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The cetrarioid core group revisited (*Lecanorales: Parmeliaceae*)

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Abstract: The cetrarioid core group has been the focus of numerous taxonomic and phylogenetic studies in recent years, yet the phylogenetic resolution and support among these clades remains unclear. Here we use four commonly employed loci to estimate if their use increases phylogenetic resolution and support. The present study largely confirms the topologies of previous studies, but with increased support. Approximately half of the genera in the cetrarioid core were not monophyletic. *Melanelia sorediella* was clustered within *Cetrariella*, and the combination *Cetrariella sorediella* (Lettau) V. J. Rico & A. Thell comb. nov. is made. Additionally, the genus *Flavocetrariella* was supported as part of *Nephromopsis* and is considered to be a synonym of the latter. Finally, a comparison of genetic distances shows that the maximum intrageneric genetic distance encompassed by many cetrarioid genera is lower than that of many other genera in *Parmeliaceae*.

Key words: *Cetraria*, *Cetrariella*, *Flavocetrariella*, genus concept, lichens, *Melanelia*, molecular systematics, *Nephromopsis*, phylogeny, taxonomy

Introduction

Parmeliaceae (*Lecanorales*: Ascomycota) represents the most species-rich family of lichen-forming fungi, encompassing approximately 2500 species (Kirk *et al*. 2008). Within the *Parmeliaceae*, several growth forms are recognized (Crespo *et al*. 2007), with the cetrarioid growth form being one of the most researched groups. The cetrarioid growth form refers to an erect foliose/subfruticose thallus with marginal apothecia and pycnidia, and cetrarioid taxa typically produce the *Cetraria*-type lichenan (Kärnefelt 1979; Goward 1985; Thell *et al*. 2002; Crespo *et al*. 2007). Cetrarioid taxa are mostly corticolous or terricolous and are restricted primarily to the Northern Hemisphere, with high diversity in eastern Asia and North America (Kärnefelt 1979; Hale 1990; Elix 1993; Randlane *et al*. 2001; Thell *et al*. 2002). Both the cetrarioid growth form and production of the *Cetraria*- type lichenan have been demonstrated to be polyphyletic. Nevertheless, a large number of cetrarioid taxa form a monophyletic group (Thell *et al*. 2002; Crespo *et al*. 2007). This group, the 'cetrarioid core', comprises approximately 90 species classified into over 14 genera (Randlane *et al*. 1997; Thell *et al*. 2002, 2004, 2009).

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Despite the intensive taxonomic and phylogenetic consideration this group has received in recent years (Thell *et al.* 2009), relationships among species and genera still remain unclear. Previous phylogenetic studies in this group have relied on morphological (Kärnefelt *et al.* 1992; Saag & Randlane 1995), a combination of molecular, morphological and/or chemical characters (Thell *et al.* 2002; Saag *et al.* 2002), and strictly molecular characters (Thell & Miao 1998; Thell *et al.* 2005, 2009). The nuclear ribosomal internal transcribed spacer (ITS) has been the molecular marker most frequently employed, with a small proportion of taxa also containing mitochondrial small subunit (mtSSU), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-tubulin, and group I intron (nuclear ribosomal small subunit) sequences (Thell & Miao 1998; Thell *et al.* 2002, 2004, 2009). Several smaller groups towards the tips of the phylogeny frequently receive strong support, but the relationships of these assemblages to one another mostly lack support.

Here we have examined whether the use of four widely employed markers in *Parmeliaceae* [ITS, mtSSU, nuclear ribosomal large subunit (nuLSU) and the RNA polymerase II largest subunit (RPB1)] would aid in resolving deeper relationships among cetrarioid clades. The ITS has been suggested as a useful marker for species-genus level relationships, while nuclear rDNA genes have been suggested as being useful across a broad taxonomic range, including the genus level (Bruns *et al.* 1991). Similarly the mtSSU has been suggested as an appropriate marker for resolving genus level relationships (Crespo *et al.* 2001). Finally, RPB1 has been shown to be quite phylogenetically informative at a shallow taxonomic scale (Schoch *et al.* 2009). Recently, these four loci were employed to aid in the generic delimitations of parmelioid lichens (Crespo *et al.* 2010), and here we assess their utility in cetrarioid lichens.

In addition, we used genetic distance estimates as a proxy to compare generic concepts of the cetrarioid core with other genera in *Parmeliaceae*. Lumbsch (2002), following an approach by Castresana (2001), employed a quantitative framework within which to address the objectivity of genera in several euascomycete families. Later, Nilsson *et al.* (2008) and del Prado *et al.* (2010) used a similar methodology to estimate the range of genetic distances at lower taxonomic levels (genera and species), with del Prado *et al.* (2010) focusing on intra- and interspecific genetic distances of parmelioid core taxa within *Parmeliaceae*. The study of Lumbsch (2002) suggested that several genera in the cetrarioid core were excessively split, relative to other genera of *Parmeliaceae* examined. Since that time, sequences from additional taxa have been generated and generic concepts among parmelioid taxa have changed, resulting in some splitting (Blanco *et al.* 2004*a*; Divakar *et al.* 2010) and some lumping (Blanco *et al.* 2004*b*, 2005) with a new generic classification of parmelioid taxa recently presented (Crespo *et al.* 2010). Consequently, a re-assessment is timely.

Materials and Methods

Taxon sampling

Our sampling was focused on the cetrarioid core genera, where we selected species for which at least two of the target loci (see below) had been sequenced. In addition, we included a small number of taxa for which only ITS sequences were available; most of these taxa were included because they were the type species of the genus or the genus had no representatives with two or more loci sequenced. Ideally we would have obtained the missing sequences for these taxa, but we either did not have fresh material or DNA from them, or were unable to obtain sequences from the material we had. We still included these taxa, however, as it has been demonstrated that taxa with missing sequence data can still be placed accurately in a phylogeny (Wiens 2003, 2006). Finally, we included a number of outgroup taxa as well as taxa previously shown to be closely allied with the cetrarioid core. Outgroup taxa were chosen based on recent phylogenetic studies in *Parmeliaceae* (Crespo *et al.* 2007, 2010). Altogether 72 taxa were analyzed, including 58 species of the cetrarioid core (Table 1).

Molecular methods

DNA isolates from previous studies (summarized in Thell *et al.* 2009) were used in the present study. Samples were PCR amplified and sequenced using the mrSSU1, mrSSU2, mrSSU2R and mrSSU3R primers

TABLE 1. *Taxa, voucher specimens, herbaria and GenBank accession numbers for the sequences used in the present study. Collection information is provided only for taxa with sequences newly generated in this study*

Species	Collection	GenBank Accession Number			
		ITS	mtSSU	nuLSU	RPB1
Alectoria sarmentosa		DQ979998	DQ899291	DQ899290	DQ923678
Bryoria fremontii		DQ980004	DQ923627	DQ923656	DQ923684
B. fuscescens		EF042903	AF351158	EF042912	EF092101
Cornicularia normoerica		DQ980009	DQ923632	DQ923661	DQ923687
Everniopsis trulla		EF105411	EF108289	EF108290	EF105429
Flavoparmelia caperata		AY581059	AF351163	AY578922	EF092107
Imshaugia aleurites		AY611126	AF351167	AY607840	EF092114
Menegazzia terebrata		DQ980019	DQ899305	DQ899304	DQ923694
Pannoparmelia angustata		AY785272	AF351170	AY785265	EF092131
Parmelia saxatilis		AF058037	AF351172	AY300849	DQ923695
Platismatia glauca		AF058035	AY756404	AY756342	AY756410
P. norvegica		DQ980022	DQ923644	DQ923671	DQ923696
Pseudephebe pubescens		AY611125	AF351180	AY607839	EF092148
Psiloparmelia denotata		EF105415		EF105426	EF105436
Ahtiana pallidula	USA, Montana, *Hauck* (private hb.)	AY353709	JN000225	JN000248	JN000278
A. sphaerosporella		AF141859			
Allocetraria ambigua	China, Sichuan, *Obermayer* 08141 (GZU)	AF404128	JN000226	JN000249	JN000279
A. flavonigrescens	China, Sichuan, *Obermayer* 08140 (GZU)	AF404127	JN000227	JN000250	JN000280
A. globulans	China, Sichuan, *Obermayer* 08137 (GZU)	AF404126	JN000228	JN000251	JN000281
A. madreporiformis	Austria, Tyrol, *Obermayer* 7746 (M)	AF416461	JN000229		
A. sinensis	China, Sichuan, *Obermayer* 08148 (GZU)	AF404125		JN000252	
A. stracheyi		AF404130	EU435374		
Arctocetraria andrejevii		DQ980001	DQ923623	DQ923652	DQ923680
A. nigricascens	Canada, Melville Island, *Westberg* 1614 (LD)	AF254628	JN000230	JN000253	JN000282
Cetraria aculeata		GQ375385	AY643091	AY607825	
C. annae		EU401759	EU435376		
C. ericetorum ssp. *ericetorum*	Finland, Varsinais-Suomi, *Puolasmaa, Stenroos & Thell* FIN-9929 (TUR)	AF228292	JN000231		JN000283

TABLE 1. *Continued*

Species	Collection	GenBank Accession Number			
		ITS	mtSSU	nuLSU	RPB1
C. islandica ssp. *islandica*	Sweden, Scania, SK9607 (LD) (mtSSU,nuLSU,RPB1; ITS from GenBank)	AF228299	JN000232	JN000254	JN000284
C. kamtczatica	USA, Alaska, *Ahti* 63296 (H)	EU401763	JN000233	JN000255	
C. laevigata	Russia, Sakha Republic, *Ahti* 64755 (H)	EU401764	JN000234	JN000256	JN000285
C. muricata	Spain, Castilla & León, *Feuerer* (LD-1197733)	EU410409	JN000235		JN000286
C. nigricans	Canada, Nunavut, *Westberg* 2377 (LD)	AF254629	JN000236	JN000257	JN000287
C. obtusata	Austria, Tyrol, *Feuerer* 9132/1 (TUR)	AF457922	EU435378	JN000258	JN000288
C. odontella	Finland, EH Sysmä, *Haikonen* 23297 (H)	AF228304	EU435367	JN000259	JN000289
C. sepincola		EU401766	EU435371		
Cetrariella commixta	Finland, Tavastia australis, *Haikonen* 19093 (H)	AF451796	JN000237	JN000260	JN000290
C. delisei		DQ980005	DQ923628	DQ923657	
C. fastigiata	Finland, Lapland, *Haikonen* 24443 (H)	EU401768	EU435370	JN000261	
Cetreliopsis asahinae	Bhutan, *Søchting* 8060 (LD)	DQ394386		JN000262	
C. laeteflava		EU401770	EU435372		
C. rhytidocarpa		DQ980008	DQ923631	DQ923660	
Dactylina arctica		AF115760	DQ986786	DQ986802	DQ986859
Esslingeriana idahoensis		AF227513			
Flavocetraria cucullata	Austria, *Kärnefelt* 1996 (LD) (nuLSU, RPB1; ITS, mtSSU from GenBank)	FJ914812	EU435382	JN000263	JN000291
F. minuscula		EU401772	EU435381		
F. nivalis		DQ980011	EU435383	DQ923663	DQ923688
Kaernefeltia californica		DQ004571			
K. merrillii	Spain, Madrid, *Amo, Argüello, Ferencova & Feuerer* (LD-1038537)	DQ395292	EU435380	JN000264	JN000292
Masonhalea richardsonii		AF254634	DQ972979	DQ973031	DQ973054
Melanelia hepatizon		DQ980016	EU435364	DQ923667	DQ923692

TABLE 1. *Continued*

Species	Collection	GenBank Accession Number			
		ITS	mtSSU	nuLSU	RPB1
M. sorediella		GU994558	GU994646	GU994606	GU994707
M. stygia		AY611097	DQ923640	AY607809	DQ923693
Nephromopsis komarovii	Russia, Primorsky Krai, *Skirina* 10972 (LD)	AF451779	JN000238	JN000265	JN000293
N. laureri	Italy, Trentino-Alto Adige, *Feuerer & Thell* (HBG)	AF451786		JN000266	JN000294
N. leucostigma	Bhutan, Thimpu District, *Søchting* 9151 (LD)	AF451777	JN000239	JN000267	JN000295
N. nephromoides	The Philippines, Misamis Occidental, *Ejem* (H)	DQ004574	JN000240	JN000268	JN000296
N. ornata	Russia, Primorsky Krai, *Kudryavtseva* 10980 (LD)	AF451783	JN000241	JN000269	
N. pallescens	Bhutan, *Søchting* 8206 (LD)	AF451784	JN000242	JN000270	JN000297
N. stracheyi		AF451785	EU435373		
Tuckermanella coralligera	USA, New Mexico, *Worthington* 28821 (ASU)	AF457924	JN000243	JN000271	JN000298
T. fendleri	USA, Arizona, *Westberg* 543 (LD)	AF451791	JN000244	JN000272	
T. weberi		AF451792			
Tuckermannopsis americana		AF072233			
T. chlorophylla		DQ980025	DQ923647	DQ923674	DQ923697
T. ciliaris		FJ005090	DQ986870	DQ986755	DQ986827
T. inermis	USA, Alaska, *Holt* 23441(LD, dupl)	EU401762	EU435377	JN000273	JN000299
T. orbata	USA, Montana, *Hauck* (private hb.)	DQ004572	JN000245	JN000274	JN000300
Usnocetraria oakesiana	Germany, Bavaria, *v. Brackel* (IV)	EU401757	EU435375	JN000275	JN000301
Vulpicida juniperina		AF058038	AY340535	AY340577	
V. pinastri		AY332522	DQ923648	DQ923675	DQ923698
V. tubulosus	Austria, Tyrol, *Feuerer & Thell* (HBG)	AF404132	JN000246	JN000276	JN000302
V. viridis	USA, Connecticut, *Feuerer* (HBG)	DQ004573	JN000247	JN000277	

(Zoller *et al*. 1999) for the mtSSU, the nu-LSU-155-5′ (Döring *et al*. 2000) and LR6 (Vilgalys & Hester 1990) primers for nuLSU, and the gRPB1-A forward (Stiller & Hall 1997) and fRPB1-C reverse (Matheny *et al*. 2002) primers for RPB1. The 25 µl PCR reactions consisted of 1 µl of each PCR primer (10 µM), 2·5 µl dNTP mix (10 mM), 5 µl BSA, 2·5 µl PCR buffer, 2µl Taq, approximately 2 µl DNA and 8·5 µl water. The PCR cycling conditions were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, a locus-specific annealing temperature for 1 min, and 72°C for 1 min, followed by a single 72°C final extension for 7 min. An annealing temperature of 53°C was used for mtSSU, 57°C for nuLSU, and 55°C for RPB1. Samples were visualized 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) and incubated at 45°C for at least 24 h.

Samples were cycle-sequenced in 10 µl volumes and included 1·5 µl Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, CA), 2·5 µl Big Dye buffer, 0·4 µl primer (10 µM), 1 µl Gelased PCR product and water. 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 precipitated with ethanol and EDTA, re-suspended in Hi-Di formamide (Applied Biosystems) and sequenced in an Applied Biosystems 3730 DNA Analyzer. Sequences were then assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI).

Phylogenetic analyses

Sequences in Table 1 were aligned using a combination of automated alignment (Muscle 3.6: Edgar 2004) and manual refinement in Mesquite 2.73 (Maddison & Maddison 2010) and Se-Al v. 2.0a11 (Rambaut 1996). Ambiguous regions and introns were removed and the alignment is deposited in TreeBase.

A maximum likelihood (ML) analysis was performed for each locus in RAxML 7.2.6 (Stamatakis 2006) using the GTRGAMMA model with 25 rate parameter categories. Support was then estimated by performing 1000 bootstrap pseudoreplicates (Felsenstein 1985) and clades with support of 70 or greater were considered strongly supported. Individual gene trees were compared to assess whether strongly supported topological conflict existed. Loci were then concatenated and two data sets created: the first data set (full) included all taxa in Table 1, while the second data set (3+) included only taxa with 3 loci or more present. These different data sets were used to examine relationships among taxa, and determine how support was affected by increased data representation. Partitioned ML analyses were performed on these data sets as described above, except that for the full data set, the GTRCAT model of evolution was used first to infer the tree topology which was subsequently evaluated under the GTRGAMMA model.

Bayesian analyses using Markov Chain Monte Carlo sampling (Larget & Simon 1999) were also performed. Substitution models for individual loci were selected by using jModelTest 0.1.1 (Posada 2008), which employs PhyML 3.0 (Guindon & Gascuel 2003) to estimate the likelihood of the data under 24 models of evolution with a fixed topology. AICc values for each model were then calculated and compared, and the model with the lowest AICc value was selected (GTR+Γ+I for ITS, mtSSU and nuLSU; K80+I for RPB1 first position; JC for RPB1 second position; SYM+G for RPB1 third position in the full analysis and K80+Γ for the 3 gene analysis). Partitioned Bayesian analyses were then performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) in the CIPRES Science Gateway v. 3.0 (Miller *et al*. 2009). To obtain acceptance rates between approximately 0·1 and 0·7, which are suggestive of adequate levels of mixing (Ronquist *et al*. 2005, 2009), the temperature was set to 0·035–0·04. Two parallel analyses with four chains each were run for 5 M generations, sampling every 200 generations. The initial 25% (6251 trees) were discarded as burn-in, and convergence among parallel runs was assessed by creating bivariate plots of bipartitions in the program AWTY (Wilgenbusch *et al*. 2004; Nylander *et al*. 2008).

Intrageneric genetic distance

We also wanted to determine how generic concepts within the cetrarioid core compared with other well-defined *Parmeliaceae* genera. To accomplish this, we estimated the maximum genetic distance encompassed within individual cetrarioid genera/clades and compared these estimates with those of other *Parmeliaceae* genera. The ITS sequences listed in Table 1 were added to the ITS alignment from the 2+gene data set from the ParSys project (Crespo *et al*. 2010), and overlapping species between the two alignments were removed (leaving one representative per species), along with any additional ambiguous regions. Genera outside the cetrarioid core were included only if more than two species were present in the alignment. *Dactylina arctica* and *Esslingeriana idahoensis* were not considered to be part of the cetrarioid core in Thell *et al*. (2009), but closely allied with it. We included these species in the data set to determine whether the maximum genetic distance within the cetrarioid core was increased when these genera were included. Pairwise genetic distances were then computed under the HKY85 model in PAUP* 4.0b10 (Swofford 2002).

Results

No evidence for significant incongruence between loci was detected. The final concatenated alignment consisted of 2628 unambiguously aligned characters, and the number of characters and proportion of variable characters per locus can be found in Table 2. As illustrated in Table 2, ITS and

TABLE 2. *The number of unambiguously aligned characters for individual loci in the full and 3+ data sets, along with the number and proportion of variable characters*

Data set	Locus	Number of taxa	Total number of characters	Variable characters	Proportion of locus that is variable	Variability contributed to combined data set
full						
	ITS	72	438	217	49·5%	30·0%
	mtSSU	63	720	133	18·5%	18·4%
	nuLSU	58	864	156	18·1%	21·6%
	RPB1	49	606	217	35·8%	30·0%
	Combined	72	2628	723	27·5%	
3+						
	ITS	58	438	210	48·0%	29·4%
	mtSSU	56	720	132	18·3%	18·5%
	nuLSU	56	864	155	17·9%	21·7%
	RPB1	49	606	217	35·8%	30·4%
	Combined	58	2628	714	27·2%	

RPB1 had the greatest number and proportion of variable sites, and contributed the most variability to the combined data set.

The topology recovered in both the full (Fig. 1) and 3+ (Fig. 2) analyses are generally in agreement with previous topologies. However, support values are higher in the present study, and the backbone, though still weakly supported, has increased support relative to previous studies. Support values were not universally increased in the 3+ analyses (relative to the full analyses), but support for a large number of clades was increased.

Approximately half of the genera in the cetrarioid core (as currently circumscribed) do not appear to be monophyletic (i.e. *Ahtiana*, *Cetraria*, *Cetrariella*, *Flavocetraria, Kaernefeltia, Nephromopsis*, *Tuckermannopsis*, *Vulpicida*), though support for the non-monophyly of these genera is often lacking. *Dactylina arctica, Esslingeriana idahoensis, Melanelia hepatizon* and *M. stygia* were strongly supported as part of the cetrarioid core.

Figure 3 illustrates the maximum ITS genetic distance encompassed by various *Parmeliaceae* genera and clades. With the exception of the *Nephromopsis* + *Cetreliopsis* clade (which together form a monophyletic group – see Figs 1 & 2), nearly all investigated cetrarioid core genera fall in the lower half of the distribution of maximum ITS genetic distance among congeners. The greatest ITS genetic distance among taxa in the cetrarioid core was between *Nephromopsis nephromoides* and *Allocetraria madreporiformis*; the inclusion of *Dactylina arctica*, *Esslingeriana idahoensis*, *Melanelia hepatizon* and *M. stygia* as part of the cetrarioid core did not increase the maximum genetic distance between two taxa. The maximum genetic distance between taxa in the cetrarioid core was found to be close to that among congeners in the genera *Cetrelia* and *Relicina*. The '*Nephromopsis*' clade (which corresponds to the cetrarioid core clade B of Thell *et al.* 2009) was found to encompass approximately the same maximum genetic distance as was found in *Parmotrema*, and that of the '*Cetraria*' clade (which corresponds to the cetrarioid core clade A of Thell *et al.* 2009) was found to be between that of *Xanthoparmelia*, but less than that of the '*Nephromopsis*' clade.

Discussion

Increased resolution

This study demonstrates that while ITS sequences contained the greatest amount of variability in the four markers examined

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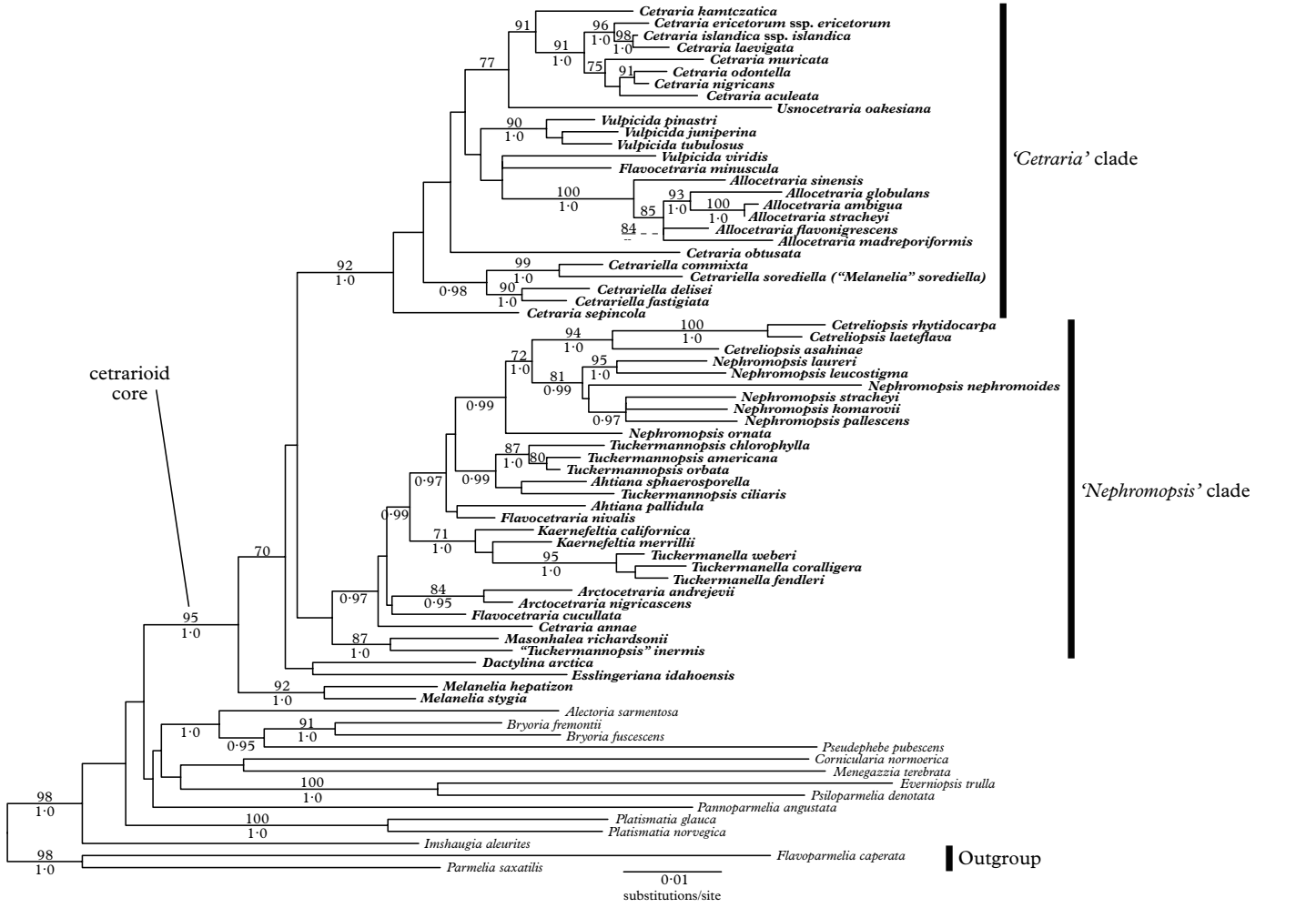

FIG. 1. The ML phylogram for the full data set. Bootstrap proportions of 70 and greater are listed above branches and Bayesian posterior probabilities of 0·95 or greater are listed below branches. Clades A and B are labelled *sensu* Thell *et al.* (2009). Taxa belonging to the cetrarioid core group are in bold, while those outside the group are in normal font.

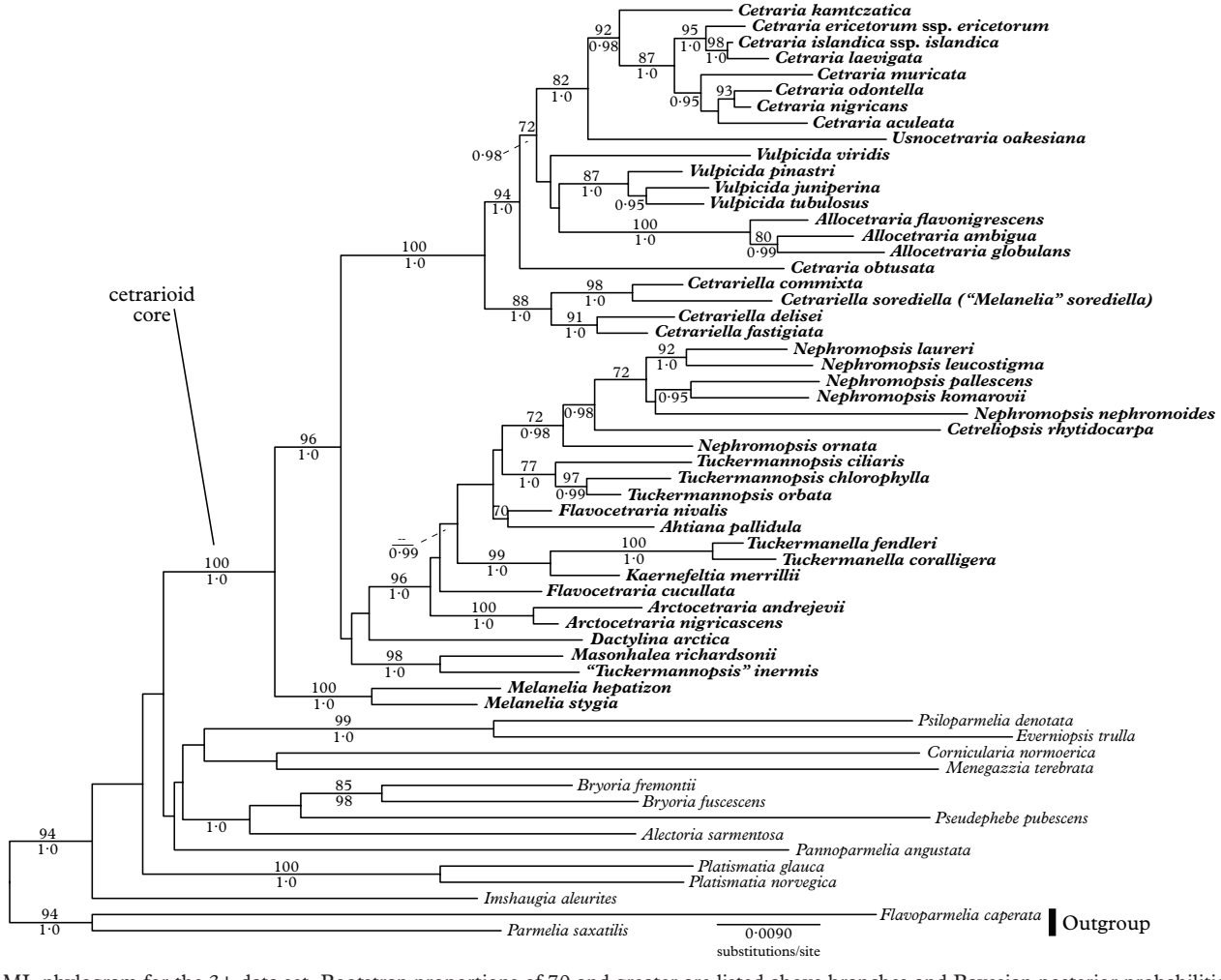

FIG. 2. The ML phylogram for the 3+ data set. Bootstrap proportions of 70 and greater are listed above branches and Bayesian posterior probabilities of 0·95 or greater are listed below branches. Taxa belonging to the cetrarioid core group are in bold, while those outside the group are in normal font.

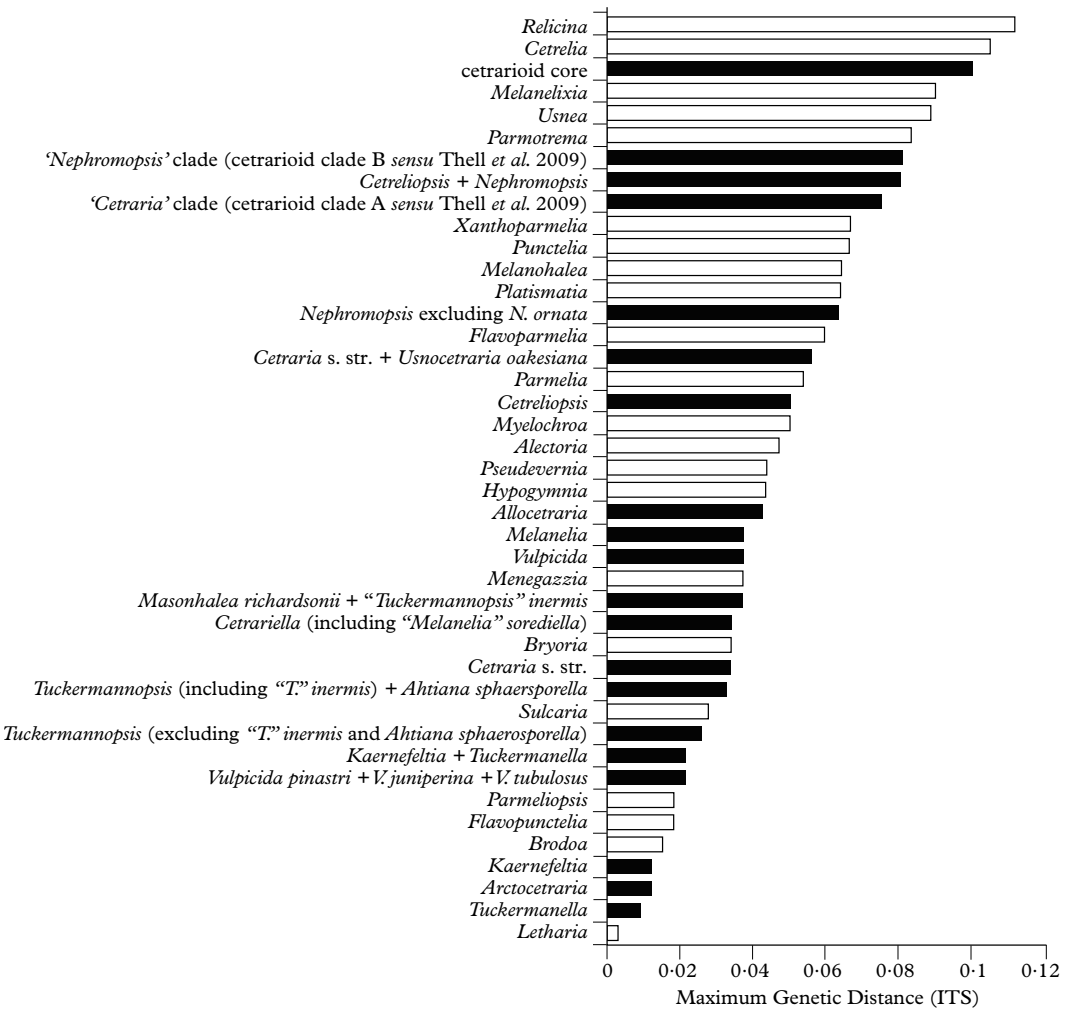

FIG. 3. Maximum ITS intra-clade genetic distance calculated for a range of *Parmeliaceae* clades and genera. Values for taxa belonging to the cetrarioid core group are in black, while those outside the group are white.

(even with many ambiguous regions of the alignment excluded), RPB1 also contributed a large amount of variation, and little needed to be excluded due to ambiguity in the alignment. At the phylogenetic scale investigated here, mtSSU contributed relatively little information and individual gene trees showed very low bootstrap support throughout the tree (data not shown). To further resolve this clade, it may be best to continue to focus on protein-coding genes, such as RPB1, RPB2, TEF1α, and Mcm7; the last has recently been shown to have exceptional phylogenetic power (Aguileta *et al.* 2008; Schmitt *et al.* 2009).

Phylogenetic relationships within the cetrarioid core

Previous studies have excluded *Dactylina arctica*, *Esslingeriana idahoensis*, *Melanelia hepatizon* and *M. stygia* from the cetrarioid

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core, instead suggesting that they are closely allied with it (Thell *et al.* 2002, 2009), while others (Crespo *et al.* 2007) placed *D. arctica*, *M. hepatizon* and *M. stygia* in the cetrarioid clade (*E. idahoensis* was not investigated in that study). Their inclusion in the cetrarioid core seems justified as these taxa have been considered to have the cetrarioid growth form (Thell *et al.* 2002), though some studies have considered *M. hepatizon* and *M. stygia* to have a parmelioid growth form (Crespo *et al.* 2007). These taxa are also strongly supported as being sister to (Fig. 1) or included in (*D. arctica* in Fig. 2) what was previously referred to as the cetrarioid core. Finally, support for the previous cetrarioid core is lacking in the present study (Figs 1 & 2), a result also found in Crespo *et al.* (2010). For these reasons, we suggest their inclusion in the cetrarioid core. Consequently, the cetrarioid core group now comprises 17 genera with *c.* 100 species. See Randlane & Saag (1993), Randlane *et al.* (1997), and the 'Third World List of Cetrarioid Lichens' website (http://esamba.bo.bg.ut.ee/checklist/cetrarioid-checklist/home.php), for more information on taxa included in the cetrarioid core.

"Tuckermannopsis" inermis appears distantly related to *Tuckermannopsis ciliaris* (Fig. 1), the type species of *Tuckermannopsis*. Instead, "*T.*" *inermis* is strongly supported as being sister to *Masonhalea richardsonii* (Figs 1 & 2). Thell *et al.* (2009) recovered this same relationship and noted the similarities between these two taxa: lateral apothecia, conspicuous pseudocyphellae (which differ in their location and pattern), and an arctic distribution. We debated transferring *"T." inermis* to *Masonhalea* despite their differences in gross morphology. This lack of gross morphological similarity would have probably surprised and disconcerted some researchers, but many genera, such as *Cladia*, *Coenogonium*, *Icmadophila* and *Xanthoparmelia* (Rambold *et al.* 1993; Blanco *et al.* 2004*b*; Rivas Plata *et al.* 2006; Crespo *et al.* 2010; Parnmen *et al.* 2010), are composed of species with a broad range of morphologies. Ultimately, we have refrained from formally combining this taxon and prefer to wait for a second collection from which DNA can be obtained to confirm its phylogenetic position. If it is confirmed, taxonomists will be faced with the choice of erecting a new monospecific genus for *"T." inermis*, or combining it with *Masonhalea*, thereby creating a genus of morphologically heterogeneous species.

Generic concepts

The non-monophyly of so many genera in the cetrarioid core ultimately results in a situation in which either new genera must be created to accommodate orphaned species (e.g. "*Cetraria*" *annae*, "*Cetraria*" *obtusata*, "*Cetraria*" *sepincola*, "*Flavocetraria*" *minuscula*, "*Nephromopsis*" *ornata*), or these orphaned taxa be included in previously described genera. We have refrained from making numerous taxonomic changes here, partly because the non-monophyly of these genera is weakly supported in many cases. A number of other taxa (such as *Cetraria subalpina*, *Tuckermannopsis platyphylla* and several *Melanelia* species) should be included in future studies to clarify further generic delimitations. However, to aid in the decision of whether to create new genera or lump orphaned taxa into existing genera, we chose to compare the genus concepts within the cetrarioid core with other *Parmeliaceae* genera by estimating the maximum intrageneric genetic distance within a number of genera. Our comparison of intrageneric distances was not intended to be exhaustive. Additionally, numerous genera were not included and only a fraction of the species encompassed within each genus was included; nevertheless, we feel this provides at least a preliminary estimate of the range of genetic variation cetrarioid genera contain relative to other *Parmeliaceae* genera.

A similar study was previously performed by Lumbsch (2002), in which 58 *Parmeliaceae* ITS sequences were included, which allowed for intrageneric distance estimates for 15 *Parmeliaceae* genera (six from the cetrarioid core). In the present study, we have included 231 ITS sequences, from 22

Parmeliaceae genera outside the cetrarioid core and 20 genera or other assembleges (e.g. *Kaernefeltia* + *Tuckermanella*, *Tuckermannopsis* s. str. + *Ahtiana sphaerosporella*) of the cetrarioid core.

Differences in genetic distances may result from several factors (discussed in Lumbsch 2002), including differences among the ages of genera, the rates of molecular evolution within genera, the rates of morphological evolution within genera, and the historic tendencies of taxonomists (to split or lump). Because of these potential sources of variation in intrageneric genetic distances, we do not advocate a strict approach to generic delimitation based on genetic distance, rather, we simply wanted to estimate how genetically disparate generic delimitations were, and how those in the cetrarioid core compared to other *Parmeliaceae* genera. Our non-statistical comparison of maximum intrageneric ITS distances suggests that cetrarioid taxa are more narrowly circumscribed than other currently recognized groups of *Parmeliaceae*. We also note that some of these results are in conflict with those of del Prado *et al.* (2010), a study which included 491 ITS sequences from parmelioid genera. For instance, in the present study, *Parmotrema* was found to encompass a greater amount of genetic distance than *Xanthoparmelia*, a result which is the opposite of that in del Prado *et al.* (2010). These discrepancies may be due to a number of factors including taxon selection and the differing methods employed. We feel, however, that we still have a high proportion of the intrageneric diversity represented in the cetrarioid core genera; therefore our estimates within these groups may be more accurate than our estimates of non-cetrarioid core genera. The discrepancy with del Prado *et al.*'s (2010) results suggests that we may have underestimated the maximum intrageneric genetic distance of some genera outside the cetrarioid core. If this is the case, the ranks of cetrarioid core genera in Fig. 3 could drop lower than they currently are (relative to non-cetrarioid genera), further illustrating the point that cetrarioid core genera may be more finely divided (based on maximum intrageneric genetic distance) than other *Parmeliaceae* genera.

Additional independent support for this view is the current lack of anatomical or cell-wall chemical characters correlated with the current generic distinctions. In the case of the parmelioid core of the family, researchers have found and stressed differences in ascospores, conidia, and cell-wall polysaccharides correlated with the molecularly revealed clades when revising generic-level taxonomies (Crespo *et al.* 2011). Where such correlations are absent, some lichenologists would question whether generic rank was appropriate as such differences would be expected for generic separations in both crustose lichens and especially non-lichenized ascomycetes. Further critical studies of anatomical features of the ascomata and conidiomata in the cetrarioid lichens would therefore be desirable before formalizing a revised generic system.

Taxonomy

Cetrariella sorediella (Lettau) V. J. Rico & A. Thell comb. nov.

MycoBank No.: MB 561568

Cetraria commixta f. *sorediella* Lettau, *Hedwigia* **60:** 119 (1918) (basionym).—*Cetraria fahlunensis* var. *sorediella* (Lettau) Räsänen, *Kuopion Luon.Ystäv. Yhdist. Julk., ser. B*, **2** (6)**:** 38 (1952).—*Melanelia commixta* var. *sorediella* (Lettau) Hafellner & Türk, *Stapfia* **76:** 153 (2001).—*Melanelia sorediella* (Lettau) V. J. Rico *et al.*, *Lichenologist* **37:** 205 (2005).

Rico *et al.* (2005) did not accept the placement of *Cetrariella commixta* in *Cetrariella*, instead referring to it as "*Melanelia*" *commixta*. Regardless of its generic name, this taxon appears sister to *Melanelia sorediella*, which in turn forms a clade sister to *Cetrariella*, a result which was strongly supported in the Bayesian analysis of the full data set (Fig. 1) and in both analyses of the 3+ data set (Fig. 2). The retention of these two taxa in *Melanelia* is not an option as they are distantly related to *M. stygia*, the type species of *Melanelia*. Rather than introduce another genus for "*M.*" *sorediella* and *C. commixta*, we propose to add "*M.*" *sorediella* to *Cetrariella*

and support the inclusion of *C. commixta* in *Cetrariella* (rather than *Melanelia*).

Flavocetrariella D. D. Awasthi

*Comp. Macrolich.India, Nepal Sri Lanka***:** 161 (2007). Type species: *F. leucostigma* (Lév.) D. D. Awasthi.

Awasthi (2007) introduced the new generic name *Flavocetrariella* for the species previously known as *Nephromopsis leucostigma* and *N. melaloma*, and suggested a relationship with *Flavocetraria*. In our molecular study the type species (*F. leucostigma*) is clustered within *Nephromopsis* and this result is in concordance with Thell *et al.* (2005, 2009); we therefore recommend the treatment of *Flavocetrariella* as a synonym of *Nephromopsis* Müll. Arg. 1891 and the retention of *F. leucostigma* and *F. melaloma* in *Nephromopsis*.

Conclusions

In this study, we have illustrated the potential the RPB1 gene offers for resolving the cetrarioid core group. The data set analyzed has confirmed many previously reported relationships, but often with increased support values. Approximately half of the genera in the cetrarioid core were not monophyletic, and two taxonomic changes have been made to help address this issue. Finally, maximum intrageneric genetic distance estimates suggested that many genera in the cetrarioid core group are narrowly defined relative to other *Parmeliaceae* genera. Consequently, justification for their continued retention needs to be sought through critical studies of ascomatal and conidiomatal features, in particular of a larger proportion of the taxa belonging to the cetrarioid core.

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