

Xerochrysum gen. nov. and *Bettsia*, genera encompassing xerophilic species of *Chrysosporium*

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Abstract: On the basis of a study of ITS sequences, Vidal *et al.* (*Rev. Iber. Micol.* **17**: 22, 2000) recommended that the genus *Chrysosporium* be restricted to species belonging to *Onygenales*. Using nrLSU genes, we studied the majority of clades examined by Vidal *et al.* and showed that currently accepted species in *Chrysosporium* phylogenetically belong in six clades in three orders. Surprisingly, the xerophilic species of *Chrysosporium*, long thought to be a single grouping away from the majority of *Chrysosporium* species, occupy two clades, one in *Leotiales*, the other in *Eurotiales*. Species accepted in *Leotiales* are related to the sexual genus *Bettsia*. One is the type species *B. alvei*, and related asexual strains classified as *C. farinicola*, the second is *C. fastidium* transferred to *Bettsia* as *B. fastidia*. Species in the *Eurotiales* are transferred to *Xerochrysum* gen. nov., where the accepted species are *X. xerophilum* and *X. dermatitidis*, the correct name for *C. inops* on transfer to *Xerochrysum*. All accepted species are extreme xerophiles, found in dried and concentrated foods.

Key words:

Eurotiales
food spoilage
Leotiales
molecular systematics
Onygenales
taxonomy

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INTRODUCTION

The genus *Chrysosporium* was revived by Carmichael (1962) as a way to rationalise the taxonomy of a number of genera of hyphomycetes characterised by the production of aleurioconidia “on undifferentiated hyphae either at the tip, along the sides, or in an intercalary position.” The aleurioconidia “were released by the disintegration of part, or all, of the spore-bearing mycelium” (Carmichael 1962). Although “aleurioconidium” is considered to be an obsolete term, it remains the most precise available for thick-walled conidia produced on pedicels along a stipe, seceding only with difficulty. Many of the species included in *Chrysosporium* by Carmichael were of medical importance, being dermatophytic or keratinolytic or both. However, one new species described by Carmichael, *C. inops*, was isolated from orange concentrate, so was likely to be xerophilic. Some species, including *C. inops*, also produce thick-walled intrahyphal conidia, both swollen cells (chlamydoconidia) and unswollen cells (arthroconidia), which intergrade (Carmichael 1962, Barron 1968).

Pitt (1966) described two new species of *Chrysosporium*, *C. fastidium* and *C. xerophilum*, from dried fruit: both appeared to be related to *C. inops*. These species were subsequently shown to be among the most xerophilic organisms known, capable of growth below 0.7 water activity (a_w) (Pitt & Christian 1968, Pitt 1975). In the following years, it was considered that Carmichael's concept of

Chrysosporium was too broad: some genera including *Sporothrix*, *Sporotrichum*, and *Geomyces* were separated out again. *Sporothrix* is a medically important dermatophyte (de Hoog *et al.* 2000), *Sporotrichum* has been restricted to the asexual states of some lignicolous basidiomycetes (Stalpers 1984), and *Geomyces* is a genus of saprophytes (Barron 1968) and also includes a xerophilic species (Hocking & Pitt 1988).

Van Oorschot (1980) monographed *Chrysosporium*, retained Carmichael's concept of the genus, and accepted 22 species. However, the medium used in descriptions by van Oorschot (1980) was a cherry decoction agar, of unspecified but undoubtedly quite high a_w , so that growth of the xerophilic species was far from optimal. The basic circumscription of *Chrysosporium* still included most hyphomycete fungi that produce aleurioconidia. Skou (1972) described a new sexual genus, *Bettsia*. This includes a single species, *B. alvei*, which produces a xerophilic *Chrysosporium* asexual morph, *C. farinicola* (Skou 1975). *Bettsia alvei* produces small, dark, distinctive cleistothecia, different from sexual morphs related to other *Chrysosporium* species. Skou (1992) described several new xerophilic species and varieties of *Chrysosporium* from mason bees (*Osmia* spp.) and beehives.

Using ribosomal ITS sequence data, Vidal *et al.* (2000) divided *Chrysosporium* into nine species groups and concluded that this genus should be restricted to asexual species in *Onygenales* that possess keratinophilic but not cellulolytic capabilities.

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Our study of the extremely xerophilic ascomycete *Xeromyces bisporus* indicated a close relatedness to the xerophilic *Chrysosporium* species *C. xerophilum* and *C. inops*. Phylogenetic analysis of the 28S rRNA gene securely placed these species within *Eurotiales*, as strictly asexual species (Pettersson *et al.* 2011). *Chrysosporium xerophilum* is capable of more rapid growth over a wider a_w range than *C. inops* (Leong *et al.* 2011).

This paper reports a critical assessment of the xerophilic species currently classified in *Chrysosporium*, i.e. *C. fastidium*, *C. farinicola*, *C. inops*, and *C. xerophilum*, together with the seven species described by Skou (1992).

MATERIALS AND METHODS

Cultural studies

Culture conditions

The xerophilic species studied grow very poorly, if at all, on conventional media such as Czapek yeast extract agar (CYA) or malt extract agar (MEA; Pitt & Hocking 2009). Optimal growth occurs on very concentrated media, such as malt extract yeast extract 50 % glucose agar (MY50G, 0.89 a_w). Taxonomic descriptions are therefore based on growth on that medium (Pitt & Hocking 2009). Plates were three-point inoculated with a needle-point of dry spores, and incubated at 25 °C for 7 d, colonies were measured, and plates were then reincubated for a further 7 d. Microscopical observations were made at both times.

Growth and morphology of cultures at 25 °C were also examined on G25N and Casein Czapek 50G agar after 14 d, the latter previously used for differentiating foodborne *Chrysosporium* species (Kinderlerer 1995).

Effect of water activity and temperature on growth rate

Agar media based on MY50G were prepared at 0.99, 0.89, 0.78, 0.73 and 0.71 a_w . At the two lowest water activities, glucose and fructose were mixed in equal amounts. The water activities of the media were monitored at the beginning and end of the trial using the dew point technique in the AquaLab CX-2 (Decagon Devices, Pullman, WA, USA). Mycelial suspensions were prepared in sterile 50 % glycerol from cultures grown on MY50G at 25 °C for 3 wk. Triplicate plates were single point inoculated with 5 μ L mycelial suspension and incubated at 20, 25, 30 and 37 °C for 50 d. Plates were wrapped in polyethylene film (household cling film) to prevent evaporation and placed in double zip-lock polyethylene bags in stacks no higher than six plates tall. To calculate the linear growth rate of the strains, colony diameters were measured on two perpendicular axes regularly at appropriate intervals, and the mean diameters recorded.

Molecular studies

Sequences used

The phylogenetic relationships of the species studied were assessed by analysis of 28S rRNA sequences, including representative species from the majority of clades

described by Vidal *et al.* (2000). Ex-type strains were used where available, obtained from CBS (KNAW-CBS Fungal Biodiversity Centre, Utrecht), and FRR (CSIRO Animal, Food and Health Sciences, North Ryde, NSW). Additional sequences were also downloaded from GenBank based on two principles. First, conserved regions of nrLSU were extracted from *Chrysosporium* species representing each strongly supported clade in Vidal *et al.* (2000) and used to find similar sequences in GenBank with BLAST (Altschul *et al.* 1990). Second, to be able to place the xerophilic *Chrysosporium* species to order and class, a wide sample of species representing most ascomycete orders and classes was included. Accession numbers for these additional sequences are noted in the TreeBASE file (see Results, Phylogenetic analysis).

Sequencing

For DNA extraction, nonxerophilic strains were grown on MEA and xerophilic strains on MY50G at 25 °C for 2 wk. Fresh mycelia were harvested by scraping colony surfaces into Eppendorf tubes containing 200 mM TRIS pH 8.5, 250 mM NaCl, 25 mM EDTA pH 8.0 and 1 % SDS. Mycelia were then mechanically sheared using a VWR pestle-homogeniser (Argos Technologies, Elgin, IL). An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, after which tubes were centrifuged at 13 000 g , for 20 min at 4 °C. The aqueous phase was collected and DNA precipitated by adding an equal volume of ice cold isopropanol, followed by 10 min incubation at -20 °C. The pellets were collected by centrifugation at 8,000 g , washed with 95 % ethanol, dried, dissolved in Tris EDTA buffer and quantified by NanoVue machine (GE Lifesciences, Uppsala).

PCR was performed with primers 5.8SR and LR6 (Vilgalys & Hester 1990) using Phusion High Fidelity DNA polymerase (Finnzymes, Helsinki) under conditions recommended by the manufacturer.

Before sequencing, the amplified fragments were purified using the QIAquick PCR Purification kit (QiaGen, Hilden, Germany). Sequencing reactions were performed at Macrogen (Seoul, Korea) using primers LR3 (Vilgalys & Hester 1990) and LR0R (Vilgalys, unpubl.) in addition to 5.8SR and LR6 also used in the prior PCR amplification. Geneious Pro v. 5 (Drummond *et al.* 2010) was used for assembly of chromatograms and for multiple alignments using the MAFFT plugin (Kato *et al.* 2002) with manual editing and optimisation of obtained alignments performed when necessary. Ambiguously aligned parts were identified by visual inspection of the alignments in Geneious and excluded from the analyses using an Exclude statement when running MrBayes. Unique sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

The aligned matrices were subjected to Bayesian analyses using MrBayes v. 3.2 (Ronquist & Huelsenbeck 2003).

To identify the most suitable substitution model for the Bayesian analyses, we used MrModeltest v. 2.3 (Nylander 2004), utilizing the Akaike Information Criterion. The analysis was performed with two sets of four chains (one cold and three heated) and the Stoprule option, stopping the analyses

Table 1. List of *Chrysosporium* and related species examined.

Strain ¹	Species	Notes ^{1,2}	Origin	GenBank No.
CBS 593.71	<i>Amauroascus aureus</i>	Clade IV Neotype of <i>Gymnoascus aureus</i>	Decayed wood, <i>Cryptomeria japonica</i> , Mt. Yudono, Japan	KC989708
CBS 342.64	<i>Aphanoascus terreus</i>	Clade II (no living ex-type culture available)	Lawn soil, India. Anamorph, <i>C. indicum</i> (H.S. Randhawa & R.S. Sandhu) Garg	KC989709
CBS 272.66	<i>Arthroderma ciferrii</i> (Isotype)	Clade VII	Soil, Arkansas, USA. Anamorph, <i>C. georgiae</i> (Varsavsky & Ajello) Oorschot	KC989711
CBS 473.77	<i>Arthroderma tuberculatum</i> (Type)	Clade VII	Feather of <i>Turdus americanus</i> , Urbana, IL, USA	KC989710
FRR 4958	<i>C. botryoides</i> (Type ³)	= CBS 492.91	Nest of <i>Osmia cornifrons</i> , Nagano region, Japan	KC989712
CBS 643.79	<i>C. carmichaelii</i> (Type)	Clade VI	Michigan, USA	KC989713
CBS 688.71	<i>Bettsia alvei</i> (Type)		Pollen in honeycomb, Zeeland, Denmark	KC989714
FRR 2000	<i>C. farinicola</i>	= UAMH 4688	Prunes, Young, NSW, Australia	KC989715
CBS 298.95	<i>C. farinicola</i>		Prune processing equipment, UK	KC989716
FRR 77	<i>C. fastidium</i> (Type)	= IMI 126288, CBS 154.67, ATCC 18053, ATCC 36783, UAMH 2369	Dried prunes, Leeton, NSW, Australia	KC989717
FRR 376	<i>C. fastidium</i>		Dried prunes, Australia	KC989718
FRR 81	<i>C. fastidium</i>	Identified as <i>C. fastidium</i> , appearance and LSU sequence of <i>C. farinicola</i> (Table 3, Fig. 1)	Dried prunes, Australia	KC989725
J211	<i>C. fastidium</i>	Identified as <i>C. fastidium</i> , appearance and LSU sequence of <i>C. farinicola</i> (Table 3, Fig. 1)	Dried prunes, Australia	KC989734
FRR 4953	<i>C. globiferum</i> (Type)	= CBS 454.91	Nest cells of <i>Osmia cornifrons</i> , Nagano region, Japan	KC989719
FRR 4957	<i>C. globiferum</i> var. <i>articulatum</i> (Type)	= CBS 493.91	Nest cells of <i>Osmia cornifrons</i> , Nagano region, Japan	KC989720
FRR 4956	<i>Chrysosporium globiferum</i> var. <i>niveum</i> (Type)	= CBS 455.91	Nest cells of <i>Osmia cornifrons</i> , Nagano region, Japan	KC989721
FRR 4954	<i>C. hispanicum</i> (Type)	= CBS 486.91	Nest cells of <i>Osmia cornuta</i> , Valladolid, Spain	KC989722
FRR 4955	<i>C. holmii</i> (Type)	= CBS 487.91	Nest cells of <i>Osmia rufa</i> , Glostrup, Denmark	KC989723
FRR 2357	<i>C. inops</i>		Chopped Chinese dates, Australia	KC989724
CBS 297.95	<i>C. inops</i>		Chinese five spice powder, Sheffield, UK	JF922024
FRR 4952	<i>C. medium</i> (Type)	= CBS 488.91	Nest cells of <i>Osmia rufa</i> , Tåstrup, Denmark	KC989726
FRR 4949	<i>C. medium</i> var. <i>spissescens</i> (Type)	= CBS 489.91	Nest cells of <i>Osmia rufa</i> , Tåstrup, Denmark	KC989727
CBS 388.68	<i>C. merdarium</i> (Type species)		Soil	KC989728
FRR 4950	<i>C. minus</i> (Type)	= CBS 490.91	Nest cells of <i>Osmia rufa</i> , Karlslunde, Denmark	KC989729
FRR 4951	<i>C. pyriforme</i> (Type)	= CBS 491.91	Nest cells of <i>Osmia rufa</i> , Sweden	KC989730
CBS 634.79	<i>C. sulfureum</i>	Clade VI	Cheese rind, Switzerland	KC989731
CBS 640.79	<i>C. synchronum</i> (Type)	Clade VIII Sordariomycetes	Commercially grown <i>Agaricus bisporus</i> , Edmonton, Alberta, Canada	KC989732
CBS 171.62	<i>C. tropicum</i> (Isotype)	Clade I	Woollen overcoat, Guadalcanal, Solomon Is.	KC989733
CBS 153.67	<i>C. xerophilum</i>	= ATCC 18052, IMI 126287, UAMH 2368	Dried prunes, Sydney, NSW, Australia	JF922023
CBS 437.88	<i>C. zonatum</i> (Type)	Clade I	Horse dung, Kuwait	KC989735
CBS 407.71	<i>Nannizziopsis vriesii</i>	Clade IV Status: Isotype of <i>Rollandina vriesii</i>	<i>Ameiva</i> (lizard) skin and lung, Netherlands	KC989736
CBS 708.79	<i>Renispora flavissima</i>	Clade IV	Soil in barn housing <i>Myotis velifer</i> , Kansas, USA	KC989737
CBS 120.77	<i>Uncinocarpus reesii</i> (Type)	Clade III	Feather, Australia	KC989738

¹Culture collections of CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), FRR (CSIRO Animal, Food and Health Sciences, North Ryde, Australia); J (Dept of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden).

²Clade numbers refer to the groupings of Vidal *et al.* (2000).

³All types studies were ex-type cultures.

at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100; the first 25 % of trees were removed as burn in.

Matrices and the resultant tree are available in TreeBASE, as accession number 14206.

RESULTS

The striking result of the broadly based LSU analysis is that the genus *Chrysosporium* as revived by Carmichael (1962) and maintained by van Oorschot (1980) includes six clades in three orders (Fig. 1). The type species of the genus is *C. corii*, the correct name for which is *C. merdarium*, but no cultures derived from the extant type material of these two names could be located. The LSU sequence examined was from CBS 388.68, derived from the type of *C. merdarium* var. *roseum*. That occupied an isolated position in *Leotiales* close to *Geomyces pannorum* and *Pseudogymnoascus roseus*, well separated from other species. Vidal et al. (2000) examined the ITS sequence from CBS 408.72, listed by CBS as *C. merdarium*, but also stated to be derived from the type of *Gymnoascus uncinatus*. Not surprisingly, that is located in *Onygenales*. However, the epitypification of the type species of *Chrysosporium* is beyond the scope of this paper. The *Chrysosporium* spp. not isolated from dried foods or bees were all incapable of growth on MY50G agar, and again outside our scope.

Of immediate relevance, however, the LSU indicated that the xerophilic species associated with food or bees are also polyphyletic, belonging to two clades, one in *Eurotiales*, where most xerophilic fungi belong, and the other in *Leotiales*.

Morphological and physiological characteristics support this separation: the seven species in the *Leotiales* clade viz. *C. farinicola*, *C. fastidium*, *C. hispanicum*, *C. holmii*, *C. medium*, *C. minor*, and *C. pyriformis*, have a distinctly different proportion of the conidial types characteristic of the xerophilic *Chrysosporium* species, and generally have a somewhat faster radial growth rate on MY50G-based media at 20–30 °C than those species grouped in *Eurotiales* (*C. botryoides*, *C. globiferum*, *C. inops*, and *C. xerophilum*; Table 2). Species in the *Leotiales* clade did not grow at 37 °C, but had an optimum temperature around 25 °C; growth rates at 0.89 a_w and 20 °C were only somewhat slower than at 25 °C and similar to those at 30 °C. Thus, this group appears to be tolerant of cooler temperatures: in contrast, species in the *Eurotiales* clade displayed a preference for warmer temperatures with most rapid growth typically at 30 °C and slower growth at 20 °C. All species apart from *C. inops* were capable of growth at 37 °C. In addition to a broader temperature range, these species also showed a somewhat greater tolerance of high and low water activities: most species showed linear growth or at least germination within the range 0.99–0.71 a_w at 25–30°C. Such growth at extremes of water activity was sporadically observed among species in the *Leotiales* clade: within 50 d, germination, but not linear growth, was noted at 0.73–0.71 a_w for certain species only and growth at 0.99 a_w was only observed at 20 °C. Species from the two clades also differed greatly during growth on Czapek Casein 50G agar (Table 3). Species

belonging to *Eurotiales* yielded dense, white, slightly floccose colonies with reverse colours in cream/pale apricot to yellow/khaki, and colony diameters > 40 mm within 14 d (18 mm for *C. inops*); whereas, species within *Leotiales* yielded sparse, hyaline colonies with little aerial mycelium, 8–26 mm diam.

The xerophilic species in the *Leotiales* clade include *C. farinicola*, the asexual morph of *Bettsia alvei*. Based on sequence identity and a striking number of physiological and morphological similarities in the asexual morph, the xerophilic species in the *Leotiales* clade, apart from *C. fastidium*, are placed in synonymy, regardless of presence/absence of sexual morph. Under the new Code (McNeill et al. 2012), the strictly asexual *Chrysosporium* species in *Leotiales* should be renamed in *Bettsia*, the oldest available generic name. The correct epithet is *B. alvei*, as its basionym *Pericystis alvei* dates from 1912, while the basionym of *C. farinicola*, *Ovularia farinicola*, was introduced in 1928 (Skou 1975, van Oorschot 1980). Retention of *C. fastidium* as a separate species, *B. fastidia*, is warranted based on the distinct yellow-brown colours of the mycelium, somewhat slower growth rate on MY50G, and its placement as a sister clade to *B. alvei* (Fig. 1).

The xerophilic *Chrysosporium* species grouped together in *Eurotiales* are all asexual morphs. In the absence of any available name, the new generic name *Xerochrysum* is given to them here. The LSU tree indicated that *C. inops* is distinct, but other species were not separated satisfactorily (Fig. 1). Based on similarities in morphology, physiology and molecular data, the remaining species are synonymised under the oldest name, *C. xerophilum*.

The genus most closely related to *Xerochrysum* is *Xeromyces* (Fig. 1), which contains one species, the extreme xerophile *X. bisporus*. *Xeromyces* is distinguished from *Xerochrysum* because it is primarily a sexual genus, in which fresh isolates readily produce characteristic asci containing two D-shaped ascospores; unlike *Xerochrysum*, it does not produce chlamydo- or aleurioconidia, instead producing a rare *Frasierella* asexual morph.

TAXONOMY

Although molecular analyses have shown that species in *Bettsia* and species now placed in the new genus *Xerochrysum* are widely separated phylogenetically, striking similarities exist in microscopic appearance. However, in culture these two groups of species are not difficult to separate. Growth rates of *Bettsia* species are faster than those of *Xerochrysum* species, although some overlap exists (Table 3). The other major distinguishing feature is the proportion of aleurioconidia to chlamydoconidia and arthroconidia: *Bettsia* species produce predominantly aleurioconidia whereas *Xerochrysum* species produce predominantly chlamydoconidia and arthroconidia (see Figs 2–5).

Bettsia

Bettsia is characterised by the formation of small black cleistothecia with a distinctive three-celled appendage. Asci are evanescent, and ascospores have smooth, dark



Fig. 1. Phylogeny based on 28S sequences of *Chrysosporium* species including xerophilic species isolated from foods or bee pollen. Classification according to Lumbsch & Huhndorf (2010) in brackets. *Leotiomyces* is supported in two clades and includes taxa noted by to have uncertain taxonomic affinity. Two strains, J211 and FRR 81, were originally identified as *C. fastidium*, but upon examination their morphology was that of *C. farinicola*.

walls. The type and only species is a hyphomycete asexual morph, with aerial hyphae bearing solitary aleurioconidia and intercalary chlamydoconidia.

Skou (1972) erected the genus *Bettsia* as a new name for *Pericystis* Betts 1912 (*non Pericystis* J. Agardh 1848). The type species is *B. alvei*. Molecular analysis (LSU) indicates

that *Bettsia* lies in *Leotiales*, with a possible relationship to the genus *Pseudeurotium*.

One other species with no known sexual morph is accepted here: *B. fastidia*.

Table 2. Radial growth rate (mm/d) of xerophilic *Chrysosporium* species on malt yeast media with water activity adjusted with glucose or a mixture of glucose and fructose at 37, 30, 25 and 20 °C.

Temp	<i>a_w</i>	<i>B. alvei</i>	<i>C. farinicola</i>	<i>C. hispanicum</i>	<i>C. holmii</i>	<i>C. medium</i>	<i>C. minus</i>	<i>C. pyriforme</i>	<i>C. fastidium</i>	<i>C. xerophilum</i>	<i>C. botryoides</i>	<i>C. globiferum</i>	<i>C. inops</i>
		CBS688.71, T ¹	FRR2000	FRR4954, T	FRR4955, T	FRR4952	FRR4950	FRR4951	FRR77, T	CBS153.67	FRR4958, T	FRR4953, T	CBS297.95
37 °C	0.99	ng ²	ng	ng	ng	ng	ng	ng	ng	ng	ng	ng	ng
	0.89	ng	ng	ng	ng	ng	ng	ng	ng	1.79±0.08 ³	0.80±0.22	1.12±0.06	ng
	0.78	ng	ng	ng	ng	ng	ng	ng	ng	0.39±0.10	0.39	0.38±0.05	ng
	0.73	ng	ng	ng	ng	ng	ng	ng	ng	0.14±0.02	germ ⁴	germ	ng
	0.71	ng	ng	ng	ng	ng	ng	ng	ng	0.13±0.01	ng	germ	ng
30 °C	0.99	ng	ng	ng	ng	ng	ng	ng	ng	0.90±0.11	germ	0.44±0.16	0.07±0.02
	0.89	0.81±0.01	2.41±0.13	2.18±0.03	2.31±0.09	1.66±0.09	1.08±0.02	2.12±0.17	0.85±0.12	1.40±0.09	0.96±0.02	1.22±0.06	0.69±0.01
	0.78	0.27±0.03	0.41±0.24	0.28±0.06	0.18±0.02	0.33±0.15	0.16±0.09	0.33±0.20	0.34±0.03	0.35±0.05	0.38±0.06	0.36±0.02	0.23±0.06
	0.73	ng	ng	ng	ng	ng	ng	ng	germ	0.24±0.05	0.14±0.01	0.15±0.01	0.08±0.01
	0.71	ng	ng	ng	ng	ng	ng	ng	ng	0.20±0.02	0.13±0.00	0.14±0.01	germ
25 °C	0.99	ng	0.10±0.02	0.05±0.01	0.11±0.02	ng	ng	ng	ng	0.61±0.12	0.10±0.01	0.28±0.07	0.26±0.09
	0.89	2.01±0.06	2.84±0.35	2.64±0.05	2.81±0.28	2.13±0.18	2.56±0.30	2.75±0.43	1.66±0.01	0.90±0.09	0.74±0.02	0.83±0.02	0.66±0.02
	0.78	0.82±0.02	0.46±0.15	0.29±0.02	0.24±0.01	0.50±0.13	germ	0.21±0.01	0.57±0.03	0.29±0.01	0.30±0.04	0.30±0.05	0.18±0.01
	0.73	ng	germ	germ	ng	ng	ng	germ	0.13±0.00	0.20±0.00	germ	0.12±0.01	0.11±0.04
	0.71	ng	ng	germ	ng	ng	ng	germ	germ	0.13±0.01	germ	0.09±0.01	germ
20 °C	0.99	0.10±0.02	0.59±0.34	0.50±0.37	0.50±0.26	0.48±0.14	0.17±0.01	0.36±0.28	0.09±0.03	0.55±0.13	0.08±0.00	0.17±0.09	0.21±0.02
	0.89	1.96±0.01	2.10±0.37	1.96±0.18	2.34±0.15	1.91±0.10	1.69±0.34	2.04±0.32	1.47±0.02	0.58±0.08	0.50±0.02	0.54±0.07	0.41±0.03
	0.78	0.54±0.05	germ	germ	ng	ng	ng	0.27±0.07	0.54±0.02	0.24	0.18±0.01	0.17±0.03	0.19±0.13
	0.73	ng	ng	ng	ng	ng	ng	ng	0.13±0.01	0.10±0.01	germ	germ	germ
	0.71	ng	ng	ng	ng	ng	ng	ng	germ	ng	germ	germ	ng
Proposed taxonomy		<i>Bettsia alvei</i>							<i>Bettsia fastidia</i>	<i>Xerochysium xerophilum</i>			<i>X. dermatidis</i>

¹Ex-type culture.

²No growth observed within 50 d.

³Standard deviation of three replicates.

⁴Germination and micro-colony formation within 50 d.

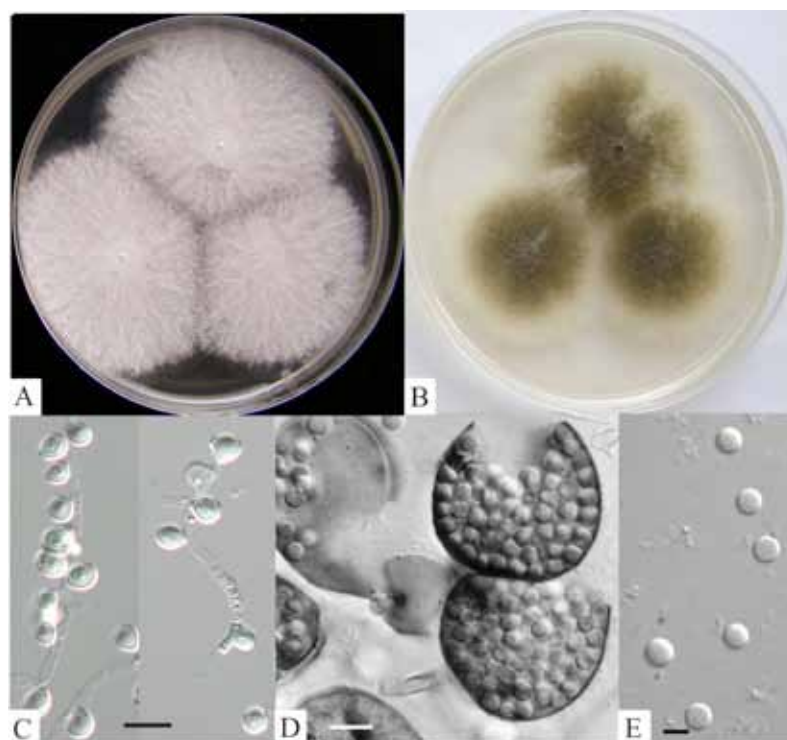


Fig. 2. *Bettsia alvei*. **A.** Colonies of a strictly asexual strain, FRR 2000, on MY50G, after 14 d at 25 °C. **B.** Colonies of a sexually reproducing strain, FRR 380, on MY50G, after 14 d at 25 °C. **C.** Aleurioconidia. **D.** FRR 380, mature cleistothecia containing ascospores. **E.** Ascospores. Bars: C = 10 µm, D = 25 µm, and E = 5 µm.

Bettsia alvei (Betts) Skou, *Friesia* **10**: 7 (1972).
(Fig. 2)

Basionym: *Pericystis alvei* Betts, *Ann. Botan.* **26**: 795 (1912).

Synonyms: *Ascospaera alvei* (Betts) Olive & Spiltoir, *Mycologia* **47**: 243 (1955).

Ovularia farinicola Burnside, *Michigan Acad. Sci., Arts Lett.* **8**: 59 (1928).

Chrysosporium farinicola (Burnside) Skou, *Friesia* **11**: 70 (1972).

Chrysosporium hispanicum Skou, *Mycotaxon* **43**: 244 (1992).

Chrysosporium holmii Skou, *Mycotaxon* **43**: 243 (1992).

Chrysosporium medium Skou, *Mycotaxon* **43**: 248 (1992).

Chrysosporium medium var. *spissescens* Skou, *Mycotaxon* **43**: 249 (1992).

Chrysosporium minus Skou, *Mycotaxon* **43**: 250 (1992); as “*minor*”.

Chrysosporium pyriforme Skou, *Mycotaxon* **43**: 246 (1992); as “*pyriformis*”.

Description: No growth on CYA at 5, 25 or 37 °C or on MEA at 25 °C. Colonies on G25N 1–20 mm diam, of low, white mycelium, or if the sexual morph is present, centrally grey from the production of immature cleistothecia; margins fimbriate; reverse pale, but grey centrally if the sexual morph is produced. Colonies on MY50G growing relatively rapidly at 7 d, 15–35 mm diam, low, plane, persistently white, or showing sectors becoming translucent or greyish if the sexual morph is present, reverse pale beneath white areas, but darker beneath grey sectors; at 14 d, 40–65 mm diam, colonies remaining white if only the asexual morph is present, but dark grey to black in the presence of the sexual morph; reverse pale or dark.

Reproduction on MY50G predominantly solitary aleurioconidia. Fertile hyphae remain mostly undifferentiated and dissolve in age. In isolates producing the sexual morph areas or sectors of translucent growth develop on MY50G.

At maturity such areas become grey and then black as small cleistothecia form. Cleistothecia formed on MY50G at 25 °C, dark brown to black, usually maturing only after several weeks at 15–25 °C, 25–60 µm diam, with walls thin and smooth, and without internal structure; initials a row of three short cells, 12–18 x 6–8 µm overall, adhering to the cleistothecial wall as a distinctive appendage; ascospores not liberated readily, spherical, 5–6 µm diam, with dark walls, smooth to minutely roughened.

Type: **Denmark**: **Zeeland**: Ringsted, Skee, a lyophilised culture, from pollen in honeycomb, 1971, *J. P. Skou* (CBS 688.71 – neotype designated by Skou 1972; IMI 160840 – ex-neotype culture).

Distinctive features: *Bettsia alvei* is distinguished from *B. fastidia* by persistently white growth in the absence of the sexual morph. In addition, while only some isolates of *B. alvei* produce the sexual morph, no sexual morph is known for *B. fastidia*.

Physiology: Optimal growth at approx. 0.89 a_w and 25 °C. No growth at 37 °C, good growth at 20 °C. Growth at 0.99 a_w observed at 20 °C only; minimum a_w estimated to be approx. 0.70–0.73 based on germination of certain strains at 0.71 a_w .

Ecology: *Bettsia alvei*, often under the name *Chrysosporium farinicola*, has been isolated from a range of low water activity substrates, including honeycomb, prunes and prune processing equipment, sultanas, mixed dried fruit, chocolate, jelly crystals and coconut from Australia, the United Kingdom, Sri Lanka, Czechoslovakia and Denmark (Pitt & Hocking 2009).

References: Skou (1975), and van Oorschot (1980).

Table 3. Distinguishing features of species in *Bettsia* and *Xerochrysium*.

Species	CYA, MEA	G25N (mm)	Colony size (mm) and morphology on MY50G	Dominant microscopic morphology from MY50G	Colony size (mm) and morphology on CZC50G
<i>Bettsia alvei</i>	No growth	1–20 (7 d)	15–35 (7 d); 40–65 (14 d) Colonies persistently white with a pale reverse; or black with a black reverse in the presence of cleistothecia	Solitary aleurioconidia	8–26 mm (14 d) Sparse, hyaline
<i>B. fastidia</i>	No growth	1–5 (7 d)	15–22 (7 d); 35–42 (14 d) Colonies dull yellow to yellow brown; reverse yellow brown	Solitary aleurioconidia	21–26 mm (14 d) Sparse, hyaline
<i>Xerochrysium dermatitidis</i>	No growth - 3 mm	1–9 (7 d)	4–10 (7 d); 12–20 (14 d) White or translucent	Chlamydo- and arthroconidia	18 mm (14 d) Dense, white, slightly floccose, reverse cream-yellow reverse, occasionally red (Kinderlerer 1995)
<i>X. xerophilum</i>	No growth - 3 mm	1–9 (7 d)	9–20 (7 d); 20–38 (14 d) White; reverse white or pale yellow to apricot, khaki	Chlamydo- and arthroconidia	40–43 mm (14 d) Dense, white, slightly floccose, reverse cream-yellow to khaki-yellow

***Bettsia fastidia* (Pitt) Pitt, comb. nov.**

Mycobank MB806122

(Fig. 3)

Basionym: *Chrysosporium fastidium* Pitt, *Trans. Br. mycol. Soc.* **49**: 467 (1966).

Description: No growth on CYA at 5, 25 or 37 °C, or on MEA. Colonies on G25N 1–5 mm diam, of low, dense, white mycelium. Colonies on MY50G at 7 d 15–22 mm diam, low, plane and sparse, pale yellow or brown, reverse yellow brown; at 14 d, 35–42 mm diam, low and plane, margins sparse and fimbriate, white, centres more dense, dull yellow; reverse yellow to pale brown.

Reproduction on MY50G predominantly by smooth walled aleurioconidia borne singly on short pedicels or less commonly sessile, spheroidal (oblate or prolate) to broadly ellipsoidal, 6–9 × 5–8 µm, in age released by dissolution of the pedicels; terminal chlamydoconidia, spherical to pyriform or pedunculate, 8–12 × 6–10 µm, also produced, but intercalary chlamydoconidia and arthroconidia rare. No sexual morph known.

Type: **Australia:** *New South Wales:* a dried Petri dish culture, from dried prunes, 1964, *J. I. Pitt* (UAMH 2368 – holotype; FRR 77, CBS 154.67, ATCC 18053, ATCC 36783, and IMI 126288 – ex-holotype cultures).

Distinctive features: *Bettsia fastidia* forms dull yellow to yellow-brown colonies; conidia produced on G25N or MY50G are predominantly aleurioconidia with few intercalary chlamydoconidia or arthroconidia. This species is readily distinguished from *B. alvei*, as the latter species show no yellow or brown colouration, and by slower growth than *B. alvei* on MY50G.

Taxonomy: Van Oorschot (1980) considered *C. fastidium* a synonym of *C. farinicola*. However, our examination of the strain she examined, FRR 77 (CBS 154.67), showed that that culture was incorrectly labelled. The two species are readily distinguished by colony colour and by LSU sequence.

Physiology: A mesophilic obligate xerophile, *B. fastidia* has a minimum for growth of 0.69 a_w (Pitt & Christian 1968), and displays optimal growth at approx. 0.89 a_w and 25 °C. No growth at 37 °C, good growth at 20 °C. Growth at 0.99 a_w observed at 20 °C only.

Ecology: This species has been repeatedly isolated from prunes (dried and high moisture) and prune processing machinery in New South Wales, Australia. There are no records of isolation from other substrates or other countries, indicating that this is a rare species with a restricted habitat.

***Xerochrysium* Pitt, gen. nov.**

Mycobank MB807003

Type species: *Xerochrysium dermatitidis* (A. Agostini) Pitt 2013 (syn. *Glenosporella dermatitidis* A. Agostini 1930).

Description: *Xerochrysium* is a genus of *Eurotiales* known only as asexual morphs which is erected to accommodate xerophilic species formerly placed in *Chrysosporium*. The known species grow poorly, if at all, on standard media including CYA, MEA and potato dextrose agar. Growth is slow on G25N. Optimal growth is obtained on MY50G, with a water activity below 0.9. Microscopically species are characterised by the production of aleurioconidia, but also by the formation of chlamydoconidia and arthroconidia, such that mature colonies are often made up predominantly

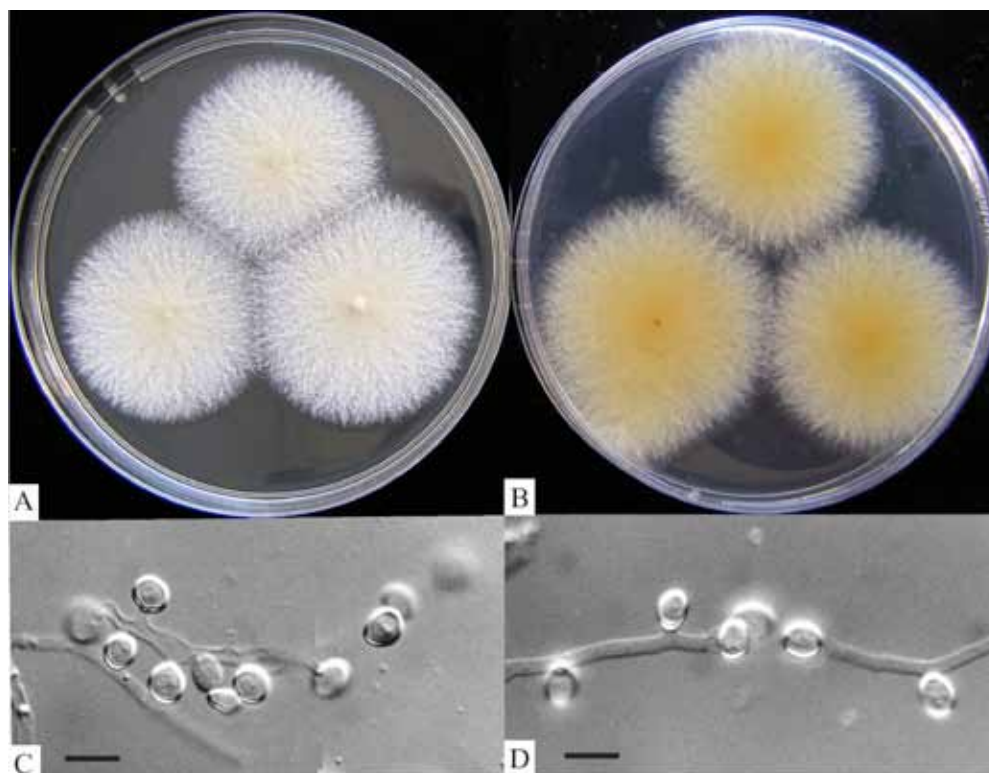


Fig. 3. *Bettsia fastidia* (FRR 77). **A–B.** Obverse and reverse of colonies on MY50G, after 14 d at 25 °C. **C–D.** Aleurioconidia borne on pedicels. Bars = 10 µm.

of these spore types, with little undifferentiated mycelium evident.

Two species are accepted here: *X. dermatitidis* and *X. xerophilum*.

Xerochrysum dermatitidis (A. Agostini) Pitt, **comb. nov.**

MycoBank MB807005

(Fig. 4)

Basionym: *Glenosporella dermatitidis* A. Agostini, *Atti Ist. Bot. Univ. Pavia* 2: 93 (1930).

Synonym: *Chrysosporium inops* J.W. Carmich., *Can. J. Bot.* 40: 1156 (1962).

Description: After 7 d at 25 °C, colonies on CYA and MEA absent or up to 3 mm diam. Colonies on G25N 1–9 mm diam, varying from low and translucent to deep and floccose, white; reverse pale to amber or duller yellow brown. No growth on CYA at 5 or 37 °C. Colonies on MY50G at 7 d, 4–10 mm diam, at 14 d, 12–20 mm diam, varying from low, sparse and translucent to moderately deep, dense and with a floccose surface, white or if translucent, uncoloured; reverse uncoloured to pale yellow brown.

Reproductive structures on G25N or MY50G at 7 d primarily short chains of chlamydoconidia and arthroconidia, borne by retrogressive differentiation from hyphal tips and as intercalary chains; some terminal chlamydoconidia also present; at maturity on MY50G (2–4 wk), clusters of chlamydoconidia and arthroconidia also present, formed by retrogressive differentiation of groups of short branching hyphae with a lateral stipe as their common origin. Chlamydoconidia spherical, 4–7(–10) µm diam; arthroconidia cylindrical or doliiform (barrel

shaped), 3–8 × 3–6 µm, those of greater width intergrading with chlamydoconidia; aleurioconidia uncommon, ellipsoidal to pyriform, 5–8 µm diam or longer. Large chlamydoconidia, up to 25 µm diam, with walls up to 2 µm thick, produced by some isolates. Sexual morph unknown.

Type: Italy: Pavia: Pavia, lyophilised culture obtained from human skin, 1930, A. Agostini (CBS 132.31 – **lectotype designated here**; IMI 96729, UAMH 802, and FRR 2376 – ex-lectotype cultures).

Nomenclature: Based on CBS 132.31, received as *Glenosporella dermatitidis*, Carmichael (1962) transferred this species to *Chrysosporium*. However, as he had already used the epithet “dermatitidis” in *C. dermatitidis*, he transferred *G. dermatitidis* as *C. inops* nom. nov. On transfer to the new genus *Xerochrysum*, this epithet is again available, so the new combination is correctly *X. dermatitidis*.

Distinctive features: In culture, *Xerochrysum dermatitidis* and *X. xerophilum* are very similar: *X. dermatitidis* grows more slowly on standard media and on CZC50G (Table 3). In young colonies (7 d), terminal chlamydoconidia are often the dominant conidium type in *X. dermatitidis*. Aleurioconidia are rare.

Physiology: Van Oorschot (1980) reported that this species had a minimum growth temperature of 20 °C, an optimum of 25 °C, and a maximum of 30 °C. However, her data were obtained on media of very high a_w , and limits are undoubtedly wider under optimal conditions. No growth at 37 °C, and maximum growth at approx. 0.88 a_w and 25 °C (Leong *et al.* 2011). Maximum a_w for growth is 0.99 and minimum a_w < 0.71, as germination was observed at 0.71 a_w and 30 °C (Table 2). It is clear, contrary to

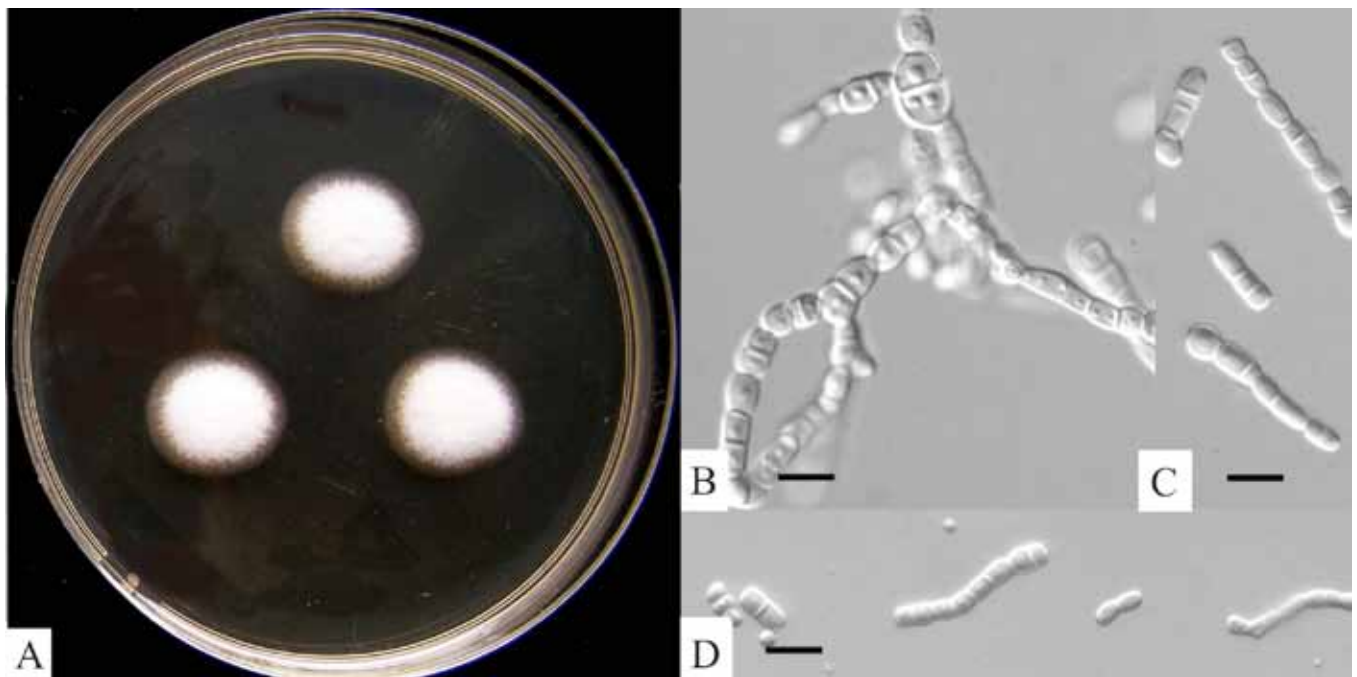


Fig. 4. *Xerochrysius dermatitidis* (FRR 2353). **A.** Colonies on MY50G, after 14 d at 25 °C. **B–D.** Chains of arthroconidia borne by hyphal transformation. Bars: B–C = 10 µm, D = 20 µm.

previous reports (Pitt & Christian 1968, van Oorschot 1980), that *X. dermatitidis* is an extreme xerophile.

Ecology: As noted by de Hoog *et al.* (2000), *X. dermatitidis* is not a dermatophyte, but a xerophile. With the exception of the original isolation by Agostini (1930) from human skin, all isolates known have come from dried or concentrated foods. Sources have included spice powders, chopped dates, gelatine confections, chocolate and nuts (Pitt & Hocking 2009).

***Xerochrysius xerophilum* (Pitt) Pitt, comb. nov.**
Mycobank MB807006
(Fig. 5)

Basionym: *Chrysosporium xerophilum* Pitt, *Trans. Br. mycol. Soc.* **49**: 468 (1966).

Synonyms: *Chrysosporium botryoides* Skou, *Mycotaxon* **43**: 252 (1992).

Chrysosporium globiferum Skou, *Mycotaxon* **43**: 253 (1992).

Chrysosporium globiferum var. *articulatum* Skou, *Mycotaxon* **43**: 254 (1992).

Chrysosporium globiferum var. *niveum* Skou, *Mycotaxon* **43**: 255 (1992).

Description: After 7 d at 25 °C, colonies on CYA and MEA absent or up to 3 mm diam. Colonies on G25N 1–9 mm diam, varying from low and translucent to deep and floccose, white; reverse pale. No growth on CYA at 5 or 37 °C. Colonies on MY50G at 7 d 9–20 mm diam, at 14 d, 20–38 mm diam, varying from low, sparse and translucent to moderately deep, dense and with a floccose surface, white or if translucent, uncoloured; reverse uncoloured to yellow-apricot, khaki.

Vegetative hyphae rare in mature colonies on G25N or MY50G, having transformed into chlamydoconidia and arthroconidia; chlamydoconidia spherical, 6–9 µm diam, arthroconidia 4–5 × 3–9 µm (width sometimes exceeding length), the two spore types intergrading. Aleurioconidia borne on hyphal tips or laterally on short pedicels, often subsequently transformed into arthroconidia as well, cylindroidal or doliiform. Terminal conidia 10–11 µm diam, on pedicels smaller, ellipsoidal to pyriform, 5–7 × 7–8 µm.

Type: Australia: New South Wales: Sydney, a dried Petri dish culture, from spoiled high moisture prunes, 1962, *J.I. Pitt* (UAMH 2368 – holotype; CBS 153.67, FRR 503, ATCC 18053, and IMI 126287 – ex-holotype cultures).

Distinctive features: *Xerochrysius xerophilum* grows more rapidly than *X. dermatitidis* (Table 3). In addition, *Xerochrysius xerophilum* produces higher numbers of aleurioconidia and at maturity, is distinguished by the almost complete differentiation of vegetative hyphae into intercalary chlamydoconidia and arthroconidia; even aleurioconidium pedicels often becoming thick-walled spores.

Taxonomy: Based on a morphological study, Boekhout *et al.* (1989) concluded that *Chrysosporium xerophilum* was a synonym of *Sporotrichum pruinosum*. However, as they reported that their strain grew well on such high water activity media as 2% MEA and phytone yeast extract agar, and that its optimal growth temperature was 36–39 °C, completely at odds with the original description (Pitt 1965), it is clear that the strain they studied was incorrectly identified.

Physiology: *Xerochrysius xerophilum* grows over a broad range of a_w , with maximum and minimum a_w for growth of

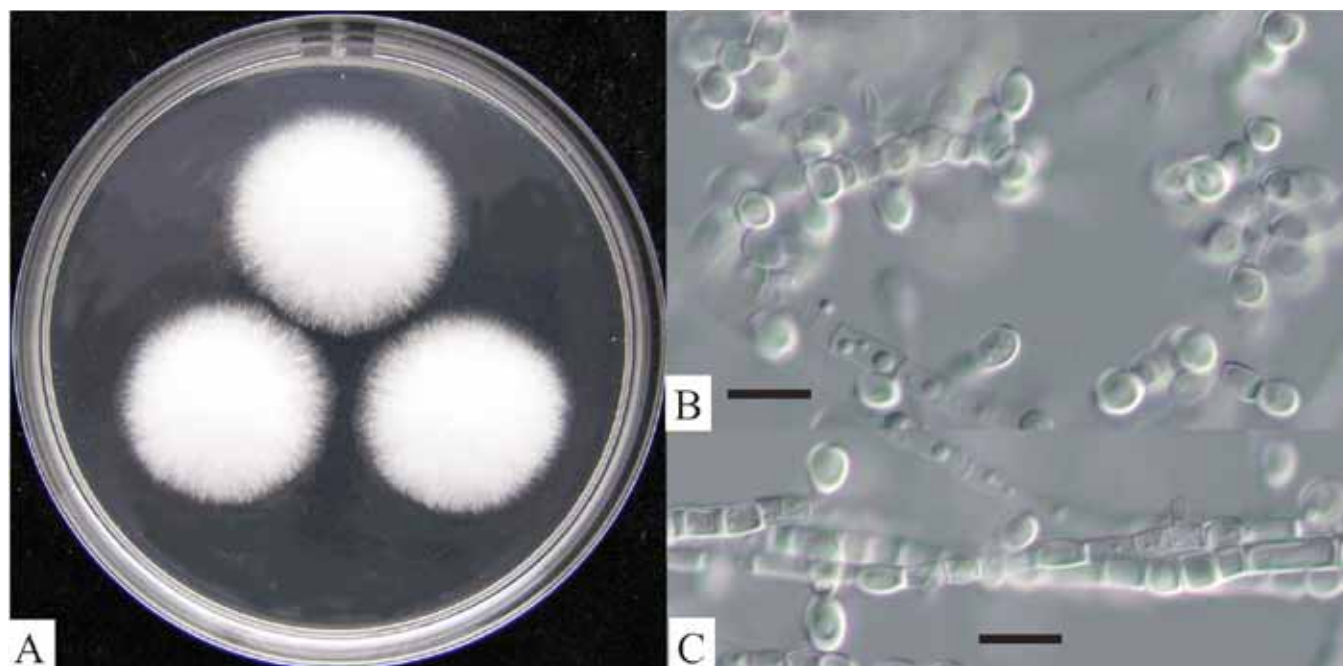


Fig. 5. *Xerochrysiium xerophilum* (FRR 503). **A.** Colonies on MY50G, after 14 d at 25 °C. **B–C.** Chains of arthroconidia borne by hyphal transformation. Bars = 10 µm.

approx. 0.99 and 0.66, respectively, and optimum around 0.94 (Table 2, Leong *et al.* 2011). Optimum temperature for growth is 30–37 °C, and growth occurs at 20 °C.

Ecology: Originally isolated from prunes, *X. xerophilum* has also been found in maize stored for long periods, chocolate and coconut (Pitt & Hocking 2009).

DISCUSSION

The result of the broadly based LSU analysis, that *Chrysosporium* as currently circumscribed includes six clades in three orders, is perhaps not surprising, as the basic asexual morph structure on which the genus is based, the aleurioconidium, is a simple conidial form that could readily have arisen more than once. More surprising is that the xerophilic species belong to two clades, in radically different orders. For a long time it was believed that xerophily, a highly evolved characteristic involving the many genes required to synthesise and retain small molecules such as glycerol, and operate all cellular processes in a more or less thick syrup, had arisen only once, and that all xerophilic species could be found in *Eurotiales* (or ascomycetous yeasts from a lineage close to *Eurotiales*). A very small number of exceptions have been identified in relatively recent years: *Trichosporonoides nigrescens* (Hocking & Pitt 1981) produces clamp connections, indicating a basidiomycetous affinity, while the isolated genus *Wallemia* has also been shown to be a basidiomycete by molecular methods and microscopy (Zalar *et al.* 2005, Padamsee *et al.* 2012). However, no species

in *Leotiales* has previously been shown to have xerophilic properties.

The ecology of these species is also of interest. Carmichael (1962) described the first xerophilic species to be placed in *Chrysosporium*, *C. inops*, from concentrated orange juice, and *C. xerophilum* and *C. fastidium* were described from prunes (dried plums) soon afterwards (Pitt 1966). When recombining *Ovularia farinicola* into *Chrysosporium*, Skou (1975) drew attention to the main natural source of these fungi, beehives, bee pollen and honey. At the same time, it is clear that some of these species are also common food spoilage fungi: *Xerochrysiium dermatitidis* (as *C. inops*), *X. xerophilum*, and *B. alvei* have been repeatedly isolated from concentrated or dried foods. *Bettsia alvei* has been recorded from honeycomb, prunes and processing equipment, dried vine fruits, chocolate, and copra, from several widely separated countries, and *X. dermatitidis* and *X. xerophilum* are also known to be widespread in similar types of products, sometimes causing spoilage. Although repeatedly isolated in Australia from prunes and prune processing equipment, *B. fastidia* has not been found elsewhere (Pitt & Hocking 2009). Despite the narrow ecological niche of *B. fastidia*, all of these species, as well as the extreme xerophile *Xeromyces bisporus*, are associated with food or feed, and require highly concentrated media such as MY50G for effective isolation (Pitt & Hocking 2009). As their colonies on this medium are somewhat similar in appearance, being pale to white and low, a common key to species in these three genera is given below.

Key to xerophilic fungi in the genera *Bettsia*, *Xerochrysum*, and *Xeromyces*

- 1 Colonies on MY50G producing D-shaped ascospores in pairs **Xeromyces bisporus**
Colonies on MY50G not producing D-shaped ascospores; instead aleurioconidia and/or chlamydoconidia observed 2
- 2 (1) Colonies on MY50G 35–65 mm after 14 d at 25 °C; colony and reverse white, yellow-brown, or black;
aleurioconidia abundant, chlamydoconidia rare. No growth at 37 °C. Growth on CZC50G poor, yielding sparse,
hyaline colonies 3
Colonies on MY50G 12–38 mm after 14 d at 25 °C; colony and reverse white; chlamydoconidia and/or
arthroconidia abundant, aleurioconidia occasionally present. Growth at 37 °C present or absent.
Colonies on CZC50G dense, white; reverse in yellow-khaki shades, occasionally red 4
- 3 (2) Colonies on MY50G 40–65 mm after 14 d, white or black, reverse pale or black **Bettsia alvei**
Colonies on MY50G 35–42 mm after 14 d, pale yellow or brown with a yellow brown reverse **Bettsia fastidia**
- 4 (2) Growth on MY50G typically 15 mm or less after 14 d (up to 20 mm); no growth at 37 °C **Xerochrysum dermatitidis**
Growth on MY50G 20–38 mm after 14 d; growth at 37 °C **Xerochrysum xerophilum**

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