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Two new *Chrysomyxa* rust species on the endemic plant, *Picea asperata* in western China, and expanded description of *C. succinea*

JING CAO[1], CHENG-MING TIAN[1], YING-MEI LIANG[2] & CHONG-JUAN YOU[1*]
[1]*The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, China*
[2]*Museum of Beijing Forestry University, Beijing 100083, China*
**Corresponding author: chongjuanyou@bjfu.edu.cn*

Abstract

Two new rust species, *Chrysomyxa diebuensis* and *C. zhuoniensis*, on *Picea asperata* are recognized by morphological characters and DNA sequence data. A detailed description, illustrations, and discussion concerning morphologically similar and phylogenetically closely related species are provided for each species. From light and scanning electron microscopy observations *C. diebuensis* is characterized by the nailhead to peltate aeciospores, with separated stilt-like base. *C. zhuoniensis* differs from other known *Chrysomyxa* species in the annulate aeciospores with distinct longitudinal smooth cap at ends of spores, as well as with a broken, fissured edge. Analysis based on internal transcribed spacer region (ITS) partial gene sequences reveals that the two species cluster as a highly supported group in the phylogenetic trees. Correlations between the morphological and phylogenetic features are discussed. Illustrations and a detailed description are also provided for the aecia of *C. succinea* in China for the first time.

Keywords: aeciospores, molecular phylogeny, spruce needle rust, taxonomy

Introduction

Picea asperata Mast.is native to western China, widely distributed in Qinghai, Gansu, Shaanxi and western Sichuan. The species is currently not listed as threatened, but population numbers have been declining due to deforestation (Fu *et al*. 1999). Spruce needle rusts and cone rusts in the genus *Chrysomyxa* Unger are damaging pathogens on *Picea*. They may retard growth and cause severe premature defoliation thus contributing to tree death, and thereby causing enormous economic losses. In 2008, the incidence area of spruce needle rusts in Tianzhu County, Gansu Province was 28000 hm^2, and volume growth loss was on average 20% per year (Cao 2000, Zhang 2005, Liu & Nan 2011).The three spruce rust pathogens, *Chrysomyxa deformans* (Diet.) Jacz, *C. qilianensis* Wang. Wu et Li and *C. rhododendri* De Bary were listed as National Forest Dangerous Pest in China in 2013.

Among the 23 *Chrysomyxa* species described worldwide (Kirk *et al*. 2008), 14 species have been reported in China (Tai 1979, Wang 1987, Cao & Li 1996, 1999, Cao 2000, Cummins &Hiratsuka 2003, Zhang 2005). Most species are heteroecious, and macrocyclic producing spermogonia and aecia on needles and cones of Pinaceae hosts, mainly on *Picea* (Cummins & Hiratsuka 2003), and uredinia and telia on Ericaceae and Pyrolaceae, mainly on *Rhododendron*, *Ledum*, *Chamaedaphne*, *Vaccinium*, *Moneses* and *Pyrola* (Crane 2001, Feau *et al*. 2011).Others species are microcyclic, producing only telia on the conifer host, or demicyclic, having all spore states except uredinia (Crane 2005b, Feau *et al*. 2011). Of the eight *Chrysomyxa* species reported on needles of *Picea* spp. in China, *C. abietis* (Wallr.) Unger, *C. deformans* (Dietel) Jacz., and *C. weirii* H.S. Jacks., are microcyclic, producing only telia, while the other five species (*C. rhododendri* (DC.) de Bary, *C. succinea* (Sacc.) Tranzschel, *C. woronini* Tranzschel, *C. ledi* de Bary and *C. qilianensis* Wang. Wu et Li) produce aecial stage on spruce needles (Zhao 1958, Cao & Li 1996, 1999, Fu *et al*. 2008).

Chrysomyxa species are characterized by peridermium-type aecia, uredinia surrounded by an inconspicuous peridium consisting of one or more layers of thin-walled pseudo-parenchymatous cells, and catenulate, one-celled teliospores (Berndt 1999, Crane 2001). Aeciospore and urediniospore ornamentation has been confirmed as taxonomically useful at the species level (Hiratsuka & Kaneko 1975, Sato & Sato 1982, Lee & Kakishima 1999, Crane 2001). Gross morphology of the aecia and aecial perdium may be useful taxonomical characters for several species, but the morphology of teliospores and basidiospores are fairly consistent within a species.

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

Fungal taxon	Host plant	specimen no.	Date of collection	Geograpphic origin	GenBank or BOLD accession no.(ITS)
C. diebuensis	*Picea asperata*	BJFC-R00556*	2012/8/9	Gansu,China	KX225393[a]
	Picea asperata	BJFC-R00507*	2012/8/6	Gansu,China	KX225394[a]
	Picea asperata	BJFC-R00220*	2012/8/9	Gansu,China	KX225395[a]
C. zhuoniensis	*Picea asperata*	BJFC-R00521*	2012/8/7	Gansu,China	KX225396[a]
	Picea asperata	BJFC-R00522*	2012/8/7	Gansu,China	KX225397[a]
C. qilianensis	*Picea crassifolia*	BJFC-R02303*	2014/7/24	Qinhai,China	KX225398[a]
C. qilianensis	*Picea crassifolia*	BJFC-R02304*	2014/7/24	Qinhai,China	KX225399[a]
C. qilianensis	*Picea crassifolia*	HMAS-52077*	1984/7/25	Gansu,China	KX225400[a]
C. succinea	*Picea wilsonii*	BJFC-R02306*	2015/8/5	Shaanxi,China	KX462882[a]
C. succinea	*Picea wilsonii*	BJFC-R02307*	2015/8/5	Shaanxi,China	KX462883[a]
C. succinea	*Rhododendron fauriae*	HMAS-6142*	1934/7/27	Japan	KX462884[a]
C. 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]
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]
C. weirii	*Picea* sp.	QFB 25018	2007-05-12	Guelph, Ontario, Canada	CHITS014-08[ab]

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

Taxonomic studies and DNA barcoding of *Chrysomyxa* species have been conducted in Europe, Japan and North America (Pethybridge 1918, Takahashi & Saho 1985, Crane 2001, 2003, 2005a, 2005b, Tillman-Sutela *et al*. 2004, Kaitera *et al*. 2010, Feau *et al*. 2011), but morphological taxonomy at the species level is confusing due to overlapping features. Therefore, comprehensive taxonomic studies including molecular data are required. During the survey of *Chrysomyxa* in China, two new species, *C. diebuensis* and *C. zhuoniensis*, were found on *P. asperata*, which is endemic to China. The two new species differ from known *Chrysomyxa* species in several morphological characteristics, for example, the dimension of aeciospores, surface ornamentation and aecial peridium. *C. diebuensis* is characterized by larger aeciospores with nailhead to peltate processes on the spore surface, and the other new species, *C. zhuoniensis*, was characterized by a distinct broad longitudinal smoother cap at ends of aeciospores, with a broken, skirt-like edge. Phylogenetic analysis of ITS sequence data indicated that they are new species with strong support, respectively. Therefore, the objective of this study is to clarify the taxonomy of the two new *Chrysomyxa* species on the same spruce species in China based on morphological comparison and molecular phylogenetic analyses. Furthermore, expanded descriptions and illustrations are also provided for the aecia of previously described species *C. succinea* in China for the first time.

Materials and Methods

Fresh specimens were collected in Gansu Province, China during 2012–2014, and deposited in the Mycological Herbarium, Museum of Beijing Forestry University (BJFC), Beijing, China. Some dried specimens of *Chrysomyxa* on *Picea* sp. in China, borrowed from Herbarium Mycologicum Academiae Sinicae, Beijing (HMAS), were also included in the study. Host plants, locality of collection and accession numbers for sequence data from GenBank database and Barcode of Life Database (BOLD, www.barcodinglife.org) are listed in Table 1.

Microscopic analysis

For light microscopy (LM) observations, spores were mounted in a drop of lactophenol or lactophenol-cotton blue solution on a microscopic slide. For each specimen, approximately 30 spores were randomly selected and measured using a DM2500 upright microscope (Leica, Germany). To prepare samples for surface structure examination using scanning electron microscopy (SEM), aeciospores were adhered onto aluminum stubs covered with double-sided adhesive tape, coated gold using the SCD-005 Sputter Coater, and then observed using a Hitachi S-3400N scanning electron microscope (Tokyo, Japan) operated at 15 kV.

DNA extraction, PCR and sequencing

DNA extraction procedures followed the method of Tian *et al*. (2004). The internal transcribed spacer (ITS) regions of rDNA were amplified with the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-CAGGAGACTTGTACA CGGTCCAG-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 Master Mix (Cwbio, Beijing, China), and 6.5 µl of ddH_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). *Chrysomyxa weirii* obtained from BOLD was used as an outgroup (Feau *et al*. 2011).

Phylogenetic analysis

The raw sequences obtained and acquired were aligned using ClustalX1.83 (Thomson *et al*. 1997) and MAFFT v.7 (Katoh & Standley 2013). Sequence alignments were deposited at TreeBase (http://www.treebase.org/) under the accession number 19504. 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). Other measures calculated were tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC). The BA was performed using MrBayes 3.1 (Ronquist *et al*. 2005) with Markov chain Monte Carlo (MCMC) and Bayesian posterior probabilities (Larget & Simon 1999). Default parameters were selected, and the evolutionary model was set to the GTR model with gamma-distributed rate variation across sites and a proportion of invariable

sites (Ronquist *et al*. 2005). The simultaneous Markov chains were run with 1,000,000 generations, and the trees were sampled every 100th generation.

Results

Morphology

Based on aeciospores and aecial perdium morphology, the two rust fungi on *P. asperata* were identified as two new *Chrysomyxa* species, *C. diebuensis* and *C. zhuoniensis*. Detailed descriptions of the morphology of the two species are given in the taxonomy section.

Morphology of the two rust fungi was compared with descriptions of other known *Chrysomyxa* species in taxonomic references (Pethybridge 1918, Takahashi & Saho 1985, Crane 2001, 2003, 2005b, Tillman-Sutela *et al.* 2004, Kaitera *et al*. 2010, Feau *et al*. 2011). The two rust fungi differed from other *Chrysomyxa* species in aeciospore surface ornamentation and aecial peridium. *C. diebuensis* has unique nailhead to peltate processes on the aeciospore surface, while most *Chrysomyxa* species possess aeciospores with annulate warts. Although morphological characteristics of aeciospores and aecia of *C. zhuoniensis* were similar to some known *Chrysomyxa* species, i.e., *C. nagodhii*, *C. cassandrae* and *C. succinea*, it differed from them in having a well-defined and smoother longitudinal cap along one side of spore surface.

Molecular phylogeny

The rDNA ITS phylogenetic trees included the 40 samples listed in Table 1. The final data matrix included 669 total characters, with 405 constant characters and 104 parsimony-uninformative variable characters. MP analysis with the remaining 160 parsimony-informative characters resulted in nine equally parsimonious trees with the following parameters: tree length (TL) = 422; consistency index (CI) = 0.815; retention index (RI) = 0.898; and rescaled consistency index (RC) = 0.732. The average standard deviation of split frequencies calculated by BA was 0.009474. Tree topologies, which were obtained based on MP and Bayesian inference, yielded consistent topologies. The phylogenetic results showed that the two new *Chrysomyxa* species on *P. asperata* formed two distinct lineages with a BT value and Bayesian posterior probability of 100/1.00 and 96/0.94, respectively (Fig. 4), and they are phylogenetically distinct from morphologically similar species, i.e., *C. qilianensis* is on *P. crassifolia* Kom., *C. succinea* on *P. wilsonii* Mast..

Molecular work revealed that the rust species on *P. wilsonii* and *C. succinea* on *R. fauriae* formed a monophyletic group with high support values (Fig. 4), suggesting that the rust fungus on *P. wilsonii* is the aecial stage *C. succinea*. Expanded descriptions and illustrations for the aecia of *C. succinea* in China are provided in the taxonomy section.

Taxonomy

Chrysomyxa diebuensis C. J. You & J. Cao, ***sp. nov.*** (Fig. 1)

MycoBank no.:—MB819569

Etymology:—*diebuensis*, referring to the location of the type specimen.

Diagnosis:—Chrysomyxa diebuensis differs from all other *Chrysomyxa* species on *Picea* in possessing aeciospores with nailhead to peltate processes and larger spores.

Type:—CHINA. Gansu Province: Diebu County, on *Picea asperata* Mast. (Pinaceae), 9 August 2012, coll. Y.M. Liang & T. Yang (Holotype: BJFC-R00556). Gansu Province: Diebu County, I on *Picea asperata* Mast. (Pinaceae), 9 August 2012, coll. Y.M. Liang &T. Yang (Paratype: BJFC-R00220).

Spermogonia, uredinia and telia unknown.

Aecia discrete, not confluent, tubular or tongue-like. Aeciospores yellowish or orange, globoid, subgloboid, ellipsoid, or slightly ovoid, 27–43 × 22–33 µm, with wall 4.5 µm thick (Figs 1A, 1B), nailhead or peltate ornamentation, 0.3–0.6 µm in height, 0.8–1.2 µm in width, with flat, smooth and broad heads (Figs 1C, 1D). Aecial peridium persistent, cells overlapping, round, square, or polygonal, outer surface shallowly concave, coarsely striate, inner surface flat or slightly convex, densely warted (Figs 1E, 1F).

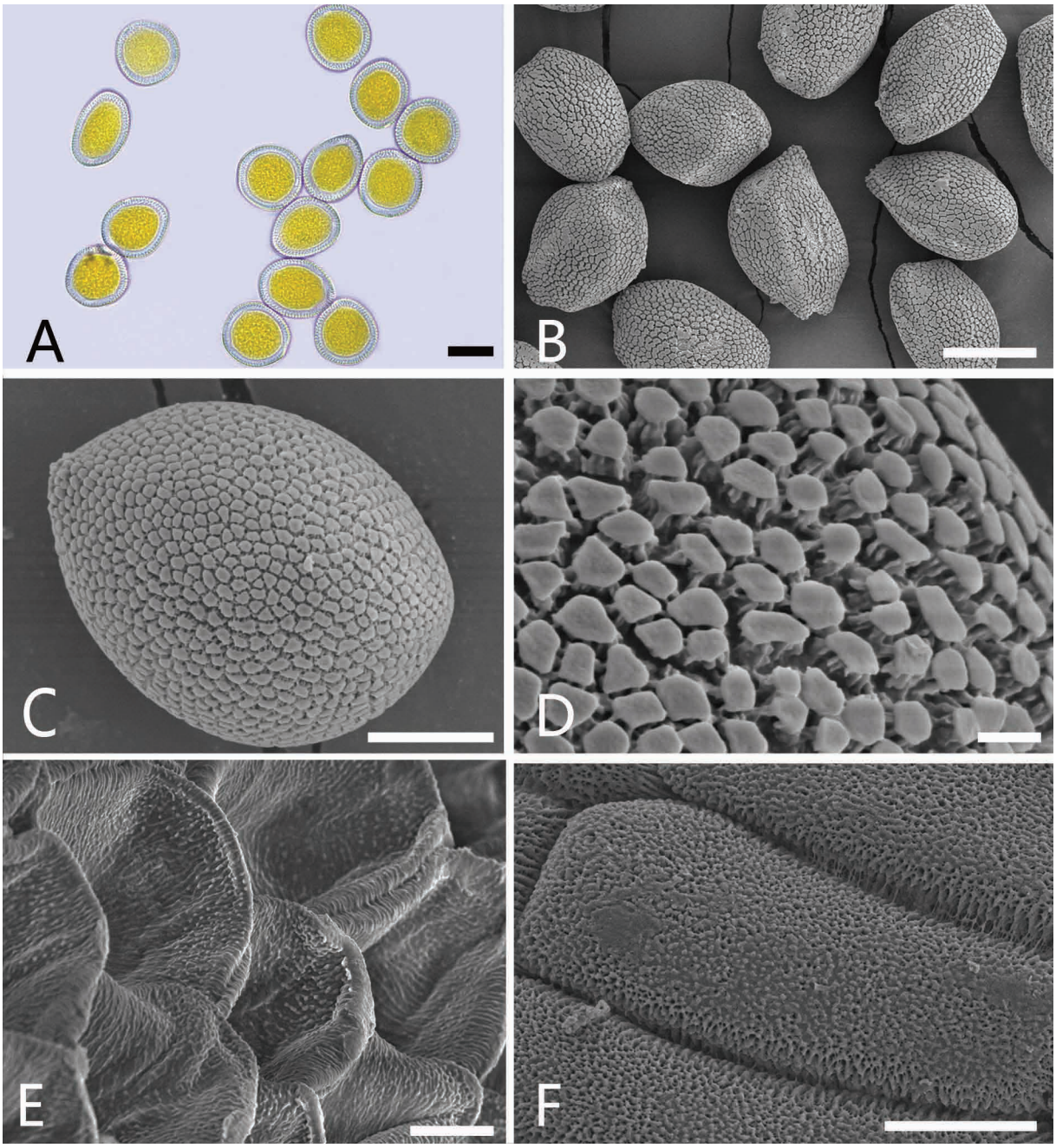

FIGURE 1. *Chrysomyxa diebuensis* (BJFC-R00556, holotype). A. Aeciospores observed by LM. B. Aeciospores observed by SEM. C. Surface ornamentation of single aeciospores. D. Aeciospores with nailhead processes surface. E. Aecial peridium with coarsely striate outer surface. F. Aecial peridium with warted inner surface. Scale bars: A = 30um; B = 20 µm; C= 10um; D = 2 µm; E and F = 10 µm.

Notes:—*Chrysomyxa diebuensis* can be distinguished from the other five *Chrysomyxa* species producing aecial stage on spruce needle, except for *C. qilianensis* in its unique nailhead to peltate processes on aeciospores surface. Most other *Chrysomyxa* species have aeciospores with annulate warts on spore surface (Table 2). Furthermore, the peridial cells of *C. diebuensis* are remarkably different to those of some known *Chrysomyxa* species (Figs 1C, 1D).

Chrysomyxa qilianensis was described first by Wang *et al*. in 1987, which is one of the most prevalent *Chrysomyxa* species on *P. crassifolia* in China (Zhang 2005, Liu & Nan 2011). It is characterized by densely verrucose aeciospores, with single central spine arised frequently from an individual flat columnar verruca (Figs 3C, 3D). *Chrysomyxa diebuensis* is distinct in aeciospore morphology from *C. qilianensis*. The aeciospores of *C. diebuensis* are larger (27–43 × 22–33 µm) (Figs 1A, B) than those of *C. qilianensis* (26–34 × 17–24 µm) (Fig. 3B). SEM clearly demonstrates the morphological differences, especially of the aeciospore surface ornamentation and peridial cells shape. The processes of *C. diebuensis* aeciospores are nailhead to peltate, without the central spine supported on peltate base, and the peridial cells are round or square, which are remarkably distinct from the rectangular cells of *C. qilianensis* (Figs 3E, 3F).

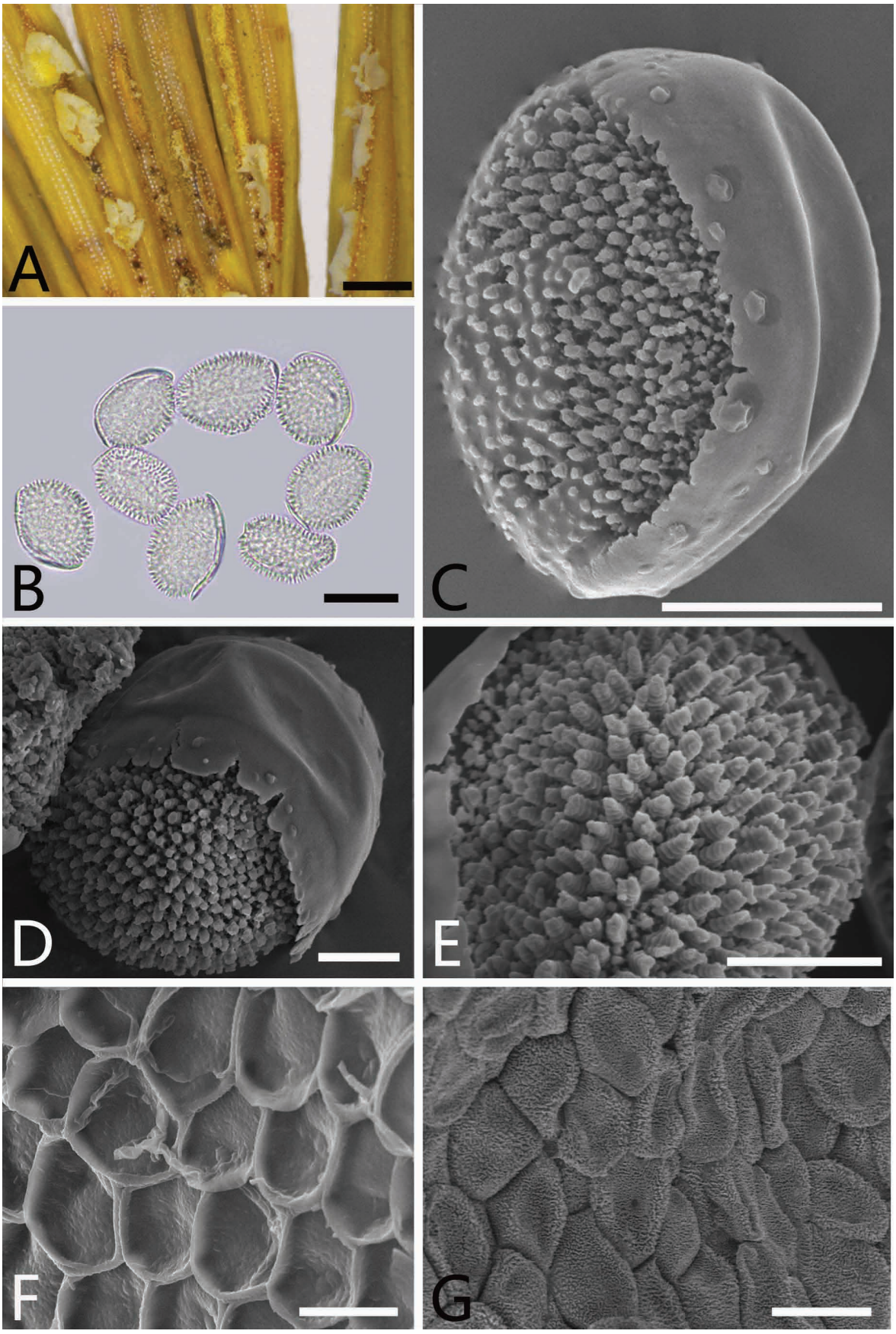

FIGURE 2. *Chrysomyxa zhuoniensis* (BJFC-R00521, holotype). A. Gross features of infected needles. B. Aeciospores observed by LM. C–D. Single aeciospores, showing longitudinal smooth cap with broken, fissured edge. E. Crowded, cylindrical warts with uneven tops. F. Aecial peridium with smooth outer surface. G. Aecial peridium with densely warted inner surface. Scale bars: A = 500um; B = 20 µm; C= 10um; D and E = 5 µm; F and G = 30 µm.

Chrysomyxa zhuoniensis C. J. You & J. Cao, ***sp. nov.*** (Fig. 2)

MycoBank no.:—MB819570

Etymology:—*zhuoniensis*, referring to the location of the type specimen.

Diagnosis:—*Chrysomyxa zhuonisis* differs from all other *Chrysomyxa* species on *Picea* in possessing aeciospores with a distinct broad longitudinal smooth cap at ends of spores.

Type:—CHINA, Gansu Province: Zhuoni County, on *Picea asperata* Mast. (Pinaceae), 7 August 2012, coll. Y.M. Liang &T. Yang (Holotype: BJFC-R00521). Gansu Province: Zhuoni County, on *Picea asperata* Mast. (Pinaceae), 7 August 2012, coll. Y.M. Liang & T. Yang (Paratype: BJFC-R00522).

Spermogonia, uredinia and telia unknown.

Aecia discrete, not confluent, tongue-like, even in width, 0.2–0.5 mm, up to 3 mm long, mostly epiphyllous (Fig. 2A). Aeciospores ellipsoidal, or ovoid, 24–37 × 17–28 µm, wall plus warts 1.9–3.4 µm (Fig. 2B), with a distinct broad, shallow, and smooth cap at one or both ends, with a broken, fissured edge, warts crowded, annulate, tapered or irregular in shape, 4–6 annuli, with uneven tops (Figs 2C, 2D, 2E); aecial peridium persistent, cells polygonal, round or square, outer surface deeply convave, with sharply defined edges, slightly rough surface, inner surface flat to convex, with raised edges, warts distinct and densely crowded (Figs 2F, 2G).

Notes:—There are two *Chrysomyxa* species, *C. nagodhii* and *C. cassandrae* in North America, that resemble *C. zhuoniensis* (Table 3). They both have aeciospores with a conspicuous longitudinal cap, but differ in the surface appearance of the cap (Crane 2005b), with *C. nagodhii* having a rougher cap with a smooth edge than *C. zhuoniensis*, and *C. cassandrae* with a more broad shallow warted cap with a broken edge. *C. zhuoniensis* differs from the other five known *Chrysomyxa* species occurring on spruce needles in China in its smoother longitudinal cap at ends of aeciospore, with a broken and fissured edge (Fig 2C, 2D) (Table 2). *C. ledi* psossesses aeciospore with a narrow longitudinal groove, features not seen in *C. zhuoniensis*. *C. qilianensis* and *C. woroninii* which lack a cap on the aeciospore, and the present species *C. zhuoniensis*, with a conspicuous cap at ends of aeciospore, appears to be distinctly different in aeciospore characters, and *C. rhododendri* and *C. succinea* possess aeciospores with a poorly defined longitudinal smooth stipe (Tai 1979, McBeath 1984, Wang 1987, Cao & Li 1996, 1999, Cao 2000, Crane 2005b, Zhang 2005, Kaitera *et.al.* 2010).

TABLE 2. Comparison of aeciospore morphology of *Chrysomyxa* on spruce needles in China.

species	Aeciospore		Aecial peridium	
	size	ornamentation	Inner surface	Outer surface
C. qilianensis	26–34× 17–24 µm	Ellipsoidal, oblongis with single echinae on peltate base	Inner surface flat and covered densely with warts	Outer surface shallowly concave striate wall smooth to slightly rough
C. succinea	18–34× 13–23 µm	Ellipsoid or subglobose with a poorly defined longitudinal smooth stipe	Inner walls convex,thicker coarsely striate transversally,verrucose	slightly rough Outer walls longitudinal section thin concave or plane;smooth
C. ledi	20–38× 15–28 µm	With a distinct narrow longitudinal groove; warts crowded, annulate, tapering; wall hyaline, 0.8 µm thick;	Inside of cells shallowly concave; shallowly and ensely warted; warts often arranged in undulating rows;	Outside of cells deeply concave; more or less smooth
C. rhododendri	18–30× 16–22 µm	With one or both ends flat or with a small stripe containing irregular shallow bumps,	sometimes appearing labyrinthine; Inner surface convex with shallow warts; surface convex with shallow warts;	On outside,cells shallowly concave,smooth
C. woroninii		Verrucose, with loculous warts	Inner surface densely warted	
C. diebuensis	27–43× 22–33 µm	With the processes of the type are nailhead to peltate ; the nailhead approximately 0.3–0.6 µm in height, 0.8–1.2 µm in width and broad heads are flat	Inner surface flat; covered densely with warts	Outer surface shallowly concave coarsely striate; wall smooth to slightly rough
C. zhuoniensis	24–37× 17–28 µm	With a distinct broad shallow cap at one or both ends of spores; the broad longitudinal smooth, with a broken, fissured edge; with uneven tops; wall plus warts 1.9–3.4µm.	Inner surface flat; covered densely crowded;	Outer surface cells deeply convave, with sharply defined edges, slightly rough surface

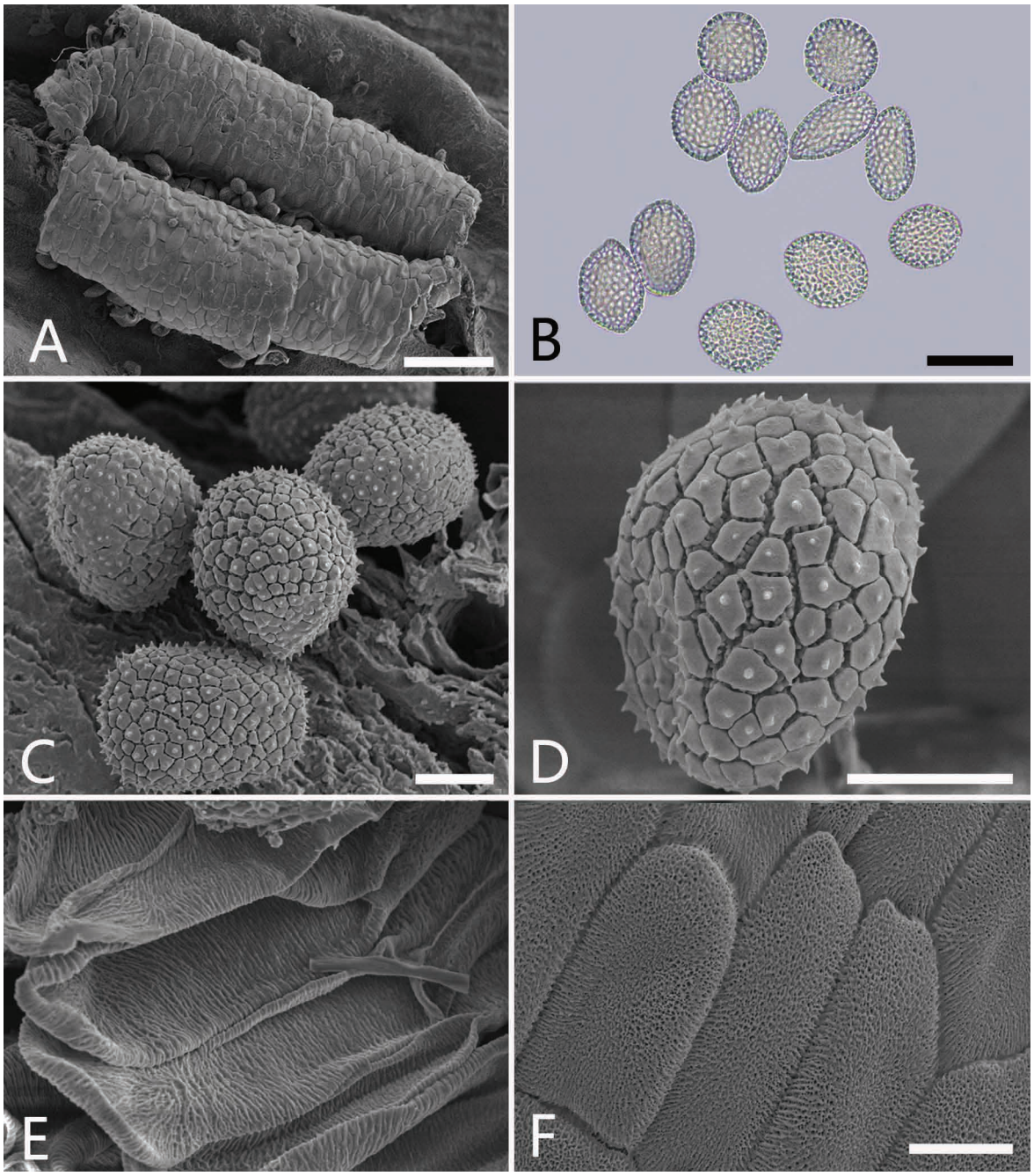

FIGURE 3. *Chrysomyxa qilianensis* (HMAS-52077, holotype). A. Infected needles of *P. crassifolia* with aecia. B. Aeciospores observed by LM. C. Aeciospores observed by SEM. D. Aeciospores with single echinae on peltate base. E. Aecial peridium with coarsely striate outer surface. F. Aecial peridium with warted inner surface. Scale bars: A = 100um; B = 30 μm; C = 10um; D and E = 10μm; F = 10 μm.

Chrysomyxa succinea (Sacc.) Tranz., Consp. Ured. URSS, Moscow, p.70, 314. 1939. (Fig. 5)

Specimens examined:—CHINA, Shaanxi Province: Ningshan County, on *Picea wilsonii* Mast. (Pinaceae), 10 August 2015, coll. J. Cao (BJFC-R02306; BJFC-R02307).

Spermogonia unknown.

Aecia hypophyllous, in 2 rows on yellow lesions, elongate, laterally compressed. Aeciospores globose, subglobose, ellipsoidal or slightly ovoid, 18–34 × 13–23 μm, wall plus warts 1.0–2.8 μm thick (Fig. 5A); aeciospores with a broad shallow cap at one or both ends, the broad longitudinal area with a broken, skirt-like edge (Fig. 5B); warts cylindrical, annulate, with smooth or rough tops (Figs 5C, 5D); peridium persistent, cells overlapping, polygonal, round or square, outer surface flat, smooth (Fig. 5E); inner surface with raised edges, warts distinct and densely crowded (Fig. 5F).

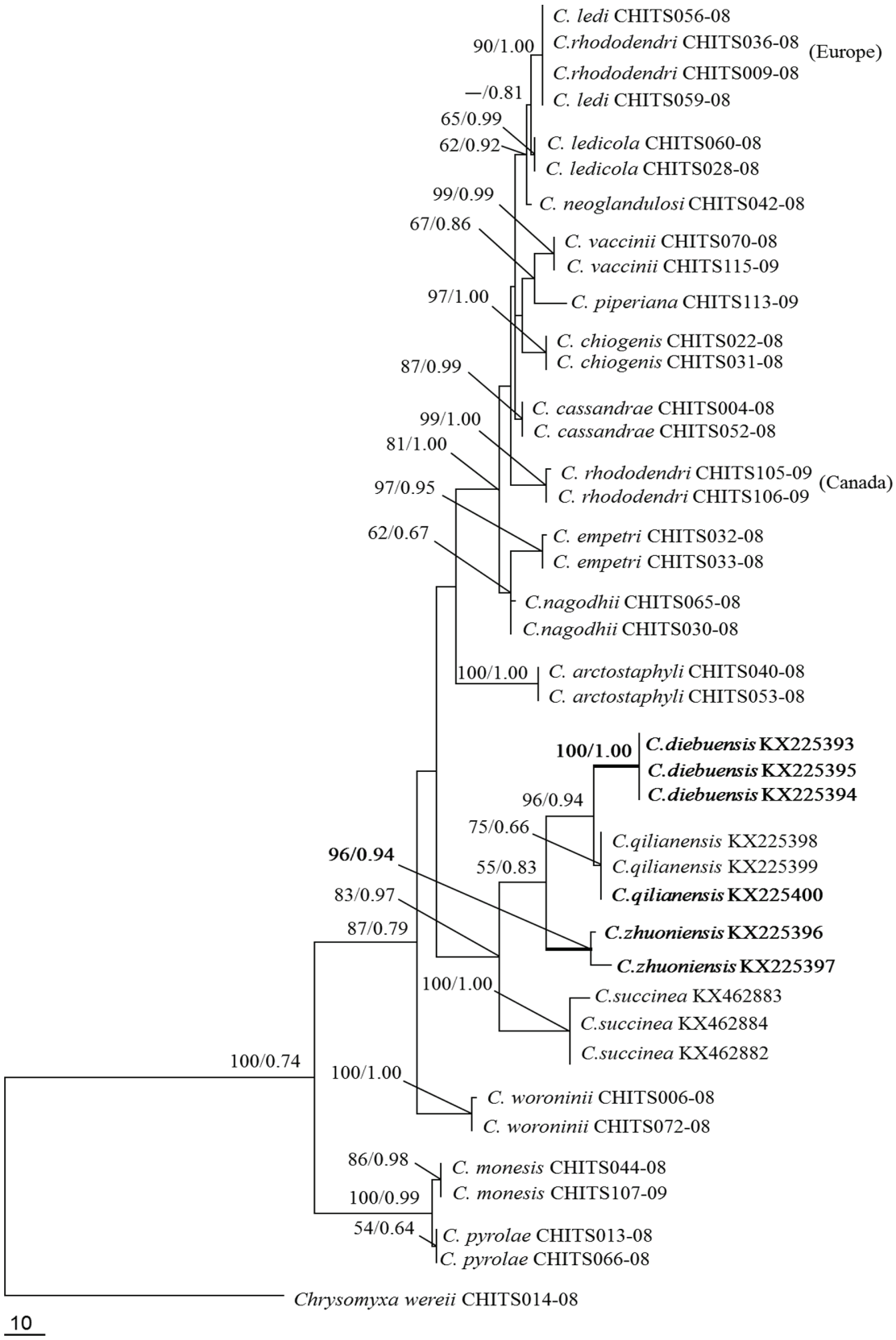

FIGURE 4. Phylogram constructed by maximum parsimony and Bayesian analyses based on ITS sequences. Bootstrap values were calculated from 1,000 replications. Parsimony bootstrap (before the slash marks) and Bayesian posterior probabilities (after the slash marks) greater than 50% are shown. *Bars*: 10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

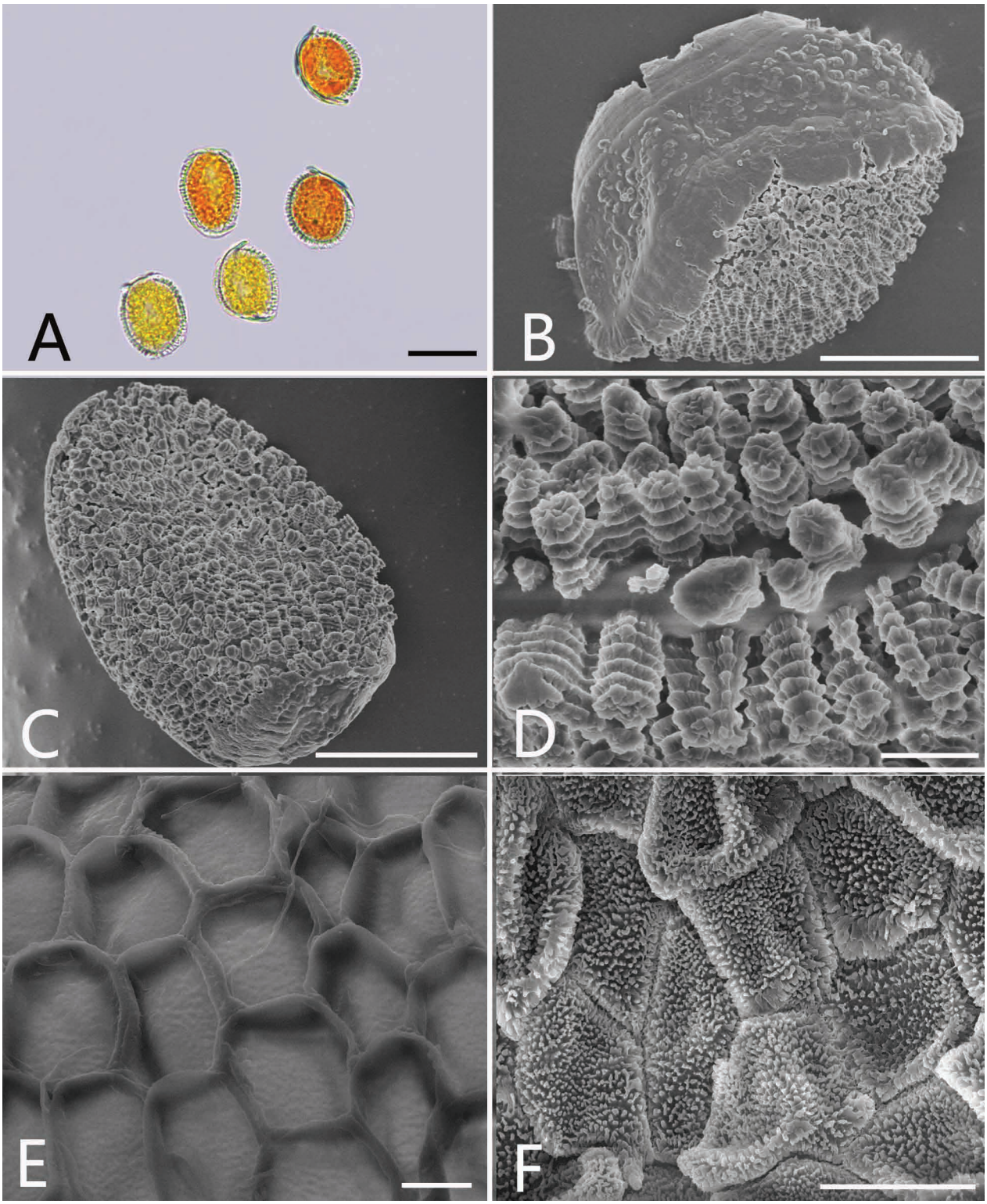

FIGURE 5. *Chrysomyxa succinea* (BJFC-R02306). A. Aeciospores observed by LM. B. Aeciospores observed by SEM,showing broad longitudinal area or cap;C. crowded,cylindrical warts with smooth or rough tops. D. Aeciospores with warts cylindrical, 4–6 annuli.E. Aecial peridium with smooth outer surface. F. Aecial peridium with densely warted inner surface. Scale bars: A = 20um; B and C = 10μm; D = 1μm; E = 10 μm; F = 20 μm.

Notes:—*Chrysomyxa succinea* was described by Saccardo (1880) as *Gloeosporium succineum*, and then transferred to *Chrysomyxa* by Tranzschel (1939). Detailed morphological descriptions of uredinia and telia stage were provided by Crane (2005b), but no confirmed material of the spermogonia and aecia stage from inoculations was examined (Hiratsuka *et al*.1992, Crane 2005b). During our survey, Chinese specimens collected on needles of *P. wilsonii* were morphologically identified and proved by molecular phylogenetic analysis to be the aecial stage of *C.*

succinea. The type specimen of *C. succinea* is not available, but morphological examinations of the three paratype specimens (HMAS 6142, HMAS 6145, HMAS 58633) showed that the morphological characteristics fit well with the description by Crane (Crane 2005b). The present study also demonstrated that *P. wilsonii* is the first host record for *C. succinea* in China.

TABLE 3. Comparison of the aecial stage of *C. nagodhii* , *C. cassandrae* and ***C. zhuoniensis.***

	C. nagodhii	*C. cassandrae*	***C. zhuoniensis***
Aeciospore ornamentation	Longitudinal cap slightly rough with smooth edge	Longitudinal cap with broad flat warts; edge broken and fissured	Longitudinal cap smooth; edge broken and fissured
	Warts tapered, 4 annuli	Warts cylindrical, 3 annuli	Warts tapered, 4–6 annuli
Outer peridium surface	Cells deeply concave, with sharply defined edges; slightly rough surface	Cells shallowly concave with poorly defined edges, smooth to slightly rough surface	Cells concave, with sharply defined edges, smooth surface
Inner peridium surface	Cells shallowly concave with a raised edge; warts shallow, irregular, discrete and fine or forming shallow ridges, sometimes appearing reticulate	Cells flat to convex; warts distinct and densely crowded, appear fingerlike; edges striate or rugulose	Cells flat to convex, with a raised edges, warts densely crowded

Discussion

The new species *C. diebuensis* was characterized by larger aeciospores (27–43 × 22–33 μm) with nailhead to peltate processes on spore surface (Figs 1A, 1D). The other new species, *C. zhuoniensis*, was characterized by a distinct broad longitudinal smooth cap at ends of aeciospores, with a broken, skirt-like edge (Figs 2C, 2D). Molecular phylogenetic analyses using sequence data of rDNA ITS region indicated that *C. diebuensis* and *C. zhuoniensis* were two distinct lineages with high BT and Bayesian posterior probability (100/1.00 and 96/0.94, respectively) (Fig. 4). A morphological examination revealed that the two new species were clearly distinguishable from other known *Chrysomyxa* species in their aeciospore ornamentation and aecial perdium morphology, characters which have been frequently used as important criterion for species recognition (Hiratsuka & Kaneko 1975, Sato & Sato 1982, Lee & Kakishima1999, Crane 2001, 2005b).

Phylogenetic analyses revealed that *C. diebuensis* was more closely related to *C. qilianensis* (Fig. 4), however, it differed from *C. qilianensis* in its unique nailhead to peltate processes on aeciospore surface, without the central spine arised from the peltate base. In addition, *C. diebuensis* differed from *C. qilianensis* in its larger aeciospore and round or square peridial cells. The ITS sequence data showed *C. zhuoniensis* clustered closely with *C. succinea* (Fig. 4), but *C. zhuoniensis* was clearly distinguished from *C. succinea* in its larger aeciospores and longitudinal smooth cap on the aeciospores; the latter had aeciospores with a poorly defined longitudinal smooth stipe, not a cap (Fig. 5). Although *C. zhuoniensis* resembled *C. nagodhii* and *C. cassandrae* in the longitudinal cap on the aeciospores, it was distinguished by the smoother cap with a broken and fissured edge (Table 3), and molecular phylogenetic analyses also indicated that *C. zhuoniensis* was clearly distinct from *C. nagodhii* and *C. cassandrae* (Fig. 4).

Feau *et al*. (2010) revealed the ITS locus to be the most appropriate DNA barcode candidate for *Chrysomyxa*, because it was usually in agreement with taxonomic species based on morphological characters. Similar to the finding in Feau *et al*. (2010), our molecular work also demonstrated that *C. rhododendri* from Europe and *C. ledi* from North America had high sequence similarity or even identity, suggesting that they are conspecific. Although the morphological trait delimitations of *C. rhododendri* (Europe) and *C. ledi* remain particularly subtle, mainly differed in aecial peridia and aeciospore ornamentation, as well as the distinct uredinial host specificities (*C. rhododendri* from Europe occurs on *Rhododendron* sp., *C. leidi* occurs on *Ledum* sp.). Furthermore, *C. rhododendri* from Europe and North America were clearly different morphologically and were separated into two unrelated clades in the rDNA ITS phylogeny, as well as the differences in life cycles, which supported that *C. rhododendri* from Europe and North America should be considered to be two allopatric races or cryptic species (Crane 2001, Feau *et al*. 2010). However, molecular phylogenetic studies on *C. rhododendri* and *C. ledi* in China have not been conducted, and further taxonomic investigation and multigene phylogenies are required, which should help to identify evolutionary patterns that drive speciation within the genus *Chrysomyxa*.

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References

Berndt, R. (1999) *Chrysomyxa* rust: morphology and ultrastructure of D-haustoria, uredinia, and telia. *Canadian Journal of Botany* 77: 1469–1484.
https://doi.org/10.1139/b99-113

Cao, Z.M., Yang, J.X., Tian, C.M. & Li, Z.Q. (1996) Tree rusts in Qinling Mountains (in Chinese with English summary). *Journal of Northwest Forestry College* 11: 91–98.

Cao, Z.M. & Li, Z.Q. (1999) *Rust fungi of Qinling Mountains* (in Chinese). China Forestry Publishing House, Beijing.

Cao, X.W. (2000) Studies of the pathogen and alternative host of *Picea asperata* Mast. and *Picea crassifolia* Kom.. *Journal of Central South forestry University* 20(2): 78–80.

Crane, P.E. (2001) Morphology, taxonomy and nomenclature of the *Chrysomyxa ledi* complex and related rust fungi on spruce and Ericaceae in North America and Europe. *Canadian Journal of Botany* 79: 957–982.
https://doi.org/10.1139/cjb-79-8-957

Crane, P.E. (2003) *Caeoma cassiopae* sp. nov., a rust on *Cassiope tetragona* in the Canadian Rocky Mountains. *Mycologia* 95(1): 156–159.
https://doi.org/10.1139/b99-113

Crane, P.E., Yamaoka, Y., Engkhaninum J. & Kakishima, M. (2005a) *Caeoma tsukubaense*n. sp., a *rhododendron* rust fungus of Japan and southern Asia, and its relathionship to *Chrysomyxa rhododendri*. *Mycoscience* 46: 143–147.
https://doi.org/10.1007/s10267-004-0228-2

Crane, P.E. (2005b) Rust fungi on *rhododendrons* in Asia: *Diaphanopellis forrestii* gen. et sp. nov., new species of *Caeoma*, and expanded descriptions of *Chrysomyxa dietelii* and *C. succinea. Mycologia* 97(2): 534–548.
https://doi.org/10.3852/mycologia.97.2.534

Cummins, G.B. & Hiratsuka, Y. (2003) *Illustrated genera of rust fungi, 3rd edn*. The American Phytopathological Society Press, St. Paul.

Feau, N., Vialle, A., Allaire, M., Maier, W. & Hamelin, R., C. (2011) DNA barcoding in the rust genus *Chrysomyxa* and its implications for the phylogeny of the genus. *Mycologia* 103 (6): 1250–1266.
https://doi.org/10.3852/10-426

Felsenstein, J. (1985) Phylogenies and the comparative method. *The American Naturalist* 125 (1): 1–15.
https://doi.org/10.1086/284325

Fu, L.G., Li, N. & Rober, R.M. (1999) *Flora of China* 4: 25–32. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis.

Fu, W.B., Yuan, H., Zhang, Y.S., Sun, X.X., Wang, Y.K., Li, j. j., Yin, C. L.& Zhang, H.R. (2000) On cone and seed life table of *Picea crassifolia* Kom. *Journal of Beijing Forestry University* 22(5): 74–77.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hiratsuka, N. & Kaneko, S. (1975) Surface structure of *Coleosporium* spores. Rep. *Reports of the Tottori Mycological Institute* 12: 1–13.

Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T.,Harada, Y., Hiratsuka, T. & Nakayama, K. (1992) *The rust flora of Japan*. Tsukuba Shuppankai, Tsukuba.

Kaitera, J., Tillman-Sutela, E. & Kauppi, A. (2010) *Chrysomyxa ledi*, a new rust fungus sporulating in cone scales of *Picea abies* in Finland. *Scandinavian Journal of Forest Research* 25(3): 202–207.
https://doi.org/10.1080/02827581.2010.488657

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
https://doi.org/10.1093/molbev/mst010

Kirk, P.M., Cannon, P. F., Minter, D.W. & Stalpers, J.A. (2008) *Dictionary of the Fungi.*10th ed. Wallingford, UK.

Larget, B. & Simon, D.L. (1999) Markov Chain Monte Carlo algorithms for the Bayesian Analysis of phylogenetic trees. *Molecular*

Biology and Evolution 16 (6): 750–759.
https://doi.org/10.1093/oxfordjournals.molbev.a026160

Lee, S.K. & Kakishima, M. (1999) Aeciospore surface structures of *Gymnosporangium* and *Roestelia* (Uredinales). *Mycoscience* 40: 109–120.
https://doi.org/10.1007/BF02464289

Liu, W. N. & Nan, T.C. (2011) Studies of the pathogen and alternative host of rusts on *Picea crassifolia* Kom. *Anhui Agricultural Science Bull* 17(13): 127–129.

McBeath, J.H. (1984) Symptomatology on spruce trees and spore characteristics of a bud rust pathogen. *Phytopathology* 74: 456–461.
https://doi.org/10.1094/Phyto-74-456

Pethybridge, H.G. (1918) *Chrysomyxa abietis* in Ireland. *The irish naturalist* 27(8): 130.

Ronquist, F., Huelsenbeck, J.P. & van der Mark, P. (2005) *MrBayes 3.1 Manual*.

Sato, T. & Sato, S. (1984) Aeciospore surface structure of the Uredinales. *Transactions of the Mycological Society of Japan* 23: 51–63.

Sutherland, J.R. (1981) Effects of inland spruce cone rust, *Chrysomyxa pirolata* Wint., on seed yield, weight and germination. *Canadian Journal of Forest Research* 1: 8–9.

Swofford, D.L. (2002) *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sinauer, Sunderland, MA.

Tai, F.L. (1979) *Sylloge fungorum sinicorum* (in Chinese). Science Press, Beijing.

Takahashi, O. & Saho, H. (1985) Notes on the Japanese rust fungi IV. Dissemination of *Chrysomyxa abietis* (Wallroth) Unger. *Transactions of the Mycological Society of Japan* 26: 433–439.

Thomson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.
https://doi.org/10.1093/nar/25.24.4876

Tian, C.M., Shang, Y.Z., Zhuang, J.Y., Wang, Q. & Kakishima, M. (2004) Morphological and molecular phylogenetic analysis of *Melampsora* species on poplars in China. *Mycoscience* 45: 56–66.
https://doi.org/10.1007/S10267-003-0150-Z

Tillman-Sutela, E., Kauppi, A., Hilli, A. & Kaitera, J. (2004) Fungal injury to seed tissues of Norway spruce, *Picea abies* (L.) Karst. *Trees* 18: 151–156.
https://doi.org/10.1007/s00468-003-0291-x

Vander Auwera, G., Chapelle, S. & De Wachter, R. (1994) Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of the oomycetes. *FEBS letters* 338 (2): 133–136.
https://doi.org/10.1016/0014-5793(94)80350-1

Wang, Y.C. (1987) A new spruce needle rust fungus. *Acta Mycologica Sinica*. 6(2): 86–88.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Snisky, J.J., White, T.J. (Ed.) *PCR protocols: a guide to methods and applications*. San Diego, USA, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, X.L. (2005) Preliminary study on leaf rust of *Picea crassifolia* in Lianhuashan National Nature Reserve of Gansu Province. *Journal of Gansu Forestry Science and Technology* 31(3): 32–48.

Zhao, Z.Y. (1958) Three spruce rusts in Tianshan forest farm. *Journal of Xinjiang Agricultural Sciences* (5): 190–192.