The Lichenologist **50**(2): 153–160 (2018) doi:10.1017/S0024282918000051

The British endemic *Enterographa sorediata* is the widespread *Syncesia myrticola* (*Roccellaceae, Arthoniales*)

Damien ERTZ, Brian J. COPPINS and Neil A. SANDERSON

Abstract: *Enterographa sorediata* is a corticolous, crustose lichen endemic to the southern part of Great Britain where it is confined to old-growth woodlands. This lichen is rarely fertile and mainly characterized by a sorediate thallus producing protocetraric acid. However, phylogenetic analyses using nuLSU, *RPB2* and nuITS sequences suggest that *E. sorediata* belongs to the genus *Syncesia* and is conspecific with *S. myrticola*. This is corroborated by the chemistry and the recent observation of a thallus with both fully developed *S. myrticola*-like apothecia and soralia. This provides further evidence of the difficulties involved in correctly placing sorediate sterile morphs of crustose lichens into particular genera without using molecular data. An updated distribution map of *S. myrticola* for Great Britain and Ireland is provided, showing that the sorediate morph extends more inland whereas the fertile morph is more coastal.

Key words: Arthoniomycetes, biodiversity, lichen, phylogeny, sorediate morph, taxonomy

Accepted for publication 8 *October* 2017

Introduction

Enterographa sorediata Coppins & P. James is a crustose, corticolous lichen species confined to old-growth woodlands in the southern part of Great Britain (Sanderson 2002). It is considered Near Threatened and a high priority species for biodiversity action plans (Woods & Coppins 2012). *Enterographa sorediata* is characterized by a pale grey or brownish grey thallus with rounded to elongate soralia, producing pinkish (when fresh), farinose soredia, and the production of protocetraric acid in the thallus (Coppins & James 1979; Sparrius 2004) (Fig. 1). Since its description in 1979, the validity of the species has never been questioned despite its similar appearance to sorediate *Schismatomma* s. lat. species but which differ in chemistry and/or colour. The species was accepted in the revision of the genus by Sparrius (2004) and in the recent lichen flora of Great Britain and Ireland (Sanderson *et al.* 2009). In the original description, "immature or ? aborted" ascomata were reported, as well as "(?immature), 8–10 × 3–4 µm, 4-5 septate, fusiform, curved" ascospores (Coppins & James 1979). However, Sparrius (2004) examined the abundant type material and did not observe ascospores, despite the presence of immature ascomata similar to those of *Enterographa crassa* (DC.) Fée. Only the type seems to have had ascospores and most of the material collected since the description of the species is sterile or, when it does have *E. crassa*-like apothecia, no well-developed ascospores can be found (N. A. Sanderson, unpublished data).

The present study originally aimed at verifying the generic position of *Enterographa sorediata* but will demonstrate that the species unexpectedly belongs to the genus *Syncesia* and is conspecific with *S. myrticola* (Fée) Tehler.

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Materials and Methods

The external morphology was studied using an Olympus SZX12 stereomicroscope. Macroscopic photographs

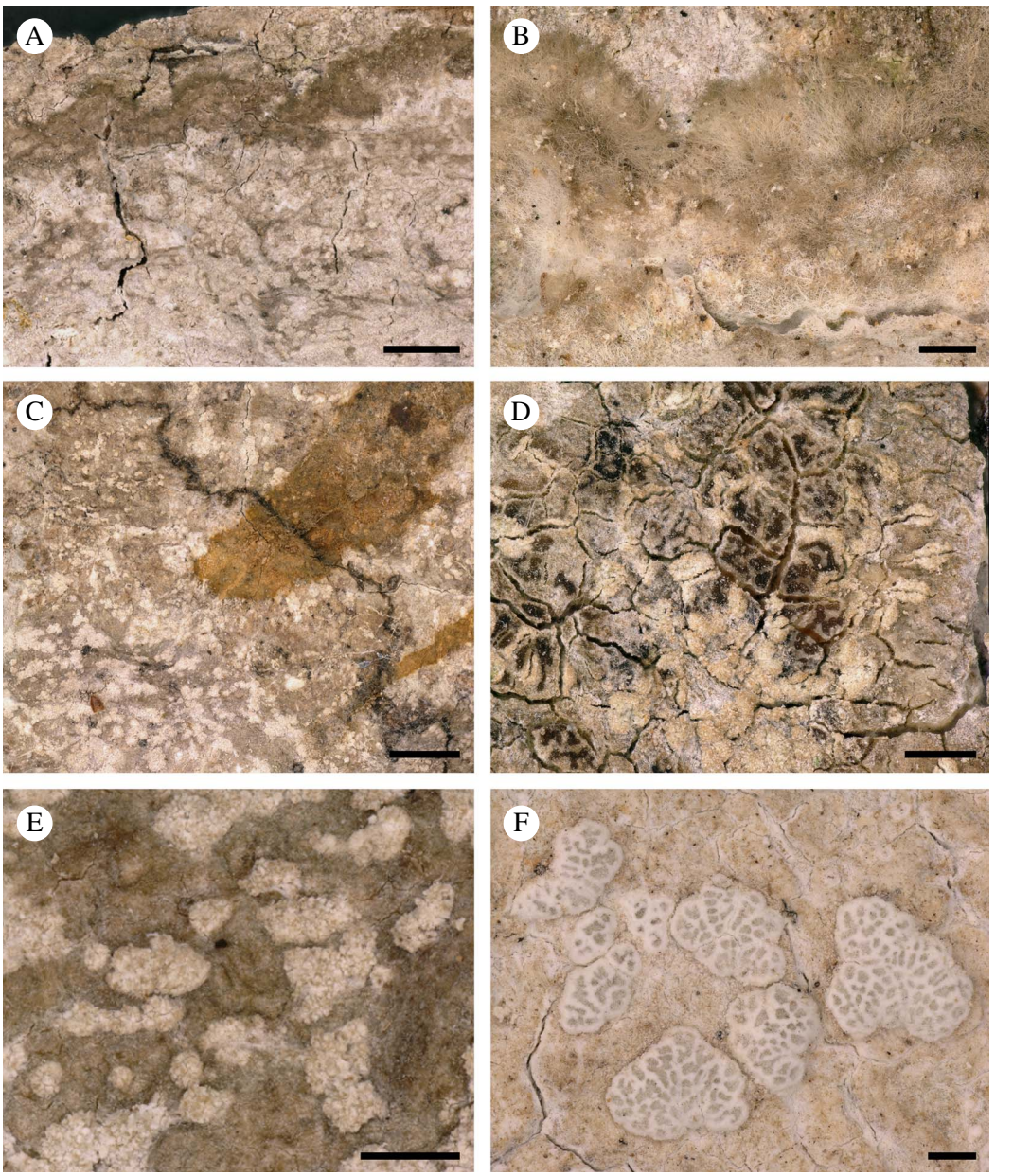

FIG. 1. *Syncesia myrticola*. A, sorediate thallus with an easily discernible byssoid prothallus above, actively overgrowing other thalli (*Sanderson* 2181); B, byssoid prothallus (*Sanderson* 2238); C, sorediate thalli separated by a black borderline, and the darker areas correspond to an orange colouration due to the reaction with paraphenylenediamine (*Sanderson* 2238); D, thallus and ascomata of *Enterographa crassa* overgrown by a thin thallus of *S. myrticola* with soralia (*Sanderson* 2238); E, soralia (*Sanderson* 2201); F, ascomata (*Ertz* 16273). Scales: A & C = 2 mm; B, D–F = 0·5 mm. In colour online.

were taken with a Keyence VHX-5000 digital microscope and a VH-Z20R/W/T lens. Hand-cut sections of ascomata and thallus were mounted in water or in a solution of 5% potassium hydroxide (K) and studied using an Olympus BX51 compound microscope. Colour reactions of the thallus were studied using 5% potassium hydroxide solution (K), common household bleach (C), paraphenylenediamine (PD) and long-wave UV (366 nm). Lichen secondary metabolites were identified using thin-layer chromatography (TLC) in solvents B and G (Orange *et al*. 2001). The extracts from *Enterographa crassa* (DC.) Fée, *Lepraria membranacea* (Dicks.) Vain. and *Flavoparmelia caperata* (L.) Hale were used as controls for confluentic, roccellic and protocetraric acids, respectively. We have not re-examined the type specimen of *E. sorediata* but detailed photographs are available on the data portal of the Natural History Museum (http://data.nhm.ac.uk/dataset/collection-specimens).

Molecular techniques

Well-preserved and freshly collected specimens of *E. sorediata* lacking any visible symptoms of fungal infection were used for DNA isolation. A small number of soredia were used for direct PCR as described in Ertz *et al*. (2015). A targeted fragment of *c*. 1·1 kb at the 5′end of the nuLSU rDNA was amplified using primers LIC15R (Miadlikowska *et al*. 2002) with LR6 (Vilgalys & Hester 1990), a fragment of *c*. 1 kb of the *RPB2* protein-coding gene was amplified using primers *fRPB2*-7cF and *fRPB2*-11aR (Liu *et al*. 1999), and a fragment of *c*. 0·6 kb of the nuITS rDNA using primers ITS1F and ITS4 (White *et al*. 1990). The nuITS rDNA was also amplified for seven *Syncesia* specimens using DNA extracts available from Ertz *et al*. (2015). The yield of the PCRs was verified by running the products on a 1% agarose gel using ethidium bromide. Both strands were sequenced by Macrogen® using amplification primers. The primer LR3 was also used for the sequencing of nuLSU (Vilgalys & Hester 1990; Vilgalys' website, http://www.botany.duke.edu/fungi/mycolab). Sequence fragments were assembled with Sequencher version 5.3 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to 'MegaBLAST' searches to verify their closest relatives and to detect potential contaminations.

Taxon selection and phylogenetic analyses

Because the closest relatives of *E. sorediata* based on 'MegaBLAST' searches were *Syncesia* species, 31 members of *Syncesia* for which the nuLSU + *RPB2* sequences were available were selected from Ertz *et al*. (2015) and their sequences retrieved from GenBank (Table 1). *Dendrographa leucophaea* (Tuck.) Darb. was chosen as outgroup. A dataset of 14 nuITS sequences was also assembled in order to verify whether *E. sorediata* differs from *Syncesia myrticola*. The sequences were aligned using MAFFT v6.814b (Katoh *et al*. 2002) within Geneious and improved manually using Mesquite 3.04 (Maddison & Maddison 2015). Terminal ends of sequences and ambiguously aligned regions were delimited manually and excluded from the datasets.

Bayesian analyses were carried out on the nuLSU + *RPB2* and the nuITS datasets separately using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method in MrBayes v.3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES web portal (Miller *et al*. 2010). Best-fit evolutionary models were estimated using Akaike Information Criterion (AIC; Akaike 1973) as implemented in jModelTest2 2.1.6 (Darriba *et al*. 2012). The GTR + I + G and the HKY + I models were selected for the nuLSU + *RPB2* and nuITS datasets, respectively. For both datasets, two parallel MCMCMC runs were performed, each using four independent chains and 40 million generations, sampling trees every 1000th generation. Tracer v.1.6 (Rambaut & Drummond 2007) was used to ensure that stationarity was reached by plotting the log-likelihood values of the sample points against generation time. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from 60 002 post-burn-in trees of the 80 002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. Posterior probabilities ≥95 were considered to be significant. Phylogenetic trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Results and Discussion

The nuLSU + *RPB2* dataset consisted of 34 specimens and 1736 unambiguously aligned sites. The resulting phylogenetic tree (Fig. 2) surprisingly placed *Enterographa sorediata* within *Syncesia*, a predominantly neotropical genus of which 15 species (representing *c*. 70% of the genus) were recently sequenced in the framework of a phylogeny of *Roccellaceae* (Ertz *et al*. 2015). *Enterographa sorediata* is even nested within the clade of *Syncesia myrticola*, the generic type and the only *Syncesia* species found in Europe. In the north of its range, *S. myrticola* is present in south-west England, Wales and Ireland along the Atlantic coast, with a distribution extending southwards to the western Mediterranean area, the Canary Islands and the Azores, being confined to coastal habitats and growing on trees and rocks (Tehler 1997). While species circumscription in tropical *Syncesia* is still somewhat problematic, *S. myrticola* is phylogenetically well delimited with specimens forming a distinct well-supported clade within the genus (Ertz *et al*. 2015). Therefore, the phylogenetic results suggest that *E. sorediata* should be conspecific with *S. myrticola*.

TABLE 1. *Specimens and DNA sequences used in this study, with their respective voucher information (h = private herbarium). GenBank Accession numbers in bold refer to sequences generated by this project.*

		GenBank Accession number		
Taxon	Voucher	nuITS	nuLSU	*RPB2*
Dendrographa leucophaea	Mexico, *Tehler* 9104 (S)		HQ454522	HQ454662
Enterographa sorediata	Great Britain, *Sanderson* 2181_A (h)	**MF737086**	**MF737098**	**MF737100**
E. sorediata	Great Britain, *Sanderson* 2181_B (h)	**MF737087**		
E. sorediata	Great Britain, *Sanderson* 2182 (h)	**MF737088**		
E. sorediata	Great Britain, *Sanderson* 2201 (h)	**MF737089**	**MF737099**	**MF737101**
E. sorediata	Great Britain, *Sanderson* 2238 (h)	**MF737090**		
Syncesia depressa	Panama, *van den Boom* 43811 (h)		KJ524325	KJ524414
S. farinacea	Galapagos Islands, *Tehler* 8720 (S)		EF081452	DQ987695
S. farinacea	Panama, *van den Boom* 43836 (h)		KJ524326	KJ524415
S. farinacea	Panama, *van den Boom* 43837 (h)		KJ524327	KJ524416
S. glyphysoides	Guadeloupe, *Ertz* 15905 (BR)		KJ524328	KJ524418
S. graphica	Galapagos Islands, *Ertz* 12059 (BR)		HQ454641	HQ454781
S. hawaiiensis	Hawaii, *Tehler* 10139_19 (S)		KJ524329	KJ524419
S. hawaiiensis	Hawaii, *Tehler* 10154_25 (S)		KJ524330	KJ524420
S. hawaiiensis	Hawaii, *Tehler* 10156 (S)	KF036029		
S. intercedens	Rwanda, *Ertz* 11059 (BR)	**MF737091**	HQ454644	HQ454784
S. leprobola	Galapagos Islands, *Tehler* 8622 (S)		EF081453	DQ987696
S. leprobola	Galapagos Islands, *Ertz* 12054 (BR)		HQ454642	HQ454782
S. leprobola	Panama, *van den Boom* 43790 (h)		KJ524331	KJ524421
S. leprobola	Panama, *van den Boom* 43792 (h)		KJ524332	KJ524422
S. leprobola	Panama, *van den Boom* 43818 (h)		KJ524333	KJ524423
S. madagascariensis	Madagascar, *Ertz* 12966 (BR)		HQ454645	HQ454785
S. mascarena	Reunion, *Ertz* 17987 (BR)		KJ524334	KJ524424
S. mascarena	Reunion, *Ertz* 18052 (BR)	**MF737092**	KJ524335	KJ524425
S. myrticola	France, *Tehler* 9533 (S)		HQ454647	HQ454787
S. myrticola	Azores, *Tehler* 10252 (S)	KF036030		
S. myrticola	Madeira, *Ertz* 10555 (BR)	**MF737093**	HQ454643	HQ454783
S. myrticola	Gomera, *Ertz* 16273 (BR)	**MF737094**	KJ524336	KJ524426
S. myrticola	Gomera, *Ertz* 16274 (BR)	**MF737095**	KJ524337	KJ524427
S. myrticola	Azores, *Ertz* 16659 (BR)	**MF737096**	KJ524338	KJ524428
S. myrticola	Azores, *Ertz* 16857 (BR)	**MF737097**	KJ524339	KJ524429
S. aff. *palmensis*	Gabon, *Ertz* 10000 (BR)		HQ454640	HQ454780
S. aff. *palmensis*	D.R. Congo, *Ertz* 14230 (BR)		KJ524340	KJ524430
S. aff. *palmensis*	D.R. Congo, *Ertz* 14766 (BR)		KJ524341	KJ524431
S. psaroleuca	Panama, *van den Boom* 44378 (h)		KJ524342	KJ524432
S. socotrana	Socotra, *Tehler* 9333 (S)		HQ454648	HQ454788
S. socotrana	Socotra, *Tehler* 9347 (S)		HQ454531	HQ454671
Syncesia sp.	Panama, *van den Boom* 44526 (h)		KJ524343	KJ524433
Syncesia sp.	Panama, *van den Boom* 44529 (h)		KJ524344	KJ524434

In order to verify the results with a more variable locus, we also sequenced nuITS (ITS1 + 5.8S + ITS2; Table 1) to generate a dataset consisting of 14 specimens and 597 unambiguously aligned sites. The resulting phylogenetic tree (Fig. 3) placed *E. sorediata* firmly into *S. myrticola*, leaving no doubt that both taxa are conspecific. This result is in agreement with the published chemistry, both taxa containing protocetraric acid. Roccellic acid is an additional compound present in *S. myrticola* and was not mentioned in the protologue of *E. sorediata*. This may be explained by the high likelihood that the TLC used for that paper (Coppins & James 1979) was probably carried out on an aluminium plate. Hence fatty acids would not have been detected. Our recent specimens of *E. sorediata* examined (viz. *Sanderson* 2201, 2181, 2182, 2238) all contain roccellic

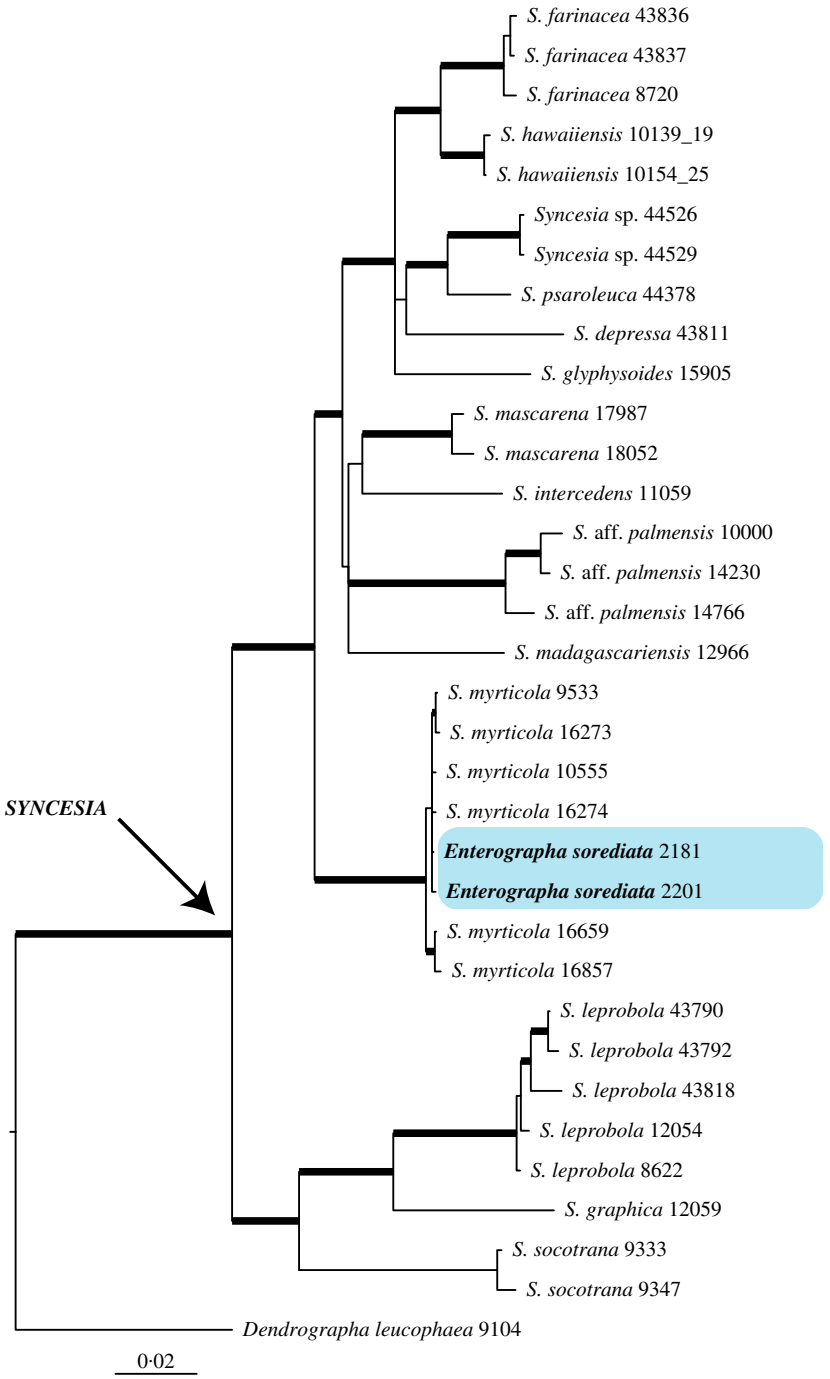

FIG. 2. Phylogenetic relationships within *Syncesia* using 33 samples based on a dataset of nuLSU and *RPB2* sequences that resulted from a Bayesian analysis using MrBayes. Internal branches considered strongly supported (PP ≥ 95) are represented by thicker lines. The newly sequenced samples, corresponding to *Enterographa sorediata*, are highlighted. Author collection numbers following the species names act as specimen and sequence identifiers (see Table 1).

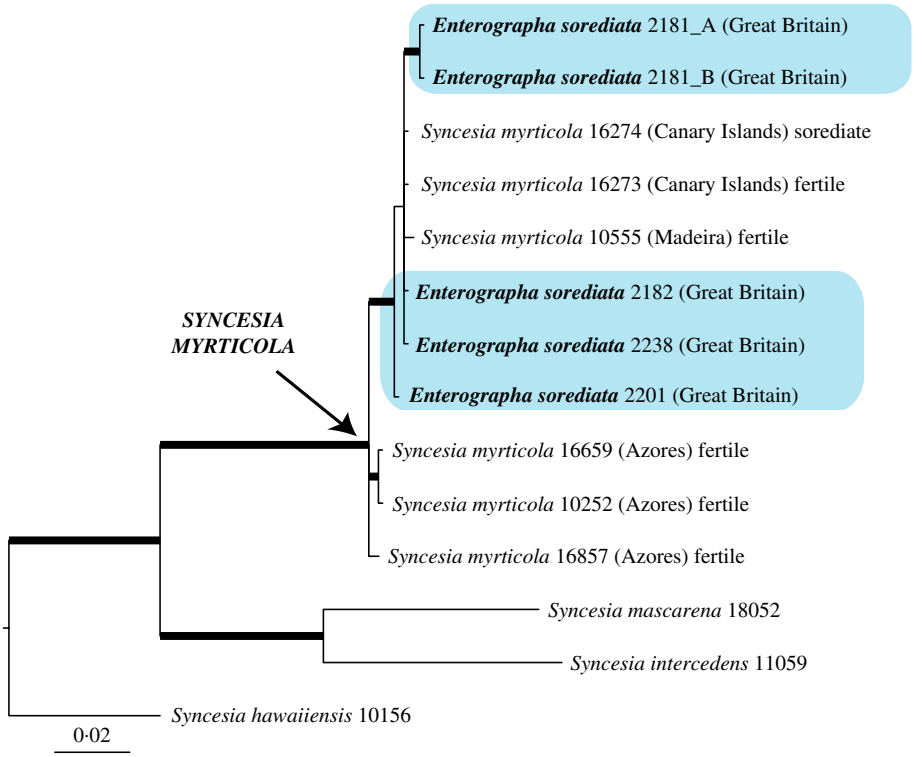

FIG. 3. Phylogenetic relationships within *Syncesia* based on a dataset of nuITS sequences that resulted from a Bayesian analysis using MrBayes. Internal branches considered strongly supported (PP ≥ 95) are represented by thicker lines. The newly sequenced samples, corresponding to *Enterographa sorediata*, are highlighted. Author collection numbers following the species names act as specimen and sequence identifiers (see Table 1).

acid. The phylogenetic results are, however, in contradiction with the ascomata and ascospores reported for *E. sorediata* in the original description. Because *S. myrticola* easily overgrows neighbouring thalli of other crustose species (a byssoid prothallus often appears when the species is actively colonizing, such as in Fig. 1 A & B, but in longer established populations the prothallus is often no longer visible, being replaced by a dark brown to black borderline as in Fig. 1C), we believe that the immature or aborted ascomata and immature ascospores reported in the original description are those of *Enterographa crassa* overgrown by sorediate morphs of *S. myrticola* (Fig. 1). A TLC of the mixed specimen *Sanderson* 2238 (Fig. 1) notably revealed confluentic acid, the main substance present in *E. crassa* but absent in *S. myrticola*. Similar confusing mixtures were also recorded for *Dendrographa decolorans* (Turner & Borrer) Ertz & Tehler growing into *E. crassa* (Sanderson 2002: 4, sub. *Schismatomma decolorans*). A thallus with both fully developed *S. myrticola*-like apothecia and soralia had been recently observed on an ancient *Fraxinus* during a biodiversity survey at Carrigawaddra, County Kerry, Ireland (Sanderson 2016). The possibility of this being *E. sorediata* was discounted in the field owing to the presence of typical *S. myrticola* apothecia. A sorediate, non-fertile thallus of *S. myrticola* has already been published from the Canary Islands but the authors had not made any link with *E. sorediata* (Ertz *et al*. 2015). Since the DNA sequencing was carried out, a specimen of the sorediate morph has been collected in the New Forest from dry bark on an old *Quercus* with typical *S. myrticola* pycnidia and a

developing apothecium. As a consequence, we lectotypify *Enterographa sorediata* on the sorediate thalli and suggest reducing *E. sorediata* into synonymy with *S. myrticola*. We provide an updated distribution map of *Syncesia myrticola* for Great Britain and Ireland, showing that the sorediate morph extends more inland whereas the fertile morph is more coastal (Fig. 4). The sorediate morph has a marked habitat specificity inland, being found almost entirely on the dry side of oaks (*Quercus*) that are more than 250 years old, with rare records from other ancient tree species such as *Alnus* (Sanderson *et al.* 2009) and a *Fraxinus* (recent field observations in Roydon Woods, a nature reserve adjacent to the New Forest). This dispersal strategy with a sterile soredia-producing morph is otherwise common in the *Roccellaceae*, as described in earlier papers (e.g. Tehler & Irestedt 2007; Tehler *et al.* 2010, 2013).

Syncesia myrticola (Fée) Tehler

Flora Neotropica, Monograph **74:** 18 (1997).—*Chiodecton myrticola* Fée, *Essai crypt. Écorc.* 63 (1824); type: not seen.

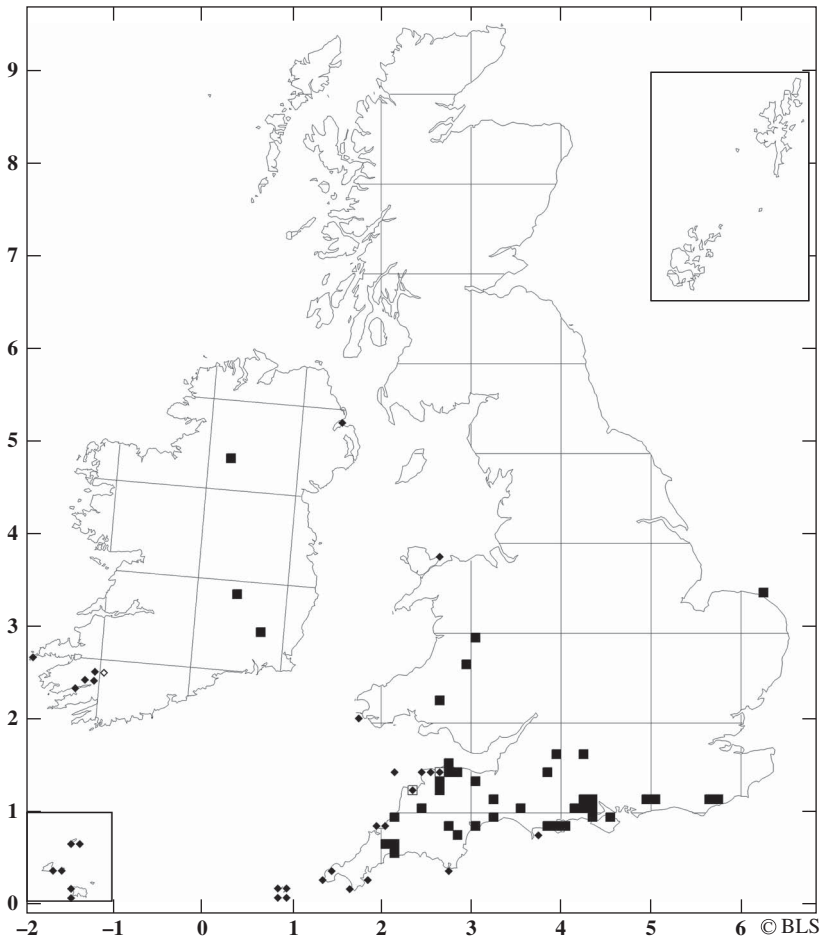

FIG. 4. Distribution of *Syncesia myrticola* in Great Britain and Ireland (© BLS). Solid square = purely sorediate morph; solid diamond = purely fertile morph; open square with solid diamond = both purely sorediate morph and purely fertile morph; open diamond = fertile morph with soralia.

Syn. nov.: *Enterographa sorediata* Coppins & P. James, *Lichenologist* **11:** 40 (1979); type: Great Britain, Anglia, New Forest, Brockenhurst, Hollands Wood, on *Quercus*, 23 November 1976, *F. Rose* (BM—lectotype, sorediate thalli, designated here).

Sequenced specimens of 'Enterographa sorediata'. **Great Britain:** *England*: **V.C. 11,** South Hampshire, New Forest SSSI: Busketts Wood, Great Stubby Hat, Grid Ref. SU30922 11185, in glade in pasture woodland, on dry bark on ancient *Quercus robur*, 2016, *Sanderson* 2181 (private herbarium); Busketts Wood, The Ridge, Grid Ref. SU31447 11281, in glade in pasture woodland, on dry bark on ancient *Quercus robur*, 2016, *Sanderson* 2182 (private herbarium); Bignell Wood, Grid Ref. SU28518 13346, *Quercus-Fagus-Ilex* pasture woodland, on acid bark on suppressed leaning *Quercus robur*, 2016, *Sanderson* 2201 (private herbarium); Allum Green, Grid Ref. SU27943 07798, *Quercus* in *Fagus-Quercus-Ilex* pasture woodland, dry bark of old *Quercus robur*, 2017, *Sanderson* 2238 & *Cross* (private herbarium).

The authors are indebted to Janet Simkin and the British Lichen Society for providing the distribution map of *Syncesia myrticola*. Cyrille Gerstmans and Wim Baert are warmly thanked for technical assistance.

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