Sticta torii sp. nov., a remarkable lichen of high conservation priority from northwestern North America

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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** (6): 105–114. Oslo. ISSN 2002-4495.

Sticta torii Simon & Goward sp. nov. is an isidiate lichen on Alnus, Malus and Picea branches in hypermaritime regions of northwestern North America. It is a rare species, currently known from only a few localities along a narrow longitudinal range in Southeast Alaska, British Columbia and Oregon. It differs from S. fuliginosa and S. sylvatica in its smaller, more irregular lobes and generally well-developed fringe of marginal isidia, and from S. beauvoisii in its smaller size, less elongate lobes and distinctly arbuscular to penicillate marginal isidia. Also diagnostic are the cyphellae, the basal portions of which bear cells with numerous papillae – a feature shared with a small group of closely allied neotropical species including the recently described S. arbuscula and S. arbusculotomentosa. These latter species, however, bear tomentum over the upper surface, unlike S. torii, which is glabrous.

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Introduction

The lichen-forming fungal genus *Sticta* (Schreb.) Ach. (Peltigerales, Lobariaceae) is primarily tropical to subtropical in distribution and encompasses more than 200 recognized species (Lücking et al. 2016). Eleven species are currently reported for North America (Esslinger 2016), excluding *S. oroborealis* Goward & Tønsberg and *S. wrightii* Tuck. which have recently been transferred to the segregate genus *Dendriscosticta* (Moncada et al. 2013). Here we formally describe *Sticta torii* Simon & Goward sp. nov., currently known from only one locality each in hypermaritime Alaska (USA) and British Columbia (Canada) and three localities in Oregon (USA).

Material and Methods

The present study is based on detailed examination and ITS rDNA sequencing of five vouchers, viz., Dillman 2003-911 (CONN), Di Meglio 135/146/156 (OSC) and Goward 02-179 (UBC). Three morphologically distinct samples (Björk 8443/19815 and Di Meglio 115) were found to be genetically similar, and were integrated in the molecular analysis. However, these samples were not included in the following taxonomic treatment and are hereinafter referred to as "S. aff. torii". Morphological features were studied using a dissecting microscope (Olympus SZ60), and a compound microscope (Olympus BX53) equipped with an AmScope camera MU900. Macromorphological structures of the holotype (Dillman 2003-911) were photographed using a Macroscopic Solutions Macropod Pro (Macroscopic Solutions, LLC, CT, USA) coupled with a Canon 6D camera. The resulting pictures were processed in Zerene Stacker (Zerene Systems LLC, Richland, Washington, USA). Microscopic measurements were made at 400× magnification in water. Descriptive terminology and layout follows the standardized approach developed by Moncada et al. (2014).

Secondary chemistry was investigated through thin layer chromatography (TLC) using solvent G following Orange et al. (2001), and standard spot tests as described by Brodo et al. (2001).

DNA was isolated using Nucleospin Plant II Midi kits (Macherey-Nagel, Bethlehem, Pennsylvania, USA) following the manufacturer's guidelines. The internal transcribed spacer (ITS was amplified and sequenced using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The PCR conditions were as follows: 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, and 70°C for 1 min, with a final extension of 70°C for 10 min. PCR products were cleaned using the ExoSAP-IT protocol (USB Corporation, Cleveland, Ohio, USA) and sequenced on a ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). BLAST search (Altschul et al. 1997), using the MegaBLAST algorithm, was carried out at the NCBI website against the nt database.

In order to assess the phylogenetic affinities of S. torii and S. aff. torii to other Sticta species, we first constructed a single-locus ITS dataset by including the eight newly generated DNA sequences and all ITS sequences used in the comprehensive study on Sticta conducted by Moncada et al. (2014). Sequences were aligned using MAFFT run in auto mode (Katoh et al. 2002; Katoh et al. 2009) and eventually manually adjusted using Geneious 10.0.7 (Biomatters Ltd., Auckland, New Zealand). The matrix was submitted to the GUIDANCE webserver (Penn et al. 2010a,b) in order to identify ambiguously aligned regions; regions with confidence scores below 0.93 were removed, which resulted in a 805 bp alignment (initially 1,286 bp). We conducted a maximum likelihood analysis using RAxML 8.2.3 (Stamatakis et al. 2008, Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010; www.phylo.org) using the rapid hill-climbing algorithm and bootstrapping with 1000 pseudoreplicates under a GTR+G model of evolution. After determining the phylogenetic placement of the eight newly generated sequences within Sticta, we pruned the alignment to only members of the S. fuliginosa clade (sensu Moncada et al. 2014), with a single sequence retained per species, and selected S. humboldtii and S. limbata as outgroups. Sequences were submitted to the GUIDANCE webserver and realigned using PRANK (Löytynoja and Goldman 2005, 2008). Since the resulting matrix contained relatively few ambiguous portions (GUIDANCE alignment score: 0.965136), the matrix was analyzed with all sites included (677 bp). A maximum likelihood analysis was performed for this subset using RAXML 8.2.3 with the same parameters as described above. The resulting tree was visualized with FigTree 1.4.3 (Rambaut 2012).

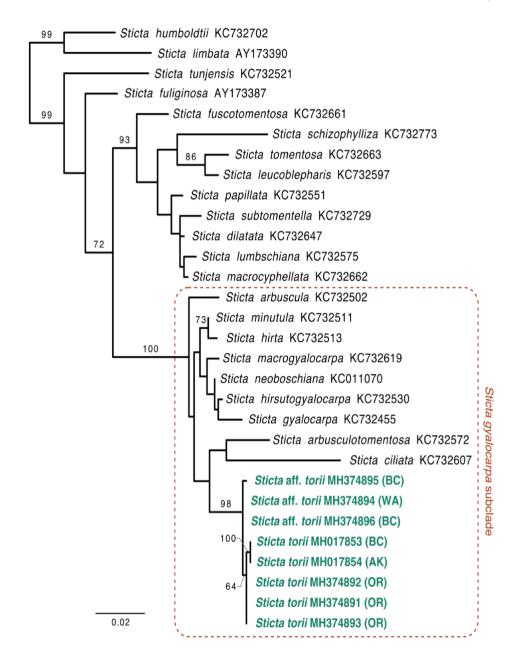


Figure 1. Single most likely phylogenetic tree based on a single-locus ITS dataset of the *Sticta fuliginosa* clade *sensu* Moncada et al. (2014), with *S. humboldtii* and *S. limbata* as the outgroup. Bootstrap values of ≥70% are indicated above branches. *Sticta torii* and the *S. gyalocarpa* subclade are highlighted. Locality abbreviations: Alaska (AK), British Columbia (BC), Washington (WA).

Results and Discussion

The five newly generated sequences of *S. torii* (ITS accessions: MH017853, MH017854, MH374891, MH374892 and MH374893) are 100% identical over their overlapping length, except for the two northernmost specimens (MH017853 and MH017854), which differed from the other samples by one substitution. *Sticta torii* and *S.* aff. *torii* differed from each other only in 1 or 2 substitutions plus one indel. While this small genetic difference is congruent with ecology and morphology (*S. torii*: hypermaritime, presence of arbuscular, marginal isidia; *S.* aff. *torii*: not hypermaritime, absence of arbuscular, marginal isidia), we could not completely rule out that *S. torii* and *S.* aff. *torii* are actually conspecific. The nomenclatural identity of *S. torii* will be anchored to the type, and the status of *S.* aff. *torii* will be dealt with in a forthcoming paper (Di Meglio et al. in prep.).

Comparisons against sequences available in GenBank indicated that the sequence of S. torii and S. aff. torii are 96% identical to that of S. minutula (KC732511), which represented the first BLAST match. Our phylogenetic inferences resolve with 98% BS support the eight samples as a clade in a strongly supported lineage within the S. fuliginosa clade, i.e., in the S. gyalocarpa subclade (sensu Moncada et al. 2014; Fig. 1), which includes species with rather variable morphologies: S. arbuscula Moncada & Lücking, S. arbusculotomentosa Moncada & Betancourt, S. ciliata Taylor, S. gyalocarpa (Nyl.) Trevis., S. hirsutogyalocarpa Moncada & Lücking, S. hirta (Nyl.) Trevis., S. macrogyalocarpa Moncada & Lücking, S. minutula Moncada & Lücking, and S. neoboschiana Moncada & Lücking. Currently, the S. gyalocarpa subclade consists only of neotropical species, with the exception of S. ciliata, which occurs also in Africa, Macaronesia and Western Europe (Moncada et al. 2014; Magain and Sérusiaux 2015). A diagnostic feature common to all of these species including S. torii is the presence, in the basal cyphellary membrane, of dehiscent cells bearing abundant papillae, as noted by Moncada (2012); this character had first been described by Vainio (1890) for S. laevis (Nyl.) Vain., but subsequently overlooked. The subclade contains two species, S. arbuscula and S. arbusculotomentosa, that are morphologically similar to S. torii in the presence of marginal, arbuscular isidia that, at maturity, branch upward from distinct, partly cyphellate stalks (Fig. 2); however, whereas the upper cortex of S. torii is glabrous, that of these species is distinctly tomentose.

Sticta torii can hardly be confused with any other species occurring in North America. The morphologically most similar species in the region is S. beauvoisii Delise, which is readily distinguished macroscopically by its larger size (2–5 cm diam.), cylindrical to coralloid isidia that lack a distinct basal stem, and microscopically by the non-papillate cells of the cyphellary basal membrane. Below we provide a key for distinguishing S. torii from other species of Sticta in northwestern North America.

Taxonomy

Sticta torii Simon & Goward, sp. nov.

MycoBank: MB826709

Diagnosis: Differing from *Sticta fuliginosa* in the arbuscular to penicillate isidia arising predominantly from the lobe margins, and from *Sticta beauvoisii* in the presence of abundant papillae on the cells of the cyphellary basal membrane.

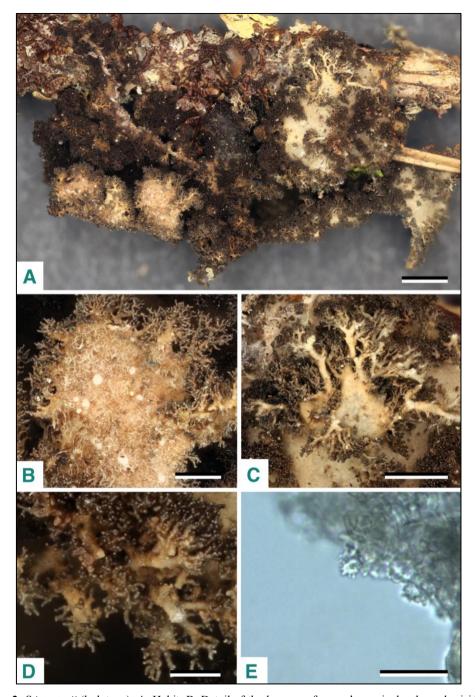


Figure 2. *Sticta torii* (holotype). A. Habit. B. Detail of the lower surface and marginal, arbuscular isidia. C. Detail of propagule stalks and isidia. D. Detail of coralloid/arbuscular isidia. E. Detail of papillae on cells of the cyphellary basal membrane. Bars: 3 mm (A), 0.5 mm (B), 1 mm (C), 0.25 mm (D), $25 \text{ }\mu\text{m}$ (E).

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Type: **U.S.A.** *Alaska*: Alexander Archipelago, Tongass National Forest, Coronation Island, Egg Harbor, Nation Point, beach fringe forest edge, on rocky headland at the point of entry into harbour, exposed to Chatham Straits NE aspect, NAD 27: 55°55.372' N, 134°19.596' W, elev. < 3 m, twigs of *Picea sitchensis*, 2003-08-12, K. Dillman 2003-911 (CONN 00225971, holotype [GenBank: MH017854, ITS]).

Description: Primary photobiont cyanobacterial (Nostoc). Stipe absent. Thallus irregular in outline, up to 1.5–2.0 cm diam., uni-lobed to at length becoming polyphyllous, brittle, rather fragile. Lobes rounded in outline to more often short-elongate, ascending except often weakly downturned toward the tips, imbricate; secondary lobes often involute; margins sometimes bearing expanded dorsiventral outgrowths of the isidia (and then appearing lacerate), not thickened; lobe internodes 1-4 mm long, 2-7 mm broad. Upper surface plane, grey or medium to dark brown when fresh, not much changing in herbarium, matt to somewhat shiny; margin concolorous; surface without papillae, pruina and cortical hairs, but occasionally with sparse maculae toward lobe tips. Cilia absent. Apothecia not observed. Isidia present, abundant, principally marginal, often continuous along margin, at times also laminal, and then branching upward, at length becoming arbuscular from a well-developed stalk; isidial 'branches' spreading and vertically oriented, up to 0.5 (0.6) mm long and 0.1–0.2 mm broad, darker than the thallus, grevish to more often dark brown, shiny, round in section, except basal portions of stalk often somewhat dorsiventral. Medulla rather compact, white, K-, C-, KC-, P-. Lower surface even, cream to brown-colored. Lower tomentum dense, sparse toward margin, thin, pubescent, soft, cream to brown-colored. Rhizines absent, Cyphellae sparse, 41–60 per cm², scattered throughout, rounded to irregular, urceolate with a wide pore, erumpent to occasionally plane, white to pale cream, without tomentum; pore 0.1-0.5 (-1.0) mm diam.; basal membrane smooth, sometimes pruinose, white, K-, C-, KC-, P-.

Upper cortex paraplectenchymatous, $25-\overline{55}$ µm thick, homogeneous, consisting of 2–4 cell layers with cells 8–14 µm diam., their walls 1–3 µm thick and their lumina rounded to isodiametric, 6–12 µm diam. Photobiont layer 35–65 µm thick, its cells 9–12 µm diam. Medulla 110–240 µm thick, its hyphae 3.5 µm broad, without crystals. Lower cortex paraplectenchymatous, 25–45 µm thick, with 3–4 cell layers; cells 7–13 µm diam., their walls 1–3 µm thick. Hairs of lower primary tomentum 60–150 µm long, in fascicles of 6–12, unbranched, cylindrical hyphae with intertwined apices; occasionally moniliform when close to margins, appearing like a rosary of isodiametric cells. Cyphellae cavity 60–135 µm deep; cells of basal membrane most or less isodiametric, 6–8 µm wide, with papillae.

Chemistry: No substances were detected by TLC.

Etymology: This taxon is named in honor of our friend and colleague Tor Tønsberg on the occasion of his 70th birthday and in recognition of his outstanding, vigorous contribution to lichenology (Fig. 3B).

Ecology and distribution: Sticta torii is so far known as growing on the outermost branches of Alnus rubra, Malus fusca and Picea sitchensis in beach fringe localities more or less exposed to storms and the open ocean. It appears to be restricted to hypermaritime areas in which subfreezing temperatures are infrequent in winter. The Alaskan locality has been ice-free for approximately 16,000 years BP (Lesnek et al. 2017). Co-occurring epiphytic lichens at the British Columbia site include Arctomia borbonica, Cavernularia hultenii, Collema furfuraceum, Erioderma sorediatum, Fuscopannaria laceratula, F. leucostictoides, Lobaria anomala, Lobaria anthraspis, Nephroma laevigatum, Pannaria malmei, Pseudocyphellaria "mallota", P. hawaiiensis, Sticta limbata and Usnea longissima. The two paratype specimens from BC were collected at the same locality in

different years on dwarfed *Picea sitchensis* branches in a sheltered north-facing site just above high-tide line on a small rocky islet. Islets provide roosting sites for passerine birds – warblers, kinglets, sparrows, etc. – passing through in migration (Ian Cruickshank, pers. comm.) and may partly account for the occurrence there of a species not noted elsewhere in the area despite intensive searching.

Conservation: Based on available data, *S. torii* is exceedingly rare, apparently more so even than the federally endangered *Heterodermia sitchensis* Goward & Noble (COSEWIC 2006), another foliose lichen growing on *Picea sitchensis* in hypermaritime regions (Goward 1984). Whereas that species is currently known from eleven localities, *S. torii* is so far known from only five localities along a latitudinal range of c. 1300 km. Taken together, these observations qualify *S. torii* as endangered [EN: B1ab(iii)] under IUCN criteria (Scheidegger & Goward 2002; Dahlberg and Mueller 2011; IUCN 2012). It is worth noting that hypermaritime portions of southeastern Alaska and British Columbia have received considerable search effort for lichens (Geiser et al. 1998; COSEWIC 2010, Dillman et al. 2012, Spribille et al. in prep.). Thus, the endangerment status of *S. torii* is unlikely to be a mere artefact of insufficient documentation.

Comments: Recent phylogenetic studies on the genus Sticta have revealed unexpected taxonomic diversity (e.g., Moncada et al. 2014). In northwestern North America, the formal description of the new species S. torii leaves several further, morphologically similar species in the genus still undescribed. In a recent phylogenetic study on European members of the S. fuliginosa morphodeme (sensu Moncada et al. 2014), Magain and Sérusiaux (2015) included British Columbia material that had tentatively been assigned to S. sylvatica, but which appears to belong to an undescribed species that includes further unpublished accessions from North and South America and Macaronesia, plus one published accession from China (Lohtander et al. 2002; AY124095). Magain and Sérusiaux (2015) also called attention to a BC specimen of S. "fuliginosa" incorporated in the phylogeny of Cornejo et al. (2009; ITS accession DQ419943), which represents an additional, distinct lineage.

Additional specimens examined: CANADA, British Columbia: Tofino Area, 5 km south of town, beach fringe at forest edge, on rocky islet N aspect, 49°07'N, 125°54'W, elevation < 3 m, twigs of *Picea sitchensis*, 2002-06-21, T. Goward 02-179 (UBC [GenBank: MH017853]); Tofino Area, 5 km south of town, as above. 2001-07-25, T. Goward 01-438 (UBC); U.S.A., Alaska: Alexander Archipelago, Tongass National Forest, Coronation Island, Egg Harbor, east side, beach fringe forest edge, 55.909112° N, 134.313087° W, elevation 2 m, twigs of Malus fusca, 2009-07-24, K. Dillman 2009-115 (CONN, UPS). Oregon: Rock Creek Campground, immediate coast, approximately 16 km south of Yachats, Oregon, 44°11'03.46" N 124°06'44.87" W, elev. 9.7 m, riparian zone near Rock Creek, mixed forest of Picea sitchensis, Salix sp. and Alnus rubra, approximately 0.5 km from the Pacific Ocean, on Alnus rubra bole, approximately 1.5 m above ground, muscicolous and some attached directly to cork cambium, 2016-09-15, J. Di Meglio 135 (OSC; [GenBank: MH374891]); Cape Lookout Trail, immediate coastal headlands, 12.7 km south of Netarts, 45°20'37.84" N 123°58'41.79" W, elevation 267 m, mixed forest of Picea sitchensis, Pseudotsuga menziesii and Alnus rubra, on Alnus rubra bole directly over the Pacific Ocean positioned on a cliff, southern exposed site, muscicolous with some specimens attached directly to cork cambium, 2016-12-30, J. Di Meglio 146 (OSC; [GenBank: MH374892]); Cape Perpetua, immediate coastal headlands, off of Forest Service road #5553, 5 km south of Yachats, 44°17'14.75" N 124°06'18.98" W, elevation 236 m, late seral mixed forest of Picea sitchensis, Pseudotsuga menziesii, Tsuga heterophylla, and Alnus rubra, on large Alnus rubra bole sharing substrate with small population of Pseudocyphellaria rainerensis, attached to epiphytic bryophytes and micro-liverworts, 2016-12-31, J. Di Meglio 156 (OSC; [GenBank: MH374893]).

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Figure 3. A. Habitat of *Sticta torii* at the type locality, on Coronation Island (AK, USA). B. Tor Tønsberg (on Coronation Island), from whom *Sticta torii* derives its name. Photographs by K. Dillman.

Key to species of Sticta in northwestern North America

	Thallus sorediate (except older soralia occasionally somewhat isidiate), soredia essentially restricted to lobe margins, white-grey
2	Isidia more or less dispersed over upper surface; thallus up to 2–4 (–6) cm across
3	Thallus usually consisting of few sparsely branched, more or less rounded lobes
3	Thallus forming narrow, frequently branched, truncated lobes
4	Thallus small, up to 1–2 cm across; lobes not distinctly elongate; isidia coralloid to arbuscular-penicillate, in age supported by a well-developed basal stalk Sticta torii
4	Thallus larger, up to 2–5 cm across; lobes distinctly elongate; isidia cylindrical to coralloid, not supported by a well-developed basal stalk

Acknowledgments: This study was supported by NSF DEB-1354631 to BG. AS is a PhD student at the University of Liège and acknowledges financial support from FRIA, a grant of the Fonds de la Recherche Scientifique - FNRS (F.R.S.-FNRS). Curtis Björk and Olivia Lee are thanked for herbarium research in support of this study. We thank Robert Lücking for valuable comments that helped improve the manuscript.

References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipaman, T. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389–3402.
- Cornejo, C., Chabanenko, S., & Scheidegger, C. 2009. Phylogenetic analysis indicates transitions from vegetative to sexual reproduction in the *Lobaria retigera* group (Lecanoromycetes, Ascomycota). *Lichenologist* 4: 275–284.
- COSEWIC. 2006. COSEWIC assessment and update status report on the seaside centipede Lichen Heterodermia sitchensis in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 28 pages.
 - http://www.sararegistry.gc.ca/virtual_sara/files/cosewic/sr_seaside_centipede_lichen_e.pdf
- COSEWIC. 2010. COSEWIC assessment and status report on the Oldgrowth Specklebelly Pseudocyphellaria rainierensis in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. Vii + 38 pages.
 - http://www.sararegistry.gc.ca/virtual_sara/files/cosewic/sr_Oldgrowth%20Specklebelly_0810_e.pdf
- Dahlberg, A. & Mueller, G. M. 2011. Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species. *Fungal Ecology* **4**: 147–162.
- Dillman, K. L., Ahti, T., Björk, C. R., Clerc, P., Ekman, S., Goward, T., Hafellner, J., Pérez-Ortega, S.,
 Printzen, C., Savić, S., Schultz, M., Svensson, M., Thor, G., Tønsberg, T., Vitikainen, O., Westberg, M.
 & Spribille, T. 2012. New records, range extensions and nomenclatural innovations for lichens and lichenicolous fungi from Alaska, U.S.A. *Herzogia* 25: 177–210.
- Esslinger, T. L. 2016. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada, Version 21. *Opuscula Philolichenum* **15**: 136–390.
- 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.
- Goward, T. 1984. *Heterodermia sitchensis*, a new lichen from the Pacific Northwest of North America. *The Bryologist* 87: 366–368.
- Geiser, L. H., Dillman, K. L., Derr, C. C. & Stensvold, M. C. 1998. Lichens and allied fungi of Southeast Alaska. *In*: M.G. Glenn, R.C. Harris, R. Dirig & M.S. Cole, (eds) *Lichenographia Thomsoniana*: *North American lichenology in honour of John W. Thomson*. Mycotaxon Ltd., Ithaca, NY, pp. 201-243.
- Guindon, S. & Gascuel, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood". Systematic Biology 52: 696–704.
- IUCN. 2012. IUCN red list categories and criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.
- Katoh, K., Asimenos, G. & Toh, H. 2009. Multiple alignment of DNA sequences with MAFFT. Methods in Molecular Biology 537: 39–64.
- Lohtander, K., Oksanen, I. & Rikkinen, J. 2002. A phylogenetic study of *Nephroma* (lichen-forming Ascomycota). *Mycological Research* **106**: 777–787.
- Löytynoja, A. & Goldman, N. 2005. An algorithm for progressive multiple alignment of sequences with insertions. Proceedings of the National Academy of Sciences of the United States of America 102: 10557–10562.
- Löytynoja, A. & Goldman, N. 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. *Science* **320**: 1632–1635.
- Lücking, R., Hodkinson, B. P. & Leavitt, S. D. 2016 [2017]. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota—Approaching one thousand genera. *The Bryologist* 119: 361–416.
- Magain, N. & Sérusiaux, E. 2015. Dismantling the treasured flagship lichen *Sticta fuliginosa* (Peltigerales) into four species in Western Europe. *Mycological Progress* 14: 1–33.

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- Miller, M. A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov 2010, New Orleans, LA, 1–8.
- Moncada, B. 2012. El género Sticta (Schreb.) Ach. en Colombia: Taxonomía, Ecogeografia e Importancia. (Doctoral dissertation). Universidad Nacional de Colombia. Retrieved from Repositorio institucional UN Universidad Nacional de Colombia (ID 11296), on 14 March 2018.
- Moncada, B., Lücking, R. & Betancourt-Macuase, L., 2013. Phylogeny of the Lobariaceae (lichenized Ascomycota: Peltigerales), with a reappraisal of the genus *Lobariella*. *Lichenologist* **45**: 2–263.
- Moncada, B., Lücking, R. & Suárez, A. 2014. Molecular phylogeny of the genus *Sticta* (lichenized Ascomycota: Lobariaceae) in Colombia. *Fungal Diversity* **64**: 205–231.
- Moncada, B., Suárez, A. & Lücking, R. 2015. Nueve especies nuevas del género *Sticta* (Ascomycota liquenizados: Lobariaceae) del morfotipo fuliginosa sensu lato de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas Físicas y Naturales* 39: 55–60.
- Orange, A., James, P. W. & White, F. J. 2001. *Microchemical methods for the identification of lichens*. London: British Lichen Society, 101 pages.
- Penn, O., Privman, E., Ashkenazy, H., Landan, G., Graur, D. & Pupko, T. 2010a. GUIDANCE: a web server for assessing alignment confidence scores. *Nucleic Acids Research* **38**: W23–W28.
- Penn, O., Privman, E., Landan, G., Graur, D. & Pupko, T. 2010b. An alignment confidence score capturing robustness to guide-tree uncertainty. *Molecular Biology and Evolution* 27: 1759–1767.
- Posada, D. & Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**: 793–808.
- Rambaut, A. 2012. FigTree. 1.4.3. Available at http://tree.bio.ed.ac.uk/software/figtree/
- Scheidegger, C. & Goward, T. 2002. Monitoring lichens for conservation: Red lists and conservation action plans. *In*: Nimis, P. L., Scheidegger, C., Wolseley, P. (eds), *Lichen Monitoring Monitoring Lichens*. Dordrecht: Kluwer Academic Publishers, pp. 163–180.
- Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57: 758–771.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312-1313.
- White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **18**: 315–322.