วิวัฒนาการเชิงโมเลกุลเบื้องต้นของราที่ก่อให้เกิดไลเคนวงศ์ทริพิทิเลียซิอิ

Preliminary molecular phylogeny of lichen-forming fungi family Trypetheliaceae

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บทคัดย่อ

ไลเคนวงศ์ทริพิทิเลียซิอิเป็นไลเคนชนิดครัสโตสพบได้ทั่วไปในเขตร้อนทั่วโลก ได้ทำการเก็บรวบรวมตัวอย่างไลเคนจากแหล่งต่างๆ ในประเทศไทย นำมาแยกเป็นเชื้อบริสุทธิ์จากแอสโคสปอร์ สามารถเลี้ยงราในวงศ์นี้ได้เป็นจำนวน 21 ไอโซเลต จากการวิเคราะห์หาความสัมพันธ์ของราก่อให้เกิด ไลเคนด้วยการสร้างต้นไม้วิวัฒนาการเชิงโมเลกุลด้วยตำแหน่ง internal transcribed spacer (ITS) พบว่า ไลเคนวงศ์นี้มีความสัมพันธ์เป็นแบบ polyphyletic จากตำแหน่งที่ปรากฏของไลเคนสกุล *Trypethelium* ช่วยยืนยันว่าไลเคนสกุลนี้มีความสัมพันธ์เป็นแบบ polyphyletic ซึ่งไม่ขึ้นกับลักษณะทางสัณฐานวิทยา

ABSTRACT

Trypetheliaceae is a family of crustose lichens, widely occurring in tropical regions. Lichens thalli were collected from various localities in Thailand and 21 lichen cultures were successfully isolated by the ascospore discharge technique. Phylogenetic analysis of lichen-forming fungi based on internal transcribed spacer (ITS) region represented as a polyphyletic family from *Trypethelium* indicated that placement confirmed a polyphyletic genus which did not depend on morphological characters.

คำสำคัญ: วิวัฒนาการเชิงโมเลกุล, ราที่ก่อให้เกิดไลเคน, วงศ์ทริพิทิเลียซิอิ

Keywords: molecular phylogeny, lichen-forming fungi, Trypetheliaceae

INTRODUCTION

Trypetheliaceae is a family of crustose pyrenocarpous cosmopolitan lichens, especially in tropical and subtropical regions, currently 13 genera and approximately 200 species around the world have been recorded (Kirk *et al*., 2008; Aptroot *et al*., 2008). This lichen group was characterized by a thallus corticated or ecorticate, *Trentepohlia* photobiont, ascomata perithecia, solitary or grouped, separate or fused ostioles, hamathecium hyaline or yellow with thin hyphae branched and anastomosing, bitunicate asci, ascospores 8 ascospore per ascus, colorless, transversely septate to muriform (Aptroot *et al*., 2008). The morphology was unable to delimited species due to similar or conflicting of some characters when compared with traditional classifications, and it is also difficult to identify to genus or species level by traditional taxonomic only. Currently molecular taxonomy influences lichen identification, previous reports based on molecular data confirmed that the Trypetheliaceae separated from Pyrenulaceae (De Prado *et al*., 2006) and reported that *Astrothelium* and *Trypethelium* were non-monophyly genera (Nelsen *et al*., 2009). This lichen family has been less few phylogenetic studies on ITS data, only two ITS sequences were reported in GenBank (www.ncbi.nlm.nih.gov). The aim of this study was to determine the phylogenetic relationships within the family Trypetheliaceae and add to the new databases.

MATERIALS AND METHODS

1. Taxonomic sampling, morphological characters and lichen-forming fungi isolation

Thalli of the lichens family Trypetheliaceae focused on the genera *Astrothelium, Laurera, Polymeridium, Pseudophyrenula* and *Trypethelium* collected from various locations in Thailand. Identifications based on morphological characters were investigated using microscopic techniques. Twenty-one lichen-forming fungal cultures were successfully obtained via ascospore germination on Malt-Yeast-Extract medium as described by Sangvichien *et al*. (2011). The cultures were grown at room temperature (25-30°C) for 9 weeks and then harvested for DNA extraction.

2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from lichen-forming fungal cultures using the CTAB precipitation and chloroform extraction protocol described by Cubero and Crespo (2002). The ITS region was amplified using the primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White *et al*., 1990). Amplifications were performed in 50 µl containing a reaction mixture of 5 µl 10x *Pfu* Buffer with $MgSO_4$, 2 mM of dNTP mix, 20 µM of each primer, 1.25 U of *Pfu* DNA Polymerase (Thermo) and 5 µl of DNA solution. The PCR program as following: initial denaturation for 1 min at 94 ºC and 38 cycles of 94 ºC for 1 min, 51 ºC for 1 min, 72 ºC for 1 min, and a final extension at 72 ºC for 5 min. The products were cleaned with QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. The purified PCR products were sent to The Field Museum (Chicago, U.S.A) for DNA sequencing.

3. Phylogenetic analysis

Twenty-one ITS sequences were aligned using Clustal W (Thompson *et al*., 1994). Two sequences of *Pyrenula macrospora* (JQ927455) and *Pyrenula nitida* (JQ927458) as outgroups were added from GenBank. A phylogenetic tree was constructed in maximum likelihood (ML) analysis by RAxML 7.2.8 (Stamatakis, 2006) with the GTRGAMMA model and bootstrap values were calculated by 1,000 replications. RAxML analyses were performed on CIPRES Science Gateway Web server (Miller *et al*., 2010).

RESULTS AND DISCUSSION

The phylogenetic tree of twenty-one lichen-forming fungi based on ITS sequences divided into 2 clades corresponding to ascospore types, including clade I (muriform ascospore) and clade II (transversely septate ascospore) (Figure 1). Clade I contained the genus *Laurera* and clade II formed complexes of the genus. Clade II was separated into 4 lineages (A-D), in which the genus *Trypethelium* was distributed into all lineages while other genera *Astrothelium, Polymeridium,* and *Pseudophyrenula* separated into lineages B, C and D respectively. Morphological characters of lichen thalli and ascospore showed that these were unrelated among the genus within lineages B, C and D (Table 1). The ML tree demonstrated Trypetheliaceae as a polyphyletic family with the genus *Trypethelium* separated into many lineages with high bootstraps value support. *Trypethelium* did not form a monophyletic group as previously reported (Nelsen *et al*., 2009). Our study confirms this genus as a polyphyletic group. Phylogenetic relationships among the genus did not depend on morphological characteristics alone but molecular data determined the relationships for inter and intra genera level. The results for the genera *Opegrapha* and *Arthonia* cannot define the genus by morphological characters alone, on the contrary molecular data assists to identify differences between both genera (Ertz *et al*., 2009).

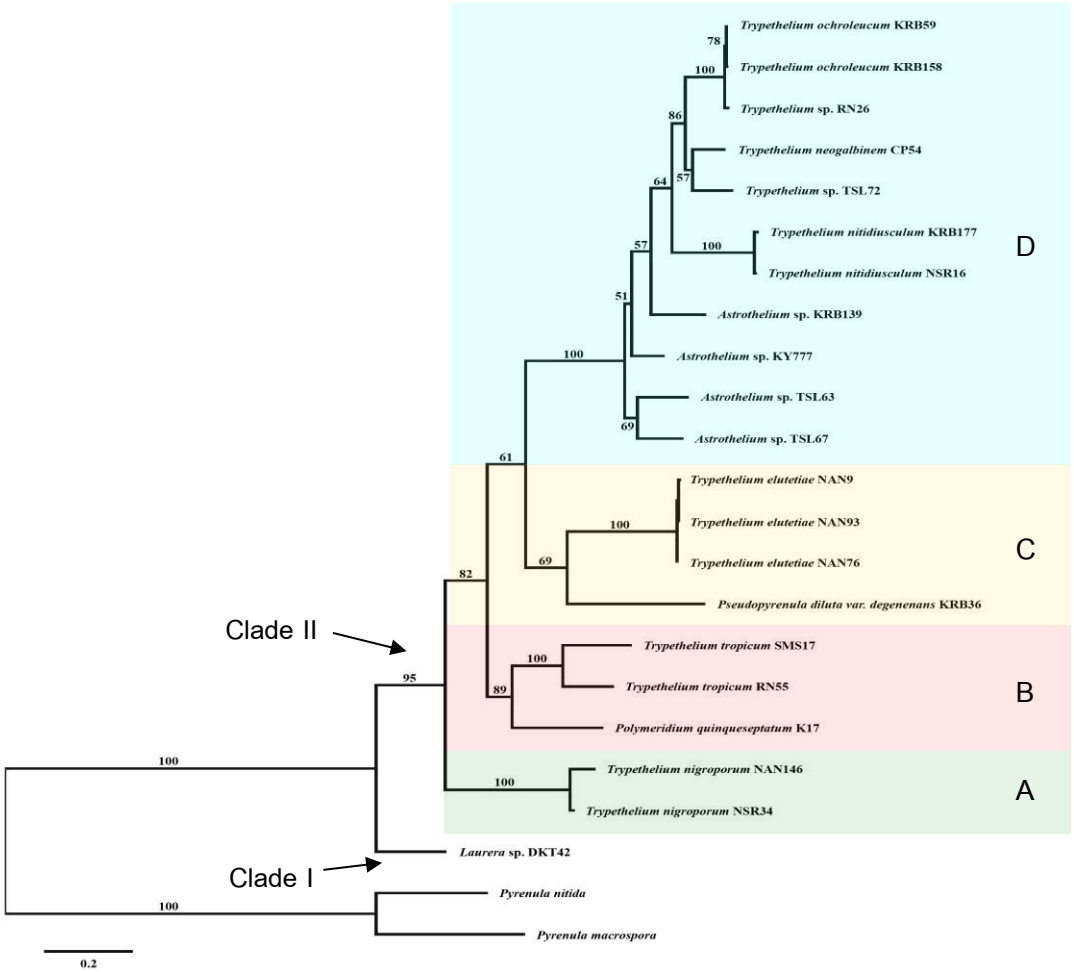

Figure 1 The Maximum likelihood tree of family Trypetheliaceae based on ITS sequences.

Table 1 Morphological character of lichen thallus and ascospore in family Trypetheliaceae.

Genus	Thallus develop	Perithecia ostiole	Ascospore type	Septate of ascospore	Clade / Lineage
Astrothelium	corticate	share	transeptate	3-5	I / D
Laurera	corticate	separate	muriform	-	II
Polymeridium	ecorticate	separate	transeptate	4-8	I / B
Pseudophyrenula	ecorticate	separate	transeptate	3	I / C
Trypethelium	corticate	separate	transeptate	3-13	I / A-D

CONCLUSION

The phylogeny of lichenized fungi family Trypetheliaceae based on ITS sequences was appeared as a polyphyletic. *Trypethelium* was confirmed as a polyphyletic genus by molecular data. Molecular data helps to determine the phylogenetic relationships of lichen-forming fungi. The result also indicated that this family needs further studies on other molecular markers such as RNA polymerase II subunit (RPB1) or elongation factor EF-1α (TEF1α) regions and focus on each genus to understand and clearify the phylogenetic relationships.

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