Paper III

The taxonomy of the lichen *Fuscidea cyathoides* (Fuscideaceae, Umbilicariomycetidae, Ascomycota) in Europe

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Abstract: Based on morphometric and molecular methods the taxonomy of the infraspecific taxa of *Fuscidea cyathoides* (Ach.) V. Wirth & Vězda, var. *corticola* (Fr.) Kalb and var. *sorediata* (H. Magn.) Poelt, has been assessed. No formal taxonomic recognition should be attributed to the morphological and ecological variation. Accordingly, var. *corticola* and var. *sorediata* are synonymized with *F. cyathoides* var. *cyathoides*. New synonyms at the specific level are *Fuscidea fagicola* (Zschacke) Hafellner & Türk and *F. stiriaca* (A. Massal.) Hafellner.

Key words: *Fuscidea fagicola*, *Fuscidea stiriaca*, molecular phylogeny, infraspecific taxonomy, lichen varieties, secondary chemistry.
Introduction

Substrate specificity is a strong feature in *Fuscidea*, however there have been occasional reports of corticolous specimens of mainly saxicolous species. For example, *F. recensa* (Stirt.) Hertel, V. Wirth & Vězda, is capable of inhabiting both rock and bark in Scandinavia (Tønsberg 1992), but corticolous specimens have not been formally recognized (Nordin *et al.* 2010). In Britain and Ireland, species of *Fuscidea* inhabit rock or, more rarely, bark, occasionally wood, and 10 of the 11 species are either exclusively saxicolous (8 spp.) or exclusively corticolous/lignicolous (2 spp.), and only *F. cyathoides* (Ach.) V. Wirth & Vězda is capable of inhabiting both rock and bark (Gilbert *et al.* 2009). Substrate ecology and the presence/absence of soredia have been suggested as important characters for formal recognition of taxonomic entities at the species level (Hafellner & Türk, 2001; Hafellner, 2002) and the varietal level (Fries, 1831; Magnusson, 1925; Zschacke, 1927) (see Table 1).

Magnusson (1925), for example, discussed seven saxicolous forms of *F. cyathoides* (as *Lecidea rivulosa* Ach.) and introduced var. *infuscata* H. Magn., separated from var. *cyathoides* based on habitat and thallus colour (see Supplementary Material Table S1). None of the *F. cyathoides* forms is longer recognized and Oberhollenzer & Wirth (1984) synonymized var. *infuscata* with var. *cyathoides* (see Taxonomy below).

In *F. cyathoides*, corticolous material has been attributed taxonomic rank at both infraspecific and specific levels. According to Fries (1831), *F. cyathoides* var. *corticola* (Fr.) Kalb (as *Biatora rivulosa* b. *corticola* Fr.) is distinct from var. *cyathoides* in possessing a different thallus colour, i.e., black-brown when dry and greenish when wet, while var. *cyathoides* is grey when dry and umber-brown when wet. Although some authors (e.g. Oberhollenzer & Wirth 1984; Gilbert *et al.* 2009) consider the corticolous variety as merely *F. cyathoides* on bark, others (e.g. Santesson *et al.* 2004; Inoue 1981) recognize this taxon as *F. cyathoides* var. *corticola*. 
Zschacke (1927) recognized the absence of black prothallus, the larger and flatter thallus as well as the larger apothecia as diagnostic characters for distinguishing \( F. \) fagicola (as Lecidea fagicola Zschacke) from \( F. \) cyathoides (as \( L. \) rivulosa). When comparing the so-called \( Fagus \)-type of apothecia of var. corticola, i.e. apothecia from specimens growing on \( Fagus \) in southern Europe, with those on \( Betula \), the so-called \( Betula \)-type, Oberhollenzer & Wirth (1984) did not find any significant variation. Based on this result they concluded that \( L. \) fagicola most certainly is synonymous with var. corticola. Hafellner & Türk (2001) transferred \( L. \) fagicola to Fuscidea and placed \( F. \) cyathoides var. corticola in synonymy without any explanatory discussion.

Hafellner (2002) made the combination \( F. \) stiriaca (A. Massal.) Hafellner based on the basionym Biatora stiriaca A. Massal., which is treated as a synonym of var. cyathoides by Magnusson (1925) (as Lecidea rivulosa var. corticola (Fr.) Jatta) and by Vainio (1934) (as \( L. \) rivulosa f. corticola (Fr.) Vain), and synonymized \( F. \) fagicola with \( F. \) stiriaca. The sorediate form, var. sorediata (H. Magn.) Poelt, is saxicolous and rare. It was, for example, accepted by Santesson et al. (2004) and Gilbert et al. (2009).

Molecular approaches changed the concept of species delimitation (as discussed in Resl et al. 2016) and provided a new approach to assess the status of sorediate lichens. In the studies of \( Pseudevernia furfuracea \) (L.) Zopf by Ferencová et al. (2010), Mycoblastus alpinus (Fr.) Kernst./\( M. \) affinis (Schærer) Schauer by Spribille et al. (2011), several species of Dirina Fr. by Tehler et al. (2013) and Rinodina degeliana Coppins/R. subparieta (Nyl.) Zahlbr. by Resl et al. (2016), no taxonomic relevance was given to the presence of soredia.

For example, Spribille et al. (2011) confirmed the hypothesis of Tønsberg (1992) that Mycoblastus alpinus and \( M. \) affinis are conspecific using a combined matrix of two protein coding (EF1-\( \alpha \), MCM7) and ITS genes. These two species differ in their morphology.
(esorediate, richly fertile vs. sorediate, sterile or sparingly fertile) and chemistry (usnic acid absent/thallus grey vs. usnic acid present in the (yellowish) soralia.

Here we aim to revise the taxonomy of *F. cyathoides s. lat.*, providing a morphological, chemical, and phylogenetic investigation of all three currently recognized varieties, and clarify the taxonomy of *F. cyathoides*, including the related *F. fagicola* and *F. stiriaca*.

**Material and Methods**

**Taxon sampling**

Herbarium material was provided by BG, HO, MSC, LD, UPS, TUR, and H-Ach, as well as from private collections. As var. *sorediata* is scarce in Europe, we only managed to obtain one fresh specimen.

**Morphology**

To determine morphological differences between varieties, the anatomy and morphology of the apothecia and thalli were examined by light microscopy on hand-cut sections mounted in water with 10% KOH using a Carl Zeiss Axiskoskop 2 microscope. 20 specimens of *F. cyathoides*, including all the three varieties, were investigated. The following morphological characters were studied: overall diameter of the apothecia and the areoles, height of the ephymenium and the hymenium, length and width of the ascospores, and the colour of the thalli (Table 2). The ratio between length and width of spores was calculated. The characters were examined using an unconstrained linear ordination, Principal Components Analysis (PCA), to explore the morphological variation. We performed the analysis with centering and standardization of characters in CANOCO 5 (Ter Braak & Šmilauer 2012); the first two axes were displayed as a scatterplot.

**Secondary chemical compounds**
Lichen substances were analysed by thin-layer chromatography (TLC), using the methods of Culberson & Kristinsson (1970), Culberson (1972), and Menlove (1974). All three solvents (A, B’ and C) were used, with glass plates in solvent C for the detection of fatty acids. Selected specimens were also run in solvent G for a detailed study of β-orcinol depsidone fumarprotocetraric acid and possible occurrences of the related substances protocetraric and succinprotocetraric acids (see Culberson et al. 1981).

**DNA extraction, PCR amplification and sequencing**

DNA from 10 specimens of *Fuscidea cyathoides* were analysed together with six other *Fuscidea* species for three genes. Altogether, we generated 36 new sequences, in addition to sequences of *Fuscidea* downloaded from GenBank (Table 3). DNA was extracted from apothecia or soredia with thallus using the DNeasy Plant Mini Kit (Qiagen). Although phylogenies based on ITS alone have been considered sufficient for infraspecific taxonomic investigations (e.g., Davydov et al. 2010; Solheim et al. 2013), we conducted a concatenate data set of three markers from two different genomes (mtSSU, nuITS and nuLSU).

The mtSSU fragment was made with the primers mrSSU1 and mrSSU3R (Zoller et al. 1999), while ITS and LSU were amplified by ITS1f (Gardes & Bruns 1993), ITS4 (White et al. 1990) and nu-nuLSU-1125-3’ (Vilgalys & Hester 1990). The PCR master mix included: 1x Buffer II GeneAmp® 10x PCR (Applied Biosystems), 2.5 μM MgCl₂ (Applied Biosystems), 20 μM dNTPs (Promega), 0.6 μM of each primer, 0.036 U AmpliTaq® DNA Polymerase (Applied Biosystems), 5.0 μl of genomic DNA extract, and distilled water to a total volume of 25 μl.

The PCR reactions were performed using the C1000™ Touch thermal cycler (Bio-Rad Laboratories) with the following programs: mtSSU: initial denaturation at 94°C for 5 min, touchdown of six cycles: 94°C for 30s, 62–56°C for 30 s, and 72°C in 1 min 45 s, followed by 34 cycles of 94°C for 30 s, 56°C for 30 s, 72°C in 1 min 45 s, and a final elongation at 72°C
for 10 min, LSU and ITS: as for mtSSU, except for the annealing temperature, where
the touchdown ranged from 63–57°C for six cycles, ending at 57°C for 34 cycles.
PCR products were visualized on a 1 % Red Gel-stained agarose gel under UV light,
and purified using Exosap-IT (GE Healthcare). The PCR products were sequenced using
the PCR primers with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems),
and run on an ABI Prism 3700XL DNA analyser (Applied Biosystems) at the DNA
Sequencing Laboratory, University of Bergen, Norway. The sequences were assembled
in SeqMan II version 4.05 (DNASTAR).

Phylogenetic analyses
Geneious version 8.1.8 (Biomatters Ltd.) was used to align the mtSSU, LSU, and ITS
sequences with 65% similarity option on (Gap penalty = 14.5, Gaps extension penalty = 5),
followed by manual adjustment. Candelariella vitellina (Hoffm.) Müll. Arg. was used
as outgroup, and two sequences, Umbilicaria proboscidea (L.) Schrader and U. crustulosa
(Ach.) Frey, as a sister group to Fuscidea.
To identify suitable substitution models for all fragments, i.e., mtSSU, LSU, ITS1, 5.8S
and ITS2, a likelihood ratio test (Huelsenbeck & Crandall 1997) was performed using
the software jModelTest version 2.1.7 (Posada 2008). For mtSSU, the model GTR+G was
selected, GTR+I+G for LSU, SYM+G for ITS1, K80+I for 5.8S, HKY+G for ITS2,
and GTR+I+G for the concatenate data set.
To detect potential conflicts between the data sets, we inspected the internodes
of the phylogenetic trees with bootstrap values >70%. These were generated using
the neighbor-joining model with a maximum likelihood distance (e.g., Reeb et al. 2004).
Bootstrap scores were calculated using 2,000 non-parametric replicates in the Jukes-Cantor
distance model implemented in Geneious version 8.1.8 (Biomatters Ltd.).
Phylogenetic relationships were estimated from the data sets both from each gene separately and the concatenated using MrBayes version 3.2.1 (Ronquist & Huelsenbeck 2003) to sample trees using a Markov chain Monte Carlo (MCMC) method in the Bayesian inference (BI). Tree sampling performed under the MCMC analysis was run for 4,000,000 generations with four parallel chains starting from a random tree, using the default temperature of 0.2. Gaps were coded as a fifth character state. Sampling frequency of trees was every 10th generation, including branch lengths. The first 40,000 trees (i.e., 10% of the total number of trees) were deleted as “burn-in”. A majority-rule consensus tree with average branch lengths was constructed from 360,000 trees and visualized in Geneious (Biomatters Ltd.). Significant posterior probabilities were equal to or above 95%.

Weighted maximum parsimony (MP) and maximum likelihood (ML) analyses were carried out in PAUP*4.0b10 (Swofford 2002) to construct MP and ML trees with bootstrap support. A first heuristic search was run to find MP trees using random sequence additions with 500 replicates, and tree bisection-reconnection branch swapping (TBR). The MulTrees and steepest descent options were on, and the collapse zero-length branches option was off. Gaps were coded as a fifth character state. To estimate the branch support for the MP trees, 1,000 bootstrap replicates with 10 random additions of the taxa were performed. A second heuristic search with 500 replicates under the ML criterion and the selected substitution model was run using the MP trees from the previous heuristic search as starting trees. Branch support for the ML trees was estimated by 100 bootstrap replicates with 10 random additions of the taxa. High bootstrap support was considered to be equal or above 70%.
Results

Morphological examination

The morphological examination (Table 2) showed that only corticolous specimens had greenish to green thalli. The colour of saxicolous specimens varied from grey to brown. Corticolous specimens more frequently developed tuberculate apothecia (i.e., 90% of examined specimens) than saxicolous ones (i.e., 20% of examined specimens). Var. *sorediata*, represented by only one specimen, had smaller and fewer apothecia (see Table 2).

The ascospores of all the three varieties were bean-shaped. However, those of the corticolous specimens were narrower (mean 4.53 ± 1.82 μm) than saxicolous ones (mean 4.79 ± 1.21 μm), but were similar in the mean of spores length, i.e., 10.17 ± 4.79 μm and 10.24 ± 3.11 μm, respectively (see Table 2).

PCA based on morphological characters of *F. cyathoides* did not separate corticolous and saxicolous specimens along the two first ordination axes representing 28.77% and 24.11% variation, respectively (Fig. 1). One character, namely the ratio between the length and width of ascospores, had a larger range for var. *cyathoides* than var. *corticola*. The height of hymenium and epihymenium, and width of ascospores, were both positively correlated with the size of areoles and tuberculate apothecia.

Secondary chemical compounds

Analysis of secondary chemical compounds in the fumarprotocetraric acid chemosyndrome did not reveal any chemical differences between the specimens. The major and diagnostic constituent was fumarprotocetraric acid; a trace of the satellite substance protocetraric acid was present in most specimens, whereas succinprotocetraric acid was not detected in any of the specimens tested.
Phylogeny of *Fuscidea cyathoides*

As no conflicts were detected between the data sets of different genes, they were combined and the final aligned sequence matrix comprised 26 taxa with 2,187 characters of which 1,618 were constant and 361 informative. The GenBank accession numbers are given in Table 3. The majority-rule consensus tree from the BI is displayed in Fig. 1. The average −ln likelihood of the tree was 8,081.83 and the average standard deviation of split frequencies was 0.0025, indicating that two independent runs of the Markov chain search converged. The calculated likelihood parameters of the MCMC analysis are summarized in Table S2.

A heuristic search using the parsimony criterion resulted in 100 MP trees of length 1,041 with consistency index = 0.7080, homoplasy index = 0.2920, retention index = 0.6984, and rescaled retention index = 0.4945. A second heuristic search under the ML criterion and the GTR+I+G model using the MP trees as starting trees resulted in three equally best ML trees (−lnL = 8,104.6499). The consensus ML tree was incongruent with the BI tree in the position of *F. kochiana* (Hepp) V. Wirth & Vězda, and five specimens (A. Aptroot 55063, M. Zahradníková MZ05 (BG-L-96931), G. Thor 18066, G. Thor 18061 and R. Haugan 1389) within the *F. cyathoides* group. All incongruences are marked with a circle in Fig. 2.

All the samples of *Fuscidea* included here formed a monophyletic group. All three varieties of *F. cyathoides* were clustered in one subgroup with PP = 0.99, MP = 97%, ML = 99% support. Within this subgroup, no clear classification into the varieties of corticolous and saxicolous specimens was discovered. The specimens from Central Europe, i.e. the Czech Republic and Slovakia, formed a group separate from northwest Europe, i.e., Norway and the Republic of Ireland.
Discussion

Neither the chemistry nor the molecular data show evidence for differentiation within *F. cyathoides*. Our results suggest that the bean-shaped spores becoming brownish when mature and the production of fumarprotocetraric acid are the only diagnostic characters for the recognition of *F. cyathoides*.

Our findings agree with Bylin *et al.* (2007), where corticolous and saxicolous specimens of *F. cyathoides* were grouped together, but with less sampling and MP bootstrap support lower than 80%. Moreover, *Fuscidea stiriaca* was clustered with var. *cyathoides* (MP = 100%).

The included representatives with apothecia of both the *Fagus*- and *Betula*-types show no morphological nor genetic differences. The observed variation between these apothecia types is not significant (see Figs. 1 and 2), confirming the statement of Oberhollenzer & Wirth (1984). In the PCA, the *Betula*-type (BG-L-89616) and the *Fagus*-type (*JV* 11397, *JV* 11411 and JM 6488) are not separated from each other; furthermore, specimens BG-L-89616 and *JV* 11411 are found to overlap. We consider the colour and the presence of crystals in the apothecia as adaptations to localities with direct light exposure. It should be noted that Fahselt (1981) found that levels of perlatolic and fumarprotocetraric acids in populations of *Cladonia stellaris* (Opiz) Pouzar & Vězda and *C. rangiformis* Hoffm., respectively, were influenced by light intensity. Massalongo (1852) suggested the bean-shaped spores and the tuberculate apothecia to be diagnostic for *F. stiriaca* (as *Biatora stiriaca*) (see Fig. 1). This cannot be supported, since both characters are also present in var. *cyathoides*.

In the present study, var. *sorediata* has the smallest apothecia (see Table 2), but this feature is considered to be as result of a biological energy saving strategy (see Tønsberg 1992), and should not be used as a diagnostic character for species forming species pairs sensu Poelt (1970, 1972).
To conclude, no significant genetic difference between specimens reflecting the morphological and ecological variations was found in *F. cyathoides*. Therefore, we synonymize var. *corticola* and var. *sorediata* with the typical form. *Fuscidea fagicola* and *F. stiriaca* are synonymized with *F. cyathoides*.

**Taxonomy**

*Fuscidea cyathoides* (Ach.) V. Wirth & Vězda


– *Lecidea subrivulosa* Vain., *Acta Societiatis pro Fauna et Flora Fennica* 57: 316 (1934); type: Russia [Finlandia]: in Somerikonvuoret in Suursaari v. Hoglandia, in rupe porphyrica, 1875, Vainio (TUR-Vainio 24352 – holotypus [!]). – *Fuscidea subrivulosa* (Vain.) P. James,
P. James, Poelt & V. Wirth, *Bibliotheca Lichenologica* 16: 154 (1981), nom. inval., Art. 41.4
(Melbourne).

– *Biatora rivulosa* b. *corticola* Fr., *Lichenographia Europaea Reformata*: 272 (1831); type:
var. *corticola* (Fr.) Kalb, *Herzogia* 4: 57 (1976). **Syn. nov.**

– *Lecidea fagicola* Zschacke, *Verhandlungen des Botanischen Vereins der Provinz
Brandenburg* 69: 11 (1927); type: Frankreich, Corsica: Vizzavona, H. Zschacke (B –
holotypus [lost, see Oberhollenzer & Wirth, *Beihefte zur Nova Hedwigia* 79: 554 (1984)];
Frankreich, Corsica, Distr. Evissa: Silva Aitone, in valle rivi Aitone, c. 1300 m. Fagicola,
by Oberhollenzer & Wirth, in *Beihefte zur Nova Hedwigia* 79: 554 (1984)). – *Biatorinella
fagicola* (Zschacke) Deschâtres & Werner, *Bulletin de la Société Botanique de France* 121:
**Syn. nov.**

– *Biatora stiriaca* A. Massal., *Ricerche sull´ autonomia del licheni crostosi*: 125 (1852); type:
Italia, vive sui faggi nelle Stiria, legit. Welwic. (VER – holotypus [!]). – *Lecidea stiriaca*
(A. Massal.) Jatta, *Sylloge Lichenum Italicorum* 39: 328 (1900). – *Fuscidea stiriaca*
(A. Massal.) Hafellner, *Fritschiana* 33: 42 (2002). **Syn. nov.**

– *Lecidea rivulosa* var. *sorediata* H. Magn., *Göteborgs Kunglige Vetenskaps- och Vitterhets-
Samhälles Handlingar*, Ser. 4, 29: 29 (1925); type: Sweden, Västergötland: par. Frölunda,
Näset, on sunny boulder, 24 August 1924, A. H. Magnusson 9237 A (UPS, L-763155 –
lectotypus, designated here). – *Fuscidea cyathoides* var. *sorediata* (H. Magn.) Poelt,
Thallus crustose, very variable, rimose-cracked, to reticulate, delimited, occasionally sorediate; over-all colour in saxicolous habitats from light grey to dark grey or brown, in corticolous habitats greyish or brownish green to olive green. Areoles discrete, irregular, convex, highly variable in size, becoming secondarily cracked. Soralia rarely present, yellowish, sometimes tinged with brown, bursting from the apices of the areoles. Prothallus distinct, dark brown or black visible, ramifying the thallus, often forming mosaics. Photobiont green, coccoid, globose to broadly ellipsoid. Apothecia immersed to sessile, constricted at base, roundish, up to 1.4 mm in diam., to 1.9 mm when tuberculate, dark grey-brown to black; margin paler or concolorous with disc, rounded to strongly flexuose; disc black, mostly flat. Epihymenium brown; hymenium pale or faintly brownish; hypothecium hyaline. Asci clavate, of the Fusidea-type. Ascospores simple, colourless, sometimes elliptical when young, bean-shaped when mature, brownish (6–)10–11(–14.5) × (3–)4–5(–7) μm. Pycnidia abundant, brown, immersed, to emergent with a thin thalline rim. Conidia bacilliform 3–4 × 1.5–2 μm.

Chemistry. Fumarprotocetraric acid (major), protocetraric acid (trace, usually present). Spot tests: K+ orange yellow, Pd+ rust-red; UV–.

Distribution and Ecology. Fusidea cyathoides is mainly saxicolous on coarse-grained, nutrient-deficient, siliceous rocks; occasionally it is corticolous on trunks and branches of Acer, Alnus, Betula, Castanea, Fagus, Quercus and Sorbus.

The typical form (saxicolous esorediate) of Fusidea cyathoides has been reported from Austria (Hafellner & Türk 2001), Belgium and Luxembourg (Diederich & Séerusiaux.
2000), the British Isles (Hawksworth et al. 1980; Gilbert et al. 2009), China (Wei 1991), Croatia (Partl 2009), Czech Republic (Vězda & Liška 1999), Denmark (Søchting & Alstrup 2008), Estonia (Randlane & Saag 1999), Finland (Nordin et al. 2010), France (Roux 2012), Germany (Wirth 1987), Greenland (Thomson 1997), Italy (Puntillo 1996), Morocco (Egea 1996), Norway (Nordin et al. 2010), Poland (Faltynowicz 1993), Portugal (van den Boom & Giralt 1999; Llimona & Hladun 2001), Romania (Ciurchea 1998), Russia (Urbanavichus & Andreev 2010), Serbia (Savić & Tibell 2006), Slovakia (Pišút et al. 1998), Slovenia (Suppan et al. 2000), Spain (Llimona & Hladun 2001), Sweden (Nordin et al. 2010), Switzerland (Clerc 2004), Turkey (Yıldız et al. 2002), and Ukraine (Kondratyuk et al. 2010). The saxicolous sorediate form is known from the British Isles (Hawksworth et al. 1980), Denmark (Søchting & Alstrup 2008), France (Roux 2012), Poland (Faltynowicz 1993), Norway (Poelt & Buschardt 1978) and presently published material, and Sweden (Nordin et al. 2010) (see Fig. 5). The records from North America and Tasmania (Richardson & Richardson 1982; Egan 1987) were later rejected as they were based on misidentifications (Kantvilas 2001; Fryday 2008).

The corticolous form has been reported from Albania (Svoboda et al. 2012), Austria (Hafellner & Türk 2001), Belgium and Luxembourg (Diede & Sérusiaux 2000), Bosnia-Herzegovina (Christensen 1994), Croatia (Partl 2009), Denmark (Søchting & Alstrup 2008), France (Roux 2012), Germany (Cezanne et al. 2004), Italy (Tretiach & Nimis 1994), Poland (Faltynowicz 1993), Portugal (van den Boom & Giralt 1999; Llimona & Hladun 2001), Montenegro (Knežević & Mayrhofer 2009), Norway (Nordin et al. 2010), Russia (Urbanavichus & Andreev 2010), Slovakia (Bielczyk et al. 2004), Slovenia (Suppan et al. 2000), Spain (Llimona & Hladun 2001), W. Scotland (Gilbert et al. 2009), Sweden (Nordin et al. 2010), Switzerland (Clerc 2004), Ukraine (Coppins et al. 2005) (see Fig. 6), as well as from Taiwan (Aptroot & Sparrius 2003).
Specimens examined (saxicolous, esorediate): **Czech Republic**: Central Bohemia, Distr. Beroun, Brdy Mts, Neřežín - Malá Víska: upper part of Krkavčina Mt., forested (*Picea, Betula, Larix* etc.) rocky hill, 49°45′55″N, 13°53′36″E, alt. 570–600 m, on siliceous boulder, 17.11.2012, *J. Malíček* 4916; Distr. Beroun, Brdy Mts, Neřežín, Jindřichova skála Mt., 1 km SE of Malá Víska, rock with E-exposed boulder scree, 49°46′05″N, 13°52′55″E, alt. 550–580 m, on siliceous boulder, 17.11.2012, *J. Malíček* 4928; Western Bohemia, Distr. Rokycany, Brdy Mts, Strašice - Lipovsko Mts (651 m), 3 km SE of town, rock with boulder scree on S-exposed slope, 49°42′53″N, 13°47′11″E, alt. 620–640 m, on siliceous rock, 8.11.2012, *J. Malíček* (4866); Moravský kras, Mohelno, 49°06′08.80″N, 16°11′05.20″E, alt. 344 m, in deciduous forest on shaded siliceous rock, 7.5.2011, *J. Halda* 662/2011 (JHP/13294).

**Norway**: Hordaland, Fjell, Sotra, SW from Landro, Ingholet, 100 m from cemetery, 60°25′6″N 4° 58′31.2″E, alt. 35–45 m, saxicolous on SW-facing vertical siliceous stone wall, 20.3.2011, *M. Zahradníková* MZ 30 (BG-L-96933); Fjell, Sotra, W of the road between Skålvik and Sekkingstad, S of road jct to Algrøyna, 60°20′06.6″N, 4°59′42.0″E, alt. 40–70 m, saxicolous on siliceous rock wall in coastal heath, 15.11.2010, *M. Zahradníková* MZ 5 (BG-L-96931); Nordland, Nesna, Island Tomma, Valhaugen, 66°17′44.63″N, 12°49′15.31″, alt. 35 m, saxicolous on bedrock in open, treeless situation, 20.6.2016, *T. Tønsberg* 46572 & A. Botnen (BG-L-99904).

**Ireland**: Co. Kerry, Macgillycuddy’s Reeks, Gaddagh River valley NE of Carrauntoohil (Corrán Tuathail) [1039 m], c. 14 km WSW of Killarney, 52°00′50.0″N, 9°42′49.0″W, alt. 225 m, on boulders near the brook, 4.9.2003, *J. Halda* & Z. Palice 7903. **U.K., Scotland**: South Aberdeenshire: V.C. 92, Braemar, Invercauld Estate, Craig Leek, NE-E facing crags, partly limestone, 57°01′24.0″N, 3°39′60.0″W, alt. 425 m, on siliceous rock in pasture below crags, 24.5.2005, *A.M. Fryday* 9012 (MSC0050557).

Specimens examined (saxicolous, sorediate): Norway: Nordland, Nesna, Tomma, Valhaugen, 66°17’44.77”N, 12°49’15.31”E, alt. 35–40 m, saxicolous in shallow crevice (with seeping water) in bedrock in open, treeless situation, 20.6.2016, T. Tønsberg 46570 (BG-L-99902).

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We thank the curators of HO, LD, UPS, H-Ach, and MSC for loan of material, Zdeněk Palice, Jan Vondrák, Jiří Malíček (all from the Academy of Science of the Czech Republic) and Josef Halda (Museum and Gallery of Orlické hory Mts., Czech Republic) for providing fresh material from Central Europe, M.R.D. Seaward (University of Bradford, England) for language improvements, Louise Lindblom, Beate Helle, and Per M. Jørgensen (all University of Bergen, Norway) for technical help with the molecular work (LL), technical help with the preparation of the distribution maps (BH) and help with botanical nomenclature (PMJ), respectively, and Kim Abel (Røyken, Norway) for taking the photos. The project was funded by the University Museum of Bergen, University of Bergen, including grants from the Grolle Olsen fund. This work is a part of the doctoral thesis of MZ. The molecular work was done in the Biodiversity Laboratories at University of Bergen.

Appendix A: Supplementary material

Supplementary data associated with this article can be found in the online version at http://###
References


Figure 1. Biplot of the two first principal component axes, showing morphological variation of the studied specimens of *F. cyathoides*. Abbreviations of the variables: *Apom* = diam. (mm) of apothecia; *Apotub* = diam. (mm) of tuberculate apothecia; *Aream* = diam. (mm) of areolum; *Hym* = width (ȝm) of hymenium; *Epi* = width (ȝm) of epihymenium; *Lspore* = length (ȝm) of ascospores; *Wspore* = width (ȝm) of ascospores; *Lwspore* = ratio of *wspore : lspore*. 
Figure 2. Phylogenetic relationships of esorediate and sorediate, saxicolous, and corticolous specimens of *Fuscidea cyathoides*, shown here as a 50% majority rule consensus tree of a B/MCMC analysis based on the concatenate data set (−ln = 8,081.83) of mtSSU, LSU and ITS. Posterior probabilities (PP) are displayed above the branches; MP and ML bootstrap values are displayed under the branches; asterisks indicate value of 100%. A circle indicates incongruent topology with the ML tree.
Figure 3. *Fuscidea cyathoides*. A: saxicolous and sorediate specimen, B: saxicolous and esorediate; C−D: corticolous. A, Norway (TT 46570, BG-L-99902); B, Norway (TT 46572, BG-L-99904); C, on *Fagus sylvatica*, Slovakia (JV 11397); D, on *Alnus incana*, Norway (TT 26205, BG-L-70280). Scale: A, B, D = 2 cm, C = 0.5 cm. Photos by Kim Abel.
Figure 4. Distribution of saxicolous, esorediate and sorediate forms of *Fuscidea cyathoides* based on the examined material and the literature.

Figure 5. Distribution of corticolous forms of *Fuscidea cyathoides* based on the examined material and the literature.
Table 1. Overview of *Fuscidea cyathoides* nomenclature.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Name of species at Species level</th>
<th>Variety level</th>
<th>Basionym</th>
<th>Synonyms</th>
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</thead>
<tbody>
<tr>
<td>Acharius E.</td>
<td>1798</td>
<td><em>Lichen cyathoides</em> Ach.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fries E.</td>
<td>1831</td>
<td></td>
<td></td>
<td><em>Biatora rivulosa</em> var. <em>corticola</em> Fr.</td>
<td><em>Lecidea Lightfootii</em> Ach.; <em>Lecidea rivulosa</em></td>
</tr>
<tr>
<td>Massalongo A.</td>
<td>1852</td>
<td><em>Biatora stiriaca</em> A. Massal.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Zschacke H.</td>
<td>1927</td>
<td><em>Lecidea fagicola</em> Zschacke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalb K.</td>
<td>1976</td>
<td><em>Fuscidea cyathoides</em> var. <em>corticola</em> (Fr.) Kalb</td>
<td></td>
<td><em>Biatora rivulosa</em> var. <em>corticola</em> Fr.</td>
<td><em>Biatorinella rivulosa</em> var. <em>corticola</em></td>
</tr>
<tr>
<td>Hafellner J. &amp; Türk R.</td>
<td>2001</td>
<td><em>Fuscidea fagicola</em> (Zschacke) Hafellner &amp;</td>
<td></td>
<td><em>Lecidea fagicola</em> Zschacke</td>
<td></td>
</tr>
<tr>
<td>Hafellner J.</td>
<td>2002</td>
<td><em>Fuscidea stiriaca</em> (A. Massal.) Hafellner</td>
<td></td>
<td><em>Biatora stiriaca</em> A. Massal.</td>
<td><em>Fuscidea fagicola</em> (Zschacke) Hafellner &amp;</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><em>cyathoides</em></td>
<td>ZP 7903</td>
<td>Ireland</td>
<td>dark grey</td>
<td>(0.23–)0.45(±0.24)(–0.90)</td>
<td>(0.63–)0.86(0.12)(–1.04)</td>
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<tr>
<td></td>
<td>JM 4928</td>
<td>Czech Rep.</td>
<td>dark grey, brown</td>
<td>(0.41–)0.68(±0.23)(–0.95)</td>
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<td>MSC0050557</td>
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<td>(0.63–)0.9(0.29)(–1.44)</td>
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<tr>
<td></td>
<td>JPH13294</td>
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<td>brown-grey, brown</td>
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<td>(0.45–)0.93±0.32(–1.35)</td>
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<td></td>
<td>BG-L-96931</td>
<td>Norway</td>
<td>grey-brown-grey</td>
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<td>BG-L-99904</td>
<td>Norway</td>
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<td>(0.66–)0.79±0.16(–1.12)</td>
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<td>BG-L-96933</td>
<td>Norway</td>
<td>light grey, grey, white</td>
<td>(0.14–)0.56±0.41(–1.35)</td>
<td>(0.45–)0.76±0.29(–1.13)</td>
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<tr>
<td></td>
<td>LD-1132977</td>
<td>Sweden</td>
<td>dark grey-brown</td>
<td>(0.45–)0.14±0.45(–1.94)</td>
<td>(0.45–)0.74±0.24(–1.13)</td>
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<tr>
<td><em>sorediata</em></td>
<td>BG-L-99902</td>
<td>Norway</td>
<td>mouse-grey, light grey</td>
<td>(0.25–)0.55±0.31(–1.05)</td>
<td>(0.16–)0.33±0.17(–0.70)</td>
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<tr>
<td>corticola</td>
<td>BG-L-98938</td>
<td>Slovakia</td>
<td>light grey</td>
<td>(0.50–)1.15±0.46(–1.76)</td>
<td>(0.54–)0.72±0.25(–0.90)</td>
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<td></td>
<td>JV 11476</td>
<td>Slovakia</td>
<td>dark greenish brown</td>
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<td>(0.32–)0.48±0.12(–0.59)</td>
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<td>BG-L-98916</td>
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<td>grey-brown</td>
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<td>(0.45–)0.78±0.31(–1.40)</td>
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<td>JM 6488</td>
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<td>(0.72–)0.9±0.12±0.17(–1.17)</td>
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<td>(0.50–)1.03±0.3x(–1.44)</td>
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Table 3. List of voucher specimens with their collection details and GenBank Accession numbers, in addition to included sequences from GenBank. Newly generated sequences are indicated in **bold**.

<table>
<thead>
<tr>
<th>Species</th>
<th>Variety</th>
<th>Country</th>
<th>Substrate</th>
<th>Collection/Accession number</th>
<th>GenBank Accession Number</th>
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<tr>
<td>Candelariella vitellina</td>
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<td>Scotland</td>
<td>siliceous rock</td>
<td>AY853315</td>
<td>AY853345</td>
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<td>Fuscidea australis</td>
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<td>Norway</td>
<td>siliceous rock</td>
<td>HO:546713</td>
<td>KJ766396</td>
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<td>Fuscidea cyathoides</td>
<td></td>
<td>Czech Rep.</td>
<td>siliceous rock</td>
<td>J. Malick 1389</td>
<td>KJ766396</td>
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<tr>
<td>Fuscidea corticola</td>
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<td>Norway</td>
<td>Betula pubescens</td>
<td>BG-L-89616</td>
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<tr>
<td>Fuscidea elixii</td>
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<td>New South Wales</td>
<td>Acacia melanoxylon</td>
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<td>KY874024</td>
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<td>Fuscidea enigmaticus</td>
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<td>Norway</td>
<td>Betula sp.</td>
<td>BGL-91025</td>
<td>KY874025</td>
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<td>Fuscidea kochina</td>
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<td>Betula sp.</td>
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<td>KY874025</td>
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<td>Fuscidea pusilla</td>
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<td>Betula sp.</td>
<td>G. Thor 18068</td>
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<td>Fuscidea stiriaca</td>
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<td>France</td>
<td>Fagus sylvatica</td>
<td>A. Apro 5063</td>
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<table>
<thead>
<tr>
<th>Species</th>
<th>Variety</th>
<th>Country</th>
<th>Substrate</th>
<th>Collection/Accession number</th>
<th>GenBank Accession Number</th>
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<td>Betula sp.</td>
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<td>KY874025</td>
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<td>Umbilicaria crustulosa</td>
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<td>Betula sp.</td>
<td>BGL-91025</td>
<td>KY874025</td>
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</table>
Appendix A: Supplementary material

Table S1. Infraspecific taxa of *Fuscidea cyathoides* (as *Lecidea rivulosa* Ach.) according to Magnusson (1925).

<table>
<thead>
<tr>
<th><em>F. cyathoides</em> var. <em>cyathoides</em> (as <em>Lecidea rivulosa</em> Ach.)</th>
<th>Thallus</th>
<th>Apothecia</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. lobarata</em> Nyl.</td>
<td>thick, with convex areoles</td>
<td>up to 2 mm, margin lobate</td>
<td>locality rich in nitrogen</td>
</tr>
<tr>
<td><em>F. depressa</em> Leight.</td>
<td>areolate-rimose, areoles flat or subtly concave</td>
<td>sessile</td>
<td></td>
</tr>
<tr>
<td><em>F. obscuroir</em> Cribb.</td>
<td>areolate-rimose, dark grey with brownish-black hypothallus</td>
<td>sessile</td>
<td></td>
</tr>
<tr>
<td><em>F. depauperata</em> Leight.</td>
<td>thin and fading, hypothallus blackish</td>
<td>sessile</td>
<td></td>
</tr>
<tr>
<td><em>F. falsaria</em> Ach.</td>
<td>thick with verrucose areoles, greyish-brown</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. cyathoides</em> Ach.</td>
<td>rimose, whitish-grey</td>
<td>sessile, concave, flexuous with thin greyish pruina</td>
<td>very shaded</td>
</tr>
<tr>
<td><em>F. sylvatica</em> Anzi.</td>
<td>smooth or finely rimose-areolate, whitish or greyish when fresh, intersecting hypothalline lines visible</td>
<td>rare, sessile, small</td>
<td>shaded</td>
</tr>
<tr>
<td><em>var. infuscata</em> H. Magn.</td>
<td>thick, cracky with plane areoles, dark brown</td>
<td>appressed, only slightly rising above thallus, usually flat</td>
<td>in open situation along the coast</td>
</tr>
</tbody>
</table>

Table S2. Calculated likelihood parameters of individual data sets of the MCMC analysis. The values indicate the mean and, in brackets, the variance.

<table>
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<th>Parameters</th>
<th>mtSSU</th>
<th>LSU</th>
<th>ITS1</th>
<th>5.8 S</th>
<th>ITS2</th>
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</thead>
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<tr>
<td>Frequency A</td>
<td>0.3317 (0.0002)</td>
<td>0.2553 (0.0002)</td>
<td>0.1852 (0.0006)</td>
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<td>Frequency C</td>
<td>0.1521 (0.0001)</td>
<td>0.2272 (0.0001)</td>
<td>0.2965 (0.0009)</td>
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<td></td>
</tr>
<tr>
<td>Frequency G</td>
<td>0.2118 (0.0002)</td>
<td>0.2980 (0.0002)</td>
<td>0.2836 (0.0009)</td>
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</tr>
<tr>
<td>Frequency T</td>
<td>0.3045 (0.0002)</td>
<td>0.2196 (0.0001)</td>
<td>0.2347 (0.0007)</td>
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</tr>
<tr>
<td>Gamma shape (G)</td>
<td>0.2740 (0.0016)</td>
<td>1.7940 (1.3397)</td>
<td>0.2411 (0.0045)</td>
<td>0.3632 (0.0105)</td>
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<tr>
<td>Proportion of invariant sites (I)</td>
<td>0.6428 (0.0007)</td>
<td>0.6428 (0.0007)</td>
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<tr>
<td>R-matrix [A-C]</td>
<td>0.1071 (0.0006)</td>
<td>0.06532 (0.0002)</td>
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<tr>
<td>R-matrix [A-G]</td>
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<td>0.1732 (0.0007)</td>
<td>0.1976 (0.0011)</td>
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<td>R-matrix [A-T]</td>
<td>0.0968 (0.0004)</td>
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<td>R-matrix [C-G]</td>
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<td>0.0476 (0.0001)</td>
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<td>R-matrix [C-T]</td>
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<td>0.5986 (0.0014)</td>
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<tr>
<td>R-matrix [G-T]</td>
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<td>0.0654 (0.0002)</td>
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<tr>
<td>Kappa (K)</td>
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<td>3.9198 (0.4114)</td>
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</table>