Kirschsteiniothelia thujina (Peck) D. Hawksw. (*Kirschsteiniotheliaceae*), reported from Europe for the first time

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Summary: A dothideomycete repeatedly collected on stubs of dead branches of *Abies alba* in the French Pyrénées and once on the Spanish side of the Pyrénées was identified as *Kirschsteiniothelia thujina* based on morphological and ecological characters. This fungus was known from North America and had not been reported from Europe. A sample was isolated in artificial culture and three loci of genes sequenced. A phylogenetic analysis based on the results confirmed its placement in *Kirschsteiniothelia* and revealed its affinities with other taxa known in that genus. The peculiar ecology of *K. thujina* is briefly commented.
Keywords: *Abies alba*, Ascomycota, bluestain of wood, *Dothideomycetes*, ITS, phylogeny, taxonomy, 18S, 28S.

Résumé : un dothidéomycète fréquemment récolté sur moignons de branches mortes d'*Abies alba* dans les Pyrénées françaises et une fois du côté espagnol des Pyrénées a été identifié comme *Kirschsteiniothelia thujina* sur la base de données morphologiques et écologiques. Cette espèce était connue d'Amérique du Nord, mais n'avait pas été signalée d'Europe. Un spécimen a été cultivé et trois loci de gènes séquencés. Une analyse phylogénétique fondée sur les résultats a confirmé l'appartenance de cette espèce au genre *Kirschsteiniothelia* et révélé ses affinités avec d'autres espèces du même genre. L'écologie particulière de *K. thujina* est brièvement commentée.
Mots-clés : *Abies alba*, Ascomycota, bleuissement du bois, *Dothideomycetes*, ITS, phylogénie, taxinomie, 18S, 28S.

Introduction

The genus *Kirschsteiniothelia* D. Hawksw. was introduced by Hawksworth (1985) to accommodate fungi formerly regarded as the *Microthelia incrustans* group in the Dothideales.

Species assigned to this genus are saprobic on dead wood, have black, subglobose, erumpent ascomata that remain slightly immersed with a flattened base, ostiolate apex and a thick pseudoparenchymatous peridium of thick-walled cells arranged in palisadic configuration at base angles; the hamathecium is composed of cellular pseudoparaphyses and the asci are bitunicate, fissitunicate, obclavate, 4- to 8-spored; ascospores are two-celled, brown, ellipsoid or soleiform, usually with the septum submedian and constricted, smooth, verruculose or striate, lacking a gelatinous sheath. The generic type, *K. aethiops* (Berk. & Curtis) D. Hawksw., is known to have a dendryphiopsis-like asexual morph. The six species recognized by Hawksworth were placed in the *Pleosporales* (Hawksworth, 1985).

A further species was added to the genus, *K. elaterascus* Shearer (Shearer, 1993) that deviated by a freshwater habitat and asci considerably stretching upon dehiscence. In a recent update on the genus supported by DNA sequences analysis, Boonmee *et al.* (2012) showed that *K. elaterascus* was best placed in the *Morosphaeriaceae* as *Morosphaeria elaterascus* (Shearer) S. Boonmee & K.D. Hyde, and that *K. maritima* (Linder) D. Hawksw. should be moved to the new genus *Halokirschsteiniothelia* S. Boonmee & K.D. Hyde as *H. maritima* (Linder) S. Boonmee & K.D. Hyde, in the *Mytilinidiales*. Subsequently, Zhang *et al.* (2013) moved *M. elaterascus* to *Helicascus* Kohlm. as *H. elaterascus* (Shearer) Huang Zhang & K.D. Hyde. Boonmee *et al.* (2012) also introduced two new species of *Kirschsteiniothelia* from Thailand, viz. *K. lignicola* S. Boonmee & K.D. Hyde and *K. emarceis* S. Boonmee & K.D. Hyde, that were assigned to this genus based on morphological traits, presence of a dendryphiopsis-like asexual morph and molecular evidence. While the genus *Kirschsteiniothelia* as previously conceived had been shown to be polyphyletic (Suetrong *et al.*, 2009), the newly circumscribed genus resulted in a phylogenetically supported clade with the two new species from Thailand clustering with the type species *K. aethiops* including a strain obtained from its asexual morph *Dendryphiopsis atra*. It should be noted that this name was recently synonymized with its sexual morph *K. aethiops* (Wijayawardene, 2014). As this clade is distant from the *Pleosporales* and distinct from the most closely related families, the new family *Kirschsteiniotheliaceae* S. Boonmee & K.D. Hyde was introduced by Boonmee *et al.* (2012). Unfortunately, molecular data were lacking for the four remaining species accommodated in *Kirschsteiniothelia* by Hawksworth (1985), viz *K. acerina* (Rossman & Wilcox) D. Hawksw., *K. recessa* (Cooke & Peck) D. Hawksw., *K. striatispora* (Aebi, Harr & Müller) D. Hawksw. and *K. thujina* (Peck) D. Hawksw.

A dothideomycete conforming to the description of *K. thujina* has been repeatedly collected by one of us (JF) over the last years on the French side of the Pyrénées in the departments of Ariège, Aude and Hautes-Pyrénées and once on the Spanish side, always in coniferous forests, mostly *Abies alba* forests at mountainous elevations of 1,000 to 1,300 msm. It was first fortuitously discovered on the old weathered stubs of dead branches arising from living or dead trunks of *Abies alba* and a systematic search revealed that it occurs very frequently on such substrates. Removing these stubs from the trunk needs the use of a wood saw because the heartwood of the stub at the junction with the trunk is extremely hard and once the stub is detached the cutting surface most often shows an intriguing greyish blue discolouration of the wood. Interestingly, a bluestain in *Abies balsamea* wood in relation with a fungus known as *Amphisphaeria thujina* (Peck) Sacc. had been reported from Canada by Pomerleau & Etheridge (1961). The authors proved the relationship by comparing cultures originating from fresh bluestained wood and cultures originating from ascospores of the fungus. As their description of *Amphisphaeria thujina* (as *Kirschsteiniella thujina* (Peck) Pomerl. & D.E. Ether.) agrees very well with our observations and the description of the type specimen by Hawksworth (1985), we could safely conclude that the fungus staining the wood greyish blue that we repeatedly found in the French Pyrénées was *K. thujina*.

Aims of this study therefore are (1) to provide a detailed morphological description of *K. thujina* with colour illustrations; (2) to connect *K. thujina* with a living culture; (3) to infer the taxonomic status of *K. thujina* from phylogenetic analysis; and (4) to report the occurrence of *K. thujina* in Europe for the first time.

Material and methods

Morphological characterization. — The observations were carried out on material rehydrated in water. Measurements of asci and ascospores were made in water and ascospore measurements processed with the free software Piximetre 5.2 (http://ach.log.free.fr/Piximetre/). In the formula given by this soft-

ware the values into brackets represent the extreme values (20%) that are not taken into account for the calculation, N represents the number of ascospores measured, Q the quotient length/width, Me the mean values of length × width and Qe the mean value of quotient length/width.

Observations of microscopic structures of asci and hamathecium were performed in black Pelikan ink and ascospores were observed in water, in dilute India ink to search for appendages or gelatinous sheath and in heated chloral-lactophenol to observe the epispore ornamentation. Free-hand sections of the peridium were mounted in heated chloral-lactophenol.

Photomacrographs were taken with a Nikon Coolpix 995 digital camera either directly mounted on a stand or, for higher magnifications, through the eyepiece of an Olympus SZ60 stereomicroscope, by the means of a 30 mm diameter adapter. Photomicrographs were taken with the same camera mounted on the trinocular port of a Leitz Orthoplan microscope. The digitalised photographs were processed with Adobe Photoshop Elements 10 and the plates assembled with the same software.

The cited material was deposited in the herbarium of Beijing Forestry University (BJFC) and duplicates are kept in the personal herbarium of JF. The initials JF refer to Jacques Fournier.

Fungal isolation. — Ascomata of the fresh specimens were cut, the contents removed with a sterilized forceps, crushed in a sterilized vial and the released spores were pasted on the surface of water agar. Germinated spores were removed 24 hours later and plated on potato-dextrose agar (PDA). Cultures were deposited in Beijing Forestry University Culture Collection (BJFCC).

DNA extraction, amplification and sequencing. — The culture of *K. thujina* (BJFCC201054) was grown on potato-dextrose agar (PDA) and malt extract agar (MEA) and total genomic DNA was extracted from mycelia following the protocols as outlined by Zhang *et al.* (2009). 18S (SSU), 28S (LSU) and internal transcribed spacer (ITS) nurDNA gene sequence data were used to study the phylogenetic relationships among *K. thujina* and other closely related pleosporalean taxa. DNA amplification and sequencing follow the protocol used by Zhang *et al*. (2009).

Sequence alignment and phylogenetic analyses. — Sequences generated by three pairs of different primers (LROR/LR5 for 28S, NS1/NS4 for 18S, and ITS1/ITS5 for ITS) were analyzed and compared to other sequences obtained from the GenBank. The ingroup and outgroup taxa were selected by referring to the relevant publications, such as Boonmee *et al.* (2012) and Hyde *et al.* (2013). A BLAST query was also performed to find possible sister groups of the newly sequenced taxon. Alignments were carried out with MEGA 5 (Tamura *et al.*, 2011) and analyses were performed in PAUP V. 4.0B10 (Swofford, 2002). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. A combined 18S, 28S and ITS nurDNA dataset was analysed in this analysed in this study. Maximum likelihood (ML) was conducted using heuristic searches as implemented in PAUP, with the default options method. For the ML analysis, the best-fit model of nucleotide evolution (GTR+I+G) was selected by the Akaike information criterion (AIC) (Posada & Buckley, 2004) in MrModeltest 2.3. Bootstrap analysis with 1,000 replicates was used to test the statistical support of the branches. With model parameters estimated from the data, a heuristic search with ten random taxon addition sequences and TBR branch swapping was performed. The same substitution model was used in the Bayesian analyses performed with MrBayes v. 3.1.2. The Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) approach was used to calculate posterior probabilities. A preliminary Bayesian inference (BI) analysis using MrBayes software revealed that the Markov Chain Monte Carlo (MCMC) steady state was reached after less than 200,000 generations (the average standard deviation of split frequencies was constantly below 0.01). A conservative burn-in of 2,000 dendrograms was chosen and a full analysis of 2,000,000 generations was carried out with sampling every 100 generations. Trees were viewed in TREEVIEW. The nucleotide sequences reported in this paper for *K. thujina* strain BJFCCC201054 were deposited in GenBank (ITS = KM982716, SSU = KM982717, LSU = KM982718).

Taxonomy

Kirschsteiniothelia thujina (Peck) D. Hawksw., *Bot. J. Linn. Soc.*, 91: 198 (1985) — MB104405. Plates 1–5

≡ *Sphaeria thujina* Peck, *Rep. (Annual) New York State Mus. Nat. Hist.*, 27: 110 (1875).

≡ *Amphisphaeria thujina* (Peck) Sacc., *Syll. fung.*, 1: 726 (1882).

≡ *Kirschsteiniella thujina* (Peck) Pomerl. & D. E. Ether., *Mycologia*, 53: 160 (1961).

≡ *Splanchnonema thujinum* (Peck) M. E. Barr, *Mycotaxon*, 49: 141 (1993).

Ascomata scattered or loosely clustered in small groups (A), rarely in contact, erumpent (D) becoming superficial with the base remaining slightly immersed in the substrate (H), hemispherical to broadly

Table 1 – Ascospores dimensions in seven collections from France and one from Spain compared with the values reported by Pomerleau & Etheridge (1961) and Hawksworth (1985) from North American material

	Extreme values (µm)	Q = quotient l/w N = number of measurements	Mean values
JF 14091	(27.5–) 30.8–34.7 (–36.6) × (11.1–) 12.1–15.6 (–16.3)	Q = (2–) 2.1–2.8 (–3.1); N = 50	Me = 32.9 × 13.7 µm; Qe = 2.4
JF 13120	(34.0–) 35.3–40.4 (–43.0) × (14.2–) 14.8–18.1 (–19.7)	Q = (1.9–) 2.1 –2.47 (–2.5); N = 50	Me = 37.6 × 16.4 µm; Qe = 2.3
JF 10152	(33.3–) 34.3–39.6 (–41.9) × (12.3–) 12.9–15.9 (–18.1)	Q = (2–) 2.3–3 (–3.3); N = 50	Me = 36.9 × 14.5 µm; Qe = 2.6
JF 07093	(33.5–) 35.2–41.3 (–46.1) × (12.6–) 13.6–16.1 (–17.8)	Q = (2.3–) 2.4–2.9 (–3.1); N = 50	Me = 38.5 × 14.7 µm; Qe = 2.6
JF 04196	(33.8–) 34.9–40.4 (–42) × (10.6–) 11.4–14.4 (–16)	Q = (2.5–) 2.6–3.3 (–3.8); N = 50	Me = 37.3 × 12.9 µm; Qe = 2.9
JF 04122	(31.4–) 34.7–38.9 (–43) × (12.6–) 14.1–16.4 (–17.1)	Q = (2.1–) 2.2–2.6 (–2.7); N = 50	Me = 36.8 × 15.3 µm; Qe = 2.4
JF 04097	(33.2–) 36.1–40.7 (–41.8) × (14) 14.5–16.9 (–18.9)	Q = (2–) 2.2–2.7 (–2.8); N = 50	Me = 38.2 × 15.6 µm; Qe = 2.5
JF 00232	(30–) 32.3–38.3 (–41.4) × (12.7–) 13.3–15.5 (–16.5)	Q = (1.8–) 2.2–2.7 (–3); N = 50	Me = 35.3 ×14.5 µm; Qe = 2.4
Pomerleau & Etheridge 1961	(29–) 36–48 (–55) × 12–16 (–19)	-	Me = 42 × 14 µm; Qe = 3
Hawksworth 1985	(29–) 36–50 (–55) × (12–)15–17 (–19)	-	Me = 43 × 16 µm; Qe = 2.7

conical with the base flattened (B–F), often slightly flattened laterally, 320–420 µm high × 340–600 µm diam, apex ostiolate, rounded to truncate, rarely papillate, sometimes split (D) or with a concave depression (C), wall black, coarsely furrowed or roughened. Peridium leathery, dark brown, 50–65 µm thick on sides, 40–45 µm thick at base, pseudoparenchymatous, composed of moderately thick-walled angular cells 8–12 µm in greatest dimension that form a vertically orientated palisadic tissue well-developed at base angles (K), the outermost cells heavily melanised forming an opaque crust on sides but much less developed at the base; apex 65–80 µm thick, entirely composed of highly melanised meandering cells (J). Dark brown smooth hyphae 3–4 µm diam arising from the ascomatal base spreading into the underlying wood that is deeply stained greyish blue (G, I).

Asci bitunicate, fissitunicate, obclavate, short-pedicellate (L, M), with 4–8 ascospores biseriate in lower part, uniseriate above, 155–180 µm long × 33–40 µm broad, with a low truncate apical ocular chamber visible in immature asci (O). **Pseudoparaphyses** cellular (N), 2–3.5 µm wide, sparingly septate, anastomosing and branching, embedded in a mucilage. **Ascospores** (34.0–) 35.3– 40.4 (–43.0) × (14.2–) 14.8–18.1 (–19.7) µm, Q = (1.9–) 2.1–2.47 (–2.5); N = 50 (Me = 37.6 × 16.4 µm; Qe = 2.3), ellipsoid-fusiform with rounded ends, straight to slightly asymmetrical in side view (P-R), thick-walled, wall 1.8–2 µm, one-euseptate, constricted at the septum, septum submedian to rarely almost median (Q = 0.48–0.68, N = 30), cell above the septum slightly swollen, content densely guttulate, wall olive-brown turning dark brown, minutely verruculose (S), without mucilaginous sheath or appendages visible in India ink (R).

Asexual morph on the natural substrate not seen.

Culture. Ascospores germinating on MEA after 1–2 d; colony radius at 26–28°C on MEA 16–19 mm after 10 d, 35–45 mm after 3 mo. Colony irregularly circular, first blackish, surface covered with a thin white mat of aerial hyphae slightly extending over the margin (A, B), turning blackish brown with black margin at 3 mo (D), composed of brown to dark brown, sparsely septate verruculose hyphae up to 4.5 µm wide (C) and hyaline to pale reddish brown hyphal elements at the periphery, straight to coiled, 1–2.5 µm wide (F); reverse blackish (E); odour not detected. No conidiogenous structures observed.

Specimens examined: France: Ariège: Prat Communal, Loumet, 950 m, mixed *Abies* forest, on stubs of dead decorticated branches of *Abies alba*, 3 Sept. 2004, J. Fournier, JF 04196; Soulan, Soulan state forest, Col de la Crouzette, 1200 m, *Picea abies* plantation, on stubs of dead decorticated branches of *Picea abies*, 3 Jun. 2004, J. Fournier, JF 04122 (BJFC201084). Aude: Belcaire, road D 20 to Niort, Niave forest, 1300 m, mixed *Abies* forest, on stubs of dead decorticated branches of *Abies alba*, 14 May 2004, J. Fournier, JF 04097 (BJFC201085); same location and host, 24 Oct. 2013, J. Fournier, JF 13210 (culture BJFCC201054); Roquefeuil, Bois du Pinet, 1000 m, mixed *Abies* forest, on dead decorticated wood of *Abies alba*, 29 Sept. 2000, J. Fournier, JF 00232 (BJFC201086); Roquefeuil, Coume Frède, 1100 m, mixed *Abies* forest, on stubs of dead decorticated branches of *Abies alba*, 4 May 2004, J. Fournier, JF 07093. Hautes Pyrénées: Campan, Prayolle, 1200 m, mixed *Abies* forest, on stubs of dead decorticated branches of *Abies alba*, 23 Aug. 2014, J. Fournier, JF 14091 (BJF201087). Spain: Huesca: Villanúa, Fuente del Paco, 1400 m, mixed *Abies* forest, on stubs of dead decorticated branches of *Abies alba*, 10 Oct. 2010, J. Fournier, JF 10152.

Discussion

The fungus described and illustrated here conforms well to the genus *Kirschsteiniothelia* by the morphology of its ascomata and its pseudoparaphysate hamathecium associated with bitunicate obclavate asci with large, brown unequally two-celled ascospores. It is morphologically similar to *K. aethiops*, the type species, from which it deviates primarily by larger asci and ascospores, apparent host specificity for coniferous wood and constant association with a greyish blue stain of the underlying wood. These characters that deviate from *K. aethiops* conform well to *K. thujina* as described by Pomerleau & Etheridge (1961) and Hawksworth (1985). However, the ascospores measured from European material consistently average shorter than those from North America (Table 1), though they remain within the wide size range given by the authors cited above. Interestingly, though averaging shorter, their width agrees well with that recorded from North American specimens and as a result their shape is more broadly ellipsoid. Based on this observation, it cannot be ruled out that the collections from Europe represent a different taxon but the degree of divergence from the North American taxon should be evaluated based on molecular analyses that would become possible when sequences are available. Accordingly, an epitype should originate from North America where the taxon was described first. Meantime it seems more appropriate to regard our collections as conspecific with the material from North America than to introduce a new taxon based on a minor difference in ascospore morphology only.

Plate 1. A living trunk of *Abies alba* ca. 40 cm diam with stubs of dead branches on which commonly occurs *K. thujina* (Aude, Belcaire, road D 20 to Niort, Niave forest, 1300 m).

A collection from France was isolated, a culture deposited and three loci sequenced (18S, 28S and ITS). The morphology of the culture agrees well with that reported by Pomerleau & Etheridge (1961) and like these authors we failed to obtain the asexual morph *in vitro*. The phylogenetic analysis based on three loci (Plate 5) shows that *K. aethiops*, *K. lignicola* and *K. thujina* form a robust clade with *K. thujina* basal to them. Although morphologically similar to the above species, *K. emarceis* deviates in having sparse hairs on the surface of ascomata and appears distantly related in our dendrogram and in that of Boonmee *et al.* (2012).

It is noteworthy and somewhat unexpected that cultures of this fungus do no feature any blue stain. A detailed microscopic examination of the blue-stained wood carried out by Pomerleau & Etheridge (1961) and resumed in this study showed that the wood is invaded

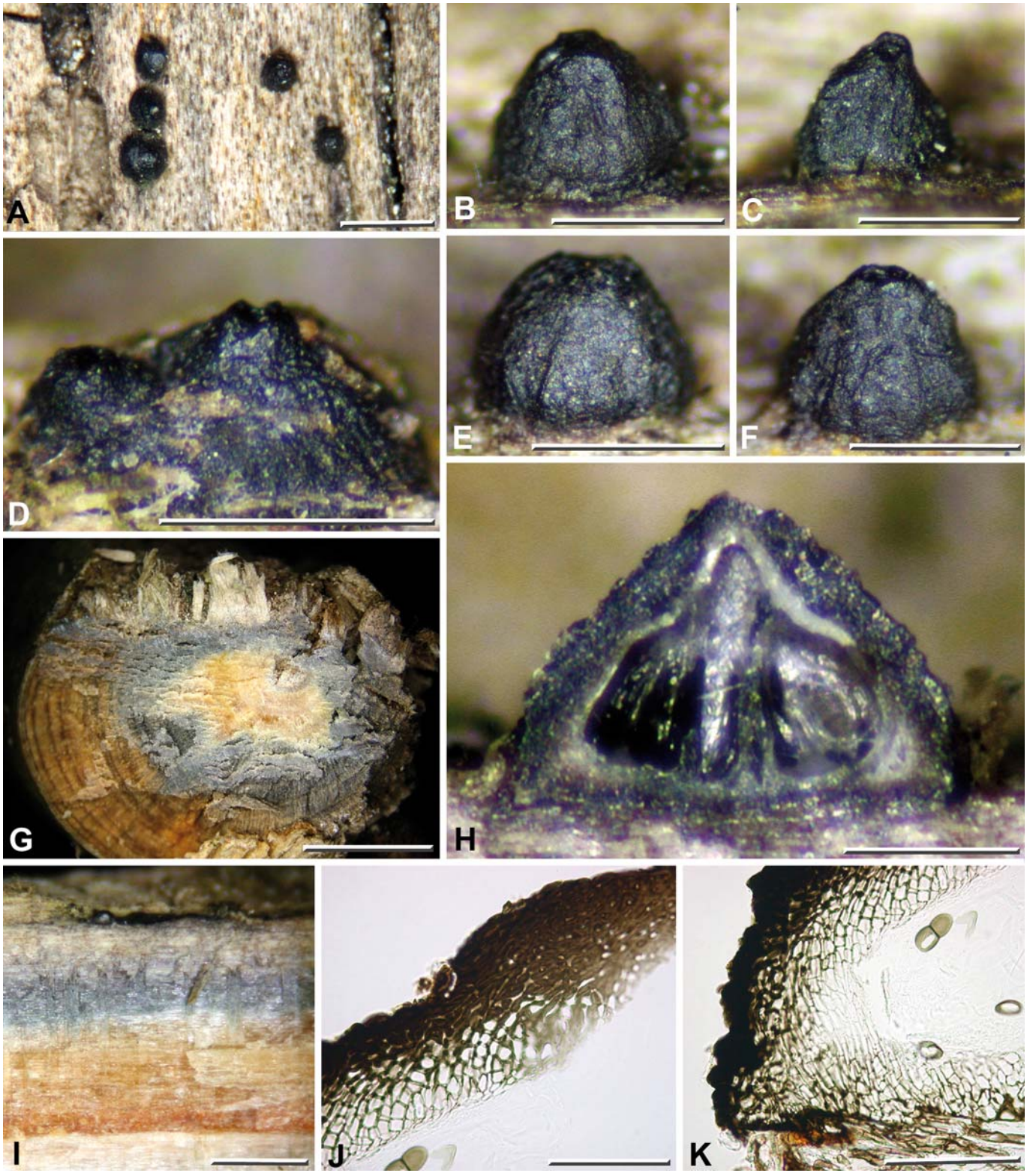

Plate 2 – *Kirschsteiniothelia thujina*. JF 13210.

A: Ascomata in surface view; B–F: Ascomata in side view; G: Wood in cross section showing the blue stain; H: Ascoma in vertical section; I: Wood in longitudinal section showing the blue stain; J, K: Peridium in vertical section in chloral-lactophenol, respectively at the apex and at the base. Scale bars: A, I = 1 mm; B–F = 0.5 mm; H = 0.2 mm; G = 5 mm; J, K = 50 µm.

Plate 3 – *Kirschsteiniothelia thujina*. JF 13210.

L, M: Immature and mature asci in black Pelikan ink; N: Pseudoparaphyses in black Pelikan ink; O: Apex of an immature ascus showing the ocular chamber, in black Pelikan ink; P, Q: Ascospores in water; R: Ascospore in dilute India ink; S: Ascospore focused on upper side showing the minutely verruculose ornamentation, in chloral-lactophenol. Scale bars: L–N = 20 µm; O–S = 10 µm.

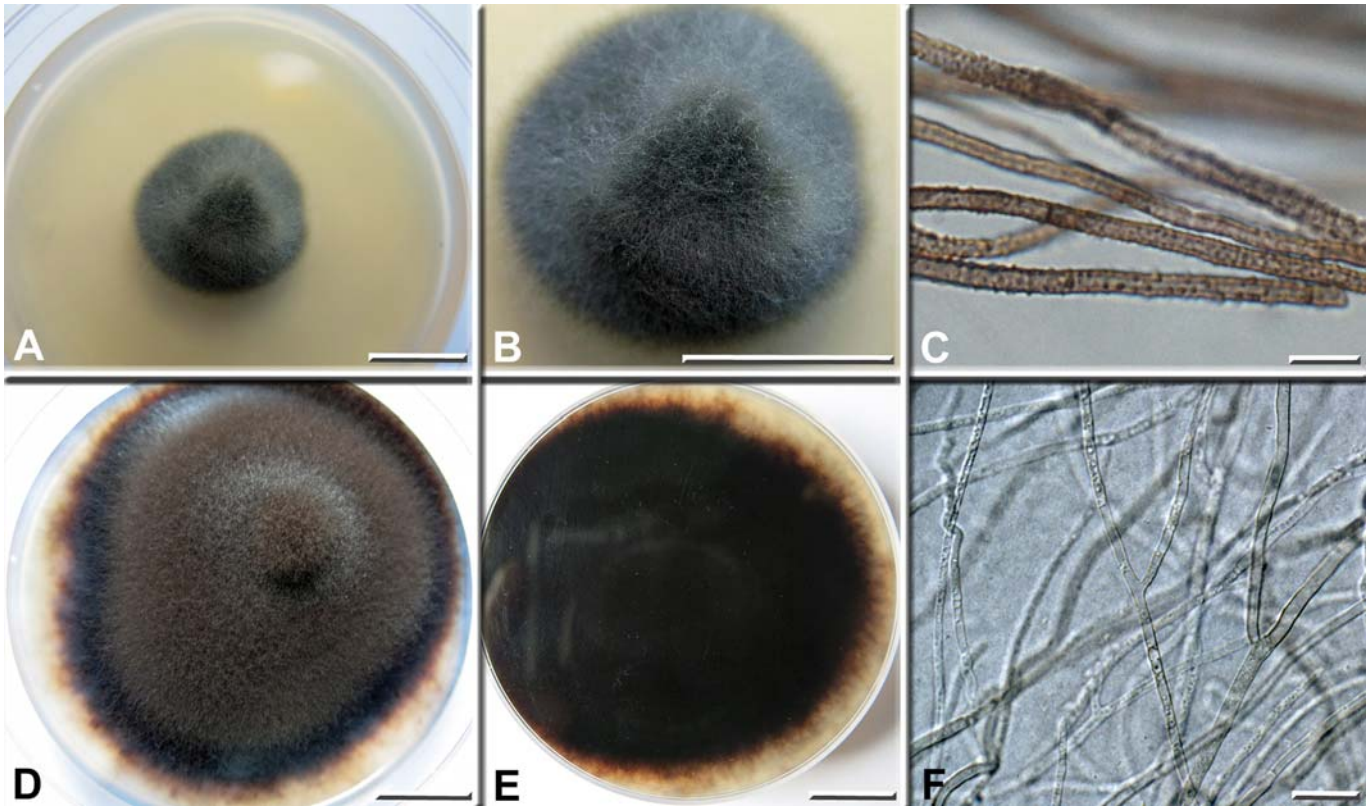

Plate 4 – *Kirschsteiniothelia thujina*. JF 13210, BJFCCC201054 culture on MEA
A: Colony at 10 days; B: Colony at 10 days in close-up; C: Brown hyphae showing the verruculose ornamentation; D, E: Colony at 90 days, top view and reverse respectively; F: Peripheral hyphae. Scale bars: A, B, D, E = 1 cm; C, F = 10 µm.

by a network of dark brown hyphae that do not release blue pigments. The perception of a bluish colour with the naked eye most likely results from a visual illusion involved by the refraction of light on the wood altered by the fungus.

Many fungi, especially species of *Ceratocystis* Ellis & Halst. spp. and *Ophiostoma* Syd. & P. Syd. are known to cause a blue or green stain, mostly in coniferous wood, and their biology and their damaging action have been largely documented (Seifert *et al*., 2013). In contrast to *K. thujina*, these fungi are pathogens and saprobes of recently killed wood, restricted to sapwood in which they form typically radiating patterns when seen on a cross section of a trunk.

Wang *et al.* (2004) accommodated five species formerly placed in *Amphisphaeria* Ces. & De Not. in *Kirschsteiniothelia* based on morphological similarities. The status of these species has not been confirmed by phylogenetic studies but interestingly, among them, *K. dolioloides* (Rehm) Y. Z. Wang, Aptroot & K. D. Hyde is reported to occur on coniferous wood (*Pinus*) in Switzerland and to have asci 150–200 × 21–23 µm and ascospores 31–41 × 12–16 µm. This might well be the first collection of *K. thujina* from Europe but unfortunately a blue stain of the wood was not recorded and we did not re-examine this specimen.

Based on the numerous collections of *K. thujina* reported from France and one from Spain in this study it seems likely that the fungus is present all over Europe in favourable environments but has remained unnoticed. In the explored sites in the Pyrénées, a minimum elevation of 1000 m seems required but at higher latitudes it can be expected at lower altitudes. Unlike *K. aethiops* that has been almost exclusively recorded on deciduous wood (Hawksworth, 1985), *K. thujina* appears restricted to coniferous trees including *Cupressaceae* and *Pinaceae*. From our experience the fungus rarely occurs on dead decorticated trunks but is much more frequent on stubs of dead branches still attached to the trunk (Plate 1), regardless of whether the trunk is living or dead, standing or fallen. This highly specialised affiliation to a substrate that is probably rarely investigated and difficult to remove from the trunk because of the very hard texture of the wood joining it to the trunk likely accounts for the rarity of records of *K. thujina*.

As the fungus is inconspicuous and grows usually 1.5 m above the soil level, the best way to find it is to cut some stubs of branches at their base with a saw and search for the blue stain on the section surface. The blue stain is not visible on the surface of the stub and may be located deep in the wood but it is the signature of the fungus that can usually be spotted with a hand lens on the wood surface.

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References

Boonmee S., Ko Ko T.W., Chukeatirote E., Hyde K.D., Chen H., Cai L., McKenzie E.H.C., Jones E.B.G., Kodsueb R. & Hassan B.A. 2012. — Two new *Kirschsteiniothelia* species with a *Dendryphiopsis* anamorphs cluster in *Kirschsteiniotheliaceae* fam. nov. *Mycologia*, 104 (3): 698-714. doi: 10.3852/11-089

Hawksworth D.L. 1985. — *Kirschsteiniothelia*, a new genus for the *Microthelia incrustans* group (*Dothideales*). *Botanical Journal of the Linnean Society*, 91:181-202. doi: 10.1111/j.1095-8339.1985.tb01144.x

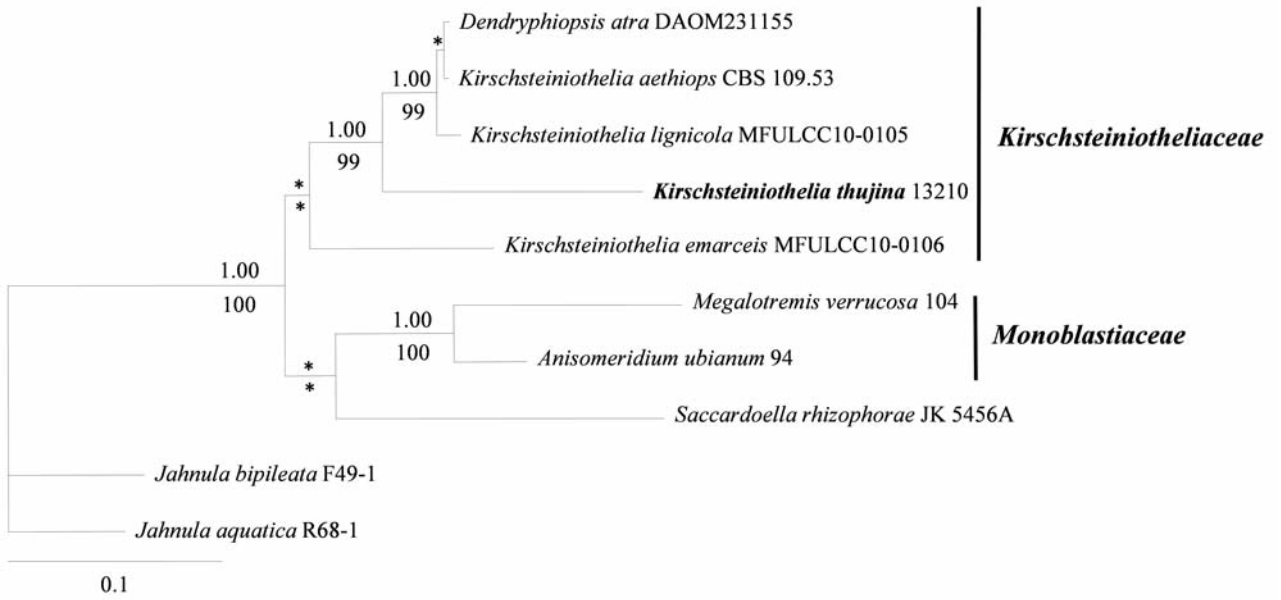

Plate 5. Phylogram of the MrBayes analysis generated from 18S, 28S and ITS *nur*DNA sequences. Posterior probabilities inferred from MrBayes analyses ≥ 70% are shown above the branches, and MP bootstrap support values ≥ 50% are shown below the branches.

Hyde K.D., Jones E.B.G., Liu J.K., Ariyawansa H., Boehm E., Boonmee S., Braun U., Chomnunti P., Crous P.W., Dai D.Q., Diederich P., Dissanayake A., Doilum M., Doveri F., Hongsanan S., Jayawardena R., Lawrey J.D., Li Y.M., Liu Y.X., Lücking R., Monkai J., Muggia L., Nelsen M.P., Pang K.L., Phookamsak R., Senanayake I.C., Shearer C.A., Suetrong S., Tanaka K., Thambugala K.M., Wijayawardene N.N., Wikee S., Wu H.X., Zhang Y., Aguirre-Hudson B., Alias S.A., Aptroot A., Bahkali A.H., Bezerra J.L., Bhat D.J., Camporesi E., Chukeatirote E., Gueidan C., Hawksworth D.L., Hirayama K., De Hoog S., Kang J.C., Knudsen K., Li W.J., Li X.H., Liu Z.Y., Mapook A., McKenzie E.H.C., Miller A.N., Mortimer P.E., Phillips A.J.L., Raja H.A., Scheuer C., Schumm F., Taylor J.E., Tian Q., Tibpromma S., Wanasinghe D.N., Wang Y., Xu J.C., Yacharoen S., Yan J-Y. & Zhang M. 2013. — Families of *Dothideomycetes*. *Fungal Diversity*, 63 (1): 1-313. doi: 10.1007/s13225-013-0263-4

Pomerleau R. & Etheridge D.E. 1961. — A bluestain in balsam fir. *Mycologia*, 53 (2): 155-170.

Posada D. & Buckley T.R. 2004. — Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, 53: 793-808. doi: 10.1080/10635150490522304

Seifert K.A., De Beer Z.W. & Wingfield M.J. (eds.) 2013. — *Ophiostomatoid Fungi: Expanding frontiers*. CBS Biodiversity series, 12. Utrecht, 337 pp.

Shearer C.A. 1993. — A new species of *Kirschsteiniothelia* (*Pleosporales*) with an unusual fissitunicate ascus. *Mycologia*, 85 (6): 963-969. doi: 10.2307/3760680

Suetrong S., Schoch C.L., Spatafora J.W., Kohlmeyer J., Volkmann-Kohlmeyer B., Sakayaro J.J., Phongpaichit S., Tanaka K., Hirayama K., Jones E.B.G. 2009. — Molecular systematics of the marine *Dothideomycetes*. *Studies in Mycology*, 64: 155-173. doi: 10.3114/sim.2009.64.09

Swofford D.L. 2002. — PAUP*: Phylogenetic Analysis Using Parsimony (and other methods). Version 4b10. Sinauer Associates, Sunderland, USA.

Tamura K., Peterson D., Peterson N., Stetcher G., Nei M. & Kumar S. 2011. — MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28: 2731-2739. doi: 10.1093/molbev/msr121

Wang Y.-Z, Aptroot A. & Hyde K.D. 2004. — *Revision of the Ascomycete genus Amphisphaeria*. Fungal Diversity Research Series 13. Hong-Kong, 168 pp.

Wijayawardene N.N., Crous P. W., Kirk P.M., Hawksworth D.L., Boonmee S., Braun U., Dai D.-Q., D'Souza M.J., Diederich P., Dissanayake A., Doilom M., Hongsanan S., Gareth Jones E.B., Groenewald J.Z., Jayawardena R., Lawrey J.D., Liu J.-K., Lücking R., Madrid H., Manamgoda D.S., Muggia L., Nelsen M.P., Phookamsak R., Suetrong S., Tanaka K., Thambugala K.M., Wanasinghe D.N., Wikee S., Zhang Y., Aptroot A., Ariyawansa H.A., Bahkali A.H., Bhat J., Gueidan C., Chomnunti P., De Hoog G.S., Knudsen K., Li W.-J., Mckenzie E.H.C., Miller A.N., Phillips A.J.L., Piatek M., Raja H.A., Shivas R.S., Slippers B., Taylor J.E., Tian Q., Wang Y., Woudenberg J.H.C., Cai L., Jaklitsch W.M. & Hyde K.D. 2014. — Naming and outline of *Dothideomycetes*–2014 including proposals for the protection or suppression of generic names. *Fungal Diversity*, 69 (1): 1-55. doi: 10.1007/s13225-014-0309-2.

Zhang Y., Schoch C.L., Fournier J., Crous P.W., De Gruyter J., Woudenberg J.H.C., Hirayama K., Tanaka K., Pointing S.B., Spatafora J.W. & Hyde K.D. 2009. — Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re-evaluation. *Studies in Mycology*, 64: 85-102. doi: 10.3114/sim.2009.64.04

Zhang H., Hyde K.D., Abdel-Wahab M.A., Abdel-Aziz F.A., Ariyawansa H.A., Ko Ko T.W., Zhao R.L., Alias S.A., Bahkali A.H. & Zhou D.Q. 2013. — A modern concept for *Helicascus* with a *Pleurophomopsis*-like asexual state. *Sydowia*, 65 (1): 147-166.

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