Plant and Fungal Systematics 70(2): 75–84, 2025
DOI: https://doi.org/10.35535/pfsyst-2025-0008

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

First European record of *Sticta arenosella* and new Central European records of *Sticta fuliginoides*

Julia Bernet[1]*, Christian Vonarburg[2] & Mónica A. G. Otálora[1]

Article info

Received: 2 Aug. 2025
Revision received: 1 Oct. 2025
Accepted: 20 Oct. 2025
Published: 9 Dec. 2025

Associate Editor

Martin Kukwa

Abstract. The genus *Sticta* has recently undergone significant taxonomic revisions in Europe, yet central regions such as Switzerland were not included in those revisions. To assess the diversity of the species complex *Sticta fuliginosa* in Switzerland, we used an integrative taxonomic approach combining morphological and genetic analyses. We sampled both the well-known localities, as well as newly discovered localities along the Doubs River. Our results showed that *S. fuliginosa* is presently absent from Switzerland. Instead, we confirmed the presence of *S. fuliginoides*, and reported the first occurrence of *S. arenosella*, a species previously known only from North and South America. Morphological, phylogenetic, and haplotype network analyses confirmed that the Swiss specimen identified as *S. arenosella* is morphologically and genetically indistinguishable from its holotype. Additionally, we also documented the presence of apothecia on *S. fuliginoides* for the first time. This expands the known phenotypic variation in this species and allows for a more complete taxonomic description. Finally, we provide a taxonomic key for the Central European species of *Sticta* to facilitate future research and monitoring.

Key words: biogeography, haplotype network, integrative taxonomy, lichens, phylogeny, taxonomic key

Introduction

Lichens represent one of the most diverse groups of fungi, with over 19,000 described species (Lücking et al. 2021). Within this diverse group, the genus *Sticta* (Schreb.) Ach. is one of the largest genera of macro lichens, characterized by its prominent thalli, and its crateriform cyphellae (large pores) on the lower surface, which make it easy to observe and collect, even by non-specialists (Moncada et al. 2014b, 2020). The genus is sub cosmopolitan, comprising both widespread common species and rare or endemic taxa, including some with disjunct oceanic distributions (Moncada et al. 2020; Di Meglio & Goward 2023). Due to its ecological significance, *Sticta* has been a focal group for lichenologists (Galloway 1994). The species in this genus are particularly sensitive to environmental changes, making them valuable bioindicators of ecosystem health (Arsenault & Goward 2016). Almost all the species present in Europe are currently considered threatened (Scheidegger & Clerc 2002; Liška et al. 2008; Wirth et al. 2011; Nascimbene et al. 2013).

Until recently, species recognition in Europe followed the framework proposed by Delise (1825), who recognized four species: *S. canariensis* (Flörke) Delise (syn. *S. dufourii* Delise), *S. fuliginosa* (Hoffm.) Ach., *S. limbata* (Sm.) Ach., and *S. sylvatica* (Huds.) Ach. However, a phylogenetic study using an integrative taxonomic approach (Magain & Sérusiaux 2015) led to a major revision of this classification. The study demonstrated that the species *S. fuliginosa*, which was assumed to be a cosmopolitan taxon (Ekman et al. 2019; Moncada et al. 2020; Di Meglio & Goward 2023), is polyphyletic, with taxa belonging to several distinct phylogenetic lineages. Currently, *S. fuliginosa* is known as a complex that comprises four distinct species just in Europe alone: *S. fuliginosa* s.str., *S. fuliginoides* Magain & Sérusiaux, *S. ciliata* Taylor and *S. atlantica* Magain & Sérusiaux (Sanderson 2016; Ekman et al. 2019). When considering other regions of the world, there are over 12 additional species (Moncada et al. 2020; Di Meglio & Goward 2023). Within this complex, *S. fuliginoides* and *S. fuliginosa* s.str. are the most common and the only cosmopolitan species, while the remaining species appear to have more restricted distributions, sometimes even endemic to a single island (Moncada et al. 2020; Di Meglio & Goward 2023). However, a recent investigation of specimens from southern South America showed that two of the species previously considered endemic, in fact, occur on more than one continent (Ossowska et al. 2024). *Sticta arenosella* Di Meglio

[1] Institute of Integrative Biology, ETH Zürich, Universitätsstrasse 16, Zürich 8092, Switzerland
[2] Else-Züblin-Strasse 101, Winterthur 8404, Switzerland
* Corresponding author e-mail: julia.m.bernet@gmail.com

& Goward, once considered restricted to North America, also occurs in southern Chile (South America). Similarly, *S. cellulosa* Kaasal., which was described to occur only in Africa, has also been recorded in Bolivia (South America) (Ossowska et al. 2024). Such findings highlight the need for broader geographic sampling to gain a more accurate understanding of the distributional range and taxonomic diversity within the species complex.

Regional European studies of the *S. fuliginosa* species complex in Britain (Sanderson 2016) and Scandinavia (Ekman et al. 2019) confirmed the presence of three species in both regions: *S. fuliginosa* s.str., *S. fuliginoides*, and *S. ciliata*. These studies also demonstrated the absence of the hyperoceanic species *S. atlantica*, with records limited to northwestern Ireland and the Azores (Magain & Sérusiaux 2015). Although *S. fuliginoides* appears to be the most widely distributed species in Europe, occurring in both continental and coastal areas (Ekman et al. 2019), its distribution has so far only been studied in Western Europe: England, France, Ireland, Wales, Scotland, Sweden, and Norway (Magain & Sérusiaux 2015; Sanderson 2016; Ekman et al. 2019). However, large parts of Europe, particularly central and southern regions, remain uninvestigated, leaving significant gaps in our understanding of the species' actual range.

To expand our knowledge of the *S. fuliginosa* species complex in Central Europe, we studied current localities across Switzerland, covering four distinct regions: the Jura, the Northern Alps, the Western Central Alps, and the Southern Alps (Fig. 1). We assessed anatomical features of the thallus and cyphellae to differentiate species (Magain & Sérusiaux 2015; Sanderson 2016; Ekman et al. 2019). In addition, we reconstructed the phylogeny of the Swiss *S. fuliginosa* species complex using methodological approaches and genetic markers employed in previous studies on that species complex (Moncada et al. 2014b; Magain & Sérusiaux 2015; Ossowska et al. 2024), incorporating GenBank sequences representing the identified lineages. Lastly, we constructed haplotype networks to assess intraspecific genetic diversity.

Materials and methods

Sample collection

Our sampling encompassed all current Swiss localities reported since 1989 in the Webatlas of Swisslichens (Stofer et al. 2019), as well as newly documented occurrences from a 2024 field survey of the Doubs River lichen flora (Fig. 1). In this survey, small lichen fragments were collected from one individual at each site. In total, 13 specimens were included for the molecular analyses, and were deposited in the fungal collection of the Z+ZT herbaria (Table 1, Table S1). Following preliminary genetic analyses, North American specimens of *S. arenosella*, including its holotype, and *S. torii* were requested on loan from the OSU herbarium to conduct comparative morphological assessments.

Morphological analysis

Based on recent studies on the *S. fuliginosa* species complex (Moncada et al. 2013, 2014a, b, 2020; Simon et al. 2018; Ekman et al. 2019; Di Meglio & Goward 2023), particular attention was given to the study of morphological and anatomical features. These include isidia shape and position, the build of the upper cortex, the presence or absence of papillae on the surface cells of the cyphellae, as well as apothecia characteristics. Microscopic

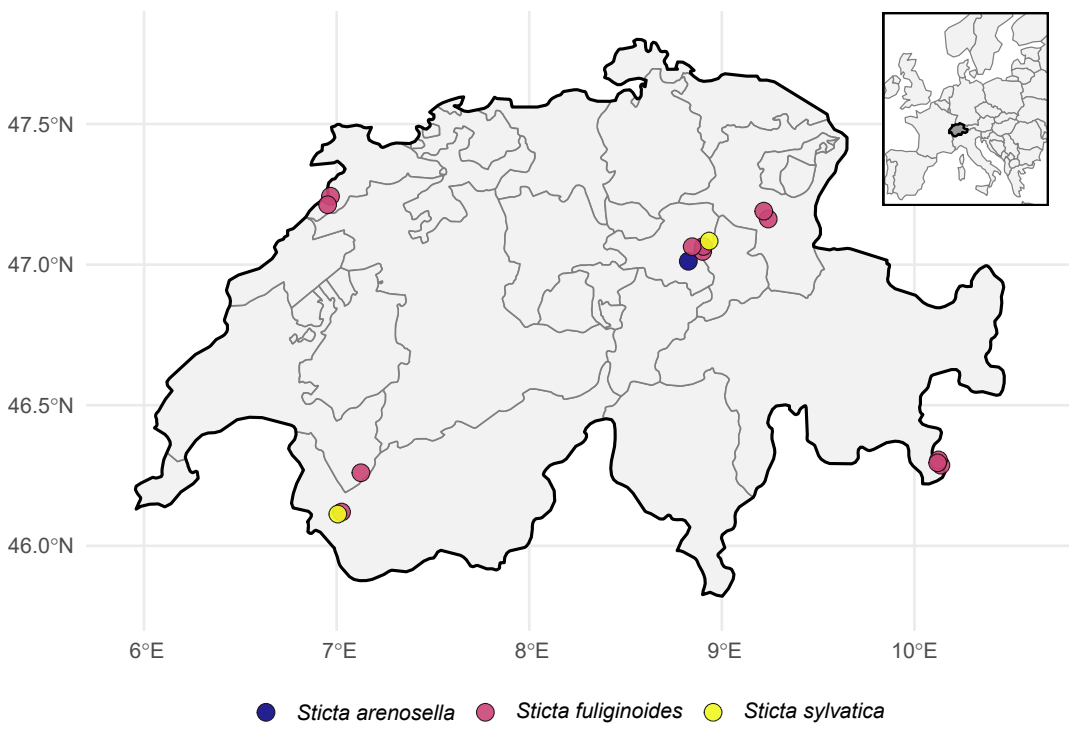

Figure 1. Geographic distribution of isidiate *Sticta* specimens collected for this study. Each point represents a unique collection site. The base map was created with Natural Earth, www.naturalearthdata.com

Table 1. List of 51 *Sticta* specimens used in the phylogenetic analysis, including voucher information and corresponding GenBank accession numbers for the ITS, LSU, and mtSSU markers. A total of 121 sequences were retrieved from GenBank. Newly generated sequences are marked in bold. These sequences were used to construct the concatenated multi-locus phylogeny presented in Figure S1.

Species	Voucher	Location	GenBank nrITS	GenBank nrLSU	GenBank mtSSU
Sticta arenosella	Di Meglio 115	USA (WA)	MH374894	OP156872	OP161480
Sticta arenosella	Di Meglio 172	USA (OR)	MT183691	–	–
Sticta arenosella	Di Meglio 173	USA (OR)	MT183692	–	–
Sticta arenosella	Di Meglio 178	USA (OR)	MT183693	–	–
Sticta arenosella	UlS 6518	Chile (MA)	PP273997	–	–
Sticta arenosella	Bjork 18443	Canada (BC)	MH374895	–	–
Sticta arenosella	Bjork 19815	Canada (BC)	MH374896	–	–
Sticta arenosella	**ChV1285**	**Switzerland (SZ)**	**PV822412**	**PV827584**	**PV827602**
Sticta atlantica	LG3747	Ireland (Kerry)	KT281734	KT281645	KT281690
Sticta atlantica	LG3858	Portugal (Azores)	KT281737	KT281648	KT281693
Sticta canariensis	LG3741	Ireland (Kerry)	KT281733	KT281644	KT281689
Sticta ciliata	LG3099	Portugal (Azores)	KT281715	KT281627	KT281671
Sticta ciliata	LG3781	Ireland (Kerry)	KT281716	KT281628	KT281672
Sticta ciliata	LG1605	Rwanda	KT281717	KT281629	KT281673
Sticta fuliginoides	LG3551	France (Brittany)	KT281729	KT281640	KT281685
Sticta fuliginoides	LG1421	France (Vosges)	KT281701	KT281613	KT281659
Sticta fuliginoides	LG3012	Spain (Canary Is.)	KT281722	KT281634	KT281678
Sticta fuliginoides	LG3733	Ireland (Kerry)	KT281732	KT281643	KT281688
Sticta fuliginoides	LGS4	UK (Devon)	KT281738	KT281649	KT281694
Sticta fuliginoides	Moncada 5352	Colombia	KC732709	–	–
Sticta fuliginoides	UK170808e	Tanzania (Kilimanjaro)	OP999494	–	–
Sticta fuliginoides	UK170844c	Tanzania (Kilimanjaro)	OP999499	–	–
Sticta fuliginoides	UK170858e	Tanzania (Kilimanjaro)	OP999505	–	–
Sticta fuliginoides	UK171438f	Tanzania (Kilimanjaro)	OP999552	–	–
Sticta fuliginoides	UK171468d	Tanzania (Kilimanjaro)	OP999558	–	–
Sticta fuliginoides	UK171485c	Tanzania (Kilimanjaro)	OP999564	–	–
Sticta fuliginoides	UK171504c	Tanzania (Kilimanjaro)	OP999577	–	–
Sticta fuliginoides	UK171577i	Tanzania (Kilimanjaro)	OP999597	–	–
Sticta fuliginoides	**ChV1392**	**Switzerland (JU)**	**PV822401**	**PV827571**	**PV827589**
Sticta fuliginoides	**ChV1388**	**Switzerland (JU)**	**PV822402**	**PV827570**	**PV827588**
Sticta fuliginoides	**MGO011024**	**Switzerland (SZ)**	**PV822403**	**PV827572**	**PV827590**
Sticta fuliginoides	**ChV1470**	**Switzerland (SG)**	**PV822404**	**PV827573**	**PV827591**
Sticta fuliginoides	**ChV1471**	**Switzerland (SZ)**	**PV822405**	**PV827574**	**PV827592**
Sticta fuliginoides	**ChV1472**	**Switzerland (SZ)**	**PV822406**	**PV827575**	**PV827593**
Sticta fuliginoides	**ChVA0001**	**Switzerland (SG)**	**PV822407**	**PV827576**	**PV827594**
Sticta fuliginoides	**MD5788**	**Switzerland (GR)**	**PV822408**	**PV827577**	**PV827595**
Sticta fuliginoides	**MD5786**	**Switzerland (GR)**	**PV822409**	**PV827578**	**PV827596**
Sticta fuliginoides	**MD5787**	**Switzerland (GR)**	**PV822410**	**PV827579**	**PV827597**
Sticta fuliginoides	**ChV1475**	**Switzerland (VS)**	**PV822411**	**PV827580**	**PV827598**
Sticta fuliginoides	**ChV1478**	**Switzerland (VD)**	–	**PV827583**	**PV827599**
Sticta fuliginosa	LG1952	South Africa	KT281703	KT281615	KT281661
Sticta fuliginosa	LG3100	Portugal (Azores)	KT281704	KT281616	KT281662
Sticta fuliginosa	LGS9	UK (Devon)	KT281739	KT281650	–
Sticta fuliginosa	LG3010	Spain (Canary Is.)	KT281721	KT281633	KT281677
Sticta fuliginosa	LG3537	France (Brittany)	KT281727	KT281638	KT281683
Sticta fuliginosa	LG795	Madagascar	KT281695	KT281609	KT281653
Sticta fuliginosa	LG989	France (Reunion)	KT281698	KT281610	KT281656
Sticta fuliginosa	LG1611	Rwanda	KT281702	KT281614	KT281660
Sticta fuliginosa	LG3729	Ireland (Kerry)	KT281731	KT281642	KT281687
Sticta limbata	LG3170	Canada	KT281710	KT281622	–
Sticta limbata	LG2230	Spain (Canary Is.)	KT281706	KT281618	KT281664
Sticta limbata	LG2690	UK (Scotland)	KT281707	KT281619	KT281665
Sticta limbata	LG2749	Spain (Canary Is.)	KT281708	KT281620	KT281666
Sticta limbata	LG3105	Portugal (Azores)	KT281709	KT281621	KT281667
Sticta limbata	LG3544	France (Brittany)	KT281728	KT281639	KT281684
Sticta limbata	LG3868	Portugal (Azores)	KT281711	KT281623	–
Sticta sylvatica	LG 3536	France (Brittany)	KT281726	KT281637	KT281682
Sticta sylvatica	LG3723	UK (Somerset)	KT281730	KT281641	KT281686

Table 1. Continued.

Species	Voucher	Location	GenBank nrITS	GenBank nrLSU	GenBank mtSSU
Sticta sylvatica	LG3780	Ireland (Kerry)	KT281735	KT281646	KT281691
Sticta sylvatica	LG3837	France (Vosges)	KT281736	KT281647	KT281692
Sticta sylvatica	**ChV1473**	**Switzerland (SZ)**	–	**PV827582**	**PV827601**
Sticta sylvatica	**ChV1476**	**Switzerland (VS)**	**PV822413**	**PV827581**	**PV827600**
Sticta torii	Goward 02-179	Canada (BC)	MH017853	–	–
Sticta torii	CONN00225971	USA (AK)	MH017854	–	–
Sticta torii	Di Meglio 146	USA (OR)	MH374892	OP156874	OP161478
Sticta torii	Di Meglio 135	USA (OR)	MH374891	OP143656	–
Sticta torii	Di Meglio 156	USA (OR)	MH374893	OP156875	OP161479
Sticta torii	Di Meglio 145	USA (OR)	MT183695	OP156873	OP161477

preparations were examined using a compound microscope (Olympus BX51), and micrographs were captured with a digital camera (Olympus UC90, Olympus Corp., Tokyo, Japan). Macroscopic images were obtained with a stereomicroscope equipped with a digital camera (Leica M165 C and Leica MC170 hD, Leica Microsystems, Heerbrugg, Switzerland).

Molecular methods

Total genomic DNA was extracted from a small section of thallus material using the NucleoSpin™ – Plant II – Kit (Macherey-Nagel™, Düren, Germany) according to the manufacturer's instructions with a slight modification in the final step: for the final elution 50 µL of water were used. Total DNA was used for PCR amplifications of nrITS (internal transcribed spacer region), the nrLSU partial region (28S ribosomal RNA gene) and the mitochondria small subunit (mtSSU). The nrITS was amplified using the primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), nrLSU with the primers LR0R and LR5 (Vilgalys & Hester 1990), and mtSSU with the primer pairs mtSSU1 and mtSSU3R (Zoller et al. 1999). Amplifications were performed using the Platinum™ II Hot-Start PCR Master Mixes (2X) (Invitrogen™, ThermoFisher Scientific Inc., Massachusetts, USA) following manufacturer's instructions. PCR products were subsequently purified using the enzymatic method ExoSAP-IT™ (ThermoFisher Scientific Inc., Massachusetts, USA). The purified PCR products were submitted for sequencing to Macrogen Europe (Netherlands). Sequences were compared with others in the GenBank database using the BLAST algorithm to verify the amplification of the correct regions, and to retrieve additional data to construct the alignments.

Phylogenetic methods

To reconstruct the phylogeny, 121 known and relevant sequences of *Sticta* were downloaded from GenBank (Table 1). *Sticta canariensis* was chosen as the outgroup, based on the phylogeny obtained by Magain and Sérusiaux (2015). First, alignments were constructed separately for each locus with MAFFT v7.525 (Katoh & Standley 2013) and trimmed using TrimAL v1.5.rev0 (Capella-Gutiérrez et al. 2009). Phylogenetic relationships were then inferred using maximum likelihood (ML) with IQ-TREE multicore v2.4.0 (Minh et al. 2020). The individual data sets were then analyzed separately to check for potential conflicting phylogenetic signals. No incongruences were found, and therefore the sets were combined to build a concatenated tree. Branch support for each ML reconstruction was assessed using standard bootstrapping with 1,000 pseudo-replicates. Values ≥70% were considered as significant support. The trees were then visualized and edited with FigTree v1.4.4 (Rambaut 2018).

To study the genealogical relationships among the species associated with the new taxon found in Switzerland, a nrITS haplotype network was constructed. The haplotype network was constructed with the R (R Core Team 2021) package pegas (Paradis 2010) and includes 14 GenBank sequences alongside the newly generated sequences.

Use of AI tools – ChatGPT (OpenAI, San Francisco, CA, USA) was used for debugging code (e.g., to resolve error messages and minor syntax issues encountered when producing the phylogenetic tree and the haplotype network). All scripts were authored and validated by the authors, and all figures/results were independently reproduced.

Results

Morphology

Through morphological examination, two distinct taxa within the *S. fuliginosa* complex were identified within our sampling: *S. fuliginoides* and *S. arenosella*.

Sticta fuliginoides, as originally described by Magain & Sérusiaux (2015) and later revised in two European studies (Sanderson 2016; Ekman et al. 2019), is characterized by having medium-sized, rounded upright and trumpet-shaped young lobes with recurved margins (Fig. 2), a stratified upper cortex and the presence of papillae on the cyphellae surface cells (Fig. 2A, B). Additionally, previously unreported apothecia were observed in this taxon in a single population at the Doubs River. The apothecia are laminal, sessile and loosely attached, 1 mm in diameter, displaying a pale orange margin with whitish hairs. The discs are concave, the hymenium is colored brown to red-brown, 60–90 µm high, and the epihymenium is orange-brown. The mature asci are 55 µm long and contain 8 spores. These ascospores are septate, measuring 16.4–25 × 6.2–8 µm (Fig. 2).

Sticta arenosella, is a species from North America described by Di Meglio & Goward (2023), with both

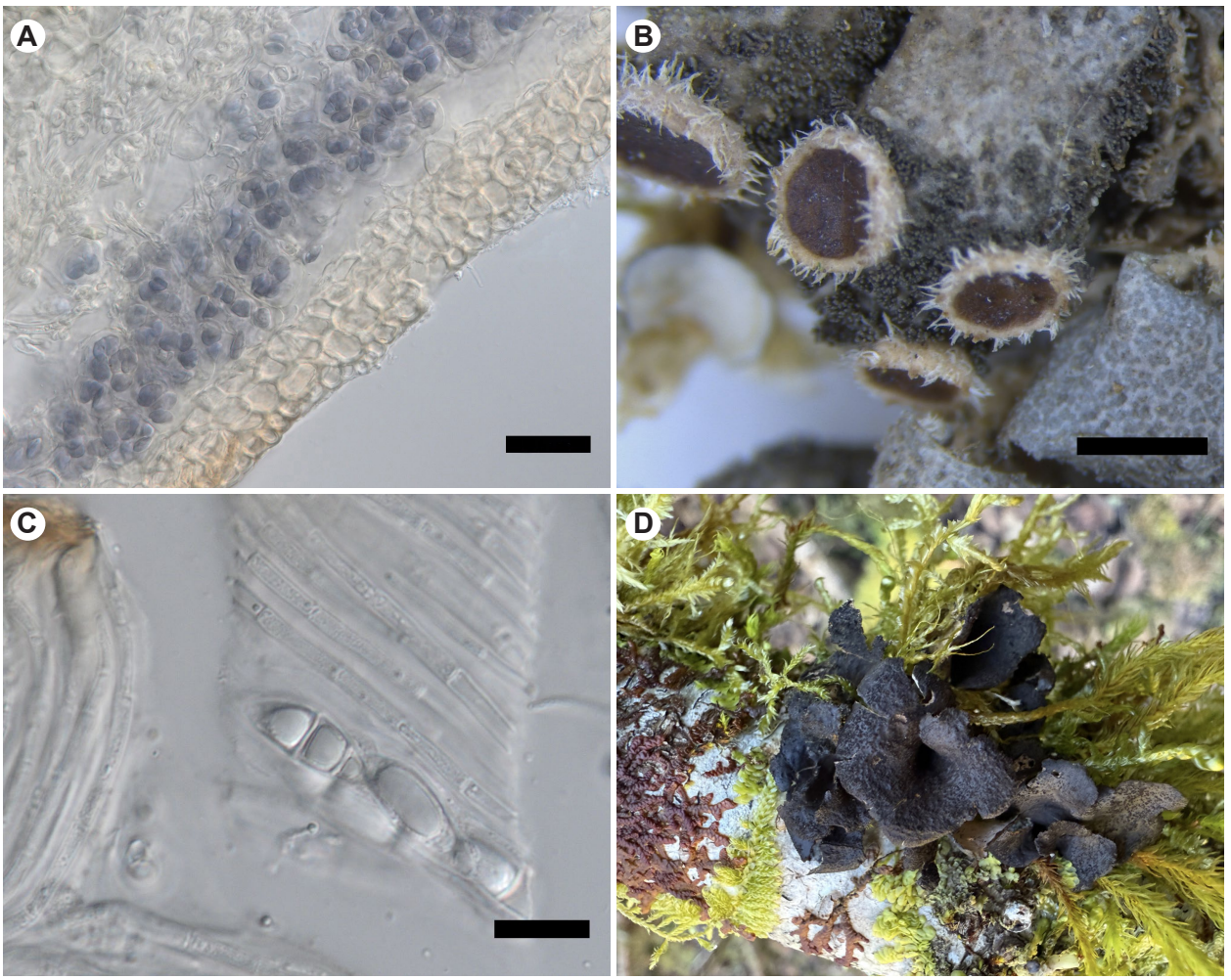

Figure 2. *Sticta fuliginoides* (ChV1392). A – stratified upper cortex; B – apothecium with distinct marginal cilia; C – septate ascospores; D – rounded upright and trumpet-shaped young lobes with recurved margins. Scales: A = 20 µm; B = 1 mm; C = 10 µm.

laminal and marginal, arbuscular to stalked isidia, a non-stratified cortex, and papillae on the cyphellae cells (Fig. 3). The morphological analysis of the North American specimens of *S. torii* and *S. arenosella* was fundamental in assigning the Swiss specimen to *S. arenosella*. Based solely on the original species descriptions, the Swiss specimen was initially identified as *S. torii*, primarily due to the presence of stalked marginal isidia (Fig. 3A). *Sticta arenosella* was originally ruled out because, according to the protologue, its isidia are predominantly granular, laminal, and only occasionally stalked and marginal (Di Meglio & Goward 2023). However, the examination of the *S. arenosella* holotype revealed copiously branched isidia, with cylindrical elongated terminal portions, and with basal portions that are narrow-stalked, supporting the reclassification of the Swiss specimen. A detailed comparison of the key macroscopic and microscopic features distinguishing the two taxa is provided in Table 2.

Phylogenetic analyses

We generated 13 nrITS, 15 nrLSU, and 15 mtSSU sequences from Swiss *Sticta* specimens. The final alignments included 66 sequences for nrITS (476 sites), 51 for nrLSU (804 sites), and 48 for mtSSU (712 sites). These were then combined into a concatenated alignment of 51 samples and 1,992 sites, by including only those specimens for which at least two of the three loci were available. Given that the nrITS alignment is more comprehensive, encompassing a broader geographical and taxonomic representation than the other markers (due to the number of GenBank sequences), the nrITS-based ML-tree

Table 2. Comparative morphological traits of isidiate *Sticta* species relevant for species delimitation in this study. The presence of papillae on cyphellary membranes, the cortex structure, and the isidial morphology are summarized based on microscopic and macroscopic observations. Differences in isidial aggregation and cortex organization provide key diagnostic features among *S. fuliginoides*, *S. torii*, and *S. arenosella*.

Species	Papillae	Cortex	Isidia
Sticta fuliginoides	present	stratified	granular
Sticta arenosella	present	not stratified, but with small cells throughout the cortex	granular to arbuscular to stalked isidia, both laminal and marginal
Sticta torii	present	not stratified	stalked marginal isidia

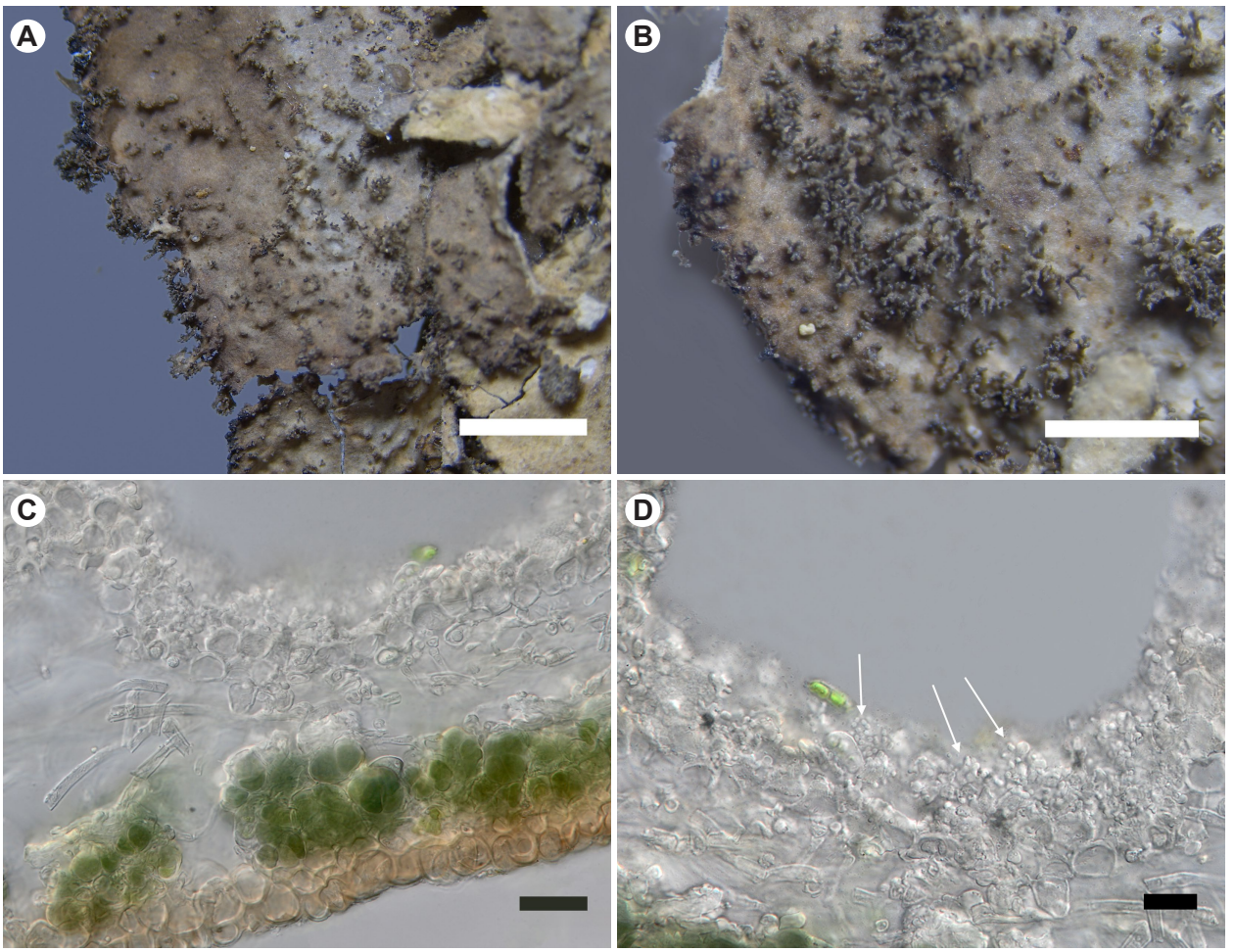

Figure 3. *Sticta arenosella* (voucher: ChV1285). A, B – marginal, stalked isidia; C – cross-section of the lichen with unstratified cortex; D – papillate surface cells on the cyphellary membrane. Scales: A, B = 2 mm; C = 20 µm; D = 10 µm.

is shown in Figure 4. The ML-tree from the concatenated dataset is provided as supplementary material (Fig. S1).

In the nrITS tree, the new Swiss samples were assigned to three distinct well-supported monophyletic lineages. These lineages are in clear agreement with morphological identifications and correspond to *S. arenosella*, *S. fuliginoides* and *S. sylvatica*. Given the high genetic similarity between *S. arenosella* and *S. torii*, and some discrepancies between the phenotypic and phylogenetic assignment, we constructed a haplotype network to better understand the genealogical relationships within this lineage. Five haplotypes were identified across the two species (Fig. 5), differing from each other by a single mutation, except for haplotype V which differed by two mutations. Haplotype I includes two geographically distant specimens of *S. arenosella*: the holotype from North America and the Swiss specimen. Haplotypes III and IV correspond exclusively to *S. torii* specimens from North America. Haplotype II includes additional *S. arenosella* specimens from North America, while Haplotype V corresponds to a specimen from Chile (South America).

In addition, the obtained topology revealed that some GenBank accessions have not been updated to reflect their current taxonomic identities. In fact, the voucher DNA6226 (KC732709) from GenBank labeled as *Sticta fuliginosa* clustered within *Sticta fuliginoides*. Similarly, the vouchers *Sticta* sp. cf. *torii* Bjork 19815 (MH374896) and *Sticta* sp. cf. *torii* Bjork 18443 (MH374895) clustered with *Sticta arenosella*.

Discussion

The phylogeny recovered in this study is largely consistent with previous works on the *S. fuliginosa* complex (Magain & Sérusiaux 2015; Di Meglio & Goward 2023). Our results confirm that *S. fuliginosa* is presently absent from Switzerland. This finding supports earlier studies from other parts of Europe, which indicated that *S. fuliginosa* has an oceanic distribution, primarily restricted to coastal regions of Scandinavia, France, the United Kingdom, and Macaronesia (Magain & Sérusiaux 2015; Sanderson 2016; Ekman et al. 2019). However, our study confirms the presence of *S. fuliginoides* in Switzerland and, more broadly, across Central Europe. Consequently, previous Swiss records attributed to *S. fuliginosa* should be reassigned to *S. fuliginoides*. This result supports previous findings that *S. fuliginoides* is the most widely distributed species in Europe, with populations occurring both along the Atlantic coast and in continental regions (Ekman et al. 2019). Although *S. fuliginoides* has a more continental distribution compared to *S. fuliginosa*, it still requires high humidity. *Sticta fuliginoides* occurs across most of Switzerland's biogeographic regions and its populations are typically found in moist habitats, particularly near water sources such as rivers and streams in

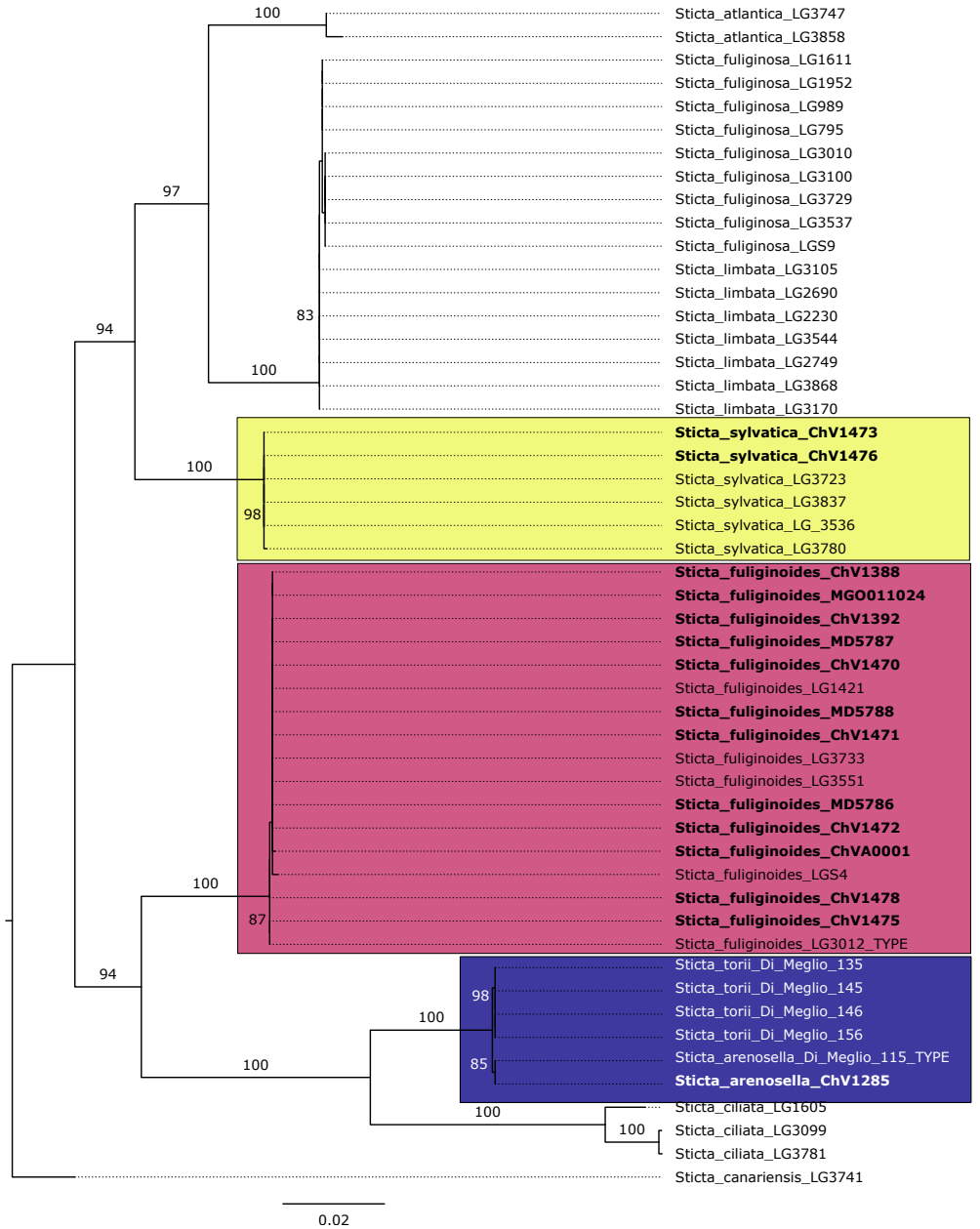

Figure 4. Maximum likelihood phylogenetic tree of *Sticta* species based on nrITS sequence data, reconstructed using IQ-TREE. Bootstrap support values are shown at the nodes. Specimens collected in Switzerland as part of this study are highlighted in bold. Clade colors indicate major phylogenetic lineages recovered in the analysis.

broadleaf forests. The species is predominantly epiphytic, commonly found on the main trunks of *Fagus sylvatica* L., *Acer pseudoplatanus* L., and other leaf trees. However, *S. fuliginoides* can also occur on moss-covered rocks, as observed in the Central Alps (Valais) and the Southern Alps (Grisons). *Sticta fuliginoides* often co-occurs with other cyanolichens like *Collema flaccidum* (Ach.) Ach., *Leptogium saturninum* (Dicks.) Nyl., *Lobaria pulmonaria* (L.) Hoffm., *Pannaria conoplea* (Ach.) Bory, *Parmeliella triptohylla*, (Ach.) Müll. Arg., *Peltigera collina* (Ach.) Schrad. or *Sticta sylvatica*.

In addition, both morphological and phylogenetic analyses revealed the occurrence of a species belonging to a lineage not reported before in Europe: *S. arenosella*, a member of the *Gyalocarpa* subclade sensu, which also includes *S. torii* (Moncada et al. 2014b; Simon et al. 2018; Di Meglio & Goward 2023). Although initial morphological identification based solely on the literature suggested that the Swiss specimen was *S. torii*, phylogenetic and haplotype analyses indicated that it is genetically more closely related to *S. arenosella*. Upon examining the holotype of *S. arenosella*, we revised our morphological assessment, as the Swiss specimen exhibits the same isidia features as the holotype.

Sticta arenosella was first described in 2023 from North America (Di Meglio & Goward 2023). Initially, it was considered rare and endemic to northwestern North America, having been reported from coastal lowlands ranging from British Columbia to Oregon. The authors also noted that the species occurs in a humid inland region with oceanic influence of British Columbia (Di Meglio & Goward 2023). A year later, a new record was reported from southern Chile, in the Department of Magallanes y Antártica Chilena (Ossowska et al. 2024), indicating

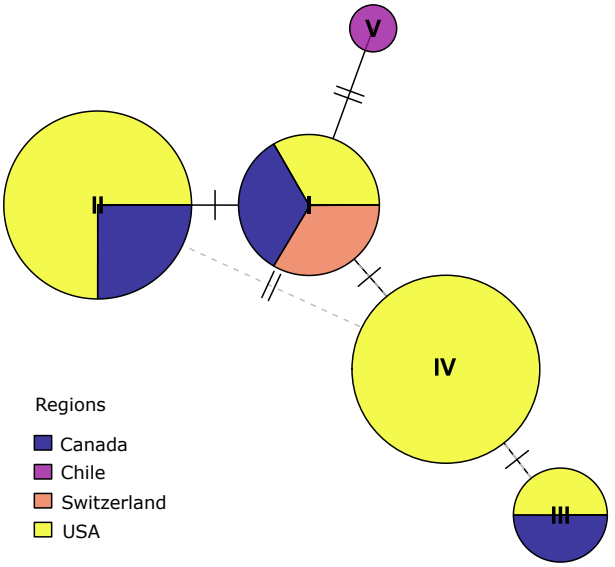

Figure 5. Haplotype network based on nrITS sequence data showing relationships among five haplotypes identified across *S. arenosella* and *S. torii*. Circle sizes are proportional to haplotype frequencies within the dataset. Each line represents a single mutational step. A complete list of specimens corresponding to each haplotype is provided in Supplementary Table S2.

that the species exhibits an amphitropical disjunction. The Chilean specimen was collected in a lowland *Nothofagus* forest, located away from the coast, but near large lakes (Ossowska et al. 2024). The Swiss specimen of *S. aresonella* was collected in 2022 in the municipality of Unteriberg, Canton Schwyz, Switzerland at approx. 1,200 m a.s.l. (Fig. 1). The site is in a north-east exposed *Abieti-Fagenion*. It lies in a valley closed off to the south. The thalli were growing in the middle trunk area of a beech tree. Other species of the *Lobarion* community were observed in the immediate vicinity. Our discovery of *S. arenosella* in Central Europe, specifically in the Northern Alps of Switzerland, further extends its known distribution beyond North and South America and into regions with a suboceanic influence.

This finding suggests that *S. arenosella* displays both amphitropical and intercontinental disjunctions (Fig. 6) and, although it is not common, has a very broad ecological niche. Similar distribution patterns have been observed in other lichen-forming fungi, and in spore-dispersed organisms, such as mosses and ferns and have been confirmed by molecular evidence (Moncalvo & Buchanan 2008; Otálora et al. 2010; Sérusiaux et al. 2011; De Paz et al. 2012; Lewis et al. 2014; Weigelt et al. 2015; Leavitt et al. 2018; Lebreton et al. 2025).

The nrITS marker has been shown to be effective in distinguishing taxa within the genus *Sticta* and revealing previously overlooked morphological and anatomical characteristics (Moncada et al. 2014a; Magain & Sérusiaux 2015). One example is the presence of papillae on the surface cells of the cyphellae, which distinguishes *S. fuliginosa* from *S. fuliginoides*. However, in other cases, where morphology clearly distinguishes taxa, this marker exhibits minimal variation or even no variation at all (Moncada et al. 2014b; Di Meglio & Goward 2023; Ossowska et al. 2024). Our results show that the nrITS region displays intraspecific variation within *S. arenosella*, with five distinct haplotypes identified (Fig. 5). The variation is, however, not fully structured by geography. While specimens from the Americas show slight differentiation, represented by three different haplotypes, the European specimen shares a haplotype with North American individuals. Thus, we report a case in which two biogeographically distant populations share the same nrITS haplotype (Fig. 5). To draw more robust conclusions about biogeography and distribution with this species, new approaches should be developed. For example, incorporating additional genetic markers such as microsatellites, or applying genomic techniques, as has already been used in other lichen-forming fungal genera (Boluda et al. 2018; Grewe et al. 2018; Barcenas-Peña et al. 2023).

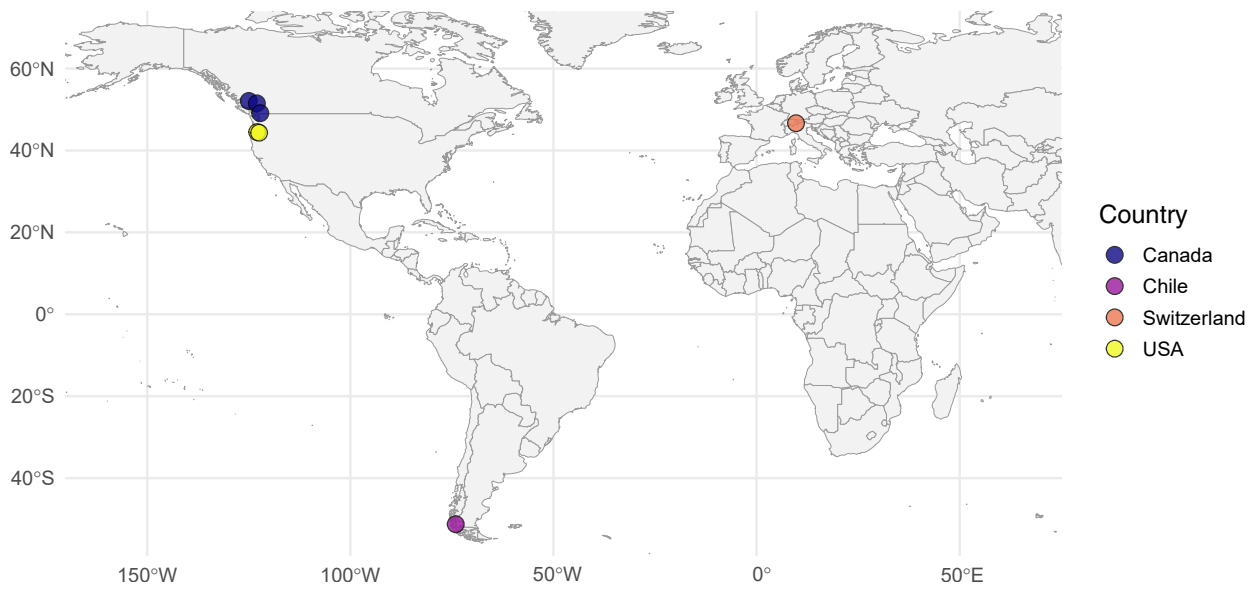

Figure 6. Global distribution of *Sticta arenosella*. Previously known localities are based on literature and herbarium records, while the new Swiss record documented in this study is shown in orange. The map highlights the disjunct distribution pattern of the species, with the Swiss specimen representing the first report of *S. arenosella* from Europe. Base map retrieved using Natural Earth, www.naturalearthdata.com

Identification key to Central European *Sticta* species

A key to the Central European species of *Sticta* is provided below. It is based on the key by Ekman et al. (2019) and has been supplemented with information on *S. arenosella*, based on Di Meglio & Goward (2023) and our own observations.

1 Thallus with soralia . . . . . ***Sticta limbata***
Thallus without soralia, mostly with isidia 2

2(1) Thallus with green-algal lobes or thallus bluish gray to gray-brown with laciniate lobes. . . . ***Sticta canariensis***
Thallus ± dark brown, without green-algal lobes and without laciniate lobes 3

3(2) Thallus lobes strap-shaped, dichotomously branched, upper surface shiny when dry, lower surface black (at least near the base). . . . . ***Sticta sylvatica***
Thallus ± rounded or irregular, not dichotomously branched, upper surface matte, lower surface ± brown to pale brown 4

4(3) Cyphella surface cells without papillae . . . . . ***Sticta fuliginosa***
Cyphella surface cells with papillae 5

5(4) Stratified cortex (the top layer of the cortex has smaller cells than the layers underneath). . . . ***Sticta fuliginoides***
Unstratified cortex (the cell sizes of the cortex are ± homogeneous) . . . . . ***Sticta arenosella***

Conclusion

In conclusion, our integrative approach that combines morphological and phylogenetic analyses, as well as haplotype networks, has provided new information on the distribution and evolutionary relationships within the *Sticta fuliginosa* species complex. Our results clearly indicate that *S. fuliginosa* s.str. is presently absent from Switzerland and the local *S. fuliginosa* complex comprises *S. fuliginoides* and *S. arenosella*. Looking forward, several avenues warrant further exploration to fully elaborate the dynamics and conservation status of Swiss *Sticta* populations. One key question is whether these populations represent relictual survivors from a historically broader distribution or if they are instead the result of more recent colonization events. To disentangle these scenarios, comparable sampling across other regions of Europe will be necessary, along with the use of additional molecular markers such as microsatellites or genome sequencing, which might enable a more detailed study of the genetic structure of the species.

Considering the new findings on the diversity and distribution of *Sticta* in Switzerland, it is recommended that the national Red List be updated accordingly. Incorporating this new species information will ensure that conservation assessments are based on the most current and comprehensive data, ultimately aiding the protection of these ecologically significant lichens.

Acknowledgements

We thank Michael Dietrich, Gisela Bauert and Ursula Tinner for the help collecting the Swiss specimens in the field, and Mathias Vust for accompanying us during the sampling at the Valais sites. Additionally, we thank the curator of OSU for the loan of herbarium material. The Legat "Eduard August Rübel" supported the study financially as part of the project "Seltene Makroflechten entlang dem Doubs".

ORCID

Julia Bernet https://orcid.org/0009-0003-3569-9958
Mónica A.G. Otálora https://orcid.org/0000-0001-7081-3937

Supplementary electronic materials

Figure S1. Maximum likelihood phylogenetic tree based on concatenated nrITS, nrLSU, and mtSSU sequence data, reconstructed using IQ-TREE. Bootstrap support values are shown at the nodes. Specimens collected in Switzerland as part of this study are highlighted in bold. The combined multilocus dataset provides increased resolution for assessing phylogenetic relationships among *Sticta* species. Download file

Table S1. List of Swiss specimens collected for this study including herbaria voucher numbers, localities and collection dates. Download file

Table S2. Specimens assigned to each of the five haplotypes identified in the ITS haplotype network (Fig. 5), with species names, voucher numbers, and localities as used in the analysis. Download file

References

Arsenault, A. & Goward, T. 2016. Macrolichen diversity as an indicator of stand age and ecosystem resilience along a precipitation gradient in humid forests of inland British Columbia, Canada. *Ecological Indicators* 69: 730–738. https://doi.org/10.1016/j.ecolind.2016.04.015

Barcenas-Peña, A., Divakar, P. K., Crespo, A., Nuñez-Zapata, J., Lumbsch, H. T. & Grewe, F. 2023. Reference-Based Restriction-Site-Associated DNA sequencing data are useful for species delineation in a recently diverged asexually reproducing species complex (*Parmeliaceae*, Ascomycota). *Journal of Fungi* 9: 1180. https://doi.org/10.3390/jof9121180

Boluda, C. G., Rico, V. J., Divakar, P. K., Nadyeina, O., Myllys, L., McMullin, R. T., Zamora, J. C., Scheidegger, C. & Hawksworth, D. L. 2018. Evaluating methodologies for species delimitation: the mismatch between phenotypes and genotypes in lichenized fungi (*Bryoria* sect. *implexae*, *Parmeliaceae*). *Persoonia* 42: 75–100. https://doi.org/10.3767/persoonia.2019.42.04

Capella-Gutiérrez, S., Silla-Martínez, J. M. & Gabaldón, T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* (Oxford, England) 25: 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Delise, D. 1825. *Histoire de Lichens*. Genre Sticta. Caen.

De Paz, G. A., Cubas, P., Crespo, A., Elix, J. A. & Lumbsch, H. T. 2012. Transoceanic Dispersal and Subsequent Diversification on Separate Continents Shaped Diversity of the *Xanthoparmelia pulla* Group (Ascomycota). *PLOS ONE* 7: 1–12. https://doi.org/10.1371/journal.pone.0039683

Di Meglio, J. R. D. & Goward, T. 2023. Resolving the *Sticta fuliginosa* Morphodeme (Lichenized Ascomycota: *Peltigeraceae*) in Northwestern North America. *The Bryologist* 126: 90–110. https://doi.org/10.1639/0007-2745-126.1.090

Ekman, S., Tønsberg, T. & Jørgensen, P. M. 2019. The *Sticta fuliginosa* group in Norway and Sweden. *Graphis Scripta* 31: 23–33.

Galloway, D. J. 1994. Studies on the lichen genus *Sticta* (Schreber) Ach.: II. Typification of taxa from Swartz's Prodromus of 1788. *Bulletin of the Natural History Museum*. *Botany series* 24: 35–48.

Gardes, M. & Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Grewe, F., Lagostina, E., Wu, H., Printzen, C. & Lumbsch, H. T. 2018. Population genomic analyses of RAD sequences resolves

the phylogenetic relationship of the lichen-forming fungal species *Usnea antarctica* and *Usnea aurantiacoatra*. *MycoKeys* 43: 91–113. https://doi.org/10.3897/mycokeys.43.29093

Katoh, K. & Standley, D. M. 2013. (MAFFT) multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. https://doi.org/10.1093/molbev/mst010

Leavitt, S. D., Kirika, P. M., Amo de Paz, G., Huang, J.-P., Hur, J.-S., Elix, J. A., Grewe, F., Divakar, P. K. & Lumbsch, H. T. 2018. Assessing phylogeny and historical biogeography of the largest genus of lichen-forming fungi, *Xanthoparmelia* (*Parmeliaceae*, Ascomycota). *The Lichenologist* 50: 299–312. https://doi.org/10.1017/S0024282918000233

Lebreton, E., Ertz, D., Lücking, R., Aptroot, A., Carriconde, F., Ah-Peng, C., Huang, J.-P., Chen, K.-H., Stenger, P.-L., da Silva Cáceres, M. E., van den Boom, P., Sérusiaux, E. & Magain, N. 2025. Global phylogeny of the family *Gomphillaceae* (Ascomycota, Graphidales) sheds light on the origin, diversification and endemism in foliicolous lineages. *IMA Fungus* 16: e144194. https://doi.org/10.3897/imafungus.16.144194

Lewis, L. R., Rozzi, R. & Goffinet, B. 2014. Direct long-distance dispersal shapes a New World amphitropical disjunction in the dispersal-limited dung moss *Tetraplodon* (Bryopsida: *Splachnaceae*). *Journal of Biogeography* 41: 2385–2395. https://doi.org/10.1111/jbi.12385

Liška, J., Palice, Z. & Slavíková, Š. 2008. Checklist and red list of lichens of the Czech Republic. *Preslia* 80: 151–182.

Lücking, R., Hodkinson, B. P. & Leavitt, S. D. 2017. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* 119: 361–416. https://doi.org/10.1639/0007-2745-119.4.361

Lücking, R., Leavitt, S. D. & Hawksworth, D. L. 2021. Species in lichen-forming fungi: balancing between conceptual and practical considerations, and between phenotype and phylogenomics. *Fungal Diversity* 109: 99–154. https://doi.org/10.1007/s13225-021-00477-7

Magain, N. & Sérusiaux, E. 2015. Dismantling the treasured flagship lichen *Sticta fuliginosa* (Peltigerales) into four species in Western Europe. *Mycological Progress* 14(10): 97. https://doi.org/10.1007/s11557-015-1109-0

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A. & Lanfear, R. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015

Moncada, B., Lückig, R. & Coca, L. F. 2013. Six new apotheciate species of *Sticta* (lichenized Ascomycota: *Lobariaceae*) from the Colombian Andes. *The Lichenologist* 45: 635–656. https://doi.org/10.1017/S0024282913000376

Moncada, B., Aguirre, J. & Lücking, R. 2014a. Ecogeography of the genus *Sticta* (lichenized Ascomycota: *Lobariaceae*) in Colombia. *Revista de Biología Tropical* 62: 257–272. https://doi.org/10.15517/rbt.v62i1.3564

Moncada, B., Lücking, R. & Suárez, A. 2014b. Molecular phylogeny of the genus *Sticta* (lichenized Ascomycota: *Lobariaceae*) in Colombia. *Fungal Diversity* 64: 205–231. https://doi.org/10.1007/s13225-013-0230-0

Moncada, B., Lücking, R. K. & Lumbsch, H. T. 2020. Rewriting the evolutionary history of the lichen genus *Sticta* (Ascomycota: *Peltigeraceae* subfam. *Lobarioideae*) in the Hawaiian islands. *Plant and Fungal Systematics* 65: 95–119. https://doi.org/10.35535/pfsyst-2020-0005

Moncalvo, J.-M. & Buchanan, P. K. 2008. Molecular evidence for long distance dispersal across the southern hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycological Research* 112: 425–436. https://doi.org/10.1016/j.mycres.2007.12.001

Nascimbene, J., Nimis, P. L. & Ravera S. 2013. Evaluating the conservation status of epiphytic lichens of Italy: A red list. *Plant Biosystems – An International Journal Dealing with all Aspects of Plant Biology* 147: 898–904. https://doi.org/10.1080/11263504.2012.748101

OpenAI. 2025. ChatGPT (April 6 version) (Large language model). https://chat.openai.com/

Ossowska, E. A., Schiefelbein, U. & Kukwa, M. 2024. First records of *Sticta arenosella* and *S. cellulosa* from South America based on molecular and morphological data. *Plant and Fungal Systematics* 69: 77–84. https://doi.org/10.35535/pfsyst-2024-0008

Otálora, M. A. G., Martínez, I., Aragón, G. & Molina, M. C. 2010. Phylogeography and divergence date estimates of a lichen species complex with a disjunct distribution pattern. *American Journal of Botany* 97: 216–223. https://doi.org/10.3732/ajb.0900064

Paradis, E. 2010. pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics* 26: 419–420. https://doi.org/10.1093/bioinformatics/btp696

R Core Team 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rambaut, A. 2018. FigTree – tree.bio.ed.ac.uk. https://tree.bio.ed.ac.uk/software/figtree/ [accessed on 30 Mar. 2025]

Sanderson, N. 2016. The field identification of the species within *Sticta fuliginosa* s.lat. in Britain. *British Lichen Society Bulletin* 119: 2–14.

Scheidegger, C. & Clerc, P. 2002. *Rote Liste der gefährdeten Arten der Schweiz: Baum- und erdbewohnende Flechten*. Hrsg. Bundesamt für Umwelt, Wald und Landschaft BUWAL, Bern, und Eidgenössische Forschungsanstalt WSL, Birmensdorf, und Conservatoire et Jardin botaniques de la Ville de Genève CJBG. BUWAL-Reihe Vollzug Umwelt.

Sérusiaux, E., Villarreal A., J. C., Wheeler, T. & Goffinet, B. 2011. Recent origin, active speciation and dispersal for the lichen genus *Nephroma* (Peltigerales) in Macaronesia. *Journal of Biogeography* 38: 1138–1151. https://doi.org/10.1111/j.1365-2699.2010.02469.x

Simon, A., Goward, T., Di Meglio, J., Dillman, K., Spribille, T. & Goffinet, B. 2018. *Sticta torii* sp. nov., a remarkable lichen of high conservation priority from northwestern North America. *Graphis Scripta* 30: 105–114.

Stofer, S., Scheidegger, C., Clerc, P., Dietrich, M., Frei, M., Groner, U., Keller, C., Meraner, I., Roth, I., Vust, M. & Zimmermann, E. 2019. SwissLichens – Webatlas der Flechten der Schweiz (Version 3). www.swisslichens.ch [accessed on 8 Apr. 2025]

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Weigelt, P., Daniel Kissling, W., Kisel, Y., Fritz, S. A., Karger, D. N., Kessler, M., Lehtonen, S., Svenning, J.-C. & Kreft, H. 2015. Global patterns and drivers of phylogenetic structure in island floras. *Scientific Reports* 5: 12213. https://doi.org/10.1038/srep12213

White, T. J., Bruns, T., Lee S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, J. W. (eds), *PCR Protocols. A Guide to Molecular Methods and Applications*. Acad. Press, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wirth, V., Hauck, M., von Brackel, W., Cezanne, R., de Bruyn, U., Dürhammer, O., Eichler, M., Gnüchtel, A., John, V., Litterski, B., Otte, V., Schiefelbein, U., Scholz, P., Schultz, M., Stordeur, R., Feuerer, T. & Heinrich, D. 2011. Rote Liste und Artenverzeichnis der Flechten und flechtenbewohnenden Pilze Deutschlands. *Naturschutz und Biologische Vielfalt* 70: 7–122.

Zoller, S., Scheidegger, C. & Sperisen, C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* 31: 511–516. https://doi.org/10.1006/lich.1999.0220