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ORIGINAL ARTICLE

Spelling out *Jaapia* species

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Abstract *Jaapia* is a wood-saprobic genus of corticioid fungi for which two species have been recognized: *J. argillacea* Bres. and *J. ochroleuca* (Bres.) Nannf. & J. Erikss. Whereas the first one is easily recognized by its characteristic spores, the descriptions of the second indicated variable spores, which once led us to believe that *J. ochroleuca* could be a species complex rather than a single species. Eleven new ITS nrDNA sequences of *J. ochroleuca* were aligned with two obtained from GenBank and four of *J. argillacea*. The molecular results, parsimony analysis and KP2 distances clearly delimitate one highly supported *Jaapia* clade, with two subclades that correspond to the two described species. Morphological studies, including the holotype and isotype of *J. ochroleuca*, show significant differences between the clades concerning the size and shape of spores. The present study corroborates two species in this genus and also confirms that *J. ochroleuca* is a well-defined species in which spores show great morphological variability. Based on the *Jaapia* "species hypothesis", the *J. ochroleuca* reference sequence has been selected. A comprehensive key to two *Jaapia* species is also provided.

Keywords Basidiomycota · Agaricomycetes · Jaapiales · Corticioid fungi · Taxonomy · Barcoding · ITS · Morphological diagnostic characters

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Introduction

Jaapia was described by Bresadola (1911) to accommodate *J. argillacea* Bres., and for 20 years, this monotypic genus was accepted by several authors (von Höhnel 1912; Wakefield and Pearson 1920; Bourdot and Galzin 1923, 1928; Rogers 1935). Nannfeldt and Eriksson (1953) considered *Coniophora ochroleuca* Bres. as the second species of *Jaapia*, and the genus was emended by them as "Fructifications effuse, resupinate, with indefinite margin, whitish or pale yellowish, loose and floccose; hyphae clamped and richly branching; cystidia present; basidia long, slightly sinuous and often constricted with four sterigmata," and its characteristic spores as: "… relatively large and more or less fusiform, with a very conspicuous, more or less peg-like apiculus; spore-wall at least after detachment becoming thick and two-layered, the inner layer staining strongly with Cotton Blue and Congo Red; in one species (*J. argillacea*) the inner layer finally surrounds as chlamidospore-like body, which does not fill out the spore completely but leaves an empty space at each (or one) end; in the other species (*J. ochroleuca*) there is generally no intermembranal space."

On the basis of the peculiar morphology of its spores, *Jaapia* was considered to be closely related to *Coniophora* DC. (von Höhnel 1912), *Pellicularia* Cooke (Rogers 1935) or even *Coniobotrys* Pouzar, a new genus described by Pouzar (1958) with *Coniophora ochroleuca* as type species. Nannfeldt and Eriksson (1953) and Donk (1964) referred it to Coniophoraceae, and Eriksson and Ryvarden (1976) regarded *Jaapia* as member of the broad concept of the family

Cortiaceae. Larsson (2007), in his phylogenetic classification for corticioid fungi, included it in an "incertae sedis" as *Jaapia* family. Binder et al. (2010) described the family Jaapiaceae in Jaapiales, an order for the lone genus *Jaapia*; according to these authors, Jaapiales was supported as the sister group of the remainder of the Agaricomycetidae.

Nowadays, this wood-saprobic fungal genus includes two taxa, *Jaapia argillacea* and *J. ochroleuca* (Bres.) Nannf. & J. Erikss. *Jaapia argillacea* seems to be a rare species with a wide distribution (Nannfeldt and Eriksson 1953); known in Europe from Denmark, France, Germany, Norway, Sweden, and the United Kingdom, (Bresadola 1911; Wakefield and Pearson 1920; Nannfeldt and Eriksson 1953; Chistiansen 1960; Eriksson and Ryvarden 1976; Boidin and Gilles 1990; Legon et al. 2005), as well as USA (Rogers 1935; Nannfeldt and Eriksson 1953; Ginns and Lefebvre 1993), and Canada (Eriksson and Ryvarden 1976; Ginns and Lefebvre 1993). *Jaapia ochroleuca* is even rarer than *J. argillacea* (Nannfeldt and Eriksson 1953; Eriksson and Ryvarden 1976), but also seems to be widely distributed. It is known in Europe from Austria, Belgium, Caucasus, Czechoslovakia, Denmark, Estonia, France, Finland, Germany, Italy, Macedonia, Norway, Portugal, Spain, Sweden, Ukraine, and the United Kingdom (Bresadola 1911; Bourdot and Galzin 1928; Rogers 1943; Nannfeldt and Eriksson 1953; Eriksson and Ryvarden 1976; Hjortstam et al. 1981; Dueñas and Telleria 1984; Boidin and Gilles 1990; Melo and Telleria 1991; Legon et al. 2005; Bernicchia and Gorjón 2010) as well as the Azores Archipelago (Telleria et al. 2009). Moreover, it has been reported from America: USA (Rogers 1943; Nannfeldt and Eriksson 1953; Ginns and Lefebvre 1993), Canada (Rogers 1943; Nannfeldt and Eriksson 1953) and Argentina (Gorjón et al. 2012); as well as from Africa, in Kenya (Hjortstam 1987), and Asia, in China (Dai 2011).

Whereas *Jaapia argillacea* is easily recognized by its characteristic spores narrowly fusiform, 16–25×5–7 μm, at first thin-walled but later with a thick and cyanophilous secondary wall, with the ends empty (Eriksson and Ryvarden 1976), the descriptions of the spores of *J. ochroleuca* show great morphological variability, particularly in spore width and therefore in length/width ratios (Brinkmann 1898; Bourdot and Galzin 1928; Rogers 1943; Nannfeldt and Eriksson 1953; Eriksson and Ryvarden 1976; Boidin and Gilles 1990; Bernicchia and Gorjón 2010) which once led us to believe that *J. ochroleuca* could be a species complex rather than a single one.

In corticioid fungi, the spore size has been considered to be a useful character with high diagnostic value; such is the case in separating several species pairs, such as *Corticium roseum* Pers. vs. *Corticium meridioroseum* Boidin & Lanq. (Boidin and Lanquetin 1983) or *Hyphoderma argillaceum* (Bres.) Donk vs. *Hyphoderma magnargillaceum* Boidin & Gilles (Boidin and Gilles 1991) or the species in the genus *Vuilleminia* (Boidin and Lanquetin 1983; Ghobad-Nejhad et al. 2010) and the genus *Hyphodermella* (Telleria et al. 2010).

Nevertheless, the delimitation of corticioid species is sometimes difficult, since morphological characters do not always help to distinguish species. Nowadays, molecular data are considered as effective tool for species discrimination and, recently, the ITS region was proposed (Schoch et al. 2012) as the first barcoding for fungi. However, the taxonomic coverage of the International Nucleotide Sequence Databases (INSD: EMBL/GenBank/DDBJ) (Cochrane et al. 2011) needs to be improved (Brock et al. 2009), and only when all described species of fungi are represented on GenBank (currently it has less than 7 % of the circa 1.5 million estimated species), will researchers be able to judge a "known" or "unknown" species. It has also been pointed out (Brock et al. 2009) that the number of insufficiently identified sequences in the INSD, lacking voucher information and named as "environmental unknowns," has increased greatly within the last 5 years (Kõljag et al. 2013).

For improving the molecular identification of fungi, new tools have been developed in the UNITE database (http://unite.ut.ee), based on a two-tier clustering process on different similarity thresholds (97–99 %); both levels represent molecular operational taxonomic units (MOTUs). The first clustering represents the genus level, and the second represents the species range. Kõljag et al. (2013) introduced the term "species hypothesis" (SH) for the second cluster, and for each SH cluster, a sequence was chosen as a reference sequence.

The aims of this study were to use morphological and molecular data to characterize and identify the voucher specimens under *Jaapia argillacea* and *J. ochroleuca* all over the world, as well as to analyze the significance of spore size and shape in the taxonomy of this genus. Also, based on the *Jaapia* "species hypothesis," the *J. ochroleuca* reference sequence was selected.

Materials and methods

Sampling and morphological studies

A total of 40 specimens, five of *Jaapia argillacea* and 35 of *J. ochroleuca*, from BIO-Fungi, GB, LISU, MA-Fungi, O, PC, and UPS herbaria were studied, including the holotype (S) and isotype (UPS) of *J. ochroleuca* (Table 1). Measurements were made from microscopic sections mounted in 3 % aqueous solutions of KOH and examined at magnifications up to 1250× using an Olympus BX51 microscope. The length and width of 30 spores were measured from each sample, and mean values and length/width ratios (Q) were calculated (Table 1). Type specimens of *J. ochroleuca* were studied in

Table 1 Specimens studied with measurements of spores and length/width (Q) ratios

Species / specimens	Country	Spore sizes (μm)	Mean spore sizes (μm)	Q=L/W	GenBank number
Jaapia argillacea Bres.		**(16−)18−27× (5−)6−8(−9)**			
GB-008 0537, KHL 4871	Sweden	(16−) 18–23 (−25)×6−7	20.4×6.6	3.09	–
GB-008 0538, KHL 11734	Finland	18−21×6.5−8	19.3×7.25	2.66	EU118636
GB-008 0539, KHL 5123	Norway	(16−) 19–20×7−8	19.2×7.6	2.52	–
GB-008 0543, KHL 8433	Sweden	17−21 (−25)×6–8 (−9)	20.8×7.3	2.84	EU118637
MA-Fungi 85217, 6685MD	Spain	17−27×(5−) 6−8	21.6×6.84	3.15	–
J. ochroleuca (Bres.) Nannf. & J. Erikss		**10−20×5.5−9**			
BIO-Fungi 11223	Spain	14−16×7−8	14.6×7.65	1.90	–
BIO-Fungi 12590	Spain	12−14 (−16)×6−8	13.1×6.85	1.92	LN824165
GB-008 0540, K. Hjortstam 17910	Norway	14−16 (−18)×7−8(−9)	15.2×7.8	1.94	–
GB-008 0541, HJM 12824	Norway	13−16 (−18)×7–8 (−9)	14.9×7.9	1,88	–
GB-008 0542, KHL 3148	Sweden	12−14×6−7	13.2×6.3	2.09	–
LISU 178581, 9081IM	Portugal	13−15×6−7	13.4×6.75	1.98	–
LISU 178600, 9100IM	Portugal	12−14 (−15)×6−7	13.3×6.85	1.92	LN824163
MA-Fungi 76104, 11955MD	Portugal	12−13×6−7	12.8×6.15	2.08	LN824164
MA-Fungi 5783, 1914Tell.	Spain	11−14 (−17)×7−8	13×7.7	1.68	–
MA-Fungi 23942, 9269Tell.	Portugal	11−14×6–7.5	12.9×7.1	1.81	LN824166
O 58785, I. Lindblad 652	Norway	14−20×7−10	16.9×8.3	2.03	LN824162
O 64242, L. Ryvarden 42617	Norway	12−13×6−7	12.5×6.6	1.89	LN824161
O 97204, J. Eriksson 5990	Norway	11−15×5.5−7	13.6×6.35	2.14	–
O 97205, J. Eriksson 6061	Norway	13−15×5.5−6.5	14.1×5.85	2.41	LN824160
O 149877, E. Bendiksen & K. Høiland 55-113	Norway	(13−) 14–18×6−7	15.45×6.4	2.41	LN824157
O 173145, E. Bendiksen & K. Høiland 64-142	Norway	13−15.5×6−8	14.15×6.3	2.24	–
O 178496	Norway	13−15 (−16)×7−8	14.3×7.5	1.90	LN824158
O 223748, F. Oldervik 333.03	Norway	13−19×6−8	15.9×6.95	2.28	LN824159
PC0084335, herb. Bourdot 5864, Galzin 3722	France	11−13×6−7	12×6,45	1.86	–
PC0084336, herb. Bourdot 5864, Galzin 3722	France	(10−) 11–15×6−7	12.5×6.2	2.01	–
PC0084337, Galzin 11352	France	10−12×6–7.5	11.5×6.55	1.75	–
PC0084338, herb. Bourdot 6643, Galzin 4374	France	11−13×6−7	11.9×7.1	1.67	–
PC0084339, herb. Bourdot 6019, Galzin 3925	France	11−15×6.5−8	13×7.2	1.80	–
PC0084340, herb. Bourdot 5864, Galzin 3722	France	11−13×6−7	12.1×6.5	1.86	–
S, F88243, ex herb. Bresadola, **holotype**	Germany	12−16 (−18)×7.5−9 (−10)	14.6×8.1	1.90	–
UPS (F-176760) 498994, J. Strodal, B. & J. Eriksson[a] 5990	Norway	–	–	–	–
UPS (F-176761) 498995, J. Strodal, B. & J. Eriksson 5992	Norway	14−16×5−7	14.7×6.15	2.39	–
UPS (F-176762) 498996, B. & J. Eriksson 5846	Norway	14−15.5×6−7	14.7×6.65	2.21	–
UPS (F-176763) 498997, T. Nathorst-Windahl 4018	Sweden	12-15(−17)×6–7.5	14.55×6.5	2.23	–
UPS (F-176764) 498998, J. Eriksson 6545	Sweden	12−14×5.5−7	12.6×6.35	1.98	–
UPS (F-176765) 498999, D.P. Rogers 702	USA	12−16×5–6 (−7)	14.7×5.75	2.55	–
UPS (F-176766) 499000, ex herb. Toronto 12875	Canada	13−15×5−6	13.8×5.6	2.46	LN824167
UPS (F-176767) 499001, ex herb. Bourdot[b] 5864	France	–	–	–	–
UPS (F-176768) 499002	Slovakia	12−14×6−7	12.9×6.4	2.01	–
UPS (F-176769) 499003, W. Brinkmann, fragm. ex Herb. Bresadola, **isotype**	Germany	13−15 (−18)×6–7 (−8)	14.4×6.75	2.13	–

[a] This is the same sample as O 97204

[b] This is the same sample as PC0084340

order to compare their spore data with those of the specimens included in this study.

DNA extraction, amplification and sequencing

DNA was isolated from herbarium specimens using the DNeasy™ Plant Mini Kit (Qiagen, Valencia, California, USA), following the instructions of the manufacturers. Lysis buffer incubation was done overnight at 55 °C following Whiting et al. (1997). The primer pair ITS1F and ITS4 was used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flanking parts of the SSU and LSU genes (White et al. 1990; Gardes and Bruns 1993). Amplifications were done using illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK) as described in Winka et al. (1998), following thermal cycling conditions in Martín and Winka (2000). Negative controls lacking fungal DNA were run for each experiment to check for contamination of reagents. Results of amplifications were assayed from 5 μl aliquots by gel electrophoresis of 2 % Pronadisa D-1 Agarose (Lab. Conda, Spain). For enhancing PCR amplification of those recalcitrant specimens, new DNA isolation was done using FTA® Indicating Micro Cards (Cat N° WB120211, Whatman, Maidstone, England) as described in Telleria et al. (2014).

Prior to sequencing, the amplification products were cleaned using a QIAquick gel PCR purification kit (QIAGEN, Valencia, California, USA). Both strands were sequenced separately using the primers mentioned above at Macrogen (South Korea). Sequences were edited and assembled using Sequencher™ version 4.2 (Gene Codes Corporations, Ann Arbor, Michigan, USA). BLASTN searches (Altschul et al. 1997) with the MEGABLAST option were used to compare the obtained sequences against the sequences in the National Center of Biotechnology Information (NCBI) nucleotide database. Moreover, a preliminary identification of the new sequences was done through UNITE database (http://unite.ut.ee) species hypotheses (SHs) search (Kõljag et al. 2013). The consensus sequences have been lodged in the EMLB/GenBank/DDBJ databases with the accession numbers indicated in Table 1.

Molecular identification

An automatic *Jaapia* PlutoF multiple sequence alignment was obtained through UNITE. The SH clusters were merged with the new sequences obtained in this study and manually adjusted using Se-Al v.2.0a11 (Rambaut 2002). Alignment gaps were indicated as "−" and ambiguous nucleotides were marked as "N". Sequences under *Gloeophyllum abietinum* (Bull.) P. Karst. (AJ420948, Germany; KM098122 and JX524620, China) were included as outgroup, since in the BLASTN search, *Gloeophyllum* sequences scored next highest after the *Jaapia* sequences mentioned above; moreover, according to the 5.8S and LSU nrDNA analyses (Larsson 2007), species of Gloeophyllaceae are closely related to *Jaapia* species.

The alignment was analyzed under a heuristic search, using the program PAUP 4.0b10 (Swofford 2003). Branch lengths equal to zero were collapsed to politomies. Nonparametric bootstrap support (Felsenstein 1985) for each clade was tested with the fast-step option, using 10,000 replicates. The phylogenetic tree was viewed with the program FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) and edited with Adobe Illustrator CS3 11.0.2. (Adobe Systems Incorporated).

From *Jaapia* sequences, genetic distances were also calculated in PAUP*4.0b10 using the Kimura 2-Parameter model (Kimura 1980), which is widely used in DNA barcoding analyses (e.g., Neigel et al. 2007); interspecific and intraspecific genetic divergences were obtained and the quotient between these distances was calculated to determine whether a barcoding gap exists (Hebert et al. 2004). Nonparametric bootstrap support for each clade was calculated as indicated above.

Results

In this study, eleven sequences were generated from the specimens studied using the primer pair ITS1F/ITS4. On the other hand, the automatic *Jaapia* PlutoF multiple sequence alignment (UCL7_001618) obtained through UNITE has six sequences distributed in two SH clusters. The sequence GU187524 (*J. argillacea*) published in Binder et al. (2010) is the reference sequence of the species hypothesis SH235459.06FU cluster; recently this sequence (GU187524) was also selected as the reference sequence of the species *J. argillacea*, and it appears in the INSD database with the number NR_119766 (Schoch et al. 2014). The SH235459.06FU cluster also includes the sequences EU118636 (*J. argillacea*) and EU118637 (*J. ochroleuca*) from Larsson (2007), and the sequence KC514904 (*J. argillacea*) from an unpublished paper. The second SH cluster, SH190037.07FU, includes two sequences from environmental samples, JX981904 and JX981905, identified as "uncultured fungus."

The final alignment had 20 sequences, including the *Jaapia* sequences from the UNITE databases (all sequences in EMLB/GenBank/DDBJ), and the three *Gloeophyllum abietinum* sequences as outgroup. After heuristic search, 100 trees were retained (tree length = 117; consistency index, CI= 0.9402; homoplasy index, HI=0.0598; retention index, RI= 0.9676); one of the 100MPTs is shown in Fig. 1, with bootstrap values above the branches. The *Jaapia* sequences formed a highly supported clade (BS=100 %). Two main

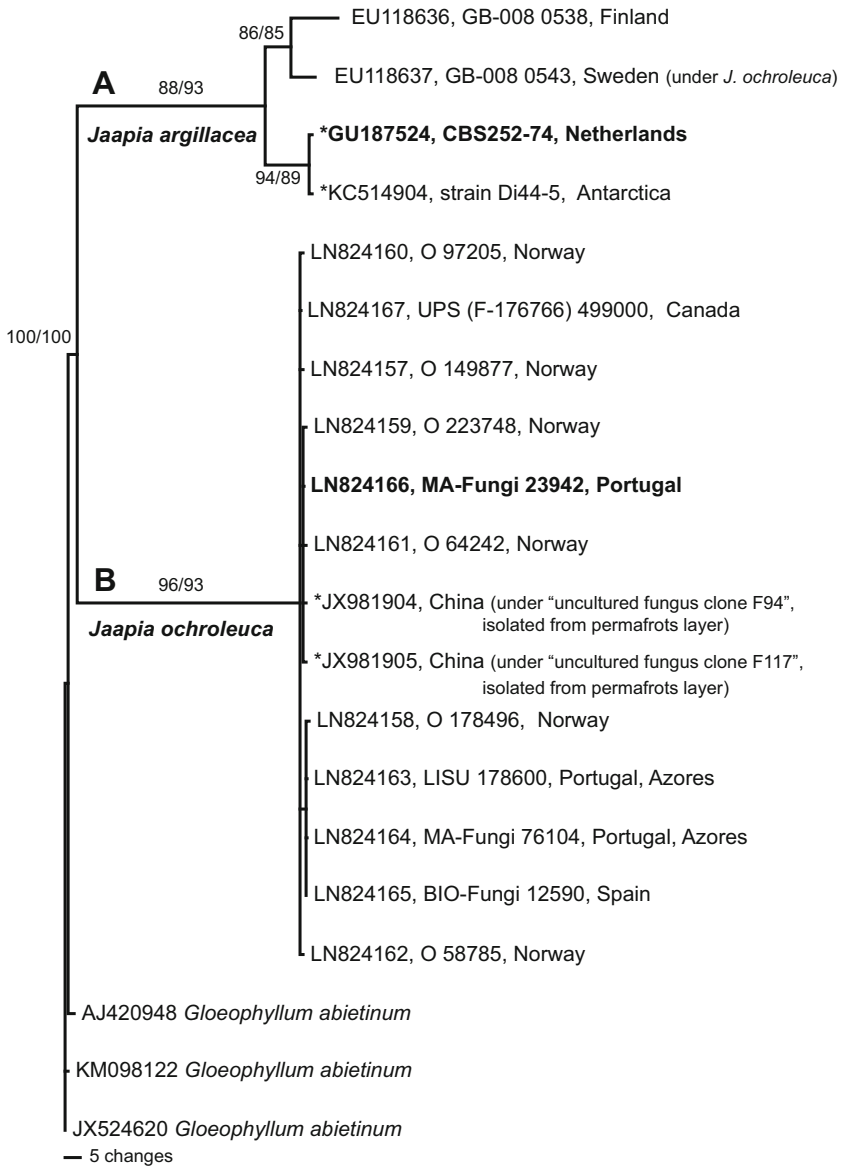

Fig. 1 One of the 100 most parsimonious trees inferred from a heuristic search of ITS nrDNA sequences of *Jaapia* species. Three *Gloeophyllum abietinum* sequences served as outgroup. The bootstrap values (%) after the parsimony and the Kimura 2-Parameter model analyses are indicated above or below the branches. The proposed reference sequence to each *Jaapia* species is indicated in boldface. Sequences located at the EMBL/GenBank/DDBJ and obtained from pure cultures or environmental samples are indicated with an *asterisk*

subclades were detected, both with very high bootstrap values. Subclade A (BS=88 %) included the four sequences that grouped in the SH235459.06FU cluster, under *J. argillacea* (three sequences) and under *J. ochroleuca* (one sequence). Subclade B (BS=96 %) consisted of the 11 sequences generated in this study and two environmental sequences that formed the SH cluster SH190037.07FU; these environmental sequences were isolated in China from the permafrost layer.

On the other hand, the K2P pairwise distance among the *Jaapia* sequences (517 positions) shows more genetic variability (range 0.00198–0.03028, average distance 0.01641) within sequences from the subclade A; whereas it shows a low value within the sequences of subclade B (range 0.00000–0.00983, average distance 0.0012). The average intraspecific K2P value for all specimens is 0.00274 and the average of interspecific value is 0.03811 (range 0.02832–0.05137). The quotient between the averages of interspecific and intraspecific distances is 13.91, which is greater than the widely used threshold for barcoding studies (10×, Hebert et al. 2004).

Morphological studies show significant differences between the clades concerning the size and shape of spores (Table 1, Fig. 2). The specimens of *Jaapia argillacea* in clade A show long and homogeneous fusiform spores, (16−)18−27×(5−)6−8(−9) μm, Q=2.52−3.15, always with protoplasm contracting in very conspicuous apical ends (Fig. 3). The specimens of *J. ochroleuca* in clade B exhibit a wide range of spore sizes, 10–20×5.5−9 μm [12–16 (−18)×7.5−9 (−10) μm in Bresadola's holotype, S, F88243]. Concerning spore shape, the specimens also show some variability with fusiform (Q=2.08−2.55) or broadly fusiform (Q=1.68−1.98) spores, sometimes with

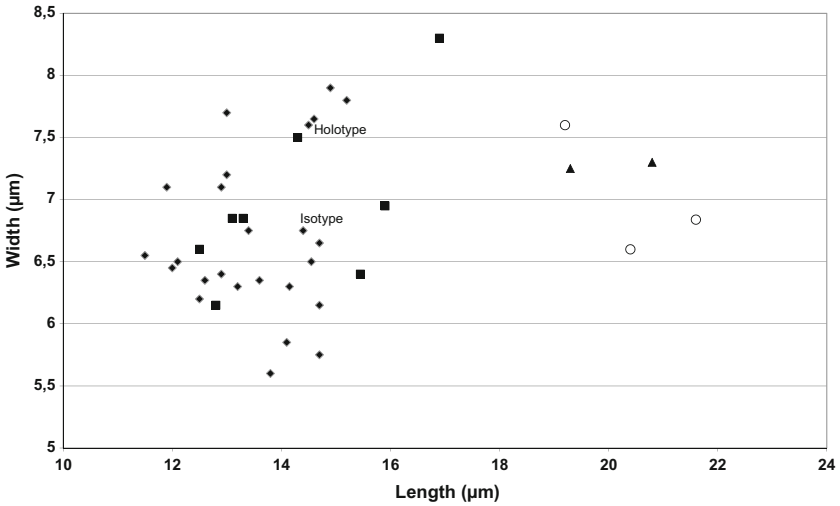

Fig. 2 Spore sizes measured as average values for 30 spores per specimen. ▲ *Jaapia argillacea* sequenced, ○ *J. argillacea* not sequenced, ■ *Jaapia ochroleuca* sequenced, ♦ *J. ochroleuca* not sequenced

protoplasm contracting leaving the spore ends collapsed, as in the UPS (F-176765) 498999 collection (Fig. 4a).

From the present study, two species are confirmed, and morphological characters are summarized in the following comprehensive key.

Key to species

1. Basidioma resupinate, effuse, argillaceous to yellowish, soft; hymenophore flocculose; margin indeterminate. Hyphal system monomitic, hyphae with clamps, thin-walled, 5–6.5 µm wide; cystidia cylindrical, projecting, thick-walled in the basal part thinner in apical direction, 125–160×(7−)8−11 µm; basidia cylindrical, sinuous, long, 45–60×8.5−10 µm, with four sterigmata; old basidia often with adventitious septa; spores (16−)18−27×(5−)6−8(−9) µm, fusiform (Q=2.52−3.15), smooth, cyanophilous, first thin-walled and after the protoplasm contracts, surrounding itself with a secondary thick wall and leaving the spore ends empty.

J. argillacea

Figures 3 and 5.

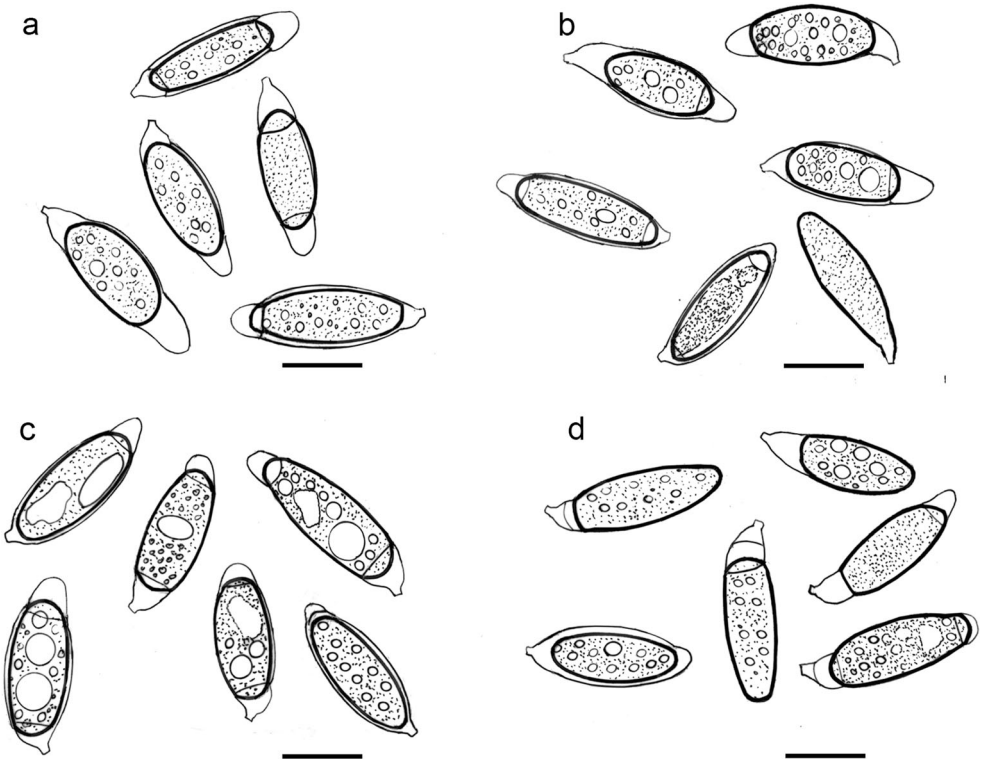

Fig. 3 Spores of *Jaapia argillacea.* **a** GB-008 0537, KHL 4871; **b** GB-008 0538, KHL 11734; **c** GB-008 0539, KHL 5123; **d** GB-008 0543, KHL 8433 (*bar* 10 µm)

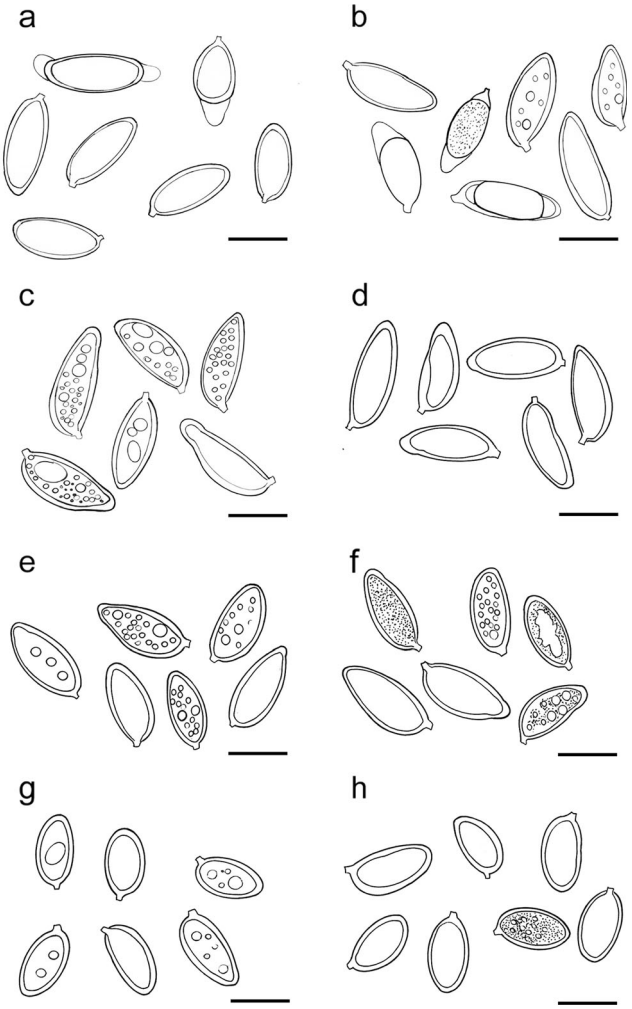

Fig. 4 Spores of *Jaapia ochroleuca*. **a** UPS (F-176765) 498999, D.P. Rogers 702; **b** UPS (F-176766) 499000, ex herb. Toronto 12875; **c** O 149877, E. Bendiksen & K. Høiland 55-113; **d** O 97205, J. Eriksson 6061; **e** O 223748, F. Oldervik 333.03; **f** MA-Fungi 76104, 11955MD; **g** MA-Fungi 5783, 1914Tell.; **h** PC0084338, herb. Bourdot 6643, Galzin 4374 (*bar* 10 μm)

1. Basidioma resupinate, effuse, yellowish to ocraceous, soft; hymenophore flocculose to pilose; margin indeterminate. Hyphal system monomitic; hyphae with clamps, thin- to thick-walled, (5−)6−11 μm wide; cystidia cylindrical, projecting, thick-walled in the basal part thinner in apical direction, 150−200−350×7−13(−17) μm; basidia claviform to cylindrical, sinuous 27–50×6.5−8.5 μm, with four sterigmata; old basidia often with adventitious septa; spores 10–20×5.5−9 μm, fusiform (Q= 2.08−2.55) or broadly fusiform (Q=1.68−1.98), smooth, cyanophilous, thick-walled, sometimes with protoplasm contracting leaving the spore ends collapsed.

J. ochroleuca

Figures 4 and 6.

Discussion

Parsimony analysis, as well as the KP2 distances, clearly delimitate two well-defined and highly supported clades (Fig. 1). Both clades are supported by morphology, specifically by the spore size (Fig. 2) and correspond to the two described species in the *Jaapia* genus.

Whereas *Jaapia argillacea* (Bresadola 1911) is a well-defined species with characteristic spores, *J. ochroleuca* has been described with a broad range of spore width. The protologue of *Coniophora ochroleuca* (Brinkmann 1898) describes the spores as "subamygdaliformes, 13–16×6–8 μm," which accords with the data on Bresadola's holotype label. Rogers (1943), based on the European and American specimens, holotype included, characterized the spores as "navicular-fusiform, obliquely apiculate, 9.5−18×4–8 μm." Nannfeldt and Eriksson (1953) concluded that they are "fusiform, with a two-layered thick wall, 12–18×4–6 μm," likely a mistake because they studied a lot of specimens, even the holotype. Unfortunately, these wide values will remain an enduring mistake, passed on from one author to the next

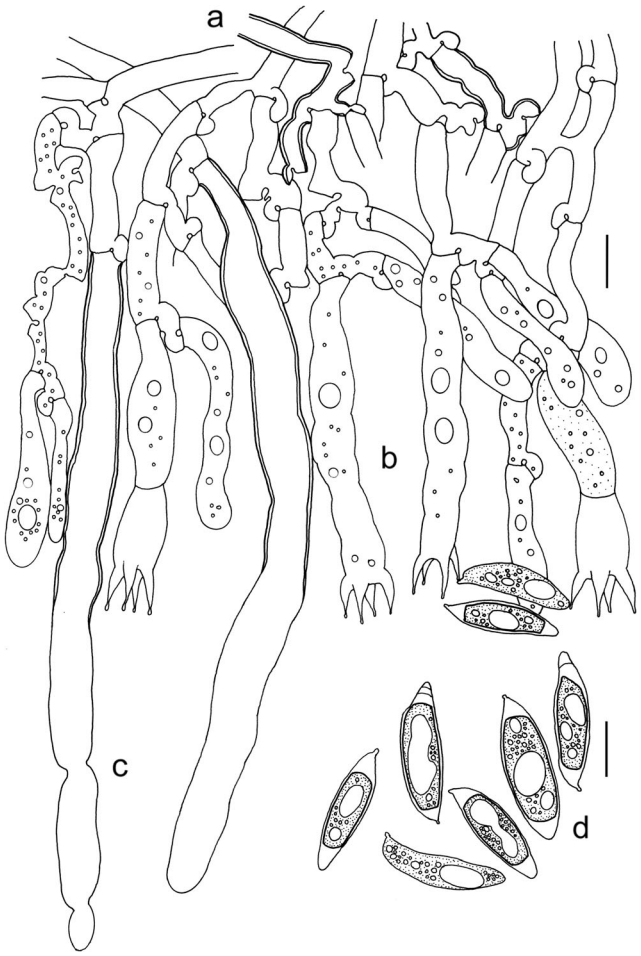

Fig. 5 *Jaapia argillacea* (MA-Fungi 85217, 6685MD). **a** basal hyphae, **b** basidia, **c** cystidia, **d** spores (*bar* 10 μm)

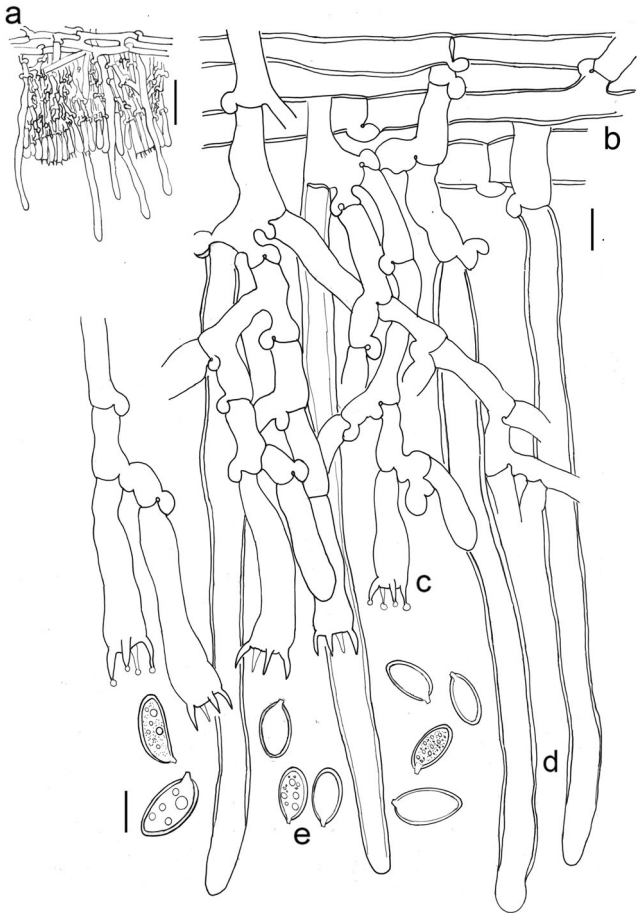

Fig. 6 *Jaapia ochroleuca* (Typus, S, F88243). **a** section through the basidioma (*bar* 100 μm), **b** basal hyphae, **c** basidia, **d** cystidia, **e** spores (*bar* 10 μm)

(Eriksson and Ryvarden 1976; Bernicchia and Gorjón 2010), with the exception of Boidin and Gilles (1990), who defined it as "largement fusiforme, 11–15×6–8 μm."

Our study confirms, based on morphological and molecular data, that *Jaapia ochroleuca* is a well-defined species with variable spores: fusiform or broadly fusiform, 10–20×5.5–9 μm.

The barcode for fungi (Schoch et al. 2012), ITS nrDNA sequences, allowed us to evaluate the accuracy of the identification of *Jaapia* specimens made by early authors. Sequences obtained in this study are based on well-identified specimens and are named not only through BLAST search; they show a good match to the two molecular operational taxonomic units (MOTUs) detected through UNITE search, and to the species level, allowing distinction between *J. argillacea* (Fig. 1 subclade A: SH235459.06FU cluster) and *J. ochroleuca* (Fig. 1 subclade B: species hypothesis SH190037.07FU cluster). A reference sequence already exists for *J. argillacea* (GU187524 in INSD and UNITE databases; named NR119766 as proposed in Schoch et al. 2014). ITS sequences generated from MA-Fungi 23942 will be proposed for deposit in the RefSeq Targeted Loci (RTL) database at NCBI (http://www.ncbi.nlm.nih.gov/bioproject/177353).

Sequences from well-identified isolates are master keys for identifying unknown and/or "environmental" sequences located in international sequence databases, giving a more accurate estimation of worldwide fungal diversity. For example, sequences JX981904 and JX981905 isolated from permafrost soil in China clearly belong to the species *J. ochroleuca.*

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