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Notes on rust fungi in China 6. Distribution of *Puccinia punctiformis* and occurrence of its albino teliospores

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Abstract—A rust fungus producing systemic infections on *Cirsium arvense* in China was confirmed as *Puccinia punctiformis,* although previously in China this fungus had been treated as *P. calcitrapae* var. *centaureae*. Its morphology is described based on Chinese specimens and its wide distribution in China is clarified by herbarium specimens. In field observations in Jilin Province, albino teliospores were found. The identity of the albino spores with *P. punctiformis* was confirmed by molecular analyses.

Key words—abnormal spore, *Compositae, Pucciniomycetes,* taxonomy, *Uredinales*

Introduction

Puccinia punctiformis is a common rust fungus that has been reported on *Cirsium arvense* in many parts of the world (Wilson & Henderson 1966, Cummins 1978, Hiratsuka & al. 1992, Azbukina 2005, Termorshuizen & Swertz 2011). This fungus causes a systemic infection and produces spermogonia, aecia, uredinia, and telia on the single host, although aecia and uredinia are morphologically similar (Wilson & Henderson 1966, Cummins 1978, Hiratsuka & al. 1992). The fungus survives in rhizomes and produces a sweet odor during formation of spermogonia. However, its life cycle has not been investigated sufficiently (Wilson & Henderson

1966, French & Lightfield 1990, Wandeler & Bacher 2006, Termorshuizen & Swertz 2011). Because its host plant, *C. arvense,* is a noxious perennial weed the fungus has been investigated as a biological control agent (Thomas & al. 1994, Wandeler & Bacher 2006), although with little success (Termorshuizen & Swertz 2011).

In China, the rust on *C. arvense* has been treated as *Puccinia calcitrapae* var. *centaureae* (DC.) Cummins (Tai 1979, Wei & Wang 1986, Zhuang 2003). Recently, abundant infection of a rust fungus on *C. arvense* was observed in the campus of Jilin Agricultural University, Changchun, Jilin Province, China. Dark brown telia on the surface of plant leaves were frequently observed, but spermogonia and aecia/uredinia were rare. The rust on *C. arvense* was morphologically similar to *P. punctiformis* and, therefore, we carried out morphological observations to confirm its identity. We also borrowed rust specimens deposited as *P. calcitrapae* var. *centaureae* on *C. arvense* in HMAS Fungarium, Beijing, China. The results of our morphological observations of a rust fungus on *C. arvense* and its distribution in China are reported. During field surveys, a rust fungus on *C. arvense* with albino systemic telia was found on leaves of one plant in June 2017. Its identity with a rust fungus producing dark brown telia on *C. arvense* was confirmed by molecular analyses.

Materials & methods

Rust specimens on *Cirsium arvense* were collected in Changchun, Jilin Province, China, from 2015 to 2017 and used for morphological observations. These specimens 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). Rust specimens on *C. arvense* were also borrowed from the Fungarium, Institute of Microbiology Chinese Academy of Sciences (HMAS), for comparative morphology.

Light (LM) and scanning electron (SEM) microscopy were used to examine morphological characters including the size and shape of sori and spores, following the method reported by Ji & al. (2017b).

The identities of the dark brown and albino teliospores on *C. arvense* collected in the campus of Jilin Agricultural University, were confirmed through molecular analyses as described by Ji & al. (2017a). Total genomic DNA was separately extracted from dark brown and albino teliospores, and rDNA-ITS region was amplified and sequenced. Sequence data were deposited in GenBank.

Fig. 1. *Puccinia punctiformis:* spermogonial and aecial stages. A, C. Systemic spermogonia produced over whole leaf surface of Cirsium arvense; B. Vertical section of a spermogonium; D. Aeciospores with brown walls; E. Spermogonia and uredinoid aecia on the lower leaf surface; F. Vertical section of a uredinoid aecium; G. Uredinoid aecium (SEM); H. Aeciospores with spines (SEM). Scale bars: B, F, G = 50 µm; D = 30 µm; H= 5 µm.

Results & discussion

Spermogonial, aecial, and telial stages of the rust on *C. arvense* collected in Jilin Province were identified as *P. punctiformis* based on the morphological similarity with the descriptions by Wilson & Henderson (1966), Cummins (1978), Hiratsuka (1980), Hiratsuka & al. (1992), Azbukina (2005), and Termorshuizen & Swertz (2011) (Figs 1, 2). These stages were systemically scattered over the entire leaf surface. Following the spermogonial stage, pedicellate spores were produced in aecia without a peridium or sori called uredinoid aecia (Figs 1E, F). However, uredinia produced by infection of aeciospores were not confirmed in the specimens because of the similarity of aecial and uredinial stages. Most specimens on the synonymous *Cephalonoplos segetum* [≡ *Carduus segetum*], *Cirsium setosum*, and *C. arvense* [≡ *Carduus arvensis*], deposited in HMAS under the name *P. calcitrapae* var. *centaureae*, were morphologically identified as *P. punctiformis* based on their telia covering most of the leaf surface and teliospores that were shorter than those of *P. calcitrapae* var. *centaureae* (33–42 × 20–24 µm; Cummins 1978, Hiratsuka 1980, Hiratsuka & al. 1992). These *P. punctiformis* specimens were collected from a broad area of China.

Albino teliospores found on leaves of a single *C. arvense* plant were completely hyaline, including the walls (Figs 3C, D). Around this plant many plants producing the normal dark brown systemic telia were also found. Teliospores in dark brown telia were brown to dark brown. Except for the wall color, telia and teliospores of the albino form were morphologically similar to the dark brown form. The teliospore surfaces in both forms were finely verrucose (Figs 2F, 3F), but there were fewer and smaller verrucae in the albino form. The rDNA ITS sequences obtained from the two forms showed 99.7% similarity, with a sequence difference of 2 bp among 586 bp (albino form: HMJAU8555, GenBank MF397930; dark brown form: HMJAU8556, GenBank MF397931). The albino fungus was therefore confirmed as *P. punctiformis*. There were no other sequence data of this rust in GenBank. The occurrence of albino spores has been reported in smut fungi (Fischer & Holton 1957, Thomas 1984) and mushrooms (Murakami & Takemaru 1990). Albino spores apparently are rarely found in rust fungi, although there are reports of albino aeciospores in *Peridermium* (Mielke & Peterson 1967, Christenson 1969) and albino urediniospores in *Puccinia graminis* f. *tritici* Erikss. & Henning (Newton & Johnson 1927). This is the first report of albino teliospores in a rust fungus. We suspect that the albino

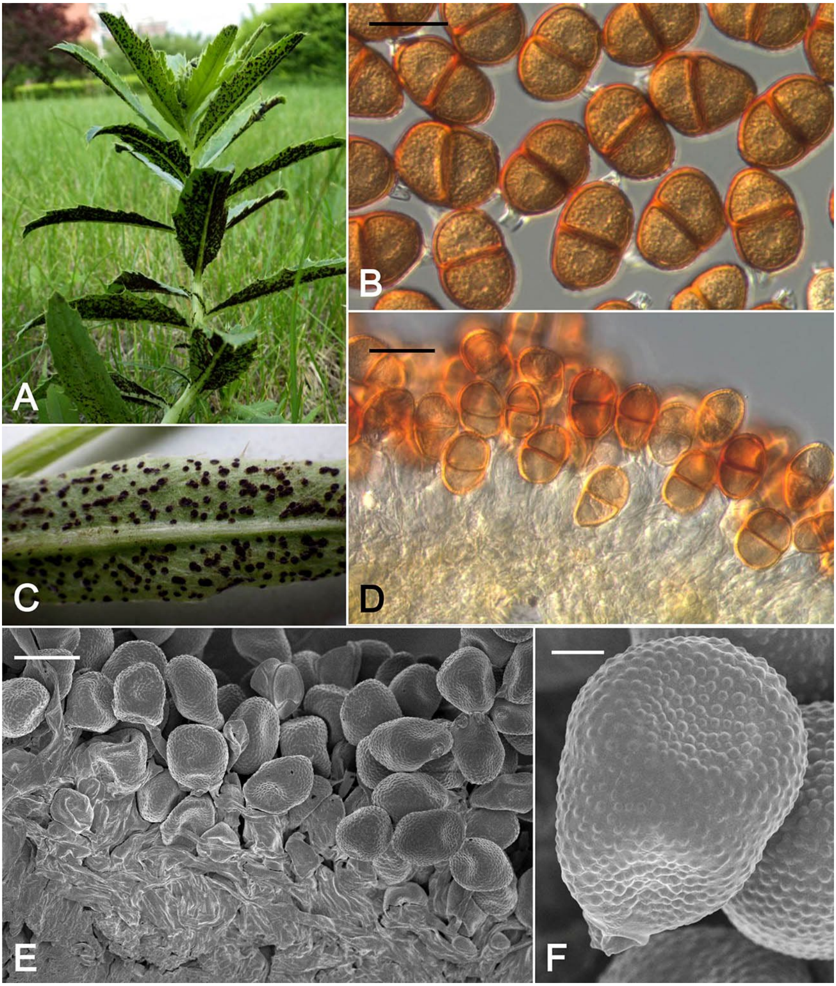

Fig. 2. *Puccinia punctiformis* telial stage. A, C. Systemic telia produced on the lower leaf surface of *Cirsium arvense;* B. Teliospores with dark brown walls; D. Vertical section of a telium; E. Telium (SEM); F. Teliospores with small verrucae (SEM). Scale bars: B, E = 30 µm; D = 40 µm; F = 5 µm.

form resulted from a mutation in a dark brown form, although further investigation is required. As neither teliospore form germinated after maturation, it is difficult to determine the functional differences between the two forms, including basidial formation and infection of plants.

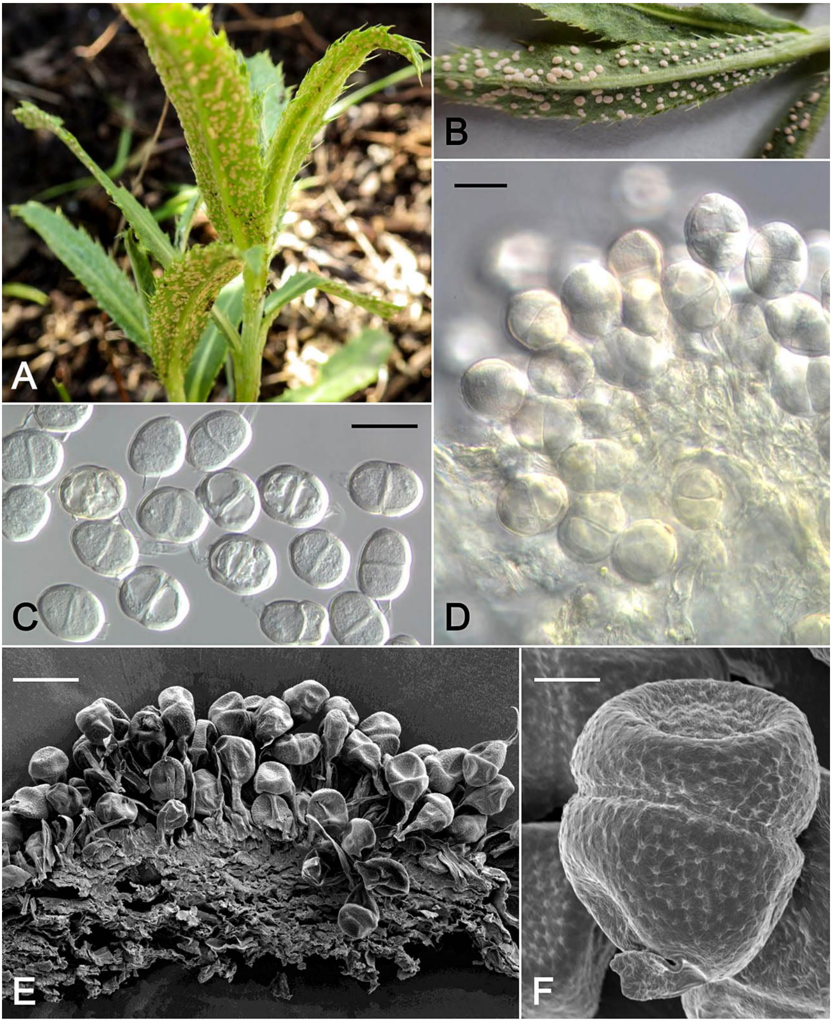

Fig. 3. *Puccinia punctiformis:* albino form. A, B. Systemic telia produced on the lower leaf surface of *Cirsium arvense;* C. Teliospores with hyaline walls; D. Vertical section of a telium; E. Telium (SEM); F: Teliospores with small verrucae (SEM). Scale bars: C, D = 35 µm; E = 30 µm; F = 5 µm.

Morphological features of this rust, including the albino form, and its distribution is provided in the following description, based on specimens collected in China.

Puccinia punctiformis (F. Strauss) Röhl.,
Deutschl. Fl., Ed. 2, 3(3): 131, 1813. FIGS 1–3

Spermogonia systemic with sweet odor, amphigenous, minute, scattered over whole leaf surface, orange-yellow, flask-shaped, type 4 of Cummins & Hiratsuka (2003). Aecia produced after spermogonia, systemic, amphigenous, pulverulent, brown, uredinoid, scattered over whole leaf surface. Aeciospores pedicellate, globoid, 18.5–25 × 16.5–23.5 µm (av. 22.5 × 20.5 µm); walls brown, mostly echinulate, 0.8–1.8 µm (av. 1.1 µm) thick; germ pores mostly 3, scattered. Uredinia and urediniospores could not be confirmed. Telia systemic, amphigenous, dark brown to black, scattered over whole leaf surface, first covered by epidermis, later pulverulent. Teliospores pedicellate, ellipsoid to broadly ellipsoid, 28–37.5 × 20–29 µm (av. 32.5 × 23.5 µm); walls brown, 1.5–3 µm (av. 2 µm) thick, verrucose with round and small verrucae; pedicels hyaline, deciduous, short. Albino telia white, similar to dark brown telia except in color. Albino teliospores pedicellate, ellipsoid to broadly ellipsoid, 27–38 × 16–28.5 µm (av. 32.5 × 24.5 µm); walls hyaline, 1–3 µm (av. 2 µm) thick, sparsely verrucose with minute verrucae; pedicels hyaline, deciduous, short.

SPECIMENS EXAMINED—On *Cirsium arvense* (L.) Scop.: **CHINA: JILIN PROVINCE**, 12 June 2015 (HMJAU8586); 3 September 2015 (HMJAU8587); 11 September 2015 (HMJAU8594); 12 June 2016 (HMJAU8593); 21 June 2016 (HMJAU8589); 24 June 2016 (HMJAU8592); 27 June 2016 (HMJAU8590); August 2016 (HMJAU8588); 21 May 2017 (albino telia: HMJAU855, GenBank MF397930; dark brown telia: HMJAU8556, GenBank MF397931); 4 September 2017 (HMJAU8591); 25 June 1950 (HMAS42950); **HENAN PROVINCE**, 13 June 1935 (HMAS08023); **JIANGSU PROVINCE**, April 1926 (HMAS12086); June 1932 (HMAS12023); 21 May 1929 (HMAS03343); 10 April 1924 (HMAS14498); 9 June 1932 (HMAS14381); 18 July 1930 (HMAS08025); **SHANDONG PROVINCE**, 13 June 1936 (HMAS14500); **SHANXI PROVINCE**, 30 June 1936 (HMAS14499); 23 August 1958 (HMAS36600); 26 May 1956 (HMAS36602); 21 July 1957 (HMAS36601); 16 September 1974 (HMAS36604); 5 May 1974 (HMAS36603); **SICHUAN PROVINCE**, 16 April 1938 (HMAS14497); 14 September 1989 (HMAS63912); **GUIZHOU PROVINCE**, April 1994 (HMAS73852); **GANSU PROVINCE**, 17 June 1943 (HMAS12022); 3 July 1958 (HMAS22374); **BEIJING MUNICIPALITY**, 5 November 1947 (HMAS12943); 1 August 1973 (HMAS36605); 27 May 1959 (HMAS49654); 16 June 2004 (HMAS172340, 172341); **LIAONING PROVINCE**, 13 July 1955 (HMAS17902); 2 May 1950 (HMAS22365); **XINJIANG PROVINCE**, 22 July 1959 (HMAS34490); 3 August 1977 (HMAS37762); 15 June 1959 (HMAS49655); **HEBEI PROVINCE**, 16 August 1964 (HMAS35363); 28 June 1964 (HMAS49658); **SHAANXI PROVINCE**, 26 June 1973 (HMAS54388); 15 June 1973 (HMAS54389); 4 April 1937 (HMAS22369); 28 April 1940 (HMAS22370); **TIBET PLATEAU**, 1 August 2012 (HMAS244560–244566).

HOSTS & DISTRIBUTION IN CHINA—On *Cirsium arvense* [= *Cephalonoplos segetum* (Bunge) Kitam.; = *Cirsium setosum* (Willd.) Besser ex M. Bieb.]: Beijing, Gansu, Guizhou, Hebei, Henan, Jiangsu, Jilin, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, Tibet [Xizang], Xinjiang (Wei & Wang 1986, Zhuang 2003).

Puccinia punctiformis is widely distributed in China, where it was first recorded in 1924. This rust will presumably be found anywhere its host is present. However, in our Jilin Province field survey suggests that its occurrence and life cycle are affected by temperature and growing condition of its host.

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