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Standard Paper

A new species of *Synarthonia* from Luxembourg, and a new combination in the genus *Reichlingia* (*Arthoniaceae*)

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Abstract

A new species of *Synarthonia*, *S. leproidica*, is described from Luxembourg. Phylogenetic analyses of mtSSU and *RPB2* sequences were used to determine the generic affiliation of this sterile species. *Synarthonia leproidica* differs from all other species of the genus by the combination of a leproid thallus and the production of psoromic acid. It is the sister species to *S. muriformis* in our phylogenetic analyses. The discovery of the new species suggests that other strictly sorediate lichen species might have been overlooked in Europe, even in intensely explored countries such as Luxembourg. Phylogenetic analyses further confirm the placement of *Reichlingia anombrophila* in the genus *Reichlingia* and of *Synarthonia astroidestera* in the genus *Synarthonia*. *Arthonia atlantica* is transferred to the genus *Reichlingia* as *R. dendritica*.

Key words: *Arthoniales*, biodiversity, lichen, phylogeny, taxonomy

(Accepted 11 March 2020)

Introduction

The family *Arthoniaceae* has a worldwide distribution and includes lichenized, lichenicolous and saprobic species (e.g. Tehler 1990; Sundin & Tehler 1998; Sundin 1999; Grube 2001, 2007; Frisch *et al.* 2014*a*). It is the largest family in the order *Arthoniales* and the seventh largest family in terms of number of lichenized species (Lücking *et al.* 2017). The family is undergoing major changes following molecular analyses, with large genera being split into smaller entities (e.g. Frisch *et al.* 2014*a*, 2015; Van den Broeck & Ertz 2016; Van den Broeck *et al.* 2018).

In 2011, a lichenological field meeting was organized in Luxembourg as part of the annual field meeting of the association 'Werkgroep Bryologie en Lichenologie' of Flanders (Belgium). A total of nine localities were visited in the Ardenne district (Oesling), a massif of markedly siliceous rocks dating back to the Cambrian, Ordovician and Lower Devonian, covered by extensive forests. In the course of three days, a total of 253 lichens and lichenicolous fungi were recorded (Van den Broeck *et al.* 2013). A striking species having a leproid thallus with a trentepohlioid photobiont was collected on siliceous rocks and could not be identified. The localities were revisited in 2018 to collect additional fresh specimens for sequencing, in order to determine its generic position. The present study aims to describe this new species in the genus *Synarthonia* with the support of molecular data. An additional sequence obtained from British material of *Arthonia atlantica* P. James, which belongs to the same subclade of *Arthoniaceae* as the new species, was added to the phylogenetic analyses to determine its generic affiliation. The phylogenetic positions of *Reichlingia anombrophila* (Coppins & P. James) Frisch and *Synarthonia astroidestera* (Nyl.) Ertz & Van den Broeck are shown for the first time.

Materials and Methods

Voucher specimens are deposited in the herbaria BR and E, and in the private collections of N. Sanderson and P. Diederich. The external morphology was studied and measured using an Olympus SZX12 stereomicroscope. Macroscopic images were captured with a Keyence VHX-5000 digital microscope and a VH-Z20R/W/T lens. Hand-cut sections and squash preparations of thallus were mounted in water, 5% aqueous potassium hydroxide solution (K), or Lugol's iodine solution (1% I_2) without (I) or with K pretreatment (KI) and observed using an Olympus BX51 compound microscope. Measurements refer to dimensions in K. Microscopic images were captured using an Olympus BX51 compound microscope fitted with an Olympus SC50 digital camera. Colour reactions of the thallus were studied using K, common household bleach (C), K followed by common household bleach (KC), crystals of para-phenylenediamine dissolved in ethanol (PD) and longwave UV (366 nm). Lichen secondary metabolites were identified using thin-layer chromatography (TLC) in solvents B, C and G (Orange *et al.* 2010).

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Table 1. Species names, voucher specimens, countries of origin and GenBank Accession numbers, of sequences generated in this study, for the specimens included in the phylogenetic inference (Fig. 1). GenBank Accession numbers for the other taxa in Fig. 1 are listed in Van den Broeck *et al.* (2018).

Species name	Voucher	Country	Accession numbers	
			mtSSU	*RPB2*
Reichlingia anombrophila	*Coppins* 25590 (E)	Great Britain	MT141111	MT140891
R. dendritica	*Sanderson* 2598 (hb. Sanderson)	Great Britain	MT141112	-
Synarthonia astroidestera	*Ertz* 17487 (BR)	France	MT141113	MT140892
S. leproidica	*Ertz* 17302 (BR)	Luxembourg	MT141114	-
S. leproidica	*Ertz* 22685 (BR)	Luxembourg	MT141115	MT140893
S. leproidica	*Ertz* 22686 & *Diederich* (BR)	Luxembourg	MT141116	-
S. leproidica	*Ertz* 22687 (BR)	Luxembourg	MT141117	MT140894

Molecular techniques

Well-preserved and freshly collected specimens lacking any visible symptoms of fungal infection were used for DNA isolation. Hand-cut sections of the apothecia or a small number of soredia were used for direct PCR as described in Ertz *et al.* (2015). The lichen material was washed with acetone and then rinsed with water to remove remnants of pigments. The material was placed directly in microtubes with 20 µl H_2O. Amplification reactions were prepared for a 50 µl final volume containing 5 µl 10× DreamTaqBuffer (Thermo Scientific, www.thermoscientific.com/onebio), 1.25 µl of each of the 20 µM primers, 5 µl of 2.5 mg ml^{-1} bovin serum albumin (Thermo Scientific), 4 µl of 2.5 mM dNTPs (Thermo Scientific), 1.25 U DreamTaq DNA polymerase (Thermo Scientific) and the lichen material. A targeted fragment of *c.* 0.8 kb of the mtSSU rDNA was amplified using primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999). 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). The yield of the PCR reactions was verified by running the products on a 1% agarose gel using ethidium bromide for visualization. Both strands were sequenced by Macrogen® using amplification primers. Sequence fragments were assembled with Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to 'Megablast' searches in GenBank to verify their closest relatives and to detect potential contaminations.

Taxon selection and phylogenetic analyses

Seven new mtSSU sequences from four species and four *RPB2* sequences from three species were obtained for this study (Table 1). For the phylogenetic analyses, the sequences were included in the mtSSU and the *RPB2* datasets published by Van den Broeck *et al.* (2018), consisting of taxa representing all major clades currently accepted in the *Arthoniaceae* except for the more distantly related *Bryostigma* clade (Frisch *et al.* 2014*a*). The sequences were aligned using MAFFT v.7.402 (Katoh *et al.* 2002) on the CIPRES Web Portal (Miller *et al.* 2010) and manually corrected for errors using Mesquite 3.04 (Maddison & Maddison 2015). *Arthothelium norvegicum* Coppins & Tønsberg was chosen as outgroup species. Terminal ends of sequences and ambiguously aligned regions were delimited manually and excluded from the datasets.

To examine topological incongruence among datasets, a maximum likelihood (ML) analysis was carried out on each of the single-locus datasets. We used RAxML v.8.2.12 (Stamatakis 2014) with 1000 replicates of ML bootstrapping (ML-BS) under the GTRGAMMA model of sequence evolution. Analyses were run on the CIPRES Web Portal (Miller *et al.* 2010). All topological bipartitions were compared for the two loci. A conflict was assumed to be significant when differing topologies for the same set of taxa (one being monophyletic and the other being non-monophyletic) were each supported with bootstrap values ≥ 70 (Mason-Gamer & Kellogg 1996). Based on this criterion, the same conflict as already highlighted by Van den Broeck *et al.* (2018) was detected regarding the sister-group relationship of *Synarthonia* with either *Coniocarpon* or *Reichlingia*. As this conflict had no impact on the monophyly of either *Reichlingia* or *Synarthonia*, and thus on our conclusions regarding the generic affiliations of the newly sequenced species, the mtSSU and *RPB2* datasets were concatenated.

The combined two-locus dataset of 56 samples consisted of 1503 unambiguously aligned sites, 639 for mtSSU and 864 for *RPB2*. Bayesian analyses were carried out on the concatenated two-locus dataset using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method in MrBayes v.3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES Web Portal (Miller *et al.* 2010). Best-fit evolutionary models for each partition were estimated using the Akaike Information Criterion (AIC) as implemented in jModelTest2 (Darriba *et al.* 2012). The GTR + I + G model was selected for the mtSSU dataset as well as for the *RPB2*/1st position, while the TVM + I + G model was selected for the *RPB2*/2nd and *RPB2*/3rd positions. Two Bayesian MCMCMC runs were executed in parallel, each using four independent chains and 120 million generations, sampling trees every 1000th generation. Tracer v.1.6.0 (Rambaut *et al.* 2013) was used to ensure that convergence was reached by plotting the log-likelihood values of the sample points against generation time. Convergence between runs was also verified using the PSRF (Potential Scale Reduction Factor), confirming that values for all parameters were equal to 1.000. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 180 002 post burn-in trees out of the 240 002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. In addition, a maximum likelihood (ML) analysis was performed using RAxML v.8.2.12 (Stamatakis 2014) with 1000 ML bootstrap iterations (ML-BS) and the GTRGAMMA model.

The Bayesian tree did not contradict the RAxML tree topology for the strongly supported branches. Therefore, only the Bayesian tree is shown, with the PP values added above the internal branches

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and the ML-BS values added below (Fig. 1). Internodes with ML-BS ≥ 70 and PP ≥ 95 were considered strongly supported (Alfaro *et al.* 2003; Lutzoni *et al.* 2004). Phylogenetic trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Results and Discussion

Phylogenetic analyses

The Bayesian tree obtained from the combined two-locus analysis of 56 samples is shown in Fig. 1. The main well-supported lineages of *Arthoniaceae* are in accordance with the results obtained by Van den Broeck *et al.* (2018). The genus *Synarthonia* is placed in a well-supported lineage (ML-BS = 96 and PP = 1) with the genus *Coniocarpon* and the *Reichlingia* group, both also being well-supported monophyletic groups (ML-BS = 98–100 and PP = 1) (Fig. 1). The new species is nested within the genus *Synarthonia*, as sister taxon to *S. muriformis*. It belongs to the core group of *Synarthonia* (i.e. from *S. astroidestera* to *S. inconspicua* (Stirt.) Van den Broeck & Ertz) characterized by species having notably white pruinose ascomata (as defined by Van den Broeck *et al.* 2018). This core group is well supported in our phylogenetic tree (ML-BS = 99 and PP = 1). Our new sequences further confirm the placement of *Arthonia astroidestera* in the genus *Synarthonia*. This species was transferred from *Arthonia* to *Synarthonia* by Van den Broeck *et al.* (2018) based on morphology and chemistry alone. The newly sequenced *Arthonia atlantica* clusters with species of *Reichlingia* in a strongly supported lineage (ML-BS = 77 and PP = 1) and is thus transferred to this genus (see Taxonomy section). *Arthonia anombrophila* was recently transferred to the genus *Reichlingia* by Frisch *et al.* (2020) based on unpublished sequences from a Norwegian specimen. Our new sequences from British material confirm its placement in *Reichlingia*. Relationships within the genus *Reichlingia* are poorly supported. The generic affiliation of *A. anglica* published by Ertz *et al.* (2009; sub '*Arthonia* sp. 1') is in need of further studies with more data, and the identity of the sequenced specimen requires confirmation because the sample comes from tropical Africa while the type locality of *A. anglica* is in Great Britain.

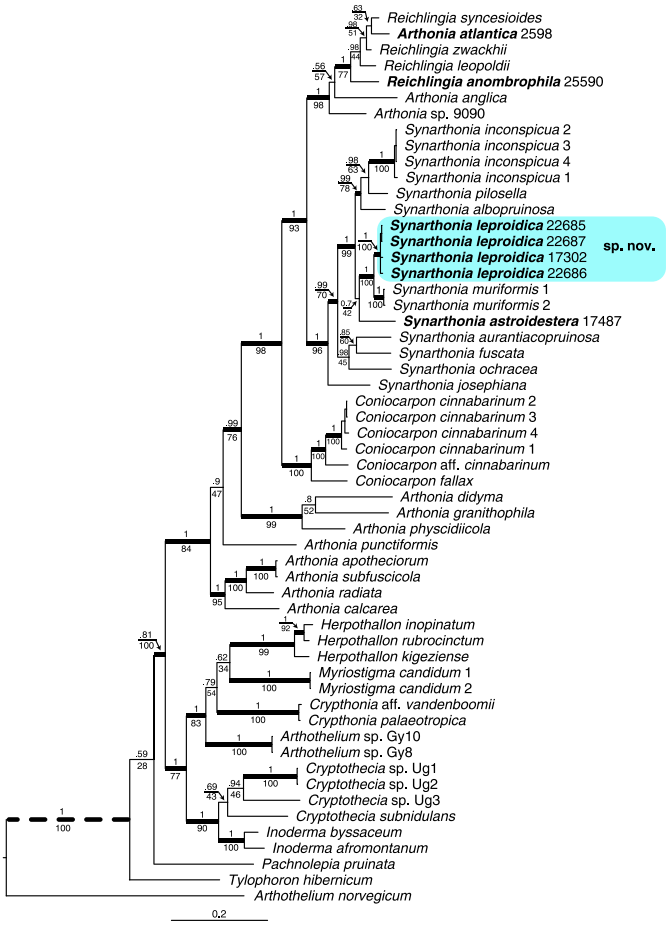

Fig. 1. Phylogenetic relationships among a selected group of *Arthoniaceae* resulting from a Bayesian analysis based on a dataset of 56 samples of mtSSU and *RPB2* sequences. *Arthothelium norvegicum* was chosen as outgroup. MrBayes posterior probabilities are shown above branches, and RAxML bootstrap values are shown below branches. Thicker lines highlight internodes considered as strongly supported by both analyses. Names of samples for which sequences were generated in this study are indicated in bold. The new species of *Synarthonia* is highlighted with a shaded box. In colour online.

Taxonomy

Synarthonia leproidica Ertz, Aptroot & Diederich sp. nov.

MycoBank No.: MB 834820

A species of *Synarthonia* characterized within the genus by a pale greyish leproid thallus with a dark brown violaceous tinge at the surface, psoromic acid as a thallus compound, a saxicolous habit and a phylogenetic position sister to *Synarthonia muriformis*.

Type: Luxembourg, Lellingen, Vallée du Lellgerbaach, Op Bärel, 49°59′11″N, 6°01′16″E, 323 m elev., chênaie-charmaie de bas de pente exposée nord-nord-ouest, petite paroi siliceuse éclairée au bord d'un chemin forestier, sur face rocheuse subverticale plus ou moins abritée de la pluie, 8 September 2018, *Ertz* 22686 & *Diederich* (BR—holotype!; hb. Diederich—isotype!).

(Figs 1 & 2)

Thallus saxicolous, crustose, leproid, forming patches of *c.* 0.5–5 mm diam., or confluent and often covering large areas up to *c.* 10 cm diam., up to 1(–2) mm thick, pale greyish, with dark brown violaceous tinge at the surface, and a greenish tinge inside when abraded (in thicker parts of the thallus). *Soredia* (14–)20–38 µm diam., formed of individual or short chains of photobiont cells surrounded by hyaline to dark brown hyphae of 2(–3) µm diam.; soredia without projecting hyphae, with many hyaline crystals of *c.* 0.5–1.5 µm diam. visible on the hyphae in polarized light that dissolve in K. *Photobiont* trentepohlioid, containing orange pigments, visible as individual globose cells, (6–)9–15(–25) µm diam. or in short chains of *c.* 2–4 cells, with individual elliptical to rectangular algal cells of 13–20(–29) × (5–)9–16 µm.

Ascomata and *conidiomata* unknown.

Chemistry. Thallus K−, C−, PD+ bright orange-yellow, UV−; hyphae I+ pale orange, KI+ pale orange. TLC revealed the presence of psoromic acid (specimen *Ertz* 17302 tested in solvents B and G; *Ertz* 22685, 22686, 22687 tested in solvent C).

Etymology. The specific epithet refers to the leproid thallus.

Distribution and ecology. So far known only from three localities in Luxembourg, where it inhabits sheltered siliceous schistose

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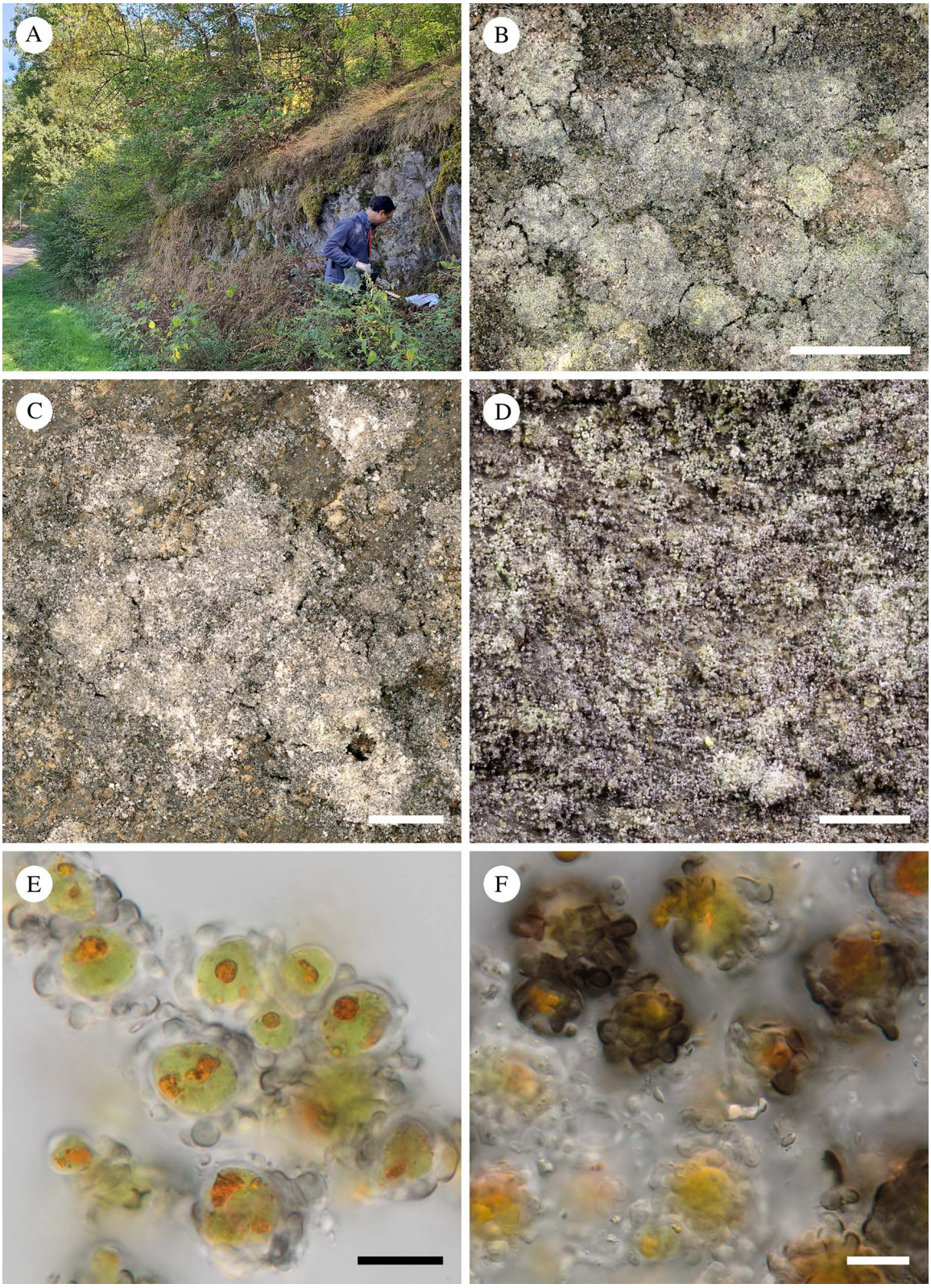

Fig. 2. *Synarthonia leproidica* (B & E, *Ertz* 22687; C & F, *Ertz* 22686 – holotype; D, *Diederich* 18666). A, habitat in Lellingen (Luxembourg). Type locality where *S. leproidica* is abundant on the rocks. B–D, thallus. E & F, soredia in K. Scales: B = 2.5 mm; C = 0.8 mm; D = 1 mm; E & F = 10 µm. In colour online.

rock faces in rather open conditions near forest edges. The associated lichen vegetation is species poor and also includes *Psilolechia lucida* (Ach.) M. Choisy and *Lepraria* spp.

Discussion. Synarthonia leproidica is a saxicolous sterile leproid crustose lichen with a trentepohlioid photobiont and a greyish thallus with a dark brown violaceous tinge reacting PD+ bright

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orange-yellow. Our molecular data surprisingly place the species within the genus *Synarthonia* (Fig. 1). The new species is at present the only known leproid member of the genus and also the only saxicolous *Synarthonia* (Van den Broeck *et al.* 2018). *Synarthonia sikkimensis* S. Joseph & G. P. Sinha is the only known species of the genus having a sorediate thallus. In that species, the thallus is never leproid, but discrete small soralia are present near the margins of the thallus. Moreover, *S. sikkimensis* differs from the new species by a well-developed rhizomorph-like prothallus and the absence of secondary metabolites in the thallus (Joseph & Sinha 2015). Psoromic acid is a substance present in the new species but also in several other species of *Synarthonia* (e.g. *S. borbonica* (Ertz *et al.*) Van den Broeck & Ertz, *S. psoromica* S. Joseph & G. P. Sinha, *S. muriformis* Van den Broeck *et al.*). In our phylogenetic analyses, it is notable that the closest relative of *S. leproidica* is *S. muriformis* which shares psoromic acid but its chemistry is more varied with the presence of evernic acid and two unknown UV+ white unidentified secondary compounds (Van den Broeck *et al.* 2018).

Siliceous rock outcrops in the Luxembourg part of the Ardennes are surprisingly rich in sterile *Arthoniales*. *Dendrographa latebrarum* (Ach.) Ertz & Tehler and *Sparria endlicheri* (Garov.) Ertz & Tehler are other strictly sterile *Arthoniales* while others are lichenized hyphomycetes such as *Milospium deslooveri* Diederich & Sérus. and *Reichlingia leopoldii* Diederich & Scheid. The discovery of the new species suggests that other strictly sorediate lichen species might have been overlooked on siliceous rocks in European regions, even in intensely explored countries such as Luxembourg.

Snippocia nivea (D. Hawksw. & P. James) Ertz & Sanderson is another strictly sterile member of the *Arthoniaceae* known from Western Europe containing psoromic acid but it differs from the new species by a corticolous pale pink thallus (James 1971) and is unrelated to the genus *Synarthonia* in molecular studies (Ertz *et al.* 2018). *Roccellographa sorediata* (Sparrius *et al.*) Coppins & Fryday is a common saxicolous, usually sterile, ±entirely sorediate lichen also producing psoromic acid, but it differs from the new species by a milk white to grey thallus with a conspicuous black prothallus and inhabits coastal rock faces (Fletcher 2009).

Additional specimens examined. **Luxembourg:** Lellingen, Vallée du Lellgerbaach, Op Bärel, 49°59′25″N, 6°02′03″E, 350 m elev., 2011, *Ertz* 17302 (BR) & *Diederich* 17236 (hb. Diederich); *ibid.*, 2018, *Ertz* 22685 (BR) & *Diederich* 18665 (hb. Diederich); same locality as type, 2018, *Diederich* 18666 (hb. Diederich); Bourscheid, sentier autour du château, 49°54′21″N, 6°04′49″E, 362 m elev., petite paroi de rocher siliceux ombragée sous le mur d'enceinte du château, sur face rocheuse subverticale ± abritée de la pluie, 2018, *Ertz* 22687 (BR) & *Diederich* 18663 (hb. Diederich).

Reichlingia

The genus *Reichlingia* was originally monotypic and described as a lichenicolous fungus (Diederich & Scheidegger 1996) but is now generally considered to be a lichenized hyphomycete (Diederich & Coppins 2009). The type species has a byssoid thallus and produces dark brown, multicellular, branched conidia grouped in irregular sporodochia-like conidiomata on its upper surface (Diederich & Coppins 2009). The genus has been emended to include three fertile species of *Arthoniaceae* (Frisch *et al.* 2014*b*) and *Arthonia anombrophila* was recently transferred to *Reichlingia* (Frisch *et al.* 2020). Here, DNA sequence data support *A. atlantica* as an additional species in this genus and the phylogenetic position of *R. anombrophila* is shown for the first time (see 'Phylogenetic analyses' above). The morphology of both species fits well with the current concept of the genus *Reichlingia*, although *R. anombrophila* differs from the other species by having an immersed or partly superficial, compact thallus.

Reichlingia dendritica (Leight.) Ertz & Sanderson comb. nov.

MycoBank No.: MB 834821

Stigmatidium dendriticum Leight., in *J. Bot., Lond.* **13**, 257 (1875).—*Arthonia dendritica* (Leight.) Cromb., *J. Bot., Lond.* **14**, 362 (1876), non (Ach.) Duf. (1818).—*Enterographa dendritica* (Leight.) P. James, *Lichenologist* **3**, 97 (1965).—*Arthonia atlantica* P. James, *Lichenologist* **4**, 318 (1970); type: not seen.

Notes. *Arthonia dendritica* (Leight.) Cromb. was illegitimate because of the earlier homonym *Arthonia dendritica* (Ach.) Duf. (=*Phaeographis dendritica* (Ach.) Müll. Arg.). Therefore, James (1970) published the replacement name *Arthonia atlantica* P. James for accepting the species in *Arthonia*. Following our phylogenetic results where *A. atlantica* is the sister taxon of *Reichlingia syncesioides* Frisch & G. Thor (Fig. 1), a new combination is made in *Reichlingia* using the epithet from the basionym (*Stigmatidium dendriticum* Leight.), while *Arthonia atlantica* becomes one of its homotypic synonyms.

Sequenced specimen. **Great Britain:** *Wales*: **V.C.48,** Merionethshire, Coed Maentwrog NNR, Coed Glan-yr-afon, Grid Ref. SH67465 41609, 70 m elev., rock face in oceanic pasture woodland, overhanging slate outcrop, 2019, *Sanderson* 2598 (hb. Sanderson).

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