

First Description of *Coleosporium plectranthi* Causing Perilla Rust in Korea

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(Received on December 9, 2006; Accepted on February 7, 2007)

Perilla rust is a damaging disease in perilla cultivation in Korea. Its causal agent was identified as *Coleosporium plectranthi* based on descriptions of morphological characteristics of spores and spore-producing fruiting structures (in uredinial and telial stages from perilla and in aecial stage from the alternate host pine) collected in 15 locations in Korea during the disease survey from 2004 to 2006. These characteristics were yellow or orange uredinium; globose or ellipsoid urediniospore of $20.8\ \mu\text{m} \times 18\ \mu\text{m}$ in size; verruca of $0.3\ \text{mm} \times 1.2\ \text{mm}$; orange telium; one-celled, oblong ellipsoid teliospore of $63.1\ \mu\text{m} \times 19.7\ \mu\text{m}$ with one-layered crusts or four-celled (when mature), internal basidium of $64.2\ \mu\text{m} \times 19.7\ \mu\text{m}$; ellipsoid to globoid basidiospore of $20.3\ \mu\text{m} \times 12\ \mu\text{m}$; type 2 spermogonium; yellow, broadly ellipsoid peridial cell of $35.6\ \mu\text{m} \times 23.1\ \mu\text{m}$; and broadly ellipsoidal or subglobose aeciospore of $25.9\ \mu\text{m} \times 18.8\ \mu\text{m}$. Phylogenetic analysis of 28S rDNA sequences revealed the closest relatedness to those of the genus *Coleosporium*, a monophyletic group distinguished from other rust fungi and divided into two main lineages, one of which was *C. plectranthi* grouped with high bootstrap value (96%). In pathogenicity test, both aeciospores and urediniospores caused rust development on perilla leaves. This is the first description of *C. plectranthi* causing perilla rust with the first findings of its telial stage on perilla and the first rust disease on the aecial host in *Pinus densiflora*. These aspects would provide basic information for the development of control measures of the disease.

Keywords : *Coleosporium plectranthi*, morphology, perilla rust, 28S rDNA sequences.

Perilla (*Perilla frutescens* var. *japonica* Hara.), a member of the family Labiatae, is an annual herbaceous plant native to Asia. Its seeds are used for cooking oil and also directly as a dried powder for seasoning side dishes, for which the plant is mostly cultivated in open fields in Korea. On the other

hand, the perilla with green leaves cultivated mainly in greenhouses is used as a vegetable and for wrapping cooked meats with seasoning and flavoring materials. The cultivation area for vegetable uses is estimated to be around 850 ha, while that for perilla seed production is around 24,000 ha in 2005 according to the Agriculture and Forestry Statistical Yearbook (Anonymous, 2006).

For the perilla production, leaf diseases are cumbersome and damaging the crop. The perilla leaf diseases caused by fungi include leaf spot, gray mold, anthracnose, rust, etc (The Korean Society of Plant Pathology, 2004; Kim et al., 2001; Kim et al., 2002; Moon et al., 1998), among which rust is frequently encountered and widely spread in Korea. Perilla rust pathogen was firstly recorded in Korea as *Coleosporium perillae* P. Sydow (Takimoto, 1916), whose species name was changed to *Coleosporium plectranthi* Barclay (Hiratsuka et al., 1992). However, the authors listed host plant species, but not made description of the causal fungus. Since then there have been no mycological or pathological illustrations of the perilla rust reported in Korea up to the present time, although the disease has been almost epidemic and widely distributed throughout Korea. Therefore, this paper reports the mycological and pathological descriptions of the causal fungus of perilla rust in Korea, which may be helpful to develop its control strategies.

Materials and Methods

Survey of Perilla Rust and Collection of Specimens.

Perilla rust was surveyed and specimens of uredinial and telial stages were collected in 14 locations of Gyeonggi, Gwangwon, Gyeongbuk, Jeonbuk, and Jeonnam provinces in Korea from 2004 through 2006 (Table 1). Also a survey was made to find the rust on the aecial host of perilla rust, *Pinus densiflora*, and a specimen was collected from Gwacheon-si, Gyeonggi province. The occurrence of perilla rust was frequently surveyed especially in the areas of Gyeonggi province. The collected specimens were used for identification by comparing with the previous descriptions

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Table 1. Specimens collected from *Perilla frutescens* var. *japonica* (perilla) and *Pinus densiflora* (pine) and used for identification of *Coleosporium plectranthi* in this study

Specimen no. ^a	Stages	Location sampled	Date sampled	Host
CO1	Uredinium and telium	Namwon-si, Jeonbuk	10 Sep. 2005	Perilla
CO2	Uredinium and telium	Dalseo-gu, Gyeongbuk	10 Sep. 2005	Perilla
CO3	Uredinium	Gunwi-gun, Gyeongbuk	11 Sep. 2005	Perilla
CO4	Uredinium	Yecheon-gun, Gyeongbuk	11 Sep. 2005	Perilla
CO5	Uredinium	Yecheon-gun, Gyeongbuk	11 Sep. 2005	Perilla
CO6	Uredinium	Bonghwa-gun, Gyeongbuk	11 Sep. 2005	Perilla
CO7	Uredinium	Yongin-si, Gyeonggi	11 Sep. 2005	Perilla
CO8	Uredinium	Suncheon-si, Chonnam	20 Sep. 2005	Perilla
CO9	Uredinium	Suwon-si, Gyeonggi	6 Nov. 2004	Perilla
CO10	Uredinium	Samcheok-si, Gwangwon	20 Sep. 2005	Perilla
CO11	Uredinium	Suwon-si, Gyeonggi	10 Sep. 2005	Perilla
CO12	Uredinium	Gwangju-si, Gyeonggi	29 Sep. 2005	Perilla
CO13	Uredinium	Gwangju-si, Gyeonggi	29 Sep. 2005	Perilla
CO14	Uredinium	Yangpyeong-gun, Gyeonggi	22 Apr. 2006	Perilla
CO15	Spermogonium and aecium	Gwacheon-si, Gyeonggi	29 Apr. 2006	Pine

^aCO9 and CO14 were collected from perilla plants grown in greenhouses, and others from open fields.

of the fungus (Hiratsuka and Kaneko, 1975; Hiratsuka et al., 1992; Kaneko, 1981). Two fresh specimens, one from uredinal stage and the other from aecial stage, were used for pathogenicity test.

Morphological characteristics. Fresh specimens of perilla rust collected from 14 locations mentioned above, consisting of all uredinal and two telial stages (Table 1), were observed by light microscopy to examine mycological characteristics of uredinia, urediniospores, telia, teliospores, and basidiospores derived from teliospores (inner basidia). Morphology and surface structures of uredinia and urediniospores were also investigated by scanning electron microscopy (SEM) to support the light microscopic features of the fungus. For SEM, uredinia and urediniospores were dusted on double-sided adhesive tape on specimen holders, coated with gold using a sputter coater (Hitachi E-1010 Ion Sputter, Japan) and then observed under a scanning electron microscope (Hitachi S-3000N, Japan) at 15 kV. The mycological data were compared to the previous descriptions of the related rust fungi (Hiratsuka and Kaneko, 1975; Hiratsuka et al., 1992; Kaneko, 1981). As for another supporting data to confirm its identity, the specimens from red pine needles (*Pinus densiflora*) of the aecial host, including spermogonia and aecia, were also examined microscopically.

28S rDNA sequencing. Genomic DNAs were extracted from urediniospores collected from perilla leaves as previously described (Hong and Jung, 2004). The D1/D2 domain of 28S rDNA was amplified by PCR using the

primer pair 5'-ACCCGCTGAAYTTAAGCATAT-3' and 5'-CTCCTTGGTCCGTGTTTCAAGACGG-3' (Van der Auwera et al., 1994): initial denaturation at 94°C for 3 min; 30 cycles, each consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min; final extension at 72°C for 10 min. PCR products were purified using Wizard PCR prep kit (Promega, Madison, WI) following the manufacturer's guide. Nucleotide sequences were determined with BigDye terminator cycle sequencing kits (Applied Biosystems, Foster City, CA) using the same primers as used in PCR amplification. Sequences were proofread, edited and merged into composite sequences with the help of the PHYLIT program version 3.2 (Chun, 1995). The sequence was deposited at GenBank sequence database (accession no. EF095711).

Phylogenetic analysis. Nucleotide sequence of the *C. plectranthi* rDNA region was aligned with those of *Coleosporium* and their related species (Maier et al., 2003). Phylogenetic tree was reconstructed by neighbor-joining algorithm (Saitou and Nei, 1987) based on the distance matrix calculated by Kimura's two-parameter model (Kimura, 1980) using PHYLIP package (Felsenstein, 1993). Robustness of the tree was estimated by bootstrap analysis (Felsenstein, 1985) of 1,000 resampled datasets using the same program package.

Pathogenicity Test. Fresh aeciospores from needles of *Pinus densiflora* collected in Gwacheon-si, Gyeonggi province and urediniospores from perilla leaves from Yangpyeong-gun, Gyeonggi were suspended in sterile

water and inoculated on young perilla plants at 5-6th true leaf stage grown in a greenhouse. Spore suspensions were sprayed on perilla leaves and the inoculated plants were placed at 21-23°C for inoculation by aeciospores and at 24-28°C for inoculation by urediniospores, respectively, for two days in humidified chambers. The inoculated perilla plants were then placed at 24-25°C in the greenhouse for examining the disease development.

Results and Discussion

Occurrence and symptoms of perilla rust. The perilla rust was observed in all the areas surveyed. The perilla rust occurred anytime during the growing season of perilla in fields as well as in greenhouses, which usually started late June and mid August, respectively. Initial symptoms and signs were tiny yellowish projections on the underside of the leaves usually spreading inwardly from terminal leaf edge, accompanying yellow to brown flecks formed on

their opposite upper side and covering the whole leaves with spore masses within 2-3 weeks after the initial symptoms and signs (Fig. 1A-D). The pustules were enlarged with time to be visible as yellowish spore masses at the beginning but whitish later, around which leaf tissues became dry and dead, sometimes broken to make a hole. In a plant, infection usually started from lower leaves and spread toward upper leaves, causing the whole plant dusted with rust spore masses (Fig. 1A, B, C). The rust spore masses appeared to be all yellowish to orange uredinia and urediniospores. However, telia and teliospores, which have not been reported yet in Korea, were observed in two specimens (on leaf edges) under the light microscope. The infected plants usually defoliated early. Infected leaves seemed not to be qualified for the fresh vegetable product in the commercial market. Also a survey for the rust on the aecial host was conducted only to find the pine needle rust, of which the symptoms and signs were dark brown flecks and white to yellowish pustules (aecial stage) developed on

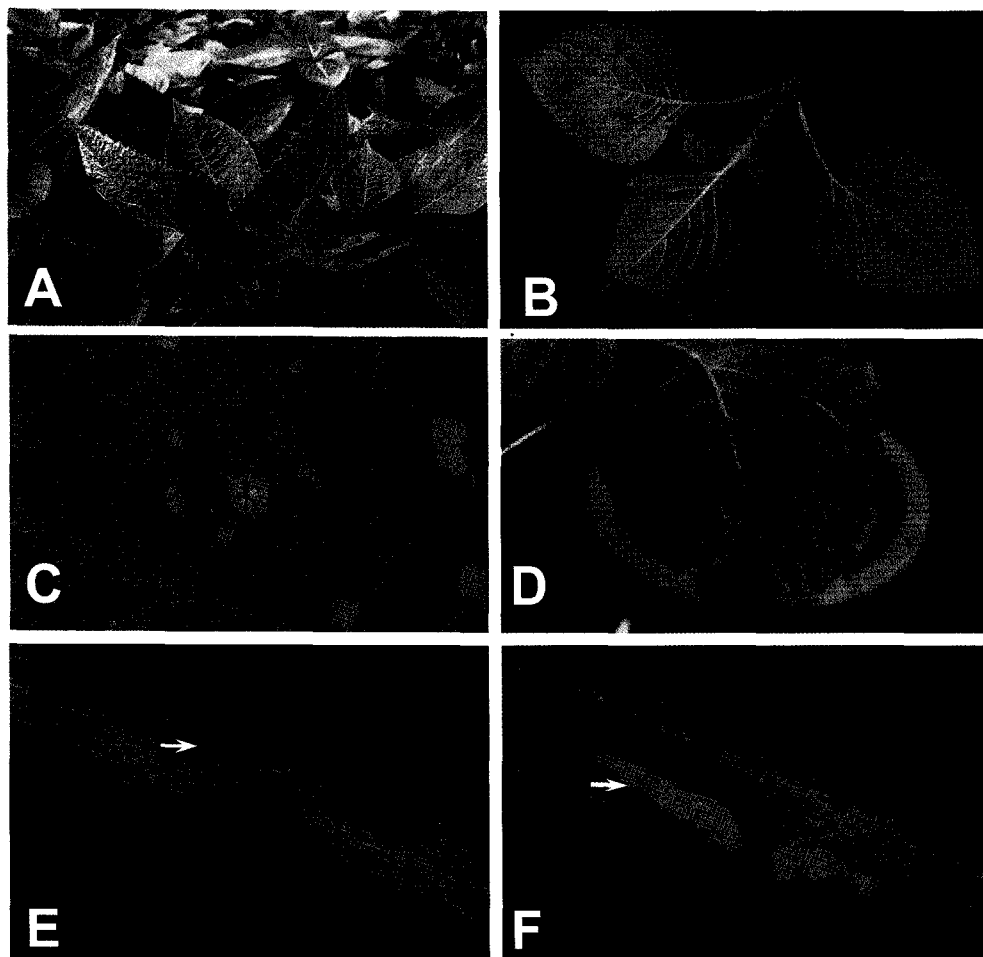


Fig. 1. Signs and symptoms of perilla rust, showing spore masses (uredinia) (arrow) formed on the underside of perilla leaves (A, B), their close-up features (C), and initial symptoms of flecks (arrow) on the upper side of the leaf (formed by artificial inoculation) (D), and pine needle rust symptoms of flecks (arrow) (E) and signs of pustules (aecia) (arrow) (F).

pine needles (Fig. 1E, F). Like the telial stage, the aecial stage of the perilla rust fungus was also firstly found on the alternate host (*P. densiflora*) in Korea.

Morphological characteristics. The host of uredinial and telial stage is *Perilla frutescens* var. *japonica*. Fig. 2 shows light microscopic features of urediniospores (Fig. 2A) and teliospores (Fig. 2B), and scanning electron micrographs of a uredinium (Fig. 2C) and urediniospores (Fig. 2D). Uredinia were yellow or orange when young, but faded to whitish when old. Urediniospores were globose or ellipsoid: $20.8\ \mu\text{m}$ ($17.7\text{--}23.8\ \mu\text{m}$) \times $18\ \mu\text{m}$ ($15.5\text{--}20.6\ \mu\text{m}$) in size. Verrucae were $0.3\ \text{mm}$ ($0.2\text{--}0.5\ \text{mm}$) wide and $1.2\ \text{mm}$ ($0.3\text{--}2.1\ \text{mm}$) high. Telia were orange; teliospores one-

celled, oblong ellipsoid, one-layered crusts with $63.1\ \mu\text{m}$ ($53.8\text{--}72.3\ \mu\text{m}$) \times $19.7\ \mu\text{m}$ ($16.8\text{--}22.6\ \mu\text{m}$) in size or four-celled (when mature), and internal basidia, with $64.2\ \mu\text{m}$ ($56.1\text{--}74.3\ \mu\text{m}$) \times $19.7\ \mu\text{m}$ ($16.2\text{--}23.4\ \mu\text{m}$) in size. Basidiospores were ellipsoid to globoid, $20.3\ \mu\text{m}$ ($18.3\text{--}22.4\ \mu\text{m}$) \times $12\ \mu\text{m}$ ($11.2\text{--}13.1\ \mu\text{m}$) in size.

Spermogonia and aecia were found on needles of *P. densiflora* (Fig. 1F). Spermogonia were type 2. Peridial cells were yellow, broadly ellipsoid, $35.6\ \mu\text{m}$ ($31.1\text{--}47\ \mu\text{m}$) \times $23.1\ \mu\text{m}$ ($18.6\text{--}25.8\ \mu\text{m}$). Aeciospores were broadly ellipsoidal or subglobose, $25.9\ \mu\text{m}$ ($23.3\text{--}28.3\ \mu\text{m}$) \times $18.8\ \mu\text{m}$ ($16.9\text{--}20.8\ \mu\text{m}$), verrucose, with short rod-like knobs, verrucae $0.5\text{--}1.5\ \mu\text{m}$ high. The characteristics of the fungus in the uredinial and telial stages, and those of the aecial stage on

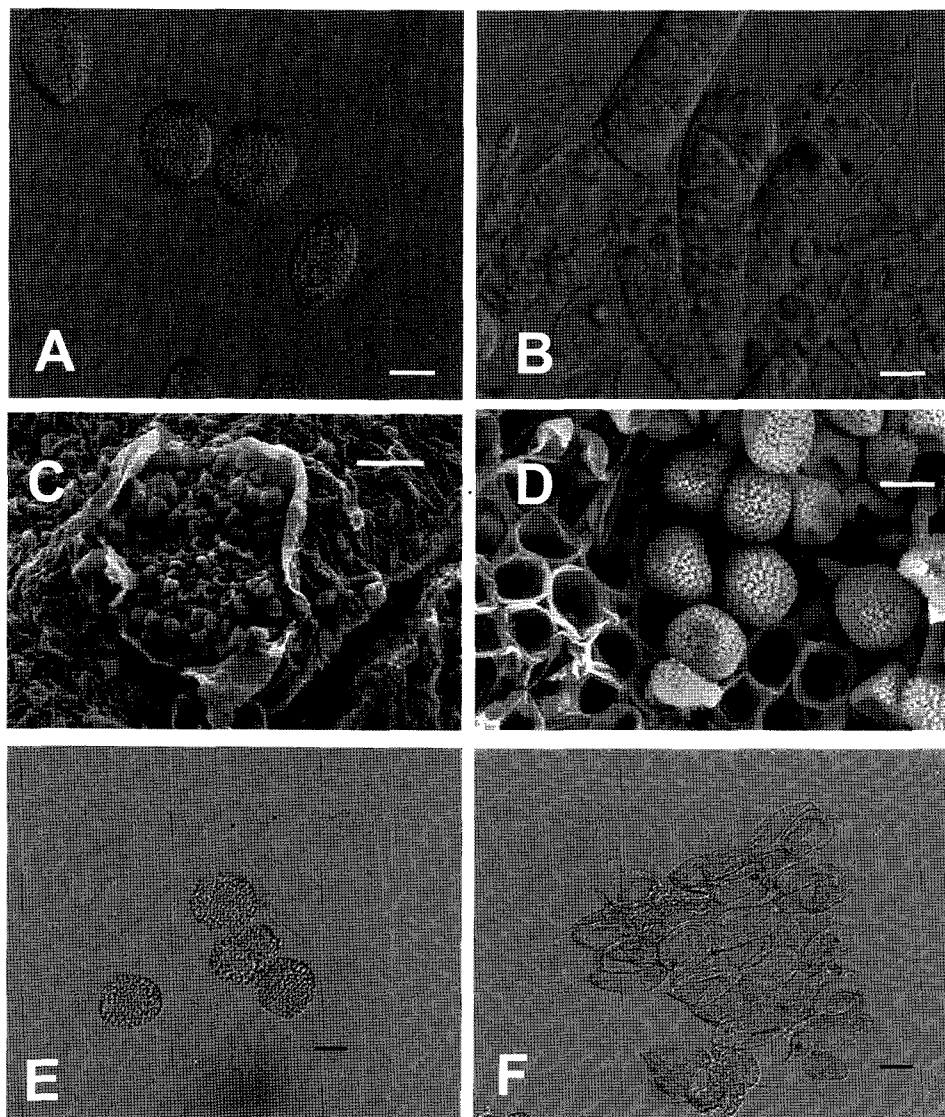


Fig. 2. Light micrographs of urediniospores (A), teliospores (B), scanning electron micrographs of uredinium (C) and urediniospores (D) from perilla leaves, and light micrographs of aeciospores (E) and peridial cells (F) from pine needles. Bars = $10\ \mu\text{m}$ (A, B, D, E), $50\ \mu\text{m}$ (C), and $30\ \mu\text{m}$ (F).

the pine are matched well with previous descriptions of *Coleosporium plectranthi* Barclay (Hiratsuka and Kaneko, 1975; Hiratsuka et al., 1992; Kaneko, 1981), although their sizes in the present study were somewhat smaller than the Japanese fungi (or in the literature). Specimens were deposited in the local herbarium (Table 1).

Phylogenetic analysis. The partial nucleotide sequences of 28S rDNA gene (Accession Number, EF095711) revealed the closest relatedness to those of the *Coleosporium* species listed in GenBank with similarities ranging between 97.4% and 99.4% (data not shown). Phylogenetic analysis with *Coleosporium* and related species revealed that the genus *Coleosporium* made a monophyletic group distinguished from other rust fungi including *Chrysomyxa*, members of Cronatiaceae and Pucciniastraceae (Fig. 3). The monophyletic group of *Coleosporium* species was divided into two main lineages, and *Coleosporium plectranthi*, was grouped with high bootstrap value (96%) with one lineage having four *Coleosporium* species, *C. cacaliae*, *C. campanulae*, *C. senecionis*, and *C. tussilaginis* that all had identical sequences in the 28S rDNA gene analyzed. The other lineage was composed of heterogeneous sequences of *Coleosporium asterum*. These aspects also support that the present rust fungus cannot be other *Coleosporium* species listed but *C. plectranthi*.

Pathogenicity. When perilla plants were inoculated with aeciospores, the first symptoms and signs similar to natural

ones appeared on terminal areas of the inoculated leaves in 9 to 15 days after inoculation (Fig. 1D). On the other hand, with the inoculum of urediniospores, infection occurred with the similar symptoms and signs somewhat earlier than with the aecial inoculum (5 to 10 days after inoculation). As this disease progressed, perilla leaves were populated with spore masses composed of uredinia and urediniospores. The uredinia and urediniospores formed by the inoculations were identical to natural ones based on the descriptions mentioned above.

In our study, both aecio- and urediniospores were shown to be pathogenic to perilla, which are known to serve as primary and secondary inocula, respectively, in a macrocyclic disease cycle of the rust fungus. Since basidiospores from the teliospores formed on perilla cannot be a pathogen to perilla because it is a heteroecious rust, the aecial stage on the red pine trees (*Pinus densiflora*) may have been served as an important primary inoculum source to initiate the disease on perilla before farmers engaged in its greenhouse cultivation for leaf production on a large scale. However, at present the involvement of aeciospores in the development of perilla rust may be relatively low because perilla is widely grown in greenhouses in which the disease is prevalent during the wintertime and urediniospores readily over winter to serve as the continuous secondary inoculum in the next year (as primary inoculum). Also the urediniospores must be the inoculum of the secondary infections responsible for rust epidemic in perilla. Probably for this reason, pine needle rust caused by *C. plectranthi*

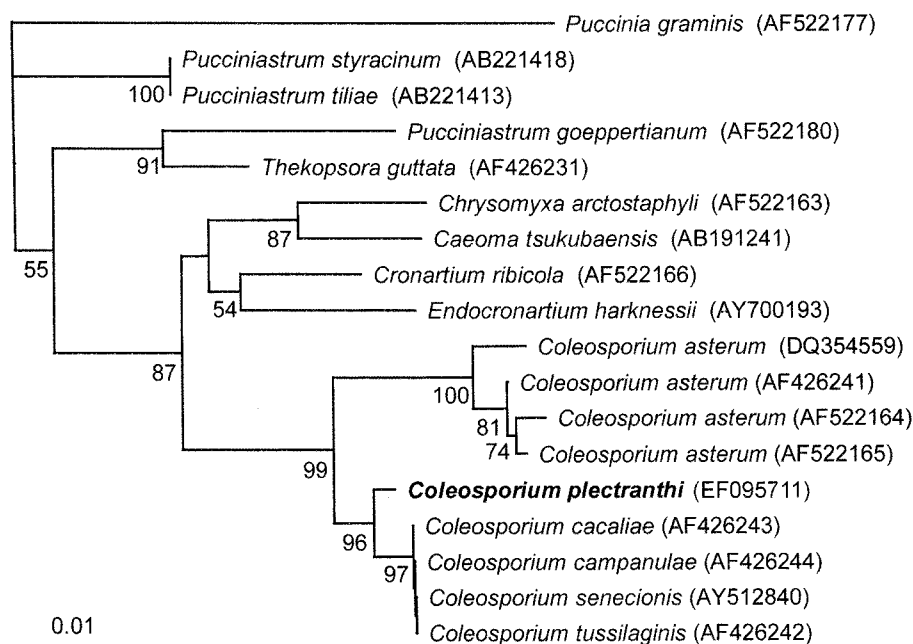


Fig. 3. Neighbor-joining tree of 28S rDNA gene of *Coleosporium* and related species. Bootstrap values were represented under each branch. Sequence of *Puccinia graminis* was used as an outgroup.

has rarely been found in nature during this study even though its uredinal stage is popular on perilla.

Because the perilla rust is an important disease in perilla, several researches on chemical control (development of fungicides) and breeding of resistance cultivars have been conducted, and the control measures have been practiced. Little study, however, has been made on cultural control practices of the rust partly due to the lack of its mycological and plant pathological characteristics. In our study, the perilla rust pathogen was fully described in its mycological characteristics for its identification. Its pathogenicity was confirmed by artificial inoculation to give a view of its natural life cycle related to the disease development. These aspects would give basic information to develop cultural control measures of the disease.

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