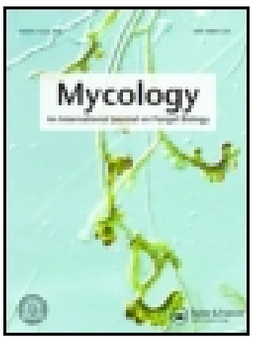

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Gyalectoid *Pertusaria* species form a sister-clade to *Coccotrema* (Ostropomycetidae, Ascomycota) and comprise the new lichen genus *Gyalectaria*

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The phylogeny and taxonomic placement of three species currently placed in the genus *Pertusaria* with gyalectoid ascomata were studied using maximum likelihood and Bayesian analysis of four molecular loci (mitochondrial SSU, nuclear LSU rDNA and the protein-coding, nuclear *RPB1* and *MCM7* genes). A total of 40 new sequences were generated for this study and aligned with 84 sequences retrieved from Genbank. Our results show that the gyalectoid *Pertusaria* species are only distantly related to *Pertusaria* s.str. They form a strongly supported sister-group relationship to *Coccotrema.* Consequently, the new genus *Gyalectaria* Schmitt, Kalb & Lumbsch is described in Coccotremataceae to accommodate these species and the new combinations *G. diluta* (C. Björk, G. Thor & T. Wheeler) Schmitt, T. Sprib. & Lumbsch, *G. gyalectoides* (Vezda) Schmitt, Kalb & Lumbsch, and *G. jamesii* (Kantvilas) Schmitt, Kalb & Lumbsch are proposed. The order Pertusariales is reduced to synonymy with Agyriales.

Keywords: Agyriales; Coccotremataceae; *Gyalectaria*; lichen-forming fungi; *MCM7*; new genus, Pertusariaceae; Pertusariales; phylogeny

Introduction

The morphology of the fruiting bodies of lichen-forming Ascomycota, the ascomata, is known to be phylogenetically unstable and similar fruiting body types have been shown to have evolved several times independently in separate clades (Schmitt et al. 2009a). Lichen-forming pyrenomycetes (with perithecia), for example, have been shown to belong to different classes, such as Dothideomycetes, Eurotiomycetes, and Lecanoromycetes (Del Prado et al. 2006, Lumbsch and Huhndorf 2007, Lumbsch et al. 2004, Lumbsch et al. 2005b, Lutzoni et al. 2001, Lutzoni et al. 2004, Miadlikowska et al. 2006, Schmitt et al. 2005). Ostropomycetidae, a subclass in Lecanoromycetes (Hibbett et al. 2007), is a perfect example for the diversity of ascoma morphologies. Within this suborder there are a number of taxa having perithecioid fruiting bodies, such as Coccotremataceae, Porinaceae, Protothelenellaceae and Thelenellaceae (Grube et al. 2004, Lumbsch et al. 2001, Lumbsch et al. 2007b, Schmitt et al. 2005, Schmitt et al. 2001). Other families are characterized by apothecioid, even stalked ascomata, such as Arctomiaceae, Baeomycetaceae, Ochrolechiaceae or Trapeliaceae (Lumbsch et al. 2005a, Lumbsch et al. 2007a, Miadlikowska et al. 2006, Schmitt et al. 2006). Some families have intermediate, urceolate to gyalectoid ascomata, including Gyalectaceae (Kauff and Lutzoni 2002, Kauff and Büdel 2005) or show a remarkable variability of ascoma-types, including perithecioid to apotheciate or hysterothecioid forms, such as Pertusariaceae or Graphidaceae (incl. Thelotremataceae) (Lumbsch and Schmitt 2002, Mangold et al. 2008, Schmitt and Lumbsch 2004, Staiger et al. 2006).

The classification of families and genera is currently poorly understood in Ostropomycetidae and this is especially true for the genus *Pertusaria,* the largest genus in Pertusariaceae. The genus is in urgent need of re-circumscription, because it has been found to be polyphyletic with at least three distinct and unrelated clades being recognized (Lumbsch and Schmitt 2001, 2002, Lumbsch et al. 2006, Schmitt and Lumbsch 2004, Schmitt et al. 2006). Within the large and heterogeneous group "*Pertusaria*", there is a small group of three species with gyalectoid ascomata, i.e. having an open disc that is sunken (urceolate) with a well-developed emergent margin (Figure 1B,C). These species are very different morphologically from other groups in *Pertusaria* and resemble members of the genus *Gyalecta.* In fact gyalectoid *Pertusaria* spp. are often confused with species of *Gyalecta* in the field; however, they are readily distinguished by simple ascospores and a different ascus-type. Gyalectoid *Pertusaria* spp. are rarely collected and occur in New Guinea, Australasia and southern South America, and one species has been described from North America (Montana/British Columbia) (Archer 2004,

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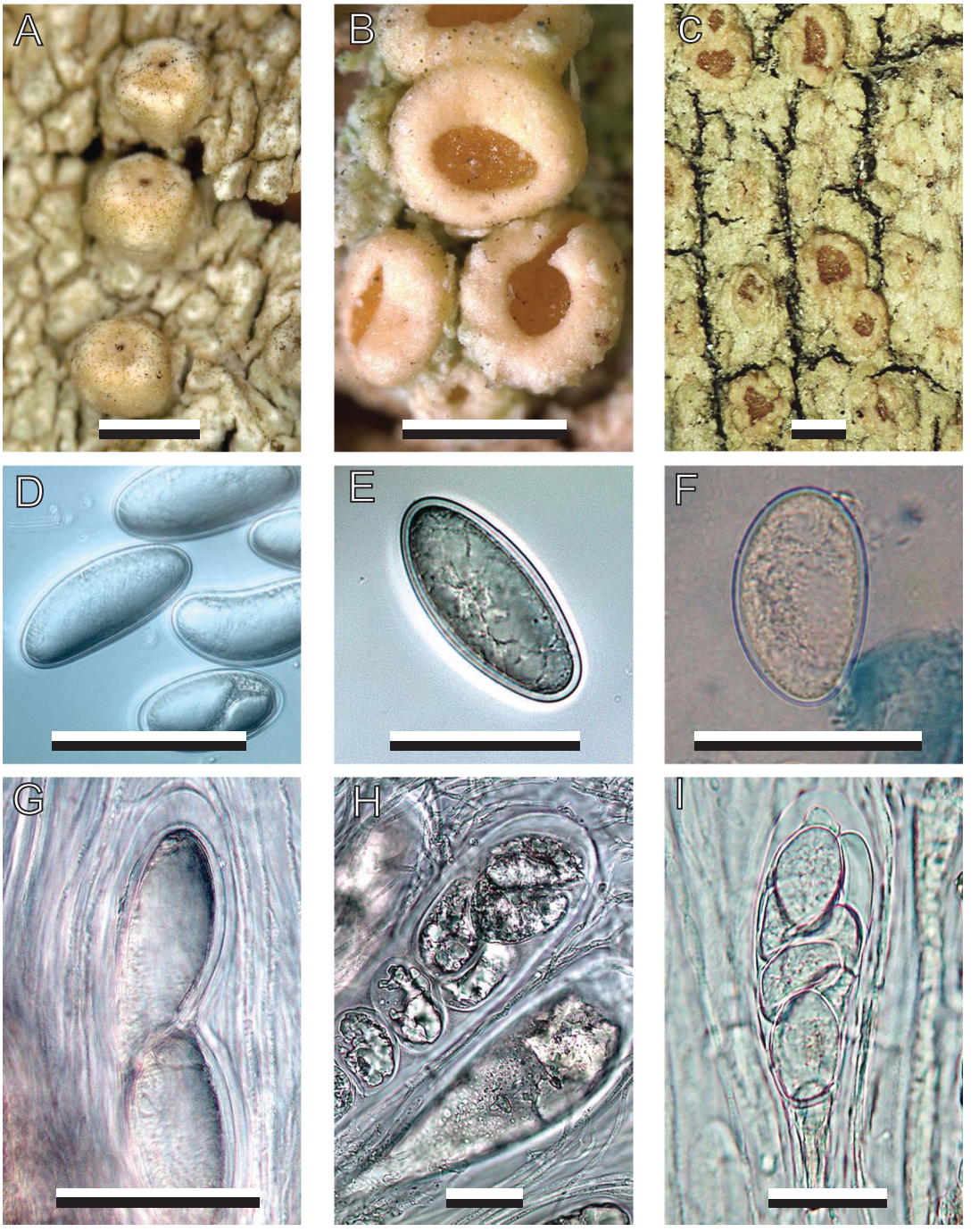

Figure 1. Morphological characters of *Coccotrema cucurbitula* (A,D,G), *Gyalectaria gyalectoides* (B,E,H) and *Gyalectaria jamesii* (C,F,I). A–C: ascomata; D–F: ascospores; G–I: ascus morphology. Scale bar: A–C 1 mm, D–I 100 µm. C,F,I taken by K. Kalb.

Galloway 2007, Kantvilas 1990, Spribille et al. 2009, Weber 1971). The taxonomic placement of these species has not been studied in detail, but Spribille et al. (2009) indicated that the placement of these species in *Pertusaria* remains uncertain. We have now assembled molecular data from these three species and additional similar taxa to study (a) whether the three gyalectoid *Pertusaria* species are closely related to each other and (b) their phylogenetic placement in Pertusariales. We have used a multi-locus approach to address these questions, including ribosomal sequences of nuclear and mitochondrial DNA and *RPB1* sequences that have previously been shown to be useful in elucidating phylogenetic relationships in this group of lichenized fungi (Lumbsch et al. 2007b, Schmitt and

Lumbsch 2004). In addition, we obtained sequences of the single-copy, protein-coding gene *MCM7,* which has recently been shown to be useful in uncovering evolutionary relationships in Ascomycota (Aguileta et al. 2008, Schmitt et al. 2009b).

Materials and methods

Taxon sampling

Data on 32 species were assembled using sequences of mtSSU rDNA, nuLSU rDNA, and the protein-coding, single-copy genes *RPB1* and *MCM7*. Specimens and sequences used for the molecular analyses are listed in Table 1. Sequences of *Everniopsis trulla* and *Parmeliopsis hyperopta* were used as outgroup based on their placement in the sister-group of Ostropomycetidae, Lecanoromycetidae (Schmitt et al. 2009b).

DNA extraction, amplification and sequencing

We extracted total genomic DNA from the lichen samples using the Qiagen Plant Mini Kit (Qiagen). PCR reactions

Table 1. Species and specimens used in the current study with GenBank accession numbers (newly obtained sequences in bold). Classification follows Lumbsch and Huhndorf (2009).

Name	Taxonomic group/phylogenetic lineage	Source	mtSSU	nuLSU	RPB1	Mcm7
Agyrium rufum	Agyriaceae	Sweden, Wedin 7931 (UPS)	EF581823	EF581826	EF581822	**GU980988**
Arctomia delicatula	Arctomiaceae	–	AY853307	AY853355	DQ870929	GQ272388
Arctomia teretiuscula	Arctomiaceae	–	DQ007349	DQ007346	DQ870930	GQ272389
Aspicilia contorta	Megasporaceae	USA, Wetmore, MIN 808806	DQ986876	DQ986782	DQ986852	**GU980989**
Aspicilia hispida	Megasporaceae	–	DQ780273	DQ780305	DQ870933	DQ780273
Coccotrema cucurbitula	Coccotremataceae	Argentina, Wirtz 11d (F)	AF329161	AF274092	DQ870939	**GU980990**
Coccotrema maritimum	Coccotremataceae	Canada, Schmitt, 13 June 2004 (F)	AF329163	AF329164	N/A	**GU980991**
Coccotrema pocillarium	Coccotremataceae	USA, Printzen, 12 Sep 1999 (ESS)	AF329166	AF274093	DQ870940	**GU980992**
Dibaeis baeomyces	Icmadophilaceae	–	AY300883	AF279385	DQ842011	N/A
Everniopsis trulla	Parmeliaceae (outgroup)	–	EF108289	EF108290	EF105429	GQ272396
Gyalectaria diluta	–	Canada, Spribille 23882 (F)	**GU980974**	**GU980982**	N/A	N/A
Gyalectaria gyalectoides	–	Fiji, Lumbsch 19837a (F)	**GU980975**	**GU980983**	**GU981006**	**GU980993**
Gyalectaria jamesii	–	Australia, Lumbsch 19983c (MIN)	**GU980976**	**GU980984**	**GU981007**	N/A
Icmadophila ericetorum	Icmadophilaceae	–	DQ986897	DQ883694	DQ883723	N/A
Lobothallia radiosa	Megasporaceae	Switzerland, Lumbsch, 9 Aug 2004 (F)	DQ780274	DQ780306	DQ870954	GQ272397
Ochrolechia parella	Ochrolechiaceae	Turkey, Lumbsch, 19625g (MIN)	**GU980977**	AF274097	DQ870959	GQ272421
Ochrolechia subpallescens	Ochrolechiaceae	USA, Lumbsch 19900a (MIN), 19903b (MIN)	**GU980978**	**GU980985**	**GU981008**	**GU980994**
Ochrolechia upsaliensis	Ochrolechiaceae	USA, Lumbsch 19916e (MIN)	**GU980979**	**GU980986**	**GU981009**	**GU980995**
Parmeliopsis hyperopta	Parmeliaceae (outgroup)	–	AY611167	AY607823	EF092142	GQ272426
Pertusaria amara	Pertusariaceae (s. lat.)	USA, Lumbsch 19925a (MIN)	AY300900	AF274101	DQ870965	GQ272423
Pertusaria californica	Pertusariaceae	USA, Lendemer L-5810 (hb Lendemer)	N/A	N/A	**GU981010**	**GU980996**
Pertusaria carneopallida	Pertusariaceae	Norway, Haugan 7560, L-151383 (O)	N/A	**GU980987**	**GU981011**	N/A
Pertusaria corallina	Pertusariaceae (s. lat.)	Germany, Dürhammer 1276 (hb. Dürhammer)	AY300901	AY300850	DQ870967	**GU980997**
Pertusaria hemisphaerica	Pertusariaceae (s. lat.)	Germany, Schmitt, 15 April 2004 (MIN)	DQ973000	AF381556	DQ902341	**GU980998**
Pertusaria hermaka	Pertusariaceae	Australia, Mangold, 22 March 2005 (MIN)	DQ780299	DQ780334	N/A	**GU980999**
Pertusaria lactea	Pertusariaceae (s. lat.)	Germany, Lumbsch, Sept 2000 (F)	AF381564	AF381557	DQ870971	**GU981000**
Pertusaria paramerae	Pertusariaceae	Turkey, Halici & Kocakaya, MGH 0.4367	**GU980980**	DQ780328	**GU981012**	**GU981001**
Pertusaria pustulata	Pertusariaceae	Japan, Yamamoto 14112707 (AKITA)	DQ780297	DQ780332	**GU981013**	**GU981002**
Pertusaria scaberula	Pertusariaceae (s. lat.)	USA, Lumbsch 19254b (MIN)	AF431959	AF274099	DQ870980	**GU981003**
Pertusaria subventosa	Pertusariaceae (s. lat.)	Australia, Lumbsch 19070a (F)	AY300905	AY300854	DQ870981	**GU981004**
Pertusaria velata	Pertusariaceae (s. lat.)	USA, Lumbsch 19913c (MIN)	**GU980981**	AY300855	DQ870982	**GU981005**
Thamnolia vermicularis	Icmadophilaceae	–	AY853345	AY961599	DQ915599	N/A

(25 µl) contained PuReTaq Ready-To-Go PCR beads (GE Healthcare), 1.25 µl of each primer (10 mM), 19.5 µl H_2O and 3 µl DNA template. We used the primers mrSSU1 (Zoller et al. 1999) and MSU7 (Zhou and Stanosz 2001) for amplification of mtSSU, nuLSU-0155-5′ (=AL1R) (Döring et al. 2000) and nuLSU-1125-3′ (=LR6) (Vilgalys and Hester 1990) for nuLSU, gRPB1-A (Stiller and Hall 1997) and fRPB1-C (Matheny et al. 2002) for *RPB1*, and Mcm7-709for and Mcm7-1348rev (Schmitt et al. 2009b) for *MCM7*. PCR cycling conditions for most PCRs were as follows: initial denaturation 94°C for 10 min, followed by 38 cycles of 94°C for 45 s, 50°C for 30 s, 72°C for 1 min, and final elongation 72°C for 5 min. We used 54°C annealing temperature for nuLSU and *RPB1*. Amplification products were stained with EZ-Vision DNA dye (Amresco) and viewed on 1% low melt agarose gels. We sequenced the fragment using Big Dye 3.1 chemistry (Applied Biosystems) and the same primers as for PCR. Cycle sequencing was executed with the following program: initial denaturation for 1 min at 96°C followed by 32 cycles of 96°C for 15 s, 50°C for 10 s, 60°C for 4 min. Sequenced products were precipitated with 25 µl of 100% EtOH mixed with 1 µl of 3 M NaOAC and 1 µl of EDTA, before they were loaded on an ABI PRISM™ 3730 DNA Analyzer (Applied Biosystems). We assembled partial sequences using SeqMan 4.03 (Lasergene) and edited conflicts manually. Fungal mitochondrial small subunit rDNA sequences contain highly variable sequence portions. Since standard multiple alignment programs become less reliable when sequences show a high degree of divergence, we employed an alignment procedure that uses a Hidden Markov Model (HMM) method as implemented in the software PRANK (Loytynoja and Goldman 2005, 2008). We eliminated unreliably aligned sites from the alignment using the program Aliscore 2.0 (Misof and Misof 2009). Aliscore settings were: window size of six positions, and gaps treated as ambiguous characters (-N option invoked).

Sequence alignments and phylogenetic analyses

We analyzed the alignments using maximum parsimony, maximum likelihood, and Bayesian inference. To test for potential conflict, we performed parsimony bootstrap analyses on each individual data set, and examined 75% bootstrap consensus trees for conflict (Lutzoni et al. 2004). Maximum parsimony analyses were performed using the program PAUP* (Swofford 2003). Heuristic searches with 200 random taxon addition replicates were conducted with tree bisection reconnection (TBR) branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein 1985) was performed based on 2000 replicates with random sequence additions.

We analyzed the concatenated alignment using MrBayes 3.1 (Huelsenbeck and Ronquist 2001). The analyses were performed assuming the general time reversible model of nucleotide substitution (Rodriguez et al. 1990), including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G). This model was determined as best fitting model using the program MrModeltest v2 (Nylander 2004). A run with 10,000,000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 1000th tree was saved into a file. The first 1000 trees were deleted as the "burn in" of the chain. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to ensure that stationarity was achieved after the first 300,000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist 2001). Additionally, we used AWTY (Nylander et al. 2007) to compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining trees, a majority rule consensus tree with average branch lengths was calculated using the "sumt" option of MrBayes. Posterior probabilities were obtained for each clade. Only clades with bootstrap support equal or above 70% under MP and ML, and posterior probabilities ≥0.95 in the Bayesian analysis were considered as strongly supported.

The ML analysis was performed using the program RAxML (Stamatakis 2006) using the default rapid hill-climbing algorithm. The model of nucleotide substitution chosen was GTRMIX. The data set was partitioned into eight parts (mtSSU, nLSU and each codon position of *RPB1* and *MCM7*), so each gene partition was treated as an independent data set. Rapid bootstrap estimates were carried out for 2000 pseudoreplicates. Phylogenetic trees were visualized using the program Treeview (Page 1996).

In our phylogenetic analyses, the gyalectoid *Pertusaria* spp. clustered outside *Pertusaria* s.str., hence contradicting current classification. Thus, we tested whether our data are sufficient to reject monophyly of *Pertusaria* s.str. + gyalectoid *Pertusaria* spp. For the hypothesis testing, we used two different methods: (i) Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 2001) and (ii) expected likelihood weight (ELW) test (Strimmer and Rambaut 2002). The SH and ELW test were performed using Tree-PUZZLE 5.2 (Schmidt et al. 2002) with the combined data set, comparing the best tree agreeing with the null hypotheses and the unconstrained ML tree. These trees were inferred in Tree-PUZZLE using the GTR+I+G nucleotide substitution model.

Morphological studies

The specimens were studied using a Nikon SMZ1500 Zoom and a Zeiss Stemi 2000-C stereomicroscope. Microscopic characters were measured in water with a Zeiss

Axio Imager compound microscope and images were captured using a Spot Insight QE digital camera and a Diagnostic Instruments Insight 2MP colour camera, each equipped with Spot 4.5 acquisition software. Illustrations were made using Adobe Photoshop. Sections of the apothecia were prepared by hand cutting with a razor blade. Measurements are based on water mounts prior to the application of 10% KOH and Lugol's iodine.

Chemical studies

Secondary metabolites were extracted overnight in two separate solvents, methanol and acetone, and analyzed using high-performance liquid chromatography (HPLC) following a standardized protocol (Feige et al. 1993).

Results and discussion

Forty-three new sequences were generated for this study, including six nuLSU, eight mtSSU, eight *RPB1* and 18 *MCM7* sequences (Table 1). The Bootstrap consensus trees method (Lutzoni et al. 2004) did not identify any conflicts (i.e. well supported differences in the topology). Hence, a multi-gene data set was analyzed. A matrix of 2891 unambiguously aligned nucleotide position characters with 909 positions in the nuLSU, 747 mtSSU, 612 *RPB1* and 573 *MCM7* data set was used for the analyses. The number of constant characters was 1636. The ML analyses of the combined data set yielded a ML tree with a likelihood value of Ln = –24188.6057. Parameters of the partitions were as follows: mtSSU – Π_A: 0.327, Π_C: 0.161, Π_G: 0.219, Π_T: 0.293, alpha: 0.376; nuLSU – Π_A: 0.254, Π_C: 0.222, Π_G: 0.307, Π_T: 0.217, alpha: 0.194; 1st_posRPB1 – Π_A: 0.313, Π_C: 0.227, Π_G: 0.336, Π_T: 0.124, alpha: 0.529; 2nd_posRPB1 – –Π_A: 0.351, Π_C: 0.194, Π_G: 0.224, Π_T: 0.231, alpha: 0.460; 3rd_posRPB1 – Π_A: 0.253, Π_C: 0.225, Π_G: 0.252, Π_T: 0.270, alpha: 2.322; 1st_posMCM7 – Π_A: 0.271, Π_C: 0.257, Π_G: 0.308, Π_T: 0.164, alpha: 0.379; 2nd_posMCM7 – Π_A: 0.333, Π_C: 0.221, Π_G: 0.159, Π_T: 0.287, alpha: 0.139; 3rd_posMCM7 – Π_A: 0.258, Π_C: 0.248, Π_G: 0.216, Π_T: 0.278, alpha: 2.542. In the B/MCMC analysis of the combined dataset, the likelihood value in the sample had a mean of LnL = –25112.

The topology of the trees from the ML and Bayesian analyses did not show any conflict and hence only the ML tree is shown here (Figure 2). ML bootstrap support equal or above 70% and posterior probabilities equal or above 0.95 are indicated by numbers at branches.

The three gyalectoid *Pertusaria* species (here indicated as *Gyalectaria*) form a well-supported monophyletic

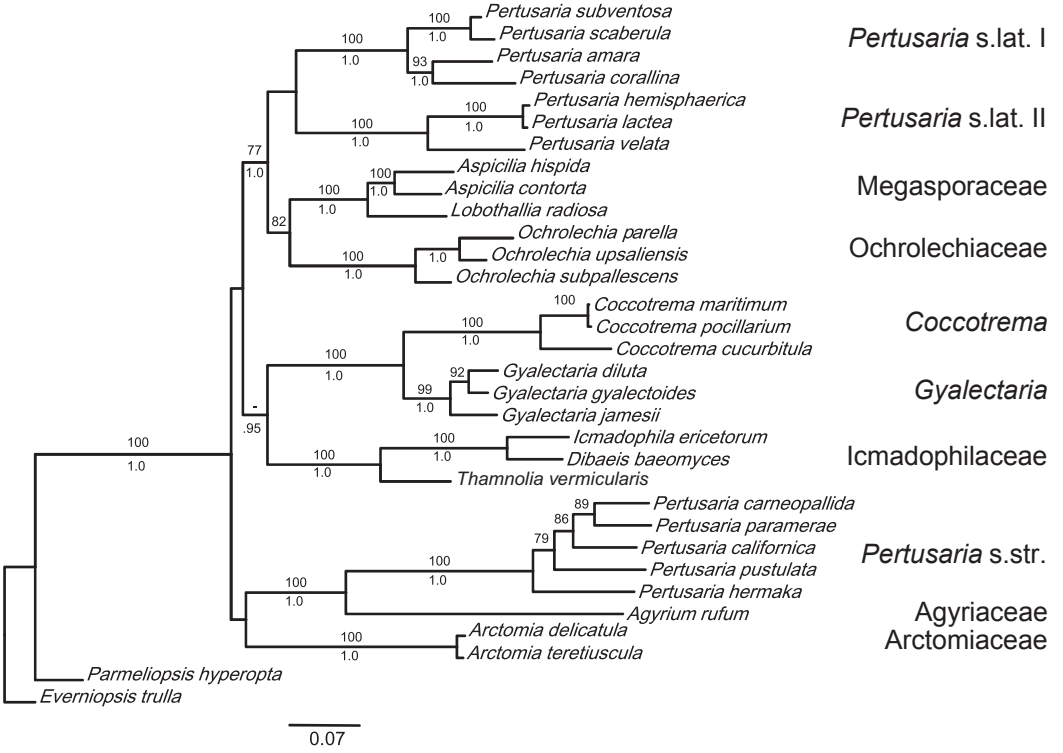

Figure 2. Phylogenetic placement of gyalectoid *Pertusaria* spp. (here indicated as *Gyalectaria*) as inferred from a concatenated alignment of mtSSU, nuLSU, *RPB1* and *MCM7* sequences. This is the optimal tree under maximum likelihood. Values above branches are likelihood bootstrap support values above 70%, and values below branches are posterior probabilities equal or above 0.95.

group with *P. diluta* and *P. gyalectoides* having a well-supported sister-group relationship (Figure 2). The gyalectoid *Pertusaria* species have a well-supported sister-group relationship with the genus *Coccotrema*. The placement of the clade consisting of *Coccotrema* and the gyalectoid *Pertusaria* spp. within Pertusariales is uncertain and lacking support. In general, the backbone of the topology within the ingroup (Ostropomycetidae) lacks support. Several well-supported monophyletic groups, such as the *Varicellaria* and *Variolaria* groups of *Pertusaria* (designated in Figure 1 as *Pertusaria* s.lat. I and II), *Pertusaria* s.str., *Ochrolechia,* and the families Arctomiaceae, Icmadophilaceae, and Megasporaceae, can be distinguished, but the relationships among these clades remain uncertain. The only exception is the strongly supported sister-group relationship of *Agyrium* and *Pertusaria* s.str. *Pertusaria carneopallida* is morphologically similar to the gyalectoid *Pertusaria* spp. in having eight-spored asci and single ascospore walls (Spribille et al. 2009). Our analyses show, however, that *P. carneopallida* falls within the *Pertusaria* s.str. clade with strong support (Figure 2). This is not entirely surprising because earlier molecular phylogenies show that other species with thin walled ascospores and eight-spored asci, such as *P. oculata* and *P. pupillaris* also fall within the *Pertusaria* s.str. group (Schmitt and Lumbsch 2004).

Hypothesis testing by both the SH and ELW tests for significant results ($p \leq 0.0001$ in both analyses), rejected a placement of the gyalectoid *Pertusaria* spp. in *Pertusaria* s.str.

We could not detect any phenolic compounds in *P. gyalectoides* and *P. jamesii* using HPLC.

The results of our phylogenetic analysis demonstrate that the gyalectoid *Pertusaria* species do not belong to Pertusariaceae s.str., but are closely related to *Coccotrema.* We, therefore, propose a new genus, *Gyalectaria*, to accommodate these three species. The new genus is placed in Coccotremataceae. Morphological characters that support a close relationship of the new genus *Gyalectaria* and *Coccotrema* include similar ascus types, eight-spored asci and thin walled ascospores (Figure 1D–I). *Coccotrema* and *Gyalectaria* differ in fruiting body morphology and chemistry. In *Coccotrema,* ascomata are perithecioid, opening only with an apical pore (Figure 1A) and the pore has periphysoids (Brodo 1973; Henssen 1976). The stictic acid chemosyndrome is often present (Brodo 1973; Messuti and Vobis 2002). In the gyalectoid *Pertusaria* species, ascomata are urceolate and the discs are clearly visible (Figure 1B,C), periphysoids are lacking, and no secondary metabolites can be found (with the exception of an unidentified unknown in *G. diluta*; see Spribille et al. 2009).

Gyalectaria is an additional monophyletic group of species formerly included in the large genus *Pertusaria* that is not closely related to *Pertusaria* s.str. The other currently known, unrelated clades are the *Variolaria* group ("Pertusaria s. lat I" in Figure 2) and the *Varicellaria* group ("Pertusaria s. lat II" in Figure 2) (Schmitt and Lumbsch 2004). The *Pertusaria* s.str., the two *Pertusaria* s.lat. and the *Gyalectaria* clades are distinguished by molecular, morphological and chemical characters. Members of *Pertusaria* s.str., for example, have a *Pertusaria*-type ascus in which the ocular chamber is clearly visible (see Figure 3 in Schmitt and Lumbsch 2004). Eight-spored taxa with single ascospore walls, such as *P. carneopallida*, *P. oculata* and *P. pupillaris*, can be readily distinguished from members of *Gyalectaria* using this character. *Pertusaria* s.str. has a rich chemistry, including chlorinated xanthones, depsides and depsidones. Members of the *Variolaria* clade (Figure 2: *Pertusaria* s. lat. I) typically have a strongly amyloid ascus without recognizable apex structures, and only one thin-walled spore per ascus. They often contain depsones (picrolichenic acid), depsides and depsidones, but may also lack phenolic substances (Schmitt and Lumbsch 2004). Members of the *Varicellaria* group (Figure 2: *Pertusaria* s. lat. II) have a strongly amyloid ascus containing one or two thick-walled spores, and frequently contain lecanoric acid (Schmitt and Lumbsch 2004).

The current study corroborates the high plasticity of taxa formerly included in the large genus *Pertusaria*, and emphasizes the need for a rigorous revision of the group. We feel that the description of a new genus is justified in the case of *Gyalectaria*, which is a small and well circumscribed unit. However, in our opinion, we need extended and geographically more balanced taxon sampling to circumscribe the more speciose *Variolaria* and *Varicellaria* groups, as well as additional, more informative molecular markers to elucidate early evolution in Agyriales (incl. Pertusariales).

Taxonomic consequences

As a consequence of our analyses, we propose a new genus in Coccotremataceae to accommodate the three gyalectoid *Pertusaria* spp., which are unrelated to *Pertusaria* s.str. The diagnosis and the new combinations are made below. Furthermore, our results confirm previous findings that *Agyrium rufum,* an unlichenized, saprophytic fungus and the type species of the genus *Agyrium* is closely related to Pertusariaceae s.str. (Lumbsch et al. 2007a). Consequently, we suggest merging the orders Agyriales and Pertusariales. The older name Agyriales should be used to include Agyriaceae and families currently included in Pertusariales.

Gyalectaria Schmitt, Kalb & Lumbsch, gen. nov. [MB 515571].

Genus fungorum lichenisatorum ad Coccotremataceas pertinens, thallo crustaceo, algas chlorococcales continenti. Apothecia hemiangiocarpia, disco urceolato, excipulo

cupulato, prosoplectenchymatico, gelatina hymeniale amyloidea, ascis 4-8-sporis, paraphysibus ramosis anastomosantibusque, ascosporis simplicibus, hyalinis. Pycnidia ignota.

Type species: *Gyalectaria jamesii* (Kantvilas) Schmitt, Kalb & Lumbsch.

Etymology. The generic name consists of the first part *Gyalect-* derived from the morphologically similar genus *Gyalecta* and the second part *-aria* derived from the second part of the generic name *Pertusaria,* to which the species have been placed previously.

The genus contains three species that are combined into *Gyalectaria* below.

Gyalectaria diluta (C. Björk, G. Thor & T. Wheeler) Schmitt, T.Sprib. & Lumbsch, comb. nov. [MB 515572]. Bas.: *Pertusaria diluta* C. Björk, G. Thor & T. Wheeler, Bryologist 112: 126 (2009).

Gyalectaria gyalectoides (Vezda) Schmitt, Kalb & Lumbsch, comb. nov. [MB 515573]. Bas.: *Pertusaria gyalectoides* Vezda, in Weber, Bryologist 74: 191 (1971).

Gyalectaria jamesii (Kantvilas) Schmitt, Kalb & Lumbsch, comb. nov. [MB 515574]. Bas.: *Pertusaria jamesii* Kantvilas, Lichenologist 22: 296 (1990).

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