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Peltigera shennongjiana, a new cyanolichen from Central China

Liu-Fu HAN, Jing-Yuan YANG, Shu-Qing BEI and Shou-Yu GUO

Abstract: As part of our ongoing research on *Peltigera*, we recognize a morphologically and phylogenetically distinct new species, *Peltigera shennongjiana* L. F. Han & S. Y. Guo, from the Shennongjia region of Central China. It is distinguished from other members of the *P. canina*-group by the presence of abundant phyllidia and flat, branched lobules along the margin or laminal cracks, short lobes, and a pruinose, usually greyish upper surface. The various populations sampled share identical ITS nr DNA sequences, of which the ITS2 regions are characterized by a unique secondary structure. Furthermore, we provide a detailed comparison of the characteristics of *P. shennongjiana* with morphologically similar species and a key to *Peltigera* species reported from China.

Key words: ITS, lichen flora of China, *Peltigerales*, secondary structure, Shennongjia Mountain

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Introduction

The genus *Peltigera* Willd. (Lecanoromycetes: *Peltigerales*) includes terricolous and muscicolous, foliose lichens that normally occupy moist habitats and are widespread on most continents (Miadlikowska & Lutzoni 2000; Martínez *et al*. 2003; Han *et al*. 2013, 2015; Manoharan-Basil *et al*. 2016; Magain *et al*. 2017). *Peltigera* is characterized by the absence of a lower cortex, with numerous rhizines and veins running along the undersurface, and with most species containing cyanobacteria, with some containing green algae and producing cephalodia that contain cyanobacteria (Miadlikowska & Lutzoni 2000; Magain *et al*. 2017). *Peltigera* is one of the earliest circumscribed lichen genera with three species formally described by Linnaeus (1753), two of them reclassified by Willdenow (1787) in the new genus *Peltigera* (*P. aphthosa* (L.) Willd. and *P. canina* (L.) Willd., the type of the genus). Although the genus is readily recognized in the field by its large, rather soft, leafy appearance, it is nonetheless often difficult to distinguish species since the diversity and variation is still poorly understood.

The traditional taxonomy of *Peltigera* relied on vegetative features of the thallus, and sometimes on profiles of secondary chemistry (Miadlikowska & Lutzoni 2000). The genus has been relatively well studied in the Northern Hemisphere and may include at least 90 species worldwide (Goward *et al*. 1995; Kirk *et al*. 2008; Han *et al*. 2013, 2015; Manoharan-Basil *et al*. 2016). *Peltigera* was among the earliest lichen-forming taxa subjected to molecular phylogenetic studies (e.g. Goffinet & Miadlikowska 1999) and sequences of ITS and LSU nrDNA have been commonly used in phylogenetic analyses (e.g. Goffinet *et al*. 2003; O'Brien *et al*. 2009; Sérusiaux *et al*. 2009; Han *et al*. 2013, 2015; Manoharan-Basil *et al*. 2016).

Phylogenetic inferences from LSU nrDNA sequences combined with morphological and chemical characters led to an infrageneric

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classification of the genus by Miadlikowska & Lutzoni (2000). A study assessing the reproductive isolation of *Peltigera* based on sequence data of the three loci (ITS nrDNA, β-tubulin and *RPB1*) indicated that the genus is more diverse in western North America than originally perceived, and that morphological diversity probably reflects the presence of undescribed species rather than evidence of hybridization or intraspecific variation (O'Brien *et al.* 2009). Recently, eight new species from the Eastern Hemisphere (Papua New Guinea and China) were described (Sérusiaux *et al.* 2009; Han *et al.* 2013, 2015) with *c.* 40 species reported in Asia in total (Martínez *et al.* 2003; Han *et al.* 2013, 2015; Niu *et al.* 2016).

Thirty species of *Peltigera* have been recorded from China (Chen 1986; Vitikainen 1986; Wei 1991; Stenroos *et al.* 1994; Wu & Liu 2012; Han *et al.* 2013, 2015; Niu *et al.* 2016). Of these, 15 species belong to section *Peltigera* (i.e. the *P. canina*-group). Two species, *P. meridiana* Gyeln and *P. subincusa* (Gyeln.) Inum, which were reported from Taiwan (*Catalogue of Life in Taiwan* 141456 & 141463, http://taibif.tw/zh/namecode/), require critical evaluation but voucher specimens are not available for examination. As part of an ongoing study of *Peltigera* species in China, we discovered an apparently new species based on its morphological characteristics, and we confirmed its monophyly using nrDNA ITS sequence data. We formally describe *Peltigera shennongjiana* below. Recently, the ITS2 region has been widely used for low-level phylogenetic analyses and distinguishing closely related species. Case studies have revealed that a compensatory base change (CBC) in the helix II or helix III ITS2 secondary structure between two organisms correlated with sexual incompatibility (Müller *et al.* 2007). Usually, if a CBC or more than four hemi-compensatory base changes (hCBCs) are found between organisms classified within the same genus, they are likely to belong to different species (Hershkovitz & Lewis 1996; Coleman 2007; Müller *et al.* 2007). Including ITS2 secondary structure information along with the primary sequence data can provide a further dimension for resolving the inherent problems in distinguishing closely related fungal species, including for lichen forming fungi (Piercey-Normore *et al.* 2006; Han *et al.* 2015). In the present study, we also compared the secondary structure of the nrDNA ITS2 of the new species with the potentially closest related species based on the sequence similarity, *P.* '*neorufescens*', (GenBank AY257916, Miadlikowska & Lutzoni 2000).

Materials and Methods

Specimens and morphology

All specimens were collected from forest in the Shennongjia region of Central China and were deposited in the Herbarium Mycologicum Academiae Sinicae-Lichenes (HMAS-L) and the Herbarium of Hebei Normal University (HBNU). Specimen examination was undertaken with a Motic SMZ-140 dissecting microscope and an Olympus CH compound microscope. Asci and ascospores were observed in cross-sections of apothecia cut by hand with a razor blade and mounted in water. Thin-layer chromatography (TLC) was performed for all specimens studied, following Orange *et al.* (2010) and using solvent systems C and G. For the terminology used in the descriptions, we followed Vitikainen (1994, 2004).

DNA extraction, PCR amplification and sequencing

Lobe tips were removed from 40 specimens selected for DNA extraction (including eight of the new species and 32 of known species in the *Peltigera canina*-group). DNA was extracted using the DNAsecure Plant DNA Kit (Tiangen, China) following the manufacturer's protocol. Amplification of the ITS region followed methods described in Han *et al.* (2013, 2015). The entire ITS region (ITS1, 5.8S and ITS2) of the nrDNA repeat tandem was targeted for PCR using the primers ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A -3', 22 nt; Gardes & Bruns 1993) and ITS4 (5'- TCC GCT TAT TGA TAT GC -3', 20 nt; White *et al.* 1990). The amplification reaction was performed in a 25 µl volume containing 0·75 units of TransStart Taq Polymerase (Tiangen, China), 2·5 µl of the manufacturer's buffer, 0·5 µl of each primer (5 µM), 2 µl (2 5 mM) deoxyribonucleotide triphosphate (dNTP mix), and 1 µl of genomic DNA. The conditions for thermocycling of the ITS region were as follows: 95 °C for 3 min followed by 35 cycles at 94 °C for 30 s, 54 °C for 30 s and 72°C for 1 min, with a final extension at 72 °C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide and sequenced by Genewiz Inc. (Beijing).

Forty newly obtained sequences were submitted to GenBank (see Table 1 & Fig. 1A & B for Accession numbers). The starting and end spacers of ITS1 and ITS2

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TABLE 1. *GenBank Accession numbers and voucher information for samples of* Peltigera *collected from China and used in the phylogenetic analysis in Fig. 1A & B. New species sequence information in bold.* ⋆ = *Type.*

Species	Sample No.	Voucher (locality, latitude and longitude, collectors and collecting no.)	GenBank Acc. no.
Peltigera canina	Guo 9	China: Mt Shennongjia (31·49°N 110·32°E), *Guo & Han* 21012	KT257166
P. canina	Seq 1	China: Mt Shennongjia (31·66°N 110·55°E), *Guo & Han* 20942	KX354693
P. canina	Seq 2	China: Mt Daxinganling (51·6°N 125·7°E), *Guo* 22507	KX354694
P. continentalis	Seq 12	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20943	KX354704
P. degenii	Seq 8	China: Mt Shennongjia (31·28°N 110·18°E), *Guo & Han* 21113	KX354700
P. degenii	Seq 9	China: Mt Shennongjia (31·28°N 110·18°E), *Guo & Han* 20958	KX354701
P. degenii	Seq 10	China: Mt Shennongjia (31·28°N 110·18°E), *Guo & Han* 20960	KX354702
P. degenii	Seq 11	China: Mt Shennongjia (31·28°N 110·18°E), *Guo* 21129	KX354703
P. didactyla	Seq 18	China: Mt Daxinganling (51·6°N 125·7°E), *Guo* 22813	KX354710
P. didactyla	Seq 19	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20955	KX354711
P. elisabethae	Seq 14	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20906	KX354706
P. elisabethae	Seq 15	China: Mt Shennongjia (31·27°N 110·13°E), *Guo & Han* 20987	KX354707
P. extenuata	Seq 20	China: Mt Daxinganling (51·51°N 121·51°E), *Guo* 22802	KX354712
P. extenuata	Seq 21	China: Mt Shennongjia (31·30°N 110·26°E), *Guo & Han* 20939	KX354713
P. horizontalis	Seq 16	China: Mt Daxinganling (51·56°N 120·51°E), *Guo* 23015	KX354708
P. malacea	Seq 17	China: Mt Daxinganling (51·53°N 123·2°E), *Guo* 22711	KX354709
P. monticola	Guo 10	China: Mt Shennongjia (31·46°N 110·45°E), *Guo & Han* 20920	KT257167
P. monticola	Seq 23	China: Mt Daxinganling (51·6°N 125·7°E), *Guo* 22504	KX354715
P. monticola	Seq 24	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20917	KX354716
P. monticola	Seq 25	China: Mt Shennongjia (31·30°N 110·26°E), *Guo & Han* 20911	KX354717
P. monticola	Seq 26	China: Mt Shennongjia (31·26°N 110·09°E), *Guo* 21119	KX354718
P. neckeri	Seq 13	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20941	KX354705
P. polydactylon	Seq 29	China: Mt Shennongjia (31·28°N 110·18°E), *Guo & Han* 20907	KX354721
P. polydactylon	Seq 30	China: Mt Shennongjia (31·28°N 110·18°E), *Guo* 21141	KX354722
P. ponojensis	Seq 22	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20980	KX354714
P. praetextata	Seq 3	China: Mt Shennongjia (31·26°N 110·18°E), *Guo & Han* 20944	KX354695
P. praetextata	Seq 4	China: Mt Shennongjia (31·27°N 110·13°E), *Guo & Han* 20993	KX354696
P. praetextata	Seq 5	China: Mt Shennongjia (31·49°N 110·32°E), *Guo & Han* 20971	KX354697
P. praetextata	Seq 6	China: Mt Shennongjia (31·28°N 110·18°E), *Guo & Han* 20982	KX354698
P. praetextata	Seq 7	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20996	KX354699
P. rufescens	Seq 27	China: Mt Shennongjia (31·29°N 110·18°E), *Guo & Han* 20936	KX354719
P. rufescens	Seq 28	China: Mt Daxinganling (51·51°N 121·51°E), *Guo* 22811	KX354720
P. shennongjiana	**Guo 1**	**China: Mt Shennongjia (31·49°N 110·32°E), *Guo & Han* 20979**	**KT257168**
P. shennongjiana	**Guo 2**	**China: Mt Shennongjia (31·40°N 110·56°E), *Guo & Han* 20830⋆**	**KT257169**
P. shennongjiana	**Guo 3**	**China: Mt Shennongjia (31·40°N 110·56°E), *Guo & Han* 20859**	**KT257170**
P. shennongjiana	**Guo 4**	**China: Mt Shennongjia (31·49°N 110·32°E), *Guo & Han* 20999**	**KT257171**
P. shennongjiana	**Guo 5**	**China: Mt Shennongjia (31·66°N 110·55°E), *Guo & Han* 20945**	**KT257172**
P. shennongjiana	**Guo 6**	**China: Mt Shennongjia (31·40°N 110·56°E), *Guo & Han* 20900**	**KT257173**
P. shennongjiana	**Guo 7**	**China: Mt Shennongjia (31·40°N 110·56°E), *Guo & Han* 20885**	**KT257174**
P. shennongjiana	**Guo 8**	**China: Mt Shennongjia (31·49°N 110·32°E), *Guo & Han* 20974**	**KT257175**

were determined by comparison with sequences available from GenBank (e.g. AY257890 *Peltigera continentalis* Vitik. and JX181776 *P. membranacea* (Ach.) Nyl.). The 3′ end of the small subunit (SSU) gene and the 5′ end of the large subunit (LSU) gene were excluded from the analyses. Sequences of the new species were aligned with representatives of the most similar taxa for which the ITS sequences were accessible from GenBank. The representative taxa were selected mainly according to morphological characters, sequence similarity from Blast searches in GenBank, and references (e.g. Miadlikowska & Lutzoni 2000; Goffinet *et al.* 2003;

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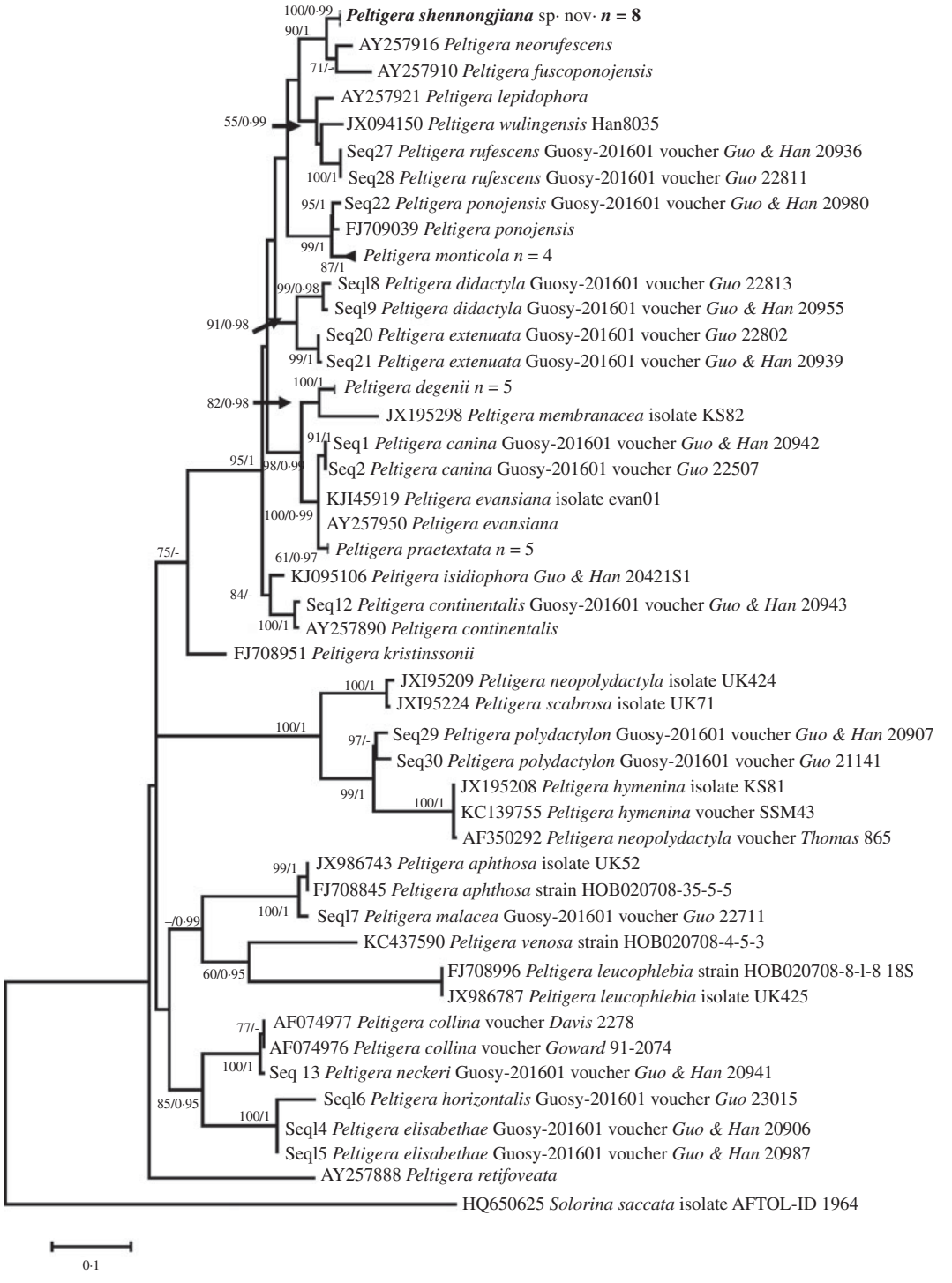

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Miadlikowska *et al.* 2003; Sérusiaux *et al.* 2009; Anstett *et al.* 2013; Han *et al.* 2013, 2015). The Accession numbers of the representative taxa obtained are provided in the trees (Fig. 1A & B).

Phylogenetic analysis and sequence comparing

The entire ITS sequences of the eight samples examined and 56 selected representatives were aligned using both ClustalW and Muscle implemented in MEGA v6 (Tamura *et al.* 2013). Ambiguously aligned regions, *sensu* Lutzoni *et al.* (2000) and Manoharan-Basil *et al.* (2016), were delimited manually and excluded from the phylogenetic analyses. We used PAUP* 4.0b10 (Swofford 2002) to detect the constant and parsimony-informative characters.

Phylogenetic relationships were inferred using the maximum probability method based on the GTR + G +I model in MEGA6, and Bayesian inference (Huelsenbeck & Ronquist 2001) also based on the GTR model with rates = Invgamma. The nucleotide substitution model parameters were estimated for the whole ITS region using the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.3 (Nylander 2004) and MEGA6. The analyses involved two data matrices containing 64 nucleotide sequences with 540 positions, and 20 nucleotide sequences with 412 positions, respectively. The secondary structures of the ITS2 sequences for the new species and its most related species were predicted by the ITS2 Database III (Koetschan *et al.* 2010) and illustrated with PseudoViewer3 (Byun & Han 2009).

Results and Discussion

Phylogenetic analysis and secondary structure of ITS2

The entire ITS region was successfully sequenced for 40 samples (including eight for the new species) of the collections from the Shennongjia forest region, Central China. Sequence length for the eight samples of the new species was an almost constant 637–638 bp for the entire ITS region (ITS1: 252–253 bp, 5.8S: 158 bp, ITS2: 226–227 bp). The ITS sequences of the new species, as well as those of 30 reference taxa of *Peltigera* in the *P. canina*- and related groups, were included in the phylogenetic analysis. The alignment of sequences from 64 taxa comprised 540 characters.

The Maximum Likelihood (ML) tree comprising all known species in Central China with bootstrap values (1000 replicates) and Bayesian posterior probabilities (BPP) at branches is shown in Fig. 1A. In the phylogenetic tree, the new species formed a clade with a group consisting of *P.* '*neorufescens*' and *P.* '*fuscoponojensis*' (Miadlikowska & Lutzoni 2000) with relatively strong support (ML = 90%, BPP = 1).

In order to demonstrate clearly the relationship between the new species and closely related taxa, an additional phylogenetic analysis including only eight ITS sequences of the new species and 12 reference sequences of *Peltigera* in the *P. canina*-group was performed. The data matrix comprised 20 taxa and 412 characters, of which 345 (83·74%) were constant and 58 (14·08%) were parsimony-informative.

The aligned dataset was analyzed using MrBayes for the Bayesian inference and MEGA6 for the maximum likelihood method. The ML tree with bootstrap values (1000 replicates) and Bayesian posterior probabilities (BPP) at branches is shown in Fig. 1B. In the phylogenetic tree, the new species formed a clade with the group consisting of *P.* '*neorufescens*' and *P.* '*fuscoponojensis*' with relatively strong support (ML = 95%, BPP = 0·96). The samples of the new species formed a clade with support of 99% (ML) and 1 (BPP).

A high-quality secondary structure model for the ITS2 sequence of the new species (KT257169, holotype) was derived from template GI32364223 (AY257916 *Peltigera* '*neorufescens*'). The structure shown in Fig. 2 is the computed structure. The calculated

FIG. 1A. Phylogenetic relationships of species in the *Peltigera canina*- and related groups sharing high similarity with *Peltigera shennongjiana* sp. nov. inferred from the ITS region. Support is indicated for branches with Maximum Likelihood (ML) bootstrap values > 50% (left of slash) and Bayesian posterior probabilities > 0·95 (right of slash). The analysis was based on 64 ITS sequences with 540 nucleotides (63 sequences from 31 species in *Peltigera*, with *Solorina saccata* as outgroup). Sequences obtained from GenBank are provided with Accession numbers and information for the remainder is given in Table 1. Individuals representing identical ITS sequences are indicated with '*n*'. The new species is indicated in bold.

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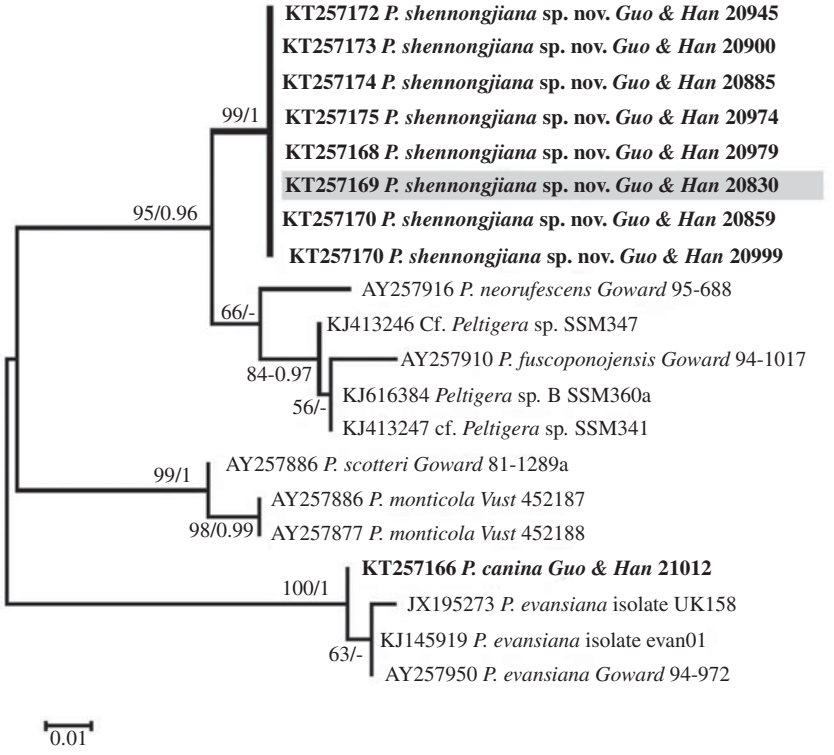

FIG. 1B. Phylogenetic relationships inferred from ITS region sequences of *Peltigera shennongjiana* sp. nov. and the most closely related species from the analysis in Fig. 1A. Support is indicated for branches with Maximum Likelihood (ML) bootstrap values > 50% (left of slash) and Bayesian posterior probabilities > 0·95 (right of slash). Branch lengths are calculated from the number of substitutions per site. Sequences obtained from GenBank are provided with Accession numbers and those from this study are indicated in bold. The holotype specimen is highlighted.

free energy (used to predict nucleic acid secondary structure) is −28·60 kcal mol^{-1} and −78·2 kcal mol^{-1} for *P. shennongjiana* and *P.* '*neorufescens*', respectively. Percentages of helix transfer are /100/100/89/96/ for four helices. The obvious differences of the ITS2 secondary structure can be observed when compared with the structural model of the closely related species. There are four and one hCBCs in the conserved part of helix III and helix IV, respectively, as well as three CBCs of helix IV for the new species and its template *P.* '*neorufescens*', which we interpret as support for the new taxon as a distinct species.

Therefore, we consider the clade comprising the phyllidiate specimens from Central China to represent a distinct species and so provide a formal description.

The Species

Peltigera shennongjiana L. F. Han & S. Y. Guo sp. nov.

MycoBank No.: MB 813433

The new species is allied to *Peltigera wulingensis* L.F. Han & S.Y. Guo. It can be distinguished from other members of the *P. canina*-group by the presence of abundant isidia, phyllidia and flat, branched lobules along the margin or laminal cracks. Thallus usually pruinose and tomentose above. Apothecia and lobes erect; discs brown; spores 3–6 septate, 32·0–58·0 × 2·0–6·0 µm.

Type: China, Hubei Province, Shennongjia forest region, Laojunshan Mountain, Jiuchong, 31°40′N, 110° 56′E, on mosses over rock and soil, alt. 900 m, 23 April 2014, *Shou-Yu Guo & Liu-Fu Han* 20830 (HMAS-L—holotype; GenBank KT257169).

(Fig. 3)

Thallus foliose, thin, rather fragile and easily broken apart when dry, approximately

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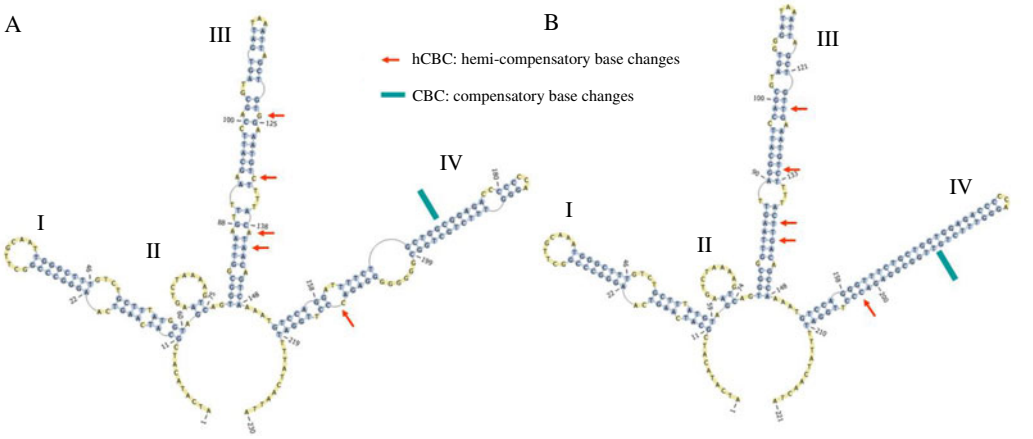

FIG. 2. The secondary structure models for an ITS2 sequence of the new species *Peltigera shennongjiana* (KT257169, holotype) (A), and one of its closest relatives *P.* '*neorufescens*' (AY257916) (B). The four helices are labelled (I-IV) and differences in ITS2 secondary structure are indicated. In colour online.

circular in outline, to 15 cm diam. Lobes flattened or crisped, imbricate or separate, 0·6–1·0 cm wide and 2·5 cm long. Lobe extremities rounded, often dentate and ascending, occasionally with tufts of tiny grey-whitish to pale brown hairs. Lateral margins usually becoming lacerated, with abundant phyllidia and flattened lobules. Phyllidia along the margin, few on laminal cracks, branched, becoming dorsiventral, spreading laterally, forming branched lobules. *Upper surface* grey, dark grey to greyish brown or dark brown when dry, blackish green to greyish brown when wet, pruinose; tiny white-grey tomentum present on the marginal parts of the lobes, which are otherwise smooth and occasionally somewhat shiny towards the centre; *soredia* absent. *Medulla* white, thin, loose. *Photobiont Nostoc*. *Lower surface* ecorticate, tomentose, white or pale near lobe tips, pale brown towards the centre, with anastomosing, elevated and narrow brown veins. *Rhizines* more or less abundant, white to brown, irregular, simple, bushy or rarely fasciculate, to 7·0 mm long.

Apothecia terminal on lobes, erect, saddle-shaped, to 4·0 mm diam. Apothecial margin smooth to crenulate. *Disc* red-brown to dark brown, rarely black, smooth, somewhat shiny. *Paraphyses* simple, septate. *Asci* clavate, 40·0–76·0 × 5·5–12·0 µm, *Peltigera*-type, colourless or pale green, 8-spored. *Ascospores* acicular, 3–6-septate, 32·0–58·0 × 2·0–6·0 µm.

Pycnidia not seen.

Chemistry. One unnamed triterpenoid was found in specimens of the new species by TLC in both solvent systems C (R_f = 39) and G (R_f = 50). The substance could not be identified despite comparing it with triterpenoids from reference samples of other species in the *P. canina*-group.

Etymology. The specific epithet refers to the type locality in Hubei Province, Central China.

Ecology and distribution. The new species grows on moss over rock and soil in a mountain forest. At present, the specimens of *Peltigera shennongjiana* are known only from Hubei Province, Central China.

Discussion. The specimens of *Peltigera shennongjiana* were obtained from the transitional region of the northern subtropical and warm temperate zone, which is also the transitional zone of the south-western high mountains

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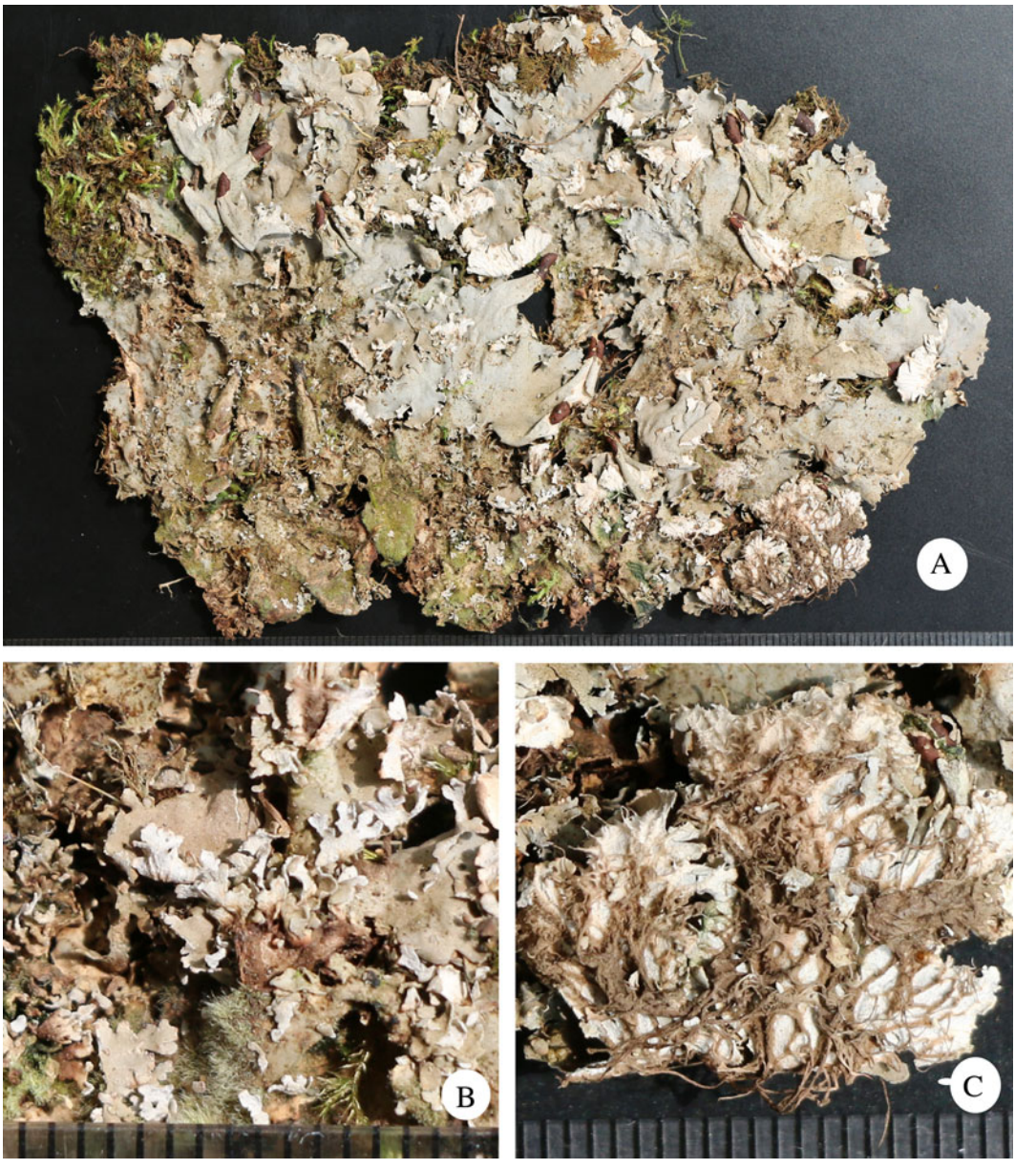

FIG. 3. *Peltigera shennongjiana* (holotype, *Guo & Han* 20830). A, upper surface and apothecia (dry); B, upper surface and marginal phyllidia (dry); C, lower surface and rhizines (dry). Scales = 1 mm. In colour online.

and the low hilly land of Central China. It is readily distinguished by its abundant phyllidia and branched lobules along the margin or laminal cracks, short lobes, pruinose and usually greyish upper surface, and the presence of an unnamed triterpenoid substance.

Peltigera shennongjiana resembles *P. wulingensis* in the presence of phyllidia along the

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TABLE 2. *A comparison of* Peltigera shennongjiana *with morphologically similar species.*

Character	*P. shennongjiana*	*P. wulingensis*	*P. canina*	*P. evansiana* Gyeln.	*P.* '*neorufescens*'	*P.* '*fuscoponojensis*'
Apothecial disc colour	Red-brown to dark brown	Not seen	Brown	Brown to pale brown	Brown	Not seen
Apothecium orientation	Vertical	Not seen	Vertical	Vertical	Vertical	Not seen
Apothecium habit	Folded downward	Not seen	Folded downward	Folded downward	Folded downward	Not seen
Pycnidium	Not seen	*Peltigera* type	*Peltigera* type	Not seen	*Peltigera* type	*Peltigera* type
Upper cortex tomentum	Tiny white-grey	Thin white-grey	Grey, dense at margin	Grey, mainly at margin	Present	Present
Upper cortex scabrosity	Absent	Present	Absent or sparse	Absent or sparse	Absent	Absent
Pruina on upper surface	Present	Not seen	Present	Present	Not seen	Present
Soralia	Absent	Absent	Absent	Absent	Absent	Absent
Isidia or phyllidia	Present	Present	Absent	Present	Absent	Absent
Veins	Raised, narrow	Slightly raised or flattened	Raised or flattened	Raised	Raised	Raised
Rhizines	Bushy, long	Rarely branched, short	Confluent, simple or other	Separate, not fasciculate	Confluent, fibrillose	Separate, simple
Chemistry	Terpenoids present	Terpenoids present	Not detected by TLC	Not detected by TLC	Not detected by TLC	Not detected by TLC
Distribution	Asia	Asia	Worldwide	Asia and North America	North America	North America
Reference	This study	Han *et al.* 2013	Miadlikowska & Lutzoni 2000	Miadlikowska & Lutzoni 2000; Han *et al.* 2015	Miadlikowska & Lutzoni 2000	Miadlikowska & Lutzoni 2000

lobe margins and laminal cracks but differs by the broader lobes (1·5–2·5 cm vs. 0·3–0·8 cm); meanwhile, *P. wulingensis* lacks branched lobules and apothecia (Han *et al.* 2013). For the ITS sequence data, however, the identity between them was 92%, with only 61% of query cover and a high E value ($1e^{-145}$).

DNA sequences that support a close relationship may also exhibit similar secondary structures. The new species is sister to a clade including *P.* '*neorufescens*' in the phylogenetic trees (Fig. 1A & B) and has similar secondary structure models for ITS2 sequences (Fig. 2.). The new species might also resemble *P.* '*neorufescens*', which is also tomentose and esorediate, but it is readily distinguished from the latter by the pruina and phyllidia, and the presence of terpenoids. Previous studies have distinguished species of *Peltigera* on the basis of monophyly in single locus (ITS) phylogenies as well as diagnostic morphological features (Goffinet & Miadlikowska 1999; Goward & Goffinet 2000; Goffinet *et al.* 2003; Miadlikowska *et al.* 2003; Han *et al.* 2013, 2015). In this study, we additionally provide the secondary structure models for ITS2 sequences, which should also be regarded as useful characters to differentiate species of lichens (Piercey-Normore *et al.* 2006; Liu & Guo 2009; Cao *et al.* 2011; Guo 2013; Han *et al.* 2015). The threshold range between intraspecific and interspecific percentages of helix transfer was 90–95% for at least one of the four helices (I–IV), especially in the helices II and III (Schultz *et al.* 2005; Guo 2013). In our case, the new species lacks three pairs of the compensatory bases in the conserved part of helix IV, and there was one percentage of helix transfer less than 90% and 5 hCBCs in the conserved part of helix III and helix IV for the new species and its template *P.* '*neorufescens*'.

In conclusion, the distinctive morphology and monophyly of the phyllidiate specimens from Hubei Province support their recognition as a new species.

Additional specimens examined (all samples are housed in HMAS-L and HBNU). **China:** *Hubei Province*: Shennongjia forest region, Laojunshan Mountain, Jiuchong, 31°40′N, 110°56′E, on mosses over rock and soil, alt. 900 m, 2014, *Shou-Yu Guo & Liu-Fu Han* 20859, 20885, 20900; Yunpanling, 31°66′N, 110°55′E, on mosses over soil, alt. 1800 m, 2014, *Shou-Yu Guo & Liu-Fu Han* 20945; Dalongtan, 31°49′N, 110°32′E, on mosses over rock and soil, alt. 2100 m, 2014, *Shou-Yu Guo & Liu-Fu Han* 20974, 20979, 20999.

A summary of the characteristics of *Peltigera shennongjiana* and morphologically similar species is provided in Table 2, and a key to all 31 species from mainland China (15 from Shennongjia) is provided below.

Key to species of *Peltigera* in mainland China

1 Main photobiont a green alga; cephalodia containing a cyanobacterium present 2
Main photobiont a cyanobacterium; cephalodia containing a cyanobacterium absent 5

2(1) Thallus small, averaging <2 cm across, and attached by a single point along the margin; cephalodia present on lower surface; rhizines absent . . . . . **P. venosa**
Thallus large, averaging <2 cm across; cephalodia present on upper surface . . 3

3(2) Apothecia horizontal; lower surface veins distinct . . . . . **P. nigripunctata**
Apothecia vertical; lower surface veins distinct or indistinct 4

4(3) Lower surface veins indistinct; rhizines separate and distinct; apothecia corticated on lower surface . . . . . **P. aphthosa**
Lower surface veins distinct; rhizines forming an intricately branched and anastomosing mat; apothecia ecorticated or poorly corticated on lower surface . . . . . **P. leucophlebia**

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5(1) Upper surface tomentose, at least at the margins 6
Upper surface smooth or scabrose, lacking tomentum 21

6(5) Upper surface sorediate; soralia orbicular and laminal 7
Upper surface esorediate 8

7(6) Lobes elongate and confluent, rhizines fasciculate to fibrillose, brush-like at the tips . . . . . **P. extenuata**
Lobes round and separate, rhizines simple to loosely branched, not fibrillose . . . . . **P. didactyla**

8(6) Isidia or phyllidia present 9
Isidia or phyllidia absent 16

9(8) Isidia laminal, dorsiventral, peltate, more or less appressed; lobes usually under 1 cm wide; apothecia uncommon . . . . . **P. lepidophora**
Isidia not peltate, more or less vertical 10

10(9) Isidia cylindrical and laminal, rarely dorsiventral; lobe tips downturned . . . . . **P. evansiana**
Isidia phyllidiate, laminal or marginal, dorsiventral 11

11(10) Isidia mainly laminal, clustered isidia grow in pits of the upper surface . . . . . **P. isidiophora**
Isidia marginal 12

12(11) Upper surface scabrose, dull, epruinose; apothecia not seen . . . . **P. wulingensis**
Upper surface smooth, somewhat shiny towards the centre, pruinose or epruinose; apothecia saddle-shaped 13

13(12) Upper surface pruinose; lobes less than 1 cm wide 14
Upper surface epruinose; lobes often over 1 cm wide 15

14(13) Flat branched lobules along the margin or laminal cracks; rhizines simple, fasciculate, penicillate or flocculent, often confluent . . . . . **P. shennongjiana**
Lobules not branched; rhizines fasciculate, little branched, discrete **P. monticola**

15(13) Lower side dark brown to black; rhizines richly branched . . . . . **P. continentalis**
Lower side pale to brownish; rhizines simple . . . . . **P. praetextata**

16(8) Upper surface scabrose; veins with tomentum near centre of thallus . . . . . **P. kristinssonii**
Upper surface smooth 17

17(16) Lobe tips upturned 18
Lobe tips downturned 20

18(17) Upper surface epruinose; rhizines mostly discrete 19
Upper surface often pruinose; rhizines becoming confluent towards thallus centre . . . . . **P. rufescens**

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19(18 Lower surface white or pale; veins narrow and raised; rhizines white, simple or irregularly branched . . . . . **P. ponojensis**
Lower surface whitish or pale tan; veins absent or with few and very broad veins, rhizines sparse, fasciculate . . . . . **P. malacea**

20(17) Rhizines squarrosely branched; medulla *c.* 150 µm thick . . . . **P. membranacea**
Rhizines simple to confluently branched; medulla 300–500 µm thick . . . . **P. canina**

21(5) Marginal and laminal soredia present; rhizines bushy or penicillate branched; flattened veins . . . . . **P. collina**
Soredia never present 22

22(21) Upper surface scabrose 23
Upper surface smooth 25

23(22) Upper surface indistinctly scabrose or in patches; lower surface with simple or fasciculate rhizines, elongate; apothecia vertical . . . . . **P. neopolydactyla**
Upper surface distinctly scabrose; lower surface with fasciculate rhizines, proportionally short 24

24(23) Lower surface with indistinct veins; apothecia vertical . . . . . **P. scabrosa**
Lower surface with darker colour, more prominent veins; apothecia horizontal . . . . . **P. dolichospora**

25(22) Isidia, phyllidia or schizidia present 26
Isidia, phyllidia or schizidia never distinctly present 28

26(25) Veins broad and indistinct; apothecia horizontal; with schizidia or frequently lobulate . . . . . **P. elisabethae**
Veins broad or narrow, distinct; apothecia vertical 27

27(26) Veins narrow and raised, pale or pale brown towards centre; marginal lobules poorly developed . . . . . **P. degenii**
Veins broad and flattened, dark brownish towards centre; margins often dilacerate and lobulate . . . . . **P. polydactylon**

28(25) Rhizines arranged in concentric circles; apothecia horizontal . . . **P. horizontalis**
Rhizines not arranged in concentric circles; apothecia vertical 29

29(28) Upper surface epruinose 30
Upper surface pruinose 31

30(29) Lower surface brownish, rhizines <5 mm long, veins indistinct . . **P. hymenina**
Lower surface brown to black, rhizines >5 mm long, veins distinct and wide . . . . . **P. neopolydactyla**

31(29) Veins distinct and wide; apothecial disc brown to dark brown . . . . **P. pruinosa**
Veins indistinct; apothecial disc dark brown to black . . . . . **P. neckeri**

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Supplementary Material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S0024282919000355

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