

A chemotaxonomic study of *Lethariella zahlbruckneri* and *L. smithii* (lichenized Ascomycota: Parmeliaceae) from Hengduanshan Mountains

Dong-ling NIU, Li-song WANG, Ying-jun ZHANG and Chong-ren YANG

Abstract: A taxonomic investigation of *Lethariella smithii* and *L. zahlbruckneri* has established that they are conspecific. Three chemical races are recognized: race 1 containing norstictic acid, race 2 norstictic acid and placodiolic acid, and race 3 placodiolic acid as major medullary substances.

Key words: chemical race, HPLC, lichens, synonym

Introduction

Lethariella Motyka was originally described as a subgenus of *Usnea* Adans., but subsequently Krog elevated *Lethariella* (Mot.) Krog to generic level and subdivided it into three subgenera based mainly on the development of the axis (Krog 1976). In his monograph, Obermayer (1997) accepted 10 species in the genus *Lethariella*, although later (Obermayer 2001) he reduced *L. mieheana* Obermayer to a synonym of *L. sinensis* J.C. Wei & Y.M. Jiang. Among the ten species, seven are known only from the Hengduanshan Mountains (namely, Yunnan, Sichuan and Tibet) and adjacent areas. Since this genus has been traditionally used for herbal teas (Wang *et al.* 2001) by the local people living in these areas, it has recently attracted considerable attention for chemical studies (Sun *et al.* 1990; Kinoshita *et al.* 2004, 2005). In addition, Wang, one of

the co-authors, perceived that there were some taxonomic problems in this genus during a taxonomic and floristic survey of the Hengdushan Mountains. Therefore, a chemotaxonomic study of this genus was undertaken, and the holotypes of *L. zahlbruckneri* (Du Rietz) Krog and *L. smithii* (Du Rietz) Obermayer and other related specimens were critically examined. In this paper, the taxonomy of *L. zahlbruckneri* is clarified, and *L. smithii* included as a synonym.

Materials and Methods

Materials

The specimens used for this study were collected from south-west China, and housed in the Cryptogamic Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN-L). Type specimens from UPS, BM, H and GZU were also examined.

Observation of external morphology and anatomy

Air-dried specimens were examined under a stereomicroscope to study the external morphology. For anatomical studies, sections were hand-cut with a razor blade under a stereomicroscope, mounted in GAW (Glycerol:Alcohol:Water=1:1:1), and observed under a light microscope.

Chemistry

The following herbarium specimens were used for high-performance liquid chromatography (HPLC): holotypes of *L. zahlbruckneri* and *L. smithii*, *Zang* 76-163, *Wang* 01-20561(a), *Yang* 88-133, *Wang* 04-23341

D. L. Niu, Y. J. Zhang (corresponding author) and C. R. Yang: State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Science, Kunming 650204, P. R. China. Email: zhangyj@mail.kib.ac.cn

D. L. Niu: Ningxia University, Yinchuan 750021, P. R. China and Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. China.

L. S. Wang: Key Lab of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650204, P. R. China

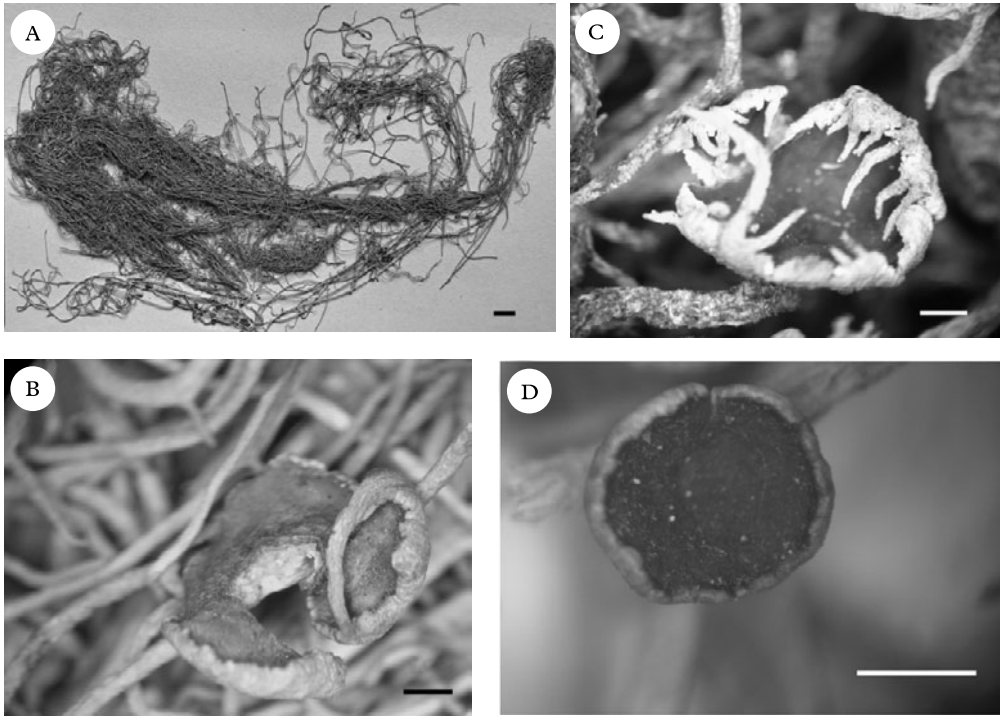


FIG. 1. *Lethariella zahlbruckneri*. A, habitus (holotype); B–D, apothecia; B, thalline exciple with long fibrils (Zang 76-163, KUN-L); C, thalline exciple with short fibrils (Wang 00-19983, KUN-L); D, thalline exciple without fibrils (holotype). Scales: A=1 cm; B–D=1 mm.

and Wang 06-26068. For more details, see the list of specimens examined.

A single fragment of each specimen was soaked in methanol at room temperature overnight. HPLC was carried out with a Waters Alliance 2695 liquid chromatograph at 25°C. Chromatographic conditions: column, Zorbax SB-C18 (4.6 × 250 mm, 5 µm); mobile phase: aqua bidest containing 1% orthophosphoric acid (solvent A) and methanol (solvent B); the solvents were degassed for 30 min in an ultrasonic vibrator prior to use. After injection of 10 µl of the sample, the concentration of solvent B in A was programmed as follows: 30% (0 min)–70% (15 min)–100% (35 min)–100% (48 min)–30% (53 min)–30% (63 min). Flow rate: 0.7 ml/min; PAD: range 210–400 nm (254 nm). Identification of the lichen substances was carried out by HPLC, and by comparison with the authentic samples isolated by our group.

Results and Discussion

Lethariella zahlbruckneri (Du Rietz) Krog

Norveg. *J. Bot.* **23**: 88 (1976).—*Usnea zahlbruckneri* Du Rietz, *Sv. Bot. Tidskr.* **20**: 92 (1926).—*Letharia zahl-*

bruckneri (Du Rietz) Zahlbr., *Symb. Sin.* **3**: 200 (1930); type: China, prov. Sze-ch'uan, 1922, Harry Smith n. 5014 (UPS—holotype).

= *Usnea smithii* Du Rietz, *Sv. Bot. Tidskr.* **20**: 92 (1926).—*Letharia smithii* (Du Rietz) Zahlbr., *Symb. Sin.* **3**: 200 (1930).—*Lethariella smithii* (Du Rietz) Obermayer, *Bibliotheca Lichenologica* **68**: 45–66 (1997); type (details) (UPS—holotype).

(Fig. 1)

Thallus corticolous or lignicolous, pendulous, 5–50 cm long; branches orange to greyish brown, grey or off-white towards the base of the thallus, terete or somewhat flattened at the ramification, 0.3–0.7 mm diam.; branching submonopodial to dichotomous or irregularly dichotomous; surface rough, moderately to strongly ridged, obviously or inconspicuously longitudinally grooved, with short side branches (acute-angled to right-angled); isidia and soredia absent. *Cortex* 20–40 µm, *medulla* 130–210 µm with

loosely interwoven hyphae, central axis solid, circular, 200–450 µm diam.

Apothecia lateral, orbicular, disc 3.0–5.0 × 3.5–6.0 mm diam., disc almost flat, brown or dark brown, epruinose; thalline *exciple* smooth or with inconspicuous ridges, concolorous with the thallus, some thalline *exciples* with fibril-like branchlets. *Ascospores* 8 per ascus, ellipsoid, 5–6 × 7–8 µm, simple, colourless.

Chemistry. Three chemical races have been recognized. Race 1 containing canarione, atranorin, norstictic acid, gyrophoric acid (trace); race 2 containing canarione, atranorin, norstictic acid, placodiolic acid; and race 3 with canarione, atranorin and placodiolic acid.

Habitat. On bark and wood of conifers, including *Juniperus* sp., *Larix* sp. and *Picea* sp. at high elevations (3800–4300 m).

Distribution. South-western China (eastern Tibet, western Sichuan, north-western Yunnan).

Distinguishing features. The unique chemical compositions of the three races readily distinguish this species from the other pendulous species of the genus. *Lethariella canariensis* (Ach.) Krog lacks aromatic acids in the medulla, has a smooth surface and is restricted to Macaronesia. *Lethariella sinensis* (syn. *L. mieheana*) is easily confused with *L. zahlbruckneri*, but the former species has psoromic acid as its major medullary substance (Obermayer 2001), which differs structurally from placodiolic acid present in *L. zahlbruckneri*. Psoromic and placodiolic acids have similar HPLC retention times but are readily separated by the UV absorption as well as by TLC. *Lethariella mieheana*, which is a synonym of *L. sinensis* (Obermayer 2001), has a variable morphology, including some specimens with soredia. We have also observed soredia in the holotype of *L. mieheana* that have been overlooked in previous descriptions. In addition, *L. sinensis* (inc. *L. mieheana*) has a more extensive distribution than *L. zahlbruckneri*. Its distri-

bution includes Qinghai except for Yunnan, Tibet, Sichuan. Therefore, on the basis of present study *L. sinensis* is not considered to be synonymous with *L. zahlbruckneri*.

Remarks. Du Rietz (1926) distinguished *Usnea zahlbruckneri* and *U. smithii* by the colour of the thallus, namely, greyish brown in *Usnea smithii* rather than orange as in *U. zahlbruckneri*. He could not find any difference in chemistry between these two species and considered them to be very closely related. Krog (1976) transferred *U. zahlbruckneri* to *Lethariella* and synonymized *U. smithii* under it because she considered that the holotype of *U. smithii* contained the same orange pigment, canarione, in the cortex as *L. zahlbruckneri*, but that the orange pigment was lost as a consequence of partial decomposition of the thallus of *U. smithii* either before collection or during transport. An unidentified substance with Rf classes 6–6–6 in the medulla of the holotype of *L. zahlbruckneri* was also reported by Krog (1976). Subsequently, Obermayer (1997) concluded that this substance was placodiolic acid, which might be the result of a few intermixed branches of *L. smithii*.

Representative chromatograms (Fig. 2A & B) of *L. smithii* and *L. zahlbruckneri* show that placodiolic acid, atranorin and canarione were found in the holotypes of both species. In addition, norstictic acid was also found in the latter. These results are at variance with the TLC analyses by Obermayer (1997), who concluded that *L. zahlbruckneri* has norstictic acid as the only major substance in the medulla whereas *L. smithii* contains placodiolic acid as the major substance. Repeated analyses of two different fragments of the thallus produced similar results. However, we do not exclude the possibility that the type material of *L. zahlbruckneri* is a mixture of two chemotypes. Sun *et al.* (1990) also reported placodiolic acid from a chloroform extract of *L. zahlbruckneri*, and most other herbarium specimens we examined contained mainly placodiolic acid in the medulla. A single exception was a specimen collected from Tibet, which contained norstictic acid with

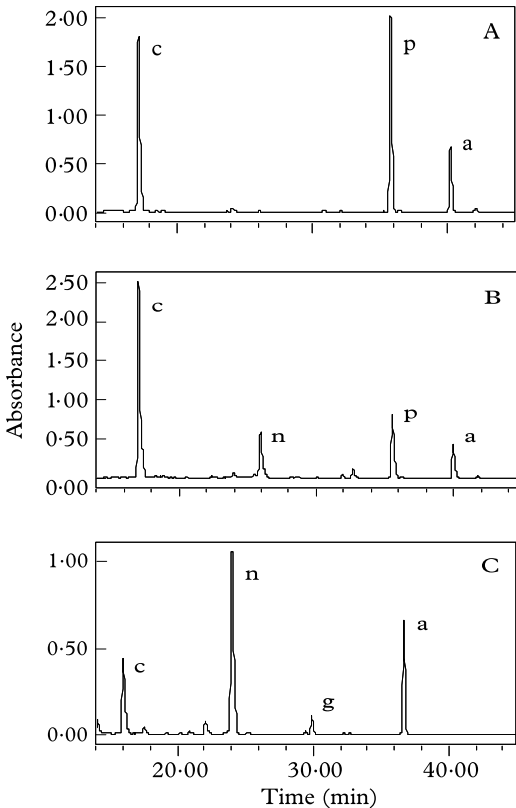


FIG. 2. Representative chromatograms of *Lethariella* spp. A, *L. smithii* (holotype); B, *L. zahlbruckneri* (holotype); C, *L. zahlbruckneri* (Zang 76-163). a: atranorin; c: canarione; p: placodiolic acid; n: norstictic acid; g: gyrophoric acid.

a trace of gyrophoric acid (Fig. 2C) as reported by Obermayer (1997). Since these chemical differences do not correlate with any morphological characters, we believe that they should be regarded as chemical races within a single species.

According to previous reports, *L. zahlbruckneri* is mainly distributed in Tibet and Sichuan provinces, China (Krog 1976; Obermayer 1997). Specimens were found in Yunnan province for the first time in the present study. The known distributional ranges differ for the chemical races (Fig. 3). Race 1 is distributed in Tibet, and Obermayer (1997) previously reported *L. zahlbruckneri*, which belongs to race 1, from Sichuan; race 2 is present in Sichuan,

and race 3 occurs mainly in Sichuan and Yunnan. Chemical race 2 is sympatric with races 1 and 3 (Sichuan), which shows their apparently identical ecological requirements. All three chemical races have apothecia, therefore, it is possible that their main method of reproduction is sexual. We speculate that gene flow by spore dispersal may have occurred between races 1 and 3, so that race 2 may be a hybrid between races 1 and 3, and could be regarded as a variant of either chemical race 1 or 3. Chemical differences appear to be polymorphisms within single populations.

Lethariella zahlbruckneri usually grows on conifers at high elevations (3800–4300 m) where it is irradiated with intense ultraviolet light from the sun, and the colour of the thallus is probably influenced by sunlight. According to our field observations, thalli of *L. zahlbruckneri* in exposed microhabitats are usually bright orange, whereas those in partial shade tend to have a pale orangish coloration. The greyish brown holotype of *L. smithii* is probably the shade form.

Apothecia of species of the genus *Lethariella* were previously considered to lack fibrils on the thalline exciple (Fig. 1D), however, fibril-like branchlets on the thalline exciple were observed in a specimen of *L. zahlbruckneri* from Tibet (Fig. 1B) and other herbarium specimens (Fig. 1C). These fibril-like branchlets varied from being short, unbranched and erect to relatively long, branched and flexuous. This is the first time that such appendages have been observed on the apothecia of *Lethariella* specimens. Previously, fibrils were considered to be confined to the genus *Usnea*, and to be uniform in size, but in *Lethariella* these fibril-like branchlets are variable in size, even within a single species, and occurred sporadically, so we have not used the character in species delimitation. This phenomenon was also recorded in the genus *Protousnea* (Calvelo *et al.* 2005).

Selected specimens examined. Chemical race 1: **China:** Prov. Tibet: Leiwuqi, 1976, Zang 76-163 (KUN-L).

Chemical race 2: **China:** Prov. Sichuan: mellan Tsago-gamba och Tamaba, 4000 m s. m., 1922, Harry Smith 5014 (UPS).

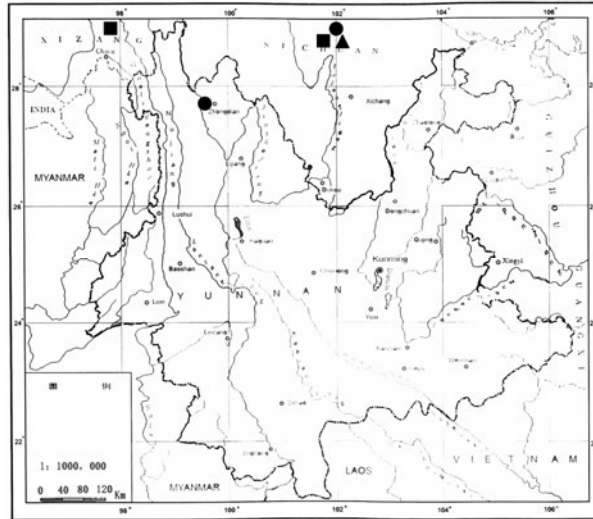


FIG. 3. Distribution of the chemical races (■ race 1; ▲ race 2; ● race 3) of *Lethariella zahlbruckneri*.

Chemical race 3: **China:** *Prov. Sichuan:* Xiaojin county, Rilong village, on *Juniperus*, 3800 m, 30°14'653"N, 102°46'975"E, 2001, Wang 01-20561 (a) (KUN-L); Xiaojin county, Rilong village, shuang-qiaogou, on bark of *Larix*, 3800 m, 2006, Wang 06-26068 (KUN-L); mellan Merge och Sangkar, lager II pa *Larix*, 4000 m s. m., 1922, Harry Smith n. 5017 (UPS). *Prov. Yunnan:* Zhongdian county, Dongwang village, seyangzhongchang, on tree, 4100 m, 1988, Yang 88-133 (KUN-L); Xianggelila County, Gezan Village Kuangchang, on *Juniperus* sp., 4100 m, 28°32'N, 99°56'E, 14 vi 2004, Wang 04-23341 (KUN-L).

Other specimen: **China:** *Prov. Yunnan:* Zhongdian county, Wengshui village daxueshan, on stump of *Sabina* sp., 4001 m, 28°34'481"N, 99°50'55"E, 2000, Wang 00-19983 (KUN-L).

We are indebted to the keeper and curators of the following herbaria for the loan of specimens: BM, GZU, H, and UPS. We thank Dr Walter Obermayer for sending us literature for this study and for helpful discussion. We are also grateful to Prof. Hiroshi Harada for discussions and for help in the field, and for commenting on the manuscript. This work was supported by National Natural Science Foundation of China (NO. 30670011).

REFERENCES

- Calvelo, S., Stocker-Wörgötter, E., Liberatore, S. & Elix, J. A. (2005) *Protousnea* (Parmeliaceae, Ascomycota), a genus endemic to southern South America. *Bryologist* **108**: 1–15.
- Du Rietz, G. E. (1926) Om slaktena *Evernia* Ach., *Letharia* (Th. Fr.) Zahlbr. emend. DR. och *Usnea* Ach. subgenus *Neuropogon* (Nees et Flot.) Jatta. *Svensk Botanisk Tidskrift* **20**: 89–93.
- Kinoshita K., Narui T., Koyama K., Takahashi K., Culberson C. F. & Nishino Y. (2005) Secondary metabolites from *Lethariella seranderi*. *Lichenology* **4**: 7–12.
- Kinoshita, K., Takatori, K., Narui, T., Culberson, C. F., Hasumi, M., Nishino, Y., Koyama, K. & Takahashi, K. (2004) A novel secondary metabolite from *Lethariella seranderi*. *Heterocycles* **63**: 1023–1026.
- Krog, H. (1976) *Lethariella* and *Protousnea*, two new lichen genera in Parmeliaceae. *Norwegian Journal of Botany* **23**: 83–106.
- Obermayer, W. (1997) Studies on *Lethariella* with special emphasis on the chemistry of the subgenus *Chlorea*. *Bibliotheca Lichenologica* **68**: 45–66.
- Obermayer, W. (2001) On the identity of *Lethariella sinensis* Wei & Jiang, with new reports of Tibetan *Lethariella* species. *Bibliotheca Lichenologica* **78**: 321–326.
- Sun, H., Niu, F., Lin, Z., Cao, D., Li, B. & Wu, J. (1990) Chemical constituents of four medicinal lichens. *Acta Botanica Sinica* **32**: 783–788 [in Chinese].
- Wang, L.-S., Narui, T., Harada, H., Culberson, C. F. & Culberson W. L. (2001) Ethnic uses of lichens in Yunnan, China. *Bryologist* **104**: 345–349.

Accepted for publication 30 August 2007