Phytotaxa 309 (1): 055–065
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.309.1.5

A new rust species of *Diaphanopellis* on *Rhododendron oreodoxa* from Southern China

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Abstract

A novel rust species *Diaphanopellis purpurea* on *Rhododendron oreodoxa* collected in Southern China was identified and described. Light and scanning electron microscopy observations indicated that this rust species was morphologically distinct from other known *Diaphanopellis* species and *Chrysomyxa* species in teliospore morphology and urediniospore surface structure. *Diaphanopellis purpurea* can be phylogenetically separated from other *Chrysomyxa* species based on analysis of internal transcribed spacer (ITS) partial gene sequences. The aecial stage of the new species was also confirmed.

Keywords: Molecular phylogeny, phylogeny, *Pucciniales*, taxonomy

Introduction

Rust genus *Diaphanopellis* was established by Crane with the type species *Diaphanopellis forrestii* P. E. Crane occurring on *Rhododendron selense* Franch (Crane 2005, Kirk *et al*. 2008). *Diaphanopellis* is characterized by the teliospores enclosed in hyaline sheaths, and the uredinia surrounded by a peridium with ornamented cells (Barclay 1891, Balfour-Browne 1955, Crane 2005). Most rusts infecting *Rhododendron* belong to the genus *Chrysomyxa*, which are morphologically different from *Diaphanopellis* species in having uredinial peridium and distinct teliospores. *Chrysomyxa* species produce catenulate teliospores without gelatineous layers and uredinia covered by an inconspicuous peridium (Berndt 1999).

During an investigation of rust fungi in China, we collected a rust species on *Rhododendron oreodoxa* Franch., producing urediniospores and teliospores that were different from these of *Diaphanopellis forrestii* and other known *Chrysomyxa* species. In this paper, we described and illustrated this rust as a new species. We also performed phylogenetic analysis of rDNA to confirm the aecial stage of this new taxon discovered on *Picea purpurea*.

Materials and methods

Specimen collection and herbaria

Fresh specimens were collected in Sichuan and Yunnan Province in China and deposited at the Mycological Herbarium, Museum of Beijing Forestry University (BJFC) in China. Herbaria were borrowed from the Mycological Herbarium, Chinese Academy of Sciences (HMAS). Host plants, locality of collection and accession numbers for sequence data from GenBank and Barcode of Life Database (BOLD, www.barcodinglife.org) were listed in Table 1.

Morphological characteristics observation

Spores and hand sections of telia were mounted in lactophenol or lactophenol-cotton blue solution on the microscopic slides. For each specimen, approximately 30 spores were randomly selected and measured using a DM2500 upright microscope (Leica, Germany). The surface macro-structures of samples were examined by using scanning electron microscopy (SEM). Aeciospores and urediniospores were adhered onto aluminum stubs covered with double-sided

Accepted by Samantha Karunarathna: 14 May 2017; published: 9 Jun. 2017

adhesive tape, coated with gold using the SCD-005 Sputter Coater (Hitachi, Tokyo, Japan), and then observed using a S-3400N scanning electron microscope (Hitachi, Tokyo, Japan) operated at 10–15 kV.

DNA extraction, PCR and sequencing

Genomic DNA was extracted using the modified method of Tian *et al*. (2004). The internal transcribed spacer (ITS) and 5.8S region of rDNA was amplified with primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*. 1990, Gardes & Bruns 1993). Amplifications were performed in 25 μL of PCR solution containing 1 μL of DNA template, 2.5 μL of sense primer (2 μM), 2.5 μL of antisense primer (2 μM), 12.5 μL of 2×Es Taq MasterMix (Cwbio, Beijing, China), and 6.5 μL of dd H_2O.The PCR conditions were as follows: 94°C for 3 min, 35 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 1 min, and a final step of 72°C for 10 min. PCR products were purified and cloned for sequencing (TSINGKE, Beijing, China).

Phylogenetic analysis

The raw sequences obtained were aligned using ClustalX1.83 and MAFFT v.7 (Thomson *et al*. 1997, Katoh & Standley 2013). We compiled two datasets for phylogenetic analyses: (A) an rDNA ITS dataset that consisted of sequence data obtained from this study, and rust species of Coleosporiaceae from BOLD (Feau *et al*. 2011) and GenBank (Table 1), and (B) a rDNA ITS dataset, which included the related rust genera with *Chrysomyxa*, representing major lineages of Pucciniaceae, Pucciniastraceae, Coleosporiaceae, Cronartiaceae and Melampsoraceae (Table 3). Phylogenetic trees obtained from analyses (A) and (B) were rooted with *Melampsora epitea* (AY471646) and *Puccinia melampodii* (EU659697) respectively. Maximum parsimony (MP) analysis was carried out using the heuristic search option with 1,000 random-addition sequences and tree bisection and reconnection as the branch-swapping algorithm implemented in PAUP v.4.0b10 (Swofford 2002). In the MP analyses, gaps were treated as missing data, and all characters were equally weighted. Clade stability was assessed using a bootstrap analysis with 1,000 replicates (Felsenstein 1985). Tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC) were also calculated. Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) using the Markov Chain Monte Carlo (MCMC) method, the best-fit substitution models were estimated using Modeltest ver. 3.7 based on the implementation of the Akaike information criterion (AIC) (Posada and Crandall 1998). GTR + I + G was selected as the best evolutionary model for the rDNA ITS datasets, and the Markov chains were run for 1,000,000 generations. The trees were sampled every 100 generations, resulting in 10,000 total trees. Sequence alignments were deposited at TreeBase (http://www.treebase.org/) under the accession number 19266.

TABLE 1. Sequence data analyzed in this study or obtained from GenBank and BOLD. (new species in bold).

Fungal taxon	Host plant	Specimen no	Date of collection	Geograpphic origin	GenBank or BOLD accession no.(ITS)
Diaphanopellis purpurea	*Picea purpurea*	BJFC-R02299*	2014-07-21	Sichuan,China	KX225401[a]
	Picea purpurea	BJFC-R02300*	2014-07-21	Sichuan,China	KX225402[a]
	Rhododendron oreodoxa	HMAS-55188*	1987-05-01	Sichuan,China	KX225403[a]
	Rhododendron oreodoxa	BJFC-R01698*	2014-05-21	Sichuan,China	KX225404[a]
	Rhododendron oreodoxa	BJFC-R01699*	2014-05-21	Sichuan,China	KX225405[a]
Chrysomyxa arctostaphyli	*Picea mariana*	DAOM 229628	1986-06-30	Klondike Loop, Yukon, Canada	CHITS040-08[b]
	Arctostaphylos uva-ursi	DAOM 183586	1982-06-16	Kenora district, Ontario, Canada	CHITS053-08[b]
C. cassandrae	*Picea mariana*	QFB 25005	2004-09-10	Abitibi, Quebec, Canada	CHITS052-08[b]
	Chamaedaphne calyculata	QFB 25007	2006-08-06	Le ´vis, Quebec, Canada	CHITS004-08[b]
C. chiogenis	*Gaultheria hispidula*	QFB 25026	2007-06-22	Charlevoix, Quebec, Canada	CHITS022-08[b]
	Gaultheria hispidula	Only DNA extraction	2007-07-25	Charlevoix, Quebec, Canada	CHITS031-08[b]
C. empetri	*Empetrum nigrum*	QFB 25033	2007-08-04	Radisson, Quebec, Canada	CHITS032-08[b]
	Empetrum nigrum	QFB 25060	2007-09-05	Charlevoix, Quebec, Canada	CHITS033-08[b]

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TABLE 1. (Continued)

Fungal taxon	Host plant	Specimen no	Date of collection	Geograpphic origin	GenBank or BOLD accession no.(ITS)
C. ledi	*Ledum palustre*	DAOM 138900	1966-09-05	Bialowieza Forest, Poland	CHITS056-08[b]
	Picea abies	DAOM 162213	1975-07-28	Pudasjärvi, Jonku, Finland	CHITS059-08[b]
C. ledicola	*Ledum groenlandicum*	Only DNA extraction	2005-06-17	Waswanipi River, Quebec, Canada	CHITS060-08[b]
	Picea glauca	QFB 25034	2007-08-04	Chisasibi, Quebec, Canada	CHITS028-08[b]
C. monesis	*Moneses (= Pyrola) uniflora*	DAOM 221985	1957-06-03	Graham Island, British	CHITS044-08[b]
	Picea sitchensis	DAVFP 10017	1956-09-01	Columbia, Canada	CHITS107-09[b]
C. nagodhii	*Rhododendron groenlandicum*	Only DNA extraction	2007-06-21	Manicouagan, Quebec, Canada	CHITS065-08[b]
	Picea mariana	QFB 25054	2007-07-25	Charlevoix, Quebec, Canada	CHITS030-08[b]
C. neoglandulosi	*Ledum glandulosum*	DAOM 229530	1999-08-21	Okanagan, British Columbia, Canada	CHITS042-08[b]
C. piperiana	*Ledum macrophyllumc*	DAFVP 14998	1963-06-06	Hope, British Columbia, Canada	CHITS113-09[b]
C. pyrolae	*Picea glauca*	QFB 25055	2006	Lac St-Jean, Quebec, Canada	CHITS013-08[b]
	Pyrola sp.	QFB 25056	2008-05-31	Bic, Quebec, Canada	CHITS066-08[b]
C. rhododendri	*Picea abies*	WM 1183	1999-08-22	Obere Chlusi, Bernese Oberland, Switzerland	CHITS009-08[b]
	Rhododendron ferrugineum	QFB 19829	1972-07-12	Simplon, Valais, Switzerland	CHITS036-08[b]
	Ledum lapponicum	DAFVP 14606	1962-08-10	Summit Pass, British Columbia, Canada	CHITS105-09[b]
	Ledum lapponicum	DAFVP 14607	1962-07-27	Summit Pass, British Columbia, Canada	CHITS106-09[b]
C. vaccinii	*Vaccinium parvifolium*	DAOM 45774	1952-07-08	Graham Island, British Columbia, Canada	CHITS070-08[b]
	Vaccinium parvifolium	DAVFP 18160	1968-05-18	Victoria Island, British Columbia, Canada	CHITS115-09[b]
C. woroninii	*Ledum groenlandicum*	QFB 25009	2006-06-26	Charlevoix, Quebec, Canada	CHITS006-08[b]
	Picea abies	DAOM 230441	1996-07-16	Sodankylä, Ruosselkä, Finland	CHITS072-08[b]
Coleosporium campanulae	*Campanula* sp.	HMBF-41501	—	China	KP017555[a]
Coleosporium phellodendri	*Phellodendron amurense*	HMBF-12	—	China	KP017556[a]
Coleosporium phellodendri	*Phellodendron chinense*	BJFC-R00700	2006-07-14	Shaanxi,China	KX225406[a]
Melampsora epitea	*Salix bebbiana*	SB2002-3	—	Minnesota,USA	AY471646[ab]

BJFC: Museum of Beijing Forestry University, Beijing, China; DAFVP: Forest Pathology Herbarium, Canadian Forest Service, Pacific Forestry Centre,Victoria, British Columbia, Canada; DAOM: Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; HMAS: Herbarium Mycologicum Academiae Sinicae, Beijing, China; QFB: Rene Pomerleau Herbarium, Canadian Forest Service, Laurentian Forestry Centre, Quebec, Canada.

[a]stands for sequences used in the current study in GenBank; [b]stands for sequences from BOLD; [ab]stands for sequences used as outgroup.

***** stands for specimens used in this study.

—stands for no information available.

Results

Morphology

The telia of the rust fungus on *R. oreodoxa* were abundant, pulvinate without stalk cells, and had a waxy or gelatinous appearance. Teliospores were covered by gelatineous sheaths (Figs 2B, 2C), which is the key characteristics for identification of the genus *Diaphanopellis*. The uredinia were surrounded by a conspicuous peridium with square and ornamented cells, the outter surface of peridium was deeply convave and smooth, while the inner surface was densely warted.

This taxon clearly differs from *D. forrestii* (Crane 2005) in its urediniospore surface structure and smaller-size teliospores. Moreover, the morphology of uredinia peridium is different in these two species. Detailed morphological comparisons of specimens of the two species, including measurements in the uredinial and telial stages, are presented in Table 2.

TABLE 2. Comparison of morphological characteristics of *Diaphanopellis forrestii* and *Diaphanopellis purpurea*.

	Diaphanopellis forrestii	***Diaphanopellis purpurea***
Spermogonial stage	Unknown	Unknown
Aecial stage	Unknown	Aecia amphigenous, ligulate. Aeciospores ellipsoidal, oblong,globose, or subglobose; 16–28× 11–24 µm, densely echinulate, a small numbers of central spines arise frequently; Peridium outer surface deeply convex, striate or rugulose; Peridium inner surface flat and smooth
Uredinial stage	Urediniosporaes size: 19–35×12–29 µm	Urediniosporaes size: 22–38×20–30 µm
	Urediniosporaes surface: densely warted, one side covered by a shallowly warted, longitudinal cap with a ragged edge;	Urediniosporaes surface: densely warted; one side covered by longitudinal cap,cap with bumps and broken edge;
	Warts crowded, annulate, cylindrical or irregular in shape with broad tops, interspersed with much smaller, shallower warts	Warts coronate,fingerlike,cylindrical or irregular in shape; the heads are acutely and minutely dentate or tubercular.
	Peridial cells outer wall surface deeply concave, smooth or finely warted;	Peridial cells outer wall surface deeply concave, with sharply defined edges, smooth to slightly rough surface
	Peridial cells inner wall surface densely warted; sometimes appearing labyrinthine	Peridial cells inner wall surface flat or slightly convex, warts shallow, irregular, and discrete
Telial stage	The telial stage occurs on *R. selense* Franch	The telial stage occurs on *R. oreodoxa* Franch
	Teliospores catenulate, covered by a hyaline, thin-walled sheath	Teliospores catenulate, covered by a hyaline, thin-walled sheath
	Teliospores size:17–33× 6–20 µm	Teliospores size:10–28× 5–12 µm

TABLE 3. Sequence data of related rust genus analyzed in this study or obtained from GenBank and BOLD. (new species in bold).

Fungal taxon	Host plant	Specimen no	Date of collection	Geograpphic origin	GenBank accession no. ITS
Diaphanopellis purpurea	*Picea purpurea*	BJFC-R02299*	2014-07-21	Sichuan,China	KX225401
	Picea purpurea	BJFC-R02300*	2014-07-21	Sichuan,China	KX225402
	Rhododendron oreodoxa	HMAS-55188*	1987-05-01	Sichuan,China	KX225403
	Rhododendron oreodoxa	BJFC-R01698*	2014-05-21	Sichuan,China	KX225404
	Rhododendron oreodoxa	BJFC-R01699*	2014-05-21	Sichuan,China	KX225405
Chrysomyxa arctostaphyli	*Picea mariana*	DAOM 229628	1986-06-30	Klondike Loop, Yukon,Canada	GU049458[b]
	Arctostaphylos uva-ursi	DAOM 183586	1982-06-16	Kenora district, Ontario,Canada	GU049459[b]

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TABLE 3. (Continued)

Fungal taxon	Host plant	Specimen no	Date of collection	Geograpphic origin	GenBank accession no. ITS
Chrysomyxa woroninii	*Ledum groenlandicum*	QFB 25009	2006-06-26	Charlevoix, Quebec, Canada	GU049495[b]
	Picea abies	DAOM 230441	1996-07-16	Sodankylä, Ruosselkä, Finland	GU049494[b]
Coleosporium phellodendri	*Phellodendron amurense*	BJFC-QL12	—	—	KP017556[b]
	Phellodendron chinense	BJFC-R00700	2006-07-14	Shaanxi,China	KX225406[b]
Coleosporium campanulae	*Campanula* sp.	HMAS-41501	—	China	KP017555[b]
	—	BJFC-ZL001	—	China	JQ219672[b]
Melampsora epitea	*Salix* sp.	BPI-US0022745	1950	Abisko,Sweden	AY471648[b]
	Salix arctica	BPI-FC2002-8	—	Fort Conger, Ellesmere Island, Nunavut Canada	AY471634[b]
Melampsora populnea	—	—	2006-09-23	France	EU808037[b]
	—	—	2006-09-23	France	EU808036[b]
Pucciniastrum tiliae	*Tilia japonica*	IBA7878	—	Aomori,Japan	AB221454[b]
	Tilia japonica	IBA7670	—	Miyazaki,Japan	AB221453[b]
Pucciniastrum boehmeriae	*Boehmeria tricuspis*	TSH-R21290	—	Aomori,Japan	AB221449[b]
	Boehmeria tricuspis	TSH-R21289	—	Aomori,Japan	AB221450[b]
Melampsoridium betulinum	*Birch* sp.	—	—	—	EU391657[b]
	—	—	—	—	JN581986[b]
Melampsoridium hiratsukanum	*Alnus incana (alder)*	—	—	Trentino region,Italy	KC888944[b]
	Alnus rhombifolia	PDR1480181	2010-08-08	Santa Cruz County, California,USA	KC313889[b]
Thekopsora ostryae	*Ostrya japonica*	BJFC -GS-78	2012-08-09	Gansu,China	KC415787[b]
	Ostrya japonica	BJFC -GS-129	2012-08-11	Gansu,China	KC415796[b]
Thekopsora areolata	*Picea abies*	—	—	Buskerud, Ovre Eiker, Hokksund,Norway	EF363336[b]
	Picea abies	—	—	Ostfold, Eidsberg, Ramstad,Norway	DQ087230[b]
Cronartium ribicola	*Ribes odoratum*	—	—	USA	KF387533[b]
	—	—	2010-06-01	USA	JN587805[b]
Cronartium flaccidum	*Vincetoxicum nigrum*	—	—	France	JN802139[b]
	Melampyrum nemorosum	—	—	—	AY566270[b]
Puccinia melampodii	*Parthenium hysterophorus*	—	—	—	EU659697[ab]

BJFC: Museum of Beijing Forestry University, Beijing, China; BPI: U.S. National Fungal Herbarium; DAOM: Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; HMAS: Herbarium Mycologicum Academiae Sinicae, Beijing, China; QFB: Rene Pomerleau Herbarium, Canadian Forest Service, Laurentian Forestry Centre, Quebec, Canada; TSH: Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan.

[b]stands for sequences from GenBank.

[ab]stands for sequences used as outgroup.

***** stands for specimens used in this study.

—stands for the information is not found.

Molecular phylogeny

The final alignment consisted of 36 ingroup taxa contained 666 total characters, with 398 constant characters and 93 parsimony-uninformative variable characters. MP analysis yielded a single parsimonious tree (TL = 430, CI = 0.798, RI = 0.887, and RC = 0.707), resulting in 18 terminal clades (Fig. 3). Bayesian analysis generated a tree of the same topology. The ITS tree indicated that the rust fungus on *P. purpurea* and *R. oreodoxa* formed a monophyletic group in Coleosporiaceae with strong bootstrap support (100) and Bayesian probabilties (1.00) (Fig. 3). They were also distinct

from other known *Chrysomyxa* species. Moreover, the current rust fungus was phylogenetically distinct from other *Coleosporium* species, which also belong to the family of Coleosporiaceae.

The ITS dataset (Fig. 4) of 9 rust genera yielded a highly resolved phylogenetic framework. The data matrix contained 35 fungal specimens. Of 719 total characters, 332 characters were constant and 37 were parsimony-uninformative variable characters. MP analysis of sequence data yielded a single parsimonious tree (TL = 845, CI = 0.611, RI = 0.882, and RC = 0.307). Bayesian analysis resulted in one of identical topology. The topology of the ITS phylogram indicated that the present rust fungus on *P. purpurea* and *R. oreodoxa* formed a sister relationship to *Chrysomyxa* species, and they clustered as a distinct clade from *Chrysomyxa* species and *Coleosporium* species, all of which belong to the family of Coleosporiaceae. The present rust fungus represents a new genus because it is different from *Chrysomyxa, Coleosporium* and *Melampsora*.

Life cycle

Sequence data of the rust fungus on *P. purpurea* and that on *R. oreodoxa* were identical, and they formed a monophyletic group with strong bootstrap support and Bayesian probabilities (Fig. 3). This suggested that the fungus on *P. purpurea* is the aecial state of the new species *Diaphanopellis purpurea*. The aecial stage of *D. forrestii* was not discovered (Crane 2005). Molecular comparisons and inoculations to obtain its aecial states would facilitate further investigations of the two species.

Diaphanopellis purpurea produced amphigenous and ligulate, single or confluent aecia on needles of *P. purpurea*. Aeciospores were variable in shape from globose to ellipsoidal or oblong, densely echinulate, a small numbers of central spines arose frequently on a separated flat columnar verruca. Aecial peridium cells were rectangle, larger than the spores, outer surface was deeply convex, striate or rugulose, while inner surface was flat and smooth. The aeciospore wall ornamentation of this new species is unique and different from all other described *Chrysomyxa* species on *Picea*, and distinct from other rust fungi with verruose or annulate aeciospores. The connection of the aecial and telial stages of new species *D. purpurea* was established by phylogenetic analysis of ITS data.

Taxonomy

Diaphanopellis purpurea C. J. You & J. Cao, *sp. nov.* (Figs. 1, 2)

MycoBank :—MB819572

Etymology:—Epithet "*purpurea*" refers to the aecial host of the holotype.

Diagnosis:—Differs from morphologically similar species, *Diaphanopellis forrestii* in the surface structure of uredinospore and uredium peridium. In addition, it differs from other known *Chrysomyxa* species because of teliospore morphology.

Type:—CHINA, Sichuan Province: Kangding County, 29°59′36″N 101°53′46″E, alt. 3181 m, I on *Picea purpurea* Mast. (Pinaceae), 21 July 2014, C. J. You (Holotype, BJFC-R02299); CHINA, Sichuan Province: Kangding County, 29°59′36″N 101°53′46″E, alt. 3181 m, I on *Picea purpurea*: 21 July 2014, C. J. You (Isotype: BJFC-R02300).

Other Specimens examined:—CHINA, Sichuan Province: Kangding County, 22°59′24″N 101°52′39″E, alt. 3227 m, II, III on *Rhododendron oreodoxa* Franch., 21 May 2014, Y. Bai (BJFC-R01698); Yunnan Province: the National Forest Park of Shangari-La, 29°45′35″N 99°59′41″E, alt. 3565 m, I on *Picea purpurea*: 15 July 2014, C.J. You (BJFC-R02301; BJFC-R02302): CHINA, Sichuan Province, Kangding County, 29°59′21″N 101°52′40″E, alt. 3263 m, 21 May 2014, Y. Bai (BJFC-R01699); Sichuan Province, Kangding County, May 1987, Y. L. Guo (HMAS-55188).

Spermogonia unknown.

Aecia amphigenous, ligulate, 0.3–1.1 mm wide. Aeciospores ellipsoidal, oblong, globose, or subglobose, 16–28 × 11–24 µm, with yellow inclusion, densely echinulate, a small numbers of central spines arise frequently on a separated flat columnar verruca; wall hyaline, 0.4–0.6 µm thick, wall plus spines 1.2–3.4 µm thick; Aecial peridium dehiscing at apex, later shredding, leaving a fringe around sorus; Peridial cells rectangle, larger than the spores, outer surface deeply convex, striate or rugulose, inner surface flat, wall smooth. Uredinia subepidermal, erumpent, Aecidium-type, covered by a conspicuous peridium; Peridial cells polygonal, round or square, similar in size or larger than the spores; Outer peridium surface cells deeply concave, with sharply defined edges, smooth to slightly rough surface; inner surface flat or slightly convex, warts shallow, irregular, and discrete. Urediniospores catenulate, globose, subglobose to polygonal or ovoid, occasionally ellipsoidal, 22–38 × 20–30 µm, densely warted, warts coronate, fingerlike, flame-shaped or irregular in shape, the heads are acutely and minutely dentate or tubercular; wall 1.3–1.6 µm thick, wall

plus warts 2.1–3.4 µm. Telia in large groups, gelatinous, orange or aurantiaca, erumpent; from round to elongated or irregular, 130–300 × 100–280 µm, often sunken in the center; raised, slightly constricted at the base, without stalk cells. Teliospores catenulate, 10–28 × 5–12 µm, thin-walled, finely tuberculate, enclosed in a loose hyaline sheath with a thin wall, not laterally adherent.

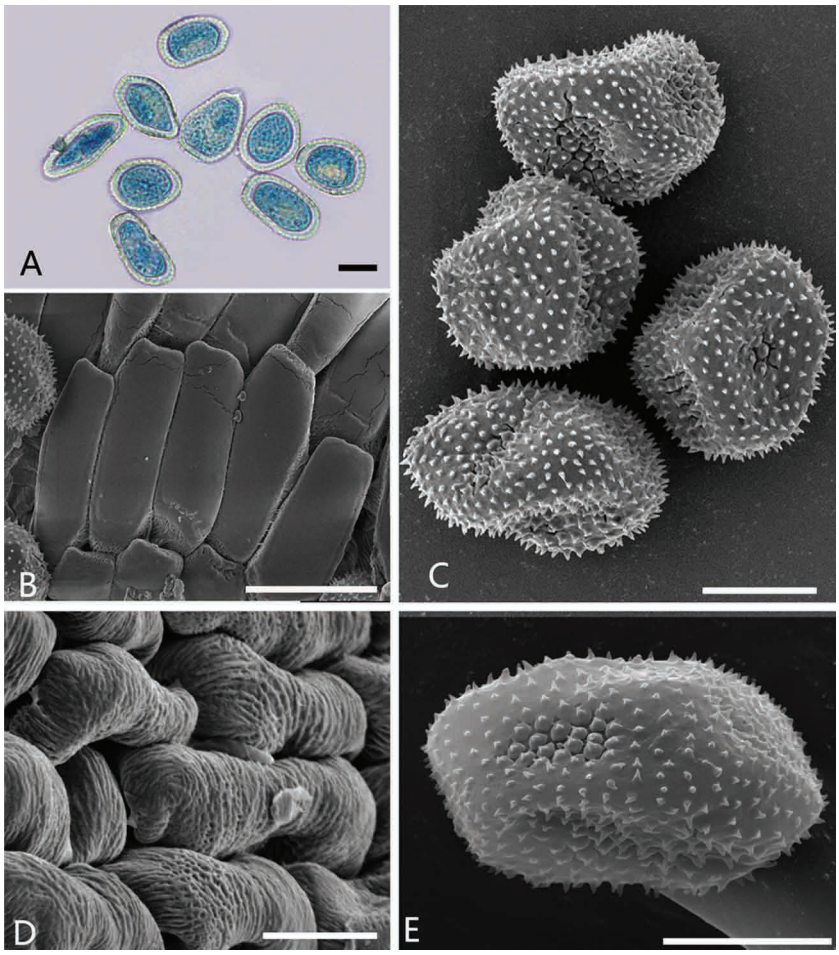

FIGURE 1. *Diaphanopellis purpurea* on *Picea purpurea* (BJFC-R02299). A. Oblong-ellipsoid aeciospores (LM); B. Aecial peridium with smooth inner surface; C. aeciospores; D. Aecial peridium with deeply convex, striate or rugulose outer surface; E. Densely echinulate on aeciospores surface. Scale bars: A, C, D, E = 10µm; B = 20 µm.

Discussion

Diaphanopellis purpurea on *R. oreodoxa* has all the characteristics of *Diaphanopellis*, its teliospores are covered by transparent sheaths (Figs 2B, 2C) and its telia morphology is similar to *Diaphanopellis forrestii*. It is distinctly different from *Chrysomyxa* - the common pathogen infecting *Rhododendron*, which produces teliospores covered by hyaline sheath and uredinial anamorph in *Aecidium* rather than *Caeoma* (Crane 2005).

Diaphanopellis forrestii on *R. selense* was described by Crane (2005) and it was characterized by catenulate, densely warted urediniospores, one side covered by a narrowly warted, longtitudinal cap with a ragged edge. The peridial cells of uredinia were larger than the urediniospores, outter surface deeply convave, smooth, or slightly warted, inner surface densely warted, sometimes labyrinthine, side walls striate (Crane 2005). The type specimen of *D. forrestii* was unavailable, but detailed morphological examinations of the 2 paratype specimens (HMAS 46927, HMAS 46933) were provided, and the morphological characteristics fitted well with the original description by Crane (2005). We were unable to obtain the ITS sequences of the species since these specimens were too old for DNA extraction.

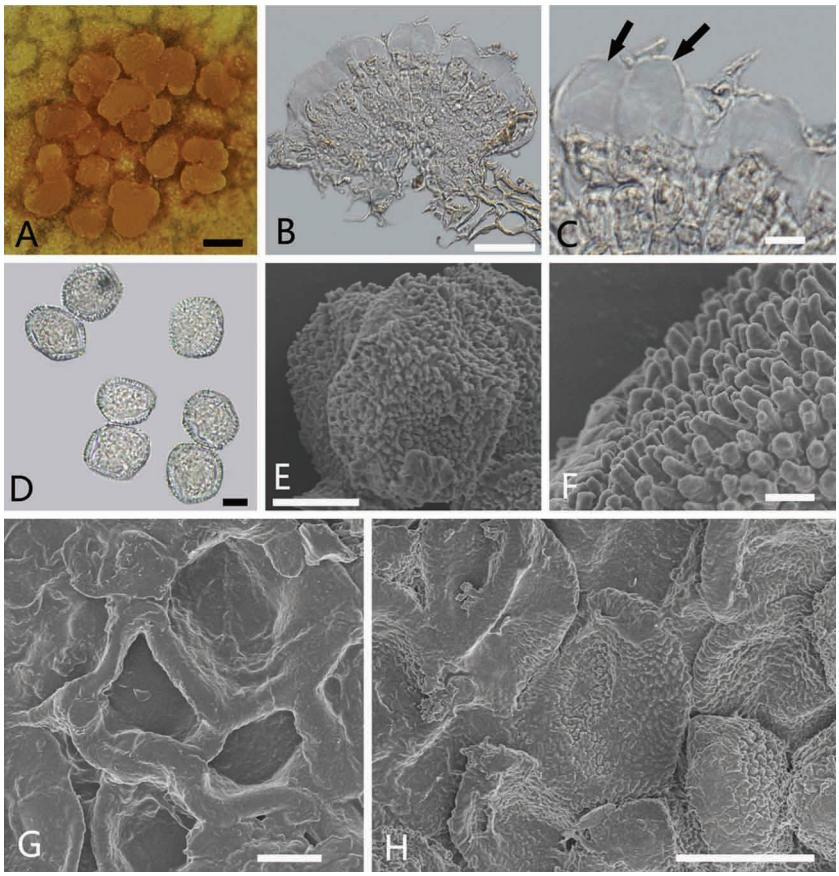

FIGURE 2. *Diaphanopellis purpurea* on *Rhododendron oreodoxa* (BJFC-R01698). A. Telia in large groups, gelatinous, orange, erumpent; B. Showing transparent sheaths extending beyond teliospores; C. Cross section of telium, showing transparent sheaths around teliospores; D. Globose to subglobose urediniospores; E. Urediniospores; F. Urediniospores showing warts coronate, fingerlike, or irregular in shape; G. Concave outer surface of peridial cells ; H. Warted inner surface of peridial cells. Scale bars: A = 200 µm; B = 50 µm; C, D, E, G = 10 µm; F = 0.5 µm; H = 20 µm.

The new species *D. purpurea* is clearly distinct from *D. forrestii* by its unique urediospore-surface structure, which is frequently used as important criterion for species identification, and by its smaller teliospores (Figs 2B, 2C). The urediniospores (Figs 2D, 2E, 2F) are densely verrucose, crowded cylindrical, flame-shaped warts, lacking narrow and irregular cap on spore surface, and the inner surface of peridial cells (Figs 2G, 2H) are discrete, shallow and irregular warts, which are different from the densely warted inner surface of *D. forrestii*. In addition, the teliospore size (10–28 × 5–12µm) of *D. purpurea* is smaller than that of *D. forrestii* (17–33 × 6–20µm; Crane 2005). The detailed morphological differences between the two *Diaphanopellis* species are listed in Table 2. Although the sequence data of *D. forrestii* is currently unavailable, the clearly distinct morphological characteristics between the two species suggest that *D. purpurea* is a new species. As Crane (2005) mentioned, these unique features of *Diaphanopellis* including peridium of uredinia and the gelatinous layer over the telium may be adaptations to avoid desiccation at high altitudes and climate changes.

Diaphanopellis purpurea differs from other known *Chrysomyxa* species in its aeciospores surface ornamentation and aecial peridium. The new species is characterized by its echinulate processes on aeciospores surface, while most *Chrysomyxa* species produce aeciospores with annulate warts on spore surface. The densely echinulate aeciospores with a small numbers of central spines arising frequently on a separated flat columnar verruca (Figs 1C, 1E) closely resemble the ornamentation on urediniospores of *Coleosporium phellodendri* Kom. on *Phellodendron* (Kaneko 1981). However, in phylogenetic analyses (Fig. 3) the new species on *P. purpurea* grouped in a distinct clade from two *Coleosporium* species and other *Chrysomyxa* species.

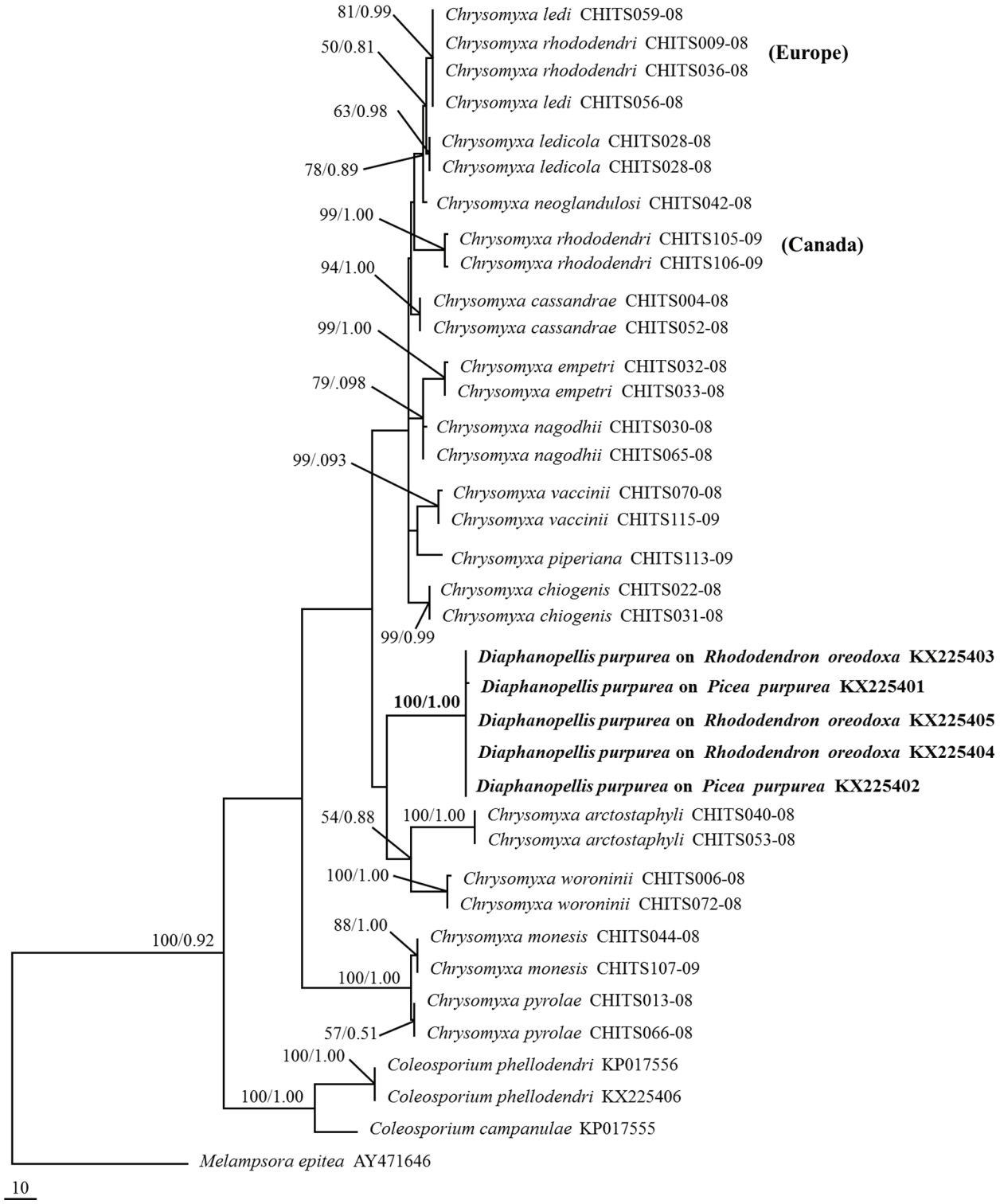

FIGURE 3. Phylogenetic tree constructed by maximum parsimony and Bayesian analyses based on ITS sequences of rust species of Coleosporiaceae. Bootstrap values were calculated from 1,000 replications. Parsimony bootstrap (before the slash marks) and Bayesian posterior probabilities (after the slash marks) greater than 50% are shown. Bars: 10 nucleotide substitutions. New species are shown in bold.

In the rDNA ITS phylogeny (Fig. 3), the present rust fungus on *P. purpurea* and *R. oreodoxa* was phylogenetically distinct from the other *Chrysomyxa* species considered in this study. The new species was phylogenetically distinct from morphologically similar species, i.e., *Chrysomyxa cassandrae* Tranzschel. and *Chrysomyxa rhododendri* de Bary. Moreover, it was nested in all *Chrysomyxa* group in the rDNA ITS phylogeny, however, at genus rank phylogenetic work based on molecular data found the present rust fungus to be a new rust genus, different from *Chrysomyxa* (Fig. 4).

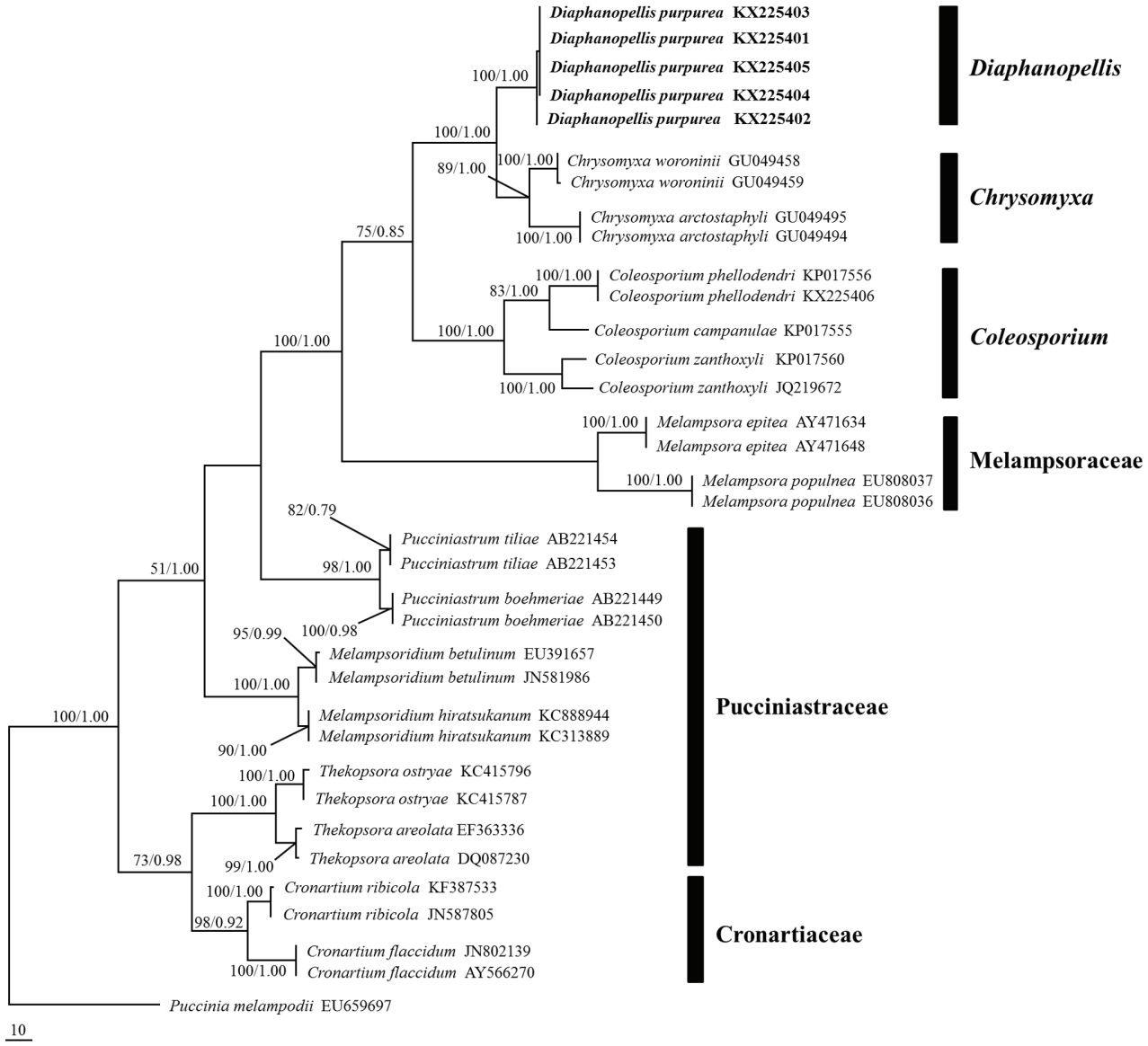

FIGURE 4. Phylogenetic tree constructed by maximum parsimony and Bayesian analyses based on ITS sequences of related rust genera. Bootstrap values were calculated from 1,000 replications. Parsimony bootstrap (before the slash marks) and Bayesian posterior probabilities (after the slash marks) greater than 50% are shown. Bars: 10 nucleotide substitutions. New species are shown in bold.

The monophyletic status of *Chrysomyxa* was supported by using 28S sequence data from the two closely related *Chysomyxa* species (*C. ledi* and *C. rhododendri*) (Maier *et al*. 2003). However when integrated into a larger phylogenetic framework based on ITS and 28S phylogenetic signals, *Chysomyxa* was proved to be polyphyletic. *Chysomyxa* species were distributed in three distinct clades, moreover, some close relationships between *C. pyrolae*, *C. monesis* and the *Coleosporium* genus and between *C. weirii* and the *Melampsora* genus were demonstrated (Feau *et al*. 2010).The new species *D. purpurea* is nested in *Chrysomyxa* group, with a weakly supported sister group relationship with the genus *Chrysomyxa*, it indicates that *Diaphanopellis* is polyphyletic, while the two genus are defined by clearly distinct morphological characteristics. Further taxonomic investigation and multigene phylogenies are required to identify evolutionary patterns that drive speciation within the genus *Chrysomyxa* and *Diaphanopellis*.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 31300540), and The Fundamental Research Funds for the Central Universities, China (No. BLX2012032) and. We would like to thank the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS), for providing herbarium materials in this study.

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