 121, 124	
<b>Sakon Nakhon</b> : Nam Pung dam	SNK	13	7	SNK1, 8, 15, 31, 33, 36, 39	
<b>Tak:</b> Doi Hua Mot, Umphang district	TAK	53	8	TAK8, 12, 17, 28, 32, 34, 49, 55	
Trat: Koh Chang island	TRA	28	9 9 VERSITY	TRA91, 95, 97, 98, 102, 105, 119, 126, 127	
<b>Ubon Ratchathani:</b> Phu Chong Nayoi National Park	UBN	93	39	UBN13, 33, 35, 37, 43, 46, 86, 90, 98, 100, 107, 111, 113, 116, 127, 130, 133, 137, 144, 146, 147, 150, 153, 157, 158, 165, 166, 170, 180, 185, 194, 212, 214, 220, 223, 224, 227, 228, 230	
Total 965 313					

**Table 5** (continued). The information of study sites, number of lichen samples, numberof isolates and the number of mycobiont isolates.



Figure 11 Development of ascospore and formation of mycobiont colony on MYA medium.

A. ascospore germ tube elongation, B. small mycobiont colony development after 1-2 weeks, C. the mycobiont colony formation after 4 weeks and D. mature mycobiont colonies after 9 weeks.

## 4.3 Taxonomic study and identification

## 4.3.1 Lichen taxonomy

The lichen family Trypetheliaceae in this study was investigated based on morphological characters of thallus, perithecia, ascospores and spot test (10% KOH), which could be classified into eight genera in Thailand. Taxonomic characters of each genus are as follows:

## 4.3.1.1 Astrothelium

Thallus corticated, green to yellow, usually smooth or bullate. Ascomata solitary or aggregate in pseudostromata, raised or immersed in thallus, sometimes contained yellow to orange pigment, KOH+ red color. Perithecia shared ostiloes, apical. Hamathecium is not inspersed or inspersed with oil droplets, hyaline and anastomosing. Ascospores 8 spore per ascus, hyaline, transversely septates, 3-10 septates, thickwalled and lumina usually with diamond shaped (Figure 12, A-B).

## 4.3.1.2 Bathelium

Thallus corticated, green to olive green, smooth or wart. Pseudostroma brownish to dark brown, inside contained brown to yellow pigment, KOH+ orange to dark brown, perithecia apical ostiole, aggregated in pseudostroma tissue. Hamathecium not inspersed, hyaline and anastomosing. Ascospores 8 spore per ascus, hyaline, muriform or transversely septates, 5-7 septates, thick-walled (Figure 12, C-D).

#### 4.3.1.3 Campylothelium

Thallus ecorticate, white, smooth. Pseudostroma solitaly, raised or semiimmersed in thallus, perithecia thick-walled, carbonized, lateral ostioles, KOH negative. Hamathecium not inspersed, hyaline and anastomosing. Ascospores muriform, hyaline, 8 spore per ascus, IKI+ violet, thin-walled (Figure 12, E-F).

#### 4.3.1.4 Laurera

Thallus corticated, olive green to brownish, smooth or wart. Perithecia globose, single, thick-wall, carbonized, apical ostiole, raised or immersed in thallus, pseudostroma presence or absence, black or yellow pigment, KOH positive red or negative. Hamathecium not inspersed or fully inspersed with hyaline oil droplets and anastomosing. Ascospores muriform, hyaline, 2-8 spore per ascus, thick-walled (Figure 12, G-H).

## 4.3.1.5 Marcelaria

Thallus corticated, green, smooth, not contained pruinose or yellow pigment KOH+ red. Pseudstroma irregular, yellow pigment, raised, perithecia globose, apical ostiole, aggregated in pseudostroma, yellow pigment with KOH+ red. Hamathecium not inspersed, hyaline and anastomosing. Ascospores muriform, hyaline, 8 spore per ascus, thick-walled (Figure 12, I-J). In addition, the pseudostroma contains anthraquinone pigment (yellow color) and KOH positive used to delimit the new genus separates from *Laurera* (Aptroot *et al.*, 2013).

#### 4.3.1.6 Polymeridium

Thallus ecorticate, white, without pruinose, smooth. Perithecia solitary, black, globose, thick-wall, carbonized, apical ostiole, raised or immersed in thallus, KOH negative. Hamathecium not inspersed or inspersed, hyaline and anastomosing. Ascospores 8 spore per ascus, hyaline, transversely septates, 3-7 septates, thin-walled (Figure 12, K-L).

## 4.3.1.7 Pseudopyrenula

Thallus ecorticated, white to brown, smooth. Perithecia solitary, black, globose, carbonized, apical ostiole, raised. Hamathecium anastomosing, inspersed with yellow oil droplets, KOH+ red. Ascospores 8 spore per ascus, hyaline, transversely septates, 3 septates, thick-walled (Figure 12, M-N).

### 4.3.1.8 Trypethelium

Thallus corticated, green to yellowish, not contained pruinose or yellow pigment KOH+ red. Perithecia globose, thick-wall, carbonized, apical ostiole, solitary or aggregated in pseudostroma, raised or immersed in thallus. Pseudostroma tissue contained yellow pigment with KOH+ red or without pruinose. Hamathecium not inspersed or inspersed with hyaline oil droplets, anastomosing. Ascospores 8 spore per ascus, hyaline, transversely septates, 3-16 septates, thick-walled and lumina mosly globose shaped (Figure 12, O-P).



Figure 12 Taxonomic characters of each genus within Trypetheliaceae.

(A-B) *Astrothelium*, A. thallus and ascomata with polycarpic, B. mature ascospores with lumina diamond shaped, (C-D) *Bathelium*, C. thallus and ascomata, D. Pseudostroma inside with orange pigment, (E-F) *Campylothelium*, E. ascomata with lateral ostiole, F. mature ascospores with IKI+ violet, (G-H) *Laurera*, G. thallus and ascomata, H. muriform ascospore.



(I-J) *Marcelaria*, I. thallus and ascomata with yellow-orange pigment, J. pseudostroma with KOH+ positive, (K-L) *Polymeridium*, K. thallus and ascomata, L. ascospore thin wall, (M-N) *Pseudopyrenula*, M. thallus and ascomata, N. hamathecium inspersed with yellow oil droplets, (O-P) *Trypethelium*, O) thallus and ascomata, P. transversely septate ascospore and lumina globose shaped.

The morphological characters were different among generic level (Table 6). Genus *Campylothelium* is similar to genera *Polymeridium* and *Pseudopyrenula* by ecorticate thallus character but differ to lateral ostiole (*Polymeridium* and *Pseudopyrenula*, apical ostiole). *Polymeridium* and *Pseudopyrenula* are different to the ascospore wall with thin and thick wall, respectively. Only genus *Bathelium* shows the perithecia wall character with yellow pigment and positive reaction with KOH changes to orange-brown color. The *Astrothelium* is similar to genus *Trypethelium* by thallus corticate, ascospore transeptate and thicked but different by shared perithecia ostiole. The morphological characters among genus *Laurera* and *Marcelaria* are very similar to muriform ascospore and thallus, which only yellow pigment on perithecia (KOH+ red) was found in *Marcelaria*.

Table	6	Comparison	of	the	major	characteristics	for	genus	delimitation	within
Trypet	helia	aceae.								

Genus	Thallus	Ostiole	Ascospore	Ascospore	Spot tested on
	type	type/site	type/wall	septation	perithecia
Astrothelium	corticate	shared/apical	transeptate/ thick	3-10	None/Red
Bathelium	corticate	single/apical	muriform or	5-7	Orange/brown
			transeptate/ thick		
Campylothelium	ecorticate	single/lateral	muriform/ thick	-	None
Laurera	corticate	single/apical	muriform/ thick	-	None
Marcelaria	corticate	single/apical	muriform/ thick	-	Red
Polymeridium	ecorticate	single/apical	transeptate/ thin	3-7	None
Pseudopyrenula	ecorticate	single/apical	transeptate/ thick	3	None
Trypethelium	corticate	single/apical	transeptate/ thick	3-16	None/Red

## 4.3.2 Lichen identification

In this study, Trypetheliaceae was identified to species based on morphological and chemical characters, of which divided into at least 61 species, including 47 species (5 new species and 17 new records) and 14 unidentified species. Representative species at least 1-3 mycobiont isolates or lichen specimens were selected for phylogenetic analysis. Total species were compared to previous reports in Thailand shown in Table 7. The descriptions of sixty-one species were described as follows;

## 1. Astrothelium aenascens Aptroot. (Figure 13, A-B)

Thallus crustose, corticated, greenish grey, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate, immerded in pseudostroma tissue and share with common ostiole. Ostiole apical, black. Pseudostroma raised, contain yellow to orange pigment. Hamathecium hyaline, inspersed with oil droplets, contain crystal, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 24-30 x 9-9.7 µm. Chemistry: Thallus UV+ orange, KOH+ yellow. Pseudostroma UV+ red-orange, KOH+ red. TLC: lichexanthone, parietin. Isolation No.: HRK93, HRK98

# Astrothelium flavocoronatum Luangsuphabool, Aptroot & Sangvichien., sp. nov. (Figure 13, C-D)

Thallus crustose, corticate, yellow to green, smooth. Algae trentepohlioid. Ascomata perithecia, pyriform, carbonized, semi-immersed to emergent, solitary, usually consisting of two cavities that are joined with a common ostiole. Ostiole apical, black, surrounded by yellow layer. Pseudostroma raised above the thallus, covered with thallus cortex or naked and carbonized. Hamathecium hyaline, clear, paraphyses anastomosing, 0.85-1 µm thick. Asci clavate, 105-110 x 18.5-19 µm. Ascospores 8 per ascus, hyaline, transversely 3-septate, narrowly ellipsoid, 22-28 x 8-9.5 µm, lumina diamond-shaped to rounded. Chemistry: Thallus UV-, KOH+ yellow. Pseudostroma around ostiole UV+ orange, KOH+ red. TLC: parietin, emodin. Isolation No.: KY859, TSL63

Etymology. The specific epithet refers to the yellow tissue surrounded ostiole.

Notes. This new species is similar to the neotropical *A. diplocarpum* Nyl. in having anthraquinone pigment suround the ostiole neck, but differs in having smaller ascospores (9-septate, 90-110 x 22-28 µm in *A. diplocarpum*) (Harris, 1995; Aptroot *et al.*, 2008). Also *A. macrocarpum* (Fée) Aptroot & Lücking (*A. galbineum* Kremp.) is similar in having a pseudostroma with anthraquinones and ascospore characters, but differs in having solitary perithecia or two locules embedded in a pseudostroma (2-4 perithecia aggregated in a pseudostroma in *A. macrocarpum*).

# 3. Astrothelium macrocarpum (Fée) Aptroot & Lücking (A. galbineum Kremp.) (Figure 13, E-F)

Thallus crustose, corticate, green to yellow-green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate, immerded in pseudostroma. Ostiole black and share with common ostiole. Pseudostroma raised, contain yellow pigment. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 17-25 x 6.5-8.5 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV+ red, KOH+ red. TLC: lichexanthone, parietin. Isolation No.: NSR6, UBN37, UBN43, UBN113

**CHULALONGKORN UNIVERSITY** 

# Astrothelium macrostiolatum Luangsuphabool, Aptroot & Sangvichien., sp. nov. (Figure 13, G-H)

Thallus crustose, corticate, olive green, smooth or somewhat warted, shiny. Algae trentepohlioid. Ascomata perithecia, pyriform, carbonized, common ostiole with two cavities, solitary or immersed in pseudostroma. Ostiole apical, black. Pseudostroma white, mostly covered by thallus but leaving a large whitish ostiolar area free. Hamathecium hyaline, inspersed with oil droplets, paraphyses anastomosing. Ascospores 8 per ascus, hyaline, transversely 9-11 septate, fusiform, 82-97.5 x 17-19 µm, lumina diamond-shaped to rounded. Chemistry: Thallus UV-, KOH+ yellow. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: None (PHL84)

Etymology. The specific epithet refers to the large whitish ostiolar area.

Notes. This new species is similar to *A. eustomum* (Mont.) Müll. Arg. in thallus and pseudostroma characters and also *A. diplocarpoides* Müll. Arg. and *A. diplocarpum* Nyl. by having large ascospores. However, it differs from those in having more septate ascospores, an inspersed hamathecium and lack of secondary metabolites: 3-5-septate ascospores, clear hamathecium, and containing lichexanthone in *A. eustomum*; 5-7-sepetate ascospores and containing lichexanthone in *A. diplocarpoides*; and 9-septate ascospores, clear hamathecium and containing anthraquinones in *A. diplocarpum* (Harris, 1984; Aptroot *et al.*, 2008; Aptroot and Lücking, 2016).



**Figure 13** Morphological characters of thallus and ascospores of *A. aenascens* (A-B), *A. flavocoronatum* (C-D), *A. macrocarpum* (E-F), and *A. macrostiolatum* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

G

Η
# 5. *Astrothelium neglectum* Luangsuphabool, Aptroot & Sangvichien., sp. nov. (Figure 14, A-B)

Thallus crustose, corticate, greenish, smooth or somewhat warted, shiny. Algae trentepohlioid. Ascomata perithecia, pyriform, carbonized, fused ostiole with two cavities, single to 2-8 aggregate groups immersed in pseudostroma. Ostiole apical, black. Pseudostroma gray to yellowish, raised above the thallus, round to irregular. Hamathecium hyaline, inspersed with oil droplets, paraphyses anastomosing. Ascospores 8 per ascus, hyaline, transversely 3-septate, narrowly ellipsoid, 17-23 x 6-7 µm, lumina diamond-shaped to rounded. Chemistry: Thallus UV+ yellow, KOH+ yellow. Pseudostroma UV+ brown-orange, KOH-. TLC: lichexanthone. Isolation No.: TAK8, TAK12, TAK17

Etymology. The specific epithet refers to the fact this species has been overlooked before.

Notes. The new species is similar to *A. eustomum* (Mont.) Müll. Arg. in thallus, pseudostroma and ascospore characters, but differs by containing lichexanthone in the thallus, whereas this substance in *A. eustomum* is only presence on the ostioles (Harris, 1984; Aptroot *et al.*, 2008; Aptroot, 2009b).

จุฬาลงกรณมหาวทยาลย

# Astrothelium neovariolosum Luangsuphabool, Aptroot & Sangvichien., sp. nov. (Figure 14, C-D)

Thallus crustose, corticate, greenish, smooth or somewhat warted, shiny. Algae trentepohlioid. Ascomata perithecia, pyriform, carbonized, fused ostiole with two cavities, single to 2-8 aggregate groups immersed in pseudostroma. Ostiole apical, black. Pseudostroma gray to yellowish, raised above the thallus, round to irregular. Hamathecium hyaline, inspersed with oil droplets, paraphyses anastomosing. Asci clavate, 115-125 x 12-13.5 µm. Ascospores 8 per ascus, hyaline, transversely 3-septate, narrow ellipsoid, 17-23 x 6-7 µm, lumina diamond-shaped to rounded. Chemistry: Thallus UV+ yellow, KOH+ yellow. Pseudostroma UV+ brown-orange, KOH-. TLC: lichexanthone. Isolation No.: KY777, KY848

Etymology. The specific epithet refers to the morphologically closely to species *A. variolosum*.

Notes. The new species is most similar to *A. variolosum* (Ach.) Müll. Arg. in having a white to grey pseudostroma and ascospore characters, but differs by hamathecium inspersed (hamathecium not inspersed in *A. variolosum*) (Aptroot *et al.*, 2008; Aptroot, 2009b).

จุฬาลงกรณมหาวทยาลย

60

# 7. Astrothelium siamense Luangsuphabool, Aptroot & Sangvichien. sp. nov. (Figure 14, E-F)

Thallus crustose, corticate, olive green to yellow, smooth, shiny. Algae trentepohlioid. Ascomata perithecia, pyriform, carbonized, common ostiole with two cavities, solitary to aggregated groups immersed in pseudostroma. Ostiole apical, black. Pseudostroma white, raised above the thallus, round to irregular. Hamathecium hyaline, inspersed with oil droplets, paraphyses anastomosing. Ascospores 8 per ascus, hyaline, transversely 4-7 septate, fusiform, 31-49 x 10.5-12 µm, lumina diamond-shaped to rounded. Chemistry: Thallus UV+ yellow, KOH+ yellow. Pseudostroma UV+ yellow-orange, KOH-. TLC: lichexanthone. Isolation No.: KRB105, KRB139

Etymology. The specific species refers to "Siam" the traditional name for Thailand, which the species was collected.

Notes. This new species is similar to *A. variolosum* (Ach.) Müll.Arg., but differs in having larger ascospores (3-septate, 20-26 x 7-9 μm in *A. variolosum*) (Aptroot *et al.*, 2008).

#### 8. Bathelium albidoporum (Makhija & Patw.) R. C. Harris. (Figure 14, G-H)

Thallus crustose, corticate, olive green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary or 2-3 carpic, immerded in pseudostroma tissue. Ostiole apical, black. Pseudostroma aboved on thallus, black, middle zone contain yellow to orange pigment, KOH+ red. Hamathecium hyaline, clear, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 5-7-septate, 30-38 x 7.5-9 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH+ red. TLC: parietin and unknown anthraquinone. Isolation No.: KRB179, KRB203, NAN143, NAN146, NSR34, NSR54, NSR57, PNG29, PJK24, UBN127, UBN144, UBN166, UBN230



**Figure 14** Morphological characters of thallus and ascospores of *A. neglectum* (A-B), *A. neovariolosum* (C-D), *A. siamense* (E-F), and *B. albidoporum* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 9. Bathelium madreporiforme (Eschw.) Trevisan. (Figure 15, A-B)

Thallus crustose, corticate, green to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate, immerded in pseudostroma tissue. Ostiole apical, black. Pseudostroma raised, brown, middle zone contain yellow to orange pigment, KOH+ red. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 32-36.5 x 9-10.5 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH+ red. TLC: parietin and unknown anthraquinone. Isolation No.: NAN79, NAN95, KY517, UBN98, UBN133, UBN147

#### 10. Bathelium tuberculosum (Makhija & Patw) R. C. Harris. (Figure 15, C-D)

Thallus crustose, corticate, olive green, dull. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary or 2-3 carpic, immerded in pseudostroma. Ostiole apical, black. Pseudostroma raised, black to brown, middle zone contain yellow to orange pigment, KOH+ red. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 4 spores per ascus, muriform, 100-130 x 23-33 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH+ red. TLC: parietin and unknown anthraquinone. Isolation No.: no mycobiont isolation (PNG48).

# 11. Bathelium sp.1 (Figure 15, E-F)

Thallus crustose, corticate, olive green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate and immerded in pseudostroma. Ostiole apical, brown. Pseudostroma raised, black to brown, shiny, inside zone contain yellow to orange pigment, KOH+ red. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 64-79 x 15-17 µm. Chemistry: Thallus UV+ white, KOH-. Pseudostroma UV-, KOH+ red. TLC: parietin and unknown anthraquinone. Isolation No.: DKT35, DKT42, DKT58, DKT71, DKT73, DKT87, DKT94, DKT98, DKT108, DKT109, PHL4, PHL7

#### 12. Campylothelium nitidum Müll. Arg. (Figure 15, G-H)

Thallus crustose, ecorticate, white, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole lateral, black. Pseudostroma raised, black to brown. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, IKI+ violet, 56-59 x 17-18 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: DKT115, UBN107, UBN111, UBN130, UBN150, UBN153

#### 13. Laurera alboverruca Makhija & Patw. (Figure 16, A-B)

Thallus crustose, corticate, green to white-grey, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary or 2-3 carpic immerded in pseudostroma. Ostiole apical, grey. Pseudostroma raised, white to grey, algae layer above on pseudostroma tissue, white color surround ostiole. Hamathecium hyaline, inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 69-169 x 23-33 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: lichexanthone. Isolation No.: PHL82, PHL89

# 14. Laurera cf. aurantiaca Makhija & Patw. (Figure 16, C-D)

Thallus crustose, corticate, olive green to yellowish with white patches, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary, immerded in pseudostroma. Ostiole apical, brown. Pseudostroma raised, cream, identical with thallus. Hamathecium inspersed with yellow oil droplets, KOH+ red, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 210-221 x 30-32 µm. Chemistry: Thallus UV-, KOH+ yellow. Pseudostroma UV-, KOH-. TLC: unknown anthraquinone. Isolation No.: no mycobiont culture (KRB53).



**Figure 15** Morphological characters of thallus and ascospores of *B. madreporiforme* (A-B), *B. tuberculosum* (C-D), *Bathelium* sp.1 (E-F), and *C. nitidum* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 15. Laurera cf. columellata Makhija & Patw. (Figure 16, E-F)

Thallus crustose, corticate, green to yellow, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, columella, 1-2 carpic, immerded in pseudostroma. Ostiole apical, black. Pseudostroma raised, cream to white, identical with thallus. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 160-200 x 23-33 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: lichexanthone. Isolation No.: CM156, CM168, PHL128

#### 16. Laurera keralensis Upreti & Ajay Singh. (Figure 16, G-H)

Thallus crustose, corticate, green to yellow, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monicarpic to polycarpic. Ostiole apical, black. Pseudostroma raised, black, cracked. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 48-92 x 15-20 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: HRK42, UBN212, UBN214 and no mycobiont isolation (TSL107).

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



**Figure 16** Morphological characters of thallus and ascospores of *L. alboverruca* (A-B), *L.* cf. *aurantiaca* (C-D), *L.* cf. *columellata* (E-F), and *L. keralensis* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 17. Laurera megasperma (Mont.) Riddle. (Figure 17, A-B)

Thallus crustose, corticate, greenish, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monicarpic, immerded in pseudostroma. Ostiole apical, black. Pseudostroma identical with thallus, algae layer above on pseudostroma tissue. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 4 spores per ascus, muriform, 175-300 x 25-48 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: TSL4, TSL39, TSL59, TSL122

#### 18. Laurera meristospora (Mont. & Bosch) Zahlbr. (Figure 17, C-D)

Thallus crustose, corticate, green to yellow, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary, immerded in pseudostroma. Ostiole apical, black. Pseudostroma identical with thallus. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 170-220 x 32-40 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: meristosporic acid. Isolation No.: KY472, TSL136

## 19. Laurera meristosporoides P.M. McCarthy & Vongshew. (Figure 17, E-F)

Thallus crustose, corticate, greenish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monicarpic, immerded in pseudostroma. Ostiole apical, brown. Pseudostroma white to cream, identical with thallus, algae layer above on pseudostroma tissue. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 78-95 x 18-20 µm. Chemistry: Thallus UV+ yellow (lichexanthone), KOH+ yellow. Pseudostroma UV-, KOH-. TLC: lichexanthone. Isolation No.: no mycobiont isolation (CM170).



**Figure 17** Morphological characters of thallus and ascospores of *L. megasperma* (A-B), *L. meristospora* (C-D), *L. meristosporoides* (E-F), and *L. phaeomelodes* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 20. Laurera phaeomelodes (Müll. Arg.) Zahlbr. (Figure 17, G-H)

Thallus crustose, corticate, greenish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, immerded in pseudostroma. Ostiole apical, black. Pseudostroma raised, black. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 38-53 x 12-14.5 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: no mycobiont isolation (CP31, TSL118).

#### 21. Laurera sikkimensis Makhija & Patw. (Figure 18, A-B)

Thallus crustose, corticate, green to yellowish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monicarpic, immerded in pseudostroma. Ostiole apical, black. Pseudostroma brown. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 24-25.5 x 132-161 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: PHL21, PHL53

# 22. Laurera subdiscreta (Nyl.) Zahlbr. (Figure 18, C-D)

Thallus crustose, corticate, green to yellowish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole apical, black. Pseudostroma raised, thick wall. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 30-50 x 10-20 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: CP5, PBR31, SMS73, UBN86, UBN90, UBN165, UBN170, UBN180, UBN220, UBN227, UBN228



**Figure 18** Morphological characters of thallus and ascospores of *L. phaeomelodes* (A-B), *L. subdiscreta* (C-D), *L. subphaerioides* (E-F), and *L. varia* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 23. Laurera subphaerioides Upreti & Ajay Singh. (Figure 18, E-F)

Thallus crustose, corticate, yellowish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole apical, black. Pseudostroma immersed in thallus, thick wall. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 64-70 x 21-22 µm. Chemistry: Thallus UV+ yellow (lichexanthone), KOH-. Pseudostroma UV-, KOH-. TLC: lichexanthone. Isolation No.: no mycobiont isolation (RN20).

#### 24. Laurera varia (Fée) Zahlbr. (Figure 18, G-H)

Thallus crustose, corticate, green to yellowish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, flat top, disc like, solitary, immersed in pseudostroma. Ostiole apical, black. Pseudostroma embed in thallus, yellow, KOH+ red, cracked surround ascomata. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 83-93 x 24-30 µm. Chemistry: Thallus UV+ yellow, KOH+ yellow. Pseudostroma UV+ red, KOH+ red. TLC: parietin. Isolation No.: CBR51, UBN35

# 25. Laurera verrucoaggregata Makhija & Patw. (Figure 19, A-B)

Thallus crustose, corticate, thick, green to yellowish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, immerded in pseudostroma. Ostiole apical, black. Pseudostroma raised, black with white annular around ostiole area. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 35-50 x 13-15.5 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: no mycobiont isolation (UBN215).



**Figure 19** Morphological characters of thallus and ascospores of *L. verrucoaggregata* (A-B), *L. vezdae* (C-D), *M. benguelensis* (E-F), and *M. cumingii* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 26. Laurera vezdae Makhija & Patw. (Figure 19, C-D)

Thallus crustose, corticate, green to yellowish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, immersed in pseudostroma. Ostiole apical, brown. Pseudostroma embed in thallus, black. Hamathecium hyaline, clear, branch and anastomosing. Ascospore hyaline, 2 spores per ascus, muriform, 130-150 x 30-31 µm. Chemistry: Thallus UV+ white, KOH+ orange. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: PNG61

# 27. *Marcelaria benguelensis* (Müll. Arg.) Aptroot, Nelsen & Parnmen. (Figure 19, E-F)

Thallus crustose, corticate, smooth, olive green, surface contain yellow-orange pruinose. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate, immerded in pseudostroma. Ostiole black and ostiole region narrow. Pseudostroma raised, contain yellow pigment. Hamathecium inspersed with hyaline oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 62-80 x 15.5-18.5 µm. Chemistry: Thallus UV+ yellow-orange, KOH+ red. Pseudostroma UV+ yellow-orange, KOH+ red. TLC: lichexanthone, parietin. Isolation No.: DCD4, PJK8, PJK9, UBN13, UBN158

#### 28. Marcelaria cumingii (Müll. Arg.) Aptroot, Nelsen & Parnmen. (Figure 19, G-H)

Thallus crustose, corticate, smooth, olive green to yellow, without yellow-orange pruinose. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate, immerded in pseudostroma. Ostiole black and ostiole region broad. Pseudostroma raised, contain yellow pigment. Hamathecium inspersed with hyaline oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, muriform, 50-70 x 13-17.5 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV+ yellow-orange, KOH+ red. TLC: lichexanthone, parietin. Isolation No.: CM192, DCD2, DCD3, DCD5, DCD7, DCD12, DCD19, DCD94, DCD95, DKT30, DKT36, DKT45, DKT54, DKT67, DKT82, DKT92, DKT95, DKT104, DKT116, KJB19, KJB69, K11, SNK1, SNK8, SNK31, SNK33, SNK36, SNK39, SP118, SP124, TSL28, NAN25, PBR24, RN104, UBN137, UBN194



**Figure 20** Morphological characters of thallus and ascospores of *P. albidum* (A-B), *P. albocinereum* (C-D), *P. catapastum* (E-F), and *P. quinqueseptatum* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 29. Polymeridium albidum (Müll. Arg.) R.C. Harris. (Figure 20, A-B)

Thallus crustose, ecorticate, white, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 18-23 x 5.5-6.7 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: no mycobiont isolation (KY856, PHL163).

#### 30. Polymeridium albocinereum (Kremp.) R.C. Harris. (Figure 20, C-D)

Thallus crustose, ecorticate, white, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, inspersed, branch and anastomosing. Ascospore hyaline, narrow ellipsoid, 8 spores per ascus, 5-9-septate, 24-30.5 x 6-6.7 µm, cell locule cylindrical. Chemistry: Thallus UV+ white, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: PHL191 and no mycobiont isolation (PHL193).

# 31. Polymeridium catapastum (Nyl.) R.C. Harris. (Figure 20, E-F)

Thallus crustose, ecorticate, white-brown, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium inspersed, branch and anastomosing. Ascospore 8 spores per ascus, 3-septate, 24.5-27 x 6-7.5 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: no mycobiont isolation (KY825, PHL169).



**Figure 21** Morphological characters of thallus and ascospores of *Polymeridium* sp.1 (A-B), *Polymeridium* sp.2 (C-D), *Pseudopyrenula diluta var. degenerans* (E-F), and *P. subnudata* (G-H).

Scales: thallus = 1 mm; as cospore =  $10 \mu m$ .

#### 32. Polymeridium quinqueseptatum (Nyl.) R.C. Harris. (Figure 20, G-H)

Thallus crustose, ecorticate, white-brown, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, fully inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, narrow ellipsoid, 8 spores per ascus, 5-7-septate, 21-23 x 6-6.5 µm, cell locule rounded. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: K17, KRB125

#### 33. Polymeridium sp.1 (Figure 21, A-B)

Thallus crustose, ecorticate, white, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, narrow ellipsoid, 8 spores per ascus, 5-7-septate, 22-24 x 5-5.5 µm, cell locule cylindrical. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: CBR16

# 34. Polymeridium sp.2 (Figure 21, C-D)

Thallus crustose, ecorticate, white, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, inspersed with a little oil droplets, branch and anastomosing. Ascospore hyaline, narrow ellipsoid, 8 spores per ascus, 7-septate, 22-24 x 6-7 µm, cell locule cylindrical. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: CP112



**Figure 22** Morphological characters of thallus and ascospores of *T.* cf. *aeneum* (A-B), *T. albopruinosum* (C-D), *T. andamanicum* (E-F), and *T. cinereorosellum* (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 35. Pseudopyrenula diluta var. degenerans Vain. (Figure 21, E-F)

Thallus crustose, corticate, smooth, brown to greenish with white patches. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium inspersed with yellow oil droplets, KOH+ red, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 21.5-23 x 7.8-8 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: KRB36

#### 36. Pseudopyrenula subnudata Müll. Arg. (Figure 21, G-H)

Thallus crustose, corticate, white-grey to brownish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium inspersed with yellow oil droplets, KOH+ red, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 22-23 x 6.5-8 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: CP123

# 37. Trypethelium cf. aeneum (Eschw.) Zahlbr. (Figure 22, A-B)

Thallus crustose, corticate, greenish to yellow, smooth, yellow pruinose. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 18-26 x 7-9.5 µm. Chemistry: Thallus UV+ yellow, KOH+ red. Pseudostroma UV+ yellow-orange, KOH+ red. TLC: anthrauinone. Isolation No.: KY655, TSL72



Figure 23 Morphological characters of thallus and ascospores of *T. eluteriae* (A-B), *T. microstomum* (C-D), *T. neogabeinum* (E-F), and *T. nitidusculum* (G-H). Scales: thallus = 1 mm; ascospore =  $10 \mu m$ .

#### 38. Trypethelium albopruinosum Makhija & Patw. (Figure 22, C-D)

Thallus crustose, corticate, smooth, orange pruinose. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, contain orange pruinose. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 23.5-24.5 x 7.8-9.3 µm. Chemistry: Thallus UV+ yellow to orange, KOH+ red. Pseudostroma UV-, KOH-. TLC: parietin. Isolation No.: no mycobiont isolation (KY730).

#### **39.** *Trypethelium andamanicum* Makhija & Patw. (Figure 22, E-F)

Thallus crustose, corticate, smooth, yellow to green with pink patches. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate in pseudostroma. Ostiole black. Pseudostroma semi-raised, yellowish to orange. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 7-9-septate, 21-31 x 6-7 μm. Chemistry: Thallus UV+ yellow to orange, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: KRB172, KRB176

# 40. Trypethelium cinereorosellum Kremp. (Figure 22, G-H)

Thallus crustose, corticate, smooth, greenish-grey to yellow. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary, immerded in pseudostroma. Ostiole black. Pseudostroma raised, white to grey. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 7-septate, 51-62 x 13-15 µm. Chemistry: Thallus UV-, KOH+ yellow. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: TSL23, TSL67

#### 41. Trypethelium eluteriae Spreng. (Figure 23, A-B)

Thallus crustose, corticate, greenish to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, aggregate, immerded in pseudostroma. Ostiole black. Pseudostroma raised, yellow. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 9-13-septate, 33-63 x 8-12 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV+ yellow to orange, KOH+ red. TLC: perietin, emodin and unidentified anthraquinones.

Isolation No.: CP69, CP70, CP72, CP73, CP78, CP81, CP86, CP89, CP98, CP100, 113, CM190, DKT66, KJB1, KJB2, KJB70, KJB74, KRB72, KRB74, KRB76, KRB78, KRB79, KRB81, KRB82, KRB83, K52, K76, KY710, KY716, KY743, KY764, KY781, KY783, KY784, KY808, KY811, KY814, KY842, L45, L48, NBR7, NAN5, NAN9, NAN16, NAN18, NAN23, NAN39, NAN50, NAN59, NAN71, NAN72, NAN76, NAN86, NAN90, NAN93, NAN104, NAN118, NAN119, NAN124, NAN126, NAN127, NAN129, NAN130, NAN131, PB20, PB24, PB25, PB42, PBR2, PBR3, PBR4, PBR5, PBR27, PBR28, PNG1, PJK14, PJK15, PJK16, PJK17, PJK18, PJK20, PJK21, PL35, PL45, PL99, SNK15, SP46, SP119, PL121, SMS74, TAK28, TAK34, TAK49, TAK55, TLN3, TLN19, TRA95, TRA102, TRA119, UBN146, UBN157, UBN185, UBN224

#### 42. Trypethelium microstomum Makhija & Patw. (Figure 23, C-D)

Thallus crustose, corticate, smooth, orange pruinose. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary. Ostiole black and not share. Pseudostroma raised, contain orange pruinose. Hamathecium hyaline, inspersed with oil droplates, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 9-12-septate, 56-60 x 11.5-12 μm. Chemistry: Thallus UV+ yellow, KOH+ yellow-brown. Pseudostroma UV-, KOH+ brown. TLC: lichexanthone. Isolation No.: PHL61 and no mycobiont isolation (PHL77).

#### 43. Trypethelium neogabeinum R.C. Harris. (Figure 23, E-F)

Thallus crustose, corticate, smooth, greenish-grey. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic and aggregate in pseudostroma. Ostiole black and not share. Pseudostroma raised, yellow orange pruinose. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 22-24.5 x 9-9.5 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV+ orange, KOH+ red. TLC: parietin. Isolation No.: CP48, CP54, TSL149, UBN33

#### 44. Trypethelium nitidusculum (Nyl.) R.C. Harris. (Figure 23, G-H)

Thallus crustose, corticate, smooth, olive green. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, white, without pruinose. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 21.5-23.5 x 7-8.5 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: NSR14, NSR16, NSR17, KRB42, KRB177

## 45. Trypethelium ochroleucum var. subdissocians (Nyl.) Hue. (Figure 24, A-B)

Thallus crustose, corticate, greenish-grey to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic and immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, concolour with thallus, white to brown pale color. Hamathecium hyaline, inspersed with oil droplates, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 19-21 x 6.5-7.2 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: CBR12, CBR13, RN26, KRB59, KRB91, KRB158, KY759



**Figure 24** Morphological characters of thallus and ascospores of *T. ochroleucum var. subdissocians* (A-B), *T.* aff. *papulosum* (C-D), *T. pseudoplatystomum* (E-F), and *T. tropicum* (G-H).

Scales: thallus = 1 mm; ascospore =  $10 \ \mu$ m.

#### 46. Trypethelium aff. papulosum (Nyl.) Makhija & Patw. (Figure 24, C-D)

Thallus crustose, corticate, white-green to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary, immersed in thallus. Ostiole black and not share. Pseudostroma immersed in thallus, identical with thallus. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 22-27 x 6.5-7.3  $\mu$ m. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV+ yellow, KOH. TLC: lichexanthone. Isolation No.: KRB128 and no mycobiont isolation (KRB175).

#### 47. Trypethelium pseudoplatystomum Makhija & Patw. (Figure 24, E-F)

Thallus crustose, corticate, green to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma semi-raised, yellow pale. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 10-14-septate, 42-43 x 7-8 µm. Chemistry: Thallus UV+ white, KOH-. Pseudostroma UV-, KOH+ brown. Isolation No.: UBN46

## 48. Trypethelium tropicum (Ach.) Müll. Arg. (Figure 24, G-H)

Thallus crustose, corticate, olive green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, solitary, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised on thallus, black, concolour with thallus. Hamathecium hyaline, inspersed with oil droplets, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 21.5-23.5 x 6-7 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: CP111, CP119, KRB80, KRB118, PNG2, PNG3, RN55, SMS17, TRA91, TRA98, KY780, KY832, KY845



**Figure 25** Morphological characters of thallus and ascospores of *T. ubianense* (A-B), *T. virens* (C-D), *Trypethelium* sp.1 (E-F), and *Trypethelium* sp.2 (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 49. Trypethelium ubianense (Vain.) Zahlbr. (Figure 25, A-B)

Thallus crustose, corticate, dark green to greenish-grey, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, white. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 7-9-septate, 27-32 x 8-9.5 µm. Chemistry: Thallus UV+ white, KOH-. Pseudostroma UV+ white, KOH-. TLC: no substances detected. Isolation No.: SMS72, TRA125

#### 50. Trypethelium virens Tuck. (Figure 25, C-D)

Thallus crustose, corticate, yellow-green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma semi-raised, yellow to pale yellow. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 7-9-septate, 34-43 x 11-13 µm. Chemistry: Thallus UV-, KOH-. Pseudostroma UV-, KOH+ yellow-brown. TLC: no substances detected. Isolation No.: CM161

# 51. Trypethelium sp.1 (Figure 25, E-F)

Thallus crustose, corticate, white-green, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic, solitary, immersed in thallus, naked at top area. Ostiole black and not share. Pseudostroma black to brown. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 18-23 x 6.5-7 µm. Chemistry: Thallus UV+ white, KOH+ orange. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: KRB155



Figure 26 Morphological characters of thallus and ascospores of *Trypethelium* sp.3 (A-B), *Trypethelium* sp.4 (C-D), *Trypethelium* sp.5 (E-F), and *Trypethelium* sp.6 (G-H). Scales: thallus = 1 mm; ascospore =  $10 \mu m$ .

#### 52. Trypethelium sp.2 (Figure 25, G-H)

Thallus crustose, corticate, yellow-green to brown, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic, solitary. Ostiole black and not share. Pseudostroma black, not identical with thallus. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 16-18 x 6-7 µm. Chemistry: Thallus UV-, KOH+ yellow. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: KRB183 and no mycobiont isolation (KRB207).

#### 53. *Trypethelium* sp.3 (Figure 26, A-B)

Thallus crustose, corticate, green to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised to semi-raised, white to pale yellow. Hamathecium hyaline, clear, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 18-24.5 x 7.5-8 µm. Chemistry: Thallus UV+ white, KOH+ yellow. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: KRB58

# 54. Trypethelium sp.4 (Figure 26, C-D)

Thallus crustose, corticate, green to dark green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, immersed in pseudostroma. Ostiole brown and not share. Pseudostroma raised, yellow-brown. Hamathecium hyaline, inspersed with oil droplates, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 9-10-septate, 38-40 x 9.5-10 µm. Chemistry: Thallus UV+ yellow, KOH+ brown. Pseudostroma UV+ yellow, KOH+ brown. TLC: no substances detected. Isolation No.: TRA127, TRA130

#### 55. Trypethelium sp.5 (Figure 26, E-F)

Thallus crustose, corticate, greenish to yellow, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, yellow to brown. Hamathecium hyaline, inspersed with oil droplates, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 9-10-septate, 29-33 x 6-6.5 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH-. Isolation No.: SMS7

#### 56. Trypethelium sp.6 (Figure 26, G-H)

Thallus crustose, corticate, grey-green to yellow, smooth and raise at pseudostroma. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, immersed in pseudostroma. Ostiole brown and not share. Pseudostroma raised, yellow-brown and white nearly margin. Hamathecium hyaline, not inspersed, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 9-14-septate, 49-52 x 11-12 µm. Chemistry: Thallus UV+ white, KOH-. Pseudostroma UV+ yellow, KOH+ red. Isolation No.: KRB87, KRB99, KRB100

# 57. Trypethelium sp.7 (Figure 27, A-B)

Thallus crustose, corticate, olive green, smooth to somewhat bullate. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, aggregate, immersed in pseudostroma. Ostiole brown and not share. Pseudostroma raised, white. Hamathecium hyaline, inspersed with oil droplates, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 23-23.5 x 7.5-8.5 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV+ yellow, KOH-. Isolation No.: PHL20



**Figure 27** Morphological characters of thallus and ascospores of *Trypethelium* sp.7 (A-B), *Trypethelium* sp.8 (C-D), *Trypethelium* sp.9 (E-F), and *Trypethelium* sp.10 (G-H). Scales: thallus = 1 mm; ascospore = 10 μm.

#### 58. Trypethelium sp.8 (Figure 27, C-D)

Thallus crustose, corticate, green to pale green, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, sometime presence columella, monocarpic to polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, white or naked. Hamathecium hyaline, inspersed with oil droplates, branch and anastomosing. Ascospore hyaline, 8 spores per ascus, 3-septate, 24-28 x 8-8.5 µm. Chemistry: Thallus UV+ yellow, KOH+ yellow. Pseudostroma UV-, KOH-. TLC: lichexanthone. Isolation No.: PHL119 and no mycobiont isolation (PHL130, PHL146).

#### 59. Trypethelium sp.9 (Figure 27, E-F)

Thallus crustose, corticate, olive green, thick, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic, aggregate, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, brown to white. Hamathecium hyaline, fully inspersed with oil droplates, branch and anastomosing. Ascospore 8 spores per ascus, 3-septate, 23-26 x 7.5-8.5 µm. Chemistry: Thallus UV+ yellow, KOH-. Pseudostroma UV-, KOH+ yellow. Isolation No.: DKT105, DKT110

# 60. Trypethelium sp.10 (Figure 27, G-H)

Thallus crustose, corticate, olive green to greenish, smooth. Algae trentepohlioid. Ascomata perithecia, black, carbonized, monocarpic to polycarpic, immersed in pseudostroma. Ostiole black and not share. Pseudostroma raised, white. Hamathecium hyaline, clear, branch and anastomosing. Ascospore 8 spores per ascus, 3-septate, 21-24 x 7-9 μm. Chemistry: Thallus UV+ yellow, KOH+ yellow. Pseudostroma UV+ yellow-orange, KOH-. Isolation No.: KRB106, KRB107

# 61. Trypethelium sp.11 (Figure 28)

Thallus crustose, corticate, yellow-brown to green, cracked, smooth and raise at pseudostroma. Algae trentepohlioid. Ascomata perithecia, black, carbonized, polycarpic. Ostiole black and not share. Pseudostroma raised, black. Hamathecium hyaline, clear, branch and anastomosing. Ascospore 8 spores per ascus, 17-23-septate, 75-101 x 18-20. Chemistry: Thallus UV+ yellow, KOH+ yellow-brown. Pseudostroma UV-, KOH-. TLC: no substances detected. Isolation No.: no mycobiont isolation (KRB90).



**Figure 28** Morphological characters of thallus and ascospore of *Trypethelium* sp.11. Scales: thallus = 1 mm; ascospore = 10 μm.

> จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University
| Species                  | Species in this study       | Number of |  |
|--------------------------|-----------------------------|-----------|--|
| (Vongshewarat, 2000)     |                             | isolates  |  |
| -                        | Astrothelium aenascens      | 2         |  |
| Astrothelium cinnamomeum | -                           | -         |  |
| Astrothelium eustomum    | -                           | -         |  |
| -                        | Astrothelium flavocoronatum | 2         |  |
| Astrothelium macrocarpum | Astrothelium macrocarpum    | 4         |  |
| -                        | Astrothelium macrostiolatum | -         |  |
| -                        | Astrothelium neglectum      | 3         |  |
| - 1                      | Astrothelium neoveriolosum  | 2         |  |
| _                        | Astrothelium siamense       | 2         |  |
| Bathelium albidoporum    | Bathelium albidoporum       | 13        |  |
| Bathelium madreporiforme | Bathelium madreporiforme    | 6         |  |
| Bathelium tuberculosum   | Bathelium tuberculosum      | -         |  |
| -                        | Bathelium sp.1              | 12        |  |
| Campylothelium nitidum   | Campylothelium nitidum      | 6         |  |
| <u> </u>                 | Laurera alboverruca         | 2         |  |
| -                        | Laurera cf. aurantiaca      | -         |  |
| -                        | Laurera cf. columellata     | 2         |  |
| Laurera keralensis       | Laurera keralensis          | 3         |  |
| Laurera megasperma       | Laurera megasperma          | 3         |  |
| Laurera meristospora     | Laurera meristospora        | 2         |  |
| Laurera meristosporoides | Laurera meristosporoides    | -         |  |
| Laurera phaeomelodes     | Laurera phaeomelodes        | -         |  |
| -                        | Laurera sikkimensis         | 1         |  |
| Laurera subdiscreta      | Laurera subdiscreta         | 12        |  |

Table 7List of lichen species in family Trypetheliaceae in Thailand based onmorphological characters and number of isolated of each species.

Species	Species in this study	Number of	
(Vongshewarat, 2000)		isolates	
Laurera subphaerioides	Laurera subphaerioides	-	
-	Laurera varia	2	
-	Laurera verrucoaggregata	-	
-	Laurera vezdae	1	
Marcelaria benguelensis	Marcelaria benguelensis	5	
-	Marcelaria cumingii	37	
Polymeridium albidum	Polymeridium albidum	-	
Polymeridium albocinereum	Polymeridium albocinereum	1	
Polymeridium catapastum	Polymeridium catapastum	-	
Polymeridium pleiomerioides		-	
Polymeridium quinqueseptatum	Polymeridium quinqueseptatum	2	
- 8	Polymeridium sp.1	1	
-	Polymeridium sp.2	1	
Pseudopyrenula diluta var.	Pseudopyrenula diluta var.	1	
degenerans	degenerans		
-	Pseudopyrenula subnudata	2	
-	Trypethelium cf. aeneum	2	
Trypethelium albopruinosum	Trypethelium albopruinosum	-	
Trypethelium andamanicum	Trypethelium andamanicum	4	
Trypethelium celatum	-	-	
Trypethelium cinereorosellum	Trypethelium cinereorosellum	2	
Trypethelium concatervatum	-	-	
Trypethelium eluteriae	Trypethelium eluteriae	126	
Trypethelium luteum	-	-	
Trypethelium microstomum	Trypethelium microstomum	1	

 Table 7 (continued). List of lichen species in family Trypetheliaceae in Thailand based

 on morphological characters and number of isolated of each species.

Species	Species in this study	Number of
(Vongshewarat, 2000)		isolates
Trypethelium myriocarpum	-	-
-	Trypethelium neogabeinum	4
-	Trypethelium nitidusculum	5
Trypethelium ochroleucum	-	-
Trypethelium ochroleucum	Trypethelium ochroleucum var.	7
var. subdissocians	subdissocians	
-	Trypethelium aff. papulosum	1
- 7	Trypethelium pseudoplatystomum	1
Trypethelium tropicum	Trypethelium tropicum	13
-	Trypethelium ubianense	3
<u> </u>	Trypethelium virens	1
- 8	<i>Trypethelium</i> sp.1	1
-	Trypethelium sp.2	1
- จุหาลง	Trypethelium sp.3	1
<u>-</u> Chulalo	<i>Trypethelium</i> sp.4	1
-	<i>Trypethelium</i> sp.5	1
-	<i>Trypethelium</i> sp.6	3
-	<i>Trypethelium</i> sp.7	1
-	<i>Trypethelium</i> sp.8	2
-	<i>Trypethelium</i> sp.9	2
-	Trypethelium sp.10	2
-	Trypethelium sp.11	-
33 species	61 species	313 isolates

 Table 7 (continued). List of lichen species in family Trypetheliaceae in Thailand based

 on morphological characters and number of isolated of each species.

## 4.4 Molecular study of family Trypetheliaceae

One hundred and eighty-one lichen samples (165 mycobionts and 16 lichen thallus fragments) were selected for DNA analysis. Six hundred and eleven of new sequences were generated from 4 loci (169 of ITS, 135 of nuLSU, 181 of mtSSU, and 126 of RPB1), as approximately 600 bp for ITS and nuLSU, 750 bp for mtSSU and 900 for RPB1. All of nucleotide sequences were analyzed and compared to a variable sequences of lichen species in GenBank database by nucleotide blast (www.ncbi.nlm.nih.gov/BLAST/). The results of nucleotide blast showed that nucleotide sequences were similar to order Trypetheliales for nuLSU, mtSSU and RPB1, while ITS region was similar to various orders as Botryosphaeriales, Caliciales, Capnodiales, Chaetothyriales, Pleosporales, Trypetheliales, and Tubeufiales (Appendix C).

According to the large amount of samples were genera Astrothelium, Laurera, Marcelaria and Trypethelium. These genera were separated for phylogenetic analysis, which combined with necleotide sequences as available GenBank database. The phylogeny was revealed to relationships and placement among species within each genus.

### 4.4.1 Molecular phylogeny of genus Astrothelium

Sixteen lichen specimens of genus *Astrothelium* and GenBank sequences database (Table 8) were phylogenetic analysed based on four loci (ITS, nuLSU, mtSSU and RPB1). The concatenated dataset had 3138 nucleotide positions with GTR+I+G model. Molecular data supported the presence of seventeen lineages of *Astrothelium* (Figure 29), showing seven lineages from Thailand, which comfirmed by 100 persentage bootstrap values and phenotypes agreeing to different species. Lineage A and C were different to other Thai material as presence anthroquenone pigment and ascospore 3-septates. Lineage C, D, E and F were formed monophyletic group by molecular data support but conflicted within morphological characters as white color of pseudostroma and absence anthroquinone (lineage D-F) and yellow color

of pseudostroma (anthroquinone) (lineage C). Lineage G was different from other species in this study as presence ascospore 4-7 septates. Five lineages (A and D-G) were proposed for the taxa new to science as *A. flavocoronatum*, *A. macrostiolatum*, *A. neglectum*, *A. neovariolosum* and *A. siamense* (see above 4.3.2 Lichen identification). In addition, *A. aenascens* (lineage C) was found for a new record from Thailand, while a common species *A. macrocapum* (lineage B) was formed monophyletic group (Figure 29).

	GenBank accession number			
Taxon (Country)	ITS	nuLSU	mtSSU	RPB1
A. cinnamomeum (Costa Rica)	DQ782839	AY584652	AY584632	DQ782824
A. crassum MPN98 (Peru)		GU327710	GU327685	-
A. crassum MPN335 (Brazil)		KM453761	KM453827	-
A. laevigatum MPN43 (Peru)		KM453768	KM453833	-
A. leucoconicum MPN42 (Peru)	Variation	KM453764	KM453830	-
A. leucosessile MPN258 (Panama)		KM453762	KM453828	-
A. macrocarpum MPN260 (Panama)	โมหาวิทยา	KM453763	KM453829	-
A. obtectum MPN422 (Brazil)	orn Unive	KM453767	KM453832	-
A. robustum MPN754 (Costa Rica)	-	KM453760	KM453826	-
A. scorioides MPN770 (Fiji)	-	KM453766	KM453831	-
A. versicolor MPN259 (Panama)	-	KM453769	KM453834	-
A. versicolor MPN703 (Brazil)	-	KM453765	-	-

 Table 8 Nucleotide sequences of genus Astrothelium were downloaded from GenBank.



Figure 29 Phylogenetic relationships of the genus *Astrothelium* based on maximum likelihood and Bayesian inference analyses using four loci (ITS, nuLSU, mtSSU and RPB1).

The ML bootstrap values  $\geq$  70% and posterior probabilities  $\geq$  0.95 were shown at the branches, respectively. The morphological and chemical characters were indicated the following species as: A. Pseudostroma absence anthraquinone pigments with KOH nagative, P. Pseudostroma with yellow anthraquinone pigments, KOH positive (red color), T. Ascospore, 3 septates, M. Ascospore, > 3-septates, W. Pseudostroma with white color, Y. Pseudostroma with yellow pigments, N. Hamathecium without oil droplets, O. Hamathecium inspersed with hyaline oil droplets.



**CHULALONGKORN UNIVERSITY** 

#### 4.4.2 Phylogeny of genera Laurera and Marcelaria

Molecular phylogeny of Laurera and Marcelaria was co-analyzed because both genera used to be a synonym as Laurera. The genus Marcelaria was separated from Laurera based on presence of anthroquinone pigment on thallus. Eighty-four new DNA sequences (42 speciemens) were generated with two DNA loci (nuLSU and mtSSU) and aligned with DNA downloaded from GenBank (Table 9). The sequence alignment consisted of a total of 1321 nucleotide positions and calculated with best-fit model as GTR+I+G. The phylogeny was divided speciemens of this study into two main clades and four lineages by high bootstrap values (Figure 30). Clade I showed morphological characters conflict between sister-species, which showed various pseudostroma colors (yellow and black) and lichen substances, consisted of lineage A (M. cumingii, M. benguelensis, L. keralensis and L. varia) and lineage B (L. subdiscreta and L. vezdae). In addition, Marcelaria cumingii and M. benguelensis showed similar placement, which were different to presence of anthroquinone pingment on thallus. Clade II was absent anthroquinone pigment on thallus that consists of lineage C (L. alboverruca, L. cf. aurantiaca, L. cf. columellata, L. megasperma, L. meristospora, L. sikkimensis and L. subdiscreta) and lineage D (L. verrucoaggregata), which only L. verrucoaggregata have ascospore smaller than other species.

Tayon	GenBank accession number			
	nuLSU	mtSSU		
Laurera gigantospora	KM453786	KM453851		
L. megasperma	KM453787	KM453852		
L. megasperma	FJ267702	GU561847		
L. aff. megasperma	KM453785	KM453850		
L. sanguinaria	KM453788	KM453853		
Marcelaria cumingii	KM453789	KM453854		
M. purpurina	KM453790	KM453855		
Mycomicrothelia hemispherica	GU327719	GU327695		
M. miculiformis	GU327720	GU327696		

Table 9 The nucleotide sequences of genera Laurera, Marcelaria and outgroup weredownloaded from GenBank.

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

M	VCD	
Marcelaria cumingii UBN137	YGP	
Marcelaria cumingii SNK1	YGP	
Marcelaria cumingii DDD24	YGP	
Marcelaria bangualansis UDN159		
Macelaria banquelensis UDN156		
- Marcelaria benguelensis 15K0	VRP	
Marcelaria kenguelensis DED4	VRP	
Marcelaria benguelensis ToRS	YRP	
Marcelaria cumingii MPN323A	1	
Marcelaria cumingii SNK8	YGP	
Marcelaria cumingii Si KK36	YGP	
Marcelaria cumingii SNK33	YGP	
Marcelaria cumingii CM192	YGP	
Marcelaria cumingii DCD5	YGP	
–Marcelaria cumingii K11	YGP	Lada I
96/0.99 - Marcelaria cumingii SP124	Y G P	Claue I
Marcelaria cumingii DKT48	Y G P	
Marcelaria cumingii DKT30	Y G P	
92/0.98 -Marcelaria cumingii DCD2	Y G P	
Laurera keralensis TSL104	BGA	
A 99/0.98 99/1.00 Laurera keralensis UBN212	BGA	
Laurera keralensis HRK42	BGA	
Laurera keralensis UBN214	BGA	
Marcelaria purpurina MPN323A		
100/1.00 Laurera varia UBN35	Y G P	
78/- Laurera varia CBR51	Y G P	
Laurera subdiscreta CP5	BGA	
Laurera subdiscreta PBR31	BGA	
Laurera subdiscreta UBN165	BGA	
Laurera vezdae PNG61	BGA	
$\mathbf{D}$ 100/1.00 Laurera ct. columellata CM168	BGA	
<b>D</b> <i>Laurera</i> ct. columeilata CN1156	BGA	
Bill Laurera sikkimensis PHL55	BGA	
100/1.00	BGA	
C Laurera meristospora K14/2		
99/1.00 - Laurera measurera TSL 50	BCA	
99/0.99	DUA	Clada II
99/1.00 Laurera megasperma TSL 39	BGA	
810.99 Laurera megasnerma TSL4	BGA	
Laurera megasperma Rivas Plata 2093 (F)	2011	
	BGA	
<sup>10000.99</sup> <sup>72/-</sup> Laurera alboverruca PHL82	BGA	
Laurera aff. megasperma Rivas Plata 2108 (F)		
<b>D</b> 91/0.99 <b>C</b> Laurera sanguinaria Canez 3133		
75/0.98 Laurera gigantospora Lucking 33037 (F)		
Laurera verrucoaggregata UBN215	B G A	
100/1.00 Mycomicrothelia miculiformis		
Mycomicrothelia hemispherica Outgroup		
0.04		

Figure 30 Phylogenetic relationships of genera *Laurera* and *Marcelaria* in Thailand based on two loci (nuLSU and mtSSU).

ML-bootstrap values above 70% and posterior probabilities equal or above 0.95 indicated at branches. The morphological and chemical characters were indicated the following species: B. Pseudostroma black, Y. Pseudostroma yellow, G. Thallus, negative chemical reaction with KOH solution, R. Thallus, positive chemical reaction with KOH (red color), A. Pseudostroma, negative chemical reaction with KOH solution, P. Pseudostroma, positive chemical reaction with KOH+ red color.



CHULALONGKORN UNIVERSITY

#### 4.4.3 Phylogeny of genus *Trypethelium*

In this study, *Trypethelium* was the highest diversity in Thailand; about 25 species was collected and identified by morphology. The phylogenetic relationships among species were investigated based on two DNA loci (nuLSU and mtSSU) and DNA sequences downloaded from Ganbank databases (Table 10), and also combined with taxonomic characters. Fifty-two specimens were represented species for DNA analysis, which one hundred and four new DNA sequences were generated for this study. A total of 1479 nucleotide positions was aligned and calculated with GTR+G as a best-fit model. The phylogenetic tree indicated that genus *Trypethelium* was divided into two main clades, included five lineages supported by high bootstrab values, which presenced various morphotypes (Figure 31). Clade I did not agree with morphological and chemical characters, while Clade II showed the taxonomic characters related to molecular data.

Clade I was divided into three lineages that consisted of lineages A-C. Lineage A has several of pseudostroma, ascospore and chemical characters, which indicated behide each species in Figure 80. Almost species in this lneage was presence ascospore 3-septates, absence yellow color on pseudostroma (anthraquinone pigment) and negative with KOH reaction, excepted *T. microstomum*, *T. cinereorosellum* and *T.* cf. *aeneum*, *T. neogabeinum* produced ascospores over 3-septates and yellow color on pseudostroma (anthroquinone pigments, KOH positive red color), respectively. Lineage B was formed monophyletic group that agreed with the taxonomy of *T. tropicum* (3-septated and absence anthroquinone pugments). Lineage C was included *Trypethelium* sp.4, *Trypethelium* sp.11, *T. ubianense* and *T. virens* that showed monophyletic group as shared the ascospores over 3-septates and lacking anthroquinone pigments.

Clade II was separated into two lineages (D and E) by strong bootstrab values, which all menbers showed specific characteristic as ascospores more than 3-septates and presented yellow color of anthroquinone pigments on pseudstroma. Lineage D was a species group that taxonomic agreed with *T. eluteriae* speices, which related to *T*. aff. *platyleucostomum*, *T.* aff. *platystomum*, *T. subeluteriae*. In addition, Molecular data indicated the *T. eluteriae* group from Thai materials that presented higher diversity than previous recognized, which might be separated at least three subgroups. For lineage E was a species diversity, which showed the relationships closely to lineage of *T. eluteriae* group.

Table 10 Nucleotide sequences of genus *Trypethelium* and outgroup were downloadedfrom GenBank.

T	GenBank accession number		
Taxon	nuLSU	mtSSU	
Trypethelium aeneum CBS132743	- 10	KC592290	
Trypethelium aeneum MPN62	KM453802	KM453866	
Trypethelium cinereorosellum MPN191	KM453809	KM453873	
Trypethelium eluteriae MPN111	-	KM453874	
Trypethelium eluteriae	GU327726	GU327704	
Trypethelium eluteriae CBS132375	-	KC592291	
Trypethelium eluteriae F19112	เล้ย <u>-</u>	DQ328990	
Trypethelium eluteriae F19113k	DQ329018	DQ328989	
Trypethelium aff. eluteriae MPN382	KM453803	KM453867	
Trypethelum floridanum F16306	-	Q329008	
Trypethelum floridanum F17090b	-	DQ329007	
Trypethelium inamoenum MPN228	KM453810	KM453875	
Trypethelum marcidum	GU327727	GU327705	
Trypethelium marcidum MPN304	KM453811	KM453876	
Trypethelium neogalbineum MPN711	KM453812	KM453877	
Trypethelium nitidiusculum MPN217	KM453813	KM453878	
Trypethelium nitidiusculum AFTOL-ID2099	GU327728	GU561848	
Trypethelium nitidiusculum	-	GU327706	

Tayon	GenBank accession number		
14,011	nuLSU	mtSSU	
Trypethelium pupula MPN224	KM453815	KM453880	
Trypethelium ochroleucum MPN313	KM453814	KM453879	
Trypethelium aff. ochroleucum MPN704	KM453804	KM453868	
Trypethelium aff. ochroleucum MPN713	KM453805	KM453869	
Trypethelium papulosum	GU327729	GU327707	
Trypethelium aff. platyleucostomum MPN349	KM453806	KM453870	
Trypethelium aff. platystomum MPN54	KM453807	KM453871	
Trypethelium aff. scorioides MPN336	KM453808	KM453872	
Trypethelium subeluteriae F17611	-	DQ329009	
Trypethelium subeluteriae MPN49C	KM453818	KM453882	
Trypethelium tropicum MPN130	KM453819	KM453883	
Trypethelium tropicum	GU327730	GU327708	
Trypethelium virens MPN497	KM453820	KM453884	
Trypethelium virens CBS132374	-	KC592292	
Trypethelium sp. Lumbsch 20551a	KM453817	-	
Trypethelium sp. Lucking 30515	KM453816	KM453881	
Capnodium coffeae	FJ190609	DQ471162	
Dothidea insculpta	DQ247802	FJ190602	
Pyrgillus javanicus	KT808612	KT808549	

Table 10 (continued). Nucleotide sequences of genus *Trypethelium* and outgroup weredownloaded from GenBank.





Figure 31 A maximum likelihood tree of genus *Trypethelium* based on nuLSU and mtSSU regions.

Bootstrap values above 70% and posterior probabilities equal or above 0.95 indicated at branches. The morphological and chemicals characters indicated the species as follows: A. Ascospores, 3-septates, B. Ascospores, > 3-septates C. Pseudostroma with carbonized, black color, W. Pseudostroma with white color, N. Negative chemical reaction with KOH solution, P. Positive chemical reaction with KOH (red color).



จุฬาลงกรณมหาวิทยาลัย Chulalongkorn University

#### 4.4.4 Phylogeny and diversity of *Trypethelium eluteriae* group in Thailand

Acorrding to the phylogeny of genus *Trypethelium* studied, *T. eluteriae* showed complex diverse species diversity, which divided at leastinto three subgroups in Thailand. Fifty-two lichen specimens of *T. eluteriae* were selected for phylogenetic analysis based on ITS and mtSSU regions.

One hundred and four DNA sequences were new generated for this study (52 each for ITS and mtSSU). A total of 1372 nucleotide positions had for DNA sequences alignment and phylogenetic analysis with best-fit model selected as GTR+G. Molecular phylogeny confirmed three clades of Thai *T. eluteriae* group, which were supported by strong bootstrab values (Figure 32). These three clades have the morphological characters which are similar to *T. eluteriae* as they presenced greenish thallus, yellow pseudostroma (KOH+ red), and character of ascospore and size (Figure 33). *Trypethelium eluteriae* group in Thailand was divided into three species that reveal to overlapping of taxonomy when compared with the literature, while chemical character used to delimite each that correlated with molecular data as *T. eluteriae* (Clade A), *T. subeluteriae* (Clade B) and *T. platystomum* (Clade C) (Table 11 and Figure 34). Two species were the new records in Thailand as *T. platystomum* and *T. subeluteriae*.

**Chulalongkorn University** 



Figure 32 Phylogeny of the *Trypethelium eluteriae* group based on partial ITS and mtSSU rDNA sequences.

Branches with posterior probabilities from a Bayesian tree sampling equal or above 0.95 and ML-bootstrap values equal or above 70% indicated at branches.



Figure 33 Morphology of thallus and ascospores in the *T. eluteriae* group.

A-B) *T. eluteriae*, C-D) *T. platystomum*, E-F) *T. subeluteriae*. Scale bars: A, C, E = 1 mm, B, D, F = 20 μm.

		Ascospores			
Species	Width	Length	No. of	Pseudostroma	KOH
	( <b>µ</b> m)	( <b>µ</b> m)	septa		
T. eluteriae	8-12	33-63	9-13	yellow to orange (red)	red
T. platystomum	11-14	42-80	8-16	yellow to orange (red)	red
T. subeluteriae	8-12	35-64	8-13	yellow-orange to brown (red)	red

 Table 11 Morphological characters of T. eluteriae, T. platystomum and T. subeluteriae.



Figure 34 TLC plates of *T. eluteriae* group with anthraquinone pigment.

(A= solvent A and B= solvent C); 1) *T. eluteriae*, 2) *T. subeluteriae*, 3) *T. platystomum*. The two yellow major pigments from above are parietin (P) and emodin (E), respectively, and unknown major orange pigment (O).

4.4.5 Phylogenetic relationships of lichen-forming fungi of Trypetheliaceae in Thailand

One hundred and twenty-six taxa of Trypetheliaceae were represented for phylogenetic analysis based on four DNA loci (ITS, nuLSU, mtSSU and RPB1). Five hundred and four new DNA sequences were generated in this study (Appendix D). Nucleotide sequences were aligned as a total 3472 positions and phylogenetic calculated with GTR+I+G as a best-fit model for nucleotide substitution. The ML tree indicated that the phologenetic pleacment within Trypetheliaceae and revealed to the relationships among genera and species. The family Trypetheliaceae was distinguished to two main clades and comprised ten lineages A-J (Figure 35).

Clade I was separated as four lineages (A-D) by 100 persentates supported by high bootstrab values, which showed the relationships of genera *Astrothelium*, *Bathelium*, *Laurera* and *Trypethelium*. Lineage A was the highest diversity that included genera *Astrothelium*, *Laurera* and *Trypethelium*, which showed various morphology of ascospore and pseudostroma and chemistry. Lineage B showed the positions of *Astrothelium* and *Trypethelium*, which did not correlate with morphological and chemical characters. For lineages C and D separated individual genera as *Bathelium* and *Laurera*, which closely related with *Astrothelium* and *Trypethelium* (lineage A and B). Both lineages (C and D) have a carbonized and black pseudostroma that different from sister lineages (A and B), which presented white, brown to yellow color of pseudostroma. In addition, the conflict of two ascospore characters within *Bathelium* was indicated by molecular data that agreed with traditional taxonomy as closely the relationships between muriform (*B. albidoporum*) and transeptate (*B. madreporiforme*) (lineage D).

Clade II was divided into six lineages (E-J) consist of servaral lichens genera as *Bathelium, Campylothelium, Laurera, Marcelaria, Polymeridium, Pseudopyrenula* and *Trypethelium*, excepted genus *Astrothelium* was only placement in lineage A and B (Clade I). Lineage E was showing the relationship between *Laurara* and *Trypethelium* including *L. varia* and *Trypethelium* s.str. (*T. andamannicum, T. eluteriae, T.* 

platystomum, T. pseudoplatystomum, Trypethelium sp.5 and Trypethelium sp.6). Lineage F was comprise of two species, Marcelaria cumningii was closely related to L. keralensis, although the taxonomy was conflict and form a sister-group with Trypethelium s.str. (lineage E). All taxa of two letter lineages presenced anthroquinone pigments (yellow color) on thallus or pseudostroma, except a *L. keralensis* have black pseudostroma and lacking anthroquinone. In addition, the generic type of Laurera (L. varia) showed the generic placement within Trypethelium s.str. group. For lineage G was revealed to genetic placement genus Bathelium (Bathelium sp.1), which did not only delimit in Clade I and related to Laurera and Tyrpethelium. This lineage had various taxonomic characters such as color of pseudostroma, ascospore types and anthraquinone presence or absence. Lineage H and I were the small group of genera Campylothelium and Pseudopyrenula, respectively, which were confirmed by molecular phylogeny and specific taxonomic characters (Table 6). Finally, lineage J was comprise of genus Polymeridium and T. tropicum, which this lineage agreed with ascospore hyaline, 3-septates, apical ostiole and only conflicted from a thallus types as coticate/ecorticate.

In this study, Trypetheliaceae was delimited to 56 species, including 8 genera (*Astrothelium, Bathelium, Campylothelium, Laurera, Marcelaria, Polymeridium, Pseudopyrenula* and *Trypethelium*). The phylogeny showed great conflicts between molecular evidence and traditional genus level classification (Table 6), except for two genera of *Campylothelium* and *Pseudopyrenula* were correlated with previous generic concept. *Marcelaria* and *Polymeridium* were each formed a small monophyletic group, which closely to sister-species as *L. keralensis* and *T. tropicum*, respectively. Genera *Astrothelium, Bathelium, Laurera* and *Trypethelium* were from polyphyletic genus, which separated from several lineages within family Trypetheliaceae, excepted *Astrothelium* found only in Clade I (lineage A and B). In addition, the *Trypethelium* s.str. did not form monophyletic group, which related to genus *Laurera* as a conflict on ascospore types (Figure 36).





Figure 35 Phylogenetic tree lichen-formin fungi family Trypetheliaceae in Thailand based on four loci (ITS, nuLSU, mtSSU rDNA and RPB1).

The ML-bootstrap values and Bayesian posterior probabilities were shown under or above branches with  $\geq$  70% and  $\geq$  0.95, respectively. The groups of mycobiont substances profile were indicated by different color.



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



**Figure 36** Overall of phylogenetic relationships of genera within family Trypetheliaceae based on four loci (ITS, nuLSU, mtSSU and RPB1). Each genera indicated by different color.

121

Although, the morphological and chemical characters of lichens thallus did not correlat to the phylogenetic relationships within the family, secondary metabolites from mycobionts culture were well supported in some groups with phylogeny. The chemotypes were related to phylogeny groups as pink naphthoquinones pigments presenced in all taxa in lineage E and F at Rf values 0.31 and 0.37 (Figure 37; 1-10). Two yellow unknown pigments were found from lineage G at Rf values 0.18 and 0.30 (Figure 37; 11-16). Also, lineage J showed the relationship of genus *Polymeridium* and *Trypethelium tropicum* based on molecular data and two pink unknown substances at Rf values 0.39 and 0.49 (Figure 37; 17-21).



จุฬาลงกรณีมหาวิทยาลัย Chulalongkorn University





Trypethelium platystomum, 2. T. subeluteriae, 3. T. eluteriae, 4. Trypethelium sp. 6, 5.
 Laurera varia, 6. T. andamandicum, 7. Trypethelium sp. 5, 8. T. pseudoplatystomum, 9.
 L. keralensis, 10. Marcelaria cumingii, 11. Bathelium sp.1, 12. Trypethelium ubianense,
 13. Laurera vezdae, 14. Trypethelium sp.4, 15. T. virens, 16. L. subdiscreta, 17.
 Polymeridium albocinereum, 18. P. quinqueseptatum, 19. Polymeridium sp.2, 20.
 Polymeridium sp.1, and 21. T. tropicum.

# 4.5 Chemical study

The representatives of lichen-forming fungi were selected from taxonomic and phylogenetic analysis. Fifty-one species of lichen mycobionts were grown on MYA medium, they were represented species for secondary metabolites study (Figure 38).



Figure 38 The character of mycobiont colonies grows on MYA medium for 9 weeks.
1. Astrothelium aenascens, 2. A. flavocoronatum, 3. A. macrocarpum, 4. A. neglectum,
5. A. neovariolosum and 6. A. siamense.



Figure 38 (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 7. *Bathelium albidoporum*, 8. *B. madreporiforme*, 9. *Bathelium* sp.1, 10. *Campylothelium nitidum*, 11. *Laurera alboverruca* and 12. *Laurera* cf. *columellata*.



Figure 38 (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 13. *Laurera keralensis*, 14. *L. megasperma*, 15. *L. meristospora*, 16. *L. sikkimensis*, 17. *L. subdiscreta* and 18. *L. varia*.



Figure 38 (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 19. *Laurera vezdae*, 20. *Macelaria cumingii*, 21. *Polymeridium albocinereum*, 22. *P. quinqueseptatum*, 23. *Polymeridium* sp.1 and 24. *Polymeridium* sp. 2.



Figure 38 (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 25. *Pseudopyrenula diluta var. degenerans*, 26. *P. subnudata*, 27. *Trypethelium* cf. *aeneum*, 28. *T. andamanicum*, 29. *T. cinereorosellum* and 30. *T. eluteriae*.



Figure 38 (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 31. *Trypethelium microstomum*, 32. *T. neogabeinum*, 33. *T. nitidusculum*, 34. *T. ochroleucum var. subdissocians*, 35. *T. papulosum* and 36. *T. platystomum*.



Figure 38 (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 37. *Trypethelium pseudoplatystomum*, 38. *T. subeluteriae*, 39. *T. tropicum*, 40. *T. ubianense*, 41. *T. virens* and 42. *Trypethelium* sp.1.


**Figure 38** (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 43. *Trypethelium* sp.2, 44. *Trypethelium* sp.3, 45. *Trypethelium* sp.4, 46. *Trypethelium* sp.5, 47. *Trypethelium* sp.6 and 48. *Trypethelium* sp.7.



**Figure 38** (continued). The character of mycobiont colonies grows on MYA medium for 9 weeks. 49. *Trypethelium* sp.8, 50. *Trypethelium* sp.9 and 51. *Trypethelium* sp.10.

### 4.5.1 Mycobionts extraction and secondary metabolites study

The mycobiont colonies of fifty-one species were extracted by n-hexane, dichloromethane and methanol. Crude extracts were concentrated by rotary evaporator and dried at room temperature. Each crude extract was calculated for percent yields. No compounds extracted from axenic culture mycobiont were found in low polarity of organic solvent. The results showed that percentage yields of methanol fraction (0.294-2.745 g/100g), while the dichloromethane fractions were less dried weight yields (0.003 – 0.646 g/100g) and some samples cannot extract by  $CH_2Cl_2$  (Table 12). Forty ( $CH_2Cl_2$  fraction) and fifty-one (MeOH fraction) of crude samples were detected by TLC, which the results showed Rf values 0-0.75 and 0-0.81 from extracted by  $CH_2Cl_2$  and MeOH, respectively (Appendix E).

Spacios	Colonies	Crude CH <sub>2</sub> Cl <sub>2</sub>	Crude MeOH
opecies	weight (g)	yield (g)	yield (g)
Astrothelium aenascens	2.0059	าลัย 0	0.724
A. flavocoronatum	2.4861	0.009	0.294
A. macrocarpum	3.1985	0.058	0.607
A. neglectum	2.7690	0.005	0.961
A. neovariolosum	2.1756	0.003	0.364
A. siamense	3.6773	0.022	1.267
Bathelium albidoporum	2.2489	0	1.638
B. madreporiforme	2.3263	0.013	1.536
Bathelium sp.1	2.3043	0.004	0.737
Campylothelium nitidum	2.8225	0.013	0.853
Laurera alboverruca	3.1528	0.010	0.670
L. cf. columellata	1.9709	0.005	0.929

 Table 12 Total amount of mycobiont colonies and crude extracts of lichen-forming fungi

 family Trypetheliaceae.

Creation	Colonies	Crude CH <sub>2</sub> Cl <sub>2</sub>	Crude MeOH	
Species	weight (g)	yield (g)	yield (g)	
L. keralensis	1.9918	0	0.724	
L. megasperma	2.0031	0.050	0.699	
L. meristospora	2.7548	0	0.741	
L. sikkimensis	1.7573	0.006	0.660	
L. subdiscreta	2.5913	0.008	1.930	
L. varia	1.5167	0.45	1.154	
L. vezdae	0.6627	0.06	1.811	
Marcelaria cumingii	0.7286	0.08	2.745	
Polymeridium albocinereum	2.0295	0.064	0.818	
P. quinqueseptatum	1.8158	0	0.468	
Polymeridium sp.1	3.2707	0.006	0.560	
Polymeridium sp.2	2.4622	0.016	0.357	
Pseudopyrenula diluta var.	1 5017	0	0 700	
degenerans				
P. subnudata GHULALONG	2.5581	ERSITY O	1.478	
Trypethelium cf. aeneum	2.1918	0.041	1.027	
T. andamanicum	1.7725	0.085	1.224	
T. cinereorosellum	1.9691	0.046	0.564	
T. eluteriae	2.6479	0.177	0.903	
T. microstomum	1.9042	0.032	0.457	
T. neogabeinum	4.0638	0	0.317	
T. nitidusculum	3.0847	0.003	0.580	
T. ochroleucum var.		0.000	0.660	
subdissocians	3.0000	0.003	0.009	
T. aff. papulosum	4.1839	0.012	0.860	

Table 12 (continued). Total amount of mycobiont colonies and crude extracts of lichen-forming fungi family Trypetheliaceae.

Species	Colonies	Crude CH <sub>2</sub> Cl <sub>2</sub>	Crude MeOH	
opecies	weight (g)	yield (g)	yield (g)	
T. platystomum	1.6659	0.270	1.567	
T. pseudoplatystomum	2.3085	0.108	0.836	
T. subeluteriae	1.5400	0.260	1.383	
T. tropicum	1.9210	0.016	0.510	
T. ubianense	2.2024	0.032	0.663	
T. virens	2.5627	0	0.843	
Trypethelium sp.1	3.3435	0	0.419	
Trypethelium sp.2	1.7560	0.068	0.877	
Trypethelium sp.3	2.2652	0	0.433	
Trypethelium sp.4	1.0905	0.0183	1.962	
Trypethelium sp.5	2.6506	0.0377	1.505	
Trypethelium sp.6	2.2856	0.0438	0.468	
Trypethelium sp.7	1.4869	0.6456	1.621	
Trypethelium sp.8	2.2077	าลัย 0.014	0.883	
Trypethelium sp.9	2.5353	ERSITY 0	0.674	
Trypethelium sp.10	2.3982	0.004	0.813	

Table 12 (continued). Total amount of mycobiont colonies and crude extracts of lichen-forming fungi family Trypetheliaceae.

## 4.6 Antimicrobial activity

The secondary metabolites of lichen-forming fungi from representative species of family Trypetheliaceae were investigated for antibacterial (Escherichia coli and Staphylococcus aureus) and antifungal activities (Candida albicans) by TLCbioautography method. The results showed that twenty-three species presented antimicrobial ativity at Rf values 0 to 0.68 (Figures 39-42 and Table 13). Candida albicans was inhibited by crude extractes of eighteen species, which eight species showing from both solvent extraction (CH<sub>2</sub>Cl<sub>2</sub> and MeOH) as Laurera cf. columellate, L. megasperma, Trypethelium andamanicum, T. cinereorosellum, T. eluteriae, T. pseudoplatystomum, T. subeluteriae, and Trypethelium sp.7, while seven species (Astrothelium negletum, L. sikkimensis, L. varia, Macelaria cumingii, T. platystomum, Trypethelium sp.2, and Trypethelium sp.5) and three species (T. ubianense, T. virens, and Trypethelium sp.8) inhibited yeast by only crudes CH<sub>2</sub>Cl<sub>2</sub> and MeOH extracts, respectively (Figures 39 and 40). For antibacterial activity, compounds of lichen-forming fungi family Trypetheliaceae did not inhibit Gram negative bacteria (E. coli), in contrast they showed good inhibition for Gram positive bacteria (S. aureus) as eighteen species. Both crude extracts from CH<sub>2</sub>Cl<sub>2</sub> and MeOH fraction inhibited S. aureus that showed in seven species (L. varia, T. eluteriae, T. platystomum, T. pseudoplatystomum, T. subeluteriae, T. ubianense, and Trypethelium sp.7), while five species (A. neglectum, M. cumingii, T. andamanicum, Trypethelium sp.2, and Trypethelium sp.5) and six species (A. flavocoronatum, C. nitidum, Polymeridium sp.1, Pseudopyrenula subnudata, Trypethelium sp.1, and Trypethelium sp.8) inhibited Gram positive bacteria by CH<sub>2</sub>Cl<sub>2</sub> and MeOH extraction, respectively (Figures 41 and 42). Antibacterial and antifungal activities showed the highest inhibit from four lichen species as T. eluteriae, T. pseudoplatystomum, T. subeluteriae and Trypethelium sp.7, which could inhibit tested microorganisms in all of solvent extraction. The summary of antimicrobial activity and Rf values were shown in Table 13.



**Figure 39** The antimicrobial activity of mycobionts substances (CH<sub>2</sub>Cl<sub>2</sub> fraction) tested against *C. albicans* by TLC-bioautography and bioactive compounds were indicated by clear zone.

1. Astrothelium neglectum, 2. Laurera cf. columellata, 3. L. megasperma, 4. L. sikkimensis, 5. L. varia, 6. Marcelaria cumingii, 7. Trypethelium andamanidum, 8. T. cinereorosellum, 9. T. eluteriae, 10. T. platystomum, 11. T. pseudoplatystomum, 12. T. subeluteriae, 13. Trypethelium sp.2, 14. Trypethelium sp.5 and 15. Trypethelium sp.7.



**Figure 40** The antimicrobial activity of mycobionts substances (MeOH fraction) tested against *C. albicans* by TLC-bioautography and bioactive compounds were indicated by clear zone.

1. L. cf. columellata, 2. L. megasperma, 3. T. andamanidum, 4. T. cinereorosellum, 5. T. eluteriae, 6. T. pseudoplatystomum, 7. T. subeluteriae, 8. T. ubianense, 9. T. virens, 10. Trypethelium sp.7 and 11. Trypethelium sp.8.



**Figure 41** The antimicrobial activity of mycobionts substances (CH<sub>2</sub>Cl<sub>2</sub> fraction) tested against *S. aureus* by TLC-bioautography and bioactive compounds were indicated by clear zone.

1. A. neglectum, 2. L. megasperma, 3. L. varia, 4. Marcelaria cumingii, 5. T. andamanidum, 6. T. eluteriae, 7. T. platystomum, 8. T. pseudoplatystomum, 9. T. subeluteriae, 10. T. ubianense, 11. Trypethelium sp.2, 12. Trypethelium sp.5 and 13. Trypethelium sp.7.



Figure 42 The antimicrobial activity of mycobionts substances (MeOH fraction) tested against *S. aureus* by TLC-bioautography and bioactive compounds were indicated by clear zone.

1. A. flavocoronatum, 2. C. nitidum, 3. L. varia, 4. Polymeridium sp.1, 5. Pseudopyrenula subnudata, 6. T. eluteriae, 7. T. platystomum, 8. T. pseudoplatystomum, 9. T. subeluteriae, 10. T. ubianense, 11. Trypethelium sp.1, 12. Trypethelium sp.7 and 13. Trypethelium sp.8.

Charles	C. albicans		S. aureus	
Species	CH <sub>2</sub> Cl <sub>2</sub>	MeOH	$CH_2CI_2$	MeOH
Astrothelium flavocoronatum	-	-	-	0.15
A. neglectum	0.34-0.47	-	0.22-0.48	-
Campylothelium nitidum	-	-	-	0.14
Laurera cf. columellata	0-0.06	0, 0.48	-	-
L. megasperma	0-0.46	0, 0.38	-	-
L. sikkimensis	0-0.09	2	-	-
L. varia	0-0.37		0-0.54	0.71
Marcelaria cumingii	0.25, 0.43	-	0.13-0.25,	-
			0.43	
Polymeridium sp.1		- 7/1	-	0.14
Pseudopyrenula subnudata		- 6	-	0.15
Trypethelium andamanicum	0-0.29	0.08, 0.20	0-0.28	-
T. cinereorosellum	0-0.09	0, 0.40	-	-
T. eluteriae	0-0.26	0.23-0.48	0-0.27, 0.52	0.21-0.65
T. platystomum	0-0.33	ทยาลย	0-0.30	0.22-0.53
T. pseudoplatystomum	0-0.29	0.22-0.49	0-0.43	0.25-0.59
T. subeluteriae	0-0.47	0.21, 0.47	0-0.44	0.22, 0.62
T. ubianense	-	0, 0.34	0.43	0.54
T. virens	-	0, 0.31	-	-
<i>Trypethelium</i> sp.1	-	-	-	0.14
Trypethelium sp.2	0.28-0.48	-	0.26-0.49	-
Trypethelium sp.5	0-0.07, 0.21,	-	0.25	-
	0.29			
Trypethelium sp.7	0-0.45	0.24, 0.57,	0-0.42	0.74
		0.68		
Trypethelium sp.8	-	0.19	-	0.19

 $\label{eq:table_$ 

## 4.7 Antioxidant activity

The DPPH free radical scarvenging was detected with substances from lichenforming fungi family Trypetheliaceae for antioxidant activity by TLC bioautography. The results showed that nineteen lichen species inhibited the DPPH free radical at Rf values 0 to 0.64. The crudes from methanol extraction were good antioxidant activity, which showed inhibition with all of nineteen species as *A. neglectum*, *B. albidoporum*, *Bathelium* sp.1, *L. megasperma*, *L. subdiscreta*, *L. varia*, *L. vezdae*, *M. cumingii*, *T. andamanicum*, *T. cinereorosellum*, *T. eluteriae*, *T. platystomum*, *T. subeluteriae*, *T. ubianense*, *T. virens*, *Trypethelium* sp.2, *Trypethelium* sp.4, *Trypethelium* sp.5 and *Trypethelium* sp.8. Also, the crudes from dichloromethane extraction were similar to result with MeOH crudes extracts, except the six species of *L. varia*, *M. cumingii*, *T. eluteriae*, *T. ubianense*, *T. virens*, and *Trypethelium* sp.8 could not inhibit for DPPH solution (Figure 43-44 and Table 14). Two species of *A. neglectum* and *T. platystomum* showed a high antioxidant activity from CH<sub>2</sub>Cl<sub>2</sub> and MeOH extraction at Rf values 0-0.21, 0.49 (CH<sub>2</sub>Cl<sub>2</sub>), 0-0.08 (MeOH) and 0-0.15, 0.33, 0.46 (CH<sub>2</sub>Cl), 0-0.22 (MeOH) respectively (Figure 42; 1 and 9, Figure 44; 1 and 12).

> จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



**Figure 43** The TLC-bioautography of mycobiont substances ( $CH_2CI_2$  fraction) detected for free radical scavengers using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution.

1. A. neglectum, 2. B. albidoporum, 3. Bathelium sp.1, 4. L. megasperma, 5. L. subdiscreta, 6. L. vezdae, 7. T. andamanicum, 8. T. cinereorosellum, 9. T. platystomum, 10. T. subeluteriae, 11. Trypethelium sp.2, 12. Trypethelium sp.4 and 13. Trypethelium sp.7.



**Figure 44** The TLC-bioautography of mycobiont substances (MeOH fraction) detected for free radical scavengers using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution.

1. A. neglectum, 2. B. albidoporum, 3. Bathelium sp.1, 4. L. megasperma, 5. L. subdiscreta, 6. L. varia, 7. L. vezdae, 8. M. cumingii, 9. T. andamanicum, 10. T. cinereorosellum, 11. T. eluteriae, 12. T. platystomum, 13. T. subeluteriae, 14. T. ubianense, 15. T. virens, 16. Trypethelium sp.2, 17. Trypethelium sp.4, 18. Trypethelium sp.7 and 19. Trypethelium sp.8.

Species	Rf values for antioxidant activity		
Species –	Dichloromethane	Methanol	
Astrothelium neglectum	0-0.21, 0.49	0-0.08	
Bathelium albidoporum	0-0.64, 0.28	0	
Bathelium sp.1	0-0.08	0	
Laurera megasperma	0, 0.46	0	
L. subdiscreta	0	0	
L. varia		0	
L. vezdae	0-29	0	
Marcelaria cumingii		0	
Trypethelium andamanicum	0-0.07	0-0.22	
T. cinereorosellum	0-0.04	0	
T. eluteriae	ALL	0	
T. platystomum	0-0.15, 0.33, 0.46	0-0.22	
T. subeluteriae	กรณ์มหาว0นยาลัย	0-0.06	
T. ubianense	ngkorn University	0	
T. virens	-	0	
<i>Trypethelium</i> sp.2	0, 0.28, 0.43	0	
<i>Trypethelium</i> sp.4	0	0	
<i>Trypethelium</i> sp.7	0-0.13	0.21	
Trypethelium sp.8	-	0-0.04, 0.17	

**Table 14** Antioxidant activity and Rf values from different solvent extraction of lichen-forming family Trypetheliaceae.

# CHAPTER V

# Discussion

Members of the lichen family Trypetheliaceae were collected from various habitats in Thailand and a total nine hundred and sixty-five lichen specimens (28 sites from 24 provinces) were obtained. Three hundred and thirteen lichen-forming the fungus partners from about two in three could not be isolated, some specimens did not discharge ascopores or the spores failed to geminate. Successful ascospore discharge and ascospore germination of tropical lichens were correlated to the season of lichens collection, freshness of specimens, temperature, humidity, maturity of ascomata and ascospores, while rate of germination related to species distributions (Sangvichien *et al.*, 2011).

The taxonomic study of Trypetheliaceae in Thailand resulted in classification into 8 genera, consists of Astrothelium, Bathelium, Campylothelium, Laurera, Marcelaria, Polymeridium, Pseudopyrenular and Trypethelium, which agreed with the major characters of each genus (Harris, 1984; Aptroot et al., 2008; Aptroot et al., 2013). This family was identified, based on morphology, to contain at least 61 species (47 species and 14 unidentified species), consisting of 7 species of Astrothelium, 4 species of Bathelium, 1 species of Campylothelium, 14 species of Laurera, 2 species of Marcelaria, 6 species of Polymeridium, 2 species of Pseudopyrenula and 25 species of Trypethelium. Seventeen species were reported as new records in Thailand including A. aenascens, L. alboverruca, L. cf. aurantiaca, L. cf. columellata, L. sikkimensis, L. varia, L. verrucoaggregata, L. vezdae, M. cumingii, Pseudopyrenula subnudata, T. cf. aeneum, T. neogabeinum, T. nitidusculum, T. aff. papulosum, T. pseudoplatystomum, T. ubianense, and T. virens. In addition, five lichen species were candidated new species of the genus Astrothelium based on morphological characters. The genus Trypethelium exhibited the highest species diversity in Thailand with 25 species, especially T. eluteriae exhibited the highest species distribution that found in all habitats as privously reported by Vongshewarat (2000). The genus Laurera with 14 species

found in this study that showed high diversity than in the previous studies (Vongshewarat *et al.*, 1999; Vongshewarat, 2000; Aptroot *et al.*, 2007), which had been reported as the center of diversity of *Laurera* as in Southeast Asia and the Indian subcontinent (Letrouit-Galinou, 1957; Awasthi, 2000).

Molecular studies based on nucleotide sequences were successful for DNA amplification in 165 mycobionts and in 16 lichen thallus, whereas 5 specimens failed in the molecular methodology. DNA sequences data were compared with GenBank databases. All sequences of nuLSU, mtSSU, and RPB1 loci were similar to the order Trypetheliales, whilst of nuLSU and mtSSU varied according to available databases, while RPB1 was found to match only two sequences of Astrothelium cinnamomeum and Bathelium degenerans in GenBank. The ITS sequences showed high similarity to the order Trypetheliales with A. cinnamomeum, Polymeridium subcinereum, and T. aeneum while some ITS sequences matched to other lichen orders with short sequences. Although molecular data of nuLSU and mtSSU was found to be higher than two previous loci in GenBank, there are still poorly for species diversity and shortly of DNA sequences for comparison (200-400 bp for nuLSU and 300-700 bp for mtSSU) (Nelsen et al., 2014). One problem on molecular studies of lichens concerns the difficulty of DNA extraction from lichens thalli. This was not only occurs in the Trypetheliaceae but also has been found in other lichen families such as Graphidaceae and Pyrenulaceae (Staiger et al., 2006; Weerakoon et al., 2012; Nelsen et al., 2014). DNA extraction from direct lichen thallus specimens risk contamination from other organisms, rapid DNA degradation and low quality of genomic DNA (Hofstetter et al., 2007b; Arnold et al., 2009; Weerakoon et al., 2012; Gueidan et al., 2016); hence, the DNA isolation from lichen mycobiont culture was necessary to ensure the reliable of DNA sequences (Ertz et al., 2009).

Molecular phylogeny of the genus *Astrothelium* demonstrated clear bootstraps supporting 7 species in Thailand, consisting of one common species, one new record (*A. aenascens*) and five new species to science (*A. flavocoronatum*, *A. macrostiolatum*, *A. neglectum*, *A. neovariolosum* and *A. siamense*). Thai *Astrothelium* showed conflict of morphological characters such as the pseudostrama characters, ascospore septation and lichen substances (anthraquinones) with phylogeny, which is in agreement to findings in some species of tropicals lichens of the genera *Chapsa* and *Lecanora* which evolved of morphological variation independently or adapted for environment conditions (Papong *et al.*, 2012; Parnmen *et al.*, 2012).

Three new species of A. macrostiolatum, A. neovariolosum and A. siamense were closely species that are shared morphological characters with a green thallus, white pseudostroma lacking anthraquinones and an inspersed hamathecium with oil droplets. However, they differ in ascospore characters. These three new species form a sister-group relationship with A. aenascens, which was similar to inspersed hamathecium, but the latter differs from those species in having a pseudostroma containing anthraquinones. Astrothelium flavocoronatum was very closely related to A. macrocarpum (syn.: A. galbineum Kremp.) and A. aenascens in having an ascomata containing the anthraquinones pigments and in the ascospore characters, but the new species differ in having ascomata with two locules (several locules with one to several ostioles in A. macrocarpum) and a non-inspersed hamathecium (inspersed in A. aenascens), and also molecular data support the new species assignment. Astrothelium neglectum, was distinct from A. neovariolosum and A. siamense, both species were similar to the new species as a green thallus, white pseudostroma and the presence of lichexanthone. However, ascospore characters and the hamathecium lack oil droplets differ between these species. The genus Astrothelium has been studied mostly in the Neotropics, while a few species which have been reported in Southeast Asia and Indian subcontinent are believed to be endemic species (Harris, 1984; Makhija and Patwardhan, 1989; Harris, 1995; Aptroot et al., 2008; Lima et al., 2013; Weerakoon and Aptroot, 2014). In this study, the new species and a new records from Thailand indicat that this genus has more species diversity than previous recognized (Vongshewarat, 2000; Aptroot et al., 2007). In addition, A. macrocarpum (syn.: A. galbineum Kremp.) has been reported as a common species of Astrothelium in Thailand (Vongshewarat, 2000; Aptroot et al., 2007), and this was also found in this study. Two species of A. conicum var. pallidum and A. ochrothelizum were reported to synonymous with A.

*galbineum* (Harris, 1984), while *A. galbineum* and *A. ochrothelizum* were separated by ascomata characters (Makhija and Patwardhan, 1989). Recently, this species was described as include name a synonym of *A. macrocarpum* (Fée) by Aptroot & Lücking (Aptroot and Lücking, 2016). Interestingly, molecular data suggested that Thai *A. macrocarpum* did not form a monophyletic species and separated Thai specimens into two groups and also proved to be distinct from Southern America sample. The diversity of *A. macrocarpum* was still uncertain based on morphological characters; hence, increasing use of molecular studies with more samples might be necessary to clarify this species delimitation.

Phylogenetic analysis of genera Laurera and Marcelaria demonstrated the relationships between both genera. Recently, Marcelaria was separated and assigned as a new genus from Laurera based on taxonomic characters as presence yellow pigments belonging to the anthraquinones on pseudostrama and/or thallus surface. This was supported by DNA sequences from a few specimens but without comparison with the generic type of Laurera (Aptroot et al., 2013; Nelsen et al., 2014). In this study, molecular evidence revealed the genus Marcelaria is very closely related with L. keralensis and L. varia (generic type) in Clade I, This clade exhibited various pseudostroma characters i.e. that smooth and containing yellow anthraquinone pigment (M. cumingii, M. benguelensis and L. varia) and black without yellow pigments (L. keralensis). In addition, two species, M. cumingii and M. benquelensis were indicated by molecular evidence to be similar to genetic placement, although they were different in the presence or absence of anthraquinones pigment on the thallus surface (Aptroot et al., 2013). Therefore, the results suggested that both species were conspecific; hence, the name of *M. benguelensis* (Müller, 1885) should be reduced to the older name as *M.* cumingii (Montagne, 1845). Chemistry of the lichen thallus were uncertain characters for delimitation of specific relationships within Marcelaria and Laurera, which produced a pruinose (anthraquinone pigments) was depended on stress condition as UV radiation (Solhaug et al., 2003; Solhaug and Gauslaa, 2004). Clade II was divided into lineage C and D that showed distinction of Laurera species in clade I. Two species of lineage B

correlated to the presence of black, carbonized and a lack of yellow anthraquinone pigment, formed a sister-group within lineage B. In contrast, lineage C shared the morphotype as thallus greenish, white pseudostroma and without anthraquinone pigment, while lineage D (*L. verrucoaggregata*) was similar to thallus greenish but differed by producing black-cabonized perithecia. The results of phylogeny and taxonomy of the *Laurera* group within clade II, strongly differed with the results in clade I and might be agreement with Nelsen *et al.* (2014) and reported as a form of the genetic placement within *Astrothelium* clade.

The molecular phylogeny of genus *Trypethelium* was divided into two main clades, which Clade I comprised of morphology and chemistry complexes, whilst agreeing with monophyletic characters. Each taxa was supported for delimits of species by high bootstrap values, except T. eluteriae (lineage D) which was placed into three lineages. Taxonomic characters were in conflict among sister-species or sister-groups within lineage A that consists of several phenotypes as ascospore septation (3 or more than 3-septa), pseudostrama with pruinose (yellow color or lacking) and lichen substances (presence or absence of anthraquinones). Molecular data reveals conflict of morphology as phenotypic divergence between closely taxa as found in many lichen groups may related to the influence of environment (Rivas Plata and Lumbsch, 2011; Papong et al., 2012). Trypethelium tropicum (lineage B) was distinct from other species that form monophyletic species with carbonized and black color of pseudostrama. *Trypethelium* species in lineage C also formed a monophyletic group, which produced ascospores with more than 3-septa and lacking anthraquinone pigment. Clade II was distinguished by phenotypic characters such as producing large ascospores (more than 3-septa) and pseudostroma with yellow color (KOH+ red anthraquinone pigment). Interestingly, lineage D showed considerable species diversity with at least three different groups, and these groups were very similar on taxonomic characters and in agreement with T. eluteriae, but also formed sister-groups to T. subeluteriae and T. platystomum. Intraspecific variation might be present in T. eluteriae; hence, this species needs further investigation.

The diversity of Trypethelium eluteriae group was therefore investigated by molecular studies including many specimens from several localities in Thailand. The results showed that the Thai T. eluteriae group was of a higher diversity than previous estimated (Vongshewarat, 2000; Aptroot et al., 2007). Three species were confirmed as two new records in Thailand as T. platystomum and T. subeluteriae. Previously, T. subeluteriae was reported as a synonym of T. platystomum (Aptroot et al., 2008), while in some literatures they were regarded as separate species (Makhija and Patwardhan, 1992; Harris, 1995). In fact, both species can be delimited on ascospores size and septation, whilst T. eluteriae has smaller ascospores than the latter two speices. Conflicting separation of these species was found in the case of immature ascospores, and seemed to be conspecific species as T. eluteriae. In this study, molecular phylogeny revealed two species separation, although difficult to identify by morphological characters of the thallus and ascospores because of overlapping data but found to be different based on lichen substances profiles. Trypethelium subeluteriae produced parietin, emodin and unknown anthraquinones, T. eluteriae contains parietin and emodin, while T. platystomum did not produce these three latter substances but was found to produce another unidentified anthraquinones. This result indicated that secondary metabolites play an important role in lichen taxonomy (Hawksworth, 1976; Lumbsch, 1988).

The phylogenetic analysis of family Trypetheliaceae was revealed to be a complex of relationships at generic level and which conflicted strongly with various types of pseudostroma formation, perithecia, ostiole, ascospore size and septation and chemical substances of lichen thallus. Five genera of *Astrothelium*, *Bathelium*, *Laurera*, *Polymeridium* and *Trypethelium* formed the polyphyletic genus. *Astrothelium* was found only in lineages A and B that related to *Laurera* and *Trypethelium* with several phenotypes as ascospore (transeptate and muriform), perithecia ostiole (shared and single) and anthraquinone pigment (presence or absence). *Laurera* and *Trypethelium* were separated into several lineages (clades I and II). Genus *Laurera* showed polyphyletic relationship with *Astrothelium*, *Bathelium*, *Marcelaria* and *Trypethelium*,

which did not depend on taxonomic characters. Genus Trypethelium exhibited a highly genetic relationship and was closely related to all genera within the family, excepted Campylothelium, Marcelaria and Pseudopyrenula, which form non-monophyletic and showed a taxonomic relationship similar to the genus Laurera. The genus Bathelium seemed to be a monophyletic group in lineage D (clade I) but was also found to form a small group with lineage G (clade II) that was closely related to the genera Laurera and Trypethelium, but with conflict between different pseudostroma characters and ascospore septation. Polymeridium was strongly bootstrap supported to be closely related to T. tropicum that also formed a non-monophyletic genus; although, were conflicted with thallus structure (corticate and ecorticate), ascospore wall (thick and thin) and number of ascospore sepation. Monophyletic genera were supported for Campylothelium, Marcelaria and Pseudopyrenula, and this correlated with the morphology concepts for each genus. A recent molecular study has reported that the genera Astrothelium, Laurera, Polymeridium and Trypethelium did not form Campyrothelium, monophyletic genera, while Bathelium s.str., Marcelaria, Psuedopyrenula and Trypethelium s.str. (T. eluteriae group) each formed monophyletic groups (Nelsen et al., 2014). In this study, the molecular phylogeny confirmed all generic placements agreeing with the previous study (Nelsen et al., 2014). However, Bathelium and Trypethelium s.str. (T. eluteriae group) produced different results and exhibited the relationship as a polyphyletic group with (Laurera and Trypethelium (lineage G)) and Laurera varia, respectively. According to the taxonomy, this results indicated that traditional generic classification of Trypetheliaceae did not correlate with genotypes, and is more complex than previous estimates (Harris, 1995; Del Prado et al., 2006; Aptroot et al., 2008; Nelsen et al., 2009). Also, this problem was encountered in other tropical families such as Pyrenulaceae and Graphidaceae (Parnmen et al., 2012; Gueidan et al., 2016). The influence of environmental conditions play an important role in for phenotypic adaptation which has evolved independently several times; hence, the thallus structure, ascospore type, secondary metabolites were developed to increase photosynthesis capacity, ascospore dispersal and germination, and UV radiation

protection, respectively (Murtagh *et al.*, 2002; Solhaug *et al.*, 2003; Beckett *et al.*, 2008; Mangold *et al.*, 2008; Rivas Plata and Lumbsch, 2011; Papong *et al.*, 2012; Parnmen *et al.*, 2012; Nelsen *et al.*, 2014). The generic delimitation within family Trypetheliaceae will necessary have to be revised for synapomorphy characters of individual groups that are related to molecular phylogeny.

Although, lichen taxonomic classifications did not support the phylogenetic relationships, the chemistry of mycobiont cultures correlated to phylogeny within some groups exhibiting morphotype conflicts. The new chemotypes can be separated into three groups that were strongly correlated with phylogeny (Figure 86). In addition, the relationships among genera Marcelaria, L. keralensis, L. varia and Trypethelium s.str. were changed and from a monophyletic group, supported by mycobiont substances profiles and molecular data. Also, T. tropicum might be included in the genus Polymeridium and from a monophyletic group based on mycobiont chemotypes. All taxa of lineage G, shared genotypic characters that related to the compounds from axenic culture of mycobionts, which comprised of Bathelium sp.1, L. subdiscreta, L. vezdae, T. ubianense, T. virens and Trypethelium sp.4. The complexes of morphology and chemistry of lichens depend on stress of the environment; whereas the conditions for culturing the mycobionts were controlled. The major of chemical phenotypes were independently producted and relate to genotypes that were without the effects of environment. Stocker-Wörgötter et al. (2004) reported that mycobiont cultures isolated from various chemotypes of Ramalina farinacea showed similarity of chemisrty profiles related to the molecular evidence. The chemical production by mycobionts was controlled by a combination of culture conditions such as culture medium, light or temperature (Hamada et al., 1996; Stocker-Wörgötter et al., 2004; Stocker-Wörgötter et al., 2009; Fazio et al., 2012). This study is the first report to relate to molecular phylogeny and mycobiont substances. The secondary metabolites produced from in vitro culture of lichen-forming fungi might be an importance role for generic classification and lichen identification in the future.

For the chemical study, fifty-one of representative mycobionts species from the lichen family Trypetheliaceae were extracted followed by n-hexane, dichloromethane and methanol, respectively. The percent yield of methanol extracts was higher than dichloromethane extracts and methanol proved a successful extraction solvent for all representative species; n-hexane did not extract any components. Antimicrobial and antioxidant activities were investigated by TLC bioautography methods and exhibited various capacities of secondary metabolites obtained from different solvent extractions. Candida albicans was inhibited by crude dichloromethane extracts (15 species) from the genera Astrothelium, Laurera, Marcelaria and Trypethelium and the numbers were higher than for the methanol extracts (11 species). Antibacterial activity proved to be most effective against Staphylococcus aureus with a variable range of extracts, but there was no activity against Escherichia coli. This result was consistent with previous several studies that reported lichen substances to be more active inhibitors of Gram positive bacteria than Gram negative bacteria (Saenz et al., 2006; Santiago et al., 2010; Mitrovic et al., 2011; Santiago et al., 2013; Vivek et al., 2014). The inhibition of Gram positive bacteria (S. aureus) shown for different genera and species may depends on organic solvent extraction of which methanol extracts demonstrated the most active compounds from Astrothelium, Campylothelium, Polymeridium, Pseudopyrenula and Trypethelium, while the dichloromethane fraction exhibited the bioactivity from the three genera Astrothelium, Marcelaria and Trypethelium. The results demonstrated that methanol has a wide ability as a solvent for antibacterial extraction from several genera within the Trypetheliaceae. For the study of antioxidant activity the inhibition of DPPH free radical was observed with mycobiont substances from different solvent extraction (dichloromethane and methanol). Five genera including Astrothelium, Bathelium, Laurera, Macelaria and Trypethelium exhibited antioxidant activity from crude methanol extracts (19 species) and crude dichloromethane extracts (13 species). The methanolic extract was of a higher efficiency as a free radical scavenger than the dichloromethane extracts. These results indicated that secondary metabolites which are produced from axenic cultures of Trypetheliaceae as medium to high polarity groups, and showed a good result for antibacterial and antioxidant activities (methanol extract) and antifungal activity (dichloromethane extract). The effects of different solvents to extract potential bioactive compounds have been reported from several lichens species such as Laurera benguelensis, Peltigera polydactyla, Ramalina farinacea, R. nervulosa and Xanthoparmelia mexicana (Karagöz et al., 2009; Manojlovic et al., 2010a; Kumar et al., 2014; Sundararaj et al., 2015). The high polarity solvent extraction showed strong antibacterial and antioxidant activities that related to groups of flavonoid and phenolic compounds (Bhattarai et al., 2008; Karagöz et al., 2009; Kosanic et al., 2011; Pavithra et al., 2013; Rashmi and Rajkumar, 2014; Sundararaj et al., 2015), while antifungal activity was found from dichloromethane extraction as similar to previously reported (Nanayakkara et al., 2005; Goel et al., 2011; Shivanna and Garampalli, 2015). For Trypetheliaceae, secondary metabolite products have been reported from lichen thallus and mycobiont culture and were anthraquinone, napthoquinone, phenalenone, xanthone and these derivatives, and these were responsible for the antimicrobial and antioxidant activities exhibited (Mathey et al., 1980; Manojlovic et al., 2010a; Manojlovic et al., 2010b; Sun et al., 2010; Takenaka et al., 2013). In this study, the bioactive compounds of mycobiont culture may be as similar or different in chemical composition with those previously identified. Secondary metabolites produced from the lichen-forming fungi of the family Trypetheliaceae need to investigate for their chemical constituents and for biological activity for pharmacology and biotechnology application in the future.

# CHAPTER V

# Conclusion

The lichen family Trypetheliaceae was found in all habitats in Thailand, 965 lichen specimens were collected from 28 study sites (24 provinces). The ascospore discharge technique was used for ascospore isolation, of which 313 isolates were successful for ascospore germination and cultivation on MYA medium. The mycobionts colonies were completely development within 9 weeks, which was sufficient for DNA analysis and chemical studies.

Trypetheliaceae was classified into 8 genera in Thailand including genera *Astrothelium, Bathelium, Campylothelium, Laurera, Marcelaria, Polymeridium, Pseudopyrenula*, and *Trypethelium.* Sixty-one species were identified based on morphological characters, consisting of 17 new records, 14 unidentified species and 5 species were proposed as new species of the genus *Astrothelium.* The genus *Trypethelium* exhibited the highest species diversity with at least 25 species recorded. *Trypethelium eluteriae* was the dominant species and widely distributed in Thailand.

The nucleotide sequences were analyzed from mycobionts and some thallus fragments, 611 new sequences generated from ITS, nuLSU, mtSSU and RPB1 regions, showed high percentage similarity to the Trypetheliales. Phylogenetic analysis of the genus *Astrothelium* based on four DNA loci (ITS, nuLSU, mtSSU and RPB1) confirmed five new species in Thailand, and the results were in agreement with morphological characters, as *A. flavocoronatum*, *A. macrostiolatum*, *A. neglectum*, *A. neovariolosum* and *A. siamense*. Molecular data showed that *A. macrocarpum* might be a non-monophyletic species, although the morphology correlated to the species. Genus *Marcelaria* was very close to genus *Laurera* based on two DNA loci sequences (nuLSU and mtSSU), which did not correlate to the pseudostroma characters and anthraquinone pigments. Two species of *M. cumingii* and *M. benguelensis* were confirmed as synonym species based on phylogeny, which these species will be reduced to *M. benguelensis* and changed to *M. cumingii*. Molecular phylogeny of nuLSU and mtSSU revealed the

complexes of morphology and chemistry within genus *Trypethelium*, which were not related to genotypic characters. In addition, the *Trypethlium eluteriae* group showed species diversity of at least 3 species. The distinctions of these three species were confirmed by molecular data and chemical profiles as *T. eluteriae* (parietin and emodin), *T. platystomum* (absence parietin, emodin), and *T. subeluteriae* (parietin, emodin and unknown orange pigment), while ascospore size as major character to separate these species showed clear overlap and sometimes as synonym species. Two species of *T. platystomum* and *T. subeluteriae* were added as new records in Thailand.

Phylogenetic analysis of family Trypetheliaceae demonstrated that Astrothelium, Bathelium, Laurera, Polymeridium and Trypethelium each form polyphyletic genera, which did not depended on taxonomic characters, while each of three genera Campylothelium, Marcelaria and Pseudopyrenula form monophyletic genus. The traditional taxonomic classification was unreliable for generic delimitation within Trypetheliaceae. Secondary metabolites were produced from axenic culture of lichenforming fungi that might be able to resolve for conflicts between morphology and phylogeny. Three groups were delimited as monophyletic groups based on each of mycobiont chemotypes and genotypes as; 1) genus Marcelaria, L. keralensis, L. varia and Trypethelium s.str., 2) genus Polymeridium and T. tropicum and 3) Bathelium sp.1, L. subdiscreta, L. vezdae, T. ubianense, T. virens and Trypethelium sp.4. The mycobiont substances played an importance role for lichen classification. The family Trypetheliaceae needs to be revised for generic classification and lichen identification by combination of phylogeny, morphology, lichen and mycobiont chemistry with a large number of samples for clearification and understanding of the relationships within this family in the future.

In this study, the combination of molecular phylogeny, morphology and chemistry of family Trypetheliaceae recognized 62 species in Thailand, consisting of 5 new species (*A. flavocoronatum*, *A. macrostiolatum*, *A. neglectum*, *A. neovariolosum* and *A. siamense*), 18 new records (*A. aenascens*, *L. alboverruca*, *L. cf. aurantiaca*, *L. cf. columellata*, *L. sikkimensis*, *L. varia*, *L. verrucoaggregata*, *L. vezdae*,

*Pseudopyrenula subnudata*, *T.* cf. *aeneum*, *T. neogabeinum*, *T. nitidusculum*, *T. aff. papulosum*, *T. platystomum*, *T. pseudoplatystomum*, *T. subeluteriae*, *T. ubianense* and *T. virens*) and 14 unidentified species (1 species for *Bathelium*, 2 species for *Polymeridium* and 11 species for *Trypethelium*).

The methanolic extraction was the best organic solvent for chemical extraction of lichen-forming fungi family Trypetheliaceae and resulted in a high percentage crude yield from all samples. Antibacterial activity was strongly effective against *S. aureus* using crude methanol extracts from representatives of the genera *Astrothelium*, *Campylothelium*, *Polymeridium*, *Pseudopyrenula* and *Trypethelium*, while all samples did not have activity against to Gram negative bacteria. Crude extracts from dichloromethane of *Astrothelium*, *Laurera*, *Marcelaria* and *Trypethelium* showed strong antifungal activity for inhibition of *C. albicans*. The DPPH free radical was highly inhibited by methanol crude extracts from *Astrothelium*, *Bathelium*, *Laurera*, *Macelaria* and *Trypethelium*. The broad spectrum of antibactial, antifungal and antioxidant activities were shown in *Astrothelium neglectum*, *Laurera varia*, *Marcelaria cumingii*, *Trypethelium andamanicum*, *T. eluteriae*, *T. platystomum*, *T. subeluteriae*, *T. ubianense*, *Trypethelium* sp.2, *Trypethelium* sp.7 and *Trypethelium* sp.8.

According to this study, secondary metabolites produced by axenic mycobiont culture not only have majority roles for lichen classification but also exhibited various biological activities. Thus, these mycobiont substances need to investigate for chemical composition structures, generic classification and other biotechnology application.

### REFERENCES

Ahmadjian, V. 1967. A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology, and identification. <u>Phycologia</u> 6(2-3): 127-160.

Ahmadjian, V. 1993. The lichen symbiosis. New York: The John Wiley & Sons.

- Ahmajian, V., and Heikkilä, H. 1970. The culture and synthesis of *Endocarpon pusillum* and *Staurothele clopima*. Lichenologist 4: 259-267.
- Aptroot, A. 2009a. Diversity and endemism in the pyrenocarpous lichen families Pyrenulaceae and Trypetheliaceae in the Malesian flora region. <u>Blumea</u> 54(1-3): 145–147.
- Aptroot, A. 2009b. Trypetheliaceae. In P. M. McCarthy (eds.), <u>Flora of Australia, Volume</u> <u>57, Lichens 5</u>, 535-552. Melbourne: CSIRO Publishing.
- Aptroot, A., and Lücking, R. 2016. A revisionary synopsis of the Trypetheliaceae. Lichenologist 48(4): (in press).
- Aptroot, A., Lücking, R., Sipman, H. J. M., Umaña, L., and Chaves, J. L. 2008.
   Pyrenocarpous lichens with bitunicate asci. A first assessment of the lichen biodiversity inventory in Costa Rica. <u>Bibliotheca Lichenologica</u> 97: 1-162.
- Aptroot, A., Nelsen, M. P., and Parnmen, S. 2013. Marcelaria, a new genus for the *Laurera purpurina* group in the Trypetheliaceae (Ascomycota: Dothideomycetes). <u>Glalia</u> 5(2): 1–14.
- Aptroot, A., Saipunkaew, W., Sipman, H. J. M., Sparrius, L. B., and Wolseley, P. A. 2007.
   New lichens from Thailand, mainly microlichens from Chiang Mai. <u>Fungal</u> <u>Diversity</u> 24: 75-134.
- Arnold, A. E., Miadlikowska, J., Higgins, K. L., Sarvate, S. D., Gugger, P., Way, A., et al.
   2009. A phylogenetic estimation of trophic transition networks for ascomycetous fungi: Are lichens cradles of symbiotrophic fungal dversification? <u>Systematic Biology</u> 58(3): 283–297.
- Asahina, Y., and Shibata, S. 1954. <u>Chemistry of Lichen Substances</u>. Ueno, Tokyo: Society for the promotion of Sciences.

- Awasthi, D. D. 1991. A key to the microlichens of India, Nepal and Sri Lanka. <u>Bibliotheca</u> <u>Lichenologica</u> 40: 1-340.
- Awasthi, D. D. 2000. <u>Lichenology in Indian Subcontinent: A Supplement to "A Handbook</u> <u>of Lichens"</u>. Dahra Dun, India: Bishen Singh Mahendra Pal Singh.
- Babula, P., Adam, V., Havel, L., and Kizek, R. 2009. Noteworthy secondary metabolites naphthoquinones–their occurrence, pharmacological properties and analysis. <u>Current Pharmaceutical Analysis 5(1): 47–68.</u>
- Bachmann, H., and Portmann, P. 1981. <u>Agent for oxidative dyeing of hair</u>. Germany: PCT Publication.
- Backorová, M., Jendželovský, R., Kello, M., Backor, M., Mikeš, J., and Fedorocko, P.
  2012. Lichen secondary metabolites are responsible for induction of apoptosis in
  HT-29 and A2780 human cancer cell lines. <u>Toxicology in Vitro</u> 26: 462–468.
- Basile, A., Rigano, D., Loppi, S., Santi, A. D., Nebbioso, A., Sorbo, S., et al. 2015.
   Antiproliferative, antibacterial and antifungal activity of the lichen *Xanthoria* parietina and its secondary metabolite parietin. <u>International Journal of Molecular</u> <u>Sciences</u> 16(4): 7861–7875.
- Beckett, R. P., Kranner, I., and Minibayeva, F. V. 2008. Stress physiology and the symbiosis In T. H. Nash III (eds.), <u>Lichen Biology</u> 2nd Edition, 134-151. New York: Cambridge University Press.
- Behera, B. C., and Makhija, U. 2002. Inhibition of tyrosinase and xanthine oxidase by lichen species *Bulbothrix setschwanesis*. <u>Current Science</u> 82(1): 61-66.
- Bhattarai, H. D., Paudel, B., Hong, S. G., Lee, H. K., and Yim, J. H. 2008. Thin layer chromatography analysis of antioxidant constituents of lichens from Antarctica. <u>Journal of Natural Medicines</u> 62(4): 481-484.
- Bogo, D., Matos, M. F. C., Honda, N. K., Pontes, E. C., Oguma, P. M., da Santos, E. C., et al. 2010. *In vitro* antitumor activity of orsellinates. <u>Zeitschrift für</u> <u>Naturforschung C</u> 65(1-2): 43–48.
- Boonpragob, K., Crittenden, P. D., and Lumbsch, H. T. 2013. Lichens: from genome to ecosystems in a changing world. <u>MycoKeys 6</u>: 1–2.

- Brandão, L. F. G., Alcantara, G. B., Matos, M. d. F. C., Bogo, D., Freitas, D. d. S.,
  Oyama, N. M., et al. 2013. Cytotoxic evaluation of phenolic compounds from
  lichens against melanoma cells. <u>Chemical and Pharmaceutical Bulletin</u> 61(2):
  176-183.
- Bridge, P. D., and Hawksworth, D. L. 1998. What molecular biology has to tell us at the species level in lichenized fungi. <u>The Lichenologist</u> 30(4-5): 307-320.
- Brisdelli, F., Perilli, M., Sellitri, D., Piovano, M., Garbarino, J. A., Nicoletti, M., et al. 2013.
  Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an *in vitro* study. <u>Phytotherapy Research</u> 27(3): 431-437.
- Brodo, I. W. 1978. Changing concepts regarding chemical diversity in lichens. <u>The</u> <u>Lichenologist</u> 10: 1-11.
- Brunauer, G., Hager, A., Grube, M., Türk, R., and Stocker-Wörgötter, E. 2007. Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. <u>Plant Physiology and Biochemistry</u> 45(2): 146-151.
- Buaruang, K., Mongkolsuk, P., and Manoch, L. 2009. Morphology and anatomy of lichen Family Parmeliaceae at Phu Hin Rongkla national park. <u>Journal of Microscopy</u> <u>Society of Thailand</u> 23(1): 20-24.
- Bucar, F., Schneider, I., Ogmundsdóttir, H., and Ingólfsdóttir, K. 2004. Anti-proliferative lichen compounds with inhibitory activity on 12(S)-HETE production in human platelets. <u>Phytomedicine</u> 11(7-8): 602-606.
- Buçukoglu, T. Z., Albayrak, S., Halici, M. G., and Tay, T. 2013. Antimicrobial and antioxidant activities of extracts and lichen acids obtained from some *Umbilicaria* Species from central Anatolia, Turkey. <u>Journal of Food Processing</u> <u>and Preservation</u> 37(6): 1103–1110.
- Büdel, B. 1992. Taxonomy of lichenized prokaryotic blue-green algae. In W. Reisser (eds.), <u>Algae and symbioses: plants, animals, fungi, viruses, interactions</u> <u>explored</u>, 301-324. Bristol: Biopress Limited.

- Büdel, B., and Scheidegger, C. 2008. Thallus morphology and anatomy. In T. H. Nash III (eds.), <u>Lichen Biology</u> 2<sup>nd</sup> Edition, 40-68. Cambridge: Cambridge University Press.
- Burlando, B., Ranzato, E., Volante, A., Appendino, G., Pollastro, F., and Verotta, L. 2009.
   Antiproliferative effects on tumour cells and promotion of keratinocyte wound healing by different lichen compounds. <u>Planta Medica</u> 75(6): 607–613.
- Candan, M., Yilmaz, M., Tay, T., Kivanç, M., and Türk, H. 2006. Antimicrobial activity of extracts of the lichen *Xanthoparmelia pokornyi* and its gyrophoric and stenosporic acid constituents. <u>Zeitschrift für Naturforschung C</u> 61(5-6): 319–323.
- Choi, J. S., Chung, H. Y., Jung, H. A., Park, H. J., and Yokozawa, T. 2000. Comparative evaluation of antioxidant potential of alaternin (2-hydroxyemodin) and emodin. <u>Journal of Agricultural and Food Chemistry</u> 48: 6347–6351.
- Choi, J. S., Lee, H. J., Park, K. Y., Ha, J. O., and Kang, S. S. 1997. *In vitro* antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora*. <u>Planta Meddica</u> 63(1): 11-14.
- Chrismas, M. 1980. Ascospore discharge and germination in *Xanthoria parietina*. <u>The</u> <u>Lichenologist</u> 12: 403-406.
- Cohen, P. A., and Towers, G. H. N. 1995. Anthraquinones and phenanthroperylenequinones from *Nephroma laevigatum*. <u>Journal of Natural</u> Products 58: 520-526.
- Collins, C. R., and Farrar, J. F. 1978. Structural resistances to mass transfer in the lichen *Xanthoma parietina*. <u>New Phytologist</u> 81(1): 71-83.
- Crawford, S. D. 2015. Lichens used in traditional medicine. In B. Rankovic (eds.), <u>Lichen</u> <u>secondary metabolites</u>, 27-80. Switzerland: Springer International Publishing.
- Crespo, A., Bridge, P. D., and Hawksworth, D. L. 1997. Amplification of fungal rDNA-ITS regions from non-fertile specimens of the lichen-forming genus *Parmelia*. <u>The Lichenologist</u> 29(3): 275-282.

- Crittenden, P. D., David, J. C., Hawksworth, D. L., and Campbell, F. S. 1995. Attempted isolation and success in the culturing of a broad spectrum of lichenforming and lichenicolous fungi. <u>New Phytologist</u> 130(2): 267-297.
- Cubero, O. F., and Crespo, A. 2002. Isolation of nucleic acids from lichens. In I. Kranner,R. Beckett and A. Varma (eds.), <u>Protocols in Lichenology</u>, 381-391. Springer-Verlag Berlin Heidelberg.
- Culberson, C. F. 1969. <u>Chemical and botanical guide to lichen products</u>. Chapel Hill: University of North Carolina Press.
- Culberson, C. F. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. Journal of <u>Chromatography</u> 72(1): 113-125.
- Culberson, C. F., and Armaleo, D. 1992. Induction of a complete secondary-product pathway in a cultured lichen fungus. <u>Experimental Mycology</u> 16: 52–63.
- Culberson, C. F., Culberson, W. L., and Arwood, D. A. 1977. Physiography and fumarprotocetraric acid production in the *Cladonia chlorophaea* group in North Carolina. <u>The Bryologist</u> 80(1): 71-75.
- Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. <u>Nature Methods 9(8)</u>: 772.
- Deduke, C., Timsina, B., and Piercey-Normore, M. D. 2012. Effect of environmental change on secondary metabolite production in lichen-forming fungi. In S. S. Young and S. E. Silvern (eds.), <u>International Perspectives on Global Environmental Change</u>, 197-230. InTech.
- Del Prado, R., Schmitt, I., Kautz, S., Palice, Z., Lücking, R., and Lumbsch, H. T. 2006.
   Molecular data place Trypetheliaceae in Dothideomycetes. <u>Mycological</u> <u>Research</u> 110(5): 511-520.
- Delmail, D., Grube, M., Parro, D., Cook-Moreau, J., Boustie, J., Labrousse, P., et al.
  2013. Halotolerance in lichens: symbiotic coalition against salt stress. In P.
  Ahmad, M. M. Azooz and M. N. V. Prasad (eds.), <u>Ecophysiology and Responses</u> of Plants under Salt Stress, 115-148. New York: Springer

Diezmann, S., Cox, C. J., Schonian, G., Vilgalys, R. J., and Mitchell, T. G. 2004.

- Phylogeny and evolution of medical species of *Candida* and related taxa: a multigenic analysis. Journal of Clinical Microbiology 42(12): 5624–5635.
- Elix, J. A. 1996. Biochemistry and secondary metabolites. In T. H. Nash III (eds.), <u>Lichen</u> <u>Biology</u>, Cambridge: Cambridge University Press.
- Elix, J. A., and Stocker-Wörgötter, E. 2008. Biochemistry and secondary metabolites. In
   T. H. Nash III (eds.), <u>Lichen Bology</u> 2<sup>nd</sup> Edition, Cambridge: Cambridge
   University Press.
- Emmerichet, R., Giez, I., Lange, O. L., and Proksch, P. 1993. Toxicity and antifeedant activity of lichen compounds against the polyphagous herbivourous insect *Spodoptera littoralis*. <u>Phytochemistry</u> 33: 1389–1394.
- Ernst-Russell, M. A., Elix, J. A., Chai, C. L. L., Willisb, A. C., Hamadac, N., and Nash III,
  T. H. 1999. Hybocarpone, a novel cytotoxic naphthazarin derivative from
  mycobiont cultures of the lichen *Lecanora hybocarpa*. <u>Tetrahedron Letters</u>
  40(34): 6321–6324.
- Ertz, D., Miadlikowska, J., Lutzoni, F., Dessein, S., Raspé, O., Vigneron, N., et al. 2009.
  Towards a new classification of the Arthoniales (Ascomycota) based on a threegene phylogeny focussing on the genus *Opegrapha*. <u>Mycological Research</u> 113(1): 141-152.
- Esimone, C. O., Grunwald, T., Nworu, C. S., Kuate, S., Proksch, P., and Uberla, K. 2009. Broad spectrum antiviral fractions from the lichen *Ramalina farinacea* (L.) Ach. <u>Chemotherapy</u> 55(2): 119–126.

Fahselt, D. 1994. Secondary biochemistry of lichens Symbiosis 16: 117-165.

- Fazio, A. T., Adler, M. T., Bertoni, M. D., and Maier, M. S. 2012. Culture studies on the mycobiont of *Caloplaca erythrantha* (Tuck.) Zahlbr. (Teloschistaceae): high production of major lichen secondary metabolites. <u>The Lichenologist</u> 44(4): 533– 542.
- Fazio, A. T., Adler, M. T., Bertoni, M. D., Sepúlveda, C. S., Damonte, E. B., and Maier, M.S. 2007. Lichen secondary metabolites from the cultured lichen mycobionts of

*Teloschistes chrysophthalmus* and *Ramalina celastri* and their antiviral activities. <u>Zeitschrift für Naturforschung C</u> 62(7-8): 543-549.

- Fazio, A. T., Bertoni, M. D., Adler, M. T., Ruiz, L. B., Rosso, M. L., Muggia, L., et al. 2009.
  Culture studies on the mycobiont isolated from *Parmotrema reticulatum* (Taylor)
  Choisy: metabolite production under different conditions. <u>Mycological Progress</u> 8: 359–365.
- Feige, G. B., and Jensen, M. 1992. Basic carbon and nitrogen metabolism of lichens. In
   W. Reisser (eds.), <u>Algae and Symbioses: Plants, Animals, Fungi, Viruses,</u>
   <u>Interactions Explored</u>, 277–299. Bristol: Biopress Limited.
- Fernández-Brime, S., Llimona, X., Lutzoni, F., and Gaya, E. 2013. Phylogenetic study of *Diploschistes* (lichen-forming Ascomycota: Ostropales: Graphidaceae), based on morphological, chemical, and molecular data. <u>Taxon</u> 62(2): 267–280.
- Fröberg, L., Baur, A., and Baur, B. 1993. Differential herbivore damage to calcicolous lichens by snails. <u>The Lichenologist 25(1): 83–95</u>.
- Gaikwad, S., Verma, N., Sharma, B. O., and Behera, B. C. 2014. Growth promoting effects of some lichen metabolites on probiotic bacteria. <u>Journal of Food</u> <u>Science and Technology</u> 51(10): 2624–2631.
- Galloway, D. J. 2007. <u>Flora of New Zealand lichens, including lichen-forming amd</u> <u>lichenicolous fungi</u> 2<sup>nd</sup> Edition. Lincoln: Manaaki Whenua.
- Gardes, M., and Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes application to the identication of mycorrhizae and rust. <u>Molecular Ecology</u> 2: 113-118.
- Gardes, M., White, T. J., Fortin, J. A., Bruns, T. D., and Taylor, J. W. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. <u>Canadian Journal of Botany</u> 69: 180–190.
- Gargas, A., DePriest, P. T., Grube, M., and Tehler, A. 1995. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. <u>Science</u> 268(5216): 1492-1495.

- Gargas, A., and Taylor, J. W. 1992. Polymerase chain reaction (PCR) primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. <u>Mycologia</u> 84(5): 589-592.
- Gauslaa, Y., and Solhaug, K. A. 2001. Fungal melanins as a sun screen for symbiotic green algae in the lichen *Lobaria pulmonaria*. <u>Oecologia</u> 126: 462–471.
- Gilbert, O. L. 2004. The lichen hunters. Lewes, East Sussex: Book Guild.
- Goebel, F., and Kunze, G. 1827. Pharmaceutische Waarenkunde. Eisenach Gonidien. Österreichische botanische Zeitschrift 40: 323-328.
- Goel, M., Sharma, P. K., Dureja, P., Rani, A., and Uniyal, P. L. 2011. Antifungal activity of extracts of the lichens *Parmelia reticulata*, *Ramalina roesleri*, *Usnea longissima* and *Stereocaulon himalayense*. <u>Archives of Phytopathology and Plant Protection</u> 44(13): 1300–1311.
- González-Tejero, M. R., Molero-Mesa, J., Casares-Porcel, M., and Martínez-Lirola, M. J. 1995. New contributions to the ethnopharmacology of Spain. <u>Journal of</u> <u>Ethnopharmacology</u> 45: 157–165.
- Gueidan, C., Aptroot, A., Cáceres, M. E. d. S., and Binh, N. Q. 2016. Molecular phylogeny of the tropical lichen family Pyrenulaceae: contribution from dried herbarium specimens and FTA card samples. <u>Mycological Progress</u> 15(1): 7.
- Halama, P., and Van Haluwin, C. 2004. Antifungal activity of lichen extracts and lichenic acids. <u>BioControl</u> 49(1): 95–107.
- Hale, M. E. 1979. How to Know the Lichens 2<sup>nd</sup> Edition. Dubuque, Iowa: W. C. Brown Co.
- Hale, M. E. 1983. <u>The Biology of Lichens</u> 3<sup>rd</sup> Edition. London: Edward Arnold.
- Hamada, N., Miyagawa, H., Miyawaki, H., and Inoue, M. 1996. Lichen substances in mycobionts of crustose lichens cultured on media with extra sucrose. <u>The</u> <u>Bryologist</u> 99(1): 71-74.
- Harris, R. C. 1984. The family Trypetheliaceae (Loculoascomycetes: lichenized Melanommatales) in Amazonian Brazil. <u>Acta Amazonica</u> 14(1-2): 55-80.
- Harris, R. C. 1995. More Florida Lichens. Including the  $10 \not{c}$  Tour of the Pyrenolichens. New York: Published by the author.

- Hawksworth, D. L. 1976. Lichen Chemotaxonomy. In D. H. Brown, D. L. Hawksworth and R. H. Bailey (eds.), <u>Lichenology: Progress and Problems</u> 139-184. London: Academic Press.
- Hawksworth, D. L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. <u>Mycological Research</u> 95(6): 641-655.
- Hawksworth, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited <u>Mycological Research</u> 105(12): 1422-1432.
- Hawrksworth, D. L., and Hill, D. J. 1984. <u>The lichen-forming fungi</u>. Glasgow & London, UK: Blackie.
- Heng, L., Li, C., Kim, J. C., Liu, Y., Jung, J. S., Koh, Y. J., et al. 2013. Biruloquinone, an acetylcholinesterase inhibitor produced by lichen-forming fungus *Cladonia macilenta*. Journal of Microbioly and Biotechnology 23(2): 161–166.
- Hertel, H. 1988. Problems in monographing Antarctic crustose lichens. <u>Polarforschung</u> 58(2-3): 65-76.
- Hidalgo, M. E., Fernández, E., Quilhot, W., and Lissi, E. 1994. Antioxidant activity of depsides and depsidones. <u>Phytochemistry</u> 37(6): 1585-1587.
- Hofstetter, V., Miadlikowska, J., Kauff, F., and Lutzoni, F. 2007a. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: A case study of the Lecanoromycetes (Ascomycota). <u>Molecular Phylogenetics and Evolution</u> 44: 412–426.
- Hofstetter, V., Miadlikowska, J., Kauff, F., and Lutzoni, F. 2007b. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: A case study of the Lecanoromycetes (Ascomycota). <u>Molecular Phylogenetics and Evolution</u> 44: 412-426.
- Honda, N. K., Pavan, F. R., Coelho, R. G., de Andrade, L. S. R., Micheletti, A. C., Lopes, T. I., et al. 2010. Antimycobacterial activity of lichen substances. <u>Phytomedicine</u> 17(5): 328–332.
- Honegger, R. 2008. Mycobionts. In T. H. Nash III (eds.), <u>Lichen Biology</u> 2<sup>nd</sup> Edition, 2739. Cambridge: Cambridge University Press.

- Huang, Z., Tao, J., Ruan, J., Li, C., and Zheng, G. 2014. Anti-inflammatory effects and mechanisms of usnic acid, a compound firstly isolated from lichen *Parmelia saxatilis*. Journal of Medicinal Plant Research 8(4): 197-207.
- Huneck, S. 1999. The significance of lichens and their metabolites. <u>Naturwissenschaften</u> 86(12): 559-570.
- Hyde, K. D., Liu, J. K., Binder, M., Aryawansha, H., Boehm, E., Boonmee, S., et al. 2013. Families of Dothideomycetes. <u>Fungal Diversity</u> 63: 1–313.
- Ingólfsdóttir, K., Chung, G. A. C., Skúlason, V. G., Gissurarson, S. R., and Vilhelmsdóttir,
   M. 1998. Antimycobacterial activity of lichen metabolites *in vitro*. <u>European</u>
   <u>Journal of Pharmaceutical Sciences</u> 6(2): 141–144.
- Ingólfsdóttir, K., Gudmundsdóttir, G. F., Ogmundsdóttir, H. M., Paulus, K., Haraldsdóttir, S., Kristinsson, H., et al. 2002. Effects of tenuiorin and methyl orsellinate from the lichen *Peltigera leucophlebia* on 5-/15-lipoxygenases and proliferation of malignant cell lines *in vitro*. Phytomedicine 9(7): 654–658.
- Ingólfsdóttir, K., Wiedemann, B., Birgisdóttir, M., Nenninger, A., Jónsdóttir, S., and Wagner, H. 1997. Inhibitory effects of baeomycesic acid from the lichen *Thamnolia subuliformis* on 5-lipoxygenase *in vitro*. <u>Phytomedicine</u> 4(2): 125–128.
- Jahns, H. M. 1973. Anatomy, morphology, and development. In V. Ahmadjian and M. E. Hale (eds.), <u>The lichens</u>, 3-58. New York: Academic Press.
- Jahns, H. M. 1988. The lichen thallus. In M. Galum (eds.), <u>Handbook of lichenology</u> V. 1, Florida, USA: CRC Press.
- Karagöz, A., Dogruöz, N., Zeybek, Z., and Aslan, A. 2009. Antibacterial activity of some lichen extracts. Journal of Medicinal Plants Research 3(12): 1034-1039.
- Kasalicky, T., Döring, H., Rambold, G., and Wedin, M. 2000. A comparison of ITS and LSU nrDNA phylogenies of *Fulgensia* (Teloschistaceae, Lecanorales), a genus of lichenised ascomycetes. <u>Canadian Journal of Botany</u> 78(2): 1580-1589.
- Kashiwada, Y., Nagao, T., Hashimoto, A., Ikeshiro, Y., Okabe, H., Cosentino, L. M., et al.
   2000. Anti-AIDS agents 38. Anti-HIV. Activity of 3-O-acyi ursolic acid derivatives.
   Journal of Natural Product 63(12): 1619-1622.

- Kathirgamanathara, S., Ratnasooriyab, W. D., Baekstromc, P., Andersend, R. J., and Karunaratnea, V. 2006. Chemistry and bioactivity of Physciaceae lichens *Pyxine consocians* and *Heterodermia leucomelos*. <u>Pharmaceutical Biology</u> 44(3): 217-220.
- Kim, J. W., Song, K. S., Yoo, I. D., Chang, H. W., Yu, S. H., Bae, K. G., et al. 1996. Two phenolic compounds isolated from *Umbilicaria esculenta* as phospholipase A<sub>2</sub> inhibitors. <u>The Korean Journal of Mycology</u> 24(3): 237–242.
- Kirk, P. M., Cannon, P. F., Minter, D. W., and Stalpers, J. A. 2008. <u>Ainsworth & Bisby's</u> <u>Dictionary of the Fungi</u> 10<sup>th</sup> Edition. Wallingford, U.K. : CABI Publishing.
- Kofler, L. 1970. A method to use lichen spores in quantitative studies on gemination. Bryologist 73: 602-606.
- Kosanic, M., Manojlovic, N., Jankovic, S., Stanojkovic, T., and Rankovic, B. 2013. *Evernia prunastri* and *Pseudoevernia furfuraceae* lichens and their major metabolites as antioxidant, antimicrobial and anticancer agents. <u>Food and</u> <u>Chemical Toxicology</u> 53: 112–118.
- Kosanic, M., Rankovic, B., Stanojkovic, T., Rancic, A., and Manojlovic, N. 2014.
   *Cladonia* lichens and their major metabolites as possible natural antioxidant, antimicrobial and anticancer agents. <u>LWT-Food Science and Technology</u> 59(1): 518–525.
- Kosanic, M., Rankovic, B., and Sukdolak, S. 2010. Antimicrobial activity of the lichen *Lecanora frustulosa* and *Parmeliopsis hyperopta* and their divaricatic acid and zeorin constituents. <u>African Journal of Microbiology Research</u> 4: 885–890.
- Kosanic, M., Rankovic, B., and Vukojevic, J. 2011. Antioxidant properties of some lichen species. Journal of food science and technology 48(5): 584–590.
- Kraichak, E., Parnmen, S., Lücking, R., and Lumbsch, H. T. 2014. *Gintarasia* and *Xalocoa*, two new genera to accommodate temperate to subtropical species in the predominantly tropical Graphidaceae (Ostropales, Ascomycota). <u>Australian</u> <u>Systematic Botany</u> 26(6): 466-474.
- Kumar, J., Dhar, P., Tayade, A. B., Gupta, D., Chaurasia, O. P., Upreti, D. K., et al. 2014.
   Antioxidant capacities, phenolic profile and cytotoxic effects of saxicolous
   lichens from Trans-Himalayan cold desert of Ladakh. <u>PLoS ONE</u> 9(6): e98696.
- Kumar, K. C., and Müller, K. 1999. Lichen metabolites. 2. Antiproliferative and cytotoxic activity of gyrophoric, usnic, and difractaic acids on human keratinocyte growth. Journal of Natural Products 62(6): 821–823.
- Larena, I., Salazar, O., González, V., Julián, M. C., and Rubio, V. 1999. Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. <u>Journal of Biotechnology</u> 75(2-3): 187-194.
- Lauterwein, M., Oethinger, M., Belsner, K., Peters, T., and Marre, R. 1995. *In vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid, and (-)-usnic acid against aerobic and anaerobic microorganisms. <u>Antimicrobial</u>
  <u>Agents and Chemotherapy</u> 39(11): 2541–2543.
- Lawrey, J. D. 1984. Biology of Lichenized Fungi. New York: Praeger Publishers.
- Lawrey, J. D. 1986. Biological role of lichen substances. The Bryologist 89(2): 111-122.
- Lebail, J. B. E. F. 1853. <u>Des lichens, conside re s sous le point de vue e conomique,</u> <u>me dical, et physiologique (nutrition)</u>. M.D. thesis, Faculte de Me decine de Paris.
- Lee, S. B., and Taylor, J. W. 1992. Phylogeny of five fungus-like protoclistan *Phytophthora* species, inferred from the Internal Transcribed Spacers of ribosomal DNA. <u>Molecular Biology Evolution</u> 9: 636–653.
- Letrouit-Galinou, M.-A. 1957. Revision monographique du genre *Laurera* (Lichenes, Trypéthéliacées). <u>Revue Bryologique et Lichénologique</u> 26: 207-264.
- Li, B., Lin, Z. W., and Sun, H. D. 1991. The chemical constituents of four lichens from China. <u>Acta Botanica Yunnanica</u> 13(1): 81-84.
- Lima, E. L., C., M. L., Aptroot, A., and Cáceres, M. E. S. 2013. New lichen species from Vale do Catimbau, Pernambuco, Brazil. <u>Bryologist</u> 116: 327–329.

- Lohézic-Le Dévéhat, F., Tomasi, S., Elix, J. A., Bernard, A., Rouaud, I., Uriac, P., et al. 2007. Stictic acid derivatives from the lichen *Usnea articulata* and their antioxidant activities. Journal of Natural Product 70(7): 18-20.
- Lopes, T. I. B., Coelho, R. G., Yoshida, N. C., and Honda, N. K. 2008. Radical scavenging activity of Orsellinates. <u>Chemical and Pharmaceutical Bulletin</u> 56(11): 1551–1554.
- Lumbsch, H. T. 1988. The use of metabolic data in lichenology at the species and subspecific levels. <u>Lichenologist</u> 30: 357-367.
- Lumbsch, H. T. 1998. Taxonomic use of metabolic data in lichen-forming fungi. In J. C. Frisvad, P. D. Bridge and D. K. Arora (eds.), <u>Chemical Fungal Taxonomy</u>, 345–387. New York: Marcel Dekker.
- Lumbsch, H. T. 2002. Analysis of phenolic products in lichens for identification and taxonomy. In I. Kranner, R. Beckett and A. Varma (eds.), <u>Protocols in</u> <u>Lichenology</u>, 281-295. Springer-Verlag Berlin Heidelberg.
- Lumbsch, H. T., Schmitt, I., Lücking, R., Wiklund, E., and Wedin, M. 2007. The phylogenetic placement of Ostropales within Lecanoromycetes (Ascomycota) revisited. <u>Mycological Research</u> 111(3): 257–267.
- Lutzoni, F., KauV, F., Cox, C. J., McLaughlin, D., Celio, G., Dentinger, B., et al. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. American Journal of Botany 91: 1446-1480.
- Lutzoni, F., and Vilgalys, R. 1995. *Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichens. <u>Cryptogamic Botany</u> 5: 71–81.
- Makhija, U., and Patwardhan, P. G. 1989. The lichen family Asterotheliaceae sensu Zahlbrucker in India. <u>Biovigyanam</u> 15: 61–89.
- Makhija, U., and Patwardhan, P. G. 1992. Nomenclatural notes on some species of *Trypethelium*. International Journal of Mycology and Lichenology 5: 237-251.
- Makhija, U., and Patwardhan, P. G. 1993. A contribution to our knowledge of the lichen genus *Trypethelium* (family Trypetheliaceae). <u>Journal of the Hattori Botanical</u> <u>Laboratory</u> 73: 183-219.

- Mangold, A., Martin, M. P., Lücking, R., and Lumbsch, H. T. 2008. Molecular phylogeny suggests synonymy of Thelotremataceae within Graphidaceae (Ascomycota: Ostropales). <u>Taxon</u> 58: 476–486.
- Manojlovic, N., Rankovic, B., Kosanic, M., Vasiljevic, P., and Stanojkovic, T. 2012. Chemical composition of three *Parmelia* lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites. <u>Phytomedicine</u> 19(13): 1166–1172.
- Manojlovic, N. T., Solujic, S., and Sukdolak, S. 2002. Antimicrobial activity of an extract and anthraquinones from *Caloplaca schaereri*. <u>The Lichenologist</u> 34: 83-85.
- Manojlovic, N. T., Vasiljevic, P. J., Gritsanapan, W., Supabphol, R., and Manojlovic, I.
  2010a. Phytochemical and antioxidant studies of *Laurera benguelensis* growing in Thailand. <u>Biological Research</u> 43(2): 169-176.
- Manojlovic, N. T., Vasiljevic, P. J., and Markovic, Z. S. 2010b. Antimicrobial activity of extracts and various fractions of chloroform extract from the lichen *Laurera benguelensis*. Journal of Biological Research-Thessaloniki 13: 27-34.
- Martin, K. J., and Rygiewicz, P. T. 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. <u>BioMed Central</u> <u>Microbiology</u> 5: 28.
- Martins, M. C. B., de Lima, M. J. G., Silva, F. P., Azevedo-Ximenes, E., da Silval, N. H., and Pereira, E. C. 2010. *Cladia aggregata* (lichen) from Brazilian Northeast, chemical characterization and antimicrobial activity. <u>Brazilian Archives of</u> <u>Biology and Technology</u> 53(1): 115–122.
- Marx, J. 2001. Anti-inflammaroties inhibit cancer growth but how? <u>Science 291: 581-582</u>.
- Matheny, P. B., Liu, Y. J., Ammirati, J. F., and Hall, B. D. 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (*inocybe*, agaricales).
   <u>American Journal of Botany</u> 89 (4): 688–698.
- Mathey, A. 1979. Contribution al'etude de la famille des Trypetheliacees. <u>Nova Hedwigia</u> 4: 917-935.

- Mathey, A., Roy, W. V., Vaeck, L. V., Eckhardt, G., and Steglich, W. 1994. *In situ* analysis of a new perylene quinone in lichens by Fourier-transform laser microprobe mass spectrometry with external source. <u>Rapid Communications in</u> <u>Mass Spectrometry</u> 8(1): 46-52.
- Mathey, A., Spiteller, P., and Steglich, W. 2002. Draculone, a new anthraquinone pigment from the tropical lichen *Melanotheca cruenta*. <u>Zeitschrift fur</u> <u>Naturforschung C</u> 57: 565-567.
- Mathey, A., Steffaan, B., and Steglich, W. 1980. 1,2-Naphthochinon-Derivate aus kulturen des mycosymbionten der flechte *Trypethelium eluteriae* (Trypetheliaceae). Liebigs Annalen der Chemie 1980(5): 779–785.
- McDonald, T. R., Gaya, E., and Lutzoni, F. 2013. Twenty-five cultures of lichenizing fungi available for experimental studies on symbiotic systems. <u>Symbiosis</u> 59(3): 165– 171.
- McEvoy, M., Gauslaa, Y., and Solhaug, K. A. 2007. Changes in pools of depsidones and melanins, and their function, during growth and acclimation under contrasting natural light in the lichen *Lobaria pulmonaria*. <u>New Phytologist</u> 175(2): 271–282.
- Melo, M. G., J.P., d. S., Serafini, M. R., Caregnato, F. F., Pasquali, M. A., Rabelo, T. K., et al. 2011. Redox properties and cytoprotective actions of atranorin, a lichen secondary metabolite. <u>Toxicology in Vitro</u> 25(2): 462–468.
- Millot, M., Tomasi, S., Articus, K., Rouaud, I., Bernard, A., and Boustie, J. 2007.
  Metabolites from the lichen *Ochrolechia parella* growing under two different heliotropic conditions. <u>Journal of Natural Product</u> 70(3): 16–318.
- Mitrovic, T., Stamenkovic, S., Cvetkovic, V., Tošic, S., Stankovic, M., Radojevic, I., et al.
  2011. Antioxidant, antimicrobial and antiproliferative activities of five lichen species. <u>International Journal of Molecular Sciences</u> 12: 5428-5448.
- Molina, M. C., and Crespo, A. 2000. Comparison of development of axenic cultures of five species of lichen-forming fungi. <u>Mycological Research</u> 104(5): 595-602.

- Molina, M. C., P.K., D., and González, N. 2015. Success in the isolation and axenic culture of *Anaptychia ciliaris* (Physciaceae, Lecanoromycetes) mycobiont. <u>Mycoscience</u> 56(4): 351-358.
- Molina, M. C., Stocker-Wörgötter, E., Türk, R., and Vicente, C. 1997. Axenic culture of the mycobiont of *Xanthoria parietina* in different nutritive media: effect of carbon source in spore germination. <u>Endocytobiosis and Cell Research</u> 12: 103-109.
- Molnar, K., and Farkas, E. 2010. Current results on biological activities of lichen secondary metabolites: a review. <u>Zeitschrift fur Naturforschung C</u> 65(3-4): 157– 173.
- Montagne, C. 1845. Plantae cellularesquas in insulis Philippinensibus a cl. Cuming collectas recensuit observationibus nonnullis descriptionibusque illustravit. <u>The London Journal of Botany</u> 4: 3-11.
- Moxham, T. H. 1986. The commercial exploitation of lichens for the perfume industry. In E. J. Brunke (eds.), <u>Progress in essential oil research</u>, 491–503. Berlin: Walter de Gruyter.
- Müller, J. 1885. Lichenologische Beiträge 21. Flora 68: 247-261.
- Murtagh, G. J., Dyer, P. S., Furneaux, P. A., and Crittenden, P. D. 2002. Molecular and physiological diversity in the bipolar lichen-forming fungus *Xanthoria elegans*. <u>Mycological Research</u> 106(11): 1277–1286.
- Nanayakkara, C., Bombuwala, K., Kathirgamanathar, S., Adikaram, N. K. B.,
  Wijesundara, D. S. A., Hariharan, G. N., et al. 2005. Effect of some lichen
  extracts from Sri Lanka on larvae of *Aedes aegypti* and the fungus *Cladosporium cladosporioides*. Journal of the National Science Foundation of Sri
  Lanka 33(2): 147–149.
- Nash III, T. H. 1996. Photosynthesis respiration productivity and growth. In T. H. Nash III (eds.), <u>Lichen Biology</u>, 88-120. Cambridge: Cambridge University Press.
- Nash III, T. H. 2008. Introduction. In T. H. Nash III (eds.), <u>Lichen Biology</u> 2<sup>nd</sup> Edition, 1-8. Cambridge: Cambridge University Press.

- Neamati, H., Hong, H., Mazumder, A., Wang, S., Sunder, S., Nicklaus, M. C., et al. 1997.
  Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching. <u>Journal of Medicinal Chemistry</u> 40: 942-951.
- Nelsen, M. P., Lücking, R., Aptroot, A., Andrew, C. J., Cáceres, M., Plata, E. R., et al.
  2014. Elucidating phylogenetic relationships and genus-level classification within the fungal family Trypetheliaceae (Ascomycota: Dothideomycetes). <u>Taxon</u> 63(5): 974-992.
- Nelsen, M. P., Lücking, R., Grube, M., Mbatchou, J. S., Muggia, L., Plata, E. R., et al.
  2009. Unravelling the phylogenetic relationships of lichenised fungi in
  Dothideomyceta. <u>Studies in Mycology</u> 64: 135-144.
- Nelsen, M. P., Lücking, R., Mbatchou, J. S., Andrew, C. J., Spielmann, A. A., and Lumbsch, H. T. 2011. New insights into relationships of lichen-forming Dothideomycetes. <u>Fungal Diversity</u> 51(1): 155–162.
- Nylander, W. 1866. Circa novum in studio lichenum criterium chemicum. <u>Flora</u> 49: 198-201.
- Oettl, S. K., Gerstmeier, J., Khan, S. Y., Wiechmann, K., Bauer, J., Atanasov, A. G., et al. 2013. Imbricaric acid and perlatolic acid: multi-targeting anti-inflammatory depsides from *Cetrelia monachorum*. <u>PLoS ONE</u> 8(10): e76929.
- Oksanen, I. 2006. Ecological and biotechnological aspects of lichens. <u>Applied</u> <u>Microbiology and Biotechnology</u> 73(4): 723-734.
- Okuyama, E., Umeyama, K., Yamazaki, M., Kinoshita, Y., and Yamamoto, Y. 1995. Usnic acid and diffractaic acid as analgesic and antipyretic components of *Usnea diffracta*. <u>Planta Medica</u> 61(2): 113–115.
- Olafsdottir, E. S., and Ingólfsdottir, K. 2001. Polysaccharides from lichens: structural characteristics and biological activity. <u>Planta Medica</u> 67(3): 199-208.
- Oliver, E., Crittenden, P. D., Beckett, A., and Brown, D. H. 1989. Growth of lichenforming fungi on membrane filters. <u>The Lichenologist</u> 21(4): 387-392.

- Ostrofsky, A., and Denison, W. C. 1980. Ascospore discharge and germination in *Xanthoria polycarpa*. <u>Mycologia</u> 72: 1171-1179.
- Papong, K., Boonpragob, K., Parnmen, S., and Lumbsch, H. T. 2012. Molecular phylogenetic studies on tropical species of *Lecanora* sensu stricto (Lecanoraceae, Ascomycota). <u>Nova Hedwigia</u> 96(1-2): 1-13.
- Parnmen, S., Lücking, R., and Lumbsch, H. T. 2012. Phylogenetic classification at generic level in the absence of distinct phylogenetic patterns of phenotypical variation: A case study in Graphidaceae (Ascomycota). <u>PLoS ONE</u> 7(12): e51392.
- Paudel, B., Bhattarai, H. D., Lee, H. K., Oh, H., Shin, H. W., and Yim, J. H. 2010.
  Antibacterial activities of ramalin, usnic acid and its three derivatives isolated from the Antarctic lichen *Ramalina terebrata*. <u>Zeitschrift für Naturforschung C</u> 65(1-2): 34-38.
- Pavithra, G. M., Vinayaka, K. S., Rakesh, K. N., Syed, J., Dileep, N., Prashith, K. T. R., et al. 2013. Antimicrobial and antioxidant activities of a macrolichen *Usnea pictoides* G. Awasthi (Parmeliaceae). Journal of Applied Pharmaceutical Science 3(8): 154-160.
- Petrini, O., Hake, U., and Dreyfuss, M. M. 1990. An analysis of fungal communities isolated from fruticose lichens. <u>Mycologia</u> 82(4): 444-451.
- Pittayakhajonwut, P., Sri-indrasutdhi, V., Dramae, A., Lapanun, S., Suvannakad, R., and Tantichareon, M. 2009. Graphisins A and B from the lichen *Graphis tetralocularis*. <u>Australian Journal of Chemistry</u> 62(4): 389-391.
- Pramyothin, P., Janthasoot, W., Pongnimitprasert, N., Phrukudom, S., and Ruangrungsi,
   N. 2004. Hepatotoxic effect of (+) usnic acid from *Usnea siamensis* Wainio in
   rats, isolated rat hepatocytes and isolated rat liver mitochondria. Journal of
   <u>Ethnopharmacology</u> 90(2-3): 381–387.
- Proksa, B., Adamcova, J., Sturdikova, M., and Fuska, J. 1994. Metabolites of *Pseudevernia furfuracea* (L.) Zopf. and their inhibition potential of proteolytic enzymes. <u>Die Pharmazie</u> 49(4): 282-283.

Purvis, W. 2000. Lichens. London: The Natural History Museum.

- Rankovic, B., and Kosanic, M. 2015. Lichens as a potential source of bioactive secondary metabolites. In B. Rankovic (eds.), <u>Lichen Secondary Metabolites</u>
   <u>Bioactive Properties and Pharmaceutical Potential</u>, 1-26. Switzerland: Springer International Publishing.
- Rankovic, B., Kosanic, M., Manojlovic, N., Rancic, A., and Stanojkovic, T. 2014.
   Chemical composition of *Hypogymnia physodes* lichen and biological activities of some its major metabolites. <u>Medicinal Chemistry Research</u> 23: 408–416.
- Rankovic, B., and Mišic, M. 2008. The antimicrobial activity of the lichen substances of the lichens *Cladonia furcata*, *Ochrolechia androgyna*, *Parmelia caperata* and *Parmelia conspersa*. <u>Biotechnology & Biotechnological Equipmen</u> 22: 1013– 1016.
- Rashmi, S., and Rajkumar, H. G. 2014. Preliminary phytochemical screening of different solvent extracts of lichens from Kodagu district, Karnataka. <u>Journal of</u> <u>Pharmacognosy and Phytochemistry</u> 3(4): 209-212.
- Rehner, S. A., and Samuels, G. J. 1994. Taxonomy and phylogeny of Gliocladium analysed from nuclear large subunit ribosomal DNA sequences. <u>Mycological</u> <u>Research</u> 98(6): 625-634.
- Rivas Plata, E., and Lumbsch, H. T. 2011. Parallel evolution and phenotypic divergence in lichenized fungi: A case study in the lichen-forming fungal family
   Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). <u>Molecular</u>
   <u>Phylogenetics and Evolution</u> 61(1): 45-63.
- Romagni, J. G., and Dayan, F. E. 2002. Structural diversity of lichen metabolites and their potential for use. In U. R. (eds.), <u>Advances in Microbial Toxin Research and</u> <u>Its Biotechnological Exploration</u>, New York: Kluwer Academic Plenum Publisher.
- Ronquist, F., and Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. <u>Bioinformatics</u> 19: 1572-1574.

- Ruibal, C., Gueidan, C., Selbmann, L., Gorbushina, A. A., Crous, P. W., Groenewald, J.Z., et al. 2009. Phylogeny of rock-inhabiting fungi related to Dothideomycetes.<u>Studies in Mycology</u> 64: 123–133.
- Russo, A., Piovano, M., Lombardo, L., Garbarino, J., and Cardile, V. 2008. Lichen metabolites prevent UV light and nitricoxide mediated plasmid DNA damage and induce apoptosis in human melanoma cells. <u>Life Sciences</u> 83(13-41): 468– 474.
- Saenz, M. T., Garcia, M. D., and J.G., R. 2006. Antimicrobial activity and phytochemical studies of some lichens from south of Spain. <u>Fitoterapia</u> 77(3): 156-159.
- Saklani, A., and Upreti, D. K. 1992. Folk uses of some lichens in Sikkim. <u>Journal of</u> <u>Ethnopharmacology</u> 37(3): 229-233.
- Sanchez, M. L., Bats, J. P., and Moulines, J. 1997. Thermal hydrolysis of the main depsides and depsidones contained in the lichens used in perfumery. <u>Riv.Ital.EPPOS</u>: 100-104.
- Sangvichien, E., Hawksworth, D. L., and Whalley, A. J. S. 2011. Ascospore discharge, germination and culture of fungal partners of tropical lichens, including the use of a novel culture technique. <u>IMA Fungus</u> 2(2): 143-153.
- Santiago, K., Sangvichien, E., Boonpragob, K., and dela Cruz, T. 2013. Secondary metabolic profiling and antibacterial activities of different species of *Usnea* collected in Northern Philippines. <u>Mycosphere</u> 4(2): 267–280.
- Santiago, K. A., Borricano, J. N., Canal, J. N., Marcelo, D. A., Perez, M. P., and dela Cruz, T. E. 2010. Antibacterial activities of fruticose lichens collected from selected sites in Luzon Island, Philippines. . <u>Philippine Science Letters</u> 3(2): 18-20.
- Sheen, J., Kho, Y. H., and Bae, K. S. 1993. Genomic sequence of mitochondrial genes coding for ATPase subunit 6 and small subunit ribosomal RNA from *Penicillium chrysogenum*: a key for molecular systematics on fungi. <u>Nucleic acids research</u> 21(18): 4393.

- Shivanna, R., and Garampalli, H. R. 2015. Evaluation of fungistatic potential of lichen extracts against *Fusarium solani* (Mart.) Sacc. causing Rhizome rot disease in Ginger. Journal of Applied Pharmaceutical Science 5(10): 67-72.
- Sipman, H. J. M., and Aptroot, A. 2001. Where are the missing lichens? <u>Mycological</u> <u>Research</u> 105(12): 1433–1439.
- Sisodia, R., Geol, M., Verma, S., Rani, A., and Dureja, P. 2013. Antibacterial and antioxidant activity of lichen species *Ramalina roesleri*. <u>Natural Product</u> <u>Research</u> 27(23): 2235–2239.
- Solhaug, K. A., and Gauslaa, Y. 2004. Photosynthates stimulate the UV-B induced fungal anthraquinone synthesis in the foliose lichen *Xanthoria parietina*. <u>Plant, Cell and Environment</u> 27(2): 167–176.
- Solhaug, K. A., Gauslaa, Y., Nybakken, L., and Bilger, W. 2003. UV-induction of sunscreening pigments in lichens. <u>New Phytologist</u> 158(1): 91–100.
- Staiger, B., Kalb, K., and Grube, M. 2006. Phylogeny and phenotypic variation in the lichen family Graphidaceae (Ostropomycetidae, Ascomycota). <u>Mycological</u> <u>Research</u> 110(7): 765-772.
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. <u>Bioinformatics</u> 22: 2688-2690.
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. <u>Bioinformatics</u> 30(9): 1312-1313.
- Stamatakis, A., Hoover, P., and Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. <u>Systematic Biology</u> 57(5): 758-771.
- Stocker-Wörgötter, E. 2001. Experimental studies of the symbiosis: DNA-analyses, differentiation and secondary chemistry of selected mycobionts, artificial resynthesis of two-and tripartite symbioses. <u>Symbiosis</u> 30: 207-227.
- Stocker-Wörgötter, E. 2008. Metabolic diversity of lichen-forming ascomycetous fungi: culturing, polyketide and shikimate metabolite production, and PKS genes. <u>Natural Product Reports</u> 25(1): 188-200.

Stocker-Wörgötter, E., and Brunauer, G. 2005. Culture of lichen fungi for future production of biologically active compounds. <u>Symbiosis</u> 38: 187-201.

- Stocker-Wörgötter, E., Elix, J. A., and Grube, M. 2004. Secondary chemistry of lichenforming fungi: chemosyndromic variation and DNA-analyses of cultures and chemotypes in the *Ramalina farinacea* complex. <u>The Bryologist</u> 107(2): 152-162.
- Stocker-Wörgötter, E., Hager, A., and Elix, J. A. 2009. Intraspecific chemical variation within the crustose lichen genus *Haematomma*: anthraquinone production in selected cultured mycobionts as a response to stress and nutrient supply. <u>Phytochemistry Reviews</u> 8(3): 561–569.
- Sun, H., Niu, F., Lin, Z., Cao, D., Li, B., and Wu, J. 1990. Chemical constituents of four medicinal lichens. <u>Acta Botanica Sinica</u> 32(10): 783-788.
- Sun, L. Y., Liu, Z. L., Zhang, T., Niu, S. B., and Zhao, Z. T. 2010. Three antibacterial naphthoquinone analogues from cultured mycobiont of lichen *Astrothelium* sp. <u>Chinese Chemical Letters</u> 21(7): 842–845.
- Sundararaj, J. P., Kuppuraj, S., Ganesan, A., Ponnusamy, P., and Nayaka, S. 2015. *In vitro* assesssment of antioxidant and antimicrobial activities of different solvent extracts from lichen *Ramalina nervulosa*. <u>International Journal of Pharmacy and</u> <u>Pharmaceutical Sciences</u> 7(8).
- Takenaka, Y., Naito, Y., Le, D. H., Hamad, N., and Tanahashi, T. 2013. Naphthoquinones and phenalenone derivatives from the cultured lichen mycobionts of *Trypethelium* sp. <u>Heterocycles</u> 87(9): 1897 - 1902.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6:
   Molecular Evolutionary Genetics Analysis Version 6.0. <u>Molecular Biology and</u> <u>Evolution</u> 30(12): 2725-2729.
- Tay, T., Türk, A. O., Yilmaz, M., Türk, H., and Kivanç, M. 2004. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstictic acid and protocetraric acid constituents. <u>Zeitschrift</u> <u>für Naturforschung C</u> 59(5-6): 384–388.

- Tehler, A., Farris, S., Lipscomb, D. L., and Källersjo, M. 2000. Phylogenetic analyses of the fungi based on rDNA data sets. <u>Mycologia</u> 92(3): 459-474.
- Thomas, E. A. 1939. Über die Biologie von Flechtenbildnern. Beitr. Kryptogamenfl. <u>Schweiz</u> 9(1-208).
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. <u>Nucleic</u> <u>Acids Research</u> 22: 4673-4680.
- Töbler, F. 1909. Das physiologische gleichgewicht von Pilz und Alge in den Flechten. Berichte der deutschen gesellschaft\berlin deutschen botanischen Gesellschaft 27: 421-427.
- Turbin, L. 1996. <u>The growth and physiology of lichen forming fungi</u>. Doctor of Philosophy, University of Nottingham.
- Türk, A. O., Yilmaz, M., Kivanç, M., and Türk, H. 2003. The antimicrobial activity of extracts of the lichen *Cetraria aculeata* and its protolichesterinic acid constituent. <u>Zeitschrift für Naturforschung C</u> 58(11-12): 850–854.
- Türk, H., Yilmaz, M., Tay, T., Türk, A. O., and Kivanç, M. 2006. Antimicrobial activity of extracts of chemical races of the lichen *Pseudevernia furfuracea* and their physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents. <u>Zeitschrift für Naturforschung C</u> 61: 499-507.
- Turner, N. J. 1977. Economic importance of black tree lichen (*Bryoria fremontii*) to the Indians of western North America. <u>Economic Botany</u> 31: 461-470.
- Vasiljevic, P., Najman, S., Manojlovic, N., Vukelic, M., and Juskovic, M. 2009. *In vitro* cytotoxic activity of lichen *Laurera benguelensis*. <u>Planta Medica</u> 75.
- Verma, N., Behera, B. C., and Joshi, A. 2012. Studies on nutritional requirement for the culture of lichen *Ramalina nervulosa* and *Ramalina pacifica* to enhance the production of antioxidantmetabolites. <u>Folia Microbiologica</u> 57: 107–114.

- Verma, N., Behera, B. C., Sonone, A., and Makhija, U. 2008a. Cell aggregates derived from natural lichen thallus fragments: antioxidant activities of lichen metabolites developed *in vitro*. <u>Natural Product Communications</u> 3(11): 1911–1918.
- Verma, N., Behera, B. C., Sonone, A., Sonone, A., and Makhija, U. 2008b. Lipid peroxidation and tyrosinase inhibition by lichen symbionts grown *in vitro*. <u>African</u> <u>Journal of Biochemistry Research</u> 2(12): 225–231.
- Vilgalys, R., and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. <u>Journal of Bacteriology</u> 172(8): 4238-4246.
- Vivek, M. N., Yashoda, K., Manasa, M., Prashith, K. T. R., and Vinayaka, K. S. 2014.
  Radical scavenging and antibacterial activity of three *Parmotrema* species from Western Ghats of Karnataka, India. Journal of Applied Pharmaceutical Science 4(3): 86-91.
- Vongshewarat, K. 2000. <u>Study on taxonomy and ecology of lichens family</u> <u>Trypetheliaceae in Thailand</u>. Master of Science (Biology) Thesis, Ramkhamhaeng University.
- Vongshewarat, K., McCarthy, P. M., Mongkolsuk, P., and Boonpragob, K. 1999. Addtions to the lichen flora of Thailand. <u>Mycotaxon</u> 70: 227-236.
- Weerakoon, G., and Aptroot, A. 2014. Over 200 new lichen records from Sri Lanka, with three new species to science. <u>Cryptogamie Mycologie</u> 35(1): 51-62.
- Weerakoon, G., Aptroot, A., Lumbsch, H. T., Wolseley, P. A., Wijeyaratne, S. C., and Gueidan, C. 2012. New molecular data on Pyrenulaceae from Sri Lanka reveal two well-supported groups within this family. <u>The Lichenologist</u> 44(5): 639–647.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics. In G. D. H. Innis M.A., Sninsky J.J., White T.J. (eds.), <u>PCR Protocols A Guide to Methods and Applications</u>, San Diego, California: Academic Press.

- Yamamoto, Y., Kinoshita, Y., and Yoshimura, I. 2002. Culture of thallus fragments and redifferentiation of lichens. In I. Kranner, R. Beckett and A. Varma (eds.), <u>Protocols in Lichenology</u>, 34-46. Springer-Verlag Berlin Heidelberg.
- Yamamoto, Y., Mizuguchi, R., and Yamada, Y. 1985. Tissue cultures of Usnea rubescens and Ramalina yasudae and production of usnic acid in their cultures. <u>Agricultural and Biological Chemistry</u> 49: 3347-3348.
- Yilmaz, M., Türk, A. O., Tay, T., and Kivanç, M. 2004. The antimicrobial activity of extracts of the lichen *Cladonia foliacea* and its (-)-usnic acid, atranorin, and fumarprotocetraric acid constituents. <u>Zeitschrift für Naturforschung C</u> 59(3-4): 249–254.
- Yoshimura, I., Yamamoto, Y., Nakano, T., and Finnie, J. 2002. Isolation and culture of lichen photobionts and mycobionts. In I. Kranner, R. Beckett and A. Varma (eds.), <u>Protocols in Lichenology</u>, 3-33. Germany: Springer-Verlag Berlin Heidelberg.
- Zhao, X., Zhang, L. L., Zhao, Z. T., Wang, W. C., Leavitt, S. D., and Lumbsch, H. T.
   2015. A molecular phylogeny of the lichen Genus *Lecidella* focusing on species from Mainland China. <u>PLoS ONE</u> 10(9): e0139405.
- Zhenga, X. L., Shenga, H. M., and Ana, L. Z. 2007. Phylogenetic analysis of lichenforming fungi *Rhizoplaca* Zopf from China based on ITS data and morphology. <u>Zeitschrift für Naturforschung C</u> 62(9-10): 757-764.
- Zhou, S., and Stanosz, G. R. 2001. Primers for amplification of mtSSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated nanmorphic fungi. <u>Mycological Research</u> 105(9): 1033-1044.
- Zitouni, A., Boudjella, H., Lamari, L., Badji, B., Mathieu, F., Lebrihi, A., et al. 2005.
   *Nocardiopsis* and *Saccharothrix* genera in Saharan soils in Algeria: isolation, biological activities and partial characterization of antibiotics. <u>Research in Microbiology</u> 156(10): 984-993.

 Zoller, S., Scheidegger, C., and Sperisen, C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes.
 <u>Lichenologist</u> 31(5): 511–516.



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



#### APPENDIX A

# Media

# 1. Water Agar (WA)

Agar	20	g
Distilled Water	1000	ml
Dissolve agar with distilled water 1000 ml thoroughly and s	terilizatio	on in

an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes.

# 2. Malt Yeast Extract Agar (MYA)

Malt Extra at	00	
Mait Extract	20	g
Yeast Extract	2	g
	00	
Agar	20	g
Distilled Water	1000	ml

Dissolve with distilled water 900 ml thoroughly after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes.

# 3. Nutrient Agar (NA)

Beef Extract	3	g
Bacto peptone	5	g
Agar	18	g
Distilled Water	1000	ml

Dissolve with distilled water 900 ml thoroughly after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes.

# 4. Nutrient Broth (NB)

Beef Extract	3	g
Bacto peptone	5	g
Distilled Water	1000	ml

Dissolve with distilled water 900 ml thoroughly after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes.

# 5. Mueller-Hinton Agar (MHA)

Beef Extract	2	g
Acid Hydrolysate of Casein	17.5	g
Starch	1.5	g
Agar	17	g
Distilled Water	1000	ml

Dissolve with distilled water 900 ml thoroughly after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes.

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

#### APPENDIX B

## Chemical reagents

#### 1. 1 M Tris-HCI (pH 8)

Tris base	121	g
Distilled Water	1000	ml

Dissolve Tris base with distilled water 800 ml thoroughly and adjust pH with HCl to pH 8 after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes and keep at 4 °C.

# 2. 0.5 M EDTA (pH 8)

EDTA (Ethylenediaminetetraacetic acid)	186.1	g
Distilled Water	1000	ml

Dissolve EDTA with distilled water 800 ml thoroughly and adjust pH with NaOH to pH 8 after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes and keep at 4 °C.

**JHULALONGKORN UNIVERSITY** 

# 3. TE buffer (Tris-EDTA buffer)

1 M Tris-HCI (pH 8)	10	ml
0.5 M EDTA (pH 8)	2	ml
Distilled Water	1000	ml

Mixed Tris-HCl and EDTA with distilled water thoroughly after that the water was added to reach 1000 ml. Sterilization in an autoclave at 121 °C with pressure at 15 pounds/square inch for 15 minutes and keep at room temperature.

# 4. 10X TBE buffer (10X Tris-boric acid EDTA)

Tris base	54	g
Boric acid $(H_2BO_3)$	27.5	g
EDTA	4.65	g
Distilled Water	500	ml

Dissolve Tris base Boric acid and EDTA with distilled water 500 ml thoroughly and keep at room temperature.

# 5. 1X TBE buffer

10X TBE buffer	100	ml
Distilled Water	900	ml

Mixed 10X TBE buffer with distilled water thoroughly after that the water was added to reach 1000 ml and keep at room temperature.

# 6. Chloroform/isoamyl alcohol (24:1 v/v)

Chloroform	192	2	ml
Isoamyl alcohol	8		ml
Mixed Chloroform and isoamyl alcohol thoroughly and	keep	at	room

temperature.

# 7. CTAB extraction buffer

Cetyltrimethylammonum bromide (CTAB)	1	g
1 M Tris-HCI (pH 8)	10	ml
0.5 M EDTA (pH 8)	3	ml
NaCl	5.85	g
Distilled Water	50	ml

Mixed with distilled water thoroughly after that the water was added to reach 100 ml and keep at room temperature.

# 8. CTAB precipitation buffer

Cetyltrimethylammonum bromide (CTAB)	0.5	g
NaCl	0.234	g
Distilled Water	100	ml
Mixed with distilled water thoroughly after that the water w	as adde	ed to

reach 100 ml and keep at room temperature.

# 9. Polyvinypyrrolidone (PVPP) 5% (w/v)

PVPP	5	g
Distilled Water	100	ml
Mixed with distilled water thoroughly after that the water	was add	ed to
reach 100 ml and keep at 4 °C.		

# 10. Agarose gel 1.0% (w/v)

Agarose	1	g
1X TBE buffer	100	ml
Strand G	1	μΙ

Mixed agarose with TBE buffer, were dissolved by microwave after that added strand G in agarose gel at 45  $^\circ\text{C}$  .

# 11. 0.5 McFarland Standard

BaCl <sub>3</sub> ·2H <sub>2</sub> O	1.175	g
Distilled Water	100	ml

Dissolved  $BaCl_3 \cdot 2H_2O$  with distilled water 100 ml thoroughly, after that take 0.5 ml  $BaCl_3 \cdot 2H_2O$  solution mixed with 1%  $H_2SO_4$  about 99.5 ml and keep at room temperature.

# APPENDIX C

# Nucleotide BLAST search

Table C1 Sequence affinity of lichens based on GenBank database for ITS sequences.

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
KRB139	Astrothelium cinnamomeum	Trypetheliales	248/284	87%
	(NR119609)			
KY777	A. cinnamomeum (NR119609)	Trypetheliales	361/434	83%
TSL63	A. cinnamomeum (NR119609)	Trypetheliales	349/421	83%
PHL84	A. cinnamomeum (NR119609)	Trypetheliales	347/425	82%
TAK17	A. cinnamomeum (NR119609)	Trypetheliales	466/487	96%
HRK98	A. cinnamomeum (NR119609)	Trypetheliales	346/430	80%
NSR6	A. cinnamomeum (NR119609)	Trypetheliales	249/284	88%
NSR34	Polymeridium subcinereum	Trypetheliales	407/534	76%
	(KC592279)			
NAN95	P. subcinereum (KC592279)	Trypetheliales	293/362	81%
KRB53	A. cinnamomeum (NR119609)	Trypetheliales	361/435	83%
PHL21	A. cinnamomeum (NR119609)	Trypetheliales	352/424	83%
PHL128	A. cinnamomeum (NR119609)	Trypetheliales	351/423	83%
CM156	A. cinnamomeum (NR119609)	Trypetheliales	347/423	82%
TSL67	A. cinnamomeum (NR119609)	Trypetheliales	354/425	83%
TSL136	A. cinnamomeum (NR119609)	Trypetheliales	362/434	83%
PHL191	P. subcinereum (KC592279)	Trypetheliales	296/355	83%
CP112	P. subcinereum (KC592279)	Trypetheliales	304/366	83%
CBR16	P. subcinereum (KC592279)	Trypetheliales	305/364	84%
PHL163	P. subcinereum (KC592279)	Trypetheliales	402/515	78%
PHL169	P. subcinereum (KC592279)	Trypetheliales	407/506	80%

Table C1	continued	).
----------	-----------	----

Isolates	Closest species match	Order	Overlap	Similarity	
	(accession no.)		(bp)	(%)	
K17	P. subcinereum (KC592279)	Trypetheliales	414/527	79%	
KY856	A. cinnamomeum (NR119609)	Trypetheliales	155/157	99%	
KRB36	A. cinnamomeum (NR119609)	Trypetheliales	171/177	97%	
CP123	A. cinnamomeum (NR119609)	Trypetheliales	157/158	99%	
DKT110	A. cinnamomeum (NR119609)	Trypetheliales	356/425	84%	
TSL39	A. cinnamomeum (NR119609)	Trypetheliales	277/311	89%	
KRB58	A. cinnamomeum (NR119609)	Trypetheliales	359/425	84%	
PHL61	A. cinnamomeum (NR119609)	Trypetheliales	249/283	88%	
PHL89	A. cinnamomeum (NR119609)	Trypetheliales	349/429	81%	
UBN215	P. subcinereum (KC592279)	Trypetheliales	285/345	83%	
PHL119	A. cinnamomeum (NR119609)	Trypetheliales	351/423	83%	
PHL20	A. cinnamomeum (NR119609)	Trypetheliales	358/431	83%	
CP54	T. aeneum (KC592278)	Trypetheliales	351/407	86%	
RN26	T. aeneum (KC592278)	Trypetheliales	303/367	83%	
TSL72	T. aeneum (KC592278)	Trypetheliales	309/359	86%	
KRB107	T. aeneum (KC592278)	Trypetheliales	279/318	88%	
KRB128	A. cinnamomeum (NR119609)	Trypetheliales	357/432	83%	
NSR16	A. cinnamomeum (NR119609)	Trypetheliales	305/362	84%	
KRB155	A. cinnamomeum (NR119609)	Trypetheliales	358/434	82%	
KRB183	A. cinnamomeum (NR119609)	Trypetheliales	280/324	86%	
SMS17	A. cinnamomeum (NR119609)	Trypetheliales	176/190	93%	
KRB90	T. aeneum (KC592278)	Capnodiales	163/168	97%	
SMS72	Mycosphaerella eumusae	Chaetothyriales	185/196	94%	
	(GU168033)				
DKT115	Sarcinomyces sp. (AJ972812)	Trypetheliales	303/372	81%	

Table C1 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
PNG61	P. subcinereum (KC592279)	Caliciales	180/194	93%
DKT71	P. subcinereum (KC592279)	Trypetheliales	303/365	83%
CM161	Botryosphaeria rhodina	Botryosphaeriales	184/200	92%
	(GU797380)			
TRA127	Heterodermia hypoleuca	Pleosporales	187/197	95%
	(KM397354)			
CP5	Sporormiella pulchella	Pleosporales	185/198	93%
	(GQ203789)			
TSL65	P. subcinereum (KC592279)	Trypetheliales	300/365	82%
TSL35	C. concolor (FJ959355)	Candelariales	177/187	95%
NAN5	C. fibrosa (KP226208)	Candelariales	173/183	95%
KRB99	A. cinnamomeum	Trypetheliales	169/175	97%
	(NR119609)			
KRB176	C. concolor (FJ959355)	Candelariales	176/186	95%
SMS7	Valsa mali (KT934362)	Diaporthales	182/193	94%
UBN46	C. concolor (FJ959355)	Candelariales	175/185	95%
CBR51	C. concolor (FJ959355)	Candelariales	177/186	95%
HRK42	P. subcinereum (KC592279)	Trypetheliales	417/532	78%
PJK8	Thaxteriella inthanonensis	Tubeufiales	180/192	94%
	(JN865211)			
SNK33	T. inthanonensis (JN865211)	Tubeufiales	180/192	94%

Table	C2	Sequence	affinity	of	lichens	based	on	GenBank	database	for	nuLSU
sequei	nces.										

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
KRB139	T. nitidiusculum (FJ267701)	Trypetheliales	556/589	94%
KY777	T. nitidiusculum (FJ267701)	Trypetheliales	567/588	96%
TSL63	T. nitidiusculum (FJ267701)	Trypetheliales	533/590	90%
PHL84	T. nitidiusculum (FJ267701)	Trypetheliales	561/589	95%
TAK17	A. cinnamomeum (AY584652)	Trypetheliales	566/608	93%
HRK98	T. nitidiusculum (FJ267701)	Trypetheliales	557/588	95%
NSR6	T. nitidiusculum (FJ267701)	Trypetheliales	555/591	94%
NSR34	Bathelium sp. (KM453776)	Trypetheliales	463/465	99%
NAN95	Laurera megasperma	Trypetheliales	467/488	96%
	(FJ267702)			
KRB53	T. nitidiusculum (FJ267701)	Trypetheliales	558/590	95%
PHL21	L. megasperma (FJ267702)	Trypetheliales	467/488	96%
PHL128	L. megasperma (FJ267702)	Trypetheliales	560/587	95%
CM156	L. megasperma (FJ267702)	Trypetheliales	560/590	95%
TSL67	T. cinereorosellum (KM453809)	Trypetheliales	426/455	94%
TSL136	L. megasperma (FJ267702)	Trypetheliales	558/589	95%
PHL191	P. albocinereum (KM453795)	Trypetheliales	443/460	96%
CBR16	P. albocinereum (KM453795)	Trypetheliales	444/460	97%
PHL163	P. catapastum (JN887402)	Trypetheliales	414/454	91%
PHL169	P. catapastum (JN887402)	Trypetheliales	257/308	83%
K17	P. albocinereum (KM453795)	Trypetheliales	440/460	96%
KRB36	Pseudopyrenula diluta	Trypetheliales	465/497	94%
	(KM453797)			
CP123	P. subnudata (KM453800)	Trypetheliales	449/457	98%

Table C2 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
DKT110	T. nitidiusculum (FJ267701)	Trypetheliales	559/590	95%
TSL39	L. megasperma (FJ267702)	Trypetheliales	577/589	98%
KRB58	L. megasperma (FJ267702)	Trypetheliales	556/587	95%
PHL61	T. nitidiusculum (FJ267701)	Trypetheliales	557/589	95%
PHL89	T. nitidiusculum (FJ267701)	Trypetheliales	561/590	95%
UBN215	Architrypethelium uberinum	Trypetheliales	414/450	92%
	(KM453758)			
PHL119	T. nitidiusculum (FJ267701)	Trypetheliales	558/589	95%
PHL20	T. nitidiusculum (FJ267701)	Trypetheliales	558/591	94%
CP54	T. neogalbineum (KM453812)	Trypetheliales	436/459	95%
RN26	T. aeneum (KM453802)	Trypetheliales	432/460	94%
TSL72	T. neogalbineum (KM453812)	Trypetheliales	428/465	92%
KRB107	T. nitidiusculum (FJ267701)	Trypetheliales	545/590	92%
KRB128	T. papulosum (GU327729)	Trypetheliales	378/404	94%
NSR16	T. nitidiusculum (GU327728)	Trypetheliales	424/453	94%
KRB155	T. nitidiusculum (GU327728)	Trypetheliales	426/453	94%
KRB183	T. nitidiusculum (GU327728)	Trypetheliales	419/454	92%
SMS17	T. tropicum (KM453819)	Trypetheliales	465/488	95%
KRB90	Trypethelium sp. (KM453817)	Trypetheliales	337/409	82%
SMS72	Campylothelium puiggarii	Trypetheliales	424/458	93%
	(KM453779)			
DKT115	P. proponens (JN887403)	Trypetheliales	442/457	97%
PNG61	C. puiggarii (KM453779)	Trypetheliales	425/453	94%
DKT71	Trypethelium sp. (KM453817)	Trypetheliales	427/455	94%
CM161	T. virens (KM453820)	Trypetheliales	447/457	98%

Table C2 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
TRA127	C. puiggarii (KM453779)	Trypetheliales	416/456	91%
CP5	C. puiggarii (KM453779)	Trypetheliales	416/456	91%
TSL65	T. subeluteriae (KM453818)	Trypetheliales	453/455	99%
TSL35	Trypethelium sp. (KM453816)	Trypetheliales	438/457	96%
NAN5	T. eluteriae (GU327726)	Trypetheliales	445/451	99%
KRB99	T. inamoenum (KM453810)	Trypetheliales	364/405	91%
KRB176	T. inamoenum (KM453810)	Trypetheliales	421/425	99%
SMS7	T. inamoenum (KM453810)	Trypetheliales	407/425	96%
UBN46	T. inamoenum (KM453810)	Trypetheliales	395/421	94%
CBR51	T. nitidiusculum (FJ267701)	Trypetheliales	505/601	84%
HRK42	M. cumingii (KM453789)	Trypetheliales	427/459	93%
PJK8	M. cumingii (KM453789)	Trypetheliales	458/460	99%
SNK33	M. cumingii (KM453789)	Trypetheliales	459/460	99%

จุฬาลงกรณ์มหาวิทยาลัย ใหม al ongkorn Hniversity

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
KRB139	T. nitidiusculum (GU561848)	Trypetheliales	731/739	99%
KY777	T. nitidiusculum (GU561848)	Trypetheliales	723/737	98%
TSL63	B. degenerans (DQ328988)	Trypetheliales	720/756	95%
PHL84	T. nitidiusculum (GU561848)	Trypetheliales	734/737	99%
TAK17	B. degenerans (DQ328988)	Trypetheliales	733/756	97%
HRK98	T. nitidiusculum (GU561848)	Trypetheliales	730/737	99%
NSR6	Astrothelium macrocarpum	Trypetheliales	720/729	99%
	(KM453829)			
NSR34	B. degenerans (DQ328988)	Trypetheliales	709/751	94%
NAN95	B. degenerans (DQ328988)	Trypetheliales	707/749	94%
KRB53	B. degenerans (DQ328988)	Trypetheliales	742/761	98%
PHL21	T. nitidiusculum (GU561848)	Trypetheliales	730/738	99%
PHL128	T. nitidiusculum (GU561848)	Trypetheliales	730/738	99%
CM156	T. nitidiusculum (GU561848)	Trypetheliales	730/738	99%
TSL67	B. degenerans (DQ328988)	Trypetheliales	716/761	94%
TSL136	B. degenerans (DQ328988)	Trypetheliales	740/757	98%
PHL191	P. subcinereum (KC592287)	Trypetheliales	718/740	97%
CP112	P. subcinereum (KC592287)	Trypetheliales	713/737	97%
CBR16	P. subcinereum (KC592287)	Trypetheliales	714/741	96%
PHL163	P. subcinereum (KC592287)	Trypetheliales	729/743	98%
PHL169	P. subcinereum (KC592287)	Trypetheliales	710/742	96%
K17	P. subcinereum (KC592287)	Trypetheliales	718/740	97%
KY856	P. diluta (KM453861)	Trypetheliales	691/692	99%
KRB36	P. subnudata (DQ328997)	Trypetheliales	719/742	97%

Table C3Sequence affinity of lichens based on GenBank database for mtSSUsequences.

Table C3 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
CP123	P. subnudata (DQ328997)	Trypetheliales	642/706	91%
DKT110	B. degenerans (DQ328988)	Trypetheliales	744/757	98%
TSL39	L. megasperma (GU561847)	Trypetheliales	737/737	100%
KRB58	B. degenerans (DQ328988)	Trypetheliales	740/758	98%
PHL61	T. nitidiusculum (GU561848)	Trypetheliales	730/737	99%
PHL89	T. nitidiusculum (GU561848)	Trypetheliales	733/737	99%
UBN215	B. degenerans (DQ328988)	Trypetheliales	712/759	94%
PHL119	T. nitidiusculum (GU561848)	Trypetheliales	735/741	99%
PHL20	T. nitidiusculum (GU561848)	Trypetheliales	735/737	99%
CP54	<i>T. aeneum</i> (KC592290)	Trypetheliales	731/737	99%
RN26	T. aeneum (KC592290)	Trypetheliales	726/741	98%
TSL72	<i>T. aeneum</i> (KC592290)	Trypetheliales	734/737	99%
KRB107	B. degenerans (DQ328988)	Trypetheliales	655/663	99%
KRB128	B. degenerans (DQ328988)	Trypetheliales	746/756	99%
NSR16	T. nitidiusculum (GU561848)	Trypetheliales	722/722	100%
KRB155	B. degenerans (DQ328988)	Trypetheliales	745/756	99%
KRB183	B. degenerans (DQ328988)	Trypetheliales	737/756	97%
SMS17	T. tropicum (KM453883)	Trypetheliales	669/682	98%
KRB90	T. virens (KC592292)	Trypetheliales	524/539	97%
SMS72	B. degenerans (DQ328988)	Trypetheliales	713/760	94%
DKT115	B. degenerans (DQ328988)	Trypetheliales	695/751	93%
PNG61	B. degenerans (DQ328988)	Trypetheliales	712/760	94%
DKT71	B. degenerans (DQ328988)	Trypetheliales	707/760	93%
CM161	T. virens (KC592292)	Trypetheliales	635/643	99%
TRA127	T. virens (KC592292)	Trypetheliales	630/649	97%

Table C3 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
CP5	M. purpurina (KM453855)	Trypetheliales	669/761	88%
TSL65	T. subeluteriae (DQ329009)	Trypetheliales	740/745	99%
TSL35	T. eluteriae (DQ328990)	Trypetheliales	750/771	97%
NAN5	T. eluteriae (DQ328990)	Trypetheliales	684/689	99%
KRB99	T. eluteriae (DQ328990)	Trypetheliales	746/770	97%
KRB176	T. inamoenum ( KM453875)	Trypetheliales	459/459	100%
SMS7	T. eluteriae (DQ328990)	Trypetheliales	743/770	96%
UBN46	T. eluteriae (DQ328990)	Trypetheliales	743/770	96%
CBR51	T. eluteriae (DQ328990)	Trypetheliales	740/764	97%
HRK42	T. eluteriae (DQ328990)	Trypetheliales	725/770	94%
PJK8	T. eluteriae (DQ328990)	Trypetheliales	737/773	95%
SNK33	T. eluteriae (DQ328990)	Trypetheliales	721/755	95%

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

Table	C4	Sequence	affinity	of	lichens	based	on	GenBank	database	for	RPB1
seque	nces.										

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
KRB139	A. cinnamomeum (DQ782824)	Trypetheles	735/857	86%
KY777	A. cinnamomeum (DQ782824)	Trypetheles	743/850	87%
TSL63	A. cinnamomeum (DQ782824)	Trypetheles	745/846	88%
PHL84	A. cinnamomeum (DQ782824)	Trypetheles	730/845	86%
TAK17	A. cinnamomeum (DQ782824)	Trypetheles	773/840	92%
HRK98	A. cinnamomeum (DQ782824)	Trypetheles	743/873	85%
NSR6	A. cinnamomeum (DQ782824)	Trypetheles	751/859	87%
NSR34	A. cinnamomeum (DQ782824)	Trypetheles	686/867	79%
NAN95	A. cinnamomeum (DQ782824)	Trypetheles	694/860	81%
KRB53	A. cinnamomeum (DQ782824)	Trypetheles	753/867	87%
PHL21	A. cinnamomeum (DQ782824)	Trypetheles	751/859	87%
PHL128	A. cinnamomeum (DQ782824)	Trypetheles	730/857	85%
CM156	A. cinnamomeum (DQ782824)	Trypetheles	745/863	86%
TSL67	A. cinnamomeum (DQ782824)	Trypetheles	757/870	87%
TSL136	A. cinnamomeum (DQ782824)	Trypetheles	746/859	87%
PHL191	A. cinnamomeum (DQ782824)	Trypetheles	581/754	77%
CP112	A. cinnamomeum (DQ782824)	Trypetheles	580/749	77%
CBR16	A. cinnamomeum (DQ782824)	Trypetheles	584/755	77%
PHL163	A. cinnamomeum (DQ782824)	Trypetheles	570/750	77%
PHL169	A. cinnamomeum (DQ782824)	Trypetheles	638/857	74%
K17	A. cinnamomeum (DQ782824)	Trypetheles	656/867	76%
KY856	A. cinnamomeum (DQ782824)	Trypetheles	648/855	76%
KRB36	A. cinnamomeum (DQ782824)	Trypetheles	655/857	76%
CP123	A. cinnamomeum (DQ782824)	Trypetheles	626/838	75%

Table C4 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
DKT110	A. cinnamomeum (DQ782824)	Trypetheles	739/849	87%
TSL39	A. cinnamomeum (DQ782824)	Trypetheles	731/850	86%
KRB58	A. cinnamomeum (DQ782824)	Trypetheles	734/858	86%
PHL61	A. cinnamomeum (DQ782824)	Trypetheles	677/815	83%
PHL89	A. cinnamomeum (DQ782824)	Trypetheles	734/865	85%
UBN215	A. cinnamomeum (DQ782824)	Trypetheles	695/865	81%
PHL119	A. cinnamomeum (DQ782824)	Trypetheles	721/836	86%
PHL20	A. cinnamomeum (DQ782824)	Trypetheles	719/833	86%
CP54	A. cinnamomeum (DQ782824)	Trypetheles	757/845	90%
RN26	A. cinnamomeum (DQ782824)	Trypetheles	756/857	88%
TSL72	A. cinnamomeum (DQ782824)	Trypetheles	757/861	88%
KRB107	A. cinnamomeum (DQ782824)	Trypetheles	753/850	89%
KRB128	A. cinnamomeum (DQ782824)	Trypetheles	756/849	89%
NSR16	A. cinnamomeum (DQ782824)	Trypetheles	751/854	88%
KRB155	A. cinnamomeum (DQ782824)	Trypetheles	763/846	90%
KRB183	A. cinnamomeum (DQ782824)	Trypetheles	773/861	90%
SMS17	A. cinnamomeum (DQ782824)	Trypetheles	584/750	78%
KRB90	A. cinnamomeum (DQ782824)	Trypetheles	505/606	83%
SMS72	A. cinnamomeum (DQ782824)	Trypetheles	587/760	77%
DKT115	A. cinnamomeum (DQ782824)	Trypetheles	580/739	78%
PNG61	A. cinnamomeum (DQ782824)	Trypetheles	661/855	77%
DKT71	A. cinnamomeum (DQ782824)	Trypetheles	668/866	77%
CM161	A. cinnamomeum (DQ782824)	Trypetheles	597/751	79%
TRA127	A. cinnamomeum (DQ782824)	Trypetheles	589/748	79%
CP5	B. degenerans (FJ941895)	Trypetheles	519/683	76%

Table C4 (continued).

Isolates	Closest species match	Order	Overlap	Similarity
	(accession no.)		(bp)	(%)
TSL65	A. cinnamomeum (DQ782824)	Trypetheles	668/861	78%
TSL35	A. cinnamomeum (DQ782824)	Trypetheles	642/853	75%
NAN5	A. cinnamomeum (DQ782824)	Trypetheles	667/870	77%
KRB99	A. cinnamomeum (DQ782824)	Trypetheles	661/855	77%
KRB176	A. cinnamomeum (DQ782824)	Trypetheles	656/841	78%
SMS7	A. cinnamomeum (DQ782824)	Trypetheles	652/841	78%
UBN46	A. cinnamomeum (DQ782824)	Trypetheles	670/863	78%
CBR51	A. cinnamomeum (DQ782824)	Trypetheles	661/853	77%
HRK42	A. cinnamomeum (DQ782824)	Trypetheles	652/866	75%
PJK8	A. cinnamomeum (DQ782824)	Trypetheles	637/855	75%
SNK33	A. cinnamomeum (DQ782824)	Trypetheles	583/774	75%

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

### APPENDIX D

#### Necleotide sequences of the species

#### 1. ITS region

#### 1.1 Astrothelium aenascens

## 1.2 Astrothelium flavocoronatum

#### 1.3 Astrothelium macrocarpum

# 1.4 Astrothelium macrostiolatum

TCCGTAGGTGAACCTGTAAGTCGTCGCCAATTCTCCCCATCTCGTGATTGTCTATCA CTAACAACTAATGTAGGCGGAGGGATCATTACAGAGTGACGGTAGCTTCGGCTGCCCAACTC CCATCCTATGTTTGACATATTATTGTTCTTCCGATATTCTTTAATCAGAGTATCGGAAAGGT TATTTAAATTCGTTTTGCAAATTTTGTCATCGAATGATTAAATCCAAATTAATCAAAACTTT CAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTAT GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGTATTCCT TGAGGCATGTCTGTTTGAGCGTTATTACAAACCTAAGACTTGGTCTTGTCATGAAAGCTCAC ATGATCTTGTCATGTGACTTTTCAAATAGTTTTTGGATGTTTCGAGTAATATCAATGCCACC AGATCTGGCAAACTGATAATCCTCGTTTCATCTTGTCATCTCATGTAATCCAACCC CAGATCAGACAAGAATACCCACTGAACTTAAGCATATCAATAGCCGGAGA

# 1.5 Astrothelium neglectum

## 1.6 Astrothelium neoveriolosum

#### 1.7 Astrothelium siamense

# 1.8 Bathelium albidoporum

# 1.9 Bathelium madreporiforme

# 1.10 Bathelium sp.1

# 1.11 Campylothelium nitidum

TCCGTAGGTGAACCTGCGGAGGGATCATTACGAGAAAACAGAGTGGTTTCGGCCACT CGACTTTCAAACCCCTGATTGTCGTATCATTGTATCTTCCGGCGTTATGCCGGACAGAATTT TCAAACTCGTCTTTAATCGTGTCACAAATTTTCAAAGTCCAATAAATCAAAACTTTCAACAA CGGATCTCTTGGCTCTAGCGTCGATGAAGAACGCAGCGGAAATGCGATAAGTAGTATGAATTG CAGAATTCAGTGAATCATCGAATTTTTGAACGCACATTGCGCCTTTTGGTATTCCATGAGGC ATGCCTGTTCGAGCGTTATTACGTTACTCAAGCATAGCTTGGTATTGAGTCCGAAGATCATC CGTGATCGGCTCTAAAAATGGATTTAGTCTCTGTTTGAAGTGATGTTGAGCAACCAAGTTTT GTGCTCCAAGCTTCATTCAGAAATTAGTATCTTCTATCCCCCCAAGTTTAACCTCGGATCAGG CAAGAATACCCGCTGAACTTAAGCATATCAATAAGCCGGAGGA
# 1.12 Laurera cf. aurantiaca

# 1.13 Laurera alboverruca

# 1.14 Laurera cf. columellata

# 1.15 Laurera keralensis

TCCGTAGGTGAACCTGCGGAGGGATCATTACTGAGTTGGGGGCAGCCTCGGCTGCTC CGACTTCCAACCCTTGACTTGTTGAATCTCTGTATCTTCCGGCCTTGCTTCGGCATGCCGGA AGGGACCTCCAAACTCGTTTTGAACAACTGTCATCCCTCAATGATAAATCAAAACTT TCAACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTA TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGTATTCC ATGAGGCATGCCTGTTCGAGCGTTATTTCAACTCTCAAGGTCAACTTGGTGTTGAGGCTGTT ATCCAACGGCCTCCAAAGAACTCGAGTTTTGTGAAAGCATCTCAGGCAACCAAAACTTGCTC GAGCAGCTTTCTCATCGCTAGTCTCTCTCCCAGTTTAACCTCGGATCAGGCAAGAATACCC GCTGAACTTAAGCATATCAAAAGNCGGGAGGA

### 1.16 Laurera megasperma

### 1.17 Laurera meristospora

### 1.18 Laurera sikkimensis

#### 1.19 Laurera subdiscreta

# 1.20 Laurera varia

#### 1.21 Laurera verrucoaggregata

#### 1.22 Laurera vezdae

TCCGTAGGTGAACCTGCGGAGGGATCATTAAAGAGTTAGGGGTAGCTTCGGCTGCTC CAACCTCCTAACCCTTTGTTTTGATGTACCATGTACCTTCCGGTTCGACCCTCATGGGATCT GCCGGAAGAGGTTTATAAACTCTGTTTTGAATAATGTCATCAAATCATTATTTAATAATCAA AACTTTCAACAACGGATCTCTTGGTTCTAGCGTCGATGAAGAACGCAGCGAAATGCGATAAG TAATATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGC ATTCCACAGGGCATGCCTGTTCGAGCGTTATCGCAATCTATCAAGCTTTGGCTTGGTCATGA ATCGTAGCCTTCCTTGCCATTGAAGGCTGATTCCAAAAGTGTGTGATGTTGTGAAGCGAATC TCAAGCAACCAAAGACTTTACGGATCTCGCAAGGAAAGTTTTGCCAACGTCAGTACTCA TCTCATATTCCAGTTTAACCTCGGATCAGGCAAGAATACCCGCTGAACTTAAGCATATCAAT AAGCGGAGGA

# 1.23 Marcelaria cumingii

TCCGTAGGTGAACCTGCGGAGGGATCATTACAGAGTTGGGGGTAGCTTCGGCTGCCC CGACTTCCCAACCCTATGGCTTGCTGTACTCTTGTATCTTCCGGCTCACTGCTCCGGCATGC CGGAAGGGATTTATCCAAACTCGTTTTTGAACAACTGTCGCCATTCAATAATCAAAATTGAA TTAAAACTTTCAACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAATGCGA TAAGTAGTATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTT TGGTATTCCATGAGGCATGCCTGTTCGAGCGTTATTACAAACTCTCAAGGTTATACTTTGGT ATTGAATCCCGTCGAAAGGCGGATTCTAAAGAGTGGAGTGTTGTGGAAGCATATCTCAAGCA ACCAAAAACTTATTGTTTCTGCTTTGAGCAACTTTTACCATCACTAGTATCTTTTATCCCTC AAGTTTAACCTCGGATCAGGCAAGAATACCCGCTGAACTTAAGCATATCAATAAGGCGGAGG A

# 1.24 Polymeridium albidum

TCCGTAGGTGAACCTGTAAGTTTTTCCCCTTCCTCCCATCTCATCCGATACTAACTC TTCACAGGCGGAGGATCATTACCGAGTTGGGGGTAGCCTTGGCTGCCTCGACTTCCGACCC TTGTCTTTCTGTCGTTTGTATCCTCCGGGCTCGCTTCCGGGCAGCCGGGACATTCAACTCTT TTCATCCCGTCTTTTTTTCTGATTAATAATCAAAACTTTCAACAACGGATATCTTGGCTCTA GCGTCGATGAAGAACGCAGCAAATGCGATAAGTAGTATGAATTGCAGAATTCCGTGAATCAT CGAATCTTTGAACGCACATTGCACCTTTTGGCATTCCAAGAGGTATGCCTGTTCGAGCATAA TTTGACATCTCAAGCTCATGCTTGGTATTGAGACTTGTCTTTTTTGGCAGTTTCCAAATCCG TTTCGGGTCTAGTGTCGCAACCTTGTGCAACCACCACTTGCTGCAAGTCAACGCCACTACAC CAGTCTCTCATCTTCAGTTTTACCTCGGATCAGGCAGGAATACCCGCTGAACTTAAGCAT ATCATAGNNCCGGAAGGA

# 1.25 Polymeridium albocinereum

# 1.26 Polymeridium catapastum

# 1.27 Polymeridium quinqueseptatum

### 1.28 Polymeridium sp.1

### 1.29 Polymeridium sp.2

#### 1.30 Pseudopyrenula diluta var. degenerans

TCCGTAGGTAAGTAATAATCGGAACTCTATTACTCTCATATCAGCCTTATACTAACG TTCTTGCTCCAGGTGAACCTGCGGAGGGATCATTACAGAGTTATGGGTATAACGTGCCCTGA CCTCCCAACCCTTTGATTACTTGTACAAGTTTCTTCCGGTTTTTTGCTCAAGCATACCGGA AATTATTTTATATCAAATTCGAAATAATTATGACCTCAAAATTATCACATCAATAAATTTAA AACTTTCAACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAG TAGTATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGT ATTCCTTGAGGCATGTCTGTTCGAGCGTTATATCAAACCTCTCAAGTTTATCTTGGTGATGA ATTGTTGTCGTTTGACACATTTCAAAGCTTAATTTTGATGTTGTGAACTTGATCTTAAGCGA CCAAGTTTTGCTGGTAGATTGATCTTACATCTCAGTTATATCTCACGTTTAACCTC GGATCAGATGAGGATACCCGCTGAACTTAAGCATATCAATAAGCCGGAGGA

#### 1.31 Pseudopyrenula subnudata

TCCGTAGGTGAACCTGCGGAGGGATCATTACAGCGTTATGGGTCAGTTAATTGACTA CCCTAACCTCCCCAACCCTTTGTGTACTTGTACAAGTTTCTTCCGGTTTTGCCTTGGGGCTT GCCGGAAAATATTTTATCAAAATCTCGAACAAACCATGAACTCTTTATTTTCATTACGCAAA TGAATTTTAAAAAAACTTTCAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGC GAAATGCGATAAGTAGTATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACAT TGCGCCTTTTGGTATTCCTTGAGGCATGTCTGTTCGAGCGTTATATTAAACCTATCAAGTCA ATCTTGAAGATGAATGTTAATTGTCTTCATGACACATTCCAAAAACTTATATGATGTGAGGA CATCTTGGGCAACCAAGTCTTGCAAATTGAGATTATCTTCACACCTCAGTTTAACTACGTAT TTTTTCTATGGTTTAACCTCGGATCAGACAGGATTACCTCGCTGAACTTAAGCATATCATAG NCGGAAGGA

# 1.32 Trypethelium cf. aeneum

# 1.33 Trypethelium andamanicum

# 1.34 Trypethelium cinereorosellum

# 1.35 Trypethelium eluteriae

TCCGTAGGTAAGTAAACATCGACAACATGCTCTTTTCCCCTTCAAGAATCAATAACT AACATAATCCAAGGTGAACCTGCGGAGGGATCATTACCGAGTTAAGGGTAGCTTCGGCTGCT CTGACTTCCCAACCCTATGATTTGATGTTTTCTCATGTATCTTCCGGTCTCTGTTCCGACA TGCCGGAAGATTACCAATCAAACTCGTCTTGGAACTATGTTGTCATCATTCAATACCATAAT TGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAGTATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCATTGCGC CTCTTGGTATTCCATGAGGCATGCCTGTTCGAGCGTTATTATAAACTCCTCAAGTTCTAGCT TGGCAATGAATTTTGTCCCTTGACAAATTCTAAAATATTTTGTCTGTTGTAAAAGCCTTTT GCTTTGACGTAACCAATGACTTTGCGCTCGGCAAATCTTTTACAACAAGTTTTTATCTTCTT CCACAGTTTAACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGA AGGA

# 1.36 Trypethelium microstomum

# 1.37 Trypethelium neogabeinum

# 1.38 Trypethelium nitidusculum

TCCGTAGGTGAACCTGCGGAGGGATCATTACAGAGTTATGGTAGTTTCTGCTGCCCA ACTCCCAACCCATGTTTGACAACTCATCATGTTCTTCCGACGTCTTTTCATAAAGCGTCGGA AAGATTATTAAAACTCGTCCTATGAACAATGTCTCATCATGATTTTAATGAATCAAAACT TTCAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGT ATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGTATTC CTTGAGGCATGTCTGTTTGAGCGTTATATCAACAATAAGACGAAGTCTTGTTTTGAAAGATT ATGCTTTTCTTAACTTTCTAAATCTAGATTGTGTCTTGAGTGACTAAATGCCACCAAATTTG GCTGTTTTGTCCTCTAGATATTTTAAATTTGAAGTTTAACCTCAGATCAGACAAGAATACC CACTGAACTTAAGCATATCAATAAGCGGAGGA

# 1.39 Trypethelium ochroleucum var. subdissocians

### 1.40 Trypethelium aff. papulosum

TCCGTAGGTGAACCTGCGGAGGGATCATTACAGAGTTATGGTAGCTTCGGCTGCCCA ACTCCCAACCCATGTTTGACAATTCTTGTTCTTCCGACGCTTTCCCCAAAAAAGAAACGTCG GAAAGATTTAACAACTCGTTTTGCAAATCGTGTCATCTCATTGCATAATCAATATCAAAACT TTCAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGT ATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGTATTC CTTGAGGCATGTCTGTTTGAGCGTTATATCATCACAAAAGACTCTGTCTTGTCATGAGAGGT CATTGGCAGTTTCTGTCATGTGACTCTCCCAAATACCATGTGACGTTTCGAGTGACGTTTGAT GCCACCAGATTTGGTAATCAATTAATCACACGTGACCGTCAGCAGATATTTTTCCCA GGTTTAACCTCAGAATCAGACAAGAATACCCACTGAACTTAAGCATATCAATAAGCGGAGGA

### 1.41 Trypethelium platystomum

TCCGTAGGTAAGTACAATCGGATTATCCTCTTCGATTATGAAATCTTCCGATAGCTA ATTCTTCTTTAGGTGAACCTGCGGAGGGATCATTATCGAGTTAGGGGTAGCTCCGGCTGCCT TGACTTCCCAACCCTATGATTTGATGTACTTTACTATGTCTTCCGGCCTCTGGCTCCGGTATG CCGGAAGATTTTACTGCCAACTCGCTAATCATGACGTCATCTTCAATCTTGAATTGAATAAA AACTTTCAACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAG TATTATGAATTGCAGAATTCAGTGAATCATCGAATTTTTGAACGCACACTGGCGCTCTTGGC ATTCCATGAGGCATGCCTGTTCGAGCGTTATTACAAAACCCTCAAGCCTTGGTGATGA ATTCCATCATTGATGGATTTTTAAAAATTTGCCGATGTTGTAGAGTTTAATTCGACGCAACC AAAACTTTTCTGCGTCAGAATGAGCTTTACAACACCACTCAGTAAATCCTTTTCAATAATTTAA CCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGG

### 1.42 Trypethelium pseudoplatystomum

TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTGGGGGTAGTTCGCTGCCCCG ACTTCCAACCCTTGCTTGCTGTACGCTTGTGTTTTCCGGCCTCTGCTGGCATGCCGGAAGAG ATCAACATCAACTCGTCTCCGAACTTGTCGTCTCTTGATAACGTAATCAAAAACTTTCA ACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTATGA ATTGCAGAATTCAGTGAGTCATCGAATCTTTGAACGCACATTGCGCCTCTTGGTATTCCATG AGGCATGTCTGTTCGAGCGTTATATCAAACCTCAAGCCCTGCTTGGTGATGAATGTTATCTA CCATCTCTGGAGCGCTTCTTGACATCGGCGTGACATCAGTCATCTCACGCAACCAAAACT TATGTTCTGCTGAGTGGCCTTCTTGACATGCGTTGACATCAGTCATCTCACGGTTTAA CCTCGGATCAGACAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

### 1.43 Trypethelium subeluteriae

# 1.44 Trypethelium tropicum

# 1.45 Trypethelium ubianense

TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTGTTGTTGTGGGTTAGCTCCGG CTGCCCAGACTCTCCACCTCATGTTTTGCAGATCTTCGGTACCTTCCGGTCCGACCCGTTA TGCGGGGAACGGCCGGAAGATCTTTCATCAACTCGTTTTTCTTGAACTCTGTCTCTGAACTA CCAAATCCATCAAAACTTTCAACAACGGATCTCTTGGTTCTAGCATCGATGAAGAACG CAGCGAAATGCGATAAGTAATATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC ACATTGCGCCCTTTGGTATTCCATAGGGCATGCCTGTTCGAGCGTTATTACAAATCATCAAG CTGTGGCTTGGTCATGAGCTGGCCAGTGATCTTCTGGCAGACTCCCAAAAACGTCCGACGTCG TCAAAGCGCATCTCGAGCAACCCAAAACTGTTCCTGTTCTGGCAGAACGCTCGGAAAGCTTTGCCGA CGCCAGTTTTGACTCGCTTCTAGTTTAACCTCGAATCAGGAATACCCGCTGAACTTAA GCATATCAATAAGGCGGGAGGA

# 1.46 Trypethelium virens

# 1.47 Trypethelium sp.1

# 1.48 Trypethelium sp.2

TCCGTAGGTGAACCTGTAAGTTCAGCCATTGTTCCGATTTTGGAAATTTTACAACAC TAACATATTCTTAGGCGGAGGGATCATTACAGAGTTACGGTAGCCTTCGGCTGCCAAACTCC CCCAACCCTATGTTTGACATATATTCTTGTTCTTCCGACATCTTCCATAATCGAAAATGTCG GAAAGATTATCTTAACTCGTCTTATGAACTTCTGTCTTATCACATGACTTAATGAAATCAAA ACTTTCAACAACGAATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGT AGTATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGTA TTCCTTGAGGCATGTCTGTTTGAGCGTTATATCAACCAATAAGATATTATGTCTTGTTTTGA AAGATCAATGGACCCTGCTCGGATGTTCTCGAGCAGAGTTGACTTTCTAAAAATGGTAAAGA TGTTATGAGTGATGTCCAAATGAGCCACCAGATCAGGTTCATGCACTTTCACTTCATATTAT TCGGTATCAGATCAATCATATTTTTCTCAGGTTTAACCTCAGATCAGACAAGAATACCCACT GAACTTAAGCATATCAATAAGCGGAGGA

# 1.49 Trypethelium sp.3

# 1.50 Trypethelium sp.4

# 1.51 Trypethelium sp.5

TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTGGGGGTAGTCCGCTGCCCCG ACTTCCAACCCGTGATTTGATGTACTCTTGTGTCTTCCGGCCTCTGCTGGTACGCCGGAAGA GATTATCAAACCCGTCTAATCATGTCGTCTCATTCATTACCAAATGAATCAAAACTTTCAAC AACGGATCTCTTGGTTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAAT TGCAGAATTCAGTGAGTCATCGAATCTTTGAACGCACATTGCGCCTCTTGGTATTCCATGAG GCATGTCTGTTCGAGCGTTATTTCAACCATCGAGCCCTGCTTGGTGATGATGCCGTCTCTG ACGGCCTCCAAAGCTGACGAGTGTTCTGAAGCGATCTCATGCAACCAAGACTTCTGTCCTGC TGAGTGATCTTCATAACTTCTGACATCACTTCCACGGTTTAACCTCGGATCAGATAGGAATA CCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

# 1.52 Trypethelium sp.6

# 1.53 Trypethelium sp.7

TCCGTAGGTGAACCTGCGGAGGGATCATTACAGAGTGACGGTAGCTTCGGTTGCCCA ACTCCCATCCTGTGTTTGATATATTAATCTGTTCTTCCGATACTCTTGTTATGAGAGTGTCG GAAAGTTTATCTGACTCGTTTTACAAACTTTGTCATCACCTGATTAAATCAGTTAATCAAAA CTTTCAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA GTATGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTTTTGGTAT TCCTTGAGGCATGTCTGTTTGAGCGTTATTGCAAACCTAAGATTAAGTCTTGTTATGAATTA TCATGTGACTGCGTCATGTGACTTTCCAAAGAATTTTTGGATGTTATGATGTCAATGC CACCAGATCTGGCAATCGGACAACTTTATAATGTCTTGTTATATTTCATAGGTTTAACCTCA GATCAGACAAGAATACCCACTGAACTTAAAGCCATATCAATAAGCCGGGAGGA

# 1.54 Trypethelium sp.8

# 1.55 Trypethelium sp.9

#### 1.56 Trypethelium sp.10

# 1.57 Trypethelium sp.11

# 2. nuLSU region

# 2.1 Astrothelium aenascens

ACCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TTAGTAACGGCGAGTGAAGAGGCAACAGCTCAAATTTTAAATCTGCCACCGTGCCGAGTTGT AATTTGCAGAGGATGTTATGGAATCTGTTTGGACTCAAGTCCTTTGGAAAAAGGCGCCAAGG AGAGTGACAGTCTCGTACTTTCCAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGGTTAAAAA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTGTCGTTGTTCAGCCTTT TGGTGTATTCAATGATAACCATGCTAGCATCAGTTTGAATAGCTGGATAAAAACTCGGAAAT GTAGCTCTCTCGGGAGTGTTATAGTTCGGGATAGAATGCAGCTCATTTAGACTGAGAGCACCGC TTTAAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

# 2.2 Astrothelium flavocoronatum

# 2.3 Astrothelium macrocarpum

ACCCGGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGCCATCCGGCCGAGTTG TAATTTGCAGAGGATGTTATGGAATCTGTATGGACTCAAATTCTTTGGAAAAAGATGCCATG GAGAGTGACAGACTCGTACTTTCCACTACATTTTCCACGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCAGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGTAATGACGGCATTGTTCAGCCTT TTGGTGTATTCAATGTCTTGTCAGGCTAGCATCAGTTTGGGTAGCTGGACAAAAGTGTTGGA AATGTAACTTCTCCGGAAGTGTTATAGTCCGATACAGAATGCAGCTTATTCAGACTGAGGAC CGCTTTAAGGATGCTGGCATAATGGTGGCATAAGGCCCGTCTGAAAAACACGGA

# 2.4 Astrothelium macrostiolatum

ACCCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTTAAATCTGCCACCGTGCCGAGTTG TAATTTGCAGAGGATGTTATGGAATCTGTGTGGACTCAAGTCCTTTGGAAAAAGGCGCCATG GAGAGTGACAGTCTCGTACTTTCCAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTGTCGTTGTTCAGCCTT TTGGTGTACTCAATGACAACCAGGCTAGCATCAGTTTGAAAAGCTTGGAAAA GGTACCCCCGGGGAGTGTTATAGTCCGAGACGTAATGCAGCTCATTTAGACTGAGAGACCT TGTAGCTCCCCCGGGAGTGTTATAGTCCGAGACGTAATGCAGCTCATTTAGACTGAGGACCG CTTTTAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

# 2.5 Astrothelium neglectum

ACCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCCACCCGGCCGAGTTGT AATTTGCAGAGGATGTCATGGGATTTGTGGGGGGCTTAAGTTCTTTGGAAAAAGACGCCATGG GGTGTTTGGGAATGCAGCTCCAAAGTGGGACGTAAATTTGTTCCAAAGCTCCTTCAAAGAGTCGA GTTGTTTGGGAATGCAGCTCAAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAAGAGAGTTAAA AAGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGCGTCAATGTTCAGCCG TTTTGGTGTACTCATTGATTAGTCATGCTAGCATCAATTGGGATAGTCGGATAAAAGTGTTG GAAATGTAACTCCCTCGGGAGTGTTATAGTCCGAGACAGAATGCGTCTAATCCCGATTGAGG ACCGCTTTGAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

# 2.6 Astrothelium neoveriolosum

ACCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCCACCGTGCCGAGTTGT AATTTGCAGAGGATGTTATGGAATTTGTTTGGACTCAAGTCCTTTGGAAAAAGGCGCCATGG AGAGTGACAGTCTCGTACTTTCCAATTCATTTTCCATGTATAACTCCTTCAAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAAA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGATGTCATTGTTCAGCCTTT TGGTGTATTCAATGATATCAGGCTAGCATCAGTTTGGATAAAAGTGTTGGAAAT GTAGCTCCTCCGGGAGTGTTATAGTCTGATACATAATGCAGCTTATCCAGACTGAGAGCTGC TTTAAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTTGAAACACGGA

# 2.7 Astrothelium siamense

ACCCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCCACCGTGCCGAGTTG TAATTTGCAGAGGATGTTATGGAATCTGTTTGGACTCAAGTCCTTTGGAAAAAGGCGTCATG GAGAGTGACAGTCTCGTACTTTCTAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTTATCAGAAATGGTGTTGTTCAGCCTT TTGGTGTATTCAACGATTGCCAGGCTAGCATCAGTTTGAAAAGCTGGATAAAAACTCCGGAAA TGTAGCTCCTTCGGGAGTGTTATAGTCCGGGATAGAATGCAGTTCATTTAGACTGAGGACCG CTTTAAGGATGCTGGCATAATGGTGGCCATGAGGCCCGTCTGAAAACACGGA

# 2.8 Bathelium albidoporum

ACCCCCTTGAAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACTGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGCCATCAGGCCGAGTTG TAATTTGCAGAGGATGCTTTGGATTTTGACTGGTGTAAAGTCCTTTGGAACAGGGCGCCATG GAGGATGAAAGTTCCGTACGCACCAGATCCAATTTCCATGTAAAGCTCCTTCAAAGAGTCGA GTTGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCAAAGCTAAATACCGGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAA AAGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGACTTGGCTAAAGTTCAGCCT TATGGTGTATTCTTTGGCATCAGGCTAGCATCAAATTTCGTCCAGATTGAAAAGCTTCAGGA ATGTAGCTCTTCGGAGTGTTATAGCCTGATTCAGAATGCAACTTGTCCAGATTGAGGGCCCGC TTTTAAGGATGCTGGCGTAATGGTGGCATGAGGCCCGTTTGGAAAACACGGA

# 2.9 Bathelium madreporiforme

ACCCGGCTGGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACTGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGCCATCCGGCCGAGTTG TAATTTACAGAGGATGCTTTGGATTCTGTCTGGCGTAAAGTCCTTTGGAACAGGGCGCCGTG GAGGATGAAAGTTCCGTACGCATCGGATCCAGTTTCCATGTAAAGCTCCTTCAAAGAGTCGA GTTGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCAAAGCTAAATACCGGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAA AAGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGACTTGATGCTGAAGTTCAGCCT TTTGGTGTATTCTTTGGTATCAGGCTAGCATCAATTTGAACAGCTGGATAAAAACTTCGGGA ATGTAGCTCTTCGGAGTGTTATAGCCCGATGCAGAATGCGGCTTGTTCAGATTGAGGGCCCGC TTTTGAGGATGCTGGCGTAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

# 2.10 Bathelium sp.1

ACCCCGNNTGGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTG CCTTAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGCCACAAGGCCGAGTT GTAATTTGCAGAGGATGCTTTGGATACTACTCCGTCTTAAGTCCTTTGGAACAGGGCGTCAT GGAGGGTGAAAATCCCGTGTTTACGGATGCTATTATCCATGTAAAGCTCCTTCGAAGAGTCG AGTTGTTTGGGAATGCAGCTCTAAATGGGAGGGAAATTTCTTCCCAAAGCTAAATATCTGCTA GAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAA AAAGCACGTGAAATTGTTGAAAGGGAAGCTCATGCAGTCAGACATGATTCAAAGGCTCAGCC TTATGGTGTACTCCTTTGAATCAGGCTAGCATCAGTTTGAGCAGTTGGATAAAAGTCTCGGG AATGTAGCTCCCTCGGAGTGTTATAGCCCGATTCAGCAGCAGCACTAGTTCAGGCTCGAGGTCCG CTTATAGGATGCTGGCGTAATGGTGGCATGAGGCCCGTCTGAAAAACACGGA

# 2.11 Campylothelium nitidum

# 2.12 Laurera cf. aurantiaca

ACCCGCTNACTTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCCT CAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGACACCGTTCCGAGTTGTA ATTTGCAGAGGATGTTATGGGATTTGTGTGGATTCAAGTTCTTTGGAAAAAGACGCCATGGA GAGTGACAGTCTCGTACTTTCCACCACATCTTCCATGTATAACTCCTTCAAAGAGTCGAGTT GTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGAGA CCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGTACTTTGAAAAGAGAGTCAAACAG CACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTCTCATTGTTCAGCCTTTT GGTGTATTCAATGTGGGCCAGGTTAGCATCAGTTTGGGCAGCTGGATAAAAGTGTTGGAAAT GTAGCTCTTTCGGGAGTGTTATAGTCTGATACAGAATGCAGCTTGCCTAGACTGAGGACCGC TTTTAAGGATGCTGGCATAATGGTGGCCATGAGCCCGTCTTGNAACACGGA

# 2.13 Laurera alboverruca

ACCCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCCACCGTGCCGAGTTG TAATTTGCAGAGGATGTTATGGAATCTGTGTGGACTCAAGTCCTTTGGAAAAAGGCGCCGTG GAGAGTGACAGTCTCGTACTTTCCAACACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTGTCGTTGTTCAGCCTT TTGGTGTATTCAATGACTACCAGGCTAGCATCAGATTGGAAAAGCTTGGACAAAAGCTTGGAAA TGTAACTCCTCCGGGAGTGTTATAGTCCGAGACACAATGCAGCTCATTTAGACTGAGGACCG CTTTAAGGATGCTGGCATAATGGTGGCCATGAGGCCCGTCTGAAAAACACGGA

### 2.14 Laurera cf. columellata

ACCCGCTTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTTAAATCTGACACTGTTCCGAGTTG TAATTTGCAGAGGATGTTATGGAATCTGTGTGGGACTCAAGTTCTTTGGAAAAAGACGCCATG GAGAGTGACAGTCTCGTACTTTCCATCACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTCAAAC AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCCATGATAAAGAGAGTCTAAAC AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCCAGAAATGGTCTCATTGTTCAGCCTT TTGGTGTATTCAATGATGACTAGGCTAGCATCAGATTGGACAGCTCGATCAAAAGTGTTGGAA ATGTAGCTCTTTCGGGAGTGTTATAGTCTGATACAGAATGCAGCTCGTCCAGACTGAGGACC GCTTTAAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGAAAAACACGGA

### 2.15 Laurera keralensis

ACCCCGCTTGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGCCACCCGGCCGAGTTG TAATTTGCAGAGGATGCTTTGGAATCTGCTCCGTCTCAAGTCCTTTGGAACAGGGCGTCACG GAGGGTGAAAATCCCGTACTTTCGGACCGCAGTTTCCGTGTAAAGCTCCTTCGAAGAGTCGA GTTGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCCAAAGCTAAATATCTGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAA AAGCACGTGAAATTGTTGAAAGGGAAGCTCATGCAGTCAGACATGCCGTCGGCTCAGCC TTTTGGTCAACTCCGACGACGTCAGGCTAGCATCAGTTGGGGTCGCTGGATAAAGGTCGTGG GAATGTAGCTCTTCGGAGTGTTATAGCCCGCTACGGCATGCAGCGCACCCCGACTGAGGACC GCTTACAGGATGCTGGCGTAATGGTGGCATGAAGGCCCGTCTTGAAAAACACGGA

# 2.16 Laurera megasperma

### 2.17 Laurera meristospora

ACCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTTAAATCTGACACTGTTCCGAGTTGT AATTTGCAGAGGATGTTATGGAATTTGTGTGGGACTCAAGTTCTTTGGAAAAAGACGCCATGG AGAGTGACAGTCTCGTACTTTCCACCACATTTTCCATGTATAACTCCTTCAAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAACA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATTAGAAATGGTCTCATTGTTCAGCCTTT TGGTGTATTCAATGATGGCCAGGCTAGCATCAGTTTGGACAGCTGGATAAAAGTGTTGGAAA TGTAGCTCCCCCGGGAGTGTTATAGTCCGATACAGAATGCACCGCTCGTCCAGACTGAGGACCG CTTTGAAGGATGCTGGCATAATGGTGGCATGAAGGCCCGTCTGAAAACACGGA

#### 2.18 Laurera sikkimensis

GGGTTCCGAAGTGTAATTTGTAGAGGATGTTATGGAATCTGTGTGGACTCAAGTTCT TTGGAAAAAGACGCCATGGAGAGTGACAGTCTCGTACTTTCCACCACATTTTCCATGTATAA CTCCTTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAA AGCTAAATACCGGCTAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACT TTGAAAAGAGAGTCAAACAGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATG GTCTCATTGTTCAGCCTTTTGGTGTATTCAATGATGGCTAGGCTAGCATCAGTTTGGACAGC TGGATAAAAGTGTTGGAAATGTATCTCCTCCGGGAGTGTTATAGTCTGATACAGAATGCAGC TCGTCCAGACTGAGGACCGCTTTAAGGATGCTGGCAATAGGTGGCATGAGGCCCGTCTGNA AACACGGA

#### 2.19 Laurera subdiscreta

ACCCGGCTGGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACTGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCCGCCACCAGGCTGAGTTG TAATTTGCAGAGGATGCTTTGGATCATGCTCCGCCTTGAGTCCTTTGGAACAGGGCGCCGAG GAGGGTGACAGTCCCGTATTTGCGGATGTCATGGTCCGTGTAAAGCTCCTTCGAAGAGTCGA GTTGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCCAAAGCTAAATATCTGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAA AAGCACGTGAAATTGTTGAAGGGGAAGCTCATGCAGTCAGACATGGCTCAAAAGTTCAGCCT TTTGGTGTACTCTTTTGAGCCAGGCTAGCATCAGTTGGGGCAGTTGGATAAAAGTTCCGGGA ATGTAGCTCCTCGGAGTGTTATAGCCCGATTCAGCATGCGATTCGTCCCGACTGAGGTCCGC TTATAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

### 2.20 Laurera varia

ACCCGCTGGACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACTGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTGAAATCTCCTTCTGGGCGAGTTGTA ATTTACAGAGGGTGTCTAGGAGTTGGTCTTGTCGCAAGTCCTTTGGAACAGGGCGTCATGGA GGGTGATAATCCCGTCCCCGTCTCTGACCCTCTCCGTGTTAGACCCCTTCGAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCCAAAGCTAAATACCCGGCTAGAG ACCGATAGCGTACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAACA GCACGTGAAATTGTTGAAAGGGAAGCCCATGCAGTCAGACATGGCGTGCAGGCTCAGCCTTC TGGTGTATTCCTGCATGCCAGGCTAGCATCAGTTTGGACCGCTGGATAAAGAATTTGGGAAT GTGACTCCTCGGAGTGTTATAGCCCGGATTGACATGCAGCGAGTTTTTCAGACTGAGGTCCG CTTATCCAGGATGCTGGCATAATGGTGGCATGGGCCCGTCTGNAAACACGGA

# 2.21 Laurera verrucoaggregata

ACCCGCTGTACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACGGGGATTGCC TTCGTAACGGCGAGTGAAGCGGCAAAAGCTCATATTTGAAATCAGCCATAAGGCCGAGTTGT AATTTGCAGAGGATCCTATGGATTATATCTGGATTGAAGTCCTTTGGAAAAAGGCGCCCAGG AAGGTGACAGCCCTGTACTTTCTAGCATATTTCTATGTATAGCTCCTTCAAAGAGTCGAGTT GTTTGGGAATGCAGCTCTAAATGGGAGGTAAATTTCTTCCAAAGCTAAATATCAACTAGAGA CCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAAAG CACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTATTAATGTTCAGCCTTTT GGTGTATTCATTGATGTCAGGCTAGCATCAATCCGGATAGTTGGATAAAGGTTTTGAGAATG ATCTCTTCGGAGTGTTTAGCTCGATTCGGAATGCATTAACCCAGCTGAGGTCCGCTCTTAGG ATGTTGGATCATGGTGGGTGAGGCCCGCTGAANCACGGACGTCTGAAACACGGA

# 2.22 Laurera vezdae

# 2.23 Marcelaria cumingii

# 2.24 Polymeridium albidum

TAATTTGTAGAGGATGCTTTGGATTTTGTGCTGACGTAAGTCCTTTGGAACAAGGCG TCACGGAGAGTGAAATCCTCGTACCGTCAGTCGCACCATCCGCACAAAGCTCCTTCGAAGAG TCGAGTTGTTTGGGAATGCAGCTCCAAGTGGGAGGTAAATTTCTTCCCAAGGCTAAATATCAG CTAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGT TAAAAAGCATTTGAAATTGTTGAAAGGGAAGCCCATGCAGTCAGACATGTTTCGGAGGCTCA GCCTTTTGGTGTACTCTTCCGAAACAGGCTAGCATCGGTTTGGGCCGCCGGACAAAGGCGTC GGGAATGTAGCTCCTCGGAGTGTTATAGCCCGACACAAAATGCGGCGCCCCAGACCGAGCC CCGCTTACAGGATGCTGGCATAATGGTGGCATGGGGCCCGTCTTGAAACACGGAC

### 2.25 Polymeridium albocinereum

### 2.26 Polymeridium catapastum

### 2.27 Polymeridium quinqueseptatum

# 2.28 Polymeridium sp.1

# 2.29 Pseudopyrenula diluta var. degenerans

# 2.30 Pseudopyrenula subnudata

ACCCGGCTGGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGCCACAAGGCCGAATTG TAATTTGCAGAGGATGCTTTGGTACTTGATATCTGGCAAAAGTCCTTTGGAACAGGGCGTCA TGGAGGGTGAAAATCCCGTATTCGTCAGTTTATCAATGCCATGTAAAGCTCCTTCGAAGAGT CGAGTTGTTTGGGAATGCAGCTCTAAATGGGAGGTAAATTTCTTCCAAAGCTCATTGGAAAGAGAGT TAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTT AAACAGCACGTGAAATTGTTGAAAGGGAAGCCTATACAGCCAGATATGATTGTCAAGGCTCA GCCTTATGGTCTACTCCTTGACAGATCAAGCTAACACCAGTTTGGACAGTTGGATAAAAGTTT TGGGAATGTAGCTCTTCGGAGTGTTATAGCCCGATTCAAAATGCAACTCATCCAGACTGAGG TCCGCTTATAGGATGTTGGCGTAATGGTGGTATGGGGCCCGTCTGAAAACACGGA

# 2.31 Trypethelium cf. aeneum

ACCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TTAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTTAAATCTGCCATCAGGCCGAGTTGT AATTTGCAGAGGATGTGATGGAATTTGTTTGATATCAAGTCCTTTGGAAAAAGGCGCCATGG AGAGTGAAAGTCTCGTCCGTATCACCACACTTTCTATTTATCACTCCTTCAAAGAGTCGAGT TGTTTGGGAATGCAGCTCAAAATGGGACGTAAATTTGTTCCAAAGCTAAATACTGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAACA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGACGTCCATGTTCAGCCGTA TGGTGTATTCATGGACGATCAAGCTAGCATCAATTGGGATAGCGGGATAAAAGTATTGGAAA TGTAGTTTTCTCCGGAATTCCTTATAGTCCGTTACATAATGCCGCTAATCTCGATTGGAAA CGCTAATAGGATGCTGGCGTAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

# 2.32 Trypethelium andamanicum

# 2.33 Trypethelium cinereorosellum

ACCCGCTGAACTTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTTAAATCTGACACTGTTCCGAGTTGT AATTTATAGAGGATGTTATGGAATCTGTGTGGGACTCAAGTTCTTTGGAAAAAGACGCCATGG AGAGTGACAGTCTCGTACTTTCCACCACATTTTCCATGTATAACTCCTTCAAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTCAAACA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTCTCATTGTTCAGCCTTT TGGTGTATTCAATGATGGCCAGGCTAGCATCAGTTTGGACAGCTGGATAAAAGTGTTGGAAA TGTATCTCCTTCGGGAGTGTTATAGTCTGATACAGAATGCACCGGCTCGGCCAGACTGAGAGCCG CTTTAAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGGAAACACGGA

# 2.34 Trypethelium eluteriae

ACCCGGCTGGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGCCATCCGGCCGAGTTG TACTTTGCAGAGGATGTTTTGAAATCTGTCTCGTATAAAGTCCTTTGGAACAGGGCGTCATG GAGGGTGAAAATCCCGTCTTTTCGATGACAGCTTTTCATTGTAAAACTCCTTCGAAGAGTCG AGTTGTTTGGGAATGCAGCTCTAAGTGGGGAGGTAAATTTCTTCCAAAGCTAAATATAGGCTA GAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAA ACAGCACGTGAAATTGTTGAAAGGGAAGCTTATGTAATCAGATATGATTCGCAGGTTCAGCC TTTTGGTGTATTCCTGAGGATCAGGTTAGCATCAGATTGGGTCGTCGGATAAAAGTTTTGGG AATGTAACTCTTCGGAGTGTTAAGCCCGATTCAGAATGCGACGAATTTAGACTGAGGTCCG CTTTGAAGGATGCTGACATAATGGTTGCATGAGGCCCGTCTGAAAACACGGA

# 2.35 Trypethelium microstomum

ACCCCGTTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTTAAATCTGCCACCGTGCCGAGTTG TAATTTGCAGAGGATGTTATGGAATCTGTTTGGACTCAAGTCCTTTGGAAAAAGGCGCCATG GAGAGTGACAGTCTCGTACTTTCCAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTGTTGTTCAGCCTT TTGGTGTATTCAACGACTGCCAGGCTAGCATCAGTTGAAAAGCTGGATAAAAACTCCGGAAA TGTAGCTCCTCCGGGAGTGTTATAGTCCGGGATAGAATGCAGTTCATTTAGACTGAGGACCG CTTTAAGGATGCTGGCATAATGGTGGCCATGAGGCCCGTCGAAAAACACGGA

# 2.36 Trypethelium neogabeinum

# 2.37 Trypethelium nitidusculum

ACCCGCTGAAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATTTGCCATCAGGCCGAGTTGT AATTTGCAGAGGATGTTATGGAATTTTGTATGAACTCCAAATTCTTTGGAAAAAGATGCTACG AAGAGTGAAAGCCTCGTACTGTTCAATACATTTTTCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCAAAGTGGGACGTAAATTTGTTCCAAAGCTAAATATTGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGACGTCAATGTTCAGCCTT TGGCCAACTCATTGATTGTCAAGTTAGCATCAATTTGGCTAGAATGACGTCAAATGTTGGAAA TGTAGCTTCTCCGGAAGTATTAAGTCTGATATAGAATGCAGCTTACCCAGATTGAGGTCCG CTTATAGGATGCTGACATAATGGTGGCATGAGGCCCGTTTGGAAACACGGA

# 2.38 Trypethelium ochroleucum var. subdissocians

# 2.39 Trypethelium aff. papulosum

# 2.40 Trypethelium platystomum

ACCCGCTGGACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGCCATCCGGCCGAGTTGT ACTTTGCAGAGGATGTTTTGGAATCTGTCCCATCTGAAGTCCTTTGGAACAGGGCGTCATGG AGGGTGAAAATCCCGTTTTTTTGGATACAGTTTCCGTGTAAAACTCCTTCGAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAATGGGAGGTAAATTTCTTCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAAA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTAATCAGACATGATTCTGGGGGTTCAGCCTTT TGGTGTATTCCTAAGTATCAGGCTAGCATCAGTTTCGGTCGTTGGATAAAAGTTTTGGGAAT GTAGCTCCTCGGAGTGTTATAGCCCGATTCAGCATGCGATGTACCGAGATTGAGGGTCCGCTA TGAGGATGCTGGCATAATGGTGACATAAGGCCCGTCTTGAAACACGGA

# 2.41 Trypethelium pseudoplatystomum

# 2.42 Trypethelium subeluteriae

ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCT TCAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGCCATCCGGCCGAGTTGT ACTTTGCAGAGGATGTTTTGGAATCTGTCTCGTTTAAAGTCCTTTGGAACAGGGCGTCATGG AGGGTGAAAATCCCGTCTTCTCGATGACAGTCTCCGCGTAAAACTCCTTCGAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCCAAAGCTAAATATCTGCTAGAG ACCGATAGCGGACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAAA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGACATGATGCTCGAGTTCAGCCTTT TGGTGTATTCTCGAGTGTCAGGCTAGCATCAGATTGGGTTGCTGGATAAAAGTTTCGGGAAT GTAACTCCTCGGAGTGTTATAGCCCGATTCAGAATGCGGCAAACTCAGACTGAGGTCCGCTT ATAGGATGCTGGCATAATGGTGACATAAGGCCCGTCTTGAAACACGGA

# 2.43 Trypethelium tropicum

ACCCGGTTTGAACTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC CTTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCAGCCACCGGGCCGAGTTG TAATTTGCAGAGGATGCTTTGGACTTTTGCTCCGTTCCAAGTCCTTTGGAAAAGGGCATCAT AGAGAGTGAAAATCTCGTAGGTTTGGATGCACTGTCCATGTAAAGCTCCTTCGAAGAGTCGA GTTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATATCAGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAA AAGCACGTGAAATTGTTGAAAGGGAAGCTCATGCAGCCAGACATGATGACGAGGTTCAGCCT TATGGTGTATTCCTCGACATCAGGCTAGCATCAGTTTGGACAGGCGGATAAAGGTTTTGGGA ATGTGACTCTTCGGAGTGTTATAGCCCGATTCAGCATGCGTCTTGTTCAGACTGAGGTTCGC TTATAGGATGCTGGCATAATGGTGGCATGAGGCCCGCTGGAAAACCCCGGA

# 2.44 Trypethelium ubianense

### 2.45 Trypethelium virens

ACCCGGCTGAAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCATAAGCTCAAATTTGAAATCAGCCTCAAGGCCGAGTTG TAATTTGCAGAGGATGCTTCGGATTCTGCTCCGGCCTAAGTCTTTTGGAACAAGGCGTCAAG GAGGGTGAAAATCCCGTATTTTCGGATTCCAGTCTCCATGTGAAGCTCCTTCGAAGAGTCGA GTTGTTTGGGAATGCAGCTCTAAAGGGGAGGTAAATTTCTTCCCAAAGCTAAATATCTGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAA CAGCACGTGAAATGGTTAAAAGGGAAGCTTATGCAGCCAGATATGATTCTCCAGGCTCAGCCT TATGGTGTATTCCTGTGAATCGAGTCAACATCAGTCTGGGCAGCTGGATAAAAGCTTCGGGA ATGTAGCTCCTCGGAGTGTTATAGCCCGATTCACCATGCAGCTCAGGCTCGGGCACGCT TTATAGGATGTTGACGTAATGGTGGCATGAGGCCCGTCTGAAAACACGGA

#### 2.46 Trypethelium sp.1

ACCCCGCTTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTG CCTTAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTGAAATCTGCCATCCGGCCGAGTT GTAATTTGCAGAGGATGTTATGGAATCTGTCTGGACTCAAGTTCTTTGGAAAAAGACGCCAG GGAGAGTGACAGTCTCGTTCTTTCCAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGA GTTGTTTGGGAATGCAGCTCAAAATGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAG AGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGGTTAAA AAGCACGTGAAATTGTTGAAAGGGAAGCTCATGTCATCAGAAATGACGTCAATGTTCAGCCT TTTGGTCTACTCATTGGTTGTCAAGTTAGCATCAATTTGAATAGCTGGATAAAAGTGTCGAA AATGTAGCTCTTCCGAGAGTGTTATAGTTTGATACACAATGCAGCTCATTCAGATTGAGGAC CGCTTAAAGGATGCTGACATAATGGTGGCATGAGGCCCGTTGAAAAACACGGA

### 2.47 Trypethelium sp.2

# 2.48 Trypethelium sp.3

ACCCCGCTGGAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCCCAAAGGCCGAGTTG TAATTTGCAGAGGATGTTATGGGATCTGTGTGGGACTCAAGTTCTTTGGAAAAAGACGCCATG GAGAGTGACAGTCTCGTACTTTCCAACACATTTCCCCCTGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCCATCAGAACCGGTGTCATTGTTCAGCCTT TTGGTGTATTCAATGACTGCCAGGCTAGCATCAGTTTGGATAGCTGGACAAAAGTGTTGGAA ATGTAACTCCTCCGGGAGTGTTATAGTCCGATATAGAATGCAGCTCATTTAGACTGAGGACC GCTTTAAGGATGCTGGCATAATGGTGGCATGAGGCCCGTCTGGAAACACGGA

# 2.49 Trypethelium sp.4

ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAAAAGCTTAAATTTGAAATCTGCCATCAGGCCGAGTTGT AATTTGCAGAGGATGTTTTGAAGTCTATTCCGAATTAAGTCCTTTGGAACAGGGCGTCAAGG AGGGTGAAAATCCCGTCTGTTCGGATATTGATTTCGTGTAAAACTCCTTCGAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCCAAAGCTAAATATCTGCTAGAG ACCGATAGCGCCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGGTTAAAAA GCACGTGAAATTGTTGAAAGGGAAGCTTATGCAGCCAGACATGATTCAAAAGCTCAGCCTCA TGGTGTACTCTTTTGGGTCAGGCTAGCATCGGTTTGGGCAGTTGGATAAAAGTTTCGGGAAT GTAGCTCCTCGGAGTGTTATAGCCCGATTCAGCATGCAGCTCGGCTCAGACTGAGGTCCGCTT ATAGGATGCTGGCGTAATGGTGGCATGAGGCCCGTCTTGAAACACGGA

# 2.50 Trypethelium sp.5

# 2.51 Trypethelium sp.6

ACCCGTTGGAATTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAATAGCTCAAATTTGAAATCTCCTTCGGGCGAGTTGTA ATTTACAGAGGGTGTCTAGGAGTTGGTCTCGTCGCAAGTCCTTTGGAACAGGGCGTCATGGA GGGTGAAAATCCCGTCCCCGTCTATGACCTTCTCCATGTTAGACCCCTTCGAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGAGGTAAATTTCTTCCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAACA GCACGTGAAATTGTTGAAAGGGAAGCCCATGCAGTCAGACATGATATGCAGGTTCAGCCTTT TGGTGTATTCTTGCGTATCAGGCTAGCATCAGTTTGGGTCGCTGGATAAGGAATTTGGGAAT GTGGCTCTTCGGAGTGTTATAGCCCGGATTGACATGCAGCGTATTCAGACTGAGGTCCGCTT ATAGGATGCTGGCATAATGGTGGCCTGGCATGGGCCCGTCTGAAAACACGGA

# 2.52 Trypethelium sp.7

ACCCGCTTGAAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CCTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCTACCGTGCCGAGTTG TAATTTGCAGAGGATGTTATGGAATTTGTGTGGGACTCAAGTCCTTTGGAAAAAGGCGCCATG GAGAGTGACAGTCTCGTACTTTCCAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTGTCGTTGTTCAGCCTT TTGGTGTATTCAATGATAACCAGGCTAGCATCAGATTGAATAGCTGGATAAAAACTTGGAAA TGTAGCTCCTTCGGGTGTGTTATAGTCCGAGATAGAATGCAGCTCATTTAGACTGAGGACCG CTTTGAGGATGCTGGCATAATGGTGGCCTGGCATGAGGCCCGCTGGAAAACCACGGA

# 2.53 Trypethelium sp.8

ACCCCGTTGAAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGC CTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGCCATCGTGCCGAGTTG TAATTTGCAGAGGATGTTATGGGATTTGTGTGGGACTCAAGTCCTTTGGAAAAAGGCGCCATG GAGAGTGACAGTCTCGTACTTTCCAATACATTTTCCATGTATAACTCCTTCAAAGAGTCGAG TTGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGA GACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAAA AGCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAAATGGTGTCGTTGTTCAGCCTT TTGGTGTATTCAATGACAACCAGGCTAGCATCAGTTTGAAAAGCTGGATAAAAACTTGGAAA TGTAGCTCCTTCGGGTGTGTTATAGTCCGAGATAGAATGCAGTTCATTTAGACTGAGGACCG CTTTAAGGATGCTGGCATAATGGTGGCCTGGCATGAGGCCCGTCTGAAAACACGGA

# 2.54 Trypethelium sp.9

ACCCGCTGAAATTAAGCATATCAATAAGTGGAGGAAAAGAAACCAACAGGGATTGCC TCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTTAAATCTGACACTGTTCCGAGTTGT AATTTGCAGAGGATGTTATGGAATTTGTGTGGGACTCAAGTTCTTTGGAAAAAGACGCCATGG AGAGTGACAGTCTCGTACTTTCCACCACATTTTCCATGTATAACTCCTTCAAAGAGTCGAGT TGTTTGGGAATGCAGCTCTAAGTGGGACGTAAATTTGTTCCAAAGCTAAATACCGGCTAGAG ACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTTAAACA GCACGTGAAATTGTTGAAAGGGAAGCTTATGTCATCAGAATTGGTTTCATTGTTCAGCCTTT TGGTGTATTCAATGATGGCCAGGCTAGCATCAGATTGGCTTGGATAAAAGTGTTGGAAA TGTAGCTCTCTCGGGAGTATTATAGTCTGATACAGAATGCAGCTTGTCTAGACTGAGGACCG CTTTAAGGATGCTGGCATAATGGTGGCCATGAGGCCCGTTTGGAAACACGGA

# 2.55 Trypethelium sp.10

# 2.56 Trypethelium sp.11

ACCCGCTGAACTTAAGCATATCAATAAGCGGAGCAAAAGTCACCAACAGGGATTGCG TCAGCAGGGAAGAGCGAAGCGGCAAAATCTCAAATCCTAAATAGGCCATCAGTATGAGTTGT ATTTTGCATAGGATGCCGTGTTCACTGAAGTGTCTTAAATCCTTTGGAAAGAGAGGGGCAAGG AGGGTGAAAATCCCGTTTTTGGCGGATGTCAATGTCGGGATAAAGGTCCTTGGAAGAGAGTGGG AGTTGTTTGGGAATGCAGCTGTAAGTGGGAGGTAAATTTTTTTCCAAAGGTCAAATATCTGATA GAGACCAAGAGCGCACAAGTAGAGTGGATCGAAAGATGAAAGGCGGTGTGAAAAGAGACTTAA AAAGCACGTGAAGAAGCGAAAGGGAAGCTCATGCAGTCAGACATGATTCAAAGGGTCAGCGG TATGTAGTATTCCTGGGAAGTTCATCAGCATCAGTCAGGCGACAGGAGGATTACAGGATTGGGA ATGGATTTCTTCGGAGTGGTGTAG

### 3. mtSSU region

### 3.1 Astrothelium aenascens

### 3.2 Astrothelium flavocoronatum

# 3.3 Astrothelium macrocarpum

### 3.4 Astrothelium macrostiolatum

# 3.5 Astrothelium neglectum

# 3.6 Astrothelium neoveriolosum

# 3.7 Astrothelium siamense

### 3.8 Bathelium albidoporum

### 3.9 Bathelium madreporiforme

#### 3.10 Bathelium sp.1

# 3.11 Campylothelium nitidum

### 3.12 Laurera cf. aurantiaca

### 3.13 Laurera alboverruca

# 3.14 Laurera cf. columellata

#### 3.15 Laurera keralensis

### 3.16 Laurera megasperma

### 3.17 Laurera meristospora

# 3.18 Laurera sikkimensis

# 3.19 Laurera subdiscreta

#### 3.20 Laurera varia

# 3.21 Laurera verrucoaggregata

#### 3.22 Laurera vezdae

#### 3.23 Marcelaria cumingii

### 3.24 Polymeridium albidum

# 3.25 Polymeridium albocinereum

# 3.26 Polymeridium catapastum

# 3.27 Polymeridium quinqueseptatum

### 3.28 Polymeridium sp.1

# 3.29 Polymeridium sp.2

#### 3.30 Pseudopyrenula diluta var. degenerans

# 3.31 Pseudopyrenula subnudata

### 3.32 Trypethelium cf. aeneum

### 3.33 Trypethelium andamanicum

# 3.34 Trypethelium cinereorosellum

CAGCAGTCGCGGCAACACAAGGAAGACAAAGTGTTATTCATCTTAAATCGGTTTAAG GGGTACCTAGACGGTCAATTAGGCTAATAGTAGGATCGTATTTCCTAGAGTTATACAAGCAT GGGGAAAGCAAACCTTTATATATATAACTGAAGATGAATTTCTGCTGCCGATTAAGGTTGGTAAA GGGGAAAGCAAACCTTTATATATATAACTGACGTTGAGGGACGAAGGCTTGGGGAGCAAACAG GATTAGATACCCTAATAGTGCAGGCAGGAGAATTATGAATGGCATAGATTATATGTAATGTAG TCTATAAATGAAAGTGTAAGCATTCCACCTCAAGAGTAATGTGGCAACGCAGGAACTGAAAT CATTAGACCGGTTCTGATACCAGTAGTGAAGTATGTGTTTAATTTGTTGGTCCACAAAGAA GCTGACCACAATTTGAATATTTAACTTATATATATATTTGTTGGTCCACAAAGAA GCTGACCACAATTTGAATATTTAACTTATATATATTTGTGGTAGATTCATAAAATTAAC AAGCGTTGCATTGTTGTCTTCAGTTAATGTTGGGAGACTTTGGGTAGATTCATAAAATTAAC GTAATCCTATAATCTATTTAGATATTAATAGATTAGTTCACCGCAATATTGGATATTGATAA CTGGGAGTAAGACAAGTCGTAATGACCTTAATATTGTGGGCCAATAGACGTGCCACA

# 3.35 Trypethelium eluteriae

CAGCAGTCGCGGCAACACAAGGAAGACAAGTGTTATTCATCTTAAATCGGTTTAAAG GGTACCTAGACGGTGAATTAGGCCTTAAATGGAACGTTTTTACTAGAGTTATATATGCATGA GGACTGTGTGAGTATTACCAGAGTAGAGATGTAATTTTTTGATACTGTTAAGACTGGTAAAG GCAAAAGCAAACCTTTATATATATAACTGACGTTGAGGGGACGAAGGCTTGGGGCGCAAACAGG ATTAGATACCCCAGTAGTCCAGGCAGAGAATTATGAATGTCATAGAATAGATATAATATTTA TCCTATAAATGAAAGTGTAAGCATTCCACCTCAAGAGTAATGTGGCAACGCAGGAACTGAAA ACCTTTCCACAATTTGATACCAGTAGTGAAGTATATATTTTATTCATCTTTATACCAGC GTTGCATTGTCTTCAGTTAATATTTAATAGATATATATTTTGGTTAGATTCATTAAATTAACGTAA TCCTATATTTTAAATATATATATATGTGGGAGATTTTGGTTAGATTCATTAAATTAACGTAA

### 3.36 Trypethelium microstomum

### 3.37 Trypethelium neogabeinum

### 3.38 Trypethelium nitidusculum

#### 3.39 Trypethelium ochroleucum var. subdissocians
# 3.40 Trypethelium aff. papulosum

# 3.41 Trypethelium platystomum

# 3.42 Trypethelium pseudoplatystomum

CAGCAGTCGCGGCAACACAAGGAAGACAAGTGTTATTCATCTTAAATCGGTTTAAAG GGTACCTAGACGGTAAATTAGGCCTTAAATGGAACGTTTTTACTAGAGTTATATATGCAGGA GGAATATGTGAGTATTACCAGAGTAGAGATGAAATTTTTTGATACTGTTAAGACTGGTAAAG GCAAAAGCAAACCTTTATATATTAACTGACGTTGAGGGGACGAAGGCTTGGGGAGCAAACAGG ATTAGATACCCTAATAGTCCAGGCAGAGAATTATGAATGTCATAGAATAGATATAATGTTTA TTCTATAAATGAAAGTGTAAGCATTCCACCTCAAGAGTAATGTGGCAACGCAGGAACTGAAA CCATTAGACCGTTTCTGATACCAGTAGTGAGAGTATGTTGTTTAATTTGTTAACCCTCAAAAA ACCTTACCACAATTTGAATATTTAATTGATATAATTTGTTAACCCTTCAAAAA CAGCGTTGCATTGTTGTCTTCAGTTAATTTGTTAATTTGTTAATTTA CAAGCGTTGCATTGTTGTCTTCAGTTAATGTTGGTTAGATTCATAAAATTAA CGTAATCCTATATTCTATTTAATAAAATTTAATAGATTAGTTCACCGCTATATTGGATATTGATA ACCGGGAGTAAGACTAGTCGTAATGACCTTAATATTGTGGGCTATGAGACGTGCCACA

## 3.43 Trypethelium subeluteriae

# 3.44 Trypethelium tropicum

# 3.45 Trypethelium ubianense

## 3.46 Trypethelium virens

# 3.47 Trypethelium sp.1

# 3.48 Trypethelium sp.2

# 3.49 Trypethelium sp.3

# 3.50 *Trypethelium* sp.4

# 3.51 Trypethelium sp.5

# 3.52 Trypethelium sp.6

# 3.53 Trypethelium sp.7

## 3.54 *Trypethelium* sp.8

# 3.55 Trypethelium sp.9

## 3.56 Trypethelium sp.10

# 3.57 Trypethelium sp.11



## 4. RPB1 region

#### 4.1 Astrothelium aenascens

# 4.2 Astrothelium flavocoronatum

# 4.3 Astrothelium macrocarpum

GAATGTCACGGTCATTTTGGCCATATTGAACTCGCTGTGCCCGTCTTCCATGTTGGT AAGGCTAGCTGAATCGCTAAACTATTCACTGCTCGATCACTAACTCGCTCAATCCAGGTTTC ATTGGCAAAATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGCAAGATCCTCATGGA TGAAGTCAGGCCAATCCAGGCATTCATTGAAGCCCAACGAGGACCGCAAGCGCCGTTT TGACAAGATCTGGACTCTATGCAAGACCAAGAAGAAATGCGAACGAGACCGCCAGGCCGTTT TGACAAGATCTGGACTCTATGCAAGACCAAGAAGAAATGCGAACGAGACCCTCAGGACGACG CCAATGCGGACGAGAATCCCGACCAACCACCTCTGAAGCCCTCGGCTACTCGTGGTGGATGCGGA AATGTCGCACCAGACATCAGGAAGGATGGATTGAAGCTCCTTGGCACTTGGAAATACGACAA ATCCGAAGAGGAAGATGAGGAACGTCGTATCGAGAAGAAGCACATCACGCCTCAACAGGCCT TACAGGCTTTCAACCATATTTCCAGCGAGGATCTGGAGAAGAATGGTCTCGGCAGTGACTAC GCGAAGCCGACGTGGATGATCCTCACGTGCTTCCTGTCCCACCTCCTCCAGTGCGTCCCAG TATTTCCGTCGATGGAACTGGTCAAGGCATGCGCGGCGAAGATGACCTTGACCTACAAGCTCA GCGACATCATTCGTGCAAATGCCAACGTCAAGAAATGCAAAGCAGAGGGTTCGCCAGGTCAC ATTGTTGCAGAGTTCGAGACTCTTTTGCAATATCACGTTGCAACCTACATGGACGACGACGACACAT CCGCGGGA

## 4.4 Astrothelium macrostiolatum

## 4.5 Astrothelium neglectum

#### 4.6 Astrothelium neoveriolosum

AATTTTCCCGGGCATTTGGCCACATTGAACTCGCTGTGCCCGTCTTCCATGTTGGTC AGTTTGAGTGAATCGCTAAACTATCCACAGCTCATTCACTGACTCACGGTTTCATCGG CAAAATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGCAAGATCCTCATGGATGAAG TTAGTCATGGGCTCCCCTGTGAGCTAATTTATTGTGTGTTATTTTGCTAACTTGATTTGTTT CAAAAGACCAACCCAGCATTCATTGAAGCCCTAAAGACGAGAGCCGCAAGCGCCGTTTTGA CAAGATCTGGACTCTTTGCAAGACCAAAAAGAAATGCGAACGAGACCGCAAGCGCCGTTTTGA ATGCTGATGAGAATCCCGATCAACCTTTGAAGCCCTCGGCTACTCGTGGTGGATGCGGAAAT GTTGCACCCGACATCAGGAAGGACGGACTAAAGCATTCTTGGCACTTGGAAATACGACAAATC CGAAGAGGAAGATGATGAGCGTCGCATTGAGAAGAAGCACATTACGCCTCAACAGGCCTTGC ACGCTTTCAACCATATTTCTAGTGAGGACCTAGAGAAGGTTGGTCTTGGCAGCGACTACGCG AAGCCAACTTGGATGATCCTCACCGTGCTCCCTGTTCCACCTCCTCCAGTGCGTCCAAGTAT CTCCGTCGATGGAACTGGTCAAGGTATGCGCGGTGAAGATGACTTGACCTACAAGCTCAGCG ACATCATTCGTGCAAACGCCAATGTCAAGAAATGCAAAGCAGAGGGCTCACCAGGGCACATT GTTGCAGAATTTGAGACGCTTTGCAACGATGACGAGAGGGCTCACCAGGGCACATT GTTGCAGAATTTGAGACGCTTTGCAATATCACGTTGCGACGTATATGGACACGAAAATGC CGG

## 4.7 Astrothelium siamense

# 4.8 Bathelium albidoporum

GAATGTCCCGGGCATTTCAGGCACATTGAGCTTGCTGTACCCGTGTTCCAAGTTCGT AAGGACGAATGGGACATCACGTTGGCAAAGAACTTTATTGCTGATTCACTCTAGGATTCATC GGCAAAATCAAGAAGCTTCTTGAAATATGCTGCCATCATTGTGGCAAGATCCTCATGGATGA AGTCAGTAATCGACTTAACCATAAGCTGATCTGTCGTATACAATGTGCTAACATGACGTGAC TCAAGACCAATCCAGCGTTTATCGAAGCCTTGAAATCTCGAGACCGCAAGCGTCGCTTTGAC AAGATATGGTCTCTGTGCAAAAGCAAAATGAAGTGCGAACGCGATCCTCAGGACAATCCCGA TGCCGACGAGCATACCGATCAGCCTAAGAAGCCCACGTCGACTCGAGGCGGGTGCGGAAATG TTGCACCAGACATCAGAAAAGACGGACTGAAACTACTTGGCACTTGGAAGTATGACAAATCA GAGGAGGAAGATGAAGAGCGTCACATTGAAAAGAAGTACATCACTCCTCAACAGGCCCTCGA CGCCTTCAACCACATTTCAGACGAAGACCTGCAGAAGATTGGTCTGGGCAGTGACTATGCAA AGCCAAAATGGATGATCTCACCGTTCTTCCTGTCCCGCCTCCTCTGTACGCCCAAGTATC TCTGTTGATGGAACTGGCCAGGGGTTGCGCGGTGAAGATGACTTGACATACAAACTCAGTCA CATCATTCGAGCCAACGCCAACGTCAAGAAATGCAAGGCAGAGGGCTCTACAGGTCACATAG TATCAGAATTCGAGACCCTCTTGCAGACCACGTGGCACATATATGGACACGACATCGCGG

## 4.9 Bathelium madreporiforme

GAGTGTCCGGGGCATTTTGGGCATATTGAGCTTGCTGTGCCCGTGTTCCACGTTGGT AAGGACAAATGGGACATCGCATTAGTGAAGAGCTTCATCGCTGATTCACTCTAGGTTTATC GGTAAAATCAAGAAGCTTCTTGAAATATGCTGCCACCATTGTGGCAAGATCCTCATGGATGA AGTCAGTAATCGACTTAACCATAAGCTGATCTGTTGTACACAATGTGCTGACATGACTCAAG ACCAACCCAGCGTTCATCGAAGCCTTGAAGTCCCGAGACCGCAAGCGTCGCTTTGACAAGAT ATGGACCCTGTGCAAGAGCAAAAAGAAATGCGAACGCGATCCTCAGGACAATCCTGATGCCG AAGAAAATGCCGACCAGCCTAAGAAGCCCACGTCGACGCGAGCGGGTGCGGAAATGTTGCA CCAGACATCAGGAAAGATGGATTGAAACTACTTGGCACCTGGAAATATGACAAATCAGAAGA GGAAGACGAGGAGCGTCGCATTGAAAAGAAGTATATCACTCCTCAACAAGCCCTCGATGCCT TCAACCACATTTCAGACGACGACCTGCAAAAAATTGGTCTGGGCAGCGACTACGCAAAGCCA AAATGGATGATCCTCACCGTCCTTCCTGTCCCGCCTCCAGTCCGCCCAGTATCTCTGT TGATGGAACTGGTCAGGGGTTGCGCGGTGAAGATGATCTGACATACAAACTCAGTGACATCA GAGTTCGAGCCCAACGTCAAGAAGTGCAAAGCGGAGGGCTCGCCGGGTCATATCGTATCA

## 4.10 Bathelium sp.1

GAATGCCCCGGGCATTTCGGACATATTGAACTTTCCGTACCTGTATTTCATGTTGGT AAGCATTTGTGAAACGACGATGCGCTGTTGAGTTGTTGTATTGCTAACCATGTCCAGGCTTC ATCGGCAAGATCAAGAAGCTTCTCGAAATTTGCTGTCATCATTGCGGGAAGATCCTCGTCGA CGAAGTCAGTCCTGATCTCGGCTCTGAGATAGTCGCTGGTGTACATTTTGCTAACTCTCTTG TGAATATAGACCAATCCAGCCTTCGTGGAAGCTGTGAAGACTAGAGACCGCAAGCGTCGCTT CGATAAGATCTGGGCTCTTTGTAAAACCAAGAAGAAATGCGAACGAGATCCTCAGGACAATC CAGACGCGGAACATGACCTGATCAGCCCAAGAAACCTTCGTCCACCCGAGGTGGCTGTGGA AACGTTGCCCCAGATATTAGAAAAGAAGGCTTAAAACTCCTCGGTACTTGGAAGTATGACAA ATCCGAAGAGGAGGATGAAGAGCGTCGGATTGAGAAGAAGTACATCACACCTCAACAGGCCC TCAATGCCTTCAATCATATTTCAGACGAGGATCTGCAGAAGATTGGTCTGGGCAGCGATTAT GCGAAGCCAAAGTGGATGATACTCACAGTACTTCCTGTTCCACCTCCTGTGCGCCCCAAG CATATCGGTTGATGGAACGGGGCAAGGGCTCCGCGGTGAAGACGATCTCACTTATAAACTTA GCGATATCATTCGTGCGAATTCGAACGTCAGAAATGCAAGGTCGCAGGATCTCACTTATAAACTTA GCGATATCATTCGTGCGAATTCGAACGTCAAGAAATGCAAGTCAGAAGGTTCGCCCGGGTCAC ATCATCGCCGGGTTTGAGACGCCTTTGCAATATCACGTTGGCAACGATCTCACCTTGGACACGACATT TCCCGG

## 4.11 Campylothelium nitidum

TATATGTGTCGGGGGGCTTCGGCACATTGAGCTCTCAGTTCCCGTCTTCCACGTTGG TATGAGCCTACCAAATCTCACCTCTGTACGTTATCCTCTGCTGACTATGTCTAGGTTTCATC AGCAAGATTAAGAAACTTCTGGAGATATGTTGCCATCACTGCGGCAAGATTCTTGTCGATGA AGTTAGTGACAAGCTATTATGAGCTAATTTGCTATATGCTTGATGCTGACCTCATCCGATGT TAGACTAACCCAGCCTTCATCGAAGCTCTGAAAACTAGGGATCGCAAGCGTCGCTTTGACAA GATCTGGACCCTTTGCAAGTCCAAGAAAAAATGCGAACGAGACCCTCAGGACAATCCCGATG CAGATCATGATCCTGACCAGCCTAAGAAGCCTTCGTCAACCAGGGGTGGCTGCGGAAACGTT GCGCCAGACATCAGGAAGGAAGGGTTGAAACTCCTTGGCACTTGGAAGTATGACAAGACTGA AGAGGAAGATGAAGAGCGTCGGATTGAGAAGAAGTACATAACTCCTCAACTTGCCCTCGACG CTTTCGAACTTATTTCAGACGAGGATCTGCAGAAGATTGGTCTGGGTAGCGACTACGCGAAG CCAAAGTGGATGATCCTGAAAGTACTTCCCGTCCCACCTCCTCCAGTGCGCCCGAGTATCTC CGTAGATGGAACTGGACAAGGACTTCGCGGCGAGGATGACCTGACTAAACTCAGTGACA TCATTCGTGCCAACTCCCAATGTCAAGAAATGTAGAGACGAGGGATCACCGGCTCATATCACT GCAGAGTTTGAGACGCTCTTGCAATATCATACTGCGACCTAATGAGNAAANNCNTCCGCGG

#### 4.12 Laurera cf. aurantiaca

GAATGTCCCCGGTCATTTTGGCCACATTGAACTCGCTGTGCCTGTCTTCCATGTTGG TAAGTCTGAGTGAATCGCCAAGCTTATCCACTGCTCAGTCGCTGACTCTCACTCTAGGTTTC ATCGGCAAAATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGGCAAGATCCTCATGGA TGAAGTTAGTCATGGGCTCCCCGTGAGCTAAGCTGTTGTGTGTCATTTTGCTAACTTGATTT CTTCCAAAAGACCAACCCGGCATTCATTGAAGCCCTGAAGACTAGAGACCGTAAGCGCCGTT TTGACAAGATTTGGACCCTTTGCAAGACCAAAAAGAAATGCGAACGAGACCCTCAGGACGAT CCCAACGCTGATGAGAATCCCGATCAACCTATGAAGCCCTCGGCCACTCGTGGTGGATGCGG AAATGTTGCACCAGACATCAGGAAGGATGGACTGAAGCTGCTTGGCACTTGGAAATACGACA AATCCGAAGAGGAAGATGATGAACGTCGCATTGAGAAGAAGCACATCACGCCTCAACAGGCT TTGCACGCTTTTAATCATATTTCCAGCGAGGATCTAGAGAAGGATGGTCTTGGCAGCGACTA CGCGAAGCCAACCTGGATGATACTCACTGTGCTCCCCGTTCCACCTCCTCCAGTGCGTCCAA GCATCTCCGTCGACGGAACCGGTCAAGGTATGCGTGGTGAAGATGACCTGACCTACAAGCTC AGCGACATTATTCGTGCAAACGCCAATGTCAAGAAGTGCAAAGCAGAGGGCTCGCCAGGACA CATTGTTGCCGAATTTGAGACGCTTTTGCAATATCACGTGGCAACGTACATGGACACACGACAT TCGCCGG

## 4.13 Laurera alboverruca

# 4.14 Laurera cf. columellata

## 4.15 Laurera keralensis

GAATGTCCCAGGACATTCGGACACATAGAACTTTCCGTACCGGTATTCCATGTTGGT ATGCATCGACGCAGTGCCGAGTCGCTGGGTTCTTCGTTACTGATCCTGCTTAGGTTTCATCG GGAAGATTAAGAAACTTTTAGAGATCTGTTGCCATCAGTGTGGCAAGATACTTGTGGATGAA GTCAGTGATAATTCCGCCAGCCAGCTTTAATACCACATGGAATGCTGACCACAGCTGATTTA GACGAACCCTGCTTTCATCGAAGCCTTGAAAACCCGAGACCGCAAGCGCCGGTTTGACAAGA TTTGGACCCTTGCAAAAGCAAGAAGAAGTGCGAGCGGGACCCGCAAGACAATCCTGATGCA GATCATGACCCCGATCAACCTAAGAAACCTTCGTCCTCTCGGGGCGGCTGCGGAAACGTCGC GCCAGACATCAGGAAAGAAGGGCTGAAATTACTGGGTACCTGGAAATATGACAAGTCCGAAG AAGAAGACGAAGAGCGTCGAATTGCCCCAGAAGTACATCACACCTCAGCAAGCCCTGCAAGC TTTCAATGCCATATCAGACGAAGACCTGCAGAAGATCGGCCTGGGCAGCGATTATGCGAAGC CAAAGTGGATGATTCTCACCGTGCTCCCCGTGCCCCCCTCTGTGCGGCCGAGCATATCT GTTGATGGGACTGGGCAAGGGCTCCAGAAATGCAAGTCAGAACGTCCCAGAACTTAGCGACAT CATTCGCGCGAATGCCAACGTCAAGAAATGCAAGTCAGAAGGTTCCCCAGGTCACATCATCG CGGAATTTGAGACACTTTTGCAACTTATGCAAACCTATATGGACAACGACATCGCCGG

## 4.16 Laurera megasperma

# 4.17 Laurera meristospora

GAATGTCCCGGTCATTTCGGCCACATAGAACTTGCTGTGCCCGTCTTCCATGTTGGT AAGTCCAAGTGAATCGTCAAAGTATTCACCGCTCAGTCACTGACTTCACTTTAGGTTTCATC GGCAAGATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGCAAGATCCTCATGGATGA AGTTAGTCATGGGCTCCCCGTGAGCTAATCTTTTGTGTAATATTTTGCTGACTTGATTTGTT TCCAAAAGACCAATCCAGCGTTCATTGAAGCCATGAAGACTAGAGACCGCAAGCGCCGTTTC GACAAGATCTGGACTCTTTGCAAAACCAAGAAGAAATGCGAACGAGATCCCCAAGATGATCA CAATGCTGATGAGAATCCCGATCAACCTATGAAACCCTCGGCTACACGTGGCGGATGCGGAA ATGTTGCACCAGACATCAGAAAGGACGGACTGAAGCTTCTTGGCACTTGGAAATACGACAAA TCCGAAGAGGAAGATGATGAACGTCGTATTGAGAAAAGGCTCGCCTTGGCAGCGACTACG CGAAGCCAACATGGATGATACTCACCGTGGCTCCCTGTTCCACCTCCTCCAGTGCGTCCAAGT ATCTCCGTCGATGGAACCGGTCAAGGTATGCGTGGTGAAGATGACCTGACCTACAAGCTCAG CGACACCATCGTGCAAACGCCAATGTCAAGAAATGCCAAGGCAGGGCTCGCCAGGGCACA TTGTTGCAGAATCGGAGACGCCTTTTGCAATATCACGTGGCAACATACGACAACGCCAACAT GCCG

## 4.18 Laurera sikkimensis

#### 4.19 Laurera subdiscreta

## 4.20 Laurera varia

GAATGTCCCCGGTCACTTTGGGCATATTGAACTTGCTGTACCGGTCTTCCACGTTGG TATGGTTATGCACAACGCCAACTTGCTAGTTTCTTTCTGATGTTGCCTAGGTTTCATCGGCA AGATAAAGAAGCTTCTGGAGATCTGCTGTCATCATTGTGGCAAGATCCTCATGGATGAAGTG AGTGACGATTTCTTCTACAAGTTTTATTCGCTCACTGAATGTTAACCTCGCTTGAACCAAGA CCAATCCTGCATTCGTCGAAGCCTTGAAAACCAGAGATCGCAAGCGCCGCTTTGACAAGATT TGGATGCTTTGTAAAACCAAAAAGAAATGCGAGCGGGATCCACAGGATAATCCGGATGCAGA CCATGACCCAGACCAACATAAGAAGCCTTCATCCACTCGAGGTGGTTGCGGAAACGTTGCGC CAGATATCCGGAAAGAAGGATTGAAACTTCTTGGCACTTGGAAATACGATAAATCCGAAGAG GAAGATGAAGAGCGTCGGGTTGAGAAGAAGTATATCACACCTCAGCAGGCGCTGGATGCGTT CAATACTATATCAGACGAAGACCTGGAGAAGATCGGTCTGGGCAGCGATTACGCCAGGCCAA AGTGGAACAGGACAAGGCCTCCGTGGCGAAGACGACTTGACTTACAAGCTTAGCGACATCAT TCGCGCGAATGCCAACGTCAGGACAAATGCAAGTCGGACGACTTACAAGCTTAGCGACATCAT TCGCGCGAATGCCAACGTCAAGAAATGCAAGTCGGACGACTGGACAACGAAAATCGCCG AATTCGAGACTCTTCTACAATACCATGTTGCAACTTACATGGACAACGAAAATCGCCG

## 4.21 Laurera verrucoaggregata

#### 4.22 Laurera vezdae

## 4.23 Marcelaria cumingii

## 4.24 Polymeridium albidum

#### 4.25 Polymeridium albocinereum

# 4.26 Polymeridium catapastum

GAATTTCCCCGGGCATTTGGCCACATCGAACTCGCTGCGCCTGTATTTCATGTTGGT AAGTGCTTGTCCTGAGTGACTGCCTGCCATCCTCTCCTGCTGACCCGCTATAGGTTTCATCA GCAAGATCAAGAAACTCCTCGAAATTTGCTGCCATCAATGTGGGCAAGATTCTCATGGATGAA GTCAGTAAAGACGTCATCTGTGGGTTTATTCACAATGTATGCAGTGCTGACCAGTCCTTCTA GAACAATCCGGCATTTGTCGAAGCCCTAAAAAGTCGGGATCGGAAGCGACGCTTCGACAAAA TCTGGACGCTGTGCAAGACCAAAAAGAAGTGCGAGCGCGATGCACAAGACCACCCTGATGCG AACCACGACCCTGACAAACTCAAGAAAGTGCGAGCGCGATGCACAAGACAACCCTGATGCG AACCACGACCCTGACAAACTCAAGAAACCTGTATCCATTCGAGGTGGCTGTGGAAACGTTGC ACCCGACATTCGAAAAGAGGGCCTGAAGCTCCCAAGCCACATGGAAATACGACAAGTCGGAAG AGGAAGATGAGGAGCGTCGCATCGAGAAGCGGTACATTACACCTCAGCAGGCTCTGGATGCT TTCAATCACATTTCAGACGAGGATCTACAAAAGAATGGTCTAGGGAGCGACCATGCCAAGCC CGCTTGGATGATCATCACCGTTCTTCCTGTCCCGCCGCCTCCAGTGCGCCCGAGTATCTCCG TCGATGGAACCGGCCAGGGTATGCGAGGTGAAGACGATCTGACCTACAAACTCAGTGACATT ATTCGTGCCAACACTGGCGTCAACCAATGCAAGCGGCACATGGCACCACATTACGCA AGAATTCGAGTCGCTCTTGCAGTATCATGTTGCAACGTACATGGACACGAAAATTTCCCG

# 4.27 Polymeridium quinqueseptatum

## 4.28 Polymeridium sp.1

## 4.29 *Polymeridium* sp.2

# 4.30 Pseudopyrenula diluta var. degenerans

## 4.31 Pseudopyrenula subnudata

## 4.32 Trypethelium cf. aeneum

## 4.33 Trypethelium and amanicum

#### 4.34 Trypethelium cinereorosellum

## 4.35 Trypethelium eluteriae

#### 4.36 Trypethelium microstomum

## 4.37 Trypethelium neogabeinum

## 4.38 Trypethelium nitidusculum

#### 4.39 Trypethelium ochroleucum var. subdissocians

## 4.40 Trypethelium aff. papulosum

# 4.41 Trypethelium platystomum

# 4.42 Trypethelium pseudoplatystomum

GAGAGTCTCCCGTCACTTCGGGCATATTGAACTTTCTGTACCGGTCTTCACGTTGGT ATGGATATGCACAACGCCAACTTGCCTGAATTCTTTCTGATACTGCTTAGGTTTCATCAGCA AGATCAAGAAACTTCTGGAGATCTGCTGCCATCATTGTGGCAAGATCCTCATGGATGAAGTG AGTCACGATTTCCTCTACAAGCTTTATTCGCTCAGAGAATGTTGACCTCGCTTGAAATCCAGA CCAACCCTGCGTTCGTCGAAGCCTTGAAAACCAGAGATCGCAAGCGCCGCTTTGACAAGATT TGGTCGCTTTGTAAAAGCAAAAGAAATGCGAGCGGGGATCCACAGGATAATCCTGACGAAGA CCATGACCCAGACCAACCTAAGAAGCCTTCATCTACTCGAGGTGGTTGCGGAAACGTTGCGC CAGATATCCGGAAAGAAGGATTGAAACTTCTTGGTACTTGGAAATACGATAAATCCGAAGAG GAAGACGAAGAGCGTCGGATCGAGAAGAAGTATATCACACCTCAGCAGGCGCTGGAAGCGTT CAATACTATATCAGACGAAGAACTGGGCAGCTCGGCAGCGACTACGCCAAGCCAA AGTGGATGATTCTTACCGTGCTTCCTGTGCCTCCTCCCAGTGCGCCGAGTATCTCTGTT GATGGAACAGGGCAAGGGCTCCGGGGCGAAGACGACTTGACTTACAAGCTTAGCGACATCAT TCGCGCGAATGCTAACGTCAAGAAATGCAAGTCGGAGGGCTCGCCGGGTCACATTATTGCAG AATTCGAGACTCTTCTACAATACCATGTTGCAACTTACATGGACAACGAAAATCGCGG

## 4.43 Trypethelium subeluteriae

# 4.44 Trypethelium tropicum

# 4.45 *Trypethelium ubianense*

#### 4.46 Trypethelium virens

# 4.47 Trypethelium sp.1

GAATTTTCCCGGGCATTCGGCCATATTGAGCTCGCCGTCCCCGTCTTCCATGTTGGT AAGTACATGTGAATTGCGAAACTATGCATCACTCATTTATTAATTCACTCTAGGTTTTATCG GCAAAATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGCAAGATCCTCATGGATGAA GTCAGTCATAGGCTCCCTGTGAGCTAATTCTGTCTTGTGGCATGTGCTAACTTGATTTGTTT TCAAAGACCAACCCTGCCTTCATTGAAGCTCTGAAGACTAGAGACCGTAAGCGTCGCTTTGA CAAGATCTGGACTCTTTGCAAGACCAAAAAGAAATGCGAACGAGACCCTCAGGATGATCCCA ACGCCGATGAGAATCCAGACCAACCTATGAAGCCCTCGTCCACTCGAGGCGGATGCGGAAAT GTTGCACCAGACATTAGGAAGGATGGACTGAAACTTCTCGGCACTTGGAAATACGACAAATC TGAAGAGGAAGACGAAGAACGTCGAATCGAGAAGAAGTACATTACACCTCACCAGGCTTTGG AGGCTTTCAATCACATCTCCAACGAGGATCTTGAAAAGATTGGTCTTGGCAGCGATTACGCG AAACCAACATGGATGATCCTCACTGTGCTCCTGTCCCACCCCCTCCAGTGCGTCCAAGTAT CTCCGTCGATGGTACTGGTCAAGGCATGCGCGGGTGAAGATGACTTGACATACAAGCTTAGCG ACATCATCCGTGCAAATGCCAATGTGAAGAAATGCAAAGGATGACTTGACATACAAGCTTAGCG ACATCATCCGTGCAAATGCCAATGTGAAGAAATGCAAAGGAGAAGGCTCTCCAGGTCACATT GTTGCAGAGGTTTGAGACGCTTTGCAATATCATGTTGCAACTTAATGGCAGAAAANNTTTCC G

# 4.48 Trypethelium sp.2

## 4.49 Trypethelium sp.3

# 4.50 Trypethelium sp.4

# 4.51 Trypethelium sp.5

GTAAGTTCCCCCGGGCATTCGGGCATATTGAACTTTCTGTACCGGTCTTCCACGTTG GTACGGATATGCACAATGCCAACTTACTAAATTCTTTCTAATGCTGCTTAGGTTTCATCAGC AAGATAAAGAAGCTTCTGGAGATCTGCTGCCATCATTGTGGCAAAATCCTCATGGATGAAGT GAGTGACAATGTCCTCTACAAGCTTTATTCGCTCACAGAATGTTAACCTCGCTTGAACCCAG ACCAATCCTGCATTCGTCGAAGCCTTGAAAACCAGAGATCGCAAGCGCCGCTTTGACAAGAT CTGGGCGCTTTGTAAAAGCAAAAAGAAATGCGAGCGGGATCCACAGGATAATCCTGATGCAG ACCATGACCCAGATCAACCTAAAAAGCATTCCTCGCACGCGGGATGCGGAAACGTTGCG CCAGATATCCGGAAAGAAGGATTGAAAACTTCTTGGTACTTGGAAATACGATAAATCTGAAGA GGAAGACGAAGAGGGTCGGATTGAGAAGAAGTATATCACACCTCAGCAGGCGCTGGAAGCGT TCAATACTATATCGGATGAAAGATCTGCAGAAGATTGGTCTGGGCAGCGATTACGCCAAGCCA AAGTGGATGATTCTTACCGTGCTTCCTGTGCCTCCTCCCAGTGCGTCCGAGTATCTCTGT TGATGGAACAGGGCAAGGGCTCCGTGGCGAAGACGACTTGACTTACAAGCTTAGCGACATCA TTCGCGCGAACGCTAACGTCAAGAAATGCAAGTCGGAGGGCTCGCCGGGTCACATTATTGCA GAATTCGAGACTCTTCTACAATACCATGTGGCAACTTACAAGAANNNNTTCCCGG

# 4.52 Trypethelium sp.6

GAATGTCNCCGGGCATTTTGGGCATATTGAACTTGCTGTACCGGTCTTCCACGTTGG TATGGTTATGCACAACGCCAACTTGCCAGTTTCTTTCTGATGTTGCCTAGGTTTCATCGGCA AGATAAAGAAGCTTCTGGAGATCTGCCGCATCATTGTGGCAAGATCCTCATGGATGAAGTG AGTGACGATTTCTTCTACAAGTTTTATTCGCTCACCGAATGTTAACCTCGCTTGAAACCAAGA CCAATCCTGCATTCGTCGAAGCCTTGAAAACCAGAGATCGCAAGCGCCGCTTTGACAAGATT TGGATGCTTTGTAAAACCAAAAAGAAATGCGAGCGGGATCCACAGGATAATCCGGATGCAGA CCATGACCCAGACCAACCTAAGAAGCCTTCATCCACTCGAGGTGGTTGCGGAAACGTTGCGC CAGATATCCGGAAAGAAGGATTGAAACTTCTTGGCACTTGGAAATACGATAAATCCGAAGAG GAAGATGAAGAGCGTCGGGTTGAGAAAAGTATATCACACCTCAGCAGGCGCTGGATGCGTT CAATACTATATCAGACGAAGACCTGGAGAAGATCGGTCTAGGCAGCGATTACGCCAGGCCAA AGTGGATGATTATTACCGTGCTTCCTGTGCCTCCTCCTCCAGTGCGCCCGAGTATCTCTGTT GATGGAACAGGACAAGGCCTCCGTGGCGAAGACGACTTGACTTACAAGCTTAGCGACATCAT TCGCGCGAATGCCAACGTCAAGAAATGCAAGTCGGACGGCCCGCGGGTCATATTATTGCAG AATTCGAGACTCTTCTACAATACCATGTTGCAACTTACATGGACACGAAAATTGCCG

# 4.53 *Trypethelium* sp.7

GAGTGTCCAGGACATTTTGACCACATTGAACTCGCCGTAACCGTCTTCCATGTTGGT CAGTTTCTGAGTGAATTGCTAAATTATCCACAGCTCTCAGTCACTGACTCACTTTAGGTTTC ATCGGCAAAATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGCAAGATCCTCATGGA TGAAGTTAGTCATCGGCTCCCCTGTGAGCTACTTTGTTGTGTTTTGTTTTGCTAACTTGATT TGTTTCAAAAGACCAACCCGGCATTCATTGAAGCCCTAAAGACTAGAGACCGCAAGCGCCGT TTTGACAAGATCTGGACTCTTTGCAAGACCAAAAAGAAATGCGAACGAGACCCTCAGGACGA CCCCAACGCTGACGAGAATCCCGACCAACCTTTGAAGCCCTCGGCCACTCGTGGTGGATGCG GAAATGTTGCACCAGACATCAGAAAGGATGGACTAAAGCTTCTTGGCACTTGGAAATACGAC AAATCCGAAGAGGAAGATGACGAGCGTCGCATTGAGAAGAAGCACATTACGCCTCAACAGGC CTTGCACGCTTTCAACCATATTTCCAGTGAGGATTTGGAGAAGATTGGTCTTGGCAGCGACT ACGCGAAGCCAACGTGGATGATCCTCACCGTGCTCCCTGTTCCACCTCCAGTGCGTCCA AGTATCTCCGTCGACGGAACTGGTCAAGGTATGCGCGGTGAAGATGACTTGACCTACAAGCT CAGCGACATCATTCGTGCAAATGCCAATGTCAAGAAATGCAAAGCAGAGGGCTCACCAGGC ACATTGTTGCAGAATCGAGACGCTTGCAAATCCCGC

# 4.54 *Trypethelium* sp.8

GTCCGCGGGGGATTTGCCACATTGAACTCGCTGTGCCCGTCTTCCATGTTGGTTAGT TTCTGAGTGAATTGCTAAATTATCCACAGCTCTCAGTCACTGACTCACTTAGGTTTCATCG GCAAAATCAAGAAACTTCTTGAAATTTGCTGCCACCATTGTGGGCAAGATCCTCATGGATGAA GTTAGTCATCGGCTCCCCTGTGAACTCATTTGTTGTTGTTTTATTTTGCTAACTTGATTTGTT TCAAAAGACCAACCCAGCATTCATTGAAGCCCTAAAGACTAGAGACCGCAAGCGCCGTTTCG ACAAGATCTGGACTCTTTGCAAGACCAAAAAGAAATGCGAACGAGACCCTCAGGATGACCCC AATGCTGATGAGAATTCCGACCAACCTTTGAAGCCCTCGGCCACTCGTGGTGGATGTGGAAA TGTTGCACCAGACATCAGAAAGGATGGACTAAAGCTTCTTGGCACTTGGAAATACGACAAAT CCGAGGAGGAAGATGACGAGCGTCGTATTGAGAAGAAGCACATTACGCCTCAACAGGCCTTG CACGCTTTCAACCATATTTCCAGTGAGGATCTGGAGAAGATGGTCTTGGCAGCGACCACGC GAAGCCAACGTGGATGATCCTCACCGTGCTCCCTGTTCCACCTCCTCCAGTGCGCACCAGCG GAAGCCAACGTGGAACTGGTCAAGGTATGCGCGGTGAAGATGACTTGACCTACAAGCTCAGC GACATCATTCGTGCAAATGCCAATGTCAAGAAATGCAAAGCAGAGGGCTCACCAGGGCATAT CGTTGCAGAAATTTGAGACCCTTTTGCAATATCACGTTGCGACGAGGGCTCACCAGGGCATAT CGTTGCAGAATTTGAGACCCTTTTGCAATATCACGTTGCGACCTACATGAGAAAATTTTTG TGTGG

## 4.55 Trypethelium sp.9

# 4.56 *Trypethelium* sp.10

# 4.57 Trypethelium sp.11

GAATGTCCCCGGTCATTTTGGACATATTGAACTTTCCGTCCCCGTATTTCATGTTGGTAAGG ATTTGCGGAACCTGGCCTCTTGAAATCTGTGTGTGCTCACTGTGTCTAGGCTTCATCGCCAAG ATCAAGAAGCTCCTGGAGATTTGCTGCCATCATTGTGGAAAGATTCTTGTTGATGAAACTAA TCCAGCCTTTATCGAAGCCGTCAAGACTCGAGACCGCCAAGCGCCGCTTCGATAAGATATGGA CCCTCTGCAAGACCAAGAAGAAATGCGAACGGGACCCTCAGGAAAATCCCGACGCAGATCAT GAGCCTGACCAACCCAAGAAACCTACGTCCACACGAGGCGGCTGCGGAAACGTTGCCCCGGA CATCAGGAAAGAAGGATTGAAGCTCCTCGGCACTTGGAAGTACGACAAGTCCGAAGAGGAAG ACGAGGAGCGTCGGATCGAGAAGAAATACATCACGCCTCAGCAGGCGTTGAATGCCTTCAAT CATATTTCCGACGAGGATCTTCAGAAGATTGGTCTGGGCAGCGATTATGCGAAACCAAAGTG GATGATCCTCACTGTGCTTCCCGTTCCACCTCCCCGTGCGCCCAAGCATATCGGTCGATG GAACTGGTCAAGGGCTTCGTGGCGAAGATGATCTAACTTACAAGCTTAGTGATATCATCCGT GCAAACTCCAACGTCAAGAAATGCAAGTCAGAAGGGTCTCCAGGTCACATCATCGCAGAGTT CGAGACTCTGCTGCCAATACCATGTTGCAACTTACATGGACAAAATTCGCCG

# APPENDIX E

# Mycobiont substances profiles



Figure E1 The TLC plates of lichen-forming fungi substances from CH<sub>2</sub>Cl<sub>2</sub> extraction and developed by solvent system n-hexane: ethyl acetate (7:5). 1. *Astrothelium anascens*, 2. *A. macrocarpum*, 3. *A. neglectum*, 4. *A. neovariolosum*, 5. *A. siamense*, 6. *Bathelium albidoporum*, 7. *Bathelium* sp.1, 8. *Campylothelium nitidum*, 9. *Laurera alboverruca*, 10. *L. cf. columellate*, 11. *L. keralensis*, 12. *L. megasperma*, 13. *L. sikkimensis*, 14. *L. subdiscreta*, 15. *Laurera varia*, 16. *L. vezdae*, 17. *Marcelaria cumingii*, 18. *Polymeridium albocinereum*, 19. *Polymeridium* sp.1, 20. *Polymeridium* sp.2, 21. *Trypethelium cf. aeneum*, 22. *T. andamanicum*, 23. *T. cinereorosellum*, 24. *T. eluteriae*, 25. *T. microstomum*, 26. *T. nitidusculum*, 27. *T. ochroleucum var. subdissocians*, 28, *T. aff. papulosum*, 29. *T. platystomum*, 30. *T. pseudoplatystomum*, 31. *T. subeluteriae*, 32. *T. tropicum*, 33. *T. ubianense*, 34. *Trypethelium* sp.2, 35. *Trypethelium* sp.4, 36. *Trypethelium* sp.5, 37. *Trypethelium* sp.6, 38. *Trypethelium* sp.7, 39. *Trypethelium* sp.8 and 40. *Trypethelium* sp.10.

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



Figure E2 The TLC plates of lichen-forming fungi substances from MeOH extraction and developed by solvent system CH<sub>2</sub>Cl<sub>2</sub>: MeOH (100:4). 1. Astrothelium anascens, 2. A. flavocoronatum, 3. A. macrocarpum, 4. A. neglectum, 5. A. neovariolosum, 6. A. siamense, 7. Bathelium albidoporum, 8. B. madreporiforme, 9. Bathelium sp. 1, 10. Campylothelium nitidum, 11. Laurera alboverruca, 12. L. cf. columellata, 13. L. keralensis, 14. L. megasperma, 15. L. meristospora, 16. L. sikkimensis, 17. L. Marcelaria cumingii, 21. subdiscreta, 18. Laurera varia, 19. L. vezdae, 20. Polymeridium albocinereum, 22. P. quinqueseptatum, 23. Polymeridium sp.1, 24. Polymeridium sp.2, 25. Pseudopyrenula diluta var. degenerans, 26. P. subnudata, 27. Trypethelium cf. aeneum, 28. T. andamanicum, 29. T. cinereorosellum, 30. T. eluteriae, 31. T. microstomum, 32. T. neogabeinum, 33. T. nitidusculum, 34. T. ochroleucum var. subdissocians, 35. T. aff. papulosum, 36. T. platystomum, 37. T. pseudoplatystomum, 38. T. subeluteriae, 39. T. tropicum, 40. T. ubianense, 41. T. virens, 42. Trypethelium sp.1, 43. Trypethelium sp.2, 44. Trypethelium sp.3, 45. Trypethelium sp.4, 46. Trypethelium sp.5, 47. Trypethelium sp.6, 48. Trypethelium sp.7, 49. Trypethelium sp.8, 50. Trypethelium sp.9 and 51. Trypethelium sp.10.

#### APPENDIX F

# Publication

## Publication

- Luangsuphabool, T., Piapukiew, J., and Sangvichien, E. 2013. Preliminary molecular phylogeny of lichen-forming fungi family Trypetheliaceae. <u>Thai Journal</u> <u>of Genetics</u> S1 (Special Issue 1): 303-307.
- Luangsuphabool, T., Piapukiew, J., Parnmen, S., Nelsen, M.P., Lumbsch, H.T., and Sangvichien, E. 2016. Diversity of the *Trypethelium eluteriae* group in Thailand (Ascomycota, Trypetheliales). <u>The Lichenologist</u> 48(1): 53-60.
- Luangsuphabool, T., Lumbsch, H.T., Aptroot, A., Piapukiew, J., and Sangvichien,
  E. 2016. Five new species and one new record of *Astrothelium* (Trypetheliaceae,
  Ascomycota) from Thailand. <u>The Lichenologist</u> 48(4) (In press).

# Conference proceedings

 Luangsuphabool, T., Sanglarpcharonekit, M., Piapukiew, J., and Sangvichien, E. 2012. Effect of culture medium on antioxidant activity from *Trypethelium eluteriae* (TSL 35). Proceeding of International Conference on Microbial Taxonomy, Basic and Applied Microbiology: October 4-6, 2012; Kosa Hotel, Khon Kaen, Thailand, pages 328-324.

# Academic Presentation

 Luangsuphabool, T., Sanglarpcharonekit, M., Piapukiew, J., and Sangvichien, E. 2012. Antioxidant activity of some Thai lichen-forming fungal extracts. Poster presentation at The 6<sup>th</sup> Thai Mycological Conference: March 6, 2012; Rama Gardens Hotel, Bangkok, Thailand. page 42.

- Luangsuphabool, T., Sangvichien, E., Lumbsch, T., and Piapukiew, J. 2012. Cryptic diversity in *Trypethelium eluteriae* in Thailand. Poster presentation at The 7<sup>th</sup> International Association for Lichenology Symposium: January 9-13, 2012; Chaophya Park Hotel, Bangkok, Thailand. page 155.
- Luangsuphabool, T., Piapukiew, J., Sanglarprcharonekit, M., and Sangvichien, E. 2012. Antimicrobial activity of lichen-forming fungi from genus *Trypethelium*. Poster presentation at The 7<sup>th</sup> International Association for Lichenology Symposium: January 9-13, 2012; Chaophya Park Hotel, Bangkok, Thailand. page 143.
- Sanglarpcharonekit, M., Luangsuphabool, T., and Sangvichien, E. 2013. Antisome plant pathogenic fungi activity of the crude extracts of lichen mycobionts. Poster presentation at The 7<sup>th</sup> Botanical Conference of Thailand: April 3-5, 2013; King Ramkhamhaeng the Great Auditorium, Ramkhamhaeng University, Bangkok, Thailand. page 127.
- Luangsuphabool, T., Sangvichien, E., Vongshewarat, K., Lumbsch, T., and Piapukiew, J. 2014. New understanding into the relationships of muriform ascospores in the lichen family Trypetheliaceae (Ascomycota Trypetheliales). Poster presentation at The 13<sup>th</sup> Annual Meeting of the Japanese Society for Lichenology and Akita International Symposium of Lichenology: July 12-13, 2014; Akita Collage plaza, Akita City, Japan. page 21.
- Luangsuphabool, T., Piapukiew, J., Whalley, A., Lumbsch, T., and Sangvichien,
  E. 2014. Molecular phylogeny of lichen-forming fungi genus *Astrothelium* in Thailand. Poster presentation at The 10<sup>th</sup> International Mycological Congress: August 3-8, 2014; Queen Sirikit National Convention Center, Bangkok, Thailand. page 792.
- Sanglarpcharonekit, M., Luangsuphabool, T., and Sangvichien, E. 2015. Preliminary biological activity of crude extracts from aposymbiotically culured lichen mycobionts. Poster presentation at The 9<sup>th</sup> Botanical Conference of Thailand: June 3-5, 2015; Ambassador Hotel, Bangkok, Thailand. page 179.

- Luangsuphabool, T., Piapukiew, J., and Sangvichien, E. 2015. Diversity of the lichen-forming fungi Trypetheliaceae in Thailand. Oral presentation at International Workshop and Symposium on Mycology in Southeast Asia and The 9<sup>th</sup> Thai Mycological Assocciation Conference: July 27-29, 2015; Khon Kaen University, Khon Kaen, Thailand.
- Jarupinthusophon, S., Aree, T., Luangsuphabool, T., Duong, T.H., Sangvichien, E., and Chavasiri, W. 2016. Secondary metabolites from the cultured lichen mycobiont of *Laurera cumingii*. Poster presentation at The 2016 Pure and Applied Chemistry International Conference: February 9-11, 2016; Bangkok International Trade & Exhibition Centre (BITEC), Bangkok, Thailand. page 261.
- Luangsuphabool, T., Sanglarpcharoenkit, M., Piapukiew, J., and Sangvichien, E.
  2016. Bioactivity of axenic cultures of mycobionts from the tropical lichen family Trypetheliaceae in Thailand. Poster presentation at The 8<sup>th</sup> International Association for Lichenology Symposium: August 1-5, 2016; University of Helsinki, Helsinki, Finland. page 185.

# Tentative title

 Luangsuphabool, T., Lumbsch, H.T., Sangvichien, E. and Piapukiew, J. Molecular phylogeny of the tropical lichen genera *Laurera* and *Marcelaria* (Trypetheliales, Ascomycota) in Thailand.

VITA

Mr. Theerapat Luangsuphabool was born on July 24, 1984 in Phitsanulok province, Thailand. He graduated with Bachelor Degree of Science in Biology (2007), Naresuan University and Master Degree of Science in Biotechnology (2010), Chulalongkorn University. After graduation M. Sc., he continued his Ph.D. in Program in Biotechnology, Faculty of Science, Chulalongkorn University (2011). Throughout his B.Sc. to Ph.D. studies, he had received the financial support from scholarship of the Human Resource Development in Science Project (Science Achievement Scholarship of Thailand) and CU. Graduate School Thesis Grant.

