

Dismantling the treasured flagship lichen *Sticta fuliginosa* (Peltigerales) into four species in Western Europe

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Abstract In the framework of a worldwide project on the phylogeny of the lichen genus *Sticta*, dedicated sampling was performed in four regions of Western Europe, roughly along an east–west line between N 48°02' E 07°01' and N 52°01' W 09°30', ranging from France/Vosges to Ireland/Kerry. Five clearly distinct ITS haplotypes were detected for isidia-producing species where only two were expected. Subtle anatomical and morphological characters, together with a strongly supported 4-loci molecular phylogeny, permit distinguishing, besides the easily recognized *S. canariensis* and *S. limbata*: 1) the two well-known *S. fuliginosa* and *S. sylvatica*, whose type collections have been carefully reassessed; the former is widespread in both hemispheres, while the latter is correctly identified only from continental Europe and the Andes in Colombia. The barcode ITS of *S. fuliginosa* differs by a single substitution from *S. limbata* (with a single exception), and the 4-loci phylogenetic tree does not resolve them as distinct lineages, most probably highlighting a very recent divergence and incomplete lineage sorting. 2) Three species that were formerly included in *S. fuliginosa*: the resurrected *S. ciliata* Taylor, belonging to a complex group yet to be disentangled and occurring in the Neotropics, Africa, Macaronesia, and Western Europe, and two species described as new to science, *S. fuliginoides*, found in continental Europe, the Canary Islands, eastern North America, and

Colombia, and *S. atlantica* only known from Ireland and the Azores archipelago. Molecular inferences demonstrate active divergence and dispersion within *S. ciliata* that may require recognition of further species. Fresh material can be identified with a morphological and anatomical preliminary key provided here. We propose that the taxonomy of all lichen species be urgently reviewed in the light of molecular data in an evolutionary context, particularly those used as bioindicators of environmental change and woodland management.

Keywords Lichenized fungi · Peltigerales · Phylogeny · Taxonomy

Introduction

The lichen genus *Sticta* features as one of the best-known macrolichens, easily recognized by its most usually large thalli, well-differentiated small pores on the lower surface (named cyphellae) and a typical fishy smell in wet conditions because of methylamines production. It is subcosmopolitan, being well-developed and diverse in humid, cool- to warm-temperate environments. It is thus most conspicuous, together with other genera in the Peltigerales such as *Lobaria*, *Peltigera*, and *Pseudocyphellaria*, and representatives of the families Collemataceae and Pannariaceae, in temperate humid coastal areas (northwestern coast of North America, southern Chile, Tasmania, and New Zealand) and in tropical montane forests, with an impressive diversity in the northern Andes in Colombia (Moncada and Lücking 2012; Moncada et al. 2013b, c, 2014a, b; Suárez and Lücking 2013). It occurs at low elevation as well, including in tropical areas, with less diversity and biomass, and is completely absent in desert habitats and dry forests.

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The genus *Sticta* belongs to the Lobariaceae, which now includes 13 genera (Moncada et al. 2013a; Miadlikowska et al. 2014a; Galloway 2015). Pending that species in the *S. wrightii* gr. (incl. *S. nylanderiana*, *S. praetextata*, *S. oroborealis*, and *S. wrightii*) are referred to an autonomous genus (*Dendriscosticta*), the genus is strongly supported by phylogenetic analyses with inferences from molecular data (Högnabba et al. 2009; Miadlikowska et al. 2014a; Moncada et al. 2014a). As in several other lineages in the Peltigerales, it can establish a symbiotic association with either cyanobacteria (belonging to the genus *Nostoc*) or green algae (belonging to the genus *Dictyochloropsis*), or both. A rather simple photomorph pattern occurs in several clades within *Sticta* s. str.: it is made of two different foliose thalli, fixed one to the other or not; the one associated with *Nostoc* most usually has a typically strongly maculate upper cortex (Magain et al. 2012a; Moncada et al. 2013b). Dedicated structures including *Nostoc* colonies (named cephalodia) on the surface of or inside a green thallus do not occur in the genus. *Dendriscoaulon*-like cyanomorphs such as those of the *Lobaria amplissima* group (*Ricasolia*) are now referred to *Dendriscosticta* or to stipitate species of *Sticta* (Galloway 2001, 2007; Magain et al. 2012a). Indeed, *Dendriscoaulon dendriothamnoides* and *D. dendroides* are either free-living or associated with stipitate species of *Sticta*, *S. stipitata*, and *S. filix* respectively, and it is likely that all the caulescent species of *Sticta* will have *Dendriscoaulon* states (Galloway 2007).

Prior to the studies of Moncada and Lücking (2012), Moncada et al. (2013b, c, 2014a, b) and Suárez and Lücking (2013), the genus contained 114 accepted species, with only a few recently described (Aptroot 2008; Øvstedal and Gremmen 2010; Upreti and Divakar 2010; Lumbsch et al. 2011), and a single study focussed on species delimitation with molecular data combined with morphological and anatomical characters (McDonald et al. 2003). The revision of large collections recently gathered in Colombia, especially in the paramos vegetation belt, together with our own data assembled from Macaronesia, continental Africa, as well as Madagascar and the Mascarene archipelago, highlights a very different picture: the easily recognized morphotypes (all lichenized with *Nostoc*), such as that of *S. fuliginosa* (hardly branched thallus with rounded lobes and laminal isidia), *S. weigelii* (branched thallus with narrow lobes and marginal isidia), *S. sylvatica* (branched thallus with narrow lobes and laminal isidia), or *S. sublimbata* (branched thallus with narrow lobes and marginal isidioid soralia), are resolved into several mostly unrelated lineages (Moncada et al. 2014a). Many further species can thus be distinguished on a sound phylogenetical basis.

In Europe, the genus is considered a straightforward genus with four well-known species (Lambinon 1969; Llimona and Hladun 2001; Burgaz and Martínez 2003; Jørgensen and Tønsberg 2007; Smith et al. 2009; Wirth et al. 2013): *S. canariensis* (Flörke) Delise, with its green photomorph

unique in the European context and its cyanomorph with a maculate upper surface and a crisped margin with phylidia (also known as *S. dufourii* Delise), *S. limbata* (Sm.) Ach. with marginal soralia, and the duo *S. fuliginosa* (Hoffm.) Ach.—*S. sylvatica* (Huds.) Ach., both with laminal isidia and being mainly distinguished by their branching pattern. Actually, the taxonomy of *Sticta* species in Europe has not changed since 1826 when the magistral work of Delise provided detailed descriptions for many species around the world, including *S. canariensis*, *S. dufourii*, *S. fuliginosa*, *S. limbata*, and *S. sylvatica* (Delise 1826). Quite obviously, Delise did not recognize that *Sticta canariensis* was the green photomorph of *S. dufourii*, first demonstrated by Armaleo and Clerc (1991), as he could not even imagine the dual nature of a lichen thallus that was eventually discovered decades later by Schwendener (1868).

In Europe, all species in the Lobariaceae, especially *Sticta*, enjoy a special status as they are recognized as patrimonial in most countries, and greatly suffer from air pollution and habitat disturbance. They indeed qualify for being very sensitive to human activities and are considered pertinent bioindicators and priority targets of conservation programs. Species of *Sticta* play a crucial role in the calculation of Indices of Ecological Continuity for woodland habitats in the British Isles (Coppins and Coppins 2002), as well as in the Pacific Northwest in North America (Tønsberg and Goward 2001; Doering and Coxson 2010; Radies and Coxson 2004; Radies et al. 2009). They also indicate very low levels of nitrogen deposit in forests throughout Europe (Giordani et al. 2014) and were recently included in newly designed indices for air quality in the Pacific Northwest (Root et al. 2015). However, the role of several ecological drivers (woodland extent and continuity, air pollution regime and climate) at regional scale and their interaction are complex, and no single and simple model can be designed to explain the composition and richness of epiphytic lichen vegetation, especially in the framework of climate change (Ellis 2012, 2013). Nevertheless, and especially at the margins of their distribution area, species of *Sticta* receive much attention, as demonstrated by the dedicated action plan proposed for Swedish species (Hultengren 2005).

However, there is no a priori reason to profess that the genus is less diverse in Europe than in other regions of the world, and that the Delise taxonomy, almost two centuries old, still is on the right track in the molecular age. We thus sampled the genus in four ecoregions in Western Europe, from the Vosges in France where the two most widespread species occur (*S. fuliginosa* and *S. sylvatica*), although they are quite rare in central Europe (Degelius 1935; Wirth et al. 2013), to SW Ireland where the four species are known to occur (Smith et al. 2009). The aim of this paper is to present the results of this work, together with a preliminary key for the identification of the European species of this emblematic and cherished, yet poorly known, genus.

Material and methods

This work is mainly based on the comparison of molecular and morphological and anatomical data of fresh material. The localities were chosen as representative of the continental environment where the two most familiar species (reported as *Sticta fuliginosa* and *S. sylvatica*) occur and more oceanic environments where the four European species have been recorded (reported as *S. canariensis*, *S. fuliginosa*, *S. limbata* and *S. sylvatica*). The localities are the following:

- France, Dept. Haut-Rhin, Hohneck, N 48°02' E 07°01', c. 1000 m elev., sampled by the authors; this locality is representative of the Vosges ridge where species of *Lobaria* (*L. amplissima*, *L. pulmonaria*, and *L. scrobiculata*) and *Sticta fuliginosa* and *S. sylvatica* occur, as well as other species typical of *Lobarion* communities such as *Nephroma parile*, *N. resupinatum*, and *Peltigera collina*; similar localities occur in the Black Forest on the right side of the Rhine graben (Wirth 1995; Wirth et al. 2013);
- France, Dept. Finistère (part of Brittany), sampled by B. Bouffinier, A. Gérault and F. Séité at several localities well known for their interesting epiphytic flora (Coppins 1971; Aptroot et al. 2007; <http://www.lichensmaritimes.org>), incl. Huelgoat (N 48°22' W 03°44', c. 150–200 m elev.), the Chaos de St-Herbot (N 48°20' E 03°48', c. 200–220 m) and the Cranou forest (N 48°19' E 04°05', c. 140–200 m elev.);
- United Kingdom/Somerset, Horner woods, N 51°10' W 03°35', c. 290 m elev., sampled by Pat Wolseley; Ibid./Devon, Popham Woods, Brayford, N 51°4'50" W 3°51'32", sampled by the authors; Ibid., Sampford Spiney, N 50°32'3", W 4°3'37", sampled by the authors; the SW part of England is renowned for its epiphytic lichen flora (Seaward 1998);
- Ireland/Kerry, Killarney, N 52°01' W 09°30', c. 30–40 m elev. (centered on Muckross), a renowned protected area, whose lichen flora has been thoroughly explored (Giavarini 2012); sampling by the authors was made after a major thunderstorm that fell down hundred of trees (Feb. 2014), collecting has been restricted to fallen trees.

These localities are considered representative of continental Europe and referred to as continental Europe. Macaronesia refers to the three archipelagos of the Azores, Madeira, and the Canary Islands, and of course are not considered part of continental Europe.

As all species of *Sticta* are rare and vulnerable throughout most regions of Western Europe, collecting was prudent and parsimonious and concentrated on specimens growing on fallen trees or branches, or in unsustainable conditions such as

“ready to fall” because of recent disturbance. Vouchers are preserved in the LG herbarium.

Molecular data

Extraction of DNA followed the protocol of Cubero et al. (1999). We sequenced the ribosomal nuclear loci ITS using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for all collections assembled for this study (188 specimens). Little variation was detected for the barcode ITS, except for *Sticta ciliata*, which comprised several haplotypes in the material from continental Europe and Macaronesia. We thus selected a representative specimen of each species and haplotype detected in each of the four localities prospected for this study and produced LSU, mtSSU, and *RPBI* sequences for them. We added data obtained from our own collections from Madagascar, Reunion in the Mascarene archipelago, the Albertine Rift in East Africa (Congo RDC and Rwanda), and Macaronesia (the Azores and the Canary Is.). We further added data obtained from recently collected material by B.J. Coppins in Scotland, P. K. Divakar in the Azores archipelago, B. Goffinet in USA/North Carolina and South Africa, T. Goward in Canada/British Columbia, and P. van den Boom in the Canary Islands.

LSU primers were LR0R and either LR7 (Vilgalys and Hester 1990) or LIC2044 (Kauff and Lutzoni 2002), the mitochondrial ribosomal locus mtSSU primers were SSU1 and SSU3R (Zoller et al. 1999) and for part of the protein-coding gene *RPBI*, with *RPB1AF* (Stiller and Hall 1997) and *RPB1CR* (Matheny et al. 2002). Amplicons were sequenced by Macrogen© or by the GIGA biotechnology platform of the University of Liège.

Sequences editing and alignment

Sequence fragments were assembled with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were subjected to megaBLAST searches (Wheeler et al. 2007) to detect potential contaminations. Sequences were aligned manually using MacClade version 4.08 (Maddison and Maddison 2005) and checked with the MAFFT software (Kato and Standley 2013). Ambiguous regions were delimited with the GBLOCK server (Castresana 2000), using the default parameters and manually checked; all ambiguous regions were excluded from the analyses. The data matrix is deposited in TreeBASE under the accession # 17996.

Concatenation and partitioning

Congruence of the four fungal loci was assessed by the comparison of single-locus phylogenetic trees produced with RAXML HPC2 version 7.2.8 (Stamatakis 2006; Stamatakis et al. 2008) as implemented on the CIPRES portal (Miller et al. 2010), looking

for the best ML tree and bootstrapping with 1000 pseudoreplicates in the same run, using GTRCAT model and the default settings. No significant conflict with bootstrap values ≥ 70 % was detected; therefore, the four loci were concatenated in a single data set. Two matrices were assembled, one containing 54 accessions of *Sticta* and one accession of *Pseudocyphellaria crocata* as outgroup, and a second one containing two additional ITS sequences of *Sticta "microisidiata ad. int."* from Colombia retrieved from GenBank. After removal of the ambiguous characters, the total length of each of these two matrices was 3090 characters. These matrices were partitioned in different subsets in order to optimize likelihood analyses. PartitionFinder (Lanfear et al. 2012) was chosen to determine the best partition and select the best models for the different subsets. We used the greedy algorithm and BIC as the criterion to define the best partition and compared all models available. The dataset tested for the analysis on the four loci was composed of eight subsets: *RPB1*, 1st codon position; *RPB1*, 2nd codon position; *RPB1* 3rd codon position; *RPB1* intron; mtSSU; LSU; ITS1/ITS2 and 5.8S.

Maximum likelihood and Bayesian phylogenetical analyses

For both matrices, the best likelihood tree was produced with RAxML version 7.4.2 (Stamatakis 2006) with the default settings and the GTRCAT model and bootstrapped for 1000 pseudoreplicates in the same run, using the best partition obtained by PartitionFinder. We further ran for both matrices a Bayesian analysis using MrBayes version 3.2.3 (Huelsenbeck and Ronquist 2001) on the CIPRES portal (Miller et al. 2010) using the partition and the substitution models determined by PartitionFinder and running for 20,000,000 generations and sampling every 1000th generation. Each analysis consisted of two runs of three heated chains and one cold one. We assessed the convergence using Tracer version 1.5 (Rambaut and Drummond 2007) and checking with AWTY (Nylander et al. 2008) that convergence was reached for each run and that tree topologies have been sampled in proportion to their true posterior probability distribution. The first 2000 trees were discarded as burn-in. Branches of the phylogenetic trees produced are considered strongly supported if bootstrap support is higher than 70 % for maximum likelihood analysis and posterior probabilities higher than 0.95 for the Bayesian analysis.

Parsimony analysis for ITS data

Thanks to data kindly made available to us by B. Moncada and R. Lücking, we could assemble an ITS matrix including all species, described or undescribed, from Colombia and assess their relationships with our own accessions. As the purpose of this analysis was to evaluate the position of the species dealt with in this study in the main clades identified by

Moncada et al. (2014a), we performed a maximum parsimony analysis with PAUP*4.0 (Swofford 2002). All ambiguous positions were strictly eliminated in the assembled matrix with the GBLOCKS server (Castresana 2000), using the default parameters and manually checked. An initial run was performed by using the tree bisection reconnection (TBR) branch swapping algorithm, with the steepest descent turned on and only 10 trees saved for each of the 200 random addition replicates; a second analysis was performed where all saved trees were swapped to completion with no limit to the number of trees saved.

Haplotypes networks

To analyse the genealogical relationships among the haplotypes, we constructed haplotype networks for two species (*S. ciliata* and *S. fuliginoides* sp. nov.) and for two closely related ones (*S. fuliginosa* and *S. limbata*). The haplotype networks were calculated using the program TCS version 1.21 (Clement et al. 2000), which implements the statistical parsimony estimation as described by Templeton et al. (1992). The connection limit of the networks was fixed to 90 % of the positions and gaps were treated as fifth character state.

Morphological, anatomical, and chemical characters

Morphological description as well as anatomical characters examined follow Tables 2 and 3 provided by Moncada et al. (2014a). Special attention was dedicated to the characters highlighted by Moncada and Lücking (2012) and especially the branching type, the two types of tomentum, and the membrane of cyphellae. Indeed, the presence of papillae on the cells of the surface of the cyphellae membrane was found to discriminate *S. fuliginosa* s. str. from two other species (*S. ciliata* and *S. fuliginoides* sp. nov.) that were so far engulfed in it. TLC analysis of two specimens of each species dealt with in this paper has been performed following the protocol of solvent C and visualization of spots with a 10 % sulphuric acid solution (Orange et al. 2010).

Results

Molecular data and phylogenetic analyses

We amplified ITS for 188 specimens (45 included in the 4-loci analysis: see below), and a further 43 for nuLSU, 44 for mtSSU, and 49 for *RPB1* (Table 1). PartitionFinder divided the partition into six subsets: one composed of LSU (best model: TrN+I+G), one with mtSSU (HKY+I), one with ITS1 and ITS2 (TIM+G), one with 5.8S and *RPB1* 2nd codon position (JC+I), one with *RPB1* 1st codon position (TrN+I), and one with *RPB1* 3rd codon position and *RPB1* intron (TrNef+I+G).

Table 1 Specimens and DNA sequences used in this study, with their respective voucher information

Epithet (<i>Sticta</i>)	Voucher	ITS	mtSSU	nuLSU	<i>RPB1</i>
1 <i>ambavillaria</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 992)	JQ735978	JQ736011	JQ735995	–
2 <i>atlantica</i>	Ireland, Kerry, 2014, <i>E. Sérusiaux</i> (LG 3747)	KT281734	KT281690	KT281645	–
3 <i>atlantica</i>	Portugal, Azores, Pico, 2014, <i>E. Sérusiaux</i> (LG 3858) Type	KT281737	KT281693	KT281648	KT281784
4 <i>beauvoisii</i>	USA, North Carolina, 2013, <i>B. Goffinet</i> 11137 (LG 3303)	KT281725	KT281681	KT281636	KT281787
5 <i>canariensis</i>	Spain, Canary Is., Tenerife, 2010, <i>E. Sérusiaux</i> (LG 1333)	KT281700	KT281658	KT281612	KT281752
6 <i>canariensis</i>	Ireland, Kerry, 2014, <i>E. Sérusiaux</i> (LG 3741)	KT281733	KT281689	KT281644	KT281779
7 <i>caperata</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 962)	JQ735979	JQ736012	JQ735996	KT281745
8 <i>carolinensis</i>	USA, North Carolina, 2013, <i>B. Goffinet</i> 11141 (LG 3302)	KT281724	KT281680	KT281635	KT281786
9 <i>ciliata</i>	Spain, Canary Is., Tenerife, 2011, <i>P. van den Boom</i> 45673 (LG 2751)	KT281712	KT281668	KT281624	–
10 <i>ciliata</i>	Portugal, Azores, Pico, 2011, <i>P.K. Divakar</i> (LG 3099)	KT281715	KT281671	KT281627	KT281762
11 <i>ciliata</i>	Spain, La Palma, 2013, <i>E. Sérusiaux</i> (LG 3406)	KT281713	KT281669	KT281625	KT281780
12 <i>ciliata</i>	France, Brittany, 2013, <i>A. Gérault</i> (LG 3539)	KT281718	KT281674	KT281630	KT281774
13 <i>ciliata</i>	France, Brittany, 2013, <i>A. Gérault</i> (LG 3542)	KT281714	KT281670	KT281626	KT281772
14 <i>ciliata</i>	Ireland, Kerry, 2014, <i>E. Sérusiaux</i> (LG 3781)	KT281716	KT281672	KT281628	KT281773
15 <i>ciliata</i>	Spain, Canary Is., Tenerife, 2014, <i>E. Sérusiaux</i> (LG 3830)	KT281719	KT281675	KT281631	KT281775
16 <i>ciliata</i>	Rwanda, 2010, <i>E. Sérusiaux</i> (LG 1605)	KT281717	KT281673	KT281629	KT281763
17 <i>ciliata</i> Colombia1	Colombia (Moncada et al. 2014a, as <i>S. microisidiata</i>)	KC732607	–	–	–
18 <i>ciliata</i> Colombia2	Colombia (Moncada et al. 2014a, as <i>S. microisidiata</i>)	KC732699	–	–	–
19 <i>cyphellulata</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 1023)	JQ735980	JQ736013	JQ735997	KT281748
20 <i>dichotoma</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 945)	JQ735981	JQ736014	JQ735998	KT281743
21 <i>dichotoma</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 984)	JQ735982	JQ736015	JQ735999	KT281746
22 <i>duplolumbata</i>	Rwanda, 2009, <i>E. Sérusiaux</i> (LG 919)	KT281696	KT281654	KT281651	KT281741
23 <i>duplolumbata</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 1040)	JQ735984	JQ736001	JQ736017	KT281751
24 <i>fuliginoides</i>	France, Vosges, 2010, <i>E. Sérusiaux</i> (LG 1421)	KT281701	KT281659	KT281613	KT281753
25 <i>fuliginoides</i>	Spain, Canary Is., Gomera, 2012, <i>E. Sérusiaux</i> (LG 3012) Type	KT281722	KT281678	KT281634	KT281765
26 <i>fuliginoides</i>	France, Brittany, 2013, <i>F. Sétit</i> (LG 3551)	KT281729	KT281685	KT281640	KT281777
27 <i>fuliginoides</i>	United Kingdom, Devon, 2014, <i>N. Magain</i> (LG S4)	KT281738	KT281694	KT281649	KT281785
28 <i>fuliginoides</i>	Ireland, Kerry, 2014, <i>E. Sérusiaux</i> (LG 3733)	KT281732	KT281688	KT281643	KT281781
29 <i>fuliginosa</i>	Madagascar, 2008, <i>E. Sérusiaux</i> (LG 795)	KT281695	KT281653	KT281609	KT281740
30 <i>fuliginosa</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 989)	KT281698	KT281656	KT281610	KT281747
31 <i>fuliginosa</i>	Rwanda, 2010, <i>E. Sérusiaux</i> (LG 1611)	KT281702	KT281660	KT281614	KT281754
32 <i>fuliginosa</i>	South Africa, 2010, <i>B. Goffinet</i> 10242 (LG 1952)	KT281703	KT281661	KT281615	KT281755
33 <i>fuliginosa</i>	Portugal, Azores, Pico, 2011, <i>P.K. Divakar</i> (LG 3100)	KT281704	KT281662	KT281616	KT281756
34 <i>fuliginosa</i>	Spain, Canary Is., Gomera, 2012, <i>E. Sérusiaux</i> (LG 3010)	KT281721	KT281677	KT281633	KT281776
35 <i>fuliginosa</i>	France, Brittany, 2013, <i>B. Bouffinier</i> (LG 3537)	KT281727	KT281683	KT281638	KT281766
36 <i>fuliginosa</i>	United Kingdom, Devon, 2014, <i>N. Magain</i> (LG S9)	KT281739	–	KT281650	KT281769
37 <i>fuliginosa</i>	Ireland, Kerry, 2014, <i>E. Sérusiaux</i> (LG 3729)	KT281731	KT281687	KT281642	KT281768
38 <i>limbata</i>	Canada, British Columbia, <i>T. Goward</i> 09–246 (LG 3170)	KT281710	–	KT281622	–
39 <i>limbata</i>	Spain, Canary Is., Gran Canaria, 2011, <i>E. Sérusiaux</i> (LG 2230)	KT281706	KT281664	KT281618	KT281758
40 <i>limbata</i>	United Kingdom, Scotland, 2011, <i>B. J. Coppins</i> (LG 2690)	KT281707	KT281665	KT281619	KT281759
41 <i>limbata</i>	Spain, Canary Is., Gomera, 2011, <i>P. van den Boom</i> 46085 (LG 2749)	KT281708	KT281666	KT281620	KT281760
42 <i>limbata</i>	Portugal, Azores, Pico, 2011, <i>P.K. Divakar</i> (LG 3105)	KT281709	KT281667	KT281621	–
43 <i>limbata</i>	France, Brittany, 2013, <i>A. Gérault</i> (LG 3544)	KT281728	KT281684	KT281639	KT281767
44 <i>limbata</i>	Portugal, Azores, Pico, 2014, <i>E. Sérusiaux</i> (LG 3868)	KT281711	–	KT281623	KT281761
45 <i>macrophylla</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 946)	JQ735985	JQ736018	JQ736002	KT281744

Table 1 (continued)

Epithet (<i>Sticta</i>)	Voucher	ITS	mtSSU	nuLSU	<i>RPB1</i>
46 <i>sublimbata</i>	Congo RDC, 2009, <i>E. Sérusiaux</i> (LG 885)	JQ735986	JQ736019	JQ736003	KT281771
47 <i>sublimbata</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 1038)	KT281699	KT281657	KT281611	KT281750
48 <i>sylvatica</i>	France, Brittany, 2013, <i>A. Gérault</i> (LG 3536)	KT281726	KT281682	KT281637	KT281788
49 <i>sylvatica</i>	United Kingdom, Somerset, 2014, <i>P. Wolseley</i> (LG 3723)	KT281730	KT281686	KT281641	KT281778
50 <i>sylvatica</i>	Ireland, Kerry, 2014, <i>E. Sérusiaux</i> (LG 3780)	KT281735	KT281691	KT281646	KT281782
51 <i>sylvatica</i>	France, Vosges, 2014, <i>E. Sérusiaux</i> (LG 3837)	KT281736	KT281692	KT281647	KT281783
52 <i>umbilicariiformis</i>	Rwanda, 2009, <i>E. Sérusiaux</i> (LG 925)	KT281697	KT281655	KT281652	KT281742
53 <i>variabilis</i>	France, Réunion, 2009, <i>N. Magain & E. Sérusiaux</i> (LG 1037)	JQ735987	JQ736020	JQ736004	KT281749
54 sp. 1 (1)	Spain, Canary Is., Gran Canaria, 2011, <i>E. Sérusiaux</i> (LG 2229)	KT281705	KT281663	KT281617	KT281757
55 sp. 1 (2)	Spain, Canary Is., Gomera, 2011, <i>P. van den Boom</i> 46379 (LG 2752)	KT281720	KT281676	KT281632	KT281764
56 sp. 1 (3)	Canada, British Columbia, 2009, <i>T. Goward</i> 09–246 (LG 3173)	KT281723	KT281679	–	–
57 <i>Pseudocyphellaria crocata</i> Outgroup	Spain, Canary Is., La Gomera, 2009, <i>E. Sérusiaux</i> (LG 688)	JQ735976	JQ736009	JQ735993	KT281770

GenBank accessions in bold refer to newly produced sequences

The single most likely phylogenetic tree resulting from the analysis of the matrix containing the two sequences of *microisidiata* ad. int. from Colombia (Moncada et al. 2014a) is presented in Fig. 1 with the bootstrap values of the ML analysis and the Bayesian PP values written above the branches when they are significant ($\geq 70\%$ for maximum likelihood analysis and ≥ 0.95 for the Bayesian analysis). The clade including *S. ciliata*, from the tree resulting from the analysis of the matrix without the two accessions from Colombia, is figured as an inset in Fig. 1 to highlight the difference in support values in this clade when they are not present. Branch support of other lineages were not significantly affected when these two accessions were included, while those inside the clade that comprise them were.

The phylogenetic tree resolved two strongly supported lineages: (1) the large and usually luxuriant species from Reunion including green (*S. caperata* and *S. variabilis*) and blue photomorphs (*S. macrophylla*) or both (*S. dichotoma*), and (2) the isolated *Sticta cyphellulata* from Reunion, resolved with strong support as sister to all other species including those dealt with in this paper. Three clades can be recognized in the latter lineage:

- one with *S. canariensis*, resolved with strong support as sister to a group comprising *S. beauvoisii*, *S. carolinensis*, and *S. sublimbata*;
- one including the resurrected epithet *S. ciliata* and *S. fuliginoides* sp. nov., with strong support only in the Bayesian analysis;
- one resolved in two well-supported groups including *S. duplolibmata*, *S. sylvatica*, and *Sticta* sp. 1 for the first

one, and *S. ambavillaria*, *S. atlantica* sp. nov., *S. fuliginosa*, *S. limbata*, and *S. umbilicariiformis* for the second one.

The MP phylogenetic tree of our own accessions within a large data set of all species recognized in Colombia (Moncada et al. 2014a) resolved the following relationships (data not shown): *S. atlantica* sp. nov. is resolved within the *S. humboldtii* clade, *S. canariensis* in the eponym clade, *S. ciliata* within the *S. fuliginosa* clade/subclade *S. gyalocarpa*, *S. fuliginoides* within the *S. fuliginosa* clade and in the eponym subclade, *S. fuliginosa* and *S. limbata* within the *S. humboldtii* clade, and finally *S. sylvatica* within the *S. kunthii* clade.

TCS haplotype networks (Fig. 2) were produced for the duo *S. fuliginosa/S. limbata* (49 accessions incl. those retrieved from GenBank and resolved in that group), for *S. fuliginoides* (32 accessions incl. those retrieved from GenBank and resolved in that species) and *S. ciliata* (29 accessions incl. both accessions under *microisidiata* ad. int. in Moncada et al. 2014a). Our accession of *S. ciliata* from Rwanda is excluded from the latter under the 90 % limit assigned to the analysis and is not shown.

With the most widely used lichen flora, at least for oceanic species (The Lichens from Great Britain and Ireland, Smith et al. 2009), the specimens collected in this study are resolved as follows:

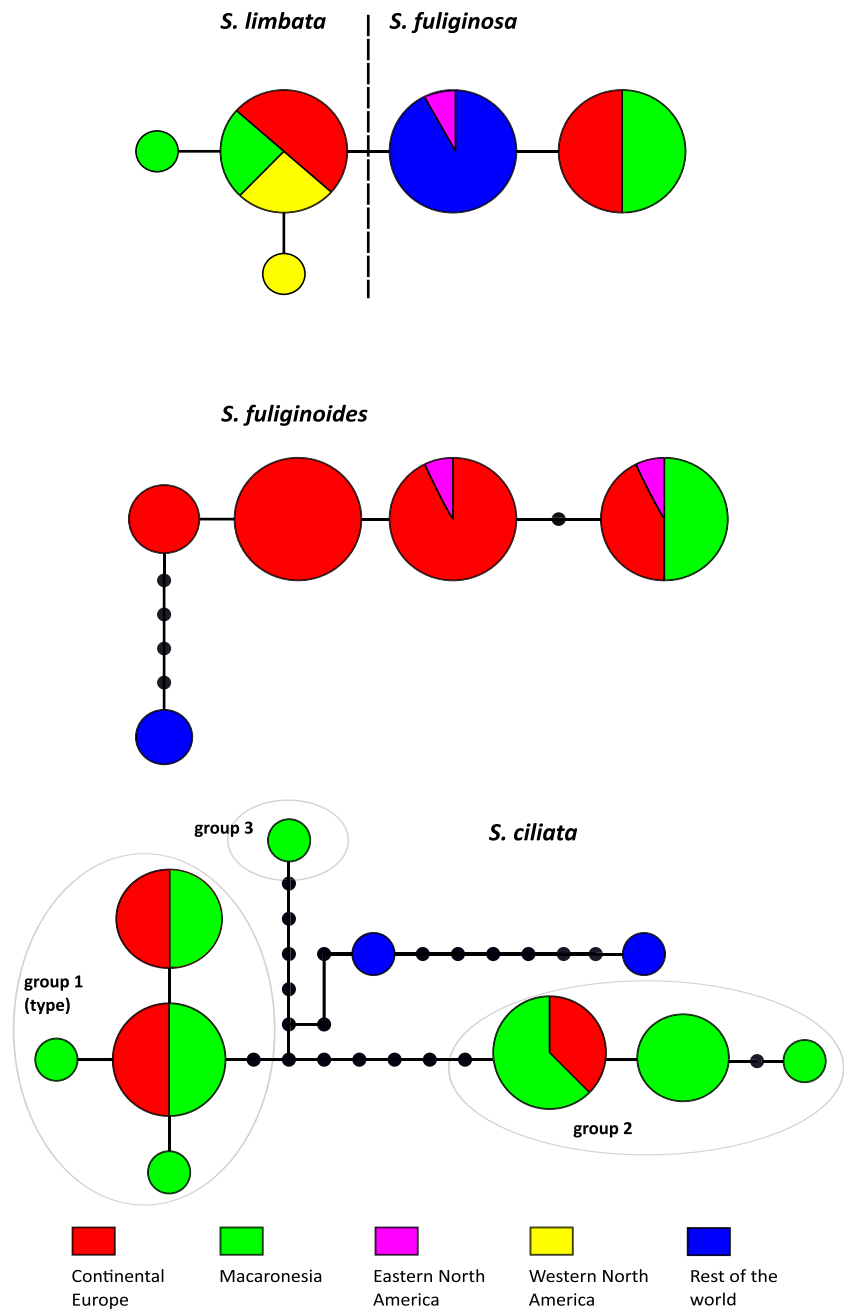
- those easily identified as *S. canariensis* are resolved as a single entity;
- those identified as *S. fuliginosa* are resolved into four distinct entities: *S. atlantica* sp. nov., the resurrected *S. ciliata*, *S. fuliginoides* sp. nov., and *S. fuliginosa* s. str.;



Fig. 1 Single most likely phylogenetic tree obtained from inferences of 4-loci molecular data (ITS, nuLSU, mtSSU, and *RPB1*). Branches in bold are those that obtained support greater or equal than 70 from maximum likelihood bootstrap and greater or equal than 0.95 for Bayesian posterior probabilities; values above branches represent ML bootstrap and

Bayesian PP values, respectively. Inset below concerns the clade comprising *S. ciliata* when both accessions from Colombia (represented only by ITS data) are excluded. The seven species present in continental Europe are highlighted with specific colors

Fig. 2 Haplotype networks for ITS accessions of *Sticta limbata* and *S. fuliginosa*, *S. fuliginoides* and *S. ciliata*. Circles represent haplotypes. The size of each circle is proportional to the number of individuals sharing this haplotype. Each bar between circles in the network represents a single mutation step, and each dot is an additional change



- those identified as *S. sylvatica* are resolved in a single entity *S. sylvatica* s. str., although *S. atlantica* sp. nov. might have been confused with it in Macaronesia.

Quite interestingly, the longest branches, representing the most genetically distinct entities, are those that were not recognized, e. g., *S. atlantica* sp. nov., *S. ciliata*, and *S. fuliginoides* sp. nov. Further, *S. fuliginosa* s. str. and *S. limbata*, two species that any lichenologist would immediately recognize as different as the first one is isidiate and the second sorediate, are not resolved as two supported entities with the 4-loci inferences. They differ by a single substitution in the ITS haplotype network.

All data retrieved from GenBank under the epithets *canariensis*, *fuliginosa*, *limbata*, and *sylvatica* have been re-evaluated and their correct taxonomical assignment is presented in Table 2.

No compounds were detected in the TLC analyses performed.

Geographical data

All data that could be confirmed on the basis of ITS data yielded a reassessment of the distribution of all species, which is summarized in Table 3.

Table 2 Status of accessions from GenBank under the four epithets used for species in continental Europe: *Sticta canariensis*, *S. fuliginosa*, *S. limbata*, and *S. sylvatica*

GenBank accession n°	Origin	Identification in original publication	Original publication	Status obtained in this study
AB239395	Japan	<i>fuliginosa</i>	Takahashi et al. 2006	<i>fuliginosa</i>
AY124095	China	<i>fuliginosa</i>	Lohtander et al. 2002	Undescribed species, also detected in the Canary Islands and Western North America
AY173391	USA/Oregon	<i>limbata</i>	McDonald et al. 2003	<i>limbata</i> but the only accession of that species with the ITS of <i>fuliginosa</i>
AY173387	Eastern USA	<i>fuliginosa</i>	McDonald et al. 2003	<i>fuliginoides</i>
AY173388	Eastern USA	<i>fuliginosa</i>	McDonald et al. 2003	<i>fuliginoides</i>
AY173389	Eastern USA	<i>fuliginosa</i>	McDonald et al. 2003	<i>fuliginoides</i>
DQ419943	Canada/B.C.	<i>fuliginosa</i>	Comejo et al. 2009	Undescribed species
DQ419944	Canary Is.	<i>canariensis</i>	Comejo et al. 2009	<i>canariensis</i>
JN857309	USA/ California	<i>limbata</i>	Fedrowitz et al. 2012	<i>limbata</i>
JX064536	Eastern USA	<i>sylvatica</i>	Hodkinson et al. 2014	<i>fuliginosa</i>
KC011069	U.K./Wales	<i>fuliginosa</i>	Moncada et al. 2013a	<i>fuliginoides</i>
KC732518	Columbia	<i>sylvatica</i>	Moncada et al. 2014a	<i>sylvatica</i>
KC732574	Columbia	<i>sylvatica</i>	Moncada et al. 2014a	<i>sylvatica</i>
KC732581	Columbia	<i>sylvatica</i>	Moncada et al. 2014a	<i>sylvatica</i>
KC732454	U.K./Wales	<i>fuliginosa</i>	Moncada et al. 2014a	<i>fuliginoides</i>
KC732709	Colombia	<i>fuliginosa</i>	Moncada et al. 2014a	<i>fuliginoides</i>
KC732607	Colombia	« <i>microisidiata</i> Magain et al. » ined	Moncada et al. 2014a	<i>ciliata</i>
KC732699	Colombia	« <i>microisidiata</i> Magain et al. » ined	Moncada et al. 2014a	<i>ciliata</i>

Taxonomy

The taxonomy of isidioid species of *Sticta* in Europe that is currently used in all modern Floras was already established by Delise (1826) in his seminal taxonomical work on the genus, and eventually confirmed by Degelius (1935) in his work on oceanic lichen species in Europe.

Unlike in other emblematic genera such as *Cladonia* and *Peltigera*, very few epithets have been validly published for European representatives of the genus. Indeed, besides those used or introduced by Delise (1826: *canariensis*, *dufourii*, *fuliginosa*, *limbata*, and *sylvatica*) only three

are available at species level (*Sticta ciliata* Tayl., *S. fimbriata* Tayl., and *S. elegans* Deak), and all have been reduced into synonymy with *S. dufourii* Delise by Galloway (1995), who was, at that time, still doubtful about the true nature of the duo of photomorphs formed by *S. canariensis* and *S. dufourii*.

Delise (1826) has been working on collections made all over the world by several most famous collectors, such as J.B.G.M. Bory de Saint-Vincent, A.M.F.J. Palisot de Beauvois (to whom *Sticta beauvoisii* was dedicated), J. A. Mougeot (to whom *Sticta mougeotiana* was dedicated, a species now referred to *Pseudocypbellaria*), C. Gaudichaud-Beaupré (to whom *S. gaudichaldia* was

Table 3 Distribution of the species of *Sticta* recognized in continental Europe by this study and confirmed out of that area by the ITS barcode

Name	France Vosges	France Brittany	U.K.	Ireland Kerry	Macaronesia	Others
<i>atlantica</i>	—	—	—	X	Azores	—
<i>canariensis</i>	—	X	X	X	Canary Is, Madeira, Azores	—
<i>ciliata</i>	—	X	—	X	Canary Is, Azores	Hawaii archipelago, Central and South America, and Rwanda
<i>fuliginosa</i>	—	X	X	X	Canary Is., Azores	Widespread in both hemispheres
<i>fuliginoides</i>	X	X	X	X	Canary Is.	Eastern North America and Colombia
<i>limbata</i>	—	X	X	X	Canary Is., Azores	Western North America
<i>sylvatica</i>	X	X	X	X	—	Colombia

dedicated), and others. After his military career, he retired in western France (Dept. Calvados) at a time when epiphytic *Lobarion* communities were luxuriant — he mentioned that he could collect *S. sylvatica* “aux environs de Paris”, a species for which he provided a quite accurate description as the thallus is said to be (p. 86, translated from Latin) “deeply lacinate with sub-truncate to rounded lobes”. More details on his life and botanical work can be found in Galloway and James (1986).

He found and described *S. dufourii*, the cyanomorph of *S. canariensis*, and further collected *S. sylvatica*, *S. limbata* and *S. fuliginosa*. His description of the latter is quite interesting as he actually describes, at least in parts, material that is here assigned to *S. ciliata*: indeed, he describes thalli with (p. 75, translated from French) “the lobes edges often eaten and undivided only in young individuals” and apothecia with cilia, developed near the thallus edges. Nowadays, in suitable habitats in Western France, *S. ciliata* is found with lacerate-strongly damaged thalli and ciliate apothecia (Fig. 5a–b). Delise clearly referred to the original description of *S. fuliginosa*, but did not realize that two species were involved. This confusion has lasted for almost two centuries.

Besides the cyanomorph of *S. canariensis*, Degelius (1935) distinguished two isidiate species (p. 171, translated from German):

- *S. sylvatica* has a larger and thicker thallus, with a shiny upper surface and deep folds to which the always very small isidia are attached.
- *S. fuliginosa* has a small, rounded, thin, not or only slightly indented thallus, with a matte or slightly shiny upper surface, mostly not wrinkled, with numerous and often large, and leaf-like isidia, which are usually scattered over the whole surface.

Within *S. fuliginosa*, Degelius further distinguished two forms: the typical one, always with a matte upper surface and an involute margin, with isidia uniformly distributed over the whole thallus; and the f. *obvoluta* (Del.) Zahlbr. (an epithet based on material from Venezuela: Delise 1826; see comments on this material in Galloway 1995: 160–161) with a slightly shiny thallus, slightly wrinkled and isidia mainly located on the folds. Although Degelius seemed confident in the characters diagnostic for distinguishing *S. sylvatica* from *S. fuliginosa*, the introduction of the intermediate form *obvoluta* confused his taxonomical treatment of the isidioid species of *Sticta* in Europe. Indeed, the distinction between these two species was a matter of debate in several earlier papers such as Harmand (1909) and Anders (1928) who concluded that these taxa should better be treated as varieties.

Nevertheless, the recognition of two taxa as delimited by Degelius characters was widely accepted, first as varieties (Poelt 1963; Lambinon 1969; Ozenda and Clauzade 1970)

and eventually as species following Poelt (1969) (Jørgensen and Tønberg 2007; Smith et al. 2009; Wirth et al. 2013). Both species were assumed to be widespread, or even subcosmopolitan. Quite interestingly, the taxonomy of this duo was never re-evaluated for European populations, although both species received much attention in environmental monitoring, for air quality as well as forests management and conservation.

Our results demonstrate that the genus in Europe is much more diversified than expected, as sampling in four regions in France, the United Kingdom, and Ireland could unveil five different species amongst specimens producing isidia. The two old and validly published names *S. fuliginosa* and *S. sylvatica* have been re-evaluated on the basis of careful examination of type collections preserved in OXF (Fig. 3). A validly published epithet, so far considered a synonym of *S. canariensis*, is resurrected (*S. ciliata*; type collection in BM; Fig. 3), and two species are described as new for science as no validly published epithets could be found for them. Each European species is described in this section, together with preliminary assessments of their distribution.

The species

Sticta atlantica Magain & Sérus., *sp. nov.* (Fig. 4)

Mycobank # 813823

Species of *Sticta* distinguished from other isidiate species in Western Europe with a thallus lichenized with a cyanobacterium by its suborbicular lobes and upper surface with typically regular or irregular swellings or ridges on which isidia start their development to form coralloid stipitate masses.

= *Sticta fuliginosa* auct. p.p., non (Hoffm.) Ach.

? = *Sticta sylvatica* auct. p.p., non (Huds.) Ach.

Type: **Portugal**, Azores archipelago, Pico Is., Parque Forestal along the road to Prainha de Cima, N 38°29'19.62" W 28°14'42.18", alt. 240 m, July 2014, plantations of *Pinus* with fragments of natural vegetation, E. Sérusiaux LG DNA 3858, (LG—holotypus; AZU—isotypus).

Thallus first flabellate or palmate as the lobes forming a single thallus develop from a central point on a short and evanescent stipe (pseudostipe) eventually becoming suborbicular or, more rarely, irregular, 4–7 cm across, sometimes forming large colonies that can reach c. 15 cm across, subcoriaceous when wet and usually papyraceous and brittle when dry; lobes with 1–2 branching, pleurotomous to polytomous, rarely irregular. *Lobes* suborbicular in typical thalli, rarely irregular in outline, 1.0–1.8 cm width, adnate to slightly ascending, imbricate and overlapping, plane or undulating, with apices rounded, plane to slightly revolute, crenate to lacerate, margin not thickened. *Upper surface* costate, irregular, with regular or irregular swellings or grooves, brownish or bluish grey (these two color types can occur side by side), of the same color when wet or dry, matte or somewhat

Fig. 3 Types collections of the three European isidiate species of *Sticta* that are already described. **a–d** *Sticta ciliata*. **a** Lectotype of *Sticta ciliata* Tayl. (BM), the lower specimen enlarged in **b**, and a well-preserved ciliate lobe enlarged in **c** (between red arrows). **d** similar thalli pictured on Faial Is., Azores (July 2014, E. Sérusiaux s.n., LG) and very much like the type of *S. fuliginosa* f. *ciliata* Degel. **e–f** *Sticta fuliginosa* (Hoffm.) Ach, epitype (OXF). **f** Detailed view of a lobe with apothecia. **g–h** *Sticta sylvatica* (Huds.) Ach., epitype (OXF)

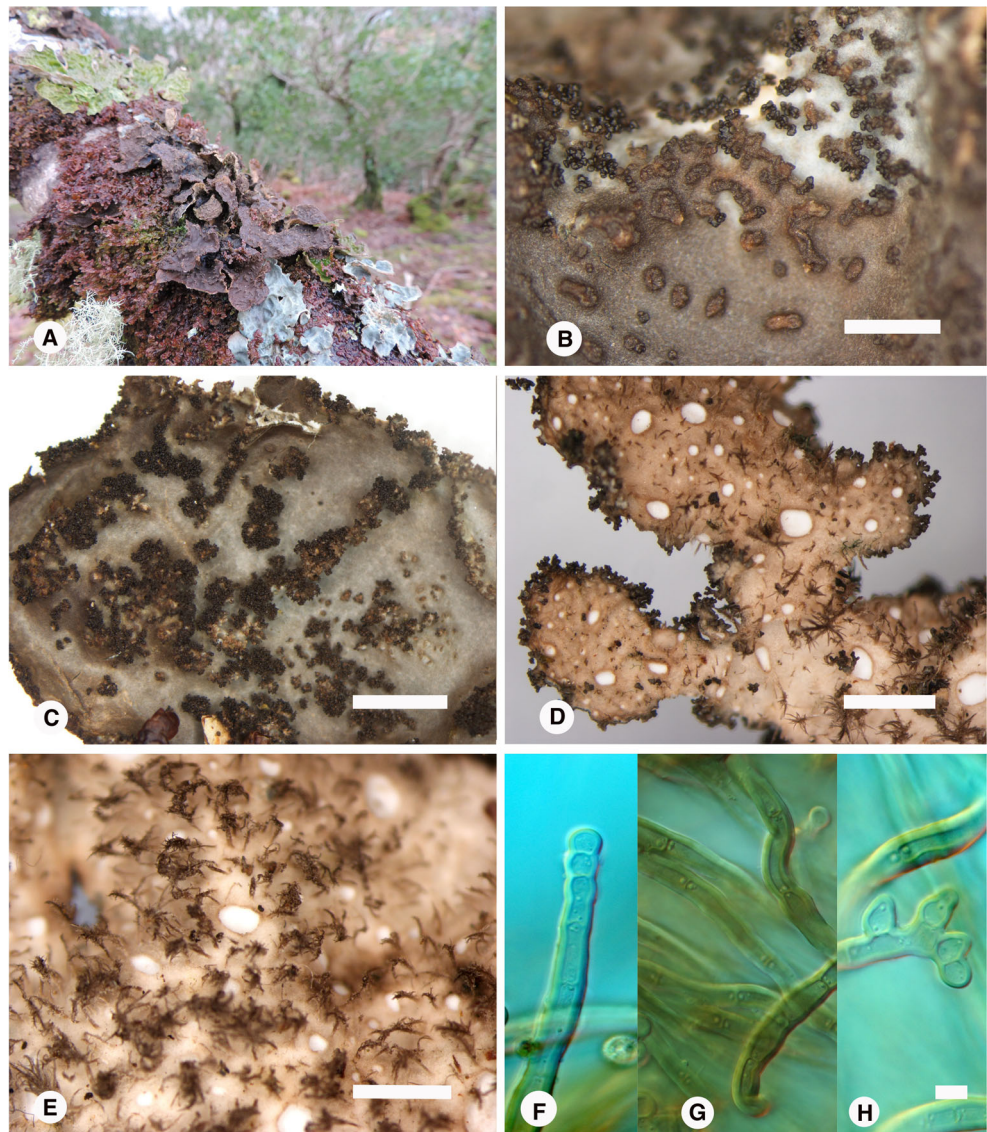


glossy, maculae yellowish rather distinct on bluish grey thalli, sparse and not confined to certain parts of the thallus. *Cilia* absent, or rarely found on regenerating lobules, whitish, simple, < 0.5 mm long. *Isidia* always present, marginal or laminal, when marginal forming a marginal string of coralloid or subsquamiform minute structures, sometimes becoming distinctly stipitate, when laminal, always starting their development on the swellings or ridges, and forming stipitate (stipe cylindrical or subapplanate, 0.2–0.4 mm long and c. 0.1 mm in diam.) coralloid to corymbose masses that can reach 1 mm in diam., coarse or delicate, usually shining, of the same color as the thallus on which they grow. *Lower surface* foveolate to scrobiculate, white, cream-colored to brownish, with primary tomentum always present, sparse towards the margin but

sometimes becoming very dense towards the center; primary tomentum made of distinct fasciculate to penicillate hairs, easily distinguished from one another in young lobes, eventually forming spongy masses, especially towards the center, soft to rough, white to grey or reddish brown. *Cyphellae* always present, abundant, round to slightly irregular, rather angular on old lobes, 25–40 per cm², but reaching 50 per cm² towards the margins, cupuliform to urceolate with wide pore, the largest ones reaching 1.5–1.8 mm in diam., much smaller towards the margin (less than 0.5 mm in diam.), prominent, with an erect, white or cream-colored margin. *Apothecia* and *pycnidia* never produced.

Upper cortex paraplectenchymatous, 25–30 μm high, with 3–4 layers of rounded to isodiametric cells, 8–12 μm in diam.

Fig. 4 *Sticta atlantica* Magain & Sérus., sp. nov. **a–b** Specimen from Ireland/Kerry (Feb. 2014, *E. Sérusiaux* s.n., LG). **a** Thallus on fallen tree. **b** Detail view of the thallus surface with irregular swellings on which isidia develop. **c–h** Type collection from Pico Is., Azores (July 2014, *E. Sérusiaux* s.n., LG). **c** Upper surface with laminal swellings and marginal coralloid isidia. **d** Lower surface with conspicuous cyphellae. **e** Lower surface with primary tomentum agglutinated in fibrillose to penicillate fascicles. **f–h** Conidiogenous cells along the primary tomentum hyphae. **g** With Woronin bodies easily distinguished. **Scale bars: b** 1 mm, **c–d** 2 mm, **e** 0.5 mm, **f–h** 10 μ m (scale in h)



and a 1.5 μ m thick wall, sometimes with an upper layer of smaller cells with their outer wall thickened to 4 μ m. *Photobiont layer* 40–50(–70) μ m high containing *Nostoc* colonies, forming rather compact, ovoid masses, tightly encompassed by hyphae forming rounded to angular cells, and thus designing a single layer wrapping up the *Nostoc* cells; no differences in the *Nostoc* cells detected between brown and bluish gray thallus. *Medulla* lax to compact, 60–80 μ m high.

Lower cortex paraplectenchymatous, 15–25 μ m high, with 2–3 layers of rounded to isodiametric cells, 8–12 μ m in diam. and a 1.5–3 μ m thick wall. *Lower primary tomentum* agglutinated in fascicles that form large, but not compact fibrillose to penicillate masses, looking-like rhizines, with free and intertwined, cylindrical hyphae, with a 2–2.5 μ m thick wall, usually with typical Woronin bodies seen near the septa; these hyphae develop a conidiogenous process either terminally or

laterally in branched and short hyphae; conidia almost always spherical, 5–7 μ m in diam with a 1 μ m thick wall. *Lower secondary tomentum* sometimes indistinct, made of budding cells on the lower cortex surface, forming moniliform assemblages of 2–10 cells, 5–10(–12) μ m in diam and with a c. 1 μ m thick wall. *Cyphellae membrane* made of a layer of rounded cells (4–8 μ m in diam.) without any papillae.

No substances detected by TLC.

Etymology: the epithet of this new species is chosen to evoke its distribution, in the hyper-oceanic localities in the NE Atlantic region.

Ecology: epiphytic on branches in *Quercus* woodland, in the most famous Tomies wood in SW Ireland (Giavarini 2012); and on branches or small trees in remnants of the laurisilva in the Azores (only known on Pico Is.). The laurisilva of the Azores and the whole Macaronesia hosts an impressive number of species of Peltigerales, with several

recently recognized species or endemics (examples with *Lobaria*: Cornejo and Scheidegger 2010, or with *Nephroma*: Sérusiaux et al. 2011).

Distribution: known from Ireland/Kerry and the Azores (Pico Is.), only abundant in the Azores, even in heavily disturbed remnants of original vegetation, where it thrives with *S. ciliata*. With the data currently available, the species has a hyper-oceanic distribution, quite similar to other rare species such as *Cladonia angustiloba* Ahti & Aptroot known from the Azores and Madeira and recently found in the Faroe Islands (Ahti and Stenroos 2012), *Degelia ligulata* P.M. Jørg. & P. James, known from W Scotland, Wales, W Ireland and Macaronesia, and *Topeliopsis azorica* (P. James & Purvis) Coppins & Aptroot known from W Scotland and the Azores (Smith et al. 2009).

Notes:

1. Amongst European species, *S. atlantica* is easily distinguished by its suborbicular lobes, with a crenate to lacerate margin and upper surface with typically regular or irregular swellings or ridges on which isidia start their development to form coralloid stipitate masses. The species can be confused with *S. fuliginosa*, which mainly differs by its larger lobes (1.5–3 cm wide in well-developed specimens) not developing small swellings on which isidia start their development, although isidia may develop only on the ridges of the scrobiculate-foveolate surface in this species.
2. Within the first phylogeny of the genus provided by Moncada et al. (2014a) based on a single locus (ITS) and focusing on species from Colombia, this new species is resolved within the *S. humboldtii* clade, a diverse group in the Andes; *S. atlantica* differs from all other species resolved in that group by at least three substitutions in ITS1 and seven in ITS2. A multi-loci phylogeny is, however, needed before the assignment of *S. atlantica* to that clade can be confirmed.
3. Two collections slightly differ from the typical haplotype, detected in the Azores as well as in Ireland: one from Ireland differs by one substitution in ITS1 and three in ITS2 while a single one from Pico differs by two substitutions and one indel in ITS1. This variation is considered as intraspecific.

Selected specimen examined: Ireland, Kerry, W of Lake Killarney, Tomies Wood, 52°02'12"N 09°43'60"W, alt. 90–100 m, Feb. 2014, disturbed *Quercus* woodland, E. Sérusiaux s.n. (LG).

Sticta canariensis (Flörke) Delise, Mém. Soc. Linn. Calvados 2: 114, 1825 (Fig. 5)

Mycobank # 406113

Pulmonaria canariensis Flörke, Mag. Ges. Naturf. Fr. Berlin 2: 127, 1890

Type: **Spain**, Canary Is., Tenerife, forêt de Laguna, Bory de Saint-Vincent (BM—lectotype !; designated by Galloway, Nova Hedwigia 61:152, 1995)

= *Sticta dufourii* Delise, Mém. Soc. Linnéenne Calvados 2: 78, pl. 6, Fig. 22 (1826)

Type: **Spain**, Canary Is., M. Defour (PC—Lenormand, lectotype; designated by Galloway, Nova Hedwigia 61:166, 1995)

= *Sticta fimbriata* Taylor, London Journal of Botany 6: 180 (1847)

Type: **Ireland**: in woods near Killarney, T. Taylor (?FH; not seen by Galloway 1995); material in BM (!) annotated *Sticta fimbriata* nobis, Hyde's Cottage 17 Aug. 1836, possibly a syntype

= *Sticta elegans* Deak, in W. Mudd, A Manual of British Lichens: 89 (1861)

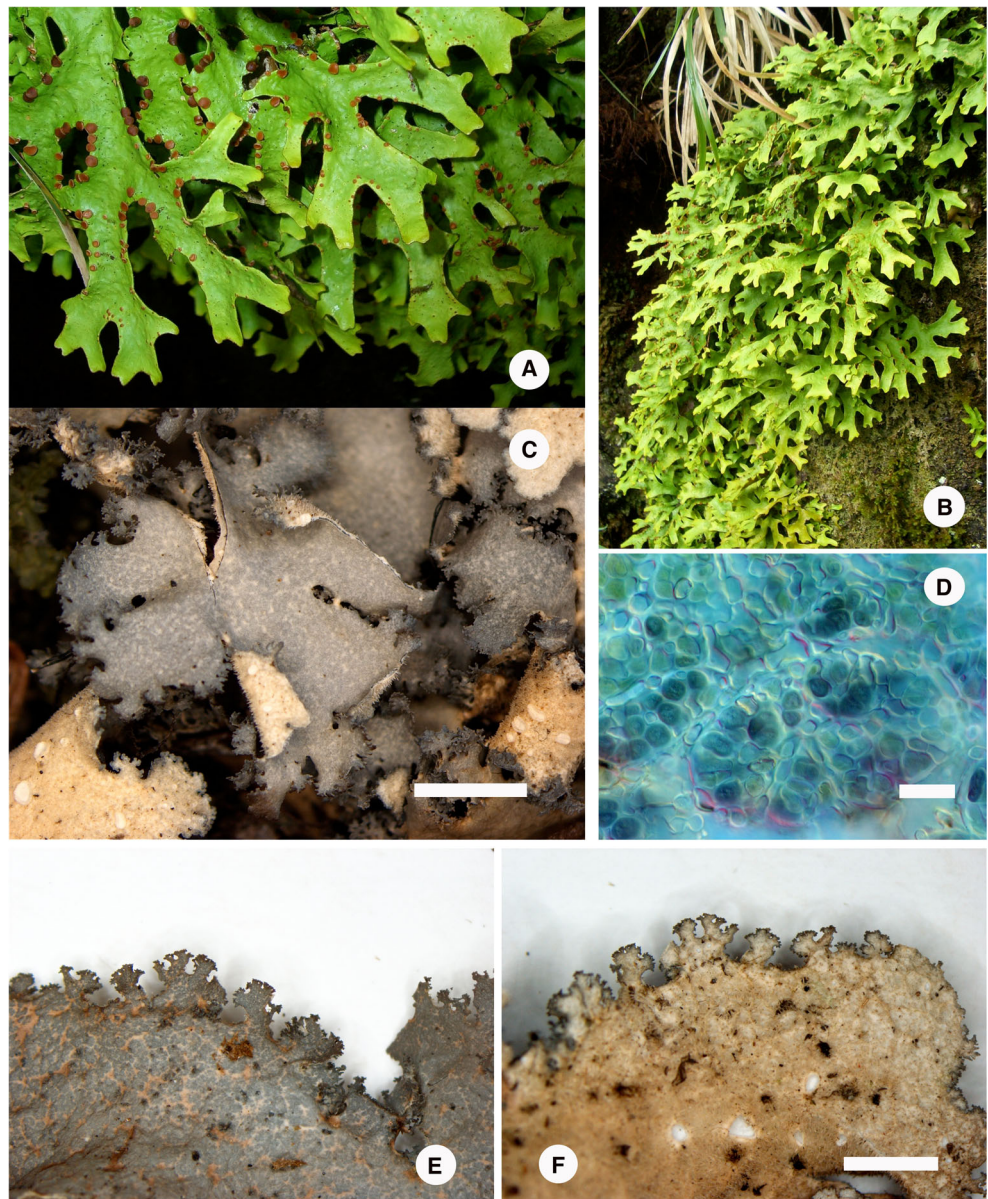
Type: **England**, on rocks and stems of ivy. Rock Walk, Ilsham, near Torquay, Devonshire, W.A. Leighton (BM—lectotype !; designated by Galloway, Nova Hedwigia 61: 169, 1995)

Cyanomorph.

Thallus usually palmate or irregular in outline, 1–6 cm in diam., in suitable conditions forming large colonies made of several thalli intermingled one with another and overlapping, usually thin and brittle when dry, but some populations develop rather stiff thalli, sometimes with a distinct, short, and robust stipe, with branched lobes (with 2–4 ramifications per main lobe), branching anisotomous or polytomous. Lobes suborbicular to ligulate in outline, overlapping, slightly ascending, especially when dry, plane to slightly involute, with rounded to irregular apices, margins regularly and minutely dissected, even at early stages of development, soon developing palmate or shortly stipitate *phyllidia*, 0.5–0.8(–1.2) mm long, dichotomously and several times branched, forming dense, +/- flattened, shortly stipitate and imbricate, minute and gently indented lace-like clusters at the thallus margins and sometimes invading parts or the whole thallus surface, of the same color as the thallus or darker. *Upper surface* smooth or rarely scrobiculate or foveolate, matte, dark bluish brown to dark brown when wet, becoming slate dark bluish when dry, typically marbled and maculate, especially on older parts, sometimes remaining dark brown, cilia absent. *Lower surface* foveolate to scrobiculate, orange to brown-colored; primary tomentum absent on the edges of young lobes and sometimes on old lobes, usually abundant throughout, pubescent to slightly spongy; no secondary tomentum detected. *Cyphellae* always present on the lower surface of main lobes, sometimes present on the lower surface of *phyllidia*, dispersed, rounded or angled, 5–15 per cm², 0.3–0.6(–1) mm in diam., immersed to erumpent, margin whitish to orange-brown. *Apothecia* and *pycnidia* never produced.

Upper cortex paraplectenchymatous, 20–25 µm high, homogeneous with 2–3 layers of rounded to isodiametric cells

Fig. 5 *Sticta canariensis* (Flörke) Delise. **a–b** Chloromorph photographed in the field in Madeira (Apr. 2007, M. Dewald, A. Hamblers & E. Sérusiaux). **a** Close-up to dichotomous lobes with numerous apothecia. **c–f** Cyanomorph, also known as *S. dufourii* (France/Brittany, Nov. 2013, A. Gérault s.n., LG). **c** Thallus with maculate upper surface and marginal phyllidia. **d** Microscopic view of *Nostoc* cells embedded into a paraplectenchamatus layer within the thallus. **e–f** Upper and lower side of the same lobe, showing the typically maculate upper surface and marginal phyllidia (that bear minute cyphellae on their lower surface). Scale bars: **c, e, f** 2 mm, **d** 10 μm



(8–15 μm in diam) or with an upper layer made of a single layer of small cells (5–8 μm in diam). *Photobiont layer* c. 50–90 μm high, *Nostoc* colonies, forming rather compact, ovoid masses, 40–70 \times 35–45 μm , tightly encompassed by hyphae forming rounded to angular cells, and thus designing a single layer wrapping up the *Nostoc* cells. *Medulla* compact, whitish, c. 50 μm high, with hyphae walls 3–4 μm . *Lower cortex* paraplectenchamatus, 20–30 μm high, with 2–3 layers of rounded to isodiametric cells (10–15 μm). *Lower primary tomentum* formed of bunches of cylindrical hyphae, 0.1–0.3 mm long, agglutinated to each other or not, usually easily separated apart in water preparation, made of elongate cells with thick walls (lumina: 8–14 \times 4–5 μm ; walls: 1.5–2 μm thick). *Cyphellae membrane* made of 1–4 layers of irregular

cells, developing ovoid to irregularly inflated cells, 6–10 \times 4–5 μm , at the cyphellae surface where they can be isolated one from another.

No substances detected by TLC.

Chloromorph

Thallus formed of bunches of elongate and dichotomously branched (branching mode rarely anisotomous or polytomous) lobes, forming large colonies in suitable conditions made of several thalli intermingled one with another and overlapping, or with lobes more adjacent than imbricate, up to 15 cm long and c. 10 cm large, 2–4 ramifications per main lobe, subcoriaceous and rather resistant. *Lobes* ligulate to mostly lacinate, horizontal or ascending when growing on subvertical substrate (tree or outcrop), plane to involute, with

apices truncate to spatulate, involute to undulate, margins entire and not thickened, internode 0.3–1.0 cm. *Upper surface* smooth, green to vivid green or yellowish green when wet, becoming pale orange brown when dry or in herbarium conditions, matte, but shiny when wet, without tomentum, maculae or cilia. *Lower surface* smooth, cream-colored to pale brown, becoming much darker towards the center of the thallus; primary tomentum present, sparse throughout, rather thick and thinner towards the margins, pubescent to arachnoid, soft, cream-colored to brownish black towards the center; no secondary tomentum detected. *Cyphellae* always present, abundant, dispersed, rounded or rarely irregular or angular, 20–40 per cm² in the mature parts of the lobes, less dense (c. 10–20 per cm² towards the apices), 0.3–0.4(–0.6) mm in diam, prominent, mostly at tomentum level, margin elevated and slightly involute, cream-colored to dark brown, basal membrane white. *Apothecia* usually abundant, rarely absent, laminal or submarginal, sessile to rarely subpedicellate, always strongly constricted at their base, without any lobe invagination underneath, 0.3–0.5(–0.7) mm in diam., with a disc chamois-colored to orange- to reddish-brown, concave when young, with prominent margin in young apothecia, cream-colored, always much paler than the disc. *Pycnidia* immersed, with a brown-black wall.

Upper cortex paraplectenchymatous, 35–45 µm thick, made of two differentiated layers, the upper one made of 1–2 cells with lumina c. 5 µm in diam. and wall c. 2 µm thick, the lower ones with larger, isodiametric and angular cells, 12–18 µm across and wall 1.5–2.5 µm thick. *Photobiont layer* 20–50 µm thick with algal cells c. 4–5 µm in diam. *Medulla* 50–120 µm thick, rather compact. *Lower cortex* paraplectenchymatous, 30–40 µm thick, sometimes much less, with 2–4 layers of cells, 8–15 µm in diam. and a c. 2 µm thick wall. *Lower primary tomentum* formed of agglutinate bunches of 12–20 unbranched, cylindrical hyphae, free or intertwined, made of elongate cells with rather thick walls (lumina: 8–15×4–5 µm; walls: 1.5–2 µm thick). *Apothecia* biatorine; excipulum made of radiating rows of isodiametric or rectangular cells, up to 100–120 µm thick; hymenium 80–130 µm high; epihymenium c. 10 µm thick, orange to pale brown, K-; ascospores 8/ascus, fusiform with pointed ends, straight, 24–30×7–9 µm ($n=10$), with 1(–3) septa, not constricted at septa. *Conidia* rarely found, bacilliform, 5–7×1–1.2 µm.

No substances detected by TLC.

Nomenclature and typification: *Sticta canariensis* was first described by Flörke (1809) on the basis of a collection made by Bory de Saint-Vincent on Tenerife. The lectotype has been chosen by Galloway (1995) and his conclusions are here confirmed. However, the species and a fortiori its epithet was not mentioned in the most famous book of Bory de Saint-Vincent (three volumes and an atlas) published in 1804 under the title "Voyage dans les quatre principales îles des mers d'Afrique" (Tenerife, Saint Helena, Mauritius, and

Reunion) (Bory de Saint-Vincent 1804; examined via Google Books, accessed on 16.1.2015). Thus, the epithet cannot be attributed to Bory de Saint-Vincent, contrarily to other epithets clearly introduced for species from Reunion and/or Mauritius and explicitly mentioned in his book, such as *Cladonia candelabrum* (Bory) Nyl. and *Sticta ambavillaria* (Bory) Ach. (respectively, as *Lichen candelabrum* and *L. ambavillarius* in Bory de Saint-Vincent 1804). The attribution of the names introduced by Flörke in 1809 on the basis of material collected by Bory de Saint-Vincent and given to him for publication has been a matter of debate in several papers: Ahti (1980) argued that these epithets must be rejected on the basis of an interpretation of the intention of Bory in making his specimens and notes available to Flörke; the sentence reads as [translated from German to English by Galloway (1995), Flörke speaking]: "He, therefore, let me have a totally free hand to name the lichens as I wished, only he wanted his diagnoses to be given alongside". Thereafter, Laundon (1984) challenged this interpretation and was followed by Galloway (1995): indeed, the epithets in Flörke's publication (1809) are not presented as synonyms, but as headings, often with descriptions. We agree with this view and further argue that if an epithet was already mentioned in Bory de Saint-Vincent (1804), it is valid under the Code of nomenclature and must be attributed to him; if not, an epithet must be attributed to Flörke, albeit on the basis of Bory's notes and collections. Therefore, the correct name for this species, which is not mentioned in Bory de Saint-Vincent (1804) is *Sticta canariensis* (Flörke) Delise.

Ecology: In Macaronesia, locally abundant in the laurisilva and in *Erica-Myrica* stands (*Fayal-Brezal*), especially in the most humid localities, growing on trees, mossy soils and rocks, both photomorphs can thrive together and usually not connected; in continental Europe, in the same habitats but preferably in damp, shaded and very humid niches, sometimes abundant, the cyanomorph being the most abundant form, chloromorph usually present as green lobules connected to the upper surface, or isidia masses, of the cyanomorph.

Distribution: A first map of the distribution of the cyanomorph was published by Degelius (1935: 193); since then the species has been found in SW Norway, where it is red-listed (Jørgensen and Tønsberg 2007). The species is mainly known from the western parts of Europe: United Kingdom and Ireland (Seaward 1998), France (Brittany, Western Pyrenees, Massif central: Roux et al. 2014), NW Spain and a single locality in SW Portugal (Burgaz and Martínez 2003), and Macaronesia (Canary Islands, Madeira, and the Azores). The species is very rare in the Mediterranean region, as it is known from two localities in Corsica (Roux 2013) and the Tyrrhenian coast in Italy (Nimis 1993); we have not found any recent (less than 20 years) record from the Mediterranean region. *S. canariensis* is also mentioned in the North America Checklist of Lichens (<http://www.ndsu.edu/pubweb/~esslinge/chcklst/chcklst7.htm#S>) with a single

reference to a collection from Ontario/Canada (Brodo 1994); this record should be re-evaluated. The distribution map available on the GBIF platform is inaccurate as it obviously includes records from other species for localities in South Africa, Central and South America (<http://www.gbif.org/species/5477265>; accessed on Feb. 21, 2015).

Notes:

1. In the European and Macaronesian context, the chloromorph is unmistakable as it usually forms splendid vivid green, large thalli with almost perfectly dichotomous lobes and laminal or submarginal apothecia. The cyanomorph is easily recognized by its maculate upper surface and its abundant, marginal, and laminal phyllydia: no other species of *Sticta* in Europe displays those characters.
2. Within the first phylogeny of the genus provided by Moncada et al. (2014a) focusing on species from Colombia, *S. canariensis* is resolved together with other species producing a chloro- and cyanomorph, or only a cyanomorph (Moncada et al. 2013b); there are two other such clades in their study (the *S. lobulata* and the *S. rhizinata* clade), and our phylogenetic tree identifies a further one, including species with large and conspicuous thalli from Madagascar and the Indian Ocean, with either only a chloromorph (*S. variabilis*), only a cyanomorph (*S. macrophylla*) or both (*S. dichotoma*: Magain et al. 2012a). The trait of associating either with a cyanobacterium, a green alga or both is thus not constrained within the evolutionary tree of the genus and may represent opportunities of shifts in symbiosis that allow emancipation of each photomorph from the other and that drive speciation, such as in the related family Pannariaceae (Magain and Sérusiaux 2014).
3. Two haplotypes can be distinguished amongst ITS data for this species, differing by a single substitution in ITS2; both haplotypes are represented in material from the Canary Islands, whilst only one is observed in continental Europe.

Selected specimens examined: **Ireland**, Kerry, S of Killarney, N 52°01'05" W 09°30'15", 30 m, Feb. 2014, park with scattered trees, E. Sérusiaux s.n. (LG). **France**, Brittany, Cranou forest, N 48°18'55" 04°05'48"W, 120 m, Nov. 2013, mixed woodland, A. Gérard s.n. (hb Gérard, LG). **Portugal**, Madeira, Ribeiro Frio, N 32°44'06" W 16°53'11", 900 m, Feb. 1988, disturbed laurisilva, E. Sérusiaux 9061 (LG). *Ibid.*, N 32°44'14" W 16°53'07", 920 m, Apr. 2007, disturbed laurisilva, M. Dewald, A. Hambuckers & E. Sérusiaux s.n. (LG). **Spain**, Canary Is., Tenerife, Peninsula de Anaga, N 28°33'46" W 16°10'19", 800 m, May 2010, disturbed laurisilva, E. Sérusiaux s.n. (LG). *Ibid.*, Punta de Anaga, Chamorga, N 28°34'33" W 16°09'18", 650–700 m,

May 2013, exposed outcrops and disturbed margins of laurisilva, E. Sérusiaux s.n. (LG).

Tayl., in Mackay J.T., *Flora Hibernica*: 152, 1836 (Fig. 6) Mycobank # 406124

Type: **Ireland**, on *Hypna* on the stems of trees in Akew Wood, Country of Kerry, T. Taylor (BM !, lectotype selected by Galloway, *Nova Hedwigia* 61: 168, 1995)

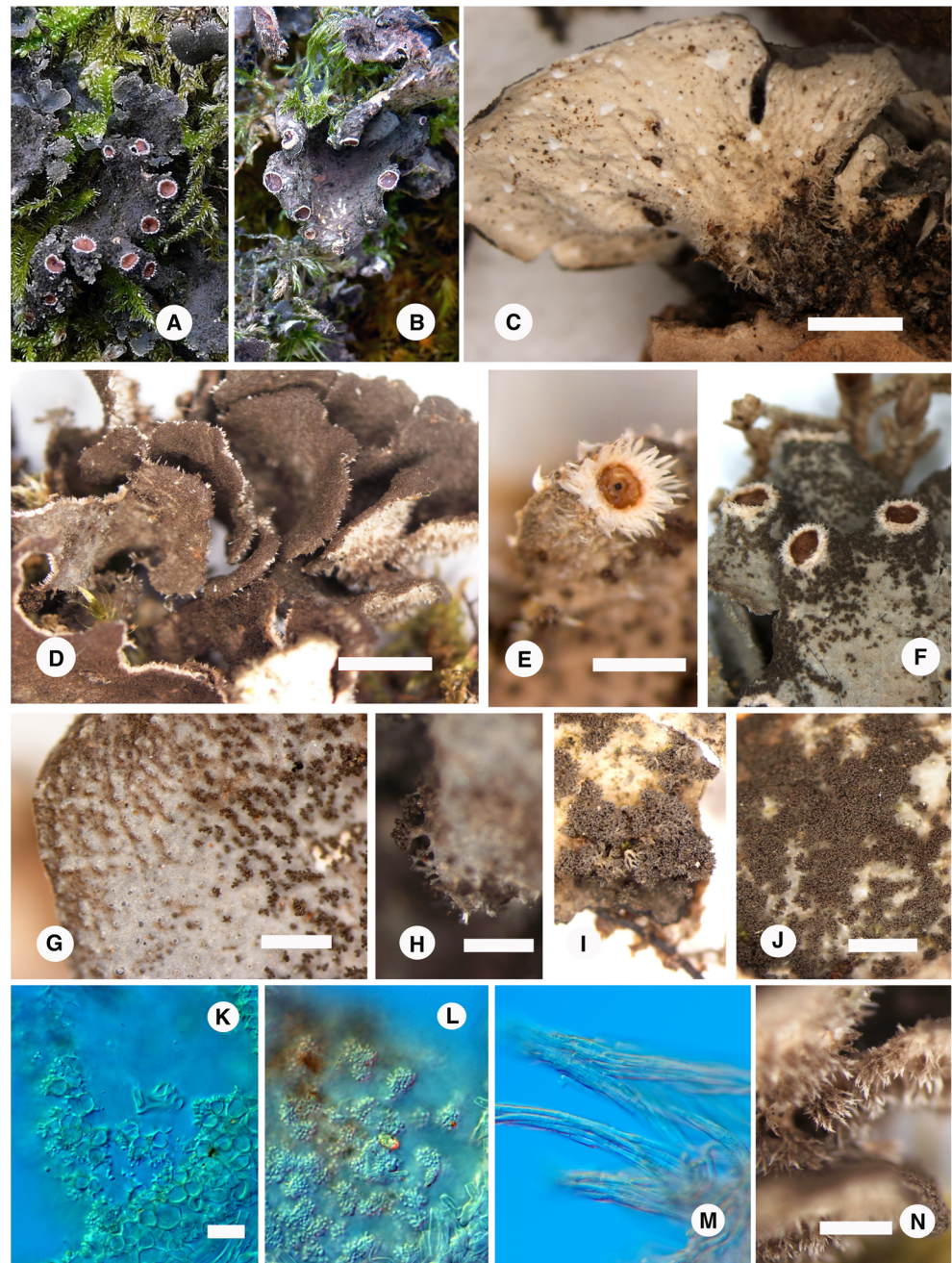
= *Sticta fuliginosa* f. *ciliata* Degel., Göteborgs Kungl. Vetenskaps- och Vitterhets-Samhälles Handlingar, Sjötte Följden, ser. B, Band 1: 19, 1941

Type: **Portugal**, Azores, Terceira, Serrata, På barken av ungt träd i skog, 20.4.1937, H. Persson (UPS, L-693709 !, here designated as the lectotype)

= *Sticta fuliginosa* auct. europ. p.p., non (Hoffm.) Ach.

Thallus first flabellate as the several lobes of a single thallus develop from a central point on a short and evanescent stipe (pseudostipe), eventually becoming suborbicular, well-developed thalli suborbicular to irregular, c. 1–3 cm in diam., rarely up to 5 cm in diam., rarely forming large colonies, papyraceous even when wet, and extremely brittle when dry; with 0–2 ramifications per main lobe, branching pleurotymous to polytymous. *Lobes* adnate to slightly ascending, imbricate and usually overlapping each other, more isolate when growing in competition with bryophytes, slightly to distinctly revolute when mature, and wet, with rounded apices and entire to crenate, lacerate, or sinuose, not tickened margin. *Upper surface* smooth when young, becoming slightly, but distinctly scrobiculate to foveolate, and thus with a distinct microrelief somewhat highlighted by scars of broken isidia, greyish to dark chocolate brown, remaining of the same color when dry, but usually paler, matte, or moderately glossy, sometimes with a whitish pruina made of minute and flattened granules, giving a minutely dotted appearance under high magnification. *Cilia* always present on young lobes, typically abundant on fast-growing thalli in suitable conditions, lasting on parts of older thalli, especially on their regenerating parts, simple to agglutinate, with usually pointed ends, white to translucent, 0.2–0.5 mm long. *Isidia* always present, abundant, laminal, typically branched and coralloid, usually with a short (<0.1 mm) but distinct stipe, forming coralloid masses 0.2–0.5 mm across, matte to slightly glossy, subglobose extremities forming the propagules, 25–50×20–40 μm and somewhat darker or bluish under high magnification. *Lower surface* foveolate to scrobiculate, whitish to cream-colored, darker towards the center, with sparse to dense and developed throughout primary tomentum; primary tomentum pubescent, hirsute to fasciculate, whitish to cream-colored and becoming dark brown to black towards its base. *Cyphellae* abundant, 15–40 per cm², irregular to angular, rarely round, cupuliform to rarely plane, erumpent, margin indistinct or elevated and slightly involute, or rarely irregular, white to cream-colored, 0.3–1.2 mm in diam. with a pore 0.2–0.8(–1.0) mm in diam. *Apothecia* rarely present but abundant when they are, 0.2–

Fig. 6 *Sticta ciliata* Tayl. **a–b** Fertile thalli photographed in the field in France/Brittany, photographs by Michel David and Bernard Bouffinier; see <http://www.lichensmaritimes.org/>. **c** Lateral view of a young palmate lobe. **d** Upper view of young dark brown lobes, with ciliate margins and nested one in the other. **e–f** Apothecia with ciliate margins. **g** Upper surface irregularly folded/pitted with scars of broken isidia. **h–j** Isidia clearly stipitate when mature. **k–l** Cells of the cyphella membrane with numerous papillae on their surface. **i** Surface view of upper side of such cells. **n** Primary tomentum forming squarrose rhizine-like fascicles. **c, d, g, h, n** Azores, July 2014, *E. Sérusiaux* s.n., LG; **e, j, k, l, m** France/Brittany, Nov. 2013, *A. Gérault* s.n., LG; **f** Tenerife, Mar. 2011, *P. van den Boom* 45673, LG; **i** Tenerife, May 2015, *E. Sérusiaux* s.n., LG. Scale bars: **c–d** 2 mm, **e, f, g, j** 1 mm, **h, i, n** 0.5 mm, **k, l, m** 10 μ m (scale in k)



1.2 mm in diam., submarginal, sessile, developed over a distinct invagination, disc reddish brown to dark brown, glossy, slightly concave especially when young, always with a crown of white to pale orange cilia. *Pycnidia* sometimes present, immersed in the thallus lobes, outer wall and opening without any distinct color.

Upper cortex paraplectenchymatous, 20–25 μ m thick, made of 1–3 layers of rounded to isodiametric cells, c. 8–12 μ m in diam. with a 1–2 μ m thick wall. *Photobiont layer* 25–40(–50) μ m high, with compact masses of *Nostoc* colonies tightly encompassed by hyphae forming rounded to angular cells, and thus designing a single layer wrapping up the

Nostoc cells. *Medulla* white, lax, 50–80 μ m thick. *Lower cortex* paraplectenchymatous, 15–20 μ m thick, with 1–2 layers of rounded to isodiametric cells, c. 8–15 μ m in diam. with a 1–2 μ m thick wall. *Lower primary tomentum* agglutinated in fascicles, with free to slightly intertwined, cylindrical hyphae, with a c. 2 μ m thick wall. *Lower secondary tomentum* made of budding cells on the lower cortex surface, forming moniliform assemblages of 2–20 cells, 5–10(–12) μ m in diam. and with a c. 1 μ m thick wall. *Cyphellae membrane* made of a layer of rounded cells (4–8 μ m in diam.) with outer side (= the side of the cyphella membrane in contact with the

environment) covered with 6–15(–20) minute papillae, 1–2×1–1.5 µm; these papillae tend to disintegrate in old thalli. *Apothecia* lecanorine, reaching 2.5–3.0 mm in height (measured from the lower cortex of the lobe invagination up to the epihymenium); excipulum pale orange to brownish, 50–60 µm thick; hairs always present on outer parts of the excipulum, 0.2–0.3 mm long, formed of long and agglutinated hyphae with walls 1.5–2.0 µm thick; hymenium 70–90 µm thick with epihymenium pale orange to dark orange brown, K-; ascospores 8/ascus, fusiform with pointed, straight or slightly curved, 26–46×7–8.5 µm ($n=15$), with 1(–3) septa, not constricted at septa, with a minute but distinct mucoid appendage on the distal end. *Pycnidia* without any color, producing numerous bacilliform conidia, 2–3×1–1.2 µm.

No substances detected by TLC.

Nomenclature and typification:

1. Examination of the lectotype collection of *S. ciliata* in BM (Fig. 3a–c) leaves no doubt about its identity; see also <http://plants.jstor.org/specimen/bm000974832?s=t>. Although the epithet was reduced into synonymy with *S. canariensis* by Galloway (1995), the scanty collection have several characters that make it referable to the taxon here recognized as distinct from both the cyanomorph of *S. canariensis* and *S. fuliginosa*: indeed it has small unlobed thalli with a short stipe and a ciliate margin (Fig. 3c), a non-maculate upper surface and genuine isidia. A sentence in the original description (Mackay 1836: 152) reads as "[...] yet occasionally flat process issues suddenly from the edge, on which is borne a circular ciliate thalloid expansion, being the evolution of a bud". This could be interpreted as the description of a young apothecium with a ciliate margin; there is, however, no such structure on the type material.
2. Degelius (1941) reported *Sticta fuliginosa* from several islands in the Azores archipelago (Flores, São Jorge, São Miguel, and Terceira). From two localities in Terceira, he mentioned a "very peculiar form" that he had "not seen before". He further stated that "the specimens are very small, up to about 0.5 cm broad (young ?) and bearing in the margin rather numerous, 0.2–0.3 mm long, white, usually simple cilia which often form haptera at the tips. (...) Evidently, this aberrant form is genotypically settled and not produced by the environment. The specimens are numerous and the cilia occur very constantly. Neither young nor old specimens in the normal type have cilia. I shall call this ciliate form f. *ciliata*". *S. ciliata* is a common species in the Azores archipelago where it thrives in natural habitats as well as artificial ones. Populations such as those described by Degelius (1941) occur in the Azores: they are made of small, very numerous thalli exuberantly growing on planted trees, mainly along the roads, and all thalli are bordered by tiny cilia (Fig. 3d, photograph taken on Faial island, 7. 2014, on *Platanus* trees at c. 530 m elev.). They have been carefully sampled and all belong to *S. ciliata*. Although the type collection is not designated in the original publication, a collection filed as the holotype has been found in UPS and examined via detailed photographs made available by the curators of the herbarium; the specimen is well-developed and clearly belongs to *S. ciliata* Tayl. and is here designated as the lectotype of *Sticta fuliginosa* f. *ciliata* Degel.
3. Moncada et al. (2014a) used the unpublished epithet *microisidiata* Magain et al. for material collected in Colombia. This epithet was used by the authors as an ad. int. name for collections from the Canary Is. and Rwanda before the epithet *ciliata* was re-discovered. As mentioned below, *S. ciliata* belongs to a clade yet to be distangled and occurring in the Neotropics, Hawaii archipelago (B. Moncada and R. Lücking, *pers. comm.*), Africa (Albertine Rift in Rwanda), Macaronesia and Western Europe.

Ecology: epiphytic on trees, usually over growing mosses, in well-preserved forests stands in France/Brittany and in Ireland/Kerry; also found over saxicolous mosses in the same localities in France/Brittany; in Macaronesia, on trees and mossy rocks within the laurisilva and in *Erica-Myrica* stands (*Fayal-Brezal*), especially in the most humid localities.

Distribution: currently known from France/Brittany and Ireland/Kerry, but expected to occur elsewhere in suitable localities in Western Europe, such as Scotland and the Western Pyrenees; in Macaronesia, known from Tenerife, Gomera and La Palma in the Canary Islands, Pico and Faial in the Azores and expected to occur in Madeira as well. Material from Colombia and Rwanda are resolved within the clade, and are thus tentatively considered as *S. ciliata*.

Notes:

1. Fresh material of that species can be easily recognized by its delicate and most usually ciliate thallus margin, especially for young thalli, and abundant tiny papillae over the cells of the cyphellae membrane. However, great care is required as regeneration lobules of all other isidiate species can have marginal cilia, albeit very rarely, and our observations of papillae on the cells of the cyphellae membrane are conclusive only for fresh and well-preserved material.

In a European context, the only other species that produces papillae on the cells of the cyphellae membrane is *S. fuliginoides* sp. nov., easily characterized by its non-ciliate thallus margin and a mushroom-like form for single lobes thalli at early stages of development; further *S. fuliginoides* sp. nov. never produces apothecia. Although apothecia are known in the type material of *S. fuliginosa* and putatively from a recent collection from France/Brittany (see under that name for further

comments), apothecia with ciliate margins are restricted to *S. ciliata* in the material examined for this study. Fertile specimens have been found in France/Brittany, the Canary Is. and the Azores.

2. Within the first phylogeny of the genus provided by Moncada et al. (2014a) focusing on species from Colombia, *S. ciliata* is resolved within the *S. fuliginosa* clade, and more precisely to the *S. gyalocarpa* subclade; material of *S. microisidiata* from Colombia is resolved in it; this subclade comprises several rather well-known species such as *S. hirta* (Nyl.) Trevis. and *S. gyalocarpa* (Nyl.) Trevis. and recently described ones such as *S. arbuscula* Moncada and Lücking (2012). Quite interestingly, the latter species also has numerous tiny papillae on cells of the cyphellae membrane.

The sequences of *S. ciliata* produced for this study with four loci (ITS, LSU, mtSSU and *RPB1*) are very distinctive; nevertheless, our phylogenetic analyses show clear affinities with *S. fuliginoides* sp. nov., the only other species with papillae on cells of the cyphellae membrane included in this study. As mentioned under *S. fuliginosa*, the material referred to that epithet by Moncada et al. (2014a) actually belongs to *S. fuliginoides* sp. nov.; therefore, the clade named *S. fuliginosa* in their study does not include the eponym species.

3. A surprising and unexpected variation is detected within this species, and further, the 4-loci phylogenetic tree (Fig. 2) does not resolve it identically as the TCS haplotype network (Fig. 3). As a matter of fact, the phylogenetic tree resolves two lineages with rather long branches, but weak support: one includes two accessions from the Canary Islands, and the other one includes accessions from continental Europe, Macaronesia (Canary Is. and the Azores), Rwanda, and Colombia. The latter clade is divided into two: one accession from France/Brittany is sister to all others, with strong support when both accessions from Colombia are excluded. Indeed, these two accessions contribute weakly to that branch as they are represented by ITS data only.

Interestingly, the TCS haplotype network (Fig. 2) yields another story about the relationships between haplotypes within that species. Here, ITS sequences from 29 accessions are included and the algorithm used for the construction of the haplotype network does not exclude any region: the whole variation within ITS sequences is considered. The accession from Rwanda is excluded from the network under the 90 % limit constraint; eight haplotypes occur in continental Europe and Macaronesia whilst both accessions from Colombia are included in the network but clearly distinguished from those from continental Europe and Macaronesia. Three haplotype groups can be easily distinguished: (1) one with the single accession from Ireland, and thus assumed to represent the type

population, further comprising material from France/Brittany and the Azores; (2) a second one differing by eight mutational steps and present in the same geographical area plus the Canary Islands (Tenerife and La Palma); and (3) a third one also differing by eight mutational steps from the typical one, and represented by a single accession from Tenerife in the Canary Islands. The pattern is thus very different as the two main lineages found in the 4-loci phylogenetic tree are not recovered: the most distinct ITS haplotype (#3) represented by a single accession from Tenerife is included in the first lineage whilst its sister accession in the 4-loci phylogenetic tree is resolved in a different haplotype group (#2) in the TCS construction. Examination of the positions in ITS1 and ITS2 that support the haplotype network show that they are excluded in the 4-loci phylogenetic analysis as they belong to most variable and thus ambiguous regions in the data matrix.

In any case, no morphological, nor anatomical diagnostic characters could be detected to distinguish the two main lineages of the 4-loci phylogenetic tree, nor for the three main haplotypes observed in the TCS network. More work is thus needed to further assess the variation encountered. We therefore consider the variation in continental Europe and Macaronesia to be infraspecific. Populations discovered in Rwanda and in Colombia may represent two further species.

4. A short sequence (15–16 bp) in ITS2 should allow identification of the three main haplotypes within *S. ciliata* in continental Europe and Macaronesia, the diagnostic positions are underlined:

(1) Type	<u>CCTCGT</u> <u>GCC</u> <u>ATCGT</u> <u>AT</u>
(2)	<u>CCTCGC</u> <u>CT</u> <u>ATCGT</u> <u>AC</u>
(3)	<u>ICTCGC</u> <u>CT</u> <u>ATCGT</u> <u>AC</u>

Selected specimens examined:

Ireland, Kerry, S of Killarney, N 52°01'05" W 09°30'15", 30 m, Feb. 2014, *Quercus* forest, E. Sérusiaux s.n. (LG). **France**, Brittany, Hôpital Camfrout, N 48°19'59" W 04°01'22", 15 m, Nov. 2013, disturbed forest, A. Gérard s.n. (hb Gérard, LG). **Portugal**, Azores, Pico Is., 2011, P. K. Divakar s.n. (MAF, LG); *ibid.*, N 38°29'19.62" W 28°14'42.18", 240 m, July 2014, plantations of *Pinus* with fragments of natural vegetation, E. Sérusiaux s.n. (LG); *ibid.*, Faial Is., N 38°35'56.75" W 28°40'53.85", 530 m, July 2014, on *Platanus* by the road, E. Sérusiaux s.n. (LG). **Spain**, Canary Is., Tenerife, Las Montanas de Anaga, N 28°33.53' W 16°10.19", Mar. 2011, 775 m, laurisilva, P. van den Boom 45673 (hb. van den Boom); *ibid.*, N 28°34'22" W 16°09'55", 690 m, May 2014, laurisilva, E. Sérusiaux s.n. (LG); *ibid.*, La

Palma, Cubo de la Galga, N 28°45'11" W 17°46'13", 480–500 m, May 2013, disturbed laurisilva, E. Sérusiaux s.n. (LG).

Sticta fuliginoides Magain & Sérus., sp. nov. (Fig. 7)
Mycobank # 813824

Species of *Sticta* distinguished from other isidiolate species in Western Europe with a thallus lichenized with a cyanobacterium by its thallus developing as a single lobe vase- or trumpet-like, with typically revolute margins, and cells of the cyphella membrane covered with papillae.

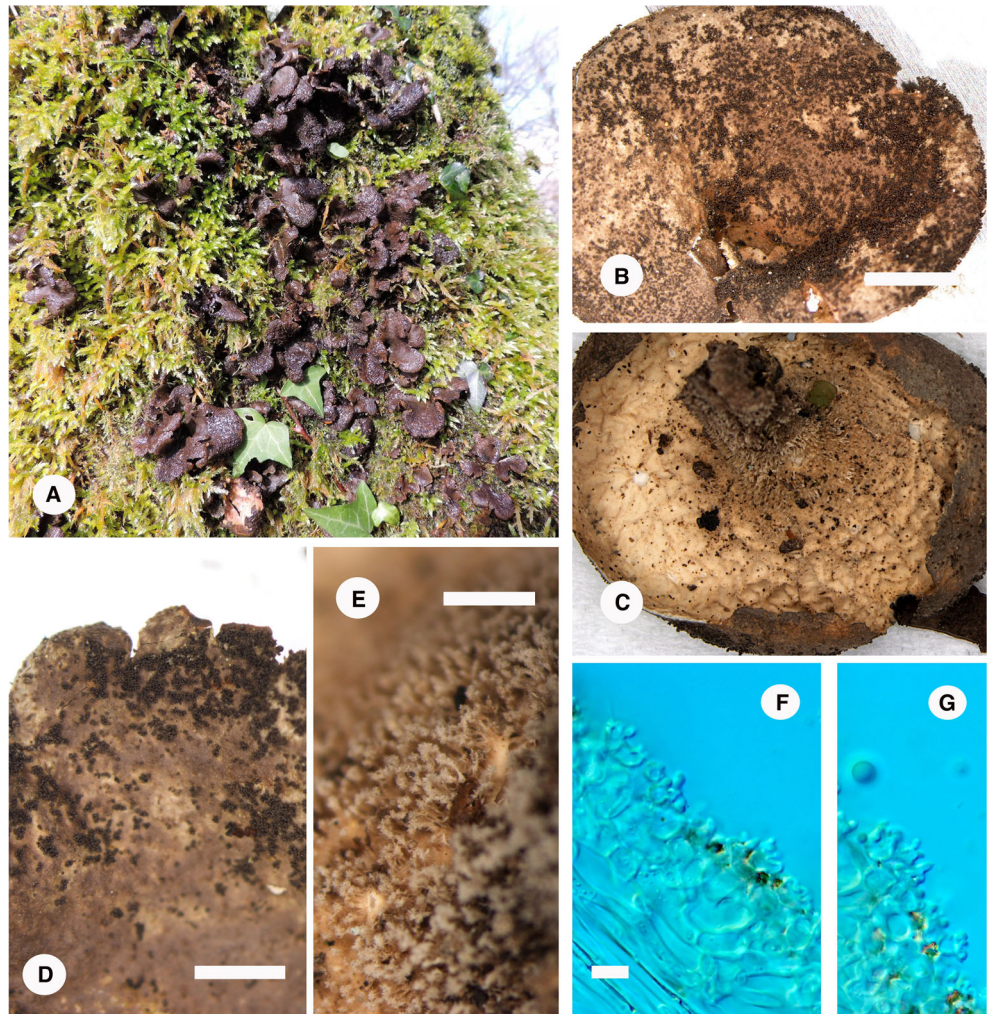
= *Sticta fuliginosa* auct. p.p., non (Hoffm.) Ach.

Type: **Spain**, Canary Is., Gomera, Parque Nacional de Garajonay, SW of the summit (Alto de Garajonay), N 28°05' 52" W 017°14'49", 1240 m, May 2012, degraded laurisilva, E. Sérusiaux LG DNA 3012 (LG—holotypus; TFC—isotypus).

Thallus first developed on a short, but robust stipe (up to 1 cm high) as a single lobe vase- or trumpet-shaped, typically with revolute margins, and thus looking like a young agaric mushroom, either in humid or dry conditions; eventually developing several flabellate lobes with an irregular or mostly suborbicular outline, usually 2–3 cm in diam., up to 5 cm in suitable conditions, rarely larger; several thalli growing

together may mimic large thalli, subcoriaceous but rather brittle when dry. *Lobes* with no or 1–2 branching, adnate to ascending, especially when young, imbricate and overlapping each other, plane to slightly involute with rounded to obtuse or even truncate lobes (especially in old thalli) that typically have a strongly revolute entire margin, old thalli can have a crenate to lacerate and hardly revolute margin. *Upper surface* smooth to slightly scrobiculate, especially in old lobes on which scars of broken isidia can mimic macules, dark brown or rarely pale greyish, in wet or dry conditions, matte or slightly glossy. *Cilia* most usually absent, sometimes seen on small regenerating lobes, simple, white, < 0.5 mm long. *Isidia* always present, abundant, laminal, dispersed throughout, first simple and globose or with a flattened top, eventually forming upright coralloid masses, < 0.2 mm high, sometimes forming substipitate lobules on old thalli, of the same color as the thallus or darker, matte to slightly glossy. *Lower surface* smooth or costillate to scrobiculate, sometimes strongly, pale orange to brownish, darker towards the center on old thalli, with sparse to dense and developed throughout primary tomentum, absent in some thalli; primary tomentum pubescent

Fig. 7 *Sticta fuliginoides* Magain & Sérus., sp. nov. **a** Large population of well-developed thalli on tree (photograph taken in the field, Feb. 2014, Ireland/Kerry). **b–c** Upper and lower view of a young vase-like thallus, showing the lower reticulate surface, typically revolute margin and stipe covered with primary tomentum. **c** Upper surface with isidia. **d** Lower primary tomentum. **e–f** Cells of the cyphella membrane with several papillae on their upper surface. **b–g** Type collection (La Gomera, May 2012, E. Sérusiaux s.n., LG). Scale bars: **b**, **c** 2 mm (scale in b), **d** 1 mm, **e** 0.5 mm, **f**, **g** 10 μ m (scale in f)



to hirsute, or fasciculate, especially towards the center, whitish to dark brown towards the center. *Cyphellae* usually abundant, rarely sparse, 20–30 per cm², round or angular, urceolate with a wide pore, erumpent to prominent, margin elevated and involute, whitish to cream-colored, rarely brownish, 0.4–0.6 mm in diam., larger ones on old thalli can reach 1.5 mm in diam. *Apothecia* and *pycnidia* never produced.

Upper cortex paraplectenchymatous, 25–40 µm thick, usually differentiated in two layers, the upper one made of a single row of elongate to rounded cells, 5–7×3–4 µm with a c. 1 µm thick wall and the other one made of 3–5 layers of rounded to isodiametric cells, 8–12 µm in diam. with a 1.5 µm thick wall. *Photobiont layer* 20–30 µm thick with compact masses of *Nostoc* encompassed by hyphae forming rounded to angular cells and thus designing a single layer wrapping up the *Nostoc* cells. *Medulla* white, lax, 40–60 µm high. *Lower cortex* paraplectenchymatous, 30–40 µm thick, made of 3–5 rows of isodiametric cells, up to 20–22 µm across in the stipe, with a 2–3 µm thick wall. *Lower primary tomentum* agglutinated in fascicles, with more than 20 unbranched or slightly branched, flexuose hyphae that are either free or more frequently intertwined, with a c. 1.5 µm thick wall. *Lower secondary tomentum* always present albeit it can be hardly detected under high magnification, made of free, unbranched moniform hyphae, <20 µm long, with 1 µm thick wall, with budding apices, producing individual rounded cells, 6–8 µm in diam., acting as diaspores. *Cyphellae membrane* made of a layer of rounded cells (5–9 µm in diam.) with outer side (= the side of the cyphella membrane in contact with the environment) covered with 4–6(–8) minute papillae, 1–2.5×1–1.5 µm; these papillae tend to disappear in old thalli; detached cells of the membrane seem to produce up to 15–20 papillae per cell.

No substances detected by TLC.

Etymology: the epithet of this new species was chosen to evoke the confusion that has prevailed for two centuries in the perception of the taxonomic variation in isidiate species of *Sticta* in Europe.

Ecology: on mossy trees and rocks within forests or at their edges, incl. parkland conditions in oceanic climate, found at the montane zone (*Fagus sylvatica* and *Abies alba* mixed forest) at c. 1000 m elev. down to *Quercus*-dominated forest at sea level; in Macaronesia, on trees and mossy rocks within the laurisilva and in *Erica-Myrica* stands (*Fayal-Brezal*), especially in the most humid localities.

Distribution: as this species has been confused with *S. ciliata* and *S. fuliginosa* for two centuries, its distribution might be underestimated. ITS data confirm it occurs in continental Europe, the Canary Islands, eastern North America, and Colombia.

Notes:

- Sticta fuliginoides* sp. nov. can be distinguished in its early stages of development by its single lobe thalli vase- or trumpet-like, with typically revolute margins. The latter character is maintained when thalli are more developed and branched and have thus lost their vase- or trumpet-like shape. Mature thalli remain rather small (less than 5 cm in diam) and this character, associated with the revolute margin, provide good diagnostic evidence for the distinction from *S. fuliginosa*. Papillae are present on the cells of the membrane of cyphellae and, at least in fresh or recently collected material, provide a further character to distinguish it from *S. fuliginosa*. *S. ciliata* also produces such papillae, usually much more abundant than in *S. fuliginoides*, but most usually has a ciliate margin and produces apothecia in suitable localities, whereas *S. atlantica* has an upper surface with typically regular or irregular swellings or ridges on which isidia start their development to form coralloid stipitate masses.
- Within the first phylogeny of the genus provided by Moncada et al. (2014a) focusing on species from Colombia, *S. fuliginoides* sp. nov. is assigned to the *S. fuliginosa* clade, that comprises three subclades, the first one with only *S. fuliginoides* sp. nov. Indeed, all accessions included as *S. fuliginosa* in Moncada et al. (2014a) represent this species. The other two subclades comprises no less than 20 species, incl. *S. ciliata* (as *microisidiata*, see under *S. ciliata*).
- Five haplotypes can be distinguished within the ITS data: they differ from each other by one or two indels in ITS1 and one or two indels plus one substitution in ITS2. The haplotype network indeed demonstrates that they are closely related (Fig. 2). Interestingly the accessions from the USA/North Carolina (accessions AY173387–9, as *S. fuliginosa*: McDonald et al. 2003) belong to two haplotypes while all accessions from the Canary Islands (Gomera and La Palma, where the species is rather common) belong to a single haplotype. The two accessions from the U.K./Wales (accessions KC011069 and KC732454, as *S. fuliginosa*: Moncada et al. 2014a) form a unique haplotype. The single accession from Colombia (KC732709, as *S. fuliginosa*: Moncada et al. 2014a) is unique and differs from the closest haplotype by one indel in ITS1 and three indels in ITS2. Although this variation should be evaluated with more loci within a phylogenetic framework, we consider for the time being that all these accessions can be referred to as *S. fuliginoides*.

Selected specimens examined:

Ireland, Kerry, S of Killarney, N 52°01'05" W 09°30'15", 30 m, Feb. 2014, *Quercus* forest, E. Sérusiaux s.n. (LG). **France**, Brittany, Huelgoat, N 48°22'03" W 03°44'06", Nov. 2013, mossy outcrop in mixed forest, F. Séité s.n. (LG, herb. Séité). Ibid., Dept. Haut-Rhin, Hohneck, N 48°02' E 07°01', c. 1000 m elev, open mixed forest, June 2010, E. Sérusiaux s.n. (LG). **United Kingdom**, Devon, Popham

Woods, Brayford, N 51°4'50", W 3°51'32", open mixed forest, Jan. 2014, N. Magain s.n. (LG).

Sticta fuliginosa (Hoffm.) Ach. Methodus Lichenum, p. 280, 1803. (Fig. 8)

Mycobank # 122533

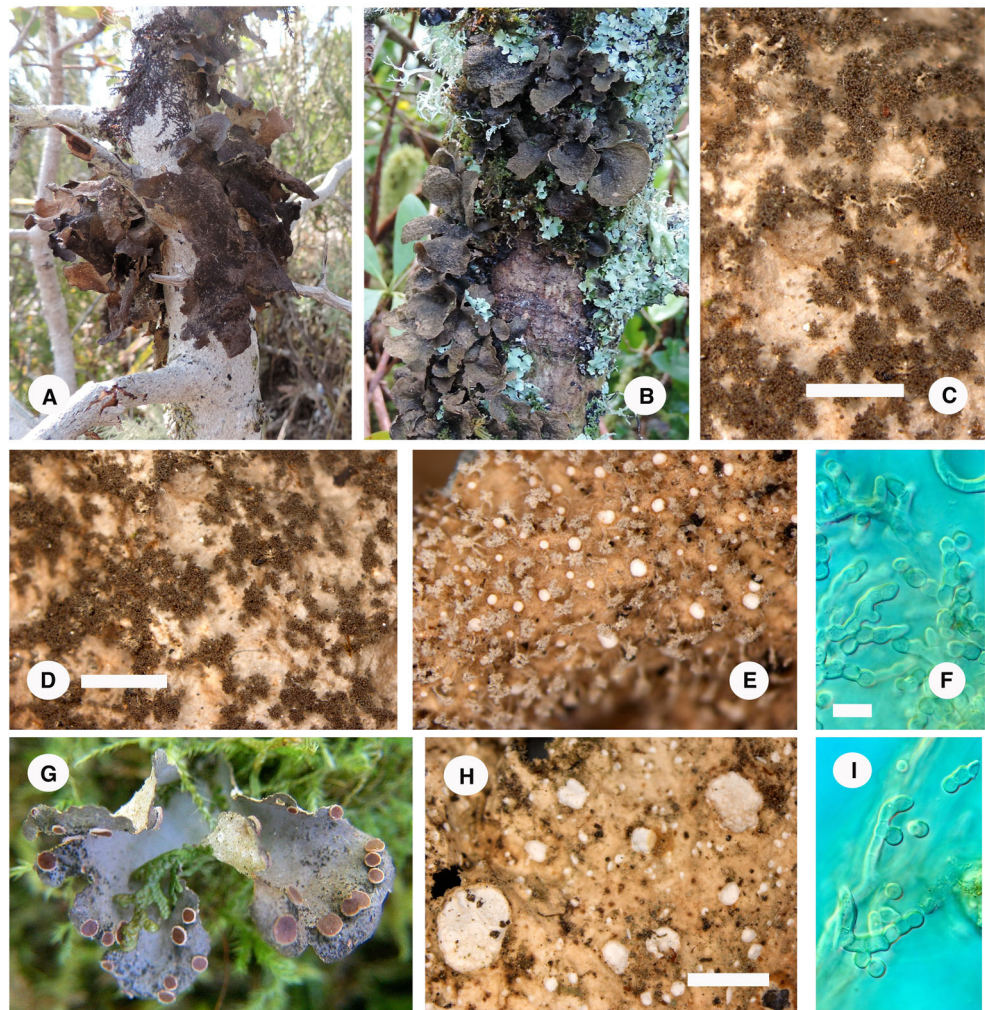
Bas.: *Lobaria fuliginosa* Hoffm., Deutschland Flora, vol. 2, p. 109, 1796.

Type: **United Kingdom**, Wales, Cader Idris; Icon. In Dillenius, Historia Muscorum, tabl. 26, Fig. 100A, 1742 (lectotype designated by Laundon, Lichenologist 16: 218–219, 1984); corresponding specimen in herb. Dillenius (OXF !, epitype, designated by Jørgensen and Tønsberg, Nordic Lichen Flora 3: 146, 2007).

Thallus first developing as a bunch of several palmate, elongate or flabellate lobes, but without any distinct stipe, eventually developing typically (sub-)orbicular and overlapping lobes, usually reaching 5–7 cm in diam in Europe, much larger (up to 10–12 cm across) in tropical areas, rather fragile and papyraceous, rather brittle when dry. *Lobes* with 0–2 branching, adnate or ascending, especially when well-developed, overlapping each other, branching polytomous or

irregular, most usually (sub-) orbicular, rarely irregular to ligulate, dissected in old thalli, apices plane and slightly undulating, sometimes slightly revolute, margin entire to crenate or even lacerate. *Upper surface* smooth to more usually scrobiculate to faveolate, undulating, greyish to brown in wet or dry conditions, maculae and cilia most usually absent; maculae irregularly developed, only seen in European material. *Isidia* always present, abundant, laminal, dispersed throughout, mainly developing on ridges of the upper surface (margins of the shallow depressions on the upper surface) first simple, glossy and rounded to applanate, but soon developing vertical coralloid masses, sometimes substipitate, up to 0.5 mm in height, darker than the thallus. *Lower surface* costillate to scrobiculate, sometimes strongly, white or pale orange to brownish, darker towards the center on old thalli, with sparse (especially in European material) to dense and developed throughout primary tomentum, absent in some thalli; primary tomentum pubescent to hirsute, especially towards the center, whitish to dark brown towards the center. *Cyphellae* abundant, 20–40 per cm² but more abundant (up to 45–60 per cm² towards the margins), round or angular,

Fig. 8 *Sticta fuliginosa* (Hoffm.) Ach. **a** Large specimen wrapping up a small tree in Madagascar (photograph taken in the field, Oct. 2014, E. Sérusiaux). **b** typical thalli on trunk of *Alnus* in Ireland/Kerry (photograph taken in the field, Feb. 2014, E. Sérusiaux). **c–d** Upper surface with development of isidia (Rwanda, Sep. 2010, E. Sérusiaux s.n., LG). **e, h** Lower surface with primary tomentum well-developed (**e** Azores, July 2014, E. Sérusiaux s.n., LG) or poorly developed (**h** Ireland/Kerry, Feb. 2014, E. Sérusiaux s.n., LG). **h, i** Conidiogenous cells and conidia all along the fascicles (Madagascar, Oct. 2008, E. Sérusiaux s.n., LG). **g** Photograph produced by François Sèité (group <http://www.lichensmaritimes.org/>) of a lobe of *Sticta fuliginosa* with apothecia, representing the only recent reliable data on apothecia production by this species. Scale bars: **c, d, e, h** 1 mm (no scale shown in **e**), **f, i** 10 µm (scale in **f**)



cupuliform to urceolate with a wide pore, prominent to suprasessile, margin erect, slightly revolute, whitish to cream-colored, or brownish, 0.4–1.1 mm in diam., larger ones are more angular and can reach 1.5–2.1 mm (cyphellae up to 3.0 mm in diam. seen in material from the Azores). *Apothecia* and *pycnidia* never seen in the material examined; one picture recently made in France/Brittany (Fig. 8g) most probably represents a thallus of this species with apothecia; otherwise apothecia only known from the type material (Fig. 3f): 1.5–2.5 mm in diam., disc pale orange brown, flat, with a raised, paler, hairy margin (hairs small, pale to translucent, fluffy and not stiff); hymenium and ascospores not examined.

Upper cortex paraplectenchymatous, 30–40 µm thick, usually not differentiated in two layers, although the upper cells are usually smaller and more applanate than the others, 5–12 µm in diam. and with a c. 2 µm thick wall. *Photobiont layer* 45–55 µm thick with compact masses of *Nostoc* encompassed by hyphae forming rounded to angular cells. *Medulla* white, lax to compact, 60–80 µm high. *Lower cortex* paraplectenchymatous, 45–50 µm thick, made of 3–4 rows of isodiametric cells, 8–14 µm in diam. and with a 2–2.5 µm thick wall. *Lower primary tomentum* agglutinated in fascicles, with more than 20 unbranched, cylindrical, free or sometimes intertwined hyphae, with cells 15–22×5–6 µm and with a c. 1.5 µm thick wall; hyphae budding along their length or their extremities, and producing isolated cells acting as conidia, either rounded or slightly ellipsoid, 4–6 µm, incl. a c. 1 µm thick wall. *Lower secondary tomentum* not seen. *Cyphellae membrane* with outer cells never producing papillae.

No substances detected by TLC.

Nomenclature and typification: the epitype specimen was designated by Jørgensen and Tønsberg (Nordic Lichen Flora 3: 146, 2007) and can be examined online at the Oxford Herbarium web site (http://herbaria.plants.ox.ac.uk/bol/MUSCORUM/image/HM-sheet_072.jpg/Zoom?width=760px&height=760px). This specimen has been examined (Fig. 2) and corresponds to one of the four species now recognized within isidiate populations of *Sticta* in Western Europe (Jørgensen and Tønsberg 2007; Smith et al. 2009; Wirth et al. 2013). Further, we could produce ITS sequences for fresh material corresponding to that species in all sampled localities (France/Brittany and Vosges, U.K./Devon and Somerset and Ireland/Kerry). They are all strictly identical: we thus consider that they represent *S. fuliginosa*. The type material features a thallus with abundant apothecia; apothecia have not been found in any collections assembled for this study; a picture recently made by F. Sèité in France/Brittany (Fig. 8g) most probably represents a thallus of this species with apothecia but unfortunately the population was not sampled.

Ecology: on mossy trees and rarely on rocks within forests or at their edges, incl. parkland conditions, locally common but surprisingly rare in France/Brittany, and interestingly not

found in the Vosges; in Macaronesia, on trees and mossy rocks within the laurisilva and in *Erica-Myrica* stands (*Fayal-Brezal*), especially in localities with frequent mist.

Distribution: Our molecular data support the presence of this species in France/Brittany, U.K./Devon and Ireland/Kerry as well as in the Canary Islands (Gomera), the Azores archipelago (Pico Is.), Reunion, Madagascar, Rwanda (Fig. 154, as *S. fuliginosa* in *Macrolichens of East Africa* by Swinscow and Krog 1988 actually represents this species) and South Africa; accessions retrieved from GenBank further support its presence in the eastern USA (as *S. sylvatica* in Hodkinson et al. 2014), Japan and New Zealand. *S. fuliginosa* could thus be a widespread species in both hemispheres.

Notes:

1. This species can be distinguished by its rather large (up to 5–7 cm in diam.), suborbicular and slightly ascending thalli, with a plane, undulating margin, rarely revolute; it might be confused with *S. fuliginoides* sp. nov., which can be recognized by its typically revolute lobe margin, especially in young thalli, and presence of papillae on cells of the cyphella membrane. The distinction with *S. ciliata* is usually easy as this species has a very delicate, usually much smaller thalli with a ciliate margin, whilst *S. atlantica* has distinct, albeit irregular, swellings over its thallus surface.
2. *Sticta fuliginosa*, as circumscribed here, is not included in the first phylogeny of the genus provided by Moncada et al. (2014a) focusing on species from Colombia; indeed, all accessions represent *S. fuliginoides* sp. nov. (see under that species). Therefore and quite unfortunately the so-called *S. fuliginosa* clade does not include this species. *S. fuliginosa* is resolved into the *S. humboldtii* clade, together with the eponym species, *S. limbata* and other undescribed species.
3. Only two haplotypes (based on ITS) can be recognized in the many collections available to us from continental Europe, Macaronesia and Africa and they show a clear distribution partition (Fig. 2): haplotype (1) was detected in material from France/Brittany, U.K./Devon and Somerset, Ireland, the Canary Islands and the Azores; haplotype (2) differs from the former by a single substitution in ITS2, and is not sympatric with haplotype (1) as it has been detected in USA/North Carolina (JX064536, as *S. sylvatica*: Hodkinson et al. 2014), Rwanda, South Africa, Madagascar, Reunion, Japan (AB239345: Takahashi et al. 2006), and New Zealand (AF350310: Thomas et al. 2002).

Quite interestingly haplotype (2) differs from the main haplotype of the sorediate taxon *S. limbata* by a single substitution in ITS1, and moreover a single accession of *S. limbata* from Western North America (AY173391: McDonald et al. 2003) has the same haplotype as (2) of

S. fuliginosa. Although we could not locate this specimen at MIN, we have no reason to believe that the identification was wrong: McDonald et al. (2003) would not have named this specimen *S. limbata* if soredia were absent and isidia present. It is the only exception to the use of the ITS barcode for the distinction of these two widely used epithets for two species of *Sticta* in the northern hemisphere.

Two accessions from U.K./Wales (accessions KC011069 and KC732454, as *S. fuliginosa*: Moncada et al. 2014) are strictly identical with *S. fuliginoides* sp. nov., haplotype (1), as well as one accession from USA/North Carolina (accession AY173387, as *S. fuliginosa*: McDonald et al. 2003).

The barcode ITS sequence can thus be used to distinguish *S. fuliginosa* from *S. limbata* with one exception. The 4-loci phylogenetic tree, however, does not resolve both species (identified with the single diagnostic character of isidia versus soredia) as single entities, providing a nice example of incomplete lineage sorting for a recent divergence.

- An accession (AY124095) from China named *S. fuliginosa* (Lohtander et al. 2002) is almost identical with a collection from British Columbia (Canada) identified as *S. cfr. sylvatica* by its collector (*T. Goward*, # 2009–246) and other unnamed collections from the Canary Islands. So far, no validly published epithet has been found for this species which will be examined in another forthcoming paper. It is referred as sp. 1 in the phylogenetic tree (Fig. 1). Another accession represents a further different species as its ITS diverges significantly from *S. fuliginosa* as well as from all other species dealt with in this paper: DQ419943 from Canada/British Columbia (Comejo et al. 2009).

Selected specimens examined:

Ireland, Kerry, S of Killarney, N 52°01'05" W 09°30'15", 30 m, Feb. 2014, *Quercus* forest, E. Sérusiaux s.n. (LG). **France**, Brittany, Trégarvan, N 48°15'08" W 04°13'24", 20 m, Nov. 2013, mixed swamp forest, B. Bouffinier s.n. (LG, herb. Bouffinier). **Madagascar**, Angavokely Forest Station, S 18°55'38" E 47°44'15", 1770–1780 m, Oct. 2008, degraded ericaceous shrub, 10.2008, E. Sérusiaux s.n.; *ibid.*, 10.2014, E. Sérusiaux s.n. (LG). **Portugal**, Azores, Pico Is., 2011, P. Divakar s.n. (LG). **Reunion**, Bebour forest, 21°7'41" S 55°34'55"E, 1370–1380 m, mixed montane forest, Nov. 2009, N. Magain & E. Sérusiaux s.n. (LG). **Rwanda**, Nyungwe National Park, Rwasekoko, S 02°31'29" E 029°20'26", 2300–2350 m, Sept. 2010, *Erica* thickets, E. Sérusiaux s.n. (LG). **South Africa**, Western Cape Province, S 33°59.14' 20°49.39' E, 447 m, Oct. 2010, afro-montane forest, B. Goffinet 10242 (UCONN). **Spain**, Canary Is., Gomera, El Bailadero, N 28°06'28" W 17°12'50", 1090 m, May 2012, Fayal-Brezal tickets, E. Sérusiaux s.n. (LG). **United**

Kingdom, Devon, Sampford Spiney, N 50°32'3", W 4°3'37", on mossy stone walls, Jan. 2014, N. Magain s.n. (LG)

Sticta limbata (Sm.) Ach. Meth. Lich.: 280, 1803. (Fig. 9)

Lichen limbatus Sm. & Sowerby, in Smith J. E. and Sowerby J., English botany 16: 1104, 1803.

Mycobank # 406275

Type: **United Kingdom**, Wales, Haford, Cardiganshire, J. E. Smith (BM—lectotype !; designated by Galloway D., Lichenologist 26: 259, 1994).

Thallus usually developing as a bunch of several palmate, elongate or flabellate lobes, without any distinct stipe, eventually developing typically (sub-)orbicular and overlapping lobes, c. 1–3 cm in diam., rarely larger, even in suitable conditions, fragile and papyraceous, rather brittle when dry. *Lobes* with 0–1(–2) branching, adnate or ascending, especially when well-developed, overlapping each other, branching polytomous or irregular, most usually (sub-) orbicular, rarely irregular to ligulate, apices slightly undulating, plane or usually slightly revolute, margin entire to crenate and sinuose, and entirely covered with soralia forming a somewhat swollen edge. *Upper surface* smooth, plane or undulating, pale to dark brown or greyish in wet or dry conditions, faintly maculate when greyish, matte or slightly glossy, cilia absent. *Soralia* always present, erupting from the medulla just underneath the upper cortex, on the upper surface, mostly towards the margins or on the lobe margins, typically first forming a star-like structure with its center slightly convex and made of immature soredia, bordered by small, pale to whitish triangular lobes, made of the dissected and disrupted upper cortex; those star-like soralia eventually developing in large, coalescent masses, invading the thallus surface and especially the margins along most of their lengths; scars of the upper cortex mostly persistent at the edges of the extensive soralia, or within them; soredia rather coarse to farinose, typically bluish grey, rarely brownish. *Lower surface* costillate to scrobiculate, white or pale orange to brownish, rarely darker towards the center on old thalli, with sparse to dense and developed throughout primary tomentum, absent in some thalli; primary tomentum pubescent to hirsute, especially towards the center, whitish, rarely dark brown towards the center. *Cyphellae* abundant, 20–40 per cm², but slightly more abundant towards the margins, round or angular, cupuliform to urceolate with a wide pore, prominent to suprasessile, margin erect, slightly revolute, whitish to cream-colored, or brownish, 0.3–1.0 mm in diam. *Apothecia* and *pycnidia* never produced.

Upper cortex paraplectenchymatous, 30–40 μm thick, not differentiated in two layers, although the upper cells are usually smaller and more appanate than the others, 5–12 μm in diam. and with a c. 2 μm thick wall. *Photobiont layer* 35–50 μm thick with compact masses of *Nostoc* encompassed by hyphae forming rounded to angular cells. *Medulla* white, lax to compact, 65–75 μm high. *Lower cortex* paraplectenchymatous, 45–50 μm thick, made of 3–4 rows

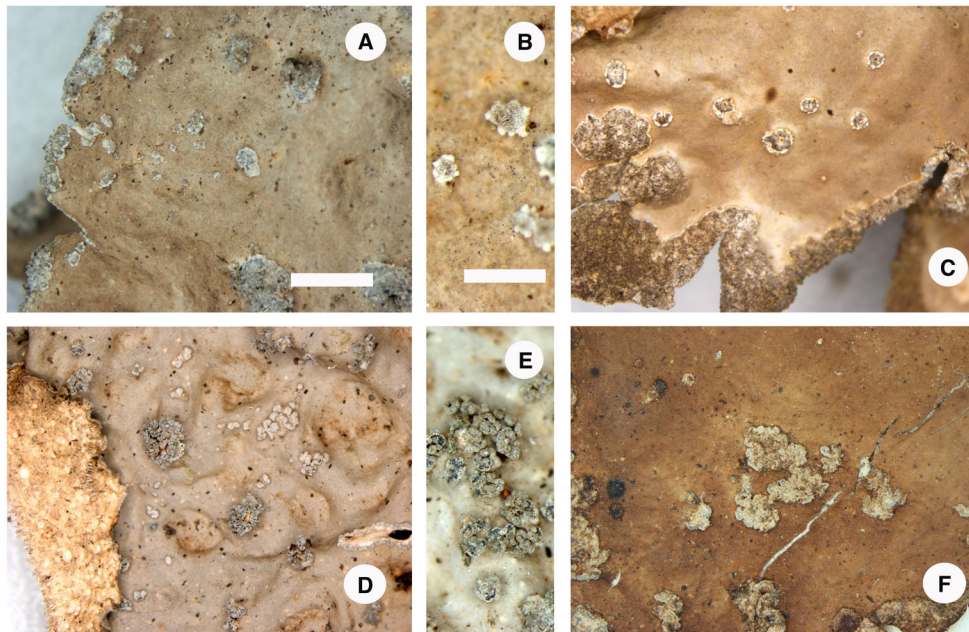


Fig. 9 *Sticta limbata* (Sm.) Ach. and *Stictia umbilicariiformis* Hochst. ex Flot.: comparison of soralia production. **a–c** *S. limbata* with typical soralia emerging from under the cortex and with triangular remnants of that roof on their margins. **d–f** *Stictia umbilicariiformis* with typical dactyls emerging from laminal bulges, and developing broken pustules,

never producing genuine soralia. **f** Upper surface of the type material with flattened and compacted pustules. **a, c** Gomera, Sep. 2011, *P. van den Boom* 46085, LG, **c** Ireland/Kerry, 02.2014, *E. Sérusiaux* s.n., LG, **d, e** Rwanda, Oct. 2009, *E. Sérusiaux* s.n., LG, **f** Ethiopia, type collection. Scale bars: **a, c, d, f** 2 mm (scale in a), **b, e** 1 mm (scale in b)

of isodiametric cells, 8–14 μm in diam. and with a 2–2.5 μm thick wall. *Lower primary tomentum* agglutinated in fascicles, with more than 20 unbranched, cylindrical, usually intertwined hyphae, with cells 15–22 \times 5–6 μm and with a c. 1.5 μm thick wall; no budding cells seen. *Lower secondary tomentum* not seen. *Cyphellae membrane* with outer cells never producing papillae.

No substances detected by TLC.

Nomenclature and typification: the lectotype has been designated by Galloway (1994: 259).

Ecology: on mossy trees and rocks within forests or at their edges, incl. parkland conditions, probably less demanding than other species of the genus as the species can be seen in urban parks in the Western Pyrenees (France and Spain); in Macaronesia, on trees and mossy rocks within the laurisilva and in *Erica-Myrica* stands (*Fayal-Brezal*), especially in the most humid localities.

Distribution: *Sticta limbata* has been reported from many parts of the world; besides Europe, Macaronesia and North America (Galloway and Thomas 2004), it is mentioned in South America (Galloway 1994), East Africa (Swinscow and Krog 1988), Australia (Galloway 2001) and New Zealand (Galloway 2007). Considering the ITS sequences currently available on GenBank and those available to us, and the confusion with *S. umbilicariiformis* (see below), we consider the species to be correctly identified only from continental Europe, Macaronesia, and North America (material examined only from the West Coast).

Notes:

1. In a European context, *S. limbata* is easily distinguished as it is the only sorediate species. In a wider context, it has been confused with *S. umbilicariiformis* Hochst. ex Flotow, a species described from Ethiopia, and whose nomenclature and typification are detailed below. *S. umbilicariiformis* is easily distinguished from *S. limbata* by its mainly laminal pustules, erupting from irregularly rounded swellings to form dactyls (sensu Krog and Swinscow 1979) or complex hollow isidioid structures, never forming genuine soredia, and its large (up to 12–15 cm in diam.), foveolate thallus. The type collection has been examined and matches recent and luxuriant collections recently gathered in the Volcanoes National Park in Rwanda (the so-called Virunga range). The 4-loci phylogenetical tree produced for this study (Fig. 2) clearly demonstrates that two species are involved. Further *S. umbilicariiformis* can produce apothecia on thalli with soralia (incl. the type collection). Non-sorediate specimens have been sampled and cannot be resolved as a different entity, either with 4-loci phylogenetical inferences nor with ITS haplotype network construction (data not shown).

Sticta umbilicariiformis Hochst. ex Flot. [as "*umbilicariaeiformis*"], in Flotow J. von, *Linnaea* 4: 16, 1843.

= *Stictina limbata* var. *umbilicariiformis* (Hochst. ex Flot.) Nyl., *Synop. Lich.* 1: 347, 1860.

= *Sticta limbata* var. *umbilicariiformis* (Hochst. ex Flot.) Zahlbr. Cat. Lich. Univ. 3: 392, 1925.

Type: **Ethiopia**, “In truncis Ericarum lateris borealis montis alpini Silke” (H-Nyl. s.n. !; material here selected as the lectotype).

Typical material of *S. umbilicariiformis* is currently known only from the Albertine Rift in East Africa, but the species is likely to be present elsewhere in East Africa. Indeed, Swinscow and Krog (1988) failed to distinguish it from *S. limbata*, which is assumed to be “widespread in cooler parts of the tropics and in temperate regions”. Earlier workers, however, did use the epithet *umbilicariiformis*, either as a variety (Zahlbruckner and Hauman 1936) or as a species (Frey 1967) to name this species in the Ruwenzori range and in the Virunga volcanoes (both montane ranges included in the Albertine Rift). The description of *S. limbata* from Chile (Galloway 1994) points to soredia in erose, marginal soralia, or in scattered, rounded, pustular laminal soralia usually close to lobes margins, coarsely granular [...], a description that would better fit *S. umbilicariiformis* than *S. limbata*; the same remark applies to the description of *S. limbata* in the Sonoran Desert Region (North America: Galloway and Thomas 2004), Australia (Galloway 2001) and New Zealand (Galloway 2007). *S. umbilicariiformis* might thus be widespread in both hemispheres.

2. Within the first phylogeny of the genus provided by Moncada et al. (2014a) focusing on species from Colombia, *S. limbata* is resolved within the *S. humboldtii* clade, comprising the eponym species and *S. fuliginosa* together with several undescribed ones.
3. As detailed in the section on *S. fuliginosa*, this species and *S. limbata* are very closely related and the ITS of the latter differs from the non-European accessions of *S. fuliginosa* by a single substitution in ITS1. There is, however, one exception as an accession of *S. limbata* from the Pacific Northwest (AY173391: McDonald et al. 2003) has the ITS of the non-European material of *S. fuliginosa*. One accession of *S. limbata* from USA/California (accession JN857309, Fedrowtiz et al. 2012) and two accessions of the same species that we could produce from material from Canada/British Columbia all have the same ITS as our accessions from continental Europe and Macaronesia. Indeed, all ITS sequences of *S. limbata* have strictly the same ITS, except for one substitution in ITS1 for an accession from the Pacific Northwest (in an otherwise invariable region) and one indel in ITS2 (also in an otherwise invariable region) for an accession from Tenerife (Fig. 3).

Selected specimens examined for *S. limbata*: **Canada**, British Columbia, 2009, T. Goward 09–246 (LG, hb.

Goward). **France**, Brittany, Brest, Nov. 2013, A. Gérault s.n. (LG, hb. A. Gérault). **Ireland**, Kerry, S. of Killarney, N 52°01'05" W 09°30'15", 30 m, Feb. 2014, *Quercus* forest, E. Sérusiaux s.n. (LG). **Portugal**, Azores, Pico Is., 2011, P. Divakar s.n. (LG). Ibid., Lagoa do Paúl, N 38°26'10.69" W 28°12'28.07", 950 m, July 2014, natural thickets dominated by *Juniperus brevifolia*, E. Sérusiaux s.n. (LG). **Spain**: Canary Is., Gomera, Garajonay National Park, N 28°07.39' W 17°12.65', 1050 m, Sept. 2011, degraded laurisilva, P. van den Boom 46085 (hb. van den Boom). Ibid., Gran Canaria, NE of Artenara, N 28°01'22" W 15°37'25", 1400 m, May 2011, *Pinus canariensis* open woodland, E. Sérusiaux s.n. (LG). **United Kingdom**, Scotland, Argyll, N 56°14'44" W 05°38'19", 35 m, 07. 2011, *Corylus* thicket, B.J. Coppins s.n. (LG).

Selected specimen examined for *S. umbilicariiformis*: **Rwanda**, Volcanoes National Park, Sabinyo, S 01° 24.01' E 029° 35.15', 2880–2900 m, Oct. 2009, dense thickets, E. Sérusiaux s.n. (LG).

Sticta sylvatica (Huds.) Ach. Methodus Lichenum, p. 281, 1803. (Fig. 10)

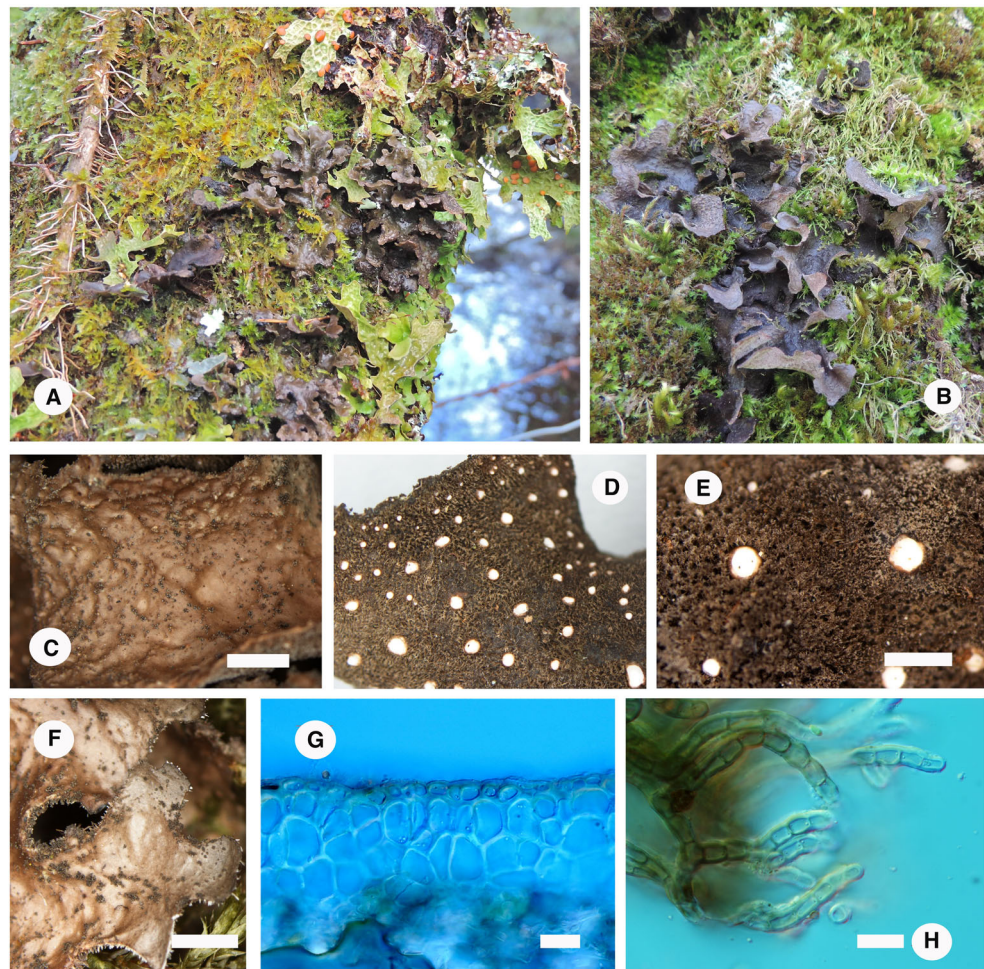
Mycobank # 406401

Bas.: *Lichen sylvaticus* Hudson, Flora Anglica, vol. 2: 721, 1778.

Type: Icon. In Dillenius, Historia Muscorum, tabl. 27, Fig. 101, 1742 (lectotype, designated by Jørgensen and Tønsberg, Nordic Lichen Flora 3: 145, 2007); corresponding specimen in herb. Dillenius (OXF !, epitype, designated by Jørgensen and Tønsberg, Nordic Lichen Flora 3: 145, 2007).

Thallus usually rounded or fan-shaped in outline, rarely irregular, 1–5 cm in diam. but able to form larger thalli or colonies of thalli in suitable conditions, up to 10–20 cm across, with several thalli intermingled and overlapping each other, subcoriaceous, but brittle when dry, branching mostly dichotomous with 1–3(–5) ramifications per main lobe; stipe absent. *Lobes* slightly ascending and dichotomously branched (more rarely polytomous or irregular), typically involute and thus forming slightly concave fan-shaped forms, 0.4–0.8 cm wide, lacinate or slightly flabellate, overlapping, ascending when dry, especially for young lobes, with rounded to irregular apices, margins slightly swollen or not, rarely dissected or irregularly lacerate. *Upper surface* usually dark brown when wet, paler and sometimes bluish grey when dry, usually glossy, typically foveolate or scrobiculate, with shallowly reticulated ridges, but not maculate. *Cilia* usually absent, but frequently detected between the main lobes, where they are scattered and mimic primary tomentum, simple, white to pale, less than 0.5 mm long. *Isidia* always present, developing mainly on lobes margins or on ridges of the upper surface, sometimes covering large parts of the thallus, globulose and developing coralloid masses, up to 0.5 mm high and in diam., formed of an accumulation of globose or irregular inflated entities, very brittle and easily detached from the thallus, dark

Fig. 10 *Sticta sylvatica* (Huds.) Ach. **a** Thalli on leaning *Quercus* (Ireland/Kerry, Feb. 2014, photograph taken in the field). **b** Well-developed and ascending thalli on *Fagus* (France/Vosges, June 2014, photograph taken in the field). **c, f** Upper glossy surface, typically foveolate (**c**) and with scattered marginal cilia (**f**). **d, e** Dark (almost black) lower surface with conspicuous cyphellae and abundant primary tomentum. **g** Section through the upper paraplectenchymatous cortex, with upper layer made of much smaller cells. **h** Primary tomentum. **c–h** France/Brittany, Nov. 2013, A. Gérault s.n. (LG). Scale bars: **a, d** 2 mm (scale in **c**), **e** 0.5 mm, **f** 1 mm, **h** 10 μ m



brown to almost black. *Lower surface* uneven or slightly foveolate, brown to dark brown, to almost black; primary tomentum sometimes absent on the edges of lobes but most usually abundant throughout and dark brown to almost black, forming a spongy mat when well-developed. *Cyphellae* always present on the lower surface, dispersed, sometimes more or less aggregated, rounded or slightly irregular, 20–40 per cm^2 or >40 per cm^2 near the lobe edges, 0.8–1.1 mm in diam., erumpent and slightly involute, outer wall sometimes brown or dark brown, and pore 0.5–0.7 mm in diam. *Apothecia* and *pycnidia* never produced.

Upper cortex paraplectenchymatous, 30–40 μm high, made of 3–4 layers of rounded to isodiametric cells, c. 10–15 μm in diam. with a 1.5–2 μm thick wall; outer layer distinct, made of a single row of smaller cells (c. 5–8 μm in diam.) with thicker walls (c. 3 μm). *Photobiont layer* c. 20–25 μm thick, made of *Nostoc* colonies, forming a continuous layer under the upper cortex, sometimes quite thin and discontinuous, not or very rarely agglomerated in compact, ovoid masses, tightly encompassed by hyphae forming rounded to angular cells and wrapping the *Nostoc* cells. *Medulla* white and lax, 50–60 μm thick. *Lower cortex* paraplectenchymatous, 20–30 μm high,

with 2–4 layers of isodiametric cells 8–15 μm in diam., with 2 μm thick walls. *Lower primary tomentum* formed of bunches of cylindrical hyphae, 0.1–0.4 mm long, agglutinated to each other except at their tips and typically intertwined, made of elongated cells with thick and dark brown walls (lumina: 6–15 \times 4–6 μm ; walls: 1.5–2 μm thick); cells sometimes slightly inflated, especially near the branching nodes; apical cells distinctly shorter (c. 5–7 μm long), slightly moniliform and probably able to be dispersed as single entities.

Cyphellae with basal membrane white, made of 1–2 layers of rounded or rarely irregular cells, 9–14 μm in diam., sometimes budding and producing papillae c. 4 μm in diam.

No substances detected by TLC.

Nomenclature and typification: the epitype specimen was designated by Jørgensen & Tønsberg (Nordic Lichen Flora 3: 146, 2007) and can be examined online at the Oxford Herbarium web site (http://herbaria.plants.ox.ac.uk/bol/MUSCORUM/image/HM-sheet_073.jpg/Zoom?width=760px&height=760px). This specimen has been examined (Fig. 3) and corresponds to the populations from Western Europe assigned to this epithet in the British Isles (Smith et al. 2009), Germany (Wirth et al. 2013) and Norway and

Sweden (Jørgensen and Tønsberg 2007). Further, we could produce ITS sequences for the four sampled localities: France/Brittany and Vosges, U.K./Somerset and Ireland/Kerry. They are all strictly identical: we thus consider that they represent *S. sylvatica*.

Ecology: on mossy trees within forests or at their edges, incl. parkland conditions in oceanic climate, found in the montane zone (*Fagus sylvatica* and *Abies alba* mixed forest) at c. 1000 m elev. down to *Quercus*-dominated forest at sea level.

Distribution: *Sticta sylvatica* has been mentioned from all continents except Australia: Europe (Austria: Hafellner and Türk 2001; British Isles: Smith et al. 2009; Germany: Wirth et al. 2013; Norway and Sweden: Jørgensen and Tønsberg 2007; Portugal and Spain: Burgaz and Martínez 2003; Macaronesia: Canary Is. and Madeira: Hafellner 1995 and Azores: <http://www.azoresbiportal.angra.uac.pt>, accessed on Feb. 11th, 2015), NY, USA (Harris 2004) and Sonoran region (Galloway and Thomas 2004), South Africa (Doidge 1950), Micronesia in the Pacific Ocean (Elix and McCarthy 1998), Taiwan (Aptroot et al. 2002), and Peru (Soukup 1965). In their account for the Sonora region, Galloway and Thomas (2004) further added Greenland and the Mediterranean region. Considering the ITS sequences currently published (Moncada et al. 2014a) and those available to us, we consider the species to be correctly identified only from continental Europe and the Andes in Colombia. The large and recent collections available to us from Macaronesia and Africa (incl. Madagascar, Mauritius and Reunion) did not yield any specimen referable to *S. sylvatica*. We consider that data from Macaronesia either refers to *S. fuliginosa* or *S. atlantica*.

Notes

1. *Sticta sylvatica* is easily distinguished by its dichotomously branched and involute lobes, with a dark brown upper surface which is typically glossy and foveolate to scrobiculate, and coralloid dark isidia developing mainly at the lobes margins and on ridges on the upper surface. *S. atlantica* might be confused with it and mainly differs by its isidia always developing on swellings on the upper surface, or along small ridges; further *S. sylvatica* has a brown to black lower surface usually densely covered by a primary tomentum forming a spongy mat when well-developed, while *S. atlantica* has a primary tomentum made of distinct fasciculate to penicillate hairs, easily distinguished from one another in young lobes, eventually forming spongy masses but never dark brown to black.
2. Within the first phylogeny of the genus provided by Moncada et al. (2014a) focusing on species from Colombia, *S. sylvatica* is resolved within the *S. kunthii* clade, the most derived group in that phylogeny. More data are, however, needed to assess its relationships within that clade.

3. All ITS sequences from the four localities sampled are strictly identical. Nearly identical ITS sequences were retrieved from GenBank for three collections from Colombia (Moncada et al. 2014a). One accession (KC732581) is identical with our material from Western Europe, except for one indel in ITS2; accession KC732518 differs by five substitutions and one indel in ITS1 and three substitutions in ITS2 and may thus represent a distinct species; the third accession (KC732574) is incomplete and cannot be properly assessed.

One ITS accession from NC, USA (JX064536) referred to *S. sylvatica* by its authors (Hodkinson et al. 2014) is strictly identical with *Sticta fuliginosa* as circumscribed in this paper. A collection from British Columbia (Canada) identified as *S. cfr. sylvatica* by its collector (*T. Goward*, # 2009–246) is almost identical to an accession (AY124095) from China (Lohtander et al. 2002, sub *S. fuliginosa*) and other collections from the Canary Islands (Gomera and Gran Canaria). These sequences are very different and are referred to sp. 1 in our phylogenetic tree. This taxon is unnamed and is not related to *S. sylvatica*. Therefore, and with the data currently available to us, *S. sylvatica* does not occur in North America.

Selected specimens examined: **Ireland**, Kerry, S of Killarney, N 52°01'05" W 09°30'15", 30 m, 2.2014, *Quercus* forest, E. Sérusiaux s.n. (LG). **France**, Brittany, Cahos de St-Herbot, N 48°20'02" W 03°48'06", 160 m, mixed forest, 11. 2013, A. Gèrault s.n. (LG, herb. Gèrault). Ibid., Haut-Rhin, Hohneck, N 48°02' E 07°01', c. 1000 m, June 2014, open mixed forest, E. Sérusiaux s.n. (LG). **United Kingdom**, Somerset, Horner woods, N 51°10' W 03°35', c. 290 m, Feb. 2014, P. Wolseley s.n. (LG).

Identification of species of *Sticta* in continental Europe

ITS barcode

ITS has been chosen as the Barcode for Fungi (Schoch et al. 2012) and is now widely used as such, although several studies have highlighted the low resolution of this loci in several groups (see for example Magain et al. 2012b for *Peltigera* sect. *Polydactylon* and Carlson et al. 2014 for *Trametes*) or the challenge to reconcile morphological and chemical results with ITS data (see for example Mark et al. 2012 for *Vulpicida* and Pino-Bodas et al. 2011, 2012a, b, 2013a, b for *Cladonia*).

Within the genus *Sticta*, ITS provides an appropriate mean to detect and delimitate species (Moncada et al. 2014a). In the geographical framework of this work (continental Europe), ITS sequences alone provide a quick answer to the task of delimitating species, with one exception detected in the duo

formed by *S. fuliginosa* and *S. limbata*, two species that cannot be resolved as single entities with 4-loci inferences in a phylogenetic framework.

We identify a 20 bp segment in ITS1 that provides a shorter and accurate way for the recognition of all species of the emblematic and cherished lichen genus *Sticta* in Europe. Sleepless nights? This title by Tripp and Lendemer (2014) was a quite enigmatic and provocative one to propose guidelines for the description of new species on the basis of molecules only. At least so far, it is not the case with the flagship genus *Sticta*, but other genera in the Peltigerales do present this challenge (Magain et al. 2012b; Miadlikowska et al. 2014b).

Autapomorphic positions (one to three) are underlined for each species (except for *S. canariensis* which has no such position in this fragment):

<i>Sticta atlantica</i>	CGGGGGG CTTCGGCTCC <u>CTTC</u>
<i>Sticta canariensis</i>	CGGGGG CTTCGGCTCC <u>TTC</u>
<i>Sticta ciliata</i>	CGGG AG CCTCGGCTCCTGTC
<i>Sticta fuliginoides</i>	CGGGGG CTTCGGCCCTGTC
<i>Sticta fuliginosa</i>	CG AGGGGGCTCCGGCTCC <u>TTC</u>
<i>Sticta limbata</i>	CG AGGGGGCTTCGGCTCC <u>TTC</u>
<i>Sticta sylvatica</i>	CGGGGG CTTCGGCCCCGCAC

Tentative key to species of *Sticta* in continental Europe when lichenized with *Nostoc*

This tentative key is based on morphological and anatomical data obtained from recently collected specimens for which DNA could be extracted and the ITS barcode amplified. It is tentative because it does not include the variation observed in herbarium material, especially depauperate specimens coming from non-optimal environmental conditions. That variation is large and confusing. Further blind tests to identify small fragments of specimens for which ITS is known to have failed in several cases, especially for the recognition of *S. ciliata* and for the distinction of the duo *S. fuliginoides*/*S. fuliginosa*. As it is impossible to extract DNA from collections of *Sticta* older than 2 years, the whole phenotype variation could not be confronted with ITS data. Examination of anatomical characters, especially the papillae on the cyphella membrane, in old herbarium specimens is difficult and may require careful preparation with Phloxin (1 % in water) after pre-treatment in KOH (5 % in water); absence of such papillae must be carefully evaluated. We are, however, convinced that further studies of the genus throughout Europe will bring in more morphological and anatomical information that should allow much easier identification of the recognized species in the

field and herbarium material. By all means, for living populations, collecting a single lobe and producing its ITS barcode is the easiest and most reliable way of identification.

1 Thallus always producing soralia, mainly at lobes margins *S. limbata*

(*S. limbata* is the only species with soralia in continental Europe; otherwise it has rather large, suborbicular lobes with plane and undulating margins)

1* Thallus never with soralia, always with isidia or phyllidia

2 Thallus with upper surface strongly maculate; margins always and upper surface usually with typical phyllidia *S. canariensis* (cyanomorph)

(The cyanomorph of *S. canariensis* is easily characterized by the combination of strongly maculate upper surface and production of phyllidia)

2* Thallus with upper surface never strongly maculate; isidia most usually coralloid, never developing into typical phyllidia

3 Papillae on cyphella membrane

4 Thallus rounded, monophyllous (and then typically mushroom-like) or with several rounded lobes, hardly dissected, dark brown, rarely pale greyish; margin typically involute, rarely sparsely ciliate on regenerating lobules; thallus rather robust; papillae on cyphella membrane few per cell; apothecia unknown *S. fuliginoides*

(*S. fuliginoides* almost always have an involute margin and mushroom-like habitus when young)

4* Thallus rounded to palmate, with lobes rounded (when young) to truncate (when old), dark dull brownish, lead grey or pale greyish; margin usually not involute, most usually ciliate, especially when young; thallus very fragile and easily broken when dry; papillae on cyphella membrane numerous per cell, easily seen in fresh material; apothecia present in well-developed specimens, typically ciliate *S. ciliata*

(*S. ciliata* is easily recognized by its fragile and irregular thallus with marginal cilia; it is the only fertile species amongst all cyanomorph species of *Sticta* in Europe)

3* Papillae absent on cyphella membrane

5 Thallus distinctly branched, almost always dichotomously, typically glossy; lobes involute, shallowing and usually with ascending margins; lower surface usually dark, especially towards the center, strongly contrasting with the white cyphellae *S. sylvatica*

(*S. sylvatica* typically have dichotomously branched thallus, with involute lobes and a black lower surface)

5* Thallus not distinctly branched, although lobes can be lacerate or dissected, not typically glossy; lobes rounded, not shallowing; lower surface usually pale, or brownish, not strongly contrasting with the white cyphellae

6 Lobes surface with distinct swellings, irregular in shape but always present, with isidia developing on their upper parts *S. atlantica*

(*S. atlantica* has small and irregular swellings on its upper surface and isidia develop on them)

6* Lobes surface with no or irregular swellings, but rather typically reticulate or scrobiculate, and isidia developing on the edges of these dimples *S. fuliginosa*

(*S. fuliginosa* has no straightforward diagnostic character and can be recognized because it does not have the diagnostic ones of others: no cilia, no mushroom-like appearance, no swellings on upper surface, no dichotomously branched thallus and no papillae on cells of the cyphella membrane)

Conclusions

With a dedicated sampling in four localities in continental Europe and assembling relevant data from collections available to us, especially from Macaronesia and Africa, we could detect seven species of *Sticta* in continental Europe, almost doubling the α -diversity of the genus for that region. Indeed, besides the very distinct *S. canariensis* and *S. limbata*, we could detect five distinct ITS entities for isidia-producing specimens that would be identified as only two species in all modern floras, namely *S. fuliginosa* and *S. sylvatica* (Lambinon 1969; Llimona and Hladun 2001; Burgaz and Martínez 2003; Jørgensen and Tønsberg 2007; Smith et al. 2009; Wirth et al. 2013). Thanks to relevant tables of morphological and anatomical characters provided by Moncada et al. (2014a), subtle anatomical and morphological characters came to light and, together with a strongly supported 4-loci molecular phylogeny, permit to distinguish: *S. atlantica* sp. nov., *S. ciliata* Tayl., *S. fuliginosa*, *S. fuliginoides* sp. nov. and *S. sylvatica*.

Most probably, such a drastic shift into the molecular era for a lichen genus that has been so much used in biomonitoring studies and biodiversity conservation policy is not a unique case. Indeed, several studies have already demonstrated how poorly supported is the taxonomy of many emblematic taxa when they are challenged by detailed molecular studies. Recent studies show either that the currently accepted species are not recovered as monophyletic and traditional diagnostic morphological characters are highly homoplasious (Leavitt et al. 2015), or that many independent lineages can be distinguished in a single species complex for which no morphological and chemical characters can be recognized. The best examples for the latter case are to be found in *Cladonia* (Pino-Bodas et al. 2011, 2012a, b, 2013a, b), and examples of the former can be found in the *Vulpicida juniperus* group (Mark et al. 2012), the *Peltigera polydactylon* clade (Magain et al. 2012b), or the *Tephromela atra* group (Muggia et al. 2014). By all means, the most emblematic case is the basiolichen *Cora pavonia*, very much characteristic of montane forests and paramos in Central and South America, which has been shown on molecular basis to contain at

least 126 species, over 110 of them being yet undescribed (Lücking et al. 2014).

We could demonstrate that the rationale of the taxonomy of *Sticta* in Europe, settled almost two centuries ago by Delise (1826) and for the most part still in use, could not resist a modern analysis. Now, as for several other lichen genera, species of *Sticta* are widely used as valuable bioindicators for environment and climate monitoring and for forests management and protection. It is unlikely that all seven species have a strictly identical ecology and their identification should improve the use and interpretation of ecological data, especially at fine scales.

Further, beyond the new taxonomy that our study could propose for a well-known and cherished genus, interesting evolutionary patterns came to light:

- (1) the two and most easiest species to distinguish one from another (*S. fuliginosa* vs *S. limbata*) also are the phylogenetically closest in our dataset: their intraspecific variation is very low and their ITS barcode differs only by one substitution. Further one accession of *S. limbata* appears to have the ITS of *S. fuliginosa*. Moreover, the 4-loci phylogenetic analysis could not resolve both species as single entities. Incomplete lineage sorting may easily explain this pattern for this species duo that must have diverged recently.
- (2) On the other hand, the largest variation is observed within the resurrected *S. ciliata*, that forms a complex of most probably cryptic species occurring in the Neotropics, Hawaii archipelago, Africa, Macaronesia and Western Europe.

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