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Galbinothrix, a new monotypic genus of *Chrysotrichaceae* (*Arthoniomycetes*) lacking pulvinic acid derivatives

Andreas Frisch[1*, 2], Göran Thor[2], Kwang Hee Moon[3] & Yoshihito Ohmura[4]

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Abstract. *Galbinothrix caesiopruinosa* is described from Japan and Korea. The new genus and species is placed in *Chrysotrichaceae* by its ascoma morphology and by a phylogenetic analysis of mtSSU and nLSU sequence data using Bayesian and maximum likelihood inference. The monotypic genus *Galbinothrix* is superficially similar to *Chrysothrix caesia* in having dark brown ascomata covered by a thin bluish grey pruina, reddish brown ascomatal pigment in the epithecium and proper exciple, the greyish green to yellowish olive thallus, and usnic acid as the main secondary thallus compound. It differs from this species and all other *Chrysotrichaceae* by its large, oblong, thick-walled ascospores with a distinct epispore, the narrowly clavate to almost tubular asci, and the never clearly granular to leprose thallus.

Key words: *Arthoniales*, *Ascomycota*, East Asia, taxonomy, lichenized fungi

Introduction

Chrysotrichaceae (Zahlbruckner 1905) was described as a monotypic family. *Chrysothrix* (Montagne 1852), the only genus originally included in the family, shows close affinities to *Arthoniaceae* but differs, among other characters, by the chlorococcalean instead of trentepohlioid photobionts and by the characteristic yellow pigments consisting of pulvinic acid derivatives in the ascomata and thallus (Zahlbruckner 1905; Laundon 1981; Elix 2009; Fletcher & Purvis 2009). Recent phylogenetic studies have shown that *Chrysotrichaceae* thus delimited is paraphyletic and further includes taxa lacking pulvinic acid derivatives, which were – or would have been – previously classified in the genus *Arthonia* in *Arthoniaceae* (Nelsen et al. 2009; Frisch et al. 2014). Such taxa include *Chrysothrix caesia* (Ertz & Tehler 2011), *Arthonia mediella* and the newly described *Melarthonis piceae* (Frisch et al. 2014).

In the course of recent fieldwork in Japan and Korea, we frequently collected a species of *Arthoniales* from smooth-barked deciduous trees which showed a superficial resemblance to *Chrysothrix caesia* but proved unrelated upon closer examination. Here we describe this species in its own genus as new to science, and we demonstrate its phylogenetic position relative to the other taxa lacking pulvinic acid derivatives in *Chrysotrichaceae* by Bayesian and ML analysis of mtSSU and nLSU sequence data.

Material and methods

Lichen sampling and investigation

Type material is deposited at the herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS), with duplicates in the National Institute of Biological Resources, Incheon, Korea (KB), Museum of Evolution, Uppsala, Sweden (UPS), and hb Frisch. Additional specimens collected in Japan are kept in TNS, UPS and hb Frisch, while specimens from Korea are deposited at KB, UPS and hb Frisch. Morphology was examined using an Olympus SZX7 dissecting microscope. Sections for anatomical examination were cut by hand, mounted in water or lactic cotton blue (LCB), and examined with an Olympus BX51 light microscope. All measurements were made on preparations mounted in LCB.

Secondary lichen compounds were identified by TLC (Orange et al. 2010) and HPTLC (Arup et al. 1993) using solvents B' and C. The amyloidity of the thallus and apothecia was examined using 1% aqueous iodine solution without (I) or with pre-treatment with 10% aqueous potassium hydroxide (KI). The color reaction of the thallus and apothecia was tested using 10% aqueous potassium hydroxide (K), potassium hypochlorite as

[1] Department of Natural History, NTNU University Museum, Erling Skakkes gate 47a, 7012 Trondheim, Norway
[2] Department of Ecology, Swedish University of Agricultural Sciences, P.O. Box 7044, SE-750 07 Uppsala, Sweden
[3] National Institute of Biological Resources, Incheon 22689, South Korea
[4] Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, 305-0005, Japan
[*] Corresponding author e-mail: andreas.frisch@ntnu.no

applied in common household bleach (C), 10% aqueous potassium hydroxide followed by potassium hypochlorite (KC), and 1,4-phenylendiamine in 96% ethanol (Pd). The UV reaction of the thallus and apothecia was tested under UV_{365} light. Calcium oxalate crystals were identified by applying 10% sulphuric acid to squash preparations of thallus samples.

DNA extraction, PCR amplification and sequencing

Molecular work was performed in Japan at the National Museum of Nature and Science (Tsukuba) and in Korea at the National Institute of Biological Resources (Incheon). Genomic DNA was extracted using the DNEasy Plant Mini Kit following the protocol of the manufacturer. Each PCR reaction of 20 µl contained 1–3 µl DNA extract, depending on the DNA concentration in the extract, and 1 µl (mtSSU, nLSU) of each primer (10 pmol/µl). The primers used for PCR amplification were mtSSU1 and mtSSU3R for mtSSU (Zoller et al. 1999), and LIC24R and LR5 for nLSU (Vilgalys & Hester 1990, Miadlikowska & Lutzoni 2000). PCR cycling conditions for mtSSU were 95°C (4 min) followed by 9 cycles of 95°C (30 sec), 62°C to 54°C (45 sec) with annealing temperatures lowered by 1°C between cycles, and 72°C (45 sec) followed by 30 cycles at 54°C annealing temperature and final extension at 72°C (7 min). For nLSU annealing temperatures started

Table 1. Vouchers used for the phylogenetic tree and the GenBank accession numbers of the sequences. New sequences are indicated in bold.

Species	Voucher	mtSSU	RPB2	nLSU
Alyxoria varia	Sweden; *Frisch 11/Se1* (UPS)	KJ851006	KJ851147	KJ851027
Arthonia apatetica	Sweden; *Svensson 2017* (UPS)	KJ850992	KJ851125	KJ851045
Arthonia apotheciorum	Sweden; *Frisch 11/Se23* (UPS)	KJ850970	KJ851148	–
Arthonia calcarea	France; *Ertz 7539* (BR)	EU704064	EU704028	–
Arthonia eos	Japan; *Thor 26000* (UPS)	KJ850987	KJ851134	KJ851053
Arthonia mediella	Sweden; *Frisch 11/Se22* (UPS)	KJ851014	KJ851133	KJ851032
Arthonia neglectula	Sweden; *Frisch 10/Se90* (UPS)	KJ850989	KJ851118	KJ851037
Arthonia punctiformis	Sweden; *Thor 21658* (UPS)	KJ850973	KJ851113	KJ851044
Arthonia radiata	Sweden; Frisch 10/SE29 (UPS)	KJ850968	KJ851108	–
Arthonia sp. (Ug10)	Uganda; *Frisch 11/Ug218* (UPS)	KJ850986	KJ851131	KJ851068
Arthonia sp. (Ug9)	Uganda; *Frisch 11/Ug212* (UPS)	KJ850985	KJ851129	KJ851064
Bryostigma muscigenum	Sweden; *Thor 26206* (UPS)	KJ850991	KJ851124	KJ851052
Chiodecton natalense	Uganda; *Frisch 11/Ug324* (UPS)	KF707647	KF707660	KF707641
Chrysothrix caesia	USA; *Amtoft* (AFTOL-ID 775)	FJ469671	FJ469670	FJ469668
Chrysothrix candelaris	Sweden; *Frisch 11/Se45* (UPS)	KF707649	KF707663	KF707640
Combea mollusca	South Africa; *Tehler 7725* (S)	AY571384	DQ987626	EF081383
Coniocarpon cinnabarinum	Uganda; *Frisch 11/Ug297* (UPS)	KJ850977	KJ851104	KJ851059
Dichosporidium boschianum	Fiji Islands; *Lumbsch 19815a* (F)	GU327692	–	GU327716
Dichosporidium brunnthaleri	Uganda; *Frisch 11/Ug8* (UPS)	KJ851011	KJ524362	KJ524283
Dimidiographa longissima	Florida; *Ertz 9155* (BR)	EU704069	EU704033	EU704097
Dothidea sambuci	*AFTOL-ID 274*	AY544739	DQ522854	AY544681
Enterographa crassa	France; *Ertz 5041* (BR)	EU704056	EU704020	EU704088
Felipes leucopellaeus	Sweden; *Frisch 10/Se34* (UPS)	KJ850984	KJ851130	KJ851033
Fouragea filicina	Rwanda; *Ertz 7994* (BR)	EU704067	EU704031	EU704095
Galbinothrix caesiopruinosa	Japan; *Frisch 12/Jp282* (UPS)	**MK107828**	–	–
Galbinothrix caesiopruinosa	Japan; *Frisch 13/Jp247* (TNS)	**MK107822**	–	**MK107829**
Galbinothrix caesiopruinosa	Japan; *Frisch 13/Jp253* (TNS)	**MK107823**	–	**MK107830**
Galbinothrix caesiopruinosa	Korea; *Frisch 16/Kr526* (UPS)	**MK107825**	–	**MK107832**
Galbinothrix caesiopruinosa	Korea; *Frisch 16/Kr527* (UPS)	**MK107824**	–	**MK107831**
Galbinothrix caesiopruinosa	Korea; *Frisch 16/Kr564 dpl.* (UPS)	**MK107826**	–	**MK107833**
Galbinothrix caesiopruinosa	Korea; *Frisch 16/Kr585* (UPS)	**MK107827**	–	**MK107834**
Herpothallon rubrocinctum	Mexico; *Rudolphi 5* (UPS)	KF707643	KF707655	–
Inoderma byssaceum	Japan; Thor 25952 (UPS)	KJ850962	KJ851089	KJ851040
Lecanactis abietina	Belgium; *Ertz 5068* (DUKE)	AY548813	AY552018	AY548812
Lecanographa amylacea	Sweden; *Thor 26176* (UPS)	KF707650	KF707659	KF707639
Melarthonis piceae	Japan; *Thor 25995* (UPS)	KJ851016	–	KJ851080
Nyungwea pallida	Uganda; *Frisch 11/Ug24* (UPS)	KJ851023	KJ851145	KJ851066
Opegrapha vermicellifera	Belgium; *Ertz 7562* (BR)	EU704077	EU704041	EU704105
Opegrapha vulgata	Belgium; *Ertz 7564* (BR)	EU704080	EU704044	EU704108
Phacographa zwackhii	Sweden; *Frisch 11/Se3* (UPS)	KJ851021	–	KJ851048
Pleospora herbarum	*AFTOL-ID 940*	FJ190610	DQ247794	DQ247804
Reichlingia leopoldi	Belgium; *Ertz 13294* (BR)	JF830774	HQ45472	HQ454582
Tylophoron hibernicum	Uganda; *Frisch 11/Ug220* (UPS)	KJ850966	KJ851097	KJ851065
Zwackhia viridis	Luxembourg; *Ertz 7619* (BR)	EU704078	EU704042	EU704106

at 62°C and were lowered to 56°C. Sequencing of the PCR products was done at the National Museum of Nature and Science on a 3130xl Genetic Analyzer (Applied Biosystems) and at the National Institute of Biological Resources by Macrogen Inc., Korea.

Alignment

RPB2 sequences were added from GenBank for better backbone support of the *Arthoniales* tree. The sequences of the three gene loci were aligned separately using the general MAFFT settings as implemented in the Guidance Web Server (Penn et al. 2010) and manually corrected. Introns, longer insertions, and ambiguously aligned regions were excluded prior to analysis. The final concatenated alignment comprised 2654 nucleotide positions, 725 for mtSSU, 1048 for nLSU and 881 for RPB2. Of these, 1272 (mtSSU 399, nLSU 355, RPB2 518) were variable and 1061 (mtSSU 331, nLSU 255, RPB2 475) were parsimony-informative. A partitioned dataset was used for the phylogenetic analyses to enable independent parameter estimation for the three gene loci. The RPB2 dataset was further partitioned into two coding regions separated by a short intron region (369–26–486 bps, respectively). Coding regions were partitioned according to codon positions to allow for the higher evolutionary rates of the 3rd codon position.

ML and Bayesian analyses

A general-time-reversible model with a proportion of invariable sites (GTR-I-Γ) was found to best explain the sequence evolution for the mtSSU, nLSU and RPB2 data set using the Akaike information criterion (AIC; Akaike 1973) implemented in MEGA5 (Tamura et al. 2011). Bayesian inference (Huelsenbeck et al. 2001; Holder & Lewis 2003) and maximum likelihood (ML) were used to infer phylogenetic hypotheses. Prior to concatenation the single-gene alignments were tested for conflicting tree topologies. Serious conflict was assumed when deviant tree topologies were supported by ≥70% bootstrap values (BS) and ≥0.95 posterior probabilities (PP).

Bayesian analysis was performed with MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003) implemented in the CIPRES Science Gateway (Miller et al. 2010). A GTR-I-Γ model of sequence evolution was applied to the partitioned dataset, and the model parameters were estimated during the run for each gene partition separately starting from a default flat Dirichlet distribution. The analysis was run for 10,000,000 generations in eight chains and every 500th generation was sampled. The first 50% of trees were discarded as burn-in and a 50% majority rule consensus tree was calculated.

Maximum likelihood was performed with the RAxML-HPC Black Box v. 8.2.10 implemented in the CIPRES Science Gateway (Miller et al. 2010) using rapid bootstrapping and full ML analysis under the GTR+GAMMA approximation allowing for a proportion of invariable sites. The analysis was stopped automatically after 102 bootstrap replicates using the bootstopping option implemented in RAxML (Pattengale et al. 2009).

Results

Phylogeny

We generated 13 new sequences for *Galbinothrix caesiopruinosa*: 7 mtSSU and 6 nLSU (Table 1). Sequencing of RPB2 failed for all samples using the general primers RPB2-7cF and RPB2-11aR (Liu et al. 1999). *Galbinothrix* is recovered as sister to the remaining *Chrysotrichaceae*, separated from the other genera in the family – *Chrysothrix* and *Melarthonis* – and *Arthonia mediella* by long branches.

Only five (mtSSU) and four (nLSU) variable nucleotide positions have been identified for the analyzed specimens of *G. caesiopruinosa*. Two specimens from Japan [13/Jp247 and 13/Jp253 (Type!) from Chichibu-Tama-Kai National Park, Nagano] differ in three of these nucleotide positions (mtSSU) from the other samples, but generally the collections from Japan and Korea are not separated in our analyses.

Taxonomy

Galbinothrix Frisch, G. Thor, K. H. Moon & Y. Ohmura, gen. nov.

MycoBank MB 828448.

Diagnosis: The monotypic genus *Galbinothrix* is characterized within *Chrysotrichaceae* by its thin and discontinuous to fissured-areolate thallus lacking pulvinic acid derivatives; dark brown, adnate, thinly to densely bluish grey pruinose ascomata with well-developed reddish brown pigmented proper exciple and epithecium; and usnic and isousnic acids as secondary lichen compounds. *Galbinothrix* is superficially similar to *Chrysothrix caesia*, but differs from this species and from all other *Chrysotrichaceae* by its larger oblong ascospores (27–36 × 5–7 µm), with distinct epispore, and the narrowly clavate to almost tubular asci (75–115 × 19–26 µm).

Generic type: *Galbinothrix caesiopruinosa* Frisch, G. Thor, K. H. Moon & Y. Ohmura

Galbinothrix caesiopruinosa Frisch, G. Thor, K. H. Moon & Y. Ohmura, sp. nov. (Figs 1A–E)

MycoBank MB 828449.

Diagnosis: The only species of *Galbinothrix* with the same diagnostic characters as the genus.

Type: Japan, Shinano Prov., Nagano Pref., Minamisaki-gun, Kawakami-mura, Chichibu-Tama-Kai National Park, along Chikumagawa River, 35°57′30.5″N, 138°42′05.3″E, on smooth bark of *Alnus* sp. in riverine forest, elev. 1414 m, 28 May 2013, Frisch 13/Jp253 & Ohmura (TNS – holotype; KB, UPS and hb Frisch – isotypes).

Description. Thallus rather variable, pale to dark greyish green to yellowish olive, thin and discontinuous to fissured-areolate, matte, consisting of discrete to confluent patches ca 0.1–0.3 mm diam., up to 0.15 mm thick, with uneven to shallowly warty to distinctly bullate to almost subgranular surface, partly endophloeodal; prothallus not observed. Photobiont layer 40–80 µm thick; photobiont chlorococcoid, the cells 5–19 × 4–17 µm; hyphae 2.0–3.0 µm wide, adspersed with pale granular crystals. Calcium oxalate crystals not observed. Ascomata with

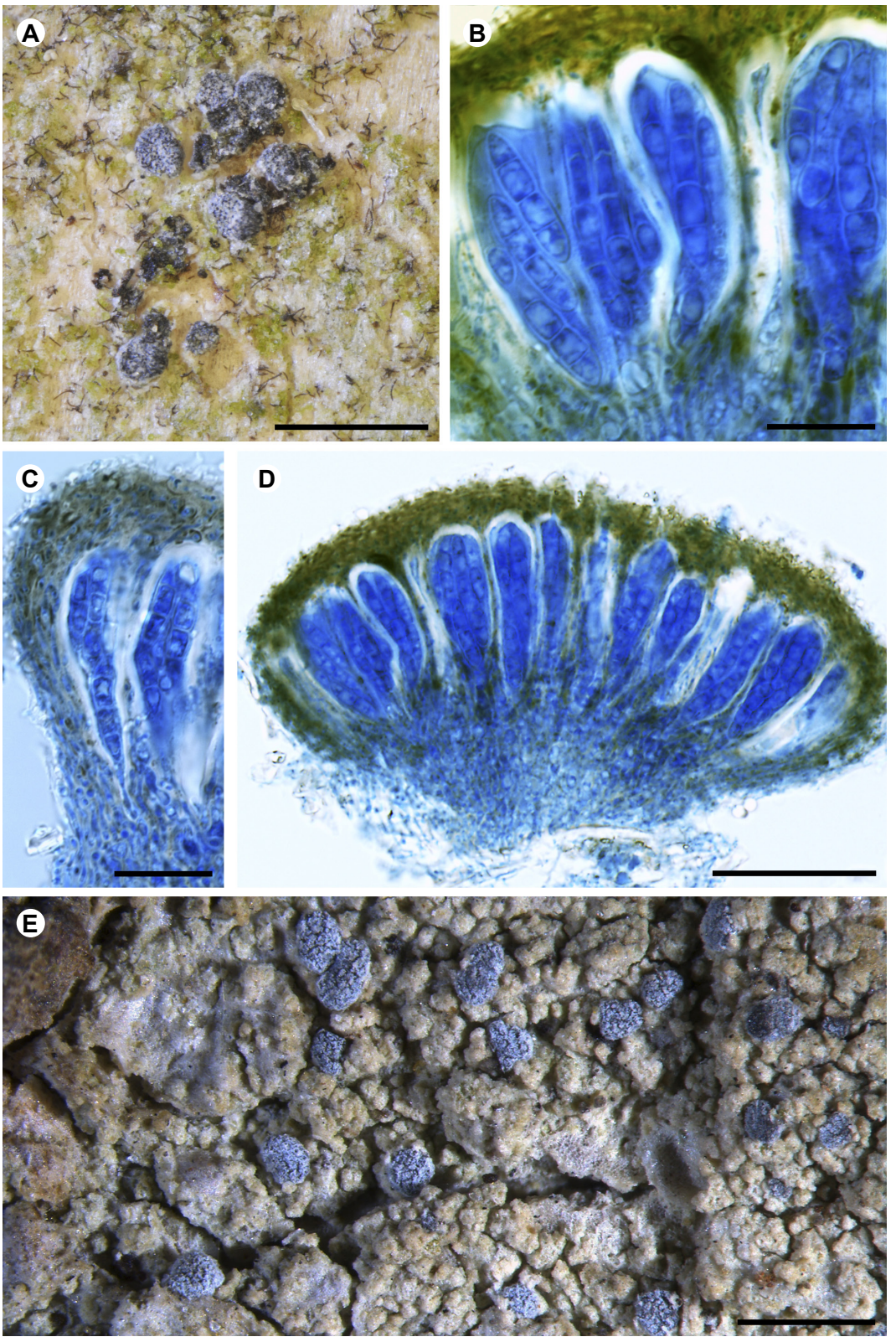

Figure 1. *Galbinothrix caesiopruinosa*. A – thallus and apothecia; B – asci and ascospores; C – margin of apothecium, showing the brownish conglutinated proper exciple and epithecium; D – transversal section through apothecium (A–D, Frisch 16/Kr367, hb Frisch); E – Thallus and apothecia (E, Frisch16/Kr585, hb Frisch). Scales: A = 1 mm, B = 25 µm, C = 20 µm, D = 100 µm, E = 50 µm.

constricted base adnate, emarginate in surface view, weakly convex, rounded to slightly angular, 0.2–0.35 mm diam., up to 0.12 mm tall, covered by thin to dense coarse bluish grey pruina, dark brown beneath. Proper exciple 7–20 µm wide, reddish brown, conglutinated, of compacted paraphysoidal hyphae, inspersed with pale granular crystals 1–3 µm wide, some hyaline leafy crystals 2–10 µm in size attached to the outer edge (not dissolving in sulphuric acid, dissolving in KOH). Epithecium reddish brown, 15–18 µm tall, conglutinated, of compacted tips of the paraphysoids (crystals as in the proper exciple). Hymenium hyaline to pale reddish brown,

60–70 µm tall, the asci closely spaced. Hypothecium hyaline to pale reddish brown, 25–80 µm tall, of 1.5–2.0 µm wide, branched and anastomosed, short-celled [3–5(–7) µm] hyphae to subparaplectenchymatic. Paraphysoids densely branched and anastomosed, with lumina ca 1 µm wide, in parallel strands in between the asci; tips 1.5–2.5 µm wide, extending horizontally above the asci, with reddish brown pigment along the outer walls and in the gelatinous matrix. Asci narrowly clavate to almost tubular, 75–115 × 19–26 µm. Ascospores 8, stacked in the asci, hyaline, oblong, (2–)3-septate, slightly constricted at the septa, (27–)30–34(–36) × (5–)5–6(–7) µm (n=20; l: mean=32.2, SD=2.05 w: mean=5.7, SD = 0.51), with distinct, ca 0.5 µm wide epispore (total wall-thickness ca 1 µm); ascospore septation starting with the median septum, with the two secondary septa appearing simultaneously. Pycnidia not observed.

Chemistry. Thallus containing usnic (major) and isousnic (minor to trace) acids (16/Kr367, 526, 585, 586, 614 tested), C–, KC–, K–, Pd–, UV–; thallus patchily I+ pale vinose/ KI+ pale blue. Ascomatal gel I+ vinose/ KI+ blue. Asci with KI+ blue ring in tholus. Brown pigments in the epithecium turning olive-green in K.

Etymology. The generic name is formed from the Latin *galbinus* (= greenish yellow) and the suffix *thrix* to indicate the color of the thallus and the relationship with *Chrysothrix* in *Chrysotrichaceae*. The epithet *caesiopruinosa* indicates the bluish grey pruinose ascomata.

Ecology and distribution. Most collections of *G. caesiopruinosa* were made from thin-stemmed deciduous trees with smooth bark, including species of *Acer*, *Alnus* and *Fraxinus*. It was collected once from smooth-barked *Abies* and once from fissured bark of *Salix.* The species appears to be most common in rather shady and humid riverine forests, but was likewise collected in humid ravines and river valleys from exposed, planted trees along roadsides and in parking lots (*Acer* spp. including *A. mono*). *Galbinothrix caesiopruinosa* is known from Hokkaido and central Honshu in Japan as well as from the eastern mountain range of the Korean peninsula. It appears to be a rather common and widespread species in suitable habitats in Eastern Asia.

Specimens examined. JAPAN. Hokkaido. Abashiri Prov., Monbetsu-gun, Engaru-cho, Ikutahara-Kiyosato, 43°51′06.3″N, 143°29′18.7″E, on bark of *Salix* sp. in riverine forest, 290 m. 29 May 2012, Frisch 12/Jp150 & Ohmura (TNS, hb Frisch); ibid., on bark of *Alnus* sp., Frisch 12/Jp151 (hb Frisch); Honshu, Shimotsuke Prov., Tochigi Pref., Nikko City, 20 km WNW of Nikko, Yumoto village, at entrance to house with exhibition of Nikko National Park, 36°48.290′N, 139°25.384′E), open mixed coniferous/deciduous forest, on bark of *Acer mono*, 1500 m. 28 Sept. 2017, Thor 35459 (UPS); Kozuke Prov., Gunma Pref., Katashina mura, 4.2 km ESE of Marunuma kogen ski resort, Mt. Oku-Shirane, along trail on N slope, 36°48.951′N, 139°22.704′E, deciduous forest, on *Alnus* sp., 1810 m, 30 Sept. 2017, Thor 35713 (UPS); ibid., 4.4 km E of Marunuma kogen ski resort, Mt. Oku-Shirane, along trail on N slope, 36°48.998′N, 139°22.772′E, open mixed coniferous/deciduous forest, on *Alnus* sp., 1780 m, 2 Oct. 2017, Thor 35847 (UPS); ibid., on *Abies* sp., 1780 m, Thor 35834 (UPS); Shinano Prov., Nagano Pref., Minamisaki-gun, Kawakami-mura, Chichibu-Tama-Kai National Park, along Chikumagawa River, 35°57′10.4″N, 138°42′36.7″E, on smooth bark of thin-stemmed tree along road, 1453 m, 28 May 2013, Frisch 13/Jp247 & Ohmura (TNS, hb Frisch); Kai Prov., Yamanashi Pref., Yamanashi-city, Makioka-cho, Yakiyama Pass, 35°49′22.8″N, 138°38′48.3″E, on *Acer* sp., 1530 m, 5 July 2012, Frisch 12/Jp282 & Ohmura (TNS). KOREA. Gangwon-do, Pyeongchang-gun, Yongpyeong-myeon, Nodong-ri, Mt. Gyebang, Nodong Valley trail, 37°42.282′N, 128°29.098′E, on young deciduous tree in forest, 820 m, 2 Oct. 2016, Frisch 16/Kr521, Kashiwadani, Moon & Printzen (hb Frisch); ibid., on young *Acer* sp., Frisch 16/Kr520, Kashiwadani, Moon & Printzen (hb Frisch); ibid., 37°42.316′N, 128°29.149′E, on thin-stemmed deciduous tree in forest, 820 m, Frisch 16/Kr526, Kashiwadani, Moon & Printzen (KB, hb Frisch); ibid., on thin-stemmed *Fraxinus* sp., Frisch 16/Kr527, Kashiwadani, Moon & Printzen (KB, hb Frisch); ibid., Jinbu-myeon, Odaesan-ro, Mt. Odae, Sangwon Temple, 37°47.096′N, 128°33.491′E, on deciduous tree, 950 m, 3 Oct. 2016, Frisch 16/Kr564, Kashiwadani, Moon & Printzen (hb Frisch); ibid., along Odae Stream, 37°44.335′N, 128°35.164′E, on young *Acer* sp. in parking area, 680 m, 4 Oct. 2016, Frisch 16/Kr585, 586, Kashiwadani, Moon & Printzen (KB, hb Frisch); Inje-gun, Buk-myeon, Yongdae-ri, Mt. Seorak, en route from Baekdam-sa Temple to Youngsi-am Temple, along Baekdam Valley, 38°09.567′N, 128°22.462′E, on thin-stemmed *Quercus* sp. in light forest near stream, 475 m, 14 June 2016, Frisch 16/Kr367 & Moon (hb Frisch); ibid., 38°10.029′N, 128°22.732′E, on deciduous tree in forest, 490 m, 6 Oct. 2016, Frisch 16/Kr614, Kashiwadani, Moon & Printzen (KB, hb Frisch); ibid., 38°09.471′N, 128°22.405′E, on *Acer* sp. in forest, 520 m, Frisch 16/Kr616, Kashiwadani, Moon & Printzen (hb Frisch); ibid., where the road crosses the river ca 1.5 km NW of Baekdam Temple, 38°09.85–10.25′N, 128°22.50′E, open mixed coniferous/deciduous forest, on deciduous tree, 450–550 m, 21 Oct. 2006, Thor 20692 (UPS).

Discussion

The new genus and species is well accommodated in *Chrysotrichaceae* on account of its morphological characters, including: (1) the adnate rounded ascomata with an epithecium of horizontally extending tips of the paraphysoids, (2) branched and netted paraphysoids, (3) asci related to the *Arthonia*-type (see below), (4) hyaline, predominantly 3-septate ascospores, and (5) the chlorococcalean photobionts. In the molecular phylogeny in Figure 2, *Galbinothrix caesiopruinosa* is shown to be genetically distant from the other genera currently accepted in *Chrysotrichaceae*, including *Chrysothrix* and *Melarthonis*, as well as from *Arthonia mediella*, based on mtSSU and nLSU sequence data. The latter species is kept here in *Arthonia* as it will be treated with related taxa in a forthcoming publication.

Galbinothrix caesiopruinosa is readily distinguished from other *Chrysotrichaceae* by the combination of the following characters: (1) crustose rimose to areolate thallus lacking pulvinic acid derivatives, (2) reddish brown well-defined proper exciple and epithecium, (3) large oblong ascospores, 27–36 × 5–7 µm, with distinct perispore, and (4) narrowly clavate to almost tubular asci of 75–115 × 19–26 µm. Despite their elongated shape and rather large size, the asci of *Galbinothrix* are best classified as a variant of the *Arthonia*-type as

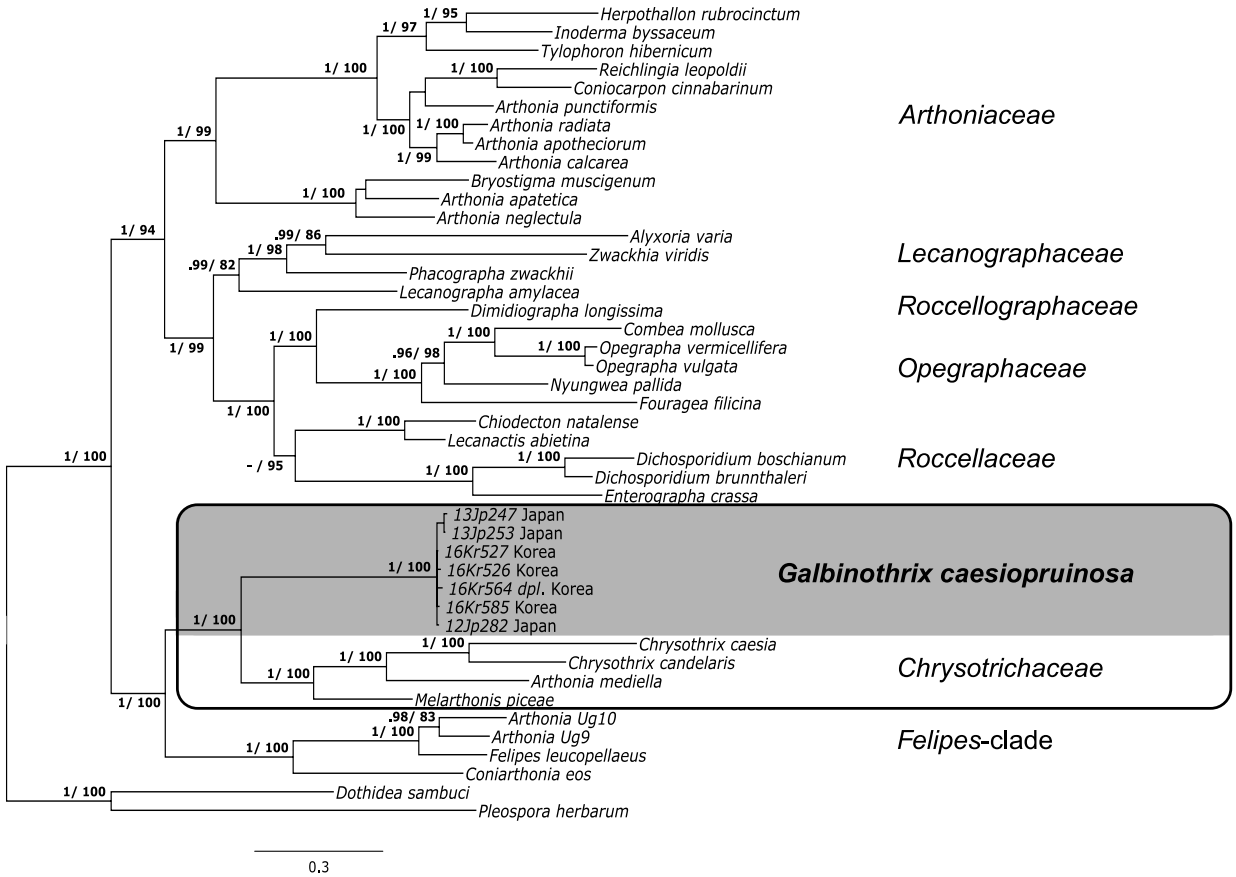

Figure 2. Bayesian 50% majority-rule consensus tree showing the position of *Galbinothrix caesiopruinosa* at the base of *Chrysotrichaceae* in *Arthoniales*. Support values from the Bayesian (first) and the RAxML (second) analysis are indicated on the branch nodes.

defined by Grube (1998). *Chrysothrix caesia* is the only species in the family that shows superficial similarity with *Galbinothrix* in lacking pulvinic acid derivatives, the brownish, white pruinose ascomata, and reddish brown pigment in the proper exciple, epithecium, and hypothecium. In addition to the leprose thallus containing zeorin along with usnic acid, this species and all other *Chrysotrichaceae* differ from *Galbinothrix* by their much smaller, thin-walled ascospores lacking a distinct epispore (in the range of 9–23 × 2.5–6 µm; outer wall and septa ≤ 0.5 µm), and clavate to broadly clavate asci measuring 20–45 × 8–15 µm (own measurements; Laundon 1981; Kalb 2001; Jagadeesh Ram et al. 2006; Elix & Kantvilas 2007).

Melarthonis differs additionally by the greenish, minutely granular thallus, the strongly convex black ascomata with ± anticlinal excipular hyphae having tips up to 2 µm wide and pigmented dark brown, and narrowly obovoid to spindle-shaped ascospores, 10.0–14.0 × 2.5–4.0 µm. *Arthonia mediella* differs by having black, strongly convex ascomata; small, narrowly obovoid to spindle-shaped ascospores (10.0–17.0 × 3.0–5.0 µm); and capitate tips of the interascal filaments with distinct dark brown caps.

Galbinothrix caesiopruinosa is a uniform species except for its thallus, which varies from pale to dark greyish green in shady localities, while thalli from more exposed sites are distinctly yellowish olive. Some specimens have a rather inconspicuous thallus consisting of low, dispersed, areola-like warts, while in others the thallus forms a continuous, distinctly fissured-areolate crust. The areolae can be more or less flat and thin, or distinctly bullate to almost coarsely subgranular.

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