School of Doctoral Studies in Biological Sciences
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# Contribution to the taxonomy and biodiversity of crustose lichens from the family Teloschistaceae

Ph.D. Thesis

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**Annotation**

The PhD thesis is composed of five published papers and one manuscript accepted for publication. The main research goal was the investigation of the taxonomy and diversity of crustose Teloschistaceae in Europe and Northern Asia with special emphasis on the genus *Pyrenodesmia*. The first paper investigated the delimitation of the genus *Pyrenodesmia* and its relationships with the closely related *Caloplaca haematites* and *C. xerica* groups and some other species. The second paper proposed standardized methods for the measurement of the morphological characters of the crustose Teloschistaceae and suggested a phenotype evaluation process for the delimitation of species that are difficult to phenotypically distinguish. The method was practically employed in the third paper for the separation of three seemingly cryptic species from the genus *Pyrenodesmia*, which were formally described in the paper. Another new *Pyrenodesmia* species was described in the fourth paper. Investigations presented in the fifth paper substantially extended the known geographical distribution of several species of crustose Teloschistaceae in Europe and Northern Asia. The accepted manuscripts proposes the description of the new species of crustose Teloschistaceae in Siberia and the Russian Far East. Our studies provide a basis for future summarizing papers on the taxonomy and diversity of the genus *Pyrenodesmia* in the Holarctic and the family Teloschistaceae in Russia.

**Declaration**

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

České Budějovice, …….. .....

Ivan Frolov

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**List of papers and author's contribution**

The thesis is based on the following papers:

**Paper 1**

**Frolov I.**, Vondrák J., Košnar J., Arup U. 2020. Phylogenetic relationships within *Pyrenodesmia* sensu lato and the role of pigments in its taxonomic interpretation. Journal of Systematics and Evolution. doi: 10.1111/jse.12717.

*Ivan Frolov made 70 % of the study: he designed the study, participated in the collection of the material, production, analysis, and interpretation of the data, and wrote the manuscript.*

**Paper 2**

Vondrák J., **Frolov I.**, Arup U., Khodosovtsev A. 2013. Methods for phenotypic evaluation of crustose lichens with emphasis on Teloschistaceae. Chornomorskiy botanichniy zhurnal 9: 382–405.

*Ivan Frolov made 20 % of the study: he participated in the study design and editing of the manuscript.*

**Paper 3**

**Frolov I.**, Vondrák J., Fernández-Mendoza F., Wilk K., Khodosovtsev A., Halıcı M.G. 2016. Three new, seemingly-cryptic species in the lichen genus *Caloplaca* (Teloschistaceae) distinguished in two-phase phenotype evaluation. Annales Botanici Fennici 53: 243–262.

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**Paper 4**

Vondrák J., **Frolov I.**, Říha P., Hrouzek P., Palice Z., Nadyeina O., Halıcı G., Khodosovtsev A., Roux C. 2013. New crustose Teloschistaceae in Central Europe. The Lichenologist 45: 701–722.

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Vondrák J., **Frolov I.**, Davydov E.A., Urbanavichene I., Chesnokov S., Zhdanov I., Muchnik E., Konoreva L., Himelbrant D., Tchabanenko S. 2016. The extensive geographical range of several species of Teloschistaceae: evidence from Russia. The Lichenologist 48: 181–189.

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**Paper 6**

**Frolov I.**, Vondrák J., Konoreva L.A., Chesnokov S.V., Himelbrant D.E., Arup U., Stepanchikova I.S., Prokopiev I.A., Yakovchenko L., Davydov E.A. (accepted manuscript). Three new species of crustose Teloschistaceae in Siberia and the Far East. The Lichenologist.

*Ivan Frolov made 70 % of the study: he designed the study, participated in the collection of the material, produced and analyzed the data, participated in the interpretation of the data, and wrote the manuscript.*

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**Co-author agreement**

Jan Vondrák, the supervisor of this Ph.D. thesis, on behalf of all the co-authors, fully acknowledges the contribution of Ivan Frolov.

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Jan Vondrák, Ph.D.

# Contents

# 1 Introduction

## 1.1 Lichen symbiosis and the taxonomy of lichens as complex organisms

Lichens are iconic examples of symbiosis. Together, fungi and algae form characteristic, self-constructed, and self-replicating thalli that can be maintained for several thousand years and resemble neither of the symbionts, a feature thought to be unique among symbiotic structures (Spribille et al. 2016, Spribille 2018). As a result, until relatively recently lichens were considered to be single organisms and treated separately from fungi and algae under the taxonomic unit *Lichenes*. The symbiotic nature of lichens was first revealed by Schwendener (1867).

At present, lichens are usually defined as symbiotic organisms, composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont, which is most often either a green alga or cyanobacterium (Nash III 2008). Most general textbooks refer to lichens as either a classic case of mutualism or, alternatively, as an example of controlled parasitism (Ahmadjian 1993, Nash III 2008).

Nevertheless, attempts to reconstruct lichen thalli from the axenic cultures of appropriate myco- and photobionts have rarely produced structures that resemble natural thalli (Honegger 1993). Gradually this has led to the understanding of lichens as meta-organisms hosting many diverse cohabitants that contribute in different ways to the prosperity of the holobiont (Aschenbrenner et al. 2016, Spribille 2018). For example, it has been demonstrated that bacteria have an important ecophysiological role in the health of the lichen thallus (Grube et al. 2015, Aschenbrenner et al. 2016). Spribille et al. (2016) found basidiomycetes yeasts in the cortex of several fruticose lichen species. The authors related phenotypic differences between the lichen species to the different amounts of various yeasts in the cortex. Therefore, these yeasts may quite accurately be considered as a third component of the lichen symbiosis. Černajová and Škaloud (2019) found that basidiomycetes yeasts are common in some species of *Cladonia* and even described a new genus and species of such yeasts from *Cladonia* thalli. However, Lendemer et al. (2019) detected basidiomycete yeasts in only nine taxa of 339 investigated lichen species spanning 57 families and 25 orders, which suggests that these yeasts are probably not ubiquitous across lichens.

Lichenization is one of the major lifestyles among fungi. Lichenized fungi are not known in nature without photosynthetic partners and make up 17% of the known 110,000 fungal species (Lücking et al. 2016). Phylogenetic evidence suggests that lichenization evolved several times independently. Between 20 and 30 independent lichenization events took place in the evolution of Ascomycota and Basidiomycota, but only nine lineages, which include more than 99% of lichens, can be considered successful (Lücking et al. 2016). The Code of Botanical Nomenclature anchored the

name of the lichen to the fungus. Hence, lichen systematics and taxonomy are currently based on the mycobiont.

### 1.2 Species concepts and their application in lichenology

The concept of "species" plays an important role in biology as a central unit for systemizing the diversity of organisms (Mayr 1942, Coyne and Orr 2004, Lumbsch and Leavitt 2011, Leavitt S.D. et al. 2015). Taking this into account, as well as the fact that current estimates of the number of extant species range from approximately two million to over one hundred million (Caley et al. 2014), the importance of accurate species delimitation cannot be overestimated. Nevertheless, the conceptualization of the term "species" remains the topic of long standing debate, and today has as much relevance as ever (Freudenstein et al. 2017, Zachos 2018). The numerous existing means of species definition and the apparent inability of biologists to agree on something so fundamental has been described as the "species problem" (Hey 2001, Freudenstein et al. 2017). The species problem is the result of the difficulty found in identifying the units of biological classification corresponding to the units of evolution (Dupré 2001, Hey 2001). When scientists categorize organisms, species appear to them as real natural entities, but when biologists try to understand species evolutionarily, they are revealed as changeable and without sharp boundaries (Hey 2001, Zachos 2018). This has led some authors to view species not as real natural entities. They argue that species are just names and the lines between species discussed by scientists do not reflect any fundamental underlying biological boundaries (Gregg 1950). Other philosophers pragmatically claim that even if species do not exist as natural units they are conceptually real and can be used for the convenience of biological classification (Dupré 2001). Some authors suggest formalizing species delimitation to simplify the legislation of biodiversity conservation (Garnett and Christidis 2017). Nevertheless, at present, most biologists agree that living nature is aggregated into really existing, discrete, evolutionarily independent entities – species (Coyne and Orr 2004).

Depending on the particular author, the number of species concepts varies from 22 to 34 (Mayden 1997, Wilkins 2012, Zachos 2016). For example, Wilkins (2012) distinguishes 7 "basic" concepts with 19 derivatives. Different subgroups of biologists advocate different species concepts and some of them are at least partially incompatible (de Queiroz 2007).

One of the earliest and the most prominent species concepts is the biological species concept, according to which the species is an interbreeding natural population isolated from other such groups (e.g., Mayr 1942). In the framework of this concept, distinct species do not need to have diagnosable morphological differences. It is successfully applied in non-lichenized Basidiomycetes, due to the possibility of using mating tests, when sexual compatibility between populations is confirmed by pairing

homokaryons from each population and forming dikaryotic mycelium in Petri dishes (Dai et al. 2003). In contrast, the dikaryotic stage of most lichens along with non-lichenized Ascomycetes is limited to the ascogeneous tissues and mating tests are difficult to apply in these fungi (Lumbsch and Leavitt 2011). A rare example of employing the biological species concept in lichenology was provided by Culberson et al. (1993), who investigated the breeding system of chemotypes of the rock-inhabiting species complex *Ramalina siliquosa* by analyzing the progeny of matemals in situ. They found that the progeny of different chemotypes tend to be chemically identical to their respective maternal individuals even in zones where maternal thalli with different chemotypes grew together. The authors concluded that these chemotypes were apparently ecologically differentiated sibling species, which could be distinguished only by chemistry.

The typological or morphological concept defines species as the smallest groups of individuals that are consistently and persistently distinct and distinguishable by ordinary phenotype-based methods (Wilkins 2012). The clusters of phenotypes within a set of specimens differentiate the species. Species named in this manner are called morphospecies, or classical species, or Linnaean species, because the concept has been used for a long time, since Linnaeus and even earlier (Wilkins 2012), being probably, the most "natural" and instinctively understandable to the common person. A phenotype-based approach to species recognition is still widely applied to lichenized fungi and relies on the use of morphological and chemical features (Lumbsch and Leavitt 2011). Morphological characters used in the species delimitation of lichens include diverse vegetative and reproductive structures. They are, for example, the form, colour, and size of thalli, attachment organs, and other supplementary formations (cilia, hairs, etc.), the presence and form of pseudocyphellae and maculae, the type of reproduction (ascomata or vegetative diaspores), and the form and location of reproductive structures (Lumbsch and Leavitt 2011). Several studies have proposed glossaries of morphological terms and discussed detailed methods and standardized procedures for phenotypic evaluation (Printzen 1995, Ekman 1996, Vondrák et al. 2013a, Frolov et al. 2016, https://glossary.lias.net).

Chemistry also plays an important role in phenotype-based species delimitations in lichenized fungi. The feature was introduced into lichenology by Nylander (1866) who proposed using simple spot tests with calcium hypochlorite and potassium hydroxide for the identification of secondary metabolites in lichen thalli. Currently, lichenologists know of 800 such metabolites (Huneck 1999, Schumm and Elix 2015) and biochemical appraisals are obligatory for most taxonomical studies (Hawksworth 1976). Moreover, species delimitation in lichen groups lacking explicit morphological characters is often based on secondary metabolites. For example, it is widely used in the genus *Lepraria* characterized by the complete absence of sexual reproduction and

hence ascomata, which are usually one of the most important morphological features of the genus (Lendemer 2011). Secondary chemistry is also a very useful tool for species delimitations in genera with complex morphology such as the crustose *Ochrolechia* (Kukwa 2011) or the fruticose *Cladonia* (Stenroos 1989, Timsina et al. 2014). The presence or absence of specific substances, or their replacement by another substance, is widely used to distinguish species when correlated with geographic differences (Lumbsch and Leavitt 2011). If chemistry is the only difference between populations, some authors distinguish them as species, while others merely as chemical races. Also, closely related substances are arranged into chemosyndromes, which are used as additional characters in species delimitation (Culberson and Culberson 1976). For instance, chemosyndromes are widely used in the taxonomy of the family Teloschistaceae (Søchting 1997, 2001, Vondrák et al. 2019a).

At the end of the 20$^{th}$ century, biology entered into the era of molecular genetic data, which currently play a prominent role in species delimitation and the investigation of phylogenetic relationships between organisms. As a result, phenotypically based species concepts have faced the problem of discrediting the characters traditionally used for species delimitation. Evolutionary biologists have always pointed out that species do not necessarily have morphological differences, since phenotypic features may be similar in distinct species due to the selective advantage of maintaining a specific phenotype during parallel or convergent evolution (Mayr 1942, Lumbsch and Leavitt 2011). Lichens display few taxonomically useful characters and many of them are widely variable; the homology of character states within and between groups is difficult to assess (Printzen 2010). Consequently, molecular genetic data has gained great influence in the species delimitation of lichens (Grube and Winka 2002, Printzen 2010, Lumbsch and Leavitt 2011, Leavitt 2015).

It has been discovered that related species may share phenotypic features inherited from a common ancestor but, at the same time, these features may also be found in unrelated clades where they have appeared independently (Crespo and Pérez-Ortega 2009). The critical evaluation of the taxonomical relevance of phenotypic data has shown that while traditional phenotypically based delimitation was supported by molecular data for some groups, it was inadequate for others. This works both for morphological and chemical data. For example, the red-fruited species of *Cladonia* described on the basis of morphology were not supported by the molecular data (Steinova et al. 2013). Both correlation and discrepancy of morphology and molecular data were found in the genus *Solenopsora* (Guttova et al. 2014). The chemically different, but otherwise very similar, *Parmeliopsis ambigua* and *P. hyperopta* or *Blastenia hungarica* and *B. subathallina* were also distinguished by molecular data (Tehler and Kallersjo 2001, Vondrák et al. 2019a). In other cases no correlation between secondary metabolites and phylogenetic lineages was found, indicating

chemical polymorphism among interbreeding populations; e.g., in the genus *Thamnolia* (Nelsen and Gargas 2009), in some species of the genus *Cladonia* (Konoreva et al. 2019), and in the crustose *Teloschistaceae* where the pigment Sedifolia-grey often occurs in unrelated species (Vondrák et al. 2012).

It has been shown that species recognition based on morphological features often strongly underestimates the number of species due to the existence of species complexes undivided in the framework of the morphological concept (Leavitt S.D. et al. 2015). About 80 cryptic lineages were estimated to be hidden under widely distributed or disjunct species in a single family Parmeliaceae (Crespo and Lumbsch 2010). Species complexes will be discussed below in detail.

At the same time, the use of morphology may lead to an overestimation of species diversity, and in such cases, molecular data question the separation of some phenotypically characterized species. Leavitt et al. (2011) demonstrated that single individual lineages within the molecular phylogeny of the genus *Xanthoparmelia* contain up to eight traditional species described on the base of morphology and chemistry. In the genus *Bryoria* section *Implexae* with ten phenotypic species, only two molecular lineages with conspecific sequences inside were found (Velmala et al. 2014). In this case, the authors decided to follow the morphological species concept and retained all ten species in the section. Later Boluda et al. (2019) analyzed the *Implexae* section using a larger set of specimens and more sophisticated phylogenetic methods and made a similar conclusion: no analyses supported the monophyly of the currently accepted morphospecies, but rather suggested the reduction of these to four phylogenetic species. Unlike Velmala et al. (2014), these phylogenetic species were accepted by Boluda et al. (2019), whereas the rest were reduced to synonymy.

The species delimitation of lichenized fungi using molecular sequencing data may be considered within the framework of the monophyletic species concept or most likely within the framework of the "diagnostic approach" of the phylogenetic species concept (according to de Queiroz 1998). The monophyletic concept defines species as a population or group of populations characterized by one or more apomorphic features (in de Queiroz 1998). Unlike the monophyletic concept, the "diagnostic approach" of the phylogenetic species concept emphasizes diagnosability, regardless of whether the diagnostic characters are apomorphic or not (de Queiroz 1998). Some lichenologists use the term "integrative taxonomy approach" when analyzing a complex of features to assess species boundaries – molecular, morphological, chemical, ecological, and distributional characters (Alors et al. 2016, Boluda et al. 2019, Frisch et al. 2020).

Obviously, the pluralism of species concepts results in different conclusions concerning the boundaries and numbers of species. De Queiroz (1998, 2007) designed his general lineage concept (GLC), or unified species concept, to solve the problem of pluralism. He argues that existing species concepts are not as different as they seem.

All of them have a common element – defining species as separately evolving segments of metapopulation lineages. Other properties that underlie alternative species concepts may be treated as secondary properties of the species. This means that under all concepts, a species is a separately evolving lineage, but under the biological concept, the lineage also has to be reproductively isolated; under the morphological concept, it also has to be phenetically distinguishable etc. The different secondary properties found among lichens lead to incompatibility between species concepts because they gradually arise at different times during the process of speciation, and not necessarily in a regular order. To solve this conflict the GLC defines the common element (species as a separately evolving lineage) as the only necessary property of species. All other properties, only necessary in the alternative concepts, may be used as species properties in the frame of the GLC, but they are no longer defining (necessary) properties of the species. De Queiroz (2007) points out that the concept of lineage in this case means an ancestor-descendant series of metapopulation, but not a clade or a monophyletic group.

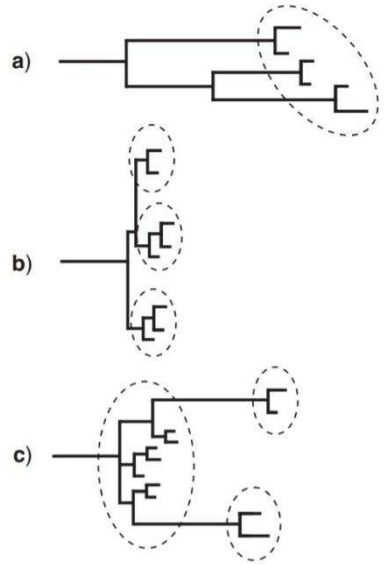


**Fig. 1. Situations in which species circumscription can be challenging.** Branch length indicates degree of historical change (lineage formation), whereas lateral distance among clades indicates degree of phenotypic differentiation; dashed ovals indicate that the species would be circumscribed in the frame of the phenophyletic approach. (A) Lineage formation with little or no phenotypic change – unless phenotypic differences can be found the lineages should not be recognized as species; (B) Phenotypic change with little historical signature – unless there is evidence that a particular phenotypic type is arising independently in different populations, these should not be recognized as species; (C) Progenitor–derivative species (a subset of a former metapopulation lineage becomes distinct for a novel phenotype and forms a new lineage while leaving the ancestral type with the plesiomorphic features) – there is no reason to reject species status for paraphyletic assemblages of populations, they should not be denied species status in the intervening period while waiting for genetic coalescence to occur. From Freudenstein et al. (2017).

Recently the GLC was also proposed for use in lichenology (Leavitt S.D. et al. 2015) as an instrument disentangling the pluralism of species concepts. However, some authors argue that this approach embraces rather than overcomes pluralism and cannot be considered as a truly universal species concept (Zachos 2018). Freudenstein et al. (2017) also criticize GLC and believe that lineage, as the only necessary species property, is not sufficient. The first reason is that it explicitly conceptualizes only the "width" and not the "length" of species, which means that it would be possible, for example, to regard a continuous population lineage from a single-celled ancestor to an elephant as a single species. Then, it suggests the existence of nascent species, which may be valuable for the study of speciation processes. However, such "species" may also be just "evolutionary ephemera". Finally, the authors assert that a necessary part of the species concept must be role – the ways in which individuals interact with their environment, which is manifested in morphology. As a result, Freudenstein et al. (2017) suggest the "phenophyletic" view as a clarification of the evolutionary species concept (Simpson 1961): a species is a lineage or group of connected lineages with a distinct role. This approach, for example, allows paraphyletic assemblages of populations to be species, as long as they share a role. However, sibling non-interbreeding lineages would represent the same species if their roles do not differ. Decisions on species delimitation, which would be made in the framework of the phenophyletic approach in "difficult" groups are illustrated in Fig. 1.

### 1.3 Species complexes in lichenized fungi

It is likely that for species that originated long ago and passed through the process of genetic coalescence almost any species concept would work (de Queiroz 2007, Freudenstein et al. 2017). The challenging parts of the tree of life are near the points at which species originate, where so-called "species complexes" are formed. Here the details of a concept and associated empirical operations of species delimitation really matter (Freudenstein et al. 2017). A species complex is a group of closely related species often with unclear boundaries between them. As a rule, the complex is monophyletic and lineages have been diversified rather recently, but might also be separated for a long time without evolving morphological differences. Species complexes include cryptic and sibling species, semi-cryptic species, species pairs etc.

The definition of cryptic species varies from species that are difficult to detect based on phenotypic features (but potentially detectable) to those that are indistinguishable by such features (Freudenstein et al. 2017). Terms "cryptic" and "sibling species" are closely related and have been used interchangeably by numerous authors. However, in a more strict sense sibling species are confined to cryptic species that share the most recent common ancestor (Lumbsch and Leavitt 2011). Most lichenologists merge the two terms under "cryptic species".

Cryptic species have been revealed in diverse groups of organisms, e.g., ants (Fox et al. 2017), geckos (Rato et al. 2016), spiders (Leavitt D.H. et al. 2015), and snails (Johnson et al. 2015). The phylogenetic studies of lichenized fungi also indicate that numerous distinct lineages may be hidden under a single species name (Crespo and Lumbsch 2010). One of the first records of cryptic species in lichens belongs to the genus *Letharia*: Kroken and Taylor (2001) analyzing the sympatric species pair *L. vulpina* and *L. columbiana* discovered at least six phylogenetic species inside the pair. Later Altermann et al. (2014) repeated the study using more accurate sampling and population assignment tests. They provided additional evidence for those six candidate phylogenetic species with subtle morphological support for some of them; however, the equivalence of these genetic clusters with species-level lineages was uncertain because of the limited phylogenetic signal.

Many cryptic lichen species have been discovered during the study of taxa considered to be cosmopolitan. In some cases, molecular data confirmed the existence of worldwide-distributed species, for example the omnipresent *Flavoplaca flavocitrina* (Vondrák et al. 2016) and the bipolar *Xanthomendoza borealis* (Lindblom and Søchting 2008) in the family Teloschistaceae and the bipolar *Cetraria aculeata* in the family Parmeliaceae (Fernández-Mendoza et al. 2011, Fernández-Mendoza and Printzen 2013). At the same time, molecular analyses have demonstrated that many lineages at a species level may be hidden under a similar morphology of currently accepted seemingly cosmopolitan taxa. One of the first studies obtaining such results was an investigation of the cosmopolitan lichen *Parmelia saxatilis* by Crespo et al. (2002). Using rDNA ITS and β-tubulin gene sequence analyses the authors revealed within the species two monophyletic groups differing in their geography and ecology. One group occurred in the Arctic and Antarctic regions in more Atlantic sites, whereas specimens from more mesic environments in the Mediterranean region belonged to the second group. Leavitt et al. (2011b, 2013) revealed six lineages under a single species *Rhizoplaca melanophthalma* collected from five continents. Two of them had broad intercontinental distributions, while the other four occurred only in western North America. One lineage was supported with chemical data; others did not correlate with phenotypic features. In another study by Leavitt et al. (2018), 10 supported candidate species-level lineages were identified within *Psora decipiens*, and at least some of them did not correlate to any non-molecular characters. Nearly all lineages consisted of specimens originating from different continents, but all Australian specimens were recovered within a monophyletic clade consisting exclusively of Australian samples. From the examples above it is clear that related cryptic species may arise both sympatrically and allopatrically.

Many cryptic lineages do not demonstrate any phenotypic differences. For instance, Zakeri et al. (2019) circumscribed six candidate species within the *Aspiciliella*

*intermutans* complex, which showed just a low correlation with morphological and ecological features and no correlation with secondary metabolites. In other cases, the re-examination of morphology against the background of a molecular phylogeny reveals subtle, previously overlooked, or considered unimportant phenotypical features (Crespo and Lumbsch 2010). Sometimes different ecological preferences were also detected for related cryptic species, e.g. the occurrence of *Parmelia serrana* mainly on bark against *P. saxatilis* mainly on rock (Molina et al. 2004).

Vondrák et al. (2009) working on the genus *Flavoplaca* (Teloschistaceae) proposed a term "semi-cryptic species" for taxa which exhibit some phenotypical, geographical, or ecological differences, but which are still difficult to distinguish without molecular investigation. Semi-cryptic species are often not sister species. Several authors have accepted the term for taxa of *Hydropunctaria* (Orange 2012), *Sulzbacheromyces* (Coca et al. 2018), and *Xanthoparmelia* (Hodkinson and Lendemer 2011).

A thorough investigation of putatively cryptic species could reveal phenotypically clear taxa. One of the most striking examples of such a study is a description of 70 new species in the basidiolichen genus *Cora* previously hidden under a single taxon (Lücking et al. 2017). The authors argue that the newly proposed species may be recognized by a combination of phenotypic and ecogeographical features. Another example is an investigation of *Parmelina quercina*, a foliose lichen with worldwide distribution in areas with Mediterranean climate (Argüello et al. 2007). It was shown that this taxon is in fact a complex of four species, which are not even cryptic, but morphologically easily recognizable. Moreover, two of them have a disjunct distribution in North America (*P. coleae*) and Australia (*P. elixia*).

Vondrák et al. (2013a) proposed a two-phase method for the investigation of such taxa with obscure phenotypic differences. It consists of the preliminary examination of all available characters using a small number of samples and a subsequent detailed study in which the potentially diagnostic characters selected in the first step are tested using more samples. Frolov et al. (2016) applied the method for the delimitation of three seemingly cryptic species in the genus *Pyrenodesmia* (Teloschistaceae).

Usually, a cryptic species complex forms a monophyletic group. However, it has been found that distantly related lineages may also show high morphological convergence (Crespo and Lumbsch 2010). For example, it has been demonstrated that the Australian lineage of *Parmelina quercina* s.lat. actually belonged to a clade unrelated to *P. quercina* s.str. and was described as the new genus *Austroparmelina* with several distinct species within it (Crespo et al. 2010a). Such examples are not only known for lichens, but also for other organisms, e.g. salamanders (Steinfartz et al. 2000).

The recognition of cryptic lineages on a species level strongly depends on the chosen concept. Some authors welcome such recognition and consider that it opens the door to the formal description of thousands of species of voucherless organisms detected through environmental sequencing techniques (Lücking and Moncada 2017). Other biologists argue against the recognition of phenotypically identical cryptic species sharing the most recent common ancestor (Freudenstein et al. 2017). Lichenologists usually abstain from making taxonomic decisions on cryptic species. They describe only semi-cryptic (Vondrák et al. 2009, Coca et al. 2018 etc.) or seemingly cryptic species revealing clear differences after a thorough revision of phenotype, geography, and ecology (Molina et al. 2004, Argüello et al. 2007, Frolov et al. 2016 etc.). However, Leavitt et al. (2013b) have suggested the formal descriptions of five cryptic species within the *Rhizoplaca melanophthalma* complex supported only with coalescent-based genetic analysis of multiple genetic loci. In other organisms, true cryptic species have also been described applying "molecular taxonomy" based on diagnostic nucleotides in DNA sequences, e.g. in lichenicolous fungi (Lücking and Moncada 2017) or in marine slugs and snails (Jörger and Schrödl 2013, Johnson et al. 2015). In this case, a voucher of extracted DNA can be used as a holotype. Such taxa, however, were not accepted by the recent ICBN (Shenzhen Code, Art. 40.5, Ex. 6).

Species pairs are an example of species complexes, which is only known in lichenized fungi. These are two closely related species with similar morphology but exhibiting different reproductive modes (Poelt 1963, Crespo and Pérez-Ortega 2009). One taxon (the so-called "primary species") has sexual reproductive structures (apothecia) and the second taxon (the so-called "secondary species") reproduces by means of vegetative structures (soralia or isidia). Secondary species usually have a wider range of distribution. Species pairs have been discovered in different families of lichenized Ascomycetes: Lecideaceae (Buschbom and Mueller 2006), Lobariaceae (Cornejo et al. 2009), Parmeliaceae (Miadlikowska et al. 2011, Widhelm et al. 2016, Grewe et al. 2018), Physciaceae (Myllys et al. 2001), and Roccellaceae (Myllys et al. 1999).

The majority of the known species pairs was described as two distinct species in the frame of morphological species concept before the advent of molecular studies into lichenology. Several hypotheses have tried to explain the phenomenon of species pairs before the molecular era. According to Poelt (1963), a secondary asexual taxon originated from one primary sexual taxon as the result of a one-off event. Tehler (1982) suggested the occurrence of multiple change events of vegetative lineages from the sexual lineage; he considered vegetative lineages as dead ends.

Molecular evidence has repeatedly illustrated that both taxa of a species pair form a single monophyletic group (Crespo and Pérez-Oretga 2009). However, within the group neither the apotheciate nor the vegetative forms are monophyletic (Myllys et al.

2001, Cornejo et al. 2009), which apparently excludes the origin of one of these reproductive modes arising from a one-off event. Also, it has been shown that asexual lineages in the genus *Lepraria*, exclusively consisting of sterile species, are genetically diverse and are the result of multiple speciation events (Ekman and Tønsberg 2002, Lendemer 2011). This means that the secondary asexual taxa of such species pairs are not necessary dead ends. In contrast to the original hypotheses, a study by Cornejo et al. (2009) revealed transitions from the vegetative state towards apotheciate morphs in *Lobaria retigera* group. Buschbom and Mueller (2006) showed that the transition from the vegetative to the sexual state happens more frequently than vice versa within the *Porpidia flavocoerulescens* and *P. melinodes* species complex. Tripp (2016) documented numerous origins of sexual reproduction from asexual ancestors in the evolution of several lichens groups. Cornejo et al. (2009) found highly supported lineages within the pair *Lobaria kurokawae-L. retigera* consisting of both apotheciate and vegetative specimens originating from the same geographical region.

Buschbom and Mueller (2006), using molecular data, proposed a new hypothesis according to which the primary species of the pair could be either a vegetative or a sexual taxon. The authors associated changes in the reproductive mode with trade-offs in the fitness of the symbiosis. When relationships between symbionts are optimal, the prevailing reproductive mode is asexual. Sexual reproduction will be preferred in suboptimal conditions in order to allow the mycobiont to escape from the photobiont, find new partners, and increase variability through recombination.

Surprising results were obtained in the latest study of the species pair *Usnea antarctica-U. aurantiacoatra*. Previously, multilocus DNA sequence data could not delimit these two species (Seymour et al. 2007, Wirtz et al. 2012) suggesting that they might be conspecific. However, Grewe et al. (2018) using a population genomic analyses of restriction-site-associated DNA sequencing resolved these species and demonstrated that asexual *U. antarctica* and sexual *U. aurantiacoatra* are reciprocal monophyletic. It is probable that the conclusions made on the species pair phenomenon will continue to change with the use of more sophisticated methods.

### 1.4 Molecular phylogenetic methods applied in the species delimitation of lichenized fungi

At present, it is clear that phenotype-based species delimitations often do not reflect the complex immanent patterns of biological diversity. In contrast, the delimitation of species based on molecular data is not without its own problems either (Lumbsch and Leavitt 2011). Sometimes the selected loci are not variable enough to distinguish among closely related species or multiple copies of a target locus are present due to a relaxation of concerted evolution or the existence of paralogous copies of protein-coding genes (Lumbsch and Leavitt 2011).

There may be problems with the molecular data themselves, particularly in recently diverged lineages (Knowles and Carstens 2007). Phylogenetic trees inferred on the basis of different genome loci of the same species can be inconsistent with each other and therefore potentially also with the species tree (Nichols 2001). Naciri and Linder (2015) recognize seven processes that can influence genome heterogeneity and hence the gene trees incongruence. Two of them have also been announced in lichenological papers: hybridization and incomplete lineage sorting.

Hybridization is one of the most common mechanisms generating reticulated relationships between closely related species in several plant genera, e.g. *Quercus* (Lagache et al., 2013), *Salix* (Percy et al., 2014) etc. Recently, a possible role of hybridization has also been shown in the speciation of the North American subalpine lichen *Rhizoplaca shushanii* using both mitochondrial and nuclear genomic data (Keuler et al. 2020).

Hybridization is hardly distinguishable from incomplete lineage sorting (ILS), another common reason for gene tree incongruence. ILS occurs when ancestral polymorphism persists through a speciation event, and ultimately distinct alleles become fixed among descendants sporadically and not in accordance with the branching order of the descendants (Maddison 1997). The lineage sorting depends on $N_e$ – the effective population size (Naciri and Linder 2015). According to Rosenberg (2003) $5.3N_e$ generations are needed for a species to acquire monophyly at 99 % of its loci given that all loci in the sister species are also monophyletic. This means that for a species of 1 million individuals with a generation time of 10 years, full monophyly will only be reached 50 M years after speciation (Naciri & Linder 2015). Several lichenological studies explain gene tree incongruence by pointing to incomplete lineage sorting (Nelsen and Gargas 2009, Pino-Bodas et al. 2011, Steinová et al. 2013, Athukorala et al. 2015, Boluda et al. 2019).

There are several ways to treat gene tree incongruence (Naciri and Linder 2015): (i) using multiple individuals of each species that cover different geographic and ecological ranges etc., (ii) using multiple loci from different genomes (nuclear, mitochondrial, chloroplast), (iii) using a coalescence framework studying closely related or sister species, (iv) analyzing loci as separate rather than concatenated data. However, Leavitt et al. (2016), studying the lichen genus *Rhizoplaca,* obtained a well-supported phylogenetic hypothesis using both concatenation and multispecies coalescent approaches, despite the tremendous incongruence between individual loci.

Below we discuss methods of species delimitation in more detail. Some of those approaches use single-locus data as an input. Most of them fall into two general categories: genetic distance- and tree-based approaches (Leavitt S.D. et al. 2015). The former methods use thresholds of pairwise genetic distances to identify species (Lumbsch and Leavitt 2011). The latter methods detect discontinuities associated

within inter- and intraspecific branching patterns (Fujisawa and Barraclough 2013). Both methods have been applied to species delimitation in lichens (e.g., Del-Prado et al. 2010, Molina et al. 2011, Alors et al. 2016, Wei et al. 2016). The tree-based methods include, for example, the general mixed Yule coalescent approach (GMYC; Fujisawa and Barraclough 2013) and the Poisson Tree Processes model (PTP; Zhang et al. 2013); genetic distance-based approaches include, e.g., Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012).

Since single-locus approaches are not able to overcome incomplete lineage sorting dealing with closely related species (Grube and Kroken 2000, Lumbsch and Leavitt 2011), using multiple independent loci is necessary to test species delimitations. There are several widely used approaches for evaluating putative species using multilocus sequence data.

First, genealogical concordance phylogenetic species recognition is used to test species delimitations (Lumbsch and Leavitt 2011). According to this method, different gene trees have the same tree topologies due to the fixation of previously polymorphic loci following genetic isolation. Strong support for clades in multi-locus phylogenies indicates species boundaries. The method has been used in several lichenological studies (e.g., Kroken and Taylor 2001, Molina et al. 2011).

The merging of coalescent theory with phylogenetics gave rise to coalescent-based species recognition (Fujita et al. 2012, Carstens et al. 2013). Within the coalescent-based framework, multiple individuals are assigned to a single species and the speciation history of species-level lineages can be inferred as a "species tree" based on multiple loci, in contrast to estimating gene genealogies from individual samples in the previous approach using the genealogical concordance of unlinked markers to test species boundaries (Leavitt S.D. et al. 2015). Coalescent-based methods do not require the reciprocal monophyly of alleles that allow for gene tree discordance (Fujita et al. 2012). Several coalescent-based species delimitation tools have been designed, such as Bayesian Phylogenetic and Phylogeography – BP&P (Yang and Rannala 2010), spedeSTEM (Ence and Carstens 2011), and Brownie (O'Meara 2010). All of them have been applied for testing species boundaries in lichenized fungi (Saag et al. 2014, Alors et al. 2016, Leavitt et al. 2016, 2018, Wei et al. 2016).

All of the above mentioned coalescent-based methods require individual sample assignments to a species or population a priori, which may be difficult in some situations: in the cases of cryptic species, incongruence between conventional species delimitation and molecular data etc. (Leavitt S.D. et al. 2015). Recently, several statistical methods have been proposed to assess individual assignment and species detection prior to coalescent-based species delimitation and species tree reconstruction (Leavitt S.D. et al. 2015). They are, for instance, algorithms BAPS (Corander et al. 2008), STRUCTURE (Falush et al. 2003) and STRUCTURAMA (Huelsenbeck et al.

2011). They have also been used for the species delimitation of lichenized fungi (Altermann et al. 2014, Miadlikowska et al. 2018).

Genome-scale investigations with high-throughput sequencing methods have just recently started to be used in lichenology (Grewe et al. 2018, Pizarro et al. 2018, Keuler et al. 2020). They allow sampling from hundreds to thousands of loci from across a species genome for a large number of species; it is now easier than ever to sequence complete genomes or transcriptomes even for non-model organisms (Leavitt S.D. et al. 2015, Resl 2017). Approaches such as anchored phylogenomics, transcriptome sequencing, and reduced-representation genomic library sequencing (RAD-Seq – restriction-site-associated DNA sequencing, GBS – genotype-by-sequencing), provide important insights into species boundaries since they allow the comparison of entire genomes, or at least large portions of genomes.

Despite the reliability of the above methods, various approaches to species delimitation may yield different estimates of species boundaries, both in lichens (e.g., Alors et al., 2016, Wei et al. 2016) and other organisms (e.g. Salter et al. 2013). Wei et al. (2016) using five of the previously mentioned methods obtained several conflicting results within the lichen group *Hypogymnia flavida-H. hypotrypa*: (i) these two conventional taxa are conspecific, (ii) they are distinct, separate species, (iii) the group consists of several species-level lineages that do not correspond to the traditional concept. Salter et al. (2013) applying six methods for trapdoor spiders delimited from 3 to 18 species-level lineages. As a result, a certain degree of subjective interpretation may be required from the researcher to find the most biologically appropriate species boundaries (Leavitt S.D. et al. 2015).

### 1.5 Generic delimitation in lichenized fungi

Unlike species, all upper taxonomic ranks including genera are usually seen as arbitrary (Arcadia 2009). Nevertheless, they play an important role in biology allowing a hierarchical approach for encompassing the great diversity of organisms (Divakar et al. 2017). There are no objective criteria for grouping species into genera. The traditional generic classification of lichenized fungi was based on morphological and chemical features, such as thallus structure, ascomatal anatomy, ascospore septation and colour, ascus type, and secondary metabolites. However, many studies have repeatedly demonstrated that phenotypic characters are unstable during evolution and hence have a limited value for generic circumscription (e.g., Crespo et al. 2010b, Ertz and Tehler 2011, Zhao et al. 2016). In a phylogenetic context, genus involves clades with a shared evolutionary history (Divakar et al. 2017). The current consensus rejects poly- and paraphyletic taxa of the levels higher than species (Lücking 2019). Even so, choosing a node delimiting a genus is also arbitrary and depends on the interpretation of the taxonomist. According to Arcadia (2009) the only objective way to define genera

is to choose a date in the past as a delimiter. If the most recent common ancestor of two species existed more recently than that date, the two species belong to the same genus, and vice versa. Kraichak et al. (2017) and Divakar et al. (2017) have developed and applied a method for generic classification that used time-calibrated chronograms inferred from multi-gene phylogenies to identify upper and lower temporal thresholds for taxonomic ranking. The method is based on the temporal banding approach proposed by Avise and Johns (1999). Divakar et al. (2017) suggested that all genera in Parmeliaceae share a common ancestor more recently than 29.45–32.55 Ma. As a result, forty-five of the currently accepted genera in Parmeliaceae were supported, three genera were resurrected, one genus was newly described and thirteen genera were reduced to synonymy. These results were revised by Lücking (2019), who argued that taxa of the same rank do not have to be comparable in age or any other single criterion, but their ranking should be based on an integrative approach, which would reflect their individual evolutionary history.

Lücking et al. (2016) proposed the most recent separate outline of lichenized fungi containing 995 genera with 19,387 species. The largest genera are *Xanthoparmelia* (820 species), *Lecanora* (550 species), *Arthonia*, *Cladonia*, and *Pertusaria* (500 species each). The average number of species per genus is 19.5, whereas 256 genera contain only one species, 377 one or two species, and 582 up to five species, which means that most genera are small. The latest classification of all fungi including lichenized fungi has been suggested by Wijayawardene et al. (2020). However, extraction of the total number of lichenized taxa from this outline is not a trivial task.

Since introducing molecular phylogenetic methods into lichenology the generic delimitation of lichens has been changed dramatically. Most changes have occurred in the largest families, such as Parmeliaceae (Crespo et al. 2010b, Divakar et al. 2017), Graphidaceae (Parnmen et al. 2012, Rivas Plata et al. 2012), Ramalinaceae (Kistenich et al. 2018), Lecanoraceae (Zhao et al. 2015), Teloschistaceae (Arup et al. 2013), Roccellaceae (Ertz et al. 2015), and Collemataceae (Otálora et al. 2014), etc. Numerous studies within these families as well as in many others demonstrate that many conventional genera are polyphyletic as a consequence of phenotypic and ecological convergence. With rare exceptions (Bendiksby et al. 2015) such genera are not accepted in the current taxonomy of lichenized fungi. As a result, the large number of genera have been split or less frequently lumped together.

Lumping together has occasionally been used to avoid poly- and paraphyly in phenotypically distinct genera. For instance, *Chondropsis*, *Karoowia*, *Neofuscelia,* and *Paraparmelia* were combined under the single genus *Xanthoparmelia* (Blanco et al. 2004a) and the distinct *Lasallia* was included in *Umbilicaria* (Davydov et al. 2017).

One of the most striking examples of genus splitting is a new taxonomy of *Hypocenomyce* s.lat. (Bendiksby and Timdal 2013). Seventeen species of the genus

formerly united based on highly similar morphology and ecology were scattered among seven genera (including four newly described), six families (including three newly described), and four orders. Another example of a remarkable convergence resulted in the split of the *Parmelina quercina* complex, giving birth to the new genus *Austroparmelina* that was formerly hidden inside the cryptic species (Crespo et al. 2010a).

Some large genera have lost most of their species after the employment of molecular data for their taxonomic revision. For example, the renewed *Melanelia* s.str. now consists of only two species, while the remaining 44 species previously belonging in *Melanelia* s.lat. are assigned to five recently described genera (Blanco et al. 2004b, Crespo et al. 2010b, Divakar et al. 2012, 2017). Following the revision of *Caloplaca* s.lat., which was previously one of the largest genera of lichenized fungi with about 1000 species, only 12 species remained assigned to *Caloplaca* s.str., while most species were spread among almost 30 newly described or resurrected genera or still have an uncertain position in Teloschistaceae (Arup et al. 2013). The number of genera encompassing species of the former *Caloplaca* s.lat. now far exceeds 30 after numerous taxonomic papers by Kondratyuk et al. (2014, 2015a, b, c, 2018, 2020, etc.).

Numerous studies investigate the phylogenetic positions of phenotypically distinct traditionally accepted informal species groups within larger conventional genera. It is often found that those groups are not related to the genera to which they were originally attributed, and numerous new genera are being described to accommodate such groups. For example, the genus *Brianaria* was suggested for the *Micarea sylvicola* group (Ekman and Svensson 2014), the genus *Bryobilimbia* for the *Lecidea hypnorum* group (Fryday et al. 2014), and the genus *Violella* for the *Mycoblastus fucatus* group (Spribille et al. 2011), etc. Sometimes new genera are proposed for newly described peculiar species, which have a distinct phylogenetic position. The genus *Ducatina* was established for Antarctic *D. umbilicata*, the only species in Trapeliaceae with a large umbilicate thallus (Ertz et al. 2017). The genus *Tenuitholiascus* was suggested for *T. porinoides*, a new species closely resembling the genus *Porina*, but entirely unrelated to the latter (Jiang et al. 2020).

The method of consistent taxonomic ranking based on the temporal banding approach was applied only for the generic classification of Parmeliaceae (Divakar et al. 2017). Other studies considered genera as monophyletic clades that could be characterized with distinct (to varying degrees) phenotypical, ecological, or geographical features. However, some studies partly or completely failed to identify diagnostic characters of the lineages at a putatively generic level. In such cases, taxonomists still either merge the groups or split them into smaller entities. For example, the otherwise paraphyletic genus *Umbilicaria* was lumped with the genus *Lasallia* and then subdivided to eight subgenera (Davydov et al. 2017). Although

*Lasallia* was readily identified based on its morphology, some other lineages had ambiguous phenotypic differences. Given that, the authors decided to treat all lineages as subgenera instead of genera. On the contrary, Bendiksby et al. (2015) in the clade of Tephromelataceae comprising *Calvitimela*, *Tephrolema,* and *Violella* retained the paraphyletic genus *Calvitimela* consisting of four phenotypically indistinguishable lineages. The authors explained this by the fact that monophyly cannot be completely rejected based on their data as well as by the strong molecular and phenotypic support of *Tephromela* and *Violella*.

For similar cases with an absence of explicit diagnostic characters Parnmen et al. (2012) proposed a new approach to choose among classification alternatives using a combination of morphology-based phylogenetic binning and a multiresponse permutation procedure to test for morphological differences among clades. Using this approach for analyzing two clades including five subclades (two + three) of the genus *Chapsa* s.lat. (Graphidaceae) they demonstrated that accepting the four clades (the first clade undivided and the second clade divided on three subclades) as different genera reflects the phenotypic pattern significantly better than accepting two or five genera (retaining two clades undivided or dividing them into all five subclades respectively).

### 1.6 Taxonomy and diversity of the family Teloschistaceae

The family Teloschistaceae, along with two much smaller families, Megalosporaceae and Brigantiaeaceae, belongs to the order Teloschistales, class Lecanoromycetes (Wijayawardene et al. 2020). Recently one more previously recognized family, Letrouitiaceae, was synonymized under Brigantiaeaceae (Kraichak et al. 2018). Arup et al. (2013) distinguished three subfamilies within Teloschistaceae – Caloplacoideae, Teloschistoideae, and Xanthorioideae. Later, Kondratyuk et al. (2015a) added one more subfamily, Brownlielloideae, which is, however, the topic of discussion since its description was probably based on a technical mistake (Vondrák et al. 2018). There are several estimates of the number of species in Teloschistaceae: from 650 to more than 1000 species (Kirk et al. 2008, Arup et al. 2013, Lücking et al. 2016). In any case, the family is among the ten largest families of lichenized fungi (Lücking et al. 2016) and has a worldwide distribution.

In addition to the characteristic “Teloschistes-type” ascus (Honegger 1978), members of the *Teloschistaceae* are usually easily recognizable by their polarilocular spores and the presence of anthraquinone pigments in the outer tissues giving them various tinges of orange, yellow, or red. However, a secondary loss of the diagnostic characters (anthraquinone pigmentation or polarilocular spores) occasionally appears (Kärnefelt 1989, Vondrák et al. 2012, Arup et al. 2013). It has been shown that adaptation to sunny habitats played an important role in the character evolution of the family (Gaya et al. 2015).

Kärnefelt (1989) performed the most comprehensive phenotypically based revision of Teloschistaceae and recognized 10 genera. Traditionally a growth form was the main feature for generic delimitation within the family. For example, the three largest conventional genera *Caloplaca*, *Teloschistes,* and *Xanthoria* were characterised by crustose, fruticose, and foliose growth respectively; the monotypic genus *Xanthopeltis* was characterized by the peltate thallus. Simple or one-septate non-polarilocular spores were used for the separation of the genera *Apatoplaca* and *Fulgensia*. Later new phenotypically based genera were suggested by Kondratyuk and Kärnefelt (1997, 2003), who used, for instance, the particular type of cortical layer and the presence of rhizines for their delimitation.

Already Kärnefelt (1989) supposed that the main genera of Teloschistaceae are not monophyletic. The first phylogenetic studies of the family applying molecular methods confirmed that the traditional genera *Caloplaca*, *Fulgensia*, *Teloschistes,* and *Xanthoria* are polyphyletic (Arup and Grube 1999, Kasalicky et al. 2000, Søchting & Lutzoni 2003, Gaya et al. 2003, 2008). As a result, all of the authors claimed that thallus morphology in Teloschistaceae was a blunt tool for the delimitation of genera. Fedorenko et al. (2009, 2012), studying the phylogeny of the xanthorioid groups, obtained several clades, which they described as the first new genera based on molecular data. These genera, however, as well as ones proposed by Kondratyuk and Kärnefelt (1997, 2003) have not generally been accepted, probably because of the incomplete documentation of methods and vouchers, nomenclatural unclarities, inadequate taxon sampling, and even paraphyly and polyphyly (Arup et al. 2013).

Arup et al. (2013) performed a comprehensive phylogenetic evaluation of a large part of the family, including molecular data on 337 species, and proposed a new taxonomy of Teloschistaceae. They recognized a total of 39 genera, of which 23 were newly described and eight were resurrected. Arup et al. (2013) demonstrated again that many phenotypic characters are extremely plastic and therefore generally unreliable as evolutionary markers. Although the authors tried to delimit genera carrying at least some non-molecular information whenever possible, it was often difficult to find perfect correlations between clades and unique combinations of phenotypic characters. As a result, the new taxonomy was mainly based on molecular data and very few of the genera could be recognized by morphological, chemical or geographical characters alone. Vondrák et al. (2018) argue that such genera without reliable diagnostic features still make sense in Teloschistaceae, since they demonstrate some trends of functional diversity – being predominantly saxicolous or having lobate thalli etc.

Other ideas about splitting Teloschistaceae were put forward in numerous papers by Kondratyuk et al. (e.g., 2014, 2015a, b, c, 2016, 2017, 2018, 2020). Their taxonomy was mostly independent of the one proposed by Arup et al. (2013) and used distinctly smaller genera, often monotypic. They further split many taxa already supposed by

Arup et al. (2013), for example, *Dufourea*, *Gyalolechia*, *Variospora*, and *Xanthomendosa* etc. The most recent outline of fungal systematics (Wijayawardene et al. 2020) accepted 63 genera within Teloschistaceae. Among them, there are all 39 genera proposed by Arup et al. (2013) and only about a half of those suggested in the later publications by Kondratyuk et al. (e.g., 2014, 2015a, b, c, 2016, 2017, 2018).

Many papers have investigated the diversity and systematics of genera/groups within Teloschistaceae, e.g., *Athallia* (Arup 2009), *Blastenia* (Arup and Åkelius 2009, Vondrák et al. 2019a), *Calogaya* (Gaya 2009, Vondrák et al. 2018), *Flavoplaca* (Arup 2006, Vondrák et al. 2009) etc. The composition of anthraquinone pigments plays a crucial role in the systematics within Teloschistaceae and Søchting (1997, 2001) proposed their classification into several chemosyndromes. For instance, the presence of chlorinated or non-chlorinated anthraquinones is an important feature for species delimitation in *Blastenia* (Vondrák et al. 2019a) and the presence of neochloroemodin is a unique character of the genus *Marchantiana* (Søchting and Arup 2018).

Several papers have explored the diversity of Teloschistaceae in various regions of the Northern Hemisphere. C.M. Wetmore dedicated a series of studies to different groups of *Caloplaca* s.lat. in North and Central America (Wetmore 1994, 1996, 2001, 2003, 2004a, 2004b, etc.). Arup (e.g., 1995) compared species composition of littoral *Caloplaca* s.lat. on the Atlantic and Pacific Coasts of North America. Some authors have investigated the family in the Arctic. The central paper for the region is a study of *Caloplaca* s.lat. in Greenland by Hansen et al. (1987). Søchting and Olech (1995) summarized data on *Caloplaca* s.lat. in polar regions. Søchting et al. (2008) investigated crustose Teloschistaceae in Svalbard and Frolov and Konoreva (2016) in the Murmansk region of Russia. Few papers dedicated to the family in arid regions of Eurasia. Vondrák et al. (2019b) investigated the species and functional diversity of Teloschistaceae in different types of landscapes in Southern Siberia. Vondrák et al. (2017) suggested a rather high diversity of the family in the Eastern Caucasus. Vondrák et al. (2016) investigated the genus Athallia in Turkey. The only major paper about diversity Teloschistaceae in East and South Asia is a comprehensive study by Poelt and Hinteregger (1993) performed in the Western Himalayas. Other papers focus mainly on the description of separate species in the region (e.g., Joshi et al. 2010, 2014, Kondratyuk 2019).

In the study by Arup et al. (2013), crucial for the taxonomy of the whole family Teloschistaceae, some lineages remained unresolved. For example, the authors resurrected the genus *Pyrenodesmia* and included several species from the *Caloplaca variabilis* group there (sensu Wunder 1974). However, they neither resolved the relationships of the genus with the *C. xerica* group nor with the rest of the species from the *C. variabilis* group (*C. albopruinosa*, *C. peliophylla*, *C. transcaspica*, etc.). As a result, those species were not assigned to any genus and were treated by the authors

under the provisional generic name "*Caloplaca*". Kondratyuk et al. (2020) ignored the problem and united the *C. variabilis*, *C. xerica* as well as *C. haematites* groups in the genus *Pyrenodesmia* without any comments. This is, however, the topic of discussion since those groups demonstrate discrepant chemistry and geography. The *C. variabilis* group and the genus *Pyrenodesmia* sensu Arup et al. (2013) consist of exclusively epi- or endolithic taxa, always with Sedifolia-grey instead of anthraquinones, and hence with black, grey, or brown apothecia and white, grey, or brownish thalli. These taxa inhabit mainly xerothermic calcareous localities in the Northern Hemisphere with the highest species diversity in the Mediterranean, Central Asia, and South-Western North America. *Caloplaca xerica* as well as *C. haematites* groups are characterized by the presence of anthraquinones in the apothecial disk and much smaller geographical ranges. Wunder (1974) performed the most comprehensive revision of the *C. variabilis* group in Central Europe, the Mediterranean, and South-Western Asia. The author accepted nine taxa whereas the other more than 40 taxa he treated as synonyms. Wetmore (1994) reported on four species occurring in North America. Later several species were described (e.g., Khodosovtsev et al. 2002, Tretiach et al. 2003, Wetmore 2009, Xahidin et al. 2010, Vondrák et al. 2013b, Frolov et al. 2016) and Muggia et al. (2008) clarified the taxonomy of the endolithic species of the group. As a result, 21 species of the *C. variabilis* group are currently known whereas only 14 of them are combined into the genus *Pyrenodesmia*. The identity and/or phylogenetic relationships of the other species are still unclear. Moreover, the group seems to comprise more species since it is still only partially known in Europe and North America and it is little known in Asia (Vondrák et al. 2012).

## 2 Aims of the study

1) To ascertain whether the epilithic species of Teloschistaceae completely lacking anthraquinones, but possessing Sedifolia-gray pigment, form a monophyletic group that merits recognition at generic rank as *Pyrenodesmia*. Further, to study the diversity of the group.
2) To propose standardized methods for measuring the anatomical and morphological features of crustose Teloschistaceae, allowing the identification of highly similar taxa using phenotypic characters.
3) To study the diversity and geographical distribution of various crustose Teloschistaceae in Russia.

# 3 Summary

Although the taxonomy and diversity of Teloschistaceae have been studied for many years (e.g., Wunder 1974, Poelt and Hinteregger 1993, Arup and Grube 1999, Arup 2006, Wetmore 2009, Arup et al. 2013, Vondrák et al. 2018 etc.), many groups within the family, one of the largest of all lichen families, are still poorly understood. This has become especially apparent after the implementation of molecular methods in the taxonomy of lichens. In addition, many geographical regions, important in terms of the group's diversity, are poorly surveyed.

One of the groups with unclear taxonomic status is *Pyrenodesmia*, the generic concept of which was not finalized in the comprehensive study by Arup et al. (2013). The authors included only part of the *Caloplaca variabilis* group in the genus, whereas the relationships of the resurrected *Pyrenodesmia* with the rest of the group as well as with the so-called *C. xerica* group remained unresolved. Moreover, it has been repeatedly demonstrated that *Pyrenodesmia* may include other species, such as *C. cretensis*, *C. demissa*, *C. obscurella*, *C. reptans*, and members of the *C. haematites* group (Muggia et al. 2008, Hodkinson and Lendemer 2012, Vondrák et al. 2012, Arup et al. 2013).

The delimitation of the genus *Pyrenodesmia* was the main aim of Paper 1. We analyzed two alignments: (i) a large Caloplacoideae alignment consisting of five concatenated DNA loci from 37 species published by Gaya et al. (2015) and 41 species sequenced by us and putatively belonging or closely related to *Pyrenodesmia* sensu Arup et al. (2013), (ii) a smaller alignment consisting of eight concatenated DNA loci from the species putatively belonging or closely related to *Pyrenodesmia*. We came to the conclusion that *Caloplaca demissa*, *C. obscurella*, and *C. reptans* are rather distant from *Pyrenodesmia*. The rest of the analyzed species formed a monophyletic clade, which was, however, internally diverse and could be divided into three homogenous groups. We recognized them on the generic level. We demonstrated that the genus *Pyrenodesmia* completely matched the *C. variabilis* group and included taxa never containing anthraquinones, but instead Sedifolia-grey. It has a Holarctic distribution with three main centers of diversity: the Mediterranean basin, Central Asia, and the arid regions of western North America. For the *C. xerica* group together with *C. cretensis* and *C. diphyodes* we have resurrected the genus *Kuettlingeria*, which was already described in the 19th century, and for the *C. haematites* group and *C. bicolor* we have described a new genus *Sanduineodiscus*. The genus *Kuettlingeria* is Holarctic, but all currently known species occur in the Mediterranean basin and only a few species extend to North America and Northern Asia. The genus *Sanguineodiscus* is known from the Mediterranean basin, adjacent regions, Northern Europe, and Central Asia. In total, 24 new combinations were proposed in the Paper 1.

To describe the diversity of the studied group we needed to understand which morphological characters we could use and how they should be measured. We realized that the most authors use the same pool of morphological characters (ascocarp anatomy, ascospore size and shape, etc.), but that their methods of measurements remained enigmatic, a serious issue as measuremet methods can influence measurement values. For example, the height of the hypothecium depends on the place where the vertical section of the apothecium is made, and the width of the exciples can be also measured in different ways resulting in different values. The width of the ascospore septum depends on the age of a spore (immature, mature, or overmature). KOH treatment also affects the width of the ascospore septum, because it causes a significant swelling of the cell walls and, thus, a widening of the overall structure. As a result, we arrived at the basic idea of Paper 2, namely proposing a list of the main phenotypic characters and standardized techniques for measuring them. We have also suggested a phenotype evaluation process for distinguishing phenotypically closely related species. This has two steps: (i) the preliminary examination of the diagnostic significance of all available characters on the small set of specimens and the selection of potentially diagnostic characters; (ii) a detailed study in which the characters selected in the first step are tested on additional specimens.

The method described above was practically employed in Paper 3 on three seemingly cryptic species from the genus *Pyrenodesmia*, which were separated according to nrITS and β-tubulin DNA phylogenies and appeared to be phenotypically indistinguishable. Three specimens, previously used in the phylogenetic analyses, were selected in each taxon for the preliminary study. In each of these specimens, we evaluated 29 continuous and about 60 discrete characters. Discrete characters constant in all specimens of any one species but different in all other specimens were selected for the second step; the most powerful continuous characters were selected for the second step by means of the linear discriminant analysis. Eleven continuous and eight discrete potentially diagnostic characters were selected for the detailed study, after which four continuous and three discrete characters were considered "fully diagnostic", i.e. allowing the correct identification of at least one species. We came to the conclusion that the three selected species are not cryptic, but can be distinguished phenotypically, and described them as *Pyrenodesmia micromarina* (I.V. Frolov et al.) I.V. Frolov & Vondrák, *P. micromontana* (I.V. Frolov et al.) Hafellner & Türk, and *P. microstepposa* (I.V. Frolov et al.) Hafellner & Türk.

We described one more *Pyrenodesmia* species in Paper 4. The new *P. molariformis* (I.V. Frolov et al.) S.Y. Kondr. is distributed in continental Eurasia and has its westernmost locality in Central Europe. It is characterized by a peculiar thallus anatomy consisting of fungal and algal stacks (sensu Vondrák and Kubásek 2013).

In addition to studying the taxonomy and diversity of *Pyrenodesmia*, we have investigated the diversity of other crustose Teloschistaceae in various regions of Russia. Although the lichen biota of the country has been quite well studied, it is less known than that of Western Eurasia, mainly because the territory is very large and some regions are difficult to access. The absence of information from such an extensive area can result in the misunderstanding of the geographical ranges of species. In Paper 5, we presented several cases where species of Teloschistaceae formerly thought to be limited to rather small territories in the western or eastern parts of Eurasia are in fact widespread in Northern Eurasia. We supported our findings with ITS nrDNA data. The range of *Caloplaca subalpina*, previously known from European mountains (Vondrák et al. 2008), was extended much further eastwards, to the Ural and Western Sayan Mountains. Vice versa, *Gyalolechia ussuriensis* formerly thought to be restricted to the Far East (Kondratyuk et al. 2011), was found from Kamchatka to the Altai Mountains. Other species (*Calogaya bryochrysion* and *Caloplaca isidiigera*) were revealed as nearly circumpolar or even nearly cosmopolitan (*Flavoplaca flavocitrina*). We also discussed the geographical ranges of taxa within species pairs (Poelt 1963). Six of the eight species studied reproduce both sexually and asexually and have wider ranges than their strictly sexually-reproducing relatives.

In Paper 6, we described three new species of crustose Teloschistaceae from Siberia and the Russian Far East. The new species presumably have a boreal north-eastern distribution in Asia: *Caloplaca saviczii* I.V. Frolov et al. from the subfamily Caloplacoideae and *Lendemeriella aureopruinosa* I.V. Frolov et al. and *Orientophila infirma* I.V. Frolov et al. from the subfamily Xanthorioideae. These species are fairly common in the region, however, they are not easily distinguishable from some wider distributed Holarctic crustose Teloschistaceae, such as *Athallia holocarpa*, *Caloplaca ahtii*, and *Lendemeriella exsecuta*. As a result, lichenologists have not yet recognized them.

To conclude, our study clarifies the delimitation of the genus *Pyrenodesmia* and its relationships with closely related species. It improves our knowledge of *Pyrenodesmia* diversity as well as the diversity of other crustose Teloschistaceae in Europe and Northern Asia. Within the framework of the present PhD thesis, the new genus *Sanguineodiscus* has been described and the genus *Kuettlingeria* has been resurrected. We have also described nine new species and proposed 28 new combinations of crustose Teloschistaceae, including four new species and seven new combinations of *Pyrenodesmia*, and found nine species new to Russia. Within the framework of our study, we have proposed standardized methods of measuring phenotypic characters of crustose Teloschistaceae and suggested a phenotype evaluation process consisting of two steps for distinguishing phenotypically closely related species. Data presented in this PhD thesis as well as data obtained during the

postgraduate study, but not presented in any manuscript yet are going to be used as a basis for the future summarizing papers on the taxonomy and diversity of the genus *Pyrenodesmia* in the Holarctic and the whole family Teloschistaceae in Russia.

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# 5 Original publications

## 5.1 Paper 1

**Frolov I.**, Vondrák J., Košnar J., Arup U. 2020. Phylogenetic relationships within *Pyrenodesmia* sensu lato and the role of pigments in its taxonomic interpretation. Journal of Systematics and Evolution. doi: 10.1111/jse.12717.

**Phylogenetic relationships within *Pyrenodesmia* sensu lato and the role of pigments in its taxonomic interpretation**

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**Abstract**

Most lichens of the family Teloschistaceae (Ascomycota) produce yellow-orange-red anthraquinone pigments. However, the genus *Pyrenodesmia* encompasses species in which anthraquinones are absent and replaced by a grey pigment Sedifolia-grey. It was shown recently that these species are related to taxa with both anthraquinones and Sedifolia-grey (*Caloplaca xerica* group, *C. haematites* group and *C. cretensis*) and to species with a brown pigment instead of both anthraquinones and Sedifolia-grey (*C. demissa*, *C. obscurella* and *C. reptans*). Nevertheless, relationships between mentioned anthraquinone-containing and anthraquinone-lacking species remained unclear. Eight DNA loci from 41 species were used here trying to resolve these uncertainties. We concluded that *C. demissa*, *C. obscurella* and *C. reptans* are rather distant from the core of *Pyrenodesmia* and we place them outside of *Pyrenodesmia* sensu lato. Within *Pyrenodesmia* sensu lato three lineages were revealed and recognized on generic level: the genus *Pyrenodesmia* sensu stricto (21 species), the genus *Kuettlingeria* (14 species) which is resurrected here, and the genus *Sanguineodiscus* (4 species) which is newly described here. The genus *Pyrenodesmia* includes taxa which never contain anthraquinones, but Sedifolia-grey. It matches with the former *Caloplaca variabilis* group. Taxa of the genera *Kuettlingeria* and *Sanguineodiscus* have anthraquinones in their apothecia and Sedifolia-grey in their thalli. The genus *Kuettlingeria* includes the former *C. xerica* group plus *C. cretensis* and *C. diphyodes*. The genus *Sanguineodiscus* includes the former *C. haematites* group and *C. bicolor*. The identity of *Kuettlingeria* (*Caloplaca*) *diphyodes* was clarified and the name *Pyrenodesmia helygeoides* was resurrected. Twenty-four new combinations were proposed.

**Key words:** anthraquinones, *Caloplaca haematites* group, *Caloplaca variabilis* group, *Caloplaca xerica* group, *Kuettlingeria*, *Pyrenodesmia* sensu stricto, *Sanguineodiscus*, Sedifolia-grey, Teloschistaceae

## Introduction

The majority of lichens belonging to the family Teloschistaceae produce yellow-orange-red anthraquinone pigments in their superficial tissues (e.g., Søchting, 1997). Some Teloschistaceae species, however, lack anthraquinones in their thalli or both thalli and apothecia. Instead of anthraquinones (or together with them) they synthesize different pigments (green, grey, brown and in some exotic species others) of unknown structure such as Cinereorufa-green (Wetmore, 1996; Arup et al., 2007) and Sedifolia-grey (e.g., Meyer & Printzen, 2000; Vondrák et al., 2012), which may have the same function as anthraquinones, namely, protection against UV radiation (e.g., Hauck et al., 2007). These pigments are not extracted in acetone and cannot be revealed by TLC, but they are detectable in sections of tissue. Sedifolia-grey is grey in section and has a violet reaction with potassium hydroxide and with nitric acid. Cinereorufa-green is green in section and has a purple reaction with nitric acid only. Species lacking anthraquinones in thalli or both thalli and apothecia do not form a monophyletic group (e.g., Wunder, 1974; Kärnefelt, 1989; Wetmore 1994; Vondrák et al., 2012; Arup et al., 2013), but belong to different lineages within Teloschistaceae, for example the genera *Blastenia*, *Caloplaca* sensu stricto and *Parvoplaca*, or have an unresolved generic position, e.g. '*Caloplaca*' *ahtii* and '*C.*' *conversa*.

*Pyrenodesmia* is another generic name originally employed for members of the Teloschistaceae characterized by the total absence of anthraquinones. Author of the genus, Massalongo (1852), used the name for a small group of lichens with clear morphological and ecological characteristics. He accommodated there four species (*P. agardhiana*, *P. chalybaea*, *P. olivacea* and *P. variabilis*) and three infraspecific taxa (*P. variabilis* β. *lilacina*, *P. variabilis* δ. *pulchella* and *P. variabilis* γ. *fusca*) lacking anthraquinones and with Sedifolia-grey both in their thallus and apothecia, which inhabit calcareous outcrops. However, later lichenologists used this generic name much wider including to the genus also species with anthraquinones (e.g., *Caloplaca monacensis* and *Flavoplaca citrina*) which are not related to the taxa of *Pyrenodesmia* sensu Massalongo as it was shown by Vondrák et al. (2009), Šoun et al. (2011), Arup et al. (2013), etc.

Zahlbruckner (1930–1931) included *Pyrenodesmia* in *Caloplaca*, but numerous later authors maintained the species without anthraquinones on limestone as a distinct infrageneric group. Wunder (1974) and Kärnefelt (1989) named it "*Caloplaca variabilis* group". Clauzade and Roux (1985) called it "subgenus *Pyrenodesmia*", however, they also included species which are not related to *C. variabilis* (e.g., *C.*

*conversa* and *C. turkuensis*). Rudolph (1955) kept the genus *Pyrenodesmia* separately, but included many unrelated species with anthraquinones (e.g., *Athallia pyracea*, *Gyalolechia flavovirescens* and *Polycauliona bolacina*). Above mentioned concepts were revised by analysis of three DNA loci by Arup et al. (2013), who resurrected the genus *Pyrenodesmia* as it was understood by Massalongo (1852). However, in the phylogeny inferred by Arup et al. (2013) the genus did not seem to be monophyletic unless the species of the so-called *Caloplaca xerica* group with orange apothecia were incorporated. As a result, the authors recognized the genus *Pyrenodesmia* sensu stricto and the informal group *Pyrenodesmia* sensu lato. In their three loci phylogenetic reconstruction *Pyrenodesmia* sensu stricto did not form a monophyletic clade, but *Pyrenodesmia* sensu lato did. The former includs the genus type *P. chalybaea* and some closely related species that lack anthraquinones, but possess Sedifolia-grey; the latter also includes species with Sedifolia-grey in the thallus, but with anthraquinones in the apothecia – the *Caloplaca xerica* group (also see Gaya et al., 2008 and Vondrák et al., 2012). Finally, Arup et al. (2013) kept in the genus *Pyrenodesmia* six species, whereas taxa of the *C. xerica* group were remained under the formal generic name '*Caloplaca*' pending more robust data. However, already in 1857 Trevisan established the separate genus *Kuettlingeria* for some species of this currently informal group.

According to some other authors, *Pyrenodesmia* sensu lato may include other species with Sedifolia-grey in the thallus, but with anthraquinones in the apothecia: the *Caloplaca haematites* group (Hodkinson & Lendemer, 2012; Vondrák et al., 2012) and *C. cretensis*, an endolithic calcareous lichen occurring in the Mediterranean (Muggia et al., 2008).

It was also demonstrated that some species lacking both anthraquinones and Sedifolia-grey, but possessing other unknown brown pigments, namely *Caloplaca demissa*, *C. obscurella* and *C. reptans*, may belong to *Pyrenodesmia* sensu lato. *Caloplaca demissa*, placed there by Arup et al. (2013), is a lobate sorediate species known only as a sterile crust on dry vertical faces of siliceous rocks in Europe and North America. *Caloplaca obscurella*, an epiphytic sorediate crust, which occurs mainly in boreal and temperate forests of Holarctic, formed a sister lineage to an unsupported clade of *Pyrenodesmia* in Vondrák et al. (2012) and was included into the *Pyrenodesmia* clade by Muggia et al. (2008). *Caloplaca reptans*, a crustose sorediate lichen which is rarely fertile, is widespread in humid habitats, on non-calcareous, sheltered rocks in Appalachian forests. Hodkinson and Lendemer (2012) showed that *C. reptans* is close to *Pyrenodesmia*.

To elucidate the taxonomy and phylogenetic relationship among taxa putatively belonging to *Pyrenodesmia*, we tried to answer following questions:

1. Do the species completely without anthraquinones, but with Sedifolia-grey, form a monophyletic group that merits recognition at generic rank as *Pyrenodesmia*?

2. Are the anthraquinone-containing groups, the *C. haematites* group and the *C. xerica* group, monophyletic and do they merit recognition at generic rank? Can we resurrect the generic name "*Kuettlingeria*" for the *C. xerica* group?

3. Are the species lacking both anthraquinones and Sedifolia-grey, *C. demissa*, *C. obscurella* and *C. reptans*, related to *Pyrenodesmia*?

## Material and Methods

### Sampling

Specimens were collected mainly by the first two authors and are deposited in PRA (J. Vondrák) and I. Frolov's personal herbarium. Other specimens for molecular investigations were kindly provided by the herbaria KW, NY, TSB, UCR, XJU and by Mehmet Gökhan Halıcı and Toby Spribille from their personal collections.

### DNA extraction and amplification

DNA was extracted with a CTAB-based protocol (Aras & Cansaran, 2006). For each sample, we sequenced as many as possible of eight DNA loci: (i) two nuclear ribosomal markers included the internal transcribed spacer regions 1 and 2 with the embedded 5.8S region (**ITS**), the nuclear ribosomal large subunit (**nucLSU**), (ii) mitochondrial ribosomal small subunit (**mtSSU**), and (iii) five protein-coding nuclear loci, parts of the largest and second largest subunits of RNA polymerase II (**RPB1** and **RPB2**, respectively), part of DNA replication licensing factor mini-chromosome maintenance complex component 7 (**MCM7**), partial sequence of transcription elongation factor 1 alpha (**EF1a**) and part of the beta-tubulin gene making up microtubules, major components of the cytoskeleton (**TUBB**). PCRs were performed in a reaction mixture containing 2.5 μM $MgCl_2$, 0.2 μM of each dNTP, 0.3 μM of each primer, 0.5 U Taq polymerase (Top-Bio, Praha, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 10 μL. Primers and annealing temperatures used are listed in Table 1. Each sequence is provided with a GenBank accession number (Table 2).

### Sequence alignment and phylogenetic reconstructions

Sequences were edited in FinchTV 1.4.0 (Geospiza, Inc.; Seattle, Washington, USA; http://www.geospiza.com) and BioEdit 7.2.5 (Hall, 1999) and aligned online by MAFFT 7 (Katoh & Standley, 2013; available at http://mafft.cbrc.jp/alignment/server/) with the L-INS-i method (Katoh et al., 2005). Alignments were checked for obvious errors and corrected in BioEdit 7.2.5 when needed. To exclude ambiguously aligned positions alignments were subsequently cleared by the *automated1* algorithm as

implemented in the trimAl software package (Capella-Gutierrez et al., 2009). Concatenated alignments are deposited in TreeBASE (Submission ID 23651).

To circumscribe *Pyrenodesmia* sensu lato, we compiled the **Caloplacoideae alignment** with five concatenated DNA loci: ITS, mtSSU, nucLSU, RPB1 and RPB2. This included 78 specimens (76 species; Table 2) and consisted of 148 single-locus sequences from 37 specimens published by Gaya et al. (2015) together with our 200 single-locus sequences from 41 specimens. The main Caloplacoideae groups and potential members of *Pyrenodesmia* sensu lato are represented in the alignment. The tree was rooted using several lineages of Teloschistoideae and Xanthorioideae as an outgroup.

To check the obtained phylogenetic scheme within *Pyrenodesmia* sensu lato we compiled the ***Pyrenodesmia* alignment** with eight concatenated DNA loci including all the loci presented in the Caloplacoideae alignment plus EF1a, MCM7 and TUBB. The *Pyrenodesmia* alignment consists of 40 specimens (=species) involving 230 single-locus sequences. Eight-loci coverage was complete for 16 specimens, seven loci were obtained for 19 specimens, six loci were obtained for three specimens, and five and/or four loci for one specimen (Table 2). The species were selected on the basis of the previously analysed Caloplacoideae alignment to include species of the *Pyrenodesmia* sensu lato clade (Fig. 1). The tree was rooted using *Caloplaca conversa* and *C. reptans* as an outgroup.

For both alignments phylogenetic reconstructions were carried out using maximum likelihood (ML) and Bayesian inference (BI). The ML phylogenetic analysis was performed using RAxML v8.2.10 (Stamatakis, 2014) and BI using MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003). Analyses were run on the CIPRES Web Portal (http://www.phylo.org/portal2/). Optimum partitioning of the data sets and the optimum substitution models per partition were calculated in PartitionFinder2 using greedy algorithm and corrected Akaike Information Criterion (Lanfear et al., 2016). In the input file for PartitionFinder we created partitions (i) for intronic and exonic fractions separately, with the three codon positions independently in the protein-coding genes, (ii) for ITS1, ITS2 and 5.8S separately in ITS and (iii) mtSSU and nucLSU were analyzed as whole fragments. PartitionFinder retained four subsets of partitions for the Caloplacoideae alignment and five subsets of partitions for the *Pyrenodesmia* alignment. Results of the analyses in PartitionFinder are shown in Table S1. In MrBayes analyses were performed using two independent runs with four MCMC chains. Trees were sampled after every 500th generation. The analyses were stopped when the average standard deviation of the split frequencies between the simultaneous runs dropped below 0.01. The first 25% of trees were discarded as the burn-in phase, and the remaining trees were used for construction of a 50% majority-rule consensus tree. The ML analysis was employed using GTR + G model. Bootstrap support was

calculated on 1000 bootstrap pseudoreplicates using rapid bootstrapping. A clade is considered to be supported with posterior probability ≥ 0.95 in BI and bootstrap value ≥ 70% in ML.

To check for topological incongruences, single gene alignments were analysed separately using BI as described above. The differences in topology of single gene trees were considered for the branches with posterior probability support values ≥ 0.95. If the incongruences were due to odd clustering of a few individual samples, repeated PCR and sequencing was used to exclude the possibility of sample contamination.

**Chemistry**

Anthraquinones in the apothecial disk and the true exciple were identified using thin layer chromatography (TLC). Although the TLC detects primarily only the major substances it is sufficient for separation of the main chemosyndromes in Teloschistaceae (sensu Søchting, 1997, 2001). We carried out the TLC in solvents A, B and C according to Orange et al. (2001).

**Results**

**Circumscription and content of *Pyrenodesmia* sensu lato: results from the five-loci Caloplacoideae dataset**

Both BI (Fig. 1) and ML (Fig. S1) analyses of the concatenated alignment showed the same result referring to clades included in *Pyrenodesmia* sensu lato, which consists of *Pyrenodesmia* sensu stricto, the *Caloplaca haematites* group, the *C. xerica* group, *C. bicolor* and *C. cretensis*. *Caloplaca obscurella* seems to be the closest lineage to *Pyrenodesmia* sensu lato.

*Pyrenodesmia* sensu lato together with *Caloplaca obscurella*, *C. reptans* and *Huea cerussata* forms a supported clade in both BI and ML reconstructions. Relationships among "*Pyrenodesmia* s.lat.+*Caloplaca obscurella*", *C. reptans* and *Huea cerussata* were not resolved. *Caloplaca demissa* is considerably distant from *Pyrenodesmia* sensu lato in our phylogenetic reconstructions and belongs to the highly supported clade which consists of the genera *Usnochroma* and *Rufoplaca* and the species *Caloplaca conversa* and *C. peludella*.

Within *Pyrenodesmia* sensu lato three main lineages could be distinguished (Fig. 1; Fig. S1; groups K, P, S), but relationships among them are not supported.

The **lineage P (*Pyrenodesmia*)** is highly supported in the BI tree (but not supported by ML; Fig. S1) and includes all species belonging to the *Caloplaca variabilis* group. Two internal branches are recognized – (P1) *C. albopruinosa* + *C. micromontana* + *Caloplaca* sp. 1 and (P2) the rest of the species. The **lineage K (*Kuettlingeria*)** is highly supported in the BI and ML trees and includes the supported clade of the *C. xerica*

group and *C. cretensis*, *C. diphyodes* and *Caloplaca* sp. 2. The **lineage S (*Sanguineodiscus*)** is well supported in the BI tree (but not supported by ML) and includes the fully supported *C. haematites* group and *C. bicolor*.

**Phylogeny within *Pyrenodesmia* sensu lato: results from the eight-loci *Pyrenodesmia* dataset**

As in the analysis of the Caloplacoideae dataset, the same three main lineages could be distinguished within the *Pyrenodesmia* sensu lato clade: **K (*Kuettlingeria*)**, **P (*Pyrenodesmia*)** and **S (*Sanguineodiscus*)**. Relationships among the lineages are not supported (Figs. 2, S2).

Unlike in the analysis of the Caloplacoideae alignment, the **lineage P** lacks support in both BI and ML analyzes of the *Pyrenodesmia* alignment. As in the Caloplacoideae tree (Fig. 1) it matches with the *Caloplaca variabilis* group and consists of two highly supported branches: (P1) involving *C. albopruinosa* + *C. micromontana* + *Caloplaca* sp.1 and (P2) involving the rest of the species.

The **lineage K** is highly supported in both analyzes. As in the Caloplacoideae tree it consists of a clade of the *C. xerica* group (also supported in both analyzes) and *C. cretensis*, *C. diphyodes* and *Caloplaca* sp. 2. The latter two species always group together. *Caloplaca cretensis* forms a highly supported clade with *C. diphyodes* and *Caloplaca* sp. 2 in the BI tree, but its relationships within the lineage K are not supported in the ML tree. The **lineage S** is supported in both analyzes. As in the Caloplacoideae tree, it consists of a clade of the *C. haematites* group (highly supported in both analyzes) and *C. bicolor*.

Eight *Pyrenodesmia* single-locus alignments were analyzed separately using BI (Fig. S3). The phylogenetic trees often have an unresolved backbone with few supported branches. Therefore, only two supported topological conflicts were observed (see below). The lineage P was monophyletic and supported in two single-locus trees, RPB1 and RPB2. Supported topological conflict was found in MCM7, with the P1 clade of *Pyrenodesmia* being more closely related to clades A and S than to the members of the P2 clade. The lineage K was resolved and supported in five loci (EF1a, MCM7, RPB1, RPB2 and TUBB), and resolved but not supported in one locus (mtSSU). Out of the four trees involving all the three analyzed taxa of the lineage S, the group was detected in MCM7 and ITS, but without support. Topological conflict was found in mtSSU, where two taxa *C. haematites* group formed well-supported clade together with two members of the lineage K (sequences of all four taxa were verified using repeated PCR and sequencing).

**Chemistry**

In the analyzed samples we identified chemosyndromes A (Søchting, 1997) and C1, C2, C5 (Søchting, 2001). Chemosyndrome A is characterized by strong dominance of parietin. Chemosydrome C belongs to syndromes with chlorinated anthraquinones. C1 is characterized by strong dominance of 7-Cl-emodin, C2 – by dominance of 7-Cl-emodin and a higher proportion of 7-Cl-citreorosein, C5 – by 7-Cl-emodin as the dominant compound in association with a substantial proportion of fragilin. Chemosyndromes are given in the taxonomical part under each species with anthraquinones in the apothecia as well as in Fig. 2.

## Discussion

### *Pyrenodesmia* sensu lato; three-generic scenario

The clade *Pyrenodesmia* sensu lato (Figs. 1, 2) encompasses species with similar morphology, chemistry, geography and ecology – crustose lichens that always contain the pigment Sedifolia-grey in outer tissues and occur mostly in xerothermic sun-lit conditions of temperate Northern Hemisphere. Nevertheless, the group has large internal variability in phenotype and genotype and could be divided into several supported lineages. In our opinion, a division into three groups (lineages K, P and S in Figs. 1 and 2) is reasonable and biologically relevant, as the lineages are monophyletic and supported at least in some of the multi-loci analyses (see the Results) and can be characterized by specific although partly overlapping sets of morphological, chemical, geographical and ecological features. The lineage P consists of the species always lacking anthraquinones both in their thalli and apothecia, growing mainly on calcareous outcrops. The lineage has the Holarctic distribution with three main centers of its diversity (regions with sets of taxa occurring only there): the Mediterranean basin, Central Asia and arid regions of western North America. Taxa of the lineages K and S normally contain anthraquinones in the apothecia (chemosyndromes A, C1, C2 and C5 in the lineage K and chemosyndrome A in the lineage S), although specimens completely lacking anthraquinones are rarely known in a few species. The lineage K is Holarctic, however all currently known species occur in the Mediterranean basin whereas just a few of them are distributed outside this region. It prefers base-rich siliceous (sometimes pure limestone) outcrops. The lineage S is absent in the North America; it is known from the Mediterranean basin, adjacent regions and Northern Europe, and Central Asia. It is the only lineage in the *Pyrenodesmia* sensu lato clade which includes both saxicolous (mainly base-rich siliceous outcrops) and corticolous species.

As these three lineages are closely related and form a monophyletic group it would be possible to unite them all within a single genus *Pyrenodesmia* including there species of the currently informal *Caloplaca haematites* and *C. xerica* groups and

therefore extending the original concept of *Pyrenodesmia* proposed by Massalongo (1852) and accepted by Arup et al. (2013) to species with anthraquinones in the apothecia. This option was chosen by Kondratyuk et al. (2020a,b). However, uniting all the lineages into one genus will result in a loss of information. In this case the large genus would have unresonably high internal variability and we would still have to talk about *C. haematites*, *C. variabilis* and *C. xerica* groups when dealing with *Pyrenodesmia* sensu lato. In our opinion, three-generic scenario corresponding to three main lineages of *Pyrenodesmia* sensu lato contains more information than one genus does; it makes most sense even if some of the genera features are partly overlapping. The most powerful and also practical difference is the lack of anthraquinones in the lineage P compared to the species from other two lineages (except *C. diphyodes*). There is also a rather strong difference between the lineage P and the lineages K and S in the substrate preferences, strongly calcareous in the former and in general calciferous or siliceous substrates in the latter two (with only a few species growing on pure limestone).

Differences between the other two lineages, K and S, are not as distinct as delimitation of the lineage P. However, uniting them into a single genus is not suitable as they do not form a monophyletic group in any of our phylogenetic reconstructions and show differences in morphology, chemistry, geography and ecology. The lineage S always has only chemosyndrome A (the lineage K has a mixture of chemosyndormes A, C1, C2 and C5 and also complete absence of anthraquinones in *C. diphyodes*); true exciple of the taxa of the lineage S is often without anthraquinones and grey (in the lineage K it is always with anthraquinones, orange); the lineage S is absent in North America (the lineage K has Holarcatic distribution) and include a corticolous species (the lineage K is exclusively saxicolous).

Moreover, during the last 170 years these three lineages were at least partly regarded as informal groups (*C. haematites*, *C. xerica* and *C. variabilis* groups) or even genera (*Pyrenodesmia* and *Kuettlingeria*). In the context of the current taxonomy of the lichenized fungi (Crespo et al., 2010; Nordin et al., 2010; Spribille et al., 2011; Ekman & Svensson, 2014; Buaruang et al., 2015) and particularly of the family Teloschistaceae (Fedorenko et al., 2012; Arup et al., 2013; Søchting et al., 2014; Kondratyuk et al., 2017a etc.) the three groups within *Pyrenodesmia* sensu lato should be treated at the genus rank as they show considerable phylogenetical and morphological differentiation.

The lineage P represents the genus *Pyrenodesmia* sensu stricto described by Massalongo (1852) and resurrected by Arup et al. (2013). The old generic name *Kuettlingeria* (Trevisan, 1857) can be used for designation of the lineage K, which includes *Caloplaca teicholyta* – current name for *Kuettlingeria visianica*, the type species of the genus *Kuettlingeria*. We were not able to find any appropriate existing

name for the lineage S, consequently it was formally described here as a new genus *Sanguineodiscus*. Differences between the three genera are summarized in Table 3.

The genus *Pyrenodesmia* sensu stricto (Fig. 3A) matches the *Caloplaca variabilis* group (Wunder, 1974; Kärnefelt, 1989; Vondrák et al., 2012). Our concept of the genus is the same as that proposed by Massalongo (1852) and Arup et al. (2013). Unlike the phylogeny by Arup et al. (2013) our analyzes demonstrate that it is well separated from the *C. xerica* group. The genus is monophyletic in all of our phylogenetic trees (Figs. 1, 2, S1, S2); however, it is supported only in the BI phylogeny of the Caloplacoideae alignment (Fig. 1). In other cases it could be subdivided on two groups corresponding to highly supported clades P1 and P2. Nevertheless, these groups do not demonstrate any perceptible differences in morphology, anatomy, chemistry or ecology. For that reason we prefer to keep them within the same genus *Pyrenodesmia*.

The genus *Kuettlingeria* (Fig. 3B) consists of a clade of the former *Caloplaca xerica* group (Vondrák et al., 2012; Arup et al., 2013) and *K. cretensis*, *K. diphyodes* and *Kuettlingeria* sp. 2. The genus *Sanguineodiscus* (Fig. 3C) consists of a lineage containing a single species *S. bicolor* and a lineage formed by the former *Caloplaca haematites* group.

**The name *Caloplaca diphyodes***

*Kuettlingeria diphyodes* is the only species of the genus lacking anthraquinones entirely. In addition, it differs from other *Kuettlingeria* species by its ecology as it grows on periodically inundated acidic siliceous boulders in watercourses.

Formerly recognized as *Caloplaca diphyodes*, it was traditionally considered as widespread in the alpine belt and in the Arctic regions in the Holarctic (e.g., Wunder, 1974; Poelt & Hinteregger, 1993; Davydov et al., 2007; McCune et al., 2015; Gröner, 2016; Hafellner & Türk, 2016). However, the type material of *Lecanora* (=*Caloplaca) diphyodes* originates from low altitude (about 600 m a.s.l.) of the Massif Central in France (Nylander, 1872). Our investigation of the material recently collected by us in the locus classicus (specimens Frolov 1430, Vondrák 15096) placed it into *Kuettlingeria* and showed that this taxon is not related to the Arctic-alpine specimens and is so far known only from a few collections from the Massif Central in France. Instead, the Arctic-alpine specimens belong to *Pyrenodesmia* sensu stricto and *P. helygeoides* is the earliest appropriate name for that species we found (see the Taxonomy).

**Species not included in *Pyrenodesmia* sensu lato**

*Caloplaca obscurella* (Fig. 3D), which is considered as the closest lineage to *Pyrenodesmia* sensu lato could be possibly included in the ingroup. Such a decision was made by Choisy (1951), who proposed the combination *Pyrenodesmia obscurella*,

and Muggia et al. (2008), who placed *C. obscurella* within their "*Pyrenodesmia*" clade. However, the chemistry of this species does not correspond with that of *Pyrenodesmia*, since both anthraquinones and Sedifolia-grey are absent. Moreover, *C. obsurella* is an epiphytic taxon distributed mainly in boreal and temperate forests which is uncharacteristic for *Pyrenodesmia* sensu lato. Distinct secondary chemistry, ecology and an outlying position in the phylogenetic trees (Figs. 1–3) led us to place *C. obscurella* outside *Pyrenodesmia* sensu lato.

*Caloplaca reptans* (Fig. 3E) is widespread in moist habitats, on non-calcareous rocks in forests of eastern North America (Hodkinson & Lendemer, 2012). It is characterised by lacking both anthraquinones and Sedifolia-grey in the thallus. Hodkinson & Lendemer (2012) observed immature apothecia with reddish-brown discs lacking anthraquinones, but did not mention Sedifolia-grey and we did not see fertile specimens. In the five-loci tree, *C. reptans* is even more distant from the *Pyrenodesmia* core than *C. obscurella* (Fig. 1) and we consider it being outside *Pyrenodesmia* sensu lato.

The genus *Huea* was erected by Dodge and Baker (1938) to encompass Antarctic species of Teloschistaceae without anthraquinones. *Huea* is known by its complicated typification (Fryday 2011) and the genus was not regarded by Arup et al. (2013). We did not study any specimens of *Huea* and do not have any information about its pigments, but we included the species *H. cerussata* into our five-loci phylogenetic analysis and confirmed the result of Gaya et al. (2015), that it is close to *Pyrenodesmia* sensu lato. Its position in the tree (Fig. 1) is however as distant from the *Pyrenodesmia* core as the position of *C. reptans*.

Our phylogenetic data do not support attribution of *Caloplaca demissa* (Fig. 3F) to *Pyrenodesmia* sensu lato. According to our five-loci phylogenetic analysis (Fig. 1) it belongs to a supported clade including the genera *Rufoplaca* and *Usnochroma* and the species *Caloplaca conversa* and *C. peludella*. As in *C. obscurella* and *C. reptans*, the chemistry of *C. demissa* differs from that of *Pyrenodesmia* sensu lato by the absence of both anthraquinones and Sedifolia-grey in the thallus (apothecia are unknown in that species).

Apparently, *C. demissa*, *C. obscurella* and *C. reptans* currently form three monotypic genera. Kondratyuk et al. (2015) already proposed the genus *Olegblumia* to accommodate *C. demissa*, but it is not legitimate, because the basionym, *Placodium demissum* Körb. ex Flotow, was not cited within the combination. Formal taxonomic proposals concerning these three taxa require further research.

Previous studies proved that the absence of anthraquinones as well as the presence of other pigments is not a phylogenetically reliable indication of relationship as the loss of anthraquinone production occurs in unrelated lineages of Teloschistaceae (e.g., Vondrák et al., 2012). However, according to our results, pigments can play a crucial

role in the taxonomy of some particular groups within the family. For example, *Pyrenodesmia* sensu lato consists of species which always possess Sedifolia-grey in thallus whereas anthraquinones are either absent or, in some species, restricted to the apothecia; species without both Sedifolia-grey and anthraquinones (for example, *Caloplaca demissa*, *C. obscurella* and *C. reptans*) do not belong there. Within *Pyrenodesmia* sensu lato, species completely without anthraquinones form a monophyletic lineage – the genus *Pyrenodesmia* sensu stricto. Two other genera of *Pyrenodesmia* sensu lato, *Kuettlingeria* and *Sanguineodiscus*, have Sedifolia-grey in their thalli and anthraquinones in their apothecia, but anthraquinones are exceptionally absent from apothecia of some individuals.

**Taxonomy**

***Kuettlingeria*** Trevis., Revista Periodica dei Lavori della Imperiale Regia Accademia di Padova 5: 72. 1857.

Fig. 3B

Type: *Kuettlingeria visianica* (A. Massal.) Trevis., Revista Periodica dei Lavori della Imperiale Regia Accademia di Padova 5: 73. 1857.

Bas.: *Blastenia visianica* A. Massal., Atti Ist. Veneto Sci. Lett. Arti, ser. 2, vol. 3 (app.): 117. 1852.

Syn.: *Kuettlingeria teicholyta* (Ach.) Trevis., Revista Periodica dei Lavori della Imperiale Regia Accademia di Padova 5: 73. 1857.

Diagnosis: Apothecial disc and true exciple yellow-orange to dark red or brown-red with anthraquinones of the chemosyndromes A, C1, C2 or C5 (sensu Søchting, 1997, 2001), exceptionally lacking anthraquinones and brown or black. Thallus and thalline exciple with Sedifolia-grey, lacking anthraquinones. Distribution Holarctic with biodiversity centre in the Mediterranean region. Saxicolous.

Description: *Morphology and anatomy:* Thallus crustose, epi- or rarely endolithic, white or grey; cortex paraplectenchymatous, usually represented by alveolate cortex (sensu Vondrák et al., 2013); some species with vegetative propagules (blastidia, soredia, isidia or minute lobules). Apothecia zeorine, rarely biatorine, sometimes appearing lecanorine, but thin true exciple is always present; thalline exciple of the same colour as thallus, disc and true exciple usually of different tinges of red and yellow, but in some individuals within a population true exciple and disc may be black, grey or brown; ascospores polardiblastic, medium to broadly ellipsoid, with medium long septum; pycnidia present or absent, grey-black; conidia bacilliform to subglobose.

*Chemistry:* Thallus and thalline exciple always without anthraquinones, but with Sedifolia-grey. Epihymenium and upper part of true exciple usually with anthraquinones (either dominated by non-chlorinated parietin,or by chlorinated 7-Cl-

emodin, fragilin or 7-Cl-citreorosein; Section 2). Sometimes epihymenium and true exciple contain both anthraquinones and Sedifolia-grey; in this case Sedifolia-grey could be seen when anthraquinones are washed out by KOH in the apothecial section. In some species, two chemotypes are known within the same species – with red colored apothecia (with anthraquinones) and rarer with black colored apothecia (without anthraquinones, only with Sedifolia-grey). *Kuettlingeria diphyodes* is the only exception in the group – the chemotype entirely without anthraquinones is only known.

*Distribution and ecology:* Northern Hemisphere. All currently known species occur in the Mediterranean regions and Macaronesia, some of them distributed also in non-Mediterranean Europe, Asia and North America. One record is known from Ecuador (*K.* aff. *soralifera* on concrete; herb. Zdeněk Palice 4836). The genus consists of exclusively saxicolous taxa, which grow both on limestone and base-rich siliceous outcrops in sun-lit conditions mostly from sea coasts to the mid-altitudinal zone. Few species (*K. diphyodes* and sometimes *K. atroflava*) grow on rather acidic siliceous boulders in streams. *Kuettlingeria percrocata* is a single species confined to the montane-alpine zone.

Remarks: Currently, 14 species are included in the genus, but this group is more diverse and contains other unnamed species (Vondrák et al., 2012). We did not consider taxa that probably belong to the genus, if their taxonomic status is unclear (e.g., *Caloplaca aetnensis* and *C. sbarbaronis*). Author of the genus *Kuettlingeria*, Trevisan (1857), included there three species – *K. lallavei*, *K. visianica* and *K. teicholyta*. Currently the former two species are synonymized under the latter species (see below under *K. teicholyta*). Apart from Trevisan, C.W. Dodge has been the only author describing or combining species names to *Kuettlingeria*. Some of them were already moved to other genera by Arup et al. (2013): *K. elegantissima* (Nyl.) C.W. Dodge to *Stellarangia*, *K. physcioides* (A. Massal.) C.W. Dodge to *Dufourea*. Other species (*K. crozetica*, *K. fuegiensis*, *K. macquariensis*, *K. rufa*, *K. rutilans* and *K. siplei*) are lichens with yellow to red thallus (Zahlbruckner, 1906a; Dodge & Baker, 1938; Dodge, 1968, 1970; Kantvilas & Seppelt, 1992), and they do not belong to *Kuettlingeria* in its present meaning.

***Kuettlingeria albolutescens*** (Nyl.) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828678

≡ *Lecanora albolutescens* Nyl., Flora (Regensburg) 64: 177. 1881 (basionym).

Type: [England]. Northumberland in Anglia, supra saxa quarcitosa ad Stocksfield, *W. Johnson* (holotype, H-NYL 29845!).

Chemistry: Chemosyndrome C5.

***Kuettlingeria areolata*** (Zahlbr.) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828679

≡ *Caloplaca cerina* var. *areolata* Zahlbr., Öst. bot. Z. 53: 289. 1903 (basionym).

Type: [Montenegro]. Bocche di Cattaro: Devesite bei Castelnuevo [Herceg Novi], altitude about 600–700 m, an Kalkfelsen, 1902, *J. Baumgartner* (holotype, W 7068!).

Chemistry: Chemosyndrome C5.

***Kuettlingeria atroflava*** (Turner) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828680

≡ *Lecidea atroflava* Turner, *Trans.* Linn. Society Lond. 9: 142. 1808 (basionym).

Type: England. Flints on the Sussex Downs, *Turner* (holotype, BM 730327!).

Chemistry: Chemosyndrome A; rarely anthraquinones completely absent.

***Kuettlingeria cretense*** (Zahlbr.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828681

≡ *Blastenia cretensis* Zahlbr., Sitzungsber. Kais. Akad. Wiss. Wien, math.-naturw. 115: 519. 1906b (basionym).

Type: Griechenland. Kreta: an Kalkfelsen auf der kleineren Insel Paximadhia, 1904, *J. Dörfler* (holotype, W!).

Chemistry: Chemosyndrome C1.

***Kuettlingeria diphyodes*** (Nyl.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828682

≡ *Lecanora diphyodes* Nyl., Flora (Regensburg) 55: 353. 1872 (basionym).

Type: [France]. Haute-Vienne: Bessines[-sur-Gartempe], sur le rochere au bord de la Gartempe, *Ripart* (holotype, H-NYL 29379!).

Remarks: see the Discussion and notes under *Pyrenodesmia helygeoides*.

Chemistry: anthraquinones completely absent.

***Kuettlingeria emilii*** (Vondrák, Khodos., Cl. Roux & V. Wirth) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828683

≡ *Caloplaca emilii* Vondrák, Khodos., Cl. Roux & V. Wirth, Lichenologist 45: 709. 2013 (basionym).

Type: Bulgaria. Black Sea coast: Kavarna, limestone cliffs on seashore 15 km NE of Kamen Brjag, 43°27′58.76″N, 28°33′55.02″E, on coastal limestone outcrop above supralittoral zone, 2007, *J. Vondrák 6600* (holotype, PRA!; isotype, KHER).

Chemistry: Chemosyndrome C5.

***Kuettlingeria erythrocarpa*** (Pers.) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828685

≡ *Patellaria erythrocarpa* Pers., Annalen der Wetterauischen Gesellschaft für die Gesammte Naturkunde 2: 12. 1811 (basionym).

Type: Galliae [France]. Ad lapides calcarios prope Dijon, *Persoon* (holotype, H-ACH 353!).

Chemistry: Chemosyndrome C5.

***Kuettlingeria furax*** (Egea & Llimona) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB832249

≡ *Caloplaca furax* Egea & Llimona, Collnea bot., Barcinon. Bot. Instit. 14: 266. 1983 (basionym).

Type: [Spain.] Lectus loco Cañada del Conejo dicto, ad pedem Sierra del Relumbrar, prope Bienservida (Albacete), altitude 350 m, ad saxa schistosa metamorphica paulo inclinata, in dominio Pyro-Querceti, super *Aspicilia* cf. *epiglypta*, 27 May 1978, *J.M. Egea et X. Llimona* ( isotype, GZU, Murc. lichenotheca no. 3039!).

Chemistry: Chemosyndrome C5.

***Kuettlingeria fuscoatroides*** (J. Steiner) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828686

≡ *Caloplaca fuscoatroides* J. Steiner, Verh. zool.-bot. Ges. Wien 69: 69. 1919 (basionym).

Type: [Greece]. Delos: Klein-Delos, auf herumliegeuden Schieferplatten, 1911, *Schiffner* (holotype, WU 41148!).

Chemistry: Chemosyndrome C5.

***Kuettlingeria neotaurica*** (Vondrák, Khodos., Arup & Søchting) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828687

≡ *Caloplaca neotaurica* Vondrák, Khodos., Arup & Søchting, Lichenologist 44: 414. 2012 (basionym).

Type: Ukraine. Crimean Peninsula: Sudak, Karadag Mts, Mt Svyataya, altitude 320 m, 44°56′03.27″N, 35°13′06.17″E, on volcanic rock, 2007, *J. Vondrák 5925* (holotype, PRA!).

Chemistry: Chemosyndrome C2; anthraquinones occasionally completely absent.

***Kuettlingeria percrocata*** (Arnold) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828688

≡ *Blastenia percrocata* Arnold, Verh. K. K. Zool.-Bot. Ges. Wien 37: 120 (1887) (basionym).

Type: [Italy]. Südtirol: Auf Sandstein der Campiler Schichten ober dem Rolle-pass bei Paneveggio, 6 Aug 1882, *Arnold*, *Arn. Lich. Exs. No 924* (lectotype, M 0102293!, selected by Wetmore, 1996, p. 312).

≡ *Blastenia arenaria* var. *percrocata* Arnold, Flora (Regensburg) 67: 309. 1884, nom. nud.

Chemistry: Chemosyndrome C5.

***Kuettlingeria soralifera*** (Vondrák & Hrouzek) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828689

≡ *Caloplaca soralifera* Vondrák & Hrouzek, Graphis Scripta 18: 8. 2006 (basionym).

Type: Czech Republic. Central Bohemia: Rakovník district, Křivoklát, Kalubice, by the small pond in the village, 50°02′56.3″N, 13°49′30.4″E, altitude 348 m, on horizontal side of concrete wall, 2004, *J. Vondrák 3332* (holotype, PRM!).

Chemistry: Chemosyndrome A.

***Kuettlingeria teicholyta*** (Ach.) Trevis

Fig. 3B

≡ *Lecanora teicholyta* Ach., Lichenographia Universalis: 425. 1810 (basionym).

Type: Gallia [France]. *Dufour* (lectotype, H-ACH 1229!, selected by Vondrák and Vitikainen 2008).

= *Blastenia visianica* A. Massal., Atti Ist. Veneto Sci. Lett. Arti, ser. 2, vol. 3 (app.): 117. 1852.

Type: [Italy. Padua ?] Viget ad saxa trachytica in horto botanico, cujus Praefecto (R. Prof. Visiani) speciem dicatam voluimos (holotype, VER!).

= *Kuettlingeria visianica* (A. Massal.) Trevis., Revista Periodica dei Lavori della Imperiale Regia Accademia di Padova 5: 73. 1857.

= *Lecidea lallavei* Clemente ex Ach. Syn. meth. lich. (Lund): 45. 1814.

= *Kuettlingeria lallavei* (Clemente ex Ach.) Trevis., Revista Periodica dei Lavori della Imperiale Regia Accademia di Padova 5: 73. 1857.

Chemistry: Chemosyndrome C5.

***Kuettlingeria xerica*** (Poelt & Vězda) I.V. Frolov, Vondrák & Arup, **comb. nov.**

MycoBank: MB828691

≡ *Caloplaca xerica* Poelt & Vězda, Mitteilungen aus der Botanischen Staatssammlung München 12: 1. 1975 (basionym).

Type: [Italy]. Südtirol: Vintschgau, Südseitige trockene, Gneishänge am Eingang in das Schlanders, Jun 1966, *J. Poelt 12073* (holotype, GZU!).

Chemistry: Chemosyndrome A; rarely anthraquinones completely absent.

***Pyrenodesmia*** A. Massal.

Fig. 3A

Type: *Pyrenodesmia chalybaea* (Fr.) A. Massal.

Diagnosis: Completely lacking anthraquinones, with Sedifolia-grey. Distribution Holarctic with biodiversity centers in the Mediterranean basin, Central Asia and arid regions of western North America. Saxicolous (mainly calcicolous).

Nomenclature: The name was resurrected by Arup et al. (2013).

Description: *Morphology and anatomy:* Thallus crustose, epi- or endolithic, white, grey or brown; cortex paraplectenchymatous, usually represented by alveolate cortex (sensu Vondrák et al., 2013), rarely well-developed and thick (known in some Central

Asian taxa); some species with vegetative propagules (blastidia, soredia, minute granules and lobules or pustulate outgrowths). Apothecia zeorine, rarely biatorine, sometimes appearing lecanorine, but a thin true exciple is always present; thalline exciple of the same colour as thallus, disc and true exciple brown, grey or black; ascospores polardiblastic with short to large long septum. Pycnidia present or absent, grey or black; conidia bacilliform to subglobose.

*Chemistry:* Thallus, apothecia and pycnidia always without anthraquinones, but with Sedifolia-grey. Unknown brown pigment (K−) sometimes present in epihymenium.

*Distribution and ecology:* Northern Hemisphere. Record from Antarctica (Øvstedal & Lewis Smith, 2001) needs conformation. Mainly in Mediterranean region, Central Asia and desert regions of western North America. There are just few taxa known in other parts of Europe, Asia and North America. The genus consists of exclusively saxicolous taxa. In Europe they grow only on calcareous outcrops (limestones and sandstones), and in Central Asia and the USA – both on calcareous and base-rich siliceous outcrops in sun-lit conditions from coast to alpine zone. *Pyrenodesmia helygeoides* often grows on acidic siliceous boulders in water.

Remarks: Currently 21 species are included in the genus, but this group is more diverse and contains many unnamed taxa (our unpublished data). We did not consider taxa that probably belong to the genus, if their taxonomic status is unclear (e.g., *Caloplaca ayachina* and *C. ochromela*). We did not study the identity of the names *C. circumalbata* var. *bicolor* (Wunder, 1974) and *Pyrenodesmia variabilis* var. *ocellulata* (Hafellner & Türk, 2016). *Pyrenodesmia duplicata* (a new combination proposed by Kondratyuk et al., 2017b), in our opinion, belongs to the genus *Kuettlingeria*, but has there an unclear taxonomic status (Redchenko et al., 2012; Motiejūnaitė et al., 2016).

***Pyrenodesmia albopruinosa*** (Arnold) S.Y. Kondr. (for details, see Kondratyuk et al., 2020a)

***Pyrenodesmia albopustulata*** (Khodos. & S.Y. Kondr.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828622

≡ *Caloplaca albopustulata* Khodos. & S.Y. Kondr., Graphis Scripta 13: 6. 2002 (basionym).

Type: Ukraine. Crimean Peninsula: Alushta district, Mt Southern Demerdji, "Dolina Prividenij", on conglomerate, 2000, *A. Khodosovtsev* (holotype, KW; isotypes, KHER, LD).

***Pyrenodesmia albovariegata*** B. de Lesd., Rev. Bryol. Lichénol., N.S. 12: 62. 1942.

Type: USA. New Mexico: Santa Fe Co., Santa Fe, Cienga Creek, 1890 m, sur roches volcanicues, 1930, *Arsène Brouard 21550* (lectotype, UPS!, selected by Wetmore 1994: 816).

Remarks: Belonging to *Pyrenodesmia* was confirmed by data on MCM7 gene (Frolov, unpublished).

***Pyrenodesmia alociza*** (A. Massal.) Arnold (for the details see Arup et al., 2013)

***Pyrenodesmia atroalba*** (Tuck.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828625

≡ *Placodium atroalbum* Tuck., Proc. Amer. Acad. Arts & Sci. 12: 172. 1877 (basionym).

Type: USA. State unknown, [river] North Platte, Rocky Mts., cretaceous sandstones, *Dr. Hayden* (lectotype, FH!, selected by Wetmore 1994: 816).

***Pyrenodesmia badioreagens*** (Tretiach & Muggia) Søchting, Arup & Frödén (for the details see Arup et al., 2013)

***Pyrenodesmia bullata*** (Müll. Arg.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828627

≡ *Callopisma bullatum* Müll. Arg., Hedwigia 31: 156. 1892 (basionym).

Type: Persien. Kuh Tscharmekam, altitude 3300 m, 1885, *Dr. Stapf* (lectotype, G 00110799!, selected by Wunder 1974: 120).

None *Placodium bullatum* Müll. Arg., Proc. R. Soc. Edinb. 11: 459. 1882.

≡ *Pyrenodesmia bullata* (Müll. Arg.) Tomin, nom. inval., Sbor. Nauchn. Trud. Akad. Nauk. Byelorussk. SSR, Inst. Biol. 1: 85. 1950.

≡ Caloplaca variabilis var. bullata (Müll. Arg.) Wunder, Biblthca Lichenol. 3: 120. 1974.

Remarks: The name *Placodium bullatum* Müll. Arg. is a basionym for *Heppsora bullata* (Müll. Arg.) Lumbsch & Mies belonging to the family Ramalinaceae (Mies & Schultz, 2004). The combination *Pyrenodesmia bullata* (Müll. Arg.) Tomin is not valid; it was mentioned as a synonym to *Caloplaca bullata* by Tomin (1950) without any comments. We failed to find any record of that combination in other papers by Tomin.

So far it is explicitly known only from the type material and we do not have any molecular data for it. Placed here on the base of morphology and chemistry (contains only Sedifolia-grey).

***Pyrenodesmia chalybaea*** (Fr.) A. Massal. (for the details see Arup et al. 2013)

Fig. 3A

***Pyrenodesmia circumalbata*** (Delile) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank MB828660

≡ *Lecidea circumalbata* Delile, Flore d'Égypte: 157 and tab. 59 (Fig. 8). 1813 (basionym).

Type: [Egypt]. Vallée de l'Égarement, *Delile* (lectotype, G 00290773!, selected by Wunder 1974: 53).

≡ *Blastenia circumalbata* (Delile) Müll. Arg., Revue mycol., Toulouse 2(2): 78 (1880).

***Pyrenodesmia concreticola*** (Vondrák & Khodos.) Søchting, Arup & Frödén (for the details see Arup et al., 2013)

***Pyrenodesmia erodens*** (Tretiach, Pinna & Grube) Søchting, Arup & Frödén (for the details see Arup et al., 2013)

***Pyrenodesmia helygeoides*** (Vain.) Arnold, Verh. zool.-bot. Ges. Wien 47: 215. 1897.

≡ *Lecanora helygeoides* Vain., Meddn Soc. Fauna Flora Fenn. 6: 148. 1881 (basionym).

Type: [Russia. Murmansk Region]: Lapponia inarensis, Köngäs [Borisoglebsky], 1878, *E. Vainio 07666* (holotype, TUR-V!).

= *Caloplaca diphyodes* auct. non Nylander (1872).

*Remarks:* We propose to use this name for most European specimens called *Caloplaca diphyodes* (see the Discussion for identity of *C. diphyodes*). The holotype of *Lecanora helygeoides* investigated by us fits the Arctic-alpine *C. diphyodes* auct. in morphology, anatomy and chemistry. A specimen Frolov 644 was collected in the Murmansk region of Russia, not far from the type locality, and it is very similar to the type. The specimen groups with Arctic-alpine specimens of *C. diphyodes* auct. in the phylogeny based on several loci (Frolov, unpublished).

***Pyrenodesmia micromarina*** (I.V. Frolov, Khodos. & Vondrák) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828641

≡ *Caloplaca micromarina* Frolov, Khodos. & Vondrák, Annales Botanici Fennici 53: 251. 2016 (basionym).

Type: Turkey. Sea of Marmara coast: Tekirdağ, in valley of small brook near Gaziköy, 40°45′21″N, 27°20′04″E, altitude 20–40 m, on stones and pebbles of calcareous sandstone, 2007, *J. Vondrák 8199* (holotype, PRA!).

***Pyrenodesmia micromontana*** (I.V. Frolov, Wilk & Vondrák) Hafellner & Türk (see Hafellner & Türk, 2016)

***Pyrenodesmia microstepposa*** (I.V. Frolov, Nadyeina, Khodos. & Vondrák) Hafellner & Türk (see Hafellner & Türk, 2016)

***Pyrenodesmia molariformis*** (I.V. Frolov, Vondrák, Nadyeina & Khodos.) S.Y. Kondr. (for details, see Kondratyuk et al., 2020b)

***Pyrenodesmia peliophylla*** (Tuck.) S.Y. Kondr. (for details, see Kondratyuk et al., 2020b)

***Pyrenodesmia pratensis*** (Wetmore) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828644

≡ *Caloplaca pratensis* Wetmore, Bryologist 112: 382. 2009 (basionym).

Type: USA. North Dakota: Oliver Co., about 3.2 km S, 6.4 km E of Hensler, The Nature Conservancy's Cross Ranch Preserve, Sangor Ghost Town, gentle slopes of mixed grass prairie, Missouri River Valley floodplain, 47°10′48″N, 100°59′42″W, altitude 515 m, on old concrete foundation, 2007, *M. K. Advaita 6100* (holotype, MIN; isotype, GZU!).

***Pyrenodesmia tianshanensis*** (Xahidin, A. Abbas & J.C. Wei) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828645

≡ *Caloplaca tianshanensis* Xahidin, A. Abbas & J.C. Wei, Mycotaxon 114: 3. 2011 (basionym).

Type: China. Xinjiang: Mt. Nan-shan in Tianshan mountain chain, Miaoergou, altitude 1280 m, on limestone, 2009, *A. Abbas & H. Xahidin 20090001* (holotype, XJU!; isotype, HMAS-L).

***Pyrenodesmia transcaspica*** (Nyl.) S.Y. Kondr. (for details, see Kondratyuk et al. 2020a)

***Pyrenodesmia variabilis*** (Pers.) A. Massal. (for the details see Arup et al., 2013)

Type: Deutschland. [Nordrhein-Westfalen]: Kalkstein zu Buren [Büren], 1856, *J. Lahm ?* (neotype, B 600011187!, selected by Wunder 1974: 97).

***Sanguineodiscus*** I.V. Frolov & Vondrák, **gen. nov.**

MycoBank: MB828647; Fig. 3C

Etymology: Included lichens often have deep red (sanguineous) apothecial discs.

Type: *Sanguineodiscus viridirufus* (Ach.) I.V. Frolov & Vondrák

Diagnosis: Apothecial disc pale to dark red with anthraquinones of the chemosyndrome A (sensu Søchting, 1997), exceptionally lacking anthraquinones and brown or black (Fig. 3C, right). True exciple often grey-black or the same color as disc. Thallus and thalline exciple with Sedifolia-grey, lacking anthraquinones. Distributed in Eurasia and Northern Africa, mainly in the Mediterranean basin and Central Asia. Saxicolous and corticolous.

Description: *Morphology and anatomy:* Thallus crustose, epilithic or epiphytic, white to dark grey; cortex paraplectenchymatous, well developed in lower part of thalline exciple, but only alveolate cortex (sensu Vondrák et al., 2013) developed in thallus; vegetative propagules not known. Apothecia zeorine, sometimes seemingly lecanorine, but thin true exciple is always present. Disc dark to pale red, but rarer some individuals have black or brown discs without anthraquinones (Fig. 3C, right). True exciple orange to red, but its outer rim often grey, darker than thallus and thalline margin. Thalline exciple of the same colour as thallus. Ascospores polardiblastic,

ellipsoid, with medium to large long septum; pycnidia often present, grey-black; conidia bacilliform.

*Chemistry:* Thallus and thalline exciple always without anthraquinones, usually with Sedifolia-grey. Epihymenium and inner rim of true exciple usually with anthraquinones. Outer rim of true exciple may contain only anthraquinones or both anthraquinones and Sedifolia-grey. Rare chemotypes with black colored apothecia (without anthraquinones, only with Sedifolia-grey) are occasionally recorded within typical populations (Fig. 3C, right).

*Distribution and ecology:* Distributed in Europe, Northern Africa and Asia, but main occurrence is in the Mediterranean basin and Central Asia. Saxicolous or corticolous. Saxicolous taxa occur on inland rain-sheltered base-rich siliceous rocks (*S. viridirufus*), seashore siliceous rocks (*S. aractinus*) in western Eurasia or on calcareous outcrops in Central Asia (*S. bicolor*). Corticolous species grow on deciduous and coniferous trees and shrubs predominantly in Mediterranean regions and Macaronesia.

Remarks: Currently four species are included in the genus, but this group is more diverse and contains unnamed taxa (both saxicolous and corticolous; Vondrák, unpublished).

***Sanguineodiscus aractinus*** (Fr.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828648

≡ *Parmelia aractina* Fr., Systema Orbis Vegetabilis (Lundae) 1: 284. 1825 (basionym).

Type: Sweden. Halland: 1825 (holotype, UPS 63456!).

Chemistry: Chemosyndrome A; rarely anthraquinones completely absent.

***Sanguineodiscus bicolor*** (H. Magn.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828649

≡ *Caloplaca bicolor* H. Magn., Lichens from Central Asia I: 132. 1940 (basionym).

Type: [China. Gansu Province]: Erh-tao-ch'uan (Nan-shan), altitude about 4100 m, 1932, *Bohlin 77 c, d* (holotype, S).

Chemistry: Chemosyndrome A.

***Sanguineodiscus haematites*** (Chaub.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828650

≡ *Lecanora haematites* Chaub., Flore Agenaise: 492. 1821 (basionym).

Type: [France]. Sur l'écorce de presque tous les arbres. CCC. Aux environs d'Agen. (type not located).

Chemistry: Chemosyndrome A.

***Sanguineodiscus viridirufus*** (Ach.) I.V. Frolov & Vondrák, **comb. nov.**

MycoBank: MB828651; Fig. 3C

≡ *Lecidea viridirufa* Ach., Lich. univ.: 204. 1810 (basionym).

Type: Helvetiae [(Switzerland)]. Ad lapides schistosos, *Schleicher 544* (holotype, H-ACH 336).

Remarks: The name refers to inland populations morphologically similar to *S. aractinus* from seashore rocks. Data from mtLSU and nucITS DNA loci suggest close relationship but separation of coastal and inland populations (Vondrák, unpublished).

Chemistry: Chemosyndrome A; rarely anthraquinones completely absent.

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Table 1. PCR primers used in this study.

| Name | Sequence (5′–3′) | Annealing temp (C) | Source |
|---|---|---|---|
| ITS 1F | CTTGGTCATTTAGAGGAAGTAA | 55 | Gardes and Bruns (1993) |
| ITS 4 | TCCTCCGCTTATTGATATGC | 55 | White et al. (1990) |
| Bt3LM | GAACGTCTACTTCAACGAG | 55 | Myllys et al. (2001) |
| Bt10LM | TCGGAAGCAGCCATCATGTTCTT | 55 | Myllys et al. (2001) |
| Mcm7-CF2 | GGTCAACGCCTACACCTG | 55 | Designed here |
| Mcm7-CR2 | GATGTCGCCACGIATCTT | 55 | Designed here |
| RPB1_191 F | ACCGTGGTATTAGGTGTGGGACTTG | 54 | Designed by F. Fernández-Mendoza |
| RPB1_1082 R | TCCATGTAGGTCGCAACGTGGAATT | 54 | Designed by F. Fernández-Mendoza |
| RPB2-CF1 | CTCTTCCAAAAGCTGACAAA | 54 or 57 | Designed here |
| RPB2-CR2 | CCCATAGCGGATTGGTAIGT | 54 or 57 | Designed here |
| nu-LSU-155-5′ | GGGTCCGAGTTGTAATTTGT | 56 | Arup et al. (2013) |
| LR5 | TCCTGAGGGAAACTTCG | 56 | Arup et al. (2013) |
| mtSSU1 | AGCAGTGAGGAATATTGGTC | 52 | Arup et al. (2013) |
| mtSSU7 | GTCGAGTTACAGACTACAATCC | 52 | Arup et al. (2013) |
| EFA_713F | GTCACCGCGATTTCATCAAGA | 58 | Designed by F. Fernández-Mendoza |
| EFA_1453 R | CCACGACGGATTTCCTTGAC | 58 | Designed by F.Fernández-Mendoza |

Table 2. Voucher information and GB accession numbers of samples included in this study; sequences with bolded accession numbers were newly obtained during this study; all samples were used in the Caloplacoideae alignment; *Pyrenodesmia* s.lat. specimens used in the *Pyrenodesmia* alignment are below the thickened line.

| Species | Locality (country, region) | Vouchers | Source | ITS | mtSSU | nucLSU | RPB1 | RPB2 | EF1a | MCM7 | TUBB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | KT29 | KT29 | KT29 | KT29 | KT29 | | | |
| *Athallia nesodes* | USA, California | DUKE s.n. | Gaya et al. 2015 | 1457 | 1495 | 1548 | 1585 | 1635 | – | – | – |
| *Blastenia* | | | | JQ30 | JQ30 | | JQ30 | | | | |
| *crenularia* | Sweden, Bohuslän | BCN s.n. | Gaya et al. 2015 | 166 | 1489 | – | 1711 | – | – | – | – |
| *Blastenia* | | | | KT29 | KT29 | KT29 | KT29 | | | | |
| *catalinae* | USA, California | DUKE s.n. | Gaya et al. 2015 | 1445 | 1477 | 1532 | 1572 | – | – | – | – |
| *Caloplaca* | | | | | JQ30 | JQ30 | | JQ30 | | | |
| *cerina* | USA, Alaska | DUKE s.n. | Gaya et al. 2015 | – | 1483 | 1549 | – | 1744 | – | – | – |
| *Caloplaca* | | | | JQ30 | JQ30 | JQ30 | JQ30 | | | | |
| *chilensis* | Chile, P.N. Fray Jorge | SGO s.n. | Gaya et al. 2015 | 1660 | 1485 | 1551 | 1709 | – | – | – | – |
| *Caloplaca* | Iceland, Sudur- | | | | JQ30 | JQ30 | JQ30 | | | | |
| *cinnamomea* | Thingeyjarsysla | DUKE s.n. | Gaya et al. 2015 | – | 1487 | 1552 | 1710 | – | – | – | – |
| *Caloplaca* | Mexico, Baja | | | | | KT29 | KT29 | KT29 | | | |
| *cinnabarina* | California | DUKE s.n. | Gaya et al. 2015 | – | – | 1538 | 1578 | 1624 | – | – | – |
| *Caloplaca* | Mexico, Baja | | | KT29 | KT29 | KT29 | KT29 | KT29 | | | |
| *conversa* 1 | California | DUKE s.n. | Gaya et al. 2015 | 1450 | 1504 | 1554 | 1589 | 1643 | – | – | – |
| *Caloplaca* | | J. Vondrák | | **MH1** | **MH1** | **MH1** | | **MH1** | **MH1** | **MH1** | **MH1** |
| *conversa* 2 | Iran, Hashtpar | 5538 (PRA) | original | **04924** | **00782** | **00750** | – | **19818** | **53698** | **19855** | **53729** |
| *Caloplaca* | | J. Vondrák | | **MH1** | **MH1** | **MH1** | | **MH1** | | | |
| *demissa* | Spain, Tenerife | 13127 (PRA) | original | **04937** | **00784** | **00752** | – | **19844** | – | – | – |
| *Caloplaca* | | P.v.d. Boom | | | | JQ30 | JQ30 | JQ30 | | | |
| *gloriae* | Spain, Almería | Herb. 38420 | Gaya et al. 2015 | – | – | 1555 | 1712 | 1752 | – | – | – |
| *Caloplaca* | Slovakia, Low Tatras | | | KT29 | JQ30 | JQ30 | JQ30 | JQ30 | | | |
| *isidiigera* | mountains | PRA s.n. | Gaya et al. 2015 | 1460 | 1492 | 1556 | 1713 | 1753 | – | – | – |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Caloplaca obscurella* | Russia, Tuapse | J. Vondrák 7641 (PRA) | original | **MH1 04938** | – | **MH1 00762** | – | – | – | – | – |
| *Caloplaca peludella* | Mexico, Baja California | DUKE s.n. | Gaya et al. 2015 | – | KT29 1505 | KT29 1555 | KT29 1590 | KT29 1644 | – | – | – |
| *Caloplaca reptans* | USA, Virginia | J. Lendemer 48186 (NY) | original | **MH1 04934** | **MH1 00796** | **MH1 00766** | **MH1 41573** | **MH1 19819** | – | – | – |
| *Caloplaca santessoniana* ad int. | Chile, P.N. Pan de Azúcar | DUKE s.n. | Gaya et al. 2015 | KT29 1463 | KT29 1508 | KT29 1558 | KT29 1592 | KT29 1647 | – | – | – |
| *Gyalolechia bracteata* | Estonia, Saaremaa | P. Alanko 121555 (H) | Gaya et al. 2015 | AF27 7666 | JQ30 1502 | JQ30 1566 | – | – | – | – | – |
| *Gyalolechia canariensis* | Spain, Canary Islands | TFC Lich: 3593, duplic. | Gaya et al. 2015 | EU63 9587 | KT29 1516 | KT29 1562 | KT29 1599 | KT29 1655 | – | – | – |
| *Gyalolechia flavorubescens* | unidentified | unidentified | Gaya et al. 2015 | AF27 9887 | AY14 3403 | AF27 9887 | – | – | – | – | – |
| *Gyalolechia stipitata* | Mexico, Baja California | DUKE s.n. | Gaya et al. 2015 | KT29 1465 | KT29 1490 | KT29 1543 | KT29 1582 | KT29 1630 | – | – | – |
| *Huea cerussata* | South America | U. Søchting s.n. | Gaya et al. 2015 | – | KT29 1519 | – | KT29 1601 | KT29 1658 | – | – | – |
| *Ioplaca pindarensis* | China, Yunnan prov. | A. Aptroot 56827 (ABL) | Gaya et al. 2015 | JQ30 1672 | – | – | JQ30 1718 | JQ30 1763 | – | – | – |
| *Kuettlingeria atroflava* 1 | USA, California | DUKE s.n. | Gaya et al. 2015 | KT29 1444 | KT29 1511 | KT29 1560 | KT29 1593 | KT29 1650 | – | – | – |
| *Leproplaca proteus* | Spain, Catalonia | BCN 13706 | Gaya et al. 2015 | EU63 9613 | KT29 1507 | KT29 1557 | – | KT29 1646 | – | – | – |
| *Leproplaca xantholyta* | Greece, Crete | T. Spribille 12970 (B) | Gaya et al. 2015 | JQ30 1670 | JQ30 1501 | JQ30 1565 | – | JQ30 1761 | – | – | – |
| *Polycauliona candelaria* | Sweden, Halland | BCN s.n. | Gaya et al. 2015 | EU63 9625 | JQ30 1528 | JQ30 1587 | JQ30 1732 | JQ30 1782 | – | – | – |
| *Pyrenodesmia chalybaea* 1 | Sweden, Gotland | BCN s.n. | Gaya et al. 2015 | JQ30 1659 | JQ30 1484 | JQ30 1550 | JQ30 1708 | JQ30 1745 | – | – | – |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rufoplaca scotoplaca* | Sweden, Bohuslän | BCN s.n. | Gaya et al. 2015 | JQ30 1665 | JQ30 1497 | JQ30 1561 | JQ30 1715 | JQ30 1756 | – | – | – |
| *Seirophora californica* | Mexico, Baja California | DUKE s.n. | Gaya et al. 2015 | KT29 1470 | KT29 1521 | KT29 1564 | KT29 1602 | KT29 1660 | – | – | – |
| *Seirophora contortuplicata* | Tajikistan | U. Søchting s.n. | Gaya et al. 2015 | KT29 1471 | KT29 1522 | – | KT29 1603 | KT29 1661 | – | – | – |
| *Seirophora lacunosa* | Spain, Alacant | BCN, herb. Gaya 391 | Gaya et al. 2015 | JQ30 1683 | JQ30 1517 | JQ30 1575 | JQ30 1724 | JQ30 1771 | – | – | – |
| *Teloschistes nodulifer* | Peru, Tarma province | DUKE s.n. | Gaya et al. 2015 | – | KT29 1524 | KT29 1566 | KT29 1605 | KT29 1663 | – | – | – |
| *Usnochroma carphinea* | Spain, Catalonia | BCN 13714 | Gaya et al. 2015 | EU63 9595 | JQ30 1482 | JQ30 1548 | JQ30 1707 | KT29 1621 | – | – | – |
| *Usnochroma scoriophila* | Spain, Almería | P.v.d. Boom Herb. 38386 | Gaya et al. 2015 | JQ30 1664 | JQ30 1496 | JQ30 1560 | JQ30 1714 | JQ30 1755 | – | – | – |
| *Variospora aurantia* | Spain, Catalonia | BCN 13326 | Gaya et al. 2015 | EU63 9602 | KT29 1479 | – | – | KT29 1616 | – | – | – |
| *Variospora cancarixiticola* | Spain, Castilla-La Mancha | MUB, holotype | Gaya et al. 2015 | EU63 9608 | KT29 1482 | – | – | KT29 1620 | – | – | – |
| *Variospora paulii* | Spain, Catalonia | BCN s.n. | Gaya et al. 2015 | EU63 9606 | KT29 1503 | – | – | KT29 1642 | – | – | – |
| *Variospora thallincola* | Sweden, Halland | BCN s.n. | Gaya et al. 2015 | JQ30 1667 | JQ30 1498 | JQ30 1563 | KT29 1595 | JQ30 1758 | – | – | – |
| *Variospora velana* | Italy, Veneto | U. Arup L07123 (LD) | Gaya et al. 2015 | KT29 1467 | KT29 1515 | – | KT29 1598 | – | – | – | – |
| *Xanthomendoza borealis* | East Antarctica, Hallett Peninsula | DUKE s.n. | Gaya et al. 2015 | KT29 1473 | KT29 1526 | KT29 1567 | – | KT29 1665 | – | – | – |
| *Xanthomendoza mendozae* | Bolivia, Potosí | DUKE s.n. | Gaya et al. 2015 | JQ30 1688 | JQ30 1523 | JQ30 1581 | – | – | – | – | – |
| *Kuettlingeria albolutescens* | Italy, Toscana | J. Vondrák 8849 (PRA) | original | **MN3 05804** | **MN3 05824** | – | **MN3 11941** | **MN3 11948** | **MN3 11926** | **MN3 11933** | **MN3 11954** |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Kuettlingeria* | | J. Vondrák | | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | | **MN3** | **MN3** |
| *areolata* | Italy, Sicily | 10843 (PRA) | original | **05805** | **05825** | **05847** | **11942** | **11949** | **–** | **11934** | **11955** |
| *Kuettlingeria* | | J. Vondrák | | **MH1** | **MH1** | **MH1** | **MH1** | | **MH1** | **MH1** | **MH1** |
| *atroflava* 2 | Greece, Meteora | 8723 (PRA) | original | **04921** | **00775** | **00744** | **41559** | – | **53700** | **19858** | **53732** |
| *Kuettlingeria* | | | | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** |
| *cretense* | Portugal | I. Frolov s.n. | original | **04925** | **00783** | **00751** | **41560** | **19821** | **53701** | **19845** | **53733** |
| *Kuettlingeria* | France, Haute-Vienne, | I. Frolov | | **MH1** | **MH1** | **MH1** | **MH1** | | **MH1** | **MH1** | **MH1** |
| *diphyodes* | topotype | 1430 | original | **04926** | **00785** | **00753** | **41561** | – | **53702** | **19846** | **53734** |
| *Kuettlingeria* | Czech Republic, | J. Vondrák | Vondrák et al. 2013 and | KC41 | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** |
| *emilii* | Pavlovské vrchy hills | 9358 (PRA) | original | 6102 | **00787** | **00754** | **41575** | **19822** | **53704** | **19847** | **53735** |
| *Kuettlingeria* | Russia, Black Sea | J. Vondrák | | **MN3** | **MN3** | | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** |
| *erythrocarpa* | coast | 6650 (PRA) | original | **05806** | **05826** | – | **11943** | **11950** | **11927** | **11935** | **11956** |
| *Kuettlingeria* | | J. Vondrák | | | **MN3** | | **MN3** | | **MN3** | **MN3** | |
| *furax* | Greece, Peloponnese | 8714 (PRA) | original | – | **05827** | – | **11944** | – | **11928** | **11936** | – |
| *Kuettlingeria* | | J. Vondrák | | | **MN3** | **MN3** | **MN3** | | **MN3** | **MN3** | **MN3** |
| *fuscoatroides* | Italy, Sardinia | 10805 (PRA) | original | – | **05828** | **05848** | **11945** | – | **11929** | **11937** | **11957** |
| *Kuettlingeria* | Ukraine, Black Sea | J. Vondrák | | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** |
| *neotaurica* | coast | 7213 (PRA) | original | **05807** | **05829** | **05849** | **11946** | **11951** | **11930** | **11938** | **11958** |
| *Kuettlingeria* | | J. Vondrák | | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** | **MH1** |
| *percrocata* | Italy, Dolomites | 4634 (PRA) | original | **04931** | **00794** | **00763** | **41563** | **19823** | **53705** | **19848** | **53736** |
| *Kuettlingeria* | | J. Vondrák | | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** | **MN3** |
| *soralifera* | France, Maritime Alps | 10813 (PRA) | original | **05808** | **05830** | **05850** | **11947** | **11952** | **11931** | **11939** | **11959** |
| *Kuettlingeria* | | in J. Vondrák | | **MH1** | **MH1** | **MH1** | **MH1** | | **MH1** | **MH1** | **MH1** |
| *teicholyta* | Ukraine, Kherson | 6943 (PRA) | original | **04935** | **00797** | **00767** | **41576** | – | **53706** | **19849** | **53737** |
| *Kuettlingeria* | | J. Vondrák | | **MN3** | **MN3** | **MN3** | | **MN3** | **MN3** | **MN3** | **MN3** |
| *xerica* | Russia, Dagestan | 14544 (PRA) | original | **05809** | **05831** | **05851** | – | **11953** | **11932** | **11940** | **11960** |
| *Kuettlingeria* | Abkhazia, NP | I. Frolov | | **MH1** | **MH1** | **MH1** | **MH1** | | **MH1** | **MH1** | **MH1** |
| sp.2 | Ritsinsky | 1456 | original | **04932** | **00786** | **00764** | **41562** | – | **53703** | **19859** | **53728** |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pyrenodesmia albopruinosa* | Italy, Verona | TSB 37658 | Muggia et al. 2008 and original | EF09 3577 | **MH1 00770** | – | **MH1 41578** | **MH1 19824** | **MH1 53708** | **MH1 19851** | KR91 2027 |
| *Pyrenodesmia albopustulata* | Turkey, Black Sea region | J. Vondrák 10463 (PRA) | Vondrák et al. 2013 and original | **MH1 04918** | **MH1 00771** | **MH1 00741** | **MH1 41564** | **MH1 19825** | **MH1 53709** | – | KC61 5301 |
| *Pyrenodesmia alociza* | Italy, Ascoli Piceno | TSB 37735 | Muggia et al. 2008 and original | EF09 0931 | **MH1 00772** | **MH1 00742** | **MH1 41587** | **MH1 19826** | **MH1 53710** | **MH1 19860** | **MH1 53739** |
| *Pyrenodesmia atroalba* | USA, Montana | T. Spribille s.n. | original | **MH1 04920** | **MH1 00774** | **MH1 00743** | **MH1 41565** | **MH1 19827** | **MH1 53711** | **MH1 19861** | **MH1 53740** |
| *Pyrenodesmia badioreagens* | Italy, Foggia | TSB 36422 | Muggia et al. 2008 and original | EF08 1035 | **MH1 00776** | **MH1 00745** | **MH1 41566** | **MH1 19828** | **MH1 53712** | **MH1 19862** | **MH1 53741** |
| *Pyrenodesmia chalybaea* 2 | Greece, Crete | J. Vondrák 4059 (PRA) | Frolov et al. 2016 and original | KC88 4498 | **MH1 00779** | **MH1 00747** | **MH1 41584** | **MH1 19830** | **MH1 53714** | **MH1 19864** | KC61 5292 |
| *Pyrenodesmia circumalbata* | Turkey, Mersin | M.G. Halici s.n. | original | **MH1 04923** | **MH1 00780** | **MH1 00748** | **MH1 41567** | **MH1 19831** | **MH1 53715** | **MH1 19865** | **MH1 53742** |
| *Pyrenodesmia concreticola* | Kazakhstan, Mangistau | J. Vondrák 9443 (PRA) | Frolov et al. 2016 and original | KC88 4506 | **MH1 00781** | **MH1 00749** | **MH1 41568** | **MH1 19832** | **MH1 53716** | **MH1 19852** | KC61 5277 |
| *Pyrenodesmia erodens* | Turkey, Kahramanmaraş | J. Vondrák 12733 (PRA) | original | **MH1 04927** | **MH1 00788** | **MH1 00755** | **MH1 41569** | **MH1 19833** | **MH1 53717** | **MH1 19853** | **MH1 53743** |
| *Pyrenodesmia helygeoides* | Switzerland, Ticino | I. Frolov 1414 | original | **MH1 04929** | **MH1 00790** | **MH1 00757** | **MH1 41570** | **MH1 19834** | **MH1 53718** | **MH1 19866** | **MH1 53744** |
| *Pyrenodesmia micromarina* | Ukraine, Black Sea coast | J. Vondrák 7236 (PRA) | Frolov et al. 2016 and original | KC61 1248 | **MH1 00791** | **MH1 00758** | – | **MH1 19835** | **MH1 53719** | **MH1 19867** | KC61 5269 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Frolov et al. | | | | | | | | |
| *Pyrenodesmia micromontana* | Russia, Southern Ural, holotype | J. Vondrák 9467 (PRA) | 2016 and original | KC34 6303 | **MH1 00792** | **MH1 00759** | **MH1 41580** | **MH1 19836** | **MH1 53720** | **MH1 19868** | KC61 5299 |
| *Pyrenodesmia microstepposa* | Czech Republic, Bohemian karst, holotype | J. Vondrák 9141 (PRA) | Frolov et al. 2016 and original | KC98 4530 | – | **MH1 00760** | **MH1 41581** | **MH1 19837** | **MH1 53721** | **MH1 19869** | KT01 3276 |
| *Pyrenodesmia molariformis* | Ukraine, Luhansk | O. Nadyeina 132 (KW) | Vondrák et al. 2013 and original | KC41 6143 | **MH1 00793** | **MH1 00761** | **MH1 41582** | **MH1 19838** | **MH1 53722** | – | **MH1 53745** |
| *Pyrenodesmia peliophylla* | USA, California | K. Knudsen 13557 (UCR) | original | **MH1 04930** | – | – | **MH1 41571** | **MH1 19839** | **MH1 53723** | **MH1 19870** | **MH1 53746** |
| *Pyrenodesmia pratensis* | USA, Wyoming | in MIN 891605 | original | **MH1 04933** | **MH1 00795** | **MH1 00765** | **MH1 41583** | **MH1 19840** | **MH1 53724** | **MH1 19871** | **MH1 53747** |
| *Pyrenodesmia tianshanensis* | China, Xinjiang, holotype | XJU 1691 | Xahidin et al. 2010 and original | GU55 2277 | **MH1 00798** | – | **MH1 41585** | **MH1 19841** | **MH1 53725** | **MH1 19872** | **MH1 53748** |
| *Pyrenodesmia transcaspica* | Kazakhstan, Mangistau | J. Vondrák 9430 (PRA) | original | **MH1 04936** | **MH1 00799** | **MH1 00768** | **MH1 41572** | **MH1 19842** | **MH1 53726** | **MH1 19873** | **MH1 53749** |
| *Pyrenodesmia variabilis* | Czech Republic, Horažďovice | J. Vondrák 5114 (PRA) | Frolov et al. 2016 and original | KC88 4500 | **MH1 00800** | – | **MH1 41586** | **MH1 19843** | **MH1 53727** | **MH1 19854** | KC61 5273 |
| *Pyrenodesmia* sp.1 | Czech Republic, Bohemian karst | J. Vondrák 9673 (PRA) | Frolov et al. 2016 and original | KC88 4525 | **MH1 00778** | – | **MH1 41579** | **MH1 19829** | **MH1 53713** | **MH1 19863** | KC98 4549 |
| *Sanguineodiscus viridirufus* | Czech Republic, Hanušovice | J. Vondrák 6702 (PRA) | original | **MH1 04919** | **MH1 00773** | – | **MH1 41574** | – | **MH1 53699** | **MH1 19857** | **MH1 53731** |
| *Sanguineodiscus bicolor* | Russia, Altai | J. Vondrák 10373 (PRA) | original | **MH1 04922** | **MH1 00777** | **MH1 00746** | **MH1 41577** | – | **MH1 53707** | **MH1 19850** | **MH1 53738** |
| *Sanguineodiscus haematites* | Ukraine, Black Sea coast | J. Vondrák 7278 (PRA) | original | **MH1 04928** | **MH1 00789** | **MH1 00756** | **MH1 41558** | **MH1 19820** | – | **MH1 19856** | **MH1 53730** |

Table 3. Characters of the genera within the *Pyrenodesmia* sensu lato clade.

| | *Pyrenodesmia* s.str. (lineage P) | *Kuettlingeria* (lineage K) | *Sanguineodiscus* (lineage S) |
|---|---|---|---|
| No. of species accepted in the paper | 21 | 14 | 4 |
| Former groups and species included | *Caloplaca variabilis* group | *Caloplaca xerica* group plus *C. cretensis* and *C. diphyodes* | *Caloplaca haematites* group plus *C. bicolor* |
| Vegetative propagules | blastidia, soredia, soredia-like minute granules, pustulate outgrowths | blastidia, soredia | not known |
| Color of apothecial disc and true exciple | brown to black | yellow-orange to dark red or brown red, exceptionally black or brown | disk pale to dark red, true exciple of the same colour or grey-black, exceptionally whole apothecium black (FIG. 3C, right) |
| Pigments | only Sedifolia-grey in thallus and apothecia | Sedifolia-grey in thallus and thalline exciple; anthraquinones in apothecial disc and true exciple (chemosyndromes A, C1, C2 and C5); in some individuals only Sedifolia-grey in apothecia | Sedifolia-grey in thallus, thalline exciple and sometimes in true exciple; anthraquinones in apothecial disc and sometimes in true exciple (chemosyndrome A); in some individuals only Sedifolia-grey in apothecia |
| Geography | Holarctic; three biodiversity centers – Mediterranean basin, Central Asia and arid regions of western North America | Holarctic; one biodiversity center – Mediterranean basin | Eurasia and Northern Africa; mainly in Mediterranean basin and Central Asia |
| Ecology | saxicolous; mainly calcareous (rarely base-rich siliceous) outcrops in xerothermic sun-lit conditions; exceptionally on acidic outcrops in wet conditions | saxicolous; mainly calciferous (sometimes pure limestone) outcrops in xerothermic sun-lit conditions; exceptionally on acidic outcrops in wet conditions | saxicolous and corticolous; mainly calciferous (rarely pure limestone) outcrops or bark of different trees and shrubs in xerothermic sun-lit conditions |

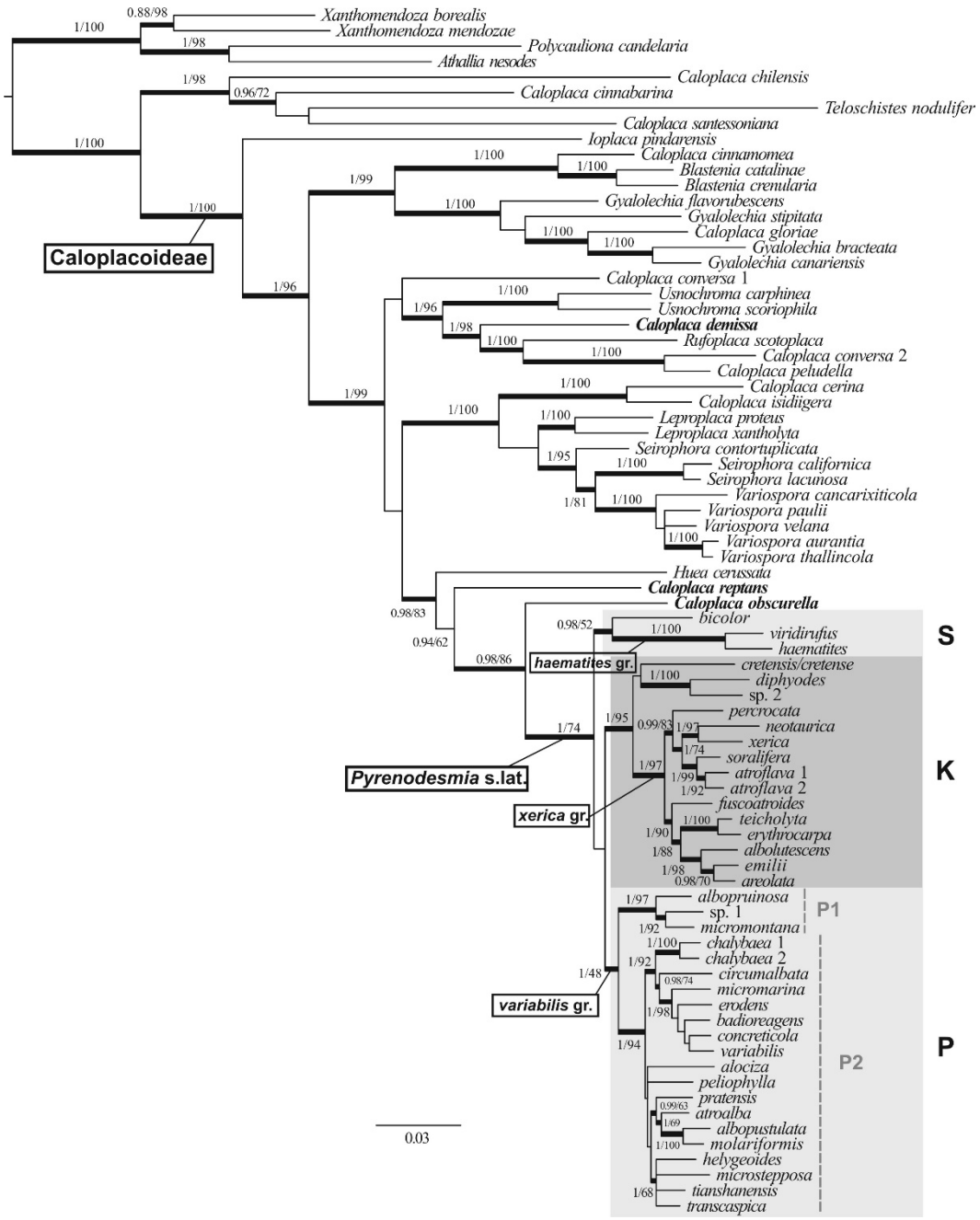



Fig. 1. Position and content of *Pyrenodesmia* s.lat. within the Caloplacoideae clade. Bayesian phylogeny of the concatenated dataset of five loci: ITS, mtSSU, nucLSU, RPB1 and RPB2. Bayesian posterior probabilities (values ≥ 0.90) and bootstrap supports from the maximum likelihood analysis (after slashes; values ≥ 70) are shown above branches. Branches supported at least in one analysis are thickened. *Caloplaca demissa*, *C. obscurella* and *C. reptans* are in bold. K, the genus *Kuettlingeria*; P, the genus *Pyrenodesmia* s.str.; S, the genus *Sanguineodiscus*.

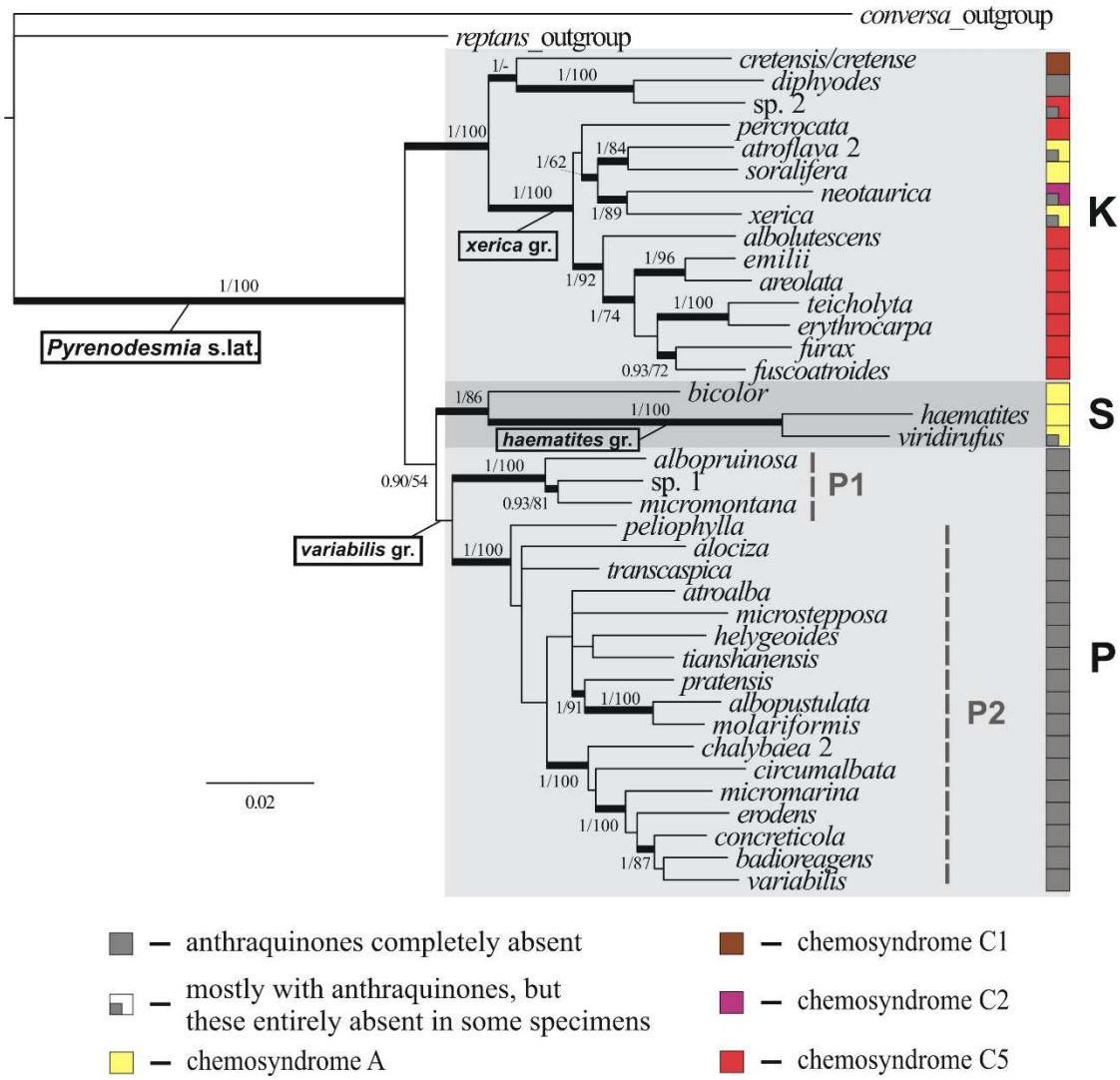



Fig. 2. The clade of *Pyrenodesmia* s.lat. Bayesian phylogeny of the concatenated dataset of eight loci: EF1a, ITS, MCM7, mtSSU, nucLSU, RPB1, RPB2 and TUBB. Bayesian posterior probabilities (values ≥ 0.90) and bootstrap supports from the maximum likelihood analysis (after slashes; values ≥ 70) are shown above branches. Branches supported at least in one analysis are thickened. K, the genus *Kuettlingeria*; P, the genus *Pyrenodesmia* s.str.; S, the genus *Sanguineodiscus*.

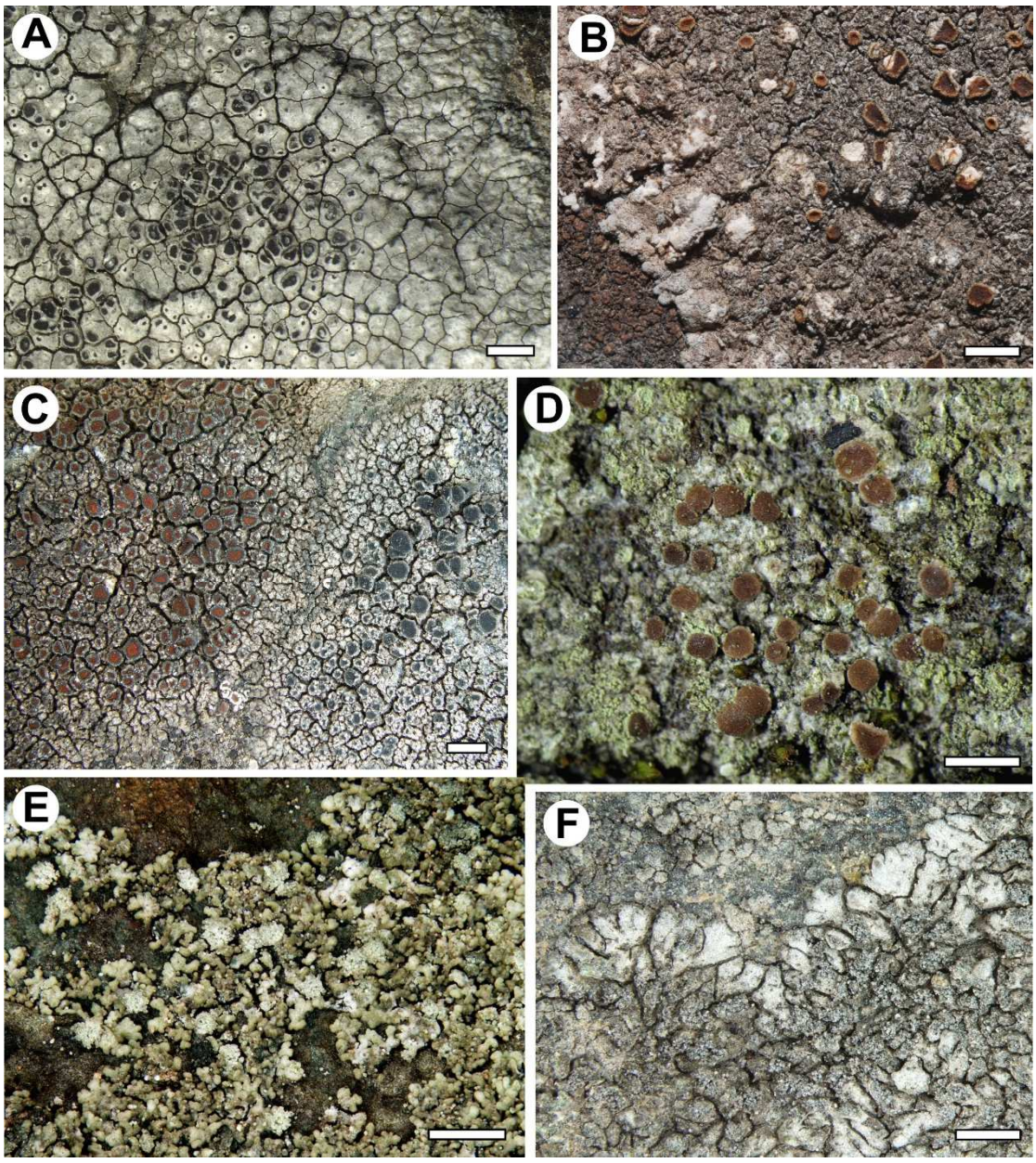



Fig. 3. Representatives of the genera in *Pyrenodesmia* sensu lato (A–C) and the species indicated to be outside *Pyrenodesmia* sensu lato (D–F). A, *Pyrenodesmia chalybaea* (PRA Vondrák 9686). B, *Kuettlingeria teicholyta* (holotype of *Blastenia visianica*, VER, photo by U. Arup). C, *Sanguineodiscus viridirufus*, thallus with red apothecia with anthraquinones on the left and thallus with black anthraquinone-lacking apothecia on the right (PRA Vondrák 9600). D, *Caloplaca obscurella* (PRA Vondrák7641). E, *Caloplaca reptans* (NY Lendemer 48186). F, *Caloplaca demissa* (PRA Vondrák19188). All scales: 1 mm.

**Supplementary Material**

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12717/suppinfo:

Table S1. Subsets of partitions and the best substitution models per subset for the Caloplacoideae and the *Pyrenodesmia* alignments: PartitionFinder results.

| **Caloplacoideae alignment** | | |
|---|---|---|
| *Subset* | *Best Model* | *Partition names* |
| 1 | GTR+I+G | ITS1, ITS2 |
| 2 | GTR+I+G | 5.8S, nucLSU |
| 3 | GTR+I+G | RPB2_codon position2, RPB1_exon2_codon position2, RPB1_exon1_codon position2, RPB1_exon1_codon position1, mtSSU, RPB2_codon position1, RPB1_exon2_codon position1 |
| 4 | GTR+I+G | RPB2_codon position3, RPB1_exon1_codon position3, RPB1_exon2_codon position3, RPB1_intron2, RPB1_intron1 |
| ***Pyrenodesmia* alignment** | | |
| *Subset* | *Best Model* | *Partition names* |
| 1 | GTR+I+G | EF1a_intron, RPB1_intron1, TUBB_intron1, ITS1, ITS2, EF1a_exon2_codon position2, EF1a_exon1_codon position3 |
| 2 | GTR+I+G | RPB1_exon2_codon position1, RPB2_codon position1, EF1a_exon1_codon position1, EF1a_exon1_codon position2, TUBB_exon2_codon position2, MCM7_codon position1, TUBB_exon1_codon position1, nucLSU mtSSU, 5.8S, EF1a_exon2_codon position1, MCM7_codon position2, EF1a_exon2_codon position3, RPB1_exon1_codon position2, |
| 3 | GTR+I+G | TUBB_exon2_codon position1, RPB2_codon position2, RPB1_exon2_codon position2, TUBB_exon1_codon position2, RPB1_exon1_codon position1 |
| 4 | GTR+G | TUBB_exon1_codon position3, TUBB_exon2_codon position3, MCM7_codon position3, TUBB_intron2 |
| 5 | SYM+G | RPB1_exon1_codon position3, RPB2_codon position3, RPB1_exon2_codon position3, RPB1_intron2 |

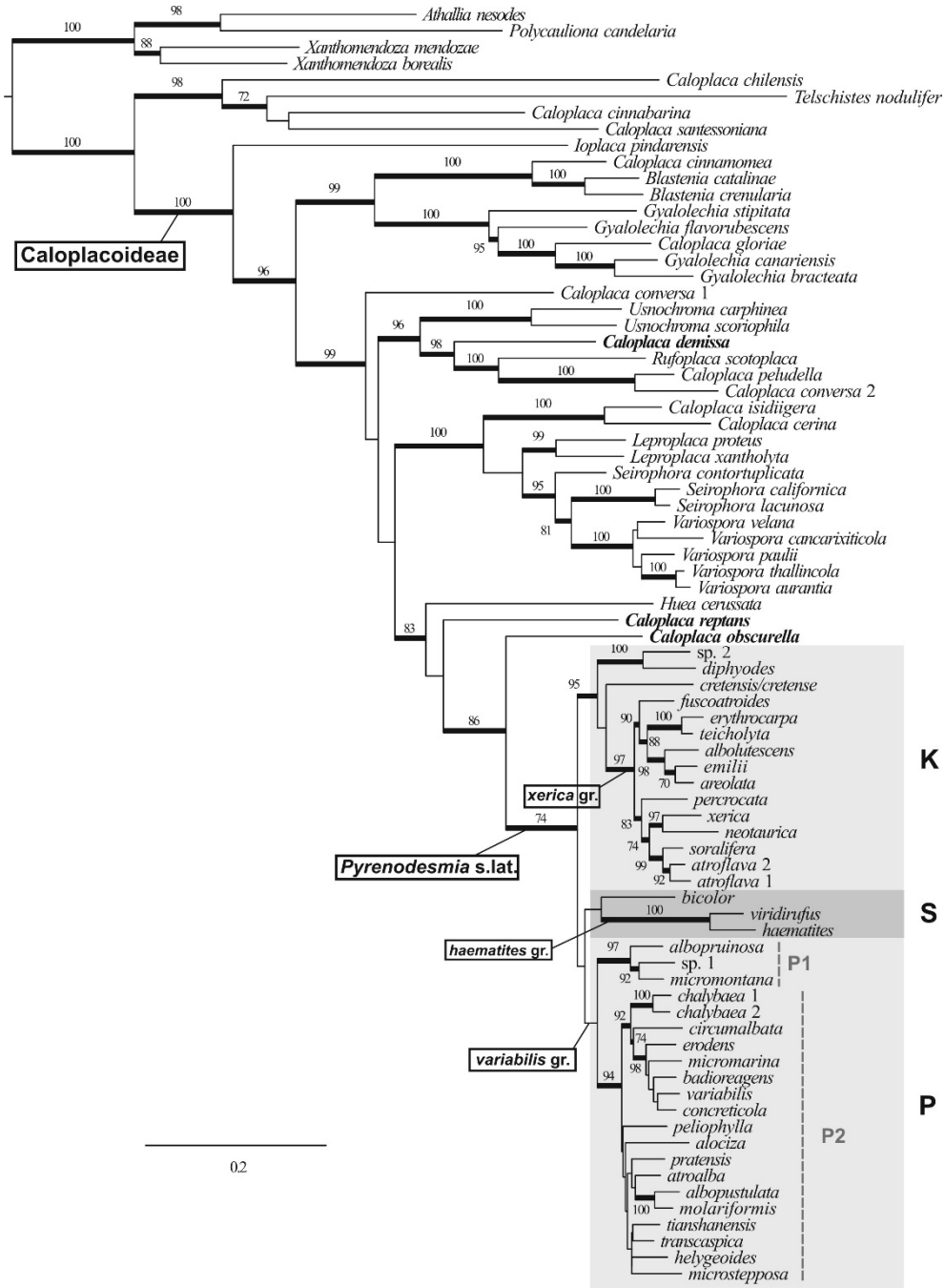



Fig. S1. Position and content of *Pyrenodesmia* s.lat. within Caloplacoideae clade. Maximum likelihood phylogeny of the concatenated dataset of ITS, mtSSU, nucLSU, RPB1 and RPB2 loci. Numbers at branches represent bootstrap values ≥ 70%. Branches with bootstrap values ≥ 70% are thickened. *Caloplaca demissa*, *C. obscurella* and *C. reptans* are in bold. K, the genus *Kuettlingeria*; P, the genus *Pyrenodesmia* s.str.; S, the genus *Sanguineodiscus*.

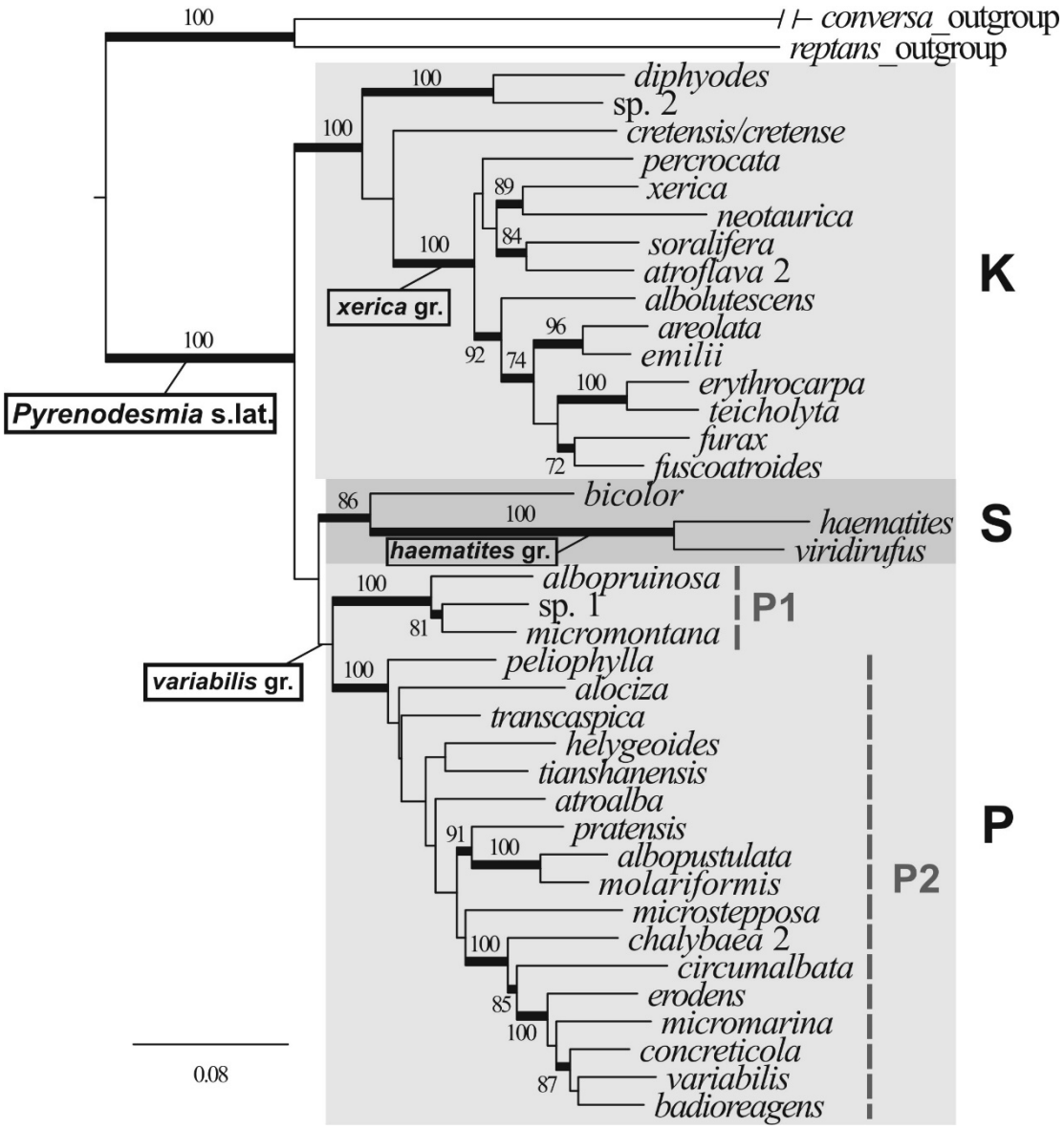
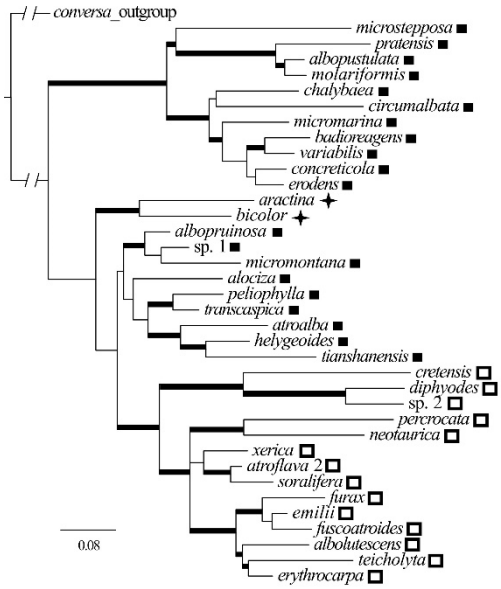
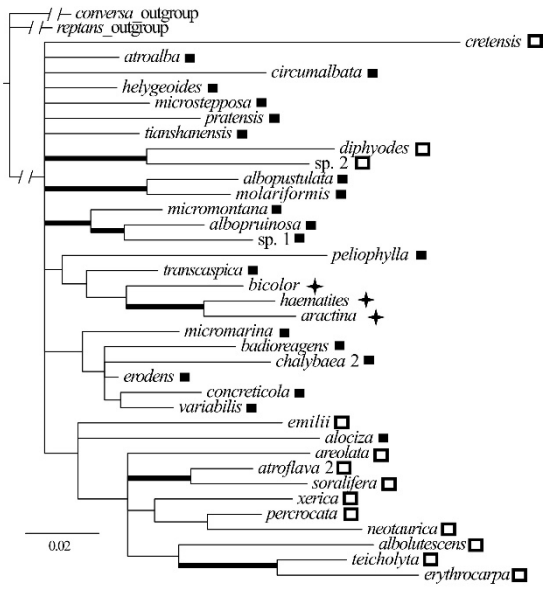


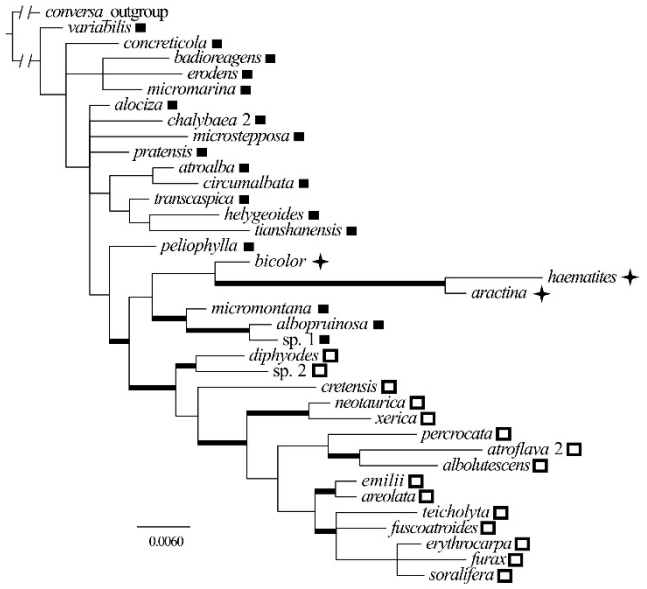

Fig. S2. The clade of *Pyrenodesmia* s.lat. Maximum likelihood phylogeny of the concatenated dataset of EF1a, ITS, MCM7, mtSSU, nucLSU, RPB1, RPB2 and TUBB loci. Numbers at branches represent bootstrap values ≥ 70%. Branches with bootstrap values ≥ 70% are thickened. Abbreviations: K, the genus *Kuettlingeria*; P, the genus *Pyrenodesmia* s.str.; S, the genus *Sanguineodiscus*.



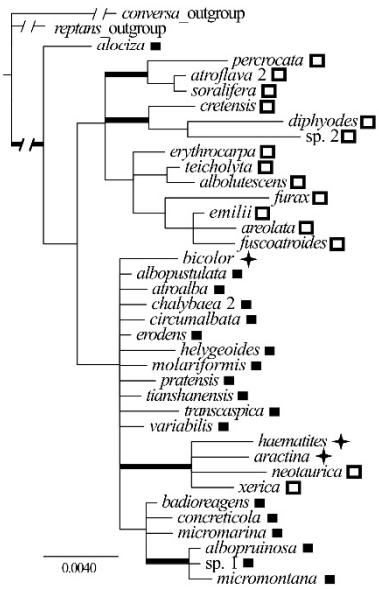

**EF1a**




**ITS**




**MCM7**




**mtSSU**

**A** □ – lineage K ***Kuettlingeria*** ■ – lineage P ***Pyrenodesmia*** ✦ – lineage S ***Sanguineodiscus***

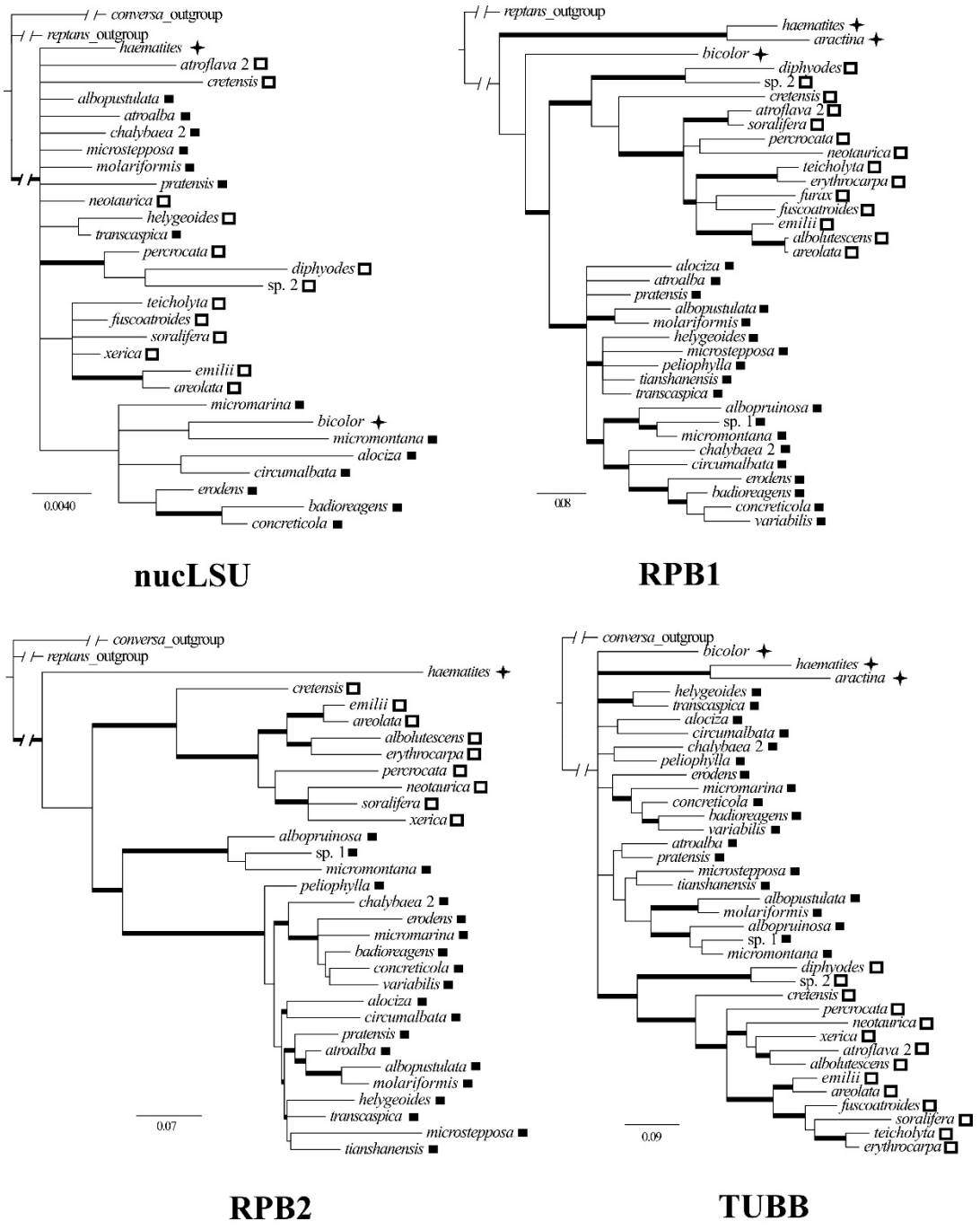



Fig. S3. The clade of *Pyrenodesmia* s.lat. Bayesian single gene phylogeny reconstructions. A, EF1a, ITS, MCM7, mtSSU. B, nucLSU, RPB1, RPB2, TUBB. Branches with posterior probabilities ≥ 0.95 are thickened.

## 5.2 Paper 2

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# Methods for phenotypic evaluation of crustose lichens with emphasis on Teloschistaceae

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## Abstract

We present methods for phenotype evaluation of crustose lichens, which enhances the chances to identify highly similar taxa using anatomical and morphological features. The basic idea is to evaluate characters with regard to completeness and reproducibility. We composed a list of about 100 basic characters and propose standardized ways of measuring them. We also present a list of morphological and anatomical terms with their standardized meaning. Basic guidelines for collecting, preparing and measuring lichen material are described. The proposed phenotypic evaluation process has two levels: (1) a pilot study is carried out on a restricted number of samples, but all available characters are evaluated; (2) a detailed study of a large number of samples that includes only potentially diagnostic features that are chosen from the pilot study according to their discriminatory weight.

**Keywords:** character evaluation, lichen anatomy, morphology, phenotype description, standardization

## Introduction

It is remarkable that, although the morphology and anatomy of lichens has been studied for over two hundred years, rather little attention has been paid to methodology. Morphological descriptions by some modern authors differ little from those made by lichenologists in the 19th century. These traditional descriptions are often incomplete (some characters are not considered), inadequate (e.g. the precise status of measurements is not specified) and not readily reproducible (methods and terminology

not fully specified). There are a few studies in which methods are discussed in detail (Ekman 1996, Printzen 1995), but they are unusual.

One unfortunate consequence of this lack of attention to details can be unjustified claims that two phylospecies cannot be distinguished by their phenotype. However, careful phenotypic evaluations can sometimes distinguish such species. Indeed, in a species-rich and taxonomically difficult group like Teloschistaceae, lack of attention to detail is likely to lead only to taxonomic confusion.

In the course of our work on crustose lichens in the Teloschistaceae, it became apparent that there was a need for some standardization of methods in the morphological investigations of these lichens. We have designed such a standard approach, and it is described below. Our priorities were to ensure completeness (all meaningful characters should be evaluated) and reproducibility (different workers should obtain similar results from any single specimen). We consider that these goals can be obtained, though at a price: our proposed methods are fairly time-consuming.

Some of our methods may be inapplicable to some other groups of lichens, and some of the comments we make below are not necessarily true for other lichen groups. However, we consider that work on most other groups of lichens would also benefit from some standardization.

The following text first defines some morphological and anatomical terms. (It also lists some synonymous terms that we prefer not to use). This is followed by some basic guidelines, which we consider fundamental. Next, we discuss the proposed methods for sample preparation. Then we discuss the evaluation of particular morphological and anatomical structures, and basic methods for the identification of pigments. Finally we discuss some matters concerning process of study, measurement accounts and databases.

**Glossary of morphological and anatomical terms in crustose lichens**

*Adnate apothecia*: slightly raised above thallus surface and slightly constricted at the base.

*Algonecral medulla*: hyaline, paraplectenchymatous tissue below the algal layer, formed by thin-walled fungal cells among dead algal cells or gaps created after the death of algal cells (fig. 2A in Vondrák et al. 2008b).

*Alveolate cortex*: "false cortex" formed by living isodiametric fungal cells among dead algal cells or gaps after dead algal cells (fig. 1D in Vondrák et al. 2009). (The term *phaenocortex* means cortical tissue of dead algal and fungal cells (Ryan et al. 2002), but this kind of tissue probably does not occur in crustose Teloschistaceae.)

*Amphithecium*: see *thalline exciple*.

*Areoles*: thallus units attached to the substrate by the entire lower surface; horizontal outline angular or rounded, ± isodiametric; areoles may be separated from

each other (scattered) or forming small separated groups or they may be adjacent (contiguous areolate thalli). Other definitions and meanings of areoles exist (e.g. Tønsberg 1992), but our definition is suitable for studies on the morphology of Teloschistaceae.

*Ascospore septum (width)*: width of cell wall in partition of 1-septate or polarilocular ascospores (figs 4–6); some authors (e.g. Wetmore 1994, 1996) use the term isthmus, but it logically refers to the thin cytoplasmatic channel within the septum. The septum has also been denoted as "equatorial thickening" or "equatorial wall-thickening" (e.g. Gaya 2009, Navarro-Rosinés 2000) but we think these terms are confusing as it may as well be used for the thick waist occurring in citriform ascospores of the *C. thallincola* group.

*Biatorine apothecia*: apothecia with well-developed or reduced true exciple, but strongly reduced or lacking thalline exciple (fig. 3). Our understanding of this term follows e.g. Wirth (1995), but differs from the definition in Smith et al. (2009). "Lecideine apothecia" are similar but have black, carbonized exciples (sensu Wirth 1995), but this term should probably not be used within Teloschistaceae since we do not know of any species with this kind of margin.

*Blastidia*: thallus outgrowths containing both algal cells and fungal hyphae; (typically c. 50– 100 µm wide, i.e. larger than typical soredia but smaller than typical isidia); rounded, upward elongated or irregularly shaped; rarely branched; ± constricted at base; not aggregated (i.e. not forming structures like soralia); without true cortex, but thin alveolate cortex may be present. This term is sometimes used for isidia with constricted bases (e.g. Tønsberg 1992), but our definition is more practical for Teloschistaceae.

*Bullate thallus*: formed of convex, seemingly inflated areoles with ± rounded horizontal outline.

*Cryptolecanorine apothecia*: a lecanorine apothecium with a disk deeply recessed in the thallus surface and an indistinct thalline rim barely differentiated from the surrounding thallus as a low bulge; in the strict sense this term only applies to immersed lecanorine apothecia but these are frequently not reliably distinguished from immersed lecideine or immersed biatorine apothecia (LIAS glossary: http://glossary.lias.net/wiki/Main_Page). We prefer to avoid this unclear term.

*Consoredia*: formed by two or more regularly rounded soredia in a larger and usually irregularly shaped aggregate where individul soredia have not broken up; similar to blastidia, but originating from soralia together with individual soredia.

*Diffuse (thallus margin)*: thallus gradually decreasing in thickness towards its margin, usually surrounded by the prothallus.

*Epihymenium*: the uppermost layer of the hymenium, between tips of asci and tips of paraphyses (fig. 1 in Printzen 1995); its width is counted as "hymenium height or

thickness – height of asci". (Some authors use the term epithecium but it is confusing; see Bungratz 2002.)

*Epinecral layer*: layer of amorphous organic matter of lichen origin on the cortex surface.

*Epithecium*: See *epihymenium*.

*Fruticulose thallus*: formed of lobes detached from substrate and often erect, or formed of extended and branched isidia. (The definition by Ryan et al. (2002), "dwarf-fruticose thallus, a smaller form of a fruticose thallus", does not reflect the situation in Teloschistaceae, where fruticulose morphotypes are mainly secondarily recruited from crustose lichens.)

*Glutinized cell wall*: walls of neighboring cells swollen and conglutinated (fig. 2A, B, in Søchting et al. 2002).

*Granules (in thallus morphology)*: vegetative diaspores in crustose thalli resembling globose isidia in shape and size, but the distinction between granules and surrounding surface is less pronounced than in isidia; granules often form the entire thallus; in this case they do not originate from the thallus surface.

*Immersed apothecia*: not raised above thallus surface.

*Isidia*: thallus outgrowths, usually more than 100 µm wide; usually globose, upward elongated, or peltate, rarely coralloid; covered by a distinct alveolate cortex or true cortex.

*Isthmus*: the thin cytoplasmatic channel within the septum. (The term has also been used incorrectly to denote ascospore septum.)

*Lecanorine apothecia*: apothecia with well-developed thalline exciple (fig. 3); true exciple reduced or absent. Most definitions only stress the presence of the thalline margin (e.g. Hensen, Jahns 1974, Ryan et al. 2002; Smith et al. 2009) and absence of true exciple is suggested. In Teloschistaceae we have not observed apothecia entirely lacking a true exciple, however true exciple is strongly reduced in some groups; we call such apothecia also lecanorine (see fig. 26 in Bungratz 2002). Lecanorine margin in immersed apothecia is sometimes called "cryptolecanorine".

*Leprose thallus*: formed of tiny granules (< 100 µm diam), not divided into thallus units. According to Lendemer et al. (2008), the thallus in a leprose species does not consist of soredia, but of granules.

*Lobes*: units of crustose thallus with non-isodiametric horizontal outline (fig. 8); usually at thallus margin and elongated outwards from the thallus center.

*Medulla*: tissue situated below algal layer or below algonecral medulla; usually formed of loose prosoplectenchyma; commonly filled by extracellular crystals of calcium salts.

*Paraplectenchyma*: tissue (sometimes called "false tissue") formed of ± isodiametric cells (length / width ratio in most cells < 2).

*Parathecium*: See true exciple. Placodioid thallus: rosette-form crustose thallus with lobes at the margin.

*Polarilocular ascospores*: bicellular with the two cells separated by a thickened, centrally perforated septum (fig. 4).

*Proper exciple*: See *true exciple*.

*Prosoplectenchyma*: tissue (sometimes called "false tissue") formed of cells distinctly longer than wide (length / width ratio in most cells > 2); ideally formed of long and thin hyphae.

*Pseudolecanorine apothecia*: indistinctly lecanorine, i.e., apothecia that have a poorly developed thalline margin that is often only poorly delimited by a cortex and generally lacks photobiont cells (LIAS glossary: http://glossary.lias.net/wiki/Main_Page) We prefer to avoid this indefinite term.

*Pycnidial tops*: spots on the thallus surface around pycnidial ostioles; these spots often have more intense pigmentation than the surrounding thallus surface; they may also contain different pigments than the surrounding thallus.

*Scleroplectenchyma & scletoprosoplectenchyma*: terms used for tissues with thick-walled cells (e.g. Ryan et al., 2002). We prefer to call them "thick-walled para- and prosoplectenchyma".

*Sessile apothecia*: raised above thallus surface and distinctly constricted at the base; apothecia with strongly constricted base may be called "sessile with incised base".

*Soralia*: areas where the surface of the thallus is eroded and where soredia are produced from the algal layer; they are usually well-delimited by surrounding thallus surface.

*Soredia*: ± globose vegetative diaspores containing both algal and fungal cells, produced in groups in soralia from the algal layer; not enclosed in cortex/alveolate cortex; may form groups – consoredia (see above).

*Squamules*: thallus units detached from substrate at the sides or at least at one side; horizontal outline ± rounded, isodiametric.

*Stalked apothecia*: raised above thallus surface, with the base prolonged into the stalk. Subhymenium: thin layer between hymenium and hypothecium consisting of ascogeneous hyphae (fig. 1 in Printzen 1995); we do not recognize this layer in Teloschistaceae.

*Suppressed apothecia*: slightly raised above thallus surface but not constricted at the base.

*Thalline exciple*: apothecial margin formed of tissue with fungal and photobiont cells; with or without cortex; (Some authors use the term amphithecium (see fig. 1 in Wetmore, 1994).

*Thallus unit*: single areole, squamule or lobe.

*True exciple*: inner apothecial margin formed of tight fungal tissue and lacking photobiont cells. ("proper exciple" or "parathecium" are synonyms for the true exciple.)

*Vegetative diaspores (=vegetative propagules)*: all parts of the thallus, which act as the reproductive units for both fungal and algal partners simultaneously; mainly soredia, blastidia, isidia and granules. (Conidia are not considered here, as they do not contain algal cells.)

*Zeorine apothecia*: apothecia with both true and thalline exciple (fig. 3); extent of true vs. thalline exciple is sometimes hard to assess externally and must be studied on vertical sections.

**Basic guidelines**

(1) Whenever material is collected for a taxonomical study, a rich sample should be taken to include, as far as possible, young as well as well-developed thalli and any aberrant morphotypes (e.g. lichens affected by snail grazing, lichenicolous fungi or trampling). Even if the sample is collected only for identification we would recommend the gathering of a rich sample if possible. Clearly, this rule should not be followed when collecting rare or endangered lichens.

(2) When collecting material, ecological and geographical characters should be noticed precisely; i.e. locality data, altitude, habitat, substrate (type of bedrock, species of phorophyte, etc.), light conditions, humidity of the micro-site and the exposure to rainwater (see the list of characters in the end of the descriptive part). Adjacent dominant lichens should be also noticed. This kind of information may be as characteristic of a species as details of anatomy and morphology.

(3) In our experience, samples that at first appear to contain only one species sometimes contain two or more taxa with similar phenotypes. Molecular barcoding (ITS sequences are used in Teloschistaceae) should be done at least from anomalous morphotypes in morphologically or anatomically heterogeneous samples. If molecular barcoding is not available, then all ± homogeneous parts of the heterogeneous samples should be evaluated separately.

(4) Only well-developed and representative material should be used for measurements of phenotype characters. For instance, specimens that are obviously young, suffering from fungal infection or grazed should be omitted. Measurements should not be taken from apothecia without spores or producing only deformed spores, as they are apt to be unreliable. In other words, apothecial characters should be measured only from apothecia containing well-developed ascospores. Apothecial characters may be roughly appraised from

apothecia without well-developed spores, but these observations are of limited value.

(5) Vertical sections should be made through the mid-point of apothecia when observing and measuring the thickness of hypothecium and hymenium and the width of the thalline exciple and true exciple. The further the section is from the mid-point, the more the measurements will deviate from the value at the mid-point (fig. 1). Sections must be made accurately perpendicular to the disc of the apothecium; this is crucial for reliable observations of apothecial tissues (true excipular hyphae, paraphyses and asci), which are mostly arranged in that plane.

**Fresh material versus herbarium material**

Baral (1992) prefers the "vital taxonomy" in Ascomycetes; using living (fresh) material for observation of vital characters (e.g. hemiamyloidity, presence of croziers in the subhymenium, ultrastructural characters), which disappear or cannot be observed in dead material. Fresh material may be used also for most of the observations within Teloschistaceae, but we found it more practical to work with "stabilized" herbarium material dried out for at least several months. (1) Tissues in our group do not change as dramatically with age of storage as demonstrated by Baral (1992). We have observed only indistinct shrinking of mycobiont cells or expanding of their walls in dead lichens. (2) Fresh material often contains only fresh and vital ascospores, which do not show one of the important characters – the width of septa (see below). (3) Fresh material is available for only a short time, but herbarium material is available for years – this enables reproducibility of phenotype evaluations. It is true that living material is essential for observation of a few characters (e.g. hemiamyloidity, presence of croziers in the subhymenium, ultrastructural characters) which disappear or cannot be observed in dead material (Baral, 1992), but we feel that the advantages of herbarium material outweigh any disadvantages.

Some characters do change in storage. While fungal cells usually do not change in shape and width of their cell-walls, algal cells strongly fade and somewhat deform (shrink) after some years as their protoplast becomes much smaller. In old material, it may be difficult to distinguish between the real algal layer and tissues without living algal cells (alveolate cortex or algonecral medulla). Oil droplets in the paraphyses may also disappear after some years. The colours of apothecia and thalli may change in old herbarium material as a result of microbial activity (usually when samples are inadequately stored in places with periodically increased humidity). However, when we investigated more than 100 year-old samples from various herbaria, the colours of thalli and apothecia often appeared to be unchanged or only slightly changed.

**Evaluation of particular structures**

*Tissues*

Yoshimura and Shimada (1980) recognized eight tissue types, when investigating seven unrelated lichen species. Although Gaya (2009) adopted this classification, it seems to be overcomplicated for morphological evaluations in Teloschistaceae. We propose an alternative simpler classification into four categories based on the shape and the width of cellwalls: (1) paraplectenchyma (of ± isodiametric cells; most cells with an aspect ratio less than two) with thin cell-walls (1 μm); (3) prosoplectenchyma (of long cells, with an aspect ratio more than two; ideally of long and thin hyphae) with thin cell-walls (1 μm). In prosoplectenchymatous tissues, we must further recognize between tissue of irregularly arranged hyphae (intricate type) and tissue of parallely arranged hyphae (palisade type). Globose cells (common in Teloschistaceae) or angular cells (very rare) may be recognized in paraplectenchyma (fig. 17 in Ryan et al. 2002). Tissues are further classified as loose or dense.

The diameter of a cell is to be understood as including its walls, but not any gelatinous envelope or extracellular crystalline sheet (if present). In tissues with thick-walled cells, the width of cell-walls should be also noted. Cell-walls in thick-walled tissues are often glutinized (see the dictionary) and it can be impossible to recognize the boundaries between the cellwalls of two adjacent cells or hyphae; in this case, the diameter of cells may be measured as the diameter of cell lumina. The cell-wall thickness has been proven to be an important character in some Teloschistaceae (e.g. Søchting et al., 2002).

Gradual transitions between tissue types are not uncommon; e.g. lower part of true exciple is usually prosoplectenchymatous, but its uppermost part is usually almost paraplectenchymatous (fig. 2). The cortex of some Teloschistaceae may be paraplectenchymatous in its upper part and gradually change into prosoplectenchymatous in the lower part. In such complicated situations, both types of tissues should be characterized separately with the note that they grade into each other.

*Size and height of apothecia*

The size of the apothecia is considered an important character in some groups of Teloschistaceae (e.g. Navaro-Rosinés, Hladun 1996, Vondrák et al. 2012a). Young apothecia are of ± the same size in samples of various crustose Teloschistaceae (c. 0,2–0,3 mm diam.) and the boundary between apothecial primordia and young apothecia is imprecise. While in some species mature apothecia stay small (1 mm). Thus we prefer to select larger (mature) apothecia in all examined samples for measurements. Samples without mature apothecia should not be measured. As a practical matter, this can be done simply by excluding samples with apothecia lacking ripe ascospores. The same rule applies to measurements of apothecial height, because young apothecia are usually low and adnate even in lichens in which they are sessile or stipitate when mature.

The height of apothecia is a little used character but it helps in some situations, e.g. *Caloplaca ferrugineoides* with ±stipitate apothecia differs from other taxa of the *Caloplaca holocarpa* group with sessile apothecia (Vondrák et al. 2012). Differences in height of apothecia are also sometimes observed between similar taxa with sessile apothecia, e.g. *Caloplaca ferruginea* versus *C. hungarica* (our unpublished data). Attachment of apothecia to the thallus is also a notable character; we propose to use six categories (after fig. 5 in Foucard 2001; descriptions in the dictionary above): (1) immersed in the thallus, (2) suppressed, (3) adnate, (4) sessile, (5) sessile with incised base, (6) stalked apothecium. Such a fine scale may be useful in distinguishing between similar taxa, e.g. *Caloplaca variabilis* s.lat. (2–5) and *C. chalybaea* (1–4).

In species with very high apothecia, the medulla may be very high below the apothecial disc (often several times; fig. 3 in Steiner, Poelt 1982). In this case, height of medulla below apothecia must be measured as a separate character from height of medulla in thallus.

*Apothecial disc*

The diameter of the disc need not be measured: it is calculated as "diameter of apothecium – minus widths of (both) margins". The colour of the disc reflects the presence of pigments in the epihymenium or/and presence of a pruina on the disc surface. The colour of the disc and the whole apothecia is strongly influenced by light conditions (e.g. normally orange apothecia are often pale yellow in strong shade), but it is an important diagnostic character in some groups (e.g. Søchting et al. 2008). Apothecia are basically yellow, orange or red (when with anthraquinones) or grey, brown or black (when without anthraquinones), but they may be covered by a pruina, which may be rusty orange, brown, olive or white (in apothecia with anthraquinones) or white (in apothecia without anthraquinones). Anthraquinone containing apothecia may also turn black in some species. The "natural blackening" of apothecia must be distinguished from darkening due to the presence of melanins in cell-walls of lichenicolous fungi (especially hyphomycetes). These, if present, are usually easily seen under the stereomicroscope as dark dots, spots or networks on the apothecial surface.

*True and thalline exciple*

Three basic types of apothecial margins occur in Teloschistaceae: (1) biatorine, (2) lecanorine and (3) zeorine - definitions are in the dictionary above. Characters of the apothecial margins are very important; e.g. lecanorine apothecia are diagnostic for the *Caloplaca cerina* group (Šoun et al. 2011) and biatorine apothecia for e.g. *Caloplaca nubigena* complex (fig. 5B in Wilk 2012) and *C. oleicola* (Vondrák et al. 2010). However, most of Teloschistaceae have zeorine apothecial margin, where the ratio of true vs. thalline exciple may vary strongly within a single taxon (e.g. Poelt, Wunder 1967).

We propose to measure the width of true and thalline exciple in the direction precisely parallel to the main thallus surface (e.g. in a horizontal direction if the thallus is growing on a horizontal substrate; fig. 3, black arrows). It means that the width of the true and thalline exciples gives us the total excipulum width in a natural view from above. The basic rule 5 (above) must be maintained. In some cases, especially when the thalline exciple cannot be veiwed below the true exciple when observed from above, the width of the thalline exciple may be alternatively measured in the direction perpendicular to the outer surface of the exciple, approximately at the mid point of apothecial height (fig.3, grey arrows).

The colour of the apothecial margin reflects pigmentation in the uppermost true exciple and the outer thalline exciple. The colour of both types of exciples may differ considerably and should be assessed separately, e.g. in the *Caloplaca xerica* group (Vondrák et al. 2012), the true exciple is in shades of yellow-orange and the thalline exciple is in shades of grey or white pruinose. Both types of exciples in zeorine apothecia have very similar colour in some Teloschistaceae, e.g. the *C. citrina* group (Vondrák et al. 2009), and cannot be clearly distinguished on the surface and the apothecia appear biatorine. In these cases the widths should be measured on vertical sections (not simply assessed in a stereomicroscope).

In the vertical section, zeorine apothecia in Teloschistaceae are more or less similar to fig. 2. The true exciple has rather isodiametric cells in the uppermost part, but its lower parts are formed by palisade prosenchyma which disappears below the hypothecium. Some species (e.g. *Caloplaca irrubescens*, Wetmore 2003), however, have a true exciple largely formed by paraplectenchyma. The presence or absence of a cortex in the thalline exciple may also provide important information, as well as its width, position, extent and structure when present (e.g. Giralt et al. 1992, Vondrák et al. 2012).

*Hymenium and hypothecium*

The height (or thickness) of the hymenium and hypothecium are usually only used as supporting characters in regional identification keys (e.g. Hansen et al. 1987, Poelt 1969, Søchting et al. 2008, Wirth 1995, Fletcher, Laundon 2009). Nevertheless, these characters should be noted, because they may be important in particular groups. For measurements of hymenium and hypothecium, the "basic rule 5" (see above) should be maintained. We propose to measure hymenium and hypothecium height in the central, highest part (fig. 1). Apart from the height of the hypothecium, its shape is also important as it may be flat at the bottom (e.g. in *Caloplaca lobulata* group) or have a conical shape with the tip facing downwards in the central part (e.g. in *Caloplaca xerica* group). It should be noted if the hypothecium fully rests on an algal layer, or if it is connected with the medulla in the central part. Another character employed in some papers (e.g. Muggia et al. 2008) is the presence vs. absence of extracellular oil drops

in the hymenium or hypothecium (inspersed tissues). Hypothecial tissues are rarely also species specific (paraplectenchyma in *C. chalybaea*, fig. 8A in Wilk 2012).

*Paraphyses*

Studies in Teloschistaceae often use the width of (usually clavate) paraphyses tips; it is considered a good character for taxa in some studies (e.g. Navarro-Rosinés, Hladun 1996). Poelt (1969), as well as our own studies, suggests that also the width of paraphyses in the lower part of the hymenium may be a good character. The ratio of "width of paraphyses tip/width of paraphyses in the lower hymenium" may be an even better character. For instance, some species from the *Caloplaca variabilis* group have thick paraphyses, which broaden only slightly at their tips. The opposite is found in species of e.g. the *C. xerica* group, which show a larger difference between thinner paraphyses and their widened tips (our unpublished data). The number of widened cells in the paraphyses below their tips may also differ among taxa (e.g. Navarro-Rosinés, Hladun 1996).

Branching and anastomosing in paraphyses also varies between species (see e.g. Navarro-Rosinés, Hladun 1996). Our experience is that branching and anastomosing is present in all carefully studied species in Teloschistaceae, though very rare in some samples. The frequency of these features should be noted (perhaps three categories; rare/regular/common). Another noteworthy character is the presence/absence of oil-drops in the upper cells of the paraphyses (e.g. Giralt et al. 1992).

*Asci*

Asci in crustose Teloschistaceae appear to be consistently of the Teloschistes-type (Honegger 1978). The shape of the asci is almost always clavate, but some species seem to have more cylindrical asci (Arup, unpublished). The size of the asci correlates with two other characters: ascospore size and height of the hymenium. In our experience, the width and length of asci are strongly dependent on the developmental stage of the ascospores inside. We have also observed increasing of the width of asci by pressure in squash preparations, thus we prefer to avoid measurements of asci in squash preparations. In conclusion, we are skeptical about the value of ascal characters, but they must be considered in pilot studies (explained below).

*Ascospores*

Ascospore shape, size and the width of the ascospore septum are commonly used characters in Teloschistaceae, as is apparent from many identification keys (Clauzade, Roux 1985, Fletcher, Laundon 2009, Nimis 1992, Wirth 1995). Unfortunatelly, ascospores are not measured consistently; some lichenologists investigate still living, immature or overmature ascospores and get unreliable measurements (fig. 4). KOH pretreatment must be avoided before measurements of ascospore characters, because it causes significant swelling of cell-walls and widening of ascospore septa (e.g. Baral 1992; our observations).

Ascospore length and width are important characters on their own, but their ratio also gives some information about shape. The ascospore shape in Teloschistaceae is usually ellipsoid in a broad sense (i.e. almost globose, broadly ellipsoid, thinly ellipsoid or fusiform), but occasionally rhomboid or citriform (fig. 6D, E) or other shapes. Note also that the ascospores are not always polarilocular, but exceptionally also simple, 1-, 2- or 3-septate, without thickened septa.

The width of the ascospore septum is a very important character for "rough" identifications of samples, because this character may determine the placement of samples in particular groups or genera. For instance, *Caloplaca crenulatella* and similar species (*Xanthocarpia* spp.; (sensu Arup et al. 2013)) have thin spore septa, but morphologically similar species from the *C. holocarpa* group (*Athallia* spp.; (sensu Arup et al. 2013) have spores with distinctly thicker septa. Thus, correct measurements of septa are essential. In Teloschistaceae the spore septum in fresh spores often looks thin, but in herbarium material, the spores are dead and the septum thickens to varying extents in different species. It normally takes about a year of dry storage for spores to die completely (become stabilized) and show the typical *Caloplaca* type of septum, but it may take as much as two years for all spores to be stabilized in a sample. Thus, in fresh samples the spores have to be killed to show the typical form and septum morphology. Measurements of samples containing only fresh or only old spores (after a strong natural plasmolysis) should not be mixed with correct measurements of dead, stabilized ascospores (fig. 4).

Although we propose to avoid measurements of fresh and old ascospores for taxonomic studies, we may sometimes need to identify fresh samples immediately. In such cases, following the simple method proposed by Steiner and Peveling (1984) and modified by us may be used. Hand-cut sections in microscopic preparations are heated (we heat samples to 100° for c. 1 second), dried out and left for about 5 minutes in dry state before a second moistening. After this treatment, samples usually show ± correct septum widths (fig. 5).

Outer ascospore wall is usually uniformly thin, but various wall-thickenings are rarely present; e.g. in the sand-glass spores sensu Navarro-Rosinés et al. (2000) and Arup (2006) or the Physcia-type ascospores with apically thickened walls (e.g. Navarro-Rosinés, Hladun 1992). Various ascospore shapes are depicted on fig. 6.

*Thallus*

The thallus provides a number of useful characters, including: size, thickness, colour and character of its units (areoles, squamules and lobes), though they are often modified by the environment. Measurements should not be made on juvenile or poorly developed thalli. Practically, thalli with well-developed apothecia (in fertile taxa) or well-developed vegetative diaspores (in rarely fertile or sterile taxa) should be used.

By the thallus size, we mean the ground area of the thalli, which is usually expressed by the diameter of thallus; this is easy to employ in radially growing lichens. In irregular thalli, we prefer to use a reasonably representative (for instance average) diameter. When thalli with a radial growth pattern grow closely together, they may merge into a large conglomerate. In this case, we propose to measure diameters of only well-circumscribed individual thalli (not conglomerates); as did Gaya (2009) in the *Caloplaca saxicola* group. Measurements of the thallus diameter are problematic in a few lichens, where the central parts are soon detached and parts of the thallus margin continue to grow forming long and cambered "belts" (e.g. *Caloplaca anularis*; Clauzade, Poelt 1972). In this case, we propose to measure width and length of the "belts" as characters showing ground area of the thalli. Measurements of thallus size in taxa with consistently poorly developed thalli, with effuse thalli, or with areoles squeezed in between thalli of other species may have little meaning.

Thallus thickness should be measured in well-developed parts of the thallus, i.e. in the area within the studied sample, where the thallus is most representative. Places, where the thallus forms thick tumors or is unusually abruptly swollen, must be avoided. Figure 7A shows that the profile of thickness along a thallus diameter may be different in various lichens. Some lichens are thickest at the centre of the thallus and others at (or near) the margins. The location of the thickest part of the thallus may itself be a noteworthy character. Figure 7 also shows how results may depend on the measurement procedure: random measurements (fig. 7B), or measurements in highest points of well developed areoles (fig. 7C; see also localizations of measurements on fig. 7A). Both measurement procedures are possible, but we prefer the latter one, because it has better discrimination power (see the differences between measurement spectra in figs 7B & 7C).

Old thallus units may be overgrown by younger thallus units (especially in squamulose thalli), so the final thallus may be formed of several layers of thalli covering each other (fig. 1C in Vondrák et al. 2008a); this multiplies the final thallus width. In this case, we recommend measuring the width of uppermost units, even if lower areoles (or squamules /lobes) are still not dead. Nevertheless, overlapping thallus units may be a noteworthy character of some taxa.

The thallus may be membranaceous (thin and not divided into units), but often consists of units well-circumscribed by crevices or broad depressions in the upper surface. The vertical and horizontal shape of these units classifies them as areoles, squamules or lobes (see definitions). Depending on the character and arrangement of the units, the thallus may be densely to scattered areolate, bullate, squamulose, lobate or fruticulose (see definitions). While the diameter is measured in isodiametric areoles and squamules, length and width are assessed in lobes. For reproducibility and consistency of data, width of lobes may be constantly measured at their base (fig. 8,

dark bars) and tips (fig. 8, pale bars). Measurements of lobes is however a difficult topic and methods may have to be modified for particular studies.

The colour of the thallus is largely determined by pigments deposited in the cortical layer, though they may be masked by a crystalline pruina (usually white) deposited on the thallus surface. The thallus may turn darker owing to the presence of melanins in cell-walls of lichenicolous fungi (especially hyphomycetes). They are usually easily detected under the stereomicroscope as dark dots, spots or networks on thallus surface. Grazing by snails causes paler thalli as the pigment itself is eaten or reduced. Thalli with strong fungal infections, grazed or otherwise abnormally affected should not be evaluated at all.

*Vegetative diaspores*

The thallus may produce vegetative diaspores, which we divide into isidia, blastidia and soredia (see definition above). Differences between soredia and blastidia and between blastidia and isidia are not always definite but rather continuous. In extreme cases, soredia, consoredia, blastidia, isidia and granules may be found on a single lichen thallus and should be assessed separately. The presence or absence of vegetative diaspores is traditionally used as a diagnostic character for species in Teloschistaceae, modern approaches however show that thalli with and without vegetative diaspores may occur in a single species (e.g. Lendemer, Morse 2010, Wetmore 1994, Wirth et al. 2011). When soredia are present, their position, shape and size should be noted, and measurements of soredia and consoredia (see definitions above) should be separated.

*Cortex, algal layer, medulla*

The cortical tissue, if present, may have various appearances, when observed in vertical sections. Based on our experience, we outline four categories (fig. 9): (1) even cortex; (2) uneven cortex; (3) cortex with cones; (4) fungal stacks. The cortex with cones is adopted from Poelt (1958) (Kögelrinden in original) and the term fungal stacks is proposed by Vondrák and Kubásek (2013). In the first two categories, the thickness of the cortex should be evaluated by random measurements (arrows in figs 9A & 9B & grey arrows in fig. 9C) , but the height of cortex cones (third category) should be appraised separately (black arrows in fig. 9C). In the fourth category, heights and widths of fungal stacks are measured. The width of fungal stacks often differs in their upper and lower part; we prefer to measure their width at the mid point of their height. A real cortex (see the definition) is not common in crustose Teloschistaceae. In European taxa, a false "alveolate cortex" (see the definition) is more frequent.

An epinecral layer (see the definition) may cover the cortical layer and its thickness may be of taxonomic importance. The boundary between a living cortical tissue and the epinecral layer is often difficult to recognize, but in our experience dead cells of the epinecral layer are not stained by lactoglycerol-cottonblue. In *Pyrenodesmia* sensu

Arup et al. 2013 (species related to the black-fruiting *Caloplaca variabilis*), the boundary is recognized as the thin layer of upper cortex / alveolated cortex containing a grey pigment (K+ slightly violet); K- epinecral layer (often very high) is located above this line.

The algal layer may form an even horizontal layer, if it is covered by an even cortex or alveolate cortex. At the opposite extreme, it may be formed by stacks (algal stacks sensu Vondrák, Kubásek 2013) enclosed by fungal stacks (fig. 9D). The thickness of the algal layer or the height of algal stacks may be a useful character. The diameter of photobiont cells should be also measured in pilot studies.

Many of the European Teloschistaceae crusts have a thin thallus, so their medulla is inconspicuous, but the medulla is much better developed in thick crusts in arid regions of the world. Its height may be an important character, but the real medulla must be distinguished from the false "algonecral medulla" (see the definition), the height of which is measured as a separate character. The height of the medulla can rarely be measured exactly, because the lower boundary of the lichen surface is often rather vague. (Often the medulla penetrates into the substrate, especially in lichens on calcareous substrata.) The medulla may contain extracellular crystals of calcium salts. Calcium (usually as oxalate or carbonate) is easily detected by the addition of concentrated sulphuric acid, which dissolves the crystals quickly and forms new rosettes of needle-shaped crystals of calcium sulphate (Timdal 1992).

*Pycnidia and conidia*

Pycnidia in Teloschistaceae always belong to the *Umbilicaria*-type or *Xanthoria*-type sensu Vobis (1980). They are formed by one or several chambers opening into a common ostiolum. The size of the pycnidia is hard to assess, because they are hardly visible on the thallus surface and to make a section through the midpoint of a pycnidium is not easy. To get a reasonable measurement of the diameter, the biggest section must be chosen from several consecutive thallus sections made around the presumed centre of the pycnidium. It is often difficult or even impossible to obtain reasonable number of measurements per specimen (see below), but low numbers of measurements give some indication of pycnidium size, which may be a valuable character. The usual diameter is 100–200 µm, but some species have smaller pycnidia (e.g. *Caloplaca pyracea*; Vondrák et al. 2012a).

The colour of the pycnidial tops (see the definition) is an important character. For instance, *Caloplaca crenularia* has orange-red anthraquinones in their pycnidial tops, whereas the similar *C. neotaurica* lacks these pigments. Both species have otherwise grey thalli without anthraquinones (Vondrák et al. 2012b).

Conidiophores (length, branching, etc.) and conidiogeneous cells (size and shape) are variable within Teloschistaceae (fig. 5B-E in Arup, Grube 1999), but they may also be variable within one pycnidium (fig. 3B in Vondrák et al. 2009). Characters of the

conidiophores (height, branching, anastomosing, etc.) as well as the shape and size of conidiogeneous cells may be useful in some groups.

Conidia are usually ellipsoid to bacilliform in crustose Teloschistaceae, but may also be subglobose (Vondrák et al. 2008a). In some taxa, there are both ellipsoid and bacilliform conidia in the same pycnidium.

**Detection of pigments**

*Anthraquinones*

The presence of anthraquinones is usually indicated by the yellow to red colour. However, when the amount of anthraquinones is low or their pigmentation is overridden by other pigments (e.g. melanins) the presence of anthraquinones should be tested (e.g. in Caloplaca concilians). These reactions are diagnostic: K+ strongly purple, K→HCl+ yellow, N+ orange, N→K+ violet-blue, N→K→HCl+ yellow, C– (Vondrák et al. 2010). The first two reactions are normally sufficient.

*Presence of chlorinated anthraquinones*

Laundon (1992) observed that apothecia of some *Caloplaca* specimens are C+ deep purple, but the reason for this reaction was not certainly known to him. Our experiments showed that the reaction with chloral is a helpful character reflecting the presence (C+ purple) or absence (C–) of chlorinated anthraquinones. Chlorinated anthraquinones (when present) may be distributed unequally within the lichen surface; usually the lowest amounts are in the thallus, with higher amounts in the apothecium discs and the strongest concentration (strongest C+ reaction) in the true exciple.

When using the C-reaction, care must be taken to use the correct concentration. Chlorinated detergents bought in drugstores are often strongly concentrated and cause a C+ red spot reaction even on samples without chlorinated anthraquinones. Therefore, we strongly recommend testing the negative reaction on apothecia of the common *Xanthoria parietina*, which never has chlorinated anthraquinones. The concentration of the C-solution must be reduced until it does not cause a red reaction on the apothecial discs of *X. parietina*.

*Acetone-insoluble pigments and other pigments*

A list of common acetone-insoluble pigments in lichens is provided by Meyer and Printzen (2000) along with their diagnostic characters. Two of them are present in some Teloschistaceae: Cinereorufa-green (= lecidea green; olive-black, in K ± unchanged, N+ red/purple/violaceous) and Sedifolia-grey (= thalloidima green; greyish, K+ sordid violet, N+ purple/violet). Wetmore (1996) shows the presence/absence of these pigments in the epihymenium, margin and thallus of numerous species. While Sedifolia-grey is commonly present in the European species, Cinereorufa-green is rather rare (e.g. in *Caloplaca crenularia*, *C. exsecuta* and *C. fuscorufa*).

Brown pigments are rarely present in Teloschistaceae crusts: (1) K+ orange pigment (Tretiach, Muggia 2006), (2) K- pigment (Vondrák et al. 2012b) or (3) pigment bleaching and forming colourless needle-like crystals in K (e.g. in *Caloplaca demissa*; our unpublished data). Other pigments, e.g. the yellow-green usnic acid in *Caloplaca* (*Usnochroma*) *carphinea*, are extremely rare.

*Amyloidity*

The reaction of various tissues with I (we use Lugol's solution without any chemical pretreatment) was used as an important character by some authors (e.g. Magnusson 1944, Søchting 1989). While the hymenium is perhaps always amyloid, the hypothecium is sometimes amyloid, and ascospores, the true exciple, cortex and medulla may be perhaps amyloid in some cases. Amyloidity is mostly not evaluated by recent authors, but we propose to investigate it in pilot studies (see further down).

**The process of phenotype evaluation**

Since it is often not clear in the beginning which characters could be useful for discrimination of the taxa, phenotype should be evaluated in two steps. (1) A pilot study – measuring of all available characters at a restricted number of specimens. This will allow us to evaluate a discrimination power of the characters and select the most powerful of them for the second step. (2) A detailed study – measuring of the selected characters on a large number of samples. The first step acquires samples containing rich and well-developed lichen material (according to the basic rule 1), because mature tissues and well developed structures are needed for thorough observations which are largely destructive. (The chosen specimens must be large enough to avoid destruction of it as it should be possible to evaluate again.) The pilot study is also time-consuming, because more than one hundred characters which are listed below should be considered. In about this step, we propose to investigate at least three geographically distinct samples of each taxon confirmed by molecular analysis or at least of each putative taxon. Choosing fewer than three specimens per taxon is inadvisable, as it introduces a risk of working with aberrant samples. In each sample, more than five measurements are advisable for each measurable character. Practically it means that at least five apothecia must be destroyed on a single specimen, because hymenium width, hypothecium width and widths of exciples should be logically measured only once per apothecial section (fig. 1; the basic rule 5). We recommend choosing apothecia of variable size and variable extent of the thalline exciple (variability in width of thalline exciple is often visible with the stereomicroscope). Measurements of paraphyses, ascospores and all cell measurements may be done in one section only, but we recommend measuring them in several sections. For thallus characters, more than five measurements are advisable per sample; each measuremen in a separate unit (i.e. areole squamule or lobe).

The time consuming process of the pilot study described above is important for getting preliminary information about (1) the variability of characters within a specimen, (2) the difference in variability among specimens of a single taxon, (3) the difference in variability between or among studied taxa.

Characters for the second step – the detailed study – are selected based on the results from the pilot study. The stepwise linear discrimination analysis (ldc) for ranking the individually best traits could be used for selection of the characters with highest discrimination power. Characters without of with low discrimination power among studied taxa are not considered in the detailed study. At least five measurements of each character on every sample included in the detailed study are advisable. Efforts to select characters based on the pilot study are seen in e.g. Ekman (1994), but we have not found details about the "pilot study" in the methods part of any taxonomic study on lichens.

In summarizing tables and texts for manuscripts, we recommend showing results of the measurements (following Ekman 1996) as (min.–) X1–X2–X3 (–max.), where min/max are extremes from all measurements, X1 is the lowest specimen arithmetic mean observed, X2 is arithmetic mean of all observations, X3 is the highest specimen arithmetic mean observed. Total numbers of assessed samples, total number of measurements, and standard deviation from all measurements should be also given for each measured character, e.g. in square parenthesis (n; N; SD).

The following list contains characters, which may be filled into the "working matrix" as follows. Each listed character represents one column of the matrix; the ten measurements in each specimen occupy ten rows. Coding of qualitative characters is explained in the list of characters in square parenthesis. Usual coding is 1 – present, 0 – absent. Some characters are coded by ordinary scale; vegetative diaspores are organized according to their size, colours to their intensity, etc. When some character is not evaluated in the respective sample, a symbol different from “0” (absence) should be filled in the respective matrix cell. The matrix based on the following list of characters can be universally used for crustose Teloschistaceae, but it is still not complete and may be modified for each studied group.

**List of characters for the pilot study**

*(1) In apothecia:* (1.1) diameter of apothecia; (1.2) height of apothecia; (1.3) attachment of apothecia to thallus (1, immersed; 2, suppressed; 3, adnate; 4, sessile; 5, stalked); (1.4) shape of disc in mature apothecia (1, concave; 2, flat; 3, convex); (1.5) surface of mature apothecial disc (1, cracked; 0, even); (1.6) thickness of medulla below disc (measurements are not acquired in groups where the thickness of medulla below apothecia does not differ from the thickness of surrounding medulla); (1.7) apothecial margin in mature apothecia (1, persistent, with expanding thalline exciple;

2, persistent, without expanding thalline exciple; 3, persistent, but reduced; 4, diminishing, subsiding below the disc); (1.8) width of true exciple; (1.9) width of cells in uppermost true exciple (or diameter in paraplectenchymatous cells); (1.10) length of cells in uppermost true exciple (in paraplectenchyma not evaluated); (1.11) width of cell-walls in upper exciple (measurements of cell-wall width are aquired in thick-walled cells, but not in thin-walled cells); (1.12) width of cells in lower true exciple (diameter in paraplectenchyma); (1.13) length of cells in lower true exciple (in paraplectenchyma not evaluated); (1.14) width of cell-walls in lower exciple (measurements of cell-wall width are aquired at thick-walled cells); (1.15) width of thalline exciple; (1.16) cortex of thalline exciple (1, restricted to lower part; 2, covering majority of or whole surface); (1.17) structure of cortex of thalline exciple (1, palisade plectenchyma; 2, intricate plectenchyma; 3, paraplectenchyma); (1.18) width of cells in cortex of thalline exciple (diameter in paraplectenchyma); (1.19) length of cells in cortex of thalline exciple (at paraplectenchyma not evaluated); (1.20) width of cell-walls in cortex of thalline exciple (measurements of cell-wall width are aquired in thick-walled cells, but not in thin-walled cells); (1.21) ratio of exciples (1, biatorine apothecia; 2, zeorine apothecia; 3, lecanorine apothecia; 0, apothecial margin strongly reduced / absent); (1.22) shape of hypothecium (1, with the central conical extension downward; 0, flat in the bottom); (1.23) width of hypothecium; (1.24) structure of hypothecium (1, paraplectenchymatous; 2, prosoplectenchymatous; 3, of variable cell shapes and sizes); (1.25) width of cells in hypothecium (diameter in paraplectenchyma); (1.26) length of cells in hypothecium (in paraplectenchyma not evaluated); (1.27) width of cell-walls in hypothecium (measurements of cell-wall width are aquired in thick-walled cells); (1.28) width of hymenium; (1.29) extracellular crystals in hymenium / hypothecium (1, present; 0, absent); (1.30) insperse hymenium / hypothecium (1, present; 0, absent); (1.31) glutinized hymenium / hypothecium (1, present; 0, absent); (1.32) width of paraphyses in lower hymenium; (1.33) width of paraphyses tips; (1.34) ratio of 1.30 / 1.29; (1.35) number of widened cells below paraphyses tips; (1.36) oil drops in upper cells of paraphyses (1, present; 0, absent); (1.37) branching / anastomosing of paraphyses (1, rare; 2, regular; 3, common; 0, absent); (1.38) height of asci; (1.39) width of asci; (1.40) ascospore length; (1.41) ascospore width (1.42) width of ascospore septa (0, in 1-septate ascospores without broadened wall in partition area); (1.43) ascospore length / width ratio; (1.44) ratio of septum width / ascospore length; (1.45) ascospores with more than 1 septum (1, present; 0, absent); (1.46) "sand-glass" type of ascospores (1, present; 0, absent); (1.47) Physcia-type ascospores (1, present; 0, absent); (1.48) rhomboid ascospores (1, present; 0, absent); (1.49) citriform ascospores (1, present; 0, absent); (1.50) more than 8 spores in asci (1, yes; 0, no); (1.51) ascospore quality (1, only deformed ascospores present; 2, well-developed ascospores present; 0, ascospores absent).

*(2) in thallus:* (2.1) diameter of thallus; (2.2) thickness of thallus; (2.3) vertical complexity of thallus (1, thin membranaceous; 2, leprose; 3, of ±flat areoles / squamules / lobes; 4, of convex areoles / squamules / lobes; 5, of highly convex units, distinctly bullate; 6, fruticulose); (2.4) thallus units (1, granules only; 2, dispersed areoles; 3, dense areoles; 5, squamules; 6, lobes; etc.); (2.5) thallus margin (1, diffuse, with distinct prothallus; 2, diffuse, without prothallus; 3, areolate; 4, squamulose; 5, lobate); (2.6) diametre of areoles / squamules / breadth of lobes; (2.7) length of lobes; (2.8) vegetative diaspores (1, granules; 2, isidia; 3, blastidia; 4, soredia; 0, absent); (2.9) position of soralia on thallus units (1, on margins; 2, concave on upper surface; 3, convex on upper surface; etc.); (2.10) extent of soredia (1, discrete, covering consistently less than 1/4 of thallus units; 2, discrete, but covering larger area; 3, confluent, but not covering whole thallus surface; 4 entire sorediate crust); (2.11) size of soralia; (2.12) size of soredia (diameter); (2.13) size of consoredia (approximated diameter); (2.14) size of blastidia (approximated diameter); (2.15) width or diameter of isidia; (2.16) height of isidia; (2.17) size of granules (approximated diameter); (2.18) cortex (1, even; 2, uneven; 3, cortex with cones; 4, fungal stacks; 0, absent); (2.19) thickness of cortex / height and width of fungal stacks; (2.20) structure of cortex (1, palisade plectenchyma; 2, intricate plectenchyma; 3, paraplectenchyma); (2.21) thickness of alveolate cortex; (2.22) width of cells in cortex / alveolate cortex (diameter in paraplectenchyma); (2.23) length of cells in cortex / alveolate cortex (in paraplectenchyma not evaluated); (2.24) width of cell-walls in cortex / alveolate cortex (measurements of cell-wall width are aquired in thick-walled cells); (2.25) glutinized cell-walls in cortex (1, present; 0, absent); (2.26) thickness of epinecral layer; (2.27) thickness of algal layer / height and width of algal stacks; (2.28) diameter of algal cells; (2.29) thickness of algonecral medulla; (2.30) thickness of medulla; (2.31) medullar tissue (1, loose thin-walled parenchyma; 2, loose thick-walled parenchyma; 3, loose thin-walled prosenchyma; 4, loose thick-walled prosenchyma; etc.); (2.32) extracellular crystals in medulla (1, present in spots; 2, present all over; 0, absent); (2.33) size of crystals in medulla; (2.34) extracellular crystals in cortex (1, present in spots; 2, present all over; 0, absent); (2.35) size of crystals in cortex; (2.36) reaction of crystals with sulphuric acid (1, positive; 0, negative; see more in text above); (2.37) presence of pycnidia (1, present; 0, absent); (2.38) width of pycnidia (approximate; min. - max.); (2.39) width of conidia; (2.40) length of conidia; (2.41) overlapping thallus units (1, present; 0, absent); (2.42) presence of pseudocyphaellae (1, poorly developed; 2, well developed; 3, absent); (2.43) lichenicolous thallus (1, young thalli lichenicolous, but mature thalli ±free-living; 2, thalli persistently lichenicolous; 0, non-lichenicolous); (2.44) lichenicolous lichens on studied thalli (1, present (should be specified); 0, absent); (2.45) lichenicolous fungal infections (1, present (should be specified); 0, absent).

*(3) pigments and colours:* (3.1) anthraquinones in apothecial disc (1, yellow; 2, orange; 3, red; etc.; 0 absent); (3.2) anthraquinones in true exciple (1, yellow; 2, orange; 3, red; 0 absent); (3.3) anthraquinones in thalline exciple (1, yellow; 2, orange; etc.; 3, red; 0 absent); (3.4) anthraquinones in thallus (1, yellow; 2, orange; 3, red; etc.; 0 absent); (3.5) Sedifoliagrey in apothecial disc (1, grey; 2, blackish; 0, absent); (3.6) Sedifolia-grey in true exciple (1, grey; 2, blackish; 0, absent); (3.7) Sedifolia-grey in thalline exciple (1, grey; 2, blackish; 0, absent); (3.8) Sedifolia-grey in thallus (1, grey; 2, blackish; 0, absent); (3.9) Cinereorufagreen in apothecial disc (1, grey; 2, blackish; 0, absent); (3.10) Cinereorufa-green in true exciple (1, grey; 2, blackish; 0, absent); (3.11) Cinereorufa-green in thalline exciple (1, grey; 2, blackish; 0, absent); (3.12) Cinereorufa-green in thallus (1, grey; 2, blackish; 0, absent); (3.13) pruina on apothecial disc (1, white, in spots; 2, white, covering majority of surface; 3, olive; 4, rusty; etc.; 0, absent); (3.14) pruina on true exciple (as in 3.13); (3.15) pruina on thalline exciple (as in 3.13);(3.16) pruina on thallus (as in 3.13); (3.17) depigmented (bleached) spots on thallus surface (1, present; 0, absent); (3.18) chlorinated anthraquinones = positive C-reaction (1, in exciple only; 2, in disc and exciple; 3, in disc, exciple and thallus; 0, absent); (3.19) other pigments in apothecia (1, present (should be specified); 0, absent); (3.20) other pigments in thallus (1, present (should be specified); 0, absent); (3.21) pycnidial tops (1, without anthraquinones, not contrasting in colour with surrounding thallus; 2, without anthraquinones, but stronger pigmented than surrounding thallus; 3, with anthraquinones and not contrasting in colour with surrounding thallus; 4, with non-chlorinated anthraquinones and brighter colour than surrounding thallus; 5, with chlorinated anthraquinones; etc.); (3.22) hypothecium amyloid (1, yes; 0, no); (3.23) true exciple amyloid (1, yes; 0, no); (3.24) cortex amyloid (1, yes; 0, no); (3.25) medulla amyloid (1, yes; 0, no).

*(4) distribution and ecology (possible coding for epilithic samples):* (4.1) latitude; (4.2) longitude; (4.3) altitude; (4.4.) macro-habitat (1, desert; 2, steppe; 3, forest-steppe; 4, forest; etc.); (4.5) micro-habitat / substrate (1, epilithic; 0, on other substrate (must be specified)); (4.6) substrate reaction (1, acidic; 2, intermediate; 3, basic); (4.7) bedrock type (e.g. 1, calcareous; 0, non-calcareous (should be specified)); (4.8) substrate stability (1, soil or loess; 2, soft rock; 3, hard but weathered rock; 3, hard rock); (4.9) substrate particles (1, pebbles; 2, stones, boulders; 3, rock); (4.10) rainwater exposure (1, sheltered below overhang; 2, on ± vertical rock face; 3, on ± horizontal rock face); (4.11) local humidity (1, moist; 2, mesic; 3, dry (should be specified)); (4.12) light exposure (1, shaded; 2, partly shaded; 3, open); (4.13) exposure to cardinal points.

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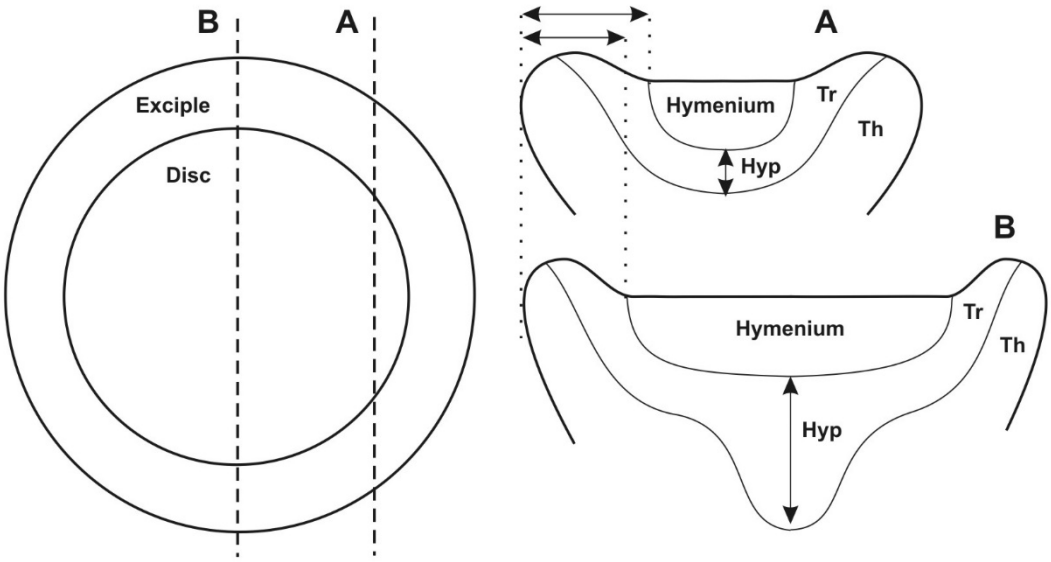


Fig. 1. Left, placements of vertical sections on apothecium viewed from above; right, vertical sections in apothecium; A, vertical section showing incorrect sizes and shapes of exciples, hymenium and hypothecium; B, vertical section through the mid-point showing correct sizes and shapes; Hyp – hypothecium; Tr – true exciple; Th – thalline exciple; arrows show measured widths of exciple and heights of hypothecium.

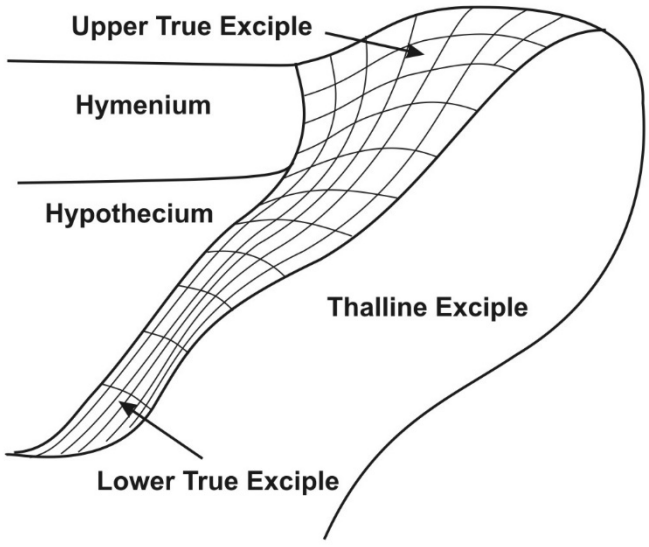


Fig 2. Vertical section of apothecial margin showing variability in cells in the true exciple.

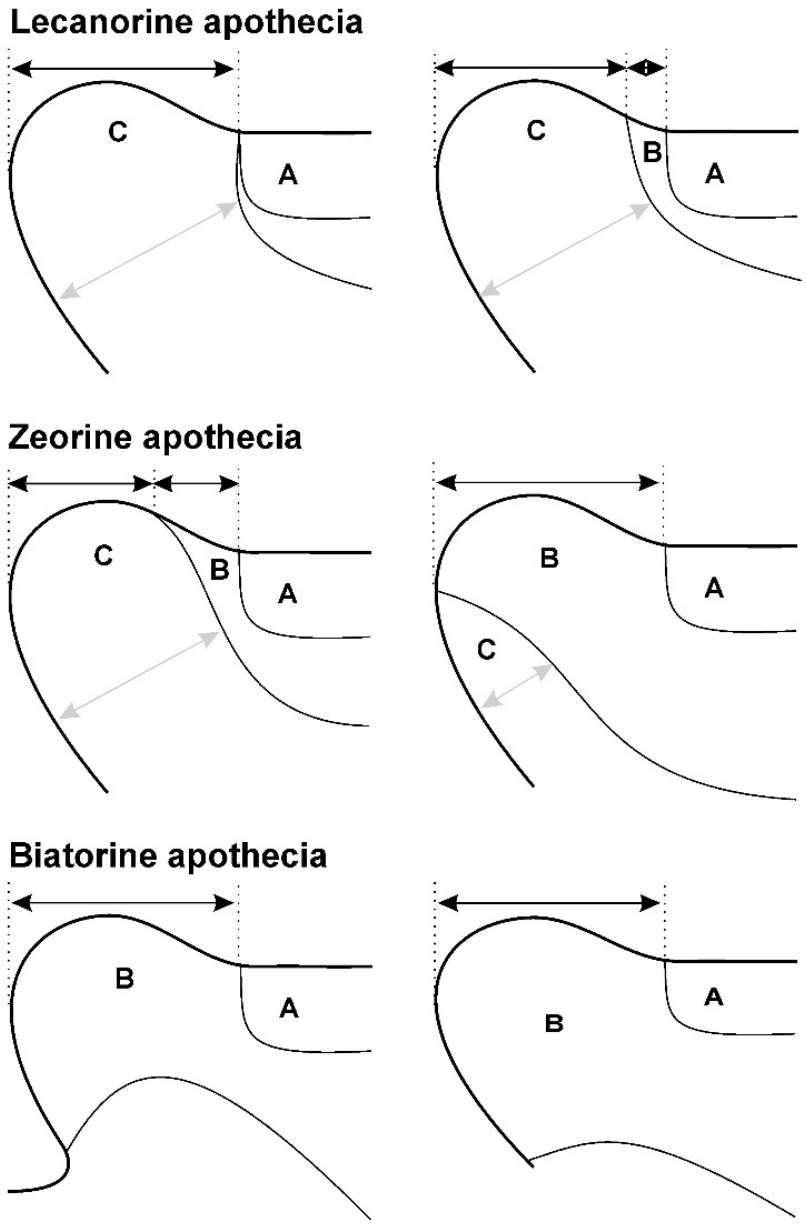


Fig 3. Vertical sections of apothecial margin; variability in lecanorine apothecia (pictures above); in zeorine apothecia (pictures in the middle); in biatorine apothecia (pictures in the bottom); A=hymenium; B=true exciple; C=thalline exciple. Black arrows; standardized measurements of widths of true and thalline exciples. Grey arrows; alternative possible measurements of the thalline exciple (explained in the text).

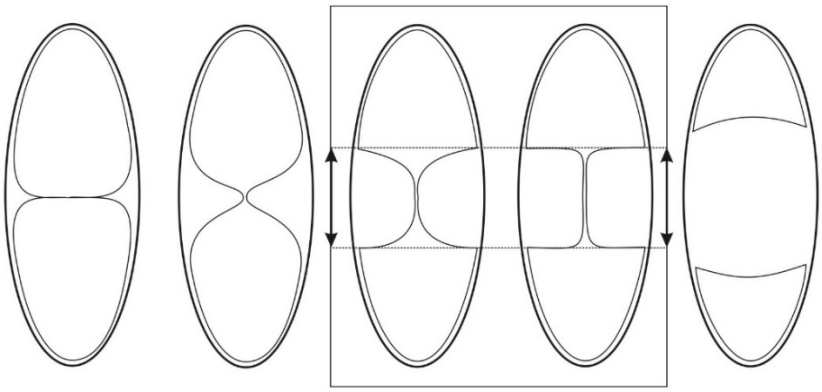

Fig. 4. Development of spores in *Caloplaca*. In young spores (two on the left side), septum width cannot be clearly defined because of rounded shape of inner spore wall. Only "stabilized" ascospores (those in square) should be measured, because their septum widths are defined by the distance of inner walls of the cells (loculi). Old spores showing strong plasmolysis (the one on the right) without the internal canal between cells should not be measured, because iner cells are getting smaller.

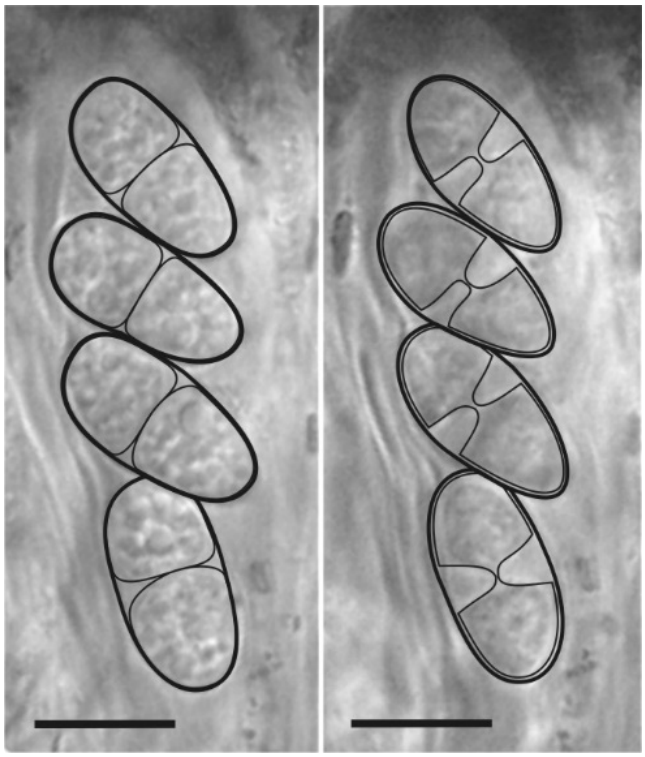

Fig. 5. Young ascospores of *Caloplaca interfulgens*; left, observed before heating and drying (indistinct wall thickenings at septa); right, after heating and kept for 5 minutes in dry state (wall thickenings at septa well-pronounced); bars = 10 µm.

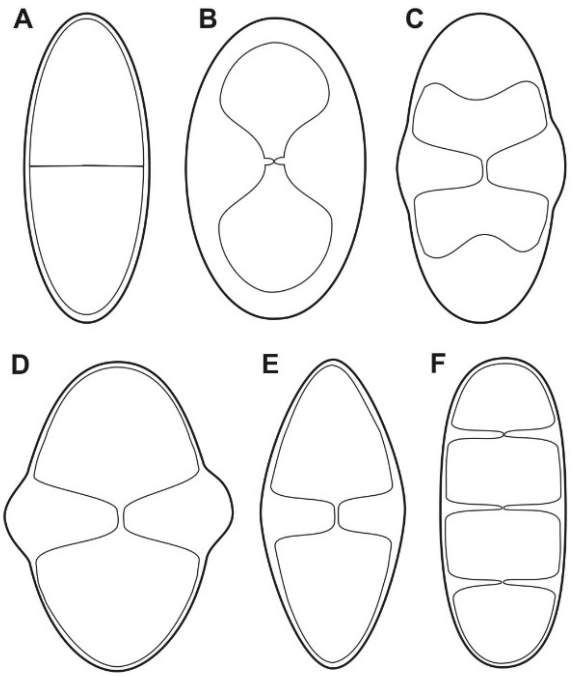


Fig. 6. Unusual ascospore shapes in Teloschistaceae; A, 1-septate without broadened wall in septum area; B, "sand-glass ascospore" with overall broadened wall; C, *Rinodina*-type; D, citriform; E, rhomboid; F, 4- septate.

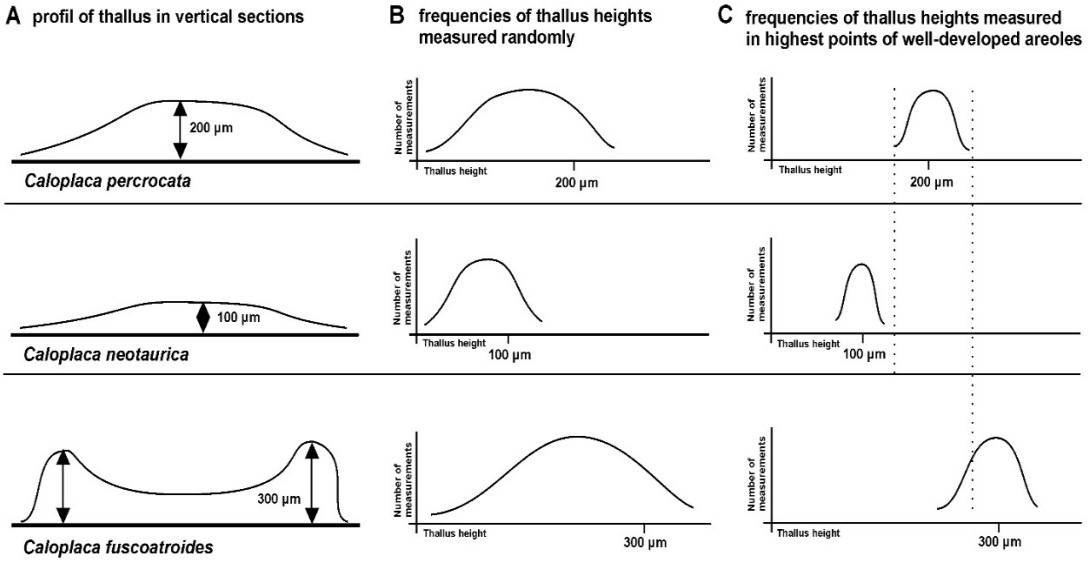


Fig. 7. Thallus height measurements in three different taxa; differences in results from random measurements vs. measurements in highest points of well developed areoles.

Fig. 8. Outline of marginal lobes in thallus of *Caloplaca anularis* viewed from above with proposed places of measurements of lobe widths. Dark grey bars - measurements at bases of lobes; pale grey bars - measurements at tips of lobes.

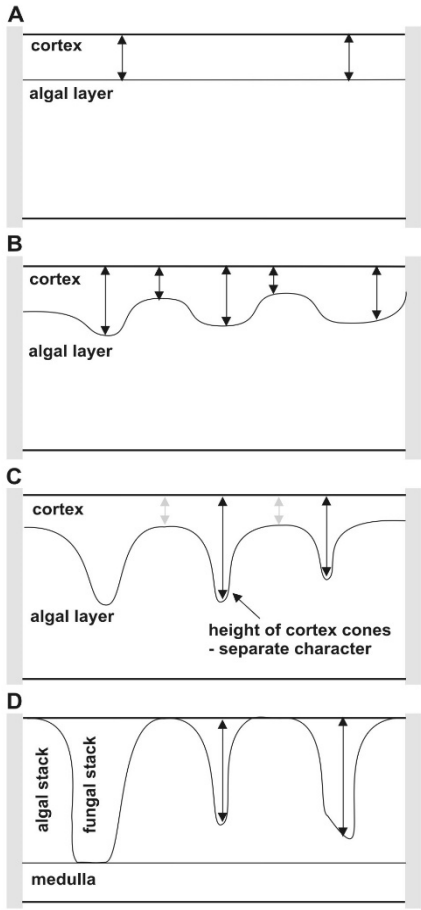


Fig. 9. Types of cortex in vertical thallus sections; A, even cortex; B, uneven cortex; C, cortex with cones; D, fungal stacks. Arrows are feasible positions of measurements (explained in the text).

## 5.3 Paper 3

**Frolov I.**, Vondrák J., Fernández-Mendoza F., Wilk K., Khodosovtsev A., Halıcı M.G. 2016. Three new, seemingly-cryptic species in the lichen genus *Caloplaca* (Teloschistaceae) distinguished in two-phase phenotype evaluation. Annales Botanici Fennici 53: 243–262.

**Three new, seemingly cryptic species in the lichen genus *Caloplaca* (Teloschistaceae) distinguished in two-phase phenotype evaluation**

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**Abstract**

We describe three new, seemingly-cryptic species in the lichen genus Caloplaca (Teloschistaceae) with black apothecia. Those species, separated in nrITS and β-tubulin DNA phylogenies, appeared to be phenotypically indistinguishable. We looked for their phenotypic differences using a two-phase method comprised of a preliminary examination in which diagnostic value of all available characters was evaluated using a small number of samples and potentially-diagnostic characters were selected, and a subsequent detailed study in which characters selected in the first phase were tested using more samples. We found 19 diagnostic characters (continuous and discrete) of which four continuous and three discrete characters could be considered "fully diagnostic", i.e. allowing for correct identification of at least one species. Hence, the three species are not cryptic, but can be distinguished phenotypically. Here, they are formally described as *Caloplaca micromarina* Frolov, Khodos. & Vondrák sp. nova, *C. micromontana* Frolov, Wilk & Vondrák sp. nova and *C. microstepposa* Frolov, Nadyeina, Khodos. & Vondrák sp. nova.

## Introduction

A large group within Teloschistaceae, containing lichens without anthraquinones, is formed by *Caloplaca variabilis* and related species, or the *Pyrenodesmia* s. lato. Pending further study, we decided to use the generic name *Caloplaca* in the present paper.

Our reconstructed genealogies based on two nuclear loci (ITS and β-tubulin) showed a number of well-supported lineages within the group (Fig. 1). Some of these lineages, often distant, were morphologically very similar. We selected three phylogenetically distinct groups of specimens that at first seemed morphologically identical to test whether these supposed taxa could be phenotypically recognized using the approach described by Vondrák et al. (2013).

All studied specimens of the three taxa share the following features: (1) anthraquinones absent; (2) thallus small, thin to indistinct, but not distinctly endolithic; (3) apothecia small, usually less than 0.5 mm diameter; (4) apothecia immersed to adnate, very rarely sessile; (5) apothecia zeorine; (6) Sedifolia-grey pigment present in epihymenium, upper part of exciple and thallus; (7) ascospore septa rather thin, to 4 µm wide; (8) occasional presence of shrunken and dead cells in tips of paraphyses (Fig. 2A and B). None of the three selected taxa are conspecific with any species treated in recent works on the group (Tretiach et al. 2003, Tretiach & Muggia 2006, Muggia et al. 2008, Vondrák et al. 2008, Xahadin et al. 2010), which is also demonstrated by our phylogenetic tree (Fig. 1).

We sought forgotten or little known names that could be used for the three taxa studied here. Among a number of potential names for black apothecial *Caloplaca* taxa (many of them listed by Wunder 1974) we found only one potentially relevant name, *C. atroalba* (Tuck.) Zahlbr. Its type is superficially identical to some of our samples. However our subsequent investigation showed that *C. atroalba* is not conspecific with any of the three taxa (see the taxonomical note under *C. microstepposa*). As we were unable to find any previously published names for the three taxa, we describe them formally here.

## Material and methods

### Sampling

Lichen samples were collected mainly by the authors from various European and western Asian localities in 2004–2012 and deposited in the herbaria GZU, KHER, KRAM, KW and PRA. In this paper, we provide full sample data, including information about locality, habitat, collecting and deposition of specimens. Citations of the older herbarium samples from GZU, KRAM, KTC and W are as full as possible. The three new taxa are compared with all similar *Caloplaca* with black apothecia

known to us; rich comparative lichen material is deposited in the herbarium PRA (http://botanika.prf.jcu.cz/lichenology/data.php). We also investigated material of *C. aegyptiaca* (holotype G), *C. albopruinosa* (GZU, KRAM, STU, TSB), C. *albovariegata* (lectotype UPS), *C. alociza* (GZU, STU, TSB), *C. atroalba* (lectotype FH; GZU; PRA-V; MIN), *C. aspicilioides* (type G), *C. badioreagens* (isotype GZU, TSB), *C. bullata* (lectotype G), *C. circumalbata* (neotype G), *C. diphyodes* (GZU; holotype H-NYL; KHER; PRA-V), *C. fulva* (isotype W, PRA-V), *C. lecideina* (GZU; isotype G), *C. paepalostoma* (isotype M, PRA-V), *C. paulsenii* (syntype TUR-V), *C. rhinodinoides* (type W), *C. transcaspica* (holotype H-NYL; KHER), and *C. variabilis* s. lato (GZU, H, KRAM, KTC, LE, PRA-V, TUR, W); images of the type specimens are available at http://botanika.prf.jcu.cz/lichenology/index.php ?pg=5&func=cat&idx=33#photos. We used literature data (Wunder 1974, Wetmore 1994, 2009, Khodosovtsev et al. 2004, Tretiach & Muggia 2006, Muggia et al. 2008) as a secondary source of information for comparisons among taxa.

**DNA extraction, amplification and sequencing**

Simple NaOH extraction (Werner et al. 2002) was used for DNA isolation. Primers for PCR amplification of ITS were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990); PCR cycling parameters for ITS follow Ekman (2001). Primers for PCR amplification of β-tubulin were Bt3LM and Bt10LM (Myllys et al. 2001); PCR cycling parameters for β-tubulin were: 94 °C for 3 min, 40 × (94 °C for 30 sec, 55 °C for 60 sec, 72 °C for 60 sec), 72 °C for 10 min, hold at 15 °C. Each sequence was provided with GenBank accession number (Table 1).

**Sequence alignment and phylogenetic reconstructions**

Two independent sources of molecular data were chosen for the phylogenetic part of this study: β-tubulin (a protein-coding nuclear gene) and ITS (a non-coding nuclear locus commonly used in phylogenetic studies). The two data sets were aligned using the L-INS-i method of MAFFT ver. 6.847b (Katoh & Toh 2008). The ITS alignment showed a high proportion of unalignable positions and therefore it was subsequently cleared by the automated1 algorithm as implemented in the trimAl software package (Capella-Gutierrez et al. 2009). To minimize the impact of computational artifacts and to get the correct reading frame for the protein-coding β-tubulin, we annotated and adjusted nucleotide and translation alignments in Geneious ver. 7.1.8 (Kearse et al. 2012). For characters of the alignments see Table 2.

Phylogenetic reconstructions were carried out both using a maximum likelihood (ML) and a Bayesian approach. Optimum partitioning of the data set and the optimum substitution models per partition were calculated with the PartitionFinder program (Lanfear et al. 2012, 2014). β-tubulin was strongly partitioned, separating the intronic

and exonic fractions first and treating all three codon positions independently in the latter. Optimum partition schemes and substitution models were estimated separately for the ML and Bayesian analyses.

Maximum likelihood reconstructions were carried out in RAxML (Stamatakis et al. 2005) through the RAxMLGUI interface (Silvestro & Michalak 2012). The analysis included three partitions: ITS with the GTR+I+G substitution model, first and second codon positions of β-tubulin exon also with the GTR+I+G model, and the third codon position of β-tubulin and the intronic fraction together with the GTR+G model. Bootstrap support was calculated on 1000 bootstrap replicates using thorough bootstrapping.

Bayesian reconstructions were carried out in MrBayes 3.2.5. (Ronquist & Huelsenbeck 2003) using the five-partition scheme in which the GTR + G model was used for ITS, K80 for the β-tubulin intron while the exon was partitioned in three codon positions with the SYM + I, JC and GTR + G substitution models used for first, second and third codon positions respectively. Four independent runs with four incrementally heated chains each were run for 80 000 000 sampling every 4000 tree to avoid autocorrelation. All sampled topologies were summarized in a strict consensus tree. In addition the nodal support for the ML topology in the Bayesian sample was calculated using function SumTrees from the Dendropy package (Sukumaran & Holder 2010).

**Phenotype evaluation**

We employed a two-phase approach consisting of (1) a preliminary study in which diagnostic value of all available characters is evaluated using a small number of samples and potentially-diagnostic characters are selected, and (2) a subsequent detailed study in which characters selected in the first phase are tested using more samples. Three samples, previously used in the phylogenetic analysis, were selected for each seemingly-cryptic taxon for the preliminary study: *C. micromarina* (Ukraine, PRA JV6420; Ukraine, PRA JV7236; Turkey, PRA JV8199); *C. micromontana* (Russia, PRA JV9467; Russia, PRA JV9523; Poland, *L. Śliwa 3118*, KRAM); *C. microstepposa* (Czech Republic, PRA JV9141; Kazakhstan, PRA JV9454; Ukraine, *O. Nadyeina 5-14*, KW). First, in each of those samples we evaluated 29 continuous and about 60 discrete characters (data available from the first author; for the list of the characters and methods of their investigation see Vondrák et al. 2013). Discrete characters constant in all samples of any one species but different in all other samples were selected for the next phase. Most powerful continuous characters were selected with the help of linear discriminant analysis (see below). Second, characters selected in the preliminary study were tested using 10 specimens of *C. micromarina*, 11 of *C. micromontana* and 11 of *C. microstepposa*. As a result, we found some characters that could be considered “partly diagnostic” and some “fully diagnostic” (i.e. allowing

correct identification of nearly 100% of samples). We considered a continuous character fully diagnostic if the interval between the smallest and the greatest means among the sample means of one species did not overlap with the corresponding interval in at least one other species. A discrete character is considered fully diagnostic if its value in all samples of any one species is different from its value in all other samples.

All morphological observations were done on hand-cut sections in water, without any chemical treatment. Measurement accuracies were 0.5 µm for cells, 1 or 10 µm for larger structures. All measurements of cells include their walls, with exception of tissues with glutinized cell-walls. In each species sample, each measurable character was measured on 10 objects (5–9 if objects were scarce) and the mean values for the sample were calculated. Subsequently, a mean value using all measurements of a given character from all samples of a species was calculated. In the results, the measurements are given in the following format: (minimum)$x_1$–$x_2$ –$x_3$(maximum), where minimum and maximum are extreme values in all samples of one species, $x_1$ is the smallest mean among the sample means of one species, $x_2$ is the mean calculated using all the values of all samples of one species, $x_3$ is the greatest mean among the sample means of one species. The total number of measurements from all samples of one species (N), the number of species samples (n), and standard deviation of x2 (SD) are given for each measured character in brackets [N, n, SD]. Morphological terminology follows Smith et al. (2009) and Vondrák et al. (2013).

**Chemistry**

Spot tests with KOH (K), sodium hypochlorite (C), and paraphenylenediamine (P) as well as examination under UV light were performed in each new species; tissues were also tested on cross sections for amyloidity in the reaction with Lugol's solution (I). Pigments insoluble in acetone were evaluated on cross sections in reactions with K and N (50% nitric acid) following Meyer and Printzen (2000). Extracellular crystals were examined in the reaction with H2 SO4 for detection of calcium salts (recrystalization into needles of calcium sulphate). Presence of acetone-soluble compounds was tested by HPLC after Feige et al. (1993) on a LichroCART 250-4 RP18-e (5 µm) column using an Agilent 1100 Series Chromatograph.

**Discriminant analysis**

Linear discriminant analysis (LDA) is a simple probabilistic classification technique which searches for a linear combination of variables that best separates two or more categories. It is applied for classifying groups of specimens (e.g. Casale et al. 2015) and sometimes for a feature selection, to identify which characters best discriminate groups of objects (e.g. Marcysiak et al. 2007).

We employed LDA (in STATISTICA: StatSoft Inc., Tulsa, USA) to evaluate the diagnostic value (discrimination power) of 29 continuous characters measured in the preliminary study (Table 3). Characters were evaluated for the three species together (Table 3, second column) and for each species pair (Table 3, columns 3–5). We arranged the characters in Table 3 according to their diagnostic value, from the best to the worst character, and chose first ten characters for the detailed study (except for "lower-/upperparaphysis-width ratio" and "conidium length"; see the section "Phenotype diagnostics").

## Results

### Phylogenetic analyses

Single-gene trees (available from the first author) as well as the concatenated two-locus tree (Fig. 1) showed positions of species treated in recent works on the group. *Caloplaca albopustulata*, *C. alociza*, *C. chalybaea*, *C. concreticola* and *C. diphyodes* form well-supported monophyletic groups. *Caloplaca badioreagens* appears monophyletic but is not supported. *Caloplaca transcaspica* is represented by only one terminal, which is not close to any other taxon. The sorediate *C. erodens* forms a clade together with non-sorediate species (called here *C. erodens* "non-sorediate") very similar to *C. albopruinosa*. *Caloplaca variabilis* is a heterogeneous taxon; we included only three samples into analyses (*C. variabilis* p.p.). Three previously unnamed taxa, "*C. micromarina*", "*C. micromontana*" and "*C. microstepposa*", which are revealed as three distinct phylogenetic species (Fig. 1), were subjected to phenotypic investigation.

### Phenotype diagnostics

On the basis of the preliminary study and the subsequent LDA analysis (Table 3), we selected eleven continuous and eight discrete, potentially diagnostic characters for the detailed study (Table 4). Of these 19 features four continuous and three discrete characters were considered "fully diagnostic" after the detailed study (with asterisks in Table 4). The character "lower-/ upper-paraphysis-width ratio" selected by LDA was not evaluated in the detailed study, because it was strongly correlated with "width of widest cell of paraphysis" (Pearson's correlation coefficient $r = 0.7380$, $p < 0.001$) and its discriminative power was lower. "Conidium length" was not used in the preliminary study, because conidia were observed only in a limited number of samples due to common absence of pycnidia. However, conidial length measured in all available specimens appears to distinguish *C. micromontana* from the other two species (Table 4).

### Species descriptions

***Caloplaca micromarina*** Frolov, Khodos. & Vondrák, sp. nova
(Figs 4A, B)

MB 803398; sequences of the holotype: KC615268 (β-tubulin), KC346301 (ITS)

Type: Turkey. Sea of Marmara coast. Tekirdağ, in valley of small brook near Gaziköy, alt. 20–40 m a.s.l., 40°45′21′′N, 27°20′04′′E, on stones and pebbles of calcareous sandstone, 11 April 2007, *J. Vondrák* (holotype PRA JV8199; isotypes GZU, LD).— Paratypes: See Appendix.

Diagnostic characters: (1) anthraquinones absent; (2) thallus epilithic, but usually less than 200 µm thick, without distinct cortex, with ± identifiable Sedifolia-grey; (3) mature apothecia usually less than 0.5 mm diameter, with black disc and true exciple, zeorine; (4) cells in uppermost true exciple 4–8 µm wide; (5) hymenium without extracellular oil drops, sometimes with stacks of crystals; (6) epihymenium and outer part of true exciple grey, with Sedifolia-grey, K+ violet; (7) width of widest cell of paraphysis 3–6 µm; (8) ascospores with rather wide septa, 2–4.5 µm.

Detailed description: Thallus epilithic, ochre, grey or with white spots on ochre/grey thallus (thallus colour often varible even within one sample or single thallus); forming small irregular spots to several cm wide or sometimes roundish spots to about 1 cm diameter; of tightly arranged, angular to rounded, flat areoles, (0.22)0.34–0.41–0.46(0.70) × (0.20)0.28–0.31– 0.33(0.55) mm [30, 3, 0.12 & 0.08]. Thickness of thallus (75)125–161–206(375) µm [90, 9, 61]. Medulla inconspicuous, to about 50 µm thick; cells hardly observable due to presence of extracellular crystals only partly dissolved and recrystallized into needles in H2 SO4 . Algal layer (25)50–55–61(100) µm thick [30, 3, 17]; algal cells globose, (8.0)12.9–14.3–16.4(20.0) µm diameter [30, 3, 3.1]. Cortex not developed; alveolate cortex usually present. Epinecral layer often present, but its boundary with alveolate cortex indistinct. Thickness of alveolate cortex together with the epinecral layer (8)15–28– 36(50) µm [90, 9, 10]. Alveolate cortex cells ± spherical, (4.5)6.0–6.4–7.0(8.0) µm diameter [30, 3, 0.9], cell-wall thickness about 1.5 µm. Vegetative diaspores absent. Extracellular crystals of calcium salts present in medulla and also forming pruina. Pruina sometimes present (white spots on thalli, often surrounding apothecia or apothecial primordia). Prothallus indistinct or distinct, ochre (paler than thallus) or grey.

Apothecia (0.20)0.28–0.34–0.40(0.55) mm diameter [100, 10, 0.07]; zeorine or rarely biatorine; mature apothecia suppressed to adnate, rarely immersed. Disc black; true exciple black; thalline exciple of same colour as thallus; white pruina often present

on disc and exciples (apothecia with and without pruina usually present in one sample). Hymenium (75)89–90–93(100) µm high [30, 3, 8], colourless, not glutinized, without extracellular oil drops, but sometimes with stacks of extracellular, often rectangular crystals (insoluble in K, recrystalized into needles in $H_2SO_4$ ); epihymenium grey. Hypothecium colourless, underlain by algal layer, with extracellular oil drops, without extracellular crystals; with a central conical extension downward, (75)89–97–93(150) µm high [30, 3, 16]; formed of thin-walled cells variable in shape. Exciple about 0–75 µm wide, formed of true exciple, (0)14–24–29(50) µm wide [30, 3, 12], and thalline exciple, (0)5–8–12(38) µm wide [30, 3, 12]. Upper part of true exciple of thin-walled cells (4.5)6.3–7.7–8.9(11.5) × (3.0)3.9–5.0– 6.2(8.5) µm [100, 10, 1.6 & 1.1]. Lower part of palisade prosoplectenchyma of thin-walled cells (6.0)9.3–10.2–10.9(14.0) × (2.0)2.3–2.4– 2.5(3.5) µm [30, 3, 2.1 & 0.4]. Thalline exciple without cortex or with indistinct alveolate cortex with numerous extracellular crystals (in $H_2SO_4$ partly dissolved and recrystalized into needles). Paraphyses (2.0)2.1–2.1–2.2(2.5) µm wide [30, 3, 0.2] in lower part, but widening gradually to (3.0)3.7–4.0–4.5(6.0) µm [100, 10, 0.7] in upper part; rarely branched and anastomosed; uppermost paraphyses cells usually dead (not stained by cotton blue), thin and shrunken (Fig. 2A and B). Asci clavate, (45)55–59–63(75) × (10)15– 16–19(23) µm [30, 3, 7 & 3]. Ascospores 8 per ascus, colourless, polarilocular, (10.5)13.2– 14.3–15.5(18.0) × (5.5)6.5–7.5–8.5(10.5) µm [90, 9, 1.5 & 1.0], with rounded ends. Septa (2.0)2.6–2.9–3.4(4.5) µm [90, 9, 0.6], cytoplasmatic channel within septum broad or thin. Ascospore length/width ratio: (1.44)1.79–1.95– 2.30(2.67) [90, 9, 0.24]; septum width/ascospore length ratio: (0.13)0.17–0.20–0.23(0.30) [90, 9, 0.03].

Pycnidia common, about 90–130 µm wide, usually with a single chamber, distinguished by their darker grey tops on thallus surface. Conidiophores of spherical, rectangular or triangular, ± isodiametric cells, about 2–5 µm diameter. Conidia ellipsoid to broadly ellipsoid, (2.5)2.8– 3.1–3.3(4.0) × (1.0)1.6–1.8–2.1 (2.5) µm [70, 7, 0.3 & 0.3].

Chemistry: Spot tests: thallus and apothecia K–, C–, P–. Thallus and apothecia UV–. In section, true exciple non-amyloid (I–); hymenium and hypothecium amyloid (I+). Uppermost cells in alveolate cortex of thallus with Sedifolia-grey (pale grey in water, K+ violet; the reaction not always observable). Concentration of Sedifolia-grey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple also with Sedifolia-grey. No substances detected by HPLC in apothecia and thallus (done in samples PRA JV6420, JV7236 and JV8199).

Similar taxa: *Caloplaca albopruinosa* (thallus distinctly endolitihic; apothecia usually pruinose, thalline exciple indistinct; montane species), *C. alociza* (hymenium with numerous extracellular oil drops, thalline exciple indistinct; thallus distinctly endolithic), *C. atroalba* & *C. microstepposa* (hymenium with numerous extracellular

oil drops, epihymenium brown, K+ brown-violet, spore septa thinner, cells in uppermost true exciple thinner; Table 4), *C. badioreagens* (endolithic thallus; with different apothecial chemistry), *C. circumalbata* (apothecia and thallus larger, thallus usually white), *C. diphyodes* (apothecia and thallus larger; apothecia sessile; spore septa wider), *C. micromontana* (spores septum thinner, upper cells in paraphyses wider, hymenium always without stacks of crystals; Table 4), *C. transcaspica* (apothecia and thallus larger, thallus usually white, apothecia sessile, hymenium sometimes with extracellular oil drops).

Phylogeny: *Caloplaca micromarina* forms a monophyletic group (Fig. 1), but its support is low in both ML and Bayesian analyses. The internal clade without one Russian specimen is well supported in the Bayesian analysis.

Distribution and ecology: *Caloplaca micromarina* is a maritime species distributed in Eastern Mediterranean. It is known from Russia, Turkey and Ukraine along the coasts of the Black Sea and the Sea of Marmara, where it grows on coastal rocks and at some distance (up to several kilometres) from the shoreline. The species occurs on calcareous conglomerates, schist and sandstone outcrops, stones or pebbles and rarely on concrete in sites with well-lit Mediterranean scrub vegetation. Co-occurring taxa are e.g. *Aspicilia contorta*, *Caloplaca conversa*, *C. crenulatella* s. lato, *C. ferrarii* s. lato, *C. neotaurica*, *Candelariella aurella*, *Diplotomma* sp. and *Lecanora dispersa* s. lato. In semi-arid conditions of the Crimean Peninsula, *C. micromarina* grows together with *C. microstepposa*, but it never occurs with *C. micromontana*.

***Caloplaca micromontana*** Frolov, Wilk & Vondrák, sp. nova
(Figs 4C, D)

MB 803399; sequences of the holotype: KC615299 (β-tubulin), KC346303 (ITS)

Type: Russia. Orenburg region: Sakmara district, village Grebeni (about 12 km NE of Orenburg), shrubby steppe on SE slope of the hill Grebeni, W of village, alt. 120–160 m a.s.l., 51°56′28′′N, 55°16′48′′E, on lime-rich schist and sandstone boulders and pebbles in scree, 7 June 2011, *I. Frolov & J. Vondrák* (holotype PRA JV9467). — Paratypes: See Appendix.

Diagnostic characters: (1) anthraquinones absent; (2) thallus epilithic or strongly reduced but not endolithic, usually < 150 µm thick, without distinct cortex, with Sedifolia-grey; (3) mature apothecia usually less than 0.4 mm diameter; (4) cells in the uppermost true exciple 3.5–7.5 µm wide; (5) hymenium without extracellular oil drops; (6) epihymenium and outer part of true exciple grey, rarely with a weak brown tinge,

with Sedifolia-grey (K+ violet); (7) width of widest cell of paraphysis 4–7.5 µm; (8) ascospores with thin septa, 1–2.5 µm.

Detailed description: Thallus epilithic, ochre or grey, forming small roundish spots to about 1 cm diameter or irregular spots to several cm wide; of tightly arranged, angular to rounded, ± flat areoles, (0.15)0.29–0.31–0.33(0.44) × (0.15)0.24–0.25–0.27(0.35) mm [20, 2, 0.16 & 0.13]; sometimes thallus diminishing and present only below apothecia or as only a few areoles. Thickness of thallus (0)95–118–123(175) µm [22, 3, 58]. Medulla inconspicuous, to about 50 µm thick; cells hardly observable due to presence of extracellular crystals only partly dissolved and recrystallized into needles in $H_2SO_4$. Algal layer (38)56–62–68(100) µm [20, 2, 32] thick; algal cells globose, (8.0)11.3–13.5– 15.0(19.5) µm diameter [30, 3, 2.9]. Cortex not developed; usually alveolate cortex present. Epinecral layer often present, but its boundary with alveolate cortex indistinct. Total thickness of alveolate cortex and epinecral layer (13)21– 21–22(25) µm [20, 2, 4]. Alveolate cortex cells ± spherical, (4.0)6.1–6.3–6.4(8.8) µm diameter [20, 2, 1.2], thickness of cell wall about 1.5 µm. Vegetative diaspores absent. Extracellular crystals of calcium salts present only in medulla. Pruina absent. Prothallus indistinct.

Apothecia (0.22)0.27–0.32–0.42(0.44) mm diameter [90, 9, 0.06]; zeorine or rarely biatorine; mature apothecia immersed to adnate, rarely sessile. Disc and true exciple brown to black; thalline exciple same colour as thallus; white pruina on disc and exciples often present (observed in seven out of eleven evaluated samples). Hymenium colourless without extracellular oil drops, rarely with crystals, (63)81–89–98(100) µm high [30, 3, 10]; epihymenium grey, rarely with weak brown tinge. Hypothecium colourless, underlain by algal layer, with extracellular oil drops, with a central conical extension downward, (55)78–85–101(125) µm high [30, 3, 21], formed of cells variable in shape. Exciple about 15–75 µm wide, formed of true exciple, (8)16–23–31(50) µm wide [30, 3, 11], and thalline exciple, (0)9–10–11(30) µm wide [30, 3, 9]. Upper part of true exciple of thin-walled cells (5.0)7.1–7.7–10.1(10.5) × (3.5)4.1–5.3–7.0(7.5) µm [103, 11, 1.2 & 1.0]. Lower part of palisade prosoplectenchyma of thin-walled cells (7.0)9.9– 11.2–12.4(17.0) × (1.5)2.1–2.5–2.7(3.5) µm [25, 3, 2.3 & 0.5]. Thalline exciple without cortex or with indistinct cortex, to 15 µm thick, with extracellular crystals (in $H_2SO_4$ partly dissolved and recrystalized into needles). Paraphyses (1.5)2.3–2.3–2.4(3.0) µm wide [30, 3, 0.4] in lower part, but widening gradually to (4.0)4.9–5.3–6.1(7.5) µm [110, 11, 0.9] in upper part; rarely branched and anastomosed; uppermost paraphyses cells usually dead (not stained by cotton blue), thin and shrunken (Fig. 2A and B). Asci clavate, (45)53–58–65(75) × (10)15– 17–18(25) µm [30, 3, 8 & 3]. Ascospores 8 per ascus, colourless, polarilocular, (11.0)14.3–14.7–15.6(19.0) × (5.5)7.2–7.9–8.6(10.0) µm [95, 10, 1.3 & 1.0], with rounded ends.

Septa (1.0)1.5–1.8–2.1(2.5) µm [95, 10, 0.4], cytoplasmatic channel within septum always rather broad. Ascospore length/width ratio: (1.45)1.72– 1.94–2.09(2.71) [95, 10, 0.22]; septum width/ ascospore length ratio: (0.07)0.09–0.12– 0.14(0.18) [95, 10, 0.02]. Extracellular crystals of calcium salts not seen in apothecia, but possibly forming pruina, which is rarely present.

Pycnidia observed in three samples with well developed thalli, about 60–100 µm wide, distinguished by their darker grey tops on thallus surface. Conidiophore cells not studied. Conidia ellipsoid, (3.0)3.6–3.7–3.8(4.5) × (1.5)1.8–1.9– 2.0 (2.0) µm [30, 3, 0.4 & 0.2].

Chemistry: Spot tests: thallus and apothecia K– (but true exciple slightly violet in apothecia with pruina), C–, P–. Thallus and apothecia UV–. In section, true exciple non-amyloid (I–); hymenium and hypothecium amyloid (I+). Uppermost cells in alveolate cortex of thallus and in cortex of thalline exciple ± with Sedifolia-grey (pale grey in water, K+ violet; the reaction not always observable). Concentration of Sedifolia-grey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple also with Sedifolia-grey. No substances in apothecia and thallus detected by HPLC (done in samples PRA JV9467, *L. Śliwa 3118* KRAM).

Similar taxa: *Caloplaca albopruinosa* (ascospore septum wider, thalline exciple indistinct, thallus distinctly endolitihic, grey or white; perhaps restricted to Europe), *C. alociza* (hymenium with numerous extracellular oil drops, thalline exciple indistinct, ascospore septum wider, thallus distinctly endolithic), *C. atroalba* & *C. microstepposa* (hymenium with numerous extracellular oil drops, epihymenium brown, K+ brown-violet, upper paraphyses cells and cells in uppermost true exciple thinner; Table 4), *C. badioreagens* (endolithic thallus; larger apothecia; with different apothecial chemistry), *C. circumalbata* (apothecia and thallus larger, thallus usually white), *C. diphyodes* (apothecia and thallus larger, ascospore septa wider, hymenium sometimes with extracellular oil drops), *C. micromarina* (spore septum wider, upper cells in paraphyses thinner, thallus thicker; Table 4), *C. transcaspica* (apothecia and thallus larger, thallus white or pale grey, apothecia always sessile, hymenium sometimes with extracellular oil drops).

Phylogeny: *C. micromontana* forms a monophyletic group supported by ML analysis (Fig. 1). It is sister to an undescribed blastidiate taxon (*Caloplaca* sp. in Fig. 1) and close to the paraphyletic *C. albopruinosa*. *Caloplaca albopruinosa* is a closely related taxon, but it is clearly different in its ITS characters. Our three ITS sequences of *C. micromontana* are almost identical (variability in less than 1% of base pairs), but all ITS sequences of *C. albopruinosa* sensu Muggia et al. (2008) differ in more than 4% of base pairs from our ITS sequences of *C. micromontana*.

Distribution and ecology: *Caloplaca micromontana* is known from inland territories of Europe and Asia. It is always recorded from mountains where it occurs in

different altitudes. In the Alps (Austria) C. micromontana is known at altitudes between 1700 and 2100 m, in the Carpathians (Poland and Slovakia) it grows between 550 and 1500 m (in Poland the taxon has been known under the name C. atroalba; e.g. Wilk 2011, 2012). Pakistanian locality is at 4100 m. Russian records are from 150–400 m (Ural Mts.) and 2200 m (Sayan Mts.). It is commonly observed that lichens of high montane to alpine habitats in central and western Europe may grow at much lower altitudes in continental southern Russia (e.g. our observations on *C. diphyodes*, *C. epithallina* and *C. percrocata*). *Caloplaca micromontana* occurs on outcrops, stones and pebbles of limestone, lime-rich schist and sandstone. Co-occurring taxa are *Acarospora moenium*, *Caloplaca crenulatella* s. lato, *C. variabilis* s. lato, *Lecanora dispersa* s. lato, *Lecidella carpathica*, *Sarcogyne regularis*, *Verrucaria* sp. *Caloplaca micromontana* can sometimes grow with *C. microstepposa*, but it never reaches coastal areas harbouring *C. micromarina*.

***Caloplaca microstepposa*** Frolov, Nadyeina, Khodos. & Vondrák, sp. nova
(Figs 2A–D, 4E, F)

MB 803400; sequences of the holotype: KT013276 (β-tubulin), KC984530 (ITS)

Type: Czech Republic. Bohemian karst. Praha, Radotín, Kosoř, protected area Černá rokle, E of village, alt. about 250–300 m a.s.l., 49°59′21′′N, 14°20′8′′E, on pebbles in sun-exposed limestone scree below SE-exposed limestone outcrop in steppe with shrubs, 3 Aug. 2011, *Z. Palice & J. Vondrák* (holotype PRA JV9141). — Paratypes: See Appendix.

Diagnostic characters: (1) anthraquinones absent; (2) thallus epilithic, usually less than 300 µm thick, without cortex (but alveolate cortex often developed), Sedifolia-grey usually absent; (3) mature apothecia up to 0.7 mm diameter, with ± brown disc and true exciple; (4) cells in uppermost true exciple narrow, 2–7 µm wide; (5) hymenium inspers, without crystals; (6) epihymenium and outer part of the true exciple brown or rarely brown-grey, containing a brown pigment and Sedifolia-grey, K+ brown-violet; (7) width of widest cell of paraphysis 3–6 µm; (8) ascospores with 1–3.5 µm wide septa.

Detailed description: Thallus epilithic, in shades of ochre, grey or grey-white, forming small roundish spots to about 1 cm diameter or irregular spots to several cm wide, sometimes mixed with thalli of other lichens; of tightly arranged, angular to rounded, flat areoles, (0.18)0.26–0.39–0.53(0.66) × (0.15)0.22–0.29– 0.39(0.44) mm [30, 3, 0.14 & 0.08]. Thickness of thallus (75)86–157–369(500) µm [102, 11, 89].

Specimens from desert of western Kazakhstan have thicker thallus than lichens from Turkey, Ukraine, Czech Republic and France. Medulla inconspicuous, to about 50 µm thick; cells hardly observable due to presence of extracellular crystals only partly dissolved and recrystallized into needles in H2 SO4 . Algal layer (25)54– 67–90(115) µm thick [30, 3, 23]; algal cells globose, about (9.0)13.5–16.0–20.6(26.0) µm diameter [30, 3, 4.6]. Real cortex not developed; alveolate cortex usually present. Epinecral layer often present, but its boundary with alveolate cortex indistinct. Alveolate cortex with epinecral layer (7)16–24–39(63) µm thick [102, 11, 9.2]. Alveolate cortex cells ± spherical, (5.0)6.2–6.4– 6.6(8.0) µm diameter [20, 2, 0.9], thickness of cell walls about 1.5 µm. Vegetative diaspores absent. Extracellular crystals of calcium salts not observed in thallus. Pruina inconspicuous or absent from thallus surface. Prothallus usually absent or poorly developed, ochre.

Apothecia (0.24)0.32–0.39–0.49(0.66) mm diameter [110, 11, 0.09]; zeorine or rarely biatorine; mature apothecia suppressed to adnate, rarely immersed or sessile. Disc brown to black; true exciple same colour as disc; thalline exciple same colour as thallus; pruina absent from apothecia. Hymenium (75)83–87– 90(100) µm high [30, 3, 7.9], colourless, not glutinose, inspers (with numerous extracellular oil drops), about 0.5–5.0 µm diameter; epihymenium usually brown but rarely brown-grey (observed in four samples). Hypothecium colourless, underlain by algal layer, with extracellular oil drops, with a central conical extension downward, (75)82–93–100(125) µm high [30, 3, 19], formed of cells variable in shape. Exciple about 10–90 µm wide, formed of true exciple, (7)17–21–24(38) µm wide [30, 3, 9.1], and thalline exciple, (0)6–10–15(55) µm wide [30, 3, 13.7]. Upper part of true exciple of thin-walled cells (4.0)5.9–6.6–7.3(10.0) × (2.0)2.6–3.5– 4.5(7.0) µm [110, 11, 1.2 & 0.9]. Lower part of palisade prosoplectenchyma of thin-walled cells (5.5)8.9–9.6–10.3(14.0) × (1.5)2.1–2.3–2.5(3.0) µm [30, 3, 0.4 & 2.3]. Thalline exciple without cortex or with indistinct alveolate cortex, with extracellular crystals (in H2 SO4 partly dissolved and recrystalized into needles). Paraphyses (1.5)2.2–2.3–2.5(3.0) µm wide [30, 3, 0.4] in lower part, but widening gradually to (3.0)3.7–4.2–4.7(6.0) µm [110, 11, 0.7] in upper part; rarely branched and anastomosed; uppermost paraphyses cells usually dead (not stained by cotton blue), thin and shrunken (Fig. 2A and B). Asci clavate, 8-spored, (45)56–60– 65(75) × (13)17–17–19(22) µm [30, 3, 7 & 3]. Ascospores 8 per ascus, colourless, polarilocular, (12.0)13.6–15.1–18.4(21.0) × (4.5)6.0–6.6–7.9 (9.5) µm [93, 10, 2.0 & 0.9], with rounded ends. Septa (1.0)1.6–1.9–2.4(3.5) µm wide [93, 10, 0.4], cytoplasmatic channel within septum always rather broad. Ascospore length/width ratio: (1.51)1.93–2.34–2.93(3.33) [93, 10, 0.45]; septum width/ascospore length ratio: (0.06)0.10– 0.12–0.17(0.23) [93, 10, 0.03]. Extracellular crystals of calcium salts absent from all apothecial parts.

Pycnidia not common (observed only in three samples), about 100–150 µm wide, distinguished by their darker grey tops on thallus surface. Conidiophore cells not evaluated. Conidia narrowly ellipsoid to broadly ellipsoid, (3.0)3.4– 3.4–3.5(4.0) × (1.0)1.4–1.7–2.0 (2.5) µm [30, 3, 0.4 & 0.3].

Chemistry: Spot tests: thallus and apothecia K–, C–, P–. Thallus and apothecia UV–. In section, true exciple non-amyloid (I–); hymenium and hypothecium amyloid (I+, but hypothecium only weakly I+). Upper cells in alveolate cortex of thallus and thalline exciple with low concentration of Sedifolia-grey (colourless or very pale grey in water, K+); K+ violet reaction observable only in two samples. Concentration of Sedifoliagrey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple with a brown pigment together with the Sedifolia-grey (usually brown or grey-brown in water, K+ brownviolet). No substances detected in apothecia and thallus by HPLC (done in samples PRA JV9344, JV9448, *O. Nadyeina 111* KW).

Similar taxa: *Caloplaca albopruinosa* (thallus endolithic, grey or white; apothecia usually white pruinose; epihymenium grey, K+ violet; ascospore septum wider, thalline exciple indistinct), *C. alociza* (thallus endolithic; margin and disc of apothecia often white pruinose; thalline exciple indistinct; ascospore septum wider), *C. atroalba* (see the taxonomic note), *C. badioreagens* (endolithic thallus; different apothecial chemistry), *C. circumalbata* (apothecia and thallus larger, thallus usually white), *C. diphyodes* (apothecia and thallus larger; ascospore septa distinctly wider), *C. micromarina* (hymenium without extracellular oil drops; epihymenium grey, K+ violet; cells in uppermost true exciple wider; ascospore septa wider; Table 4), *C. micromontana* (hymenium without extracellular oil drops; epihymenium grey, K+ violet; upper cells in paraphyses and cells in uppermost true exciple wider; Table 4), *C. transcaspica* (apothecia and thallus larger; thallus white, grey, usually pruinose; epihymenium grey).

Phylogeny: *C. microstepposa* forms a wellsupported monophyletic group together with two sequences of lichens macroscopically similar to *C. transcaspica*, with white large thalli and large apothecia (Fig. 1). These two terminals are called "*C.* aff. *microstepposa*". In our unpublished single-locus phylogenies of MCM7 and RPB2, samples of *C.* aff. *microstepposa* (e.g. Vondrák 5466) do not group with *C. microstepposa*, but form a supported clade with *C. transcaspica*.

Distribution and ecology: *Caloplaca microstepposa* is known from inland arid and semi-arid regions of Asia and from dry inland localities throughout Europe in altitudes up to 1000 m. It is common in deserts of the Mangystau region in western Kazakhstan, where it grows on soft limestone outcrops. In the steppe and forest-steppe zone of Russia and Ukraine, the lichen occurs on pebbles or outcrops of calcareous schist, calcareous sandstone and limestone. The taxon has been known from Ukrainian Donetsk Upland as *C. transcaspica* (Nadyeina 2009). In northern Turkey, it was

collected from calcareous sandstone pebbles and limestone outcrops in continental forest-steppe, open sub-Mediterranean bush, but also from sunny habitats in the zone of montane forests. In central and southern Europe (Austria, Bulgaria, Czech Republic, France, Germany, Italy, Poland, Serbia, Spain) *C. microstepposa* grows usually on calcareous pebbles and stones, rarely on limestone outcrops or concrete, often in sunny, S-exposed screes and in rocky steppes, up to 1000 m alt. Co-occurring taxa are *Aspicilia calcarea*, *A. contorta*, *Caloplaca concreticola*, *C. crenulatella* s. lato, *C. decipiens*, *C. ferrarii* s. lato, *C. interfulgens*, *C. teicholyta*, *C. variabilis* s. lato, *Candelariella aurella*, *Diplotomma* sp., *Lecanora dispersa* s. lato, *Leptogium plicatile*, *Rinodina bischoffii*, *Verrucaria muralis* and *V. nigrescens* s. lato. *Caloplaca microstepposa* reaches coastal areas in the Crimean Peninsula where it can grow together with *C. micromarina*. It also rarely occurs with *C. micromontana*, for instance in steppe foothills of Asian mountains.

Taxonomical note: We investigated the type specimen of *C. atroalba* (FH, lectotype) and some other samples identified as this taxon described from North America (samples identified/revised by C. Wetmore in GZU, MIN and several samples from herbaria T. Spribille and T. Wheeler). *Caloplaca atroalba* sensu Wetmore (1994) is a heterogeneous taxon containing *C. atroalba* s. stricto and *C. diphyodes* (our unpubl. data) and possibly other taxa. Our evaluation of the type specimen did not show any phenotypic differences from *C. microstepposa*; our observations are available inside the specimen envelope. Some recently collected samples named "*C. atroalba*" are phenotypically identical with the type of *C. atroalba* (USA. Montana, 2010, T. Spribille s.n.; USA. Montana, 2010, T. Wheeler 3152), but their β-tubulin, MCM7, RPB2 and ITS sequences do not group with *C. microstepposa* (data not shown). *Caloplaca atroalba* and *C. microstepposa* are probably true cryptic species. Revision of "*C. atroalba*" specimens will be the subject of a separate paper.

**Key to the Eurasian species related to *Caloplaca variabilis* and without anthraquinones (genus *Pyrenodesmia* sensu Arup et al. 2013)**

Epiphytic and some epilithic Teloschistaceae crusts without anthraquinons that are unrelated to *C. variabilis* are not included in the key (e.g. *Caloplaca demissa*, *C. obscurella* and *C. servitiana*).

1. Thallus with soredia, minute granules or pustulate outgrowths ..... 2
1. Thallus without soredia, minute granules or outgrowths ..... 5
2. Thallus with pustulate outgrowths, areolate, well-developed, sordid-grey to white-greyish ..... *Caloplaca albopustulata*
2. Thallus with soredia or minute granules ..... 3

3. Thallus endolithic with obscurely sublobed prothallus, thalli often form shallow bowl-shaped depressions in limestone, soredia or soredia-like minute granules often completely covering central part of thallus ..... *Caloplaca erodens*
3. Thallus epilithic, well-developed ..... 4
4. Thallus with well-developed fungal and algal stacks (sensu Vondrák & Kubásek 2013), upper surface with ridges derived from epinecral layer ……..……. .....*Caloplaca molariformis*
4. Thallus without fungal or algal stacks and ridges on upper surface..... ……..……. *Caloplaca concreticola*
(be aware of other taxonomically unclear sorediate/blastidiate species with similar appearance to *C. concreticola*, as e.g. *Caloplaca* sp. in Fig. 1)
5. Thallus distinctly endolithic ..... 6
5. Thallus epilithic ..... 9
6. Epihymenium reddish-brown in KOH..... *Caloplaca badioreagens*
6. Epihymenium violet in KOH ..... 7
7. Thalline exciple well-developed..... *Caloplaca erodens* "non-sorediate" (see comments in the text)
7. Thalline exciple indistinct ..... 8
8. Hymenium with numerous extracellular oil drops ……. *Caloplaca alociza*
(apprised isotype of *C. lecideina* (*Callopisma variabile* var. *lecideina*, G 00290968) belongs to *C. diphyodes*)
8. Hymenium without extracellular oil drops ..... *Caloplaca albopruinosa*
9. Thallus small and thin, often forms roundish spots to about 1 cm diameter, apothecia usually less than 0.5 mm diameter ..... 10
9. Thallus and apothecia distinctly larger ..... 12
10. Hymenium with extracellular oil drops, epihymenium brown or rarely brown-grey, width of cells in uppermost true exciple usually 2.5–4.5 µm, rarely 2–7 µm ..... *Caloplaca microstepposa*
10. Hymenium without extracellular oil drops, epihymenium grey, width of cells in uppermost true exciple usually 4–7 µm, rarely 3–8.5 µm..... 11
11. Spores septa usually 1.5–2.1 rarely up to 2.5 µm, widest cell of paraphyses usually 4.9–6.1 rarely 4–7.5 µm; in mountains, never close to seashore ..... *Caloplaca micromontana*
11. Spores septa usually 2.6–3.4 rarely 2–4.5 µm, widest cell of paraphyses usually 3.7–4.5 rarely 3–6 µm; close to seashore .....*Caloplaca micromarina*
12. Thallus bullate; arid regions of Asia..... *Caloplaca bullata*
12. Thallus not bullate; in different regions of Eurasia ..... 13
13. Apothecia immersed to adnate ..... 14
13. Apothecia sessile ..... 15

14. Thallus thick, consisting of flat, very tightly arranged areoles, hypothecium containing vertical rows of small round paraplectenchymatous cells; mainly in temperate and Mediterranean regions, also in high mountains, throughout Europe, the Near East, rarely in northern Africa and southern Siberia ..... *Caloplaca chalybaea*

14. Thallus thick, areoles not flat, often convex, not tightly arranged, hypotecium without vertical rows of small round paraplectenchymatous cells; in arid regions of northern Africa and Near East, very rarely in southern Europe ..... *Caloplaca circumalbata*

15. Spores septa thin (1–3 µm); in arid regions of eastern Europe and Asia, very rarely in southern Europe..... 16

15. Spores septa wide (more than 3 µm), thallus of variable colors; in temperate, arctic and alpine regions, rarely in arid regions..... 17

16. Thallus consists of peltate areoles, yellowish-brown ..... *Caloplaca tianshanensis*

16. Areoles not peltate, thallus white to white-greyish ..... *Caloplaca transcaspica* s. lato (we accomodate *C. ayachina* here until its taxonomy is not resolved)

17. Apothecial margin and sometimes also disk white pruinose, thallus of variable colors; always on calcareous substrate, avoiding humid conditions close to water; mainly in temperate and Mediterranean regions of Europe, but also in the Near East, northern Africa and southern Siberia ..... *Caloplaca variabilis* s. lato (taxon with variable morphotypes; we accommodate all of them here until the relationships between them are resolved)

17. Apothecia without pruina, thallus grey; often on siliceous substrates, often near water; mainly in montane to alpine belts in European mountains, in Arctic and continental parts of Asia also at low altitudes..... *Caloplaca diphyodes*

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## Appendix

Paratype specimens. — *Caloplaca micromarina*: **Russia**. Black Sea coast: Tuapse, coastal rocks S of Gryaznova, 44°11′13′′N, 38°53′16′′E, on schist, 2007, *J. Vondrák* (PRA JV7470); Gelendzhik, coastal rocks W of Krinitsa (near Betta), 44°23′34′′N, 38°19′22′′E, on calcareous conglomerate, 2007 *J. Vondrák* (PRA JV6537, JV6662). **Ukraine**. Crimean Peninsula: Sudak, Kurortnoe, in slopes of Karadag Mts., alt. 100–200 m a.s.l., on lime-rich schist, 2008 *J. Vondrák* (PRA JV7230); Sudak, Kurortnoe, Mt. Eczkedag SW of village, alt. about 300 m a.s.l., on lime-rich schist, 2008 *J. Vondrák* (PRA JV6412, JV6414, JV6420, JV6422); Sudak, Dachnoe, alt. about 100 m a.s.l., on lime-rich schist, 2008 *J. Vondrák* (PRA JV7229); Alushta, Mt. Ayudag, alt. about 200 m a.s.l., on base-rich volcanic rock, 2008 *J. Vondrák* (PRA JV7236). — *Caloplaca micromontana*: **Austria**. Kärnten (Carinthia): Karnische Alps, [Lubenhochwald], alt. 2000 m a.s.l., 46°34′N, 13°19′E, on limestone, 1994 *J. Poelt* (GZU 52-94); Karnische Alps, Oisternig SW from Feistritz im Gailtal, SE slope near Feistritzer Alm, alt. 1850 m a.s.l., on limestone, 1987 *J. Hafellner 17279* (GZU 45-87); Steiermark (Styria): Hochschwabgruppe, Griesmauer above Vordernberg, ridge near peak 2019, alt. 2000–2118 m a.s.l., on limestone, 1986 *J.*

*Poelt & Cl. Roux* (GZU 97-86); Niedere Tauern, Wölzer Tauern, Kasofen, 2 km N from Pusterwald, W-exposed wall directly below peak "Stinkmarmor", alt. 1860–1890 m a.s.l., on quite dry marble rocks, 1993 *A. Wilfling & M. Möslinger 540* (GZU 43-98); Bruck an der Mur, Hochschwabgruppe, Hochstein, N of Aflenz, Gipfelschrofen, alt. 1730–1740 m a.s.l., on limestone, 1993 *J. Poelt* (GZU 1-93); Nördliche Kalkalpen, Dachsteingruppe, Ramsau [am Dachstein], between Dachsteinsüdwandhütte [cabin] and Hunerscharte, below Scheiblingsteins, alt. 1900–2000 m a.s.l., on marl, 1993 *J. Poelt & M. Grube* (GZU 73-93). **Pakistan**. Baltistan: Haramosh range, "Alm" Pakora SE of pass Ganto La, pasture and rocks around alm, rocky slopes, alt. about 4100 m a.s.l., 35°41′N, 75°21′E, on limestone, 1991 *J. Poelt* (GZU 109-91). **Poland**. Western Carpathians: West Tatra Mts., Dolina Chochołowska, Polana Chochołowska, alt. 1105 m a.s.l., 49°14′16′′N, 19°47′47′′E, on limestone, 2004 *L. Śliwa 3118* (KRAM); Western Carpathians: Pieniny Właścive Mts., Pieniny National Park, limestone outcrops at Czorsztyn Castle, alt. 560 m a.s.l., 49°26′11′′N, 20°18′48′′E, on limestone on sunny S-exposed slope, 2005 *K. Wilk 3470b* (KRAM). **Russia**. Sverdlovsk Region: Rezh, 2 km SW of village Samocvet, rocky steppe on small limestone and siliceous outcrops above left bank of river Rezh, alt. 165 m a.s.l., 57°35′33′′N, 61°44′28′′E, on limestone cliff in extrazonal steppe, 2013 *J. Vondrák* (PRA JV11081 & 11088); Rezh, Aramashevo, limestone cliffs at village above left bank of river Rezh, alt. 150–200 m a.s.l., 57°36′31′′N, 61°44′11′′E, on limestone cliff in extrazonal steppe, 2013 *J. Vondrák* (PRA JV11087; distributed in Vondrák: sel. exs. of *Caloplaca*, fasc. 4); Voronezh region: Khokholsky district, near village Kostyonki, protected area "Kostyonki-Borschyovo′", alt. about 200 m a.s.l., on limestone in steppe, 2003 *I. Bogdanova* (KRAM-L-65593). Republic of Bashkortostan: Sterlitamak, about 8 km S of town, Mt. Shikhan Toratau, alt. 300–400 m a.s.l., 53°33′10′′N, 56°5′53′′E, on limestone in steppe, 2011 *O. Vondráková & J. Vondrák* (PRA JV9523); Republic of Tyva: West Sayan Mts., Ak-Dovurak, Ak-Sug, Enge-Beldir, glacier cirque in S-slope from pass ′′Sayanskiy pereval; 2200 m′′, by the road A161, close to border with Khakasia, alt. 2150–2200 m a.s.l., 51°42′0′′N, 89°53′14′′E, on base-rich schist, below S-exposed overhanging outcrop, in alpine zone, 2013 *J. Vondrák & I. Frolov* (PRA11083). **Slovakia**. Tatra Mts.: Feigsblösse [Faixová in Belianské Tatry], on limestone, 1868 *(W.) H. Lojka* (W 2013-02600). — *Caloplaca microstepposa*: **Austria**. Tirolia: Ötztaler Alpen, Landeck District, SW of Fließ, above Neuer Zoll [hotel], W-exposed dry overhang, alt. about 1000 m a.s.l., on schist, 1989 *J. Poelt* (GZU 65-89). **Bulgaria**. Eastern Rodopi Mts.: Kardzhali, Momchilgrad, Starovo, about 2 km E of village, alt. 390 m a.s.l., 41°28′N, 25°25′E, on concrete, 2004 *J. Vondrák* (PRA JV2160). **Czech Republic**. Bohemian karst: Praha, Kosoř, SW-exposed rocks in valley of Radounský potok, about 1.5 km NW of village, alt. about 250 m a.s.l., 49°59′41′′N, 14°18′26′′E, on limestone outcrops and pebbles in steppe, 2012 *I. Frolov & J. Vondrák* (PRA JV9678, JV9679, JV9675); Praha, Třebotov, rock at SW slope of Kulivá hora hill, about 1 Km SW of village, alt. 330 m a.s.l., on vertical sun-exposed limestone outcrop, 49°57′51′′N, 14°17′10′′E, 2012 *I. Frolov & J. Vondrák* (PRA JV9671, JV9724). **France**. Maritime Alps: Menton, Breil-sur-Roya, La Brigue, rocks in S-slope above valley of brook La Levensa, alt. about 1000 m a.s.l., 44°04′02′′N, 7º38′17′′E, on sun-exposed limestone, 2012 *I. Frolov, J. Malíček, J. Vondrák* (PRA JV10221). **Germany**. Bavaria: Würzburg, near Sugenheim, [alt. about 400 m a.s.l.], on marl, 1865 *H. Rehm* (W 1906-10939, 1990-00463, 2013-02598, 2013-02599). **Italy**. South Tyrol (Südtirol, Alto Adige): [Bolzano], Margola near Predazzo, S-exposed slope, [alt. about 1000 m a.s.l.], on limestone, 1883 *F. C. G. Arnold* (W 1900-9904, 1913-5686, 2013-02601). **Kazakhstan**. Mangistau region: Mangistau district, by the road between villages Shetpe and Say-Utes, about 30 km SW of Say-Utes, alt. 260 m a.s.l., 44°09′20′′N, 52°39′10′′E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9136, JV9452); Mangistau district, west chink (slope) of Ustyurt plateau, Manashy, by the road between villages Say-Utes and Shetpe, alt. 290 m a.s.l., 44°06′12′′N, 53°12′40′′E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9428); Mangistau district, East Karatau ridge, rocks by the road between Zhatybay and Shetpe, about 30 km SW of Shetpe, alt. 180 m a.s.l., 43°57′00′′N,

52°05′52′′E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9448, JV9470, JV9462); Mangistau district, village Shetpe, West Karatau ridge, about 10 km N of village, alt. 130 m a.s.l., 44°12′48′′N, 52°03′58′′E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9450, JV9454). **Poland**. Góry Świętokrzyskie: village Łukowa, hill without forest, SW of village, on limestone pebbles scattered on ground, 1976 *K. Toborowicz* (KTC-4705); Kielce County: Wesoła above Wierna Rzeka, hill without forest near railway, eastern slope, alt. about 200–300 m a.s.l., on limestone, 1976 *K. Toborowicz* (KTC-6332, as *Caloplaca atroalba* in Wilk 2011); Opatów district, about 15 km WSW of Ostrowiec Świętokrzyski, Grzegorzewice near village Waśniów, alt. about 300 m a.s.l., on dry limestone outcrops, 1976 *J. Nowak* (KRAM-L-22963); Western Carpathians: Pogórze Przemyskie, Rzeszów, about 12 km SW of Przemyśl, village Koniusza, alt. about 400 m a.s.l., on sun-exposed calcareous schist outcrops, 1981 *J. Kiszka, J. Piórecki* (KRAM-L-56053). **Russia**. Orenburg Region: Tanalik, 5 km N of village Chapaevka, W-exposed limestone outcrops in steppe, above water reservoir Iriklinskoe, alt. 330 m a.s.l., 52°05′44′′N, 58°49′36′′E, on limestone outcropes in steppe, 2013 *J. Vondrák* (PRA JV11079); Republic of Altay: Kosh-Agach district, SE part of Kuray Ridge, NE of village Chagan-Uzun, alt. 2000–3000 m a.s.l., on lime-enriched siliceous outcrop in alpine steppe, 2012 *J. Vondrák & Frolov* (PRA JV10436); Republic of Khakasia: 20 km N of town Shira, quartzite, limestone and schist outcrops in short-grass steppe, alt. 410 m a.s.l., 54°39′48′′N, 89°50′35′′E, on calcareous sandstone in steppe, 2013 *J. Vondrák* (PRA JV11107); Tashtip, steppes on hills between villages Nizhnaya Teya and Poltakov, with sandstone and schist outcrops, alt. 480 m a.s.l., 52°56′14′′N, 90°05′29′′E, on calcareous sandstone in steppe, 2013 *J. Vondrák* (PRA JV11111); Republic of Tyva: Sarig-Sep, Buren-Bay-Khaak, concrete gutter in steppe at village, alt. 840 m a.s.l., 51°11′57′′N, 95°31′33′′E, on horizontal, sun-exposed face of concrete, 2013 *J. Vondrák* (PRA JV11071). **Serbia**. Đerdap National Park: SW exposed limestone road cut along road from Mosna to Tekija, alt. about 160 m a.s.l., 44°38′18.8′′N 22°18′28.0′′E, on relatively fresh limestone outcrops, 2014 *I. Frolov* (herbarium I. Frolov 878). **Spain**. Catalonia: Barcelona, outscirts of Santa Maria de Montserrat Abbey, on way to Mt. Miranda de Sant Jeroni, conglomerate outcrops, alt. about 1000 m a.s.l., 41°36′18.3′′N, 1°48′41.1′′E, on xerothermic conglomerate outcrops, 2015 *I. Frolov* (herbarium I. Frolov 1002). **Turkey**. Çorum region: Çorum, Dodurga, Yeniköy, alt. 970 m a.s.l., 40°49′29′′N, 34°44′02′′E, on soft limestone in bottom of dry gorge in forest-steppe, 2012 *J. Vondrák* (PRA JV9779); Isparta region: near Hadschi Bey, on shore of lake Egerdin, [alt. about 1000 m a.s.l.], 1931 *V. Pietschmann* (W 1959-6621); Sinop region: Sinop, Boyabat, by the road Kastamonu-Sinop, in valley of brook at village Şeyhli Köyü, alt. 700 m a.s.l., 41°42′41′′N, 34°55′26′′E, on limestone pebbles, 2012 *J. Vondrák* (PRA JV9811); Tokat region: Tokat, Merkez, Geyras Mahallesi, small gorge above highway, alt. 800 m a.s.l., 40°14′54′′N, 36°32′47′′E, on calcareous stones in open sub-Mediterranean bush, 2012 *J. Vondrák* (PRA JV9757). **Ukraine**. Kherson region: Nikolskoe, slope above left bank of Ingulec river, alt. 10–20 m a.s.l., on S-exposed limestone outcrop, 2009 *A. Naumovich, A. Khodosovtsev, J. Vondrák* (PRA JV6943; KHER 4999); Antonovka, slope above right bank of Dniepr river, on limestone, 1992 *A. Khodosovtsev* (KHER 2594); Kairy, Dniepr river, on limestone, 2010 *A. Khodosovtsev & L. Gavrylenko* (KHER 5000). Lugansk region, Donetsk Upland: Sverdlovsk district, near village Provallya, alt. about 200 m a.s.l., steppe slopes with sandstone outcrops and isolated trees, 2005 *O. Nadyeina 63* (KW; PRA JV6952); same locality in "Provalskaya step" Reserve, 2005 *O. Nadyeina 53* (KW); Sverdlovsk district, at village Medvezhanka, in "Medvezhanskyi′" Botanical Reserve, alt. about 150 m a.s.l., on calcareous schist outcrops in steppe, 2006 *O. Nadyeina* (PRA JV6953); same locality, 2006 *O. Nadyeina 111* (KW); Sverdlovsk district, Dar'ino-Yermakovo, by the road Kharkiv–Rostov, alt. about 150 m a.s.l., on calcareous sandstone, 2006 *O. Nadyeina 109* (KW); Lutuhyno district, near village Verkhnia Orikhivka, Pershozvanivsy water-reservoir, 0.5 km N of village, alt. 200–250 m a.s.l., on calcareous stone, 2005 *O. Nadyeina 5-14* (KW); Khmelnitsk' region: National Park "Podilskyi Tovtry", 15 km SE of Kamianets Podilskyi, Kitaihorod,

canyon of river Tarnava, alt. 140 m a.s.l., 48°38′25′′N, 26°46′58′′E, on calcareous sandstone on SW slope, 2003 *P. Czarnota* (KRAM-L 48695; as *Caloplaca atroalba* in Wilk 2011); National Park "Podilskyi Tovtry", junction of rivers Smotrich and Dniestr, near village Ustia, alt. 136 m a.s.l., 48°33′54′′N, 26°39′24′′E, on limestone outcrops, 2003 *M. Kukwa* (KRAM-L-48831).

Table 1. List of sequences of *Caloplaca* used in the molecular analysis. Vouchers of the new ITS and β-tubulin sequences are set in boldface.

| species / country | voucher data | ITS accession | β-tubulin accession |
|---|---|---|---|
| *Caloplaca albopruinosa* (Italy) | Muggia et al. 2008 | EF093564 | – |
| *Caloplaca albopruinosa* (Italy) | Muggia et al. 2008 | EF093566 | – |
| *Caloplaca albopruinosa* (Italy) | Muggia et al. 2008; **TSB 37658** | EF093577 | **KR912027** |
| *Caloplaca albopruinosa* (Italy) | Muggia et al. 2008 | EF093578 | – |
| *Caloplaca albopruinosa* (Italy) | Muggia et al. 2008; **TSB 37661** | EF093568 | **KR912024** |
| *Caloplaca albopruinosa* (Italy) | Muggia et al. 2008; **TSB 37712** | EF093569 | **KR912025** |
| *Caloplaca albopruinosa* (Slovenia) | Muggia et al. 2008; **TSB 37068** | EF093573 | **KR912026** |
| *Caloplaca albopustulata* (Ukraine) | **Vondrák7128 (PRA)** | **KJ816764** | **KC615302** |
| *Caloplaca albopustulata* (Turkey) | **Vondrák10463 (PRA)** | – | **KC615301** |
| *Caloplaca albopustulata* (Ukraine) | Vondrák et al. 2008 | EU192150 | – |
| *Caloplaca alociza* (Czech Republic) | **Vondrák9298 (PRA)** | **KC611250** | – |
| *Caloplaca alociza* (Germany) | **Wirth19042 (STU)** | **KC884522** | **KC615291** |
| *Caloplaca alociza* (Spain) | **Vondrák6272 (PRA)** | **KC884520** | **KC615290** |
| *Caloplaca alociza* (Italy) | Muggia et al. 2008; **TSB 37764** | EF090928 | **KR912028** |
| *Caloplaca alociza* (Italy) | Muggia et al. 2008; **TSB 36393** | EF090935 | **KR912029** |
| *Caloplaca aractina* (Greece) | **Vondrák3806 (PRA)** | **KR912052** | **KR912044** |
| *Caloplaca badioreagens* (Italy) | Muggia et al. 2008; **TSB 36422** | EF081035 | **KR912030** |
| *Caloplaca badioreagens* (Italy) | Muggia et al. 2008 | EF081036 | – |
| *Caloplaca badioreagens* (Italy) | Muggia et al. 2008 | EF081039 | – |
| *Caloplaca badioreagens* (Italy) | Muggia et al. 2008 | EF081040 | – |
| *Caloplaca chalybaea* (Austria) | Tretiach et al. 2003 | AY313970 | – |
| *Caloplaca chalybaea* (Austria) | Tretiach et al. 2003 | AY313971 | – |
| *Caloplaca chalybaea* (Spain) | Gaya et al. 2003 (as *C. variabilis*) | AY233224 | – |
| *Caloplaca chalybaea* (Greece) | **Vondrák4059 (PRA)** | **KC884498** | **KC615292** |
| *Caloplaca chalybaea* (Czech Republic) | **Vondrák9684 (PRA)** | **KC884536** | **KC615294** |
| *Caloplaca chalybaea* (Czech Republic) | **Vondrák9686 (PRA)** | **KC884534** | **KC615293** |

| | | | |
|---|---|---|---|
| *Caloplaca concreticola* (Czech Republic) | **Vondrák9348 (PRA)** | **KJ816755** | – |
| *Caloplaca concreticola* (Czech Republic) | **Vondrák9676 (PRA)** | **KC884524** | **KC615278** |
| *Caloplaca concreticola* (Kazakhstan) | **Vondrák9443 (PRA)** | **KC884506** | **KC615277** |
| *Caloplaca concreticola* (Russia) | **Vondrák9392 (PRA)** | **KC884542** | **KC615279** |
| *Caloplaca concreticola* (Russia) | **Vondrák10465 (PRA)** | **KJ816757** | **KR912031** |
| *Caloplaca concreticola* (Ukraine) | Vondrák et al. 2008 | EU192151 | – |
| *Caloplaca concreticola* (Slovakia) | Vondrák et al. 2008 | EU192152 | – |
| *Caloplaca diphyodes* (Russia) | **Vondrák9391 (PRA)** | **KR912046** | **KC615284** |
| *Caloplaca diphyodes* (Russia) | **Vondrák8236/1 grey thallus (PRA)** | **KJ816761** | **KR912032** |
| *Caloplaca diphyodes* (Russia) | **Vondrák8236/2 pale ochre thallus (PRA)** | **KC884510** | **KC615282** |
| *Caloplaca diphyodes* (Russia) | **Frolov50 (Herb. I. Frolov)** | **KJ816756** | **KR912033** |
| *Caloplaca diphyodes* (Switzerland) | **1984, *J. Poelt* (GZU114-84)** | **KC884513** | **KC615283** |
| *Caloplaca diphyodes* (Russia) | Vondrák et al. 2012; **Vondrák8326 (PRA)** | JN641782 | **KR912034** |
| *Caloplaca diphyodes* (Russia) | Vondrák et al. 2012; **Vondrák8179 (PRA)** | JN641783 | **KR912035** |
| *Caloplaca diphyodes* (Russia) | Vondrák et al. 2012; **Vondrák8182 (PRA)** | JN641784 | **KR912036** |
| *Caloplaca erodens* (Italy) | Muggia et al. 2008 | EF090921 | – |
| *Caloplaca erodens* (Austria) | Muggia et al. 2008 | EF090922 | – |
| *Caloplaca erodens* (Italy) | Muggia et al. 2008 | EF090923 | – |
| *Caloplaca erodens* (Italy) | Muggia et al. 2008 | EF090924 | – |
| *Caloplaca erodens* (Iran) | **Vondrák5579 (PRA)** | **KC884515** | **KC615286** |
| *Caloplaca erodens* (Russia) | **Vondrák9387 (PRA)** | **KC884541** | **KC615295** |
| *Caloplaca erodens* (Turkey) | **Vondrák8574 (PRA)** | **KC884518** | **KC615289** |
| *Caloplaca erodens* (Turkey) | **Vondrák5362 (PRA)** | **KC884516** | **KC615287** |
| *Caloplaca erodens* "non-sorediate" (Hungary) | **Vondrák6380 (PRA)** | **KC884509** | **KC615281** |
| *Caloplaca erodens* "non-sorediate" (Romania) | **Vondrák6606 (PRA)** | – | **KR912037** |
| *Caloplaca erodens* "non-sorediate" (Turkey) | **Vondrák6659 (PRA)** | **KC884511** | **KC615285** |
| *Caloplaca haematites* (Spain) | **Vondrák6259 (PRA)** | **KR912053** | **KR912045** |
| *Caloplaca micromarina* (Ukraine) | **Vondrák7236/1 non-pruinose apothecia (PRA)** | **KC611248** | **KC615269** |
| *Caloplaca micromarina* (Ukraine) | **Vondrák7236/2 pruinose apothecia (PRA)** | **KC611247** | **KC615270** |
| *Caloplaca micromarina* (Ukraine) | **Vondrák6420 (PRA)** | **KC611249** | **KC615271** |
| *Caloplaca micromarina* (Russia) | **Vondrák6537 (PRA)** | **KC611246** | – |

| | | | |
|---|---|---|---|
| *Caloplaca micromarina* (Turkey) HOLOTYPE | **Vondrák8199 (PRA)** | **KC346301** | **KC615268** |
| *Caloplaca micromontana* (Austria) | **1994, J. Poelt (GZU 52-94)** | – | **KC615297** |
| *Caloplaca micromontana* (Poland) | **L. Śliwa3118 (KRAM)** | – | **KC615298** |
| *Caloplaca micromontana* (Russia) HOLOTYPE | **Vondrák9467 (PRA)** | **KC346303** | **KC615299** |
| *Caloplaca micromontana* (Russia) | **Vondrák9523 (PRA)** | KJ816762 | **KC615300** |
| *Caloplaca micromontana* (Russia) | **Vondrák11083 (PRA)** | **KR912047** | **KR912038** |
| *Caloplaca microstepposa* (Czech Republic) HOLOTYPE | **Vondrák9141 (PRA)** | **KC984530** | **KT013276** |
| *Caloplaca microstepposa* (Kazakhstan) | **Vondrák9135 (PRA)** | **KC611242** | **KC615274** |
| *Caloplaca microstepposa* (Kazakhstan) | **Vondrák9448 (PRA)** | **KJ816758** | – |
| *Caloplaca microstepposa* (Kazakhstan) | **Vondrák9452 (PRA)** | **KJ816759** | – |
| *Caloplaca microstepposa* (Kazakhstan) | **Vondrák9454 (PRA)** | **KC611243** | **KC615276** |
| *Caloplaca microstepposa* (Russia) | **Vondrák10436 (PRA)** | **KR912048** | **KR912041** |
| *Caloplaca microstepposa* (Russia) | **Vondrák11071 (PRA)** | **KR912049** | **KR912040** |
| *Caloplaca microstepposa* (Russia) | **Vondrák11107 (PRA)** | **KR912050** | **KR912039** |
| *Caloplaca microstepposa* (Turkey) | **Vondrák12732 (PRA)** | **KR912051** | **KR912042** |
| *Caloplaca microstepposa* (Ukraine) | **O. Nadyeina111 (KW)** | **KC611245** | **KT013277** |
| *Caloplaca microstepposa* (Ukraine) | **Vondrák6943 (PRA)** | **KC611244** | **KC615275** |
| *Caloplaca microstepposa* (Ukraine) | **O. Nadyeina5-14 (KW)** | **KJ816760** | – |
| *Caloplaca* aff. *microstepposa* (Greece) | Tretiach et al. 2003 | AY313969 | – |
| *Caloplaca* aff. *microstepposa* (Ukraine) | Vondrák et al. 2008; **Vondrák5466 (PRA)** | EU192156 | **KR912043** |
| *Caloplaca transcaspica* (Kazakhstan) | **Vondrák9432 (PRA)** | **KC884507** | **KC615280** |
| *Caloplaca variabilis* p.p. (Greece) | **Vondrák4219 (PRA)** | **KC884499** | **KC615272** |
| *Caloplaca variabilis* p.p. (Czech Republic) | **Vondrák5114 (PRA)** | **KC884500** | **KC615273** |
| *Caloplaca variabilis* p.p. (Hungary) | **Vondrák6357 (PRA)** | **KJ816763** | **KC615296** |
| *Caloplaca* sp. (Czech Republic) | **Vondrák9140 (PRA)** | **KC884539** | **KC984550** |
| *Caloplaca* sp. (Czech Republic) | **Vondrák9673 (PRA)** | **KC884525** | **KC984549** |
| *Caloplaca* sp. (Turkey) | **Vondrák9814 (PRA)** | – | **KT013275** |

Table 2. Summary of phylogenetic analyses.

| Alignment | Number of sequences | Length of alignment | Informative positions (all / ingroup only) |
|---|---|---|---|
| ß-tubulin | 61 | 783 | 186 / 168 |
| ITS | 79 | 537 | 188 / 179 |
| Concatenated | 84 | 1320 | 487 / 451 |

Table 3. Results from linear discriminant analysis (LDA). Ranking of the most explicative continuous characters for the data set comprising the three studied taxa and the discrimination success in distinction between pairs of taxa in the preliminary study (shown only for the most powerful characters).

| Character | Rank/ discrimination success | *microstepposa*/ *micromontana* | *micromontana*/ *micromarina* | *microstepposa*/ *micromarina* |
|---|---|---|---|---|
| Ascospore-septum width | 1/0.644 | 0.7 | 0.9 | 0.767 |
| Septum-width/ascospore-length ratio | 2/0.644 | 0.683 | 0.917 | 0.783 |
| Width of cells in uppermost true exciple | 3/0.567 | 0.867 | | 0.783 |
| Thallus width | 4/0.533 | 0.666 | 0.783 | 0.633 |
| Length of cells in uppermost true exciple | 5/0.489 | 0.717 | | 0.783 |
| Width of widest cell of paraphysis | 6/0.489 | 0.717 | 0.767 | |
| Ascospore width | 7/0.478 | | | 0.7 |
| Lower-/upper-paraphysis-width ratio | 8/0.467 | 0.7 | 0.683 | |
| Width of alveolate cortex plus epinecral layer | 9/0.463 | | 0.867 | 0.717 |
| Apothecium diameter | 10/0.444 | 0.683 | | 0.666 |
| Ascospore length/width ratio | 11/0.444 | | | 0.733 |
| Width of paraphysis bellow tip | 12/0.433 | | 0.65 | 0.666 |
| Apothecium height | 13/0.422 | | | |
| Algal-layer width | 14/0.413 | 0.683 | 0.633 | |
| Hypothecium width | 15/0.411 | | | |
| Ascus height | 16/0.411 | | | |
| Ascus width | 17/0.400 | | | |
| Areola width | 18/0.400 | 0.683 | 0.683 | |
| Areola length | 19/0.400 | | 0.683 | |
| Ascospore length | 20/0.389 | | | |
| Thalline-exciple width | 21/0.378 | | | |
| Hypothecium-cell length | 22/0.378 | | | |
| Length of cells in lower true exciple | 23/0.376 | | | |
| Width of cells in lower true exciple | 24/0.376 | | | |
| Thalline-exciple/true-exciple ratio | 25/0.367 | | | |
| Hymenium width | 26/0.367 | | | |
| Hypothecium-cell width | 27/0.367 | | | |
| Algal-cell diameter | 28/0.367 | | | |
| True exciple width | 29/0.344 | | | |

Table 4. Characters evaluated in the detailed study; eleven continuous and eight discrete characters. Continuous characters are ordered as in Table 3. Discrete characters are in alphabetical order. The character "lower-/upper-paraphysis-width ratio" selected for the detailed study was not considered, because it is strongly correlated (see the section "Phenotype diagnostics") with "width of widest cell of paraphysis" hence its discriminative power is lower. Length of conidia appears to be a useful character, but conidia were observed only in limited number of samples due to common absence of pycnidia. Asterisks indicate values or features considered "fully diagnostic", i.e. allowing for correct identification of at least one taxon. The measurements are given in the following format: (minimum)$x_1$–$x_2$–$x_3$(maximum), where minimum and maximum are extreme values in all samples of one species, $x_1$ is the smallest mean among the sample means of one species, $x_2$ is the mean calculated using all the values of all samples of one species, $x_3$ is the greatest mean among the sample means of one species. The total number of measurements from all samples of one species ($N$), the number of species samples ($n$), and standard deviation of $x_2$ (SD) are given for each measured character in brackets.

| Characters | *C. micromarina* | *C. micromontana* | *C. microstepposa* |
|---|---|---|---|
| *Ascospore-septum width (µm) | (2.0–)2.6–2.9–3.4(–4.5) [90;9;0.6]* | (1.0–)1.5–1.8–2.1(–2.5) [95;10;0.4] | (1.0–)1.6–1.9–2.4(–3.5) [93;10;0.4] |
| *Septum-width/ascospore-length ratio | (0.13–)0.17–0.20–0.23(–0.30) [90;9;0.03]* | (0.07–)0.09–0.12–0.14(–0.18) [95;10;0.02] | (0.06–)0.10–0.12–0.16(–0.23) [93;10;0.03] |
| *Width of cells in uppermost true exciple (µm) | (3.0–)3.9–5.0–6.2(–8.5) [100;10;1.1] | (3.5–)4.1–5.3–7.0(–7.5) [103;11;1.0] | (2.0–)2.6–3.5–4.5(–7.0) [110;11;0.9]* |
| Thallus width (µm) | (75–)125–160–206(–375) [90;9;60.8] | (0–)19–87–123(–175) [30;3;57.9] | (75–)86–157–369(–500) [102;11;89.3] |
| Length of cells in uppermost true exciple (µm) | (4.5–)6.3–7.7–8.9(–11.5) [100;10;1.6] | (5.0–)7.1–7.7–10.1(–10.5) [103;11;1.2] | (4.0–)5.9–6.6–7.3(–10.0) [110;11;1.2] |
| *Width of widest cell of paraphysis (µm) | (3.0–)3.7–4.0–4.5(–6.0) [100;10;0.7] | (4.0–)4.9–5.3–6.1(–7.5) [110;11;0.9]* | (3.0–)3.7–4.2–4.7(–6.0) [110;11;0.7] |
| Ascospore width (µm) | (5.5–)6.5–7.5–8.5(–10.5) [90;9;1.0] | (5.5–)7.2–7.9–8.6(–10.0) [95;10;1.0] | (4.5–)6.0–6.6–7.9(–9.5) [93;10;0.9] |
| Width of alveolate cortex plus epinecral layer (µm) | (7.5–)14.8–28.0–36.3(–50.0) [90;9;10.4] | (12.5–)20.5–21.2–22.0(–25.0) [20;2;3.9] | (7.5–)16.0–23.6–38.5(–62.5) [102;11;9.2] |
| Apothecium diameter (mm) | (0.20–)0.28–0.34–0.40(–0.55) [100;10;0.07] | (0.22–)0.27–0.32–0.42(–0.44) [90;9;0.06] | (0.24–)0.32–0.39–0.49(–0.66) [110;11;0.09] |

| | | | |
|---|---|---|---|
| Ascospore length/width ratio | (1.44–)1.79–1.95–2.30(–2.67) [90;9;0.24] | (1.45–)1.72–1.94–2.09(–2.71) [95;10;0.22] | (1.51–)1.93–2.34–2.93(–3.33) [93;10;0.45] |
| Conidium length (μm) | (2.5–)2.8–3.1–3.3(–4.0) [70;7;0.3] | (3.0–)3.6–3.7–3.8(–4.5) [30;3;0.4] | (3.0–)3.4–3.4–3.5(–4.0) [30;3;0.3] |
| Attachment of mature apothecia to thallus | usually half immersed | sessile to immersed | sessile |
| *Color reaction of epithecium and true exciple with K | violet | violet | brown-violet* |
| *Epithecium and true exciple color | grey | grey | brown* |
| Habitat | close to sea | mountains, from low steppe foothills to alpine belt | usually inland arid and semi-arid regions |
| *Inspers hymenium | absent | absent | present* |
| Pruina on apothecial disc and true exciple | sometimes present | sometimes present | absent |
| Reaction of thallus alveolate cortex with K | usually violet (90% samples), rarely K– | sometimes violet (50% samples), or K– | usually K– (80% samples), rarely violet |
| Stacks of crystalls in hymenium | present in some apothecia | absent | rarely present |

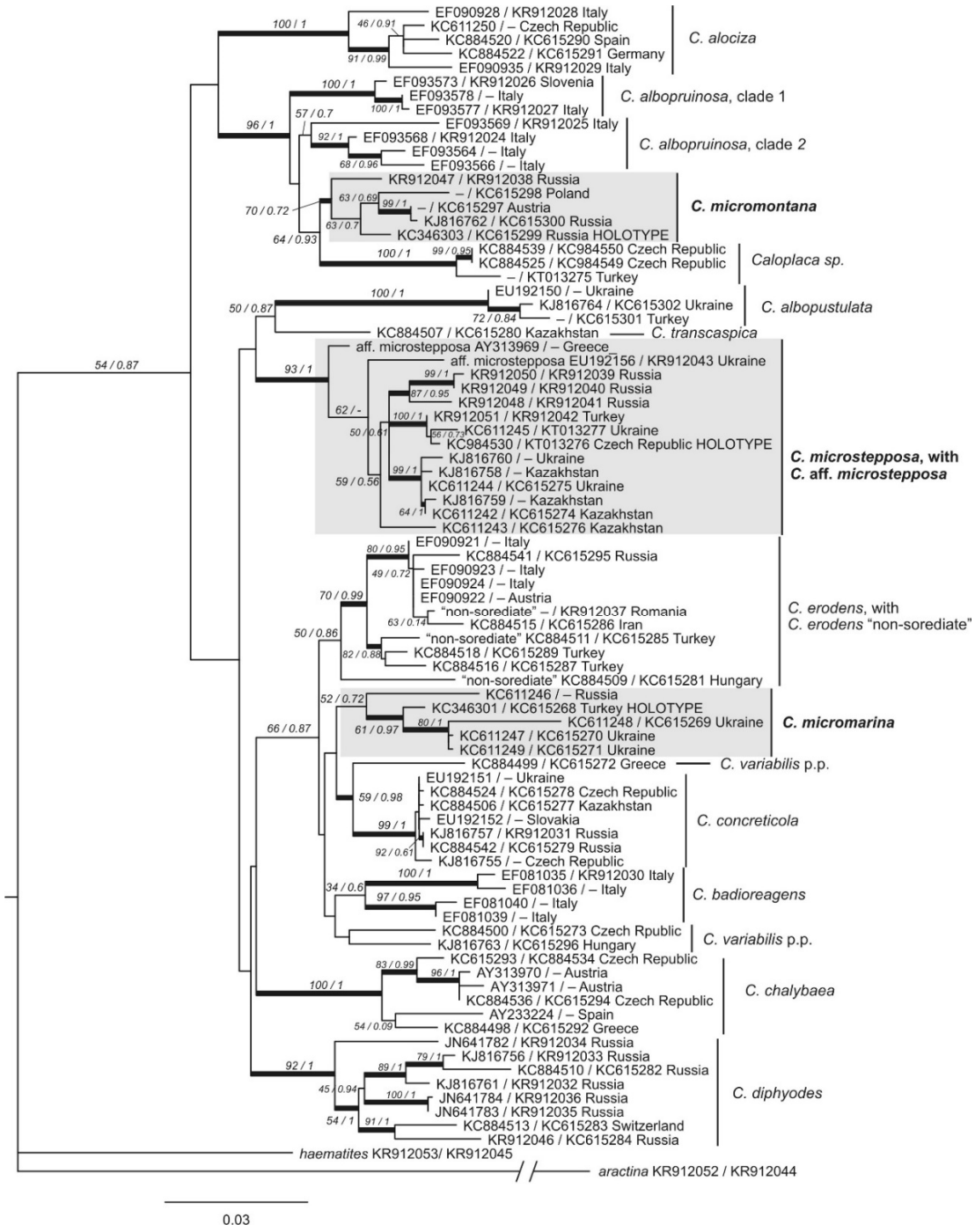


Fig. 1. Phylogenetic position of the newly proposed species of *Caloplaca* within the clade of species related to *Caloplaca variabilis*. The maximum likelihood topology based on a concatenated analysis of ITS and β-tubulin is shown and is annotated with bootstrap support and Bayesian posterior probabilities. Numbers at branches represent bootstrap values ≥ 50% (before forward slash '/') and posterior probabilities values ≥ 0.6 (after forward slash '/'). Branches with bootstrap values ≥ 70% and/or posterior

probabilities ≥ 0.95 are thickened. Specimens belonging to species described in this paper are on grey background. ITS and β-tubulin accession numbers are shown before and after forward slash '/', respectively ('–' = missing data). Names at tree tips correspond to those in Table 1.

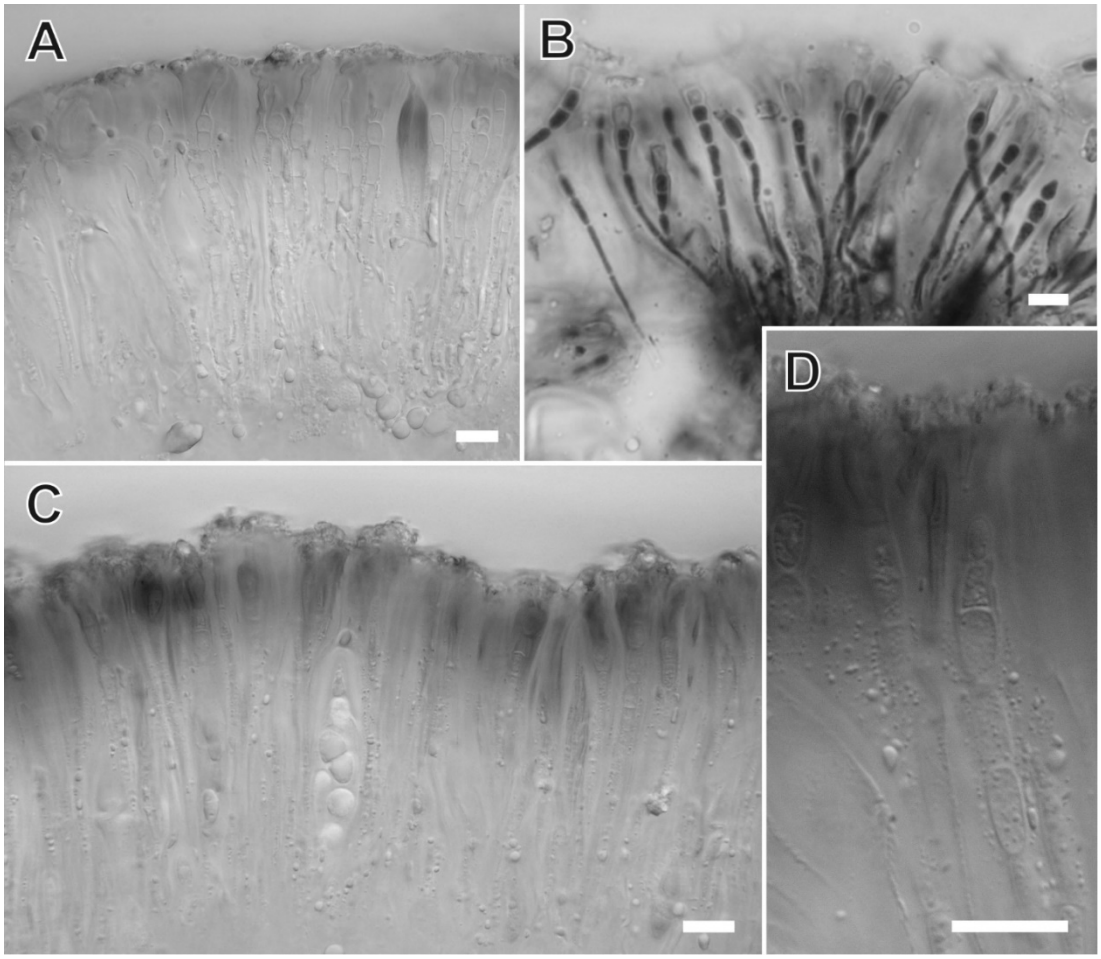


Fig. 2. *Caloplaca microstepposa* (PRA JV9454). — A: Paraphyses with dead, thin and shrunken upper cells. — B: Paraphyses stained by cotton blue; dead upper cells remained colourless. — C and D: Inspers hymenium. Bars: 10 µm.

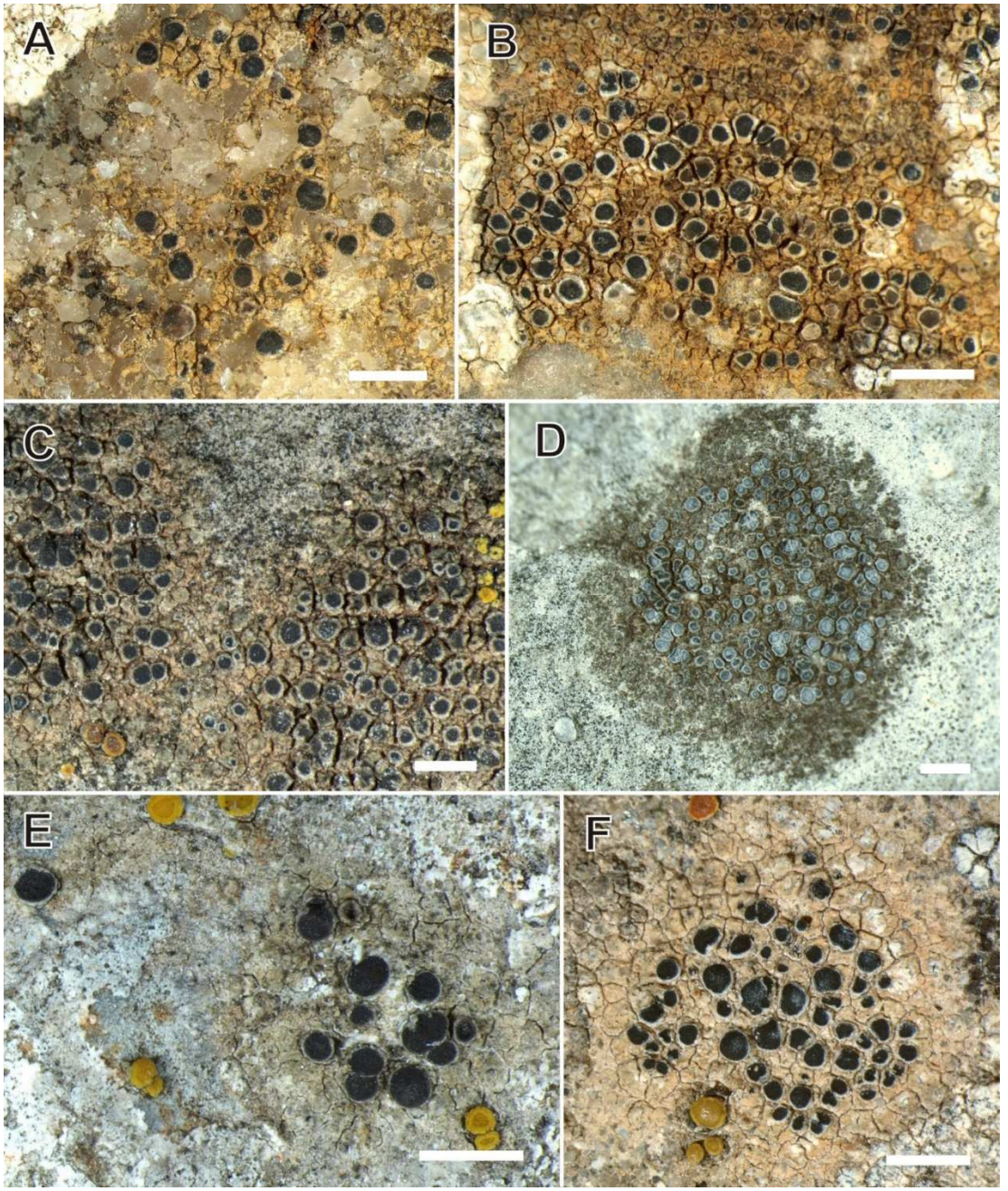


Fig. 3. — A and B: *Caloplaca micromarina* (holotype). — C: *Caloplaca micromontana* (holotype). — D: *Caloplaca micromontana* (PRA JV9523). — E: *Caloplaca microstepposa* (holotype). — F: *Caloplaca microstepposa* (PRA JV9454). Bars: 1 mm.

## 5.4 Paper 4

Vondrák J., **Frolov I.**, Říha P., Hrouzek P., Palice Z., Nadyeina O., Halıcı G., Khodosovtsev A., Roux C. 2013. New crustose Teloschistaceae in Central Europe. The Lichenologist 45: 701–722.

## New crustose Teloschistaceae in Central Europe

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**Abstract**

Central Europe in general is poor in Teloschistaceae lichen crusts (*Caloplaca* s. lat.). Diversity of these lichens is increased by the occurrence of some Arctic, Mediterranean and continental species, which are here close to the limits of their range. Examples include:

1) *Caloplaca interfulgens*, previously known from arid territories of northern Africa and western Asia, is recorded, surprisingly, from Austria, Czech Republic, Germany, Slovakia and southern Russia. In Central Europe, it is restricted to scattered xerothermic limestone outcrops.

2) *Caloplaca scabrosa*, previously known only from Svalbard, is recorded from the Sudetes in the Czech Republic. It is similar to, but not conspecific with, *C. furfuracea*. Its diagnostic characters include a blastidiate thallus and the presence of atranorin. Our results show that atranorin is absent in the majority of taxa related to *C.*

*furfuracea* with only two exceptions: the sample from Eastern Carpathians, here called *C.* aff. *scabrosa*, and in one Sudetan sample identified as *C. crenularia*.

3) *Caloplaca emilii*, newly described below, is closely related to the Mediterranean *C. areolata*. We consider *C. emilii* a Mediterranean species rarely occurring in higher latitudes in Austria, the Czech Republic and Germany. It is distinguished from *C. areolata* mainly by the presence of vegetative diaspores (blastidia); a possible role of blastidia in the distribution pattern of *C. emilii* is discussed below. Status of the names *Caloplaca areolata*, *C. isidiigera* and *C. spalatensisis*, formerly used for the new taxon, is clarified.

4) *Caloplaca molariformis*, newly described below, belongs to the *Pyrenodesmia* group (a lineage of *Caloplaca* without anthraquinones). It is a continental species, frequently collected on limestone or lime-rich tuffs in steppes or deserts in Turkey, Iran, western Kazakhstan and southern Russia, and is also known from eastern Ukraine and southern Slovakia. *Caloplaca molariformis* is characterized by its thick thallus with fungal and algal tissues arranged in high stacks.

5) *Caloplaca substerilis*, newly described below, is distinguished from the closely related *C. ulcerosa* by its endophloeodal or minutely squamulose thallus with soralia formed in bark crevices or on margins of squamules. While *C. ulcerosa* has a maritime distribution in Europe, *C. substerilis* is typically a continental species. North American continental lichens called ‘‘*C. ulcerosa*’’ are phylogenetically closer and more similar to *C. substerilis*.

The positions within Teloschistaceae of the taxa considered are demonstrated by ITS phylogenies. The distributions of *C. areolata*, *C. emilii* and *C. interfulgens* are mapped. The new species are fully described using more than a hundred phenotype characters, and diagnostic characters are indicated separately.

**Key words:** biodiversity, biogeography, ITS phylogeny, lichen phenotype evaluation, species recognition, vegetative reproduction

## Introduction

Teloschistaceae, with its 1000 or more species (Arup et al. 2013), has highest biodiversity in temperate regions (Feuerer 2011). In Central Europe, hot spots of *Caloplaca* diversity are restricted to habitats with sun-exposed calcareous or base-rich siliceous outcrops in alpine zones of the Alps and high Carpathians (e.g. Poelt 1953a, b, 1954, 1955, 1960, 1964; Wilk & Flakus 2006; Vondrák et al. 2008), or in dry and warm rocky steppes (e.g. Poelt 1975; Vondrák et al. 2007). In other Central European habitats, only the common epiphytic and epilithic species are found; the highest number of these common species is found on lime-rich artificial substrata (e.g. Vondrák & Hrouzek 2006; Svoboda et al. 2007; Vondrák et al. 2010a).

Altogether, more than one hundred *Caloplaca* species occur in Central Europe (Vondrák & Wirth 2013), but about two thirds of these are rare species, known from very few localities. In other words, the generally low *Caloplaca* species diversity in Central Europe is partly enriched by marginal occurrences of some 'exotic' taxa further distributed in the Mediterranean basin, western Asia or in the Arctic. Known examples are *C. exsecuta* (Nyl.) Dalla Torre & Sarnth., *C. haematites* (Chaub.) Zwackh, *C. pollinii* (A. Massal.) Jatta (Vondrák & Wirth 2013), *C. raesaenenii* Bredkina (e.g. Søchting & Stordeur 2001), *C. tominii* Savicz (Vondrák et al. 2011), and many others.

Here we report several taxa newly discovered in Central Europe. *Caloplaca interfulgens* and *C. scabrosa* were previously known only from very distant areas and their occurrence in Central Europe was not expected. *Caloplaca emilii*, *C. molariformis* and *C. substerilis* are newly described from elsewhere, but also occur in Central Europe.

## Materials and Methods

### Sampling

Lichen samples were collected by the authors from various European and Asian localities between 1994 and 2012. We list information regarding locality, habitat, collection and deposition of specimens. Citations of the older herbarium samples from BRA and STU (in *Caloplaca interfulgens* and *C. emilii*) are as complete as we can make them. Specimens from CBFS, PRA and GZU used for comparative studies are cited more briefly in the text.

### Phenotype evaluation

More than 100 phenotype characters were assessed before preparing descriptions of the three new taxa. The list of characters and the way in which they were studied is provided in Vondrák et al. (2013). All observations were carried out on dead, stabilized material, on handcut sections mounted in water, without any chemical treatments. Measurements are accurate to 0,5 mm for cells and 10 mm for larger structures. All measurements of cells include their walls, except for tissues with glutinized cell walls. In *Caloplaca molariformis*, the widths of algal and fungal stacks are measured at the mid-point of their vertical extent. In each sample, ten measurements were made for each measurable character. Results of the measurements are given as (min.–) $x_1$–$x_2$–$x_3$ (–max.), where min/max are extremes from all measurements, $x_1$ is the lowest specimen arithmetic mean observed, $x_2$ is the arithmetic mean of all observations, $x_3$ is the highest specimen arithmetic mean observed. In cases where measurements were made from one sample, only $x_2$ is recorded. Total number of measurements (n), number of samples asessed (N), and standard deviation from all measurements (SD) are given

in square parenthesis for each character measured [n; N; SD]. General morphological terminology follows Smith et al. (2009); the term "alveolate cortex" is adopted from Vondrák et al. (2009a).

**Chemistry**

Spot tests with KOH (K), sodium hypochlorite (C), paraphenylenediamine (P) and UV light were performed in each new species. Tissues were also tested for amyloidity by the reaction with Lugol's solution (I). Pigments insoluble in acetone were evaluated following Meyer & Printzen (2000). Extracellular crystals were examined by the reaction with concentrated H2SO4 for detection of Ca. HPLC was used for identification of acetone-soluble compounds. The anthraquinone contents were analyzed on a LichroCART 250-4 RP18-e (5 mm) column using an Agilent 1100 Series Chromatograph after Søchting (1997), but using the wavelength (240 nm). Whole absorption spectra in the range 200–600 nm were monitored. The presence of atranorin in the samples was determined after Feige et al. (1993) on the same column and chromatographic system.

**DNA extraction, amplification and sequencing**

The simple NaOH extraction (Werner et al. 2002) was used for DNA isolations. Primers for PCR amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR cycling parameters follow Ekman (2001). A total of 51 nuclear ITS sequences were newly generated (Table 1).

**Phylogenetic analyses**

Five independent phylogenetic analyses of the nuclear ITS region were made to cover the individual groups studied. All analyses followed almost the same design; differences are listed in Table 2. Sequences were aligned using the MAFFT v6 server (http://mafft.cbrc.jp/alignment/server; Katoh & Toh 2008) according to the LINS-i strategy. The resulting alignments required some manual adjustments (done in BioEdit; Hall 1999) and, in the case of the C. crenularia group, also trimming of unalignable positions (using TrimAl-automated1 algorithm, Capella-Gutierrez et al. 2009). The length of datasets submitted to further analyses ranged from 486– 535 positions. Final alignments were submitted to TreeBase http://treebase.org/treebase-web/home.html.

Molecular phylogenies were estimated by Bayesian inference as incorporated in MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Model selection was committed to the Kakusan4 algorithm (Tanabe 2011), whereas the baseml software (Adachi & Hasegawa 1996) served as the computational core. With reference to the Bayesian information criterion (Schwartz 1978), we opted for SYM or GTR models with rate variation across sites simulated by discrete gamma distribution

(G8) and autocorrelated by the AdGamma rates prior (Table 2.). The increased probability of transitions over transversions, well documented in many rDNA datasets (see e.g. Keller et al. 2007), was reflected by setting the substitution rates prior (revMatPr) to dirichlet with values 1 and 3 for these two mutational types, respectively. Each analysis comprised two independent runs, each of which encompassed four Metropolis-coupled MCMC chains with 10 000 000 generations sampled after every 1000th generation. In every run, one Markov chain was cold and three were incrementally heated by the parameter of 03. To eliminate trees sampled before reaching apparent stationarity, the first 25% of entries were discarded as burn-in and the rest were used to compute majority-rule consensus, where the relative occurrences of nodes are identified with the Bayesian posterior probabilities (Figs 2–6). Bayesian posterior probabilities ≥ 50 are shown, branches with lower posterior probabilities are collapsed.

**Nomenclature**

Arup et al. (2013) proposed a new nomenclature within Teloschistaceae and split the crustose genus *Caloplaca* into numerous genera. We do not follow the new nomenclature in this paper, because generic names are still missing for many Teloschistaceae taxa, including *Caloplaca emilii* and *C. substerilis* described here. Names of other lichen taxa follow the Index Fungorum http://www.indexfungorum.org/names/names.asp.

**Records new to Central Europe**

***Caloplaca interfulgens* (Nyl.) J. Steiner**

Verh. zool.-bot. Ges. Wien 52: 479 (902). – *Lecanora interfulgens* Nyl. Flora 56: 340 1878.

Images of some Czech and German specimens are available on the lichenological web page at the University of South Bohemia http://botanika.bf.jcu.cz/lichenology/index.php?pg=5.

*Diagnostic characters.* Thallus well-developed, consisting of yellow areoles and often with squamules at the margin. Ascospores polarilocular, c. 15–19 5,0–7,5 mm with septa up to 4 mm wide. Prothallus indistinct. Occurs on calcareous rocks.

*Similar taxa* are *Caloplaca crenulatella* s. lat. (the yellow thallus usually reduced), *C. diffusa* Vondrák & Llimona (on non-calcareous rocks, yellow thallus with thin

diffuse margin, with grey-white prothallus, squamules absent) and species of the *Caloplaca velana* complex (ascospores shorter with thicker septa).

*Distribution* (Fig. 1A). *Caloplaca interfulgens* was previously known only from deserts, semi-deserts or steppes in North Africa (Nylander 1878; Navarro-Rosinés & Hladun 1996), Mediterranean Europe (Italy: Nimis & Martellos 2008; Spain: Nimis et al. 1998), Iran, Kazakhstan (Vondrák et al. 2011) and continental Turkey (Vondrák et al. 2012a). The new records are surprisingly from less arid territories in Austria, the Czech Republic, Germany, southern Russia and Slovakia. In all Central European localities, *C. interfulgens* is restricted to limestone outcrops in xerothermic sites (often south-facing steppes).

*Phylogeny.* In the ITS phylogeny of the *Caloplaca crenulatella* group (Fig. 2), *Caloplaca interfulgens* forms a well-resolved sister clade to *Caloplaca tominii*, a sorediate species with a similar distribution pattern in Europe.

*Taxonomic note.* Although the *Caloplaca crenulatella* group has been studied recently (Navarro- Rosinés & Hladun 1996; Vondrák et al. 2011), it is still poorly understood and many lineages are not yet well characterized. Fortunately, its well-developed areolate thallus separates *C. interfulgens* from the many taxa with reduced thalli. However, some Central Asian taxa have a thallus similar to *C. interfulgens* (e.g. '*Caloplaca* sp., southern Russia' in Fig. 2) and their delimitation requires further study.

*New records:* **Austria:** *Niederösterreich:* Wien, Hainburg an der Donau, rocks on SW slopes of hill Braunberg NE of town, 48°09'10"N, 16°57'12"E, 280 m, 2012, *J. Vondrák 9550* (CBFS). **Czech Republic:** *Central Bohemia:* Bohemian karst, Beroun, Tmáň, protected area Kotýz, 1.5 km NE of village, 49°54'56"N, 14°2'55"E, 350 m, 2011, *J. Vondrák* 9153, 9155 & 9156 (CBFS); Praha, Dobřichovice, Karlík, limestone outcrops 1 km NW of ruin of Karlík, 49°56'56"N, 14°14'49"E, 300 m, 2011, *I. Frolov & J. Vondrák* 9399 (CBFS); Praha, Radotín, Kosoř, protected area Černá rokle, E of village, 49°59'21"N, 14°20'8"E, 250-300 m, 2011, *Z. Palice & J. Vondrák* 9144 (CBFS); *Southern Moravia:* Pavlovské vrchy hills, Mikulov, Klentnice, SE slope of hill Pálava, 48°51'22"N, 16°38'33"E, 350-400 m, 2012, *J. Vondrák* 9577 (CBFS). **Germany:** *Baden-Württemberg:* Schwäbishe Alb Mts, Langenau, Albeck, shallow valley W of Kornberghöfe, 520-550 m, 1984, V. Wirth 29418 (STU; hb. Wirth); *Rheinland-Pfalz:* Eifel Mts, Uxheim, Dreimühlen, limestone outcrops in dry grassland, 1992, *V. Wirth* 23937 *& R. Düll* (STU; hb. Wirth). **Russia:** *Orenburgskaya Oblast':*,surroundings of water reservoir "Iriklinskoe vodokhranilishche", vill. Chapaevka, limestone rocks on opposite slope of lake, NE of village, 52°05'12"N, 58°48'1"E, 270-290 m, 2011, *I. Frolov & J. Vondrák* 9396 (CBFS). **Slovakia:** *Cerová vrchovina upland:* Filakovo, Hajnáčka, hill Ragač, lime-rich outcrop of volcanic pyroclastics in open beech-oak forest,,48°13'25"N, 19°59'6"E, 500 m, 2012, *J.*

*Vondrák* 10137 (CBFS); *Muranská Planina Mts:* Brezno, Tisovec, hill Okruhla skala, *c*. 2 km W of town, 48°40'42"N, 19°54'57"E, *c.* 800 m, 2011, *J. Vondrák* 9260 (CBFS); *Strážovské vrchy Mts:* Ilava, Zliechov, on S-slope of Mt Strážov, 48°56'59"N, 18°27'16"E, 1000 m, 2012, *J. Vondrák* 10198 (CBFS); *Vihorlat Mts:* Sobrance, Podhorod [Podhradí], 1930, *J. Buček* (BRA, sub *Caloplaca zimmermannii*, Servít, nomen ined.).

***Caloplaca scabrosa* Søchting, Lorentsen & Arup**

Nova Hedwigia 87: 89 (2008).

Images of European samples and the isotype are available on the lichenological web at the University of South Bohemia http://botanika.bf.jcu.cz/lichenology/index.php?pg=5.

*Observation of the type specimen.* Isotype (CBFS JV9402, ex C; Søchting 5513) examined in detail.

Thallus rough and scabrous by blastidia, densely covering the thallus surface. Blastidia (40–)71(–130) mm diam. [10; 1; 29]. Thallus surface pale grey to sordid white, but tips of blastidia often dark grey. Grey thallus parts containing Cinereorufa-green (green-grey in water, K-, N+ red) in the uppermost thallus cells. Thallus divided into thin and more or less flat angular areoles, c. 0,2–1,3 mm diam. The real cortex absent, but indistinct alveolate cortex present in spots, of spherical, thick-walled cells (wall c. 1 mm thick). Thallus without anthraquinones, but with atranorin.

Apothecia biatorine, deep red (old apothecia somewhat blackened), with anthraquinones; major: parietin and 7-Cl-emodin; traces of emodin, 7-Cl-citreorosein, 7-Clemodinal and parietinic acid (C+ purple owing to chlorinated compounds). True exciple of palisade prosoplectenchyma, of cells with glutinized, c. 1 mm thick walls. Lower exciple and lower hypothecium brown-red (possibly due to small amount of anthraquinones; with weak K+ purple reaction). Ascospores polarilocular, (12,0–)14,0(–17,0) × (5,5–)6,5(–8,0) mm [10; 1; 1,3 & 0,7], with septa (4,0–) 5,0(–5,5) mm [10; 1; 0,5].

Pycnidia not present on the available isotype material. The type material is also described in Søchting et al. (2008).

*Observations of the Central European specimens.* (Fig. 7A).

Thallus rough and scabrous by blastidia, densely covering the thallus surface. Blastidia (30–)58–67–72(–130) mm diam. [30; 3; 28]. Thallus surface pale grey to white, but tips of blastidia often dark grey. Grey pigmented thallus parts containing

Cinereorufa-green (green-grey in water, K-, N+ red) in the uppermost thallus cells. Thallus divided into thin and flat angular areoles, c. 0,2–1,0 mm diam. The real cortex absent, but indistinct alveolate cortex present in spots, of spherical, thick-walled cells (wallsc. 1 mm thick). Thallus without anthraquinones, but with atranorin.

Apothecia deep red (old blackened apothecia not observed), with anthraquinones; major: parietin and 7-Cl emodin; traces of emodin, fragilin and parietinic acid (C+ purple owing to chlorinated compounds); biatorine or zeorine; thalline exciple sometimes strongly expanded in old apothecia. True exciple of palisade prosoplectenchyma, of cells with glutinized, 1–2 mm thick walls. Inner exciple and lower hypothecium brown-red (perhaps by anthraquinones). Ascospores polarilocular, (11,5–)13,0(–15,0) × (6,5–)7,5 (–9,0) mm [10; 1; 1,4 & 0,8], with septa (3,0–)4,0(–5,0) mm [10; 1; 0,5].

Pycnidia with red tops, containing chlorinated anthraquinones (C+ purple). Conidia more or less bacilliform, c. 3–4 × 1 mm.

*Importance of particular characters. Caloplaca scabrosa* shares many characters with other related taxa from the *C. crenularia* group (as defined in Fig. 3), so their diagnostic power is rather low. They include: 1) presence of Cinereorufa-green in the thallus; 2) apothecia with chlorinated anthraquinones (C+ purple); 3) structure of the true exciple; 4) brownish pigment in lower hypothecium and inner true exciple; 5) pycnidia with red caps.

Some characters are specific for *C. scabrosa*: 1) presence and size of blastidia; 2) presence of atranorin in the thallus. We have tested the diagnostic power of the presence of atranorin. We analyzed thalli of various species of the *C. crenularia* group: *Caloplaca ammiospila* (Ach.) H. Olivier (CBFS JV10223), *C. crenularia* (With.) J. R. Laundon (CBFS JV4596; 5608; hb. Z. Palice 7837; Poland, Nowak's exsiccate 203 in GZU; Sardinia, 1986, Poelt in GZU), *C. ferruginea* (Huds.) Th. Fr. (CBFS JV7224; 7256), *C. furfuracea* H. Magn. (Ural, hb. I. Frolov), and *C. hungarica* H. Magn. (CBFS JV3081). Atranorin was detected in only one sample of *C. crenularia* from the basalt outcrops in the Karkonosze Mountains, W Sudetes (Nowak, Lich. Polon. Exs. n. 203), indicating that this *C. crenularia* specimen does not belong in the main *C. crenularia* clade.

The type specimen of *Caloplaca scabrosa* differs from the Central European material in the following characters: 1) size of areoles; 2) thallus thickness; 3) extent of the thalline exciple. Based on our observations of numerous samples of the *C. crenularia* group, these characters were very variable both within and between specimens of a single species, so the differences are of little taxonomic importance. Ascospore size and septum width also differ between the type and the Central European collections, but this difference may be merely a consequence of the low number of available specimens and measurements.

*Phylogeny.* The ITS sequence of the Central European specimen of *Caloplaca scabrosa* is placed in the basal polytomy of the ITS phylogeny of the *C. crenularia* group (Fig. 3). It is perhaps closely related to the arctic-alpine *C. ammiospila* or boreo-montane *C. furfuracea*.

*Taxonomic notes.* The epixylic taxon *Caloplaca furfuracea* is very similar to *C. scabrosa*. It likewise produces blastidia (isidia according to Arup & Åkelius 2009) of the same size; tips of blastidia are also usually dark grey due to the Cinereorufa-green content in the alveolate cortex. With the exception of the ecology, the only reliable character distinguishing *C. furfuracea* from *C. scabrosa* is the absence of atranorin.

We have collected samples of a granular to blastidiate lichen in the subalpine belt of the Eastern Carpathians (''*Caloplaca scabrosa*'' in Fig. 3). These saxicolous specimens are very similar to both *C. furfuracea* and *C. scabrosa*. They appear to be closer to *C. furfuracea* in the ITS phylogeny but they share chemistry and ecology with *C. scabrosa*.

*New records:* **Czech Republic:** *Northern Moravia:* Rýmařov, Karlov, central part of Velký kotel corrie, on phyllitic overhanging rock, 1330–1340 m, 2002, *Z. Palice* 7024 (PRA); Ibid.: 50°03'20"N, 17°14'E, 1250-1300 m, 2004, *J. Vondrák* 1907, 1908 & 1909 (CBFS).

*C.* "*scabrosa*"*:* **Ukraine:** *Eastern Carpathians:* Svidovets Mts, at glacial lake at bottom of glacial cirque in N slope, 48°15'41"N, 24°13'22"E, on sun-exposed base-rich sandstone boulders close to water, *c*. 1300 m, 2007, *J. Vondrák* 6199 (CBFS).

**New species**

***Caloplaca emilii* Vondrák, Khodos., Cl. Roux & V. Wirth sp. nov.**

MycoBank No: MB 803332

Thallus grey or brown-grey, non-pruinose, of more or less flat areoles, with Sedifolia-grey and without anthraquinones. Dark grey blastidia always present at margins of thallus units. Mature apothecia zeorine, usually with brown disc and more or less yellow true exciple, Ce purple (with chlorinated anthraquinones). Ascospores broadly ellipsoid, less than 15 mm long, with thick septa. Pycnidial tops dark grey. Conidia ellipsoid, not bacilliform.

Type: Bulgaria, Black Sea coast, Kavarna, limestone cliffs on seashore 1.5 km NE of Kamen Brjag, 43°27'58.76"N, 28°33'55.02"E, on coastal limestone outcrop above

supralittoral zone, 6 April 2007, *J. Vondrák* 6600 (CBFS–holotypus, KHER–isotypus). ITS sequence of the holotype: KC416101.

Images of the German sample are available on the lichenological web page at the University of South Bohemia http://botanika.bf.jcu.cz/lichenology/index.php?pg=5.

(Figs 1B, 4, 7B)

Thallus forming irregular spots, browngrey or pale to dark grey, to several cm wide; often starting on other crustose lichens; of tightly arranged, angular to rounded, flat to slightly convex, areoles or squamules, (0,3–) 0,6–0,9–1,1 (–2,6) mm diam. [70; 7; 0,4]. Thickness of thallus 100–500 mm. Medulla well-developed only in thick thalli, but up to 400 mm thick; medullary tissue formed of loose prosoplectenchyma; medullary hyphae c. 2–3 mm wide with walls thickened up to 1 mm. Algal layer 50–140 mm thick; algal cells globose, c. 5–20 mm diam. Cortex developed in patches, up to 30 mm thick, not gelatinous; sometimes only alveolate cortex present. Epinecral layer often present, up to about 10 mm thick. Cortex cells or alveolate cortex cells spherical, thin-walled, about 4–6 mm diam. Blastidia simple, globose, dark grey, always present, produced at margins of areoles or squamules, rarely also on their upper surface, (20–)53–65–95(–210) mm diam. [60; 6; 36]. Extracellular crystals of calcium salts not observed in any thallus part. Pruina absent. Prothallus indistinct or absent. Thallus frequently affected by brown hyphomycetes resembling species of *Intralichen*.

Apothecia present in c. 50% of samples collected; rare in northern populations; (0,3–) 0,5–0,7–0,9 (–1,4) mm diam. [40; 4; 0,2]; zeorine. Disc in shades of brown (orange in young apothecia); true exciple usually yellow (contrasting with disc); thalline exciple in shades of grey; pruina absent. Hymenium colourless, without distinct gelatinous matrix and without extracellular oil drops, c. 70– 110 mm high; epihymenium ochre to greenyellow. Hypothecium colourless, rarely with extracellular oil drops, more or less flat, c. 100–300 mm high, formed of cells variable in shape; subhypothecial algal layer present (algal cells underlying entire hypothecium). Exciple c. 70–110 mm wide, formed of true exciple, c. 30–60 mm wide, and thalline exciple, c. 10–70 mm wide. Upper part of true exciple of thin-walled spherical cells c. 4–6 × 3–4 mm. Lower part of palisade prosoplectenchyma of thin-walled cells c. 5– 12 × 15–20 mm. Thalline exciple without cortex or with indistinct alveolate cortex. Paraphyses 2,0–2,5 mm wide in lower part, but widening gradually to (2,5–)3,0–3,5–4,0(–5,0) mm [30; 3; 0,5] in upper part; rarely branched and anastomosed. Asci clavate, c. 50–70 × 15–20 mm. Ascospores polarilocular, (8,0–)12,0–12,5–13,5(–15,0) × (5,0–)7,0– 7,5–8,0(–9,5) mm [50; 5; 1,5 & 0,9], septa (4,0–)5,0–5,5–6,0(–7,5) mm [50; 5; 0,9]. Ascospore length/breadth ratio: (1,0–)1,5– 1,7–1,8(–2,2) [50; 5; 0,3]; septum width/

ascospore length ratio: (0,30–)0,40–0,45– 0,47(–0,60) [50; 5; 0,1]. Extracellular crystals of calcium salts absent from all apothecial parts.

Pycnidia not common (observed in only three samples), c. 150–200 mm wide, with several partly separated chambers (Xanthoria-type), distinguished by their darker grey tops on the thallus surface. Conidiophores formed of isodiametric cells, c. 2–4 mm diam. Conidia ellipsoid, broadly ellipsoid or tear-shaped, rather uniform in size, 2,0–2,5 × 1,5 mm.

*Chemistry.* True exciple, medulla and lower cortex non-amyloid (I-); hymenium and hypothecium amyloid (I+). Uppermost cells in cortical tissue of thallus and thalline exciple contain Sedifolia-grey (grey in water, K+ violet, N+ red, $H_2SO_4$+ red, I+ blue). Content of Sedifolia-grey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple contain anthraquinones: fragilin (major) and 7-Cl-emodin (HPLC done in sample JV6597).

*Etymology.* The epithet is derived from the name of our great friend Emil Červenka, who supported the first author during difficult times.

*Similar taxa. Caloplaca areolata* (Zahlbr.) Clauzade (without blastidia), *C. chlorina* (Flot.) Sandst. and *C. isidiigera* Vězda (with blastidia but with lecanorine apothecia and bacilliform conidia), *C. concreticola* Vondrák & Khodos. (with blastidia but without anthraquinones in apothecia), *C. soralifera* Vondrák & Hrouzek (with soredia, often pruinose) and *C. xerica* Poelt & Vězda (usually with isidia, without flat areoles, with larger ascospores). A little-known blastidiate morphotype of *Caloplaca atroflava* (Turner) Mong. is a similar lichen; it is very common in Central Europe, but occurs mainly on noncalcareous rocks (orange, C- apothecia, without chlorinated anthraquinones, blastidia usually overgrowing most of thallus surface).

*Phylogeny.* In the ITS phylogeny (Fig. 4), *Caloplaca emilii* is definitely placed in the *C. xerica* group (sensu Vondrák et al. 2012b). It forms a well-circumscribed clade (PP = 1,0), sister to *C. areolata*. Both taxa form a wellsupported monophyletic group (PP = 0,99).

*Ecology and distribution. Caloplaca emilii* occurs on sun-exposed, usually horizontal, faces of limestone outcrops in fast-drying places in steppes, forest-steppes or in open Mediterranean shrub vegetation, mainly in the *Placocarpetum schaereri* (Roux 1978: 120– 130). Co-occurring lichens are *Acarospora cervina*, *Aspicilia calcarea*, *A. contorta*, *Bagliettoa calciseda*, *Caloplaca aurantia*, *C. chalybaea*, *C. coronata*, *C. crenulatella* s. lat., *C. inconnexa*, *C. lactea*, *C. teicholyta*, *C. variabilis*, *Candelariella aurella*, *Diplotomma hedinii*, *D. venustum* s. str., *Heteroplacidium fusculum*, *Lecanora muralis* s. lat., *Lobothallia cheresina* s. lat., *L. radiosa*, *Placocarpus schaereri*, *Placopyrenium canellum*, *Rinodina calcarea*, *R. ocellata*, *R. bischoffii*, *Verrucaria lecideoides*, *V. macrostoma* f. *furfuracea*, and *V. nigrescens* s. lat.

The species is already known from Germany (as the blastidiate variant of *Caloplaca areolata* in Wirth et al. 2011). Nevertheless, this lichen has a rather southern distribution in Europe; it is probably most common in the Mediterranean basin and adjacent areas, such as France, Italy, Spain, mainly in the supramediterranean and montane belts (Roux 1978: 124, as *C. areolata*). Although it is common in continental areas around the Black Sea, we do not know it from continental areas east of the Mediterranean basin. In southern areas, it sometimes grows with its close relative *C. areolata* (for example in southern France and Greece). Both taxa have similar ecology, but *C. areolata* without vegetative diaspores appears to be restricted to the Mediterranean region, whereas the blastidiate *C. emilii* also occurs in isolated localities far to the north (Fig. 1B). The ability to reproduce vegetatively may have facilitated the northward extension of its distribution. A similar situation is observed in other Mediterranean lichens from the *C. xerica* group; sorediate/blastidiate *C. albolutescens* (Nyl.) H. Olivier and *C. teicholyta* (Ach.) J. Steiner are known from much more northern territories than the closely related *C. erythrocarpa* (Pers.) Zwackh, which is without vegetative diaspores.

*Taxonomic notes. Caloplaca emilii* is well known from the Mediterranean regions of France, where it has been named *C. areolata* (Clauzade 1963, 1965, 1969; Roux 1978) or later *C. isidiigera* (Roux 1982, 1984; Boissière et al. 1989; Houmeau & Roux 1991; Roux & Gueidan 2002; Bricaud 2007). However, these names belong to other taxa; *C. areolata* lacks vegetative diaspores (see also under ecology) and *C. isidiigera* is an unrelated species with lecanorine apothecia and a (sub-)alpine distribution (Vězda 1978; Šoun et al. 2011).

*Caloplaca areolata* has recently been considered a synonym of *C. spalatensis* Zahlbr. (e.g. Nimis & Martellos 2008). This synonymization is incorrect, because *C. spalatensis* is a very different lichen which belongs to the *C. crenularia* group (images of both holotypes, deposited in the herbarium W, are available on the lichenological web page at the University of South Bohemia http://botanika.bf.jcu.cz/lichenology/index.php?pg=5).

*Paratypes:* **Austria:** *Niederösterreich:* Wien, Hainburg an der Donau, rocks on SW slopes of hill Braunberg NE of town, 48°09'10"N, 16°57'12"E, 280 m, 2012, *J. Vondrák* 9570 (CBFS). **Bulgaria:** *Black Sea coast:* Kavarna, Kamen Brjag, 43°27'59"N, 28°33'55"E, 2007, *J. Vondrák* 6600 (CBFS); *The Rhodopes:* Madzharovo, Silen, Byal Kladenets, in valley below village, 41°37'N, 25°40'E, 350 m, 2004, *J. Vondrák* 2223 (CBFS); **Czech Republic:** *Southern Moravia:* Mikulov, in town, ruin of castle Kozí Hrádek, 48°48'34"N, 16°38'17"E, 2011, *J. Vondrák* 9358 *& O. Vondráková* (CBFS); Mikulov, Klentnice, SE slope of hill Pálava, 48°51'22"N, 16°38'33"E, 350-400 m, 2012, *J. Vondrák* 9581 (CBFS); Mikulov, Klentnice, at ruin

of Sirotčí hrádek, 48°50'43"N, 16°38'25"E, *c*. 410 m, 2011, *J. Vondrák* 9357 *& O. Vondráková* (CBFS). **France:** *Provence:* Vaucluse, Gordes, entre les Devens et Lancie, sur dalle de molasse miocène au ras su sol, 43,9026°N, 5,1931°E, 275 m, 1975, *G. Clauzade* (MARSSJ 189); Vaucluse, Mirabeau, 520 m, 2005, *C. Roux* 23475 (hb. Roux). **Germany:** *Bayern:* Oberfranken, Fränkische Alb: Kleinziegenfelder Tal, Grenzstein, 1976, *V. Wirth* 6101 (STU). **Greece:** *Attica:* Poros, limestone outcrops in N-part of island, 37°31'28"N, 23°29'10"E, *c*. 200 m, 2010, *J. Vondrák* 8726, 8832 *& O. Vondráková* (CBFS). **Romania:** *Dobrogea:* Târguşor, 44°27'46.26"N, 28°28'07.59"E, 2007, *J. Vondrák* 6599 (CBFS); Tulcea, Enisala, 44°52'42.09"N, 28°51'01.27"E, 2007, *J. Vondrák* 6604 (CBFS); Tulcea, Popina Island, 44°58'03"N, 28°58'57"E, 2007, *J. Vondrák* 6596, 6597, 6598 & 7149 (CBFS). **Ukraine:** *Kherson region:* Berislav, Burgunka, 2008, *A. Khodosovtsev & G. Naumovich* (KHER, dupl. in CBFS).

***Caloplaca molariformis* Frolov, Vondrák, Nadyeina & Khodos. sp. nov.**

MycoBank No: MB 803333

Anthraquinones entirely absent. Thallus epilithic, thick, ochre or dark grey, pruinose in spots, with Sedifolia-grey in superficial fungal cells. Blastidia and/or soralia always present. Thallus formed by high algal and fungal stacks (sensu Vondrák & Kubásek 2013). Fungal stacks of colourless palisade prosoplectenchyma, of cells elongated vertically. The upper thallus surface with ridges derived from the epinecral layer, above fungal stacks (similar structure is described in South African ‘‘Fensterflechten’’ by Vogel 1955). Epihymenium and outer part of true exciple brown to grey, with Sedifolia-grey, K+ (slightly) violet to violet-brown. Ascospores c. 14–18 mm long with rather thin septa, c. 3 mm wide.

Type: Slovakia, Cerová vrchovina upland. Filakovo, Hajnáčka, Šurice, SW-slope of the hill Soví hrad, 48°13'34"N, 19°54'45"E, on lime-rich outcrop of volcanic pyroclastics in sun-exposed abandoned quarry, *c*. 250 m, 8 November 2012, *J. Vondrák* 10192 (CBFS–holotypus, isotypi to be distributed in Exsiccates of *Caloplaca*, fasc. 4). ITS sequence of the holotype: KC416142.
More images available on the lichenological web page at the University of South Bohemia http://botanika.bf. jcu.cz/lichenology/index.php?pg=5.

(Figs 5, 7C; fig. 2 in Vondrák & Kubásek 2013)

Thallus epilithic, ochre, white-grey to dark grey, usually with white pruinose spots, forming irregular spots to several cm wide; of tightly arranged, angular to rounded, more or less flat areoles or somewhat umbilicate squamules, (0,44–)0,70–0,95–1,26 (–2,05) mm diam. [100; 10; 0,35]. Marginal areoles sometimes bigger than areoles in the centre. Several small, tightly arranged areoles may merge to form larger units, but on the contrary, large areoles are sometimes divided into smaller subareoles due to secondary crevices. Thickness of the thallus, together with brown (probably necrotic) lower medulla (0,2–)0,6–1,2–2,2(–5,0) mm [30; 3; 1,0]; thickness of the thallus without lowermost brown part (0,1–)0,3–0,4–0,5(–0,9) mm [30; 3; 0,2]. The brown lower medulla usually distinct, up to 125 times thicker than the rest of the thallus. Colourless medulla also present, (50–)140–235–330(–550) mm thick [26; 3; 145]; cells hardly observable due to presence of extracellular crystals insoluble in KOH and only partly dissolved and recrystallized into needles in $H_2SO_4$. Algal cells arranged in vertical stacks, (30–) 67–91–129(–250) wide [47; 6; 44], and (100–)223–263–334(–550) mm high [47; 6; 112]. Algal cells globose, (8,0–)12,6–13,7– 14,5(–22,0) mm diam. [30; 3; 3,2]. Cortex above the algal stacks absent or indistinct, alveolate cortex present, up to c. 15–30 mm thick; upper fungal cells in algal stacks grey, containing Sedifolia-grey. Fungal stacks (measured with epinecral layer) (13–)45–86– 120(–270) wide [46; 6; 55] and (75–)180– 322–505(–750) mm high [46; 6; 165]; formed by vertically oriented palisade prosoplectenchyma; size of cells in the middle part of stacks (4,5–)9,4–11,9–13,3(–18,0) × (3,0–)3,7–4,3–4,8(6,5) mm [30; 3; 3,9 & 0,9]. In lower part of stacks, cells longer and narrower; in uppermost part, cells almost isodiametric, c. 4–7 mm diam. Epinecral layer above fungal stacks usually well-developed, (5–)20–95–200(–350) mm thick [81; 9; 72]; dead cells (colourless in cotton blue) recognizable in the lower part. Boundary between epinecral layer and upper cells of the fungal stack sometimes indistinct, but recognizable after KOH treatment as a sordid grey-violet line caused by traces of the Sedifolia-grey in uppermost fungal stack cells. Epinecral layer often forms distinct ridges on thallus surface above fungal stacks, because it is absent from surface of algal stacks (Fig. 7C). Epinecral ridges best developed in samples from deserts of Western Kazakhstan, but less distinct in samples from Slovakia and Ukraine. Fungal stacks sometimes reaching medulla at the bottom and the boundary between the stacks and medulla recognized by the crystals abundant in medulla but absent from stacks. Margins of areoles and squamules and the lower surface of squamules usually with cortex, up to c. 20 mm thick, of isodiametric cells, c. 4–7 mm diam. Vegetative diaspores are blastidia (always present) or rarely soredia; sometimes diaspores poorly developed, present only on few areoles. Blastidia simple, more or less globose, (30–)54–67–89(–150) mm diam. [52; 6; 25], dark grey, present on the margin and upper surface of areoles and squamules; detached blastidia occasionally cover the whole surface. Blastidia sometimes with appearance of consoredia, with internal soredia-like

structures. Extracellular crystals soluble in KOH and Sedifolia-grey pigment present in outer fungal cells of blastidia. Soralia rarely observed, on the upper surface between epinecral ridges; soredia c. 25–40 mm diam. White pruina always present, better developed between epinecral ridges. Prothallus indistinct or absent. Thallus frequently affected by brown hyphomycetes resembling species of Intralichen.

Apothecia (0,33–)0,42–0,55–0,72(–1,32) mm diam. [100; 10; 0,15], zeorine or rarely almost lecanorine; mature apothecia sessile, usually not abundant on thallus, sometimes absent. Richly fertile populations known only from Slovakia and Ukraine. Disc brown to black, not pruinose, sometimes cracked; true exciple concolourous with the disc, occasionally white pruinose; thalline exciple concolourous with the thallus, with white pruina. Hymenium (63–)91–102–109(–175) mm high [30; 3; 23], colourless, often with very small (<1 mm) extracellular oil drops, sometimes strongly inspersed with extracellular oil drops up to c. 2 mm diam., sometimes not inspersed; without crystals. Epihymenium brown, grey or grey-brown. Hypothecium colourless, underlain by the algal layer, usually with extracellular oil drops, without extracellular crystals; with a central conical extension downward, (75–) 153–174–185 (–275) mm high [30; 3; 48]; formed of thin-walled cells variable in shape. Exciple c. 10–160 mm wide. True exciple (10–) 18–35–54(–93) mm wide [30; 3; 22], and thalline exciple (0–)18–24–27(–68) mm wide [30; 3; 20]. Upper part of the true exciple grey-brown, brown-grey or grey, of thin-walled cells (4,0–)6,2–6,6–7,3(–10,0) × (2,0–)3,4–4,5–5,2(–8,0) mm [100; 10; 1,1 & 1,1]. Lower part colourless, of palisade prosoplectenchyma of thin-walled cells (6,0–) 7,7–8,2–8,7(–11,5) × (2,0–)2,4–2,8–3,3 (–5,0) mm [30; 3; 1,3 & 0,8]. Thalline exciple sometimes with cortex in its upper part, c. 8– 20 mm thick; cortex changing into alveolate cortex in the lower part of thalline exciple. Cells of the cortex spherical, c. 3,5–7,0 mm diam., often hardly observed due to extracellular crystals insoluble in KOH. Paraphyses (1,5–)2,1–2,3–2,8(–3,5) mm wide [100; 10; 0,4] in lower part, but widening gradually to (3,0–)3,5–4,4–5,5(–6,5) mm [100; 10; 0,8] in upper part; rarely branched and anastomosed; the uppermost cell of paraphyses usually dead and deformed. Asci clavate, (40–)58–64–69(–85) × (12–)17–20–21(–28) mm [30; 3; 1 & 10]. Ascospores polarilocular, (12,0–)14,3–16,2–18,3(–23,0) × (5,0–) 6,4–7,7–9,1(10,5) mm [70; 8; 2,3 & 1,3]; septa (2,0–)2,6–3,0–3,3(–4,0) mm wide [70; 8; 0,5]. Ascospore length /breadth ratio: (1,40–)1,98–2,12–2,27(–2,86) [70; 8; 0,32]; septum width/ascospore length ratio: (0,11–) 0,17–0,19–0,22(–0,30) [70; 8; 0,04]. Ascospores with well-developed septa often absent.

Pycnidia rare, c. 140–190 mm wide, mainly with a single chamber, present on the upper thallus surface, but also on the lower surface of squamules; superficially hardly distinguishable. Old pycnidial chambers sometimes filled by crystals insoluble in KOH. Conidiophores of spherical or triangular, more or less isodiametric cells. Conidia narrowly to broadly ellipsoid, 2,5–4,5 × 1,5–2,0 mm [14; 2; 0,2 & 0,5].

*Chemistry.* Spot tests: thallus K± violet (sometimes not observable or observable only in spots with blastidia and soredia), apothecia K-, thallus and apothecia C-, P-, UV-. Epihymenium, uppermost true exciple, uppermost fungal cells in thallus and vegetative diaspores contain Sedifolia-grey (grey or invisible in water, K+ sordid violet). The reaction above fungal stacks usually weaker than above algal stacks. Strongest reaction in superficial hyphae of vegetative diaspores. True exciple non-amyloid (I-); hymenium and hypothecium amyloid (I+). No substances revealed by HPLC (apothecia and thallus of an isotype were investigated).

*Etymology.* Areoles and squamules of the lichen thallus often resemble molars of herbivores.

*Similar taxa.* The thallus anatomy, with tissues in stacks, is very rare within the *Pyrenodesmia* subgroup of *Caloplaca*. It is present in one known species only, *Caloplaca albovariegata* (B. de Lesd.) Wetmore, which is very similar to *C. molariformis* but has no vegetative diaspores (Wetmore 1994; lectotype in UPS seen). This species was described from North America, but similar morphotypes are known in continental Eurasia (our observations). Zhou et al. (2012) reported a taxon with tissues in stacks from China and named it *C. albovariegata*, but it has a thallus surface without ridges derived from the epinecral layer and it does not resemble *C. molariformis*. Other similar taxa are *Caloplaca albopustulata* Khodos. & S.Y. Kondr. (with pustules and schisidia), *C. bullata* (Müll. Arg.) Zahlbr. (bullate thallus without vegetative diaspores), *C. concreticola* (with soralia) and *C. transcaspica* (Nyl.) Zahlbr. (without vegetative diaspores), but all these taxa have thallus tissues arranged in horizontal layers, not in stacks. They also do not have specific ridges derived from the epinecral layer. (Type specimens and other comparative material studied by the authors.)

*Phylogeny.* In the ITS phylogeny (Fig. 5), *Caloplaca molariformis* is placed in the *C. variabilis* group, closely related to *C. albopustulata*.

*Distribution and ecology. Caloplaca molariformis* is mainly distributed in steppes and deserts of Iran, Kazakhstan, continental Turkey and southern Russia, at altitudes of 50–2100 m. Two isolated localities are also known from the steppe or forest-steppe, in eastern Ukraine and southern Slovakia. The species occurs in sunny habitats on soft limestone, chalk, calcareous sandstone or tuffs with evident content of lime (always reacting with HCl). Co-occurring lichen taxa include *Acarospora* spp., *Aspicilia* spp., *Caloplaca concreticola*, *C. crenulatella* s. lat., *C. decipiens*, *C. flavocitrina*, *C. soralifera*, *C. sororicida*, *C. teicholyta*, *C. tominii*, *C. transcaspica* s. lat., *C. xerica*, *Candelariella aurella*, *Lecanora muralis* s. lat., *Lemmopsis arnoldiana*, *Lichinella* sp., *Verrucariaceae* spp. (e.g. *Staurothele frustulenta*, *Verrucaria macrostoma*, *V. nigrescens* agg.).

*Paratypes.* **Iran:** *West Azerbaijan:* Lake Urmia, rocks at road *c.* 2 km N of Saraydeh, 37°52'59"N, 45°34'26"E, 1280 m, 2007, *J. Vondrák* 5556 (CBFS); Khoy, airport, 38°25'16.17"N, 44°54'24.05"E, 1180 m, 2007, *J. Vondrák* 5801 (CBFS); Lake Urmia, rocky outcrops near coast N of Aq Gonbad, 37°49'12.02"N, 45°25'09.61"E, *c.* 1290 m, 2007, *J. Vondrák* 5846 (CBFS). **Kazakhstan:** *Mangistau province:* Mangistau district, village Shetpe, West Karatau ridge, *c.* 15 km N of village, 44°14'35"N, 52°03'19"E, 100 m, 2009, *A. Khodosovtsev* 7775-7781 *& J. Vondrák* 8262, 8247, 9477 & 9487 (CBFS, KHER); Beyneu district, village Beyneu, *c.* 50 km SW of town at road to Aktau, valley of salt river Manashi, 45°01'26"N, 54°59'56"E, 50 m, 2009, *A. Khodosovtsev & J. Vondrák* 9483 (CBFS); Mangistau district, West Aktau ridge, soft valley with rocky outcrops at river Akespe, 44°24'21"N, 51°35'59"E, 100 m, 2009, *A. Khodosovtsev & J. Vondrák* 9486 (CBFS); Mangistau district, at road between village Shetpe and Say-Utes, *c.* 30 km SW of Say-Utes, 44°09'20"N, 52°39'10"E, 260 m, 2009, *A. Khodosovtsev & J. Vondrák* 9506 (CBFS); Mangistau district, East Karatau ridge, rocks at road between Zhatybay and Shetpe, *c.* 30 km SW of Shetpe, 43°57'00"N, 52°05'52"E, 180 m, 2009, *A. Khodosovtsev & J. Vondrák* 9499 (CBFS). **Russia:** *Orenburgskaya Oblast':* Orenburg, village Mikhaylovka (*c.* 30 km SES of city), Khanskaya gora hill, S of village, above brook Berd'yanka, 51°25'48"N, 55°26'27"E, *c.* 200 m, 2011, *I. Frolov & J. Vondrák* 9456 (CBFS); Saraktash district, protected area Kamennaya, rock outcrops in S-slope above river Sakmara, 51°56'53"N, 55°58'23"E, 180 m, 2012, *I. Frolov & J. Vondrák* 10225 (CBFS); *Republic of Altay:* Kosh-Agach district, Kosh-Agach, Telengit-Sortogoy, S-slopes of Kuray Ridge (easternmost part), *c.* 6 km N of village, 50°04'24"N, 88°42'30"E, 2000-2100 m, 2012, *I. Frolov & J. Vondrák* 10224 (CBFS). **Slovakia:** *Cerová vrchovina upland:* Filakovo, Hajnáčka, Šurice, SW-foot of hill Soví hrad, 48°13'34"N, 19°54'45"E, 240-250 m, 2012, *Z. Fakovcová, A. Guttová, J. Liška, Z. Palice* 15905 *& J. Vondrák* 10190 (CBFS, PRA; topotypes). **Turkey:** *Kurdistan:* Iğdır, shale hills SE of town, 39°51'23"N, 44°05'42"E, 1060 m, 2007, *J. Vondrák* 6463 (CBFS); *Central Anatolia:* Yozgat, Boğazlıyan, Özler village, 39°04'10"N, 35°08'17"E, 1100 m, 2012, *J. Vondrák* 9751 (CBFS); Kayseri, Talas, Derevenk valley, 38°41'23"N, 35°34'52"E, 1230 m, 2012, *J. Vondrák* 9760, 9809 & 9787 (CBFS); Kayseri, south-east of Himmetdede, north-west of Kalkancık village, montane steppe with shrubs, 38°53'43"N, 35°07'01"E, 1170 m, 2012, *J. Vondrák* 9791 (CBFS). **Ukraine:** *Donetsk Upland:* Luhansk region, Lutugyno district, steppe slopes with marl outcrops near village Rozkishne, in botanical reserve "Balka Ploska", *c.* 150 m, 2007, *O. Nadyeina* 131, 132 & 134 (KW). [Specimens from Ukraine were published as *Caloplaca concreticola* in Nadyeina (2009)].

***Caloplaca substerilis* Vondrák, Palice & van den Boom sp. nov.**

MycoBank No: MB 803334

Similar to *Caloplaca ulcerosa*, but differs in thallus morphology. Thallus endophloeodal, but also forming minute areoles or squamules; sorediate; without any pigments or TLC identifiable compounds. Apothecia up to c. 0,5 mm diam., orange-red, not pruinose, without chlorinated anthraquinones, biatorine to zeorine. Ascospores broadly ellipsoid, c. 10–15 mm long, with septa c. 4–6 mm wide. Pycnidia with yellow caps containing anthraquinones. Conidia bacilliform, c. 3–4 × 1,0–1,5 mm.

Type: Czech Republic, Southern Bohemia, Novohradské hory Mts, Benešov nad Černou, Žofín, alt. 745 m, 48°40'29"N, 14°41'38"E, on bark of solitary *Ulmus glabra*, 26 May 2010, *J. Vondrák* 7920*, A. Vondráková & O. Redchenko* (CBFS–holotypus). ITS sequence of the holotype: KC416109.

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(Figs 6, 7D)

Thallus endophloeodal or partly of diffuse tiny squamules (somewhat epiphloedal areolate thallus present in samples from the Alps); sorediate; forming irregular pale grey to white spots or extensive crusts, covering large areas of trunks. Squamules 100–150 mm thick and (0,10–)0,17–0,18–0,19(–0,30) μm diam. [30; 3; 0,05]. Soralia small, usually extended in one direction (rarely rounded), usually up to 0,2 mm in length, formed in tiny cracks in the tree bark or on margins and lower surface of squamules, usually not in concave, crater-like depressions (typical for *Caloplaca ulcerosa* Coppins & P. James); soralia in older lichens often tightly arranged and may resemble a continuous sorediate crust. Soredia without pigmentation, (15–) 23–24–26(–30) mm diam. [40; 4; 4]; consoredia (30–)37–41–46(–65) mm diam. [40; 4; 8]. Fungal cells in soredia or consoredia (3,5–)5,4–5,5–5,7(–7,5) × (2,0–)3,2–3,3– 3,4(–4,5) [20; 2; 1,1 & 0,8]. Surface of soredia papillate; papillae formed of fungal cell outgrowths, up to 7 mm high. Medulla indistinct or absent. Algal layer forms majority of thallus, c. 100–140 mm thick; algal cells globose, c. 5–20 mm diam.; old cells often internally divided into several irregularly spherical autospores (cell division typical for Trebouxia; e.g. Peksa & Škaloud 2008). True cortex absent; alveolate cortex developed in patches, up to 20 mm thick, of thin-walled, more or less spherical cells. Epinecral layer indistinct. Thallus surface papillate; papillae of the same size and character as those in soredia. Extracellular crystals of calcium salts not observed in any thallus part. Pruina absent. Prothallus indistinct or absent.

Apothecia present in c. 20% of samples collected (indicated by asterisk in the list of paratypes), but fertile specimens usually with scattered apothecia. The sample from the Alps (van den Boom 15927) with many apothecia is exceptional. Apothecia mostly up to 05 mm diam.; biatorine or zeorine. Disc orange to orange-red; true exciple yelloworange to orange (usually somewhat paler than disc); thalline exciple (when visible) yellow to white; pruina absent or indistinct. Hymenium colourless, somewhat gelatinous, without extracellular oil drops, c. 60–70 μm high; epihymenium ochre. Hypothecium colourless, up to 100 mm high, more or less flat, but with downward extension through the subhypothecial algal layer in the centre, of thin-walled cells variable in shape; extracellular oil drops not seen. Exciple c. 40–80 mm wide, formed of true exciple, c. 30–70 mm wide, and thalline exciple, c. 0–30 mm wide. Upper part of true exciple of cells c. 4– 8 × 3–5 mm, with thin or more than 1 mm thick, glutinized walls. Lower part of palisade prosoplectenchyma of thin-walled cells, 6– 11 × 2–4 mm. Thalline exciple sometimes with alveolate cortex in lower part, up to 30 mm thick, of spherical cells; thalline exciple sometimes sorediate. Paraphyses 1,5–2,0 mm wide in lower part, but about three upper cells widened; branching and anastomosing not observed; paraphyses tips (3,5–)4,6–4,6– 4,6(–5,5) mm [20; 2; 0,7] wide. Asci clavate, c. 50–60 10–16 mm. Ascospores polarilocular, (10,0–)12,0–12,0–12,5(–16,5) × (5,0–) 7,5–8,0–8,0(–10,5) mm [20; 2; 1,4 & 1,3], septa (4,0–)4,5–5,0–5,5(–8,5) mm wide [20; 2; 1,3]. Ascospore length /breadth ratio: (1,2–)1,6–1,6–1,7(–2,2) [20; 2; 0,3]; septum width/ascospore length ratio: (0,26–)03,4–0,40–0,42(–0,52) [20; 2; 0,1]. Extracellular crystals of calcium salts absent from all apothecial parts.

Pycnidia more common than apothecia (observed in c. 50% of samples), c. 50–100 mm wide, with several partly separated chambers (*Xanthoria*-type), distinguished by their yellow tops containing anthraquinones. Conidiophores various in height, formed of rectangular, triangular or spherical cells, c. 3– 5 × 4–8 mm. Conidia usually bacilliform, straight or slightly curved, rarely ellipsoid or tear-shaped, (2,5–)3,2–3,4–3,5(–5,0) × (1,0–) 1,2–1,2–1,3(–1,5) mm [20; 2; 0,7 & 0,2].

*Chemistry.* Spot tests: thallus K-, C-, P-, UV± white; apothecia K+ purple, C-, UV-. True exciple non-amyloid (I-). Hymenium and the upper part of hypothecium (subhymenium) amyloid (I+). The C- reaction of epihymenium and outer cells in the true exciple suggests an absence of chlorinated anthraquinones. No compounds revealed from thallus by TLC.

*Etymology.* '*Substerilis*' reflects the usually sterile occurrence.

*Similar taxa.* Apothecial characters in the new species are identical to those of the closely related *Caloplaca ulcerosa*, but they differ in thallus characters. In *C. substerilis*, the thallus is endophloeodal or of diffuse minute squamules, with marginal soralia, while *Caloplaca ulcerosa* forms an epiphloedal nonsquamulose thallus with round to irregular soralia formed in crater-like depressions. The latter species further

differs in its shorter, ellipsoid conidia (c. 2,5–3,0 × 1,5 mm), much higher fertility and in ecology; it is a maritime species (Vondrák et al. 2009b).

White morphotypes of *C. phlogina* (Ach.) Flagey are similar (see Kondratyuk et al. 1998; Vondrák et al. 2010b); they also have papillate soredia of similar size without pigmentation, yellow pycnidial caps and an endophloedal thallus, sometimes with minute white squamules. However, *C. phlogina* differs in frequently having apothecia: these are large (mostly □0,5 mm diameter), yelloworange, with a rough surface caused by yellow anthraquinone pruina. Ascospores are significantly smaller with thinner septa: ascospores (8,5–)10,5–10,8–11,2(–13,0) × (4,0–)5,0–5,4–5,7(–7,0) mm [30; 3; 1,0 & 0,7], and septa (2,5–)3,1–3,6–3,8(–4,5) mm wide [30; 3; 0,6].

Sterile thalli may resemble a number of taxa, including: *Caloplaca obscurella* ( J. Lahm) Th. Fr. (with rounded crater-like soralia, brown apothecia), *C. sterilis* Šoun et al. (on steppe shrubs, with lecanorine apothecia), *C. subalpina* Vondrák et al. (saxicolous, with lecanorine apothecia and Sedifolia-grey in soredia), *Candelariella subdeflexa* (Nyl.) Lettau (with different apothecia and more conspicuous squamules producing conidia from the underside) and *Rinodina degeliana* Coppins (areolate-squamulose thallus with marginal soralia; presence of atranorin and zeorin).

*Phylogeny.* In the ITS phylogeny (Fig. 6), *Caloplaca substerilis* forms a well-supported clade, sister to the clade of North American *C. ''ulcerosa''*. Both taxa are sister to the European *C. ulcerosa*. Close relatives of these three taxa are not known. Ecology and distribution. *Caloplaca substerilis* occurs on nutrient-rich bark of *Acer campestris*, *A. platanoides*, *Carpinus*, *Juglans*, *Quercus*, *Populus* and *Ulmus* in well-lit conditions, sometimes overgrowing mosses on bark. Specimens from the Alps were collected on the bark of *Sambucus* and on *Picea abies* twigs. Co-occurring lichens are more or less nitrophilous *Caloplaca cerinelloides*, *C. monacensis*, *C. obscurella*, *Lecanora hagenii*, *Macentina dictyospora*, *Phaeophyscia nigricans*, *P. orbicularis*, *Physcia* spp., *Physconia* sp., *Piccolia ochrophora*, *Rinodina pityrea*, *Xanthomendoza fulva* and *Xanthoria parietina*.

*Caloplaca substerilis* shows continental bias in Europe. It appears to be quite common in the Southern Ural Mountains (most of known localities). It is probably distributed throughout eastern and central Europe in suitable woodland areas with preserved undisturbed solitary elm and poplar trees. So far it is known from Austria, Bulgaria, the Czech Republic, Russia and Slovakia.

*Taxonomic notes.* The North American taxon called *Caloplaca ulcerosa* (Wetmore 2004) is morphologically more similar to *C. substerilis* than to *C. ulcerosa* s. str. We have examined three samples of the North American taxon (GZU: Iowa, Teloschistaceae Exsiccati 95; Iowa, Wetmore 93230; South Dakota, Advaita 6490), and did not find any diagnostic difference from *C. substerilis*. It corresponds well with the ITS phylogeny, where both taxa form a monophyletic group. The distribution of

the North American taxon (Wetmore 2009) and the distribution of *C. substerilis* are similarly continental and different from the maritime distribution pattern of *C. ulcerosa* s. str. (Vondrák et al. 2009b). Provisionally, we call the North American specimens *C. "ulcerosa"* in Fig. 6.

Two ITS sequences of *C. substerilis* from the Alps form a separate lineage from the other *C. substerilis* sequences. The specimens from the Alps also differ slightly in morphology (frequent apothecia, more or less epiphloeodal thallus and absence of minute squamules) and ecology. While most samples were collected from solitary elms, poplars and oak, specimens from the Alps came from *Sambucus* bark and spruce twigs. This suggests that the populations from the Alps might represent a distinct infraspecific taxon.

*Paratypes (fertile specimens indicated by asterisk):* **Austria:** *Steiermark:* Schladming, Ramsau am Dachstein, in gorge with road from Ramsau to Weissenbach, *c*. 850 m, 2009, *J. Vondrák* 7257 (CBFS); *****Kärnten:* Gailtaler Alpen, 10 km WNW of Weissbriach, 0.5 km SE of Felstritz, open pine forest, 550 m, 1994, *P. v.d. Boom* 15927 (hb. v.d. Boom). **Bulgaria:** *The Rhodopes:* Madzharovo district, Silen, Rabovo, valley of small brook N of village, 41°37'N, 25°40'E, 250 m, 2004, *J. Vondrák* (CBFS, in sample "*Caloplaca virescens*, Exs. of *Caloplaca*, Nr 11"). **Czech Republic:** *Southern Bohemia:* Novohradské hory Mts, Benešov nad Černou, Žofín, 48°40'29"N, 14°41'38"E, 745 m, 2009, 2010, *Z. Palice* 12943 & 13676 (PRA, topotypes); Šumava Mts, Borová Lada, Knížecí Pláně, avenue of old trees along yellow-marked tourist footpath near abandoned cemetary, N48°57.61', E013°37.19', 1000-1020 m, 2005, *Z. Palice* 8928 (PRA); distr. Jindřichův Hradec, Novobystřická vrchovina, W slope of crest Homolka - Fabián - "Lesovna v Dubovici", 49°02'N, 14°58'50"E, 540 m, 2002, *M. Kukwa & Z. Palice* 6844 (PRA); *Western Bohemia:* Šumava Mts, Zhůří: valley of Pěnivý potok brook, nearby the settlement Bílý Potok, N49°06.3', E013°34.1', 770 m, 2005, *Z. Palice* 9414 *& J. Palicová* (PRA); *Southern Moravia:* Mikulov, Klentnice, protected area Soutěska, 48°51'48"N, 16°38'40"E, 400 m, 2013, *J. Vondrák* 10668, 10669, *I. Frolov & N. Pirogov* (CBFS). **Russia:** *Chelyabinskaya Oblast':* Southern Ural Mts,, Ust'-Katav, vill. Orlovka (*c*. 10 km SW of Ust'-Katav), fragments of forest with *Ulmus laevis-Ulmus glabra* in valley of small brook *c*. 2 km SE of village, 54°52'04"N, 58°06'36"E, 500 m, 2012, *J. Vondrák* 9963 (CBFS); *Orenburgskaya Oblast':*, Kuvandik, vill. Maloe Churaevo (25 km N of Kuvandik), camp *c*. 2 km W of village, steppes and *Quercus robur-Tilia cordata-Ulmus laevis* woodland areas around camp, 51°40'9"N, 57°27'14"E, 250-500 m, 2011, *J. Vondrák* 9957, 9968 & 9970 (CBFS); *****Saraktash, vill. Andreevka (*c*. 25 km NE of Saraktash), alluvial forest with *Tilia cordata*, *Populus* sp. and *Ulmus laevis*, *c*. 8 km NW of village, in valley of river Bolshoy Ik, 52°00'29"N, 56°33'39"E, 150 m, 2012, *J. Vondrák* 9967 (CBFS);

*****_Republic of Bashkortostan_: Irendik range, Sibay, vill. Gabelsha (_c_. 15 km W of Sibay), waterfall Gadelsha in upper stream of brook Khudolaz, 52°45'26"N, 58°22'34"E, 500-800 m, 2011, *J. Vondrák* 9361 (CBFS). **Slovakia:** *West Carpathians:* Muránská planina Mts, Mt Cigánka, well-lit oak forest on limestone on S slope, 48°45'18"N, 20°03'22"E, 800 m, 2010, *J. Halda & Z. Palice* 13441 (PRA).

Linda in Arcadia kindly revised the English and proposed the Latin name *molariformis*. David Svoboda provided some translations from French. Our research was supported by the program NAKI of the Ministry of Culture of the Czech Republic (DF12P01OVV025), by long-term research development project no. RVO 67985939, by the Centre for Algal Biotechnology Třeboň – ALGATECH (CZ. 1.05/21.00/03.0110), by the institutional resources of the Ministry of Education, Youth and Sports of the Czech Republic and by TÜBİTAK (111T927 coded project).

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Table 1. New *Caloplaca* ITS sequences generated during this study.

| Labels of phylogenetic tree terminals | Voucher | GenBank Accession No |
|---|---|---|
| *Caloplaca* aff. *crenularia* Canary Islands | GZU (1993, *Poelt & Sanchez-Pinto*) | KC416116 |
| *C. areolata* Croatia | CBFS JV7950 | KC416098 |
| *C. areolata* Montenegro | GZU (2008, Mayrhofer) | KC416097 |
| *C. areolata* Spain | CBFS JV6314 | KC416096 |
| *C. crenularia* Bulgaria | CBFS JV2065 | KC416112 |
| *C. crenularia* Crete 1 | GZU (*Mayrhofer* 18045) | KC416113 |
| *C. crenularia* Crete 2 | CBFS JV4137 | KC416119 |
| *C. crenularia* Hungary | CBFS JV6409 | KC416117 |
| *C. crenularia* Iran | CBFS JV5608 | KC416115 |
| *C. crenularia* Spain | CBFS JV6255 | KC416114 |
| *C. crenularia*Turkey | CBFS JV6064 | KC416118 |
| *C. emilii* Bulgaria, holotype | CBFS JV6600 | KC416101 |
| *C. emilii* Bulgaria, Rhodopes | CBFS JV2223 | KC416099 |
| *C. emilii* Czech Republic | CBFS JV9358 | KC416102 |
| *C. emilii* Czech Republic 2 | CBFS JV9357 | KC416103 |
| *C. emilii* France | Herb. Clauzade 23475 | KC416100 |
| *C. emilii* Greece | CBFS JV8832 | KC416104 |
| *C. ferrarii* s.lat. Czech Republic 1 | CBFS JV8782 | KC416139 |
| *C. ferrarii* s.lat. Czech Republic 2 | CBFS JV9150 | KC416132 |
| *C. ferrarii* s.lat. Czech Republic 3 | CBFS JV9043 | KC416137 |
| *C. ferrarii* s.lat. Czech Republic 4 | CBFS JV9151 | KC416140 |
| *C. furfuracea* Austria | PRA (*Palice* 12390) | KC416120 |
| *C. fuscorufa* Ukraine | CBFS JV6204 | KC416111 |
| *C. herbidella* Turkey | PRA (*Palice* 11832) | KC917268 |
| *C. interfulgens* Czech Republic 1 | CBFS JV9399 | KC416134 |
| *C. interfulgens* Czech Republic 2 | CBFS JV9153 | KC416131 |
| *C. interfulgens* Czech Republic 3 | CBFS JV9156 | KC416129 |
| *C. interfulgens* Czech Republic 4 | CBFS JV9155 | KC416130 |
| *C. interfulgens* Czech Republic 5 | CBFS JV9144 | KC416138 |
| *C. interfulgens* Slovakia 1 | CBFS JV9260 | KC416136 |
| *C. interfulgens* Slovakia 2 | CBFS JV9186 | KC416135 |
| *C. interfulgens* southern Russia | CBFS JV9396 | KC416133 |
| *C. interfulgens* Turkey | CBFS JV8552 | KC416125 |
| *C. interfulgens* Turkey | CBFS JV8557 | KC416126 |
| *C. interfulgens* Turkey | CBFS JV8539 | KC416127 |
| *C. lactea* Greece | CBFS JV8331 | KC416128 |
| *C. lactea* Italy | CBFS JV8679 | KC416124 |
| *C. molariformis* Kazakhstan | CBFS JV7635 | KC416146 |
| *C. molariformis* Slovakia, holotype | CBFS JV10192 | KC416142 |
| *C. molariformis* Turkey | CBFS JV9787 | KC416144 |
| *C. molariformis* Ukraine 1 | KV (Luhansk, *Nadyeina* 132) | KC416143 |
| *C. molariformis* Ukraine 2 | KV (Luhansk, *Nadyeina* 134) | KC416145 |

| | | |
|---|---|---|
| *C. scabrosa* Czech Republic | CBFS JV1908 | KC416122 |
| *C.* "*scabrosa*" Ukraine 1 | CBFS JV6198 | KC416121 |
| *C.* "*scabrosa*" Ukraine 2 | CBFS JV6199 | KC416123 |
| *C. substerilis* Austria | CBFS JV7257 | KC416107 |
| *C. substerilis* Bulgaria | CBFS (Exs. of *Caloplaca*, nr 11) | KC416108 |
| *C. substerilis* Czech Republic, holotype | CBFS JV7920 | KC416109 |
| *C. substerilis* Slovakia | PRA (*Palice* 13441) | KC416110 |
| *C.* "*ulcerosa*" USA | GZU (*Wetmore* 93230) | KC416105 |
| *C.* "*ulcerosa*" USA 2 | GZU (*Advaita* 4915) | KC416106 |
| *C.* sp. southern Russia | CBFS JV8181 | KC416141 |

Table 2. Summary of phylogenetic analyses: length of alignments (including gapped positions) and model selected for the purpose of MrBayes calculation.

| Target group | Phylogenetic tree | Length of alignment | Model |
|---|---|---|---|
| *Caloplaca crenulatella* group | Fig. 2. | 525 positions | SYM+ADGamma |
| *C. crenularia* group | Fig. 3. | 486 positions | GTR+ADGamma |
| *C. xerica* group | Fig. 4. | 501 positions | SYM+ADGamma |
| *Pyrenodesmia* group | Fig. 5. | 535 positions | SYM+ADGamma |
| *C. ulcerosa* and related taxa | Fig. 6. | 519 positions | GTR+ADGamma |

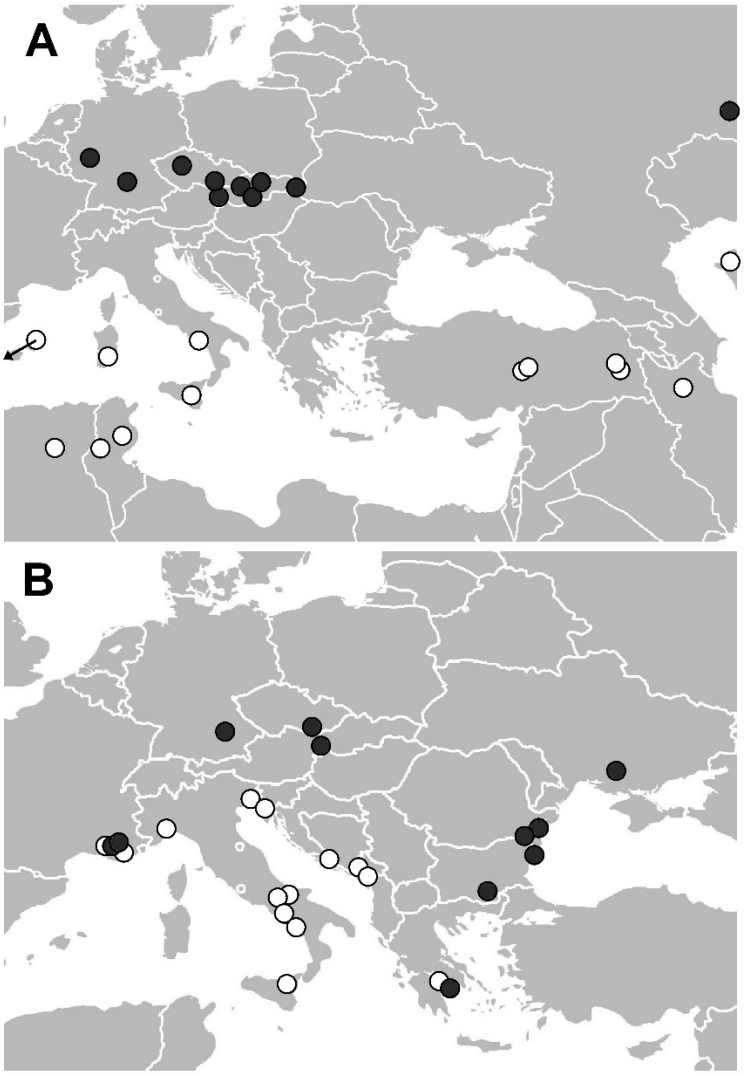


Fig. 1. Distribution maps. A, *Caloplaca interfulgens*, previously published data (white dots), new records (black dots); B, *C. emilii* (black dots), *C. areolata* (white dots).

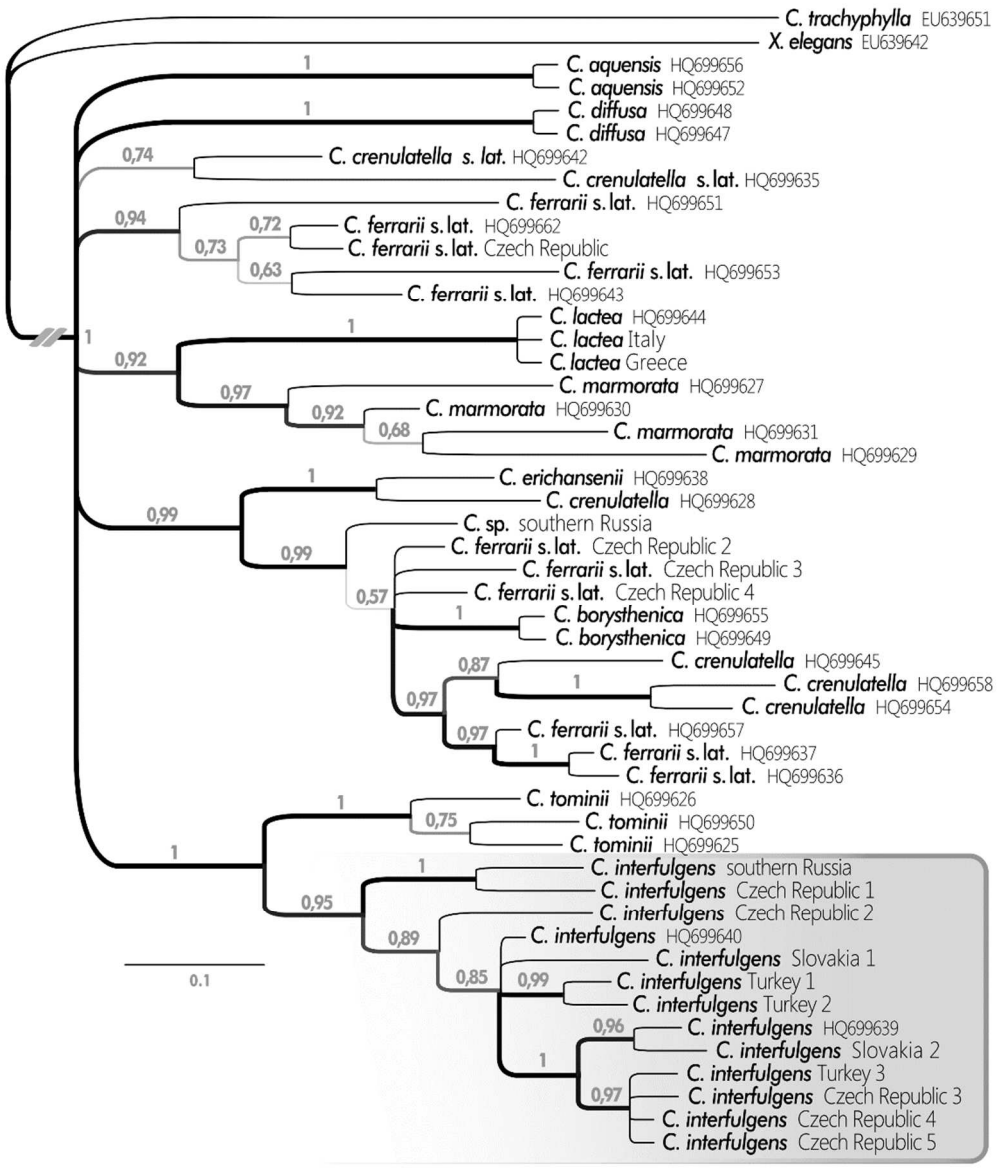


Fig. 2. Bayesian ITS phylogeny of the *Caloplaca crenulatella* group; *C. interfulgens* clade delimited by the grey square.

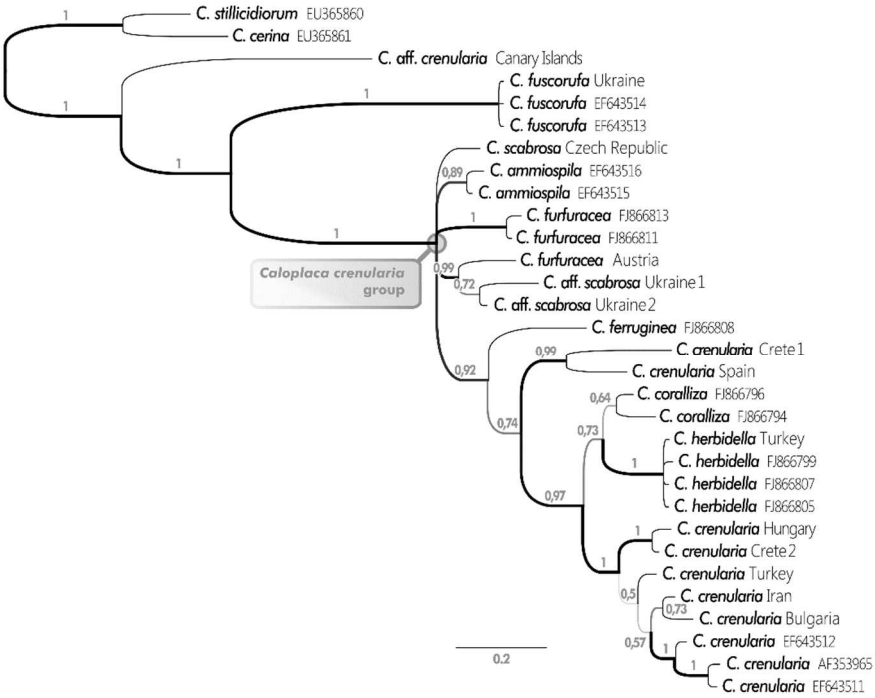


Fig. 3. Bayesian ITS phylogeny of the *Caloplaca crenularia* group including the Central European sample of *C. scabrosa* and *C. "scabrosa"* from the Eastern Carpathians.

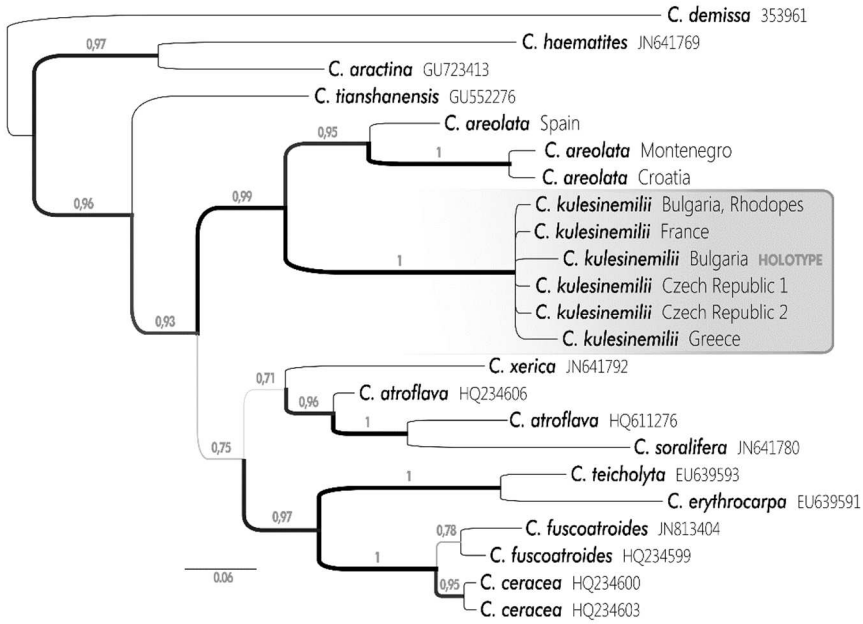


Fig. 4. Bayesian ITS phylogeny of the *Caloplaca xerica* group including *C. emilii* (in the grey square) and C. areolata.

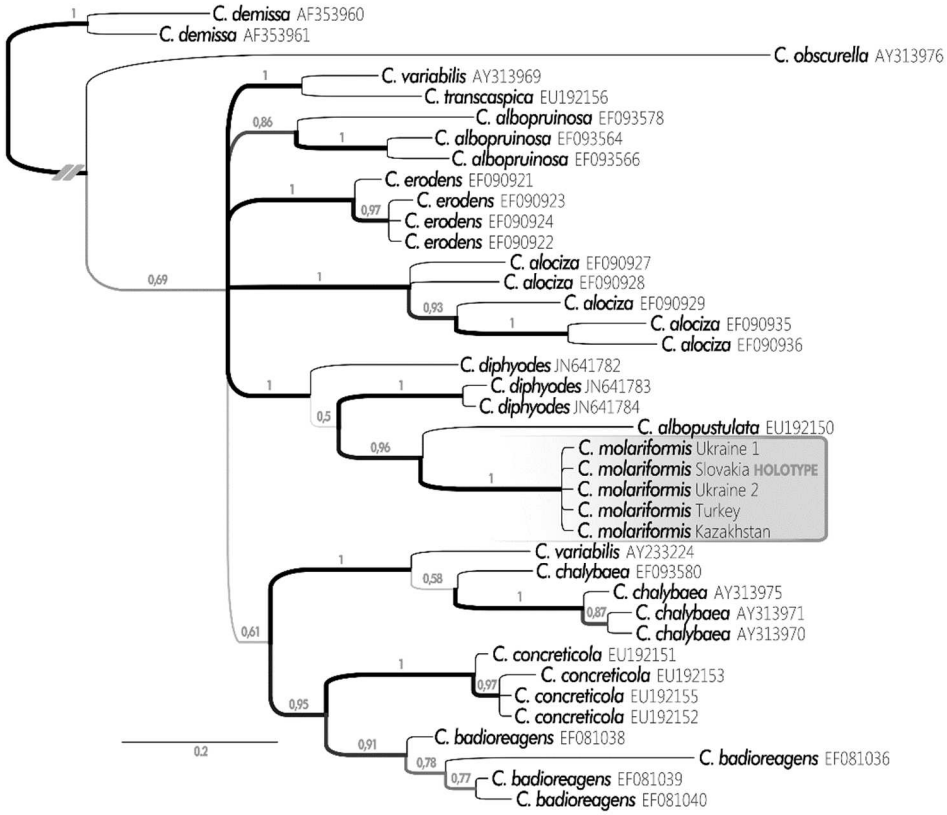


Fig. 5. Bayesian ITS phylogeny of the *Pyrenodesmia* subgroup of *Caloplaca*, including *C. molariformis* clade delimited by the grey square.

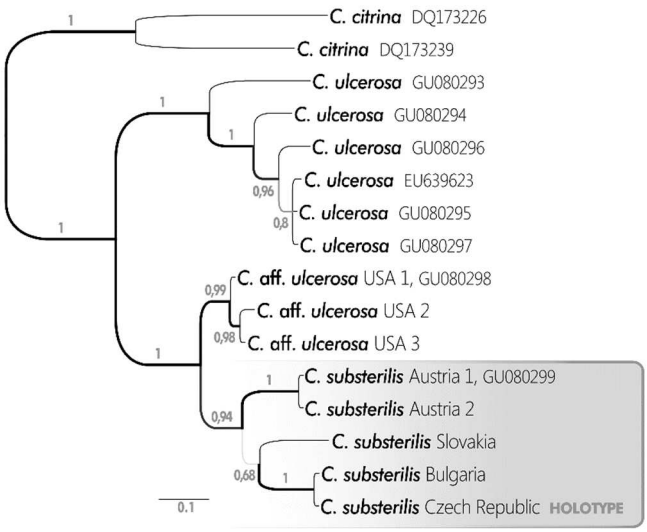


Fig. 6. Bayesian ITS phylogeny of *Caloplaca ulcerosa* and related taxa including *C. substerilis* (delimited by the grey square).

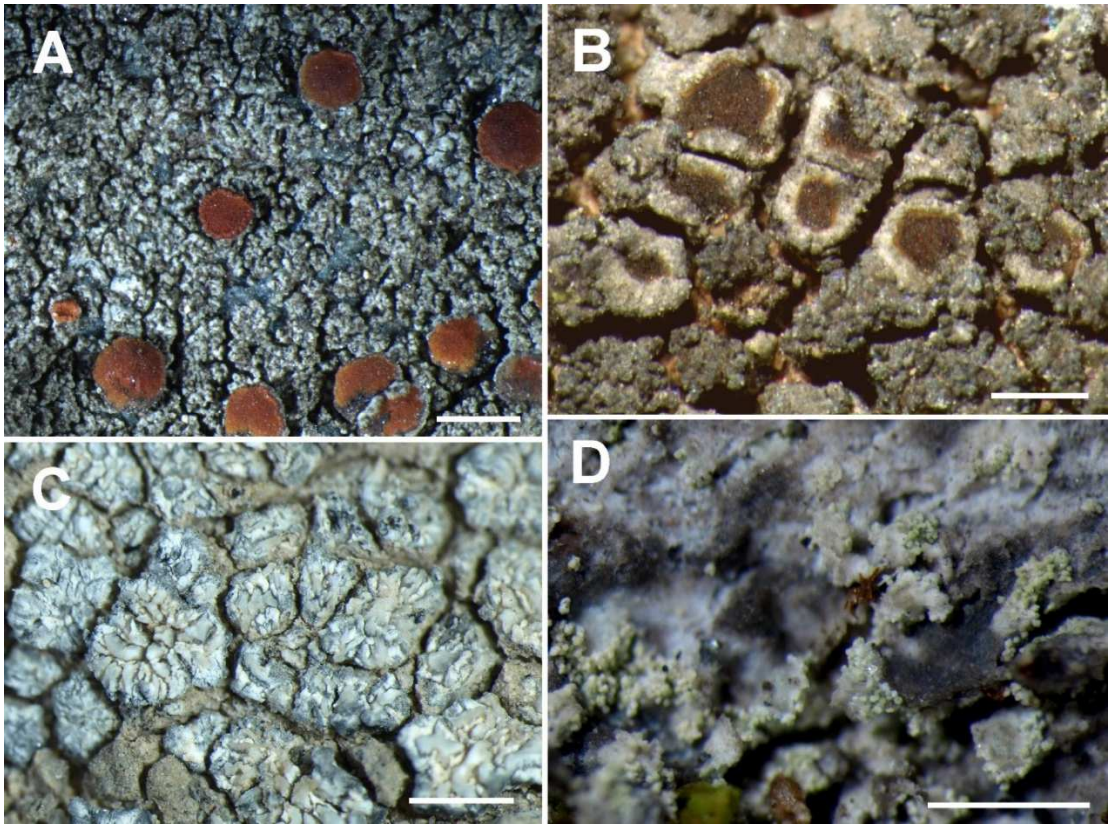

Fig. 7. A, *Caloplaca scabrosa* (CBFS JV1908); B, *C. emilii* (holotypus); C, *C. molariformis*, morphotype with well-developed epinecral ridges above fungal stacks (CBFS JV9486); D, *C. substerilis* (CBFS JV7920, holotypus). Scales: A & C = 1 mm; B & D = 0,5 mm.

## 5.5 Paper 5

Vondrák J., **Frolov I.**, Davydov E.A., Urbanavichene I., Chesnokov S., Zhdanov I., Muchnik E., Konoreva L., Himelbrant D., Tchabanenko S. 2016. The extensive geographical range of several species of Teloschistaceae: evidence from Russia. The Lichenologist 48: 181–189.

**The extensive geographical range of several species of Teloschistaceae: evidence from Russia**

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**Abstract**

The current view of the geographical ranges of lichens is often distorted by overly narrow or overly broad applications of names and by insufficient survey of most regions of the world. Here we present several cases where species of Teloschistaceae formerly thought to be limited to rather small territories in the western or eastern parts of Eurasia are in fact widespread in northern Eurasia. We support our findings with ITS nrDNA data in several new trees showing relationships in the genera *Athallia*, *Calogaya*, *Caloplaca*, *Flavoplaca* and *Gyalolechia*. The widespread species have little in common, except that most of them reproduce both sexually and asexually, and we discuss the possible influence of the combined reproduction on geographical range. *Calogaya bryochrysion*, *Calogaya saxicola*, *Gyalolechia epiphyta* and *Gyalolechia ussuriensis* are new combinations. *Calogaya alaskensis* is a younger synonym for *C. bryochrysion*. The generally arctic-alpine *Calogaya bryochrysion* also occurs on the bark of solitary trees in dry parts of the Altai Mountains. The Australian *Flavoplaca*

*cranfieldii* is a younger synonym of *F. flavocitrina*. *Gyalolechia epiphyta* has been described numerous times, from different regions and substrata, as *Caloplaca juniperi*, *C. laricina*, *C. tarani*, *Gyalolechia arizonica* and *G. juniperina*. The name *Gyalolechia xanthostigmoidea* has recently been used for *G. epiphyta*, but it represents a distinct taxon. *Gyalolechia ussuriensis* is closely related to and morphologically indistinguishable from *G. persimilis*, but they have a different ecology and distribution and we regard them as distinct species. *Caloplaca juniperina* Tomin is lectotypified.

**Key words:** *Athallia*, biogeography, *Calogaya*, *Caloplaca*, circumpolar distribution, *Flavoplaca*, *Gyalolechia*.

## Introduction

Geographical ranges of lichen species are often underestimated, mainly because of the very unbalanced intensity of lichen diversity research in various regions of the world (Arcadia 2013). Some species of microlichen (lichen crusts) have a distribution that is probably known reliably, often because of special circumstances, such as species of *Dirina* (Tehler et al. 2013), most of which are restricted to coastal sites, a habitat that can be sampled fairly effectively because of its limited area. However, for most species distributional data are scarce, which might result in seemingly implausible disjunctions in known distributions, such as in *Rinodina capensis* (Mayrhofer et al. 2014), *Sclerophora amabilis* (Tibell 1999) and many others. Another reason for underestimated geographical ranges is the poor, but all too common, taxonomic practice of redescribing a lichen when it is found in different geographical regions, without adequately considering previous work. For instance, Sheard (2010) provided some cases of crustose species that have been described and redescribed even in recent times.

The opposite problem, too extensive a reported geographical range, can be caused by insufficient taxonomic knowledge. According to the world biodiversity database GBIF (http://www.gbif.org/), some 'prominent' lichen names (e.g. *Caloplaca citrina* and *C. holocarpa*) are mapped throughout the world, but these species have not been confirmed outside temperate regions of the Northern Hemisphere (Vondrák et al. 2009, 2016). The use of mainly European literature to determine lichens from other parts of the world has led to error in these cases and probably many others.

Russia includes most of northern Eurasia between 28°E and 169°W longitude and investigations of lichen diversity within its territory are essential to discover the real distributions of lichen taxa, especially those previously known only from Europe or North America (Davydov & Printzen 2012). Although the lichen biota of Russia has been quite well studied, it is less known than that of western Eurasia, mainly because the territory is very large and some regions are difficult to access. Here we report on

selected examples, supported with molecular data, where our Russian records have changed the previous understanding of a species' range.

## Materials and Methods

### Specimens

Assessed specimens belong to nine species of *Athallia*, *Calogaya*, *Caloplaca*, *Flavoplaca* and *Gyalolechia* (Teloschistaceae). Specimens were collected by the authors from various regions of Russia. Acronyms of the author followed by the author's herbarium numbers are used to identify specimens in the figures and in Table 1. Most specimens are precisely localized by WGS 84 coordinates. Vouchers collected by IU, IZ, JV, GU (Genadii Urbanavichus) and EM are deposited in PRA, those collected by LK and SC in LE, by ED and L. Yakovchenko in ALTB, by DH in LECB, by TS (Toby Spribille) in GZU and by IF in the private herbarium of the author. All specimens were examined and identified by the first author. For the molecular analyses we sequenced the ITS of selected samples from Russia, and also from other countries if GenBank data were scarce, to produce more comprehensive phylogenetic trees (Table 1).

### Sequences and phylogenetic reconstructions

DNA was extracted with a CTAB-based protocol (Aras & Cansaran 2006). Primers for PCR amplification of ITS were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). The PCR parameters included an initial hold at 94 °C for 5 min, and then 45 cycles with denaturating at 94 °C (30 s), annealing at 62 °C with the touchdown to 56 °C during the first 7 cycles (30 s), and an extension at 72 °C (60 s).

ITS nrDNA sequence data were used in our study for practical reasons: they are easily generated; the NCBI database (GenBank) includes a number of ITS sequences for reasonable fingerprinting; ITS single-locus genealogies are usually consistent with phenotypic data (seen in numerous ITS-based studies on Teloschistaceae) and are generally congruent with the loci nrLSU and mtSSU (e.g. Arup et al. 2013). New sequences were submitted to NCBI's BLAST website (Johnson et al. 2008; http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm taxonomic identity.

The 69 sequences from this study (Table 1) were arranged into five alignments for five genera together with close GenBank sequences (Table 2). Alignments were done in BioEdit 7.2.5 free software (Hall 1999) with the use of ClustalW application (Thompson et al. 1997) and corrected by hand. Most of the GenBank data used are from Arup (2006), Arup & Grube (1999), Arup et al. (2013), Gaya et al. (2011), Himelbrant et al. (2015), Joshi et al. (2011), Kasalicky et al. (2000), Malíček et al. (2014), Powell & Vondrák (2011), Redchenko et al. (2012), Šoun et al. (2011),

Vondrák et al. (2008, 2009, 2012a, b) and Wedin et al. (2002). Maximum likelihood (ML) phylogenetic analyses were run in the application Phylogeny.fr (Dereeper et al. 2008) without Gblocks, with 250 bootstrap replicates and the GTR + I + G nucleotide substitution model. Outgroup sequences were selected from closely related genera on the basis of analyses by Arup et al. (2013) and our broader unpublished analyses.

## Results

### *Athallia alnetorum* (Giralt et al.) Arup et al.

See Arup et al. (2013) for nomenclatural details.

*Caloplaca alnetorum* Giralt et al. was combined into *Athallia* by Arup et al. (2013). It resembles some morphotypes of *Gyalolechia flavorubescens* s. lat., but according to Giralt et al. (1992) differs in ascospore size and shape of conidia. We confirm that ascospore size is diagnostic, but we observed bacilliform conidia, characteristic of *G. flavorubescens*, in some specimens of *A. alnetorum* (specimens from Latvia; Frolov 663, 664). *Athallia alnetorum* is well known in Mediterranean mountains and the Alps (e.g. Giralt et al. 1992; Vondrák & Wirth 2013). It is new to Russia from the western foothills of the Caucasus Mountains but it is also common on the Baltic Sea coast in Latvia (I. Frolov, unpublished data), thus more northern Russian records are possible. The ITS sequence of the Russian specimen is within the *A. alnetorum* clade (see Supplementary Material Figure S1, available online).

*Russian specimen*. **Russia:** *Krasnodar Krai*: Caucasus Mts, Utrish Reserve, forested mountain c. 20 km SE from Anapa, alt. 430 m, 44·7212°N, 37·4684°E, broadleaved forest, on branch of *Quercus petraea*, 2014, *I. Urbanavichene* s. n. (PRA).

### *Calogaya arnoldii* (Wedd.) Arup et al.

See Arup et al. (2013) for nomenclatural details.

A common lichen which has been called *Caloplaca saxicola* (Hoffm.) Nordin by numerous Russian lichenologists (cf. Urbanavichus 2010) but proved to be *Calogaya arnoldii* (sensu Gaya 2009; Gaya et al. 2011). *Calogaya arnoldii* and *Calogaya saxicola* (the combination proposed below) are closely related and the differences are subtle; they mostly concern shape and size of ascospores. However, both taxa are phenotypically variable, their characters overlap, and they cannot be identified with certainty from their phenotype. The Russian specimens were identified from their ITS sequences (Fig. 1). One Russian specimen from the Western Sayan Mountains

(JV12558) has an ITS sequence (KT804947) similar to *Calogaya saxicola* sensu Gaya et al. (2011) and could be considered conspecific with *C. saxicola*.

We consider the subspecies *arnoldii*, *nana*, and *obliterata* proposed by Gaya (2009) to be merely expressions of phenotype plasticity within the species *C. arnoldii*, and our opinion is reflected in the ITS tree (Fig. 1).

*Russian specimens*. **Russia:** *Republic of Adygea:* Caucasus Mts, Caucasus Reserve, Kamennoe More Ridge, the edge of a cliff above Armyanka River, 44.0164N, 39.9789E, alt. 2000–2030 m, on limestone, 2011, *G. Urbanavichus* s.n. (PRA); *Republic of Bashkortostan:* Southern Urals Mts, Shulgan-Tash Reserve, cliff above Kapova Cave at the banks of Belaya River, 53.0419N, 57.0672E, alt. 300 m, on bark of *Betula*, 2007, *G. Urbanavichus* s.n. (PRA); *Altai Territory:* Soloneshensk district, Bashchelaksky Range, velley of Shinok River, alt. 1035 m, 51.3545N, 84.5676E, on stone, 2003, *E. Davydov 6934* (ALTB). *Sverdlovsk Region:* Yekaterinburg, Rezh, Glinskoe, 0.5 km E of village Chepchugovo, 57.4858N, 61.4941E, on lime-rich schist, 2013, *J. Vondrák 12552* (PRA); *Zabaikalsky Territory:* Kodar Ridge, 56.9196N, 118.0291E, on lime-enriched siliceous rock, 2013, *L. Konoreva* s.n. (LE).

***Calogaya bryochrysion*** **(Poelt) Vondrák comb. nov.**

MycoBank No.: MB 814538

*Caloplaca bryochrysion* Poelt, *Feddes Repertorum* 58: 175 (1955); type: Germany, Wettersteingebirge, Gipfel der Alpspitze, in feinen Felsspalten an Vogelblöcken, 1954, *Poelt* (M-0024347—holotype seen).

**Syn. nov.** *Caloplaca alaskensis* Wetmore, *Bryologist* 107: 507 (2004); type: USA, Alaska, valley of Mancha Creek with Firth River, 1958, *Sharp* 6531 (MIN—holotype).— *Calogaya alaskensis* (Wetmore) Arup et al. (2013: 38).

(Fig. 5A)

The name *Caloplaca bryochrysion* was synonymized with *C. epiphyta* by Hansen et al. (1987). Søchting & Tønsberg (1997), however, considered *C. epiphyta* synonymous with *C. xanthostigmoidea* (= *Gyalolechia xanthostigmoidea*), but recognized *C. bryochrysion* as distinct. *Caloplaca xanthostigmoidea* and related taxa (now the genus *Gyalolechia*) contain fragilin and some chlorinated anthraquinones, but the type of *C. bryochrysion* has parietin as the main anthraquinone and lacks substances characteristic of *Gyalolechia* (Søchting & Tønsberg 1997). Those authors therefore

suggested that *C. bryochrysion* is related to *C. citrina*, a morphologically similar taxon with the same pigments.

We examined *Caloplaca bryochrysion* specimens from the Austrian Alps (in GZU, PRA) and also obtained an ITS sequence (JV7262 in Table 1) that groups with two *C. alaskensis* sequences (Fig. 1). We further compared the type of *C. bryochrysion* (Poelt 1955) with numerous samples of *Calogaya alaskensis* and consider both names synonymous. The epithet *bryochrysion* has priority over *alaskensis*, so a new combination is required.

Wetmore (2004) described *Caloplaca alaskensis* (now *Calogaya*) from only two localities in Alaska, but within a few years it had been reported from numerous arctic and boreal-alpine localities in North America, Europe, Svalbard and Greenland (Søchting et al. 2008). The latter authors also provided ITS sequence data showing that geographically distant samples called *C. alaskensis* belong to the same species. Recently it was also found in central Europe, in the Carpathians (Malíček et al. 2014).

We obtained five ITS sequences from five Russian samples of *Calogaya bryochrysion*. Two are from arctic-alpine habitats and typical substrata (calcareous rock, calciphilous bryophytes), but the other three are from dry continental, semi-desert habitats in the Altai Mountains. They were collected on *Populus laurifolia* and *Salix pentandra* growing along rivers in high mountains mostly covered by dry steppe communities. This corticolous population may eventually prove to be an incipient species that is already distinct from the arcticalpine population, but that is not evident from the ITS (Fig. 1) and morphological data, and so for the present we include it in *C. bryochrysion*.

*Russian specimens.* **Russia:** *Republic of Altai:* Kosh-Agach district, SE part of Kuray Ridge, NE of village Chagan-Uzun, alt. 2000 m, 50.052N, 88.709E, on bark of *Populus laurifolia*, 2012, *I. Frolov & J. Vondrák 10372* (PRA); Kosh-Agach district, left bank of Yustyd River, 2 km downstream of Boguty and Naryngol Rivers junction, alt. 2200 m, 49.7969N, 89.3619E, on bark of *Salix pentandra*, 2013, *E. Davydov 11498* (ALTB); Kosh-Agach district, Kurai, right bank of Kuraika River at 5 km N of Kurai, alt. 1670 m, 50.2669N, 87.9513E, on bark of *Populus laurifolia*, 2013, *E. Davydov 11499* (ALTB); *Republic of Tuva:* West Sayan Mts, Ak-Dovurak, Ak-Sug, Enge-Beldir, glacier cirque in S-slope from pass 'Sayanskiy'; 2200 m", at road A161, close to Republic of Khakasia border, alt. 2150–2200 m, 51.7000N, 89.8872E, on base-rich schist, 2013, *I. Frolov & J. Vondrák 11086* (PRA); *Arkhangelsk Region:* Novaya Zemlya Archipelago, NE extremity of Severny Island, Karlsen Cape, alt. 0–5 m, 76.999722N, 67.780000E, on lime-rich pebbles at seashore, 2013, *I. Zhdanov* (LE). *Zabaikalsky Krai:* Kodar ridge, Hadytkanda valley, alt. 1230m, 56·7480°N, 117·2650°E, 2015, *S. Chesnokov* 249 (LE); ibid., valley of Zolotoy brook, alt. 1410m, 56·8389°N, 117·3064°E, 2015, *S. Chesnokov* 161 (LE).

***Calogaya saxicola* (Hoffm.) Vondrák comb. nov.**

MycoBank No.: MB 815508

*Psora saxicola* Hoffm., *Descr. Adumb. Pl. Lich.* 1 (3): 82, Tab. 17, Fig. 3 (1790); type: Sweden (H-Ach 1019E "*Lecanora murorum*, Svecia"—neotype selected by Nordin 1972).

***Caloplaca isidiigera* Vězda**

See Šoun et al. (2011) for nomenclatural details.

(Fig. 2A; distribution map)

*Caloplaca isidiigera*, described from the Carpathians (Vězda 1978), is known from numerous montane-alpine sites in Europe and North America (Šoun et al. 2011). We newly report it from several localities in southern Siberia and suggest that it has a circumpolar distribution. *Caloplaca isidiigera* also occurs at low altitudes in continental Eurasia (e.g. JV9541 from the Chelyabinsk Region). An ITS sequence from the specimen from the Caucasus Mountains falls within the *Caloplaca isidiigera* clade (see Supplementary Material Figure S2, available online).

*Russian specimens.* **Russia:** *Republic of Adygeya:* Caucasus Mts, Caucasus Reserve, Kamennoe More Ridge, c. 0.85 km N from Mt. Nagoi Koshi, on limestone, 44.0304N, 40.0251E, alt. 2025 m, 3 Jul 2011, *G. Urbanavichus* s.n. (PRA); *Republic of Altai:* Altai Mts, Choya district, Karakoksha, settlement Uymen', Mt. Sagani (2036 m), about 40 km S of Karakoksha, alt. 1700–2030 m, on vertical face of base-rich rock in subalpine zone, 2012, *I. Frolov & J. Vondrák 10315* (PRA); *Republic of Bashkortostan:* Ural Mts, Irendik Range, Sibay, vill. Gabelsha (c. 15 km W of Sibay), waterfall Gadelsha in upper stream of brook Khudolaz, alt. 500–800 m, 52.7572N, 58.3761E, on shaded base-rich siliceous stone in brook, 2011, *I. Frolov & J. Vondrák 10512* (PRA); *Republic of Tuva:* West Sayan Mts, Ak-Dovurak, Ak-Sug, Enge-Beldir, glacier cirque in S-slope from pass 'Sayanskiy pereval'; 2200 m, at road A161, close to border with Republic of Khakasia, alt. 2150–2200 m, 51.7000N, 89.8872E, on S-exposed schist outcrop, below overhang, in alpine zone, 2013, *I. Frolov & J. Vondrák 11099* (PRA); *Chelyabinsk Region:* Magnitogorsk, in steppe c. 10 km S of town, alt. c. 300 m, 53.2613N, 58.9263E, on limestone boulders in steppe, 2011, *O. Vondráková & J. Vondrák 9541* (PRA); *Krasnoyarsk Territory:* West Sayan Mts, Minusinsk, at road Minusinsk — Kyzyl, 2 km E of pass 'Buybinskiy pereval', E-exposed glacier cirque

with mica-schist bedrock, alt. 1550–1600 m, 52.8491N, 93.2808E, on vertical mica-schist rock-face in subalpine zone, 2013, *I. Frolov & J. Vondrák 12653, 12654, 12697* (PRA); *Murmansk Region:* Pechenga, Kandalakskiy Reserve, Bolshoy Aynov Island, alt. 20 m, 69.8355N, 31.5691E, on siliceous stone, 2010, *A. V. Melekhin* s.n. (KPABG, det. *I. Frolov*).

***Caloplaca subalpina* Vondrák et al.**

See Šoun et al. (2011) for nomenclatural details.

(Fig. 2A; distribution map)

*Caloplaca subalpina* was previously known from subalpine and alpine zones of the Alps, the Carpathians, the Pyrenees and the Sudetes (Vondrák et al. 2008), but according to our new data, its range extends much further eastwards, to the Western Sayan Mountains. No previous reports were corticolous, but one of our collections is from birch bark, where it is accompanied by two other generally saxicolous lichens, *Caloplaca arnoldii* and *Xanthoria sorediata*. ITS sequences of two Russian samples are placed in the *Caloplaca subalpina* clade (see Supplementary Material Figure S2, available online).

*Russian specimens.* **Russia:** *Republic of Bashkortostan:* Southern Urals Mts, Irendik Range, Sibay, vill. Gadelsha (c. 15 km W of Sibay), waterfall Gadelsha in upper stream of brook Khudolaz, alt. 500–800 m, 52.7572N, 58.3761E, on vertical face of base-rich schist, with *Leproplaca obliterans*, 2011, *I. Frolov & J. Vondrák 9397* (PRA); Southern Urals Mts, Shulgan-Tash Reserve, cliff above Kapova Cave at the banks of Belaya River, 53.0419N, 57.0672E, alt. 300 m, on bark of *Betula*, 2007, *G. Urbanavichus* (PRA); *Krasnoyarsk Territory:* West Sayan Mts, Minusinsk, at road Minusinsk – Kyzyl, 2 km E of pass 'Buybinskiy pereval', E-exposed glacier cirque with mica-schist bedrock, alt. 1550–1600 m, 52.8491N, 93.2808E, on vertical mica-schist rock-face in subalpine zone, 2013, *I. Frolov & J. Vondrák 12652, 12658, 12667, 12673* (PRA).

***Flavoplaca flavocitrina* (Nyl.) Arup et al.**

See Arup et al. (2013) for nomenclatural details.

**Syn. nov.** *Caloplaca cranfieldii* S. Y. Kondr. & Kärnefelt in Kondratyuk et al., *Bibl. Lichenol.* 95: 352 (2007); type: Western Australia, Northampton, Lynton, on sandstone, 2004, *Kärnefelt & Cranfield* (*Kondratyuk* 20423, PERTH—holotype;

GZU—isotype seen).—*Flavoplaca cranfieldii* (S. Y. Kondr. & Kärnefelt) Arup et al., *Nord. J. Bot.* 31: 45 (2013).

(Fig. 2B; distribution map)

*Caloplaca flavocitrina* (Nyl.) H. Olivier was synonymized with *C. citrina* by Laundon (1965) and this view was accepted by many, including Russian authors (e.g. Stepanchikova et al. 2014). However, some recent authors have regarded *C. flavocitrina* as distinct from other yellow sorediate crusts of *C. citrina* s. lat. (cf. Vondrák et al. 2007). ITS sequence data have confirmed that it is distinct (Arup 2006; Vondrák et al. 2009). It is now placed in the genus *Flavoplaca*, which includes both sorediate and non-sorediate crusts (Arup et al. 2013).

*Flavoplaca flavocitrina* s. lat. (including *F. geleverjae*) forms a well-supported clade (BS =1, Fig. 3), sister to a clade composed of *F. austrocitrina* and *F. limonia* that acts as outgroup. *Flavoplaca flavocitrina* differs from this outgroup in 13 nucleotide substitutions in our ITS alignment. *Flavoplaca citrina*, *F. confusa* and *F. nigromarina*, three morphologically similar taxa, are less closely related to *F. flavocitrina* in ITS. *Flavoplaca geleverjae* differs from *F. flavocitrina* in five nucleotide substitutions (two of them shared with the outgroup) and it may be a distinct species (Khodosovtsev et al. 2003; Vondrák et al. 2009). The sequence EU563389 (*F.* aff. *flavocitrina*, Bulgaria) is also included in the *Flavoplaca flavocitrina* s. lat. clade, but differs from *F. flavocitrina* in five substitutions (four of them shared with the outgroup). The corresponding specimen has *F. flavocitrina* morphology.

*Flavoplaca flavocitrina* is exceptional among taxa of this genus owing to its very broad ecological range. It can grow on mineral-rich siliceous and calcareous rocks, numerous artificial substrata (e.g. tarmac, concrete), dustimpregnated wood and on base-rich bark (e.g. *Acer platanoides*, *Ulmus glabra*). No other species of *Flavoplaca* is so indifferent to substratum, and very few species anywhere in Teloschistaceae are so indifferent. It may be almost cosmopolitan in the Northern Hemisphere, which is also exceptional in *Flavoplaca*: as well as numerous European and Mediterranean records, it is known from North America (Brodo et al. 2013), Hawaii (Vondrák et al. 2009) and Siberia (this paper).

*Flavoplaca flavocitrina* also occurs in the Southern Hemisphere (Australia), where it has been known as *Caloplaca cranfieldii* (Kondratyuk et al. 2007; ≡ *Flavoplaca cranfieldii*). The isotype of *C. cranfieldii* in GZU matches *F. flavocitrina* morphologically, and the ITS sequence from the type (published by Arup et al. 2013) falls into the *F. flavocitrina* clade in our phylogenetic reconstruction (Fig. 3). We consider *C. cranfieldii* to be a synonym of *Flavoplaca flavocitrina*.

There are several reports of *Flavoplaca flavocitrina* from European Russia (Vondrák et al. 2009; Muchnik et al. 2014; Himelbrant et al. 2015). We can now add records from two Siberian localities, from siliceous rocks in natural habitats. It is definitely the most widely distributed species of *Flavoplaca* in Russia; most others are restricted to the Black Sea coast, such as *F. arcisproxima*, *F. austrocitrina* and *F. communis* (Vondrák et al. 2009), or to European Russia, such as *F. dichroa* (e.g. Vondrák et al. 2010). Identification of *Flavoplaca flavocitrina* should be confirmed by molecular barcoding (ITS sequences), because some taxa, including *F. citrina* (not confirmed from Russia), are very similar.

*Russian specimens.* **Russia:** *Republic of Altai:* Altai Mts, Turochak district, Artibash, about 5 km NW of village, SW-exposed gneiss rocks above right bank of Biya River, alt. 450 m, on vertical face of siliceous rock, 2012, *I. Frolov & J. Vondrák 12679* (PRA); *Oryol Region:* Krasnaya Zarya, Khomutovo, alt. 180 m, 52.8406N, 37.5663E, on limestone, 2014, *Muchnik* (PRA); *Zabaikalsky Territory:* Kodar Ridge, alt. 940 m, 56.9196N, 118.0291E; Ibid.: alt. 1590 m, 56.9194N, 118.0011E, on siliceous rock, 2013, *L. Konoreva* (LE, herb. S. Chesnokov).

***Gyalolechia epiphyta* (Lynge) Vondrák comb. nov.**

MycoBank No.: MB 815509

*Caloplaca epiphyta* Lynge, *Skrifter om Svalbard og Ishavet* 81: 119 (1940); type: [Greenland], Østgrønland, Jackson, Ø, 1929, *Lynge* (O-L-1279—holotype, seen in http://nhm2.uio.no/lav/web/index.html).

**Syn. nov.** *Caloplaca arizonica* H. Magn., *Bot. Not.* 1944: 69–70 (1944); type: USA, Arizona, Grand Canyon NP, Coconino Plateau, on *Juniperus monosperma*, 1926, *E. & G. DuRietz* 182/1 (UPS—holotype, not seen).— *Gyalolechia arizonica* (H. Magn.) Søchting et al. in Arup et al. (2013: 70).

**Syn. nov.** *Caloplaca juniperi* Poelt & Hinteregger, *Bibl. Lichenol.* 50: 150–152 (1993); type: Pakistan, Karakorum Mountains, Gilgit, Rakaposhi Range, Baghrot, N-facing flank opposite Sat, alt. 2600–2700 m (36°03'N, 74°35'E), on old Juniperus, 1991, *J. Poelt* (GZU— holotype, seen).

**Syn. nov.** *Caloplaca juniperina* Tomin, *Bot. Materialy, Notulae System. e Sect. Cryptog. Inst. Bot. Nomine V. L. Komarovii Acad. Sci. URSS* 9: 11–12 (1953); syntypes—Uzbekistan (Uzbekskaya SSR), northern slopes of Alay ridge, 1) Dzhaylayau Shayd, 26 vii 1948; 2) Dzhaylayau Mashelan', 10 vii 1950; 3) ibid., 15 vi 1951; all syntypes collected by *F. Shafeev* (syntype 2 in LE seen and selected here as

lectotype).—*Gyalolechia juniperina* (Tomin) Søchting et al. in Arup et al., *Nord. J. Bot.* 31: 71 (2013).

**Syn. nov.** *Caloplaca laricina* Rondon, *Rev. Bryol. Lich.* 32: 260 (1963); type: France, Hautes-Alpes, Ville-Vieille en Queyras, alt. 1400 m, [44,4136°N, 6,2498°E], on wood of *Larix decidua*, 1957, *Y. Rondon* (G00288634—type not seen).

**Syn. nov.** *Caloplaca tarani* S. Y. Kondr. et al. in Kondratyuk et al., *Acta Bot. Hungarica* 55: 48–52 (2013); type: Russia, Sakhalin Island, Smirnykhovsky district, at the base of Mt Pogranichnaya, mixed deciduous and coniferous forest, on bark of *Ulmus laciniata*. 30.05.1997, *A. A. Taran* (SAKH—holotype, Fig. 4 in Kondratyuk et al. 2013).

(Fig. 2C, distribution map; Fig. 5B)

*Gyalolechia epiphyta* is diagnosed by its blastidiate/granulose thallus and absence of true soralia (Fig. 5B), but it is quite similar to the sorediate taxa *Gyalolechia persimilis*, *G. ussuriensis* and *G. xanthostigmoidea*. *Gyalolechia epiphyta* forms a supported clade in the ITS tree (Fig. 4). Variability among 12 sequences included in the ITS tree was detected in 19 positions, but this variability is rather randomly distributed; each sequence pair within the clade is more than 98,5% identical. The exception is KC179447 (from Greenland) which contains an indel of 21 bp length that is absent in other *Gyalolechia* species. The closest relative is *G. flavorubescens* s. lat. (including *G. xanthostigmoidea* and *"Caloplaca" subflavorubescens*) which forms a supported ITS clade with considerable internal variability (Fig. 4).

*Gyalolechia epiphyta* is widely distributed in the Arctic and temperate zones of the Northern Hemisphere. In continental regions it prefers steppes and dry forests. It is usually epiphytic or epixylic (often on *Juniperus*), but also epigeic or epibryic in rock crevices in arctic-alpine habitats or in steppes. Its epilithic occurrences are common in the Arctic. It is variable in thallus morphology; in particular, the size of vegetative diaspores (blastidia, granules) varies considerably, often within a single thallus. When it grows on bark, it is commonly fertile, but specimens from soil or bryophytes are usually sterile.

The wide geographical range of *G. epiphyta* and its occurrence in different climatic zones and on different substrata has resulted in it being described as new several times under different names. We consider *Gyalolechia arizonica* synonymous with *G. epiphyta*. We have not seen its type specimen, but the ITS sequence of the specimen "*T.H. Nash* 38931 (C)" is placed within the *G. epiphyta* clade. We have also appraised several specimens of *G. arizonica* from Arizona (*T. H. Nash* 16456 in PRA-V, *T. H. Nash* 21219 in PRA-V, *O. Breuss* 27.7.1991 in W) and morphologically they fit

collections of *G. epiphyta* with coarse granules. They were collected from *Juniperus*, a typical substratum for Asian populations of *G. epiphyta*.

We have seen type specimens of *Caloplaca juniperi* from northern Himalaya and *Gyalolechia juniperina* from Central Asia and we consider them conspecific with *G. epiphyta*. Rondon (1963) described *Caloplaca laricina* from the Alps; although we did not locate its type, we appraised the specimen collected by Rondon in 1963 from *Larix* wood in Basses-Alpes, Méolans (A. Vězda: Lich. Sel. Exs. 250 in PRA-V) and it has *G. epiphyta* morphology. The photograph showing thallus morphology in the description by Rondon (1963) also represents *G. epiphyta*. The protologue of *Calplaca tarani* with a photograph of the type (Kondratyuk et al. 2013) indicates that this taxon described from the Far East is also *G. epiphyta*. We have assessed specimens collected from Kamchatka in the Far East (in the list below) that have *G. epiphyta* morphology but, unfortunately, repeated attempts to sequence these specimens were unsuccessful.

Despite *Gyalolechia epiphyta* having been described from many parts of the world under different names, we disagree with the synonymization of *G. xanthostigmoidea* (Räsänen) Søchting et al. in Arup et al. (2013: 72) with *G. epiphyta* proposed by Søchting & Tønsberg (1997). *Gyalolechia xanthostigmoidea*, described from New Brunswick in Canada (Räsänen 1933), is probably a distinct taxon more similar to *G. persimilis*/*G. ussuriensis*, because it forms soralia (Fig. 5F) and its ITS sequence (see Table 1 for specimen details) does not place it in the G. epiphyta clade (Fig. 4). Arctic-alpine, blastidiate specimens belong to *G. epiphyta*, as supported by the ITS sequence KC179447 from the Greenland specimen (Fig. 4), called "*G. xanthostigmoidea*" by Arup et al. (2013).

*Russian specimens.* **Russia:** *Republic of Altai:* Altai Mts, Kosh-Agach district, Kuray Steppe, limestone hills about 4 km W of Kuray, alt. 1470–1680 m, on wood of *Juniperus sabina*, 2012, *I. Frolov & J. Vondrák 12710* (PRA); ibid., on mosses in limestone crevices, *J. Vondrák* 10319 (PRA); Kosh-Agach district, SE part of Kuray Ridge, NE of village Chagan-Uzun, alt. 3000-3100 m, over mosses on limestone outcrop in alpine zone, 2012, *I. Frolov & J. Vondrák 10353* (PRA). *Kamchatka Krai:* Ust'-Bol'sheretsk district, Praviy Kihchik River basin, alt. 250m, 53·558224°N, 156·738025°E, on *Lonicera caerulea*, 2004, *D. Himelbrant* s. n. (PRA, ex LECB); ibid., alt. 220 m, 53·581380°N, 156·683090°E, on *Populus suaveolens*, 2004, *D. Himelbrant* s. n. (PRA, ex LECB); ibid., alt. 250 m, 53·548477°N, 156·697123°E, on *Populus suaveolens*, 2004, *D. Himelbrant* s. n. (PRA, ex LECB).

***Gyalolechia ussuriensis* (Oxner, S. Y. Kondr. & Elix) Vondrák comb. nov.**

MycoBank No.: MB 814537

*Caloplaca ussuriensis* Oxner et al. in Kondratyuk et al., *Folia Cryptogamica Estonica* 48: 21–23 (2011); type: Russia, Primorsky Krai, in the vicinity of Okeanicheskaya [= Okeanskaya] railway station, on *Acer pseudosieboldianum*, 1927, *A. Oxner* (LE—isotype seen).

(Fig. 2C, distribution map; Fig. 5D)

*Gyalolechia ussuriensis* is a humid-temperate to boreal taxon described from the Far East (Kondratyuk et al. 2011). Although it is paraphyletic in our ITS tree with *G. persimilis* (Fig. 4), we consider these taxa to be distinct because *G. persimilis* is known from quite different conditions in dry, temperate regions of western North America (Wetmore 2004) (see Fig. 2C). ITS sequences of *G. ussuriensis* also differ from those of *G. persimilis* in 15 nucleotide positions. The sequence of the Alaskan *G.* aff. *ussuriensis* (KT804988 in Fig. 4) is short, without the ITS2 region. It has affinities with both *G. persimilis* and *G. ussuriensis*, but it also has unique nucleotides in seven positions. This specimen (KT80498) may represent another taxon because it has a more reduced thallus than either *G. persimilis* or *G. ussuriensis* (compare Fig. 5C, *G. persimilis* and D, *G. ussuriensis* with E, *G.* aff. *ussuriensis*), and it has a rather specific ecology, growing on the bark of *Cupressus nootkatensis* in places not favourable for other lichens. (Note that all published specimens of *G. persimilis*/*G. ussuriensis* have been collected from broadleaved trees.) *Gyalolechia xanthostigmoidea* (Fig. 5F) is morphologically very similar to both *G. persimilis* and *G. ussuriensis*, but it is geographically distinct (Fig. 2C) and its ITS sequence KT804992 is not related to either (Fig. 4).

*Gyalolechia ussuriensis* was known only from a small territory in the Russian Far East (Kondratyuk et al. 2011), but our records from the Salair Range, Sayan Mountains and Kamchatka suggest a much broader range in humid taiga forests in Siberia.

*Russian specimens.* **Russia:** ***Altai Territory:*** Zalesovsky district, Salair Range, headwaters of Berd' River at 20 km NE from the Kordon settlement, in *Abies sibirica* - *Populus tremula* forest, alt. 430 m, 54.4166N, 85.1166E, on *Populus tremula*, 2012, *E. Davydov 11220* (ALTB); ***Kamchatka Territory:*** Mil'kovo district, Nature Reserve, S of Nikolka volcano, alt. 270 m, 55.0958N, 159.9950E; *Ibid.:* 55.1013N, 159.9894E, on *Populus suaveolens*, 2009, *D. Himelbrant & I. Stepanchikova* (LECB); ***Krasnoyarsk Trritory:*** West Sayan Mts, Minusinsk, Shushenskoe, 10 km SE of village Tanzibey, forest in valley of Bolshoy Kebezh River, alt. 440 m, 53.0830N, 93.0944E, 2013, *I. Frolov & J. Vondrák 13417* (PRA); ***Primorye Territory:*** Terney district, Northern Sikhote-Alin', 30 km WNW of Amgu settlement, alt. 570 m, 45.8963N, 137.3130E, on bark, 2014, *L. Yakovchenko & E. Davydov 11500* (ALTB).

**Discussion**

Work by the first author in and around the Mediterranean (Vondrák et al. 2009, 2012b) has previously suggested that many species of Teloschistaceae, for example those in *Flavoplaca* or the *Caloplaca xerica* group, have a narrow range. However, our recent data from Russia shows quite the opposite. Some species previously known only from Europe (e.g. *Caloplaca subalpina*) occur as far east as the Sayan Mountains in South Siberia. *Caloplaca ussuriensis*, formerly thought to be restricted to the Far East, occurs from Kamchatka to the Altai Mountains in South Siberia. Other species (*Calogaya bryochrysion* and *Caloplaca isidiigera*) are almost circumpolar, and *Flavoplaca flavocitrina* may be almost cosmopolitan.

Our earlier conclusion about narrow ranges is therefore not applicable to Teloschistaceae as a whole. It was biased by the particular characteristics of the Mediterranean region, where a combination of history, climate and geography has indeed resulted in a high degree of endemism (Blondel & Aronson 1999). In contrast, our more recent data support the fact that numerous species known from Europe or North America have been merely unrecognized in North Asia (Davydov & Printzen 2012).

Within species pairs (sensu Poelt 1970), lineages which reproduce vegetatively often have larger geographical ranges than their strictly sexual counterparts. Such contrasts in distribution can be found in, for example, *Hypogymnia* (Miądlikowska et al. 2011), *Letharia* (Kroken & Taylor 2001), and *Ramalina* (Rundel & Bowler 1976). In phylogenies of many genera within Teloschistaceae, lineages producing vegetative diaspores randomly alternate with strictly sexual lineages, that is, those with apothecia (and with or without pycnidia). This pattern was also observed, for example, by Buschbom & Mueller (2006) in a section of *Porpidia*. Species that display only vegetative distribution are very few (e.g. *Leproplaca* spp.), but most Teloschistaceae that reproduce vegetatively produce both apothecia and vegetative diaspores (Table 3), although apothecia are not common in some cases. The ability to produce both sexual and vegetative diaspores combines all the advantages of evolutionary plasticity with the ability to retain favourable allele combinations (e.g. Williams 1975; Maynard Smith 1978). Vondrák et al. (2013, pages 710–711) reported some examples where species with vegetative diaspores have wider geographical ranges than their strictly sexual relatives and here we provide additional evidence. Six of the eight species discussed reproduce both sexually (via ascospores) and asexually (by soredia/blastidia/isidia and also by conidia) and have wider ranges than their strictly sexual relatives. These are as follows:

1) The continental and arctic-alpine *Calogaya bryochrysion* is related to a clade containing strictly sexual *C. biatorina*, *C. ferrugineoides* and *C. polycarpoides* (Fig. 1) that are widely distributed in Central Asia, but they are

absent from arctic and alpine habitats. Another related sexual species, *C. pusilla*, is probably restricted to western Eurasia: our easternmost records are from Turkey (unpublished data).

2) Within the genus *Caloplaca*, three of ten species with vegetative diaspores are distributed in Eurasia and also in North America. Strictly sexual species, 15 lineages of *C. cerina* s. lat. and *C. stillicidiorum* s. lat. in Šoun et al. (2011), are usually known from rather small territories, with the exception of the lineage "*stillicidiorum* (5)".

3) Sexual species closely related to *Flavoplaca flavocitrina* are *F. havaasii*, *F. marina*, *F. maritima* and *F. ora* (Arup et al. 2013). All these have rather restricted geographical ranges.

4) *Gyalolechia epiphyta* is related to sexual *G. flavorubescens* s. lat. (Fig. 4), an entity that has a wide range, but which probably consists of several geographically more restricted taxa. *Gyalolechia ussuriensis* is related to the sexual *G. flavovirescens* known from western Eurasia, Greenland and North America, but its wide range has not been tested by molecular data, and so more species may exist within *G. flavovirescens*.

Evidence is accumulating that various Teloschistaceae species have wide geographical ranges. Many of them are characterized by dual reproductive modes (producing sexual and asexual diaspores), but a few species without vegetative diaspores may also have broad ranges. The influence of reproductive mode on the fitness, competitive success and geographical range of lichens seems a promising area for research. The evolutionary grounds for switches between reproductive modes are also a related and promising topic for future study.

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**Supplementary material**

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0024282916000116

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Table 1. New ITS nrDNA sequences for Teloschistaceae used in this study together with locations, substrata and herbarium (hb) information.

| Taxon | Sample / hb. acronym | Locality / altitude (m) / substratum | Latitude(°) | Longitude(°) | GenBank accession numbers |
|---|---|---|---|---|---|
| *Athallia alnetorum* | JV8316 / PRA | Greece, Peloponnese / 300 / *Pistacia lentiscus* | 37.55 | 23.25 | KT804927 |
| *A. alnetorum* | IU (UT-014) / PRA | Russia, Krasnodar Region, Utrish Reserve / 430 / *Quercus* twigs | 44.7212 | 37.4684 | KT804928 |
| *Calogaya arnoldii* | JV12552 / PRA | Russia, Yekaterinburg, Rezh / 180 / calcareous schist | 57.4858 | 61.4941 | KT804929 |
| *C. arnoldii* | ED6934 / ALTB | Russia, Altai Mts, Soloneshnoye / 1035 / calcareous schist | 51.3545 | 84.5676 | KT804930 |
| *C. arnoldii* | SC205 / LE | Russia, Trans-Baikal region, Kodar Ridge / 940 / base-rich rock | 56.9196 | 118.0291 | KT804931 |
| *C. arnoldii* | GU (s.n.) / PRA | Russia, Adygea, Caucasus Reserve / 2000 / limestone | 44.0164 | 39.9789 | KT804932 |
| *C. arnoldii* | GU (L-022) / PRA | Russia, Bashkortostan, Shulgan-Tash Reserve / 300 / *Betula* | 53.0419 | 57.0672 | KT804933 |
| *C. biatorina* | JV10514 / PRA | Czech R., Praha, Radotín / 300 / limestone | 49.989498 | 14.334760 | KT804934 |
| *C. bryochrysion* | JV10372 / PRA | Russia, Altai Mts, Kosh-Agach / 2000 / *Populus laurifolia* | 50.052 | 88.709 | KT804935 |
| *C. bryochrysion* | ED11498 / ALTB | Russia, Altai Mts, Kosh-Agach / 2200 / *Salix pentandra* | 49.7969 | 89.3619 | KT804936 |
| *C. bryochrysion* | ED11499 / ALTB | Russia, Altai Mts, Kuray, Kuraika basin / 1670 / *Populus laurifolia* | 50.2669 | 87.9513 | KT804937 |
| *C. bryochrysion* | IZ (s.n.) / PRA | Russia, Novaya Zemlya / 0-5 / lime-rich pebbles | 76.999722 | 67.780000 | KT804938 |
| *C. bryochrysion* | JV9529 / PRA | Svalbard / coastal / calcareous cliff | 78.38 | 16.49 | KT804939 |
| *C. bryochrysion* | JV7262 / PRA | Austria, Schladming / 2750 / bryophytes on limestone | 47.469151 | 13.625209 | KT804940 |
| *C. bryochrysion* | JV11086 / PRA | Russia, W Sayan Mts / 2150 / base-rich schist | 51.7000 | 89.8872 | KT804941 |

| | | | | | |
|---|---|---|---|---|---|
| *C.* aff. *ferrugineoides* | JV12708 / PRA | Russia, Altai Mts, Kosh-Agach / 1550 / wood of *Juniperus* | 50.240337 | 87.876771 | KT804942 |
| *C. ferrugineoides* | JV8534 / PRA | Turkey, Tuzluca / 1300 / shrubs | 40.174084 | 43.674208 | KT804943 |
| *C. ferrugineoides* | ED11221 / ALTB | China, Xinjiang, Dzhungar basin / 940 / shrubs | 46.605768 | 89.585706 | KT804944 |
| *C. persica* | JV8515 / PRA | Turkey, Lake Van / 1750 / *Juniperus* | 38.466427 | 42.502325 | KT804945 |
| *C. polycarpoides* | JV5541 / PRA | NW Iran, Khalkhal / 1680 / *Populus* | 37.679664 | 48.491388 | KT804946 |
| *C. saxicola*s.lat. | JV12558 / PRA | Russia, W Sayan Mts / 2150 / base-rich schist | 51.700754 | 89.885716 | KT804947 |
| *Calogaya* sp. | JV12707 / PRA | Russia, Altai Mts, Kosh-Agach / 2700 / limestone | 50.145324 | 88.465936 | KT804948 |
| *Caloplaca conversa* s.lat. | JV10289 / PRA | Russia, Altai Mts, Chemal / 500 / volcanic rock | 51.632759 | 85.782572 | KT804949 |
| *C. conversa* s.lat. | JV10265 / PRA | Russia, Altai Mts, Ulagan / 500 / gneiss | 50.92 | 88.19 | KT804950 |
| *C. conversa* s.str. | JV744 / PRA | Bulgaria, Madzharovo / c 400 / siliceous rock | 41.66 | 25.66 | KT804951 |
| *C. conversa* s.str. | IF1048 / hb. Frolov | Russia, Bashkirtostan, Sargaya / 700 / xerothermic serpentinite | 53.35 | 57.74 | KT804952 |
| *C. conversa* s.str. | JV5538 | NW Iran, Talesh / 1640 / siliceous stone | 37.623664 | 48.800237 | KT804953 |
| *C. conversa* s.str. | JV6461 | Turkey, Artvin / 550 / siliceous rock | 41.189884 | 41.857240 | KT804954 |
| *C. egeana* | JV6262 / PRA | Great Britain, Gibraltar rock / c 100 / limestone | 36.145139 | -5.345071 | KT804955 |
| *C. isidiigera* | GU (L-017) / PRA | Russia, Adygea, Caucasus Reserve / 2030 / limestone | 44.0304 | 40.0251 | KT804956 |
| *C. stillicidiorum* s.lat. | JV11104 / PRA | Russia, Rep. of Tuva, Ak-Sug / 1490 / siliceous stone in river | 51.618879 | 90.076022 | KT804957 |
| *C. subalpina* | GU, L-022 / PRA | Russia, Rep. of Bashkortostan, Shulgan-Tash Reserve / 300 / *Betula* | 53.0419 | 57.0672 | KT804958 |
| *C. subalpina* | JV9397 / PRA | Russia, Rep. of Bashkirtostan, Sibay / 700 / schist | 52.7572 | 58.3761 | KT804959 |
| *C. subflavorubescens* | Joshi (s.n.) / PRA | South Korea (coll. Y. Joshi) / ? / bark | ? | ? | KT804960 |

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|---|---|---|---|---|---|
| *Flavoplaca aff. austrocitrina* | JV8603 / PRA | Greece, Peloponnese, Methana / 240 / volcanic rock | 37.614744 | 23.333514 | JN813411 |
| *F. austrocitrina* | JV8712 / PRA | Greece, Nafpaktos, Monastiraki / 10 / limestone | 38.400524 | 21.931378 | JN813423 |
| *F. flavocitrina* | JV8605 / PRA | Greece, Nafpaktos, Monastiraki / 10 / limestone | 38.400524 | 21.931378 | JN813420 |
| *F. flavocitrina* | JV12679 / PRA | Russia, Rep. of Altai, Artibash / 620 / siliceous rock | 51.813403 | 87.192289 | KT804961 |
| *F. flavocitrina* | SC197 / LE | Russia, Trans-Baikal region, Kodar Ridge / 940 / base-rich rock | 56.9196 | 118.0291 | KT804962 |
| *F. flavocitrina* | SC244 / LE | Russia, Trans-Baikal region, Kodar Ridge / 1590 / siliceous rock | 56.9194 | 118.0011 | KT804963 |
| *F. flavocitrina* | SC246 / LE | Russia, Trans-Baikal region, Kodar Ridge / 1590 / siliceous rock | 56.9194 | 118.0011 | KT804964 |
| *F. flavocitrina* | JV10226 / PRA | Slovakia, Revúca, Muráň / 700 / *Quercus* | 48.770250 | 20.079807 | KT804965 |
| *F. flavocitrina* | Muchnik (s.n.) / PRA | Russia, Oryol Region, Krasnaya Zarya / 180 / limestone | 52.8406 | 37.5663 | KT804966 |
| *F. flavocitrina* | JV9178 / PRA | Slovakia, Revúca, Muráň / 700 / limestone | 48.770250 | 20.079807 | KT804967 |
| *F. geleverjae* | JV8887 / PRA | Greece, Nafpaktos, Monastiraki / 10 / limestone | 38.400524 | 21.931378 | JN813406 |
| *Gyalolechia* aff. *ussuriensis* | TS38925 / GZU | USA, Alaska, Glacier Bay NP / 70 / *Cupressus nootkatensis* | 58.35637 | –136.38144 | KT804988 |
| *G. allochroa* | TS39368 / GZU | USA, Alaska, Glacier Bay NP / 810 / vertical siliceous rock | 58.46046 | –135.56179 | KT804968 |
| *G. epiphyta* | JV5696 / PRA | NW Iran, Lake Urmia / 1280 / soil bryophytes | 37.883040 | 45.571059 | KT804973 |
| *G. epiphyta* | JV5585 / PRA | NW Iran, Lake Urmia / 1370 / soil bryophytes | 37.787453 | 45.454082 | KT804974 |
| *G. epiphyta* | JV12710 / PRA | Russia, Altai Mts, Kosh-Agach / 1550 / *Juniperus sabina* | 50.240337 | 87.876771 | KT804975 |
| *G. epiphyta* | JV5582 / PRA | NW Iran, Lake Urmia / 1280 / soil bryophytes | 37.883040 | 45.571059 | KT804976 |
| *G. epiphyta* | JV12411 / PRA | China, Xinjiang, Tianshan Grand | 43.326478 | 87.362703 | KU360123 |

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|---|---|---|---|---|---|
| *G. epiphyta* | JV12412 / PRA | Canyon / 2400 / *Picea schrenkiana* wood China, Xinjiang, Tianshan Grand Canyon / 2400 / *Picea schrenkiana* bark | 43.326478 | 87.362703 | KU360122 |
| *G. epiphyta* | JV13626 / PRA | Iran (coll. V. Tahereh) / ? / soil bryophytes | ? | ? | KT804977 |
| *G. flavorubescens* | JV5575 / PRA | NW Iran, Talesh / 1640 / *Fagus orientalis* | 37.623664 | 48.800237 | KT804969 |
| *G. flavorubescens* | JV418 / PRA | Italy, Sicily, NP Nebrodi / c 1000 / *Quercus* | 37.92 | 14.67 | KT804970 |
| *G. flavorubescens* | JV5599 / PRA | NW Iran, Talesh / 170 / *Swida* | 37.705114 | 48.887425 | KT804980 |
| *G. flavorubescens* | JV5691/1 / PRA | NW Iran, Talesh / 1150 / *Acer* | 37.656739 | 48.819694 | KT804981 |
| *G. flavorubescens* | JV5691/2 / PRA | NW Iran, Talesh / 1150 / *Acer* | 37.656739 | 48.819694 | KU360124 |
| *G. flavorubescens* | JV5700/1 / PRA | NW Iran, Talesh / 500 / *Fagus orientalis* | 37.681581 | 48.819696 | KT804982 |
| *G. flavorubescens* | JV5700/2 / PRA | NW Iran, Talesh / 500 / *Fagus orientalis* | 37.681581 | 48.819696 | KT804983 |
| *G. flavorubescens* | JV5718 / PRA | NW Iran, Talesh / 500 / *Cerasus* | 37.681581 | 48.819696 | KT804984 |
| *G. flavorubescens* | JV5723 / PRA | NW Iran, Talesh / 40 / *Cerasus* | 37.717774 | 48.960760 | KT804985 |
| *G. flavorubescens* | JV5738 / PRA | NW Iran, Talesh / 1150 / *Acer* | 37.656739 | 48.819694 | KT804986 |
| *G. flavorubescens* | JV5844 / PRA | NW Iran, Talesh / 500 / *Acer* | 37.681581 | 48.819696 | KT804987 |
| *G. flavorubescens* | JV14390 / PRA | Russia, Caucasus, Kavkazskii Zapovednik / 1600 / *Populus tremula* | 44.068597 | 40.001542 | KU360121 |
| *G. flavovirescens* | JV5537 / PRA | NW Iran, Namin / 1350 / base-rich siliceous rock | 38.426759 | 48.581384 | KT804971 |
| *G. flavovirescens* | JV5615 / PRA | NW Iran, Khalkhal / 1900 / base-rich siliceous rock | 37.611880 | 48.740579 | KT804972 |
| *G. persimilis* | JV7486 / PRA | USA, Davis, Winters / 900 / *Quercus* | 38.500055 | – 122.118556 | KT804978 |
| *Gyalolechia persimilis* | GZU, Wetm.: Tel. Exs. 33 / GZU | Mexico, Baja California / 1700 / *Quercus tuberculata* | 23.601700 | – 109.931902 | KT804979 |
| *G. ussuriensis* | JV13417 / PRA | Russia, Krasnoyarsk Region, Minusinsk / 440 / *Salix* | 53.0830 | 93.0944 | KT804989 |

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|---|---|---|---|---|---|
| *G. ussuriensis* | ED11500 / ALTB | Russia, Primorsky krai Area, Terney / 570 / *Populus* | 45.8963 | 137.3130 | KT804991 |
| *G. ussuriensis* | ED11220 / ALTB | Russia, Altai Mts, Zalesovsk / 430 / *Populus tremula* | 54.4166 | 85.1166 | KT804990 |
| *G. xanthostigmoidea* | TS32410 / GZU | Canada, Québec, Côte-Nord, Lac Gobeil / 110 / *Thuja occidentalis* | 48.232099 | –69.658427 | KT804992 |

Table 2. Alignment of the 69 sequences from this study for five genera of Teloschistaceae.

| Alignment | All sequences / new sequences | Outgroup | Alignment length | Variable positions / variable in ingroup |
|---|---|---|---|---|
| part of *Athallia* (Supplementary Material Fig. S1) | 17/2 | *Athallia pyracea* | 530 | 60/40 |
| *Calogaya* (Fig. 1) | 54/19 | *Rusavskia* | 544 | 214/200 |
| *Caloplaca* (Supplementary Material Fig. S2) | 52/12 | *Rufoplaca* and *Caloplaca conversa* | 551 | 292/133 |
| part of *Flavoplaca* (Fig. 3) | 36/13 | *Flavoplaca limonia* and *F. austrocitrina* | 596 | 67/48 |
| *Gyalolechia* (Fig. 4) | 57/28 | *Blastenia* | 560* | 289/273 |

* without 21 BP insertion in one sequence

Table 3. Modes of reproduction of species in large Teloschistaceae genera with distribution centres in northern Eurasia.

| Genus | Apothecia; no vegetative diaspores | Vegetative diaspores and apothecia | Source |
|---|---|---|---|
| *Athallia* | 12 | 1 | Vondrák (unpublished) |
| *Blastenia* | 14 | 8 | Vondrák (unpublished) |
| *Calogaya* | 10 | 2 | Gaya *et al.* 2011, Arup *et al.* 2013 |
| *Caloplaca* | 7 | 10 | Šoun *et al.* 2011 |
| *Flavoplaca* | 10 | 15 | Vondrák *et al.* 2009, Arup *et al.* 2013 |
| *Xanthocarpia* | 14 | 2 | Vondrák *et al.* 2011 |

Note: species dispersed solely by vegetative diaspores are not known in these genera. Those producing both apothecia and vegetative diaspores (third column) can be without apothecia locally, but samples with apothecia are not exceptional. Large genera without modern taxonomic revision are not treated (e.g. *Pyrenodesmia* and *Variospora*)

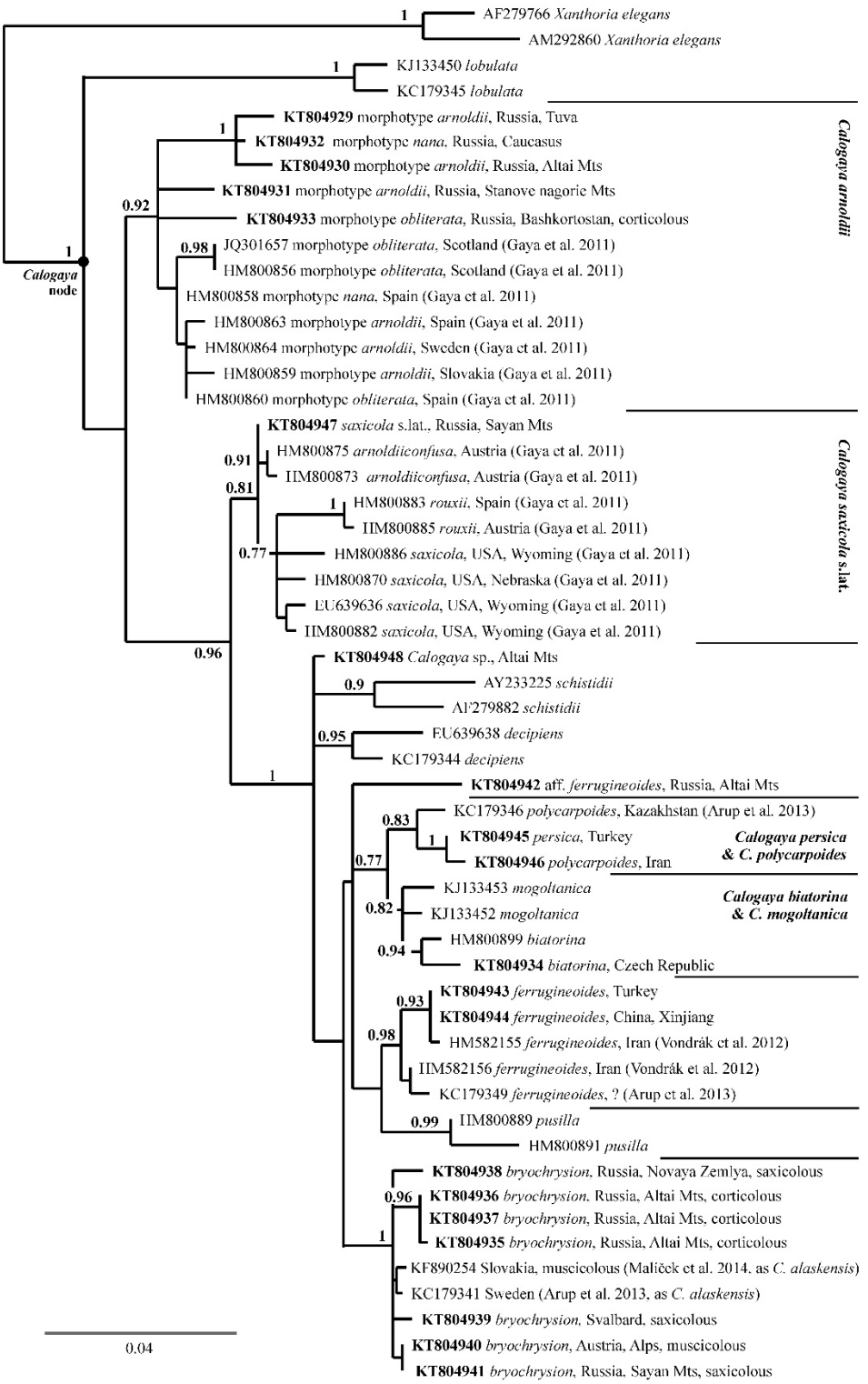


Fig. 1. Maximum likelihood ITS phylogeny of *Calogaya* showing positions of *C. arnoldii*, *C. bryochrysion* and *C. ferrugineoides*. New sequences are in bold; bootstrap supports (BS□0·7) are shown at nodes.

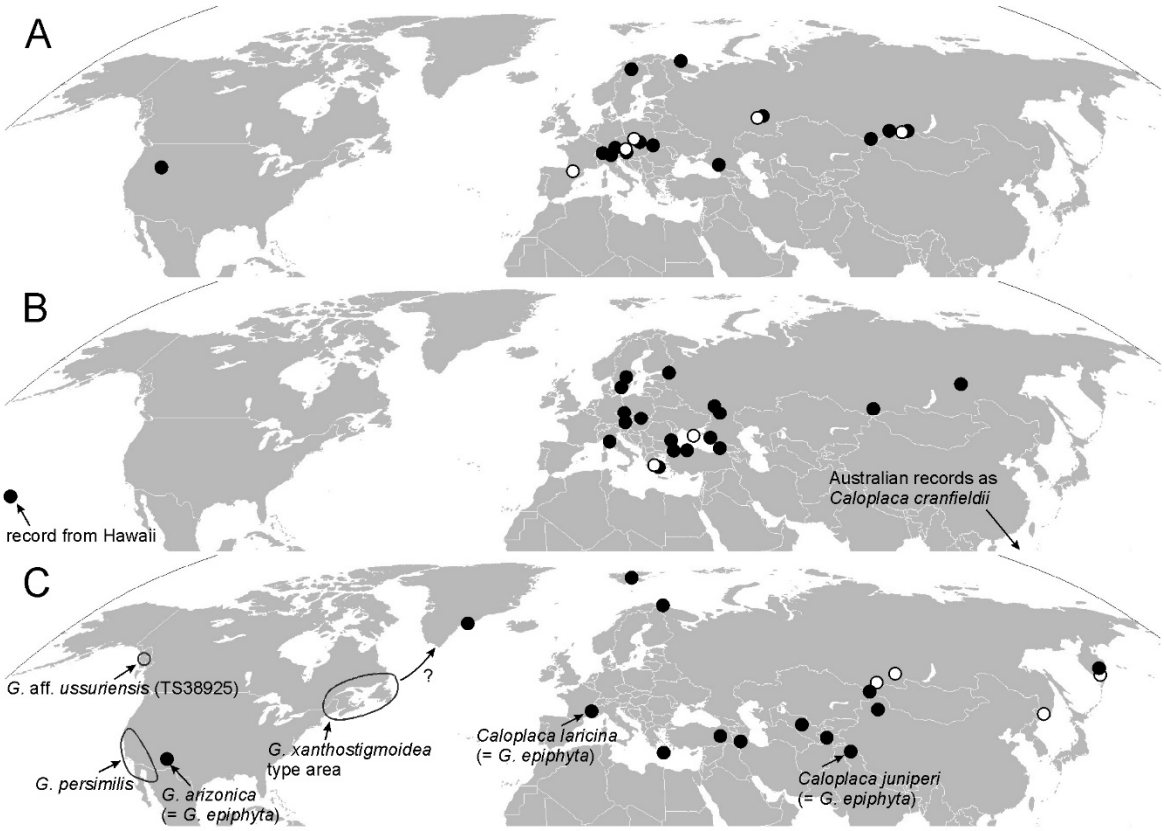


Fig. 2. Locations of specimens sequenced and confirmed in this study. A, *Caloplaca isidiigera* (black dots) and *C. subalpina* (white dots); B, *Flavoplaca flavocitrina* (black dots) and the closely related *F. geleverjae* (white dots); C, *Gyalolechia epiphyta* (black dots) and *G. ussuriensis* (white dots), with approximate ranges of other sorediate *Gyalolechia* (outlined).

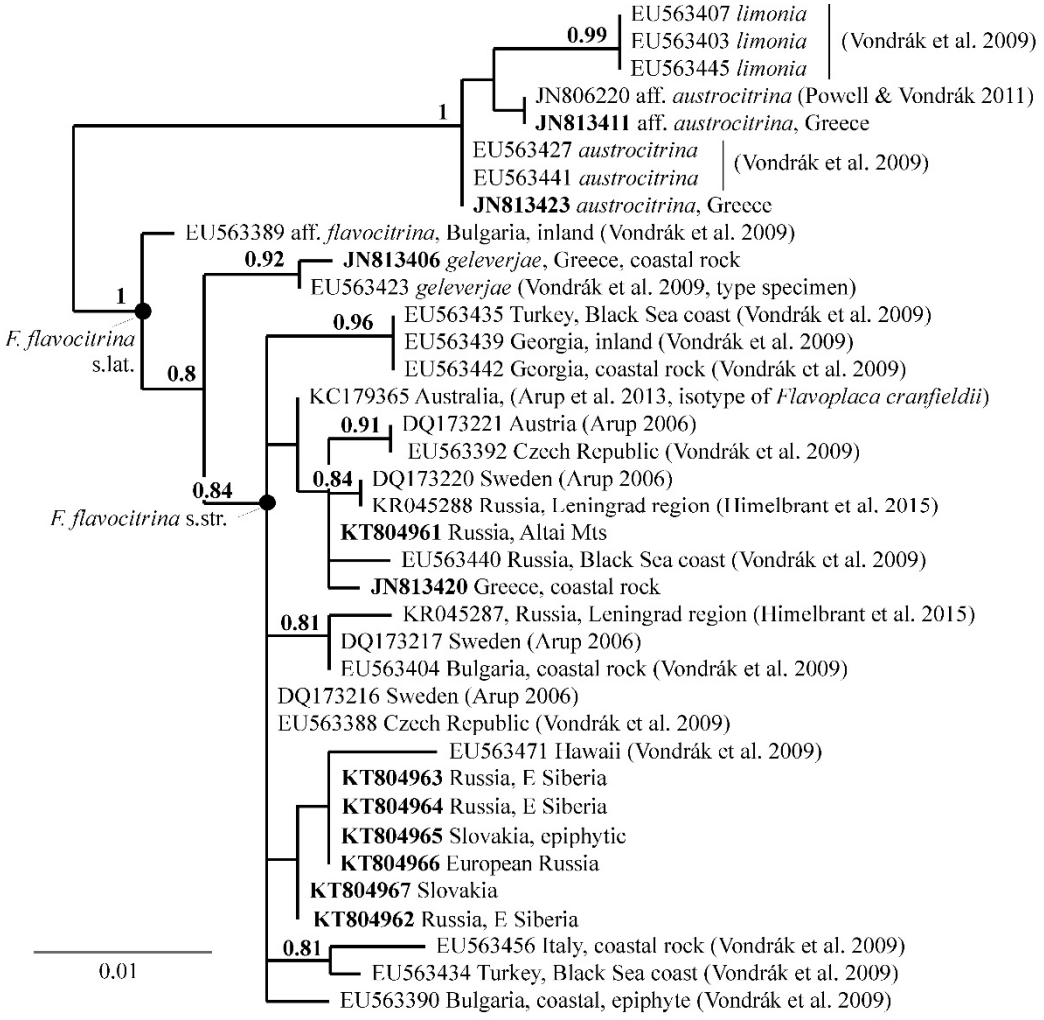


Fig. 3. Maximum likelihood ITS phylogeny of a section within *Flavoplaca* including *F. flavocitrina*. New sequences are in bold; bootstrap supports (BS ≥0,7) are shown at nodes.

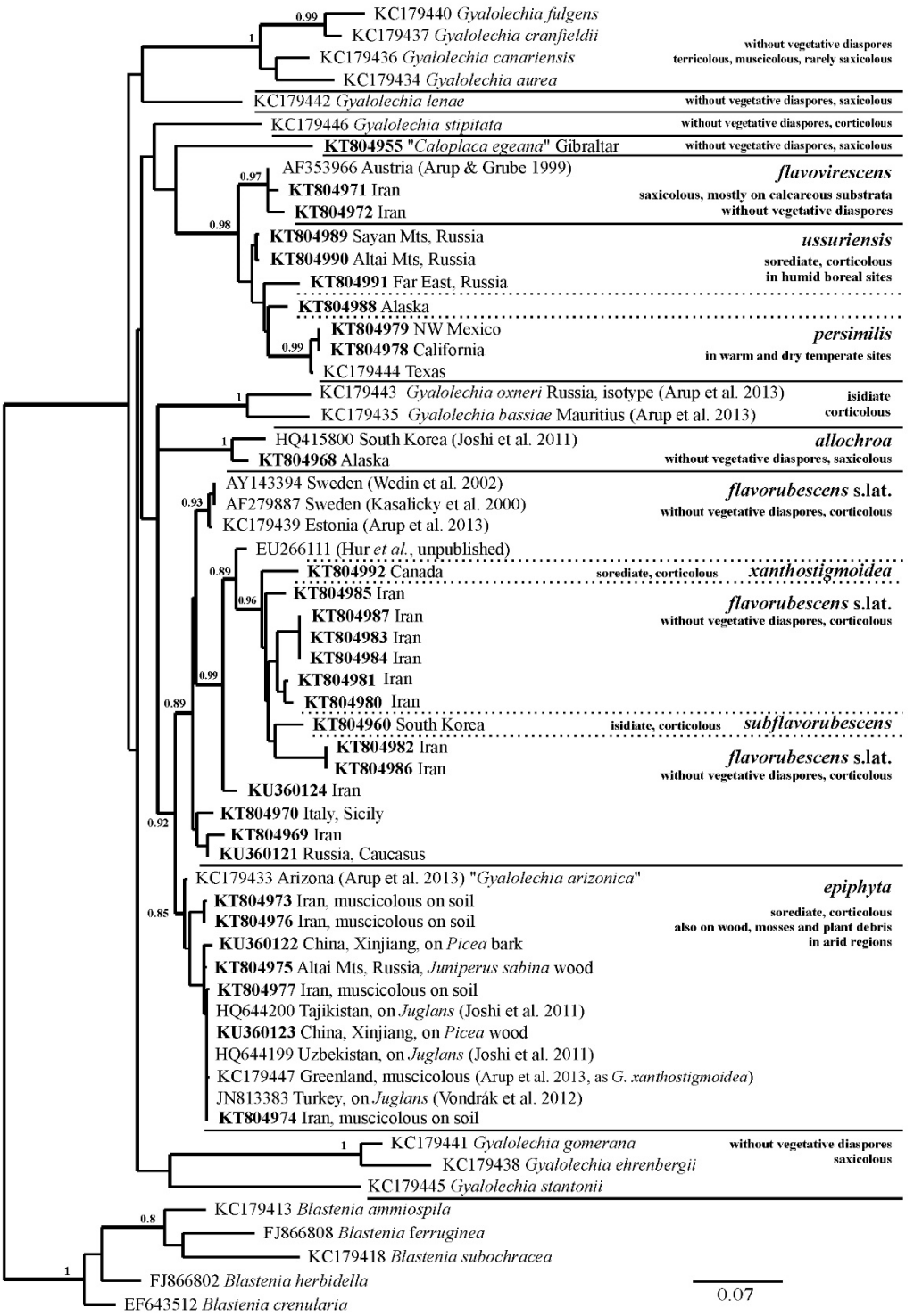


Fig. 4. Maximum likelihood ITS phylogeny of *Gyalolechia* showing positions of *G. epiphyta* and *G. ussuriensis*. New sequences are in bold; bootstrap supports (BS ≥0,7) are shown at nodes.

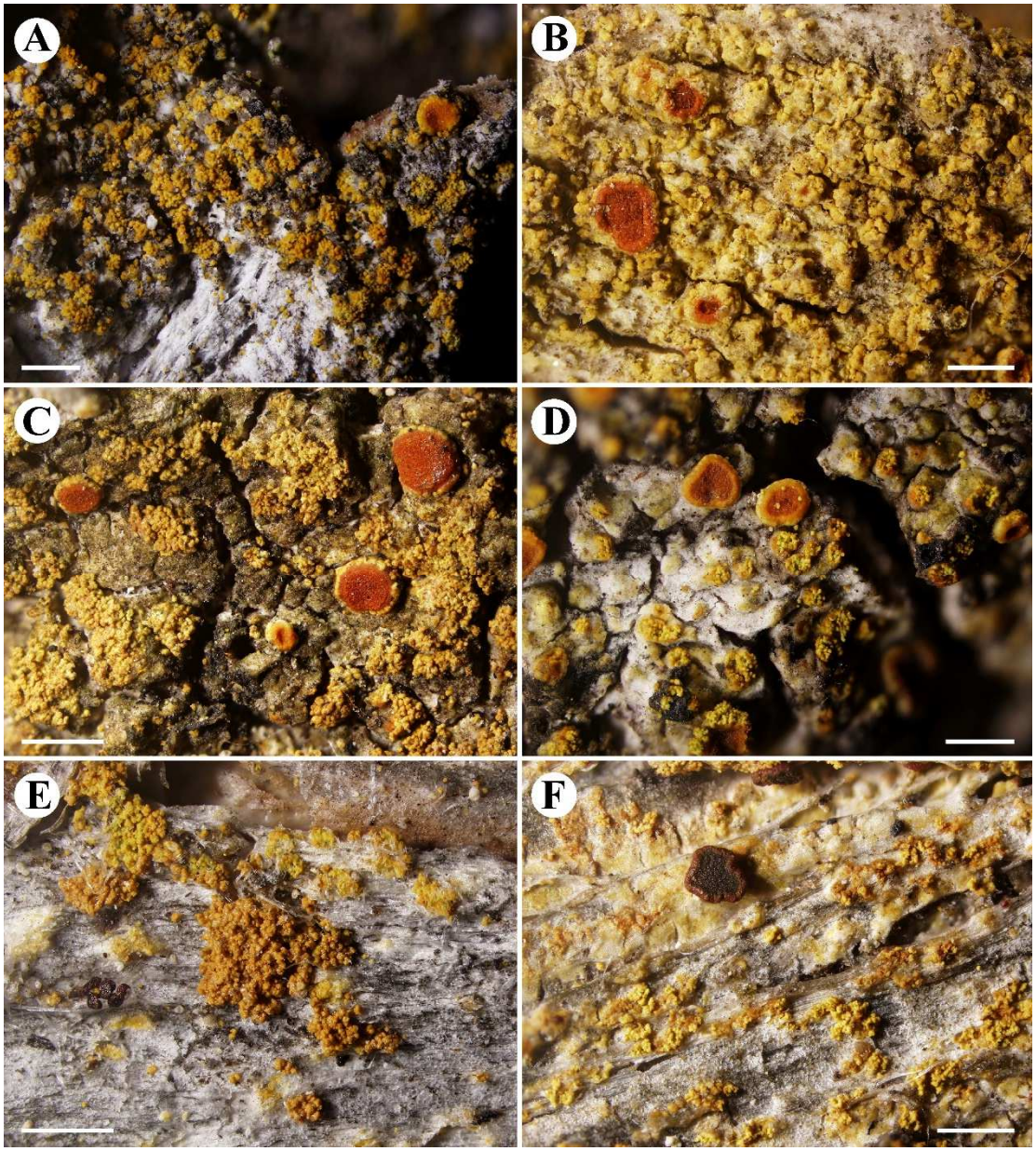


Fig. 5. A, *Calogaya bryochrysion*, corticolous specimen from Altai Mts (ED11499, KT804937); B, *Gyalolechia epiphyta* with blastidiate thallus and without soralia from Tajikistan (hb. Halda 174, HQ644199); C, *Gyalolechia persimilis* with pale yellow thallus and bright yellow soralia from California (JV7486, KT804978); D, *Gyalolechia ussuriensis* with pale yellow thallus and bright yellow soralia from the Russian Far East (ED11500, KT804991); E, *Gyalolechia* aff. *ussuriensis* with an inconspicuous endophloedal thallus and bright yellow soralia from Alaska (TS38925, KT804988); F, *Gyalolechia xanthostigmoidea* from eastern Canada (TS32410, KT804992), a taxon morphologically similar to *G. persimilis*/*G. ussuriensis*. Scales: A–F=0·5 mm.

**Supplementary Material**

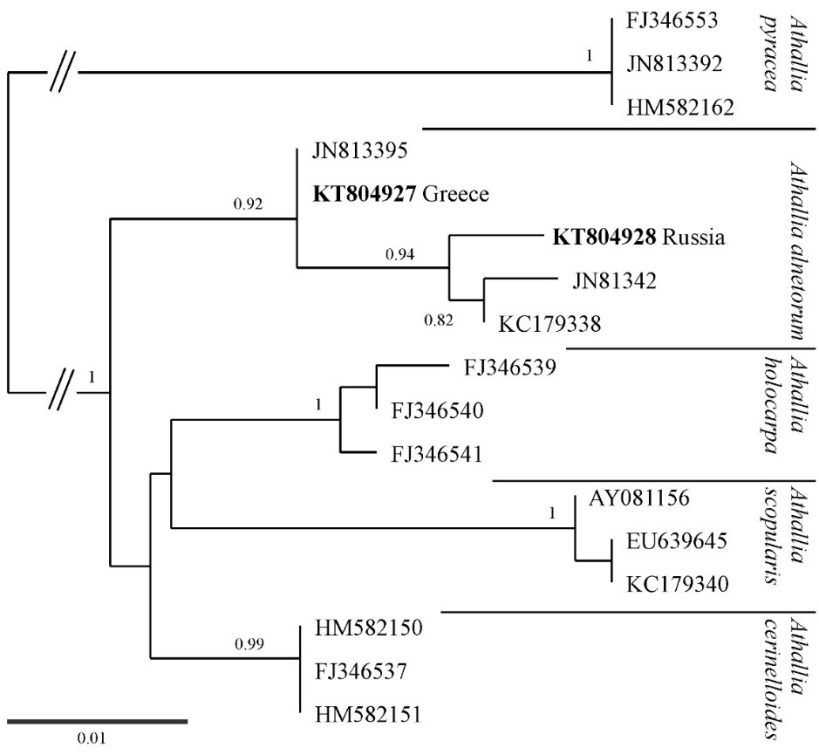


Fig. S1. Maximum likelihood ITS phylogeny of a section within *Athallia* including *A. alnetorum*. New sequences are in bold; bootstrap supports (BS□0.7) are shown at nodes.

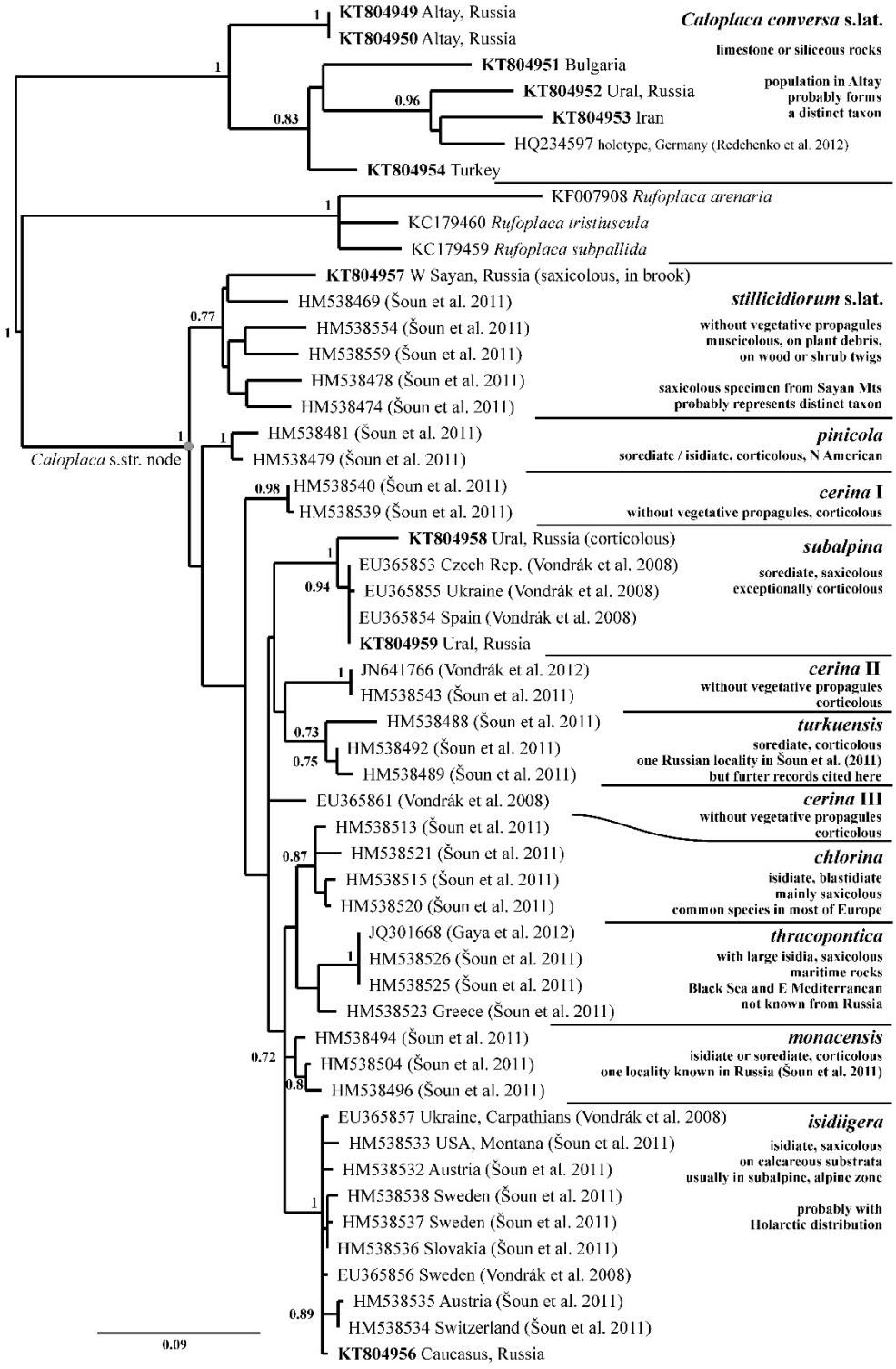


Fig. 2. Maximum likelihood ITS phylogeny of *Caloplaca* (sensu Arup *et al.* 2013) showing positions of *C. isidiigera* and *C. subalpina*. New sequences are in bold; bootstrap supports (BS 0.7) are shown at nodes.

## 5.6 Paper 6

**Frolov I.**, Vondrák J., Konoreva L.A., Chesnokov S.V., Himelbrant D.E., Arup U., Stepanchikova I.S., Prokopiev I.A., Yakovchenko L., Davydov E.A. (accepted manuscript). Three new species of crustose Teloschistaceae in Siberia and the Far East. The Lichenologist.

**Three new species of crustose Teloschistaceae in Siberia and the Far East**

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**Abstract**

Three species of the family Teloschistaceae (lichenized Ascomycota) are described as new for science from southern and eastern Siberia and the Far East. Corticolous *Caloplaca saviczii* belongs to the genus *Caloplaca* s.str.; it has *C. cerina*-like apothecia and green to grey-green, crateriform soralia with white rim. *Lendemeriella aureopruinosa* is a saxicolous taxon with thin grey thallus, small apothecia 0.3–0.6 mm in diameter, with dark orange disk usually bearing eipsamma and with often grey true exciple containing pigment Cinereorufa-green. *Orientophila infirma* is a corticolous species with endophloedal thallus and small orange apothecia,

0.2–0.3 mm in diameter, with usually inconspicuous thalline exciple. All new taxa presumably have boreal north-eastern distribution in Asia.

**Key words:** *Caloplaca* sensu lato, Kamchatka, Khabarovsk, combined phylogeny, Primorye, Russia, Sakhalin, Tuva, Yakutia

## Introduction

Crustose *Teloschistaceae*, or *Caloplaca* s.lat., include about 1000 species (Arup *et al.* 2013), of which the majority is from temperate regions (Feuerer 2011). About 18 % of this diversity (around 280 species) is known in Russia (Urbanavichus 2010; Urbanavichus & Urbanavichene 2012; Vondrák *et al.* 2013*b*, 2017, 2019; Muchnik *et al.* 2014; Frolov & Konoreva 2016, etc.) and it makes the family one of the most species-rich in the country (Urbanavichus 2014). The highest numbers of species of Teloschistaceae is characteristic for the regions with dry and warm rocky steppes with base-rich bedrock, i.e. Southern European Russia, Russian Caucasus, Southern Ural, and Southern Siberia (Urbanavichus 2014). Eastern Siberia and the Russian Far East with mainly boreal and temperate forests and acidic siliceous outcrops do not have outstanding diversity of *Teloschistaceae*. Mainly common European or circumboreal taxa were known from the region until a number of species with Asian distribution was recently described from there (Søchting & Figueras 2007; Kondratyuk *et al.* 2011, 2013, 2014, 2015). Here we provide further information to show particular uniqueness of Teloschistaceae diversity in this part of Asia. During the last several years authors of the present paper collected lichens in different regions of Siberia in a huge territory from Altai Mts to Kamchatka Peninsula and independently found several specimens of *Caloplaca* s.lat., which failed to be identified with any of the known taxa. All these specimens were gathered together, studied carefully and finally described here as three new species.

## Materials and Methods

### Sampling

Lichens were collected by the authors from various localities in Siberia and the Russian Far East in 2013–2019 and deposited mainly in LE, PRA and I. Frolov's personal herbarium. L. Konoreva and S. Chesnokov collected in the Republic of Sakha (Yakutia), Khabarovsk Territory and Trans-Baikal Territory; I. Frolov – in Sakhalin Region and the Republic of Tuva; J. Vondrák – in the Republic of Tuva; D. Himelbrant and I. Stepanchikova – in Kamchatka Territory; E. A. Davydov and L. Yakovchenko – in Primorye Territory.

**Phenotype evaluation**

Measurements of morphological characters follow Vondrák *et al.* (2013*a*). All microscopical observations are based on hand-cut sections mounted in water, mainly without chemical treatments (paraphyses and upper cells of true exciple were measured in KOH since they were not visible due to anthraquinones crystals). Spores were sometimes vivid (with badly visible septa) and thus measured after heating (Steiner & Peveling 1984). Measurements are accurate to 0.5 μm for cells and 5–10 μm for larger structures. For cells (ascospores, conidia, paraphyses etc.) ten measurements and for larger structures (hymenium, hypothecium etc.) five measurements per specimen were made, except for poor specimens with deficient material. Results are given as (min.–)$x_1$–$x_2$–$x_3$(–max.), where min/max are extremes from all measurements, $x_1$ is the lowest specimen arithmetic mean observed, $x_2$ is the arithmetic mean of all observations, $x_3$ is the highest specimen arithmetic mean observed. Total number of measurements (n), number of samples assessed (N), and standard deviation from all measurements (SD) are given in square parenthesis for each character measured [n; N; SD]. Morphological terminology follows Smith *et al.* (2009) and Vondrák *et al.* (2013*a*).

**Chemistry**

Composition of secondary metabolites was identified by HPLC analysis in apothecia of one specimen of *Orientophila infirma* and two specimens of *Lendemeriella aureopruinosa*. Air-dried lichens were used for the analysis. A crushed portion of a test sample was extracted with 0.1 mL of acetone on constant stirring for 24 h at room temperature. HPLC analyses were performed with a 1290 Series Agilent chromatograph with UV detection. For chromatographic separation, a ZORBAX Eclipse XDB-C18, 80 Å column (150×0.5 mm×5μm) was used. The mobile phase consisted of (A) aqueous formic acid (0.1%), and (B) acetonitrile. Analyses were performed at 25 °C and a flow rate of 0.1 ml/min in the isocratic elution mode. The volume of the injected sample was 1 μL. Spectra of eluting substances were recorded in UV at 250 nm. After separation, the samples were also analyzed with an quadrupole time-of-flight mass spectrometer (6538 Series, Agilent, USA). Ionization was achieved by electrospray in the negative mode. Voltage on the capillary was 2.5 kV, capillary temperature 350°C, atomizing gas pressure 45 psi, desiccant gas (nitrogen) temperature 225°C, drying gas flow rate 5 L/min. Mass spectra were recorded in the range 100–1000 m/z. The resulting chromatograms were processed with the MassHunter WorkStation v. B.04.00 software package (Agilent, USA). The substances were identified based on their chromatographic properties and molecular masses. The identification of insoluble lichen pigments follows the methods described by Meyer & Printzen (2000).

**DNA extraction, amplification and sequencing**

DNA was extracted with a CTAB-based protocol (Aras & Cansaran 2006). Amplifications were made of the internal transcribed spacer regions (nrITS) and the large subunit (nrLSU) of the nuclear ribosomal RNA genes, and the small subunit of the mitochondrial ribosomal RNA gene (mrSSU). Primers for PCR amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) for ITS, AL1R (Döring *et al.* 2000) and LR5 (Vilgalys & Hester 1990) for nrLSU, and mrSSU1 (Zoller *et al.* 1999) and mrSSU7 (Zhou & Stanosz 2001) for mrSSU. The PCR settings followed Ekman (2001) for ITS and Arup *et al.* (2013) for nrLSU and mrSSU. Obtained sequences are uploaded into the NCBI database (GenBank); accession numbers are provided (Table).

**Alignments and phylogenetic analyses**

Newly obtained sequences were edited in FinchTV 1.4.0 (Geospiza, Inc.; Seattle, Washington, USA; http://www.geospiza.com) and BioEdit 7.2.5 (Hall 1999). All datasets were aligned online by MAFFT 7 (Katoh & Standley 2013; available at http://mafft.cbrc.jp/alignment/server/) with the L-INS-i and FFT-NS-I methods (Katoh *et al.* 2005) selected automatically by the program for each datasets. To exclude ambiguously aligned positions alignments were subsequently cleared by the *automated1* algorithm as implemented in the trimAl software package (Capella-Gutierrez *et al.* 2009). Phylogenetic reconstructions were carried out using Bayesian inference (BI) in MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003). Analyses were run on the CIPRES Web Portal (http://www.phylo.org/portal2/). Optimum partitioning of the data sets and the optimum substitution models per partition were calculated in PartitionFinder2 using greedy algorithm and corrected Akaike Information Criterion (Lanfear *et al.* 2016). For a concatenated alignment of ITS, nrLSU and mrSSU in the input file for PartitionFinder we created partitions for ITS1, ITS2, 5.8S, nrLSU and mrSSU. PartitionFinder suggested two subsets: (i) ITS1, ITS2, and (ii) mrSSU, nrLSU, 5.8S with GTR+I+G model for both subsets. In MrBayes analyses were performed using two independent runs with four MCMC chains. Trees were sampled after every 500th generation. The prior settings for the combined analysis were the same for both subsets of partitions: rates of reversible rate matrix = Dirichlet (1.00,1.00,1.00,1.00,1.00,1.00), stationary state frequencies = Dirichlet, shape of scaled gamma distribution of site rates = Exponential (1.00), proportion of invariable sites = Uniform (0.00,1.00), partition-specific rate multiplier = Dirichlet (1.00,1.00), topology = All topologies equally probable a priori, branch lengths = Unconstrained:GammaDir (1.0,0.1000,1.0,1.0). Rate heterogeneity across partitions was allowed (ratepr=variable). The analyses were stopped when the average standard deviation of the split frequencies between the simultaneous runs dropped below 0.01

(370 000 generations in the combined analysis). In the combined analysis, PSRF of the model parameters values ranged from 0.999 to 1.004. The first 25% of trees were discarded as the burn-in phase, and the remaining trees (1112 trees in the combined analysis) were used for construction of a 50% majority-rule consensus tree. The alignments of the three different genes were first analyzed separately to check for incongruence between genes. A conflict was assumed to be significant if two different relationships were both supported with posterior probabilities 0.95 or higher (Buckley *et al.* 2002). Accession numbers of the sequences downloaded from the GenBank and used in the analyses are provided in Supplementary Material Table S (available online).

**Results and Discussion**

To determine position of the new species in the phylogeny of Teloschistaceae we included them into the combined analysis of nrITS, nrLSU and mrSSU dataset together with the main genera of the family. Initially, the alignments of these genes were analyzed separately to check for incongruence between genes, but no incongruences were found. The combined alignment included 157 terminal species and a total of 2410 positions before and 2093 positions after trimming. The phylogeny was rooted with taxa outside the family following Arup *et al.* (2013). A resulted tree is presented in Fig. 1.

The new species belong to the subfamilies *Caloplacoideae* and *Xanthorioideae*. One of them (*Caloplaca saviczii*) is explicitly nested inside the genus *Caloplaca* s.str. Another species (*Lendemeriella aureopruinosa*) is closely related to the *Caloplaca exsecuta* group sensu Vondrák *et al.* (2019), which is currently included into the genus *Lendemeriella* (Kondratyuk *et al.* 2020). The third taxon (*Orientophila infirma*) forms a sister lineage to the genus *Orientophila* and we described it in that genus. We discuss taxonomic positions of the new species in more detail in their diagnoses below.

We also prepared three separate ITS phylogenies for each of the new species using more sequences of these species and closely related taxa (see Supplementary Material Figs S1–S3, available online). Outgroups were chosen following the results of the combined analysis. Ingroup of the *Caloplaca* s.str. alignment included 86 sequences belonging to 13 species; five sequences were newly obtained. Ingroup of the *Lendemeriella* alignment included 27 sequences belonging to six species; 10 sequences were newly obtained. The *Orientophila* alignment included 17 sequences of nine species of *Athallia*, *Flavoplaca* and *Orientophila*; four sequences were newly obtained. All new species form well-delimited and highly supported clades (see Supplementary Material Figs S1–S3, available online).

**Taxonomy**

***Caloplaca saviczii* I.V. Frolov, Himelbrant, Stepanchikova, Konoreva & S. Chesnokov sp. nov.**

MycoBank No.: MB833718

Similar to *Caloplaca cerina*, but differs by presence of green to grey-green, crater-like soralia with thin white intact or torn rim. Soredia usually with emerald green pigment. Thallus endophloedal or of poorly developed scattered beige areoles with unclear margins. Apothecia rare, about 0.3–0.4 mm in diameter, with beige thalline exciple, when young with thick white pruina on disc and exciple. Ascospores 12–14 × 5–7 µm, with septa 4–5 µm width.

Type: Russia. Kamchatka Territory, Koryakia, Penzhina District, fluvial valley of river Katal'yanayvayam, left bank of the river, alt. 61 m, 61°24'39.7"N, 165°02'02.9"E, on bark of *Populus suaveolens* in *Chosenia arbutifolia* (60 years old) floodplain forest with *Populus suaveolens* (60 years old), *Salix schwerinii*, *Alnus fruticosa* and *Calamagrostis purpurea*, 21 August 2016, *D. Himelbrant* Kor-Ichig-34-2016 (LE-L15203—holotype; H—isotype). GenBank accession numbers of the sequences of the holotype (soralia): MN814226 (ITS), MW227509 (LSU), MW227327 (mrSSU).

(Figs 2A–C)

*Thallus* endophloedal or of poorly developed scattered elongated or roundish slightly convex beige areoles about 0.25–1.05 × 0.15–0.28 mm with unclear margins fusing with substrate. Thickness of *areoles* (45–)45–83–125(–125) µm [7, 6, 27]. *Cortex* on section colorless to beige, towards areoles surface gradually changed to epinecral layer, thickness of cortex together with epinecral layer (13–)13–26–38(–38) µm [8, 7, 9]. Cortex cells ± spherical, (4.5–)5.4–5.9–6.2(–8.0) µm in diameter [21, 3, 0.8], cell-wall thickness up to 1.5 µm. *Algal layer* (25–)25–55–56(–63) µm thick [7, 6, 11]; algal cells globose, (8.0–)12.5–12.9–13.8(–19.0) µm in diameter [44, 5, 2.6]. *Medulla* inconspicuous or algonecral, up to 40 µm thick. Cortex and algal layer sometimes distinguishable also in endophloedal thalli. Sometimes areoles form *pustules* of the same color with thick white pruina on top; diameter of pustules about 250–360 µm. Pustules covered with paraplectenchymatous cortex about 45–55 µm width with ± spherical cells about 5–9 µm in diameter poorly distinguishable due to dense crystals of different form about 1–8 µm size insoluble in K. Pustules comprise circular algal layer about 40–50 µm wide enclosing medulla which consists of few solitary algal cells interwoven with fungal hyphae about 2 µm width. These pustules then likely break to soralia. *Soralia* green to grey-green, crater-like, (0.11–)0.16–0.23–0.34(–0.56) mm in diameter [94, 10, 0.08], with thin white intact or torn rim, one or rarely two per areole, scattered on surface of bark or rarely crowded. Soralia walls (18–)34–48–60(–75) µm width [24, 12, 13], about 200 µm high, colourless on section or rarely pale beige, cells undistinguishable or deformed. *Soredia* usually emerald green (intensifying in K) rarely colourless, (13–)16–18–21(–28) µm in diameter [118, 12, 3];

*consoredia* rare, about 23–35 µm in diameter. Fungal cells in soredia (3.0–)3.8–4.3–4.9(–6.5) µm in diameter [40, 4, 0.9]; algal cells in soredia (4.0–)5.2–6.9–9.3(–12.0) µm in diameter [40, 4, 1.8]. *Prothallus* usually absent or in form of grey film.

*Apothecia* (0.21–)0.29–0.36–0.45(–0.50) mm in diameter [36, 6, 0.07], lecanorine to zeorine (true exciple visible only in cross-section of apothecia), adnate; apothecia rare, were observed on about 50% of the specimens and they were never abundant on a specimen. *Disc* orange; *thalline exciple* of same colour as thallus or darker; thick white pruina present on disc and exciple, especially in immature apothecia. *Hymenium* (88–)88–93–105(–105) µm high [4, 4, 8], colourless, not glutinized, without extracellular oil drops and crystals; *epihymenium* golden brown. *Hypothecium* colourless, in central part above clusters of algal cells or rarely with a central conical extension downward, with or rarely without extracellular oil drops, without extracellular crystals, (35–)35–93–150(–150) µm high [4, 4, 47]; formed of thin-walled cells variable in shape and orientation. *Exciple* about 30–80 µm wide, formed of poorly developed true exciple, (0–)7–9–20(–20) µm wide [4, 4, 8], and thalline exciple, (28–)28–47–68(–68) µm wide [4, 4, 20]. Upper part of true exciple of thin-walled cells about 7 × 3 µm. *Thalline exciple* with well-developed hyaline cortex, (30–)30–48–58(–58) µm wide (in the widest part) [4, 4, 12]. Cortex cells of two types: (i) ± spherical, (5.0)5.3–6.2–7.5(9.0) µm in diameter [21, 4, 1.2], (ii) elongated and oriented perpendicular to surface of cortex, (7.0–)9.3–9.8–10.3(–13.0) × (4.0–)5.2–5.2–5.3(–6.0) µm [11, 2, 1.9 & 0.6]; thickness of walls up to 2 µm. *Paraphyses* about 2 µm wide in lower part, 1–2 upper cells slightly widened and the widest upper cell (2.0–)2.9–3.3–4.5(–5.5) µm wide [36, 4, 0.8]; often branched in upper part. *Asci* clavate, (50–)56–56–57(–63) × (13–)16–17–17(–21) µm [10, 2, 4 & 3]. *Ascospores* 8 per ascus, colourless, polarilocular, (10.0–)11.6–12.0–14.2(–15.0) × (4.5–)5.3–6.1–6.6(–7.5) µm [37, 4, 1.3 & 0.8], with rounded ends. Septa (2.5–)4.1–4.6–5.3(–6.0) µm [37, 4, 0.7]. Ascospore length/width ratio: (1.60–)1.81–2.02–2.20(–2.56) [37, 4, 0.24]; septum width/ascospore length ratio: (0.25–)0.35–0.37–0.39(–0.45) [37, 4, 0.05].

Pycnidia not observed.

*Chemistry.* Epihymenium and upper true exciple with anthraquinones, K+ purple. Upper part of cortex of thallus and thalline exciple without anthraquinones, with Sedifolia-grey, K+ violet, or without it, K–. Fungal cells of soredia with an unidentified pigment, insoluble in acetone, emerald green in water, K– or K+ intensifying, C+ orange; in N slowly dark-grey with violet tinge (brownish?) and then, after adding K, orange. We did not analyse an anthraquinone chemosyndrome in the apothecia of the new species due to scarcity of the material. However, considering the taxonomic position nested in the *Caloplaca cerina*/*C. stillicidiorum*-clade we would expect an ordinary syndrome A (Søchting 1997).

*Etymology.* Named in honour of Vsevolod Pavlovich Savicz (1885–1972), the first lichenologist who studied lichens of Kamchatka and described the first *Caloplaca* s.lat. species from there – *C. kamczatika* (Savicz 1914).

*Phylogeny and taxonomic position.* According to the combined phylogeny (Fig. 1) the new species is explicitly nested within the genus *Caloplaca* s.str. (subfamily *Caloplacoideae*). Generic affiliation of *C. saviczii* is also supported by its lecanorine *C. cerina*-like apothecia. According to the ITS phylogeny (see Supplementary Material Fig. S1, available online) *C. saviczii* is well delimited from the other sorediate species of *Caloplaca* str. and nested in a large *C. cerina*/*C. stillicidiorum*-clade that is, however, not supported. In spite of its likely close relationships to *C. cerina* s.lat. and *C. stillicidiorum* s.lat. the new taxon deserves a rank of species, since it could be characterised morphologically (peculiar crater-like soralia with an unknown emerald green pigment), ecologically (occurrence in boreal floodplain forests) and geographically (possibly restricted to North-Eastern Asia). All sequences of the new species were obtained from soralia.

*Similar taxa.* The new species is monophyletic and well delimited based on the molecular results, however, it could be confused with some other epiphytic sorediate *Caloplaca* s.str. species, among which morphologically *C. hanneshertelii* S.Y. Kondr. & Kärnefelt is the closest species bearing similar crater-like soralia with whitish or greyish rim. The latter species is so far only known from south-eastern Australia and differs by better developed areoles, dark-bluish colour of soralia which are sometimes flat and not crater-like (always crater-like in *C. saviczii*), and paraphyses which are more distinctly swollen on the top, 4–6 μm (Kärnefelt & Kondratyuk 2004). Other epiphytic sorediate *Caloplaca* s.str. species (*C. chlorina* (Flot.) Sandst., *C. pinicola* H. Magn., *C. sterilis* Šoun *et al.* and *C. turkuensis* (Vain.) Zahlbr. never form crater-like soralia.

Sterile *C. saviczii* could be confused with sterile epiphytic *Caloplaca* s.lat. species with grey or greenish soralia without anthraquinones. Soralia of *C. ahtii* Søchting are distinctly smaller, dark (bluish) grey, containing Sedifolia-grey, K+ violet. Thalli of *C. alstrupii* Søchting have bluish black hypothallus borders, much more abundant smaller pustules, 100–250 μm in diameter, and very pale yellowish green soralia (Søchting 1999). *Caloplaca obscurella* (J. Lahm) Th. Fr. and *C. ulcerosa* Coppins & P. James have thin grey to white more or less continuous thallus, paler yellowish green, pale green or greyish soralia without emerald green pigment, soralia often crater-like, but sometimes not and developed along fissures in thallus (always crater-like in *C. saviczii*). *Caloplaca ulcerosa* is a European mainly maritime species (Vondrák *et al.* 2009) and *C. obscurella* is inland lichen with no reliable records east of the Urals. Soralia of *C. sorocarpa* (Vain.) Zahlbr. are barrel shaped, never crateriform.

*Ecology and distribution. Caloplaca saviczii* grows on bark of trunks of various deciduous trees and shrubs (*Chosenia arbutifolia*, *Fraxinus* sp., *Populus suaveolens*, *P. tremula*, *Salix cardiophylla* and other species of *Salix*, *Sambucus* sp., *Ulmus* sp.) in floodplain forests in taiga or rarely in coniferous forests along small streams in taiga or forest-tundra at altitudes from 60 m to 650 m above sea level. Co-occurring lichen taxa include *Arthrosporum populorum* A. Massal., *Athallia pyracea* (Ach.) Arup *et al.*, *Bacidia circumspecta* (Norrl. & Nyl.) Malme, *Caloplaca ahtii*, *C. gordejevii* (Tomin) Oxner, *C. taranii* S.Y. Kondr. *et al.*, *Catinaria atropurpurea* (Schaer.) Vězda & Poelt, *Gyalolechia ussuriensis* (Oxner *et al.*) Vondrák, *Lecidea erythrophaea* Flörke ex Sommerf., *Lecidella elaeochroma* (Ach.) M. Choisy, *Lendemeriella infirma* I.V. Frolov *et al.*, *Phaeophyscia kairamoi* (Vain.) Moberg, *Physcia alnophila* (Vain.) Loht. *et al.* etc. The species is known from Eastern Siberia (Yakutia, Russia) and the Far East (Kamchatka and Sakhalin, Russia). It seems to be quite common there in appropriate localities, but inconspicuous and therefore easily overlooked. The known localities are marked on Fig. 3.

*Additional material studied.* **Russia: *Kamchatka Territory:*** Koryakia, Penzhina District, fluvial valley of river Katal'yanayvayam, left bank of the river, 61°24'39.7"N, 165°02'02.9"E, 61 m, 2016, *D. Himelbrant* Kor-Ichig-34-2016 (LE-L15202); Koryak Nature Reserve, Parapol'sky Dol segment, fluvial valley of river Ichiginynvayam, right bank of the river, 61°24'40.8"N, 165°01'53.5"E, 70 m, 2016, *D. Himelbrant* Kor-Ichig-24-2016 (LE-L15201; LE-L15204; LE-L15205; LE-L15216; LE-L15220, duplicate will be send to H); ***Sakhalin Region, Sakhalin Island:*** Smirnykh District, 16 km SE of Pervomaysk, narrow floodplain of river Vitnica, just below Mt Vayda, 49°52'44.2"N, 143°26'59.3"E, 350 m, 2019, *I. Frolov* 2467; 15 km SE of Pilvo, near road from Smirnykh to Pilvo, wide floodplain of river Pilevka, 49°56'4.9"N, 142°18'16.0"E, 140 m, 2019, *I. Frolov* 2468, 2469, 2470; Tymovskoye District, 20 km E of Palevo, wide floodplain of river Tym', 50°37'43.7"N, 143°0'16.3"E, 260 m, 2019, *I. Frolov* 2471, 2472 (duplicates will be send to GZU, H, LE, LD and PRA); ***Republic of Sakha (Yakutia)*:** Aldan District, Tommot, river Kurung, 58°44'29"N, 126°21'5"E, 470 m, 2015, *L. Konoreva* 351 (LE-L15218); Neryungri District, Chul'man, left bank of river Chul'man, 56°51'48.4"N, 124°54'16.2"E, 649 m, 2015, *S. Chesnokov* 38 (LE-L15222); Tomponsky District, 6,5 km W of town Tyoply Klyuch, 62°47'04.3"N, 136°40'42.3"E, 295 m, 2016, *L. Konoreva* J-330 (LE-L15217).

***Lendemeriella aureopruinosa*** **I.V. Frolov, Vondrák, Arup, Konoreva, S. Chesnokov, Yakovchenko & Davydov sp. nov.**

MycoBank No.: MB833717

Thallus epilithic, in form of inconspicuous grey film or ± well-developed, continuous or areolate. Apothecia about 0.3–0.6 mm in diameter, disc dark orange to

brick colour; thalline exciple absent or inconspicuous, at the base of apothecia; true exciple of the same colour as disk or dark grey, containing Cinereorufa-green; young apothecia often with aureate epipsamma. Ascospores 11–15 × 6–7 μm, with septa 3–5 μm width.

Type: Russia. Republic of Sakha (Yakutia), Aldan District, Yllymakh, right bank of river Bes-Yuryakh, near road from Tommot to Yllymakh, alt. 640 m, 58°38'27.0"N, 126°36'38.7"E, on siliceous outcrops in *Betula* sp. – *Alnus* sp. – *Larix gmelinii* forest, 13 July 2015, *S. Chesnokov* 154 (LE-L15207—holotype; H—isotype). GenBank accession numbers of the sequences of the holotype: MN814228 (ITS), MW227504 (LSU), MW227332 (mrSSU).

(Figs 2D–F)

*Thallus* epilithic, but usually in form of inconspicuous continuous or discontinuous, rarely cracked film, greenish grey, whitish grey, brownish grey, grey, forming roundish spots about 0.7–3.5 cm in diameter; sometimes thallus ± well-developed, mainly continuous or with few areoles about 1 × 0.8 mm. Thickness of thallus (88–)88–106–331(–425) μm [8, 5, 106]. *Cortex* up to 33 μm, colorless or grey in lower part of section, often completely turning into epinecral tissue without cell structure, but sometimes vivid cells up to 5 μm in diameter distinguishable in thin lower part of section; sometimes cortex inconspicuous. *Algal layer* about 50–90 μm thick; algal cells globose, (4.0–)5.4–10.8–13.6(–18.0) μm in diameter [39, 4, 3.7]. *Medulla* up to 310 μm thick, full of crystals, probably from substrate. *Prothallus* present, dark grey.

*Apothecia* (0.20–)0.33–0.47–0.59(–0.90) mm in diameter [77, 9, 0.12], biatorine to zeorine, sessile or adnate. *Disc* dark orange, orange red or of brick colour; *true exciple* of the same colour as disk (sometimes paler) or grey or dark grey, *thalline exciple* absent or inconspicuous, occur at base of apothecia; apothecial *disc* and margin typically with thick bright orange yellow, golden pruina consisting of anthraquinones – epipsamma according to Poelt (1969), epipsamma especially distinct in young apothecia. *Hymenium* (70–)72–90–100(–113) μm high [22, 10, 12], in upper part yellowish, sometimes with grey tinge, in lower part colourless, sometimes completely colourless, not glutinized, without extracellular oil drops and crystals; *epihymenium* dark golden brown. *Hypothecium* in upper part yellowish, in lower part colourless, with or rarely without extracellular oil drops, without extracellular crystals, (75–)81–112–133(–145) μm high [22, 10, 22], formed of thin-walled cells variable in shape and orientation; algal cells present in clusters or in a layer or rarely absent below hypothecium, and in the latter case hypothecium forms a central conical extension downward. *Exciple* about 10–90 μm wide, formed of true exciple, (5–)13–33–66(–88) μm wide [21, 10, 20]. *Thalline exciple* rarely present, at base of apothecia, hardly distinguishable from thallus, up to 65 μm wide. *True exciple* sometimes with epinecral

layer about 5–18 µm wide; upper part of true exciple of thin-walled cells about (4.0–)5.0–6.5–7.8(–10.0) × (2.5–)2.8–3.4–4.6(–6.0) µm [44, 8, 1.3 & 0.9]; external part of true exciple greenish black with golden brown crystals of anthraquinones on surface or inside, rarely greenish black colour inconspicuous and golden brown colour predominant. *Paraphyses* about 2 µm wide in lower part, gradually slightly widen, rarely 2–3 upper cells significantly wider, the widest upper cell (2.0–)2.8–3.4–4.3(–5.0) µm width [70, 7, 0.8], the uppermost cells rarely small and deformed like in some species of *Pyrenodesmia* (Frolov *et al.* 2016); paraphyses sometimes with intracellular oil drops, not branched or sometimes slightly branched in upper part. *Asci* clavate, (40–)43–48–54(–65) × (11–)12–15–18(–24) µm [32, 6, 7 & 3]. *Ascospores* 8 per ascus, colourless, polarilocular, (9.0–)11.5–13.0–14.8(–18.0) × (4.5–)5.9–6.4–7.2(–8.5) µm [78, 9, 1.7 & 0.8], with rounded ends. Septa (3.0–)3.5–4.0–4.3(–7.0) µm wide [78, 9, 0.7]. Ascospore length/width ratio: (1.60–)1.75–2.10–2.42(–3.11) [78, 9, 0.30]; septum width/ascospore length ratio: (0.20–)0.27–0.30–0.35(–0.50) [78, 9, 0.05].

*Pycnidia* not observed.

*Chemistry.* Epihymenium and upper true exciple with anthraquinones, K+ purple, N+ yellow; true exciple also with green black pigment Cinereorufa-green, which especially visible as N+ purple substance after removing anthraquinones from apothecium cross-section by KOH treatment. Thalline cortex K–. Apothecia of two specimens (LE-L15208 and IF2475) were analyzed by HPLC, both contain parietin, parietinic acid, emodin, teloschistin (traces in IF2475) and fallacinal.

*Etymology.* The epithet reflects the typical presence of bright aureate pruina on young apothecia.

*Phylogeny and taxonomic position.* Our combined phylogeny (Fig. 1) shows that the just recently described genus *Lendemeriella* from the subfamily *Caloplacoideae* (Kondratyuk *et al.* 2020) is the closest lineage to the new species. Morphologically the new taxon is similar to some species of the *Caloplaca exsecuta* group sensu Vondrák *et al.* (2019), which is now a part of the genus *Lendemeriella*. For example, *L. exsecuta* (Nyl.) S.Y. Kondr., *L. nivalis* (Körb.) S.Y. Kondr. and *L. tornoensis* (H. Magn.) S.Y. Kondr. have poorly developed thalli and apothecia with yellow-orange epipsamma and Cinereorufa-green. However, according to the ITS phylogeny (see Supplementary Material Fig. S2, available online) the new species is an outgroup to the *Caloplaca exsecuta* group. In addition, the genus *Lendemeriella* itself is a topic for a discussion since the included species are rather different on their chemistry, geography and ecology. Nevertheless, to avoid taxonomical complications and considering our molecular data we tentatively describe the new species in the genus *Lendemeriella*.

*Similar taxa.* The new taxon is monophyletic and well delimited based on the molecular results. Morphologically, however, it is hardly distinguishable from the epilithic *Lendemeriella exsecuta*, which has very similar apothecia with epipsamma

(Hansen *et al.* 1987) and contains Cinereorufa-green. The latter species grows in different (but overlapping with the new taxon yet) ecological conditions – it occurs in zonal tundra and the alpine belt of high mountains and rarely in the upper part of the forest belt, while *L. aureopruinosa*, on the contrary, grows in the forest belt in mountains and rarely in the alpine belt. In addition, *L. exsecuta* usually has darker apothecia and contains 7-chloroemodin that corresponds to the chemosyndrome A2 (Søchting 2001). Morphologically the new taxon is also close with the other two poorly known epilithic species *Caloplaca lacinulata* (Hue) Zahlbr. and *C. hexaspora* (Hue) T. Okamoto described from more southern and warmer regions of South Korea and Japan by Hue (1913). *Caloplaca lacinulata*, recently rediscovered in South Korea by Joshi *et al.* (2011), differs by narrower ascospores (4.5–8.5 μm vs. 7.5–10 μm) and wider hypothecium (75–145 μm vs. 30–100 μm). In addition, we did not observe pycnidia in *L. aureopruinosa*, whereas *C. lacinulata* is known with pycnidia. *Caloplaca hexaspora* has larger apothecia (up to 1.5 mm vs. up to 0.9 mm) and ascospores with narrower septa (2–2.5 μm vs. 3–7 μm). It is also known with pycnidia. In contrary to consistently 8-spored asci in *L. aureopruinosa*, Hue (1913) reported six (rarely eight) spores per ascus to be a diagnostic feature of *C. hexaspora*. Although this character was even reflected in the epithet "*hexaspora*", six-spored asci were observed in numerous Teloschistaceae species (e.g. Vondrák *et al.* 2020). Probably both *C. lacinulata* and *C. hexaspora* are closely related to *L. aureopruinosa*, however we are not able to prove it due to lack of the molecular data for these two poorly known species.

Sometimes *L. aureopruinosa* could be confused with species of the genus *Rufoplaca*, which have similar ecology. In case of doubt several (at least ten) spores in more than one apothecia should be measured – septa thickness of *Rufoplaca* usually do not exceed 3.5 μm. Also apothecia of *Rufoplaca* do not bear characteristic epipsamma.

*Ecology and distribution. Lendemeriella aureopruinosa* grows on siliceous outcrops mainly in shady conditions of forest belt in mountains, but also on seashore and in alpine belt above timberline at altitudes from about 5 m to 1590 m above sea level. Co-occurring lichen taxa include *Calogaya arnoldii* (Wedd.) Arup *et al.*, *Caloplaca atroflava* (Turner) Mong., *Lecanora campestris* (Schaer.) Hue, *Leptogium saturninum* (Dicks.) Nyl., *Rhizocarpon petraeum* (Wulfen) A. Massal., *Rhizoplaca subdiscrepans* (Nyl.) R. Sant., *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt, *R. sorediata* (Vain.) S.Y. Kondr. & Kärnefelt, etc. The taxon seems to be quite common in Eastern Siberia (Yakutia and Trans-Baikal Territory, Russia) and the Russian Far East (Khabarovsk Territory, Primorye Territory, and Sakhalin Region). The known localities are marked on Fig. 3.

*Additional material studied*. **Russia: *Khabarovsk Territory:*** Khabarovsk District, Bolshekhekhtsirsky Nature Reserve, near lodge "kordon Bykovka", 48°14'31.9"N, 134°47'57.1"E, 559 m, 2018, *S. Chesnokov* 211 (LE-L15212); Mt. Bolshoy Khekhtsir,

48°13'11.2"N, 134°46'53.5"E, 934 m, 2018, *L. Konoreva* 399 (LE-L15211); ***Primorye Territory:*** Terney District, Sikhote-Alin, 50 km WNW of Amgu, 46°01'54"N, 137°06'58"E, 495 m, 2014, *E. Davydov* 17247 & *L. Yakovchenko*. ***Sakhalin Region, Sakhalin Island:*** Dolinsk District, 19 km SE of Dolinsk, Cape Ostryj, 47°15'03.9"N, 143°01'03.8"E, 5 m, 2019, *I. Frolov* 2473; SE outskirts of Yuzhno-Sakhalinsk, Mt Medika, 46°54'3.6"N, 142°51'17.5"E, 730 m, 2019, *I. Frolov* 2474; Makarov District, c. 1 km W of Zaozyornoe, 48°21'56.6"N, 142°39'29.6"E, 30 m, 2019, *I. Frolov* 2475 (duplicates will be send to PRA and LD); ***Republic of Sakha (Yakutia):*** Aldan District, Yllymakh, left bank of river Bol'shoy Yllymakh, 58°35'2"N, 126°41'54"E, 357 m, 2015, *L. Konoreva* 431 (LE-L15208); Neryungri District, Iyengra, left bank of river Timpton, near road A-360 from Iyengra to Tynda, 55°57'15"N, 124°55'12"E, 843 m, 2015, *L. Konoreva* 73, 68 (LE-L15209, LE-L15215); ***Trans-Baikal Territory:*** Kalarsky District, Kodar Mountains, Novaya Chara, canyon of the first brook to W of river Anarga, 56°55'10"N, 118°00'04"E, 1592 m, 2013, *L. Konoreva* 230, 239 (LE-L15213, LE-L13214); left bank of river Khadytkanda, 56°44'53.3"N, 117°15'54.0"E, 1229 m, 2015, *L. Konoreva* 284 (LE-L15210).

***Orientophila infirma*** **I.V. Frolov, Vondrák, Konoreva & S. Chesnokov sp. nov.**
MycoBank No.: MB833716

Thallus endophloedal or sometimes consists of tiny inconspicuous scattered orange areoles. Apothecia 0.2–0.3 mm in diameter, zeorine, usually scattered or sometimes more or less crowded and contiguous; disc orange or sometimes yellow in young apothecia; thalline exciple on underside of apothecia and usually inconspicuous. Ascospores 10–13 × 5–7 μm, with septa 4–5 μm width. Pycnidia immersed between fibers of substrate. Conidia ellipsoid to bacilliform.

Type: Russia. Republic of Sakha (Yakutia), Oymyakon District, Ust-Nera, 1005$^{th}$ km of R504 Kolyma Highway, brook Egelyakh (left tributary of river Nera), alt. 541 m, 64°28'21.2"N, 143°52'25.0"E, on bark of *Larix gmelinii* in *L. gmelinii* forest with *Vaccínium vitis-idaea*, lichens and mosses, 5 July 2016, *L. Konoreva* J-003 (LE-L15194—holotype; H—isotype). GenBank accession numbers of the sequences of the holotype: MN814235 (ITS), MW227507 (LSU), MW227329 (mrSSU).

(Figs 2G,H and Fig. 16D in Vondrák *et al.* 2019)

*Thallus* endophloedal (endoxylic) or sometimes represented by tiny inconspicuous scattered orange areoles about 0.10–0.16 mm in diameter. *Vegetative diaspores* absent. *Prothallus* absent.

*Apothecia* (0.13–)0.19–0.24–0.31(–0.40) mm in diameter [68, 7, 0.06], zeorine, sessile, usually scattered or sometimes more or less crowded and contiguous. *Disc* orange or in young apothecia sometimes yellow; *thalline exciple* occurs on underside of apothecia and usually inconspicuous, but sometimes visible as outer yellow rim,

paler then other parts of apothecium; *true exciple orange*, of the same colour as disk or paler, in young apothecia sometimes yellow. *Hymenium* (63–)74–78–81(–88) µm high [26, 6, 6], colourless, not glutinized, without extracellular oil drops and crystals; *epihymenium* golden brown. *Hypothecium* colourless, delimited by algal layer from below and without central conical extension downward, with small amount of extracellular oil drops, without extracellular crystals, (10–)18–27–36(–50) µm high [26, 6, 10]; formed of thin-walled cells variable in shape and orientation. *Exciple* about 15–55 µm wide, formed of true exciple, (15–)19–28–38(–53) µm wide [26, 6, 8], and thalline exciple, (28–)44–50–58(–100) µm wide [26, 6, 15]. Upper part of *true exciple* golden brown of thin-walled ± spherical cells (5.0–)6.0–6.6–7.1(–8.0) µm in diameter [51, 6, 0.9]. *Thalline exciple* with cortex which is usually alveolate, (8–)11–14–17(–23) µm wide [26, 6, 4]; cells of cortex thin-walled ± spherical (4.0–)5.2–6.6–6.9(–9.0) µm in diameter [36, 5, 1.1]. Cortex of thalline exciple sometimes with epinecral layer up to 6 µm wide. *Paraphyses* about 2 µm wide in lower part, 2–3 upper cells significantly increased and the widest upper cell (4.5–)5.3–6.0–6.3(–7.0) µm wide [51, 6, 0.7]; often branched in upper part. *Asci* clavate, (35–)41–45–49(–58) × (11–)12–14–16(–18) µm [33, 6, 5 & 2]. *Ascospores* 8 per ascus, colourless, polarilocular, (9.0–)10.1–11.0–12.3(–13.0) × (4.5–)5.3–5.6–7.3(–8.0) µm [51, 7, 1.0 & 0.6], with rounded ends. Septa (3.5–)3.9–4.4–5.3(–5.5) µm [51, 7, 0.6]. Ascospore length/width ratio: (1.63–)1.70–1.98–2.13(–2.56) [51, 7, 0.21]; septum width/ascospore length ratio: (0.29–)0.34–0.40–0.43(–0.50) [51, 7, 0.05].

*Pycnidia* immersed between fibres of substrate, of the same colour with apothecial disks, about 55–85 µm wide. Conidia ellipsoid to bacilliform, (2.5–)2.9–3.2–3.5(–4.0) × (1.0–)1.5–1.7–2.0(–2.0) µm [20, 2, 0.4 & 0.3].

*Chemistry.* Epihymenium, upper true exciple, cortex of thalline exciple, pycnidia and areoles with anthraquinones, K+ purple. Apothecia (specimen LE-L15193) contain parietin, parietinic acid, emodin, teloschistin and fallacinal, which corresponds to chemosyndrome A of Søchting (1997).

*Etymology.* The epithet reflects “weak” habitus of the lichen: often it consists of tiny scattered apothecia, which could be overlooked in cracks of bark.

*Phylogeny and taxonomic position.* The new species is nested within the subfamily *Xanthorioideae*. It demonstrates the closest relationships to *Orientophila* although forms an outgroup to all species of the genus currently available at NCBI (Fig. 1, Supplementary Material Fig. S3, available online, and Fig. 2 in Vondrák *et al.* 2019). Phenotypically *Orientophila* is very similar to species of the genera *Flavoplaca* and *Athallia* (Arup *et all.* 2013) and the new species can be assigned to any of these genera based on its morphology and chemistry alone. All known *Orientophila* species are related to the seacoast in Far East Asia, whereas *O. infirma* occurs in boreal inland

localities in Siberia. Nevertheless, to avoid taxonomical complications and considering our molecular data we decided to describe the new species in the genus *Orientophila*.

*Similar taxa.* The new taxon resembles some species of the genus *Athallia* as well as *Caloplaca ahtii* and *Lendemeriella borealis* (Vain.) S.Y. Kondr.; however, it is well characterized by its DNA sequences and some morphological and chemical differences. *Athallia cerinella* (Nyl.) Arup *et al.* has 12–16 spores per ascus. Apothecia of *A. cerinelloides* (Erichsen) Arup *et al.* are usually crowded in distinct small groups, are paler, yellow to orange-yellow and its hymenium is thinner, 55–70 µm (Arup 2009). *Athallia holocarpa* (Hoffm.) Arup *et al.* usually grows on rocks or stones, but sometimes occurs on wood and in this case could be confused with *Orientophila infirma*, however the former species has significantly larger apothecia, about 0.7 mm, up to 1 mm in diameter and thicker hypothecium, about 50–80 µm (Arup 2009). The similar *A. pyracea* has significantly larger apothecia, up to 1 mm in diameter, thicker hypothecium (70–100 µm) and the greyish thalline exciple, contrasting with orange apothecial disk; spores of *A. pyracea* are a bit longer, up to 15.5 µm (Arup 2009). *Caloplaca ahtii* usually has small grey crater-like soralia, but rarely soralia are inconspicuous or even absent and it could be confused with *O. infirma*. The former species, however, has paler yellow-orange apothecia, its young apothecia usually have conspicuous ring of thin grey thalline exciple. The pale morphotype of *Lendemeriella borealis* (see Frolov & Konoreva 2016) also resembles *O. infirma*, however it usually has well-developed whitish areolate thallus, and paler, orange-yellow apothecia with greyish to grey proper exciple containing Cinereorufa-green.

*Ecology and distribution. Orientophila infirma* grows on bark of trunks, branches and small twigs of coniferous (*Juniperus* sp., *Larix gmelinii*, *Picea obovata*) and deciduous trees and shrubs (*Populus tremula*, *Salix* spp.), once recorded on wood. It mainly occurs in light coniferous forests and floodplain forests or on solitary trees and shrubs on rocky outcrops and stone runs in taiga or rarely in forest-steppe at altitudes from 165 m to 1120 m above sea level. Co-occurring lichen taxa include *Athallia pyracea*, *Caloplaca ahtii*, *C. cerina* (Hedw.) Th. Fr., *C. saviczii*, *Parmelia sulcate* Taylor, *Physcia aipolia* (Ehrh. ex Humb.) Fürnr. etc. It is known from Eastern Siberia (Yakutia, Russia, where it seems to be quite common) and Southern Siberia (the only locality from the Republic of Tuva, Russia). The known localities are marked on Fig. 3.

*Remarks.* The species was listed for Altai-Sayan region by Vondrák *et al.* (2019) as "unknown '*Caloplaca*' sp.", specimen J. Vondrák 18687.

*Additional material studied.* **Russia: *Republic of Sakha (Yakutia)*:** Aldan District, Aldan, river Bol'shoy Kuranakh, 58°39'48.1"N, 125°29'8.9"E, 464 m, 2015, *S. Chesnokov* 58 (LE-L13373); Mt Skarnovy gol'ets, river Turuk, 58°32'47.5"N, 125°36'15.6"E, 732 m, 2015, *S. Chesnokov* 79 (LE-L15197); Bol'shoy Nimnyr, left

bank of river Bol'shoy Nimnyr, 58°02'19.4"N, 125°29'54.4"E, 863 m, 2015, *S. Chesnokov* 113 (LE-L15196, LE-L15221); Tommot, river Kurung, 58°44'29"N, 126°21'5"E, 470 m, 2015, *L. Konoreva* 351 (LE-L15219 in LE-L15218); Tommot, left bank of river Aldan, 58°55'34"N, 126°18'6"E, 409 m, 2015, *L. Konoreva* (LE-L15198); right bank of river Aldan, 58°28'31.6"N, 129°10'51.4"E, 220 m, 2015, *S. Chesnokov* 227 (LE-L15195); Tomponsky District, R504 Kolyma Highway, pass "prizhim Zayachya Petlya", 63°07'44.7"N, 139°14'54.6"E, 1017 m, 2016, *L. Konoreva* J-238 (LE-L15199); Ust-Maya District, Allakh-Jun', river Ot-Jurjakh, 61°12'35.2"N, 138°00'50.7"E, 791 m, 2017, *L. Konoreva* 215 (LE-L15193, duplicates will be send to PRA and LD); Petropavlovsk, right bank of river Aldan, 60°16'59.8"N, 134°20'00.1"E, 165 m, 2017, *L. Konoreva* 262 (LE-L15200); ***Republic of Tuva*:** Ak-Dovurak, Alash, 2 km SE of village Ak-Sug, in valley of river Mungash-Ak, 51°22'57"N, 90°28'04"E, 1120 m, 2013, *I. Frolov & J. Vondrák* 18687 (PRA).

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Table. GenBank accession numbers of the new sequences obtained in this study.

| Species | nrITS GenBank No. | nrLSU GenBank No. | mrSSU GenBank No. | Location and source |
|---|---|---|---|---|
| *"Caloplaca" obscurella* | MH104938 | MH100762 | – | Krasnodar Territory, Russia, PRA JV7641 |
| *C. saviczii* 1 | MN814226 | MW227509 | MW227327 | Kamchatka, Russia, holotype |
| *C. saviczii* 2 | MN814227 | MW227510 | MW227326 | Sakhalin, Russia, Frolov 2472 |
| *C. saviczii* | MN814223 | – | – | Yakutia, Russia, LE-L15218 |
| *C. saviczii* | MN814224 | – | – | Yakutia, Russia, LE-L15217 |
| *C. saviczii* | MN814225 | – | – | Kamchatka, Russia, LE-L15220 |
| *Lendemeriella aureopruinosa* 1 | MN814228 | MW227504 | MW227332 | Yakutia, Russia, holotype |
| *L. aureopruinosa* 2 | MN814229 | MW227511 | MW227333 | Yakutia, Russia, LE-L15209 |
| *L. aureopruinosa* 3 | MN814234 | MW227505 | MW227331 | Sakhalin, Russia, Frolov 2473 |
| *L. aureopruinosa* | MN814232 | – | – | Khabarovsk Territory, Russia, LE-L15211 |
| *L. aureopruinosa* | MG954210 | – | – | Primorye Territory, Russia, Davydov 17247 |
| *L. aureopruinosa* | MN814233 | – | – | Sakhalin, Russia, Frolov 2475 |
| *L. aureopruinosa* | MG954213 | – | – | Trans-Baikal Territory, Russia, LE-L15214 |
| *L. aureopruinosa* | MG954214 | – | – | Trans-Baikal Territory, Russia, LE-L15213 |
| *L. aureopruinosa* | MN814230 | – | – | Trans-Baikal Territory, Russia, LE-L15210 |
| *L. aureopruinosa* | MN814231 | – | – | Khabarovsk Territory, Russia, LE-L15212 |
| *L. borealis* | MW227317 | MW227512 | MW227334 | Chelyabinsk Region, Russia, Frolov 2476 |
| *Orientophila infirma* 1 | MN814235 | MW227507 | MW227329 | Yakutia, Russia, holotype |
| *O. infirma* 2 | MN814238 | MW227506 | MW227330 | Yakutia, Russia, LE-L15193 |
| *O. infirma* 3 | MN814236 | MW227508 | MW227328 | Yakutia, Russia, LE-L15196 |
| *O. infirma* | 237 | – | – | Yakutia, Russia, LE-L15198 |

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|---|---|---|---|---|
| | MG954 | | | Tyva Republic, Russia, Vondrák *et* |
| *O. infirma* | 157 | – | – | *al.* (2019), PRA JV18687 |
| *Pyrenodesmia* | KC8844 | | | Greece, Frolov *et al.* (2016), PRA |
| *chalybaea* | 98 | MH100747 | MH100779 | JV4059 |

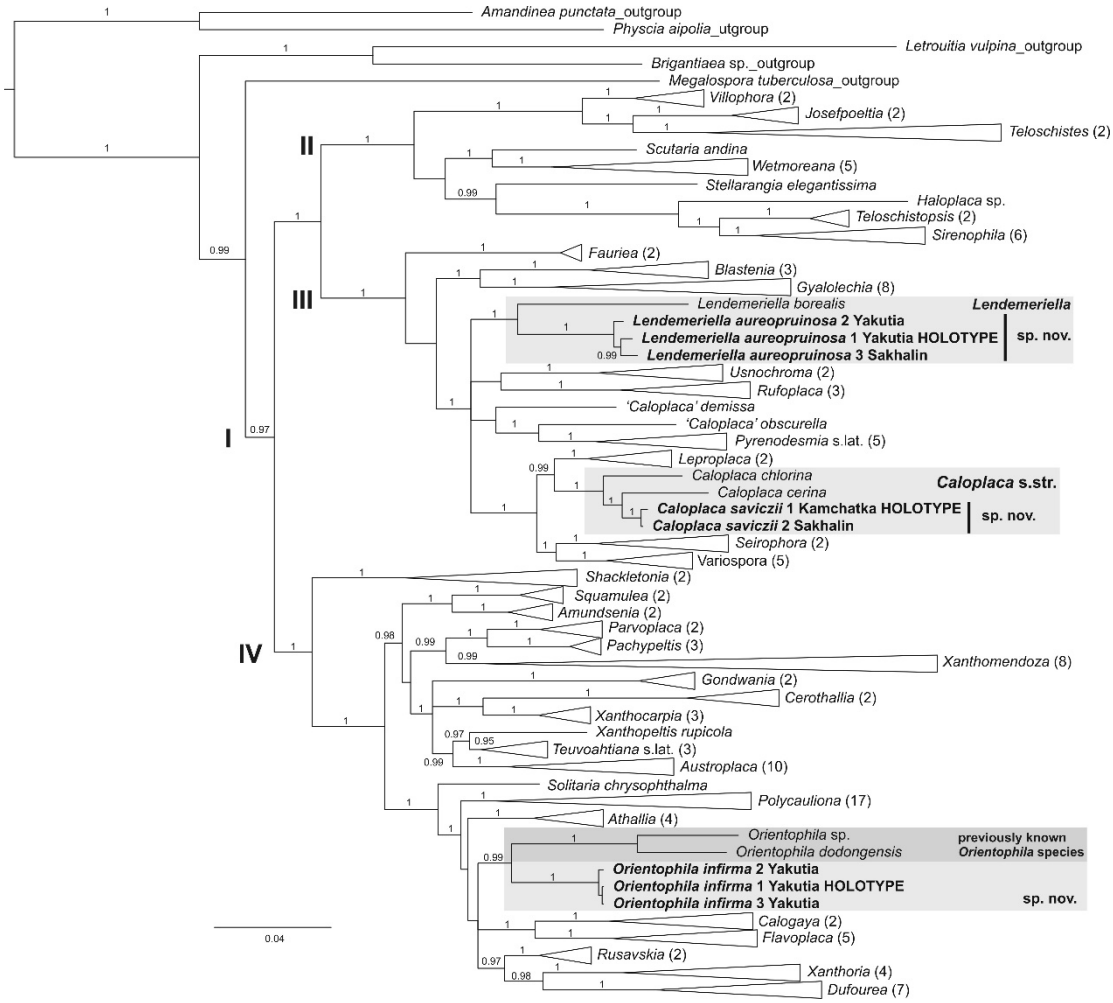


Fig. 1. Phylogeny of the family Teloschistaceae based on the combined Bayesian analysis of nrITS, nrLSU and mrSSU data. Genera are collapsed into single terminals. Numbers at branches represent posterior probability values ≥ 0.95. Numbers in parentheses correspond to the number of species of a genus used in the analysis. I – *Teloschistaceae*; II – *Teloschistoideae*; III – *Caloplacoideae*; IV – *Xanthorioideae*.

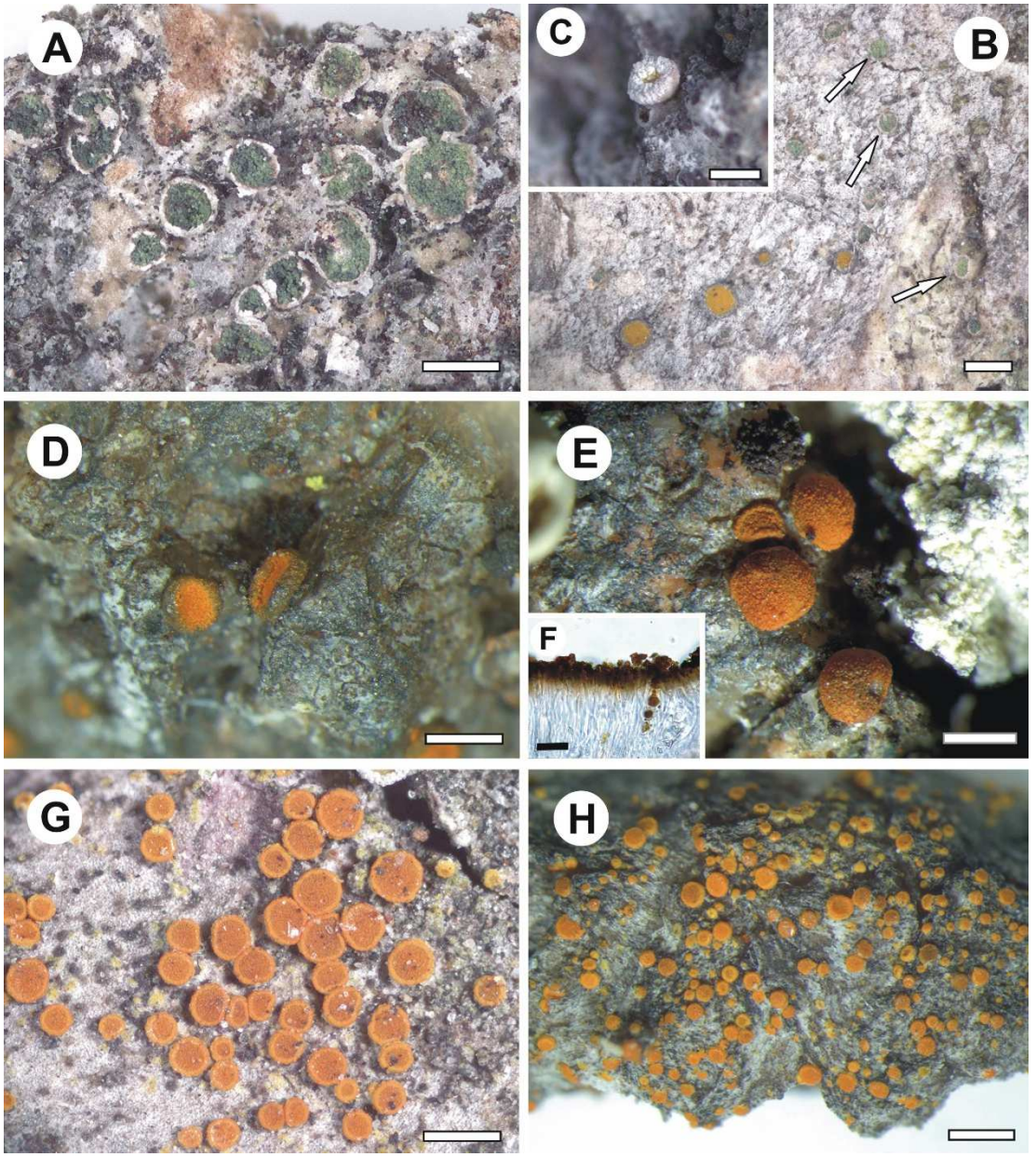


**Fig. 2.** A, *Caloplaca saviczii*, crowded crater-like soralia (holotype); B, *C. saviczii*, apothecia and scattered crater-like soralia (some of them are pointed out with arrows; isotype); C, *C. saviczii*, a young apothecium with thick white pruina and a soralium on the same areole (holotype). D, *Lendemeriella aureopruinosa*, apothecia with grey true exciple (holotype); E, *L. aureopruinosa*, apothecia with true exciple of the same colour as disk (holotype); F, *L. aureopruinosa*, epithecium, overlain with granules of epipsamma; G, *Orientophila infirma* (holotype); H, *O. infirma* (LE-L15193). Scales: A & C = 0.2 mm; B, D, E, G & H = 0.5 mm; F = 15 μm.

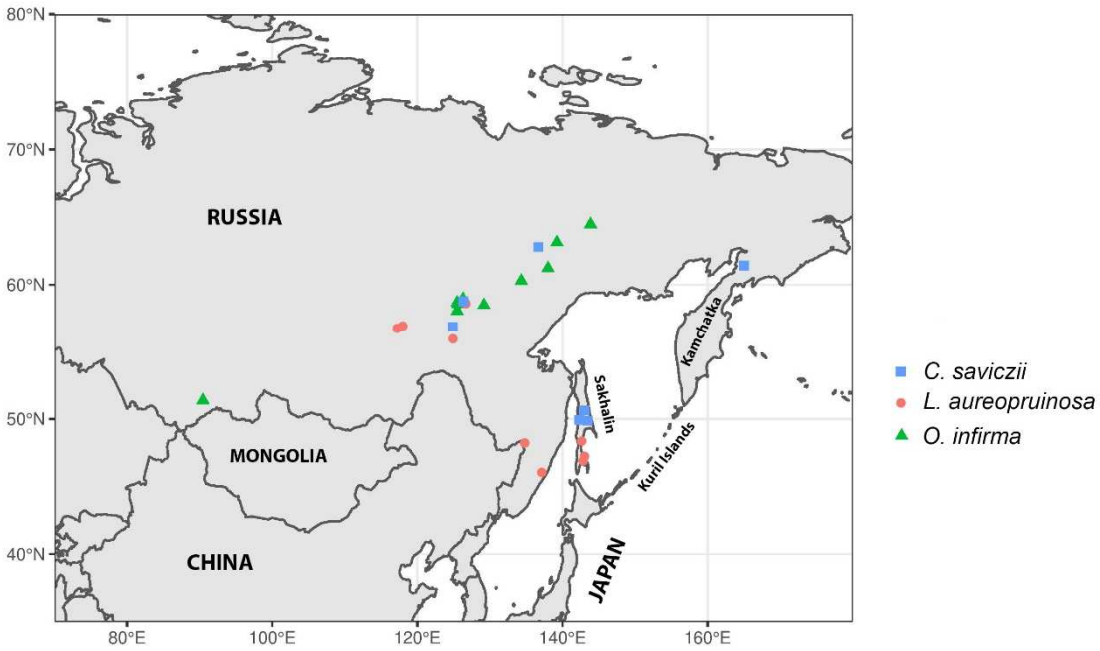


**Fig. 3.** Known localities of the newly described *Caloplaca saviczii*, *Lendemeriella aureopruinosa* and *Orientophila infirma*.

## Supplementary Material.

Supplementary Material Table S. GenBank accession numbers and accompanying information for the downloaded sequences used in the study. Specimens with two-three sequences were used in the combined analysis.

| Species | ITS GenBank No. | LSU GenBank No. | mtSSU GenBank No. | Source |
|---|---|---|---|---|
| *Amandinea punctata* | AF250780 | AY340536 | AY143399 | Arup *et al.* (2013) |
| *Amundsenia approximata* | KJ789965 | KJ789972 | KJ789974 | Søchting *et al.* (2014) |
| *A. austrocontinentalis* | JX036068 | – | KJ789975 | Søchting *et al.* (2014) |
| *Athallia baltistanica* | KR921581 | – | – | Vondrák *et al.* (2016) |
| *A. baltistanica* | KR902665 | – | – | Vondrák *et al.* (2016) |
| *A. cerinella* | FJ346537 | – | – | Arup (2009) |
| *A. cerinella* | FJ346538 | – | – | Arup (2009) |
| *A. cerinelloides* | KC179339 | KC179147 | KC179477 | Arup *et al.* (2013) |

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|---|---|---|---|---|
| *A. cerinelloides* | HM582153 | – | – | Vondrák *et al.* (2012) |
| *A. cerinelloides* | KU926977 | – | – | Vondrák *et al.* (2017) |
| *A. holocarpa* | FJ346539 | – | – | Arup (2009) |
| *A. holocarpa* | FJ346541 | – | – | Arup (2009) |
| *A. holocarpa* | FJ346540 | KC179148 | KC179478 | Arup *et al.* (2013) |
| *A. pyracea* | FJ346553 | KC179149 | KC179479 | Arup *et al.* (2013) |
| *A. scopularis* | KC179340 | KC179150 | KC179480 | Arup *et al.* (2013) |
| *Austroplaca ambitiosa* | KC179081 | KC179151 | KC179481 | Arup *et al.* (2013) |
| *A. cirrochrooides* | KC179082 | KC179152 | KC179482 | Arup *et al.* (2013) |
| *A. darbishirei* | KC179083 | KC179153 | KC179483 | Arup *et al.* (2013) |
| *A. hookeri* | KC179085 | KC179154 | KC179484 | Arup *et al.* (2013) |
| *A. lucens* | KC179087 | KC179155 | KC179485 | Arup *et al.* (2013) |
| *A. millegrana* | KC179088 | KC179156 | KC179486 | Arup *et al.* (2013) |
| *A. soropelta* | KC179089 | KC179157 | KC179487 | Arup *et al.* (2013) |
| *Austroplaca* sp. 1 | KC179093 | KC179160 | KC179490 | Arup *et al.* (2013) |
| *Austroplaca* sp. 2 | KC179090 | KC179158 | KC179488 | Arup *et al.* (2013) |
| *Austroplaca* sp. 3 | KC179092 | KC179159 | KC179489 | Arup *et al.* (2013) |
| *Blastenia ammiospila* | KC179413 | KC179161 | KC179491 | Arup *et al.* (2013) |
| *B. crenularia* | KC179415 | KC179162 | KC179492 | Arup *et al.* (2013) |
| *B. ferruginea* | KC179416 | KC179163 | KC179493 | Arup *et al.* (2013) |
| *Brigantiaea* sp. | KC179419 | KC179164 | KC179494 | Arup *et al.* (2013) |
| *Calogaya arnoldii* s.lat. | KC179343 | KC179166 | KC179497 | Arup *et al.* (2013) |
| *C. decipiens* | KC179344 | KC179167 | KC179498 | Arup *et al.* (2013) |
| *"Caloplaca" atroflava* | KC179424 | KC179171 | KC179504 | Arup *et al.* (2013) |
| *C. cerina* | KC179425 | KC179168 | KC179499 | Arup *et al.* (2013) |
| *C. cerina* | HM538546 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538547 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538548 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538542 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538543 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538544 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538545 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538485 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | EU365861 | – | – | Vondrák *et al.* (2008) |
| *C. cerina* | HM538486 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538539 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538540 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538541 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | AF353958 | – | – | Arup & Grube (1999) |

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|---|---|---|---|---|
| *C. cerina* | EU681283 | – | – | Fedorenko *et al.* (2009) |
| *C. cerina* | HM538475 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538476 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538477 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538478 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538471 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538472 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538473 | – | – | Šoun *et al.* (2011) |
| *C. cerina* | HM538474 | – | – | Šoun *et al.* (2011) |
| *C. chlorina* | KC179426 | KC179169 | KC179500 | Arup *et al.* (2013) |
| *C. chlorina* | EU365858 | – | – | Vondrák *et al.* (2008) |
| *C. chlorina* | HM538518 | – | – | Šoun *et al.* (2011) |
| *C. chlorina* | HM538520 | – | – | Šoun *et al.* (2011) |
| *"C." demissa* | AF353960 | KC179172 | KC179505 | Arup *et al.* (2013) |
| *"C." erythrocarpa* | KC179427 | KC179173 | KC179506 | Arup *et al.* (2013) |
| *C. fluviatilis* | MG954127 | – | – | Vondrák *et al.* (2019) |
| *C. fluviatilis* | MG954128 | – | – | Vondrák *et al.* (2019) |
| *C. hanneshertelii* | HM538483 | – | – | Šoun *et al.* (2011) |
| *C. isidiigera* | HM538532 | – | – | Šoun *et al.* (2011) |
| *C. isidiigera* | HM538534 | – | – | Šoun *et al.* (2011) |
| *C. isidiigera* | HM538538 | – | – | Šoun *et al.* (2011) |
| *C. monacensis* | HM538506 | – | – | Šoun *et al.* (2011) |
| *C. monacensis* | HM538507 | – | – | Šoun *et al.* (2011) |
| *C. monacensis* | HM538508 | – | – | Šoun *et al.* (2011) |
| *C. monacensis* | HM538502 | – | – | Šoun *et al.* (2011) |
| *C. pinicola* | HM538479 | – | – | Šoun *et al.* (2011) |
| *C.* aff. *pinicola* | HM538480 | – | – | Šoun *et al.* (2011) |
| *C.* aff. *pinicola* | HM538482 | – | – | Šoun *et al.* (2011) |
| *C. sterilis* | HM538528 | – | – | Šoun *et al.* (2011) |
| *C. sterilis* | HM538530 | – | – | Šoun *et al.* (2011) |
| *C. sterilis* | HM538531 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538569 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538570 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538573 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538470 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538466 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538468 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538461 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | EU365860 | – | – | Vondrák *et al.* (2008) |
| *C. stillicidiorum* | HM538462 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538456 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538459 | – | – | Šoun *et al.* (2011) |

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|---|---|---|---|---|
| *C. stillicidiorum* | HM538558 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538559 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538560 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538561 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538562 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538565 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538567 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538568 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538550 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538556 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538557 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538554 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538551 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538552 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538574 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538575 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538576 | – | – | Šoun *et al.* (2011) |
| *C. stillicidiorum* | HM538577 | – | – | Šoun *et al.* (2011) |
| *C. subalpina* | EU365854 | – | – | Vondrák *et al.* (2008) |
| *C. subalpina* | EU365853 | – | – | Vondrák *et al.* (2008) |
| *C. subalpina* | EU365855 | – | – | Vondrák *et al.* (2008) |
| *"C." teicholyta* | KC179431 | KC179176 | KC179510 | Arup *et al.* (2013) |
| *C. thracopontica* | EU365848 | – | – | Vondrák *et al.* (2008) |
| *C. thracopontica* | HM538525 | – | – | Šoun *et al.* (2011) |
| *C. thracopontica* | HM538527 | – | – | Šoun *et al.* (2011) |
| *C. thracopontica* | HM538524 | – | – | Šoun *et al.* (2011) |
| *C.* aff. *thracopontica* | HM538523 | – | – | Šoun *et al.* (2011) |
| *C. turkuensis* | HM538487 | – | – | Šoun *et al.* (2011) |
| *C. turkuensis* | HM538489 | – | – | Šoun *et al.* (2011) |
| *C. turkuensis* | HM538490 | – | – | Šoun *et al.* (2011) |
| *C. ulmorum* | HM538509 | – | – | Šoun *et al.* (2011) |
| *C. ulmorum* | HM538510 | – | – | Šoun *et al.* (2011) |
| *C. ulmorum* | HM538511 | – | – | Šoun *et al.* (2011) |
| *Cerothallia luteoalba* | KC179099 | KC179177 | KC179511 | Arup *et al.* (2013) |
| *C. yorkensis* | KC179101 | KC179178 | KC179513 | Arup *et al.* (2013) |
| *Dufourea alexerbaai* | KC179350 | KC179179 | KC179514 | Arup *et al.* (2013) |
| *D. angustata* | KC179351 | KC179180 | KC179515 | Arup *et al.* (2013) |
| *D. bonae-spei* | KC179353 | KC179181 | KC179516 | Arup *et al.* (2013) |
| *D. dissectula* | KC179355 | KC179182 | KC179517 | Arup *et al.* (2013) |
| *D. flammea* | KC179357 | KC179183 | KC179518 | Arup *et al.* (2013) |
| *D. karrooensis* | KC179358 | KC179184 | KC179519 | Arup *et al.* (2013) |
| *D. ligulata* | KC179359 | KC179185 | KC179520 | Arup *et al.* (2013) |

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| *Fauriea chujaensis* | KX793097 | KX793100 | KX793103 | Kondratyuk *et al.* (2016) |
| *F. orientochinensis* 1 | KX793096 | KX793099 | KX793102 | Kondratyuk *et al.* (2016) |
| *F. orientochinensis* 2 | KX793095 | KX793098 | KX793101 | Kondratyuk *et al.* (2016) |
| *Flavoplaca citrina* | DQ173224 | KC179186 | KC179521 | Arup *et al.* (2013) |
| *F. citrina* | KT934387 | – | – | Vondrák & Malíček (2015) |
| *F. marina* | AF353946 | KC179187 | KC179522 | Arup *et al.* (2013) |
| *F. microthallina* | KC179368 | KC179188 | KC179523 | Arup *et al.* (2013) |
| *F. oasis* | FJ346546 | KC179189 | KC179524 | Arup *et al.* (2013) |
| *F. oasis* | MG954199 | – | – | Vondrák *et al.* (2019) |
| *Flavoplaca* sp. | KC179370 | KC179190 | KC179525 | Arup *et al.* (2013) |
| *Gallowayella weberi* | KC179145 | KC179285 | KC179625 | Arup *et al.* (2013) |
| *Gondwania cribrosa* | KC179102 | KC179192 | KC179526 | Arup *et al.* (2013) |
| *G. regalis* | KC179103 | KC179193 | KC179527 | Arup *et al.* (2013) |
| *Gyalolechia arizonica* | KC179433 | KC179195 | KC179529 | Arup *et al.* (2013) |
| *G. aurea* | KC179434 | KC179196 | KC179530 | Arup *et al.* (2013) |
| *G. flavorubescens* | KC179439 | KC179197 | KC179531 | Arup *et al.* (2013) |
| *G. flavovirescens* | AF353966 | KC179198 | KC179532 | Arup *et al.* (2013) |
| *G. fulgens* | KC179440 | KC179199 | KC179533 | Arup *et al.* (2013) |
| *G. gomerana* | KC179441 | KC179200 | KC179534 | Arup *et al.* (2013) |
| *G. stantonii* | KC179445 | KC179201 | KC179535 | Arup *et al.* (2013) |
| *G. stipitata* | KC179446 | KC179202 | KC179536 | Arup *et al.* (2013) |
| *Haloplaca* sp. | KC179295 | KC179203 | KC179537 | Arup *et al.* (2013) |
| *Josefpoeltia parva* | KC179296 | KC179204 | KC179539 | Arup *et al.* (2013) |
| *J. sorediosa* | KC179297 | KC179205 | KC179540 | Arup *et al.* (2013) |
| *Lendemeriella borealis* | MG954129 | – | – | Vondrák *et al.* (2019) |
| *L. borealis* | KX216688 | – | – | Frolov & Konoreva (2016) |
| *L. borealis* | KX216687 | – | – | Frolov & Konoreva (2016) |
| *L. borealis* | KX216686 | – | – | Frolov & Konoreva (2016) |
| *L. exsecuta* | MG954227 | – | – | Vondrák *et al.* (2019) |
| *L. exsecuta* | MG954211 | – | – | Vondrák *et al.* (2019) |
| *L. exsecuta* | MG954224 | – | – | Vondrák *et al.* (2019) |
| *L. exsecuta* | MG954226 | – | – | Vondrák *et al.* (2019) |
| *L. exsecuta* | MG954130 | – | – | Vondrák *et al.* (2019) |
| *L. exsecuta* | MG954131 | – | – | Vondrák *et al.* (2019) |
| *L. exsecuta* | MG954223 | – | – | Vondrák *et al.* (2019) |

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|---|---|---|---|---|
| *L. exsecuta* | MG954225 | – | – | Vondrák *et al.* (2019) |
| *L. nivalis* | MG954222 | – | – | Vondrák *et al.* (2019) |
| *L. sorocarpa* | MG954132 | – | – | Vondrák *et al.* (2019) |
| *L. sorocarpa* | MG773658 | – | – | Vondrák *et al.* (2018) |
| *L. tornoensis* | MG954220 | – | – | Vondrák *et al.* (2019) |
| *L. tornoensis* | MG954221 | – | – | Vondrák *et al.* (2019) |
| *Leproplaca obliterans* | KC179449 | KC179207 | KC179541 | Arup *et al.* (2013) |
| *L. xantholyta* | KC179451 | KC179208 | KC179542 | Arup *et al.* (2013) |
| *Letrouitia vulpina* | KC179452 | KC179209 | KC179543 | Arup *et al.* (2013) |
| *Megalospora tuberculosa* | KC179453 | AY584650 | AY584623 | Arup *et al.* (2013) |
| *Orientophila dodongensis* | KC179373 | KC179211 | KC179545 | Arup *et al.* (2013) |
| *O. loekoesii* | KC179374 | – | – | Arup *et al.* (2013) |
| *O. subscopularis* | KC179375 | – | – | Arup *et al.* (2013) |
| *Orientophila* sp. | KC179372 | KC179210 | KC179544 | Arup *et al.* (2013) |
| *Pachypeltis invadens* | KC179108 | KC179212 | KC179548 | Arup *et al.* (2013) |
| *Pachypeltis* sp. 1 | KC179109 | KC179213 | KC179549 | Arup *et al.* (2013) |
| *Pachypeltis* sp. 2 | KC179110 | KC179214 | KC179550 | Arup *et al.* (2013) |
| *Parvoplaca tiroliensis* | KC179116 | KC179216 | KC179552 | Arup *et al.* (2013) |
| *Parvoplaca* sp. | KC179113 | KC179215 | KC179551 | Arup *et al.* (2013) |
| *Physcia aipolia* | AF250803 | AY300857 | AY143406 | Arup *et al.* (2013) |
| *Polycauliona celaria* | KC179379 | KC179217 | KC179553 | Arup *et al.* (2013) |
| *P. coralloides* | KC179380 | KC179218 | KC179554 | Arup *et al.* (2013) |
| *P. ignea* | KC179382 | KC179219 | KC179555 | Arup *et al.* (2013) |
| *P. luteominia* | KC179387 | KC179220 | KC179556 | Arup *et al.* (2013) |
| *P. phlogina* | DQ173235 | KC179221 | KC179557 | Arup *et al.* (2013) |
| *P. polycarpa* | KC179389 | KC179222 | KC179558 | Arup *et al.* (2013) |
| *P. rosei* | KC179390 | KC179223 | KC179559 | Arup *et al.* (2013) |
| *P. stellata* | KC179400 | KC179229 | KC179566 | Arup *et al.* (2013) |
| *P. tenax* | KC179401 | KC179230 | KC179567 | Arup *et al.* (2013) |
| *P. tenuiloba* | KC179402 | KC179231 | KC179568 | Arup *et al.* (2013) |
| *P. thamnodes* | KC179403 | KC179232 | KC179569 | Arup *et al.* (2013) |
| *P. verruculifera* | KC179404 | KC179233 | KC179570 | Arup *et al.* (2013) |
| *Polycauliona* sp. 1 | KC179392 | KC179224 | KC179560 | Arup *et al.* (2013) |
| *Polycauliona* sp. 2 | KC179393 | KC179225 | KC179561 | Arup *et al.* (2013) |
| *Polycauliona* sp. 3 | KC179394 | KC179226 | KC179562 | Arup *et al.* (2013) |
| *Polycauliona* sp. 4 | KC179396 | KC179227 | KC179563 | Arup *et al.* (2013) |
| *Polycauliona* sp. 5 | KC179397 | KC179228 | KC179564 | Arup *et al.* (2013) |
| *Pyrenodesmia variabilis* | AF353963 | KC179234 | KC179572 | Arup *et al.* (2013) |

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|---|---|---|---|---|
| *Rufoplaca scotoplaca* | KC179457 | KC179235 | KC179573 | Arup *et al.* (2013) |
| *R. tristiuscula* | KC179460 | KC179237 | KC179575 | Arup *et al.* (2013) |
| *Rufoplaca* sp. | KC179458 | KC179236 | KC179574 | Arup *et al.* (2013) |
| *Rusavskia elegans* | KC179406 | KC179238 | KC179576 | Arup *et al.* (2013) |
| *R. sorediata* | AY453647 | KC179239 | KC179577 | Arup *et al.* (2013) |
| *Scutaria andina* | KC179298 | KC179242 | KC179581 | Arup *et al.* (2013) |
| *Seirophora lacunosa* | KC179465 | KC179243 | KC179582 | Arup *et al.* (2013) |
| *S. scorigena* | KC179466 | KC179244 | KC179583 | Arup *et al.* (2013) |
| *Shackletonia hertelii* | KC179118 | KC179240 | KC179579 | Arup *et al.* (2013) |
| *S. sauronii* | KC179120 | KC179241 | KC179580 | Arup *et al.* (2013) |
| *Sirenophila bermaguiana* | KC179299 | KC179245 | KC179584 | Arup *et al.* (2013) |
| *S. eos* | KC179300 | KC179246 | KC179585 | Arup *et al.* (2013) |
| *S. gallowayii* | KC179301 | KC179247 | KC179586 | Arup *et al.* (2013) |
| *S. jackelixii* | KC179303 | KC179248 | KC179587 | Arup *et al.* (2013) |
| *S. maccarthyi* | KC179304 | KC179249 | KC179588 | Arup *et al.* (2013) |
| *Sirenophila* sp. | KC179306 | KC179250 | KC179589 | Arup *et al.* (2013) |
| *Solitaria chrysophthalma* | KC179408 | KC179251 | KC179590 | Arup *et al.* (2013) |
| *Squamulea squamosa* | KC179125 | KC179252 | KC179591 | Arup *et al.* (2013) |
| *S. subsoluta* | AF353954 | KC179253 | KC179592 | Arup *et al.* (2013) |
| *Stellarangia elegantissima* | KC179310 | KC179254 | KC179593 | Arup *et al.* (2013) |
| *Teloschistes flavicans* | KC179317 | KC179255 | KC179594 | Arup *et al.* (2013) |
| *T. hypoglaucus* | KC179319 | KC179256 | KC179595 | Arup *et al.* (2013) |
| *Teloschistopsis bonae-spei* | KC179322 | KC179257 | KC179596 | Arup *et al.* (2013) |
| *T. eudoxa* | KC179324 | KC179258 | KC179597 | Arup *et al.* (2013) |
| *Teuvoahtiana altoandina* | KC179094 | KC179170 | KC179503 | Arup *et al.* (2013) |
| *"Teuvoahtiana"* sp. 1 | KC179096 | KC179174 | KC179507 | Arup *et al.* (2013) |
| *"Teuvoahtiana"* sp. 2 | KC179098 | KC179175 | KC179508 | Arup *et al.* (2013) |
| *Usnochroma carphinea* | KC179468 | KC179259 | KC179598 | Arup *et al.* (2013) |
| *U. scoriophila* | KC179469 | KC179260 | KC179599 | Arup *et al.* (2013) |
| *Variospora aurantia* | KC179470 | KC179261 | KC179600 | Arup *et al.* (2013) |
| *V. aurantia* | AY233219 | – | – | Gaya *et al.* (2003) |
| *V. dolomiticola* | KC179471 | KC179262 | KC179601 | Arup *et al.* (2013) |
| *V. flavescens* | KC179473 | KC179263 | KC179602 | Arup *et al.* (2013) |
| *V. flavescens* | EU639601 | – | – | Gaya *et al.* (2008) |
| *V. glomerata* | KC179474 | KC179264 | KC179603 | Arup *et al.* (2013) |

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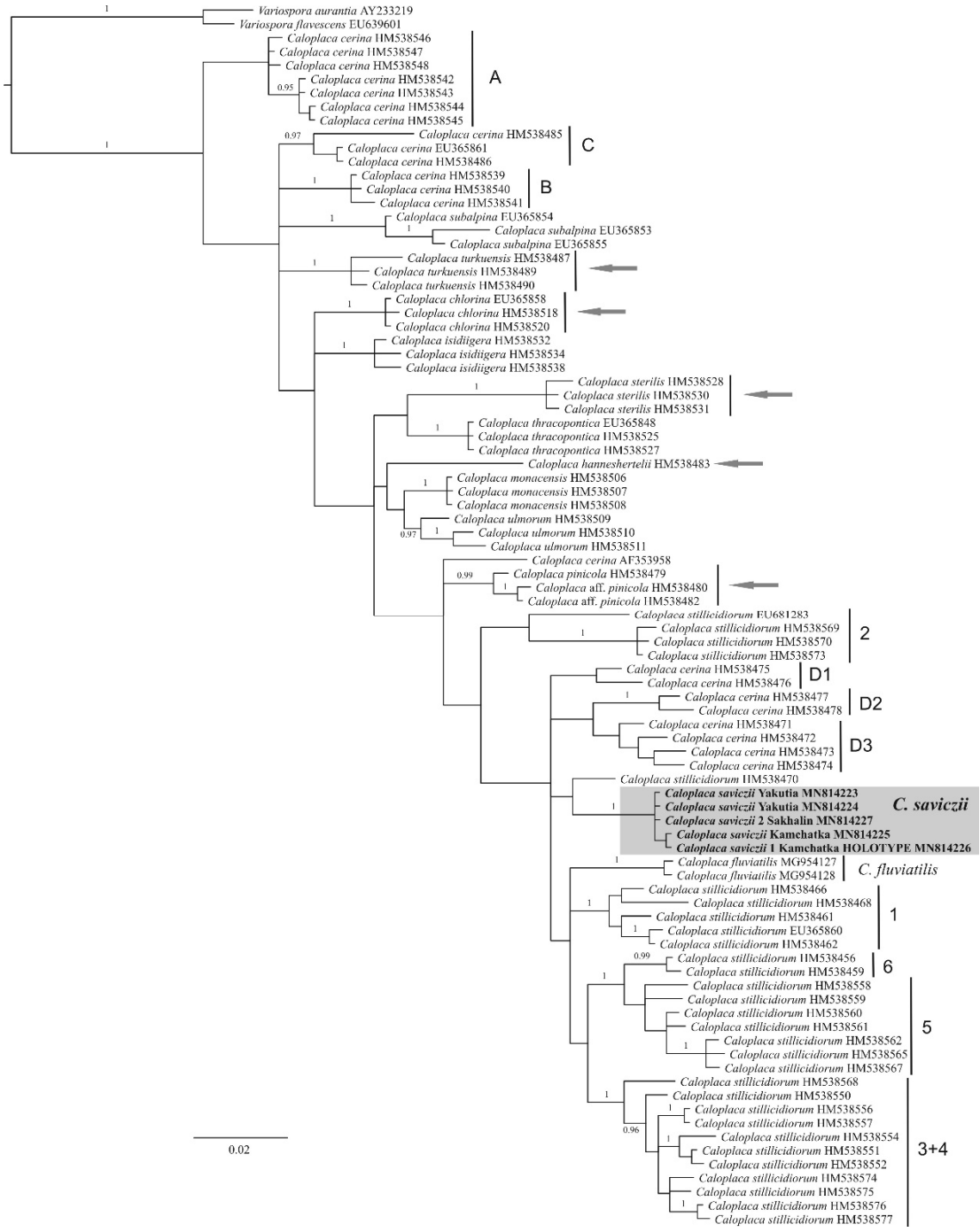
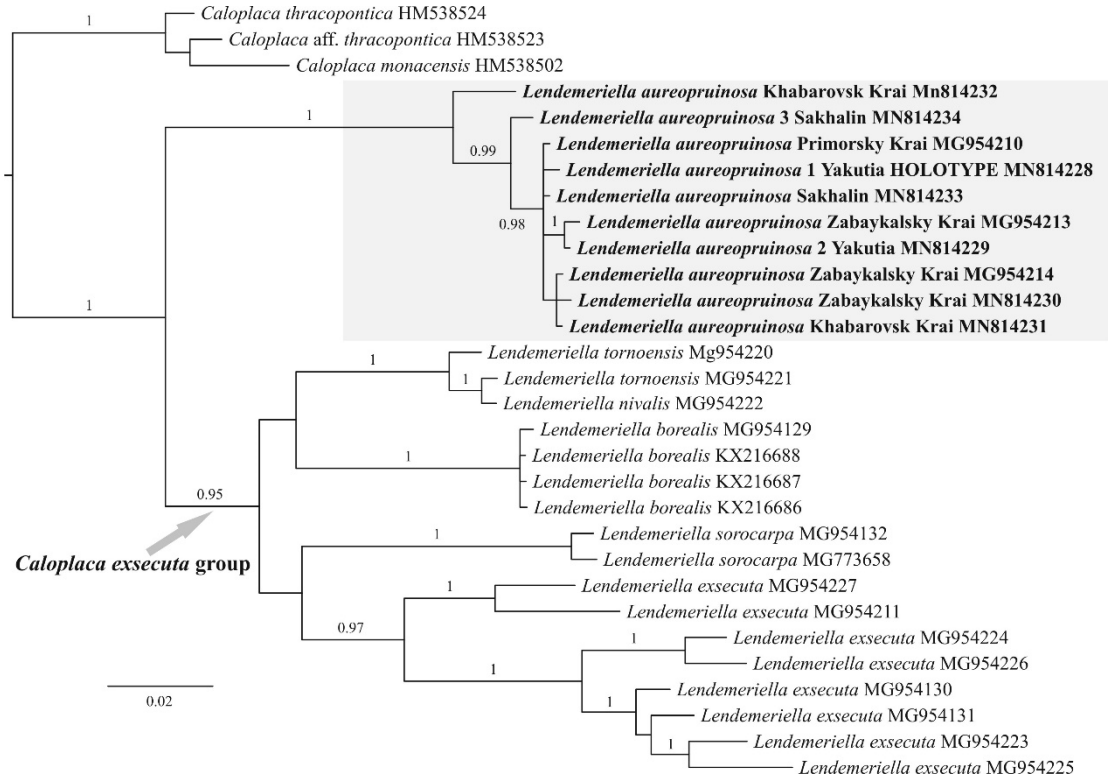
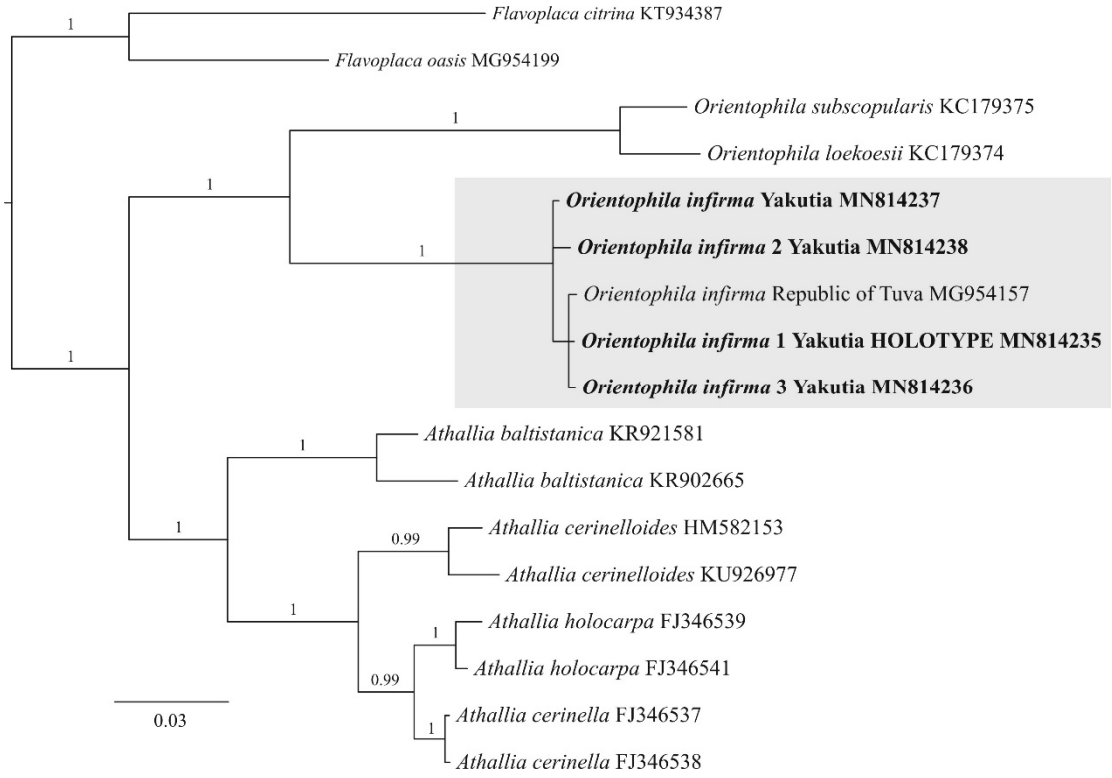
| *V. velana* | KC179476 | KC179265 | KC179605 | Arup *et al.* (2013) |
| *Villophora isidioclada* | KC179325 | KC179266 | KC179606 | Arup *et al.* (2013) |
| *Villophora* sp. | KC179328 | KC179267 | KC179607 | Arup *et al.* (2013) |
| *Wetmoreana decipioides* | KC179333 | KC179269 | KC179608 | Arup *et al.* (2013) |
| *W. texana* | KC179337 | KC179273 | KC179612 | Arup *et al.* (2013) |
| *Wetmoreana* sp. 1 | KC179334 | KC179270 | KC179609 | Arup *et al.* (2013) |
| *Wetmoreana* sp. 2 | KC179335 | KC179271 | KC179610 | Arup *et al.* (2013) |
| *Wetmoreana* sp. 3 | KC179336 | KC179272 | KC179611 | Arup *et al.* (2013) |
| *Xanthocarpia crenulatella* | KC179126 | KC179274 | KC179613 | Arup *et al.* (2013) |
| *X. epigaea* | KC179127 | KC179275 | KC179614 | Arup *et al.* (2013) |
| *X. marmorata* | KC179131 | KC179276 | KC179615 | Arup *et al.* (2013) |
| *Xanthomendoza borealis* | KC179133 | KC179278 | KC179617 | Arup *et al.* (2013) |
| *X. fallax* | AF353955 | KC179279 | KC179618 | Arup *et al.* (2013) |
| *X. hasseana* | KC179136 | KC179280 | KC179619 | Arup *et al.* (2013) |
| *X. mendozae* | KC179138 | KC179281 | KC179620 | Arup *et al.* (2013) |
| *X. poeltii* | KC179142 | KC179282 | KC179622 | Arup *et al.* (2013) |
| *X. trachyphylla* | KC179143 | KC179283 | KC179623 | Arup *et al.* (2013) |
| *X. ulophyllodes* | KC179144 | KC179284 | KC179624 | Arup *et al.* (2013) |
| *Xanthopeltis rupicola* | KC179146 | KC179286 | KC179626 | Arup *et al.* (2013) |
| *Xanthoria calcicola* | AF353944 | KC179287 | KC179627 | Arup *et al.* (2013) |
| *X.* cf. *stiligera* | KC179409 | KC179288 | KC179628 | Arup *et al.* (2013) |
| *X. parietina* | KC179411 | KC179289 | KC179629 | Arup *et al.* (2013) |
| *X. resendei* | AF101285 | KC179290 | KC179630 | Arup *et al.* (2013) |




Supplementary Material Fig. S1. Bayesian ITS phylogeny of the genus *Caloplaca* s.str. containing the newly described species *C. saviczii* (delimited by the grey rectangle). Sorediate species of the genus are indicated with arrows. Designations of the clades of *C. cerina* s.lat. and *C. stillicidiorum* s.lat. correspond to Šoun *et al.* (2011). Numbers at branches represent posterior probability values ≥ 0.95. Newly sequenced specimens are indicated in bold.




Supplementary Material Fig. S2. Bayesian ITS phylogeny of the clade containing the *Caloplaca exsecuta* group and the newly described species *Lendemeriella aureopruinosa* (delimited by the grey rectangle). Numbers at branches represent posterior probability values ≥ 0.95. Newly sequenced specimens are indicated in bold.




Supplementary Material Fig. S3. Bayesian ITS phylogeny of the clade containing the genera *Athallia* and *Orientophila* and the newly described species *O. infirma* (delimited by the grey rectangle). Numbers at branches represent posterior probability values ≥ 0.95. Newly sequenced specimens are indicated in bold.

## 6 Curriculum vitae

**Personal Data**

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Foreign languages: English (B2), Czech (colloquial)

**Current Position**

Since 2017 – senior researcher, Botanical Garden of the Russian Academy of Sciences, Ural Branch (Russia, Yekaterinburg)

**Research Interests**

Lichen diversity of the Ural Mts, systematics of the lichen family Teloschistaceae in the Holarctic

**Professional experiences**

2012–2014 – junior researcher, Ural Federal University
2010–2011 – university teacher, Orenburg State University
2010 – researcher, Institute of Plant and Animal Ecology, Russian Academy of Sciences, Ural Branch (Yekaterinburg)
2009–2010 – teacher, lyceum at the Ural State University
2006–2010 – laboratory assistant, Ural State University

**Academic career**

2000–2006 – student, Faculty of Biology, Ural State University (Russia, Yekaterinburg), Diploma "Lichen flora of the Bashkir Natural Reserve"
2011–2012 – Fellow, University of South Bohemia, České Budějovice, Czech Republic, "Modern taxonomy of a peculiar lichen group 'Caloplaca sect. Pyrenodesmia' in the Ural Mts (Russia)" (International Višegrad Fund), 12 months
2018 – Fellow, Russian Foundation for Basic Research 17-34-50115, St. Petersburg, Russia, 6 months.
Since 2012 – post-graduate student, Botany Department, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic, thesis "Contribution to the taxonomy and biodiversity of crustose lichens from the family Teloschistaceae", supervisor Jan Vondrák, Ph.D.

**Research grants**

2011–2012 – International Višegrad Fund, scholarship 51100848, "Modern taxonomy of a peculiar lichen group 'Caloplaca sect. Pyrenodesmia' in the Ural Mts (Russia)"

2013–2015 – Russian Foundation for Basic Research (RFBR) 13-04-96083 "Lichens on metal-rich substrates: ecology, bioaccumulation and a role in biomonitoring", co-worker

2014–2015 – Grant Agency of the University of South Bohemia, project 133/2014/P "Island biogeography theory tested on calciphilous lichens in Central Europe. Key study on the genus Pyrenodesmia (Teloschistales, Ascomycota)"

2014–2015 – RFBR 14-04-31024 "The critical and monographic processing of some complicated groups of lichens for the extratropical Eurasia", co-worker

2016–2018 – RFBR 16-04-01488 "Critical revision of difficult groups of crustose lichens from the genera Pyrenodesmia and Micarea in Holarctic", co-worker

2017–2018 – RFBR 17-54-04030 "Lichens of manor parks in Mogilev and Smolensk Regions, as a refuge and a reserve for conservation of lichen in Eastern Europe", co-worker

2017–2019 – RFBR 17-04-01483 "Metabolome analysis as a new approach in chemotaxonomy of lichen genus", co-worker

2018–2019 – RFBR 18-34-00332 "Taxonomic revision of the genera Arctoparmelia Hale and Flavocetraria Kärnefelt & Thell in Russia", co-worker

2019–2020, RFBR – Japan Society for the Promotion of Science (JSPS) 9-54-50010, "Morphological and molecular-phylogenetic study for the diversity of lichenized fungi of
alpine-subalpine zones in southern Far East Russia and Japan", co-worker.

2019–2021 – RFBR 19-04-00074 "Biodiversity and phylogenetic relationships of lichens of the Far East and West coast of North America on the example of the families Micareaceae and Teloschistaceae", co-worker

**Selected scientific publications**

Vondrák J., **Frolov I.**, Arup U. and Khodosovtsev A. 2013. Methods for phenotypic evaluation of crustose lichens with emphasis on Teloschistaceae. Chornomorskiy botanichniy zhurnal 9(3): 382–405.

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