Phylogeny of the cetrarioid core (*Parmeliaceae*) based on five genetic markers

**Arne THELL, Filip HÖGNABBA, John A. ELIX,
Tassilo FEUERER, Ingvar KÄRNEFELT, Leena MYLLYS,
Tiina RANDLANE, Andres SAAG, Soili STENROOS,
Teuvo AHTI and Mark R. D. SEAWARD**

Abstract: Fourteen genera belong to a monophyletic core of cetrarioid lichens, *Ahtiana, Allocetraria, Arctocetraria, Cetraria, Cetrariella, Cetreliopsis, Flavocetraria, Kaernefeltia, Masonhalea, Nephromopsis, Tuckermanella, Tuckermannopsis, Usnocetraria* and *Vulpicida*. A total of 71 samples representing 65 species (of 90 worldwide) and all type species of the genera are included in phylogentic analyses based on a complete ITS matrix and incomplete sets of group I intron, β-tubulin, GAPDH and mtSSU sequences. Eleven of the species included in the study are analysed phylogenetically for the first time, and of the 178 sequences, 67 are newly constructed. Two phylogenetic trees, one based solely on the complete ITS-matrix and a second based on total information, are similar, but not entirely identical. About half of the species are gathered in a strongly supported clade composed of the genera *Allocetraria, Cetraria* s. str., *Cetrariella* and *Vulpicida*. *Arctocetraria, Cetreliopsis, Kaernefeltia* and *Tuckermanella* are monophyletic genera, whereas *Cetraria, Flavocetraria* and *Tuckermannopsis* are polyphyletic. The taxonomy in current use is compared with the phylogenetic results, and future, probable or potential adjustments to the phylogeny are discussed. The single non-DNA character with a strong correlation to phylogeny based on DNA-sequences is conidial shape. The secondary chemistry of the poorly known species *Cetraria annae* is analyzed for the first time; the cortex contains usnic acid and atranorin, whereas isonephrosterinic, nephrosterinic, lichesterinic, protolichesterinic and squamatic acids occur in the medulla. Notes on the anatomy of *Cetraria annae* and *Flavocetraria minuscula* are also provided.

Key words: *Cetraria* s. lat., conidial shape, DNA sequences, lichen phylogeny, taxonomy

A. Thell (corresponding author) and I. Kärnefelt: The Biological Museums, Lund University, Östra Vallgatan 18-20, SE-223 61 Lund, Sweden. Email: arne.thell@botmus.lu.se

F. Högnabba, L. Myllys, S. Stenroos and T. Ahti: Botanical Museum, Finnish Museum of Natural History, P.O. Box 7, FI-000 14 University of Helsinki, Finland.

J. A. Elix: Research School of Chemistry, Building 33, Australian National University, Canberra, ACT 0200, Australia.

T. Feuerer: Hamburg Univesity, Biozentrum Klein Flottbek, Department of Botany and Botanical Garden, Ohnhorststrasse 18, D-22609 Germany.

T. Randlane and A. Saag: Institute of Ecology and Earth Sciences, University of Tartu, Lai Street 38, 51005 Tartu, Estonia.

M. R. D. Seaward: Department of Archaeological, Geographical & Environmental Sciences, University of Bradford, Bradford, BD7 1DP, UK.

Introduction

The delimitation of the Parmeliaceae

The *Parmeliaceae* is the largest (*c.* 2300 species) and most intensely studied of all lichen families. It has a characteristic ascoma ontogeny, a cupular exciple and forms a monophyletic clade, including recent segregates such as *Alectoriaceae*, *Hypogymniaceae* and *Usneaceae* (Crespo *et al.* 2007). The number of genera in the family increased from *c.* 20 in the early 1970s to *c.* 90 at the end of the last century (Elix 1993; Henssen & Jahns 1974; Kärnefelt & Thell 1992; Thell *et al.* 2004). Phylogenetic studies based on DNA sequences resulted in both synonymization and the creation of new genera. However, the number of genera has decreased over the last decade, but the taxonomy is still

far from settled, as shown by adjustments made as a result of several large phylogenetic investigations. Thallus-form groups, alectorioid, cetrarioid, hypogymnioid, parmelioid and usneoid lichens, have often been discerned in the family, which to some degree correspond to the family names previously segregated from the *Parmeliaceae* (Thell *et al*. 2004). The mainly foliose parmelioid group constitutes the major part of the family, comprising *c*. 1500 species, of which 800 belong to the largest genus in the family, *Xanthoparmelia* (Vain.) Hale (Crespo *et al*. 2007). Most of the fruticose members, mainly alectorioid and usneoid, are gathered in the genera *Alectoria* Ach., *Bryoria* Brodo & D, Hawksw. and *Usnea* Adans., the last perhaps comprising as many as 600 species (Wirtz *et al*. 2006). Monophyletic cores of the thallus-form groups have been identified with the aid of different molecular markers, but all of them nested within the *Parmeliaceae* (Blanco *et al*. 2004*a*, 2005; Crespo *et al*. 2007; Divakar *et al*. 2006; Ohmura 2002; Ohmura & Kanda 2004). The sister family of the *Parmeliaceae* is the *Lecanoraceae* wherein the genus *Protoparmelia* M. Choisy is probably most closely related to the *Parmeliaceae* (Arup *et al*. 2007; Crespo *et al*. 2007).

The cetrarioid core

The morphological group 'cetrarioid lichens' contains *c*. 135 species spread over 20 genera, of which *c*. 90 species and 14 genera form a monophyletic clade (Randlane *et al*. 1997; Thell *et al*. 2002; 2004) (Table 1). Thus, eight genera, *Asahinea* W. L. Culb. & C. F. Culb., *Bryocaulon* Kärnefelt, *Cetrelia* W. L. Culb. & C. F. Culb., *Coelopogon* Brusse & Kärnefelt, *Cornicularia* (Schreb.) Hoffm., *Dactylina* Nyl., *Esslingeriana* Hale & M. J. Lai, *Parmelaria* D. D. Awasthi and *Platismatia* W. L. Culb. & C. F. Culb., traditionally known as cetrarioid, are spread within different clades in the *Parmeliaceae*, without having any close affinities to each other. Some cetrarioid species are combined in non-cetrarioid genera, such as *Himantormia deusta* (Hook. f.) A. Thell & Søchting, *Melanelia agnata* (Nyl.) A. Thell, *M. culber-*

sonii (Hale) A. Thell and *M. hepatizon* (Ach.) A. Thell. The genus *Parmelaria* is closely related to *Parmotrema* A. Massal. s. lat. (Blanco *et al*. 2005). The genera *Esslingeriana* and *Melanelia* are sister groups to the core of cetrarioid lichens and, according to a phylogenetic study of the *Parmeliaceae* (A. Crespo *et al*., unpublished), *Dactylina* is an additional genus that is closely related to the cetrarioid core.

Molecular phylogeny vs. taxonomy

Before the era of DNA sequencing, the systematics of macrolichens was based on morphology, anatomy and secondary chemistry, with an increased emphasis on reproductive structures in the 1980s and 1990s, frequently with a focus on ascus apex characters (Hafellner 1984; Kärnefelt & Thell 1994; Thell *et al*. 1995*c*), which show little correlation with DNA sequences, both in the *Parmeliaceae* and in other lichen families (Thell *et al*. 2004; Lumbsch *et al*. 2007). Cetrarioid lichens probably show the greatest variation of asci and conidia in comparison with the other morphological groups of the *Parmeliaceae*. Somewhat unexpectedly, DNA based phylogeny of cetrarioid lichens shows a clear correlation with conidial shape (Thell *et al*. 2002).

Hitherto, taxonomic changes based on DNA sequences have not been peformed on a large scale, and have mainly been responsible for reducing the number of polyphyletic genera (Blanco *et al*. 2004*b*) or for lumping rather than splitting genera (Blanco *et al*. 2004*a*). Among cetrarioid lichens, *Tuckneraria* Randlane & A. Thell was synonymized with *Nephromopsis* Müll. Arg., and *Nimisia* Kärnefelt & A. Thell with *Himantormia* I. M. Lamb (Thell *et al*. 2007). A detailed history of the pre-DNA sequencing taxonomy of cetrararioid lichens is provided by Thell *et al*. (2002).

The aim of this paper is to present a complementary phylogeny of the monophyletic core of cetrarioid lichens and discuss the relationship of the taxonomy to phylogeny within the group. Eleven species are investigated for the first time (Table 2), *Ahtiana*

TABLE 1. *Genera and type species of the cetrarioid core of the* Parmeliaceae

Genus	Type species
Ahtiana Goward, *Bryologist* **88:** 370, 1985.	*Ahtiana sphaerosporella* (Müll. Arg.) Goward
Allocetraria Kurok. & M. J. Lai, *Bull. Nat. Sci. Mus. Tokyo, ser. B* **17:** 60, 1991.	*Allocetraria stracheyi* (Bab.) Kurok. & M. J. Lai
Arctocetraria Kärnefelt & A. Thell, *Bryologist* **96:** 402, 1993.	*Arctocetraria andrejevii* (Oxner) Kärnefelt & A. Thell
Cetraria Ach., *Meth. Lich.*: 292, 1803.	*Cetraria islandica* (L.) Ach
Cetrariella Kärnefelt & A. Thell, *Bryologist* **96:** 402, 1993.	*Cetrariella delisei* (Schaer.) Kärnefelt & A. Thell
Cetreliopsis M. J. Lai, *Quart. J. Taiwan Mus.* **33:** 218, 1980.	*Cetreliopsis rhytidocarpa* (Mont. & Bosch) M. J. Lai
Flavocetraria Kärnefelt & A. Thell, *Acta Bot. Fennica* **150:** 81, 1994.	*Flavocetraria cucullata* (Bellardi) Kärnefelt & A. Thell
Kaernefeltia A. Thell & Goward, *Bryologist* **99:** 125, 1996.	*Kaernefeltia californica* (Tuck.) A. Thell & Goward
Masonhalea Kärnefelt, *Bot. Notiser* **130:** 102, 1977.	*Masonhalea richardsonii* (Hook.) Kärnefelt
Nephromopsis Müll. Arg., *Flora* **74:** 374, 1891.	*Nephromopsis stracheyi* (Bab.) Müll. Arg.
Tuckermanella Essl. Mycotaxon **85:** 135–136. 2003.	*Tuckermanella weberi* (Essl.) Essl.
Tuckermannopsis Gyeln., *Acta Fauna Fl. Univ.*, ser. 2 (Bot.), **1** (5/6): 6, 1933.	*Tuckermannopsis ciliaris* (Ach.) Gyelnik
Usnocetraria M. J. Lai & J. C. Wei, *J. Nat. Taiwan Mus.* **60:** 45–61, 2007.	*Usnocetraria oakesiana* (Tuck.) M. J. Lai & J. C. Wei
Vulpicida Mattsson & M. J. Lai, *Mycotaxon* **46:** 427, 1993.	*Vulpicida juniperinus* (L.) Mattsson & M. J. Lai

aurescens (Tuck.) Randlane & A. Thell, '*Cetraria*' *annae* Zahlbr., *Cetraria australiensis* Kärnefelt, *Cetraria crespoae* (Barreno & Vázquez) Kärnefelt, *Cetraria laevigata* Rass., *Cetraria kamczatica* Savicz, *Cetreliopsis laeteflava* (Zahlbr.) Randlane & A. Saag, '*Flavocetraria*' *minuscula* (Elenk. & Savicz) Ahti, Poryadina & Zhurb., '*Melanelia*' *sorediella* (Lettau) V. J. Rico, van den Boom & Barrasa, *Tuckermannopsis ciliaris* (Ach.) Gyeln. and *Tuckermannopsis inermis* (Nyl.) Kärnefelt.

Material and Methods

Selected material and genetic markers

The matrix composed of 77 samples, representing 71 species, including the outgroups, were selected to investigate the phylogeny within the monophyletic core *Cetraria* s. lat., represented by 65 of the 90 species worldwide. Eleven of the species have not been previously analyzed for their DNA, 67 of the 178 sequences are new, and mitochondrial DNA is used in this group for the first time (Table 2).

Representatives from five genera were selected as outgroups. *Melanelia* and *Esslingeriana* are sister groups to the monophyletic core of cetrarioid lichens according to earlier investigations (Blanco *et al.* 2005, 2006; Thell *et al.* 2002, 2004). The three genera, *Alectoria*, *Cetrelia* and *Platismatia*, all rather distantly related to the cetrarioid core, and not closely related with each other, were selected as an external outgroup.

Earlier molecular phylogenetic studies on the cetrarioid group constituted a base for the combined, complementary data set. Amplification of ITS sequences were easily performed for all species, whereas β-tubulin, GAPDH and mtSSU sequences were much more difficult to amplify, the latter marker sequenced for the cetrarioid core on a large scale for the first time. Group I intron sequences are absent from more than half of the species investigated (Thell 1999; Thell *et al.* 2000, 2002, 2004).

DNA analysis

Extraction

MagAttract 96 DNA Plant Extraction Kit from Qiagen (08/2003) was used. The samples were rigorously shaken in a vortex machine in 300 µl extraction buffer incorporating one steel bead into each microtube; otherwise, the enclosed protocol for manual DNA purification was followed.

Amplification

25 µl PCR-reactions were prepared to amplify the nuclear ITS1-5.8S-ITS2 ribosomal DNA region. The primers ITS1F (Gardes & Bruns 1993), ITS4 (White *et al.* 1991), bt3LM, bt10LM (Myllys *et al.* 2001), gpd1LM, gpd2LM (Myllys *et al.* 2002), and mrSSU1, mrSSU3 (Zoller *et al.* 1999) were used. Ready To Go PCR beads (in 0·2 ml tubes) from Pharmacia Biotec Inc. were dissolved in 11·8 µl distilled water, 0·35 µl of a 16µM concentration of each of the primers. The ITS

TABLE 2. *Lichen material and sequences used in the analyses of the cetratoid core of the* Parmeliaceae. *Sequences with accession-numbers beginning with EU were produced in this study.*

Species	Specimen–ID	Extr.	Intron	ITS	bt	Gpd	mtSSU
Alectoria ochroleuca	Austria, Tirol, *Feuerer & Thell* s. n. (HBG)	AT976	–	AF457926	AF457926	AY249638	–
Cetrelia olivetorum	Austria, Tirol, *Feuerer & Thell* 64372 (HBG)	AT913	–	AF451763	AF449716	AY249611	
Platismatia glauca	Estonia, Tartumaa, *Thell* 9903 (TUR)	AT550	–	AF451758	AF457925	AY249593	
Esslingeriana idahoensis	Canada, British Columbia, *Goward* 961348 (UBC)	AT146	–	AF227513	–	–	–
Melanelia hepatizon	Italy, Trentino Alto Adige, *Feuerer & Thell* 64248 (HBG)	AT934	AF451776★	AF451776	DQ004576	DQ004577	EU435364
M. sorediella	Andorra, Ordino, *Rico* (MAF–Lich 10592)	AT1917	EU401769	EU401769	–	EU423865	EU435369
M. stygia	Italy, Trentino Alto Adige, *Feuerer & Thell* 64247 (HBG)	AT922	–	AF451775	AY074778	AY249607	EU435365
Ahtiana aurescens	USA, Michigan, Gogebic Co., *Wetmore* 882794 (MIN)	FH195	–	EU401756	–	–	–
A. pallidula	USA, Montana, Sanders Co., *Hauck* (private hb.)	AT1268	–	AY353709	–	AY249602	–
A. sphaerosporella	Canada, British Columbia, *Miao & Taylor* (TDI–211)	AT73	AF141859	AF141859	–	AY249604	–
Allocetraria ambigua	China, Sichuan, *Obermayer* 08141 (GZU)	AT874	–	AF404128	–	–	–
A. flavonigrescens	China, Sichuan, *Obermayer* 08140 (GZU)	AT873	–	AF404127	–	–	–
A. globulans	China, Sichuan, *Obermayer* 08137 (GZU)	AT870	–	AF404126	–	–	–
A. madreporiformis	Austria, Tyrol, *Obermayer* 7746 (M)	AT973	–	AF416460	–	–	–
A. sinensis	China, Sichuan, *Obermayer* 08148 (GZU)	AT868	–	AF404125	–	–	–
A. stracheyi	China, Sichuan, Hengduan Shan, *Obermayer* 8139 (GZU)	AT875	–	AF404129	AF449733	–	EU435368
Arctocetraria andrejevii	Greenland, Qeqertannguit, *Hansen*, exs. 836 (LD-1001631)	AT1364	–	DQ004575	–	–	–
A. nigricascens	Canada, N. W. T., Melville Isl., *Westberg* 1614 (LD)	AT793	AF254628★	AF254628	AF449728	AY249599	–
Cetraria aculeata	Spain, Castlla & León, *Feuerer* s. n. (LD-1196893)	AT1922	–	EU401758	–	–	–
C. annae	Russia, Baikal region, *Urbanavicius* (LD-1271346)	FH194	–	EU401759	–	–	EU435375

TABLE 2. *Continued*

Species	Specimen–ID	Extr.	Intron	ITS	bt	Gpd	mtSSU
C. arenaria	Canada, Ontario (TDI–220)	AT173	AF115758	AF115758	–	–	–
C. australiensis	Australia, N. S. W., Kosciusko State Park, *Feuerer* (HBG)	AT1696	EU401760	EU401760	–	EU423860	EU435366
C. crespoae	Spain, Castilla Y Léon, *Feuerer* (LD–1199338)	AT1920	–	EU401761	–	EU423861	EU435379
C. ericetorum	Sweden, Skåne, Åhus, *Thell & Marth* 9928 (TUR)	AT544	AF228296	AF228296	AF449740	AY249594	–
C. islandica	Estonia, Tartumaa, Taevaskoja, *Thell* 9901 (TUR)	AT548	AF228290	AF228290	AF449739	–	–
C. kamczatica	USA, Alaska, Noatak Nat. Preserve, *Ahti* 63296 (H)	AT1950	EU401763	EU401763	–	–	–
C. laevigata	Russia, Sakha Republic, *Ahti* 64755 (H)	AT1952	EU401764	EU401764	–	–	–
C. muricata	Spain, Castilla Y Léon, *Feuerer* (LD–1197733)	AT1921	–	EU410409	–	–	–
C. nigricans	Canada, Baffin Island, *Westberg* 2377 (LD)	AT791	–	AF457922	–	–	–
C. oakesiana	Slovenia, *Kärnefelt* 960306 (LD-1077935)	AT136	–	AF116179	AF449731	–	–
C. oakesiana	Germany, Bavaria, Oberbayern, *v. Brackel* (IVL)	AT1915	–	EU401757	–	EU423859	EU435374
C. obtusata	Austria, Tyrol, *Feuerer & Thell* s. n. (HBG)	AT990	AF449739	AF449739	–	–	EU435378
C. odontella	Finland, E. H., Sysmä, *Haikonen* 23297 (H)	AT1694	EU401765	EU401765	EU410411	EU423863	EU435367
C. sepincola	Finland, Varsinais–Suomi, *Puolasma & Thell* 0202 (HBG)	AT1248	–	EU401766	–	EU423864	EU435371
Cetrariella commixta	Finland, Tavastia australis, *Haikonen* 19093 (H)	AT720	–	AF451796	AF449735	AY249596	–
C. delisei	Iceland, S. Mulasysla, *Thell* 9714 (LD-1016978)	AT234	–	AF228305	AF449737	AY249595	EU435368
C. delisei	USA, Alaska, Noatak, *Holt* 23063 (LD-1190098)	AT1913	–	EU401767	–	–	–
C. fastigiata	Finland, KiL, *Haikonen* 24443 (H)	AT1951	–	EU401768	–	–	EU435370
Cetreliopsis asahinae	South Korea (*Hur* 040500, unpubl.)			DQ394386			
C. laeteflava	Taiwan, Kaohsiung County, *Lai* (private hb.)	FH124	–	EU401770	–	–	EU435372

Table 2. *Continued*

Species	Specimen–ID	Extr.	Intron	ITS	bt	Gpd	mtSSU
C. rhytidocarpa	Philippines (*Bawingan* CL0582, *Mol. Phyl. Evol.* 44)			DQ980008			
Flavocetraria cucullata	Russia, Sakha Republic, *Ahti* 61793 (H)	FH89	–	EU401771	–	–	EU435382
F. cucullata	Austria, Tyrol, *Feuerer & Thell* 64185 (HBG)	AT932	–	AF451793	–	AY249601	–
F. minuscula	Russia, Sakha Republic, *Ahti* 61573 (H)	FH90	–	EU401772	–	–	EU435381
F. minuscula	Russia, Sakha Republic, *Ah*ti 61682 (H)	FH91	–	EU401773	–	–	–
F. minuscula	USA, Alaska, *Zhurbenko* 04204 (H)	FH93	–	EU401774	–	–	–
F. nivalis	Iceland, S. Thingeyar sysla, *Frödén* 643 (LD-1025426)	AT700	–	AF451794	–	–	EU435383
Kaernefeltia merrillii	Spain, Madrid, El Berrueco, *Thell* 0501 et al. (LD-1038537)	AT1918	DQ395292★	DQ395292	EU410412	EU423866	EU435380
K. californica	USA, Oregon, Lincoln Co., *McCune* 27703 (LD-1045103)	AT1703	–	DQ004571	–	–	–
Masonhalea richardsonii	Canada, Yukon Territory, *Westberg* 1246 (LD)	AT792	–	AF254617	AF449730	AY249598	–
Nephromopsis ahtii	Bhutan, Paro Distr., *Søchting* 8489 (LD-1061438)	AT607	AF404123	AF404123	–	–	–
N. komarovii	Russia, Primorie, *Skirina* 10972 (LD-1000141)	AT621		AF451779	AF449722	–	–
N. laureri	Italy, Trentino-Alto Adige, *Feuerer & Thell* 64288 (HBG)	AT938	AF451786	AF451786	AF449724	DQ004529	–
N. leucostigma	Bhutan, Thimpu Distr., *Søchting* 9151 (LD-1003792)	AT604	AF451777	AF449716	DQ004583		–
N. melaloma	Bhutan, Thimpu Distr., *Søchting* 9181 (LD-1056669)	AT430	AF451778	AF449720			–
N. morrisonicola	China, Sichuan, *Obermayer* 8279 (GZU)	AT903	–	AF451780	–	–	–
N. nephromoides	The Philippines, *Ejem* s. n. (H).	AT1693	–	DQ004574	–	–	–
N. ornata	Russia, Primorye, *Kudryavtseva* 10980 (LD-1022832)	AT624	–	AF451783	AF449721	–	–
N. pallescens	Bhutan, *Søchting* 8206 (C)	AT618	–	AF451784	–	DQ004578	–
N. pseudocomplicata	China, Sichuan, *Obermayer* 08276a (GZU)	AT907	–	AF404131	AF449725	AY249603	–
N. stracheyi	Bhutan, Thimpu, *Søchting* 8095 (C)	AT606	AF451785★	AF451785	–	AY249605	EU435373

TABLE 2. *Continued*

Species	Specimen–ID	Extr.	Intron	ITS	bt	Gpd	mtSSU
Tuckermannopsis americana	Canada, British Columbia, *Goward* 961350 (UBC)	AT148	AF072233	AF072233	AF449726	–	–
T. chlorophylla	South Africa, Western Cape, *Feuerer & Thell* s. n. (HBG)	AT1022	–	AF451789	AF449727	AY249600	–
T. ciliaris	USA, N. Carolina, 2004-10-17, *Fraker* et al. (AFTOL proj.)	–	FJ005090		–	–	–
T. inermis	USA, Alaska, Noatak Nat. Pr., *Holt* 23441 (LD-1190038)	AT1912	–	EU401762	EU410410	EU423862	EU435376
T. orbata	USA, Montana, *Hauck* s. n. (private hb.)	AT1067	DQ004572★	DQ004572	–	–	–
T. platyphylla	Canada, British Columbia, *Thell & Veer* 9643 (LD-1058556)	AT43	AF072235	AF072235	–	–	–
T. platyphylla	Canada, British Columbia, *Thell* 9675 (LD-1096447)	AT75	–	AF072236	AF449741	DQ004581	–
T. subalpina	Canada, British Columbia, *Thell* 9606 (LD)	AT109	AF072237	AF072237	–	–	–
Tuckermanella coralligera	USA, New Mexico, *Worthington* 28821 (ASU)	AT1158	AF457924★	AF457924	–	DQ004582	–
T. fendleri	USA, Arizona, *Westberg* 543 (LD-1059952)	AT612	AF451791	AF451791	–	–	–
T. weberi	USA, Arizona, *Westberg* 548 (LD-1012687)	AT614	–	AF451792	–	–	–
Vulpicida canadensis	Canada, British Columbia, *Thell & Veer* 96250 (LD)	AT36	–	AF072238	–	–	–
V. juniperinus	Finland, Varsinais–Suomi, Lohja, *Pykälä* 21426 (H)	AT1695	–	EU401775	–	–	–
V. pinastri	Sweden, Scania, *Thell* 9604 (LD-1056353)	AT02	AF139031	AF139031-	–	–	–
V. tubulosus	Austria, Tyrol, Innsbruck, *Feuerer & Thell* s. n. (HBG)	AT933	AF404132★	AF404132	AF449736	–	–
V. viridis	USA, Connecticut, Tolland Co., *Feuerer* s. n. (HBG)	AT1291	DQ004573★	DQ004573	–	DQ004580	–

★Unpublished intron-sequences obtained from old ITS sequences.

fragments were amplified with a Perkin-Elmer Gene Amp PCR System 9700 thermal cycler. 12·5 µl of the concentrated DNA extractions were added to the solution. The PCR program described by Ekman (2001) was employed: after a 2 min. hold at 94°C, six cycles followed with denaturation at 94°C for 60 sec., annealing at 62°–56°C for 60 sec. (decreasing 1°C per cycle), and an extension at 72°C for 105 sec., followed by 34 cycles with denaturation at 94°C for 30 sec., annealing at 56°C for 30 sec. and extension at 72°C for 105 sec.; finally, a 10 min. hold at 72°C was performed before the PCR products were cooled to 4°C.

Purification and sequencing

The PCR products were cleaned with PCR clean-up NucleoFast 96 PCR Purification Kit from Macherey-Nagel following the user manual (2002/3/Rev. 01. p.12). 50 µl TE buffer was applied to each sample prior to the standard procedure for purification of PCR products under vacuum, which was followed, except for the second, optional, washing step, with purified water. The purified samples were collected in 50 µl water and the amount of DNA was measured in an Eppendorf BioPhotometer. The concentrations ranged between 18 and 54 ng/µl. The amount of DNA for sequencing, 1 ng/base pair, was dried for 1 hr at 65°C. Finally, the DNA–fragments were sent to Macrogen to be sequenced, using the same primers as for the amplification.

Phylogeny

The phylogenetic analyses of the manually aligned sequences were done with PAUP version 4.0b (Swofford 1998). Trees were calculated using the general heuristic search option and the TBR branch swapping method, whereas gaps were treated as missing characters. Bootstrap analyses with 1000 replicates were performed using the same settings. Support values of 50 or above are marked in the consensus trees (Figs. 1 & 2). Two phylogenies are presented, the first based on the complete ITS matrix and and a second using all the DNA sequences.

To complement the parsimony analysis, data were analysed using a Bayesian approach (Larget & Simon 1999), MrBayes 3.1 program. Posterior probabilities were approximated by sampling trees using a variant of the Markov Chain Monte Carlo (MCMC) method called Metropolis-coupled Markov Chain Monte Carlo (MCMCMC). The following priors were used: flat Dirichlets for the substitution rate matrix and nucleotide frequencies, uniform (0, 200) and (0, 1) distributions for the gamma curve shape parameter and proportion of invariable sites, an exponential distribution with mean 0·1 for the branch lengths, and a uniform distribution across tree topologies. The following settings were applied: the best-fit model (GTR+I+G) selected by AIC in MrModeltest 2.2; number of generations = 1100 000; number of simultaneous independent analyses = 2; number of simultaneous chains = 4; sample frequency = 100; 'temperature' = 0·2. No molecular clock was assumed. The initial 2500 trees were discarded as burn-in before stability was reached. Using sumt option of MrBayes, a majority-rule consensus tree was calculated from 8500 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. Phylogenetic trees were drawn using TreeView.

Anatomy

Complementary anatomical studies were performed on two rare cetrarioid species, '*Cetraria*' *annae* and '*Flavocetraria*' *minuscula*. Anatomical sections, 15 µm thick, produced using a Kryomate, Leitz freezing microtome, were mounted in lactophenol cotton-blue. The sections were studied with a Zeiss Axioscope light microscope.

Secondary chemistry

Secondary compounds of *Cetraria annae* were detected by means of high performance liquid chromatography (Elix *et al*. 2004).

Taxonomy

The taxonomy according to the latest world list of cetrarioid lichens (Randlane & Saag 2002) is strictly used, with the addition of two recently added genera in the cetrarioid core, *Tuckermanella* and *Usnocetraria* (Esslinger 2003; Lai *et al*. 2007) and two species in other genera *Cetrariella commixta* (Nyl.) A. Thell & Kärnefelt (Thell *et al*. 2004: 309) and '*Melanelia*' *sorediella* (Rico *et al*. 2005: 205). Several existing, more natural combinations than the ones used here are discussed below. New combinations are needed for a few species, after careful evaluation of the genus concept. Taxonomic changes are postponed for the third updated world list of cetrarioid lichens (T. Randlane, A. Saag, A. Thell & T. Ahti, unpublished).

Results

Results of the phylogenetic analyses

Phylogenetic analyses were performed on two data sets, the first on the complete ITS matrix, and the second based on the total matrix. The ITS analysis resulted in eight most parsimonious trees, all with a length of 1086 changes, CI=0·4171, RI=0·6463. Including gaps, the matrix was composed of 517 characters, of which 101 provided parsimony information. The analysis based on the combined data set resulted in 23 most parsimonious trees, having a length of 2923 changes, CI=0·5279, RI=0·6067. Including
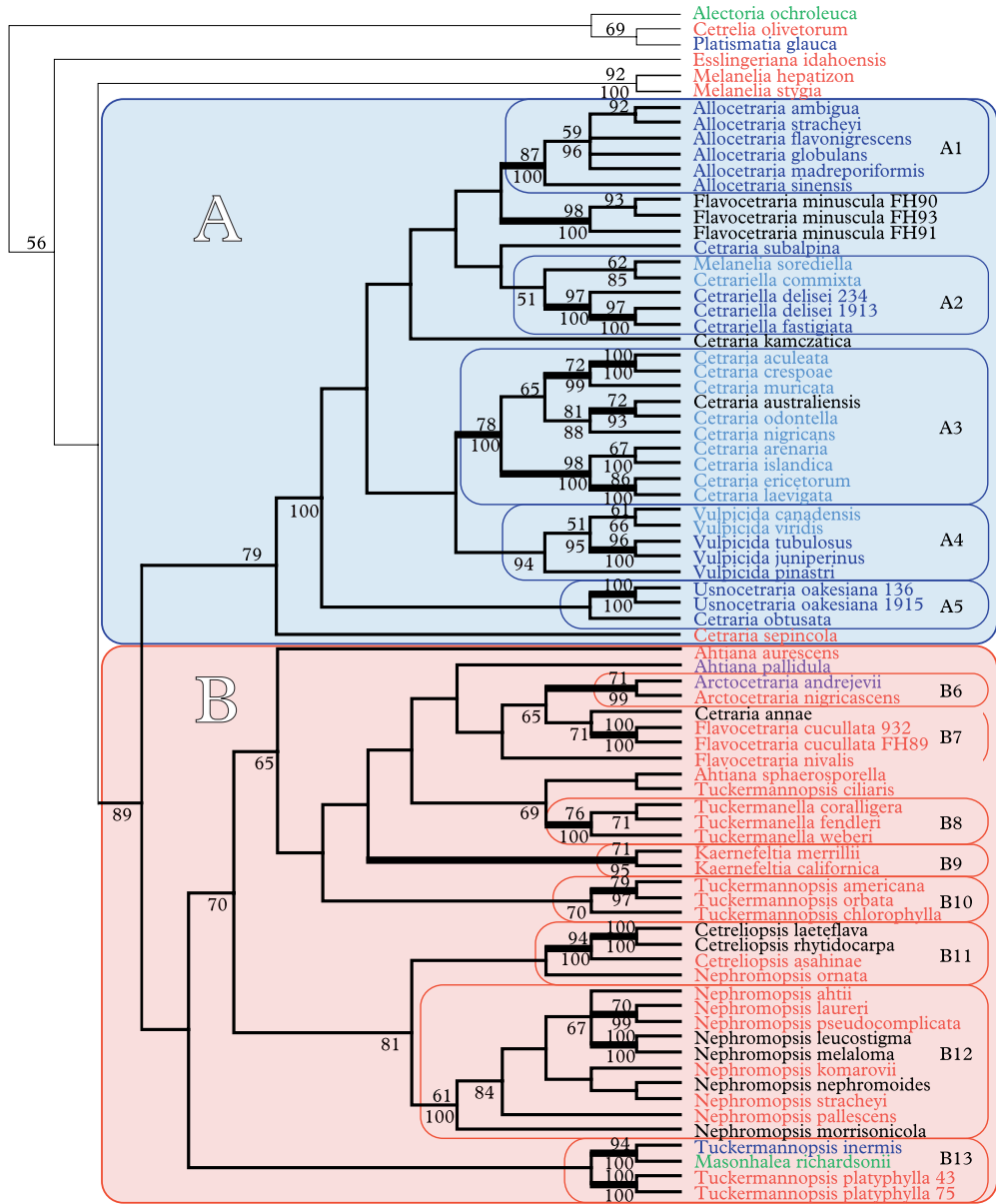
FIG. 1. Consensus tree from eight most parsimonious trees using the heuristic search option in PAUP 4.0b, based on ITS sequences. Bootstrap support values ≥ 50 from 1000 replicates are indicated above the branches. Posterior probability values ≥ 50% from the Bayesian analysis are indicated below the branches. Strongly supported clades (bootstrap support ≥ 70% and posterior probabilities ≥ 95% in the Bayesian analysis) are marked in bold.
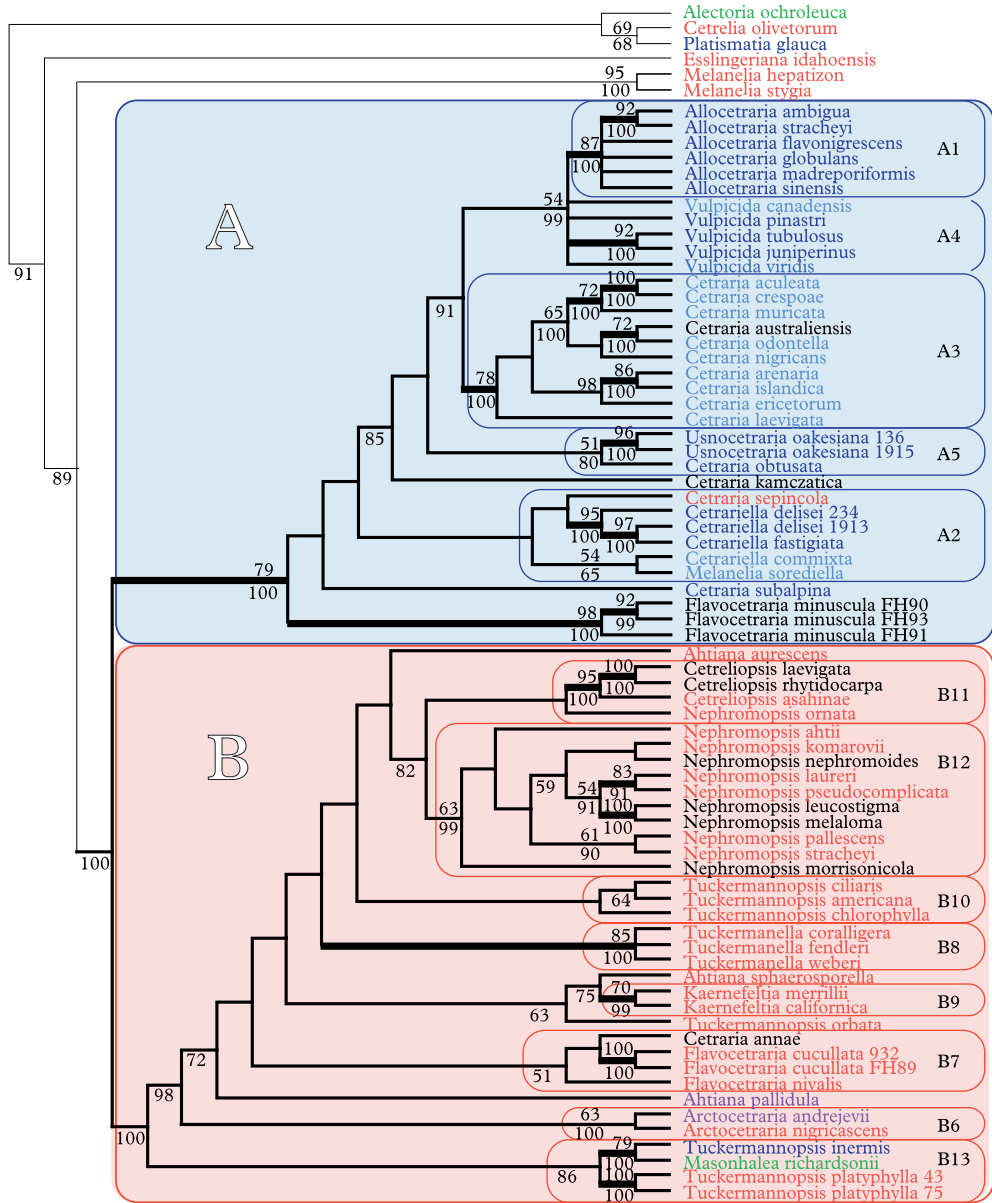
FIG. 2. Consensus tree from 23 most parsimonious trees using the heuristic search option in PAUP 4.0b, based on the combined data matrix (Table 2). Bootstrap support values ≥ 50 from 1000 replicates are indicated above the branches. Posterior probability values ≥ 50% from the Bayesian analysis are indicated below the branches. Strongly supported clades (bootstrap support ≥ 70% and posterior probabilities ≥ 95% in the Bayesian analysis) are marked in bold.

gaps, the matrix was extended to 3314 characters, of which 644 proved to be parsimony informative.

The two consensus trees, calculated by PAUP, based on the ITS and combined data set, are similar, but not congruent, in their topologies (Figs 1 & 2). All groups supported by the bootstrap analysis are present in the PAUP consensus trees, whereas a few groups with support in the Bayesian analysis are not.

By performing MrBayes multiple runs starting from random trees, the inferences for independent runs were the same. Credible sets of trees for one run (7646 trees sampled) are as follows: 50% credible set contains 3146 trees, 90% credible set contains 6746 trees, 95% credible set contains 7196 trees, 99% credible set contains 7556 trees. Posterior probability values equal or above 50% are indicated on the consensus trees.

Supported clades

Most clades of the consensus trees calculated with PAUP were supported either by the bootstrap or Bayesian analyses. The clades that received bootstrap support equal or above 70% and/or posterior probabilities equal or above 95% in the Bayesian analysis were considered as strongly supported. The position of the species within the clades A1–4 and B9 and B12 differ slightly between the PAUP and Bayesian analyses. Generally, more clades were supported by the Bayesian analyses than by the bootstrap analyses.

The cetrarioid core – ingroup

The study group selected, comprising 14 genera (Table 1), was divided into two subgroups, the *Cetraria* and *Nephromopsis* clades, both with maximum posterior probabilities in the Bayesian analysis of the combined DNA-matrix (Figs 1 & 2, clades A & B).

A. The *Cetraria* clade

This clade contains about half of the cetrarioid core and is divided mainly between five genera, *Allocetraria, Cetraria, Cetrariella*, *Usnocetraria* and *Vulpicida*, characterized by conidia with one swelling (apical or central,

i.e. citriform, sublageniform or filiform), with *Cetraria sepincola* (Ehrh.) Ach., having dumb-bell shaped conidia, as the single known exception. The variation in morphology, anatomy and secondary chemistry is large and no key character for the clade can be presented. The species included are mainly from the Northern Hemisphere (often Himalayan) in their distribution ranges. The clade is strongly supported in the bootstrap analysis, 79 in both the ITS and combined DNA matrix analyses, as well as by the Bayesian analysis of the combined DNA matrix (maximum posterior probability).

A1. The *Allocetraria* clade

The bootstrap support value for *Allocetraria* is 87 in both the ITS and in the total analysis, although the genus is nested within *Vulpicida* in the PAUP analysis based on the entire matrix (Fig. 2). Six of the nine accepted species were investigated, and they all belong to this clade. The additional taxon, *Usnocetraria oakesiana* (Ach.) M. J. Lai & J. C. Wei (formerly treated in *Allocetraria*) is absent from this clade in all our analyses, appearing as one of several singular species.

A2. The *Cetrariella* clade

Cetrariella is composed of a core of two closely related species, *C. delisei* (Schaer.) Kärnefelt & A. Thell and *C. fastigiata* (Nyl.) Kärnefelt & A. Thell, which are not separated by ITS data. The presence of *Cetrariella commixta* and '*Melanelia*' *sorediella* in *Cetrariella* is weakly supported in this study. *Cetraria sepincola* was positioned in the same clade by the analysis based on the total matrix.

A3. The *Cetraria* s. str. clade

The genus *Cetraria* s. str. received moderate bootstrap support, 78 in both analyses, but is recognized in its entirety by the Bayesian analyses. *Cetraria* s. str. is divided into two species groups, centred around *C. aculeata* (Schreb.) Fr. and *C. islandica* (L.) Ach. All known members of the group, except for *C. steppae* (Savicz) Kärnefelt, were included in the study. *Cetraria laevigata*, for which only group I intron and ITS sequences were available, appears as a sister group to

other *Cetraria* s. str. in the PAUP analysis based on the total evidence matrix, but is strongly supported as a member of the *C. islandica* group using the complete ITS matrix.

A4. The *Vulpicida* clade

Vulpicida is a chemically distinct genus and is supported as monophyletic by the Bayesian analysis of the ITS data, with posterior probability of 94 (Fig. 1). However, the genus appears to be paraphyletic in the PAUP analysis based on the entire matrix (Fig. 2) as *Allocetraria* is nested within the same clade. The positions of *Vulpicida* species remain unresolved in the PAUP analysis of the combined data set (Fig. 2). Five of the six species known worldwide were included in the analyses.

A5. *Usnocetraria oakesiana* and *Cetraria obtusata*

These two species form a separate clade according to three out of four analyses, but the support for the clade is weak (e.g. 51 bootstrap support and 80 posterior probability in the analyses of the combined matrix) and it is not definitely delimited from the other groups. The clade has a few non-molecular characters in common, i.e. presence of sublageniform conidia and secalonic acid.

B. The *Nephromopsis* clade

This clade, including the rest of the cetrarioid core, the genera *Ahtiana, Arctocetraria, Cetreliopsis, Flavocetraria, Kaernefeltia, Masonhalea, Nephromopsis, Tuckermanella* and *Tuckermannopsis*, lacks bootstrap support but is maximally supported by the Bayesian analysis based on the total matrix (Figs. 1 & 2). The group is characterized by conidia with two apical or subapical swellings, dumb-bell to disc-bar shaped conidia. However, conidia of different types have been observed in two of the species, *Ahtiana pallidula* (Riddle) Goward & A. Thell and *Arctocetraria andrejevii* (Oxner) Kärnefelt & A. Thell (Kärnefelt 1979: 59; Thell *et al.* 1995*a*: 602, fig. 15), and no additional non-DNA character that characterizes the clade is known. Furthermore, some species included in the *Masonhalea* clade (see below) still have conidia without swellings or with one apical swelling. The position of this clade varies, being excluded from the *Nephromopsis* clade according to the Bayesian analysis of the ITS matrix and appearing as a sister clade to the remaining part of the cetrarioid core (such topology having, however, posterior probability of 89).

The species included in the *Nephromopsis* clade often have a western North American or South-east Asian distribution. The genera *Arctocetraria, Cetreliopsis, Kaernefeltia, Nephromopsis* s. str. and *Tuckermanella* form monophyletic clades according to the present taxonomy, whereas *Nephromopsis ornata* (Müll. Arg.) Hue is more closely related to *Cetreliopsis* than *Nephromopsis*, thus not belonging to *Nephromopsis* s. str. The genera *Ahtiana, Flavocetraria* and *Tuckermannopsis* appear to be polyphyletic.

B6. The *Arctocetraria* clade

Arctocetraria, a genus of only two species with an arctic distribution, is moderately supported by bootstrap analyses and strongly supported by Bayesian analyses. The inclusion of *Arctocetraria* within the genus *Flavocetraria* based exclusively on ITS sequences is only weakly supported.

B7. The *Flavocetraria* clade

All three accepted species were investigated. Two 'old' taxa, *Flavocetraria cucullata* (Bell.) Kärnefelt & A. Thell and *F. nivalis* (L.) Kärnefelt & A. Thell formed a separate clade together with the rare and recently rediscovered species '*Cetraria*' *annae*. The third *Flavocetraria* species, the recently proposed '*F.*' *minuscula*, is included in another major clade of the cetrarioid core – the *Cetraria* clade.

B8. The *Tuckermanella* clade

The bootstrap support of 76 for the North American genus *Tuckermanella* is weaker in the bootstrap analysis when based exclusively on ITS sequences, but when compared with the analysis inferred from the total matrix, the support value reached 85. Maximum

support was received from the Bayesian analyses. Three of six accepted species were included.

B9. The *Kaernefeltia* clade

Kaernefeltia is an additional genus composed of two species. Like *Arctocetraria*, the genus received strong support by both bootstrap and Bayesian analyses.

B10. The *Tuckermannopsis* clade

Altogether 25 species have been combined in the genus, only eight are included in the genus in the strict sense (Randlane & Saag 2003). Four 'true' *Tuckermannopsis* species, *T. americana* (Spreng.) Hale, *T. chlorophylla* (Willd.) Hale, *T. ciliaris* and *T. orbata* (Nyl.) M. J. Lai, were investigated in this study. Three species (in various combinations) are included in the *Tuckermannopsis* clade in different analyses (Figs. 1–2). The clade is, however, only weakly supported.

B11. The *Cetreliopsis* clade

The monophyletic status of this East Asian genus is strongly supported by all the analyses. The genus could be seen as nested within *Nephromopsis* s. lat. because *N. ornata* is positioned as a sister group to *Cetreliopsis*. However, the support for the common clade of *Cetreliopsis-Nephromopsis* (B11 & B12) is lower than acceptable. Three species of seven worldwide were included in the analyses.

B12. The *Nephromopsis* s. str. clade

Eleven of the 19 species were analysed. The clade, including 10 taxa (*N. ornata* excepted), received weak support, slightly above 60, in both bootstrap analyses, but high posterior probabilities (99 and 100) in the Bayesian analyses.

B13. *Masonhalea richardsonii* and *Tuckermannopsis inermis*

Masonhalea richardsonii (Hook.) Kärnefelt, a species characterized by bacillariform conidia, and *Tuckermannopsis inermis*, with sublageniform conidia, form a clade supported by all analyses. However, the two species have no non-DNA characters in common except for their arctic distribution. Both

types of conidia, bacillariform and sublageniform, differ from that observed in the major *Nephromopsis* clade (clade B on Figs 1 & 2). The third taxon, *Tuckermannopsis platyphylla* (Tuck.) Hale, is related to these two taxa in three out of four analyses, but only with weak support.

Discussion

Conidial shape and phylogeny

The variation in conidial shape is greater in the cetrarioid core than in any other corresponding clade in the *Parmeliaceae* (Thell 1995*b*) and is the character that shows strongest correlation with DNA based phylogeny (Thell *et al.* 2002), as illustrated by the different colours in Figs. 1 & 2. The main difference between the conidia is the number of swellings: none, one or two. Conidia with two swellings are dominant among species of the major *Nephromopsis* clade (B). There are slight differences between them, sometimes discerned as dumb-bell and disc-bar shaped, but the swellings are always more or less apical and are treated as one type in this study. This type is characteristic of the genera *Flavocetraria*, *Kaernefeltia*, *Nephromopsis*, *Tuckermanella* and *Tuckermannopsis*.

The second type of conidium has one swelling that may be situated either apically or centrally. Conidia with one apical swelling are either sublageniform (bottle-shaped) if they are shorter than 12 μm or filiform if they are longer than 12 μm. Sublageniform conidia occur in three genera of the *Cetraria* clade (A): *Cetrariella*, *Usnocetraria* and *Vulpicida*, as well as in *Cetraria obtusata* (Schaer.) v. d. Boom & Sipman and *C. subalpina* Imshaug. Filiform conidia is a key character for distinguishing the genus *Allocetraria*. Conidia with a central swelling are fusiform, varying from short to oblong citriform; the latter is typical for *Cetraria* s. str., whereas the short citriform type is known from *Cetrariella commixta*, *Vulpicida canadensis* (Räsänen) J.-E. Mattsson and *V. viridis* (Swein.) J.-E. Mattsson.

The third type of conidium, without swellings (bacillariform), is rare in cetrarioid

lichens, being restricted to *Masonhalea rich-ardsonii*.

In spite of the strong correlation between conidial shape and DNA, there are notable exceptions, such as *Cetraria sepincola*, the only species with dumb-bell shaped conidia in the major *Cetraria* clade, *Arctocetraria andrejevii*, where both dumb-bell and oblong citriform conidia have been found, and *Ahtiana pallidula*, where different conidial shapes have been observed within the same pycnidium (Thell *et al*. 1995*a*). Obermayer (2008) recently reported variation in conidial shape within *Cetraria islandica*.

Notes on the genera

Ahtiana *Goward*

Ahtiana was originally proposed as a monotypic genus for *A. sphaerosporella* (Müll. Arg.) Goward, a species segregated from *Parmelia* Ach. (Goward 1985). The inclusion of two additional North American species, *A. aurescens* and *A. pallidula* (Thell *et al*. 1995*a*), based on their similar spherical ascospores and the presence of caperatic acid in the medulla, is not supported by cladistic analyses (Figs 1 & 2); however, accommodating these taxa into any other genera has no support either. Returning to a monotypic *Ahtiana*, including only *A. sphaerosporella*, would seem attractive to lower the number of polyphyletic genera, but is not confirmed in the present study. Hale (in Egan 1987) combined several species in *Tuckermanopsis* including *Ahtiana pallidula* but not *A. aurescens*, and the only alternative combination for *A. aurescens*, namely *Cetraria aurescens* Tuck., would not be tenable if one preserves a multi-genus concept within the cetrarioid core.

Allocetraria *Kurok. & M. J. Lai*

This genus includes nine species, mainly occurring at high altitudes, seven of which are endemic to the Himalayas (Kurokawa & Lai 1991; Randlane & Saag 2004). The type species, *A. stracheyi* (Bab.) Kurok. & M. J. Lai, also occurs infrequently in North America, and *A. madreporiformis* (Ach.) Kärnefelt & A. Thell is widespread in arctic and alpine areas of the Northern Hemisphere

(Randlane & Saag 2004). Some species originally described in the genera *Cetraria* and *Dactylina* have been transferred to this genus on two occasions since the original circumscription of the genus (Thell *et al*. 1995*b*; Kärnefelt & Thell 1996). One such species, *A. oakesiana* (Tuck.) Randlane & A. Thell, differs in having shorter conidia (7–12 μm long) when compared with *Allocetraria* s. str. (12–21 μm long). The exclusion of *A. oakesiana* from the genus results in a strongly supported *Allocetraria*-clade (Figs 1 & 2). As presently delimited, *Allocetraria* is characterized by filiform conidia, slightly thicker at one end, and longer than in any other genus of the *Parmeliaceae*, except for *Parmeliopsis*, which has conidia of similar length

Lai *et al*. (2007) proposed the genus *Usnocetraria* to include 11 species formerly accommodated in *Allocetraria*, *Cetraria*, *Nephromopsis* and *Tuckermannopsis*, and returned *A. madreporiformis* to *Dactylina*. However, the position of *A. madreporiformis* close to the type species of the genus *Allocetraria*, *A. stracheyi*, was confirmed in a phylogenetic analysis by Saag *et al*. (2002). Therefore, the genus *Usnocetraria* is proposed here as being mono-typic for *U. oakesiana* (see *Usnocetraria*).

Arctocetraria *Kärnefelt & A. Thell*

Arctocetraria was segregated from *Cetraria* because of differences in ascus structure, mainly the broader axial body, as well as the presence of bifusiform conidia (although the conidia are usually citriform in *A. andrejevii*), and the occurrence of norrangiformic and rangiformic acids in the medulla (Kärnefelt *et al*. 1993). The consensus tree based on ITS sequences suggests a relationship between *Arctocetraria* and *Flavocetraria*, but without bootstrap support. Both of these *Cetraria*-segregates are positioned outside the rather well supported *Cetraria* s. lat. clade in the phylogenetic trees (Fig. 1).

Cetraria *Ach.*

This traditional, well-known genus is characterized by the fatty acids, lichesterinic and protolichesterinic acids, in the medulla, and the fusiform, oblong citriform conidia,

6–10 × 1 μm (Kärnefelt 1979, 1986; Kärnefelt *et al*. 1992), often clavate, according to Obermayer (2008). The *Cetraria* s. str. clade (A3) is exclusively composed of the *C. aculeata* and *C. islandica* groups. However, in order to avoid the creation of a large number of new genera, *Cetraria* is considered to include some additional species, *C. kamczatica*, *C. obtusata* and *C. sepincola*, from the *Cetraria* s. lat. clade (A). They differ from *Cetraria* s. str. in both secondary chemistry and conidial shape, with *C. sepincola* being the most divergent in having dumb-bell shaped conidia. The name *C. subalpina* should be used instead of *Tuckermannopsis subalpina* (Imshaug) Kärnefelt, and '*Flavocetraria*' *minuscula* is probably more appropriately accommodated in *Cetraria* than in any of the other genera from clade A or in *Flavocetraria* from clade B according to the results presented here.

Cetrariella *Kärnefelt & A. Thell*

This *Cetraria* segregate was proposed for the closely related species, *Cetrariella delisei* and *C. fastigiata*, characterized by broader asci and axial bodies than in *Cetraria* s. str., sublageniform conidia, and presence of gyrophoric and hiascic acids in the medulla (Kärnefelt *et al*. 1993). Subsequently, the former *Cetraria commixta* (Nyl.) Th. Fr. was shown to be in the same clade as *Cetrariella delisei* and *C. fastigiata* following a cladistic analysis based on DNA sequences, and the new combination in *Cetrariella* was proposed (Thell *et al*. 2004). *Cetrariella commixta* had earlier been transferred to *Melanelia* together with the superficially similar *Melanelia agnata* (Nyl.) A. Thell, *M. culbersonii* (Hale) A. Thell and *M. hepatizon* (Ach.) A. Thell (Thell 1995*a*).

Another taxon, formerly known as *Cetraria commixta* f. *sorediella* Lettau, was raised to species level as *Melanelia sorediella* (Rico *et al*. 2005). This species was subjected to DNA analysis in the present study and shown to be the closest relative of *Cetrariella commixta*, which would suggest that the two taxa evidently belong to the same genus. However, the position of both *Cetrariella commixta* and '*Melanelia*' *sorediella* in the *Cetrariella*-clade

has lower support than accepted values, and furthermore these locations are not supported by any non-DNA characters. If *Cetrariella* survives as a separate genus, it could be delimited either to include additionally '*M.*' *sorediella* or retained exclusively for *C. delisei* and *C. fastigiata*. The present results support the latter solution as the phylogenetic position of *Cetrariella commixta* and '*M.*' *sorediella* remains unresolved.

The consensus tree based on ITS sequences places *Cetraria subalpina* as a sister group to *Cetrariella*, a position which is not supported in the consensus tree based on the complete matrix.

Cetreliopsis *M. J. Lai*

Cetreliopsis is a genus of large foliose species which occur in the eastern and southeastern Asia (Randlane *et al*. 2001; Randlane & Saag 2004). The genus was originally described as monotypic (Lai 1980), but was later re-evaluated and distinguished from *Nephromopsis* by the presence of pseudocyphellae on both sides of the thallus in combination with the presence of fumarprotocetaric acid in the medulla. The genus currently includes seven species with this combination of characters (Randlane *et al*. 1995; Lai & Elix 2002; Randlane & Saag 2003). Relatively few species, three out of a possible seven, were included in the present study but the genus has rather strong bootstrap support as it is currently circumscribed.

Flavocetraria *Kärnefelt & A. Thell*

Hitherto, this genus was composed of three arctic-alpine species which occur mainly in the Northern Hemisphere. Two widely known taxa, *F. cucullata* and *F. nivalis,* are presumed to be closest relatives, with strong bootstrap support in earlier analyses (Kärnefelt *et al*. 1994; Thell & Miao 1998; Thell *et al*. 2002; 2004; 2005). However, in this study, the situation is different because of the inclusion of two rare species, an amphi-Beringian '*F.*' *minuscula,* and the recently rediscovered terricolous '*Cetraria*' *annae* from Siberia. The latter is most closely related to *F. cucullata* with which it forms

a weakly supported clade, together with *F. nivalis*. Following the results based on the combined data set, the most natural position for '*Cetraria*' *annae* would be in *Flavocetraria* (for detailed information on '*C.*' *annae* see below).

According to the present analyses, '*Flavocetraria*' *minuscula* has no affinities with the genus *Flavocetraria*, but belongs to another major clade of the cetrarioid core, the *Cetraria* s. lat. clade (Fig 1 & 2); for further details on '*F.*' *minuscula* see below. Thus as it currently stands, the genus *Flavocetraria* appears to be polyphyletic.

Kaernefeltia *A. Thell & Goward*

Kaernefeltia is a genus of two closely related species which are characterized by conidia with two swellings (disc-bar shaped) and the presence of several distinctive fatty acids. *Kaernefeltia merrilli* (Du Rietz) A. Thell & Goward is the more common species, with a remarkable disjunct distribution, occurring in western North America, from Baja California to northern British Columbia and eastwards to Saskatchewan, as well as in central Spain, whereas *K. californica* (Tuck.) A. Thell & Goward is maritime along the Pacific coast in North America (Thell & Goward 1996). The genus has moderate support in the present study.

Masonhalea *Kärnefelt*

Compared with the cetrarioid core, this monotypic genus has several unique characters, i.e. a freely-rolling (vagrant) habit, decorticate patches on the lower side, a thick, prosoplectenchymatous upper cortex composed of pachydermatous hyphae and bacillariform conidia (Kärnefelt 1977*a*, 1979). The presence of alectoronic acid is rare in the cetrarioid core, but is found in some genera and species, such as *Cetrariella commixta, Nephromopsis pallescens* (Schaer.) Park and *Tuckermannopsis* s. str. (Kärnefelt & Thell 1993). The lateral position of the apothecia is a character in common with the closely related *Tuckermannopsis inermis* and some additional *Tuckermannopsis* species, as well as with the genera *Cetreliopsis* and *Nephromopsis*. The monotypic status of the genus is not supported phylogenetically according to the present study. However, another member of the well-supported *Masonhalea* clade, *T. inermis*, shares no non-molecular characters with *M. richardsonii,* except for the similar arctic, northern-Beringian distribution. The pseudocyphellae are conspicuous in both species, forming unique, large decorticate patches on the lower side of *M. richardsonii*, and a continuous line close to the margin of the lower side of *T. inermis*.

Nephromopsis *Müll. Arg.*

This genus is the second oldest of the group, originally described as monotypic (Müller 1891). The generic name was rarely used until re-established by Lai (1980) and re-delimited by Randlane *et al.* (1995). An outline of the genus, including distribution maps and keys is presented by Randlane & Saag (1998). At that time *Nephromopsis* included the genus *Cetrariopsis* Kurok. More recently, the genus *Tuckneraria* Randlane & A. Thell was synonymized with *Nephromopsis* and two new combinations were made, *Nephromopsis leucostigma* (Lév.) A. Thell & Randlane and *N. melaloma* (Nyl.) A. Thell & Randlane following a molecular analysis (Thell *et al.* 2005) so that *Nephromopsis* then included 19 species. The most closely related genus is *Cetreliopsis*, which is distinguished by the presence of pseudocyphellae on both upper and lower surfaces, and fumarprotocetraric acid in the medulla.

Tuckermanella *Essl.*

The genus *Tuckermanella* was recently described to accommodate six rather small, brown, adnate foliose species, apparently endemic to southern and western inland areas of North America (Esslinger 2003). The presence of mostly ellipsoid ascospores, submarginal and laminal pycnidia and continuous marginal pseudocyphellae distinguish this genus from *Tuckermannopsis* where most of these species had previously been accommodated. Three of the six species have been analyzed phylogenetically and form a well-supported clade nested within the large *Nephromopsis* clade.

Tuckermannopsis *Gyeln.*

This genus was little used until resurrected by Lai (1981). Since then it has been used as a 'dustbin' where various cetrarioid species (25 in total) of dubious generic position have been placed (Randlane & Saag 2003). Kärnefelt & Thell (2001) attempted to delimit the genus based on morphology and DNA sequences and listed seven species in *Tuckermannopsis* s. str., four of which have been investigated in this study: *T. americana*, *T. chlorophylla*, *T. ciliaris* and *T. orbata*. The three species excluded from our analyses are endemic to Japan, namely *T. gilva* (Asahina) M. J. Lai, *T. microphyllica* (W. L. Culb. & C. F. Culb.) M. J. Lai and *T. ulophylloides* (Asahina) M. J. Lai. In addition, the DNA of two further rare species combined in *Tuckermannopsis* but evidently not belonging to the genus in a strict sense remain to be studied, the Japanese endemic *T. platyphylloides* (Asahina) M. J. Lai and the Chinese endemic, *T. weii* (X. Q. Gao & L. H. Chen) Randlane & Saag. Two additional species, *T. inermis* and *T. platyphylla*, were included in the present study but their generic positions still remains unclear.

The delimitation of *Tuckermannopsis* s. str. is difficult to determine. The *Tuckermannopsis* clade, including *T. americana, T. chlorophylla* and *T. orbata* according to analyses based on the ITS matrix, and *T. americana, T. ciliaris* and *T. chlorophylla* according the combined DNA matrix, has no acceptable support. The species are dispersed in the different analyses, among *Ahtiana, Kaernefeltia* and *Tuckermanella* (Figs 1 & 2). *Tuckermannopsis inermis* forms a supported clade together with *Masonhalea richardsonii*, while *T. platyphylla* is related to these taxa in three out of four analyses, but with only weak support. As with *Cetraria*, the polyphyly of *Tuckermannopsis* is currently accepted in order to avoid erecting several new genera.

Usnocetraria *M. J. Lai & C. J. Wei*

In a synopsis of Chinese cetrarioid lichens in a broad sense, the genus *Allocetraria* was re-delimited and the new genus *Usnocetraria* proposed (Lai *et al.* 2007). *Usnocetraria oakesiana* was selected as the type species of the genus. This species was not closely related to the three genera where it has previously been combined, i.e. *Allocetraria, Cetraria* s. str. and *Tuckermanopsis*. Consequently, *Usnocetraria* is proposed here as being monotypic for *U. oakesiana*, a disjunct alpine species in the Northern Hemisphere (Randlane & Saag 2004). A second proposed species which was transferred from *Nephromopsis*, *U. kurokawae* (Shibuichi & Yoshida) M. J. Lai & J. C. Wei, endemic in Japan, was not supported by the present study. Nine additional taxa of different phylogenetic origins were listed under *Usnocetraria* as new combinations (Lai *et al.* 2007: 45), but are invalidly published as they lacked basionym citations. None of these species are closely related to *U. oakesiana*, whose closest relative is most likely *Cetraria obtusata*. The latter taxon occurs in the Alps and is very different from *U. oakesiana* in gross morphology, being erect fruticose, but it does have similar sublageniform conidia and contains secalonic acid in the medulla (v.d. Boom & Sipman 1994).

Vulpicida *J.-E. Mattsson & M. J. Lai*

This genus is characterized by the secondary chemistry, namely the presence of pulvinic and vulpinic acids, compounds derived from the shikimic acid pathway. The presence of these substances together with usnic acid give the thallus and medulla a shiny yellow or greenish yellow colour (Mattsson & Lai 1993; Mattsson 1993). Five of the six known species were included in the present analyses. Two of these species, *V. canadensis* and *V. viridis*, are endemic to different parts of North America and characterized by short citriform conidia. The Eurasian *V. juniperinus* (L.) J.-E. Mattsson & M. J. Lai and the disjunct European *V. tubulosus* (Schaerer) J.-E. Mattsson & M. J. Lai are closely related, whereas the widespread *V. pinastri* (Scop.) J.-E. Mattsson & M. J. Lai has a more isolated position within the genus. The sixth species, the Eurasian arctic-alpine *V. tilesii* (Ach.) J.-E. Mattsson & M. J. Lai (Randlane & Saag 2005), was not included.

The genus is supported as being monophyletic by the Bayesian analysis of the ITS data (Fig. 1), whereas the positions of the
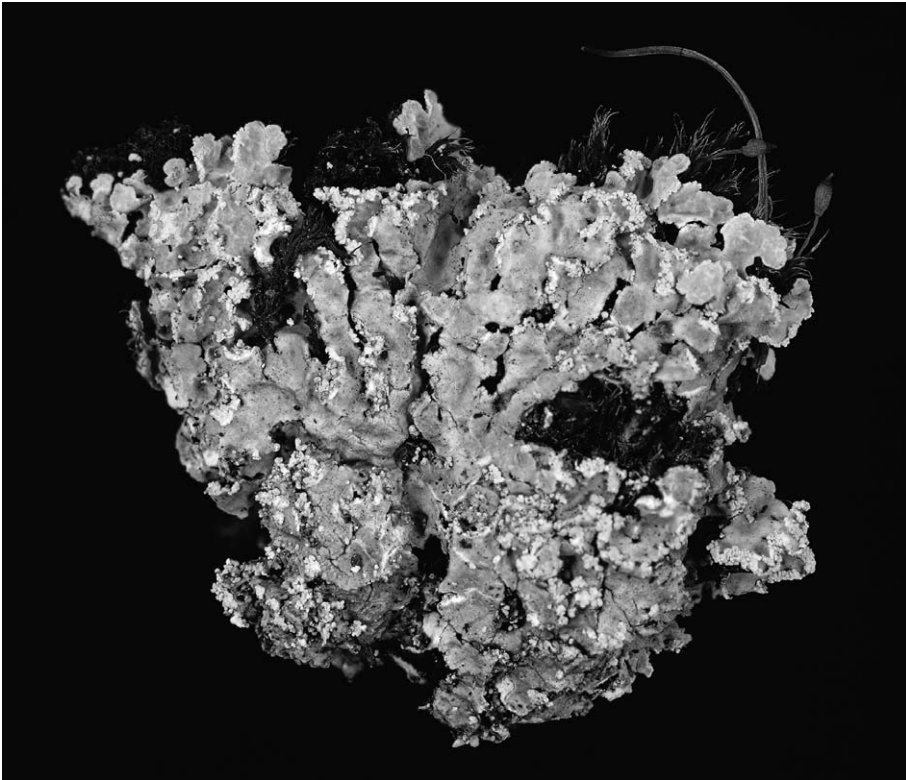
Fig. 3. *Cetraria annae*, Okinskii District, Russia, 18 viii 2007 *Urbanavichus* (LD-1271346, dupl. ex TU), × 4.

species remained unresolved in the PAUP analysis of the combined data set because *Allocetraria* was nested within *Vulpicida* (Fig. 2).

Comments on some rare and phylogenetically interesting species

Cetraria annae *Oxner*

Cetraria annae was forgotten soon after its description by Oxner (1933). However, this species was recently rediscovered after being collected again from the same area. The species is pale yellow, terricolous, and closely attached to the substratum (Fig. 3).

The thallus is foliose, to 3 cm wide, with narrow, rounded lobes, 1–3 mm wide and *c*. 300 μm thick. Lobules are present, and are often constricted at the base. The lobe margins are somewhat crenulate. The soralia are conspicuous along margins and weakly labri-

form; soredia are white, granular. Both upper and lower cortices are composed of mesodermatous paraplechtenchyma, with both layers *c*. 50 μm thick. The medulla is white, and almost as compact as the cortical layers. Algal cells occur in clusters, 5–20 together, in the upper part of the medulla. No apothecia were seen, but the scattered, marginal, black pycnidia were pronounced, but lacked conidia.

Cetraria annae is positioned phylogenetically close to *Flavocetraria cucullata*. Chemical analysis of two specimens (*Urbanavichus* 1996, 2001; TU) revealed the presence of usnic acid [major], isonephrosterinic acid [major], lichesterinic acid [minor], atranorin [minor], squamatic acid [minor], protolichesterinic acid [trace] and nephrosterinic acid [trace]. Usnic, lichesterinic and protolichesterinic acids also occur in *F. cucullata*. However, the anatomical studies provided

no confirmation of the weakly supported phylogenetic position of this species in *Flavocetraria*.

Specimens examined. **Russia:** Baikal region, River Levaya Anosovka, on riverbank rocks, 1996, *Urbanavicius* (TU, det. Randlane); Baikal Nature Reserve, in the vicinity of Lake Tschornaya, on mossy rocks, 2001, *Urbanavicius*, (TU, det. Randlane); Republic of Buriat, Okinskii district, River Zhom-Bolok, 52°42′42.3″N 99°17′37.3″E, on mossy rocks, 2007, *Urbanavicius* (TU, LD-1271346).

Cetraria australiensis *Kärnefelt*

This Australian endemic is morphologically very similar to *C. odontella* (Nyl.) Nyl. However, these two closely related species differ in their morphology, substratum and distribution, with *C. australiensis* being dorsiventral and growing on twigs or on soil in alpine habitats of Australia, whereas the lobes of the widely distributed, tuft-forming *C. odontella* are more or less isodiametric (Kärnefelt 1977*b*, 1986). One report of *C. odontella* from Australia, based on Group I and ITS sequences (Thell *et al.* 2000: AF228285), proved to be *C. australiensis* after comparison with the newly determined but identical ITS sequence performed in the present study, as well as by re-evaluating the morphology. The ITS sequences of the two species differ in two positions.

Flavocetraria minuscula *(Elenk. & Savicz) Ahti, Poryadina & Zhurb.*

Flavocetraria minuscula, known earlier as a form or variety within *F. cucullata*, was raised to species status by Zhurbenko *et al.* (2005) because of its distinctive morphological characters, being smaller than *F. cucullata* with tube-like lobes, a pruinose surface and characteristic helmet-shaped, hooked lobe tips (Fig. 4).

The anatomy of *F. minuscula* was studied in the present work but no differences were found between the amphi-Beringian *F. minuscula* and the more widely distributed *F. cucullata*. The cortical layers of each are *c.* 50 μm wide and are composed of mesodermatous paraplectenchyma, while the medulla is composed of densely interwoven, thick-walled hyphae and the algal cells are scattered in the upper part of the medulla.

Fig. 4. *Flavocetraria minuscula*, Verkhoyansk District, Russia, 31 vii 2006, *Ahti* 64946 (LD-1154094, dupl. ex H), × 4.

Flavocetraria minuscula is included in a separate clade within the *Cetraria* s. lat. clade, composed of taxa having, with one exception, conidia with one thickening. Unfortunately the conidia of *F. minuscula* have yet to be observed. Ultimately, the conidial shape may confirm the unexpected phylogenetic position of *F. minuscula* distant from *F. cucullata,* a species characterized by dumb-bell shaped conidia.

'Melanelia' sorediella *(Lettau) V. J. Rico, van den Boom & Barrasa*

'*Melanelia*' *sorediella*, formerly known as *Cetraria commixta* f. *sorediella*, was raised to

Fig. 5. *Tuckermanopsis inermis*, Noatak National Preserve, Alaska, 12 vii 2005, *Holt* 23441 (LD-1190038, dupl. ex OSU), × 7.

species level because of the presence of pycnoisidia and the absence of pseudocyphellae and apothecia (Rico *et al.* 2005). *Cetraria commixta* was previously transferred to *Melanelia* because of similarities in reproductive structures and other anatomical characters (Thell 1995*a*), and later to *Cetrariella* based on DNA analyses (Thell *et al.* 2004). The conidia of '*M*'. *sorediella* are citriform like those of the closely related *Cetrariella commixta*. Rico *et al.* (2005) hesitated about the taxonomic position of '*M*'. *sorediella*, and the present placement in *Melanelia* remains tentative due to the large phylogenetic distance between *Melanelia* and *Cetrariella*. Results of this study demonstrate that *Cetrariella commixta* and '*Melanelia*' *sorediella* should belong to the same genus while the exact phylogenetic position of these two taxa remains unresolved.

Tuckermannopsis inermis (Nyl.) Kärnefelt

Except for their similar arctic, northern-Beringian distribution, the most closely related species, *Masonhalea richardsonii*, shares no structural or chemical characters with *Tuckermannopsis inermis* (Kärnefelt 1977*a*; 1979). The pseudocyphellae are conspicuous in both species, forming unique, large decorticate patches on the lower side of *M. richardsonii*, and a continuous line close to the margin of the lower side of *T. inermis* (Fig. 5).

Conclusions

The interpretation of molecular phylogenetic studies in taxonomy is still in an initial phase. In *Parmeliaceae*, some new monophyletic groups have been detected and some superfluous genera have been synonymized

(Blanco *et al*. 2004*a*; b). However, after four decades of splitting, a trend towards larger genera has more recently been adopted in the *Parmeliaceae* (Divakar *et al*. 2005, 2006; Wirtz *et al*. 2006).

The morphological, anatomical and chemical variation in the cetrarioid core is probably as large as in the *Xanthoparmelia* clade, although the conidial variation is greater among the cetrarioid lichens. Today, *Xanthoparmelia* (Vain.) Hale is a genus of approximately 800 species comprising eight previously accepted genera, a clade supported by the presence of *Xanthoparmelia*-type lichenan in the cell walls (Prado *et al*. 2007; Thell *et al*. 2006). The second largest genus of the family, *Usnea* Adans., is characterized by a dense cord in the medulla and comprises up to 600 species after the inclusion of *Neuropogon* and *Protousnea* (Wirtz *et al*. 2006).

Polyphyletic genera in the cetrarioid core could, in some cases, be eliminated or adjusted by using alternative, previously published combinations. However, neither the whole cetrarioid core nor either of the main clades (A & B) can be defined by any presently known non-molecular characters. The general shape of conidia (with one swelling *versus* two swellings) corresponds, with some exceptions (Fig 1 & 2), to the division of the cetrarioid core into two major groups, *Cetraria* s. lat. and *Nephromopsis* s. lat. The only alternative to the multi-generic concept proposed here, where smaller monophyletic clades are accepted as genera (albeit nested within larger, paraphyletic genera), would be to name the whole cetraroid core *Cetraria*, despite the lack of any known correlating characters.

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