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地卷属 1 中国新记录种

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摘　要：通过对地卷属地衣形态和化学的研究，并结合核基因 ITS 序列的系统发育分析，报道了采自中国西北地区地卷属的 1 个中国新记录种——芽片地卷(新拟)。它的典型特征是沿着地衣体边缘或地衣体上表面裂隙具有大量的薄片状 phyllidia，且常常覆有粉霜。地衣体上表面的边缘具有白色绒毛，下表面具有丛生假根，而且假根在边缘为白色，逐渐向中心变为深色。该研究提供了该种的详细描述，并与近缘种进行了细致的讨论。

关键词：中国西北；地卷属；ITS 序列；新记录种；

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New Record of the Lichen Genus *Peltigera* from China

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Abstract: A new record species *Peltigera monticola* Vitik. was identified from the northwest of China, based on morphological and chemical analysis, and a phylgenetic analysis of nrDNA ITS sequence was also carried out. It was distinguished by its numerous laminal phyllidia, often pruinose, along the margin or cracks, marginal parts tomentose, tufted rhizomes with pale near margins and darkened towards center. Detailed description and discussion with allied species were provided.

Key words: northwest of China; *Peltigera*; ITS sequences; new record species

The genus *Peltigera* is cosmopolitan monophyletic genus with more than 91 species, which typically have muscicolous and terricolous foliose thallus[1-3]. Most of *Peltigera* species have a cyanobacterial photoboint that carries out nitrogen fixation and plays a major ecological role in nitrogen cycling of ecosystem. This genus is a common lichen group represented by 28 taxa in China[4-5], distributed in all geographical distributions of China. However, until now this genus is not studied thoroughly in China, in which the potential for finding new species is high. During our recent fieldwork in Liupanshan and Helanshan Mountains located in the northwest of China, we find one additional taxa (*Peltigera monticola* Vitik.) belonging to this genus based on morphology and molecular characters.

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1 Materials and methods

1.1 Morphological analysis

Specimens for this study were collected from Liupanshan and Helanshan Mountains, located in the northwest of China. The morphological descriptions were made from dry materials. Morphological and anatomical characters were observed and photographed using a XTS20 (Fukai Science and Technology Development Co., China) and Leica DM2500 (Leica instrument Co., Germany). The voucher specimens for this study were kept in the herbarium of College of Life Science of Ningxia University (NXAC, Yinchuan, Ningxia, China). Besides our own collection we examined the specimens of the same species deposited in William Louis & Chicita F. Culbertson Lichen Herbarium & Library (Duke University, Durham, NC, USA)

1.2 Chemical analysis

Chemical analyses were carried out using spot test[6]. These involve applying tiny amounts of a reagent to lichen and observing any resulting color change. Four reagents were used in these spot tests, K (10% aqueous potassium hydroxide), C (sodium hypochlorite solution), KC (10% K followed by C solution), P (5% alcoholic P-phenylenediamine).

1.3 Molecular analysis

DNA was obtained from dry samples. Extraction followed a modification of DOYLE and DOYLE's (1987) method[7-8]. rDNA internal transcribed spacers (ITS1-2) were amplified via polymerase chain reaction (PCR) using following primers: ITS1F[9] and ITS4[10]. PCR mixtures (25 μL) consisted of 25 μg BSA, 1 U *Taq* DNA polymerase, dNTPs (0.2 mmol/L), primers (0.5 μmol/L

Table 1 Voucher information of *Peltigera monticola* Vitik. samples with GenBank accession number and specimens from GenBank ITS sequences obtained

	Species	Vouncher	GenBank accession
NP39	*P. monticola*	Helanshan Ningxia,13-0002	KT781508
NP36	*P. monticola*	Helanshan Ningxia,13-0003	KT781509
NP41	*P. monticola*	Liupanshan Ningxia,12-0007(1)	KT781510
NP19	*P. monticola*	Liupanshan, Ningxia,12-0016	KT781512
NP20	*P. monticola*	Helanshan,Ningxia,13-0005	KT781513
NP21	*P. monticola*	Helanshan,Ningxia,13-0004	KT781514
AY257877	*P. monticola*	Switzerland, Vust452188(G)	AY257877
AY257881	*P. monticola*	Switzerland, Vust452187(G)	AY257881
AY257886	*P. scotteri*	Canada,Goward81-1289a(UBC)	AY257886
AY257874	*P. monticola*	Poland,Toborowicz13.08.1976(KTC)	AY257874
AY257872	*P. monticola*	Poland,Faltynowicz5239(UGDA-L)	AY257872
KF957622	*P. ponojensis*	Italy,3523(HB-TO)	KF957622
AY257875	*P. monticola*	Switzerland,Vust452186(G)	AY257875
AY257883	*P. ponojensis*	Canada,Goward82-1233(CANL)	AY257883
KJ413221	*P. monticola*	SSM353(UI)	KJ413221
KC139753	*P. monticola*	SSM125(UI)	KC139753
AY257873	*P. monticola*	Yugoslavia,Vitikainen 7196(H)	AY257873
FJ709039	*P. ponojensis*	HOB020708-62-1-3	FJ709039
AY257884	*P. ponojensis*	Poland, Bielczyk2116(KRAM-L)	AY257884
AY257885	*P. ponojensis*	Poland, Kiszka2.09.1988(KRAP-L)	AY257885
AY257961	*P. elisabethae* *	Poland, Bielcayk 42135(KRAM-L)	AY257961

* Specimen name followed by an asterisk represents outgroup sequence.

each), and PCR buffer, adjusted with H_2O to 25 μL. The following thermal cycler profile was employed for PCR reactions: an initial denaturation of 95 ℃ for 5 min, follow by 35 cycles of 95 ℃ for 45 s, 52 ℃ for 90 s, and 72 ℃ for 90 s, with a final extension of 72 ℃ for 10 min. Amplification products were cleaned with ExoAP and sequenced using Big Dye chemistry with an ABI 3730 automated sequencer (PE Applied Biosystems, USA). Sequences were assembled using Sequencher 4.2 (Gene Codes, USA). All newly obtained sequences were subjected to Blast searches to verify their identity and submitted to GenBank.

1.4 Phylogenetic analysis

The entire ITS sequences of 6 samples examined and 14 representatives selected (Table 1) were aligned by Muscle implemented in *MEGA* version 6[11]. The alignments were analyzed using neighbor joining (NJ), the software-based Kimura 2-parameter model was used, and gaps were excluded in the pairwise distance estimation, the support values were tested by 1 000 bootstrap replications.

2 Results and discussion

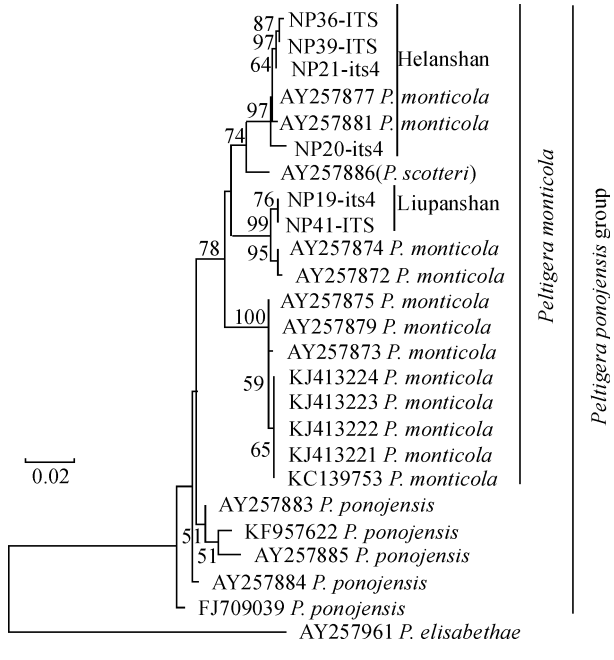
2.1 Phylogenetic analyses

Sequences of ITS alone were sufficient to identify all species from the *Peltigera canina* species complex. The *P. ponojensis* group was well defined as a monophyletic clade (Fig. 1), which has been identified before[12]. ITS sequences of representative collections showed that all studied *Peltigera* species from Liupanshan and Helanshan Mountains, belonged to the species *P. monticola* clade, support for the monophyly of this taxon was strong (BS=78%). The specimens in different geographical region were in different lineages, the specimens from Liupanshan were in a lineage, those from Helanshan in another lineage. '*P. scotteri*' is a nonmonophyletic putative species, and one of the specimens has been proved sister to *P. monicola*, so '*P. scotteri*' is not validly published[13]. The specimen of '*P. scotteri*' from Canade (Table 1) belonged to Helanshan lineage, which means this '*P. scotteri*' should be treated as a member of *P. monticola*. The specimens from Liupanshan were sister to the specimens from Poland with high support (BS=99%), the specimens from Helanshan and Switzerland were delimited as a monophyletic entity (BS=97%). Sequence data were also compared using Blast analysis of ITS1-2 regions deposited in NCBI. They were 100 % identical to *Peltigera monticola*.

2.2 The species

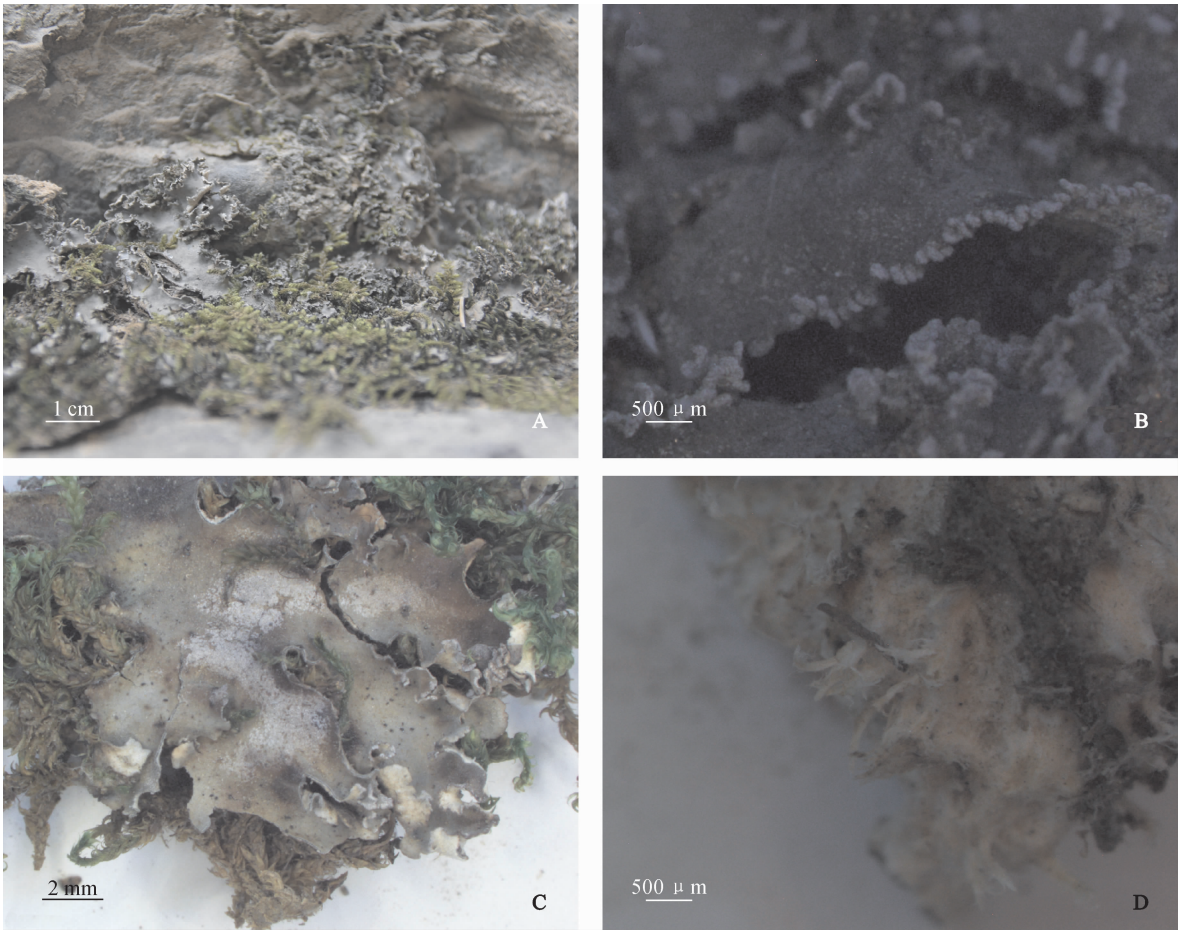
Peltigera monticola Vitik., Acta Bot. Fennica 152: 64 (1994, Fig. 2)

Thallus: foliose, small to medium-sized, 3—9 cm in diam., adnate; lobes: flattened and elongate, 0.5—3 cm long and 1—3 mm wide, with upturned, curled, with numerous flat phyllidia; upper surface: gray, partially brown when dry, tomentose marginally, dull marginally, smooth forwards central, sometimes pruinose on the surface; phyllidia: flat squamulose along margins and cracks, often pruinose; medulla: white with interwoven hyphae; photobiont: nostoc; lower surface: white margin with white raised veins, brown towards center; rhizines: tufted, pale near margins,



Support is indicated for branches by bootstrap frequencies exceeding 50% under the Neighbor Joining

Fig. 1 Phylogenetic tree of *P. monticola* and selected species of *P. ponojensis* group based on ITS nrDNA



A. general habit; B. marginal phyllidia with pruinose; C. pruinose on the surface; D. tufted rhizines

Fig. 2 *Peltigera monticola*.

darkened towards center; apothecia: not seen, said to be flat or saddle-shaped, up to 5 mm in diam.; pycnidia: not seen.

Chemistry: spot tests on the cortex and medulla K-, C-, KC-, P-.

Ecology and Distribution: *P. monticola* mainly grows among mosses over soil. Its world distribution includes North America, Europe and Asia[14]. At present this species is mainly recorded from Liupanshan which is located in Ningxia and Helanshan which is located in Ningxia and Inner Mongolia from the Northwest of China. Furthermore, it is mainly collected from forest zones in Nature Reserve of Ningxia and Inner Mongolia.

Notes: ITS results indicate that this taxon from the northwest of China is comprised of two geographically distinct lineages, but there is no obvious morphological difference between them, so two geographical populations of *P. monticola* are treated here as a taxon. The population from the northwest of China is sister to the specimens from Europe (Poland and Switzerland) with high support. We speculate it is due to their similar climatic conditions. *P. monticola* is identified by its laminal phyllidia along the margin or cracks, lobe ends with grey-whitish tomentose, tufted rhizines. The species is close to *P. ponojenis* basing on GenBank Blast, but the latter is distinguished by short of phyllidia and pale unbranched to fibrous, discrete rhizines.

Specimens examined: China, Prov. Ningxia, Guyuan, Liupanshan, Qiuqianjia, 1 718 m, on moss, 2012, 12-0016; Liupanshan, Erlonghe, 2 039 m, on moss, 2012, 12-0017; Helanshan,

Suyukou, 2 086 m, on moss, 2013, 13-0005; 13-0004; Prov. Inner Mongolia: Helanshan, South Temple, 2 000 m, on moss, 2013, 13-0002; 13-0003. — (specimens are deposited in NXAC). France: Lorraine, La Madeleine, Fusboesch, 345 m, on soil, 2008, 07. 27. 02/8. — (specimens are deposited in Duke University Herbarium).

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