ELSEVIER

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/mycres

Chorioactidaceae: a new family in the *Pezizales* (*Ascomycota*) with four genera

Donald H. PFISTER[*], *Caroline SLATER*, *Karen HANSEN*[†]

Harvard University Herbaria – Farlow Herbarium of Cryptogamic Botany, Department of Organismic and Evolutionary Biology, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received 15 June 2007
Received in revised form
1 November 2007
Accepted 29 November 2007
Corresponding Editor:
H. Thorsten Lumbsch

Keywords:
LSU
RPB2
Sarcoscyphaceae
Sarcosomataceae
SSU

ABSTRACT

Molecular phylogenetic and comparative morphological studies provide evidence for the recognition of a new family, *Chorioactidaceae*, in the *Pezizales*. Four genera are placed in the family: *Chorioactis*, *Desmazierella*, *Neournula*, and *Wolfina*. Based on parsimony, likelihood, and Bayesian analyses of LSU, SSU, and RPB2 sequence data, *Chorioactidaceae* represents a sister clade to the *Sarcosomataceae*, to which some of these taxa were previously referred. Morphologically these genera are similar in pigmentation, excipular construction, and asci, which mostly have terminal opercula and rounded, sometimes forked, bases without croziers. Ascospores have cyanophilic walls or cyanophilic surface ornamentation in the form of ridges or warts. So far as is known the ascospores and the cells of the paraphyses of all species are multinucleate. The six species recognized in these four genera all have limited geographical distributions in the northern hemisphere.

© 2007 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction

The *Pezizales*, operculate cup-fungi, have been put on relatively stable phylogenetic footing as summarized by Hansen & Pfister (2006), but many of the relationships are not recognized in a formal classification. In this study we examine a group of taxa whose relationships and familial assignments have been equivocal. In an earlier molecular phylogenetic study using SSU rDNA data of the pezizalean families *Sarcoscyphaceae* and *Sarcosomataceae*, Harrington *et al.* (1999) showed that the species of *Chorioactis*, *Desmazierella*, *Neournula*, and *Wolfina*, taxa placed alternatively in one or both of these families, might form a distinct lineage. Also, Perry *et al.* (2007) indicated a relationship of these taxa to the *Sarcosomataceae* and discussed the group as the *Chorioactis* clade. Only six species are assigned to these genera, most of which are infrequently collected. The study of these fungi is hampered by their limited and often geographically disjunct patterns of distributions. Ascomata of all of these species are externally dark, but their hymenia are light beige, yellow, rose, or fulvous. The dark outer surface of the ascomata suggests a placement in *Sarcosomataceae*; the light or bright hymenia suggests a placement in *Sarcoscyphaceae*. Korf (1970, 1972, 1973) placed the genera discussed here in the *Sarcosomataceae* but in different tribes. Prior to Korf's (1970) recognition of two families, a single family, the *Sarcoscyphaceae*, was employed, with two

* Corresponding author.
E-mail address: dpfister@oeb.harvard.edu
† Present address: Department of Cryptogamic Botany, Swedish Museum of Natural History, PO Box 50007, 10405 Stockholm, Sweden.
0953-7562/$ – see front matter © 2007 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.mycres.2007.11.016

tribes, one dark (*Urnuleae*) and the other brightly coloured (*Sarcoscyphaeae*). Eckblad (1968) included *Desmazierella* in the tribe *Sarcoscyphaeae* (comprised largely of taxa of the *Sarcoscyphaceae*) and the other two then known genera, *Wolfina* and *Chorioactis*, in the tribe *Urnuleae* (representing taxa now placed in the *Sarcosomataceae*). Taking another approach, Denison (1972) grouped the small conifer needle-inhabiting *Sarcoscyphaceae*, including *Desmazierella*, in the tribe *Pithyeae* of the *Sarcoscyphaceae*. This tribe has proven to be polyphyletic (Harrington *et al.* 1999). In the recent outline of *Ascomycota* (Eriksson 2006) *Chorioactis* and *Desmazierella* are placed in the *Sarcoscyphaceae*; *Neournula* and *Wolfina* are questionably assigned to the *Sarcosomataceae*.

Structural features of the ascospores and asci support the deposition of *Desmazierella* and *Chorioactis* in the *Sarcoscyphaceae*. Merkus (1976) found ascospore wall development and ascus type to be similar in *Sarcoscypha coccinea* and *Desmazierella acicola*. Bellemère *et al.* (1994) and Melendez-Howell *et al.* (1998) studied the asci of *C. geaster* and *D. acicola*, respectively. They concluded that *D. acicola* has *Sarcoscypha*-type asci and considered it a member of the *Sarcoscyphaceae*. *Chorioactis geaster* has asci that resemble the *Sarcoscypha*-type, but differ in aspects of wall layering and thus Bellemère *et al.* (1994) suggested that *Chorioactis* holds a special place within the *Sarcoscyphaceae*. Nevertheless, they did not implement their idea in a formal taxonomy. Earlier, Le Gal (1958) excluded *C. geaster* (as *Urnula geaster*) from the tribe *Urnuleae*, which she characterized in part by the presence of gelatinous excipular tissues. She concluded its relationship was with a very different group.

In order to resolve the contradictory placements of these taxa, we have added an additional two datasets, LSU and RPB2, to the SSU dataset, for combined analyses and conducted a comparative morphological study.

Materials and methods

Material studied

Herbarium specimens of *Chorioactis, Desmazierella, Neournula,* and *Wolfina* were used for morphological study and are cited in the taxonomy section. For molecular phylogenetic study, representative taxa were selected from the closely related families *Sarcoscyphaceae*, *Sarcosomataceae*, *Pyronemataceae*, and *Ascodesmidaceae* (lineage C of *Pezizales*; see Hansen & Pfister 2006). In addition, taxa from the families *Morchellaceae* and *Discinaceae* were included (lineage B). Taxa of *Pezizaceae* (lineage A) were used to root the tree, because more inclusive phylogenetic analyses support lineage A as a sister group to the lineages B and C. A total of 38 unique species were used in phylogenetic analyses (Table 1 and below). In some cases, up to three different specimens of the same species were sequenced to verify the sequences (LSU and RPB2) and explore intraspecific variation. Eighteen RPB2 and 20 LSU sequences are newly determined in this study. The remaining sequences were previously published by us or other authors, including all SSU sequences used: *Byssonectria terrestris* (syn. *Inermesia aggregata*; Z30241), *Chorioactis geaster* (AF104340), *Cookeina speciosa* (syn. *Cookeina sulcipes*; U62010), *Cookeina tricholoma* (AF006311), *Desmazierella acicola* (AF104341), *Disciotis venosa* (AY544711), *Donadinia* sp. (AF104342), *Eleutherascus lectardii* (DQ062997), *Galiella rufa* (AF004948), *Gyromitra californica* (AY544717), *Melastiza cornubiensis* (DQ646537), *Microstoma floccosum* (AF006313), *Morchella elata* (U42641), *Nanoscypha tetraspora* (AF006314), *Neournula pouchetii* (AF104666), *Peziza quelepidotia* (U42665), *Peziza succosa* (U53383), *Peziza vesiculosa* (AFTOL-202, specimen JV95-652), *Phillipsia domingensis* (AF006315), *Plectania rhytidia* (AF104344), *Pseudopithyella minuscula* (AF006317), *Pseudoplectania nigrella* (AF104345), *Pyronema confluens* (DQ646549), *Sarcoscypha austriaca* (AF006318), *Sarcoscypha coccinea* (AY544691), *Scutellinia scutellata* (DQ247814), *Strobiloscypha keliae* (AF006310), *Tricharina praecox* (DQ646552), *Urnula craterium* (AF104347), *Wolfina aurantiopsis* (AF104664), and *Wynnea* sp. (AF006319). Three genera (*Plectania*, *Phillipsia*, and *Sarcoscypha*) are represented by different species in the combined analyses; for example *Plectania* LSU and RPB2 sequences are from *P. nannfeldtii*, whereas the SSU sequence is from *P. rhytidia*. In such cases, only the generic name is listed on the tree figure (e.g. *Plectania* spp.). As far as possible, the different gene regions have been sequenced from the same collection of a species.

Morphological techniques

Dried preserved specimens were used, with the exception of several living specimens of *Chorioactis geaster*. Specimens were re-hydrated for 8 h or overnight and either sectioned freehand or using a freezing microtome set to make sections about 20 μm thick. Sections were stained with Congo Red in ammonia or Cotton Blue in lactic acid (0.05 g Cotton Blue (1B 495 Baumwollblau, Chroma-Gesellschaft) in 875 ml lactic acid, 63 ml glyercol, 62 ml water). For general morphological methods see Hansen *et al.* (2001). An Olympus BH-2 microscope was used. Photographs were made using Ektachrome 100 film and resulting transparencies were scanned to provide digital images. Scaled drawings were made freehand.

Molecular techniques

DNA was isolated from mostly dried or fresh (stored in extraction buffer) ascomata and extracted as outlined in Hansen *et al.* (1999, 2005). The RPB2 region between conserved motif 6 and 11 (Denton *et al.* 1998; James *et al.* 1991) was amplified using the PCR with the degenerate primers described in Hansen *et al.* (2005) and one additional new primer (2003-6F: 5′-TGGGGNYTNGTBTGYCCYGC-3′). It was amplified in a single piece or in two pieces when necessary, using the Pb7F primer to amplify the overlapping region. In a few instances, when amplification of region 6–7 failed, region 5–7 was amplified. The 5′ end of the LSU, approximately 900 base pairs, was amplified using the primers LROR and LR5 (Moncalvo *et al.* 2000). In addition, nested primers LR3 and LR3R were used for sequencing (Moncalvo *et al.* 2000). For PCR, 4 μl from the various DNA extract dilutions (1:10, 1:100) was used as a template in a reaction volume of 20 μl. Forward and reverse primers were added to a final concentration of 1 μM each. A mix of the four dNTPs and $MgCl_2$ were both added to a final

Table 1 – Collections for LSU and RPB2 sequences used in the molecular phylogenetic study

Species	Collection number (herbarium)	Geographic origin, year and collector	GenBank LSU	GenBank RPB2
Byssonectria terrestris	KS-94-04 (C)	DENMARK, Møn: 5 Apr 1994, *K. Hansen & S. Sandal*	**AY500531**	**AY500504**
Chorioactis geaster (1)	s.n. (FH) (ext #4)	JAPAN, KYUSHU: 19 Nov 1997, *S. Kurogi*	AY307945	DQ017607[a]
C. geaster (2)	ZZ 2 (FH) (ext #2)	USA, TX: Tarrant Co., 6 Nov 1997	AY307943	DQ017608[a]
C. geaster (3)	s.n. (FH) (ext #28)	USA, TX: Tarrant Co., 7 Oct 1992, *H.W. Keller & K.C. Rudy*	**AY307944**	**DQ017609**[a]
Cookeina speciosa	1D-D6 (FH)	VENEZUELA, AMAZONAS: 7 Jul 1997, *K. Samuels*	AY945862[a]	
C. tricholoma	1D-D5 (FH)	VENEZUELA, AMAZONAS: 7 May 1997, *K. Samuels*	AY945860[a]	
Desmazierella acicola (1)	RK 95.12 (Herb. Roy Kristiansen)	NORWAY, Østfold: 1 Apr 1995, *A. Gravningan*	**AY945854**[a]	**DQ017603**[a]
D. acicola (2)	RK 95.11 (Herb. Roy Kristiansen)	NORWAY, Østfold: 29 Mar 1995, *R. Kristiansen*	**DQ220328**[b]	**DQ017604**[a]
Disciotis venosa	NRRL 22213		**AY544667**	**DQ470892**
Donadinia sp.	mh 669 (FH)	USA, NY: Dutchess Co, Apr 1996, *M. & D. Potter*	**DQ220329**[b]	**DQ017593**[a]
Eleutherascus lectardii	CBS 626.71	France, 1968, *P. Lectard*	**DQ168334**	**EU360913**[d]
Galiella rufa	mh 101 (FH)	USA, GA.	**AY945850**[a]	**DQ017594**[a]
Gyromitra californica	OSC 100068		**AY544673**	**DQ470891**
Melastiza cornubiensis	KH-03-43 (FH)	NORWAY, Nordland: Rana, 20 Aug 2003, *K. Hansen & C. Lange*	**DQ646524**[c]	**EU360914**[d]
Microstoma floccosum	Weinstein 45 (FH)	MEXICO, Tlaxcala: 20 Aug 1998, *K. Griffith*	DQ220370	
Morchella elata	NRRL25405		**U42667**	**AF107810**
Nanoscypha tetraspora	mh PR61 (FH)	PUERTO RICO: 18 Jan 1996, *D.H. Pfister & F.A. Harrington*	DQ220374[b]	
Neournula pouchetii	NSW 6435 (ORG)	USA, OR: 16 Apr 1991, *N.S. Weber*	**AY307940**	**DQ017601**[a]
Peziza quelepidotia	NRRL 22205	USA	**U42693**	**AF107809**
P. succosa	KH-98-07 (C)	DENMARK, Sjælland: 6 Jul 1998, *A. Storgaard*	**AF335166**	**AY500487**
P. vesiculosa	JV 95-652 (C)	DENMARK, Jylland: 11 Nov 1995, *J. Vesterholt*	**AY500552**	**AY500489**
Phillipsia crispata	T. Læssøe AAU-44895a (C)	ECUADOR, NAPO: 5 Jul 1983, *T. Læssøe*	AY945845[a]	**DQ017599**[a]
P. domingensis	PR-1583 (FH)	PUERTO RICO: Palo Colourado Forest, 24 Feb 1990, *D.J. Lodge*	AY945844[a]	
Phillipsia olivacea	T. Læssøe AAU-43162 (C)	ECUADOR, NAPO: 2 Feb–15 Mar 1983, *T. Læssøe*	**AY945843**[a]	
Plectania nannfeldtii	KH-97-16 (FH)	USA, CA: Sierra Nevada, 8 Jun 1997, *K. Hansen*	**AY945853**[a]	**DQ017592**[a]
Pseudopithyella minuscula	mh 675 (FH)	USA, CA: San Mateo Co., 9 Feb 1997, *F.A. Harrington*	**AY945849**[a]	**DQ017600**[a]
Pseudoplectania nigrella	KH-97-28 (FH)	USA, CA: Sierra Nevada, 4 Jun 1997, *K. Hansen*	AY945852[a]	
Pyronema confluens	TL-11685 (QCNE, C)	ECUADOR, Carchi: 2004, *K. Hansen et al.*	**DQ220397**[b]	**EU360915**[d]
Sarcoscypha austriaca (1)	TL-11247 (C)	DENMARK, Jylland: 7 Apr 2004, *T. Læssøe*	**AY945855**[a]	**DQ017597**[a]
S. austriaca (2)	mh 670 (FH)	USA, NY: Duchen Co., Apr 1996, *M. & D. Potter*	**AY945856**[a]	**DQ017598**[a]
S. austriaca (3)	s.n. (FH)	USA, VT: Norwich, Apr 1998, *K. Griffith*	**AY945857**[a]	
S. coccinea	KH-04-78 (C)	DENMARK, 2004, *H. Knudsen*	AY945847[a]	
S. occidentalis	DAH-12 (FH)	USA, MA: 12 Sep 2003. *D. Hewitt, G. Riner & D. Chou*	**AY945846**[a]	**DQ017596**[a]
Scutellinia scutellata	OSC 100015		**DQ247806**	**DQ247796**
Strobiloscypha keliae	NSW 7333 (ORG)	USA, OR: 1991, *K. Kuykendall*	**DQ220437**[b]	**DQ017602**[a]
Tricharina praecox	KH-03-101 (FH)	NORWAY, Nordland: Rana, 24 Aug 2003, *K. Hansen & C. Lange*	**DQ646525**[c]	**EU360916**[d]
Urnula craterium	DHP 04-511 (FH)	USA, NC: Wake Co., 25 Apr 2004, *D.H. Pfister*	**AY945851**[a]	**DQ017595**[a]
Wolfina aurantiopsis (1)	DHP 04-599 (FH)	USA, NC: Chatham Co., 3 Jul 2003, *Grand/Vernia*	**AY945859**[a]	**DQ017605**[a]
W. aurantiopsis (2)	RPK 4337 (CUP)	USA, OH: Benua Estate, Fairfield Co., 14 Aug 1976. *S.J. Mazzer*	**AY945858**[a]	**DQ017606**[a]
Wynnea americana	s.n. (FH)	USA, NY: Tompkins Co., no date, *K.T. Hodge*	AY945848[a]	
W. sparassoides	s.n. (FH)	USA, NJ: 1992, *M. Spock*	**EU360917**	

GenBank numbers in bold are sequences included in the combined LSU, RPB2 and SSU analyses.
a New sequences for this study.
b Sequences from Perry *et al.* (2007).
c Sequences from Hansen & Pfister (2006).
d Sequences from a study now underway by Hansen and coauthors.

concentration of 0.5 mM. The high-fidelity enzymes Herculase® (1.25 μl per reaction; Stratagene, La Jolla, CA) and Pfu turbo (0.25 U per reaction; Stratagene) were used in the reaction, with 1 × Herculase® buffer. For RPB2, the PCR cycling parameters were as follows: initial denaturation at 95 °C for 3 min, and 30 cycles at 95 °C for 45 s, 55 or 60 °C for 60 s increasing the temperature by 0.3 °C s^{-1}, 72 °C for 2 min, followed by a final elongation at 72 °C for 10 min and a soak at

4 °C. For LSU, the PCR parameters were as follows: an initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 60 °C for 45 s, 72 °C for 2 min, followed by a final elongation at 72 °C for 10 min and a soak at 4 °C. For both genes, slight modifications to these parameters were made when the genes did not amplify at first. These modifications consisted mostly of lowering the annealling temperatures and increasing the time for the elongation step. The amplified products were either directly purified using the QIAquick PCR purification kit (QIAGEN, Valencia, CA) or excised from a band on the agarose gel and purified using QIAquick spin columns (QIAGEN). The PCR products were used directly in cycle sequencing reactions using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol except that the reaction volume was 8 μl, using a Peltier Thermal Cycler lowing program: 96 °C for 3 min, then 25 cycles of ramping 1 °C s^{-1} to 96 °C, 96 °C for 10 s, ramping 1 °C s^{-1} to 50 °C, 50 °C for 5 s, ramping 1 °C s^{-1} to 60 °C, 60 °C for 4 min, followed by a final 4 °C soak. Primers on both strands were used to maximize coverage and improve accuracy by comparing the sequences on both strands. The sequencing reactions were purified as in Hansen *et al.* (2005). Electrophoresis and data collecting were done on an ABI PRISM® 3100 Genetic Analyser (ABI, Foster City, CA).

Analytical methods

The sequences were edited and assembled using Sequencher 3.0 (GeneCodes, Ann Arbor, MI) and are deposited in GenBank (Table 1). Sequences were manually aligned in the Sequence Alignment Editor Se-Al v2.0a11 (Rambaut 1996; http://tree.bio.ed.ac.uk/software/seal/). Introns in the RPB2 region were too variable to align and not present in all taxa; therefore, they were removed from the dataset. The position of the introns was recognized by sequence comparisons and the conserved dinucleotide sequences at the ends of introns (GT at start and AG at end). The RPB2 exons were further converted to amino acids using MacClade 4.0 (Maddison & Maddison 2000) to confirm the alignment and the position of the introns. RPB2 was analysed using the nucleotides. The combined LSU, SSU, and RPB2 alignment are available from TreeBASE (http://www.treebase.org/treebase/) as accession number S1928. Additional sequences from species of *Sarcoscyphaceae* and *Sarcosomataceae* were included in separate analyses of the LSU and SSU data to explore the sensitivity of the tree topologies to the inclusion of additional taxa.

Phylogenetic analyses were performed using PAUP 4.0b10 for Unix (Swofford 2002) and MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001) on G5 Macintosh computers. Parsimony (MP) analyses with heuristic searches consisted of 1 K random stepwise sequence addition replicates, with tree bisection–reconnection (TBR) branch swapping, MULPARS in effect, and saving all equally most parsimonious trees (MPTs). Alignment gaps were treated as missing data and all characters were equally weighted. Robustness of individual branches was assessed by parsimony BS analyses (PB), using 500 BS replicates, each consisting of a heuristic search with 100 random addition sequence replicates, TBR branch swapping and MAXTREES unrestricted.

Prior to combined analyses the combinability of the data was explored. The separate LSU and SSU gene-region analyses were performed on the more inclusive datasets, as well as on datasets restricted to those taxa included in the combined analyses. Congruence of the separate datasets was assessed by visual inspection of the individual BS values. We considered the phylogenies to be incongruent only if they displayed strongly BS supported incongruence, using the following BS categories: unsupported, <50 %; weak, 50–74 %; moderate, 75–84 %; strong, 85–100 %. Incongruence is then considered conflict of clades with PB ≥ 85 %; that is, clades that are strongly supported in one analysis that conflict with different and strongly supported clades in the others.

ML and Bayesian analyses were performed on the combined three-gene dataset. To select the model of nucleotide substitution with the least number of parameters that best fit each dataset, hierarchical likelihood ratio tests were performed as implemented in the program MrModeltest 2.2 (Nylander 2004). All searches were performed using a GTR + I + G model of sequence evolution, and the ML model parameters calculated from one of the MPTs recovered in the MP analysis of the combined data described above. The ML analysis consisted of heuristic searches with 100 random stepwise sequence addition replicates, and TBR branch swapping. ML BS values were generated using 100 BS replicates, each consisting of a heuristic search with stepwise 'as is' sequence addition, TBR branch swapping and MAXTREES unrestricted. Bayesian analyses were performed using Metropolis-coupled MCMC (MCMCMC) methods as implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), using uniform prior probabilities and the GTR + I + G model. Each dataset in the combined analysis (LSU, SSU, and RPB2) was specified as distinct partitions. Analyses consisted of two parallel searches, with four simultaneous chains of MCMCMC, run for 5M generations, starting from random trees. The chains were sampled every 100 generations for a total of 50K trees each, sampled from the posterior distribution. Those trees sampled prior to the chains reaching a split deviation frequency of 0.005 were discarded from the sample as the 'burn-in', while the remaining trees were used to calculate the Bayesian PP of the clades.

Results

Datasets

The most inclusive LSU alignment consisted of 999 characters for 37 taxa, with 343 variable positions, including 243 that were parsimony informative. The most inclusive SSU alignment consisted of 1785 characters for 31 taxa, with 302 variable positions, including 179 that were parsimony informative. The combined LSU, SSU, and RPB2 alignment included 4401 characters for 25 taxa, with 1423 variable positions, including 1173 that were parsimony informative; 959 characters of LSU with 194 being parsimony informative; 1780 of SSU with 162 being parsimony informative; and 1662 of RPB2

with 817 being parsimony informative. The RPB2 alignment included sequences spanning regions 6–11 for all taxa, except for *Wolfina aurantiopsis*, *Phillipsia crispata*, and *Byssonectria terrestris* for which only the 6–7 region has been obtained (*ca* 720 bp).

Individual LSU, SSU, and RPB2 phylogenies

Parsimony analyses of the restricted LSU and SSU datasets resulted in 18 and 11 MPTs respectively, whereas the more inclusive datasets yielded six (LSU) and nine (SSU) MPTs. The MP analysis of RPB2 resulted in one MPT. All MPTs from all individual datasets resolve the *Sarcoscyphaceae*, *Sarcosomataceae* (excluding *Strobiloscypha*), and a clade of *Chorioactis*, *Desmazierella*, *Neournula*, and *Wolfina* (the *Chorioactis* clade) as distinct lineages (trees not shown). These lineages are highly supported by both RPB2 and LSU data (PB 90–100 %), except for the *Chorioactis* clade, which receive only weak support by the LSU (PB 66–73 %; Table 2). The *Sarcoscyphaceae* are likewise highly supported by the SSU data (PB 100 %), whereas the *Sarcosomataceae* and the *Chorioactis* clade are only weakly to moderately supported (PB 57–77 %; Table 2). However, the relationships among the lineages are unresolved or without PB support. The inclusion of additional taxa in separate analyses of the LSU and SSU datasets did not affect overall the tree topologies.

Combined LSU, SSU, and RPB2 phylogeny

No supported conflict (PB $\geq$ 75 %) was detected between the individual LSU, SSU, and RPB2 gene trees and the data were therefore combined. The RPB2 region accounts for the greatest number of potentially parsimony informative characters within the combined dataset (69.65 %), followed by the 5′ portion of the LSU rDNA (16.54 %) and the SSU rDNA (13.81 %). Parsimony analysis of the combined dataset resulted in four MPTs. The combination of the three datasets produced similar or more strongly supported lineages; all families included and the *Chorioactis* clade received 100 % PB (Table 2). The *Sarcoscyphaceae*, *Sarcosomataceae*, the *Chorioactis* clade, *Pyronemataceae*, and *Ascodesmidaceae* form a monophyletic group (PB 80 %), with *Morchellaceae* and *Discinaceae* as a sister group (Fig 1). Relationships among the lineages within this larger group are still unresolved or have weak support in MP analyses (Fig 1). The *Chorioactis* clade is resolved as a sister group to *Sarcosomataceae*, but only with weak support (PB 52 %).

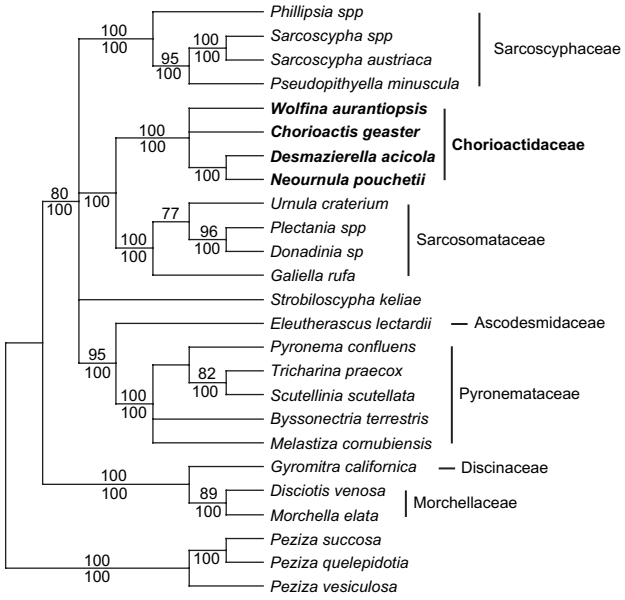

Fig 1 – Phylogenetic relationships of *Chorioactis*, *Desmazierella*, *Neournula*, and *Wolfina* (*Chorioactidaceae*) among members of the C-lineage of *Pezizales*. Strict consensus tree of four equally most parsimonious trees obtained from analysis of combined LSU, SSU, and RPB2 nucleotide sequences. Numbers at branches are parsimony BS frequencies (> 50 %). Numbers below branches are PPs (PP $\geq$ 95 %), obtained from the 50 % majority rule consensus tree of the 46 100 trees sampled from Bayesian MCMCMC analyses.

The ML analysis found a single optimal tree (-lnL = 31189.70336, Fig 2). Bayesian analyses reached an average standard deviation of split frequencies below 0.005 after approximately 4 610 000 generations, and the first 3900 trees were excluded as the 'burn-in'. ML and Bayesian analyses of the combined data identified all families and the *Chorioactis* clade with strong support (Fig 2, MLB and PP 100 %).

Table 2 – Parsimony BS support for lineages in separate and combined analyses of the different LSU, SSU, and RPB2 datasets

	Inclusive LSU	Inclusive SSU	LSU for combined	SSU for combined	RPB2	LSU, SSU, RPB2
Sarcoscyphaceae	89	100	98	100	100	100
Sarcosomataceae	95	77	96	60	100	100
Chorioactis clade	73	57	66	61	93	100
Pyronemataceae	98	99	99	100	97	100
Chorioactis clade plus *Sarcosomataceae*	–	–	–	–	<50	52
Chorioactis clade plus *Sarcoscyphaceae*	<50[a]	60[a,b,c]	–	<50[a,c]	–	–

a Plus *Strobiloscypha*.
b Plus *Pyronemataceae*.
c Plus *Ascodesmidaceae*.

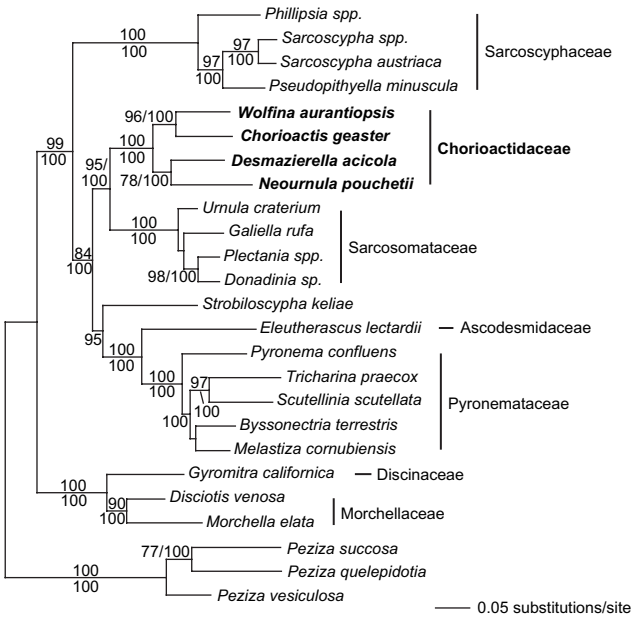

Fig 2 – Phylogenetic relationships of the *Chorioactidaceae* among members of the closely related families in the C-lineage of *Pezizales*. The tree with the highest log likelihood (-lnL = 31189.70336) obtained from ML analysis. Branch length corresponds to genetic distance (expected nucleotide substitutions per site). Numbers above branches are ML BS values (MLB > 70 %) and numbers below branches are PPs (PP ≥ 95 %).

In addition, the *Chorioactis* clade and *Sarcosomataceae* form a strongly supported monophyletic group (MLB 95 %, PP 100 %). *Pyronemataceae*, *Ascodesmidaceae,* and *Strobiloscypha keliae* are moderately supported as a sister lineage to this group (MLB 84 %, PP 100 %), followed by *Sarcoscyphaceae* as a successive sister (MLB 99 %, PP 100 %).

Morphological characters

In all taxa of the *Chorioactis* clade spores are ornamented with cyanophilic warts or ridges or, as in *Desmazierella acicola*, a cyanophilic outer layer is present. Hymenial colours range from beige, yellow, orange, fulvous, vinaceous, to maroon. Opercula are terminal or slightly eccentric and are more or less uniform in thickness, without an apical pad. Asci are narrowly pedicillate and have a simple septum at the base. The asci may taper to a pedicel or they may be constricted abruptly at the pedicel. In some species the bases of the asci are rounded. In other cases the asci have a pedicel that is inserted laterally giving the ascus a lobed or bifurcate base. Further variation of ascus morphology is described under each genus. The outer surface of the ascoma has brown, ornamented hairs; the ornamentation often takes the form of discrete warts or spines. The outer region of the excipulum is composed of small, angular, generally dark-walled cells of dense *textura intricata*. The medullary excipulum is thick and more or less corky; it is composed of non-gelatinized *textura intricata*.

Taxonomy

Based on the strong support provided by ML and Bayesian analyses of the combined LSU, SSU, and RPB2 data (Fig 2) and distinct morphological and cytological characters, we erect the following new family for the *Chorioactis* clade.

Chorioactidaceae Pfister, **fam. nov.**
MycoBank no.: MB 511346
Etym.: From the genus name *Chorioactis*.

Ascomata 3 mm usque maius quam 12 cm, hymenium albidum, roseum, rubellum, aurantium, fulvoum, senatum. Pagina externa ascomatorum fuscorum cum brunneis spineis pilis. Caro non gelatinosa composita. Ascosporae plurinucleati cum cyanophilis parietibus aut ornamentis. Paraphyses plurinucleati. In lingo aut conifis foliis.
Typus: *Chorioactis* Kupfer, *Bull. Torrey Bot. Club* **29**: 142 (1902)

Included genera: Desmazierella, Neournula, Wolfina

Ascomata 3 mm to 12 cm broad, with rose, red, orange or fulvous hymenia. Flesh white. When young the ascomata are inrolled often with a small mouth or opening, expanding as they mature, sometimes splitting in a star-like fashion, sessile, substipitate or with an elongate, buried stalk. In *Desmazierella* hymenial setae extend above the hymenial surface. Outer surface brown to nearly black, with hairs often giving it a velvety texture. *Excipulum* of *textura intricata*, which toward the outer surface becomes wider and gives the appearance of *textura angularis*, the cells generally brown in the outer layers. *Hairs* arising from within the medullary excipulum or from the outermost cells of the outer excipulum. Hairs of various length, brown with spines or warts. *Asci* long, reaching a length of 700 μm, arising from a narrow hyphal base or pedicel often abruptly expanded above the base and sometimes lobed or forked, often maturing simultaneously, with a terminal or subterminal operculum. *Ascospores* uniseriate, ellipsoid or fusioid, with a cyanophilic outer wall and/or with cyanophylic warts, longitudinal ridges, or punctae, multinucleate. *Paraphyses* filiform or moniliform, highly anastomosing, in some cases setae with warts or spines similar in appearance to the hairs are also present in and extending above the hymenium, cells multinucleate. Growing on woody debris and conifer needles.

Notes: In species of *Wolfina, Chorioactis,* and *Desmazierella*, the hairs are brown to dark brown and are ornamented with distinctive conical warts or spines, aptly described by Eckblad (1968) as 'prickled'. The hairs are illustrated by Eckblad and are shown by Bellemère *et al.* (1994) in TEM of *Chorioactis geaster*. Melendez-Howell *et al.* (1998) illustrate and discuss the ornamentation of hairs in *Desmazierella acicola* in SEM and TEM, and show the same type of ornamentation on paraphyses and hymenial setae of that species. Galán & Raitviir (1995) also show these ornamentations in *D. acicola*. These are also present and illustrated in *D. piceicola*, the only other accepted

species of the genus *Desmazierella* (Huhtinen & Mäkinen 1984). Hairs of *Neournula pouchetii* are shorter and lighter in pigmentation, but they are similarly ornamented.

Ultrastructural studies of the asci in *D. acicola* suggest they are of the *Sarcoscypha*-type (Melendez-Howell *et al.* 1998); in *C. geaster* the asci resemble those of *Sarcoscypha* but differ in aspects of wall layering (Bellemère *et al.* 1994). In light microscopic studies, the asci are distinctive morphologically in members of *Chorioactidaceae*. The asci of *C. geaster* are abruptly constricted at the base to a narrow pedicel, as illustrated by Imazeki & Otani (1975) and discussed by Pfister & Kurogi (2004). Both species of *Desmazierella* have bifurcate ascus bases (Galán & Raitviir 1995; Huhtinen & Mäkinen 1984), and Paden & Tylutki (1968) describe and illustrate the ascus bases in *N. pouchetii* (as *N. nordmanensis*) as attenuated and lobed. Furthermore, the asci in *C. geaster* mature more or less simultaneously within a single apothecium (Pfister & Kurogi 2004). Simultaneous maturation of asci is found in members of the genus *Cookeina, Sarcoscyphaceae,* and was suggested in *D. piceicola* (Huhtinen & Mäkinen 1984) and in *D. acicola* by Korf & Zhuang (1991) who speculated that this might account for the deviation in spore size noted in the literature for this species.

The number of nuclei in ascospores is considered a useful character in the delimitation of families of *Pezizales*. The spores of the *Sarcoscyphaceae* and *Sarcosomataceae* are multinucleate, but multinucleate spores are also found in families of lineage B of *Pezizales*, suggesting that multinucleate spores have evolved several times within the *Pezizales*. The cells of the paraphyses are multinucleate in members of the *Sarcoscyphaceae* and uninucleate in members of the *Sarcosomataceae* (Berthet 1964b), a condition according to Berthet found in this group of taxa and in the unrelated genus *Tarzetta* (as *Pustularia*) among the *Pezizales*. In the taxa of *Chorioactidaceae*, for which cytological information is available (*Desmazierella* studied by Berthet 1964b; *Neournula* reported by Berthet & Riousset 1965), both the spores and cells of the paraphyses are multinucleate. Thus, cytological evidence points toward a relationship with *Sarcoscyphaceae*, while molecular phylogenetic analyses point to a sister relationship with *Sarcosomataceae*.

Chorioactis Kupfer, *Bull. Torrey Bot. Club* **29**: 142 (1902). (Figs 3C, 4, 5)

Type species: *Chorioactis geaster* (Peck) Kupfer, *Bull. Torrey Bot. Club* **29**: 142 (1902). [syn. *Urnula geaster* Peck, *Ann. Rep. New York State Mus.* **46**: 39 (1893)]

Other species: no other species described.

Apothecia tough and leathery, large up to 12 cm diam when mature, with a stipe, often buried, up to 10 cm long, cylindrical, ovoid, clavate or spindle-shaped when young, at maturity splitting into four to seven rays that bear the hymenium, externally brown tomentose. *Hymenium* whitish, yellowish, saffron to salmon to butterscotch, in age chestnut. *Medullary excipulum* of *textura intricata*, white, without gelatinous contents. *Ectal excipulum* of brown-walled cells of *textura intricata* to *textura angularis*. The outer cells give rise to hairs of two types: (a) short, broad, blunt, light brown hairs with distinct prickles on the lower portions but smooth above; (b) long tapering hairs with often acute apices, walls brown and prickled. *Asci* long, up to 700 μm, 8-spored, with a terminal operculum, abruptly constricted below and connected to the subhymenium by a thin, hyphal pedicel, maturing synchronously. *Ascospores* large, up to 75 μm, fusiform, inequilateral, marked with low cyanophilic punctae, with many inclusions. *Paraphyses* at first filiform, at maturity the cells swell and become moniliform.

Distribution: Texas, USA; Miyazaki Pref., Kyusyu, Japan

Plant associates: *Ulmus crassifolia*, *Quercus gilva*, and *Symplocos myrtacea*.

Anamorph: mycelial growth reported by Imazeki & Otani (1975); Peterson *et al.* (2004) report a *Conoplea* state.

Notes: Eckblad (1968) considered Kupfer's (1902) paper, in which the genus was described, to lack a clear description and to be based on a misinterpretation of the excipular construction. We hold that Kupfer's paper indeed clearly describes the taxon and meets the requirements of the *Code*. That Kupfer misinterpreted the cellular construction of the excipulum does not influence the status of the name.

Seaver (1942) and Wolf (1958) discussed the moniliform paraphyses described for this species. Both authors attribute

Key to the genera

1 Ascomata small, up to 5 mm diam, disc whitish, beige, gray; hairs/setae projecting above the hymenium, on conifer needles.... **Desmazierella**
Ascomata large, over 1 cm diam, setose paraphyses not present in the hymenium... 2

2(1) Hymenium rose, pale pink when young, purplish when mature. Outer surface pale brown to purplish brown, with scattered brown hairs, spores with low warts. Stipitate, immersed in conifer duff... **Neournula**
Hymenium salmon, saffron, orangish, reddish, butterscotch, outer surface brown to black densely covered with brown hairs, spores with ridges or very low warts... 3

3(2) Ascomata salmon, saffron, butterscotch, at first cylindrical, fusoid, spindle-shaped, when young, splitting regularly into four to seven rays at maturity, paraphyses moniliform, spores up to 70 μm long with low cyanophilic punctuations.... **Chorioactis**
Ascomata reddish or orangish, more or less globose at first, splitting irregularly upon opening, paraphyses straight, not inflated, spores up to 45 μm long with longitudinal cyanophilic ridges... **Wolfina**

Fig 3 – Ascomata of *Chorioactidiaceae*. (A) *Desmazierella acicola*, from a collection from Denmark, photograph © Jens H. Petersen/MycoKey. (B) *Wolfina aurantiopsis*, ANM248, photograph by A. N. Miller. (C) *Chorioactis geaster*, Austin, Texas, photograph by Henry Aldrich. (D) *Neournula pouchetii*, specimen from Oregon, photograph by Lorelei Norvill. Bars = (A) 3 mm, (B) 3 cm, (C) 5 cm, (D) 2 cm.

the splitting of the ascomata to the swelling of the paraphyses. Our observations support this view, in that in mature and open apothecia the paraphyses are moniliform, whereas in immature specimens the cells of the paraphyses are unexpanded. Kupfer (1902) and Heald & Wolf (1910) discuss the construction of the excipular tissues. Heald & Wolf (1910) accurately described the prosenchymatous nature of the tissue.

Imazeki (1938), Imazeki & Otani (1975), and Otani (1980) describe this fungus from Kyusyu, Japan. Seaver (1939) commented on the odd distributional disjunction as follows: 'It would be difficult indeed to account for it [the distribution] we merely accept the facts as they are'. Peterson *et al.* (2004) used sequence data to demonstrate that the Japanese and American populations were significantly divergent and suggested that they have been separated for a minimum period of 19 million years. No morphological differences could be found between these disjunct populations.

Kurogi *et al.* (2002) looked at the conditions necessary for the development of fruit bodies in the Kyusyu population; the article includes an extensive series of photographs. Keller & Rudy (1995), Rudy & Keller (1996), and Samson & Jackson (1977) discuss the occurrence of this fungus in Texas.

Specimens of Chorioactis geaster *examined:* **Japan:** *Kyushu: Miyazak Prefecture*: Tano Experimental Forest of Miyazaki University, Tano-cho, Miyazaki-gun, on fallen trunks of *Quercus gilva*, 12 Oct 1978, *Y. Otani* (TMI 7589); Aya Town, 19 Nov 1997, *S. Kurogi* (FH) [Two collections and anamorph]. — **USA**: *Texas*: San Saba Co.: Richland Springs, Woods of *Quercus* and *Ulmus*, 18 Dec 1912, *Eleanor Hall* (FH); Tarrant Co.: Arlington, 6 Nov 1997, *K. C. Rudy* (FH); River Legacy Parks, Arlington, 8 Oct 1994, *K. C. Rudy and H. W. Keller* (FH); River Legacy Parks, Arlington, 7 Oct 1994, *K. C. Rudy and H. W. Keller* (FH); Arlington, 22 Mar 1998, *K. C. Rudy* (FH); Travis Co.: Austin, on roots of oak and elm, Dec 1932, *C. W. Goldsmith* (FH); Mansfield, Lloyd Park, Joe Pool Lake, mixed hardwoods, predominantly cedar elm, *ca* 1991, *K. Rice* (FH); Austin, ground, 24 Nov 1891 (BPI); Austin, Dec 1932, *Goldsmith G. W.* (BPI); Guadalupe Co.: Sequin, on *Ulmus crassifolia*, 2 Jan 2001, *Forrest M. Mims III* (FH); Bexar Co.: San Antonio, 5 Feb 1922, *Ellen D Schultz* (BPI); Nov 1908, *W. H. Long* (BPI); San Antonio, on *Ulmus crassifolia*, 22 Jan 1933, *W. H. Long* (BPI); Bell Co.: Midway, 30 Oct 1930, *S. E. Wolff* (BPI); Denton Co.: Denton, roots rotten, Dec 1907, *W. H. Long* (BPI); Denton, *Ulmus* sp., roots rotten, Dec 1909, *W. H. Long* (BPI).

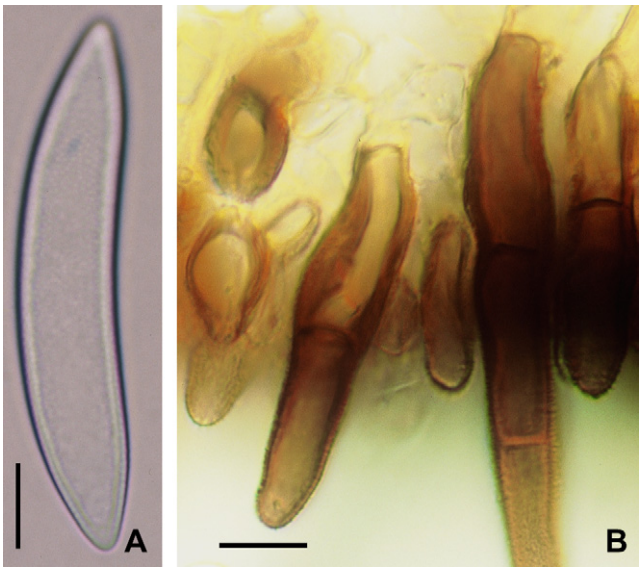

Fig 4 – *Chorioactis geaster*. (A) Ascospore. (B) Section showing hairs on the outer surface of an apothecium, stained in Congo Red in ammonia. FH, Mims 2001. Bars = (A) 10 μm, (B) 20 μm.

Desmazierella Lib., *Ann. Sci. Nat.* **17**: 83 (1829).

(Figs 3A, 6, 7)

Type species: *D. acicola* Lib., *Ann. Sci. Nat.* **17**: 83 (1829).

Other described species: *D. piceicola* Huhtinen & Y. Mäkinen, *Mycotaxon* **20**: 551 (1984). Two species described by Rick, *D. bulgarioides* and *D. foliicola*, have not been studied.

Apothecium small, up to 5 mm diam, sessile or short stipitate, cupulate to plane, setose on the outer surface, situated on a brown subiculum and sometimes in association with its anamorph. *Hymenium* buff, provided with dark brown setae, and appearing tomentose; outside clothed with dark brown, straight hair. *Medullary excipulum* of *textura intricate*. *Ectal excipulum* of *textura angularis* of light to dark brown cells, sometimes thick-walled, and with some brownish hyphal incrustations in the inner zones. *Hairs* of two types, superficial and rooting, ornamented with warts and spines. Superficial hairs are light brown, regularly septate, with blunt tips. Rooting hairs are dark brown to black, densely ornamented with long and acutely pointed warts. *Asci* cylindrical, up to 300 μm, 4- or 8-spored, slightly constricted below the tip, operculum terminal, bifurcate or lobed at the base, the base often rounded, maturing synchronously or seratum. *Ascospores* with 6–8 nuclei (Berthet 1964b), ellipsoid, hyaline, smooth with a cyanophilic covering layer, sometimes appearing apiculate (Benkert 1991), or marked with cyanophilic longitudinal ridges, 2 small guttules present. *Paraphyses* much branched and hyaline below, anastomosing along their length, becoming brown apically and roughened to granular, longer than the asci, with 2–6 nuclei per cell (Berthet 1964b; Huhtinen & Mäkinen 1984), setae are present in the hymenium and these are long, dark-brown and exceed the level of the hymenium by 500 μm or more; they are unbranched and pointed at the tip.

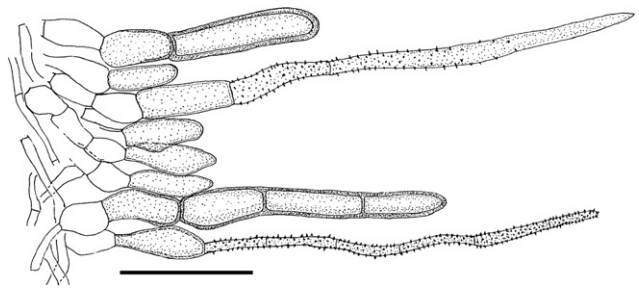

Fig 5 – *Chorioactis geaster*. Section of the outer surface of an apothecium. FH, Goldsmith. Bar = 50 μm.

Distribution: Europe, North America, Japan

Plant associates: on needles and twigs of *Pinus densiflora, P. pinaster, P. sylvestris*, and *Picea abies*.

Anamorph: *Verticicladium trifidum* (Berthet 1964a; Gremmen 1949; Hughes 1951) known in the type species.

Notes: The genus was included in the *Humariaceae* tribe *Lachneae* by Le Gal (1947). Nannfeldt (1949) recognized its affinities with the broadly inclusive family *Sarcoscyphaceae*, a position Le Gal (1953, 1963) later accepted. Benkert (1991) and Galán & Raitviir (1995) provide a detailed description and illustrations of *Desmazierella acicola*. It is generally described as having smooth spores, but there is a cyanophylic outer wall that has often been overlooked, and which may be particularly prominent as spores age as observed by Huhtinen & Mäkinen (1984) and us. *D. acicola* is distinguished from *D. piceicola* by smaller spores (20–25 × 10–12 *versus* 48–52 × 11–12 μm) and by its smooth rather than longitudinally ridged ascospores. *D. piceicola* also has 4-spored asci and occurs in autumn.

The original collection of *D. acicola* was reported on 'pin sauvage' presumably *Pinus sylvestris* (Libert 1829) and this seems to be the common host plant in Europe. Larsen & Denison (1978) list three collections from Oregon, which we have studied. None of these indicate the pine species on which the fungus was found. Significantly perhaps all were from arboreta or test gardens where it is likely that European pines were under cultivation. The pine host species is not reported for collections cited by Korf & Zhuang (1991) from the Canary Islands. *D. acicola* is reported from Japan by Otani (1980) and Imazeki *et al.* (1988) on *P. densiflora*, Japanese red pine. Shaw's (1972) listing of a collection on *Tsuga* in Washington State is unverified but is repeated by Farr *et al.* (1989) who also listed the species from China but this record could not be verified.

Hughes (1951) extensively studied the anamorph, *Verticicladium trifidum*. He states that the type collection was from *P. sylvestris* and that in his studies in Britain *V. trifidum* was always found and was collected throughout the year. Further studies by Kendrick & Burges (1962), Kowalski (1988), and van Maanen & Gourbière (1997) establish the widespread occurrence and high incidence of the anamorphic state in Europe. The later authors summarize the worldwide literature on the occurrence of *V. trifidum*. This fungus is considered to be the most important internal colonizer of *P. densiflora* in Japan (Tubaki & Saito 1969). The teleomorph seems to be encountered infrequently and perhaps under more narrow environmental conditions, but its early season fruiting and small size may account for its presumed rarity.

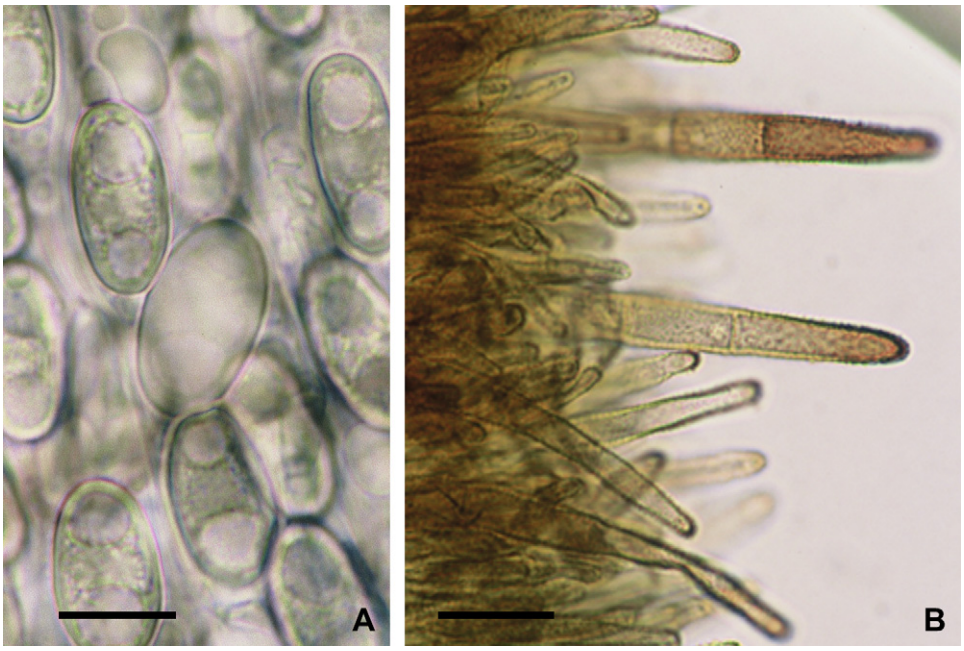

Fig 6 – *Desmazierella acicola*. (A) Ascospores. (B) Section showing hairs on the outer surface of an apothecium. Herb. Roy Kristiansen, R. Kristiansen 95.40. Bars = 20 μm.

Specimens of Desmazierella acicola *examined*: **Belgium:** Hieme. Ad folia putrescentia Pini sylvestris. Vere. Plantae Cryptogamicae no. 24, *Libert* (FH). — **Germany:** *Baden-Württemberg*: Rastatt, auf Nadeln von *Pinus silvestris*, Feb 1877, *Schröter*, Rabenhorst, Fungi europaei no. 2211 (FH). *Berlin*, auf Kiefernnadeln, Jun 1894, *P. Sydow*, Mycotheca Marchica no. 4164 (FH). *Brandenburg*: Triglitz, auf faulenden Nadeln von *Pinus silvestris*, 2 Apr and 28 May 1904, *Otto Jaap*, Fungi selecti exsiccati no. 86 (FH); Prignitz, an faulenden Nadeln von *Pinus sylvestris*, Mar 1910, *Otto Jaap*, Rehm, Ascomycetes no. 705b (FH). *Hesse-Nassau*: *ca* Johannisberg, ad Pini sylvestris folia putrida, rarissime, Vere, *Fuckel*, Fungi Rhenani no. 2681 (FH). *Saxony*: Königstein, auf faulenden Nadeln von *Pinus silvestris*, Mar 1886 and 1887, *W. Krieger*, Fungi saxonici no. 292 (FH); 20 May 1893, *W. Kreiger*, Fungi saxonici no. 292 b (FH). — **Great Britain:** *North Wales*, *W. Phillips*, Elvellacei Britainici no. 45 (FH). — **Italy:** Torre d'Isloa prope Papiam, in acubus emortuis Pini silvestris, aestate, *F. Cavara*, Fungi Longobardiae exsiccati no. 113 (FH). — **Netherlands:** *Gelderland*: Lochem, ad folia putrescentia rarus ad ramulos Pini sylvestris, May, *Th. Sprée*, Rabenhorst, Fungi europaei no. 623 (FH). — **Norway:** *Buskerud*: Nedre Eiker, Hokksund, on pine needles, 18 Apr 1995, *Berit Krømer*, det. *Roy Kristiansen 95.23* (Herb. Roy Kristiansen). *Østfold*: Hvaler, Søndre Sandøy, near Reierstangen at the seashore, on pine needles, 29 Apr 1995, *Roy Kristiansen 95.30* (Herb. Roy Kristiansen); Hvaler, Kirkøy, close to Hvaler church, on cut branches of *Pinus sylvestris*, 31 Mar 1983, *Roy Kristiansen 83.155* (Herb. Roy Kristiansen); Hvaler, Asmaløy, Gravningen, 1 Apr 1995, *Roy Kristiansen 95.11, 95.12* (Herb. Roy Kristiansen); Fredrikstad, Kråkerøy, Hellekilen, in spruce wood with some pine (*Pinus sylvestris*), 22 Apr 1995, *Roy Kristiansen 95.26* (Herb. Roy Kristiansen); Fredrikstad, Onsøy, Stegeberget, pine needles, 15 May 1996, *Roy Kristiansen no. 96.11* (Herb. Roy Kristiansen); Hvaler, Spjærøy, Bekkene, on pine needles, 30 Apr 1995, *Roy Kristiansen 95.40* (Herb. Roy Kristiansen); Hvaler, Asmaløy, Geitvika, near sea, on dead needles of *Pinus sylvestris*, 28 Apr 1992, *Roy Kristiansen 92.04* (Herb. Roy Kristiansen); Onsøy, Flåtaviken, on pine needles, on cut branches of *Pinus sylvestris*, 1 May 1983, *Roy Kristiansen 83.85* (Herb. Roy Kristiansen). — **USA:** *New York*: Tompkins Co.: Ithaca, Cornell Plantations near Test Garden, 2 May 1956, *R. P. Korf (2475)* (Herb. R. P. Korf – CUP); as above 30 May 1954, *R. P. Korf 54-10* (CUP). *Oregon*: Benton Co.: Peavy Arboretum, on dead needles of *Pinus* buried in litter, 12 Feb 1976, *A. Rossman (1108)* & *S. Carpenter* (BPI).

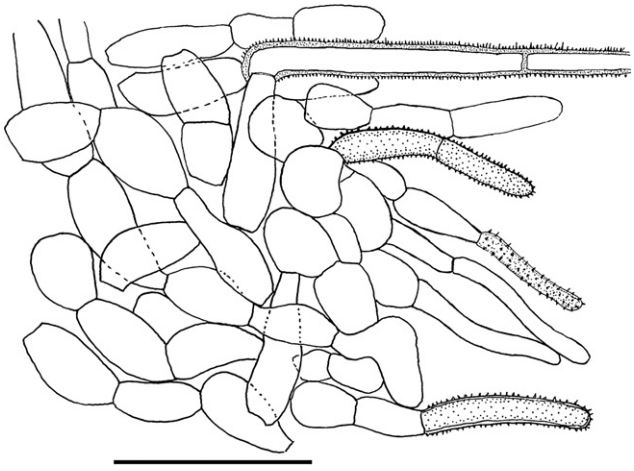

Fig 7 – *Desmazierella acicola*. Section of the outer surface of an apothecium. FH, Jaap, *Fungi selecti exsiccati* no. 86. Bar = 50 μm.

Neournula Paden & Tylutki, *Mycologia* **60**: 1160 (1968). (Figs 3D, 8, 9)

Type species: *Neournula pouchetii* (Berthet & Riousset) Paden, *Mycologia* 64: 457 (1972) [syn. *Urnula pouchetii* Berthet & Riousset, *Bull. Mens. Soc. Linn.* Lyon **34**: 253 (1965), *Neournula nordmanensis* Paden & Tylutki, *Mycologia* **60**: 1161 (1968)].

Other described species: *Neournula pouchetii* is the accepted name for the only species in the genus. The combination *Neournula helvelloides* (syn. *Donadinia helvelloides*) has been made,

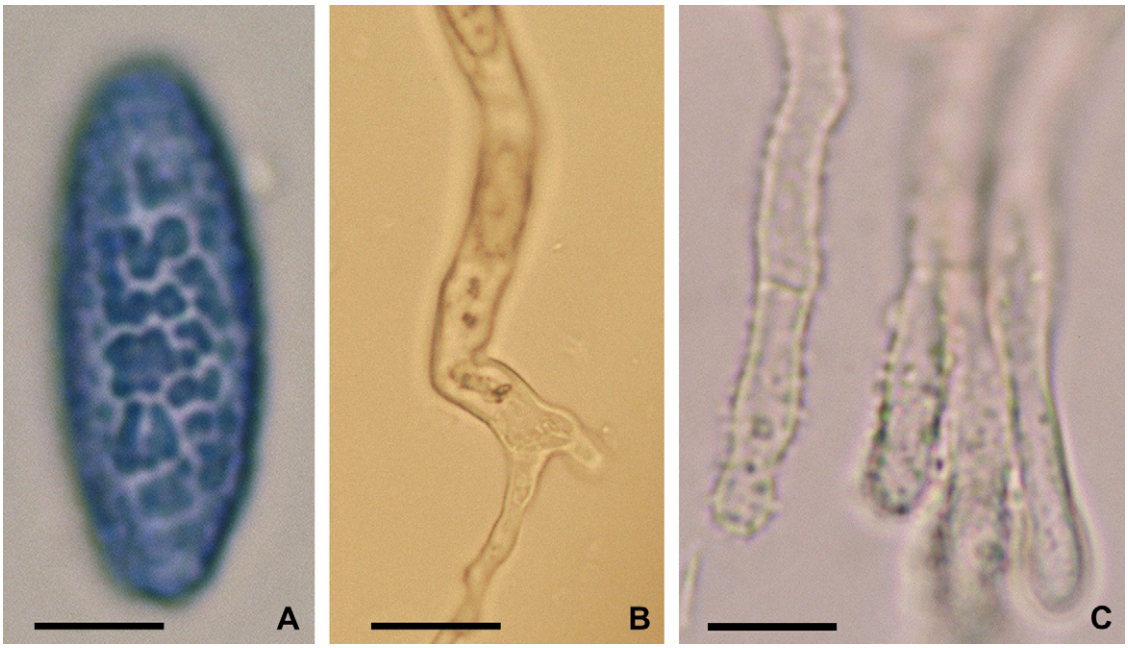

Fig 8 – *Neournula pouchetii*. (A) Ascospore, stained in Cotton Blue in lactic acid. (B) Ascus base. (C) Excipular hairs. WSU 56267. Bars = (A) 6 μm, (B) 20 μm, (C) 20 μm.

but we do not consider this to be a member of the genus *Neournula*.

Apothecia up to 4 cm diam, short stipitate, when young cylindrical–tubular with a narrow opening, expanding to form a sphere with a small opening, at maturity urceolate to goblet-shaped; margin dentate, stellate or ray-like, leathery. *Hymenium* rose, light to dull-purplish, in age dark brown, lacking carotenoids (Arpin 1969); outer surface light brown to purplish brown, lightly tomentose. *Medullary excipulum* of *textura intricata*, light coloured, without gel, the hyphae composing this layer are often encrusted with brownish amorphous material. *Ectal excipulum* of angular cells, which on the outside give rise to short, brown, hyphoid hairs that are ornamented with warts. *Asci* 280–400 μm, with a more or less eccentric operculum, ascus base blunt and lobed, connected to the subhymenium with thin hyphae, maturing seratum. *Ascospores* with 6–8 nuclei (Berthet & Riousset 1965), eguttulate or with a few small oil drops, at maturity marked with low warts sometimes forming interconnected islands and ridges, staining in cotton blue. *Paraphyses* branched, septate, anastomosing along their length, containing 1–3 nuclei per cell (Berthet & Riousset 1965).

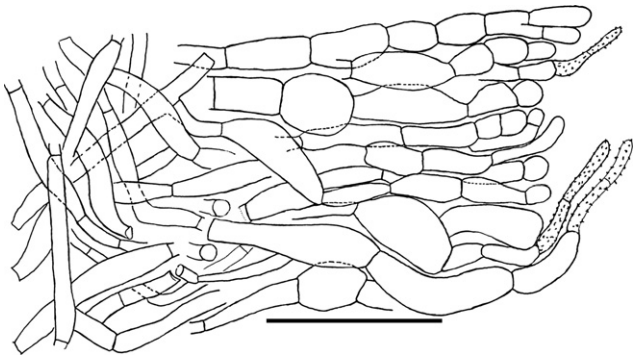

Fig 9 – *Neournula pouchetii*. Section of the outer surface of an apothecium. WSU 56281, type of *N. nordmanensis*. Bar = 50 μm.

Distribution: France, Italy, Morocco, Spain, in western North America.

Plant associates: leaf litter of *Cedrus atlantica*, *Thuja plicata*, *Tsuga heterophylla*, *Pinus monticola*, *Abies grandis*, and *Pseudotsuga menziesii*.

Anamorph: 'Slow growing, appressed–floccose, at first white, developing black stromata after about 60 d. The stromata consist of a thick layer of irregular, closely adhering dark cells over a thicker layer of hyaline hyphae. No conidial state has been observed' (Paden & Tylutki 1968: 1163).

Notes: The distribution of this fungus is remarkable. It has moved with its host, *Cedrus atlantica*, in Europe through plantations in the Mediterranean region. In North America it seems to be associated with the litter of several conifers. Malençon (1979) noted that in Morocco *N. pouchetii* co-occurs with *Geopora sumneriana*, which was similarly found by Fouchier & Neville (1998) in France.

Zhuang (Zhuang & Wang 1998) states that *Donadinia*, based on *Urnula helvelloides*, is not distinguishable from *N. pouchetii*. This position is based primarily on the presence of cyanophilic spore markings in both taxa. In molecular phylogenetic and morphological studies *Donadinia* species have been placed squarely in the core group of *Sarcosomataceae* (Bellemère *et al.* 1990; Harrington *et al.* 1999: figs. 1 and 2). Furthermore, *Donadinia* species have a gelatinous matrix associated with excipular cells, whereas members of the *Chorioactidaceae* lack such a configuration. Bellemère *et al.* (1990) compared the ascus and ascospore structure of *D. helvelloides* with other

members of *Sarcosomataceae* (*Plectania melastoma*, *P. platensis*, *Urnula craterium*, *Pseudoplectania nigrella*, *Sarcosoma globosum*, *Galiella rufa*) and conclude that its ascus structure agrees with that of *Sarcosomataceae*; they thought it to be closest to *Pseudoplectania*.

Cherubini & Perrone (1994), Fernández Vicente & Undagoitia (2001), Fouchier & Neville (1998), Pascual & Rocabruna (1988), and Péricouche (1995) have published illustrations and descriptions of European material.

Specimens of Neournula pouchetii *examined*: **France**: *Graissessac*, Terril de Garella, sous cèdres, 15 May 2005, Guy Garcia (FH)— **USA**: *Idaho*: Bonner, sec. 17, T63 N, R5W, Gold Creek, in leaf litter of *Thuja plicata*, 2 Jul 1964, *E. E. Tylutki* (holotype of *Neournula nordmanesis*, WSP 56281); Bonner, leaf litter in duff, in 200+ years climax stand of western red cedar, western hemlock, approx. 500 ft from granite creek on gentle SW slope, 3000′ approx. elev., NW 40, SW1/4, sec 28, T 62 N.R, 5W, 1 Jul 1942, *A. W. Slipp* (WSU 56267). *Washington*: Callam Co.: Fairholm, in litter of *Tsuga heterophylla*, Olympic National Forest along US 101, 2 Jun 1967, *J. W. Paden* 532 (WSU 57811).

Wolfina Seaver ex Eckblad, *Nytt Mag. Bot.* **15**: 126 (1968). (Figs 3B, 10, 11)

Type species: *Wolfina aurantiopsis* (Ellis) Seaver, *Mycologia* **29**: 680 (1937) [syn. *Peziza aurantiopsis* Ellis, *Bull. Torrey Bot. Club* **9**:18 (1882), *Lachnea aurantiopsis* (Ellis) Sacc., *Syll. Fung.* **8**:180 (1889), *Scutellinia aurantiopsis* (Ellis) Kuntze, *Rev. Gen. Pl.* **2**: 269 (1891), *Sarcosoma carolinianum* Durand, *J. Mycol.* **9**: 103 (1903)].

Other described species: *Wolfina oblongispora* (J. Z. Cao) W. Y. Zhuang & Zheng Wang; *W. papuana* Otani (1975) (excluded by Zhuang & Wang 1998).

Apothecia up to 5 cm broad, at first globose nearly closed, opening to form a deep cup, later opening out to a more or less discoid ascoma, sessile or subsessile, margin irregularly torn, corky, situated on a dark mycelial mat. *Hymenium* pale yellow or reddish; outer surface brown to black, covered with dark tomentum. *Medullary excipulum* white, of *textura intricata*, the cells enlarging toward the ectal excipulum, without gelatinized tissues. *Ectal excipulum* of angular/interwoven cells with dark walls. Outer cells of the excipulum giving rise to hairs of two types: (a) short, wide hairs, ornamented with spines or prickles, and (b) long, narrower hairs, ornamented

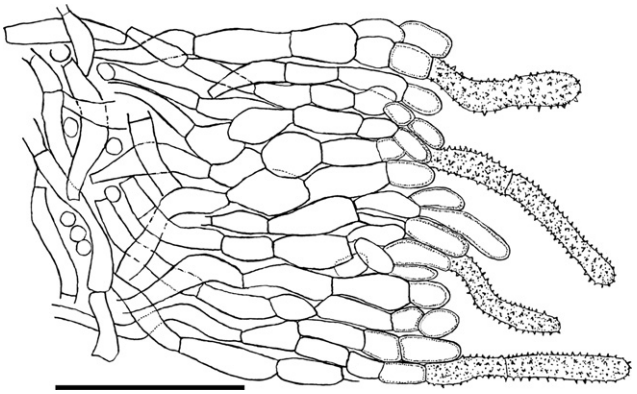

Fig 11 – *Wolfina aurantiopsis*. Section of the outer surface of an apothecium. FH, R. Thaxter 3636. Bar = 50 μm.

below and smooth above. *Asci* up to 400 μm long, with a terminal operculum, with a long tapering base that becomes hyphal-like, maturing seratum. *Ascospores* ellipsoid, marked with cyanophilic, fine, longitudinal striations, contents granular but without guttules. *Paraphyses* filiform, straight, septate, and anastomosing.

Distribution: *Wolfina aurantiopsis* in eastern North America is known from Connecticut, Florida, New Jersey, North Carolina, Ohio, Pennsylvania, and Tennessee; *W. oblongispora* in China is known from Fujian and Yunnan.

Plant associates: On rotten wood.

Notes: The genus was proposed by Seaver (1937), but without a Latin description. Later, Seaver (1942) treated *Sarcosoma carolinianum* as a synonym of *Wolfina aurantiopsis*. Eckblad (1968) validated the genus and species by providing a Latin description. Based on morphology and molecular phylogenetic analyses, *Wolfina* is most closely related to *Chorioactis*. Both also are associated with hardwoods.

We have not studied material of *W. oblongispora* (Otani 1975), but based on its description (Cao *et al.* 1992) it agrees with our generic concept of *Wolfina* except for Zhuang & Wang's (1998) characterization of the tissues of the excipulum being gelatinous. This seems a contradiction given that they describe the flesh as corky when dried rather than drying to

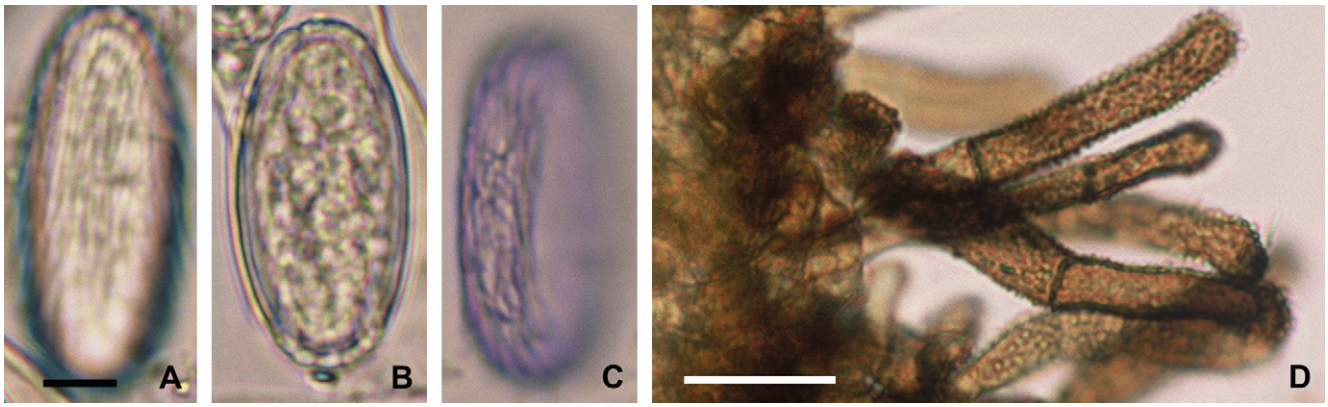

Fig 10 – *Wolfina aurantiopsis*. (A–C) Ascospores in Cotton Blue in lactic acid. (D) Section showing hairs on the outer surface. FH, DHP 06-617, Bars = (A–C) 5 μm, (D) 20 μm.

a horny consistence as gel-containing taxa often do. *W. oblongispora* has larger spores than *W. aurantiopsis* [36–45 × 15–22 μm *versus* 27–33 × 16–18 μm according to Zhuang & Wang (1998)]. The questionable identity of *W. papuana* aside, the distributional patterns of *Chorioactis* and *Wolfina* are similar in that each has a disjunct North American–Asian distribution.

Specimens of Wolfina aurantiopsis *examined:* **USA:** *Connecticut*: Marlboro, on duff and moss, *Bill Neill, det. Bill Roody* (FH, DHP 06-617). *Florida*: Coconut Grove, on wood, 1897–1898, *R. Thaxter, 3636* [det. E. J. Durand as *Sarcosoma carolinianum*] (FH). *North Carolina*: Chatham Co.: White Pine Conservancy Stand, in hardwoods, 3 Jul 2003, *L. Grand & C. S. Vernia* (FH). *Ohio*: Fairfield Co.: Benua Estate, 14 Aug 1976, *R. P. Korf 4337* (CUP). *Tennessee:* Cherokee Orchard, Great Smoky Mountains National Park, on wood, 17 Aug 1939, *R. W. Davidson & J. A. Stevenson* (BPI); Hamilton Co.: Tennessee River Gorge Trust, 15 miles NW of Chattanooga, at end of Edward's Point Road, 35° 8′ 49.9″ N, 85° 22′ 44.4″ W, 549 m elev. grapevine stem on ground, 2 cm. diam, 7 Jul 2005, *A.N. Miller (ANM428), W.S. Sundburg* (Herb. Miller, Herb. Sundburg).

Discussion

The results presented here suggest two possible treatments of the *Chorioactis* clade. It might be treated as a subfamily in *Sarcosomataceae* or the taxa might be placed in a separate family. Given the strong support provided by molecular data and distinct morphological and cytological characters, we have chosen to recognize a separate family. *Sarcosomataceae,* with the exclusion of *Chorioactidaceae*, are well delimited and are characterized by externally dark coloured ascomata, multinucleate spores, and uninucleate paraphyses (Berthet 1964b). Moreover, to a greater or lesser degree, all members of *Sarcosomataceae s. str.* have gel in the excipulum. Although *Chorioactidaceae* have multinucleate spores, in contrast to *Sarcosomataceae*, they have lighter coloured hymenia, multinucleate cells of the paraphyses (Berthet 1964b; Berthet & Riousset 1965), and ascomatal flesh that lacks gelatinous material.

Le Gal (1958) first recognized the importance of gelatinous tissues when she augmented the concept of the *Urnuleae* (which included most *Sarcosomataceae s. str.*). She concluded that all members of the tribe had gelatinous tissues. In some instances the gel was prominent and copious; in others the gel was associated with hyphal walls only. The only member of *Chorioactidaceae* mentioned by Le Gal was *C. geaster* (as *Urnula geaster*) which she unequivocally excluded from the *Urnuleae*, considering it to belong to a very different but unstated group. Eckblad (1968: 115) treated both *Chorioactis* and *Wolfina* in *Sarcoscyphaceae* tribe *Urnuleae*, along with *Desmazierella*, a placement he qualified with the following comment: 'Actually, the genus is an aberrant element in any family'. Korf & Waraitch (1971: 101) echo his statement: '*Desmazierella* surely represents a somewhat anomalous element in the *Sarcosomateae*'. *Wolfina* was recognized as being close to *Chorioactis* by Eckblad (1968) based on their similarly ornamented hairs and our study has confirmed this.

Missing from our study is the genus *Thindia*. This genus, with one species, *T. cupressi*, was compared with *Desmazierella* by Korf & Waraitch (1971). It was placed in *Sarcoscyphaceae* tribe *Sarcoscypheae*. Material of this species has not been available for molecular study. It is up to 1.5 mm in diam, yellow–orange to orange, has brown setose hairs, occurs on dead needles of *Cupressus*, and has four spored-asci, with ascospores ranging up to 37 μm (Korf & Waraitch 1971). Further, in the original description, abruptly rounded ascus bases are described and this is reminiscent of the asci of *Chorioactidaceae*. We cannot further comment on the species or the placement of the genus, but we can not rule out a placement of it among the *Chorioactidaceae*. Those with access to specimens or to the regions where this taxon was collected, Nainital Hills and the Mussoorie Hills, Uttar Pradesh, India, surely should seek and study this fungus.

Acknowledgements

Roy Kristiansen kindly provided many collections of *Desmazierella acicola* from Norway for our study. Andrew Miller, Larry Grand, and William Neill provided material of *Wolfina aurantiopsis*. Forrest Mims III sent material and observations of *Chorioactis geaster* on several occasions, as did Harold Keller and K. C. Rudy; we thank them and the following individuals for photographs: Jens H. Petersen © MycoKey, Andrew Miller, Henry Aldrich, and Lorelei Norvell. We are indebted to Genevieve Lewis-Gentry for the Latin diagnosis and to Kanchi Gandhi, Harvard University Herbaria, for nomenclatural advice. Brian Perry and Katherine LoBuglio added valuable comments. National Science Foundation DEB 9521944 and DEB 0315940 provided funding.

REFERENCES

Arpin N, 1969. Les caroténoïdes des Discomycètes: essai chimiotaxinomique. *Bulletin Mensuel de la Société Linnéenne de Lyon* **38** (Suppl): 1–169.

Bellemère A, Malherbe MC, Chacun H, Meléndez-Howell LM, 1990. L'étude ultrastructurale des asque et des ascospores de l'*Urnula hevelloides* Donadini, Berthet et Astier et les concepts de'asque suboperculé et de *Sarcosomataceae*. *Cryptogamie, Mycologie* **11**: 203–238.

Bellemère A, Meléndez-Howell LM, Chacun H, Malherbe MC, 1994. Les asques du *Chorioactis geaster* (*Ascomycetes, Pezizales, Sarcoscyphaceae*), leur déhiscence et leurs ascospores: étude ultrastructurale. *Nova Hedwigia* **58**: 49–65.

Benkert D, 1991. Bemerkenswerte Ascomyceten der DDR. 12. *Sarcoscyphaceae* und *Sarcosomataceae (Pezizales)*. *Gleditschia* **19**: 173–201.

Berthet P, 1964a. Formes conidiennes de divers discomycètes. *Bulletin de la Société Mycologique de France* **80**: 125–149.

Berthet P, 1964b. *Essai Biotaxinomique sur le Discomycètes*. Joanny Lorge, Lyon.

Berthet P, Riousset L, 1965. Un *Urnula* nouveau des cedraies provençales: *Urnula pouchetii* nov. sp. (discomycètes operculés). *Bulletin Mensuel de la Société Linnéenne de Lyons* **34**: 253–261.

Cao JZ, Fan L, Liu B, 1992. Notes on the genus *Galiella* in China. *Mycologia* **84**: 261–263.

Cherubini A, Perrone L, 1994. *Neournula pouchetii* (Berthet et Riousset) Paden. Segnalazione di un ascomicete primaverile. *Bollettino dell'Associazione Micologica et Ecologica Romana* **11**: 31–34.

Denison WC, 1972. Differentiation of tribes and genera in the family *Sarcoscyphaceae*. *Persoonia* **6**: 433–438.

Denton AL, McConaughy BL, Hall BD, 1998. Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. *Molecular Biology and Evolution* **15**: 1082–1085.
Eckblad F-E, 1968. The genera of operculate discomycetes: a re-evaluation of their taxonomy, phylogeny and nomenclature. *Nytt Magasin for Botanikk* **15**: 1–191.
Eriksson OE (ed), 2006. Outline of *Ascomycota* —2006. *Myconet* **12**: 1–82.
Farr DF, Bills GF, Chamuris GP, Rossman AY, 1989. *Fungi on Plants and Plant Products in the United States*. APS Press, St Paul.
Fernández Vicente J, Undagoitia J, 2001. Gorbeia Parke Naturaleko lau askomizeto [Cuatro ascomicetos del Parque Natural del Gorbeia]. *Belarra* **17-18**: 37–43.
Fouchier F, Neville P, 1998. *Hypotarzetta insignis, Neournula pouchetii* et *Geopora sumneriana*: Ascomycota printaniers del cedraie du Petit Luberón. *Bulletin Semestriel de la Fédération des Associations Mycologiques Méditerranéennes N.S.* **13**: 32–44, 48.
Galán R, Raitviir A, 1995. *Desmazierella acicola* Libert in Spagna. *Rivista di Micologia* **38**: 39–44.
Gremmen J, 1949. De imperfecte vorm van *Desmazierella acicola* Lib. *Fungus* **19**: 32–35.
Hansen K, Pfister DH, Hibbett DS, 1999. Phylogenetic relationships among species of *Phillipsia* inferred from molecular and morphological data. *Mycologia* **91**: 299–314.
Hansen K, Læssøe T, Pfister DH, 2001. Phylogenetics of the *Pezizaceae*, with an emphasis on *Peziza*. *Mycologia* **93**: 958–990.
Hansen K, LoBuglio KF, Pfister DH, 2005. Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: RPB2, β-tubulin, and LSU rDNA. *Molecular Phylogenetics and Evolution* **36**: 1–23.
Hansen K, Pfister DH, 2006 ['2007']. Systematics of the *Pezizomycetes* — the operculate discomycetes. *Mycologia* **98**: 1029–1040.
Harrington FA, Pfister DH, Potter D, Donoghue MJ, 1999. Phylogenetic studies within the *Pezizales* I. 18S rDNA sequence data and classification. *Mycologia* **91**: 41–50.
Heald FD, Wolf FA, 1910. The structure and relationship of *Urnula geaster*. *Botanical Gazette* **49**: 182–188.
Huelsenbeck JP, Ronquist F, 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.
Hughes SJ, 1951. Studies on micro-fungi. IX. *Calcarisporium, Verticicladium*, and *Hansfordia* (*gen. nov.*). *Mycological Papers* **43**: 3–25.
Huhtinen S, Mäkinen Y, 1984. Finnish records of discomycetes: a new species in the monotypic genus *Desmazierella (Pezizales)*. *Mycotaxon* **20**: 551–557.
Imazeki R, 1938. A rare fungus *Urnula geaster* Peck grows in Kyusyu, Japan. *Journal of Japanese Botany* **14**: 680–684.
Imazeki R, Otani Y, 1975. Rediscovery of *Chorioactis geaster* (Peck) Eckblad in Kyusyu, Japan. *Transactions of the Mycological Society of Japan* **16**: 222–229.
Imazeki R, Otani Y, Hongo T, 1988. *Nihon no kinoko*. Yama to Keikokusha, Tokyo.
James P, Whelen S, Hall BD, 1991. The RET1 gene of yeast encodes the second largest subunit of the RNA polymerase III. *Journal of Biological Chemistry* **266**: 5616–5624.
Keller HW, Rudy KC, 1995. The devil's cigar. *Mycophile* **36**: 1–2, 6.
Kendrick WB, Burges A, 1962. Biological aspects of the decay of *Pinus sylvestris* leaf litter. *Nova Hedwigia* **4**: 313–342.
Korf RP, 1970. Nomenclatural notes. VII. Family and tribe names in the *Sarcoscyphineae (Discomycetes)* and a new taxonomic disposition of the genera. *Taxon* **19**: 782–786.
Korf RP, 1972. Synoptic key to the genera of the *Pezizales*. *Mycologia* **64**: 937–994.
Korf RP, 1973. *Discomycetes* and *Tuberales*. In: Ainsworth CG, Sparrow FK, Sussman AS (eds), *The Fungi: an Advanced Treatise*. Academic Press, New York, pp. 249–319.
Korf RP, Waraitch KS, 1971. *Thindia*, a new genus of the *Sarcoscyphineae (Pezizales)*. *Mycologia* **63**: 98–103.
Korf RP, Zhuang W-Y, 1991. A preliminary discomycete flora of Macaronesia: part 11, *Sarcoscyphineae*. *Mycotaxon* **40**: 1–11.
Kowalski T, 1988. Zur Pilzflora toter Kiefernnadeln. *Zeitschrift für Mykologie* **54**: 159–173.
Kupfer EM, 1902. Studies on *Urnula* and *Geopyxis*. *Bulletin of the Torrey Botanical Club* **29**: 137–144.
Kurogi S, Nagao H, Kiyota E, 2002. Investigation of the evolution conditions and environment of endangered fungi *Chorioactis geaster*. *Miyazaki Prefectural Museum of Nature and History Bulletin* **23**: 23–45.
Larsen Jr HJ, Denison WC, 1978. A checklist of the operculate cup-fungi (*Pezizales*) of North America west of the Great Plains. *Mycotaxon* **7**: 68–90.
Le Gal M, 1947. *Recherches sur les Ornementations Sporales des Discomycètes Operculés*. Masson, Paris.
Le Gal M, 1953. Les Discomycètes de Madagascar. *Prodrome flore mycologique Madagascar* **4**: 1–465.
Le Gal M, 1958. Discomycètes du Maroc. I. Un *Urnula* nouveau: *Urnula megalocrater* Malençon et Le Gal sp. nov. Etude de l'espèce, suivie d'une révision des caractères des genres *Urnula* Fr. et *Sarcosoma* Casp. *Bulletin Trimestriel de la Société Mycologique de France* **74**: 155–177.
Le Gal M, 1963. Valeur taxinomique particulière de certains caractères chez les discomycètes supérieurs. *Bulletin Trimestriel de la Société Mycologique de France* **79**: 456–470.
Libert M-A, 1829. Description d'un nouveau genre de champignons nommé *Desmazierella*. *Annales des Sciences Naturelles* **17**: 82–83.
van Maanen A, Gourbière F, 1997. Host and geographical distribution of *Verticicladium trifidum, Thysanophora penicillioides*, and similar fungi on decaying coniferous needles. *Canadian Journal of Botany* **75**: 699–710.
Maddison DR, Maddison WP, 2000. *MacClade 4: Analysis of Phylogeny and Character Evolution* Version 4.0. Sinauer Associates, Sunderland, MA.
Malençon G, 1979. Nouvelles contributions a la flore mycologique du Maroc — II. *Bulletin Trimestriel de la Société Mycologique de France* **95**: 119–137.
Melendez-Howell LM, Coute A, Mascarell G, Bellemère A, 1998. Ultrastructure des asques et des ascospores de *Desmazierella acicola* (*Sarcoscyphaceae, Pezizales, Ascomycetes*). Interet systematique et biologique. *Mycotaxon* **68**: 53–74.
Merkus E, 1976. Ultrastructure of the ascospore wall in *Pezizales* (ascomycetes) — IV. *Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae*, and *Sarcoscyphaceae*. General discussion. *Persoonia* **9**: 1–38.
Moncalvo J-M, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R, 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* **49**: 278–305.
Nannfeldt JA, 1949. Contributions to the mycoflora of Sweden. 7. A new winter discomycete, *Urnula hiemalis* Nannf. n. sp., and a short account of the Swedish species of *Sarcoscyphaceae*. *Svensk Botanisk Tidskrift Utgifven af Svenska Botaniska Foreningen* **43**: 468–484.
Nylander JAA, 2004. *MrModeltest* v. 2.2. Evolutionary Biology Centre, Uppsala University, Uppsala.
Otani Y, 1975. Some discomycetes collected in Papua New Guinea. In: Otani Y (ed), *Reports on the Cryptogams in Papua New Guinea*. National Science Museum, Tokyo, pp. 5–41.
Otani Y, 1980. *Sarcoscyphineae* of Japan. *Transactions of the Mycological Society of Japan* **21**: 149–179.
Paden JW, Tylutki EE, 1968. *Idaho Discomycetes*. I. A new genus of the *Sarcoscyphaceae*. *Mycologia* **60**: 1160–1168.
Pascual R, Rocabruna, A, 1988. *Neournula pouchetii*. In: *Bolets de Catalunya*. Societat Catalana de Micologia, Barcelona. Lam. núm. 337.

Péricouche A, 1995. Contribution à l'étude du genre *Urnula* Fr. dans le département du Loiret (45). *Bulletin Trimestriel de la Société Mycologique de France* **111**: 300–301.
Perry BA, Hansen K, Pfister DH, 2007. A phylogenetic overview of the family *Pyronemataceae* (*Ascomycota, Pezizales*). *Mycological Research* **111**: 549–571.
Peterson KR, Bell CD, Kurogi S, Pfister DH, 2004. Phylogeny and biogeography of *Chorioactis geaster* (*Pezizales, Ascomycota*) inferred from nuclear ribosomal DNA sequences. *Harvard Papers in Botany* **8**: 141–152.
Pfister DH, Kurogi S, 2004. A note on some morphological features of *Chorioactis geaster* (*Pezizales, Ascomycota*). *Mycotaxon* **89**: 277–281.
Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
Rudy KC, Keller HW, 1996. The rare and fascinating devil's cigar, *Chorioactis geaster*. *Mycologist* **10**: 33–35.
Samson RA, Jackson RE, 1977. Mycologische waarnemingen in Texas. *Coolia* **20**: 38–43.
Seaver FJ, 1937. Photographs and descriptions of cup-fungi—XXVIII. A proposed genus. *Mycologia* **29**: 678–680.
Seaver FJ, 1939. *Urnula geaster*. *Mycologia* **31**: 367–368.
Seaver FJ, 1942. *The North American Cup-fungi (Operculates)*, Supplemented edn. The author, New York.
Shaw CG, 1972. *Host Fungus Index for the Pacific Northwest. I, Hosts*. Washington Agricultural Experiment Station, Washington State University, Pullman.
Swofford DL, 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, beta version 4.0. Sinauer Associates, Sunderland, MA.
Tubaki K, Saito T, 1969. *Endophragmia alternata* sp. nov. and other hyphomycetes on *Pinus* leaves in Japan. *Transactions of the British Mycological Society* **52**: 477–482.
Wolf FA, 1958. Mechanism of apothecial opening and ascospore expulsion by the cup-fungus *Urnula craterium*. *Mycologia* **50**: 837–843.
Zhuang WY, Wang Z, 1998. Sarcosomataceous discomycetes in China. *Mycotaxon* **67**: 355–364.