Mycoscience (2005) 46:143–147
DOI 10.1007/s10267-004-0228-2

FULL PAPER

Patricia E. Crane · Yuichi Yamaoka
Jintana Engkhaninun · Makoto Kakishima

Caeoma tsukubaense n. sp., a rhododendron rust fungus of Japan and southern Asia, and its relationship to *Chrysomyxa rhododendri*

Received: September 30, 2004 / Accepted: December 28, 2004

Abstract A rust fungus found in Japan on *Rhododendron kaempferi*, *R. kiusianum*, and *R. dauricum* has previously been identified as *Chrysomyxa rhododendri*. Light and scanning electron microscopy of fresh and herbarium materials of the rust fungus, however, show that the spore surface morphology differs from the urediniospores of *C. rhododendri*, and the spores are slightly smaller. Furthermore, the DNA sequence of the 5′-end of the large subunit of ribosomal DNA differs from that of *C. rhododendri* by 3%. Telia have not been found; therefore, it is redescribed as a new anamorphic species, *Caeoma tsukubaense*. Several specimens from North Korea, Tibet, and Nepal bearing a similar rust fungus are also included in the species.

Key words *Caeoma tsukubaense* · *Chrysomyxa rhododendri* · Ericaceae · Rust fungus · Taxonomy · Uredinales · Uredinia

Introduction

Rhododendrons are cultivated worldwide, but most species originate in Asia. In spite of the economic and horticultural importance of these plants, the diversity and basic biology of rust fungi (Basidiomycota: Uredinales) infecting Asian rhododendrons are inadequately known. Most rust fungi of plants in the family Ericaceae, including rhododendrons, are in the genus *Chrysomyxa* or are anamorphic species that probably belong in that genus. Confusion exists among the rust fungi in this group because descriptions often lack sufficient detail for identification. Scanning electron microscopy is needed to elucidate the unique spore surface morphology of many taxa.

In Japan, two rust species have been reported on hosts in three different subgenera of *Rhododendron*. *Chrysomyxa succinea* (Sacc.) Tranz. is found only on hosts in *Rhododendron* subgenus *Hymenanthes*, section *Ponticum* (e.g., *R. brachycarpum* D. Don ex G. Don) (Crane 2005). Host alternation to spruce (*Picea* spp.) has been experimentally proven for *C. succinea* (Sato 1966; Hiratsuka and Sato 1969). The rust fungi on other rhododendron hosts in Japan, including those in subgenera *Tsutsusi* and *Rhododendron*, have all been identified as *Chrysomyxa rhododendri* de Bary, the common European species (Hiratsuka 1927, 1932, 1943, 1952, 1969; Hiratsuka et al. 1992). Host alternation has not been proven for the rust fungi on these rhododendron hosts (Hiratsuka 1969). During a monographic study of the genus *Chrysomyxa* worldwide (Crane 2000, 2001, 2005), the rust fungus on *Rhododendron kaempferi* Planch. and *R. kiusianum* Makino was observed to have different spore morphology from *C. rhododendri*. A similar rust fungus was found on specimens from North Korea, Tibet, and Nepal. The objectives of this study are to redescribe this rust fungus as a new species, and to show, using morphological and molecular evidence, that it is different from *C. rhododendri*.

Materials and methods

Materials examined

Rust-infected leaves of *R. kaempferi* were collected from Mt. Tsukuba, Ibaraki, Japan, in June 2003, and monthly from March to June, 2004. Dried specimens of rust-infected rhododendrons from the following herbaria were also

P.E. Crane (✉)
Department of Renewable Resources, University of Alberta, Edmonton, Alberta, T6G 2H1, and Northern Forestry Centre, Canadian Forest Service, Natural Resources Canada, Edmonton, Alberta, T6H 3S5, Canada
Tel. +1-780-435-7328; Fax +1-780-435-7359
e-mail: pacrane@shaw.ca

Y. Yamaoka · J. Engkhaninun · M. Kakishima
Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan

Contribution no.193 from the Laboratory of Plant Parasitic Mycology, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

Table 1. A list of specimens of *Caeoma tsukubaense* examined, their spore size, and the rhododendron species on which they occur

Rhododendron species	Location	Collection date	Specimen no.[a]	Spore size (mean) (µm)
Subg. *Tsutsusi*, Sect. *Tsutsusi*				
R. kaempferi	Mt. Tsukuba, Ibaraki, Japan	June 28, 2003	TSH-R22712 (= CFB 22233)	20–28 × 14–20 (23.7 × 16.9)
	Mt. Tsukuba, Ibaraki, Japan	April 22, 2004	CFB 22258	17–22 × 16–19 (19.9 × 17.4)
	Mt. Tsukuba, Ibaraki, Japan	May 13, 2004	TSH-R 22711 (= CFB 22259)	15–24 × 13–18 (19.5 × 15.6)
	Agematsu (Kiso), Nagano, Japan	August 8, 1931	PUR F12695	20–24 (–28) × 18–21 (23.1 × 19.2)
	Mt. Dogo, Hiroshima, Japan	August 26, 1941	TNS-F 233422	22–31 × 15–20 (26.9 × 17.0)
	Beppu, Ohita, Japan	November 10, 1924	TNS-F 111557	21–24 × 15–20 (22.8 × 18.0)
R. kiusianum	Kirishima Mts., Kagoshima, Japan	October 25, 1939	PUR F12694	19–24 × 14–20 (20.9 × 16.8)
	Kirishima, Kagoshima, Japan	October 27, 2000	TSH-R1966	20–26 × 16–22 (22.8 × 18.8)
	Kirishima, Kagoshima, Japan	October 25, 1939	TNS-F 233423	18–28 × 14–20 (22.4 × 16.5)
Subg. *Rhododendron*, Sect. *Rhododendron*				
R. "Macranthum"	Japan (unknown)	May 1906	TNS-F 229163	22–30 × 14–20 (26.6 × 16.2)
R. mucronulatum f. *ciliatum*	Mosan-gun, Kanhoku, N. Korea	July 6, 1939	PUR F12697	22–26 × 12–17 (23.5 × 15.1)
R. dauricum	Ishikari, Hokkaido, Japan	August 3, 1934	HMAS 6139	20–25 × 16–22 (23.1 × 18.4)
R. lepidotum	Nyalam, Tibet	October 13, 1990	HMAS 67315	20–27 × 14–19 (22.5 × 16.1)
R. lepidotum	Jilong, Tibet	September 12, 1990	HMAS 67325	18–25 × 13–16 (22.6 × 15.3)
R. lepidotum ?	Junbeshi, Nepal	October 17, 1988	TSH-R22717 (= CFB 22320)	20–26 × 14–19 (22.4 × 16.0)

[a] CFB, Canadian Forest Service, Edmonton, Canada; PUR, Purdue University, Indiana, USA; HMAS, Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Beijing, China; TSH, Mycological Herbarium, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; TNS, Department of Botany, National Science Museum, Tsukuba, Japan

examined: CFB (Canadian Forest Service, Edmonton, Canada), PUR (Purdue University, Indiana, USA), HMAS (Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Beijing, China), TSH (Mycological Herbarium, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan), and TNS (Department of Botany, National Science Museum, Tsukuba, Japan) (Table 1).

Microscopy

Cross sections of sori were made after soaking small pieces of dried, infected leaves on moistened filter paper. Sections were made with a razor blade under a dissecting microscope. Spores and leaf sections were mounted in lactophenol or lactophenol-cotton blue for light microscopic examination. For each sample, the size of 20–25 randomly selected spores was measured under brightfield microscopy at 400×; wall thickness and wart height were measured at 1000×. For scanning electron microscopy, spores were dusted onto aluminum stubs coated with adhesive; they were then coated with gold using a Polaron sputter-coater (E5000C-PS3) and examined with a Hitachi S-510 scanning electron microscope.

Polymerase chain reaction amplification and sequencing of D1/D2 region

DNA extraction and amplification of the D1/D2 region of 28S rDNA and the internal transcribed spacer (ITS) region of rDNA were modified from the method of Virtudazo et al. (2001). Spores from a single sorus were crushed between two sterile glass slides and suspended in 20µl extraction buffer [10mM Tris-HCl (pH 8.3), 1.5mM $MgCl_2$, 50mM KCl, 0.01% Proteinase K, 0.01% sodium dodecyl sulfate (SDS)], incubated at 37°C for 60min, then at 95°C for 10min. From this crude extract, 3µl was used directly for each polymerase chain reaction (PCR) amplification. Amplifications were done using 40-µl PCR reactions, each containing 0.2µM each primer, 1 unit TaKaRa Ex Taq DNA polymerase (Takara, Tokyo, Japan), and a commercial deoxynucleaside triphosphate (dNTP) mixture (containing 2.5mM each dNTP) and Ex Taq reaction buffer (containing 2mM Mg^{2+}). For 28S rDNA amplification, the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAA

Figs. 1–8. 1–7 *Caeoma tsukubaense*. **1** Sori on the abaxial surface of a leaf of *R. kaempferi* collected at Mt. Tsukuba, Japan (TSH-R22712). **2** Cross section of a sorus showing catenulate spores (TSH-R22712). **3** Spores on *R. kaempferi* (TSH-R22712). **4** Spores on *Rhododendron* sp. collected in Nepal (TSH-R22717). **5** Scanning electron microscopy (SEM) view of spores collected in Japan (PUR F12695). **6** Surface ornamentation of a spore by SEM (PUR F12695). **7** Spores by light microscope (TSH-R22712). **8** A urediniospore of *Chrysomyxa rhododendri* on *Rhododendron intermedium* (Germany) by SEM, showing narrow annulate warts and the smoother vertical "stripe" (PUR F533). *Bars* **1** about 3mm; **2** 30µm; **3, 4** 20µm; **5, 7, 8** 10µm; **6** 5µm