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FULL PAPER

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

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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

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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).

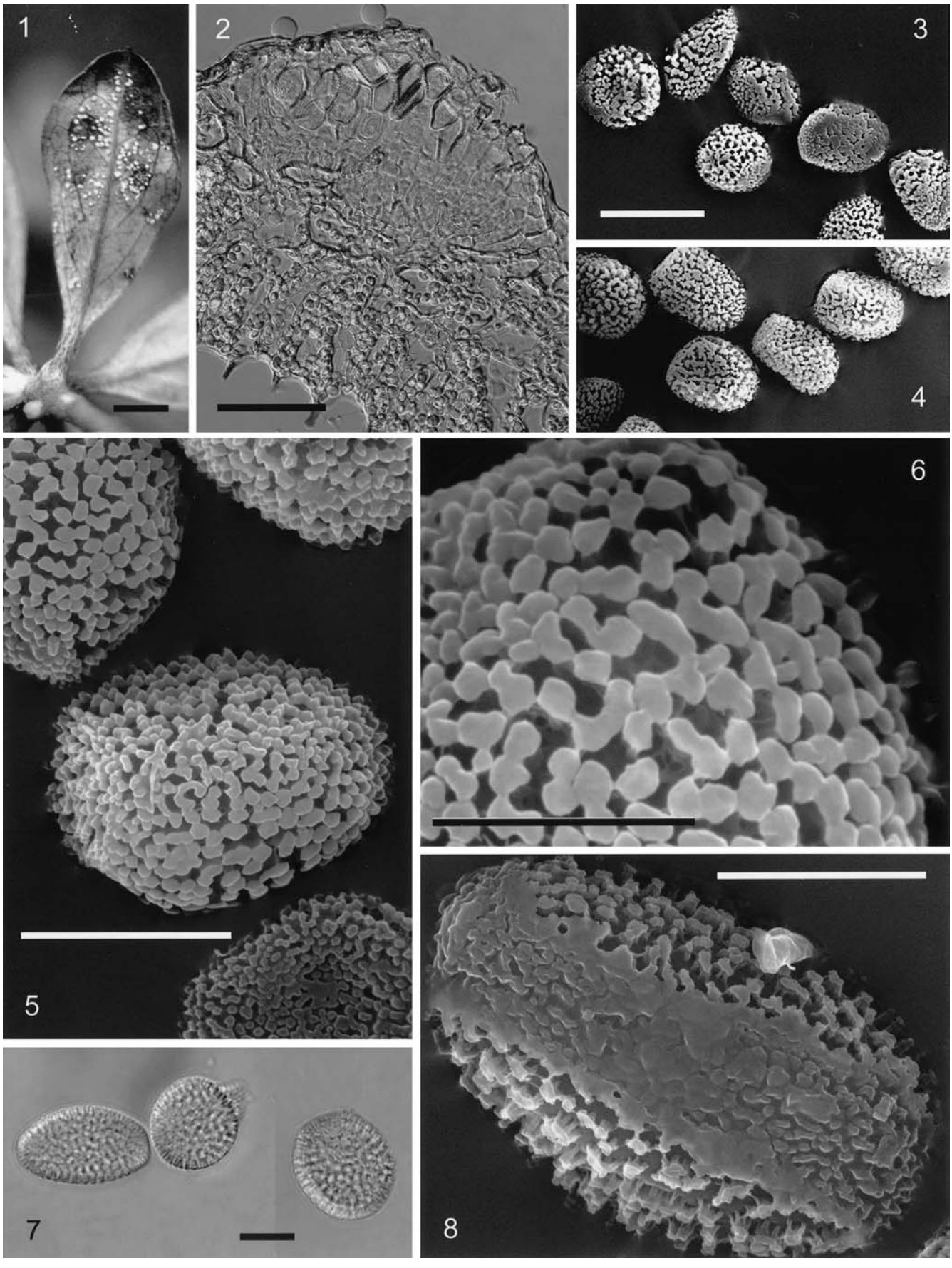
Microscopy

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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AG) and NL-4 (5′-GGTCCGTGTTTCAAGACGG) were used (O'Donnell 1993). The PCR amplification of the ITS regions was conducted with different primers specific to each region. The primers used for amplification of the ITS1 region were ITS5(5′-GGAAGTAAAAGTCGTAACAA GG-3′) (White et al. 1990) and ITS2BL (5′-CTGTGTTCTT CATCGATGTGA-3′) (Vogler and Bruns 1998). The ITS2 region, with partial 5.8S rDNA, was amplified using primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Vogler and Bruns 1998) and ITS3r (5′-ATCGATGAAGAACACAG-3′) (Gardes and Bruns 1993). PCR was carried out using a Gene Amp PCR System 9700 under the following conditions: 95°C for 3 min; 35 cycles of 95°C for 30 s, 55°C for 1 min, 72°C for 1 min; final step of 72°C for 10 min. After amplification, 3 µl of the reaction product was electrophoresced on 1% (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in TAE buffer [40 mM Tris, 20 mM sodium acetate, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4]. PCR products were purified using MicroSpin S-400 HR columns (Amersham Pharmacia Biotech, Buckinghamshire, England). Products were sequenced directly using a Dye terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, CA, USA) with the same primers used for PCR. Cycle sequencing reactions were purified, and sequence data collected using an ABI 377 Automated DNA Sequencer (Perkin Elmer). Sequences obtained were compared with those in GenBank using a BLAST search. Nucleotide sequence data were submitted to the GenBank database.

Taxonomy

Caeoma tsukubaense P.E. Crane, Yamaoka, Engkhaninun & Kakishima, sp. nov.

Figs. 1–8

Sori hypophylli, solitarii vel gregarii, magnitudine variabiles, 0.05–1 mm diametro; peridium abest. Sporae aurantiacae, catenulatae, cum cellulis intercalaribus, ovoideae, ellipsoideae vel subglobosae, 18–32 × 12–22 µm, dense verrucosae; verrucae annulatae, pariter sparsae per superficie omni sporae, discretae vel aliquando in lateribus conjunctae; tunica sporarum hyalina, difficiliter visa, tenuissima (<1 µm), cum verruca 1.2–2.5 (–3.3) µm crassa.

Holotypus: On *Rhododendron kaempferi* Planch., Mt. Tsukuba, Tsukuba, Ibaraki, Japan, May 13, 2004, Y. Yamaoka (TSH-R22711). Isotypus: CFB 22259.

Sori hypophyllous on leaves of *Rhododendron*, single or in groups, 0.05–1 mm in diameter. Spores deep orange, catenulate with intercalary cells, ovoid, ellipsoidal, or subglobose, one end often flat or pointed, 18–32 × 12–22 µm (mean ± SD = 22.8 ± 3.0 × 16.8 ± 1.9 µm), densely covered with warts; warts shallower at spore ends, annulate, variable in size and shape, sometimes joined laterally and appearing labyrinthine; spore wall hyaline, difficult to distinguish from warts, very thin (<1 µm), wall + warts 1.2–2.5 (–3.3) µm thick.

Specimens examined: See Table 1.

Results and discussion

This newly described rhododendron rust fungus was placed in the form-genus *Caeoma* because of its aperidiate sori, catenulate spore production, and verrucose spores. It is unlike *Uredo*, which produces pedicellate, echinulate spores. *Caeoma*-like uredinia are characteristic of the genus *Chrysomyxa* and several other rust genera (Cummins and Hiratsuka 2003). The sori of *C. tsukubaense* are assumed to be uredinia because of this resemblance and because they are not associated with spermogonia.

Caeoma tsukubaense appears to overwinter in living leaves of *R. kaempferi* as mycelium and sori. In March, old sori with faded spores were found on leaves of the previous year on Mt. Tsukuba. The viability of these spores was not determined. Young sori of current-year infections at first appeared gelatinous, similar to telia, but in cross sections the ornamented spores could be discerned. Telia would be expected to form in early spring if this species were typical of heteroecious species of *Chrysomyxa*. However, telia were not observed on infected plants on Mt. Tsukuba from March to June 2004 or in any of the herbarium specimens examined. A spruce tree (*Picea* sp.), the usual alternate host of heteroecious members of *Chrysomyxa*, was growing in the vicinity of infected *R. kaempferi* on Mt. Tsukuba, but remained uninfected.

Sequencing of the 5′-region of D1/D2 region of 28S rDNA yielded 585 bp (AB191241). In a BLAST search (GenBank), the sequence of *C. tsukubaense* showed greatest similarity to three sequences in the genus *Chrysomyxa*: *C. arctostaphyli* Dietel isolate DV29.1 (AF522163) (97%), *C. ledi* de Bary on *Picea abies* (L.) H. Karst. (AF426246) (98%), and *C. rhododendri* on *R. ferrugineum* L. (AF426245) (97%). The placement with *Chrysomyxa* is not surprising, given that most rust fungi of ericaceous plants are found in that genus, and that the sori of *C. tsukubaense* are morphologically similar to uredinia of *Chrysomyxa*. A difference of 3% for the D1/D2 region, a part of the rDNA often used to distinguish genera, confirms that *Caeoma tsukubaense* is a different species from *Chrysomyxa rhododendri*. Unambiguous sequence could not be obtained for the complete ITS1 region of rDNA and 5.8S region but only for the ITS2. The incomplete sequence consisted of 503 bp (AB191242). This sequence was most similar to *C. arctostaphyli* (L76488) (86%) on *Picea engelmannii*, the only ITS sequence deposited in GenBank for a *Chrysomyxa* species.

Rust fungi of North America and Asia have often been identified, based on spore size, as the same species found in Europe. However, it was recently demonstrated that North American rust fungi on *Ledum* spp. are different from the European *Chrysomyxa ledi* (Crane 2001). Similarly, although rhododendron rust fungi in Asia have often been identified as *C. rhododendri*, it now appears that *C. rhododendri* in Asia, as well as elsewhere, occurs only in northern boreal and subalpine regions (e.g., northern China, Siberia, Kamchatka) (Crane 2005). Because of similar spore size, rust fungi with uredinia-like sori on several

rhododendron species in Japan have previously been identified as *C. rhododendri* (Hiratsuka 1969; Hiratsuka et al. 1992). Interestingly, the urediniospore width cited in Hiratsuka et al. (1992) for *C. rhododendri* is considerably greater (19–26 µm) than that given by other authors (14–22 µm) (e.g., Saccardo 1888; Savile 1955; Wilson and Henderson 1966; Parmelee 1989; Crane 2001), and is also larger than the spore width of *C. tsukubaense*. Furthermore, both telia and aecia are described. It is not known whether the description in Hiratsuka et al. (1992) is based solely on Japanese specimens or on those from elsewhere. Not all rhododendron species listed in Hiratsuka et al. (1992) were examined in this study, and it is possible that other rust fungus taxa remain undiscovered on rhododendrons in Japan. Furthermore, it is possible that the rust fungus on *R. parvifolium* Adams [= *R. lapponicum* (L.) Wahlenb.] in the northern island of Hokkaido (Hiratsuka et al. 1992) is *C. rhododendri* s.s. because this is a common host in other parts of the northern hemisphere (e.g. northern Canada; Parmelee 1989).

The most obvious difference between *C. rhododendri* and *C. tsukubaense* is in spore ornamentation. The spore warts of *C. tsukubaense* are broad and often confluent, whereas the urediniospore warts of *C. rhododendri* are narrow, separate, and annulate over most of the spore. Furthermore, *C. rhododendri* spores have a flat, smoother vertical band on the spores that is lacking in *C. tsukubaense* (see Figs. 5, 8). *Caeoma tsukubaense* is most similar to two other anamorphic rust fungi: *Uredo rhododendri-capitati* Z.M. Cao & Z.Q. Li in southern China (Cao et al. 2000) and a recently recognized *Caeoma* species from the Philippines (Crane 2005). However, the spores of both are much longer (up to 46 µm) (Crane 2005) than those of *C. tsukubaense*. The Philippine rust fungus is also on a host in subgenus *Tsutsusi* and may be closely related to *C. tsukubaense*. All other ericaceous rust fungi have different urediniospore morphology than spores of *C. tsukubaense* (Crane 2000, 2001, 2003, 2005).

Specimens with the same spore morphology were found on hosts of two different subgenera of the genus *Rhododendron* (*Rhododendron* and *Tsutsusi*). Molecular evidence suggests that these subgenera are not phylogenetically closely related to each other within the genus *Rhododendron* (Chamberlain et al. 1996; Kron 1997; Kron and Johnson 1998; Kurashige et al. 1998). There is evidence that rhododendron rust fungi of similar morphology are usually confined to the same host subgenus (Crane 2005). Therefore, the samples from subgenus *Rhododendron*, including those from North Korea, Tibet, and Nepal (see Table 1), are placed tentatively within *C. tsukubaense*. Knowledge of other spore stages of this rust fungus as well as DNA sequencing of more samples will determine whether they belong to one or more similar taxa.

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