

## Notes on rust fungi in China 7. *Aecidium caulophylli* life cycle inferred from phylogenetic evidence and renamed as *Puccinia caulophylli* comb. nov.

JING-XIN JI<sup>1\*</sup>, ZHUANG LI<sup>2</sup>, YU LI<sup>1</sup>, MAKOTO KAKISHIMA<sup>1, 3,4\*</sup>

<sup>1</sup> Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, Jilin 130118, China

<sup>2</sup> College of Plant Pathology, Shandong Agricultural University, Tai'an 271000, China

<sup>3</sup> College of Forestry, Beijing Forestry University, Beijing 100083, China

<sup>4</sup> University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

\* CORRESPONDENCE TO: <sup>a</sup>1096314395@qq.com <sup>b</sup> kakishima.makoto.ga@u.tsukuba.ac.jp

**ABSTRACT**—Spermogonial and aecial stages of *Aecidium caulophylli* on *Caulophyllum robustum* (Berberidaceae) were shown by phylogenetic analyses of ITS and 28S sequence data to be identical to a uredinial and telial rust on *Milium effusum* (Poaceae). A new combination, *Puccinia caulophylli* is proposed for this species, and an epitype is designated.

**KEY WORDS**—Pucciniaceae, Pucciniales, taxonomy

## Introduction

*Aecidium caulophylli* was described in 1898 based on spermogonial and aecial stages of a specimen on *Caulophyllum robustum* (Berberidaceae) collected by V. Komarov in Amur, Siberia, Russian Far East, during June 1895 (Saccardo & Sydow 1902). This rust has been reported from China, Russian Far East, and Japan (Miura 1928, Ito 1950, Tai 1979, Harada 1984, Hiratsuka & al. 1992, Azbukina 2005). However, its uredinial and telial stages have not been clearly demonstrated, although Azbukina (1984, 2005) listed this species as spermogonial and aecial stages of *Puccinia brachypodii* var. *poae-nemoralis* (G.H. Otth) Cummins & H.C. Greene [= *P. poae-nemoralis* G.H. Otth], without any evidence.

TABLE 1. Sequence data analyzed in this study

SPECIES	HOST PLANT	LOCALITY IN CHINA	DATE	VOUCHER SPECIMEN	GENBANK ACCESSION NO.	
					ITS	28S
<i>Puccinia caulophylli</i>	<i>Caulophyllum robustum</i>	Hongyegu, Jiaohe, Jilin, Jilin	24-Jun-2015	HMJAU8531	MK785259	MK785284
	<i>C. robustum</i>	Lafa Mountain, Jiaohe, Jilin, Jilin	23-Jun-2015	HMJAU8532	MK785260	MK785284
	<i>C. robustum</i>	Bangchuigu, Jiaohe, Jilin, Jilin	1-Jul-2018	HMJAU8624	MK785261	MK785284
	<i>C. robustum</i>	Qingling, Jiaohe, Jilin, Jilin	29-Jun-2017	HMJAU8620	MK785262	MK785284
	<i>C. robustum</i>	Hongyegu, Jiaohe, Jilin, Jilin	24-Jun-2015	HMJAU8534	MK785273	MK785284
	<i>Milium effusum</i>	Fenghuang Mountain, Wuchang, Heilongjiang	5-Jul-2016	HMJAU8619	MK785271	MK785284
	<i>M. effusum</i>	Changbai Mountain, Erdaobaihe, Yanbian, Jilin	28-Jul-2015	HMJAU8618	MK785272	MK785284
	<i>M. effusum</i>	Lushuihe Forest Park, Baishan, Jilin	2-Sep-2018	HMJAU8625	MK785274	MK785284
	<i>M. effusum</i>	Bangchuigu, Jiaohe, Jilin, Jilin	12-Sep-2017	HMJAU8622	MK785275	MK785284
	<i>M. effusum</i>	Erdaobaihe, Yanbian, Jilin	2-Sep-2018	HMJAU8627	MK785276	MK785284
	<i>M. effusum</i>	Lafa Mountain, Jiaohe, Jilin, Jilin	1-Jul-2018	HMJAU8623	MK785277	MK785284
	<i>M. effusum</i>	Lushuihe Forest Park, Baishan, Jilin	3-Sep-2018	HMJAU8626	MK785278	MK785284
	<i>M. effusum</i>	Fenghuang Mountain, Wuchang, Heilongjiang	9-Sep-2017	HMJAU8621	MK785279	MK785284
<i>Puccinia adenocauli</i>	<i>Carex onoei</i>	Qingling, Jiaohe, Jilin, Jilin	12-May-2018	HMJAU8630	MK785267	MK785284
	<i>C. onoei</i>	Jilin Agricultural University, Changchun, Jilin	2-Jul-2017	HMJAU8274	MK785268	MK785284
	<i>Adenocaulon himalaicum</i>	Bangchuigu, Jiaohe, Jilin, Jilin	1-Jul-2018	HMJAU8629	MK785269	MK785284
	<i>A. himalaicum</i>	Fenghuang Mountain, Wuchang, Heilongjiang	23-Jun-2017	HMJAU8628	MK785270	MK785284
<i>Puccinia klukistanum</i>	<i>Ligustrum obtusifolium</i>	Jilin Agricultural University, Changchun, Jilin	24-Jun-2018	HMJAU8633	MK785263	MK785284
	<i>L. obtusifolium</i>	Jilin Agricultural University, Changchun, Jilin	12-Jun-2015	HMJAU8198	MK785264	MK785284
	<i>L. obtusifolium</i>	Jingyuetan Forest Park, Changchun, Jilin	25-Jun-2017	HMJAU8631	MK785266	MK785284
	<i>Cleistogenes hackelii</i>	Jilin Agricultural University, Changchun, Jilin	28-Aug-2016	HMJAU8282	MK785265	MK785284
	<i>C. hackelii</i>	Jilin Agricultural University, Changchun, Jilin	9-Jul-2016	HMJAU8280	MK785280	MK785284
	<i>C. hackelii</i>	Jingyuetan Forest Park, Changchun, Jilin	16-Sep-2017	HMJAU8632	MK785281	MK785284
<i>Gymnosporangium yamadae</i>	<i>Malus baccata</i>	Jilin Agricultural University, Changchun, Jilin	10-Sep-2014	HMJAU8096	MK785282	MK785284
<i>Gymnosporangium asiaticum</i>	<i>Pyrus</i> sp.	Wunvfeng Forest Park, Jian, Tonghua, Jilin	8-Jun-2016	HMJAU8324	MK785283	MK785284

During an investigation of rust fungi in Jilin Province, China, during 2015–2018, *A. caulophylli* was observed on *C. robustum*. Near the infected *C. robustum*, uredinial and telial stages of *Puccinia* species were also observed on plants of *Poaceae* and *Cyperaceae*. We suspected that this rust may have an heteromacrocytic life cycle, producing uredinia and telia on one of these plants. Inoculation of plants with spores is an appropriate method to clarify rust fungus life cycles (Ji & al. 2017a,b), but often plant collection may be restricted in conservation areas. As it may be difficult to supply appropriate growth conditions after transplantation, molecular analyses have been applied to clarify rust life cycles (Liu & Hambleton 2013, Ji & al. 2016, Padamsee & McKenzie 2017, Scholler & al. 2019). We report here the results of phylogenetic and morphological analyses using spermogonial/aecial specimens on *A. caulophylli* and potentially related uredinial/telial specimens on *Poaceae* and *Cyperaceae*.

## Materials & methods

### Molecular analyses

Spermogonial (0) and aecial (I) stages of *A. caulophylli* on *C. robustum* were collected in Jiaohe, Jilin Province, China, and used for molecular analyses. Rust specimens on *Milium effusum* (*Poaceae*) were collected in several areas in Jilin and Heilongjiang Provinces, China, as preliminary phylogenetic analyses suggested they represented the uredinial (II) and telial (III) stages of *A. caulophylli*. The specimens were collected during surveys of rust fungi in Jilin Province from 2015 to 2018. For comparative analyses, sequences were included from two life cycles that previously clarified by inoculations (Ji & al. 2017a,b): (1) *Puccinia klugkistiana* (Dietel) Jing X. Ji & Kakish. on *Ligustrum obtusifolium* Siebold & Zucc. (0, I) and *Cleistogenes hackelii* (Honda) Honda (II, III), collected in Changchun, Jilin Province; and (2) *P. adenocauli* (Syd. & P. Syd.) Jing X. Ji & Kakish. on *Adenocaulon himalaicum* Edgew. (0, I) and *Carex onoei* Franch. & Sav. (II, III).

Total genomic DNA was directly extracted from about 200 spores obtained from single sori on the leaves of each specimen, using similar methods reported by Ji & al. (2016, 2019). Specimens used in the experiments were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU) and all data sequenced in this experiment were deposited at GenBank (TABLE 1).

Sequences from the specimens were aligned following Ji & al. (2019). ITS and 28S sequence data retrieved from GenBank were added to phylogenetic analyses. Accession numbers for these data are shown in the phylogenetic trees (FIGS 1, 2). Phylogenetic trees were constructed with the sequences of *Gymnosporangium yamadae* Miyabe ex G. Yamada and *G. asiaticum* Miyabe ex G. Yamada as outgroup,

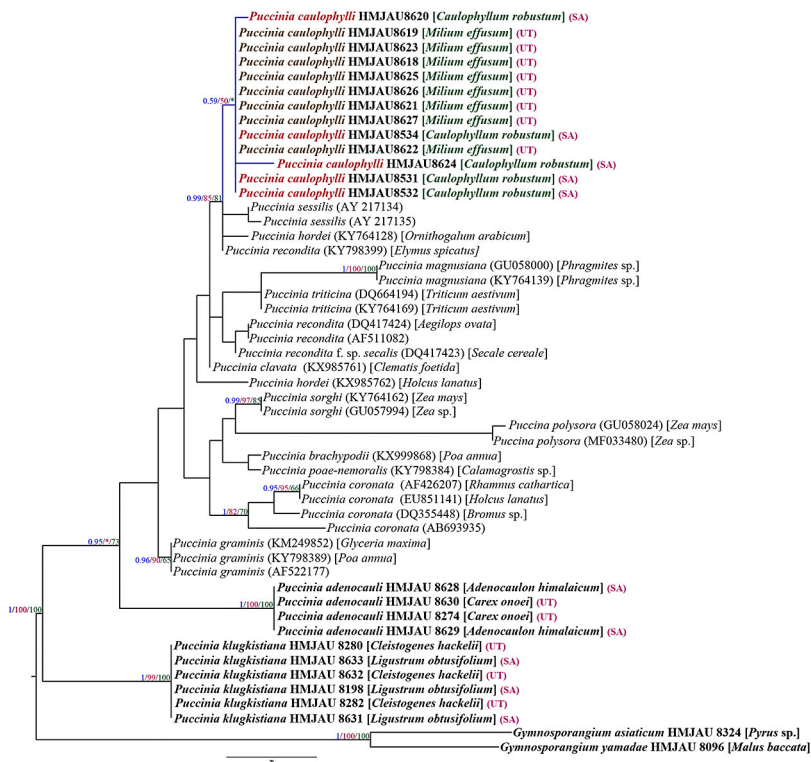


FIG. 1. Phylogenetic tree constructed by MP method based on sequences of 28S regions of rDNA. Bootstrap values of MP and ML are followed by the Bayesian posterior probabilities (BPP) on the nodes in the topology. Asterisk (\*) represents bootstrap values <50% or BPP <0.5 in the topology. Sample data are shown with species name, voucher specimen number or GenBank accession number (in parentheses), and host plant. Sequence data determined in this study are shown in bold face. SA: Spermatogonial and aecial stages, UT: Uredinial and telial stages.

according to Ji & al. (2019). The alignment and trees were deposited in TreeBase under <http://purl.org/phylo/treebase/phyloids/study/TB2:S24307> (FIG. 1) and TB2:S24308 (FIG. 2).

### Morphological observations

Light (LM) and scanning electron (SEM) microscopy were used to examine morphological characters of rust specimens including the size and shape of sori and spores following Ji & al. (2019).

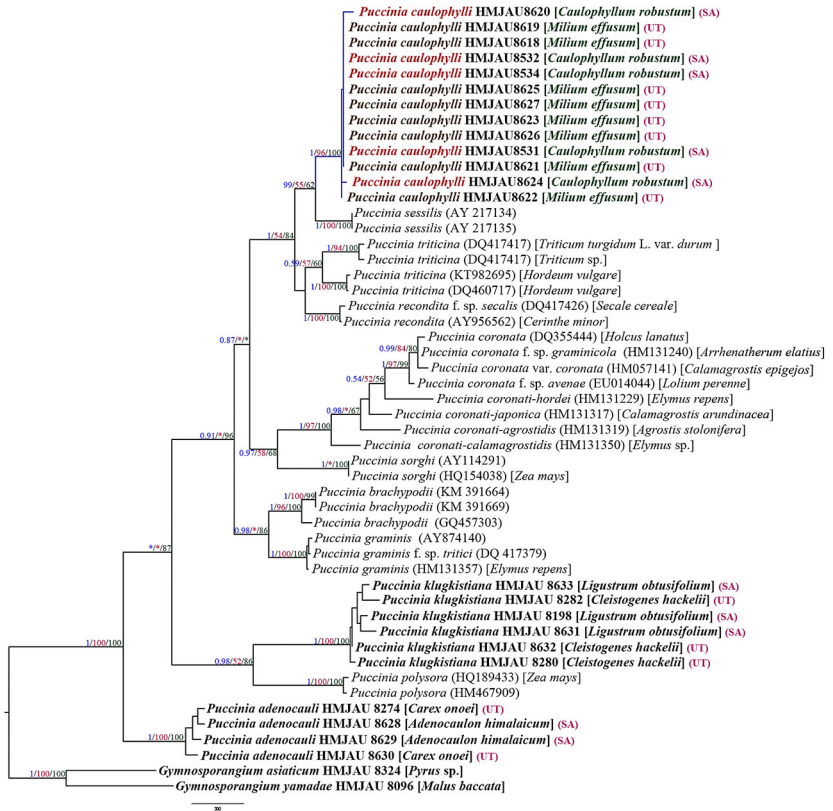


FIG. 2. Phylogenetic tree constructed by MP method based on ITS regions of rDNA. Bootstrap values of MP and ML are followed by the Bayesian posterior probabilities (BPP) on the nodes in the topology. Asterisk (\*) represents bootstrap values <50% or BPP <0.5 in the topology. Sample data are shown with species name, voucher specimen number or GenBank accession number (in parentheses), and host plant. Sequence data determined in this study are shown in bold face. SA: Spermatophyte and asexual stages, UT: Uredinial and telial stages

## Results & discussion

### Phylogeny and life cycle

The 28S dataset comprised 51 sequences of 50 taxa with 525 total characters, including 430 constant characters, 25 parsimony-uninformative variable characters, and 70 parsimony-informative characters. Parsimony analysis yielded one parsimonious tree with TL = 159, CI = 0.679, RI = 0.864

and RC = 0.587. Bayesian analysis resulted in average standard deviation of split frequencies of 0.005869. The final ITS dataset comprised 51 sequences of 50 taxa with 820 total characters, of which 336 were parsimony-informative. Parsimony analysis yielded one parsimonious tree with TL = 995, CI = 0.623, RI = 0.840 and RC = 0.523. Bayesian analysis resulted in average standard deviation of split frequencies of 0.004251. Tree topologies formed by MP, ML, and MCMC methods were identical among trees. The phylogenetic trees generated through Bayesian analysis are shown in FIG. 1 (28S) and FIG. 2 (ITS).

Both 28S and ITS phylogenetic trees placed spermogonial and aecial stages on *C. robustum* (HMJAU 8531, 8532, 8534, 8620, 8624) and uredinial and telial stages on *M. effusum* (HMJAU 8618, 8619, 8621, 8622, 8623, 8625, 8626, 8627) within a monophyletic clade (FIGS 1, 2). All stages of *P. klugkistiana* and *P. adenocauli* were placed in separate monophyletic clades, confirming that heteroecious life cycles of rust fungi can be revealed by phylogenetic analyses as demonstrated by Liu & Hambleton (2013), Padamsee & McKenzie (2017), and Scholler & al. (2019). The phylogenetic analyses also supported the life cycle connection between the rust on *C. robustum* and the rust on *M. effusum*.

Although the *Caulophyllum/Milium* rust was shown to be phylogenetically close to *P. sessilis* W.G. Schneid. ex J. Schröt., *P. triticina* Erickss., and *P. recondita* Roberge ex Desm., these three rusts inhabit different host plants. This rust is also genetically distant from *P. brachypodii* G.H. Oth and *P. poae-nemoralis*, two other telial rusts reported on *Milium* spp. (Cummins 1971, Zhuang & al. 1998, Azbukina 2005). Therefore, we conclude that this rust is distinct from other species.

### Morphology & taxonomy

From the phylogenetic analyses, the spermogonia and aecia on *A. caulophylli* and the uredinia and telia on *M. effusum* are produced by one and the same heteromacrocytic rust species. LM and SEM observations showed that overall morphology of the rust on *C. robustum* is identical with that of *A. caulophylli* described by Saccardo & Sydow (1902), Ito (1950), Harada (1984), and Hiratsuka & al. (1992) (FIGS 3, 5A,B). The morphologies of uredinial and telial specimens on *M. effusum* were similar to each other (FIGS 4, 5C–E), and the two-celled teliospores refer the rust to the genus *Puccinia* (Cummins & Hiratsuka 2003).

Specimens on *Milium effusum* are phylogenetically close to *P. sessilis*, but urediniospores on *M. effusum* are bigger than those of *P. sessilis* (20–32



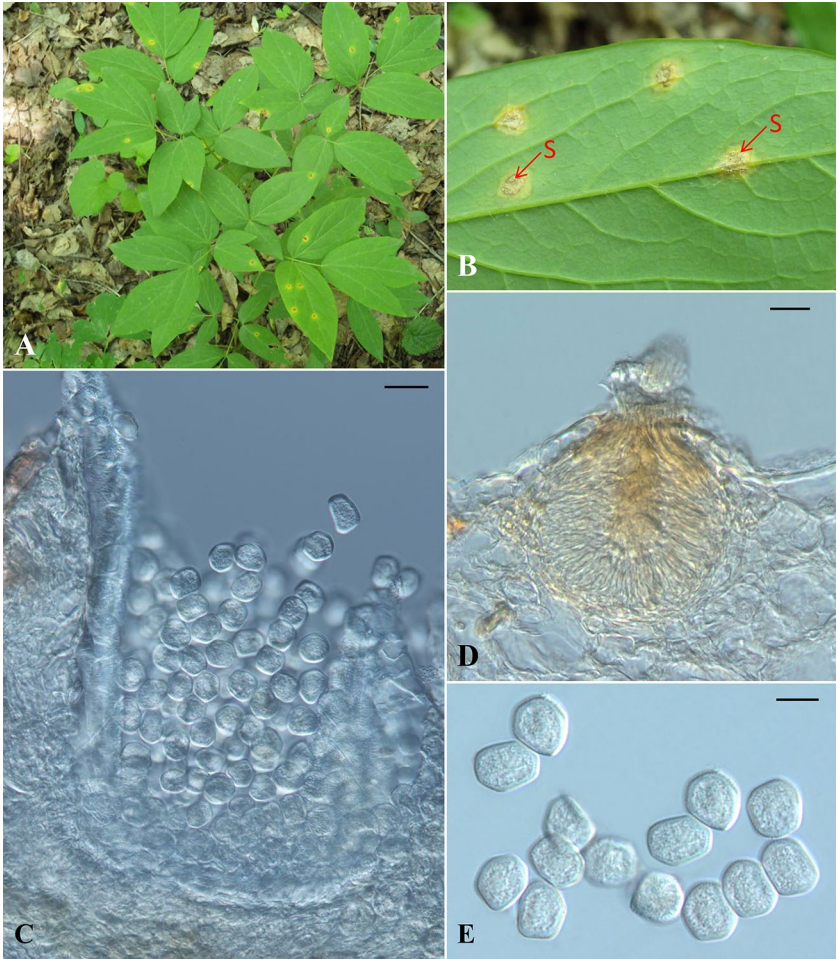


FIG. 3. *Puccinia caulophylli* on *Caulophyllum robustum*: spermogonial and aecial stages. A. Yellow lesions on the leaves producing spermogonia and aecia; B. Aecia produced around spermogonia on lower surface of the plant; C. Catenulate aeciospores surrounded by peridium in a vertical section of an aecium; D. Vertical section of a spermogonium; E. Aeciospores. Scale bars: C, D = 30  $\mu$ m; E = 15  $\mu$ m.

$\times 19\text{--}25\text{ }\mu\text{m}$ ), and teliospores are smaller than those of *P. sessilis* ( $32\text{--}58 \times 13\text{--}20\text{ }\mu\text{m}$ ). Additionally, the rust on *M. effusum* has uredinial paraphyses that have not been reported in *P. sessilis* (Hiratsuka & al. 1992, Zhuang & al. 1998). *P. brachypodii* var. *poae-nemoralis* occurs on *M. effusum* (Cummins 1971, Zhuang & al. 1998) and its telial structures and teliospores are

morphologically similar to the present rust fungus. However, urediniospores of the current rust are bigger than those of *P. brachypodii* var. *poae-nemoralis* (18–25 × 15–23 µm). The aecial stage of *P. brachypodii* var. *poae-nemoralis* occurs on *Berberis* spp. (Cummins 1971).

*Aecidium caulophylli* is a legitimate name under the INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI, AND PLANTS (Shenzhen Code, Art. F.8, 2018). However, the application of an asexual name, such as *Aecidium*, to a sexual species can cause confusion (Ono 2016). Therefore, we propose a new combination in *Puccinia* for *A. caulophylli*. The holotype specimen on *C. robustum* has only spermogonial and aecial stages of the rust; because the uredinial and telial stages define the rust genus, we designate an epitype specimen on *M. effusum*.

***Puccinia caulophylli* (Kom.) Jing X. Ji & Kakish., comb. nov.**

FIGS 3–5

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≡ *Aecidium caulophylli* Kom., in Jaczewski & al., Fungi Rossiae Exsicc. 4: no 176, 1898.

TYPES: Russian Federation, Russian Far East, Siberia, Amur, Mt. Burejenses, stages 0, I on *Caulophyllum robustum* Maxim. [≡ *C. thalictroides* subsp. *robustum* (Maxim.) Kitam.], June 1895, leg. V. Komarov (holotype, LE; isotype, NY 00610976). China, Jilin Province, Yanbian, *Erdaobaihe*, stages II, III on *Milium effusum* L., 2 September 2018, leg. J.X. Ji & M. Kakishima (epitype designated here, HMJAU 8627; MBT 386779).

**SPERMOGONIA** amphigenous, pale yellow to yellowish brown, subepidermal, type 4 of Cummins & Hiratsuka (2003). **AECIA** hypophyllous, yellow, subepidermal, erumpent, *Aecidium*-type with firmly connected peridia. **AECIOSPORES** catenulate, subglobose, ovate to ellipsoid, 14–24 × 13.5–20 µm (av. 18.5 × 15.5 µm), walls hyaline, 0.5–2 µm thick (av. 1 µm), densely verrucose.

**UREDINIA** mostly hypophyllous, pale yellow to cinnamon-brown, subepidermal, erumpent, with abundant peripheral and intermixed paraphyses. **PARAPHYSES** cylindric to capitate, 16.5–41 × 3–9.5 µm (av. 30 × 5 µm), walls hyaline, 0.5–1.5 µm thick (av. 1 µm). **UREDINIOSPORES** pedicellate, globose to subglobose, 22–35 × 21–30.5 µm (av. 30 × 27 µm), walls hyaline or pale yellow, 1–3.5 µm thick (av. 1.5 µm), echinulate, germ pores obscure. **TELIA** mostly hypophyllous, dark brown to black, subepidermal, covered by epidermis, without paraphyses. **TELIOPORES** 2-celled by transverse septum, borne singly on pedicels, clavate to oblong, with round to obtuse apex and attenuate towards base, 28.5–40.5 × 9.5–17 µm (av. 35.5 × 13 µm), walls pale brown to dark brown, 0.5–1.5 µm thick at sides (av. 1 µm), 1–5 µm thick at apex (av. 2.5 µm), smooth; pedicels short, hyaline.



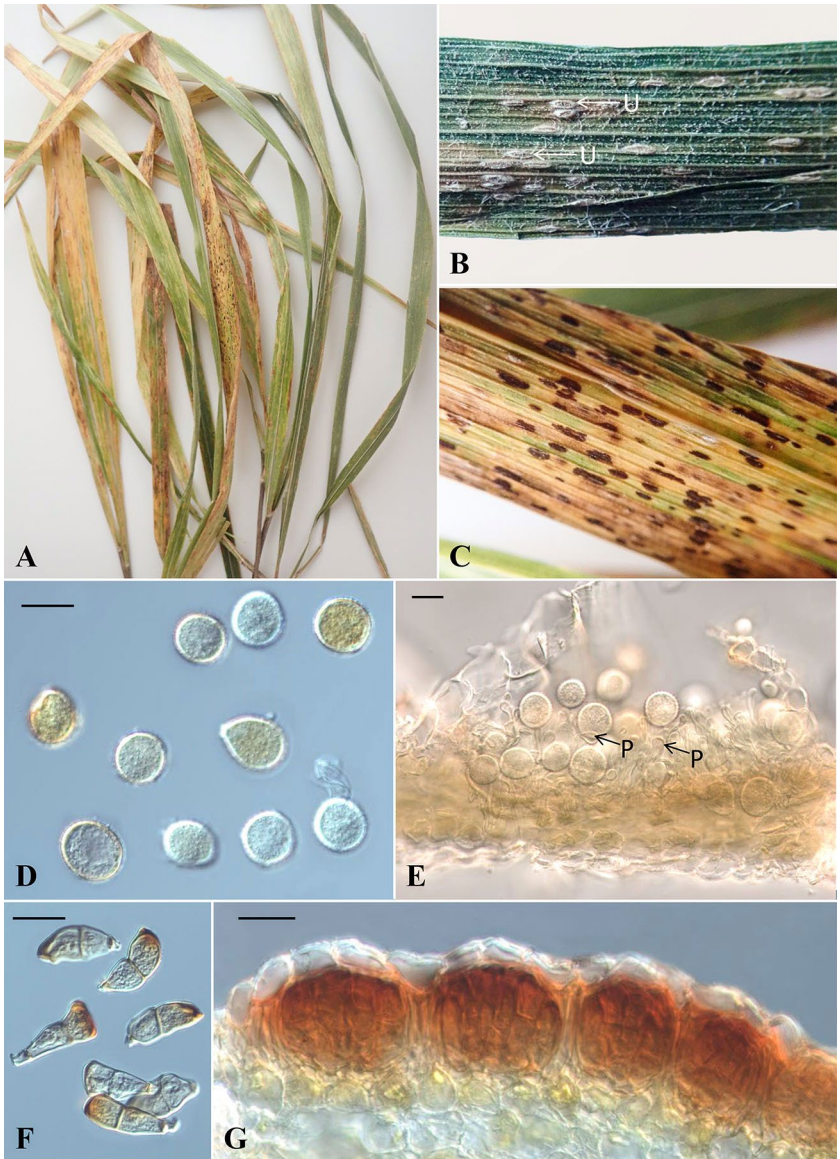


FIG. 4. *Puccinia caulophylli* on *Milium effusum*: uredinial and telial stages. A. Uredinia and telia produced on the leaves; B. Pale yellow uredinia (U) on lower leaf surface; C. Dark brown telia on lower leaf surface; D. Echinulate urediniospores; E. Vertical section of uredinium with urediniospores and paraphyses (P); F. Teliospores; G. Vertical section of telia covered by host epidermis. Scale bars: D, E = 30  $\mu$ m; F, G = 20  $\mu$ m.

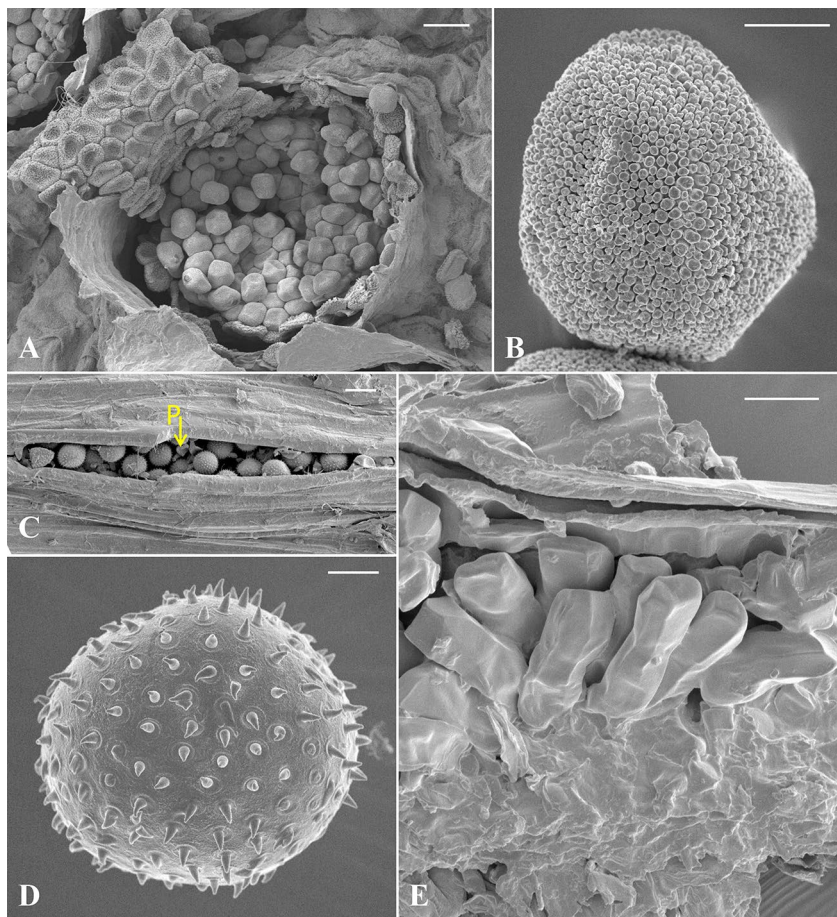


FIG. 5. *Puccinia caulophylli* observed by SEM. A. Aecium with catenulate aeciospores and peridium; B. Aeciospore with densely verrucose surface; C. Uredinium with urediniospores and paraphyses (P); D. Echinulate urediniospore; E. Vertical section of a telium covered by host epidermis. Scale bars: A = 30  $\mu$ m; B, D = 5  $\mu$ m; C, E = 20  $\mu$ m.

ADDITIONAL SPECIMENS EXAMINED—Stages 0, I on *Caulophyllum robustum*: CHINA: JILIN PROVINCE, Jilin, 23 June 2015 (HMJAU 853); 24 June 2015 (HMJAU 8531, HMJAU 8534); 29 June 2017 (HMJAU 8620); 1 July 2018 (HMJAU 8624).

Stages II, III on *Milium effusum*: CHINA: JILIN PROVINCE, Jilin, 12 September 2017 (HMJAU 8622); 1 July 2018 (HMJAU 86232). Yanbian, 28 July 2015 (HMJAU 8618); Baishan, 2 September 2018 (HMJAU 8625); 3 September 2018 (HMJAU 8626). HEILONGJIANG PROVINCE: Wuchang, 5 July 2016 (HMJAU 8619); 9 September 2017 (HMJAU 8621).

HOSTS & DISTRIBUTION—Stages 0, I on *Caulophyllum robustum*: China (Miura 1928, Tai 1979), Japan (Ito 1950, Harada 1984, Hiratsuka & al. 1992). Stages II, III on *Milium effusum*: China.

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