

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

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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

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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: 袍脐衣属, 石耳属, 全长 rDNA 大亚基, 边缘种, 单系类群

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INTRODUCTION

All the species in the family Umbilicariaceae are umbilicate thalli with lecideine or sublecidine 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 $\beta(1\rightarrow6)$ 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 $\beta(1\rightarrow6)$ 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 phynotypic 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 1 The generic classification of the Umbilicariaceae during the period of the phynotypic taxonomy

Generic treatment	Family	Authors
<i>Umbilicaria</i> Hoffm. sensu Körb. <i>Gyrophora</i> Ach. sensu Körb	<i>Umbilicaria-Gyrophora</i> family	Körber 1855
<i>Umbilicaria</i> Hoffm. sensu Körb. <i>Gyrophora</i> Ach. sensu Körb. <i>Gyrophoropsis</i> Elenk. & Savicz	Trigeneric family	Elenk & Savicz 1911
<i>Umbilicaria</i> Hoffm. <i>Umbilicaria</i> Hoffm. <i>Omphalodiscus</i> Schol. <i>Gyrophora</i> Ach. <i>Actinogyra</i> Schol.	Monotypic family Qudrigenic family	Frey 1933 Scholander 1934
<i>Lasallia</i> Mérat. <i>Agyrophora</i> Nyl. <i>Omphalodiscus</i> Schol. <i>Umbilicaria</i> Hoffm. <i>Actinogyra</i> Schol.	Quinquegeneric family	Llano 1950
<i>Lasallia</i> Mérat. <i>Umbilicaria</i> Hoffm.	<i>Lasallia-Umbilicaria</i> family	Wei 1966*
<i>Lasallia</i> Mérat. <i>Umbilicaria</i> Hoffm. <i>Llanoa</i> Dodge	Trigeneric family	Eriksson & Hawksworth 1986

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.

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. pennsylvanica* 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 criteria 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

Species	Location	Specimen number	GenBank accession numbers	Species	Location	Specimen number	GenBank accession numbers
<i>U. antarctica</i>	Antarctic	NJ053	JQ739980	<i>U. loboperipheric</i>	Jilin	JL09103	JQ739995
<i>U. aprina</i>	Jilin	S697	JQ739981	<i>U. lyngei</i>	Jilin	TBF66	JQ739996
<i>U. arctica</i>	Arctic	2190	JQ739982	<i>U. muehlenbergii</i>	Jilin	112322	JQ739997
<i>U. caroliniana</i>	Inner Mongolia	20134	KX925283	<i>U. proboscidea</i>	Inner Mongolia	2013710	KX925285
<i>U. cinerascens</i>	Shaanxi	075751	JQ739983	<i>U. squamosa</i>	Tibet	075750	JQ739998
<i>U. cylindrica</i>	Jilin	099480	JQ739984	<i>U. subglabra</i>	Jilin	099473	JQ739999
<i>U. decussata</i>	Yunnan	1338746	KX925284	<i>U. thamnodes</i>	Yunnan	113228	JQ740000
<i>U. esculenta</i>	Jilin	111863	JQ739985	<i>U. torrefecta</i>	Jilin	THF19	JQ740001
<i>U. exasperata</i>	Arctic	B2185	JQ739986	<i>U. tylorhiza</i>	Jilin	TBF63	JQ740002
<i>U. flocculosa</i>	Jilin	CBF30	JQ739987	<i>U. vellea</i>	Jilin	THF22	JQ740003
<i>U. formosana</i>	Jilin	CBF31	JQ739988	<i>U. yunnana</i>	Yunnan	116117	JQ740004
<i>U. grisea</i>	Jilin	THF21	JQ739989	<i>L. asiae-orientalis</i>	Guizhou	083278	JQ740005
<i>U. hirsuta</i>	Xinjiang	084137	JQ739990	<i>L. daliensis</i>	Yunnan	113237	JQ740006
<i>U. hypococcinea</i>	Jilin	TBF56	JQ739991	<i>L. pertusa</i>	Xinjiang	116129	JQ740007
<i>U. indica</i>	Tibet	075789	JQ739992	<i>L. pennsylvanica</i>	Shaanxi	TBF18	JQ740008
<i>U. kisovana</i>	Jilin	113215	JQ739993	<i>L. sinorientalis</i>	Fujian	0907686	JQ740009
<i>U. krascheninikovii</i>	Jilin	TBF55	JQ739994	<i>L. rossica</i>	Heilongjiang	HY11189	JQ740010

Table 3 Primers for the PCR reactions

Abbreviation	Sequence (5'-3')	Position within <i>Saccharomyces cerevisiae</i>
E9	TTGTACACACCGCCCGT	1 640–1 656 (ssu)
SL4R	TTCGATCACTCTACTTGTGC	356–375
SL2	CGGCGAGTGAAGCGGCA	106–122
CL5R	CTTACCAAAAATGGCCCACT	1 136–1 155
SL7	GATCGATTTGCACGTCAAGAA	886–905
CL7R	GATCTATTTTGCCGACTTC	1 916–1 934
SL14	TAGCTCCGGACAAACCGAT	1 736–1 754
P22R	CAATGTCAAAGTAGAGTCAAGC	2 426–2 447
CL9	ATGACGAGGCATTTGGCTA	2 286–2 304
SL18R	CTTAGAGGCGTTCAGCCAT	3 120–3 139

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

Species	Accession No.	Systematic position	Species	Accession No.	Systematic position
<i>Aspergillus fumigatus</i>	AAHF01000017	Ascomycota	<i>Rhizopus stolonifer</i>	DQ273817	Zygomycota
<i>Penicillium verruculosum</i>	AF510496		<i>Mucor racemosus</i>	MRARRHA	
<i>Phaeosphaeria</i> sp.	EF590325		<i>Zea mays</i>	AJ309824	Viridiplantae
<i>Saccharomyces cerevisiae</i>	BK006945		<i>Arabidopsis thaliana</i>	X52320	
<i>Cryptococcus neoformans</i>	CPC58255	Basidiomycota	<i>Oryza sativa</i>	AP009051	
<i>Minimedusa polyspora</i>	DQ915476		<i>Mus musculus</i>	NR 003279	Metazoa
<i>Spizellomyces</i> sp.	DQ273821	Chytridiomycota	<i>Rattus norvegicus</i>	V01270	
<i>Orpinomyces</i> sp.	AJ864475		<i>Homo sapiens</i>	NR 003287	

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). *Umbilicaria esculenta* as a template, 11 structure regions are found (Table 5). Furthermore, in term of the diagram of rDNA structure by Qu (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

Table 5 Structure region within *Umbilicaria esculenta* LSU rDNA

Region	1	2	3	4	5	6
Conserved	1–126	259–429	608–716	779–1 073	1 116–1 334	1 377–1 549
Variable	127–258	430–607	717–778	1 074–1 115	1 335–1 376	1 550–1 596
Region	7	8	9	10	11	12
Conserved	1 597–1 766	1 806–1 980	2 079–2 470	2 492–2 533	2 581–3 076	-
Variable	1 767–1 805	1 981–2 078	2 471–2 491	2 534–2 581	-	-

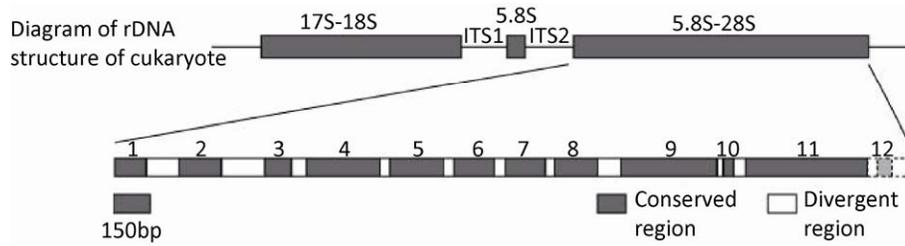


Fig. 1 Diagram of *Umblicaria esculenta* LSU rDNA structure. There are 12 structure regions, which include the conserved region, and the divergent region. Boxes with continuous lines circumscribe the presumed 12th region based on previous data.

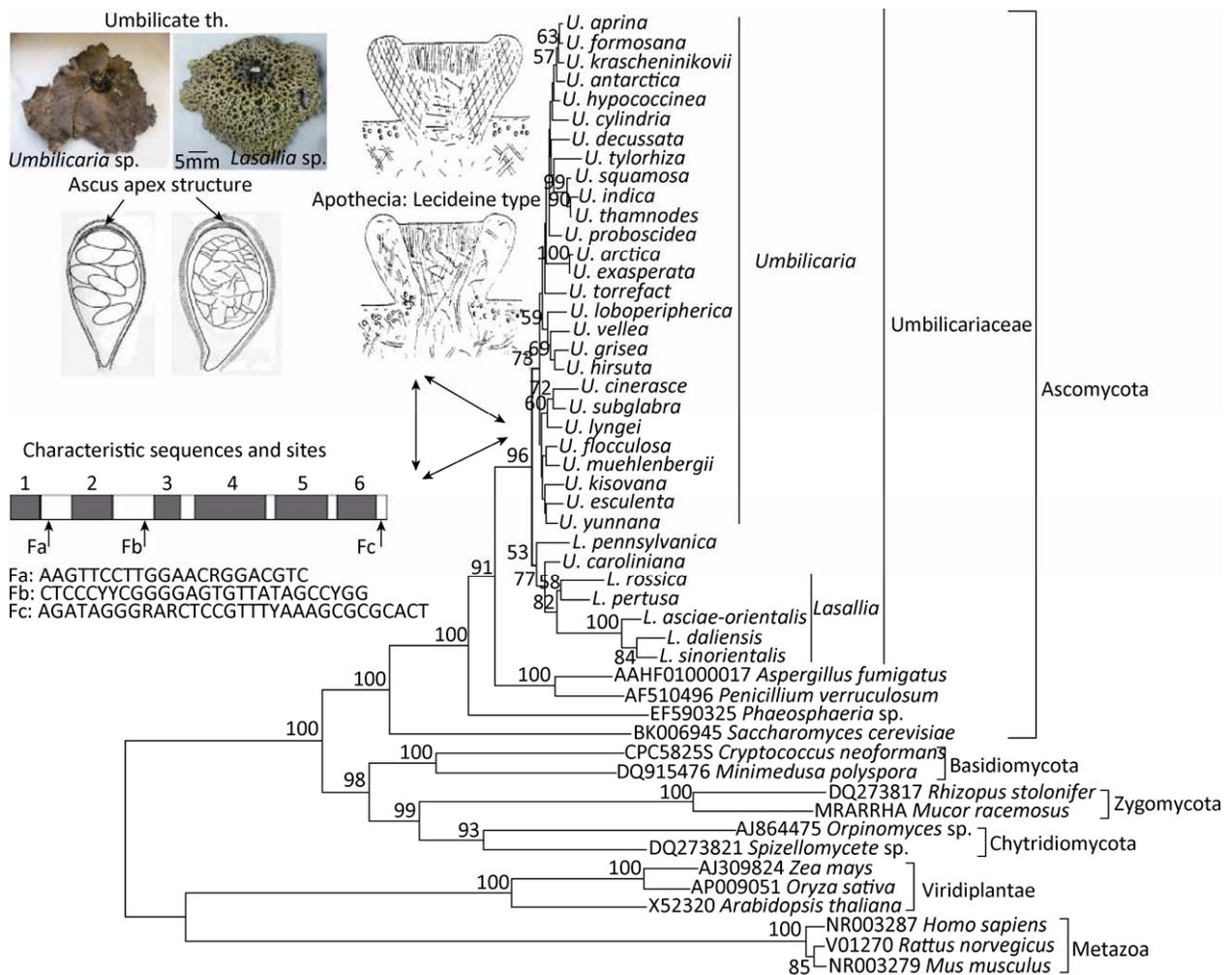


Fig. 2 The NL tree inferred from full-length LSU rDNA data. The reliability of the inferred tree was tested by 1 000 bootstrap replications; the numbers in each node represent bootstrap support values. Only bootstrap values greater than 50% are shown. The morphological, anatomical (left above) and symplesiomorphic sequences and their sites from the LSU rDNA (left below) for the family Umblicariaceae 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* (Hcfff.) 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.

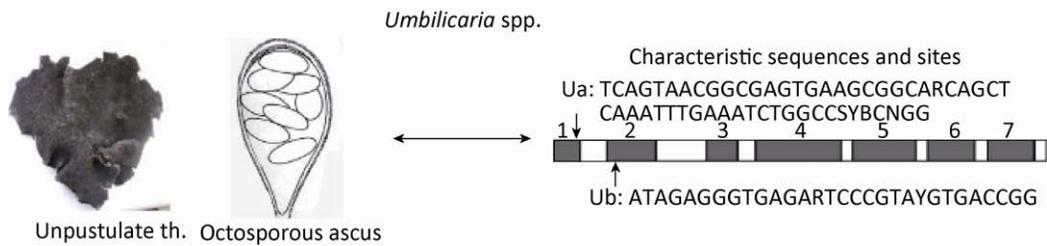


Fig. 3 The non-pustulate thallus below rich in rhizines with octosporous ascus (left and middle), the symplesiomorphic sequences Ua, Ub and their positions (right) of *Umbilicaria*.

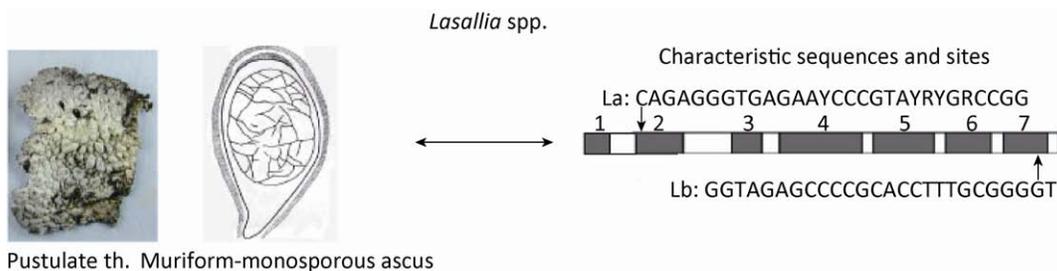


Fig. 4 The pustulate thallus with monosporous ascus (left and middle), the symplesiomorphic sequences La, Lb and their positions (right) of *Lasallia*.

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 asci 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 asci 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.

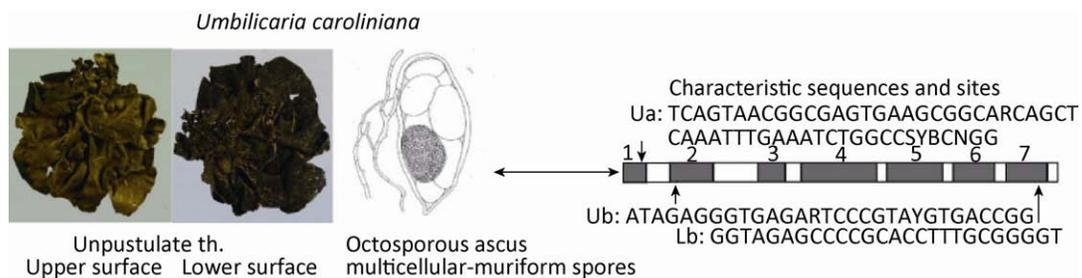


Fig. 5 The non-pustulate thallus below rich in rhizines with octosporous ascus, the symplesiomorphic sequences Ua & Ub, and Lb of *Umbilicaria caroliniana*. Thallus, upper surface (left), and lower surface (middle). Octosporous ascus with muriform spores (right).

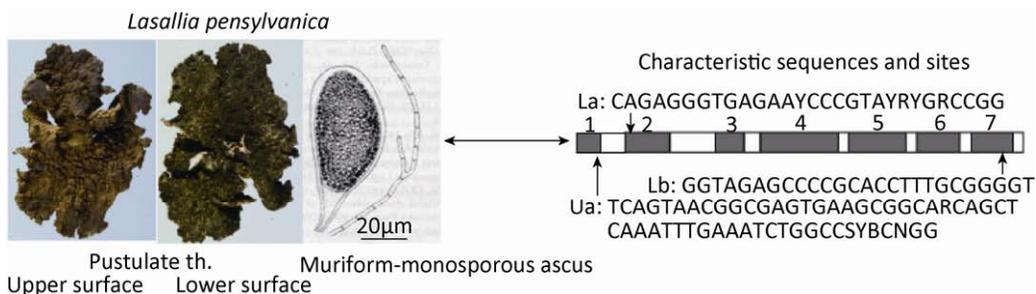


Fig. 6 The pustulate thallus with monosporous ascus, the symplesiomorphic sequences La & Lb, and Ua of *Lasallia pensylvanica*. Thallus, upper surface (left), and lower surface (middle). Monosporous ascus with muriform spores (right).

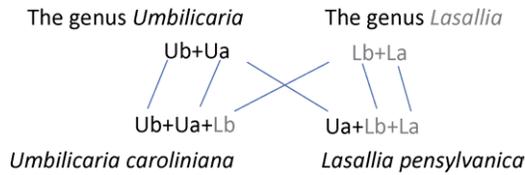


Fig. 7 *Umbilicaria caroliniana* is characterized by the non-pustulate thallus below rich in rhizines with octosporous ascus and the symplesiomorphic sequences Ub & Ua of *Umbilicaria* with Lb of *Lasallia* in passing (left below). *Lasallia pensylvanica* is characterized by pustulate thallus with monosporous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

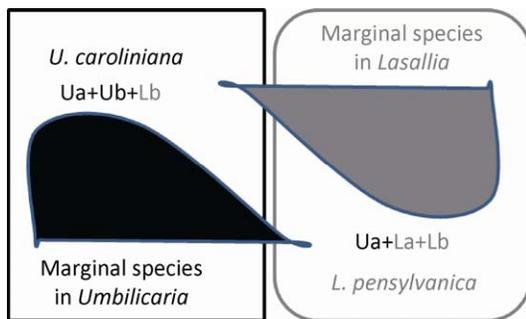


Fig. 8 The two marginal species *U. caroliniana* and *L. pensylvanica* represented by the picture.

3 CONCLUSION

The symplesiomorphic analyses of genotype and phenotype 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 below 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 thalli below 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 phenotype 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 phenotype 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 phenotype in the family Umbilicariaceae is just the evidence of the common descent theory.

4 DESCRIPTION

Umbilicariaceae F. F. Chevier

Fl. Gen. env. Paris: 640 (1826), [as "Umbilicarieae"].
Hawksworth & David, Family names, Index of fungi supplement: 73 (1989). Fee, Essai Cryptog. Ecorc. Officin. LXX, 1824 (as Umbilicariacees). [nom. inval., Art. 32.1 (b); see Art. 18.4.].

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 or off-centered umbilicus of the lower surface (Fig. 2, above left), heteromerous with upper and lower cortices mainly consisting of palisade- or scleroplektenchymatous 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 subleceideine (=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 symplesiomorphic 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**

Descr. Adumbr. Pl. Lich. 1 (1): 8 (1789).

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

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

Description:

Thallus foliose, heteromerous with upper and lower cortices, umbilicate, monophyllous to polyphyllous, non-pustulate (Fig. 3, left), strongly attached to substrate by a central or off-centered umbilicus from the lower surface, somewhat leathery when wet, brittle when dry; the upper cortex usually palisade- or scleroplektenchymatous; the medulla loose to compact and mucilaginous, sometimes not clearly differentiated from the lower cortex; the lower cortex usually scleroplektenchymatous, with or without rhizines.

Apothecia immersed or adnate to stipitate, lecideine to sublecanorine (=subleceideine), leiodiscs to omphalodiscs, or gyrose to actinodiscs; asci octosporous (Fig. 3, middle); ascospores small, 7–40×3.5–20µm, simple and hyaline or in some species muriform and brown.

The symplesiomorphic sequences are Ua and Ub, and their sites for the genus from the LSU rDNA (Fig. 3, right).

Algae: *Trebouxia* spp.

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

Thalloconidia are present in many species mainly on the lower surface of thallus and / or on the surface of rhizines.

***Umbilicaria caroliniana* Tuck.**

Proc. Amer. Acad. Arts & Sci. 12: 167 (1877).

Type: U.S.A., North Carolina, 1845, coll. M.A.Curtis No. 88, in H-Tuck (FH, holotype!).

Type observation: Thallus monophyllous to polyphyllous, membranous and fragile, with strongly undulating surface; upper surface brownish to dark brown, smooth and dull to shining; lower surface

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

Apothecia with gyrose discs.

≡ *Gyrophoropsis caroliniana* (Tuck.) Elenkin & Savicz, Trav. Mus. Bot. Acad. Petersbourg 8: 34 (1910).

≡ *Gytrophora caroliniana* (Tuck.) Schol. Nyt. Mag. Naturvid. 75: 28 (1934).

≡ *Lasallia caroliniana* (Tuck.) Davydov, Peršoh & Rambold, *Mycol Progress* (2010) 9: 264 (2010).

Misapplied name: *Umbilicaria mammulata* auct. non (Ach.) Llano in Tuck. Proc. Amer. Acad. Arts. & Set. 1: 261 (1848).

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 μ m thick; upper cortex consisting of a brown outer layer of 3–8 μ m thick, and a colourless inner layer of 5–16 μ m thick; algal layer 21–54 μ m thick; medulla 34–99 μ m thick; lower cortex consisting of a colourless inner layer of 11–30.5 μ m thick, and a brown outer layer of 5–16 μ m thick.

Apothecia 2–3mm diam., epihymenium brown,

14–42 μ m thick; hymenium 106–162 μ m thick; subhymenium yellowish, 12–55 μ m thick; asci octosporous, 95–135 \times 25–39 μ m; ascospores brown, muriform, ellipsoid, 27–39 \times 14–20 μ m; paraphyses richly branched, septate, 1–2 μ m thick, expanded at the tip.

Pycnidia 92–185 \times 92–213 μ m; pycnoconidia bacilliform, 3–5 \times <1 μ m.

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

The Symplesiomorphic 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 530m, July 26, 1983, coll. Zhao CF, No. 2963 (HMAS-L, IFP!).

Mongolia: Ara-Khangai, Aug. 2, 25, 1970, coll. Biazrov, Nos. 4236, 5748 (MOSCOW!).

Japan: Honshu, prov. Shinano, Mt. Kimpu, on rocks, alt. 2 190m, July 28, 1965, coll. S. Kurokawa No. 65172 [Kurok. Lich. Rar. et Cri. Exs. No. 99] (BM, COLO, E, FH, LE, S, TUR!); Honshu prov. Ishikari: Mt. Tomuroushi, on rocks, c. 1 700m, July 20, 1969 coll. M. Togashi [Kurok. & Kashiw. Lich. Rar. et Cri. Exs. No. 446] (S, TUR, FH, COLO!); Mt. Komagatake of Kiso, July 27, 1926, Coll. Y. Asahina [Lich. Japan Exs. No. 49] (BM!). Jilidesan, alt. 3 000m, Aug. 1898, coll. Faurie. No. 859, pr.p. (LE, MIN!). Lich. du Japan, part of 863 (pr.p.) (MIN!). Hokkaido, Mt. Tomuraushi, July 31, 193, Sato No. 19 (FH, MIN!).

Russia: Yakut prov, July, 1903, Coll. Shogolev, Mus. Bot. Acad. Sci. Petr. N.1 (LE!); Yakut, Aug, 25, 1956, coll. V. Ivanova 153 (LE!); Yakut, Aug. 3, 1956, coll. V. Ivanova 116/9 (LE!); Yakut, Tomponsk region, 1955 coll. Dobrecova as *G. pulvinaria* Savicz (LE!); Yakut, Aldan, N. 63° lat., E. 132° long, Sept. 18, 1956,

coll. V. Kuvaev, No. 242-7 (LE!); Yakut, Jugo, Charaulach, Jun. 24, 1939, coll. W. Scheludjakova (TUR!). Amursk prov, alt. 1 500m, July 19, 1910, coll. Prohorov & Kuzeneva Nos. 580, 584 (LE!), and July 23, 1915, coll. Prohorov & Kuzeneva (LE!), and Regio Amurensis in Valle fl. Zeja, ad saxa, 1915. coll. Kuzeneva, No. 2229 (US!). Siberia, July, 19, 1932, coll. V. Savicz No. 110 (LE!); SE Siberia, Baikal region, 1915, coll. Alexandrov, on east bank of Lake of Baikal (LE!); On rocks of west bank of Lake of Baikal, June 25, 1928, coll. K.A. Rassadina, 194 (LE!); July 10, 1928, coll. K.A. Rassadina (LE!); Amur region, alt. 1 500m, on rocks, July 19, 1910, coll. N. Prohorov & Kuzeneva Nos. 580, 584 (LE!).

Other specimens examined:

U.S.A.: Tennessee, [Kurok. Lich Rar. et C. Exs. 290] (LE!); [Lich. Amer. Exs. 149] (LE!). N. Carolina, [Vezda. Lich. Sel. Exs. 1071] (LE!); N. Alaska. Lich. Arctici 27 (LE!).

Literature records for ASIA:

China (Chen *et al.* 1981, p. 157 for Liaoning; Wei & Jiang, 1988, p. 79; Wei, 1991, p. 246).

Japan (Sato, 1943, p. 103; Yoshimura, 1974, p. 123; Ikoma, 1983, p. 109).

Russia (Golubkova *et al.*, 1978, p. 129 for Siberia and Far East; Sedelnikova, 1985, p. 87 & Makrei, 1990, p. 55 for Siberia).

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**

Nouv. Fl. Paris ed. 2 (1): 202 (1821).

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 or off-centered umbilicus of the lower surface.

Apothecia lecideine to sublecidine (=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: *Trebouxia* spp.

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

***Lasallia pensylvanica* (Hcchm.) Llano**

Monograph of the Umbilicariaceae 42 (1950).

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

≡*Umbilicaria pensylvanica* Hoffm. Descr. Adumb. Pl. Lich. 3(4): 5-6, Pl.69, fig. 1-2, (1801).

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 lacinate 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 scleroplectenchymatous, colourless, 23–46µm thick.

Apothecia leiodisc, occasionally with a single

fissure or button in the disc; epihymenium 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; pycnoconidia 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:

China: Jilin, Mt. Changbai, south slope, Mt. Hongtuo: alt. 1 950m, Aug. 5, 1980, coll. Hu YC, No. 2149; alt. 1 970m, Aug. 16, 1982, coll. Hu YC, Nos. 3386, 3432; alt. 1 880m, July 28, 1983, coll. Wei JC & Chen JB No. 6250; alt. 1 980–2 000m, coll. Hu YC, Nos. 4218, 4230, 4232 (HMAS-L!). Nimongol, Da Hingan Ling, Bailong forest farm, Mt. Jiguan, alt. 1 600m, on rocks, Aug. 2, 1991, coll. Chen & Jiang, Nos. A-812, A-817, A-820 (HMAS-L!) Xinjiang, Mt. Altai, near Halas lake, alt. 2 000m, on rocks, Aug. 4, 1986, coll. Gao XQ, No. 1987 (HMAS-L!).

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* Domb. 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. Biazrov, No. 6579 (MOSCOW!).

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

Japan: Honshu prov. Etchu: Mt. Etchusawa-dake on rocks, alt. 2 400m, July 27, 1976, coll. Kashiwadani (No. 13413) and K. Yoshida in Kurok.: Lich. Rar. et Cr. Exs. 293 (COLO, DUKE, FH, LE, S, TENN, US!); Musashi: Mt. Kinpu. on rocks, alt. 2 400m, Aug. 25, 1960, coll. Asahina, Togashi, Kurok., and Nuno, [Kurok. & Kashiw. Lich. Rar. et Cr. Exs. No. 316, 317] (COLO, DUKE, FH, LE, S, TUR, UPS, US!); Mt. Ohyama, coll. H. Shibuichi (No. 4719) [Kurok. & Kashiw., Lich. Rar. et Cr. Exs. 696] (COLO, DUKE, EH, LE, US!), on rocks, alt. c. 1 800m, Mt. Nagayamadake, Mt. Daisetsu National Park, Prov. Ishikari, Hokkaido, Aug. 22, 1971, Coll. I. Yoshimura (No. 12395) [Lich. Jap. Exs. No.39] (COLO, DUKE, H, LE, TUR, US!). Hokkaido, Mt. Tomuraushi, July 31, 1935, coll. M.M. Sato (FH!). Herb. of the U.S. North Pacific Exploring Expedition under commanders Ringgold and Rodgers, 1853-56 *Umbilicaria pensylvanica*, C. Wright (FH!). Near Fukushima alt. 3 000m, Aug. 1898, coll. Faurie No. 863 (LE!), Jiidesan, alt. 3 000m, Aug. 1898, Coll. Faurie, No. 859, pr. p. (LE, pr. p. min. MIN!).

Russia: Chukotka, western Chukotka, North Anuisk, July 14, 1978, coll. M.P. Andreev (HMAS-L!). North Ural, Herb. of Inst. of Biology of Ural Academy of Sciences, Aug. 4, 1962, coll. by M. Storjjeva (H, partly TUR!). Siberia, near Angara river, on rocks, Oct. 1, 1907, coll. Martjanov, No. 171 in Herb. Vain 25 as *U. caucasica* Lojka (TUR!). Sverdlovsk region, Jun. 24, 1959, coll. A. Kasarinova, S25920, C. M. COLO-146277, Pr. P. (COLO!). Sept. 16, 1926, coll. A. Oxner (COLO, UPS!). Gorno-Altai Auto-oblast. SE of Ongudai on Little Ilzumen River near Khabarovka

below Chikemansky Pass, alt. 900–1 000m; arid mountain steppe with low granite outcrops, also riparian woodland, Aug. 20, 1978, coll. T.S. Elias, W. Weber, C.S. Tomb & I.M. Krashnoborov (COLO!). Vom Gipfel des Urals bei Jekaterinburg, auf Granit Sept. 12, 1823, Herb. Chr. Steven (H!). Exped. Uralensis, Jun. 22, 1847, coll. Branth ex Herb. Acad. Petrop. (UPS!). Ural, 1900, coll. Vasili Sokolor (H!).

Other specimens examined:

[H. Lojka: Lichenotheca Universalis No.13] (S!). Ex Herb. W.G. Farlow, *Umbilicaria pensylvanica* Hoffm., Jun. 13, 1883, coll. E. Faxon (S!). Lichens collected by Carolyn W. Harris in Vicinity of Chilson Lake, Essex Co, N.Y. alt. 1 200 ft, *Umbilicaria pensylvanica* Hoffm. (S!). N. Amer. (N. Carol.) coll. H.A. Green, July, 1883, *Umbilicaria pensylvanica* Hoffm. (S!).

Literature records for ASIA:

China (Chen *et al.* 1981, p. 158; Wang & Lai, 1976, p. 228).

Mongolia (N. Golubkova, 1981, p. 137; Huneck *et al.*, 1992, p. 296).

Japan (Sato, 1943, p. 103; Yoshimura, 1974, p. 119; Ikoma, 1983, p. 109).

Russia (Golubkova *et al.*, 1978, p. 133 for Siberia and Far East; Sedelnikova, 1985, p. 89 for Siberia; Makrei, 1990, p. 55 for Siberia).

Himalaya (Singh, 1964, p. 178, as *U. pensylvanica*; Awasthi, 1965, p. 126).

Comments: It is easy to distinguish this species from *L. apulosa* 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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