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Sticta puebloensis (lichenized Ascomycota: *Peltigeraceae*) a new species of *Sticta* from the American Southwest

Joseph R. Di Meglio[1]*, Jack C. Niedbala[2] & Elisa B. Di Meglio[1]

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Abstract. In a continued effort to dismantle the *Sticta fuliginosa* morphodeme of western North America, we performed an extensive sampling throughout the mountainous regions of the southwestern states of the USA: Arizona, Colorado, and New Mexico. Recent studies have concluded the genus *Sticta* has extensive hidden biodiversity and is a large sub-cosmopolitan species-rich genus that encompasses 500+ taxa and counting. The prior treatment, incorporating all laminal isidiate species of *Sticta* into the *Sticta fulignosa* s.str. and *Sticta sylvatica* s.str. morphodemes, was incorrect. Our data does not support those previous treatments nor the broad application of European species concepts of isidiate *Sticta* in Western North America. Instead, our 4-locus phylogenetic analyses support a narrowing of that concept to a single species of *Sticta* that is neither *S. fuliginosa* s.str. nor *S. sylvatica* s.str. and is a distinct species with a specialized ecological niche. This unique proposed species is named and described here as *Sticta puebloensis* sp. nov.

Key words: Arizona, Colorado, lichens, New Mexico, phylogenetics, systematics, taxonomy

Introduction

The genus *Sticta* (Schreb.) Ach. (*Ascomycota*, *Peltigeraceae*) is a species-rich genus of subcosmopolitan epiphytic lichens that commonly occur in subtropical and tropical ecoregions, which is particularly speciose in neotropical montane ecosystems of the Americas (Galloway 1995; Moncada 2012; Moncada & Lücking 2012; Moncada et al. 2013a, b, c, 2014, 2015, 2018, 2020, 2021a, b; Suarez & Lücking 2013; Mercado-Díaz et al. 2020; Ossowska 2021; Ossowska et al. 2022a, b, 2024). Recent investigations have provided better resolution in speciation and distribution of *Sticta* in the America's, either providing a broadening or in some cases narrowing of species concepts (McDonald et al. 2003; Moncada 2012; Moncada et al. 2013a, b, c, 2015; Magain & Sérusiaux 2015; Lendemer & Goffinet 2016; Simon et al. 2018; Mercado-Díaz et al. 2020; Ossowska 2021; Ossowska et al. 2022a, b, 2024; Di Meglio & Goward 2023). Ecoregions that were once thought to have minimal species diversity in western Europe and northwestern North America have been shown to have several cryptic species hiding in flagship complexes (Magain & Sérusiaux 2015; Simon et al. 2018; Ekman et al. 2019; Moncada et al. 2021b; Di Meglio & Goward 2023). Prior to this study, there was little attention paid to the genus *Sticta* in the Southwest USA, with the most recent treatment of the region by Galloway & Thomas (2004). They included much of the Northwestern Mexican states of Baja California, Chihuahua, Sinaloa, Sonora, and the southern portions of California, Arizona, and southwestern New Mexico. However, notwithstanding their technological limitations, Galloway & Thomas (2004) executed the task well with a broad treatment of lichen flora, which incorporated a ten species treatment of *Sticta* including two new species descriptions: *Sticta nashii* Galloway, *Sticta mexicana* Galloway and two new combinations: *Sticta leucoblephara* and *Sticta xanthotropa* (Kemp.) Galloway (Galloway & Thomas 2004). Moreover, Galloway & Thomas (2004) adhered to a narrow taxonomic ideology in their treatment of *Sticta*, subsuming laminal and marginal isidiate *Sticta* from Arizona USA, and the Mexican states of Chihuahua, Sinaloa, and Sonora, into a European species construct in either the *Sticta fuliginosa* s.lat. (With.) Ach., *Sticta sylvatica* s.lat. (Hudson) Ach. or *Sticta xanthotropa* s.lat. (Kemp.) Galloway morphodemes (Galloway & Thomas 2004; Mercado-Díaz et al. 2020; DiMeglio & Goward 2023). Of particular note, our preliminary data from a broad sampling of *Sticta* from the American southwest and northwestern Mexico suggests species richness is far more diverse and quite different than what was described by Galloway & Thomas (2004).

Here, we will further discuss the previous taxonomic concepts presented by Galloway & Thomas (2004) of laminal isidiate *Sticta* of the American Southwest. This

[1] Department of Botany and Plant Pathology, Oregon State University, Cordley Hall, 2701 SW Campus Way, Corvallis Oregon 97331, USA
[2] 11108 Elvin NE Albuquerque, New Mexico 87112, Mexico
* Corresponding author e-mail: dimeglij@oregonstate.edu

work contributes to the continual dismantling of the *Sticta fuliginosa* morphodeme of western North America and presents a description of *Sticta puebloensis* Di Meglio & Niedbala, a new species from the American Southwest states of Arizona, Colorado and New Mexico.

Methods and materials

Fresh specimens were collected from the southwestern US states of Arizona, New Mexico and the mountain state of Colorado between the years 2017–2023 (Table 1). Historical specimens of *Sticta fuliginosa* s.lat. and *Sticta sylvatica* s.lat., collected from the same general locations in the southwestern USA and the Mexican state of Chihuahua, were requested and examined via loan from Arizona State University (ASU), the private herbarium of Dr. Esslinger, and the Duke University Herbarium (Table 1). Morphological observations were conducted on both freshly collected and historical specimens, with the freshly collected material deposited in the OSC and UNM herbaria.

Microscopy and photography

Specimens were examined with an Olympus BX-51 compound microscope with phase condenser, dark field and with an Olympus SZ dissecting microscope. In situ photos were captured with a Nikon Z7ii mirrorless DSLR camera with a Nikon Nikkor 50 mm 1:1 macro lens, a Laowa 60 mm 2:1 macro lens and a Laowa 25 mm 5:1 macro lens. Studio photos were stacked using Cognisyne photo-staking equipment and stacked photos were generated with Helicon Focus professional stacking software.

DNA isolation, PCR amplification and sequencing

Sigma-Aldrich Extract-N-Amp Plant PCR kit was used for DNA extraction per manufacturer's instructions, however, the PCR portion of the Extract-N-Amp kit was not used in amplification. Instead, PCR amplification was accomplished with Thermo-Scientific Dream Taq Green reaction mix adhering to the manufacturer's instructions. A total of four genes were amplified and sequenced. Gene markers, primers and thermal cycle protocols can be found in supplementary data section (Table S2).

Table 1. List of specimens loaned and collected for the study, including species, herbaria, GenBank accession, location and voucher data.

Species	Tentative ID	Herbarium Code/Name	Genbank ITS	Location	Voucher
Sticta arenosella	*S. arenosella*	OSC/UBC	MH374894	Washington, USA	Di Meglio 115: **Holotype**
Sticta fasciculata	*S. fasciculata*	OSC/UBC	MN699993	British Columbia, Canada	Di Meglio 91: **Holotype**
Sticta fuliginoides	*S. fuliginoides*	J. Hollinger Herbarium	PP319023	North Carolina, USA	Hollinger 27501
Sticta fuliginoides	*S. fuliginoides*	J. Hollinger Herbarium	PP319024	North Carolina, USA	Hollinger 28824
Sticta fuliginoides	*S. fuliginoides*	J. Hollinger Herbarium	PP319025	North Carolina, USA	Hollinger 28827
Sticta fuliginosa s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Wetmore 54477
Sticta fuliginosa s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Nash 38958
Sticta fuliginosa s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Nash 3202
Sticta fuliginosa s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Nash 39466
Sticta fuliginosa s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Nash 18560
Sticta fuliginosa s.lat.	*Sticta* sp.	ASC	N/A	Chihuahua, Mexico	Nash 37369
Sticta fuliginosa s.lat.	*Sticta* sp.	ASC	N/A	Chihuahua, Mexico	Nash 31362
Sticta fuliginosa s.lat.	*Sticta* sp.	ASC	N/A	Chihuahua, Mexico	Nash 37514
Sticta fuliginosa s.lat.	*Sticta gretae*	T. Esslinger Herbarium	N/A	New Mexico, USA	Esslinger 12042
Sticta fuliginosa s.lat.	*Sticta gretae*	T. Esslinger Herbarium	N/A	New Mexico, USA	Esslinger 12048
Sticta fuliginosa s.lat.	*Sticta gretae*	T. Esslinger Herbarium	N/A	New Mexico, USA	Esslinger 15448A
Sticta fuliginosa s.lat.	*Sticta gretae*	T. Esslinger Herbarium	N/A	New Mexico, USA	Esslinger 15494
Sticta fuliginosa s.lat.	*Sticta* sp.	DUKE	N/A	South Dakota, USA	Anderson, Lewis E. 24192
Sticta fuliginosa s.str.	*S. fuliginosa* s.str.	F. Anderson Herbarium	PP315673	Nova Scotia, Canada	F. Anderson 17132
Sticta fuliginosa s.str.	*S. fuliginosa* s.str.	S. Brinker Herbarium	PP291711	Quebec, Canada	S. Brinker 5335
Sticta fuliginosa s.str.	*S. fuliginosa* s.str.	S. Brinker Herbarium	PP291712	Quebec, Canada	S. Brinker 6179
Sticta fuliginosa s.str.	*S. fuliginosa* s.str.	S. Brinker Herbarium	PP291713	Quebec, Canada	S. Brinker 7920
Sticta globulifuliginosa	*S. globulifuliginosa*	OSC/UBC	MN699974	Oregon, USA	Di Meglio 151
Sticta gretae	*S. gretae*	OSC/UBC	MN700002	Oregon, USA	Di Meglio 78: **Holotype**
Sticta limbata	*S. limbata*	OSC	MN700026	Oregon, USA	Di Meglio 166
Sticta sp.	*S. puebloensis* sp. nov.	OSC	OP984116	New Mexico, USA	Di Meglio & Niedbala 232
Sticta sp.	*S. puebloensis* sp. nov.	OSC/UNM	OP984117	New Mexico, USA	Di Meglio & Niedbala 241: **Holotype**
Sticta sp.	*S. puebloensis* sp. nov.	OSC	OP984120	Arizona, USA	Di Meglio 312
Sticta sp.	*S. puebloensis* sp. nov.	OSC	OP984121	Arizona, USA	Di Meglio 313
Sticta sp.	*S. puebloensis* sp. nov.	OSC	OP984123	Colorado, USA	Di Meglio 315
Sticta sp.	*S. puebloensis* sp. nov.	OSC	OP984124	Colorado, USA	Di Meglio 319
Sticta sylvatica s.str.	*S. sylvatica* s.str.	BG	N/A	Norway: Hordaland	Knutsen L105705
Sticta sylvatica s.str.	*S. sylvatica* s.str.	BG	OP782381	Norway: Hordaland	Knutsen L105706
Sticta sylvatica s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Nash 30992
Sticta sylvatica s.lat.	*S. puebloensis* sp. nov.	ASC	N/A	Arizona, USA	Nash 21129b

The PCR chemistry was conducted by using 7.5 uL of Green Taq rxn mix, 6.4 mL DI water, 0.6 uL of primer and 1 uL of DNA template (Di Meglio & Goward 2023). PCR products were cleaned with Affy-Metrix ExoSAP-IT protocol as followed, 3 uL of PCR product and 1 uL of ExoSAP-IT for a total of 4 uL. Thermal cycle protocol is as followed, 15 minutes at 87°C and 15 minutes at 37°C (Di Meglio & Goward 2023). Sequence chemistry was carried out as follows; 8.8 uL of sterile DI water, 1.2 uL of sequence primer and 1uL of cleaned PCR template (Di Meglio & Goward 2023). Sanger sequencing of the PCR products was performed by Eurofins MWG Operon Inc., Kentucky, USA.

Raw sequence data was processed through Geneious Prime 2024.0.2 using the default parameters for the de novo sequence assembler. Once the contigs were aligned, they were cropped to attain similar lengths to those that were imported from GenBank, and a consensus sequence was generated. We chose our Genbank sequences based upon prior studies that are from reputable research groups and peer reviewed studies of the genus. We wanted to present a broad geographical distribution in our ITS model of *Sticta* and where our species *S. puebloensis* nested in the overall evolutionary tree. We incorporated ITS sequences from the America's, Western Europe, Central Africa, Asia and Australia/New Zealand. These sequences and associated data can be found in our supplementary data (Supplementary Table S1). The consensus sequences and the sequences imported through GenBank were assembled into a multi-alignment using default parameters with MAFFT v7 (Katoh et al. 2002; Katoh & Standly 2013). We used three different evolutionary models to infer taxonomic designation, PhyML3.0 (Guindon et al. 2010) GTR model with 1,000 bootstrap pseudo-replicates, RAxML version 8 (Stamatakis 2014) GTR CAT model used with default parameters with 1,000 bootstrap pseudo-replicates, and lastly, Mr. Bayes 3.2.6 Bayesian analysis (Ronquist & Huelsenbeck 2003) with default parameters as seen in Di Meglio & Goward (2023).

With the intention of gaining a deeper understanding of the relationships between the new species and other relatives within the *Fuliginoides* clade, a haplotype network was generated using PopArt software (Version 4.8.5 http://popart.otago.ac.nz), applying the median-joining haplotype model (Bandelt et al. 1999). The ITS sequences selected for the haplotype network analysis were based on the relationships observed in our single locus ITS topology and the concatenated topology (Table S1). The sequences used are indicated by an asterisk placed on the species name.

Results

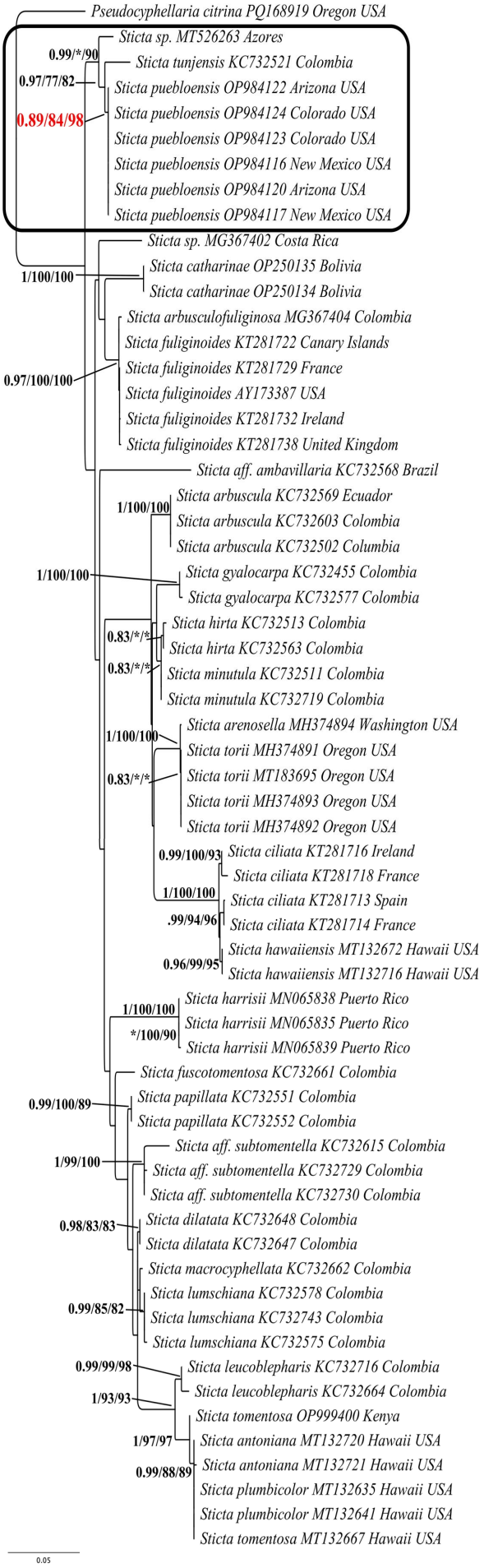
Phylogenetic analysis and haplotype network

We chose to use *Pseudocyphellaria citrina* (Gyeln) Lücking, Moncada & S.Stenroos as our rooted outgroup for the phylogeny, following previous works in *Sticta* taxonomy (Widhelm et al. 2018; Torres et al. 2021; Di Meglio & Goward 2023). We first assessed phylogenetic relationships with a global single-locus ITS 1,000 pseudo-replicate topology using PhyML ML GTR and RAxML GTR-CAT models to visualize the clade position of our six sequences of *Sticta puebloensis*. Once we understood the clade position within the global ITS dataset, the clade and adjoining subclades were then isolated and used to assemble our four loci concatenated phylogenies using 1000 pseudo-replicate PhyML ML GTR analysis, 1,000 pseudo-replicate RAxML GTR CAT and the Bayesian GTR analysis. The three analyses yielded varying results in overall topology structure and bootstrap percentages; however, we decided to use the best scoring concatenated PhyML maximum likelihood phylogeny. We considered branches within our PhyML and RAxML topologies with 70% or above as strongly supporting and 0.95 for the Bayesian analysis (Magain & Sérusiaux 2015; Di Meglio & Goward 2023).

Both single locus 1,000 pseudo-replicate ITS PhyML GTR and RAxML GTR-CAT topologies yielded an 85% bootstrap support and nested our sub-clade in the greater *Fuliginoides* clade (Supplemental Fig. S1). Based upon the four gene concatenated analysis, we infer that *Sticta puebloensis* forms a 98% bootstrap supported subclade and nests within the greater *Sticta fuliginoides* clade (Fig. 1). We assembled two other evolutionary tests, a Bayesian analysis and RAxML analysis with the concatenated alignment, the bootstrap support values of both models are included in figure 1. The other models can be found in the supplementary data section (Fig. S2, S3).

Sticta puebloensis has a unique phylogenetic placement with only a few related species in the clade. It shares a close evolutionary proximity to *Sticta tunjensis* (KC732521) Moncada & Lücking (2012), which until recently was phylogenetically isolated from Colombia, *Sticta* sp. (MT526263) Sérusiaux (Fig. 1). Several other undescribed species share this clade from Mexico and Colombia that are not included in this analysis and are currently under study (Lücking, in preparation). However, when compared to sequence data in GenBank, *S. puebloensis* had high pairwise identity with the following species: *Sticta* sp. (MT526263) Lindgren et al. (2020) at 97.5%, *Sticta fuliginoides* (KT281732) Magain & Sérusiaux (2015) at 95.0%, *Sticta* sp. (MG367395) Lindgren et al. (2020) at 95.8%, *Sticta* sp. JAM-015 (MT553253) Dal Forno at 93.4%, and *Sticta tunjensis* (KC732521) Moncada & Lücking (2012) with a pairwise identity of 95.9%, respectively.

Our haplotype network analysis illustrates that *S. puebloensis* (indicated as a red node) differs by twelve substitutions from *S. tunjensis* (yellow node), twenty from *S. fuliginoides* (green nodes), five from *Sticta* sp. MT526263 (blue node) and twenty-three from *Sticta* sp. MG367402 (orange node) (Fig. 4). The key elements of this haplotype network include node sizes (circles), which indicate the proportional number of sequences; branch lengths, which represent evolutionary divergence; and dashes on branches, which denote the number of mutational changes. The moderate genetic distance and clustering of *S. tunjensis* suggest it is on a distinct evolutionary trajectory, representing a separate species. However,


Pseudocyphellaria citrina PQ168919 Oregon USA
Sticta sp. MT526263 Azores
0.99/*/90
Sticta tunjensis KC732521 Colombia
0.97/77/82
Sticta puebloensis OP984122 Arizona USA
0.89/84/98
Sticta puebloensis OP984124 Colorado USA
Sticta puebloensis OP984123 Colorado USA
Sticta puebloensis OP984116 New Mexico USA
Sticta puebloensis OP984120 Arizona USA
Sticta puebloensis OP984117 New Mexico USA
Sticta sp. MG367402 Costa Rica
1/100/100
Sticta catharinae OP250135 Bolivia
Sticta catharinae OP250134 Bolivia
Sticta arbusculofuliginosa MG367404 Colombia
Sticta fuliginoides KT281722 Canary Islands
0.97/100/100
Sticta fuliginoides KT281729 France
Sticta fuliginoides AY173387 USA
Sticta fuliginoides KT281732 Ireland
Sticta fuliginoides KT281738 United Kingdom
Sticta aff. ambavillaria KC732568 Brazil
1/100/100
Sticta arbuscula KC732569 Ecuador
Sticta arbuscula KC732603 Colombia
Sticta arbuscula KC732502 Columbia
1/100/100
Sticta gyalocarpa KC732455 Colombia
Sticta gyalocarpa KC732577 Colombia
Sticta hirta KC732513 Colombia
0.83/*/*
Sticta hirta KC732563 Colombia
0.83/*/*
Sticta minutula KC732511 Colombia
Sticta minutula KC732719 Colombia
1/100/100
Sticta arenosella MH374894 Washington USA
Sticta torii MH374891 Oregon USA
0.83/*/*
Sticta torii MT183695 Oregon USA
Sticta torii MH374893 Oregon USA
Sticta torii MH374892 Oregon USA
0.99/100/93
Sticta ciliata KT281716 Ireland
Sticta ciliata KT281718 France
1/100/100
Sticta ciliata KT281713 Spain
.99/94/96
Sticta ciliata KT281714 France
Sticta hawaiiensis MT132672 Hawaii USA
0.96/99/95
Sticta hawaiiensis MT132716 Hawaii USA
1/100/100
Sticta harrisii MN065838 Puerto Rico
Sticta harrisii MN065835 Puerto Rico
*/100/90
Sticta harrisii MN065839 Puerto Rico
Sticta fuscotomentosa KC732661 Colombia
0.99/100/89
Sticta papillata KC732551 Colombia
Sticta papillata KC732552 Colombia
Sticta aff. subtomentella KC732615 Colombia
1/99/100
Sticta aff. subtomentella KC732729 Colombia
Sticta aff. subtomentella KC732730 Colombia
0.98/83/83
Sticta dilatata KC732648 Colombia
Sticta dilatata KC732647 Colombia
Sticta macrocyphellata KC732662 Colombia
Sticta lumschiana KC732578 Colombia
0.99/85/82
Sticta lumschiana KC732743 Colombia
Sticta lumschiana KC732575 Colombia
0.99/99/98
Sticta leucoblepharis KC732716 Colombia
1/93/93
Sticta leucoblepharis KC732664 Colombia
Sticta tomentosa OP999400 Kenya
1/97/97
Sticta antoniana MT132720 Hawaii USA
0.99/88/89
Sticta antoniana MT132721 Hawaii USA
Sticta plumbicolor MT132635 Hawaii USA
Sticta plumbicolor MT132641 Hawaii USA
Sticta tomentosa MT132667 Hawaii USA
0.05

the relatively low number of mutations compared to more distant lineages (*Sticta* sp. MT526263, *Sticta* sp. MG367402, and *S. fuliginoides*) indicates that the divergence between *S. puebloensis* and *S. tunjensis* is relatively recent. The direct branch connection further reflects their shared ancestry and recent divergence, most likely driven by geographical and ecological isolation, which has allowed the populations to accumulate genetic differences over time.

Discussion

This study represents an ongoing taxonomic revision of the genus *Sticta* from the Americas (McDonald et al. 2003; Galloway & Thomas 2004; Moncada 2012; Moncada et al. 2013a, b, c; 2014; 2015; 2018; 2020 and 2021; Suarez & Lücking 2013; Lendemer & Goffinet 2016; Simon et al. 2018; Dal Forno et al. 2018; Mercado-Díaz et al. 2020; Torres et al. 2021; Ossowska 2021; Ossowska et al. 2022a, b, 2024; Di Meglio & Goward 2023; Yánez-Ayabaca et al. 2023; Moncada et al. 2023). In the present study, we have accomplished a broad sampling of Lobarioid lichens from the Southwest USA, which has revealed an unforeseen species richness.

We conclude that *Sticta puebloensis* is a unique species and is not synonymous with *S. fuliginosa* or *S. sylvatica* as presented in Galloway & Thomas (2004), and in effect is more closely related to a species group that is still being studied based upon the phylogenetic relationships shown in the ITS topology (Fig. S1) and the concatenated topology (Fig. 1), thallus morphology (Fig. 2A–H), unique ecological requirements, and geographic isolation from a closely related phylospecies *S. tunjensis* (Fig. 3). As seen in our phylogeny, *Sticta puebloensis* shares a close evolutionary proximity to *S. tunjensis* with approximately 14 insertions and 8 deletions when aligned and compared. They also differ in the ITS haplotype network analysis by 12 substitution sites (Fig. 4). A pairwise comparative analysis was conducted between *S. puebloensis* and *S. tunjensis* ITS sequences, and based upon the analysis output, they share 95.9% similarities within the ITS.

We were able to compare morphology between *S. puebloensis* and *S. fuliginosa* s.str. from Eastern North America (Brinker 5335 PP291711, Brinker 6179 PP29712 and Anderson 17132 PP315673; Table 1) and *S. sylvatica* s.str. from Norway (Knutsen L105706 OP782381; Table 1). *Sticta puebloensis* differs in morphology significantly from both *S. fuliginosa* and *S. sylvatica. Sticta fuliginosa* s.str. is typical of having layered, palmate to flabellate lobes when immature, soon becoming suborbicular at maturity. The upper surface is usually smooth, scrobiculate to faveolate with abundant laminal isidia more or less evenly dispersed, but commonly developing on ridges of the upper surface. Cyphellae are abundant, round to angular lacking pronounced pore membrane with an erect margin, with smooth cyphellary basal membrane (Magain & Sérusiaux 2015 and Ekman et al. 2019). Whereas *S. sylvatica* s.str. is typical of rounded to fan-shaped thalli, with lacinate to slightly flabellate, concave lobes that overlap each other, dichotomously branched. The upper surface is typically uneven, foveolate to scrobiculate, with shallow reticulations on the ridges, dark brown when moist, glossy, maculae not seen. Isidia abundant and always present, globulose in shape forming coralloid masses in maturity; developing primarily on lobe margins and/or on ridges, often covering large portions of the upper surface of the thallus. The lower surface with dense primary tomentum, brown to dark brown often becoming black and spongy with maturity. The cyphellae are more or less abundant, at times more or less aggregated to dispersed, rounded to slightly irregular in shape, with a smooth cyphellary basal membrane (Magain & Sérusiaux 2015 and Ekman et al. 2019).

We can compare this to *S. puebloensis*, which has a general morphological appearance of *Sticta sylvatica* s.str. rather than *Sticta fuliginosa* s.str., neither of which are confirmed in the Southwest USA region. *Sticta puebloensis* differs in the presence of dense laminal branched to corymbose isidia, suborbicular to elongate lobes, lobe margins entire, cream to tan marginal tomentum becoming dark brown interior primary tomentum, with a papillate cyphellary basal membrane and all chemical reactions negative (Fig. 2A–H).

However, in our attempts to compare morphology between *S. puebloensis* and *S. tunjensis*, we were unable to obtain any confirmed specimens of *S. tunjensis*. We relied on the formal description and photos from Moncada & Lücking (2012) to compare key differences between *S. puebloensis* and *S. tunjensis*. The morphological differences based upon the description are striking and it would be nearly impossible to confuse the two in−situ. *Sticta tunjensis* has the general morphology of "strap-like" marginal isidiate cyano-lichens, similar to the *Sticta weigelii* (Ach.) Vain. morphodeme (Moncada et al. 2021b; Ossowska 2021), with predominately marginal isidia, flabellate lobes, pale ventral tomenta throughout, a distinct maculate laminal surface, grey green to brownish thalli, marginal cilia and a medulla K+ Yellow reaction (Moncada & Lücking 2012).

Sticta puebloensis and *S. tunjensis* differ in ecological requirements and are separated by a large geographical distance, thus existing in completely different ecosystems. *Sticta tunjensis* is found in the tropical montane sub-andine forests on soil or as an epiphyte associated with *Plagiochila* sp. (Moncada & Lücking 2012). It is found between elevations of 1,900–3,080 meters in the Cordillera Oriental, which is in the neotropical ecoregion of the northern South American countries of Colombia and Venezuela (Van der Hammen 1989; Moncada & Lücking 2012; Martini et al. 2017). In comparison, *S. puebloensis*.

Figure 1. Concatenated 1,000 bootstrap single most likely PhyML+ GTR topology with Baysian and RAxML inference of 4 loci: ITS, nuLSU, mtSSU, and RPB2_sticta. Specimens with a Genbank accession number only have ITS data and specimens with the original collection voucher numbers are concatenated sequences and can be found in the S1-Table. The bootstrap scores are as followed, Baysian/RAxML/PhyML; an asterisk (*) indicates a bootstrap score below 70%, respectively.

is found in mixed deciduous and coniferous forests growing on moss over acidic rock distributed throughout high elevation mountain ranges between 2,150–3,000 meters. It is found on north facing aspects and within deep canyons that have high humidity due to proximity to flowing water and or waterfall spray zones. These sites are found in the southwestern high deserts, the mid to lower Rocky Mountains and the northern most sky islands of the Madrean Archipelago of North America.

In conclusion, *Sticta* is a speciose genus with an incredible amount of diversity comprised of cryptic species that in the past were subsumed in other species complexes. Lichenologists have better taxonomic tools to elucidate cryptic species from these complexes that have evolved morphological similarities. The work by Galloway and Thomas (2004) of desert *Sticta* species created the foundation for future lichen studies in the regions of northwestern Mexico and the Southwest USA. The application of molecular tools and the pivotal morphological assessment by Bibiana Moncada thesis and Moncada et al. studies in *Sticta*, have given new insight on *Sticta* cryptic morphology, evolution, and diversity. It is clear with our broad sampling and specimen loans that several unknown species still exist from the *Sticta fuliginosa* morphodeme in the Southwest United States and Northern Mexico and further field sampling and study is required.

Taxonomy

Sticta puebloensis Di Meglio & Niedbala, sp. nov. (Fig. 2A–H)

Diagnosis: Species resembling *Sticta fuliginosa* s.str., but differs by elongated lobes, dense marginal to laminal corymbose isidia, cyphellary membrane papillate with budding cells. Dense shaggy ventral primary tomentum, cyphellae with distinct pore membrane.

Type: USA, New Mexico, San Miguel Co., Gallinas Canyon; mixed coniferous and deciduous forest; on granite cliffs in wet seeps with dense bryophyte cover on northern exposer adjacent to Gallinas Creek, 35°42′29.052″N, 105°27′1.4616″W, alt. 2,360 m, 20 Sep. 2019, J. Di Meglio & J. Niedbala 241 (OSC – holotype!; archival packet; UNM – isotype!; archival packet).

MycoBank MB 856971

ITS BARCODING SEQUENCE ACCESION: OP984117 (holotype)

Description. Primary photobiont cyanobacterial (*Nostoc*). Stipe absent. Thallus foliose, polyphyllus, irregular in appearance, radially spreading, incised to branched; thallus medium to large 2–6(–8) cm in diameter, overlapping, brittle when dry, subpendulous. Lobes: suborbicular when immature, becoming elongate and somewhat strap-like with maturity, irregular, branched, involute, 2.8–3.5(–6) cm long, 0.5–1.5(–2) cm wide, branched. Margins: entire or at times with small incisions, cilia sparse, isidia may or may not be present. Upper surface: isidiate, soredia not seen, rarely producing phyllidia, glabrous and smooth on younger portions of lobes, becoming slightly wrinkled or scrobiculate to shallowly pitted or foveolate towards center/older portions of thallus, light brown to dark brown with darkened margins when dry, greenish black to greyish blue-black when wet, maculae absent. Isidia: small to medium 0.2–0.5(–0.75) mm in diam. and 0.2–0.35(–0.45) in height, sooty appearance when clustered, loosely coralloid to densely branched to tightly corymbose, becoming distinctly stalked (isidaliate), darker than upper surface, brownish-black to blackish-grey, glabrous to glossy; abundant, forming at lobe margin and becoming densely clustered approximately 1–2 cm from margin, becoming dispersed often collecting on thalline ridges, becoming sparse towards center, some isidia scaring, but uncommon. Lower Surface: white–cream to buff, becoming brown to dark brown near center, smooth to slightly foveolate, densely tomentose, cyphellae common. Tomenta: dense, beginning at lobe margins and contiguous throughout lower surface, two types: primary and secondary, primary tomentum fasciculate of 10–20 strands of hyphae with free apices, 0.25–0.45(–0.5) mm in length; secondary tomentum is fine, pubescent, short ranging from 25–60(–100) μm and 2–5 cells in length. Cyphellae: common, scattered throughout lower surface, beginning as very small almost "pin prick" like at lobe margins, becoming larger centrally, 0.1–0.4(–0.7) in diam., round, rarely angular or irregular, basal membrane deeply nested into the thallus below lower cortex, distinct hairless membrane partially covering the opening creating a distinctive pore, basal membrane with sparse single papillae and budding cells. Upper cortex: paraplectenchymatous, 30–40(–43) μm thick, comprised of 3–4 cells, top cell layer is compressed and are smaller than cells beneath 4–6(–8) μm in diam., cell layers underneath are between 6–12 μm in diameter with cell wall 1 μm thick. Photobiont layer: 55–65(–68) μm thick, individual photobiont cells 8–15 μm in diameter. Medulla: 90–115(–120) μm lax hyphae at 1–1.5 μm wide. Lower cortex: paraplectenchymatous, 55–60(–63) μm thick with 4–5 cell layers, individual cells 5–15 μm in diameter with 1 μm thick cell wall.

Etymology. The name "Puebloensis" is derived from the Spanish name for village or "Pueblo" to which the indigenous peoples of the Southwestern states of North America are named. The name Puebloensis refers to the habitat *Sticta puebloensis* often associates with, deep canyons with perennial or seasonal water flow where the first and contemporary Puebloan peoples made their homes.

Ecology and substrate. *Sticta puebloensis* is rare, but locally common in high elevation mesic/humid/moist/seasonally wet microhabitats. Typically found at elevations from 2,133 meters to 2,499 meters in Colorado and New Mexico, and 2,590 meters to 2,895 meters in Arizona. Found growing on north aspect or deeply shaded acidic rock seeps with dense bryophyte coverage and high diversity of cyano-lichens. Also found in high elevation canyons within meters of flowing water near creeks, rivers, and waterfall spray zones.

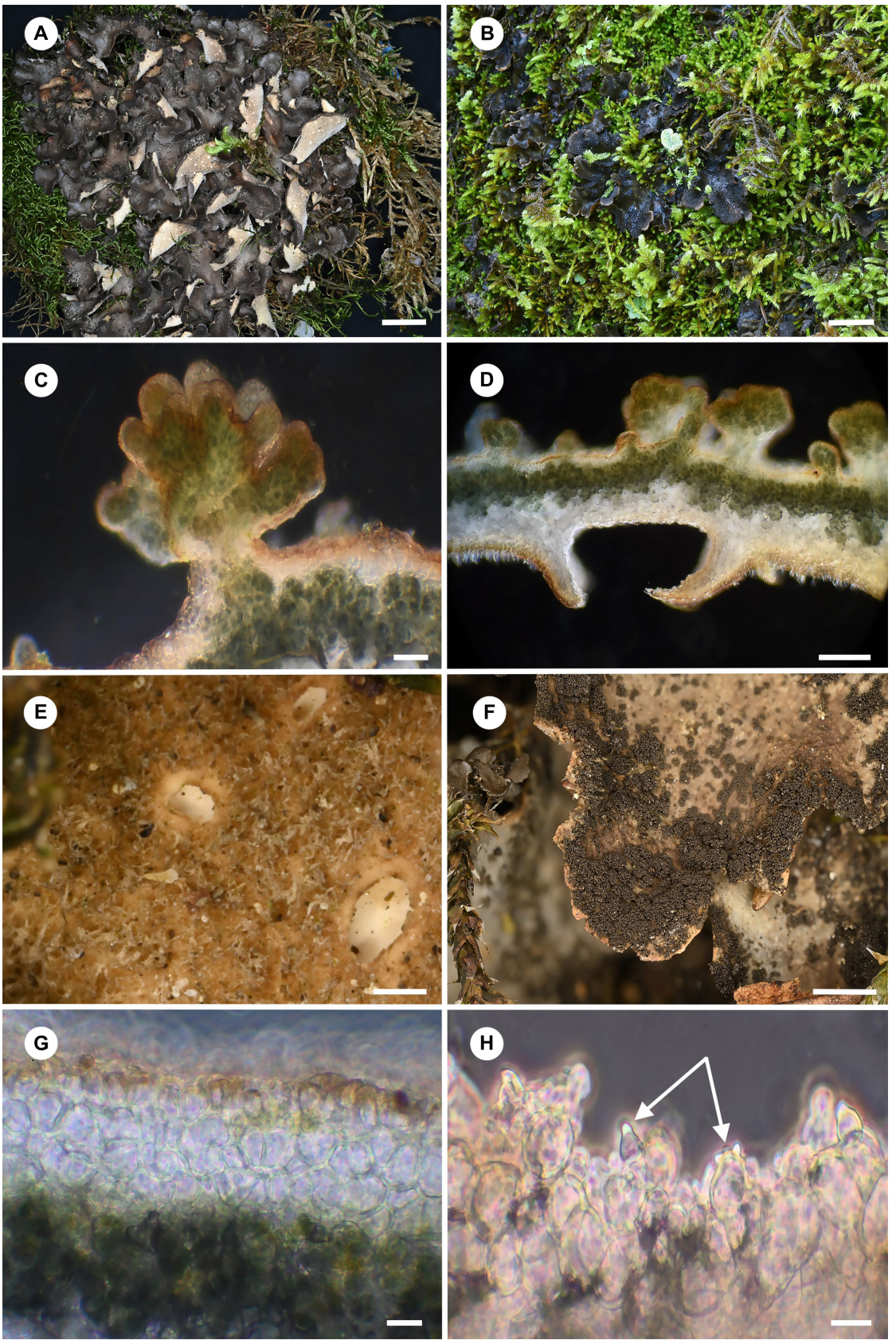


Figure 2. A – *Sticta puebloensis* habit; B – *Sticta puebloensis* holotype in-situ New Mexico, USA; C – laminal mature isidium, compact corymbose morphology with distinct isidalia at base of isidia; Dark Field; Magnification: 20×; D – thallus cross section with view of stratified thallus organization of upper cortex, photobiont dispersal, lax medulla hyphae, and lower cortex; deeply immersed cyphellae with basal membrane nested below lower cortex and distinct hairless membrane that creates a "pore" opening of cyphellae (arrow); Dark Field; Magnification: 10×; E – ventral surface with examples of coarse primary tomentum and distinct cyphellae hairless pore membrane; magnification: 2:1; F – isidia dispersal on laminal surface with aggregation of isidia from lobe margin to approximately a cm on to the interior of lobe; G – cross section of upper cortex showing the darker compressed top layer of cortical cells compared to larger cells beneath; Phase Contrast; Magnification: 40×; H – cyphellae basal membrane with single papillate and budding cells (arrows); Phase Contrast; Magnification: 40×. Scales: A = 1 cm; B = 2 cm; C = 10 µl; D = 200 µl; E = 0.25 mm; F = 0.5 cm; G = 10 µl; H = 5 µl.

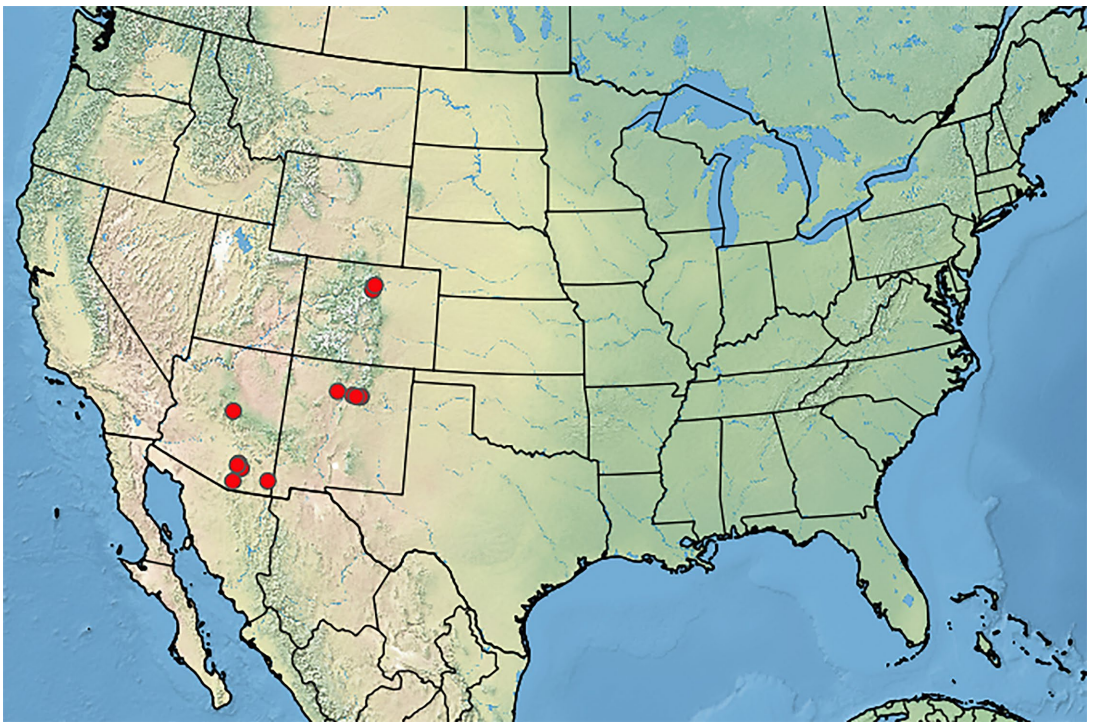


Figure 3. Distribution of *Sticta puebloensis* sp. nov. in the southwestern states of the USA North America.

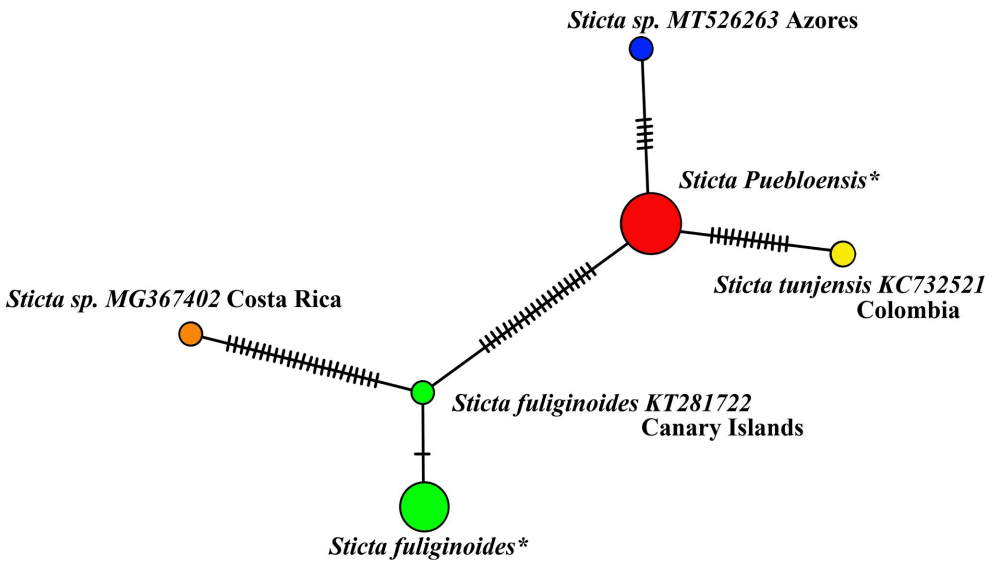


Figure 4. Median-Joining haplotype network model. Species with asterisk, location data can be found in the S1 table in the supplementary files. The branch length and the number of "dashes" indicate mutational changes and divergence. The diameter of the node is proportional to the number of individual ITS sequences in each haplotype. *Sticta puebloensis* sp. nov., red node: 6 sequences, *Sticta tunjensis*, yellow node: 1 sequence, *Sticta fuliginoides*, green node: 4 sequences, *Sticta* sp. MT526263, blue node: 1 sequence: Sérusiaux and *Sticta* sp. MG367402, orange node: 1 sequence.

Distribution. *Sticta puebloensis* is endemic to high elevation mountain canyons and Sky Islands of the American Southwest in the states of Arizona, Colorado, and New Mexico.

Similar species. There are approximately fifteen species of *Sticta* recognized in North America, not including Mexico, and seven of those species have laminal isidia and were once part of the *Sticta fuliginosa* morphodeme. There are five laminal isidiate species from the western portion: *S. arenosella* Di Meglio & Goward, *S. fasciculata* Di Meglio and Goward, *S. globulifuliginosa* Moncada & Lücking, *S. gretae* Goward & Di Meglio and *S. torii* Simon & Goward (Simon et al. 2018; Di Meglio & Goward 2023) and two laminal isidiate species from the eastern portion: *S. fuliginosa* (With.) Ach. s.str. and *S. fuliginoides* Magain & Sérusiaux (McDonald et al. 2003; Hodkinson et al. 2014; Magain & Sérusiaux 2015) of the USA and Canada that could be mistaken for *Sticta puebloensis*. The overall gross morphology may be initially confusing and possible taxonomic mistakes could be made. However, morphological characters, geographical boundaries, substrate and specific ecological requirements keep the other laminal isidiate species isolated from *S. puebloensis*, apart from *S. gretae* that has small disjunct population in the Sacramento Mountains in southern New Mexico (Table 1). *Sticta puebloensis* and *S. gretae* are easily distinguishable from each other solely based upon morphological characters and substrate. *Sticta puebloensis* is primarily saxicolous, musicolous over acid rock or on rare occasions

terricolous (Fig. 2A–B). The lobe morphology is elongate, isidia distribution is primarily marginal, submarginal to laminal, with dense shaggy ventral tomentum (Fig. 2C–F). Basal cyphellary membrane cells of *S. puebloensis* are papillate and are relatively evenly distributed across the membrane (Fig. 2H). Whereas compared to *S. gretae* which has sparse basal membrane papillate cells that differ in shape and are more "nodular". *Sticta gretae* has only been observed as being corticolous in New Mexico and the lobe morphology is quite different from *S. puebloensis* being more orbicular to suborbicular, isidia distribution is primarily laminal with marginal lobules on mature thalli; lacking primary tomentum and is extremely rare in New Mexico with only one known population. For further details on morphology for *S. gretae* please reference Di Meglio & Goward 2023. Within the southwestern region of North America, *Sticta puebloensis* would be nearly impossible to mistake with any other macro lichen and is the only one of two laminal isidiate species of *Sticta* in the region. However, with an untrained eye, confusion could occur between *S. puebloensis* and *Leptogium* sp. or *Leptogium santerninum* (Dicks.) Nyl. group due to superficial morphological similarities and shared habitat. The main differences between *L. santerninum* group from *S. puebloensis* 1) *Leptogium* species are homoiomerous and lacks a stratified thallus and is technically a "jelly-lichen". Whereas *S. puebloensis* sp. nov. has a typical heteromerous thallus architecture that includes and upper cortex, photobiont layer, medulla, and lower cortex (Fig. 2D, G). 2) *Leptogium* species lack true cyphellae, The tomentum is quite long and fine, whereas *S. puebloensis* creates a rather thick dark brown "shag carpet" layer of fasciculate primary tomentum with a shallow layer of secondary tomentum.

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Supplementary electronic materials

Figure S1. Global ITS single locus 1,000 bootstrap PhyML+ GTR topology topology. Download file

Figure S2. Concatenated 1,000 bootstrap RAxML + GTR CAT with inference of 4 loci: ITS, nuLSU, mtSSU, and RPB2_sticta. Specimens with a Genbank accession number only have ITS data and specimens with the original collection voucher numbers are concatenated sequences and can be found in the Table S1. Download file

Figure S3. Concatenated Bayesian analysis with inference of 4 loci: ITS, nuLSU, mtSSU, and RPB2_sticta. Specimens with a Genbank accession number only have ITS data and specimens with the original collection voucher numbers are concatenated sequences and can be found in the S1-Table. Download file

Table S1. Species sequence list used in the study. Species with asterisks indicate their inclusion in the haplotype network and species highlighted in bold were used in the concatenated analysis, which include GenBank ITS, nuLSU, mtSSU, and RPB2_Sticta sequences, references, voucher number, and the location where the specimen was collected. Download file

Table S2. Gene markers, Primers and thermal cycle protocols used in the study. Download file

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