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Thekopsora ostryae (Pucciniastraceae, Pucciniales), a new species from Gansu, northwestern China

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ABSTRACT

Thekopsora ostryae, a new rust fungus on leaves of *Ostrya japonica* collected from Gansu Province was described. Morphological examination using light and scanning electron microscopy showed that this new species is distinct from other species of *Thekopsora* in the characteristics of ostiolar peridial cells of uredinia and the spinules on the surface of urediniospores. Analyses of the ITS1-5.8S-ITS2 and 28S rRNA gene partial sequences showed that *T. ostryae* could be a distinct lineage in the genus *Thekopsora*.

1. Introduction

The rust genus *Thekopsora* Magnus was established in 1875 with *Thekopsora areolata* (Fr.) Magnus on *Prunus padus* L. as the type species. It was separated from *Pucciniastrum* sensu lato and related genus mainly based on the position of telia in the host epidermis and their ontogeny (Pady 1933; Hiratsuka 1936; Ziller 1974; Cummins and Hiratsuka 1983). *Thekopsora* produces telia within the epidermal cells, and germ pores 1 in each cell at the center of spores balls, while *Pucciniastrum* G.H. Otth and *Naohidemyces* S. Sato, Katsuya & Y. Hirats. produce them underneath the epidermis of host plants (Pady 1933; Hiratsuka 1936; Cummins and Hiratsuka 1983, 2003; Sato et al. 1993).

The species of *Thekopsora* are delimited based primarily on the telial host range, surface structure of uredial ostiolar cell and urediniospores size (Hiratsuka 1927, 1958; Sato et al. 1993). Thirteen species of *Thekopsora* have been described worldwide with six of them having been reported in China (Tai 1979; Cao and Li 1996, 1999; Chen 1999; Zhuang and Wei 2003; Zhuang

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Table 1 – *Thekopsora* species and GenBank accession numbers used in this study.

Fungal taxon	Host plant	Specimen no.	GenBank accession no.	
			ITS	28S
Thekopsora ostryae Y.M. Liang & T. Yang.	*Ostrya japonica*	HMBF-GS-78.1[a]	KC415796	KC416004
		HMBF-GS-78.2[a]	KC415797	KC416005
		HMBF-GS-78.3[a]	KC415798	KC416006
		HMBF-GS-129.1[a]	KC415787	KC415993
		HMBF-GS-129.2[a]	KC415788	KC415994
		HMBF-GS-155	–	–
T. rubiae Kom.	*Rubia chinensis*	HMBF-GS-52.3[a]	KC415791	–
	R. cordifolia	HMBF-GS-157.1[a]	–	KC415997
		HMBF-GS-157.2[a]	–	KC415998
		HMBF-XZ-1.1[a]	KC415802	KC416009
		HMBF-XZ-1.2[a]	KC415803	KC416010
		HMBF-XZ-1.3[a]	KC415804	–
		HMBF-QH-1.1[a]	KC415799	KC416007
		HMBF-QH-1.2[a]	KC415800	KC416008
		HMBF-QH-1.3[a]	KC415801	–
T. nipponica Hirats. f.	*Galium davuricum*	HMBF-GS-53.1[a]	KC415792	KC416001
		HMBF-GS-53.2[a]	–	KC416002
	G. aparine	HMBF-GS-54.1[a]	KC415793	KC416003
T. nipponica Hirats. f.	*G. aparine*	HMBF-GS-54.2[a]	KC415794	–
		HMBF-GS-54.3[a]	KC415795	–
		HMBF-GS-156.1[a]	–	KC415995
		HMBF-GS-156.2[a]	–	KC415996
T. guttata (J. Schröt.) P. Syd. & Syd.	*Galium odoratum*	HMBF-GS-52.1[a]	KC415789	KC415999
		HMBF-GS-52.2[a]	KC415790	KC416000
		–	–	AF426231[b]
T. minima (Arthur) Syd. & P. Syd.	*Vaccinium corymbosum*	–	–	HM439777[b]
		–	–	GU355675[b]
T. symphyti (DC.) J. Müll.	*Symphytum officinale*	–	–	AF426230[b]
T. areolata (Fr.) Magnus	*Picea excels*	–	EF363336[b]	–
		–	DQ087230[b]	–
Melampsoridium betulinum (Pers.) Kleb.	*Salix* sp.	–	EU391657[b]	DQ354561[b]

[a] The number after the point stands for different monoclonal of this specimen.
[b] Sequences from GenBank.

2005; Liu et al. 2006; Zhuang 2006). Of the 13 species, the telial stage of three species occurs on Ericaceae, three on Rubiaceae, and seven on other dicotyledonous families.

During our investigation of rust fungi in Gansu Province, northwestern China, a previously unknown species of *Thekopsora* on *Ostrya japonica* Sarg. (Betulaceae) was found. This study is conducted to confirm the taxonomic status of the new species through morphological examination and phylogenetic analyses using the internal transcribed spacer region (ITS1-5.8S-ITS2) and 28S rRNA gene partial sequences.

2. Materials and methods

2.1. *Morphological examination*

Specimens used for this study were collected in Diebu County, Gansu Province, northwestern of China, and were deposited at Mycological Herbarium, Beijing Forestry University, Beijing (HMBF) (Table 1).

For light microscopy, urediniospores and hand sections of telia were mounted in a drop of lactophenol-cotton blue solution. For each specimen, about thirty spores were randomly chosen and examined for the morphological characteristics, including urediniospore size, shape and the arrangement of germ pore, by using Leica 4D07 (Leica, Berlin, Germany). The dimensions of urediniospores were measured by Microview MVC TWAIN Image Analyzer software (Leica).

The surface structure of urediniospores and the uredinial ostiolar cells were examined using scanning electron microscope (SEM). For SEM, segments of leaves with uredinia and urediniospores were attached on aluminum stubs covered with double-adhesive tapes, and coated with platinum-palladium by Hitachi SCD-005 Sputter Coater, then examined using a Hitachi S-4200 scanning electron microscope operated at 5.0 kV (Hitachi, Tokyo, Japan).

2.2. *DNA extraction and PCR amplification*

The total genomic DNA from the urediniospores was extracted following the method described in Liang et al. (2006). Some presumed related sequences and outgroup members were obtained from GenBank. All the fungal taxa used in this study are listed in Table 1.

ITS region (ITS1-5.8S-ITS2 and 28S) of the rRNA gene was amplified by using the primer pairs ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns 1993) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990), and NL1