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Phylogenetic reassessment of the *Teloschistaceae* (lichen-forming *Ascomycota*, *Lecanoromycetes*)

Ester GAYA[a,b,*], *Pere NAVARRO-ROSINÉS*[b], *Xavier LLIMONA*[b], *Néstor HLADUN*[b], *François LUTZONI*[a]

[a]*Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA*
[b]*Departament de Biologia Vegetal (Unitat Botànica), Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain*

ARTICLE INFO

Article history:
Received 20 April 2007
Received in revised form
1 October 2007
Accepted 15 November 2007
Corresponding Editor:
H. Thorsten Lumbsch

Keywords:
Arc
Caloplaca
Fulgensia
INAASE
ITS
Letrouitiaceae
Molecular phylogenetics
Teloschistaceae
Teloschistes
Xanthoria

ABSTRACT

The *Teloschistaceae* is a widespread family with considerable morphological and ecological heterogeneity across genera and species groups. In order to provide a comprehensive molecular phylogeny for this family, phylogenetic analyses were carried out on sequences from the nuclear ribosomal ITS region obtained from 114 individuals that represent virtually all main lineages of *Teloschistaceae*. Our study confirmed the polyphyly of *Caloplaca*, *Fulgensia* and *Xanthoria*, and revealed that *Teloschistes* is probably non-monophyletic. We also confirm here that species traditionally included in *Caloplaca* subgenus *Gasparrinia* do not form a monophyletic entity. *Caloplaca aurantia*, *C. carphinea* and *C. saxicola* s. str. groups were recovered as monophyletic. The subgenera *Caloplaca* and *Pyrenodesmia* were also polyphyletic. In the subgenus *Caloplaca*, the traditionally recognized *C. cerina* group was recovered as monophyletic. Because this study is based solely on ITS, to maximize taxon sampling, the inclusion of phylogenetic signal from ambiguously aligned regions in MP (recoded INAASE and arc characters) resulted in the most highly supported phylogenetic reconstruction, compared with Bayesian inference restricted to alignable sites.

Introduction

Suprageneric treatment within the Teloschistales

The latest classification of the *Teloschistales* (Eriksson 2006) includes one large (*Teloschistaceae*) and two much smaller families (*Letrouitiaceae* and *Megalosporaceae*). *Caloplaca* is the largest genus within the *Teloschistaceae*, along with 11 other smaller genera (Table 1) according to Eriksson (2006). *Fulgensia*, *Teloschistes*, and *Xanthoria* (with *ca.* ten, 30, and 30 species, respectively) are the next largest genera, followed by mainly monotypic genera with species segregated from the four main genera within this family (e.g. *Cephalophysis*, *Huea*, *Ioplaca*, *Josefpoeltia*, *Seirophora*, *Xanthodactylon*, *Xanthomendoza*, *Xanthopeltis*).

The classification of the *Teloschistaceae* at the ordinal level has been highly debated, especially its placement within the *Lecanorales versus Teloschistales*. The order *Teloschistales* was first described by Hawksworth & Eriksson (1986), with a single family (*Teloschistaceae*). Later, two more families

* Corresponding author. Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA
E-mail address: eb62@duke.edu
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doi:10.1016/j.mycres.2007.11.005

Table 1 – Genera accepted within the family *Teloschistaceae* according to different authors

Zahlbruckner (1931, 1940)	Ozenda & Clauzade (1970)	Eriksson & Hawksworth (1986)	Kärnefelt (1989)	Hawksworth *et al.* (1995)	Eriksson (1999)	Kirk *et al.* (2001)	Eriksson *et al.* (2003)	Eriksson (2006)
•	•	*Apatoplaca*	*Apatoplaca*	*Apatoplaca*	*Apatoplaca*	*Apatoplaca*	–	–
Blastenia[*]	–	–	–	–	–	–	–	–
Bombyliospora[*]	*Bombyliospora*[#]	–	–	–	–	–	–	–
Caloplaca[*]	*Caloplaca*	*Caloplaca*	*Caloplaca*	*Caloplaca*	*Caloplaca*	*Caloplaca*	*Caloplaca*	*Caloplaca*
•	•	*Cephalophysis*	*Cephalophysis*	*Cephalophysis*	*Cephalophysis*	*Cephalophysis*	*Cephalophysis*	*Cephalophysis*
•	•	*Follmannia?*	–	–	–	–	–	–
•	*Fulgensia*	*Fulgensia*	*Fulgensia*	*Fulgensia*	*Fulgensia*	*Fulgensia*	*Fulgensia*	*Fulgensia*
•	•	•	•	•	•	•	•	*Huea*
•	•	*Ioplaca*	*Ioplaca*	*Ioplaca*	*Ioplaca*	*Ioplaca*	*Ioplaca*	*Ioplaca*
•	•	•	•	•	*Josefpoeltia*	*Josefpoeltia*	*Josefpoeltia*	*Josefpoeltia*
•	•	*Leproplaca*	–	–	–	–	–	–
Lethariopsis[**]	–	–	–	–	–	–	–	–
Protoblastenia[*]	*Protoblastenia*[§]	–	–	–	–	–	–	–
•	•	*Seirophora*	*Seirophora*	–	*Seirophora*	*Seirophora*	*Seirophora*	*Seirophora*
Teloschistes[**]	*Teloschistes*	*Teloschistes*	*Teloschistes*	*Teloschistes*	*Teloschistes*	*Teloschistes*	*Teloschistes*	*Teloschistes*
•	•	•	*Xanthodactylon*	*Xanthodactylon*	*Xanthodactylon*	*Xanthodactylon*	*Xanthodactylon*	*Xanthodactylon*
•	•	•	•	•	*Xanthomendoza*	*Xanthomendoza*	*Xanthomendoza*	*Xanthomendoza*
•	•	*Xanthopeltis*	*Xanthopeltis*	*Xanthopeltis*	*Xanthopeltis*	*Xanthopeltis*	*Xanthopeltis*	*Xanthopeltis*
Xanthoria[**]	*Xanthoria*	*Xanthoria*	*Xanthoria*	*Xanthoria*	*Xanthoria*	*Xanthoria*	*Xanthoria*	*Xanthoria*

• Genus name not included in classification because the classification preceded the protologue of the genus.
– Genus name existed at the time of the classification, but was nevertheless excluded from the proposed classification.
* Family *Caloplacaceae* according to Zahlbruckner (1926).
** Family *Teloschistaceae* according to Zahlbruckner (1898).
# Genus synonym to *Megalospora* (*Megalosporaceae*). Most of the species from this family were transferred to *Letrouitia*.
§ Currently included in the family *Psoraceae*.

were included in this order (Eriksson & Hawksworth 1991; Hafellner 1988): *Letrouitiaceae* (Hafellner & Bellemère 1981a) and *Fuscideaceae* (Hafellner 1984), even though Kärnefelt (1989, 1994) never accepted the *Fuscideaceae* as part of the *Teloschistales*. Earlier, Mattick (1951) had introduced the order *Caloplacales* in an attempt to join the taxa with polarilocular ascospores. However, this name was not validly published (Art. 36 of the *Code*).

Later on, the order *Teloschistales* was recognized as a suborder within the *Lecanorales* (Hafellner *et al.* 1994; Rambold *et al.* 1991; Rambold & Triebel 1992). Tehler (1996) included the *Fuscideaceae*, *Letrouitiaceae,* and *Teloschistaceae* within the *Lecanorales* suborder *Teloschistineae*. This classification had already been proposed by Henssen & Jahns (1973) based on ontogenic characters. Poelt (1974) also included the *Teloschistaceae* within the order *Lecanorales*, but in a different suborder, *Buelliineae*, based on morphological and anatomical features of the thallus and apothecia.

Eriksson (1999) and Eriksson *et al.* (2001, 2003, 2004) maintained the classification of the *Teloschistaceae* within the *Lecanorales*, which was confirmed by molecular data (e.g. Stenroos & DePriest 1998; see revision by Grube & Winka 2002), but without support. Nevertheless, several authors have maintained that the *Teloschistales* is a valid order (e.g. Kirk *et al.* 2001).

The inclusion of the family *Teloschistaceae* in the order *Lecanorales* was revisited in light of recent higher-level phylogenetic studies. Miadlikowska & Lutzoni (2004) demonstrated that none of their phylogenetic trees revealed the order *Lecanorales* (*sensu* Eriksson *et al.* 2003; or Tehler 1996) as monophyletic. Therefore, they emphasized the need of recognizing a monophyletic order *Lecanorales* (*s. str.*) restricted to the core of the *Lecanorales* apart from the *Peltigerales* and *Teloschistales*. Lumbsch *et al.* (2004) preferred a broader use of the order *Lecanorales*, to include *Teloschistales*, *Caliciales,* and *Peltigerales*, which was recovered as a well-supported monophyletic group. Peršoh *et al.* (2004) also accepted the *Lecanorales* in a broad sense and referred to a clade that included the suborder *Teloschistineae*, with *Caloplaca*, *Megalospora,* and *Xanthoria*. Wiklund & Wedin (2003) also considered the *Lecanorales* in a broad sense, including the *Teloschistaceae* and the *Caliciaceae*, and accepting the suborder *Teloschistineae*. However, this broad circumscription of the *Lecanorales* is redundant with the subclass *Lecanoromycetidae* and diminishes the number of ranks needed for the classification of this large subclass within the *Lecanoromycetes* (Miadlikowska & Lutzoni 2004). The recognition of the *Lecanorales*, *Teloschistales,* and *Peltigerales* within the *Lecanoromycetidae* (*sensu* Miadlikowska & Lutzoni 2004, and Miadlikowska *et al.* 2007) is in agreement with the classification adopted by a consortium between Myconet (Eriksson 2006), *The Dictionary of the Fungi* (Kirk *et al.* 2001) and GenBank (Hibbett *et al.* 2007).

The *Fuscideaceae* have recently been excluded from the *Teloschistales* based on a multilocus phylogenetic study by Reeb *et al.* (2004). Consequently, this family has a status of *incerta sedis* within the *Lecanoromycetidae* in Eriksson's (2006) classification of the *Ascomycota*. However, a new study (Miadlikowska *et al.* 2007) shows that the *Fuscideaceae* is part of the newly recognized *Umbilicariales* (Hibbett *et al.* 2007), and that the *Letrouitiaceae* remains in the *Teloschistales*. The *Letrouitiaceae* is a monotypic family (*Letrouitia*), with about 15 species. Members of this family are widely distributed in subtropical and tropical regions, and are corticolous. *Letrouitia* includes species that were classified in the species complex *Bombyliospora domingensis* (Hafellner 1981; Hafellner & Bellemère 1981a), and that were placed in a separate genus based mainly on the unique structure of asci and ascospores. Finally, the *Megalosporaceae* was classified within the *Teloschistales* (Eriksson 2005), based on Helms *et al.* (2003) and Lutzoni *et al.* (2004).

The family *Teloschistaceae* was first described by Zahlbruckner (1898) who grouped together foliose and fruticose taxa having polarilocular or 4-locule ascospores (*Xanthoria*, *Teloschistes* and *Lethariopsis*). Later, Zahlbruckner (1926) described a second family, *Caloplacaceae*, for crustose taxa with ascospores that are polarilocular, or rarely with 3-4 locules or simple. At that time he considered four genera to be part of the *Caloplacaceae*: *Caloplaca*, *Blastenia*, *Bombyliospora*, and *Protoblastenia* (Table 1). *Bombyliospora* species have been transferred to *Letrouitia* (Hafellner 1981; Hafellner & Bellemère 1981a), and *Bombyliospora* itself is now considered a synonym of *Megalospora* (*Megalosporaceae*; Hafellner & Bellemère 1981b). Currently, *Protoblastenia* is classified within the *Psoraceae*, and *Blastenia* (Massalongo 1852, 1853) is a synonym of *Caloplaca*. The recognition of the *Caloplacaceae* as a distinct family from the *Teloschistaceae* was later rejected. Fink (1910) and Malme (1926) already fused the two families into one —*Teloschistaceae*. Alternatively, crustose genera were grouped within the family *Blasteniaceae* (Dodge & Baker 1938), including the *Placodiaceae* (Räsänen 1943). Dodge (1948) used *Blasteniaceae* also to refer to the *Teloschistaceae*, even though this change of name was illegitimate, and Rudolph (1955) concurred. Furthermore, Dodge (1971) tried to introduce another family name, *Xanthoriaceae* (not validly published; Art. 36 of the Code), to encompass *Xanthodactylon*, *Xanthopeltis* and *Xanthoria*.

Kärnefelt (1989) conducted an exhaustive revision of this family and the order *Teloschistales*, and accepted ten genera within the *Teloschistaceae* (Table 1). With a few exceptions, this remains the main classification in use.

The last taxonomical treatment of the family *Teloschistaceae* was by Oxner (1993). In his flora of the Ukraine, the family *Teloschistaceae* includes *Caloplaca*, *Fulgensia*, *Protoblastenia*, *Pyrrhospora*, *Teloschistes,* and *Xanthoria*. Oxner (1993) also accepted *Caloplaca elegans* instead of *Xanthoria elegans*, as well as *C. australis* and *C. schistidii* instead of *Fulgensia australis* and *F. schistidii*, respectively.

Members of the *Teloschistaceae* are usually easily recognized by the frequent presence of anthraquinones, giving them an orange to yellow colour (K+ purple; Santesson 1970a). They include the full spectrum of thallus forms ranging from fruticose to endolithic crustose. Their photobiont belongs to the green alga *Trebouxia* or its related genera. Their apothecia usually have well-developed thallin margins. The external layer of the ascus tip is I+ (blue) and ascospores are discharged through a longitudinal slit (Kärnefelt 1989). As Kärnefelt (1989) pointed out, initially, polarilocular ascospores were thought to be a diagnostic trait for this family, but with the inclusion of other genera, such as *Apatoplaca*, transferred to *Caloplaca* by Wetmore (1994), *Cephalophysis*, *Fulgensia,* and *Xanthopeltis*, which have simple or septate spores, the main features defining the family had to be reconsidered.

As Søchting & Lutzoni (2003) pointed out, the delimitation among genera included within the *Teloschistaceae* is highly artificial and in need of revision, especially for the closely related species within *Caloplaca*, *Fulgensia*, *Teloschistes,* and *Xanthoria*. The distinction between the foliose species (*Xanthoria*) and lobed crustose or placodioid species (e.g. *Caloplaca* subgenus *Gasparrinia*) is especially tenuous (Arup & Grube 1999; Kärnefelt 1989; Poelt & Hafellner 1980; Søchting & Lutzoni 2003; Wetmore & Kärnefelt 1998).

Infrageneric treatment of Caloplaca

Caloplaca is a large and phenotypically heterogeneous genus. More than 1000 species names have been published for *Caloplaca* alone (Søchting & Lutzoni 2003). However, Kärnefelt (1989) estimated that the family *Teloschistaceae* comprises approximately 580 species, and Hawksworth *et al.* (1995) reduced the number to 525 species. Hence, the number of taxa included in *Caloplaca* and the *Teloschistaceae* are unresolved at this time.

Caloplaca comprises a group of lichens with hyaline polarilocular ascospores, occasionally plurilocular (3-4 locules) or, rarely, simple with a slight wall thickening at the equatorial region. Thalli are mostly crustose, usually with anthraquinones present in the thallus and apothecium. Several anthraquinone syndromes have been reported for this genus, sometimes together with other lichen metabolites (Santesson 1970b; Søchting 1997, 2001). Asci and ascospores have been thoroughly studied by Bellemère & Letrouit-Galinou (1982) and Honegger (1978). *Caloplaca* is cosmopolitan and found in most xeric and mesic habitats.

There have been several attempts to subdivide *Caloplaca* into smaller taxonomical units that have been recognized as separate genera over time: *Blastenia*, *Follmania*, *Gasparrinia*, *Gyalolechia*, *Huea*, *Kuttlingeria*, *Mawsonia*, *Meroplacis*, *Polycauliona*, *Pyrenodesmia*, *Triophthalmidium,* and *Xanthocarpia*. Most of these segregated genera were reclassified in *Caloplaca* as subgenera (e.g. subgenus *Gasparrinia*) or other ranks. Most of these taxonomical units were based mainly on a single character and have been considered as highly artificial (Kärnefelt 1989). Consequently, the current circumscription of *Caloplaca* is very similar to what was established more than a century ago (Søchting & Lutzoni 2003).

Wade (1965) and Clauzade & Roux (1985) provided the most comprehensive infrageneric treatments for *Caloplaca*. Wade (1965), in his study of *Caloplaca* in the British Islands, described four sections. Section *Caloplaca* comprises species with crustose thalli and apothecia with or without a thalline margin and with a continuous or discontinuous photobiont layer present under the hypothecium. This section includes species with apothecia of various pigments. Section *Triophthalmidium* includes taxa with crustose thalli, apothecia without a thalline margin and ascospores with four cells. Section *Gasparrinia* refers to taxa with placodioid or squamulous thalli and apothecia with a thalline margin. Section *Leproplaca* includes taxa with leprarioid thalli that are usually sterile.

In their lichen flora of occidental Europe, Clauzade & Roux (1985) proposed six subgenera. Three of these subgenera (*Caloplaca*, *Gasparrinia,* and *Leproplaca*) were similar to Wade's (1965) concept. However, subgenus *Caloplaca sensu* Clauzade & Roux was further divided into three groups (*C. citrina*, *C. cerina,* and *C. ferruginea* groups), and subgenus *Gaspirrinia* was subdivided into five groups (*C. aurantia, C. aurea, C. carphinea, C. persica,* and *C. saxicola*; Table 2). Subgenus *Pyrenodesmia* includes species with white–grey or nearly blackish thalli that are K− or K+ violet, and dark apothecia with a thin or absent thalline margin and an epithecium K−or K+ violet. Subgenus *Gyalolechia* regroups species with ascospores that have a thin equatorial wall thickening (< 3 μm). Subgenus *Xanthocarpia* encompasses species with thin or endolithic thalli and with four-locular or three-septate ascospores.

Subsequently, Hansen *et al.* (1987) subdivided the *Caloplaca* species from Greenland into ten groups: *Cerinae*, *Chalybeae*, *Citrinae*, *Ferrugineae*, *Nivales*, *Pauliae*, *Pyraceae*, *Saxicolae*, *Sinapispermae,* and *Trachyphyllae*; and Poelt & Hinteregger (1993) established 21 groups to accommodate the Himalayan species of *Caloplaca*.

'*Subgenus* Gasparrinia'

In most floristic studies *Caloplaca* subgenus *Gasparrinia* refers to a group of species with placodiod, squamulose, effigurated (i.e., lobate) thalli, usually with anthraquinones in the thallus and/or apothecia. This group was first described by Tornabene (1849), who listed eight species. Five of the species were later considered outside this group (Wetmore & Kärnefelt 1998). The three remaining species, which Fries (1871) referred to as *Caloplaca* section *Gasparrinia*, were *Caloplaca callopisma* (syn. *C. aurantia*), *C. cirrochroa,* and *C. murorum* (syn. *C. saxicola*). According to Poelt (1954), the most successful study made on this group was the one by Weddell (1876). Later on, the use of an infrageneric category including all lobed species was accepted by several authors (e.g. Clauzade & Roux 1985 who recognized the five species groups described below; Poelt 1969; Wade 1965; see also Table 2 and Appendix A for a summary of the systematic treatments of this subgenus according to different authors).

***Caloplaca aurantia* group.** Characterized by the presence of citriform ascospores. Recently, Sipman & Raus (2002) described a new species (*Caloplaca aegaea*) that, based on thallus features and ascospore shape, could fit within this group.

***Caloplaca aurea* group.** Species within this group have an equatorial wall thickening < 2 μm. Poelt's (1965) comparative study of *Caloplaca aurea* and *C. paulii*, with *Fulgensia* species, showed that there were intermediate states between the lobed *Caloplaca* and the genus *Fulgensia s. str*. According to Clauzade & Roux (1985), *C. scrobiculata* (syn. *C. anularis*) is also included within this group (Table 2).

***Caloplaca carphinea* group.** This group encompasses two species (Table 2) that are easily distinguished from other lobed taxa by the light yellowish, more or less greenish colour of the thallus, due to the presence of usnic acid. The group is known mainly from the Mediterranean region and Canary Islands. Although these species have been traditionally classified within *Gasparrinia*, macroscopically they are similar to *Dimelaena oreina*, which also contains usnic acid. However, the apothecial disk in the *C. carphinea* group produces emodine and parietin (Hansen *et al.* 1987; Santesson 1970b), and the ascospores are polarilocular. Breuss (1989) recognized these two taxa, previously treated as subspecies, as distinct species.

***Caloplaca persica* group.** This taxonomic entity consists of three corticolous species (Table 2) that had been previously

Table 2 – Species included in the five groups described by Clauzade & Roux (1985) within subgenus *Gasparrinia*, and in the seven groups described by Poelt (1954) within lobed species of *Caloplaca*

Clauzade & Roux (1985)		Poelt (1954)	
C. aurantia group	[a]*C. aegaea*	a - ohn. näh. Ansch.	*C. carphinea*
	C. aurantia		*C. microthallina*
	C. flavescens		*C. rubelliana*
	C. thallincola		*C. squamulosa*
			C. subsoluta
C. aurea group	*C. aurea*		*C. tenuata*
	C. paulii		*C. tominii*
	C. scrobiculata		
		b - Alpinae	*C. aurea*
C. carphinea group	*C. carphinea*		*C. australis*
	C. scoriophila		*C. paulii*
			C. pruinosa
C. persica group	*C. lobulata*		*C. schistidii*
	C. persica		
	[a]*C. polycarpoides*	c - Aurantiae	*C. aurantia*
			var. *aurantia*
C. saxicola group	*C. biatorina*		var. *heppiana*
	ssp. *biatorina*		var. *papillata*
	ssp. *gyalolechioides*		C *thallincola*
	C. cirrochroa		
	ssp. *cirrochroa*	d - Soraliferae	*C. arnoldii*
	ssp. *fulva*		var. *arnoldii*
			var. *fulva*
	C. decipiens		*C. cirrochroa*
	C. gloriae		*C. microphyllina*
	C. granulosa		*C. obliterans*
	C. littorea		*C. proteus*
	C. marina		
	C. microthallina	e - Murales	*C. alcarum*
	C. necator		*C. decipiens*
	C. obliterans		*C. marina*
	C. saxicola		*C. murorum*
	ssp. *arnoldii*		var. *laceratula*
	ssp. *biatorinoides*		var. *murorum*
	ssp. *laceratula*		*C. scopularis*
	ssp. *miniata*		
	ssp. *obliterata*	f - Granulosae	*C. granulosa*
	ssp. *pulvinata*		*C. verruculifera*
	ssp. *saxicola*		
	C. scopularis	g - Biatorinae	*C. biatorina*
	C. tenuata		var. *baumgartneri*
	C. tenuatula		var. *biatorina*
	ssp. *inconnexa*		var. *gyalolechioides*
	ssp. *verrucariarum*		var. *sympecta*
	ssp. *tenuatula*		
	var. *athallina*		
	var. *lithophila*		
	var. *pertenuis*		
	var. *pervulgata*		
	var. *tenuatula*		
	C. verruculifera		

a Not considered by Clauzade & Roux (1985).

included within the genus *Xanthoria*, and later, treated as *Caloplaca* section *Xanthoriella* because of the lack of either inferior cortex or rhizines (Steiner & Poelt 1982). In the same publication, Steiner & Poelt also suggested that the presence of other characters, such as the slightly stipitate apothecia, with a very lax or empty stipe, supported the inclusion of these three species within the same section (*Xanthoriella*). Conversely, Clauzade & Roux (1985) did not consider this section, even though they maintained these species as a separate group within *Gasparrinia*.

***Caloplaca saxicola* group.** Represents the core of the lobed-effigurate *Caloplaca* species. In their key, Clauzade & Roux (1985) included taxa of the *C. saxicola* group as well as species from other groups and even other genera (e.g. *Xanthoria*), demonstrating the unclear limits of this broad and heterogeneous group.

Poelt (1954) subdivided lobed species of *Caloplaca* into seven groups (a–g; Table 2). Nordin (1972) studied material from Northern Europe and concluded that section *Gasparrinia* was a well-delimited group. In his study, 16 species were considered without group affiliations. Verseghy (1970, 1971, 1972), in her monograph of Hungarian species, treated *Gasparrinia* at the genus level and described 13 species with several forms and varieties. Kärnefelt (1989) assembled lobed species into several groups, but without making any formal classification. In the same study, some lobed species that had not been mentioned in previous studies were also considered, e.g. *C. ochraceofulva*, *C. orthoclada,* and *C. sublobulata*. Finally, Wetmore & Kärnefelt (1998) did not delimit groups for the 19 lobed species studied from North and Central America. They considered again that subgenus *Gasparrinia* was not a natural group, and could not be treated at any taxonomical level.

Apart from the species already mentioned, there are other species with more or less well-developed lobed margins that have never been included within *Gasparrinia*, e.g. *Caloplaca cinnabarina*, *C. dolomiticola*, or *C. haematodes*. Paradoxically, some species in *Gasparrinia* have thalli that are not clearly lobate (e.g. *C. littorea*, *C. marina*, *C. microthallina,* or *C. necator*).

Regardless of the different classifications of *Caloplaca* subgenus *Gasparrinia*, it is clear that the most accute problem is directly associated with the delimitation of the genus *Xanthoria* (Søchting & Lutzoni 2003). This delimitation between *Caloplaca* and *Xanthoria* is based solely on the presence or absence of a lower cortex. *Xanthoria* thalli usually show two cortical layers, upper and lower, whereas *Caloplaca* subgenus *Gasparrinia* presents only an upper cortical layer. However, the development of a lower cortex has been observed in some lobed species, e.g. *C. scopularis* (Poelt & Romauch 1977) and *C. thallincola* (Kärnefelt 1989).

Phylogenetic studies within the Teloschistales

Previous phylogenetic studies on members of the *Teloschistales* focused mainly on the genus *Caloplaca* (Arup & Grube 1999), addressed mostly the monophyly of *Fulgensia* (Gaya *et al.* 2003; Kasalicky *et al.* 2000), discussed more specifically relationships between *Caloplaca* and *Xanthoria* (Søchting & Lutzoni 2003), and revised *Xanthomendoza* (Søchting *et al.* 2002). None of these studies included the genus *Teloschistes*. Molecular phylogenetic studies within genera of the *Teloschistales* were centered on *Caloplaca* subgenus *Pyrenodesmia* (Muggia *et al.* 2008), and the *C. aurantia* group (Søchting & Arup 2002). The genus *Xanthoria* was the subject of a systematic study by Franc & Kärnefelt (1998), a population study centered on *X. calcicola* and *X. parietina* by Lindblom & Ekman (2005), and a research project focused on the phylogeography of *X. elegans* by Dyer & Murtagh (2001) and Murtagh *et al.* (2002).

In this context, a phylogenetic study with a broad sampling across the *Teloschistaceae* was needed. The ITS was shown to provide sufficient phylogenetic confidence across this family when signal from ambiguously aligned regions is accommodated in phylogenetic analyses (Gaya *et al.* 2003). Therefore, we restricted our sequencing efforts to the ITS to maximize our taxon sampling for this study.

Our first aim was to circumscribe the *Teloschistaceae* using monophyly as a grouping criterion, to establish its relationship to the *Letrouitiaceae*, and to evaluate the monophyly of generic and sub-generic morpho-groups within the *Teloschistaceae*. With a taxon sampling biased toward the species belonging to the traditionally called subgenus *Gasparrinia*, we attempted to confirm the polyphyly of lobed species of *Caloplaca* within a broad taxon sampling of the *Teloschistaceae*, including taxa from all main groups of *Caloplaca*.

Material and methods

Taxon sampling

We selected a total of 114 specimens (Supplementary Material Appendix B) from the *Teloschistaceae*. This sampling included 82 specimens (56 species, 59 taxa) of *Caloplaca*, 11 specimens (nine species) of *Fulgensia*, 11 specimens (seven species) of *Teloschistes*, one specimen of *Xanthomendoza,* and nine specimens (six species) of *Xanthoria*. The remaining genera (mainly having few species) in the *Teloschistaceae* (Eriksson 2006) have not been included in this study as we could not obtain fresh material or because they are doubtful genera (Table 1).

In order to evaluate the infrageneric classification of *Caloplaca* based on morphological characters, the largest number of species sampled were from this genus, and sequences from at least one species per subgenus or group of species (*sensu* Clauzade & Roux 1985) were used: subgenus *Caloplaca* (*C. citrina* group, *C. cerina* group, *C. ferruginea* group), subgenus *Gasparrinia* (*C. carphinea* group, *C. aurea* group, *C. aurantia* group, *C. saxicola* group), subgenus *Gyalolechia*, subgenus *Leproplaca*, subgenus *Pyrenodesmia* and subgenus *Xanthocarpia*. The *C. persica* group that comprises the corticicolous species of subgenus *Gasparrinia* was not included due to lack of material. Nevertheless, we widely sampled the lobed taxa included in subgenus *Gasparrinia*.

In addition to these 114 sequences of taxa from the family *Teloschistaceae*, we sequenced two specimens from the genus *Letrouitia* (*Letrouitiaceae*). Unfortunately, we could not include the family *Megalosporaceae*.

In order to compare the results of this study with those from Gaya *et al.* (2003), we maintained the same outgroup that was used in that study: *Letharia columbiana*, *L. vulpina*, *Protoparmelia badia,* and *Usnea arizonica* (*Parmeliaceae*), and added *Protoblastenia rupestris* (*Psoraceae*).

Molecular data

DNA isolation and sequencing. Genomic DNA was obtained from fresh samples and herbarium specimens (BCN, C, DUKE, E, GZU, LEB, MARSSJ, MIN, MUB, SANT, TFC Lich, Aptroot herbarium - ABL, U. Arup personal herbarium and J. Etayo personal herbarium; voucher information is detailed in Supplementary Material Appendix B). DNA was isolated using the Puregene Kit (GENTRA Systems, Minneapolis) following the manufacturer's protocol for filamentous fungi. DNA concentration was determined by visual comparison with positive control (λ 100 ladder, concentration 10, 20, 40 ng) on an ethidium bromide-stained Tris–borate–ethylenediamine tetraacetate (TBE) agarose gel. Symmetric PCRs were prepared for a 50 μl final volume containing 31.7 μl sterile double-distilled water, 5 μl of 10 × Taq

polymerase reaction buffer (Boehringer–Mannheim, Indianapolis), 5 μl of 2.5 mM dNTPs, 0.3 μl Taq DNA polymerase (Boehringer–Mannheim), 2.5 μl for each of the 10 μM primers ITS1F or ITS1 or ITS5 and ITS4 (Gardes & Bruns 1993; White *et al.* 1990), 1.5 μl of 10 mg ml^{-1} bovine serum albumin (BSA; BioLabs), 0.5 μl of 50 mM $MgCl_2$ and 1 μl of template genomic DNA. PCR was performed on Perkin–Elmer GeneAmp 2400 under the following conditions: one cycle of 1 min at 95 °C linked to 40 cycles of 1 min at 95 °C, 45 s at 52 °C, and 2 min at 72 °C with the last step increased by increments of 5 s for the last 15 cycles. A final extension step of 10 min at 72 °C was added, after which the samples were kept at 4 °C. The PCR products were purified using either Cycle-Pure Kit (E.Z.N.A., Omega Bio-Tek, Doraville, GA), or low-binding regenerated cellulose 30,000 NMWL (nominal molecular weight limit) filter units (Millipore, Billerica), following the manufacturer's instructions. Both strands of the purified PCR products were sequenced using PCR primers used for the symmetric amplification and additional primers 5.8S and 5.8SR (Vilgalys & Hester 1990) or ITS2 and ITS3 (White *et al.* 1990). Sequencing reactions were prepared in 10 μl final volume using BigDye Terminator v3.1 (ABI PRISM, Perkin-Elmer Biosystems, Wellesley) and following the manufacturer's instructions. Sequenced products were precipitated with 26 μl deionized sterile water and 64 μl of 95 % ethanol before they were loaded on an ABI Prism 3730 automated DNA sequencer (Perkin–Elmer, Applied Biosystems).

Sequence alignment. Sequence fragments were subjected to BLAST searches for a first verification of their identities. They were assembled using Sequencher version 4.1 (Gene Codes Corporation, Ann Arbor) and Sequencer Navigator 1.0.1 (Applied Biosystems), and aligned manually with MacClade 4.01 (Maddison & Maddison 2001). The delimitation of ambiguous regions was done using the method described by Lutzoni *et al.* (2000). All DNA sequences have been deposited in GenBank (Supplementary Material Appendix B) and the alignment is available in TreeBASE.

Phylogenetic analyses

MP analyses were conducted using PAUP* version 4.0b10 for UNIX and Macintosh (Swofford 2002) and Bayesian analyses [MCMC with Metropolis coupling (B-MCMCMC)] were carried out using the program MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001).

MP analyses. We performed three weighted MP analyses: a first MP analysis (MP1) was executed using exclusively unambiguously aligned sites. The second MP search (MP2) included also coded (INAASE) characters, and we added coded (*arc*) characters for the third MP search (MP3).

In all analyses symmetric step matrices were created for unambiguous portions as follows. The options 'Show character status/full details/hide excluded characters' from the Data menu in PAUP* were implemented. From the resulting table, the column States showing all nucleotide states found at each of the unambiguously aligned and non-constant sites was saved as a separate text file. This file was used as an input file for the program STMatrix 2.1 (François Lutzoni & Stefan Zoller, Department of Biology, Duke University), which generates a step matrix (in Nexus format) by calculating frequencies of reciprocal changes from one state to another and converting them into costs of changes using the negative natural logarithm of the frequencies (Felsenstein 1981; Wheeler 1990). ITS1, ITS2, and 5.8S each were subjected to a specific symmetric step matrix. Gaps were used as a fifth character state for unambiguous portions of the alignment.

Ambiguously aligned regions were removed from MP searches. However, some of these ambiguously aligned sites were recoded and subjected to specific step matrices obtained with the program INAASE 2.3b (Lutzoni *et al.* 2000), incorporating the phylogenetic signal from these regions without violating positional homology. Ambiguous regions that were over 100 bp in length, highly variable (i.e., over 32 character states) or that showed major length variation among sequences of the same ambiguous region were recoded into 23 characters with the aid of the program *arc* v1.5 (Kauff *et al.* 2003; Miadlikowska *et al.* 2003) using the nucleotide option, as outlined in Reeb *et al.* (2004). Each of the 23 characters obtained with *arc*-nucleotide were subjected to a specific weight: 1.00 for character 1; 0.25 for characters 2–5; 0.10 for characters 6–15 and 0.50 for characters 16–23.

All three MP searches were performed using heuristic searches with 1 K random-addition-sequences (RAS), TBR (tree bisection–reconnection) branch swapping, Multrees option in effect, and collapsing branches with maximum branch length equal to zero. In MP1 and MP2, the high number of equally most parsimonious trees that filled the memory before completing the search required stopping these searches before their completion. For this reason, in MP1 we executed three successive searches progressively incrementing the number of trees saved per RAS. In the first round, we saved only one tree per replicate, in the second we saved 100 trees per replicate, and in the third, 1 K trees per replicate were saved. With this strategy we could detect that even when incrementing the number of trees saved per RAS, the topology of the majority rule consensus tree remained the same. Because the power of resolution was higher in MP2, resulting from the addition of INAASE characters, we could perform a search in two steps. In the first step, we saved only one tree per replicate. In the second step, we searched for all equally parsimonious trees, saving all trees only when swapping a tree equal to or shorter than the shortest tree found in the first step. MP3 was conducted in one step by saving all trees as soon as TBR swapping was initiated for each of the 1 K RAS.

Branch support was assessed by BS analyses (Felsenstein 1985) with full heuristic searches, 1 K parsimony BS replicates, using two RAS per BS replicate and by saving no more than ten trees per RAS in MP1 and MP2, and 642 parsimony BS replicates, using 50 RAS per BS replicate and by saving all trees per RAS in MP3. In all BS analyses, the same parameters as in the original MP search were used, and constant sites were excluded from all BS analyses.

Bayesian analyses. Bayesian analyses were conducted using MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). PPs were approximated by sampling trees using a B/MCMCMC method. The model of evolution for Bayesian inference was selected using a hierarchical likelihood ratio test (HLRT) (Huelsenbeck & Crandall 1997) with the program Modeltest 3.06 (Posada & Crandall 1998). HLRT implemented in Modeltest suggested that the TrN + G + I (Tamura & Nei 1993) was the model that best fitted the data, with estimation of invariable sites,

Table 3 – Synopsis of data sets used in MP and Bayesian analyses

	MP1 analysis	MP2 analysis	MP3 analysis	Bayesian analysis
Total number of characters	1067	1074	1304	1067
Number of sites excluded	1001	1001	1001	848
Number of constant sites	153	153	153	153
Number of variable characters	66	73 (7)	301 (7+228)	66
Number of ambiguously aligned sites	848	848	848	848
Number of recoded ambiguously aligned regions (INAASE, *arc*)	0	7 (7, 0)	17 (7, 10)	0
Total number of analysed characters	66	73	301	66
Number of parsimony informative characters	46	53	281	NA

NA, not applicable.

and assuming a Gamma distribution for rate heterogeneity among sites. Since the TrN model cannot be implemented in MrBayes, the GTR + G + I model was considered instead. Bayesian analyses were initiated on random trees and run for 8 M generations, sampling the Markov chains at intervals of 100 generations. To ensure that all trees from the burnin were excluded, the majority rule consensus tree was calculated with PAUP* using only the last 60 K out of the 80 K trees sampled. The exclusion was made by plotting the log-likelihood values against the time generation, and by setting the stationarity when log-likelihood values reached a stability equilibrium value (Huelsenbeck & Ronquist 2001). A second, independent, run of 8 M generations was performed as described to confirm the result from the first Bayesian analyses. We also used AWTY, as implemented in MrBayes 3.1.2, to test for convergence as a proxy for confirming that our two independent 8 M generation B/MCMCMC runs had reached stationarity. This analysis stopped at 1 M generations. The results were very similar to the first two runs, but likelihoods were slightly worse. Because topological differences were all in the expected parts of the trees with the highest level of phylogenetic uncertainty, none of these differences were seen as significant and would have changed the conclusions derived from this study. Therefore, we present here the result from one of the longest Bayesian analyses of 8 M generations.

Results

Alignments

The size of the ITS final data matrix for this study was 121 sequences by 1067 sites (Table 3). A total of 19 ambiguously aligned regions were delimited, resulting in the exclusion of 848 nucleotide sites. From the 219 remaining sites, 153 were constant, and 66 were variable. In MP1, from the 66 sites left, 46 were parsimony informative. In MP2, seven INAASE coded characters, which substituted seven ambiguously aligned regions, were combined with the 66 characters for a total of 73 non-constant characters and of those, 53 were parsimony informative. In MP3, apart from the seven INAASE characters, *arc* recoded characters were included, which substituted ten ambiguously aligned regions for a total of 230 characters (corresponding to 71.4 down-weighted characters). From the total of 301 non-constant characters of this analysis, 281 were parsimony informative (Table 3).

Comparison of resolution and support among phylogenetic analyses

The unequally weighted MP1 search yielded 13 003 equally most parsimonious trees (Table 4) of 312.13 steps, which

Table 4 – Synopsis of analytical results and internode support

Analysis type	MP1	MP2	**MP3**	Bayesian
	UNAMB	UNAMB + INA	**UNAMB + INA + A**	UNAMB
Number of resolved internodes (≥50 %)	18	38	**73**	34
Number of significantly supported internodes	8	24	**57**	12
Number of equally most parsimonious trees	13 003	30	**3**	–
Number of significantly supported internodes in common with MP3	8	23	–	10
Number of significantly supported internodes lost	49	34	–	47
Number of significantly supported internodes gained	0	1	–	2

The MP analysis, including INAASE and *arc* characters (in bold), was used as a reference for all other analyses. Nodes were considered significant if support values were ≥70 % with MP BS and ≥95 % with Bayesian analyses. UNAMB, unambiguously aligned sites; INA, addition of INAASE characters; A, addition of *arc* characters.

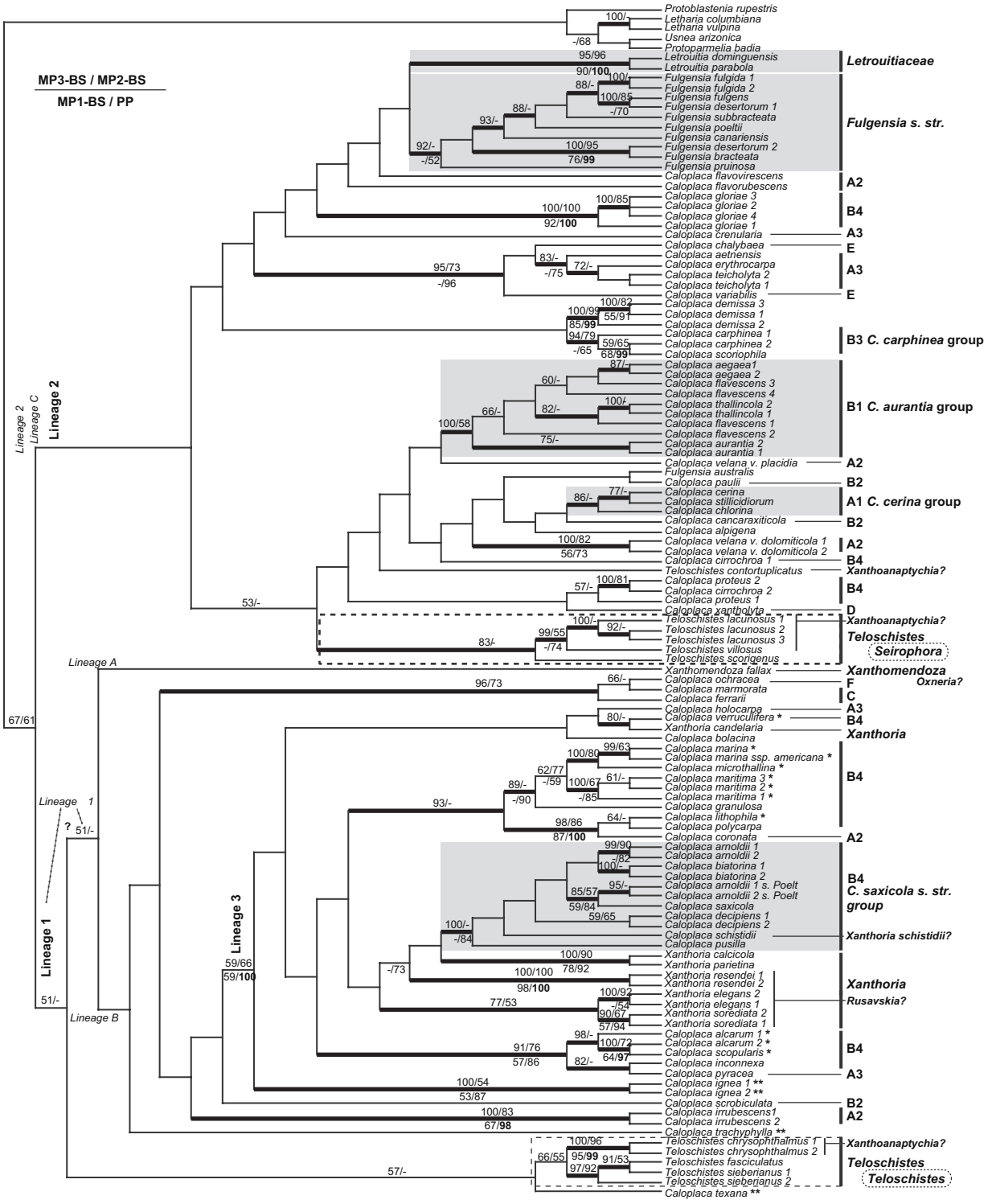

Fig 1 – Phylogenetic relationships within the *Teloschistales* (*Caloplaca*, *Fulgensia*, *Letrouitia*, *Teloschistes*, and *Xanthoria*), based on an ITS nrDNA data set for 79 species of *Teloschistaceae* and two species of *Letrouitiaceae*; taking as an outgroup four species of the family *Parmeliaceae* (*Letharia columbiana*, *L. vulpina*, *Protoparmelia badia*, and *Usnea arizonica*) and a species of the *Psoraceae* (*Protoblastenia rupestris*). Strict consensus tree of three equally most parsimonious trees generated with the parsimony analysis MP3 accommodating signal from 17 ambiguously aligned regions (INAASE and *arc* characters). Internodes with BS values from MP3 analysis (BS) ≥70 % are highlighted by thicker lines. PP values ≥95 % are marked in bold. Grey shading delimits genera and species groups previously described that have obtained statistical significance in this study. *Teloschistes* taxa are delimited by dashed lines showing the putative polyphyly of this genus. An asterisk after names shows taxa with littoral preferences belonging to *Caloplaca*. Two asterisks highlight lobed species from *Caloplaca* that had not been previously included within subgenus *Gasparrinia*. All subgenera of *Caloplaca* are indicated by capital letters and species

were part of 32 islands hit 35 times out of 1 K RAS (CI, excluding uninformative characters = 0.466; RI = 0.858). The unequally weighted MP2 search, with INAASE characters, revealed 30 equally most parsimonious trees of 587.47 steps. These trees were found in 30 islands hit 31 times out of 1 K RAS (CI, excluding uninformative characters = 0.538; RI = 0.845). A total of three equally most parsimonious trees was found in one island that was hit six out of 1 K times with the MP3 search based on combined unambiguously aligned sites, INAASE characters and *arc* characters (Fig 1). The score of the best trees was 3340.10 steps (CI, excluding uninformative characters = 0.351; RI = 0.648).

Comparing just the three parsimony treatments, we observed similar topologies throughout the three MP analyses. All three searches recovered the two main lineages 1 and 2 (Fig 1). The main discrepancies detected were in the degree of resolution within these two lineages, especially in lineage 2. The number of internodes with BS support ≥70 % went from 8, when the analysis was restricted to unambiguously aligned sites (MP1), to 24 when seven INAASE characters were added to these unambiguous sites (MP2), and to 57 when *arc* characters were added (MP3; Table 4). Only one significantly supported internode in MP2 (BS = 77 %) was not significant (<70 %) in MP3.

The Bayesian consensus tree revealed 12 well-supported internodes (PP ≥ 95 %). Forty-seven of 57 internodes with BS ≥ 70 % in MP3 received PP < 95 % in Bayesian inference, whereas only two internodes with significant PPs received MP3-BS below 70 %. Regarding the topology reconstructed, the Bayesian analysis did not resolve phylogenetic relationships between species groups in the family *Teloschistaceae*; even the two main lineages were not recovered.

Based on these results, we can assert that MP3 analysis provided the most resolved and supported phylogenetic inference for the family *Teloschistaceae*. Consequently, we will focus mainly on this topology (Fig 1) in the following sections.

Phylogenetic relationships

One of the most striking results was the phylogenetic placement of the family *Letrouitiaceae* within the *Teloschistaceae* (Fig 1). However, this relationship did not receive a single significant support value. Therefore, we cannot exclude the possibility that this family forms a monophyletic group outside the *Teloschistaceae,* within the *Teloschistales*, as implied by current classifications (e.g. Eriksson 2006) or within the *Teloschistineae* as reported by Miadlikowska *et al.* (2007) with high phylogenetic confidence but for a small taxon sampling within this suborder.

As in previous studies (Arup & Grube 1999; Gaya *et al.* 2003; Søchting & Lutzoni 2003), the same two main sister lineages were recovered within the *Teloschistaceae*. In the most phenotypically diverse clade (lineage 2), relationships among genera and species groups remained uncertain. However, several monophyletic clades recovered at genus or species complex level were strongly supported. Lineage 1 seemed to show again a slightly higher phenotypic homogeneity than lineage 2. It included most species with anthraquinones in the thallus and a mainly fruticose, foliose or placodiod habit. Again, in this lineage it was not possible to recover high support at deeper internodes.

In this study, genera *Caloplaca*, *Fulgensia,* and *Xanthoria* were again recovered as polyphyletic, confirming previous results. The genus *Teloschistes* appeared also as polyphyletic, with apparently separate origins in the two main lineages.

Regarding the genus *Caloplaca*, species belonging to subgenus *Pyrenodesmia*, subgenus *Leproplaca*, subgenus *Gasparrinia* (*C. aurantia* group, *C. aurea* group, *C. carphinea* group and *C. saxicola* group) and subgenus *Caloplaca* (*C. citrina* group, *C. cerina* group and *C. ferruginea* group) (*sensu* Clauzade & Roux 1985) were recovered in the most phenotypically diverse clade (lineage 2). The *C. cerina* group was significantly recovered as monophyletic (BS = 86 %), *C. carphinea* and *C. scoriophila* always formed a monophyletic clade with BS = 94 %, as well as the *C. aurantia* group (BS = 100 %).

Caloplaca subgenus *Gasparrinia* (most species of the *C. saxicola* group, except *C. gloriae* and one species from the *C. aurea* group) and several species from subgenus *Caloplaca* (*C. citrina* group and *C. ferruginea* group) were recovered in lineage 1, together with subgenus *Gyalolechia* and subgenus *Xanthocarpia* (*sensu* Clauzade & Roux 1985), the last two subgenera forming a robust monophyletic entity (BS = 96 %). The phylogenetic afiliation of *C. scrobiculata* (*C. aurea* group) could not be established with high confidence in any of the analyses, but was always sister to lineage 3.

Lineage 3 was recovered in all analyses, but only Bayesian PPs were statistically significant (PP = 100 %). Taxa with mainly foliose or placodioid thalli, and anthraquinones in the thallus, from the genus *Xanthoria* and *Caloplaca saxicola* group, were nested within this lineage. Only a few species with reduced thalli, with or without anthraquinones, e.g. *C. holocarpa*, *C. pyracea,* or *C. coronata* were also included in this clade. Relationships among species groups within this lineage remained unresolved, even though the monophyly of several groups was confirmed. Hence, the *C. saxicola s. str.* group, whose circumscription will be described in a forthcoming paper, appeared as monophyletic with strong evidence (BS = 100 %). *Xanthoria* was recovered as polyphyletic (Fig 1), with *X. elegans* and *X. sorediata* sharing a most

groups within these subgenera are referred by numbers: A: *Caloplaca* subgenus *Caloplaca* (A1: *C. cerina* group, A2: *C. citrina* group, A3: *C. ferruginea* group); B: *Caloplaca* subgenus *Gasparrinia* (B1: *C. aurantia* group, B2: *C. aurea* group, B3: *C. carphinea* group, B4: *C. saxicola s. lat.* group); C: *Caloplaca* subgenus *Gyalolechia*; D: *Caloplaca* subgenus *Leproplaca*; E: *Caloplaca* subgenus *Pyrenodesmia*; F: *Caloplaca* subgenus *Xanthocarpia*. This classification of subgenera and species groups follows Clauzade & Roux (1985). Names followed by question marks refer to genera proposed by Kondratyuk & Kärnefelt (2003) included in our study. Names surrounded by a dotted line indicate the proposal by Frödén & Lassen (2004) for *Teloschistes*. Lineages A, B and C from Søchting & Lutzoni (2003) and lineages 1 and 2 from Gaya *et al.* (2003) are italicized. Lineages 1 and 2 recovered in this study are in bold.

recent common ancestor (BS = 77 %), *X. calcicola* and *X. parietina* forming also a strongly supported monophyletic group (BS = 100 %), and *X. candelaria* being sister to *C. verruculifera* (BS = 80 %).

In all analyses, *Fulgensia s. str.*, as defined by Gaya *et al.* (2003), was monophyletic (BS = 92 %). *Fulgensia australis* was also part of lineage 2, but appeared related without support to *C. paulii*. Conversely, *C. schistidii* (syn. *F. schistidii*) was well nested within the *C. saxicola* group (BS = 100 %) in lineage 3.

As for *Fulgensia*, the genus *Teloschistes* was recovered in three clades. Except for *T. contortuplicatus*, the species of *Teloschistes* recovered in lineage 2 formed a monophyletic group (BS = 83 %). Species so far included within this genus with anthraquinones in the thallus were otherwise recovered in lineage 1 forming a weakly supported (BS = 66 %) group.

Finally, the genus *Xanthomendoza* was depicted in our study by only one representative: *Xanthomendoza fallax*, which was recovered as an early diverging lineage (lineage A), within lineage 1.

Discussion

Comparison of optimization criteria: MP with recoded INAASE and arc *characters* versus *Bayesian methods and MP considering only unambiguously aligned regions*

Because this phylogenetic study was based only on ITS to resolve relationships across the *Teloschistaceae*, the inclusion of phylogenetic signal from ambiguously aligned regions in MP (recoded INAASE and *arc* characters) has proved advantageous in the reconstruction of the phylogeny. This has been shown by an increase in the number of supported internodes in MP2 and MP3, compared with MP1 analysis and Bayesian inference, both restricted to unambiguously aligned regions (Table 4). Because the great majority of nucleotides could not be aligned unambiguously (848; Table 3), the Bayesian method, considered as more efficient than other phylogenetic methods (Alfaro *et al.* 2003), has not shown a greater resolving power when restricted to non-ambiguously aligned sites than MP when recovering signal from ambiguously aligned regions of the alignment. Our results agree with Reeb *et al.* (2004) in their nuclear ribosomal LSU and SSU analyses, where higher phylogenetic confidence was not revealed with Bayesian inference when the analyses were restricted to these two genes. They postulate that LSU and SSU evolved slowly, and without the phylogenetic signal recovered from ambiguously aligned regions there was not enough variation to resolve relationships with a high phylogenetic confidence, even for the Bayesian inference. However, when one gene (*RPB2*) was added to the SSU and LSU, Bayesian analyses using different models of evolution were more efficient than MP analyses even with the addition of signal from ambiguously aligned regions.

In Gaya *et al.* (2003), we suggested that large data sets of ITS sequences for the *Teloschistaceae* would greatly benefit from methods like INAASE, that have been designed to obtain phylogenetic signal from ambiguously aligned regions. We also pointed out the possibility that ITS alone could provide sufficient phylogenetic information to completely resolve relationships within the *Teloschistaceae*, and could generate high support values for most internodes if a new method was able to capture phylogenetic signal from all ambiguously aligned regions, even those with more than 32 character states, more than 100 bp in length, or with a considerable variation in length among sequences from the same ambiguous region.

By using *arc* we have been able to recover phylogenetic signal from an additional ten of the remaining 12 ambiguously aligned regions that could not be recoded using INAASE. Miadlikowska *et al.* (2003) mentioned that variation among their ITS1-HR sequences (recoded with *arc*) contributed in a great extent to species delimitation and identification, and stated that it can be useful for population studies. We prove that these characters can also be useful for higher-scale phylogenies, as in the case of the *Teloschistaceae*. Only when including INAASE and *arc* characters could we reach the level of resolution and support for the family shown here. Nonetheless, deep internodes of our topology lost significant support that was previously recovered in other phylogenetic studies restricted to fewer taxa. This is a common phenomenon for deep internodes when adding many taxa without adding more characters (see Miadlikowska *et al.* 2007). From this we deduce that the number of taxa exceeded the resolving power of ITS even when including INAASE and *arc* characters. Nevertheless, ITS remains an excellent marker to resolve, with high phylogenetic confidence, species complexes if used in combination with genes providing complementary resolution and support, such as the nuLSU (e.g. Miadlikowska *et al.* 2003).

Phylogenetic relationships within the family Teloschistaceae

The same two main lineages obtained by Arup & Grube (1999) (BS = 91 % and 99 %), Gaya *et al.* (2003) (lineage 1, PP = 99 % and lineage 2, BS = 89 %) and Søchting & Lutzoni (2003) (without significance), are consistently recovered within the family *Teloschistaceae*, even though without or with very low support in our study. The taxon sampling here was much broader and included a higher number of species. Although genera *Caloplaca*, *Fulgensia*, *Teloschistes*, and *Xanthoria* are recovered as polyphyletic, several species groups have been consistently recovered as monophyletic.

Agreeing with previous results, we can confirm that lobed species traditionally included within *Caloplaca* subgenus (*sensu* Clauzade & Roux 1985), or section (*sensu* Poelt 1969) *Gasparrinia*, do not form a monophyletic entity. *Caloplaca aurantia*, *C. carphinea*, and *C. saxicola s. str.* groups are the only groups recovered as monophyletic (with high phylogenetic confidence) within this subgenus. In this way, delimitation of the *C. saxicola s. lat.* group (*sensu* Clauzade & Roux 1985) is not in agreement with our molecular phylogeny, and neither are the *C. aurea* group and sorediate species of the *C. saxicola* group. In a cladistic study based on morphological characters, Kärnefelt (1989) also did not provide enough evidence to accept *Gasparrinia* as a separate group. Kärnefelt (1989) pointed out that the use of infrageneric categories within the lobed species could be justified.

The *C. aurantia* group had previously been shown to be separate from the rest of members of subgenus *Gasparrinia* based on molecular data by Søchting & Arup (2002). They argued that the *C. aurantia* group is distinguished by several distinct

morphological characters. The recently described species *C. aegaea* (Sipman & Raus 2002) is reported to be part of this group (Fig 1). All four taxa (*C. aegaea*, *C. aurantia*, *C. flavescens*, and *C. thallincola*) are characterized by having citriform spores, and differ from each other by the different types of cortex and by the presence or absence of calcium oxalate crystals in the cortex. Interspecific relationships have not been resolved in our study, except for the monophyly of *C. aegaea*, *C. aurantia*, and *C. thallincola*.

The *C. carphinea* group is consistently monophyletic in all analyses. One specimen of *C. carphinea* shares a most recent common ancestor with *C. scoriophila* (supported only by PP). Breuss (1989) distinguished these two species mainly by the type of cortex, paraplectenchymatous in *C. carphinea* and scleroplectenchymatous in *C. scoriophila*, and by the size of the spores, longer in *C. scoriophila*. According to this author, *C. carphinea* can be included in the mediterranean element, whereas *C. scoriophila* shows a more Atlantic distribution. Based on only three specimens, we cannot make conclusions about the monophyly of these two species.

In our analyses, the *C. aurea* group shows three putative origins. In lineage 2, the placement of *C. cancarixiticola* and *C. paulii* remains uncertain. In lineage 1, *C. scrobiculata* is always recovered without support as sister to lineage 3. Contrary to what several authors had suggested, neither *C. cancarixiticola* nor *C. paulii* show a close relationship with *Fulgensia s. str.* in this study. Kärnefelt (1989) and Poelt (1965) had proposed a morphological proximity of the *C. aurea* group with *Fulgensia* subgenus *Candelariopsis* (*sensu* Poelt 1965 and Poelt & Vězda 1977). Kärnefelt (1989) and Westberg & Kärnefelt (1998) stated that several taxa included within the *C. aurea* group (e.g. *C. paulii*) could be related to *Fulgensia canariensis* and *F. schistidii* based on cortex structure. Navarro-Rosinés *et al.* (2000) proposed morphological affinities between *C. aurea* and typical *Fulgensia* (subgenus *Fulgensia sensu* Poelt 1965), and group D from Westberg & Kärnefelt (1998). *Caloplaca cancarixiticola* was thought to be related to subgenus *Candelariopsis* and Westberg & Kärnefelt's subgroups A, B and C by Navarro-Rosinés *et al.* (2000) based on a comparison of *C. cancarixiticola* specifically to *F. australis*, *F. canariensis,* and *F. schistidii*. None of these relationships are corroborated by our study.

Clauzade & Roux (1985) considered four sorediate species within the *C. saxicola* group: *C. cirrochroa*, *C. decipiens*, *C. obliterans,* and *C. proteus*. The topology recovered in this study does not support this grouping. Hence, *C. cirrochroa* and *C. proteus* are recovered within lineage 2, whereas *C. decipiens* shares a most recent common ancestor with the taxa included in the *C. saxicola s. str.* group in lineage 1. Unfortunately, *C. obliterans* could not be included in our study. Although the two specimens of *C. proteus* are nested in a group together with one of the specimens of *C. cirrochroa*, the latter shows a second origin of uncertain position. Based on these results, we cannot verify or deny whether *C. cirrochroa* and *C. proteus* have evolved independently from different non-sorediate ancestors as stated by Poelt (1969). The phylogenetic placement within the *Teloschistaceae* of this small group of sorediate taxa remains unresolved.

Caloplaca gloriae (syn. *C. gomerana*, type lost, synonymization pending to study) was considered a species close to *C. saxicola* by Llimona & Werner (1975), and was included within the *C. saxicola* group by Clauzade & Roux (1985). However, *C. gloriae* differs, among other characters, by containing fragilin and caloploicin (Søchting & Lutzoni 2003), two compounds that are also found in *Fulgensia* (Søchting & Lutzoni 2003). In lineage 2, *C. gloriae* appears closely related to *C. flavorubescens*, *C. flavovirescens*, *Fulgensia s. str.*, and *Letrouitia*, even though without support. Gaya *et al.* (2003) had already shown a significant close relationship among *C. gloriae*, *C. flavorubescens*, *C. flavovirescens*, and *Fulgensia s. str.* Based on results from Miadlikowska *et al.* (2007), the non-supported phylogenetic placement of *Letrouitia* as shown in our Fig 1 is most likely incorrect due to the lack of characters for such a high number of taxa.

Most taxa of the *C. saxicola s. lat.* group (*sensu* Clauzade & Roux 1985), *C. scrobiculata* (*C. aurea* group), and other species that could be considered as closely related to subgenus *Gasparrinia* by being lobed and showing anthraquinones in the thallus, i.e., *C. ignea*, *C. texana,* and *C. trachyphylla*, are recovered in lineage 1. Considering the *C. saxicola s. lat.* group, this circumscription shows several potential origins. In this sense, most lobed taxa without maritime affinities constitute what we named the *C. saxicola s. str.* group. This monophyly was also recovered by Gaya *et al.* (2003) and Arup & Grube (1999). However, in Søchting & Lutzoni (2003, clade B3) the two species of the *C. saxicola* group included, shared a common ancestor with *C. holocarpa*. Within this group, we recovered *C. pusilla*, also named *C. saxicola* ssp. *pulvinata*. We will publish this synonymy in a forthcoming morphological study on the *C. saxicola s. str.* group. In our study, taxa with littoral or maritime affinities, considered by Clauzade & Roux (1985) as part of the *C. saxicola s. lat.* group, appear in separate clades, not sharing a most recent common ancestor with the *C. saxicola s. str.* group. One of these clades, groups *C. marina*, *C. maritima*, and *C. microthallina*, which are species with a highly reduced thallus, having microlobes, areoles, granules or warts, depending on the species, and all showing a paraplechtenchymatous cortex (Arup 1992, 1994, 1997). Arup & Grube (1999) and Gaya *et al.* (2003) had shown a close phylogenetic relationship between *C. marina* and *C. maritima*. *Caloplaca granulosa* is sister to this group of littoral species. This taxon shows well-developed lobes and abundant isidia. Although it is not a littoral species, it grows on steep surfaces, exposed to heavily eutrophicated rainwater runoffs.

Caloplaca alcarum and *C. scopularis* constitute another clade of littoral taxa. One of the *C. alcarum* specimens and the *C. scopularis* specimen are recovered as monophyletic, being sisters to the other specimen of *C. alcarum*. A thorough morphological study of these two species led us to discover a strong similarity in terms of ascospore shape and cortex structure (Gaya 2008). Based on the morphological characters and the phylogenetics results, we suggest the possibility that these two taxa could be in fact the same species, with a wide range of lobe development. In further studies, additional characters should be used to confirm this hypothesis. Arup (1995a) established similarities between *Caloplaca alcarum* and *C. inconspecta* (not included in this study), and *C. marina*, but *C. alcarum* was never found to be closely related to *C. scopularis*. *Caloplaca alcarum* and *C. scopularis* can be anatomically distinguished from the other clade of littoral species by having a scleroplectenchymatous or sclero-prosoplectenchymatous cortex, but never paraplectenchymatous, as in *C. marina* and *C. maritima*. *Caloplaca alcarum* and *C. scopularis* are shown here to share

a most recent common ancestor with a species from the *C. ferruginea* group (*C. pyracea*) and a species from the *C. saxicola* s. lat. group, *C. inconnexa*. Phenotypically, there are no similarities among these taxa, except for their preference for euthrophicated habitats.

Caloplaca inconnexa and *C. pyracea* are part of a group of quite problematic and not well-known *Caloplaca* species. Their circumscriptions as species vary depending on the authors. Magnusson (1946) divided *C. pyracea* into two species according to their substrate. Saxicolous specimens were classified as *C. lithophila*, whereas corticolous specimens were named *C. pyracea*. Later on, these two taxa were synonymized with *C. holocarpa* (Santesson 1984; Wade 1965), a lignicolous species of uncertain taxonomy. Arup (1994) stated that, apart from the differences of substrate preferences, *C. lithophila* and *C. pyracea* were very similar. Clauzade & Roux (1985) considered *C. lithophila* as a variety, *C. tenuatula* ssp. *tenuatula* var. *lithophila*, and *C. inconnexa* as a subspecies, *C. tenuatula* ssp. *inconnexa*, within the *C. saxicola* s. lat. group, whereas *C. holocarpa* and *C. pyracea* were placed in the *C. ferruginea* group. Clauzade & Roux (1985) distinguished *C. holocarpa* from *C. pyracea* by the presence on the latter of a grey–yellowish thallin margin and bigger apothecia. In our study, we follow the concept of Clauzade & Roux (1985) regarding the taxonomy of these species, and have included another taxon from the *C. tenuatula* complex, *C. polycarpa* (syn. *C. tenuatula* ssp. *verrucariarum*, Clauzade & Roux 1985). Despite the morphological affinities among these taxa, our analyses do not support a close relationship. Although all of them appear within lineage 3, *C. holocarpa* shows an uncertain position, *C. inconnexa* and *C. pyracea* form a well-supported monophyletic group, related to *C. alcarum* and *C. scopularis*, and *C. lithophila* and *C. polycarpa* form a weakly supported monophyletic entity, within a well-supported clade including *C. coronata*.

Caloplaca verruculifera is another littoral species that have been classified within the *C. saxicola* s. lat. group (e.g. Clauzade & Roux 1985). This species shows a close relationship with *X. candelaria* in our study, a grouping that also appeared in the phylogenetic analyses by Søchting *et al.* (2002). Both *C. verruculifera* and *X. candelaria* have few apothecia. *Caloplaca verruculifera* presents a kind of globose isidia called phyllidia (Wetmore & Kärnefelt 1998), whereas *X. candelaria* abounds in blastidiate soredia (Lindblom 1997; Poelt & Petutschnig 1992a, b). *Caloplaca verruculifera* does not form a monophyletic entity with *C. granulosa*, another isidiate species from the *C. saxicola* s. lat. complex, with which it has been frequently confused, according to Arup (1994). Poelt & Romauch (1977) distinguished these two species by the anatomy of the thallus and stated that *C. verruculifera* shows a denser medulla than *C. granulosa*, with strongly conglutinated hyphae. Søchting & Lutzoni (2003) obtained a closer relationship between *C. scopularis* and *C. verruculifera*, indicating morphological similarities between these two species in the layout of the hyphae of the cortex and the ability to produce pseudocyphellae in the upper cortex. According to these authors, these features relate *C. scopularis* and *C. verruculifera* to a fruticose species, *C. coralloides*. Results from Søchting & Lutzoni (2003) support the suggestion that *C. coralloides* could be related to a taxonomical group including *C. alcarum*, *C. scopularis,* and *C. verruculifera* (Arup 1995b). With the addition of new taxa, we can conclude that this grouping does not agree with the current phylogeny. However, we did not include *C. coralloides* in our study. Further studies considering fruticose species will be necessary to clarify the relationships among this group of taxa.

Caloplaca texana and *C. trachyphylla*, two species morphologically similar to members of subgenus *Gasparrinia*, are recovered within lineage 1, outside of lineage 3. *Caloplaca texana* is known from the United States and Mexico (Wetmore & Kärnefelt 1998), and in our topology it is sister to *Teloschistes* species within lineage 1, but with low support. *Caloplaca trachyphylla*, a species from North America, Central Asia and Greenland, has been reported to be related to *X. elegans*, *C. gloriae*, *C. verruculifera*, and even to fruticose taxa (e.g. *C. coralloides*, *C. thamnodes*) (e.g. Wetmore & Kärnefelt 1998). *Caloplaca trachyphylla* occupies an uncertain position in our topology.

Within the subgenus *Caloplaca* (Fig 1), the *C. citrina* and *C. ferruginea* groups are revealed to be polyphyletic, with potential independent origins both in lineages 1 and 2. These two groups are distinguished mainly by the presence of yellow to orange thalli and apothecia in the *C. citrina* group, and by whitish, grey, or black thalli and yellow to nearly black apothecia in the *C. ferruginea* group (Clauzade & Roux 1985). In their keys, the latter authors often included taxa from other groups or subgenera within the *C. citrina* and *C. ferruginea* groups.

The *C. citrina* group is represented in lineage 2 by *C. flavorubescens*, *C. flavovirescens,* and *C. velana*. The latter species includes two infraspecific taxa, var. *dolomiticola* and var. *placidia*. *Caloplaca flavorubescens* and *C. flavovirescens*, difficult to differentiate based only on morphological characters, were treated as subspecies within *C. flavorubescens* by Clauzade & Roux (1985). Conversely, Giralt *et al.* (1992) described two new species (*C. aegatica* and *C. alnetorum*) and a variety (*quercina*) based on the corticolous complex of *C. flavorubescens*, highlighting the great morphological diversity within this taxon. Considering these different taxonomical treatments, a molecular phylogenetic study will be necessary to reassess the morphological diversity of this complex. Regarding *C. velana*, in our study the two infraspecific taxa included within this species do not belong to the same clade, but more characters are needed to confirm this result. In further studies, it will also be necessary to include all infraspecific taxa of *C. velana* considered by Clauzade & Roux (1985). These taxa have often been synonymized and included within a broader concept of the species.

In lineage 1, the *C. citrina* group is represented by *C. irrubescens*, a species with slightly lobed orange to ocher areoles, which shows an uncertain phylogenetic position outside lineage 3, and by *C. coronata*, a blastidiate taxon that shares a most recent common ancestor with *C. lithophila* and *C. polycarpa*, two species from the *C. saxicola* s. lat. group nested within lineage 3. These two species do not form blastidia, but share the same ecology with *C. coronata*, mainly coniophilous and ornithocoprophilous, and in the case of *C. polycarpa*, by sometimes parasitizing other crustose lichens. Recently, Arup (2006) studied the relationships within the *C. citrina* group in the Nordic countries. In his study, Arup (2006) showed that there are at least five species within what has been called *C. citrina*, of which four are closely related to one another and to several non-sorediate species (e.g. *C. maritima*). In our study, we did

not include these species, but the position of *C. coronata* and *C. irrubescens* (syn. *C. subsoluta*) does not contradict Arup's results.

In lineage 2, several taxa in the *C. ferruginea* group with whitish to light grey thalli, lacking anthraquinones, and with ferruginous apothecia, are recovered, i.e., *C. aetnensis*, *C. crenularia*, *C. erythrocarpa*, and *C. teicholyta*. *Caloplaca erythrocarpa* and *C. teicholyta* share a most recent common ancestor with strong support, although the monophyletic delimitation of these species cannot be confirmed nor denied in our analyses. Each have whitish thalli, but in *C. teicholyta* apothecia are rare and the thallus is more or less lobed and covered by soredia, which gives a pulverulent aspect, whilst in *C. erythrocarpa* apothecia are abundant and the thallus has no lobes or soredia. *C. aetnensis* shows similar features, also having a whitish thallus, but in this case areoles and warts form the thallus. The morphological affinities of these three species are translated into a well-supported clade that in turn is nested with high confidence with the two species of the subgenus *Pyrenodesmia*, *C. chalybaea* and *C. variabilis*. Conversely, the phylogenetic placement of *C. crenularia*, another species of the *C. ferruginea* group, remains unsupported. This species has a dark brownish thallus and orange–ferruginous to blackish apothecia, with a sinuous margin. *C. chalybaea* has been considered here as a separate species from *C. variabilis* based on the information provided by M. Tretiach (pers. comm.) regarding a study on the phylogeny of subgenus *Pyrenodesmia*. Muggia *et al.* (2008) presented preliminary results of this study, where several distinct lineages within this group of endolithic lichens were recovered.

Based on the taxa included in our study, we can state that the *C. cerina* group (subgenus *Caloplaca*) is monophyletic with strong support. This group is recovered in lineage 2 and includes three closely related species, one of them, *C. stillicidiorum*, has often been considered a variety of *C. cerina*, the type species of the genus *Caloplaca*.

The subgenus *Leproplaca* groups leprarioid species without cortex, and is depicted in our study by only one specimen of *C. xantholyta*, recovered in lineage 2 with an uncertain phylogenetic placement. Conversely, subgenus *Xanthocarpia*, represented by *C. ochracea*, is recovered in lineage 1, forming a statistically significant monophyletic entity with *C. ferrarii* and *C. marmorata*, two taxa from subgenus *Gyalolechia* (*C. lactea* group *sensu* Navarro-Rosinés & Hladun 1996). These three species are characterized by extremely reduced and often endolithic thalli.

Regarding the genus *Fulgensia*, Westberg & Kärnefelt (1998), in their morphological study, suggested that the circumscription of *Fulgensia sensu* Poelt was probably polyphyletic. Later, Kasalicky *et al.* (2000) and Gaya *et al.* (2003) confirmed this polyphyly with molecular data. In this study, including new species, the polyphyly of *Fulgensia* is again confirmed. The affiliations of the three independent origins already shown in Gaya *et al.* (2003) are still uncertain, except for the high support in favor of the inclusion of *C. schistidii* (syn. *F. schistidii*) within *C. saxicola s. str.* The relationships within the *C. saxicola s. str.* group will be discussed in a forthcoming paper.

In our analyses, the group with the highest number of taxa, *Fulgensia s. str.* (including the type species of the genus, *F. fulgens*) confirms *Fulgensia sensu* Kasalicky *et al.* (2000). All molecular studies carried out thus far (Kasalicky *et al.* 2000; Gaya *et al.* 2003; this study) support in part the classification of the subgenera proposed by Poelt (1965). What we call *Fulgensia s. str.*, in our study, fits well with what Poelt (1965) called subgenus *Fulgensia*. Conversely, subgenus *Candelariopsis* (*F. australis*, *F. schistidii*, and *F. pruinosa*) *sensu* Poelt (1965) is not monophyletic (Fig 1), as previously reported by Gaya *et al.* (2003), Kasalicky *et al.* (2000) and Westberg & Kärnefelt (1998). In our study, two species described subsequently to the work of Poelt (1965), *F. canariensis* and *F. poeltii*, are also included and nested within *Fulgensia s. str.*

Fulgensia s. str. used to group terricolous species with yellow thalli, covered with abundant pruina, cortex darkened by the presence of crystals and spores mostly without septum. The heterogeneity of this group has increased in our analyses by the inclusion of species with different thalli and occasionally septate spores, such as *F. canariensis*. The new phylogenetic circumscription of *Fulgensia s. str.* does not correspond exactly to group D from Westberg & Kärnefelt (1998), as it includes *F. canariensis*, a species that these authors thought belonged to another group (group B). Species included in group D by Westberg & Kärnefelt (1998) were characterized mainly by having a crustose or squamulous yellow thallus, covered by abundant pruina. Spores in this group are simple or uniseptate, sometimes with a visible internal thickening (*F. pruinosa*). Conversely, *F. canariensis* shows an areolate thallus, without pruina, and the colour is waxy yellowish-orange. Spores in *F. canariensis* have a slight equatorial wall thickening. According to Westberg & Kärnefelt (1998) spore shape brings this species nearer to *F. fulgida*, but septum ontogeny might indicate certain affinity to some species of *Caloplaca* with polarilocular spores. Based on this character, Breuss (2001) combined *F. canariensis* into *Caloplaca*, and stated that this species, apart from being terricolous, can also grow on basaltic rocks. In our results, morphological or ecological differences do not determine a different origin for this species, and we have kept it within *Fulgensia*.

The two specimens of *F. desertorum* are not recovered as monophyletic. The specimen from Norway forms a robust clade with *F. bracteata*, whereas the specimen collected in northern Spain shares a most recent common ancestor with *F. fulgens*. As pointed out in Gaya *et al.* (2003), it might be possible that the identification of specimens from northern Europe was erroneous. Conversely, the grouping of *F. desertorum* and *F. fulgens* does not clarify the identity of these two species. *Fulgensia fulgida* is the only species that is revealed as monophyletic. The sister relationship between *F. fulgida* and *F. fulgens* was already shown by Kasalicky *et al.* (2000). However, *F. pruinosa* appeared as sister to *F. bracteata* with high support, whereas in our topology it is sister to the rest of the *Fulgensia s. str.*

The polyphyly of the genus *Teloschistes* is here reported for the first time based on molecular data. In our analyses, *Teloschistes* might have up to three origins, two of them in lineage 2; *T. contortuplicatus* and a monophyletic group comprising *T. lacunosus*, *T. scorigenus*, and *T. villosus*. These three species do not contain anthraquinones in the thallus, whereas in *T. contortuplicatus* the thallus is slightly pigmented, yellowish to grey coloured. Søchting & Frödén (2002) grouped *Teloschistes* species according to their anthraquinone pigmentation into

four groups and included the three species without anthraquinones mentioned above within a group of species without, or rarely with, spots of anthraquinones in the thallus. *T. contortuplicatus* was included within another group characterized by having a thallus fully or partially pigmented, rarely without anthraquinones, which is shared by two of the three *Teloschistes* species recovered in lineage 1. Consequently, the group without anthraquinones in the thallus is recovered as monophyletic, whereas the species usually pigmented do not correspond to a monophyletic entity (Fig 1). Nevertheless, the four taxa included in lineage 2 are part of a group of species that have been transferred to the genus *Seirophora* by Frödén & Lassen (2004), who stated that the separation between *Seirophora* and *Teloschistes* was supported by molecular data (P. Frödén, unpubl.). According to our results the delimitation of *Seirophora* is not yet confirmed. The third potential origin of *Teloschistes* is recovered in lineage 1, and includes species only with yellow-orangish thalli: *T. chrysophthalmus*, *T. fasciculatus*, and *T. sieberianus*. Søchting & Frödén (2002) indicated that even though the production of anthraquinones has an environmental component, as these pigments are produced only under well-lighted conditions, in some species these pigments are environmentally independent, and thalli may not contain anthraquinones even though they are well exposed to sunlight. Some of these unpigmented species are *T. lacunosus*, *T. scorigenus,* and *T. villosus*. It is possible that the phylogenetic relationships of these groups of species, morphologically so different, will be resolved with phylogenetic confidence with the inclusion of more characters. Additionally, it will be necessary to include the type species of the genus, *T. flavicans* that, according to Frödén & Lassen (2004), may belong to the group of *Teloschistes* from lineage 1, to complete a taxonomic revision of these fruticose lichens.

When comparing results to Gaya *et al.* (2003) and Søchting & Lutzoni (2003), the polyphyly of the genus *Xanthoria* is maintained. In our topology, *Xanthoria* species are recovered in lineage 3, and show a close relationship with taxa from subgenus *Gasparrinia* included in this lineage. Based on our taxon sampling, lineage B from Søchting & Lutzoni (2003), including *Xanthoria* and *Caloplaca* species with crustose thalli, is difficult to interpret. Species in lineage B are characterized by the presence of ellipsoidal conidia and by having parietin as a dominant anthraquinone, together with small amounts of fallacinal, teloschistin, parietinic acid, and emodin (table 4 in Søchting & Lutzoni 2003; chemosyndrome A, Søchting 1997). Comparing lineage B to the clade sister to *Xanthomendoza fallax* in our study (Fig 1), the morphological homogeneity described by Søchting & Lutzoni (2003) lacks consistency. The *C. holocarpa* specimen used by Søchting & Lutzoni (2003), and recovered together with taxa from the *C. saxicola s. str.* group in their clade B3, may not correspond to our understanding of *C. holocarpa*. Actually, they confirmed that their specimen could correspond to *C. tenuatula* ssp. *inconnexa sensu* Clauzade & Roux (1985). According to Claude Roux (pers. comm.) the identity of this specimen is *C. polycarpa* var. *athallina*.

In our analyses, we do not recover lineage B1 from Søchting & Lutzoni (2003) that grouped *X. elegans* and *X. parietina*. Instead, *X. elegans* appears more closely related to *X. sorediata*, and *X. parietina* is nested with *X. calcicola*. The monophyletic entity constituted by *X. parietina* and *X. calcicola* was also revealed in Arup & Grube (1999). Recently, in a population study by Lindblom & Ekman (2005), the separation between *X. parietina* and *X. calcicola* has been confirmed. Søchting & Lutzoni (2003) characterized species from their clade B1 by the presence of a foliose thallus, by a paraplectenchymatous cortex, and by fixation to the substrate by means of a lower cortex or hapteria (*sensu* Kondratyuk & Poelt 1997). After an anatomical study on *Xanthoria* species we verified that *X. elegans* and *X. sorediata* show the same type of cortex structures, proso-scleroplectenchymatous, and non-paraplectenchymatous. The relationship between these two species had already been mentioned (Kondratyuk & Poelt 1997), even though it had been established based on the primary and secondary species concept of Poelt (1963, 1970, 1972).

Poelt & Petutschnig (1992a, b) classified *X. candelaria* within the *X. fallax* group based on the presence of soredia. In our analyses, we do not recover this relationship. According to Søchting & Lutzoni (2003), phylogenetic separation of *X. candelaria*, outside the *X. fallax* group, can be morphologically explained by the presence of ellipsoidal conidia and by the absence of rhizines in *X. candelaria*.

In this study we accepted the combination of the *X. fallax* group into the genus *Xanthomendoza*. Just as for Arup & Grube (1999) and Gaya *et al.* (2003), *X. fallax* is derived from an early divergence within lineage 1; however, unlike these previous studies, *C. texana* and the *Teloschistes* group are revealed as the first divergence within lineage 1 rather than *X. fallax*. This branch could correspond to group A from Søchting & Lutzoni (2003), which, instead of *X. fallax*, included *X. borealis* and *X. poeltii*, two species also transferred to the genus *Xanthomendoza*. The *X. fallax* (or *X. ulophyllodes*) group was first established by Poelt & Petutschnig (1992a, b) and later recombined as an independent genus (*Xanthomendoza*) by Søchting *et al.* (2002). Species of *Xanthomendoza* are characterized by having true rhizines, by the slightly different cortex structure, and by having narrow, oblong, or bacilliform conidia (Lindblom 1997). Moreover, most species have chemosyndrome A_3, described by Søchting (1997). Persistence of *Xanthomendoza* in presenting a different origin from the rest of *Xanthoria* species supports its delimitation anticipated by morphological characters.

Recently, Kondratyuk & Kärnefelt (2003) have described three new genera in the *Teloschistaceae*: *Oxneria*, *Rusavskia,* and *Xanthoanaptychia*, respectively segregated from genera *Xanthomendoza*, *Xanthoria,* and *Teloschistes*. *Oxneria* corresponds to the *Xanthomendoza ullophyllodes* group, *Rusavskia* would be equivalent to the presumed natural group of *Xanthoria elegans*, and *Xanthoanaptychia* is composed of the *Teloschistes villosus* group. In Khodosovtsev *et al.* (2004), the differentiating characters of these newly described genera, from those traditionally accepted, are based mainly on the thallus habit, conidial form, presence/absence of rhizines, and upper and lower cortex structure. We do not recognize these new genera in this study because phylogenetic relationships within the *Teloschistaceae* need further resolution and support before new generic names can be ascribed to stable monophyletic groups within the *Teloschistaceae* in a contructive manner. Based on our sampling, *Oxneria* would be represented by one species (*Xanthomendoza fallax*); *Rusavskia* by *Xanthoria elegans*, *X. resendei* and *X. sorediata*; and *Xanthoanaptychia* by *Teloschistes contortuplicatus*, *T. lacunosus* and *T. villosus* (see Fig 1).

Regarding *Oxneria*, we cannot accept segregating this genus from *Xanthomendoza* based only on the type of cortex structure. Cortex structure is quite a variable character and it would be risky to use it to discriminate supraspecific entities. As an example, Kondratyuk & Kärnefelt (2003) transferred *Xanthomendoza incavata* into *Oxneria*, a species that, according to Søchting *et al.* (2002), has a cortex structure very similar to *X. mendozae*, the only species that then remained within genus *Xanthomendoza*. Moreover, considering the phylogeny obtained by Søchting *et al.* (2002), *Oxneria* would be paraphyletic.

Genera *Rusavskia* and *Xanthoanaptychia* were also differentiated based on the cortex structure and by the presence/absence of a lower cortex. Species included in *Rusavskia* revealed several origins both in our topology and in Gaya *et al.* (2003), just as for *Xanthoria* species. *Xanthoanaptychia* is also a polyphyletic genus and the species considered within *Xanthoanaptychia* correspond to some of those transferred to genus *Seirophora* by Frödén & Lassen (2004). Until publication of the phylogenetic and morphological study on the genera *Seirophora* and *Teloschistes* by P. Frödén (pers. comm.), we believe it more appropriate to maintain the traditional taxonomical categories.

Kondratyuk & Zelenko (2002) combined *C. schistidii* into *Xanthoria schistidii*. In our study, this species proves to be nested within the *C. saxicola s. str.* group. Therefore, we do not consider this transfer appropriate at this time.

Taxonomic conclusions

In our study, we observe that lineage 2 continues to show a high level of phenotypic heterogeneity, and includes several type species: *Caloplaca cerina*, *Fulgensia fulgens,* and *Letrouitia domingensis*. Several species groups are revealed with high confidence (*C. aurantia* group, *C. carphinea* group and *C. cerina* group). Because of the lack of support for intermediate and basal internodes we have decided to make no nomenclatural changes and to wait until new data help to build a more comprehensive phylogenetic classification. Nevertheless, we can make some suggestions based on our results. Accepting the generic entity of the *Fulgensia s. str.* group could lead to accepting species groups mentioned above at the genus level. These groups have several morphological features that would also support their separation. However, we need to be aware that if *C. cerina* is the type species of genus *Caloplaca*, segregation of this species group will necessitate considerable nomenclatural changes for the rest of species currently within *Caloplaca*. Regarding *Teloschistes* species included within lineage 2, the proposal by Frödén & Lassen (2004) to use *Seirophora* would be valid if the monophyly of this group could be confirmed in future studies.

Lineage 1 appears more diverse than previously thought. A possible taxonomic solution for this lineage would be to consider it as a part of the redefined genus *Xanthoria* as proposed by Gaya *et al.* (2003) and Søchting & Lutzoni (2003). Other possibilities would be to restrict *Xanthoria* to a subclade within lineage 1, leaving *Teloschistes* and *Xanthomendoza* as independent genera. Species of very different morphology, such as those in subgenera *Gyalolechia* and *Xanthocarpia,* could be maintained as a subgenus within *Xanthoria*. Finally, another option would be to consider *Xanthoria* based solely on lineage 3 and establish independent genera for the rest of the excluded clades.

Acknowledgements

We thank Claude Roux for critical comments on the manuscript, Valérie Reeb for her invaluable support in the DNA isolation and sequencing, and Ulf Arup, Cécile Gueidan, Jolanta Miadlikowska, and Ulrik Søchting for their useful discussions and help. The authors are also grateful to Ulf Arup, André Aptroot, Javier Etayo, Cécile Gueidan, and Ulrik Søchting, as well as the curators of the herbaria of BCN, C, DUKE, E, GZU, LEB, MARSSJ, MIN, MUB, SANT and TFC Lich for providing material used in this work; Frank Kauff and Stefan Zoller for writing computer programs useful for this study and Molly McMullen for the English revision. This work was carried out within the projects (PB 96-1115-C04-02, BOS 2001-0869-C04-02, CGL 2005-04322), Spanish government, and CIRIT (2001 SGR 00095, 2005 SGR 01047), Catalonian government. We gratefully acknowledge support from a PhD scholarship (FI-programa propi, Universitat de Barcelona) and a postdoctoral grant from the Fulbright Scholar Program to EG.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.mycres.2007.11.005.

REFERENCES

Alfaro ME, Zoller S, Lutzoni F, 2003. Bayes or bootstrap? A simulation study comparing the performance of bayesian markov chain monte carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* **20**: 255–266.

Arup U, 1992. *Caloplaca marina* and *C. rosei*, two difficult species in North America. *Bryologist* **95**: 148–160.

Arup U, 1994. The genus *Caloplaca* on seashore rocks in eastern North America. *Bryologist* **97**: 377–392.

Arup U, 1995a. Eight species of *Caloplaca* in coastal western North America. *Bryologist* **98**: 92–111.

Arup U, 1995b. *Littoral species of the lichen genus Caloplaca* in North America. PhD thesis, University of Lund.

Arup U, 1997. *Caloplaca maritima*, a misunderstood species in western Europe. *Lichenologist* **29**: 503–512.

Arup U, 2006. A new taxonomy of the *Caloplaca citrina* group in the Nordic countries, except Iceland. *Lichenologist* **38**: 1–20.

Arup U, Grube M, 1999. Where does *Lecanora demissa* (*Ascomycota*, *Lecanorales*) belong? *Lichenologist* **31**: 419–430.

Bellemère A, Letrouit-Galinou MA, 1982. Le développement des asques et des ascospores chez le *Caloplaca marina* Wedd. et chez quelques lichens de la famille des *Teloschistaceae* (*Caloplaca*, *Fulgensia*, *Xanthoria*): étude ultrastructurale. *Cryptogamie, Bryologie et Lichénologie* **3**: 95–137.

Breuss O, 1989. Zur Unterscheidung von *Caloplaca carphinea* und *C. scoriophila* (Lichenes, *Teloschistaceae*). *Linzer Biologische Beitrage* **21**: 583–590.

Breuss O, 2001. Über *Caloplaca canariensis* (Lichenisierte Ascomyceten, *Teloschistaceae*). *Österreichische Zeitschrift für Pilzkunde* **10**: 83–86.

Clauzade G, Roux C, 1985. Likenoj de Okcidenta Europo. *Bulletin de la Société Botanique du Centre-Ouest, Nouvelle série - Numero Spécial* **7**: 1–893.
Dodge C, 1948. Lichens and lichen parasites. *Report of the British Australia New Zeland Antarctic Research Expeditio, Series B*. **7**: 1–276.
Dodge C, 1971. Some lichens of tropical Africa. V. *Lecanoraceae* to *Physciaceae*. *Beihefte zur Nova Hedwigia* **38**: 1–225.
Dodge CW, Baker GE, 1938. The second byrd antarctic expedition-Botany. II. Lichens and Lichen parasites. *Annals of the Missouri Botanical Garden[query author stl]* **25**: 515–727.
Dyer PS, Murtagh GJ, 2001. Variation in the ribosomal ITS-sequence of the lichens *Buellia frigida* and *Xanthoria elegans* from the Vestfold Hills, eastern Antarctica. *Lichenologist* **33**: 151–159.
Eriksson OE, 1999. Outline of *Ascomycota* — 1999. *Myconet* **3**: 1–88.
Eriksson OE, 2005. Outline of *Ascomycota* — 2005. *Myconet* **11**: 1–113.
Eriksson OE, 2006. Outline of *Ascomycota* — 2006. *Myconet* **12**: 1–82.
Eriksson OE, Hawksworth DL, 1986. Outline of the ascomycetes. *Systema Ascomycetum* **5**: 185–324.
Eriksson OE, Hawksworth DL, 1991. Notes on ascomycete systematics — Nos 1252–1293. *Systema Ascomycetum* **10**: 135–149.
Eriksson OE, Baral HO, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe T, 2001. Outline of *Ascomycota* — 2001. *Myconet* **7**: 1–88.
Eriksson OE, Baral HO, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe T, 2003. Outline of *Ascomycota* — 2003. *Myconet* **9**: 1–89.
Eriksson OE, Baral HO, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe T, 2004. Outline of *Ascomycota* — 2004. *Myconet* **10**: 1–99.
Felsenstein J, 1981. A likelihood approach to character weighting and what it tells us about parsimony and compatibility. *Biological Journal of the Linnean Society* **16**: 183–196.
Felsenstein J, 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
Fink B, 1910. The lichens of Minnesota. *Contributions from the United States National Herbarium* **14**: 1–269.
Franc N, Kärnefelt EI, 1998. Phylogeny of *Xanthoria calcicola* and *X. parietina*, based on ITS sequences. *Graphis Scripta* **9**: 49–54.
Fries T, 1871. Lichenographia Scandinavica sive dispositio lichenum in Dania, Suecia, Norvegia, Fennia, Lapponia Rossica hactus collectorum. Vol. I Archilichenes discocarpos continens. Pars. I. Uppsala.
Frödén P, Lassen P, 2004. Typification and emendation of *Seirophora* Poelt to include species segregated from *Teloschistes* Norman. *Lichenologist* **36**: 289–298.
Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes. Application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.
Gaya E, Lutzoni F, Zoller S, Navarro-Rosinés P, 2003. Phylogenetic study of *Fulgensia* and allied *Caloplaca* and *Xanthoria* species (*Teloschistaceae*, lichen-forming *Ascomycota*). *American Journal of Botany* **90**: 1095–1103.
Gaya E, 2008. Taxonomical revision on the *Caloplaca saxicola* group (*Teloschistaceae*, lichen-forming *Ascomycota*). *Bibliotheca Lichenologica*, in press.
Giralt M, Nimis PL, Poelt J, 1992. Studien über den Formenkreis von *Caloplaca flavorubescens* in Europa. *Cryptogamie, Bryologie–Lichénologie* **13**: 261–273.
Grube M, Winka K, 2002. Progress in understanding the evolution and classification of lichenized ascomycetes. *Mycologist* **16**: 67–76.
Hafellner J, 1981. Monographie der Flechtengattung *Letrouitia* (*Lecanorales*, *Teloschistineae*). *Nova Hedwigia* **35**: 645–729.
Hafellner J, 1984. Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien *Lecanoraceae* und *Lecideaceae*. *Beihefte zur Nova Hedwigia* **79**: 241–371.
Hafellner J, 1988. Principles of classification and main taxonomic groups. In: Galun M (ed), *CRC Handbook of Lichenology*. *Volume III*. CRC Press, Boca Raton, FL, pp. 41–52.
Hafellner J, Bellemère A, 1981a. Elektronenoptische Untersuchungen an Arten der Flechtengattung *Letrouitia* gen. nov. *Nova Hedwigia* **35**: 263–312.
Hafellner J, Bellemère A, 1981b. Elektronenoptische Untersuchungen an Arten der Flechtengattung *Bombyliospora* und die taxonomischen Konsequenzen. *Nova Hedwigia* **35**: 207–235.
Hafellner J, Hertel H, Rambold G, Timdal E, 1994. Discussion 4: Lecanorales. In: Hawksworth DL (ed), *Ascomycete Systematics: problems and perspectives in the nineties*. *NATO Advanced Science Institutes Series A269*. Plenum Press, New York, pp. 379–387.
Hansen E, Poelt J, Søchting U, 1987. Die Flechtengattung *Caloplaca* in Grönland. *Meddelelser om Grönland. Bioscience* **25**: 1–52.
Hawksworth DL, Eriksson OE, 1986. The names of accepted orders of ascomycetes. *Systema Ascomycetum* **5**: 175–184.
Hawksworth DL, Kirk PM, Sutton BC, Pegler DN, 1995. *Ainsworth & Bisby's Dictionary of the Fungi*, 8th edn. CAB International, Wallingford.
Helms G, Friedl T, Rambold G, 2003. Phylogenetic relationships of the *Physciaceae* inferred from rDNA sequence data and selected phenotypic characters. *Mycologia* **95**: 1078–1099.
Henssen A, Jahns HM, 1973 ['1974']. *Lichenes*. Georg Thieme Verlag, Stuttgart.
Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson O, Huhndorf S, James T, Kirk PM, Lücking R, Lumbsch T, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde K, Koljalg U, Kurtzman CP, Larsson KH, Lichtward R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schuessler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White M, Winka K, Yao YJ, Zhang N, 2007. A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**: 509–547.
Honegger R, 1978. The ascus apex in lichenized fungi I. The *Lecanora-*, *Peltigera-* and *Teloschistes*-types. *Lichenologist* **10**: 47–67.
Huelsenbeck JP, Crandall KA, 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* **28**: 437–466.
Huelsenbeck JP, Ronquist F, 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*. *Applications Note* **17**: 754–755.
Kärnefelt I, 1989. Morphology and phylogeny in the *Teloschistales*. *Cryptogamic Botany* **1**: 147–203.
Kärnefelt I, 1994. *Caliciales*, *Graphidales*, and *Teloschistales*. In: Hawksworth DL (ed), *Ascomycete Systematics: problems and perspectives in the nineties*. *NATO Advanced Science Institutes Series A269*. Plenum Press, New York, pp. 393–396.
Kasalicky T, Döring H, Rambold G, Wedin M, 2000. A comparison of ITS and LSU nrDNA phylogenies of *Fulgensia* (*Teloschistaceae*, *Lecanorales*), a genus of lichenised ascomycetes. *Canadian Journal of Botany* **78**: 1580–1589.
Kauff F, Miadlikowska J, Lutzoni F, 2003. *Arc — a program for ambiguous region coding*. v.1.5. Duke University, Durham.
Khodosovtsev AY, Kondratyuk S, Makarova I, Oxner A, 2004. *Handbook of the Lichens of Russia, Vol. 9. Fuscideaceae, Teloschistaceae*. The Russian Academy of Sciences, Nauka, St. Petersburg.
Kirk PM, Cannon PF, David JC, Stalpers JA, 2001. *Ainsworth & Bisby's Dictionary of the Fungi*, 9th edn. CAB International, Wallingford.

Kondratyuk S, Poelt J, 1997. Two new Asian *Xanthoria* species (*Teloschistaceae*, lichenized *Ascomycotina*). *Lichenologist* **29**: 173–190.

Kondratyuk S, Zelenko S, 2002. New lichens and lichenicolous fungi from Israel and the near east. *Ukranian Botanic Journal* **59**: 598–606.

Kondratyuk S, Kärnefelt I, 2003. Revision of three natural groups of xanthorioid lichens (*Teloschistaceae*, *Ascomycota*). *Ukranian Botanic Journal* **60**: 427–437.

Lindblom L, 1997. The genus *Xanthoria* (Fr.) Th. Fr. in North America. *Journal of the Hattori Botanical Laboratory* **83**: 75–171.

Lindblom L, Ekman S, 2005. Molecular evidence supports the distinction between *Xanthoria parietina* and *X. aureola* (*Teloschistaceae*, lichenized *Ascomycota*). *Mycological Research* **109**: 187–199.

Llimona X, Werner RG, 1975. Quelques lichens nouveau ou interessants de la Sierra de Gata (Almeria, SE de l'Espagne). *Acta Phytotaxonomica Barcinonensia* **16**: 1–32.

Lumbsch HT, Schmitt I, Palice Z, Wiklund E, Ekman S, Wedin M, 2004. Supraordinal phylogenetic relationships of *Lecanoromycetes* based on a Bayesian analysis of combined nuclear and mitochondrial sequences. *Molecular Phylogenetics and Evolution* **31**: 822–832.

Lutzoni F, Wagner P, Reeb V, Zoller S, 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* **49**: 628–651.

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Hambleton S, Crockett M, Shoemaker R, Sung GH, Lucking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim YW, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R, 2004. Assembling the fungal tree of life: progress, classification and evolution of subcellular traits. *American Journal of Botany* **91**: 1446–1480.

Maddison WP, Maddison DR, 2001. *MacClade Manual: analysis of phylogeny and character evolution*. Version 4.01. Sinauer Associates, Sunderland, MA.

Magnusson AH, 1946. Lichens from Lycksele Lappmark and adjacent part of Norway. *Arkiv för Botanik utgivet av. K. Svenska vetenskapsakademien* **33**: 1–146.

Malme G, 1926. Lichenes blasteniospori Herbarii Regnelliani. *Arkiv för Botanik utgivet av. K. Svenska vetenskapsakedemien* **20**: 1–51.

Massalongo A, 1852. Synopsis lichenum blasteniosporum. *Flora* **35**: 561–576.

Massalongo A, 1853. Monografia dei Licheni blasteniospori. *Atti dell'I.R. Istituto Veneto di Scienze, Lettere ed Arti*, **III:** 5–131.

Mattick F, 1951. Wuchs- und Lebensformen, Bestand- und Gesellschaftsbildung der Flechten. *Botanischen Jahresvericht* **75**: 378–424.

Miadlikowska J, Lutzoni F, Goward T, Zoller S, Posada D, 2003. New approach to an old problem: Incorporating signal from gap-rich regions of ITS and rDNA large subunit into phylogenetic analyses to resolve the *Peltigera canina* species complex. *Mycologia* **95**: 1181–1203.

Miadlikowska J, Lutzoni F, 2004. Phylogenetic classification of peltigeralean fungi (*Peltigerales*, *Ascomycota*) based on ribosomal RNA small and large subunits. *American Journal of Botany* **91**: 449–464.

Miadlikowska J, Kauff F, Hofstetter V, Fraker E, Grube M, Hafellner J, Reeb V, Hodkinson BP, Kukwa M, Lücking R, Hestmark G, Otalora MG, Rauhut A, Büdel B, Scheidegger C, Timdal E, Stenroos S, Brodo I, Perlmutter GB, Ertz D, Diederich P, Lendemer JC, May P, Schoch CL, Arnold AE, Gueidan C, Tripp E, Yahr R, Robertson C, Lutzoni F, 2007 [2006]. New insights into classification and evolution of the *Lecanoromycetes* (*Pezizomycotina*, *Ascomycota*) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* **98**: 1088–1103.

Muggia L, Grube M, Tretiach M, 2008. A combined molecular and morphological approach to species delimitation in black-fruited, endolithic *Caloplaca*: high genetic and low morphological diversity. *Mycological Research* **112**: 36–49.

Murtagh GJ, Dyer PS, Furneaux PA, Crittenden PD, 2002. Molecular and physiological diversity in the bipolar lichen-forming fungus *Xanthoria elegans*. *Mycological Research* **106**: 1277–1286.

Navarro-Rosinés P, Hladun N, 1996. Las especies saxícolo-calcícolas del grupo de *Caloplaca lactea* (*Teloschistaceae*, líquenes) en las regiones mediterránea y medioeuropea. *Bulletin de la Société Linnéenne de Provence* **47**: 139–166.

Navarro-Rosinés P, Egea JM, Llimona X, 2000. *Caloplaca cancarixiticola*, a new species from south-east Spain growing on ultrapotassic rocks. *Lichenologist* **32**: 129–138.

Nordin A, 1972. Caloplaca *sect.* Gasparrinia *i Nordeuropa*. *Taxonomiska och Ekologiska Studier*. Skriv Service AB, Uppsala.

Oxner A, 1993. *Flora of the Lichens of Ukraine,* vol. 2. Naukova Dumka, Kiev.

Ozenda P, Clauzade G, 1970. *Les Lichens*. *Etude Biologique et Flore Illustree*. Masson & Cie, Paris.

Peršoh D, Beck A, Rambold G, 2004. The distribution of ascus types and photobiontal selection in *Lecanoromycetes (Ascomycota)* against the background of a revised SSU nrDNA phylogeny. *Mycological Progress* **3**: 103–121.

Poelt J, 1954. Die Gelappten arten der flechtengattung *Caloplaca* in Europa. *Mitteilungen der Botanischen Staatssammlung München* **11**: 11–31.

Poelt J, 1963. Flechtenflora und Eiszeit in Europa. *Phyton, Annales Rei Botanicae* **10**: 206–215.

Poelt J, 1965. Über einige Artengruppen der Flechtengattungen *Caloplaca* und *Fulgensia*. *Mitteilungen der Botanischen Staatssammlung München* **5**: 571–607.

Poelt J, 1969. *Bestimmungsschlüssel europäischer Flechten*. J. Cramer, Lehre.

Poelt J, 1970. Das Konzept der Artenpaare bei den Flechten. *Vorträge aus dem Gesamtgebiet der Botanik, N.F.* **4**: 187–198.

Poelt J, 1972. Die taxonomische Behandlung von Artenpaaren bei den Flechten. *Botaniska Notiser* **125**: 77–81.

Poelt J, 1974 ['1973']. Classification. Appendix A. In: Ahmadjian V, Hale ME (eds), *The Lichens*. Academic Press, New York, pp. 599–632.

Poelt J, Romauch E, 1977. Die Lagerstrukturen placodialer Kusten- und Inlandsflechten. Ein Beitrag zur ökologischen Anatomie der Flechten. In: Frey W, Hurka H, Oberwinkler F (eds), *Beiträge zur Biologie de niederen Pflanzen Systematik, Stammesgeschichte, Okologie*. Gustav Fischer Verlag, Stuttgart, pp. 141–153.

Poelt J, Vězda A, 1977. Bestimmungsschlüssel europäischer Flechten. Ergänzungsheft I. *Bibliotheca Lichenologica* **9**: 1–258.

Poelt J, Hafellner J, 1980. *Apatoplaca* - genus novum *Teloschistacearum* (*Lichenes*). *Mitteilungen der Botanischen Staatssammlung München* **16**: 503–528.

Poelt J, Petutschnig W, 1992a. *Xanthoria candelaria* und ähnliche Arten in Europa. *Herzogia* **9**: 103–114.

Poelt J, Petutschnig W, 1992b. Beiträge zur Kenntnis der Flechtenflora des Himalaya IV. Die Gattungen *Xanthoria* und *Teloschistes* zugleich Versuch einer Revision der *Xanthoria candelaria*-Gruppe. *Nova Hedwigia* **54**: 1–36.

Poelt J, Hinteregger E, 1993. Beiträge zur Kenntnis der Flechtenflora des Himalaya VII. Die Gattungen *Caloplaca, Fulgensia*

und *Ioplaca* (mit englischem Bestimmungsschlüssel). *Bibliotheca Lichenologica* **50**: 1–247.
Posada D, Crandall KA, 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics. Applications Note* **14**: 817–818.
Rambold G, Triebel D, 1992. The inter-lecanoralean associations. *Bibliotheca Lichenologica* **48**: 1–258.
Rambold G, Schuhwerk F, Triebel D, 1991 ['1992']. Die grosssystematischen Einheiten der Ordnung *Lecanorales (Ascomycetes)* und ihre ökologischen Präferenzen. *Mitteilungen der Botanischen Staatssammlung München* **30**: 385–400.
Räsänen V, 1943. Das System der Flechten. *Acta Botanica Fennica* **33**: 1–82.
Reeb V, Lutzoni F, Roux C, 2004. Contribution of *RPB2* to multilocus phylogenetic studies of the euascomycetes (*Pezizomycotina*, *Fungi*) with special emphasis on the lichen-forming *Acarosporaceae* and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32**: 1036–1060.
Rudolph ED, 1955. *Revisionary studies in the lichen family* Blasteniaceae *in North America north of Mexico*. Washington University.
Santesson J, 1970a. Neuere Probleme der Flechtenchemie. *Vorträge aus dem Gesamtgebiet der Botanik, N.F.* **4**: 5–21.
Santesson J, 1970b. Anthraquinones in *Caloplaca*. *Phytochemistry* **9**: 2149–2166.
Santesson R, 1984. *The Lichens of Sweden and Norway*. Swedish Museum of Natural History, Stockholm.
Sipman H, Raus T, 2002. An inventory of the lichen flora of Kalimnos and parts of Kos (Dodecanisos, Greece). *Willdenowia* **32**: 351–392.
Søchting U, 1997. Two major anthraquinone chemosyndromes in *Teloschistaceae*. *Bibliotheca Lichenologica* **68**: 135–144.
Søchting U, 2001. Chemosyndromes with chlorinated anthraquinones in the lichen genus *Caloplaca*. *Bibliotheca Lichenologica* **78**: 395–404.
Søchting U, Arup U, 2002. Phylogenetic position of the *Caloplaca aurantia* group. In: Ryvarden L, Schumacher T (eds), *Abstracts of the Seventh International Mycological Congress*. University of Oslo, p. 231.
Søchting U, Frödén P, 2002. Chemosyndromes in the lichen genus *Teloschistes* (*Teloschistaceae*, *Lecanorales*). *Mycological Progress* **1**: 257–266.
Søchting U, Kärnefelt I, Kondratyuk S, 2002. Revision of *Xanthomendoza* (*Teloschistaceae*, *Lecanorales*) based on morphology, anatomy, secondary metabolites and molecular data. *Mitteilungen aus dem Institut fü Allgemeine Botanik, Hamburg* **30-32**: 225–240.
Søchting U, Lutzoni F, 2003. Molecular phylogenetic study at the generic boundary between the lichen-forming fungi *Caloplaca* and *Xanthoria* (*Ascomycota*, *Teloschistaceae*). *Mycological Research* **107**: 1266–1276.
Steiner M, Poelt J, 1982. *Caloplaca* sect. *Xanthoriella*, sect. nov.: Untersuchungen über die *Xanthoria lobulata*-Gruppe. *Plant Systematics and Evolution* **140**: 151–177.
Stenroos SK, DePriest PT, 1998. SSU rDNA phylogeny of cladoniiform lichens. *American Journal of Botany* **85**: 1548–1559.
Swofford DL, 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, MA.
Tamura K, Nei M, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512–526.
Tehler A, 1996. Systematics, phylogeny and classification. In: Nash TH (ed), *Lichen Biology*. Cambridge University Press, New York, pp. 217–239.
Tornabene F, 1849. Lichenographia Sicula. Catanae. *Atti dell'-Accademia Gioenia di Scienze Naturali di Catania* **2**: 1–152.
Verseghy K, 1970. Hazai *Gasparrinia* fajok. I. *Botanikar Kozlemények* **57**: 23–29.
Verseghy K, 1971. Hazai *Gasparrinia* fajok. II. Rendszertani resz. *Botanikar Kozlemények* **58**: 21–28.
Verseghy K, 1972. Hazai *Gasparrinia* fajok. III. Rendszertani resz. (befejezes). *Botanikar Kozlemények* **59**: 13–18.
Vilgalys R, Hester M, 1990. Rapid identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
Wade AE, 1965. The genus *Caloplaca* Th. Fr. in the British Isles. *Lichenologist* **3**: 1–28.
Weddell HA, 1876. Notice monographique sur les *Amphiloma* de la flore française. *Bulletin de la Societé Botanique de France* **23**: 83–99.
Westberg M, Kärnefelt I, 1998. The genus *Fulgensia* A. Massal. & De Not., a diverse group in the *Teloschistaceae*. *Lichenologist* **30**: 515–532.
Wetmore CM, 1994. The lichen genus *Caloplaca* in North and Central America with brown or black apothecia. *Mycologia* **86**: 813–838.
Wetmore CM, Kärnefelt EI, 1998. The lobate and subfruticose species of *Caloplaca* in North and Central America. *Bryologist* **101**: 230–255.
Wheeler WC, 1990. Combinatorial weights in phylogenetic analysis: a statistical parsimony procedure. *Cladistics* **6**: 269–275.
White TJ, Bruns TD, Lee SB, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*. Academic Press, New York, pp. 315–322.
Wiklund E, Wedin M, 2003. The phylogenetic relationships of the cyanobacterial lichens in the *Lecanorales* suborder *Peltigerineae*. *Cladistics* **19**: 419–431.
Zahlbruckner A, 1898. Flechten (Lichenes). B. Spezieller Teil. In: Engler A (ed), *Syllabus der Pflanzenfamilien* 2. Berlin, p. 45.
Zahlbruckner A, 1926. Lichenes (Flechten). B. Spezieller Teil. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien 8*, Aufl. 2, Engelmann, Leipzig, pp. 61–270.
Zahlbruckner A, 1931. *Catalogus Lichenum Universalis* 7. Gebrüder Borntraeger, Leipzig.
Zahlbruckner A, 1940. *Catalogus Lichenum Universalis* 10. Gebrüder Borntraeger, Leipzig.