Generic classification based on the symplesiomorphy of genotype and phenotype of the family Umbilicariaceae (Ascomycota)

ZHANG Ying¹,³ WEI Jiang-Chun¹,²*

¹The State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
²University of Chinese Academy of Sciences, Beijing 100101, China
³College of Life Sciences, Zaozhuang University, Zaozhuang, Shandong 277100, China

Abstract: This paper includes three aspects. First, a new concept of classification has been proposed, i.e. systematic biology of lichenized fungi with three systems of storage and retrieval is a bridge between biodiversity in the nature and exploration of lichen resources. Second, the symplesiomorphic analyses based on genotype together with phenotype data have solved the generic classification in the Umbilicariaceae, which has not been solved by the analyses of molecular systematics. Third, marginal species have been found in genus differentiation of the Umbilicariaceae by symplesiomorphic analyses. The concept of the marginal species is discussed. The results show, the family Umbilicariaceae and their genera are monophyletic group respectively.

Key words: Lasallia, Umbilicaria, full-length LSU rDNA, marginal species, monophyletic group

以基因型表型共同祖征为基础的石耳科属级分类研究

张颖¹,³ 魏江春¹,²*

¹中国科学院微生物研究所真菌学国家重点实验室 北京 100101
²中国科学院大学 北京 100101
³枣庄学院生命科学学院 山东 枣庄 277100

摘 要：本文首先提出了地衣型真菌系统生物学及其 3 大存取系统是自然界生物多样性与地衣资源研发之间桥梁的概念。其次，以基因型与表型为基础的共同祖征综合分析解决了分子系统学未能解决的石耳科属级分类问题。第三，通过基因型与表型共同祖征的综合分析，发现了边缘种在石耳科属级分化中的存在。对于边缘种的概念进行了具体分析和论述。研究结果表明，石耳科及其各属均为单系类群。

关键词：疱脐衣属，石耳属，全长 rDNA 大亚基，边缘种，单系类群

*Corresponding author. E-mail: weijc2004@126.com
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INTRODUCTION

All the species in the family Umbilicariaceae are umbilicate thalli with lecideine or sublecideine type of apothecia containing ascus apex structure of *Umbilicaria* type, and most of them are saxicolous and only few corticolous. A famous and delicious edible lichen *Umbilicaria esculenta* (Miyoshi) Minks endemic to NE Asia, and the widespread species *Lasallia papulosa* Llano in the family Umbilicariaceae are rich in acetylated β(1→6) glucan, which can improve the activity of anti cancer immunity (Nishikawa et al. 1969). A sulfate (GE-3-S) prepared by chlorosulfonic acid treatment of GE-3, a partially acetylated β(1→6) glucan of the *Umbilicaria esculenta*, inhibited the cytopathic effect of human immunodeficiency virus (HIV) and suppressed the HIV-antigen expression in Molt-4 (clone 8) cells (Hirabayashi et al. 1989).

The systematic biology of lichenized fungi with three systems for storage and retrieval including that of bioinformation, fungarium and culture collections of mycobionts and photobionts plays the role of bridge between lichen diversity in the nature and R & D of lichen resources.

The generic classification of the family Umbilicariaceae based on the phytotypic characteristics has undergone great changes along with the progress of science and technology and the difference of people’s thinking mode (Table 1). The

<table>
<thead>
<tr>
<th>Generic treatment</th>
<th>Family</th>
<th>Authors</th>
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<tr>
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<td>Trigeneric family</td>
<td>Elenk &amp; Savicz 1911</td>
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<td><em>Lasallia</em> Mérat.</td>
<td>Quinquegeneric family</td>
<td>Llano 1950</td>
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<td><em>Agyrophora</em> Nyl.</td>
<td>Monotypic family</td>
<td>Frey 1933</td>
</tr>
<tr>
<td><em>Omphalodiscus</em> Schol.</td>
<td>Qudrigenric family</td>
<td>Scholander 1934</td>
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<tr>
<td><em>Actinogyra</em> Schol.</td>
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<td></td>
</tr>
<tr>
<td><em>Lasallia</em> Mérat.</td>
<td><em>Lasallia-Umbilicaria</em> family</td>
<td>Wei 1966*</td>
</tr>
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<td><em>Agrophora</em> Nyl.</td>
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Note: *The *Lasallia-Umbilicaria* system of the family Umbilicariaceae was firstly discussed in details by J.C. Wei in 1966. Poelt used *Lasallia-Umbilicaria* only in his key to European species of the family in 1962.

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Lasallia-Umbilicaria system in the Umbilicariaceae including Lasallia with pustulate thalli below lacking rhizine and containing muriform mono- or rarely bisporous asci, and Umbilicaria with non-pustulate thalli below filling or lacking rhizines and containing octosporous asci have been being recognized and accepted since 1960s.

In recent years the Lasallia-Umbilicaria system has to face the challenge of molecular taxonomy (Davydov et al. 2010; Miadlikowska et al. 2014). A new combination Lasallia caroliniana (Tuck.) Davydov, Peršoh & Rambold was proposed as a result of the phylogenetic analysis of the ITS/5.8S nrDNA, in which U. caroliniana and L. pensylvanica grouped together as a sister group of Lasallia, and then the delimitations of both the genera Lasallia and Umbilicaria were modified by the authors (Davydov et al. 2010). In addition, U. esculenta, U. mammulata, and U. muelenbergii, with non-pustulate thalli below filling rhizines or solid papillate lamellae becoming trabeculate and containing unicellular octosporous asci, grouped together as a sister group of Lasallia as well, and formed a polyphyletic group. More than 20 species of Umbilicaria s. str. in the phylogenetic tree formed a separate but paraphyletic group (Miadlikowska et al. 2014). So, the delimitation of both Lasallia and Umbilicaria proposed by Davydov et al. in 2010 was subverted by the result of a multigene phylogenetic synthesis for the class Lecanoromycetes (Miadlikowska et al. 2014).

Thus, the delimitations of genera in the Umbilicariaceae resulted from phynotypic classification (Table 1), mistaking non-pustulate U. caroliniana for pustulate with phylogenetic analyses based on ITS/5.8S nrDNA (Davydov et al. 2010), and a multigene phylogenetic synthesis for the class Lecanoromycetes (Miadlikowska et al. 2014) were not consistent with each other.

Such a method as phynotypic classification is obviously one-sided; the analyses based on non-coding sequences with an inaccurate morphological analysis are also open to question; and the similarity analysis of multigene fragments is also difficult to reflect the genetic relationship between taxa.

According to the Darwin’s theory of common descent, the organisms in the terrestrial biosphere are all descended from common parents (Darwin 1872). Therefore, all living organisms have some of the characteristics of their common ancestor. So, it may be feasible to classify the genera by both their genotypic and phynotypic features of the common ancestor that they retain, i.e. symplesiomorphy. The result of symplesiomorphic analysis may reflect the genetic relationship among taxa from their common ancestor.

Ribosome is the workshop of protein synthesis. The protein is the product of gene expression. The genotype regulates the phenotype, and the phenotype is the end product of the genotype (Heywood 1976). So, some symplesiomorphic sequences can be found from the full-length LSU rDNA.

Some characteristic sequences in the conserved regions of LSU rDNA were used for the molecular criterias of Eukarya, Archaea, and Bacteria (Qu 1986; Qu et al. 1988; Qu & Chen 1999). In addition, four symplesiomorphic sequences for the kingdom Fungi, one for Ascomycota, and another one for Basidiomycota were determined from the full-length LSU rDNA (Fu & Wei 2008).

The researches on the generic classification based on symplesiomorphy of genotype and phynotype in the family Umbilicariaceae would be carried out in this article.
1 MATERIALS AND METHODS

1.1 Materials

The lichen materials used for this study were from fungarium lichen section in CAS (Herbarium Mycologicum Academiae Sinicae-Lichenes HMAS-L). The selected samples are listed in Table 2.

1.2 DNA extraction

Before the DNA extraction, the lichen thalli were cleaned carefully in order to avoid DNA pollution. Total DNA was extracted from individual thallus using the modified CTAB method (Roger & Bendich 1988; Cubero et al. 1999).

1.3 PCR amplification and sequencing

In order to obtain the full length sequences of LSU rDNA, PCR reactions were performed with five primers pairs as shown in Table 3 (Fu & Wei 2008). PCR reaction was carried out as follows: pre-denatured at 95°C for 5min, followed by 35 cycles of amplification (95°C for 50s, 53°C for 50s, 72°C for 1min), and then followed by extension for 8min at 72°C. All the PCR products were verified by electrophoresis on 1% agarose gels and then sequenced.

1.4 Sequence analysis

The full length sequences of LSU rDNA from 34 species of Umbilicariaceae were obtained in this study (Table 2), and those of other species were from GenBank (Table 4). Major insertions, when present in the sequence, were excluded prior to the analyses. The phylogenetic analysis was executed with software MEGA (Tamura et al. 2011). Kimura 2-parameter model was taken, and gaps were retained initially while being excluded in the pairwise distance estimation. The neighbor joining (NJ) method was used in constructing the phylogenetic tree and the reliability of the inferred tree was tested by 1 000 bootstrap replications.

Table 2 Information for samples of family Umbilicariaceae

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Specimen number</th>
<th>GenBank accession number</th>
<th>Species</th>
<th>Location</th>
<th>Specimen number</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. antarctica</em></td>
<td>Antarctic</td>
<td>NJ053</td>
<td>JQ739980</td>
<td><em>U. laboperipheric</em></td>
<td>Jilin</td>
<td>JLO9103</td>
<td>JQ739995</td>
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<td><em>U. aprina</em></td>
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<td>S697</td>
<td>JQ739981</td>
<td><em>U. lymeii</em></td>
<td>Jilin</td>
<td>TBF66</td>
<td>JQ739996</td>
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<td><em>U. arctica</em></td>
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<td>2190</td>
<td>JQ739982</td>
<td><em>U. muehlenbergii</em></td>
<td>Jilin</td>
<td>112322</td>
<td>JQ739997</td>
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<td><em>U. caroliniana</em></td>
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<td>KX925283</td>
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<td>Inner</td>
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<td>JQ739983</td>
<td><em>U. squamosa</em></td>
<td>Tibet</td>
<td>075750</td>
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<td><em>U. cylindrica</em></td>
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<td>099480</td>
<td>JQ739984</td>
<td><em>U. subglabra</em></td>
<td>Jilin</td>
<td>099473</td>
<td>JQ739999</td>
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<td>Jilin</td>
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<td><em>U. flocculosa</em></td>
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<td><em>U. hirsuta</em></td>
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<td><em>U. indica</em></td>
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<td>JQ739992</td>
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<td>Shaanxi</td>
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<td><em>U. kisovana</em></td>
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<td>JQ739993</td>
<td><em>L. sinorientalis</em></td>
<td>Fujian</td>
<td>0907686</td>
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<td><em>U. krascheninikovii</em></td>
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<td>TBF55</td>
<td>JQ739994</td>
<td><em>L. rossica</em></td>
<td>Heilongjiang</td>
<td>HY11189</td>
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Table 3 Primers for the PCR reactions

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<th>Abbreviation</th>
<th>Sequence (5′-3′)</th>
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<tr>
<td>E9</td>
<td>TTGTACACCCGCGGCT</td>
<td>1 640–1 656 (ssu)</td>
</tr>
<tr>
<td>SL4R</td>
<td>TCGATCATCTCTTTGTC</td>
<td>356–375</td>
</tr>
<tr>
<td>SL2</td>
<td>CGGCCAGTGAGCGGCA</td>
<td>106–122</td>
</tr>
<tr>
<td>CL5R</td>
<td>TTTCAAAAATGGCCACT</td>
<td>1 136–1 155</td>
</tr>
<tr>
<td>SL7</td>
<td>GATCGATTGCAGTCGAA</td>
<td>886–905</td>
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<tr>
<td>CL7R</td>
<td>GATCTATTGGCGACTTC</td>
<td>1 916–1 934</td>
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<tr>
<td>SL14</td>
<td>TAGCAGGACATTTTCGAT</td>
<td>1 736–1 754</td>
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<tr>
<td>P22R</td>
<td>CAATGCTAACTAGAGTCAAGC</td>
<td>2 426–2 447</td>
</tr>
<tr>
<td>CL9</td>
<td>CTGACGAGGCATTTGGCTA</td>
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<tr>
<td>SL18R</td>
<td>CTTAGAGGCTTCAGCCAT</td>
<td>3 120–3 139</td>
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</table>

Table 4 The full-length sequences of LSU rDNA obtained from GenBank

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<td>Phaeosphaeria sp.</td>
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<td>Saccharomyces cerevisiae</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>CPC58255</td>
<td>Basidiomycota</td>
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<td>Minimedusa polyspora</td>
<td>DQ915476</td>
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<tr>
<td>Spizellomycte sp.</td>
<td>DQ273821</td>
<td>Chytridiomycota</td>
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<td>Orpinomyces sp.</td>
<td>AJ864475</td>
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<td>Rhizopus stolonifer</td>
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<td>Rattus norvegicus</td>
<td>V01270</td>
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<tr>
<td>Homo sapiens</td>
<td>NR 003287</td>
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</table>

1.5 Diagram of LSU rDNA structure

Previous studies have shown that the LSU rDNA can be divided into 12 structure regions (each region including the conserved and divergent regions) in terms of the different evolutionary rates (Qu 1986; Qu et al. 1988; Qu & Chen 1999), we also built a diagram of U. esculenta LSU rDNA structure (Fig. 1).

2 RESULTS AND DISCUSSION

The phylogenetic tree based on the full-length sequences of LSU rDNA indicated that all species examined in the family Umbilicariaceae form a monophyletic group (Fig. 2, right above). The family
Fig. 1 Diagram of *Umbilicaria esculenta* LSU rDNA structure. There are 12 structure regions, which include the conserved region, and the divergent region. Boxes with continuous lines circumscribe the presumed 12th region based on previous data.

Fig. 2 The NL tree inferred from full-length LSU rDNA data. The reliability of the inferred tree was tested by 1 000 bootstrap replications; the numbers in each node represent bootstrap support values. Only bootstrap values greater than 50% are shown. The morphological, anatomical (left above) and symplesiomorphic sequences and their sites from the LSU rDNA (left below) for the family *Umbilicariaceae* are given.
is supported by three symplesiomorphic sequences Fa, Fb, and Fc in the regions 1, 2 & 6 of the LSU rDNA (Fig. 2, left below), with umbilicate thalli, lecideine to sublecideine type of apothecia, and ascus apex structure of *Umbilicaria* (Fig. 2, left above).

Furthermore, there are two different generic groups formed within the Umbilicariaceae. The first group is *Umbilicaria* (Fig. 2, right above), and the second one is *Lasallia* (Fig. 2, right below). In addition, between the *Umbilicaria* and *Lasallia* two species *L. pensylvanica* (Hcffm.) Llano and *U. caroliniana* Tuck. grouped together as a sister group of *Lasallia* in the Umbilicariaceae (Fig. 2, right).

The genus *Umbilicaria* is supported by two symplesiomorphic sequences Ua and Ub in the regions 1 & 2 of the LSU rDNA (Fig. 3, right), with non-pustulate thalli and octosporous asci (Fig. 3, left). The genus *Lasallia* is proved by the symplesiomorphic sequences La and Lb in the regions 2 & 7 of the LSU rDNA (Fig. 4, right), with pustulate thalli and multicellular-muriform monosporous asci (Fig. 4, left).

According to the way of ascospore formation after completion of meiosis I and meiosis II, the original diploid cell has been transformed into four haploid cells, and one of them successfully forms an ascospore with the surrounding protoplast in the genus *Lasallia*, only occasionally forms two ascospores. And in the genus *Umbilicaria* the four haploid cells implement mitosis. After completion of the mitosis the four haploid cells have been transformed into 8 haploid cells and then formed 8 ascospores with the surrounding protoplast.

**Fig. 3** The non-pustulate thallus below rich in rhizines with octosporous ascus (left and middle), the symplesiomorphic sequences Ua, Ub and their positions (right) of *Umbilicaria*.

**Fig. 4** The pustulate thallus with monosporous ascus (left and middle), the symplesiomorphic sequences La, Lb and their positions (right) of *Lasallia*. 
Umbilicaria caroliniana is supported by the symplesiomorphic sequences Ua & Ub and non-pustulate thalli below rich in rhizines, containing muriform octosporous asci, belonging to Umbilicaria. In addition, it also has a characteristic sequence of Lasallia Lb in region 7 of the LSU rDNA and multicellular-muriform characteristic spores of Lasallia, but octosporous ascis characteristic for Umbilicaria (Figs. 5, 7).

Lasallia pensylvanica is supported by the symplesiomorphic sequences La & Lb and pustulate thalli below lacking rhizines containing multicellular-muriform monosporous asci, belonging to the genus Lasallia. In addition, it also has a characteristic sequence of Umbilicaria Ua in the region 1 of the LSU rDNA (Figs. 6, 7) and occasionally with thalli below few rhizines.

Thus, U. caroliniana can be seen as a marginal species within Umbilicaria, and L. pensylvanica as a marginal species within Lasallia (Fig. 8).

Both the marginal species U. caroliniana and L. pensylvanica sometimes grouped together as two sister taxa within the group of Lasallia in phylogenetic trees (Fig. 2, and Davydov et al. 2010: Fig. 1), the reason for that might be due to the software, which cannot solve the complex cases in biology.

It seems that the species with non-pustulate thalli containing octosporous ascis of Umbilicaria may be derived from the differentiation of those species with pustulate thalli containing multicellular-muriform monosporous asci of Lasallia, and among them those species containing oligocellular, especially multicellular muriform octosporous asci, such as U. caroliniana may be lagged behind other species in the long process of evolution in the Umbilicariaceae.

Fig. 5 The non-pustulate thallus below rich in rhizines with octosporous ascus, the symplesiomorphic sequences Ua & Ub, and Lb of Umbilicaria caroliniana. Thallus, upper surface (left), and lower surface (middle). Octosporous ascus with muriform spores (right).

Fig. 6 The pustulate thallus with monosporous ascus, the symplesiomorphic sequences La & Lb, and Ua of Lasallia pensylvanica. Thallus, upper surface (left), and lower surface (middle). Monosporous ascus with muriform spores (right).
The genus *Umbilicaria*  
\[ \text{Ub+Ua} \]  
*Umbilicaria caroliniana*  
\[ \text{Ub+Ua+Lb} \]  
*U. caroliniana*  
\[ \text{Ub+Ua+Lb} \]  
\[ \text{Ub+La} \]  
*Lasallia pensylvanica*  
\[ \text{Ub+La} \]  
*Lasallia pensylvanica*  
\[ \text{Ua+La+Lb} \]  
\[ \text{Ua+La+Lb} \]  
\[ \text{Ua+La} \]  
\[ \text{L. pensylvanica} \]  
\[ \text{L. pensylvanica} \]  

Fig. 7 *Umbilicaria caroliniana* is characterized by the non-pustulate thallus below rich in rhizines with octosporous ascus and the symplesiomorphic sequences Ub & Ua of *Umbilicaria* with Lb of *Lasallia* in passing (left below). *Lasallia pensylvanica* is characterized by pustulate thallus with monosporous ascus and the symplesiomorphic sequences Lb & La of *Lasallia* with Ua of *Umbilicaria* in passing (right below).

**3 CONCLUSION**

The symplesiomorphic analyses of genotype and phynotype showed that three symplesiomorphic sequences of LSU rDNA Fa, Fb, Fc and umbilicate thalli with lecideine or sublecideine type of apothecia containing ascus apex structure of *Umbilicaria* type for the family Umbilicariaceae form a monophyletic group. The other two symplesiomorphic sequences of LSU rDNA La, Lb and pustulate thalli bellow lacking rhizine with multicellular muriform monosporous asci for the genus *Lasallia* compose a monophyletic group in the Umbilicariaceae. The symplesiomorphic sequences of LSU rDNA Ua, Ub and non-pustulate thalll bellow filling rhizines or lacking rhizine with octosporous asci for the genus *Umbilicaria* make a monophyletic group as well in the same family.

In addition, the species *U. caroliniana* supported by the symplesiomorphy of genotype and phynotype for the genus *Umbilicaria*, and also has another sequence of LSU rDNA Lb, can be considered as a marginal species within *Umbilicaria*. The species *L. pensylvanica* supported by the symplesiomorphy of genotype and phynotype for the genus *Lasallia*, and also has another sequence of LSU rDNA Ua, can be seen as a marginal species within *Lasallia* as well.

Thus, the generic classification based on symplesiomorphy of genotype and phynotype in the family Umbilicariaceae is just the evidence of the common descent theory.

**4 DESCRIPTION**

**Umbilicariaceae** F. F. Chevlier


Type: *Umbilicaria* Hoffmann.

Diagnosis: The family *Umbilicariaceae* is characterized by having umbilicate thalli with lecideine to sublecideine (= sublecanoriene) apothecia containing ascus apex structure of *Umbilicaria* type similar to that of Sarrameana type (Fig. 2, left), and three symplesiomorphic sequences Fa, Fb, and Fc, and their positions in the LSU rDNA (Fig. 2, left below).
Description:
Thallus foliose, umbilicate, firmly attached to substrate by a central of off-centered umbilicus of the lower surface (Fig. 2, above left), heteromeros with upper and lower cortices mainly consisting of palisade- or scleroplechtenchymatous tissues, monophyllous or polyphyllous; upper surface plane to undulating, smooth to areolated non-pustulate or pustulate with convex pustules, grey, grey-green, grey-brown to dark brown or black-brown; lower surface brownish or grey-brown to dark brown or sooty, smooth to areolated or strongly verrucose, with or without rhizines (Fig. 2).

Apothecia lecideine to sublecideine (=sublecanorine) (Fig. 2, above middle), adnate to stipitate, leiodiscs to omphalodiscs, or gyrose discs to actinodiscs (without a common proper margin). Asci monosporous, rarely disporous, or octosporous, and ascus apex structure of Umbilicaria type (Fig. 2, left middle). Ascospores simple and hyaline to muriform and brown.

The sympleiomorphic sequences are Fa, Fb, and Fc, and their positions in the LSU rDNA (Fig. 2, left below).

Algae: Trebouxia spp.
Substrate: usually rocks, occasionally bark for few species.

Umbilicaria Hoffmann
Type: Umbilicaria hyperborean (Ach.) Hoffm. (1801).

Diagnosis: The genus Umbilicaria is characterized by having non-pustulate thalli, containing octosporous asci (Fig. 3, left), and sympleiomorphic sequences Ua and Ub and their sites for the genus from the LSU rDNA (Fig. 3, right).

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black and granulate to verrucose, with sparse and black rhizines.

Apothecia with gyrose discs.


Diagnosis: Umbilicaria caroliniana is characterized by non-pustulate thalli below filling rhizines, containing octosporous asci and symplesiomorphic sequences Ua and Ub with Lb, and their sites for the species from the LSU rDNA (Fig. 5), as a marginal species within Umbilicaria (Figs. 7, 8).

Description:

Thallus non-pustulate, monophyllous or polyphyllous, thin, membranous, fragile, with overlapping lobes crowded as cushion; upper surface undulating, brownish to brown in all tints of olive, dull to almost shining; lower surface black, granulose to verrucose, with sparse, black, richly branched at the tip of rhizines.

Thallus 125–174 \( \mu \text{m} \) thick; upper cortex consisting of a brown outer layer of 3–8 \( \mu \text{m} \) thick, and a colourless inner layer of 5–16 \( \mu \text{m} \) thick; algal layer 21–54 \( \mu \text{m} \) thick; medulla 34–99 \( \mu \text{m} \) thick; lower cortex consisting of a colourless inner layer of 11–30.5 \( \mu \text{m} \) thick, and a brown outer layer of 5–16 \( \mu \text{m} \) thick.

Apothecia 2–3 mm diam., ephymenium brown, 14–42 \( \mu \text{m} \) thick; hymenium 106–162 \( \mu \text{m} \) thick; subhymenium yellowish, 12–55 \( \mu \text{m} \) thick; asci octosporous, 95–135 \( \times \) 25–39 \( \mu \text{m} \); ascospores brown, muriform, ellipsoid, 27–39 \( \times \) 14–20 \( \mu \text{m} \); paraphyses richly branched, septate, 1–2 \( \mu \text{m} \) thick, expanded at the tip.

Pycnidia 92–185 \( \times \) 92–213 \( \mu \text{m} \); pycnoconidia bacilliform, 3–5 \( \times \) 1 \( \mu \text{m} \).

Chemistry: lecanoric, gyrophoric, and umbilicaric (trace) acids.

The Sympliomorphic sequences are Ua, Ub with Lb, and their sites for the species from the LSU rDNA (Fig. 5).

Specimens examined:

China: Neimongol, Da Hinggan Ling, Ergun Zuoqi (Genhe), Mt. Oklidui, on rocks, alt. 1 530 m, July 26, 1983, coll.Zhoa CF, No. 2963 (HMAS-L, IFP!).


Other specimens examined:


Literature records for ASIA:


Comments: This North Americano-north Asian species is characterized mainly by its cushion-shaped thallus and brownish to brown upper surface and black, granulose to verrucose with rich rhizines lower surface containing octosporous asci with muriform and brown ascospores.

North Americano-asian.

**Lasallia** Merat


**Type:** *Lasallia pustulata* (L.) Merat.

Diagnosis: The genus *Lasallia* is characterized by having thalli of pustulate umbilicate lobes containing mono or rarely disporous asci (Fig. 4, left), and symplesiomorphic sequences La & Lb and their sites for the genus from the LSU rDNA (Fig. 4, right).

Description:

Thallus foliose, umbilicate, pustulate, upper surface pustulate with convex pustules (Fig. 4, left), smooth to areolate, sometimes isidiate, squamose or sorediate, brown to dark brown or grey-brown, sometimes in all tints of red brown; lower surface lacunose, with lacunae corresponding to pustules of upper surface, smooth to areolate or papillate, verrucose or strongly verrucose, grey-brown, brown or dark brown to black brown, usually lacking rhizines, occasionally with poor and short rhizines; heteromerous with upper and lower cortices, monophyllous strongly attached to substrate by a central of off-centered umbilicus of the lower surface.

Apothecia lecideine to sublecideine (=sublecanorine), leiodiscs or with gyrose discs; asci monosporous or rarely disporous; ascospores large and multicellular muriform, 36–113×18–62µm, brownish to dark brown.

The symplesiomorphic sequences are La and Lb, and their sites for the genus from the LSU rDNA (Fig. 4, right).

Algae: *Trebuoxia* spp.

Pycnidia containing pycnoconidia are usually present mainly in the peripheral zone of upper surface of thallus.

**Lasallia pensylvanica** (Hcflm.) Llano

Monograph of the Umbilicariaceae 42 (1950).

**Type:** Amer. Septent. Muehlenberg, H-Ach. No. 571 (H, neotype!).

Type observation: Thallus smooth, with squamules on the upper surface, black below, coarsely verrucose. Apothecia with smooth discs.

Diagnosis: Lasallia pensylvanica is characterized by pustulate thalli below lacking rhizine or occasionally with few rhizines and containing monosporous asci with symplesiomorphic sequences La, Lb & Ua, and their sites for the species from the LSU rDNA (Fig. 6), as a marginal species within Lasallia (Figs. 7, 8).

Description:
Thallus umbilicate, pustulate, monophyllous, orbicular, undulating, rigid, to 6cm. diam.; upper surface smooth to more or less areolately papillate, dull to shining, warm buff to wood brown, covered with a thin layer of whitish pruina, with a few scattered black-brown curved squamules, elevated, over the umbo, occasionally with a few small superficial thalli, marginally laciniate with many dark brown to black-brown toothed lacinia; lower surface sooty-black, with concave lacunae corresponding to the pustules of upper surface, coarsely verrucose, without rhizines or occasionally with few cylindrical rhizines up to 4mm long, surface of the rhizines clearly papillate (Mongolia, Jun. 30, 1976, coll. Biazrov, No. 3178 with rare rhizines, Moscow!; Wei J.C & Jiang Y.M., Fig. 22 Ac, 1993).

Thallus 354–385μm thick; outer cortex yellowish brown, 23–51μm thick, inner cortex colourless, 4.5–18.5μm thick; algal layer 42–55μm thick; medulla 236–260μm thick, the part next to the algae brownish, below colourless; outer lower cortex brown, 9–18.5μm thick, inner lower cortex scleroleptenchymatous, colourless, 23–46μm thick.

Apothecia leiodisc, occasionally with a single fissure or button in the disc; ephymenium brown, 5.5–16μm thick; hymenium colourless, 54–106μm thick; subhymenium brownish, 18–63μm thick; paraphyses branched, septate, colourless, sometimes brownish, 1.5μm thick, inflated at the tip, ellipsoid, 1.5–5.5μm thick. Asci monosporous, 61–81×23–30μm; ascospores large, multicellular, muriform, brown, 48.5–52×25–30.5μm.

Pycnidia 148–204×143–172μm; pycno- conidia bacilliform, colourless, 3–4×<1μm.

Chemistry: lecanoric and gyrophoric acids.
The symplesiomorphic sequences are La and Lb with Ua, and their sites for the species from the LSU rDNA (Fig. 6).

Specimens examined:

Mongolia: Ara-Khangai, on rocks, Jun. 15, 1971, coll. Biazrov, No. 5768 (MOSCOW!); alt. 2 100m, Aug. 28, 1971, coll. Biazrov, No. 4228 (MOSCOW!); Sept. 7, 1979, coll. Biazrov No. 6186 (MOSCOW!). The following specimens examined were identified as Lasallia rossica Dombr. in the herbarium: Ara-Khangai; alt. 2 130m, on rocks, Aug. 16, 1972, coll. Biazrov, No. 6690 (MOSCOW!); Jun. 30, 1976, coll. Biazrov, No. 3178 (with rare rhizines, MOSCOW!); alt. 2 000m, July 1, 1980, coll. Biazrov, Nos. 4350, 4356 (MOSCOW!); Bulgan, Jun. 29, 1972, coll. Biazrov,
Nos. 5110, 5103 (MOSCOW!), July 11, 1976, coll. Blazrov, No. 6579 (MOSCOW!).

Kazakhstan: Turkestan, Kokchetav, July 13, 1896, coll. A. Tordiagei (LE!).


Other specimens examined:


Literature records for ASIA:


Comments: It is easy to distinguish this species from L. papulosa by the sooty black lower surface of the thallus and the recurved squamules. L. papulosa has applanate squamules on the upper surface and light to brownish coloured lower surface of thallus.

Multirange.

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