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Molecular phylogeny reveals the true colours of Myeloconidaceae (Ascomycota: Ostropales)

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Abstract. The lichen-forming fungal family Myeloconidaceae, with the single genus *Myeloconis*, has been suggested to share affinities with Porinaceae (Lecanoromycetes: Ostropales). We examined its position relative to this family by using molecular data from the mitochondrial small-subunit and nuclear large-subunit rDNA. Our results revealed that *Myeloconis* forms a monophyletic group nested within Porinaceae, closely related to *Porina farinosa*. Neither *Porina s.str.* nor *Clathroporina sensu* Harris form monophyletic groups; instead, two strongly supported clades were recovered, which differ in ascospore septation (septate *v.* muriform), with the clade producing muriform ascospores including *Myeloconis*. We therefore reduce Myeloconidaceae to synonymy with Porinaceae; however, because generic delimitations within Porinaceae remain unclear, we retain *Myeloconis* as a separate genus within the family. The species concept currently used in the genus, based largely on secondary metabolites and ascospore measurements, is supported by the phylogeny.

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Introduction

In their treatment of lichen-forming fungi in the Guianas in the family Porinaceae (as Trichotheliacae), Aptroot and Sipman (1993) noted collections identified as *Clathroporina enteroxantha*, with yellow medullary pigmentation. McCarthy (1995) re-examined some of these collections and concluded that they represented a new genus known also from Brazil, Australia, Melanesia and Malaysia. The genus *Myeloconis* (Fig. 1) was subsequently established to accommodate this group of four crustose species with yellow to orange medullary phenalenones (Ernst-Russell *et al*. 2000), producing perithecial ascomata with a dense perithecial wall (McCarthy and Elix 1996). In addition, these species lack an involucrellum and periphyses, but form basally anastomosing paraphyses that are simple above, asci that are thin-walled, unitunicate and lack an apical apparatus, and produce hyaline, muriform ascospores that are elongate in shape (McCarthy and Elix 1996; McCarthy 2001*a*, 2001*b*). The phenalenones often burst through the thallus (Fig. 1), together with fungal hyphae and algal cells, and these structures have been suggested to possibly fulfill a reproductive role similar to soredia (McCarthy 2001*a*). Species within the genus are distinguished on the basis of spore and perithecial size, as well as the presence or absence of various secondary metabolites. *Myeloconis* associates with trentepohlioid algae and is corticolous, occurring in lowland tropical rainforests (McCarthy and Elix 1996).

When describing *Myeloconis*, McCarthy and Elix (1996) noted that its thallus morphology and chemistry, along with the basally anastomosing hamathecium, suggested affinities with Trypetheliaceae, whereas its ascospore shape, ascus structure (unitunicate) and the otherwise mostly free paraphyses suggested a relationship with Porinaceae. They felt it more likely that *Myeloconis* was related to Porinaceae than Trypetheliaceae, but did not rule out the possibility of it belonging to an undescribed family, and left the genus as *incertae sedis* (McCarthy and Elix 1996). Later, McCarthy (2001*a*, 2001*b*) placed *Myeloconis* in a new family, Myeloconaceae (corrected to Myeloconidaceae by McCarthy 2003), and argued for its distinction from Porinaceae (as Trichotheliaceae) on the basis of its chemistry, lack of an involucrellum and the presence of a deeply pigmented wall of periclinal cells external to the exciple (McCarthy 2001*a*, 2001*b*).

Porinaceae is a medium-sized family of over 400 species world-wide (McCarthy 2001*c*, 2003, 2013), being most diverse in the tropics and decreasing in species richness toward higher latitudes, although it remains rich in New

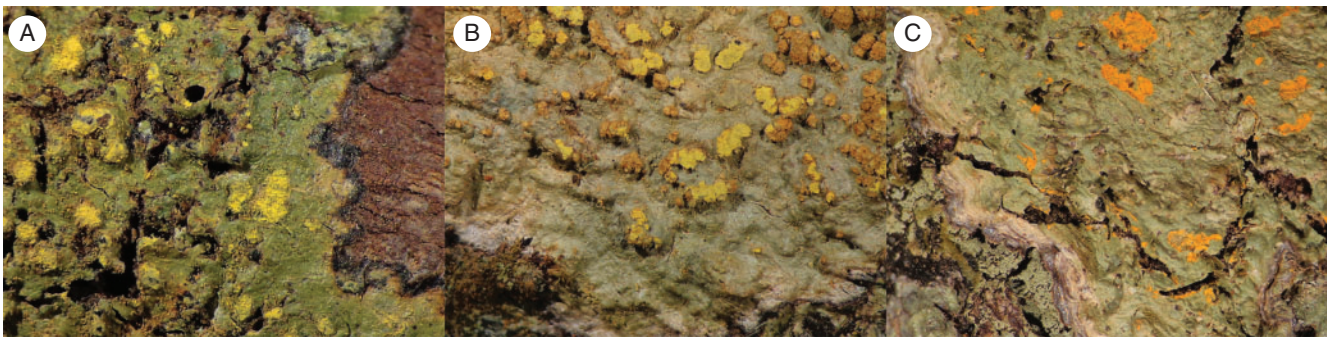

Fig. 1. A–C. Habit images of three of the four known *Myeloconis* species (A. *M. erumpens*. B. *M. guyanensis*. C. *M. fecunda*). Note the brightly coloured medullary phenalenones erupting from the thallus.

Zealand and Tasmania (McCarthy 1993, 2003; McCarthy and Kantvilas 2000). Taxa primarily occur on bark, rock and leaves, although some are known to grow over bryophytes (McCarthy and Kantvilas 2000; McCarthy 2003; Lücking 2008). All species associate with trentepohlioid photobionts (Santesson 1952; McCarthy 2003; Baloch and Grube 2006; Lücking 2008; Nelsen *et al*. 2011*a*). Porinaceae produce crustose thalli that are typically greenish, sometimes with a hypothallus, and often with calcium-oxalate crystals in the thallus that form a distinct layer (crystallostratum); in addition, they form immersed to sessile, variously coloured, perithecial ascomata with angiocarpous ascohymenial development, a distinct to vestigial, sometimes absent involucrellum, cylindrical to obclavate, thin-walled asci without tholus that are functionally unitunicate, paraphyses which are generally unbranched, variously developed or lacking periphyses, and hyaline, transversely septate to muriform ascospores (Swinscow 1962; Vězda 1968; Janex-Favre 1971; Henssen and Jahns 1974; Hafellner and Kalb 1995; Harris 1995; Lücking 2008).

The family Porinaceae (as Trichotheliaceae) was placed in the order Trichotheliales (Hafellner and Kalb 1995), and McCarthy (2001*a*, 2001*b*, 2003) suggested that also Myeloconidaceae belonged to that order. The first available molecular data, provided by Bhattacharya *et al*. (2000), suggested that *Porina* formed part of Lecanoromycetes, specifically being sister to *Stereocaulon* in the Lecanorales; this, however, stemmed from the sequences of the species used (*P. guentheri*) apparently being incorrectly labelled or from contaminants (the nuSSU appears to be part of Dothideomycetes, whereas the nuLSU is suggested to belong to Parmeliaceae and includes a rare intron). Nevertheless, the placement of Porinaceae in Lecanoromycetes was subsequently shown by Grube *et al*. (2004), who demonstrated that it belonged in Ostropomycetidae, not Lecanoromycetidae, close to taxa in Ostropales. Hibbett *et al*. (2007) later synonymised Trichotheliales with Ostropales, a change further supported by the phylogenetic analysis of Baloch *et al*. (2010), which placed Porinaceae in an expanded Ostropales (*sensu* Kauff and Lutzoni 2002).

Here we utilise molecular sequence data to (1) assess the monophyly of *Myeloconis* and (2) reconstruct the phylogenetic position of Myeloconidaceae relative to Porinaceae.

Materials and methods

Taxon selection

We sequenced fungal DNA from 10 neo- and paleotropical collections of *Myeloconis* representing three of the four known species, as well as 13 collections of *Porina s.lat.* representing species currently placed in either *Porina s.str.* or *Clathroporina* (Table 1).

Molecular methods

The Sigma REDExtract-N-Amp Plant PCR Kit (Sigma–Aldrich, St Louis, MO, USA) was used to isolate DNA, following the manufacturer's instructions, except that 10–25 µL of extraction buffer and 10–25 µL dilution buffer were used, and a 20× DNA dilution was then utilised in subsequent polymerase chain reaction (PCR) reactions. Portions of the fungal mitochondrial small subunit (mtSSU) and nuclear ribosomal large subunit (nuLSU) were amplified using the mrSSU1, mrSSU2R, mrSSU3R (Zoller *et al*. 1999) and mrSSU-2/3–5′-mpn (Nelsen *et al*. 2011*b*) primers for the mtSSU, and the AL2R (Mangold *et al*. 2008), f-nu-LSU-0116–5′/ITS4A-5′ (Nelsen *et al*. 2011*b*, 2012; reverse complement of D. L. Taylor's ITS4A in Kroken and Taylor 2001), LR3 (Vilgalys and Hester 1990) and LR3-Porina-mpn (the present study: CCA TTA CGC CMG CAT CCG TGC) primers for the nuLSU. The 10-µL PCR reactions consisted of 5 µM of each PCR primer, 2 µL diluted DNA and 5 µL REDExtract-n-Amp PCR Ready Mix (Sigma–Aldrich). The PCR cycling conditions were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 53°C (mtSSU) or 55°C (nuLSU) for 1 min, and 72°C for 1 min, followed by a single 72°C final extension for 7 min. Samples were visualised on a 1% ethidium bromide-stained agarose gel under UV light and bands were gel extracted, heated at 70°C for 5 min, cooled to 45°C for 10 min, treated with 1 µL GELase (Epicentre Biotechnologies, Madison, WI, USA) and incubated at 45°C for at least 24 h.

The 10-µL cycle sequencing reactions consisted of 1–1.5 µL of Big Dye version 3.1 (Applied Biosystems, Foster City, CA, USA), 2.5–3 µL of Big Dye buffer, 1–6 µM primer, 0.75–2 µL of GELase-treated PCR product and water. Samples were sequenced with PCR primers. The cycle sequencing conditions were as follows: 96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Samples were

Table 1. Species in the phylogenetic analysis, specimen information (provided for newly sequenced collections) and GenBank accession numbers
Alpha-numeric codes following species names refer to DNA isolate numbers. Dataset O refers to Ostropales dataset, whereas P+M refers to Porinaceae +Myeloconidaceae dataset

Family	Species	Collection	Dataset	nuLSU	mtSSU
Baeomycetaceae	*Phyllobaeis erythrella*		O	DQ986780	DQ986888
Cladoniaceae	*Pycnothelia papillaria*		O	DQ986800	DQ986783
Coccotremataceae	*Coccotrema cucurbitula*		O	AF274092	AF329161
Coenogoniaceeae	*Coenogonium luteum*		O/P+M	AF279387	AY584699
Coenogoniaceeae	*Coenogonium pineti*		O/P+M	AY300834	AY300884
Graphidaceae	*Calenia monospora*		O	AY341351	AY341365
Graphidaceae	*Diorygma antillarum*		O	JX046465	JX046452
Graphidaceae	*Diploschistes cinereocaesius*		O	DQ883799	DQ912306
Graphidaceae	*Echinoplaca epiphylla*		O	AY341354	AY648891
Graphidaceae	*Fissurina insidiosa*		O	DQ973045	DQ972995
Graphidaceae	*Gyalidea hyalinescens*		O	DQ973046	DQ972996
Graphidaceae	*Heiomasia seaveyorum*		O	JX046462	GU395553
Gyalectaceae	*Gyalecta jenensis*		O	AF279391	AY340493
Gyalectaceae	*Gyalecta ulmi*		O	AF465463	AY584706
Lecanoraceae	*Lecanora achariana*		O	DQ973027	DQ972976
Letrouitiaceae	*Letrouitia domingensis*		O	AY584648	AY584619
Megalosporaceae	*Megalospora tuberculosa*		O	AY584650	AY584623
Myeloconidaceae	*Myeloconis erumpens* MPN21	Philippines, *Rivas Plata* 1024B, BC700 (F)	P+M		KJ449320
Myeloconidaceae	*Myeloconis erumpens* MPN77	Fiji, *Lumbsch* 19756 g (F)	P+M		KJ449321
Myeloconidaceae	*Myeloconis erumpens* MPN107	Thailand, *Lücking* 24061 (F)	O/P+M	KJ449335	KJ449322
Myeloconidaceae	*Myeloconis erumpens* MPN778	New Caledonia, *Lumbsch* 8233 (F)	O/P+M	KJ449338	KJ449328
Myeloconidaceae	*Myeloconis fecunda* MPN3C	Peru, *Nelsen* s.n. (F)	O/P+M	KJ449334	KJ449319
Myeloconidaceae	*Myeloconis fecunda* MPN757	Brazil, *Cáceres and Aptroot* 11895 (F)	O/P+M	KJ449336	KJ449323
Myeloconidaceae	*Myeloconis fecunda* MPN759	Brazil, *Cáceres and Aptroot* 11308 (F)	O/P+M	KJ449337	KJ449325
Myeloconidaceae	*Myeloconis guyanensis* MPN758	Brazil, *Cáceres and Aptroot* 11739 (F)	O/P+M		KJ449324
Myeloconidaceae	*Myeloconis guyanensis* MPN760	Puerto Rico, *Mercado-Díaz* 3372 F53 (F)	O/P+M		KJ449326
Myeloconidaceae	*Myeloconis guyanensis* MPN761	Puerto Rico, *Mercado-Díaz* F25 (F)	O/P+M		KJ449327
Nephromataceae	*Nephroma parile*		O	AY584656	AY584625
Odontotremataceae	*Coccomycetella richardsonii*		O	HM244761	HM244737
Odontrotremataceae	*Odontotrema phacidioides*		O	HM244770	HM244749
Pannariaceae	*Degelia plumbea*		O	DQ912347	DQ912299
Parmeliaceae	*Pseudevernia consocians*		O	DQ986754	DQ986868
Parmeliaceae	*Tuckermannopsis ciliaris*		O	DQ986755	DQ986870
Phlyctidaceae	*Phlyctis agelaea*		O	AY853381	AY853332
Phlyctidaceae	*Phlyctis argena*		O	DQ986771	DQ986880
Porinaceae	*Porina aenea*		P+M		HM244754
Porinaceae	*Porina alba*		P+M		FJ711089
Porinaceae	*Porina atrocoerulea*		P+M		DQ168389
Porinaceae	*Porina byssophila*		P+M		HM244755
Porinaceae	*Porina cryptostoma* MPN11	Costa Rica, *Lücking* 16117a (F)	O/P+M	KJ449329	KJ449308
Porinaceae	*Porina dolichophora* MPN5B	Costa Rica, *Lücking* AS HT1 (F)	P+M		KJ449306
Porinaceae	*Porina* aff. *dolichophora* MPN7B	Costa Rica, *Lücking* PIIr(7) P7-MR (F)	P+M		KJ449307
Porinaceae	*Porina epiphylla*		P+M		FJ711103
Porinaceae	*Porina exasperatula* MPN69	Panama, *Lücking* Pan-Comp. PAN-18 (F)	P+M		KJ449316
Porinaceae	*Porina farinosa* MPN35	Panama, *Lücking* Pan-02 (F)	O/P+M	KJ449332	KJ449311
Porinaceae	*Porina farinosa* MPN36	Panama, *Lücking* Panama-Crane, Pan-03 (F)	O/P+M	KJ449333	KJ449312
Porinaceae	*Porina guianensis*		P+M		DQ168384
Porinaceae	*Porina heterospora* MPN158	Brazil, *Nelsen* s.n. (F)	P+M		KJ449318

(*Continued next page*)

Table 1. (*continued*)

Family	Species	Collection	Dataset	nuLSU	mtSSU
Porinaceae	*Porina imitatrix* MPN37	Panama, *Lücking* Pan26 (F)	P+M		KJ449313
Porinaceae	*Porina imitatrix* MPN68	Panama, *Lücking* Pan-Comp. PAN-17 (F)	P+M		KJ449315
Porinaceae	*Porina karnatakensis*		P+M		FJ711115
Porinaceae	*Porina lucida*		P+M		FJ711122
Porinaceae	*Porina nitidula*		P+M		DQ168392
Porinaceae	*Porina nucula* MPN13B	Costa Rica, *Lücking* 17007c (F)	O/P+M	KJ449331	KJ449310
Porinaceae	*Porina papillifera*		P+M		DQ168395
Porinaceae	*Porina repanda*		P+M		AY64889
Porinaceae	*Porina simulans*		P+M		DQ168379
Porinaceae	*Porina* sp. MPN70	Panama, *Lücking* Pan-Comp. PAN-19 (F)	P+M		KJ449317
Porinaceae	*Porina subepiphylla*		P+M		DQ168380
Porinaceae	*Porina subnitidula*		P+M		DQ168394
Porinaceae	*Porina tetracerae* MPN17B	Costa Rica, *Lücking* 17038b (F)	O/P+M	KJ449330	KJ449309
Porinaceae	*Porina tetracerae* MPN38	Panama, *Lücking* Panama-Cupanella, Pan-07 (F)	P+M		KJ449314
Porinaceae	*Trichothelium annulatum*		P+M		DQ168415
Porinaceae	*Trichothelium pallidisetum*		P+M		AY648900
Psoraceae	*Psora decipiens*		O	AY756343	AY567772
Sagiolechiaceae	*Rhexophiale rhexoblephara*		O	AY853391	AY853341
Sagiolechiaceae	*Sagiolechia protuberans*		O	HM244775	HM244757
Sphaerophoraceae	*Sphaerophorus globosus*		O	DQ986767	DQ986866
Stictidaceae	*Acarosporina microspora*		O	AY584643	AY584612
Stictidaceae	*Stictis radiata*		O	AF356663	AY300914
Trapeliaceae	*Orceolina kerguelensis*		O	AY212830	AF381561

precipitated and sequenced in an 3730 DNA Analyzer (Applied Biosystems), and sequences assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA) and sequences submitted to GenBank (Table 1).

Phylogenetic analyses

Initial BLASTn (Altschul *et al*. 1997) searches of *Myeloconis* sequences revealed close matches with Ostropales; consequently, we assembled a dataset (Ostropales dataset) focused on this order, including representatives of most families (Table 1). Lecanoromycetidae taxa were included as outgroup representatives. These initial analyses suggested that Myeloconidaceae was part of or closely related to Porinaceae; therefore, we constructed a second, more focused dataset (Porinaceae+Myeloconidaceae dataset), including representatives of these two families. We sequenced nuLSU and mtSSU from Porinaceae specimens and supplemented these with sequences from GenBank representing a further 16 OTUs in Porinaceae. Additionally, sequences of two Coenogoniaceae taxa were retrieved from GenBank and used as the outgroup (Table 1). Sequences were aligned in Mesquite v. 2.75 (Maddison and Maddison 2010) using a combination of manual and automated (MUSCLE 3.6: Edgar 2004) alignment in MAFFT v. 7.029b (Katoh *et al*. 2002) using the l-ins-i algorithm. Introns and manually delimited ambiguous regions were removed and the resulting alignment was deposited in TreeBase (Study ID 15391).

For both datasets, a maximum-likelihood (ML) analysis, which was partitioned by locus, was performed in RAxML 7.4.4 (Stamatakis 2006), using the GTRGAMMA model. Support was estimated by utilising 1000 fast-bootstrap pseudoreplicates (Stamatakis *et al*. 2008). A Bayesian analysis was also performed, using Markov chain Monte Carlo (MCMC) sampling (Larget and Simon 1999) in MrBayes 3.2.1 (Ronquist *et al*. 2012). We performed a reversible-jump MCMC analysis (Huelsenbeck *et al*. 2004), partitioning the dataset by gene and employing the time-reversible class of substitution models with a gamma distributed rate heterogeneity. This allowed for exploration of different submodels with the GTR+G model space, and liberated us from *a priori* model testing. Two parallel analyses were run at a temperature of 0.1 in MrBayes for 30 000 000 generations, with four chains each, sampling every 1000 generations. The program AWTY (Wilgenbusch *et al*. 2004; Nylander *et al*. 2008) was used to diagnose convergence between parallel runs by the creation of a bivariate plot of bipartitions. Furthermore, the average standard deviation of split frequencies (Lakner *et al*. 2008) dropped below 0.01, and the potential scale-reduction factor (Gelman and Rubin 1992) for all parameters was found to approach 1.0. Initial burn-in trees (initial 25%) were discarded for each run and a majority-rule consensus tree was constructed. Relationships were considered supported if they had ML bootstrap support (BS) values of 70 or greater and Bayesian posterior probabilities (PP) of 0.95 or greater. To assess potential conflict among loci, individual ML phylogenies were constructed for each locus as described above. We compared supported clades from the single-locus phylogenies using the python program compat.py 3.0 (Kauff and Lutzoni 2002, 2003); conflict among supported clades was taken as evidence for topological incongruence. All ML and Bayesian analyses were performed in the Cipres Web Portal 3.3 (Miller *et al*. 2010).

We also evaluated the monophyly of selected morphological and ecological characters used to define *Myeloconis* and genera in Porinaceae. These included substrate, ascospore septation, the presence or absence of yellow–orange medullary pigments, and the *Clathroporina*-type thallus and prothallus combination (shiny thallus with shiny, violet–black prothallus). Character states were retrieved from the literature and sequenced specimens and mapped onto the tips of the phylogeny.

Finally, we conducted a Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) to assess whether the monophyly of *Porina* could be rejected. Using the Ostropales dataset, we conducted ML searches to obtain the best-known tree under the following two topological constraints: (1) monophyletic *Porina* and monophyletic *Myeloconis*; and (2) monophyletic *Porina* (but *Myeloconis* was left unconstrained). These trees were then compared to the best-known ML tree obtained from the unconstrained analysis using the SH test as implemented in RAxML. Because assumptions for the SH test are frequently violated (Goldman *et al*. 2000), we also performed 500 replicates of the Swofford–Olsen–Waddell–Hillis (SOWH) test (Swofford *et al*. 1996) for each constraint using the Sowhat Perl script (Church *et al*. 2014), RAxML 7.9.5 (Stamatakis 2006) and Seq-Gen 1.3.2x (Rambaut and Grassly 1997).

Results

The final Ostropales alignment consisted of 1816 sites (645 mtSSU, 1171 nuLSU) and conflict was detected among the placement of *Fissurina insidiosa*, which had a supported placement with the Graphidaceae subfamily Gomphilloideae in the nuLSU analysis, whereas in the mtSSU analysis, it formed a supported relationship with the Graphidaceae subfamily Graphidoideae. We retained *F. insidiosa*, because we assumed that its position was not germane to the groups of interest, and proceeded with further analyses. The more detailed Porinaceae +Myeloconidaceae dataset consisted of 1117 sites (651 mtSSU, 466 nuLSU), and no conflict was detected among loci. Bayesian analyses of both the Ostropales and Porinaceae +Myeloconidaceae datasets demonstrated that no single substitution model was found to achieve an exceptionally high posterior probability; instead, several GTR+G submodels were sampled. Analysis of the Ostropales dataset confirmed the placement of *Myeloconis* in this order with strong support (Fig. 2). The phylogenies of the combined Ostropales and Porinaceae+Myeloconidaceae datasets recovered a strongly supported, monophyletic *Myeloconis* that was embedded within Porinaceae (Figs 2, 3). Analyses of both datasets revealed that *Myeloconis* formed a strongly supported sister relationship with *Porina farinosa* (Figs 2, 3). The SH and SOWH tests rejected a topology in which *Porina* and *Myeloconis* each formed monophyletic groups, as well one in which monophyly of *Porina* was enforced (both rejected at $P \leq 0.01$).

Analysis of the Porinaceae+Myeloconidaceae dataset further revealed that the *Myeloconis–P. farinosa* clade formed a sister group to a strongly supported clade containing the remainder of Porinaceae, including members of *Trichothelium* and the *P. epiphylla* (*Phylloporina*), *P. nucula* (*Porina s.str*.), *P. dolichophora*, *P. radiata*, *P. nitidula* and *P. rufula* groups (*Pseudosagedia* and *Segestria*) (Fig. 3). Within this clade, several well supported clades emerged, although support for relationships among these clades was weak (Fig. 3). In addition to relationships recovered in previous studies, our results highlight the non-monophyly of the *P. imitatrix* group (*Clathroporina sensu* Harris 1995, here represented by *P. exasperatula*, *P. imitatrix*, *P. tetracerae*), with *P. imitatrix* grouping with *P. alba* (*P. epiphylla* group) with strong support, whereas *P. tetracerae* grouped with *P. karnatakensis* and other members of the *P. epiphylla* group, and *P. exasperatula* with *P. dolichophora*. *Trichothelium s.lat.*, including *Pseudosagedia* (*P. atrocoerula*, *P. nitidula*, *P. papillifera*, *P. repanda*, *P. subnitidula*), was recovered as a strongly supported, monophyletic clade, but there was not support for *Trichothelium s.str.* and *Pseudosagedia* being reciprocally monophyletic; instead, *Trichothelium* appears embedded within *Pseudosagedia*. Our analysis also revealed the lack of support for groups defined by substrate preferences (foliicolous *Phylloporina* (*P. epiphylla* and *P. radiata* groups, the latter not included in the present study)), suggesting that substrate shifts have occurred multiple times within this lineage. Notably, ascospore septation appeared correlated with phylogeny, with taxa forming muriform ascospores (*Myeloconis*, *Porina farinosa*) occurring in a clade separate from those with transversely septate ascospores (Fig. 3). Within the clade containing taxa producing transversely septate ascospores, taxa with very long, narrow, tapering, multiseptate ascospores (*P. dolichophora*, *P. exasperatula*) separated from those with broader, fusiform, mostly seven-septate ascospores (*P. nucula* and relatives).

We examined the *P. farinosa* collections (Fig. 4A–C) to determine whether their anatomy and morphology were more consistent with that of *Porina* or *Myeloconis*. Ascomatal sections revealed a narrow, pale yellow, prosoplectenchymatous, basally closed exciple, covered by a thick thalline, basally expanded layer resembling an involucrellum; that layer was composed of an inner, dark-pigmented layer adjacent to the exciple, a thick crystallostratum, a thin photobiont layer, and a thin, hyaline, corticiform layer. Close to the ostiole, the covering layer lacked a photobiont layer and crystals, as well as the inner, dark-pigmented layer, and instead was formed by an orange to dark brown (bordering the ostiole) tissue covered by a hyaline, corticiform layer, which in turn covered the narrow, true exciple reaching up to the ostiole. In addition, we observed periphysoids near the ostiolar region, and ellipsoid (to fusiform), distinctly muriform ascospores with thickened walls. McCarthy (1995) described the presence of an involucrellum for species of *Porina* (*Clathroporina*) with muriform ascospores; however, his illustrations (e.g. of *C. eminentior* and *C. exocha*) depicted a covering thalline layer, including algae and crystal clusters, similar to what we found in the two specimens here identified as *P. farinosa*. To confirm this, we also investigated the sequenced sample of *P. nucula* and found its perithecial sections very similar to those of the sequenced *P. farinosa*.

Within *Myeloconis*, our sampling included three of the four known species. Representatives of *M. fecunda* formed a strongly supported, monophyletic group. This taxon is characterised by the presence of myeloconone B, and ascospores 17–26 µm in length. Our data also showed a strongly supported, monophyletic

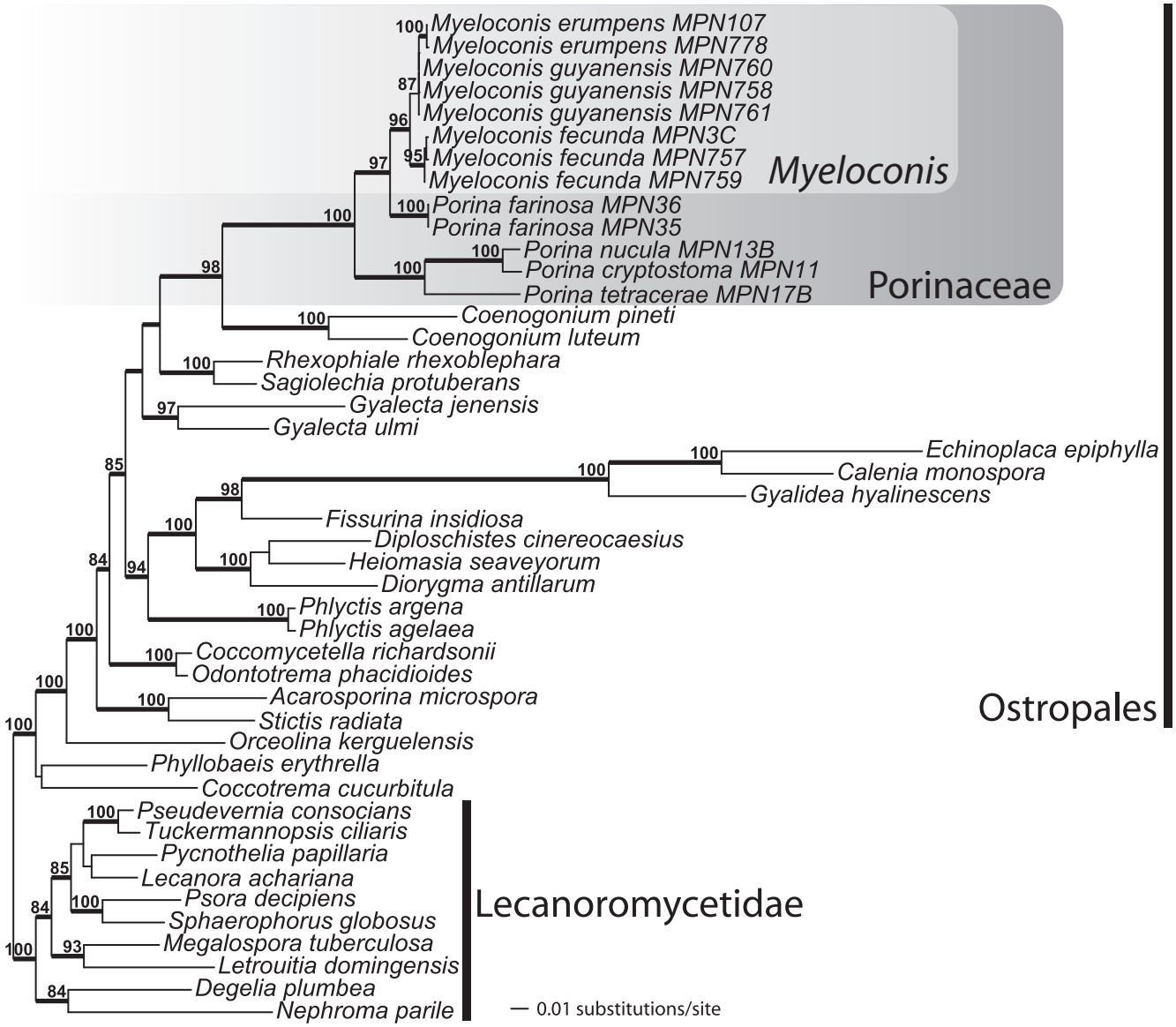

Fig. 2. Phylogenetic relationships of Ostropales as inferred from the maximum-likelihood (ML) analysis of the combined mtSSU + nuLSU dataset. Bootstrap support values ≥70 in the ML analysis are indicated above the branch, and branches with a Bayesian posterior probability ≥0.95 are thickened. Alpha-numeric codes following species names refer to DNA-isolate numbers (see Table 1).

M. erumpens, which is characterised by its large ascospores (145–207 µm long) and the presence of myeloconone A as a major component. However, this species is embedded within an unsupported, paraphyletic *M. guyanensis*, a taxon characterised by small ascospores (22–34 µm long) and the presence of myeloconone A as a major component.

Discussion

The present study confirmed that Myeloconidaceae is not related to Trypetheliaceae but belongs in Ostropales close to Porinaceae, as suggested by previous authors (McCarthy and Elix 1996; McCarthy 2001*a*, 2001*b*, 2003). However, instead of forming a separate family, our results suggested it is nested within Porinaceae. This poses a challenge to the currently accepted classification, because retaining Myeloconidaceae as a separate family would result in a paraphyletic Porinaceae. The critical taxon appears to be *Porina farinosa* (Fig. 4) which, although agreeing with *P. nucula* (the type of *Porina*) in all features except the muriform ascospores, forms a strongly supported sister relationship with *Myeloconis* (Fig. 3). The topology in Fig. 3 permits the retention of Myeloconidaceae with the inclusion of *P. farinosa*; however, we argue against this because the distinction between the two families, (perithecial anatomy and medullary chemistry) would be erased by the inclusion of *P. farinosa* in Myeloconidaceae. The only character in the present taxon sampling shared exclusively between *Myeloconis* and *P. farinosa* is the muriform ascospores, but

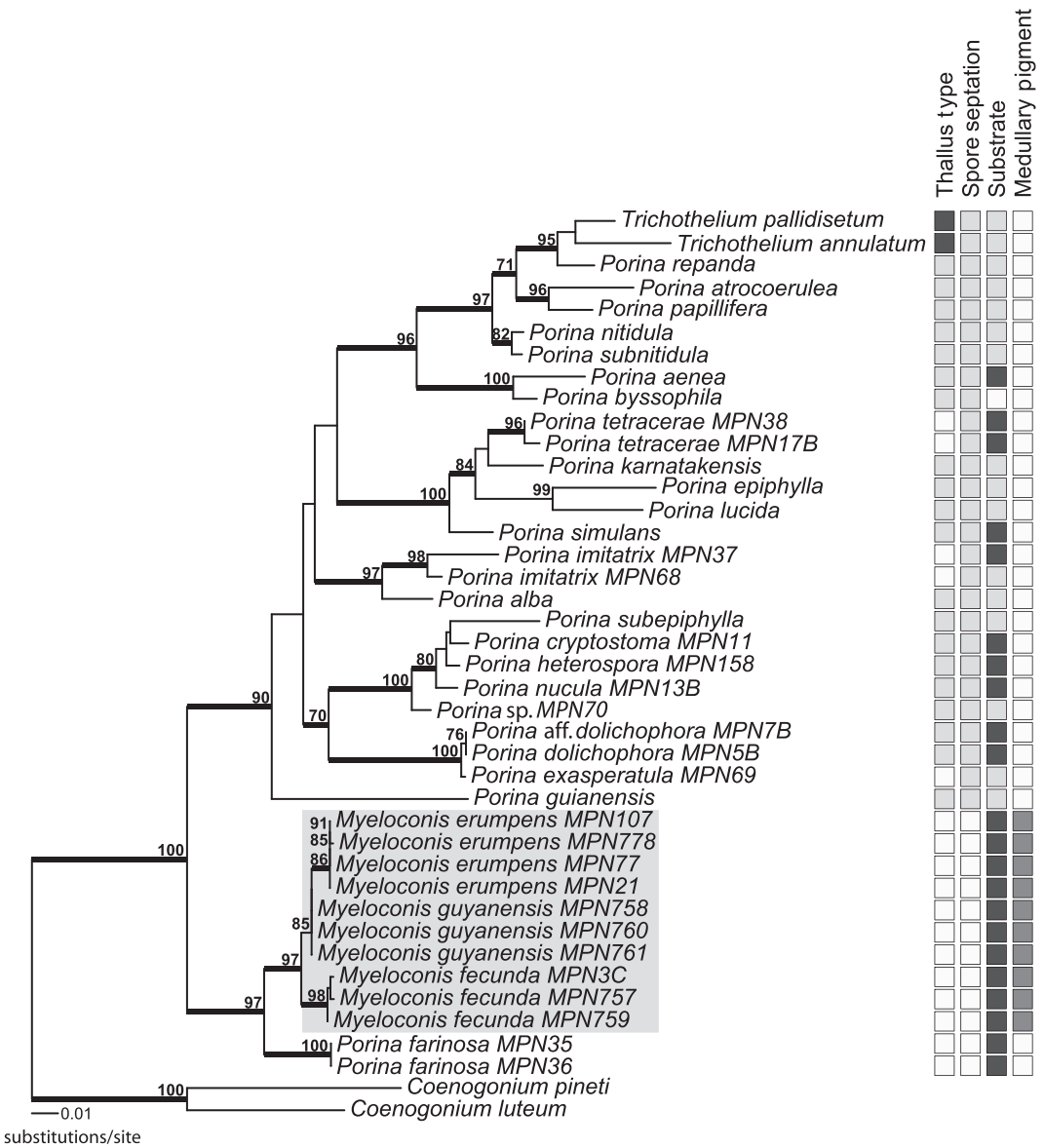

Fig. 3. Phylogenetic relationships of Porinaceae as inferred from the maximum-likelihood (ML) analysis of the combined mtSSU + nuLSU dataset. Bootstrap support values ≥70 in the ML analysis are indicated above the branch, and branches with a Bayesian posterior probability ≥0.95 are thickened. Alpha-numeric codes following species names refer to DNA isolate numbers (see Table 1). Several characters (and their states) are given on the right-hand side of the figure. Thallus type: *Porina s.str.* type (light grey), *Trichothelium* type (dark grey), *Clathroporina* type (white). Spore septation (ascospores): transversely septate (light grey), muriform (white). Substrate: stone (white), leaves (light grey), bark (dark grey). Medullary pigment (yellow–orange medullary crystals): absent (white) and present (light grey).

their shape is very different (long-fusiform *v.* ellipsoid) and the ascospores of *P. farinosa* are more consistent with those of species currently placed in *Porina s.lat.* (McCarthy 1995). We, therefore, see no alternative to reducing Myeloconidaceae to synonymy with Porinaceae (see below).

Revision of the type material of *P. farinosa* and its synonyms (McCarthy 1995) revealed that possibly three different taxa are involved. The types of *P. farinosa* C.Knight, described from Australia, and *Thelenella elaeophthalma* Vain., described from the Caribbean, have thin, distinctly verrucose thalli and prominent, hemispherical to wart-shaped perithecia. In contrast, the type of *Clathroporina superans* Müll. Arg. from Africa has a non-verrucose thallus and prominent perithecia, whereas the types of *Thelenella turgidula* Vain. and *T. irregularis* Vain., both from the Caribbean (Vainio 1896, 1915, 1923), are more similar to *Clathroporina eminentior* in their slightly glossy thallus and largely immersed, lens-shaped perithecia. Perithecial anatomy and ascospore details in the types of *P. farinosa* and *Thelenella elaeophthalma* agree with the material sequenced here; hence, we are confident with our

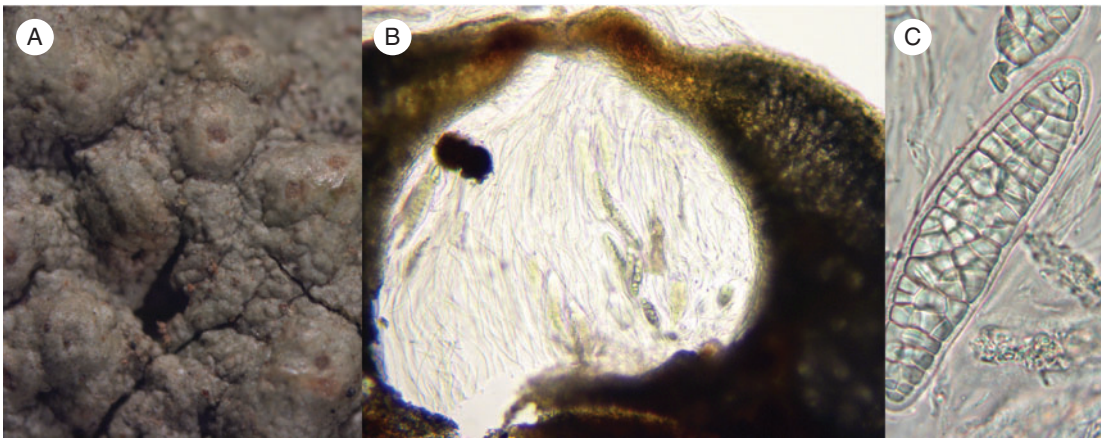

Fig. 4. Images of *Porina farinosa*. A. Habit. B. Ascomatal section. C. Thick-walled, muriform ascospore.

identification of this material as *P. farinosa*. In any case, the correct identification of this material does not affect the finding that its thallus and perithecial morphology and anatomy, as well as its chemistry, conforms to that of *P. nucula* and is different from *Myeloconis*, with which it clusters with strong support.

Generic delimitation within Porinaceae has long been debated (Santesson 1952; Hafellner and Kalb 1995; Harris 1995; Malcolm and Vězda 1995; McCarthy and Malcolm 1997; Lücking 1998, 2004, 2008), with none of the proposed classification schemes being supported by molecular data (Baloch and Grube 2006). Presently, *Porina* and *Trichothelium* are universally accepted in variable definitions, whereas the recognition of additional genera, such as *Clathroporina*, *Polycornus*, *Pseudosagedia*, *Segestria* and *Zamenhofia*, is disputed (McCarthy and Malcolm 1997; Lücking 1998, 2004, 2008; Hafellner and Türk 2001; Aptroot 2002; McCarthy 2003; Santesson *et al*. 2004; Harris 2005; Galloway 2007; Orange *et al*. 2009; Lumbsch and Huhndorf 2010). Our topology largely agrees with that of Baloch and Grube (2006), confirming the non-monophyly of several proposed segregate genera of *Porina*. With our increased taxon sampling, we were further able to confirm the non-monophyly of the *P. imitatrix–P. eminentior* group (*Clathroporina*). The largely unsupported backbone of our phylogeny would permit the possible recognition of the following five genus-level taxa: *Myeloconis*, the *P. farinosa* group, *Segestria* (species with red-walled perithecia lacking a crystallostratum), *Porina s.str.* (species with red-walled perithecia and crystallostratum, including *Clathroporina* and *Phylloporina*) and *Trichothelium s.lat.* (species with black-walled perithecia lacking a crystallostratum, including *Pseudosagedia* and *Zamenhofia*). To clarify this situation, further species of *Segestria* and *Clathroporina* must be sequenced, particularly the type species of the latter, *C. eminentior*. As *Trichothelium* appears embedded within *Porina* (Fig. 2; Baloch and Grube 2006), further work is needed to address its continued recognition or synonymisation.

In contrast to the non-monophyly observed for most other species groups or generic segregates in Porinaceae, specifically in the taxa with a crystallostratum, our data support the monophyly of *Myeloconis*. However, further work is needed to critically test species delimitation within this genus. Except for the medullary pigments, species of *Myeloconis* fit well within the family definition and are morphologically reminiscent of species currently placed in *Clathroporina sensu* Harris (1995). Although the medullary chemistry is unique for *Myeloconis*, it does not necessarily warrant separation at the family level, because similar variation is, for example, known from Graphidaceae, where species with pigmented medulla are concentrated in a single genus, *Ocellularia s.str.* (Rivas Plata *et al*. 2012, 2013). Therefore, synonymising Myeloconidaceae with Porinaceae and placing *Myeloconis* in the latter family, on the basis of molecular data, do not unduly conflict with morphological or anatomical characters. Whether increased sampling will uphold the monophyly of *Myeloconis* remains to be seen, but given these results, we argue for the retention of the genus *Myeloconis* while genera are re-delimited in Porinaceae. The situation is complicated by the close relationship of *Myeloconis* with *P. farinosa*, a taxon that shares the general morphology and anatomy with *P. nucula* and related species (McCarthy 1995, 2001*c*). If *Myeloconis* is retained in its current sense, then *P. farinosa* and other species clustering here would have to be placed in a genus different from *Porina s.str.* The only argument for this would be the muriform ascospores, a character otherwise shared with *Myeloconis*. Therefore, expanded sampling of other species with muriform ascospores, especially *Clathroporina eminentior*, is required to resolve this issue. Future work should also attempt to determine the position of the enigmatic genus *Amphorothecium*. This genus has been described as sharing many features with *Myeloconis*, and also produces large, thick-walled ascospores (McCarthy *et al*. 2001). Its apically thin-walled asci suggest it may occupy a position close to Porinaceae, Coenogoniaceae or Gyalectaceae in Ostropales.

Revised taxonomy

Porinaceae Rchb. [as 'Porineae'], Consp. Regn. Veg.: 20 (1828)

Type. Porina Ach. [nom. cons.; conserved type: P. nucula Müll. Arg.].

= Myeloconidaceae P. M. McCarthy [as 'Myeloconaceae'], Fl. Australia 58A: 227 (2001).

Type: *Myeloconis* P. M. McCarthy & Elix.

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