Bry. Div. Evo. 38 (1): 001–022
http://www.mapress.com/j/bde

Article

ISSN 2381-9677 (print edition)
BRYOPHYTE DIVERSITY & EVOLUTION
ISSN 2381-9685 (online edition)

http://dx.doi.org/10.11646/bde.38.1.1

Preliminary insights from DNA barcoding into the diversity of mosses colonising modern building surfaces

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Abstract

Schistidium species are prominent colonisers on modern building surfaces. Although the taxonomy of this genus has advanced considerably in recent years, growth of *Schistidium* on building surfaces is often slow, with the life cycle often incomplete. The availability of diagnostic morphological characters for species identification can therefore be limited; in consequence these building colonisers are often assigned to "*Schistidium* species*"*. In recent years, DNA barcoding has been used in studies of species complexes, to aid species delimitation and identification. Here we report our first findings of a DNA barcoding project on accessions of European *Schistidium* that are involved in the colonisation of modern buildings. This study gives an inital insight into the taxonomy of pioneer *Schistidium* taxa amongst the 'primary growth' on modern building surfaces and an assessment of the utility of DNA barcoding for the identification of cryptic, character poor samples and species.We show that samples with poor morphological characters due to incomplete development from modern building surfaces identified as "*Schistidium* species" fall into several clades, and re-examination of the morphology of these samples shows some morphological differences, suggesting cryptic taxa.

Keywords: Biodiversity, DNA barcoding, modern building, primary colonization, *Schistidium*

Introduction

Plant growth on man-made structures like masonry is usually unwelcome. Discoloration due to cryptogams (algae, bryophytes, fungi and lichens) on building surfaces is often considered as "damage". Previous investigations of the first colonizers of modern building surfaces ("primary growth"), especially of external thermal insulation compound systems (ETICS), revealed that after a comparatively short time, mosses can be found (Hofbauer 2007): Fungal and algal colonizers are usually first, but after only a few months to a few years, if conditions are favourable, moss growth may start. Members of the genus *Schistidium* Bruch & Schimper in Bruch, Schimper & Gümbel (1845: 93) (Grimmiaceae), commonly, and confusingly, named "Grimmias" (e.g. Bosanquet in Atherton *et al.* 2010), are prominent among these early colonizers. Other typical early emerging bryophytes on modern building surfaces include *Bryum argenteum* Hedwig (1801: 181), *Grimmia pulvinata* (Hedwig [1801: 158]) Smith (1807: 1728), *Hypnum cupressiforme* Hedwig (1801: 291), *Orthotrichum anomalum* Hedwig (1801: 162), *Orthotrichum diaphanum* Schrader ex Bridel (1801: 29) and *Tortula muralis* Hedwig (1801: 123) (Hofbauer 2007).

The genus *Schistidium* comprises about 139 (Tropicos 2015) to 156 (The Plant List 2013) accepted species, and some subordinate taxa, worldwide. Almost every year, further species are described within the genus (e.g. Ochyra & Afonina 2010, Blom *et al.* 2011, Feng *et al*. 2013, McIntosh *et al*. 2015). With few exceptions, species occur on natural rock substrates, with ecotypes that prefer moist or even wet surfaces and others that tolerate rather dry habitats. Some species are regularly found on anthropogenic hard substrates, for example concrete or tarmac (Blom 1996); relatively recent masonry structures may also be invaded (Hofbauer 2007).

The taxonomy of this critical genus has advanced considerably in recent years, with studies showing that a narrow species concept (e.g. Blom 1996, 1998, Goryunov *et al*. 2007, Milyutina 2007, Ignatova *et al.* 2009) is more appropriate than a broad concept (e.g. Bremer 1980). The narrow morphological species concept better fits patterns of nuclear ribosomal DNA spacer sequence variation within the group (e.g. Ignatova *et al.* 2009, Milyutina *et al.* 2010).

Accepted by Dietmar Quandt: 5 Feb. 2016; published: 13 Apr. 2016

The growth of *Schistidium* on buildings may be slow and its life cycle is often incomplete. Furthermore, morphology (e.g. leaves) can be atypical under the generally extreme environmental conditions on external building surfaces. The morphological characters required for species identification are frequently not present, and, as a consequence, these building colonizers are still often assigned to "*Schistidium* sp".

In recent years, DNA barcoding has been used for bryophyte identifications and taxonomic clarification (e.g. Stech & Quandt 2010, Bell *et al.* 2012). In this paper we describe initial results from a collaboration between the Royal Botanic Garden Edinburgh (RBGE) and the Fraunhofer-Institute for Building Physics (IBP) aiming to obtain DNA barcodes of mosses involved in the primary colonization of building surfaces (masonry), with special emphasis on *Schistidium*. The herbarium at RBGE (E) holds over 600 specimens of *Schistidium*. From these, specimens of *Schistidium* species thought to be involved in the colonization of European buildings were examined morphologically. A subset of these samples (representative of the different morphological types) has been DNA barcoded using the core Consortium for the Barcode of Life land plant DNA barcode loci (Hollingsworth *et al*. 2009; CBOL 2009) (*rbc*L, *mat*K) and two of the most widely used supplementary barcode loci (*psb*A-*trn*H and ITS2) to establish a reference library; we are currently verifying this reference collection.

In this current paper we look at the distribution of ITS2 sequence variation in 140 newly collected samples from masonry and the surroundings of building locations. This gives a first insight into the taxonomy of *Schistidium* species inhabiting modern building structures, as a prelude to more comprehensive work involving reference DNA barcodes from the herbarium and multiple DNA barcode markers, as described above.

Material & Methods

Fresh samples of *Schistidium* growing on different substrates were collected from Austria, Germany, Italy and Scotland (Figure 1). Four main substrates were sampled: a) growth on masonry of external thermal insulation compound systems (ETICS), with an emphasis on quite recently established growth (surfaces erected within the last four to six years); b) other anthropogenic surfaces, mostly concrete, but also e.g. tarmac (usually at least 10 to 15 years old); c) rock surfaces in urban to semi-urban situations, preferably with some proximity to the anthropogenic substrates that have been sampled; and d) natural rock surfaces in the wild.

FIGURE 1. *Schistidium* samples from different anthropogenic surfaces. A and C show cushions growing on concrete ("substrate group b"); B and D show established *Schistidium* growth on the surface material (plaster) of an ETICS ("substrate group a"). Note that the growth on plaster is quite weak and no capsules are developed, therefore morphological determination is problematic.

Only homogenous patches or cushions that may comprise only a single clone or genet (Cook, 1984) were sampled. Completely developed plants (preferred), as well as under-developed plants (mostly from ETICS), were sampled. Where possible, samples were taken during dry conditions. All samples were air dried after collecting. Samples were then frozen for several days before entering the herbarium at RBGE. Care was taken to avoid cross contamination between the samples.

Accessions used for DNA extraction and sequencing (Table 1) consist of 140 samples in total:

Nine samples of ***Schistidium apocarpum*** (Hedwig [1801: 76]) Bruch & Schimper in Bruch, Schimper & Gümbel (1845: 99) from Great Britain, Italy and Austria (from rocks, buildings, mortar and concrete);

17 samples attributed to ***Schistidium crassipilum*** H.H. Blom (1996: 224) from Austria, Germany and Great Britain (from rock, concrete, tarmac and ETICS);

Six samples of ***Schistidium dupretii*** (Thériot [1907: 63]) Weber (1976: 106) from Great Britain, Austria and Germany (from rock and concrete);

17 samples of ***Schistidium elegantulum*** H.H. Blom (1996: 233) from Great Britain, Austria Germany and Italy (from rock, concrete and ETICS);

One sample of ***Schistidium strictum*** (Turner [1804: 20]) Loeske ex Mårtensson (1956: 110) from Great Britain (from rock);

Five samples of ***Schistidium papillosum*** Culmann in Amann & Meylan (1918: 386) from Austria, Italy and Great Britain (from rock);

One sample referable to ***Schistidium pulchrum*** H.H. Blom (1996: 119) from Austria (from rock);

One sample of ***Schistidium pruinosum*** (Wilson ex Schimper [1876: 241]) Roth (1904: 398) from Great Britain;

Three samples of ***Schistidium trichodon*** (Bridel [1826: 171]) Poelt (1953: 253) from Austria and Great Britain (from limestone and concrete);

78 samples of ***Schistidium*** that could not be assigned to species using morphological data, from Austria, Germany and Italy (from rock, concrete tarmac and ETICS).

Outgroup selection was according to the ITS phylogeny in Milyutina *et al.* (2010), with the analysis rooted using ***S. pulchrum*** and ***S. grandirete*** H.H. Blom (1996: 50) accessions from GenBank.

TABLE 1. The 140 accessions of *Schistidium* sampled; species identification, DNA number, voucher information, international state code, location and general substrate and GenBank accession numbers. All vouchers are deposited at E.

Morphological identification	Clade	DNA no.	Voucher information	International country code, location	Substrate	GenBank no.
Schistidium crassipilum	1A	6608	Long 41531	GREAT BRITAIN, Cousland	concrete lid	KU321363
Schistidium crassipilum	1A	6651	Chamberlain E11	GREAT BRITAIN, West Ross	concrete	KU321364
Schistidium elegantulum	1A	6577	Hofbauer WH019	AUSTRIA, Kufstein, Tirol	concrete box	KU321365
Schistidium elegantulum	1A	6578	Hofbauer WH021	AUSTRIA, Kufstein, Tirol	concrete	KU321366
Schistidium elegantulum	1A	6671	Hofbauer WH058	AUSTRIA, Kufstein, Tirol	cement/concrete	KU321367
Schistidium elegantulum	1A	6695	Hofbauer WH039	AUSTRIA, Kufstein, Tirol	rock	KU321368
Schistidium elegantulum	1A	6570	Hofbauer WH003	AUSTRIA, Zirl, Tirol	concrete	KU321369
Schistidium elegantulum	1A	6595	Hofbauer WH064	GERMANY, Valley, Bavaria	ETICS	KU321370
Schistidium elegantulum	1A	6668	Hofbauer WH067A	GERMANY, Valley, Bavaria	ETICS	KU321371
Schistidium elegantulum	1A	6669	Hofbauer WH066	GERMANY, Valley, Bavaria	ETICS	KU321372
Schistidium elegantulum	1A	6738	Long 37713	GREAT BRITAIN, Afon Alun	limestone	KU321373

...Continued on next page

TABLE 1. (Continued)

Morphological identification	Clade	DNA no.	Voucher information	International country code, location	Substrate	GenBank no.
Schistidium elegantulum	1A	6528	Long & Kungu 42882	GREAT BRITAIN, Dumfriesshire	margin of pasture, on wall under *Acer*	KU321374
Schistidium elegantulum	1A	6713	Hofbauer WH171	GREAT BRITAIN, East Lothian	cement top of stone wall (BBS excursion)	KU321375
Schistidium elegantulum cf	1A	6572	Hofbauer WH007	AUSTRIA, Kufstein, Tirol	concrete	KU321376
Schistidium elegantulum cf	1A	6670	Hofbauer WH059	AUSTRIA, Kufstein, Tirol	concrete	KU321377
Schistidium sp.	1A	6573	Hofbauer WH010	AUSTRIA, Kufstein, Tirol	tarmac	KU321378
Schistidium sp.	1A	6581	Hofbauer WH026	AUSTRIA, Kufstein, Tirol	concrete	KU321379
Schistidium sp.	1A	6585	Hofbauer WH034	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321380
Schistidium sp.	1A	6588	Hofbauer WH043	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321381
Schistidium sp.	1A	6589	Hofbauer WH044	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321382
Schistidium sp.	1A	6672	Hofbauer WH057	AUSTRIA, Kufstein, Tirol	concrete	KU321383
Schistidium sp.	1A	6673	Hofbauer WH035	AUSTRIA, Kufstein, Tirol	rock	KU321384
Schistidium sp.	1A	6675	Hofbauer WH028	AUSTRIA, Kufstein, Tirol	concrete	KU321385
Schistidium sp.	1A	6679	Hofbauer WH020	AUSTRIA, Kufstein, Tirol	concrete	KU321386
Schistidium sp.	1A	6689	Hofbauer WH056	AUSTRIA, Kufstein, Tirol	concrete	KU321387
Schistidium sp.	1A	6690	Hofbauer WH054	AUSTRIA, Kufstein, Tirol	concrete	KU321388
Schistidium sp.	1A	6692	Hofbauer WH051	AUSTRIA, Kufstein, Tirol	rock	KU321389
Schistidium sp.	1A	6693	Hofbauer WH050	AUSTRIA, Kufstein, Tirol	rock	KU321390
Schistidium sp.	1A	6530	Hofbauer WH040	AUSTRIA, Tirol, Kufstein	concrete, old wall	KU321391
Schistidium sp.	1A	6593	Hofbauer WH061	GERMANY, Valley, Bavaria	ETICS	KU321392
Schistidium sp.	1A	6596	Hofbauer WH065	GERMANY, Valley, Bavaria	ETICS	KU321393
Schistidium sp.	1A	6597	Hofbauer WH068	GERMANY, Valley, Bavaria	ETICS	KU321394
Schistidium sp.	1A	6612	Hofbauer WH078	GERMANY, Valley, Bavaria	ETICS	KU321395
Schistidium sp.	1A	6616	Hofbauer WH083	GERMANY, Valley, Bavaria	ETICS	KU321396
Schistidium sp.	1A	6621	Hofbauer WH089	GERMANY, Valley, Bavaria	ETICS	KU321397
Schistidium sp.	1A	6623	Hofbauer WH122	GERMANY, Valley, Bavaria	ETICS	KU321398
Schistidium sp.	1A	6633	Hofbauer WH104	GERMANY, Valley, Bavaria	rock	KU321399
Schistidium sp.	1A	6644	Hofbauer WH125	GERMANY, Valley, Bavaria	tarmac	KU321400
Schistidium sp.	1A	6646	Hofbauer WH127	GERMANY, Valley, Bavaria	tarmac	KU321401
Schistidium sp.	1A	6686	Hofbauer WH123	GERMANY, Valley, Bavaria	ETICS	KU321402
Schistidium sp.	1A	6688	Hofbauer WH091	GERMANY, Valley, Bavaria	ETICS	KU321403
Schistidium elegantulum cf	1B	6529	Hofbauer WH002	AUSTRIA, Tirol, Zirl	concrete, on old garden wall	KU321404

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TABLE 1. (Continued)

Morphological identification	Clade	DNA no.	Voucher information	International country code, location	Substrate	GenBank no.
Schistidium elegantulum cf	1B	6661	Hofbauer & Dickson WH167	ITALY, Klausen	rock	KU321405
Schistidium elegantulum cf	1B	6700	Hofbauer WH166	ITALY, Triest	boulder	KU321406
Schistidium elegantulum cf	1B	6701	Hofbauer WH165	ITALY, Triest	boulder	KU321407
Schistidium sp.	1B	6716	Hofbauer WH142	AUSTRIA, Kundl, Tirol	concrete	KU321408
Schistidium apocarpum	2	6654	Long 34084	GREAT BRITAIN, Leadburn Moss	window sill	KU321409
Schistidium apocarpum	2	6655	Long 40616	GREAT BRITAIN, Selkirk	wall	KU321410
Schistidium crassipilum	2	6677	Hofbauer WH024	AUSTRIA, Kufstein, Tirol	concrete	KU321411
Schistidium crassipilum	2	6678	Hofbauer WH022	AUSTRIA, Kufstein, Tirol	concrete pole	KU321412
Schistidium crassipilum	2	6648	Hofbauer WH131	GERMANY, Valley, Bavaria	tarmac	KU321413
Schistidium crassipilum	2	6605	Long 38578	GREAT BRITAIN, Causewaybank	brickwork by water	KU321414
Schistidium crassipilum	2	6601	Long & McBeath 41444	GREAT BRITAIN, Darnchester	concrete	KU321415
Schistidium crassipilum	2	6604	Chamberlain & Kungu E04	GREAT BRITAIN, Dumfries, Dala	house roof	KU321416
Schistidium crassipilum	2	6699	Hofbauer WH139	GREAT BRITAIN, Edinburgh, Tanfield	sandstone in wall	KU321417
Schistidium crassipilum	2	6649	Chamberlain & Kungu E09	GREAT BRITAIN, Fife	concrete	KU321418
Schistidium crassipilum	2	6650	Chamberlain E10	GREAT BRITAIN, Kilsyth	concrete	KU321419
Schistidium crassipilum	2	6603	Long 42260	GREAT BRITAIN, Moniaive Town	roadside wall	KU321420
Schistidium crassipilum	2	6607	Chamberlain E07	GREAT BRITAIN, West Lothian	cement top of garden wall	KU321421
Schistidium crassipilum cf	2	6579	Hofbauer WH023	AUSTRIA, Kufstein, Tirol	concrete	KU321422
Schistidium crassipilum cf	2	6704	Hofbauer WH172	GREAT BRITAIN, Edinburgh, Ferry Road	concrete top of wall	KU321423
Schistidium crassipilum cf	2	6698	Hofbauer WH137	GREAT BRITAIN, Edinburgh, Inverleith	concrete	KU321424
Schistidium dupretii	2	7348	Long 5018	GREAT BRITAIN, Glen Tilt	rock	KU321425
Schistidium pruinosum	2	7345	Long & Chamberlain 28002	GREAT BRITAIN, Pentland Hills		KU321426
Schistidium sp.	2	6586	Hofbauer WH036	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321427
Schistidium sp.	2	6591	Hofbauer WH049	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321428
Schistidium sp.	2	6674	Hofbauer WH032	AUSTRIA, Kufstein, Tirol	concrete plastering	KU321429
Schistidium sp.	2	6676	Hofbauer WH027	AUSTRIA, Kufstein, Tirol	concrete	KU321430

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TABLE 1. (Continued)

Morphological identification	Clade	DNA no.	Voucher information	International country code, location	Substrate	GenBank no.
Schistidium sp.	2	6720	Hofbauer WH015	AUSTRIA, Kufstein, Tirol	concrete	KU321431
Schistidium sp.	2	6532	Hofbauer WH073	GERMANY, Bavaria, Vallley	ETICS, specimen 3	KU321432
Schistidium sp.	2	6613	Hofbauer WH079	GERMANY, Valley, Bavaria	ETICS	KU321433
Schistidium sp.	2	6625	Hofbauer WH095	GERMANY, Valley, Bavaria	concrete	KU321434
Schistidium sp.	2	6631	Hofbauer WH132	GERMANY, Valley, Bavaria	concrete	KU321435
Schistidium sp.	2	6632	Hofbauer WH133	GERMANY, Valley, Bavaria	concrete	KU321436
Schistidium sp.	3	6637	Hofbauer WH111	GERMANY, Valley, Bavaria	rock	KU321437
Schistidium sp.	3	6640	Hofbauer WH114	GERMANY, Valley, Bavaria	rock	KU321438
Schistidium sp.	3	6647	Hofbauer WH130	GERMANY, Valley, Bavaria	tarmac	KU321439
Schistidium trichodon	3	6534	Hofbauer WH154	AUSTRIA, Tirol, Vomp	concrete, old, near river	KU321440
Schistidium trichodon	3	6741	Long 41031	GREAT BRITAIN, Clova	limestone	KU321441
Schistidium trichodon	3	6742	Long 38391	GREAT BRITAIN, Iover Feith	limestone	KU321442
Schistidium apocarpum	3A	6584	Hofbauer WH033	AUSTRIA, Kufstein, Tirol	concrete	KU321443
Schistidium apocarpum	3A	6714	Hofbauer WH140	AUSTRIA, Kundl, Tirol	concrete	KU321444
Schistidium apocarpum	3A	6653	Preston E13	GREAT BRITAIN, Allt Comadaidh	mortar	KU321445
Schistidium apocarpum	3A	6744	Long & Buchan 41552	GREAT BRITAIN, Kale Water	rocks	KU321446
Schistidium apocarpum	3A	6743	Long & Rothero 26149	GREAT BRITAIN, Witch Linn	boulder	KU321447
Schistidium apocarpum	3A	6662	Hofbauer & Dickson WH148	ITALY, near Brixen	rock	KU321448
Schistidium apocarpum	3A	6664	Hofbauer & Porley WH146	ITALY, Schnalstal	rock	KU321449
Schistidium crassipilum	3A	6602	Long 42451	GREAT BRITAIN, Hawick	wall of flower bed	KU321450
Schistidium papillosum	3A	6533	Hofbauer WH145	AUSTRIA, Tirol, Hopfgarten	rock	KU321451
Schistidium papillosum	3A	6718	Hofbauer & Dickson WH169	AUSTRIA, Zwieselstein, Tirol	rock	KU321452
Schistidium papillosum	3A	6739	Long & Rothero 28279	GREAT BRITAIN, Craig Leek	limestone	KU321453
Schistidium papillosum	3A	6657	Hofbauer & Dickson WH156	ITALY, near Brixen	rock	KU321454
Schistidium papillosum	3A	6663	Hofbauer & Dickson WH147	ITALY, Pfossental	rock	KU321455
Schistidium sp.	3A	6587	Hofbauer WH037	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321456

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TABLE 1. (Continued)

Morphological identification	Clade	DNA no.	Voucher information	International country code, location	Substrate	GenBank no.
Schistidium sp.	3A	6592	Hofbauer WH053	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321457
Schistidium sp.	3A	6615	Hofbauer WH082	GERMANY, Valley, Bavaria	ETICS	KU321458
Schistidium sp.	3A	6638	Hofbauer WH112	GERMANY, Valley, Bavaria	rock	KU321459
Schistidium sp.	3A	6659	Hofbauer & Dickson WH153	ITALY, Kreuztal	rock	KU321460
Schistidium strictum	3A	6527	Long & Kungu 42891	GREAT BRITAIN, Dumfriesshire	south-facing crags, on wet rock ledge	KU321461
Schistidium dupretii	4A	6574	Hofbauer WH012	AUSTRIA, Kufstein, Tirol	concrete	KU321462
Schistidium dupretii	4A	7346	Long & Payne 10026	GREAT BRITAIN, Ben Lawers		KU321463
Schistidium dupretii cf	4A	6575	Hofbauer WH014	AUSTRIA, Kufstein, Tirol	concrete	KU321464
Schistidium dupretii cf	4A	6658	Hofbauer WH159	AUSTRIA, near Ackernalm, Thiersee	rock, base rich	KU321465
Schistidium dupretii cf	4A	6569	Hofbauer WH001	GERMANY, Hirschberg, Bavaria	rock	KU321466
Schistidium dupretii cf	4A	6684	Hofbauer WH101	GERMANY, Valley, Bavaria	concrete	KU321467
Schistidium sp.	4A	6583	Hofbauer WH031	AUSTRIA, Kufstein, Tirol	concrete plastering	KU321468
Schistidium sp.	4A	6531	Hofbauer WH060	GERMANY, Bavaria, Vallley	ETICS, specimen 1	KU321469
Schistidium sp.	4A	6594	Hofbauer WH063	GERMANY, Valley, Bavaria	ETICS	KU321470
Schistidium sp.	4A	6598	Hofbauer WH067	GERMANY, Valley, Bavaria	ETICS	KU321471
Schistidium sp.	4A	6599	Hofbauer WH070	GERMANY, Valley, Bavaria	ETICS	KU321472
Schistidium sp.	4A	6614	Hofbauer WH080	GERMANY, Valley, Bavaria	ETICS	KU321473
Schistidium sp.	4A	6618	Hofbauer WH086	GERMANY, Valley, Bavaria	ETICS	KU321474
Schistidium sp.	4A	6619	Hofbauer WH087	GERMANY, Valley, Bavaria	ETICS	KU321475
Schistidium sp.	4A	6620	Hofbauer WH088	GERMANY, Valley, Bavaria	ETICS	KU321476
Schistidium sp.	4A	6628	Hofbauer WH099	GERMANY, Valley, Bavaria	concrete	KU321477
Schistidium sp.	4A	6629	Hofbauer WH100	GERMANY, Valley, Bavaria	concrete	KU321478
Schistidium sp.	4A	6630	Hofbauer WH102	GERMANY, Valley, Bavaria	concrete	KU321479
Schistidium sp.	4A	6635	Hofbauer WH107	GERMANY, Valley, Bavaria	rock	KU321480
Schistidium sp.	4A	6642	Hofbauer WH118	GERMANY, Valley, Bavaria	rock	KU321481
Schistidium sp.	4A	6643	Hofbauer WH120	GERMANY, Valley, Bavaria	rock	KU321482
Schistidium sp.	4A	6665	Hofbauer WH081	GERMANY, Valley, Bavaria	ETICS	KU321483
Schistidium sp.	4A	6667	Hofbauer WH072	GERMANY, Valley, Bavaria	ETICS	KU321484
Schistidium sp.	4A	6683	Hofbauer WH109	GERMANY, Valley, Bavaria	rock	KU321485
Schistidium sp.	4A	6687	Hofbauer WH092	GERMANY, Valley, Bavaria	ETICS	KU321486
Schistidium sp.	4A	6719	Hofbauer WH090	GERMANY, Valley, Bavaria	ETICS	KU321487

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TABLE 1. (Continued)

Morphological identification	Clade	DNA no.	Voucher information	International country code, location	Substrate	GenBank no.
Schistidium sp.	4B	6571	Hofbauer WH005	AUSTRIA, Kufstein, Tirol	concrete	KU321488
Schistidium sp.	4B	6576	Hofbauer WH017	AUSTRIA, Kufstein, Tirol	ETICS	KU321489
Schistidium sp.	4B	6590	Hofbauer WH046	AUSTRIA, Kufstein, Tirol	rock, calcareous	KU321490
Schistidium sp.	4B	6694	Hofbauer WH047	AUSTRIA, Kufstein, Tirol	rock	KU321491
Schistidium sp.	4B	6696	Hofbauer WH038	AUSTRIA, Kufstein, Tirol	rock	KU321492
Schistidium sp.	4B	6600	Hofbauer WH071	GERMANY, Valley, Bavaria	ETICS	KU321493
Schistidium sp.	4B	6624	Hofbauer WH124	GERMANY, Valley, Bavaria	ETICS	KU321494
Schistidium sp.	4B	6627	Hofbauer WH098	GERMANY, Valley, Bavaria	concrete	KU321495
Schistidium sp.	4B	6634	Hofbauer WH105	GERMANY, Valley, Bavaria	rock	KU321496
Schistidium sp.	4B	6636	Hofbauer WH108	GERMANY, Valley, Bavaria	rock	KU321497
Schistidium sp.	4B	6639	Hofbauer WH113	GERMANY, Valley, Bavaria	rock	KU321498
Schistidium sp.	4B	6645	Hofbauer WH126	GERMANY, Valley, Bavaria	tarmac	KU321499
Schistidium sp.	4B	6681	Hofbauer WH119	GERMANY, Valley, Bavaria	rock	KU321500
Schistidium sp.	4B	6685	Hofbauer WH097	GERMANY, Valley, Bavaria	concrete	KU321501
Schistidium pulchrum cf	5	6660	Hofbauer & Dickson WH168	AUSTRIA, Zwieselstein	rock	KU321502

Morphological characterization

Morphological traits according to Blom (1996, 1998) were assessed and digital photographs of most specimens have been produced. Species delimination of fresh samples was performed using recent literature (e.g. Blom 1996, 1998, Casas 2001, Blom *et al.* 2006, Weibull 2006; Milyutina 2007, Erzberger & Schröder 2008, Ignatova *et al.* 2009) where possible.

DNA barcoding

Following the mixed stand concept (Koponen 1967, Blom 1996) only samples or parts of samples that clearly represented the same clone were choosen for subsampling for DNA isolation. In order to avoid potentially adhering contaminants (e.g. bryophytes, algae, cyanoprokaryota, fungi, lichens), 5 to 7 shoot tips with clean and vigorous appearance were collected for each sample, comprising only the uppermost stem and leaves.

Dry plant tissue was ground with a TissueLyser (QIAGEN) with 3mm tungsten beads. Total DNA was extracted from stem tips of recent and herbarium material using Qiagen DNeasy Mini kits (Qiagen Ltd) following the manufacturer's protocol or automated by use of a QIAxtractor® (QIAGEN) (see Forrest *et al.* submitted, for protocol).

In our final study, four DNA barcoding regions will be sequenced for this set of accessions, including three plastid (part of the *mat*K gene, part of the *rbc*L gene, and the *trn*H-*psb*A intergenic spacer region) and one nuclear (the ribosomal internal transcribed spacer, ITS2) marker. A combination of universal and lineage-specific primers have been used (Bell *et al*. 2012) on a small sample set of accessions. For the *mat*K barcode locus, moss-specific primers designed by Alan Forrest (2012) for the Barcode of Life project (matK.Moss485F and matK.Moss1336R) were used. For *rbc*L, we amplified the standard DNA barcode region using primers designed by Kress & Erickson (2007; rbcL. aF) and Fazekas *et al.* (2008; rbcL.ajf634R). For *trn*H-*psb*A we used the trnH reverse primer designed by Sang *et al.* (1997; psbA.trnHR) in combination with a forward primer located within the *psb*A gene (psbA.501F) (Forrest & Crandall-Stotler 2004), to maximize the amount of sequence data generated for the region. For ITS2, to reduce risk of fungal contamination, we used primers designed from moss species by Stech *et al.* (2003; ITS.4bryo) and Olsson *et al.* (2009; ITS.2seqF). Polymerase chain reaction (PCR) was performed according to standard protocols (Forrest *et al.* submitted). Sequences were run using the BigDye-Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc.) on an ABI3730 automated sequencer at the GenePool Sanger Sequencing Service (University of Edinburgh, UK) using the same primers as for PCR. As data collection for the three plastid barcode loci is not yet complete, only the ITS2 data is reported here, although a subset of the plastid sequences have been checked for levels of variability and made publically available. GenBank accession numbers for all new ITS sequences are given in Table 1, while GenBank numbers for the additional loci for the subset of eight test accessions are in Table 2.

TABLE 2. Details of eight *Schistidium* accessions tested for the complete set of 4 barcode loci.

Morphological identification	Clade	DNA no.	Voucher information	Country, location	Substrate	ITS2 GenBank no.	*mat*K GenBank no.	*psb*A-trnH GenBank no.	*rbc*L GenBank no.
Schistidium elegantulum	1A	6528	Long & Kungu 42882	GREAT BRITAIN, Dumfriesshire	margin of pasture, on wall under *Acer*	KU321374	KU309591	KU309583	KU309599
Schistidium sp.	1A	6530	Hofbauer WH040	AUSTRIA, Tirol, Kufstein	concrete, old wall	KU321391	KU309593	KU309585	KU309601
Schistidium elegantulum cf	1B	6529	Hofbauer WH002	AUSTRIA, Tirol, Zirl	concrete, on old garden wall	KU321404	KU309590	KU309582	KU309598
Schistidium sp.	2.	6532	Hofbauer WH073	GERMANY, Bavaria, Vallley	ETICS, specimen 3	KU321432	KU309595	KU309587	KU309603
Schistidium trichodon	3.	6534	Hofbauer WH154	AUSTRIA, Tirol, Vomp	concrete, old, near river	KU321440	KU309597	KU309589	KU309605
Schistidium papillosum	3A	6533	Hofbauer WH145	AUSTRIA, Tirol, Hopfgarten	rock	KU321451	KU309592	KU309584	KU309600
Schistidium strictum	3A	6527	Long & Kungu 42891	GREAT BRITAIN, Dumfriesshire	south-facing crags, on wet rock ledge	KU321461	KU309596	KU309588	KU309604
Schistidium sp.	4A	6531	Hofbauer WH060	GERMANY, Bavaria, Vallley	ETICS, specimen 1	KU321469	KU309594	KU309586	KU309602

Sequence alignment and phylogenetic analysis

Bidirectional sequences were assembled and edited using Sequencher version 5.1 (Gene Codes Corporation) and Geneious version 6.1.8 (Biomatters Ltd). All 142 *Schistidium* ITS2 sequences available from GenBank on 26th September 2014 were downloaded and added to our newly generated sequences. An automated alignment with default parameters for CLUSTALW for the 282 *Schistidium* accessions was generated in Geneious, giving an alignment length of 588 characters. Because of the number of taxa involved, combined with alignment ambiguity due to high levels of sequence divergence and indels between parts of the spacer, this matrix was checked manually then run through GBLOCKS 0.91b (© Castresana 2002) with semi-conservative settings (gap positions allowed within final blocks, and less strict flanking positions) to obtain a reduced matrix containing 341 unambiguously-aligned bases.

A neighbour-joining tree was generated from this GBLOCKS matrix, using a Jukes-Cantor genetic distance model with the Geneious tree builder, and a maximum parsimony tree was generated by an heuristic search in PAUP* 4.0a146 (Sinauer Associated Inc., Sunderland, MA, © Swofford 2015). Both trees were used to identify GenBank sequences that were not close matches to the barcoding accessions sequenced for this project, which were then removed from the matrix (Suppl. Table 1). It was also apparent that our samples fell into five *Schistidium* clades, and individual manual alignments were made for each of these. Parsimony and neighbour-joining trees were generated for the clades to check that relationships between taxa were congruent with other analyses, and then the separate alignments were manually recombined and standardized, to produce a final internally consistent alignment of 214 taxa and 617 characters (Treebase accession http://purl.org/phylo/treebase/phylows/study/TB2:S18627). Seventy four *Schistidium* accessions from GenBank were used in the final analyses (Table 3).

TABLE 3. GenBank accessions of *Schistidium* included in the ITS matrix.

GenBank No.	Taxon	Clade	Voucher information	Collection country coGermany, location	collection date
HM031072	*Schistidium elegantulum*	1A	Ignatov & Ignatova 06-5062 (MW)	NORWAY	
HM031071	*Schistidium elegantulum*	1B	Ignatov & Ignatov s.n. (MHA)	RUSSIA, Caucasus	05/08/2002
HM053886	*Schistidium atrofuscum*	2	Kockinger 12258 (MW)	AUSTRIA	
HM053887	*Schistidium atrofuscum*	2	Ignatov & Ignatov 05-3313 (MW)	RUSSIA, Caucasus	
HM031073	*Schistidium crassipilum*	2	Ignatov & Ochyra s.n. (MHA)	PORTUGAL,	10/03/1995
HM031070	*Schistidium crassipilum*	2	Seregin M-564 (MW)	RUSSIA, Caucasus	
HM053958	*Schistidium viride*	2	Darigo 4201 (MO)	USA, Maryland	
HM053957	*Schistidium viride*	2	Allen 27405 (MO)	USA, Missouri	
HM031060	*Schistidium boreale*	3	Ignatov 0/285 (MHA)	RUSSIA, Altai	
HM053888	*Schistidium boreale*	3	Fedosov 06-208 (MW)	RUSSIA, Anabar 1	
HM053889	*Schistidium boreale*	3	Fedosov 06-694 (MW)	RUSSIA, Anabar 2	
HM031069	*Schistidium boreale*	3	Martynenko 14 (MW)	RUSSIA, Bashkortostan	
HM053890	*Schistidium boreale*	3	Hedenas & Aronsson B1748 (S)	SWEDEN	20/07/1990
HM031067	*Schistidium canadense*	3	Maksimov & Maksimova 62-339 (MW)	RUSSIA, Karelia	
HM053915	*Schistidium canadense*	3	Allen 16385 (MO)	USA, Maine 1	
HM053914	*Schistidium canadense*	3	Allen 15716 (MO)	USA, Maine 2	
HM053917	*Schistidium canadense*	3	Allen 27860 (MO)	USA, Maine 3	
HM053916	*Schistidium canadense*	3	Allen 24480 (MO)	USA, Maine 4	
HQ890515	*Schistidium lancifolium*	3	Zare s.n. (MW)	IRAN, Cheten	
HQ890514	*Schistidium lancifolium*	3	Zare s.n. (MW)	IRAN, Veisar 1	
HM031064	*Schistidium lancifolium*	3	Ignatov & Ignatova 05-3721 (MW)	RUSSIA, Caucasus	

...Continued on next page

TABLE 3. (Continued)

GenBank No.	Taxon	Clade	Voucher information	Collection country coGermany, location	collection date
HQ890516	*Schistidium lancifolium*	3	Pisarenko 03263 (MHA)	RUSSIA, Khabarovsk	
HQ890512	*Schistidium lancifolium*	3	Pisarenko 03254 (MW)	RUSSIA, Sakhalin 1	
HQ890513	*Schistidium lancifolium*	3	Pisarenko 03261 (MW)	RUSSIA, Sakhalin 2	
HQ890517	*Schistidium lancifolium*	3	Allen 24447 (MO)	USA, Maine	
HM053953	*Schistidium trichodon var. nutans*	3	Kockinger 12261 (MW)	AUSTRIA	
HM053954	*Schistidium trichodon var. nutans*	3	Kharzinov 1721 (MW)	RUSSIA, Caucasus	
HM053882	*Schistidium andreaeopsis*	3A	Matveena s.n. (MW)	CANADA	25/07/2005
HM053881	*Schistidium andreaeopsis*	3A	Fedosov 06-63 (MW)	RUSSIA, Anabar	
HM031076	*Schistidium apocarpum*	3A	Ignatov s.n. (MHA)	GREAT BRITAIN	08/09/2004
HM031074	*Schistidium apocarpum*	3A	Ignatov & Ignatov 05-3764 (MW)	RUSSIA, Caucasus	
HM031075	*Schistidium apocarpum*	3A	Ignatov s.n. (MHA)	RUSSIA, St.Petersburg	25/10/1996
HM031077	*Schistidium apocarpum*	3A	Ignatov & Ignatov s.n. (MHA)	RUSSIA, Vologda	14/08/2001
HM053913	*Schistidium holmenianum*	3A	Matveeva s.n. (LE)	CANADA, NWT, Arctic Archipelago	27/08/2005
HM053912	*Schistidium holmenianum*	3A	Afonina s.n. (LE)	RUSSIA, Vrangel Island	20/07/1985
HM031061	*Schistidium papillosum*	3A	Ignatov & Ignatova s.n. (MW)	RUSSIA, Caucasus	27/07/2004
HM031062	*Schistidium papillosum*	3A	Ignatov & Ignatova s.n. (MW)	RUSSIA, Irkutsk	08/06/2005
HM031063	*Schistidium papillosum*	3A	Czernyadjeva 120 (MW)	RUSSIA, Kamchatka	31/08/2003
HQ890520	*Schistidium papillosum*	3A	Fedosov 1-3-177 (MW)	RUSSIA, Kommander Islands	
HM031065	*Schistidium papillosum*	3A	Fedosov Sch7 (MW)	RUSSIA, Taimyr	15/06/2004
HM053876	*Schistidium papillosum cf*	3A	Hedenas s.n. (S)	SWEDEN	11/09/2005
HM053932	*Schistidium pruinosum*	3A	Akatova s.n. (MW)	RUSSIA, Adygeya	26/06/2003
HM053933	*Schistidium pruinosum*	3A	Ignatov & Ignatova s.n. (MW)	RUSSIA, Kabardino-Balkarian	27/07/2004
HM053944	*Schistidium strictum*	3A	Blom s.n. (MW)	NORWAY	2002
HM053891	*Schistidium confertum*	4	Kockinger 12251 (MW)	AUSTRIA	
JF262179	*Schistidium confertum*	4	Kockinger 93-1312 (MW)	AUSTRIA, 2	
HM053892	*Schistidium confertum*	4	Hedenas s.n. (S)	SWEDEN	
HM053893	*Schistidium cryptocarpum*	4	Chernyadjeva s.n. (MHA)	RUSSIA, Kamchatka	
HM053919	*Schistidium marginale*	4	Kockinger 12239 (MW)	AUSTRIA, 1	
HM053920	*Schistidium marginale*	4	Kockinger 12240 (MW)	AUSTRIA, 2	
HM053921	*Schistidium marginale*	4	Ignatov & Ignatova 05-1092 (MW)	RUSSIA, Caucasus	
HM053946	*Schistidium subflaccidum*	4	Ignatov & Ignatova 05-3973 (MW)	RUSSIA, Caucasus	
HM053945	*Schistidium subflaccidum*	4	Kockinger 12254 (MW)	AUSTRIA	

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TABLE 3. (Continued)

GenBank No.	Taxon	Clade	Voucher information	Collection country coGermany, location	collection date
HM031055	*Schistidium submuticum*	4	Zolotov 14-29 (MW)	RUSSIA, Bashkortostan	
HM031056	*Schistidium submuticum*	4	Ignatov s.n. (MHA)	RUSSIA, Perm	
HM031057	*Schistidium submuticum*	4	not given	RUSSIA, St Petersburg	
HM053949	*Schistidium submuticum subsp. arcticum*	4	Fedosov 06-443 (MW)	RUSSIA, Anabar 1	
HM053948	*Schistidium submuticum subsp. arcticum*	4	Fedosov 06-476 (MW)	RUSSIA, Anabar 2	
HM053950	*Schistidium submuticum subsp. arcticum*	4	Filin s.n. (MW)	RUSSIA, Yakutia	
HM053951	*Schistidium tenerum*	4	Readfern 36434 (MO)	CANADA, Yukon	
HM053952	*Schistidium tenerum*	4	Afonina s.n. (LE)	RUSSIA, Chukotka	
HM053956	*Schistidium umbrosum*	4	Hedenas s.n. (S)	NORWAY	
HM053955	*Schistidium umbrosum*	4	Kucera 11499 (MW)	RUSSIA, Murmansk	
HM053894	*Schistidium dupretii*	4A	Kockinger 12243 (MW)	AUSTRIA	
HM053895	*Schistidium dupretii*	4A	Bezgodov 630 (MW)	RUSSIA, Perm	06/08/1995
EU343750	*Schistidium sp. 'lingulatum'*	4A	MA 26281		
HM053938	*Schistidium robustum*	4B	Hedenas s.n. (S)	SWEDEN, Gotland	19/10/1989
HM053910	*Schistidium grandirete*	5	Matveeva s.n. (LE)	RUSSIA, Putorana	
HM053911	*Schistidium grandirete*	5	Matveeva s.n. (LE)	RUSSIA, Severnaya Zemlya	04/08/2000
HM031050	*Schistidium pulchrum*	5	Fedosov 06-545 (MW)	RUSSIA, Anabar 1	
HM031051	*Schistidium pulchrum*	5	Tubanova s.n. (MW)	RUSSIA, Buryatia 1	11/07/2003
HM031052	*Schistidium pulchrum*	5	Tubanova 5 (MW)	RUSSIA, Buryatia 2	14/07/2002
HM031053	*Schistidium pulchrum*	5	Bezgodov 78 (MW)	RUSSIA, Perm	09/08/2005
HQ890521	*Schistidium pulchrum*	5	Fedosov HK-9 (MW)	RUSSIA, Taimyr	18/08/2004

To visualize relationships among these samples, maximum parsimony trees were produced using PAUP* version 4.0a146. The maximum parsimony analysis consisted of an initial heuristic search using a TBR algorithm and 1000 random addition replicates, saving five trees per replicate; the most parsimonious trees were then used for a second heuristic search with TBR with a maximum of 10000 trees. Bootstrapping was performed with 1000 replicates, each with 10 random addition replicates and saving 5 trees per replicate. A neighbour joining analysis, performed using the Geneious tree builder, was based on Jukes-Cantor distances, with a bootstrap analysis consisting of 10 000 replicates. Finally, RAxML black box 7.7.7 was used to perform rapid bootstrapping and a Maximum Likelihood search. A lineage containing two GenBank accessions of *S. grandirete* and five of *S. pulchrum*, along with one new *Schistidium* accession from Austria, was used to root the trees.

For the eight accessions used as a test of the four barcode loci, DNA sequences were aligned manually in Geneious, and sequence character data was obtained from PAUP*. Branch and bound searches were used to find the most parsimonious trees for each locus, for the three plastid loci combined and for all four loci combined. Bootstrap support was calculated in each case using 1000 branch and bound replicates.

TABLE 4. Details of the four DNA barcode loci tested for the eight *Schistidium* accessions.

Locus	Locus length (base pairs)	Alignment length (characters)	Invariable characters (number)	Invariable characters (%)	Parsimony-uninformative characters (number)	Parsimony-uninformative characters (%)	Parsimony-informative characters (number)	Parsimony-informative characters (%)	Branches in bootstrap tree (number)	Branches with > 80% bootstrap support (number)
ITS2	478-513	540	494	91.5	19	3.8	27	5.5	5	3
***mat*K**	781	781	760	97.3	12	1.6	9	1.2	4	2
***psb*A-trnH**	736-742	743	738	99.3	2	0.3	3	0.4	2	1
***rbc*L**	607	607	602	99.2	2	0.3	3	0.5	2	1
plastid	n/a	2131	2100	98.5	16	0.8	15	0.7	4	3
all loci	n/a	2671	2594	97.1	35	1.3	42	1.6	5	4

Results

Of the four DNA barcode markers that were tested for eight *Schistidium* accessions, the ITS2 locus provided the most variable characters, and also the most resolved phylogenetic tree, with *mat*K the second most useful locus; the *rbc*L and *psb*A-trnH regions were least variable (Table 4). Combining the three plastid loci still produced a less supported tree than using the ITS2 region alone, although combining all four loci produced the most robust tree. However, the ITS2 region is also the most length-variable, with substancial alignment difficulties that added considerably to the amount of time required to compile and analyse the data.

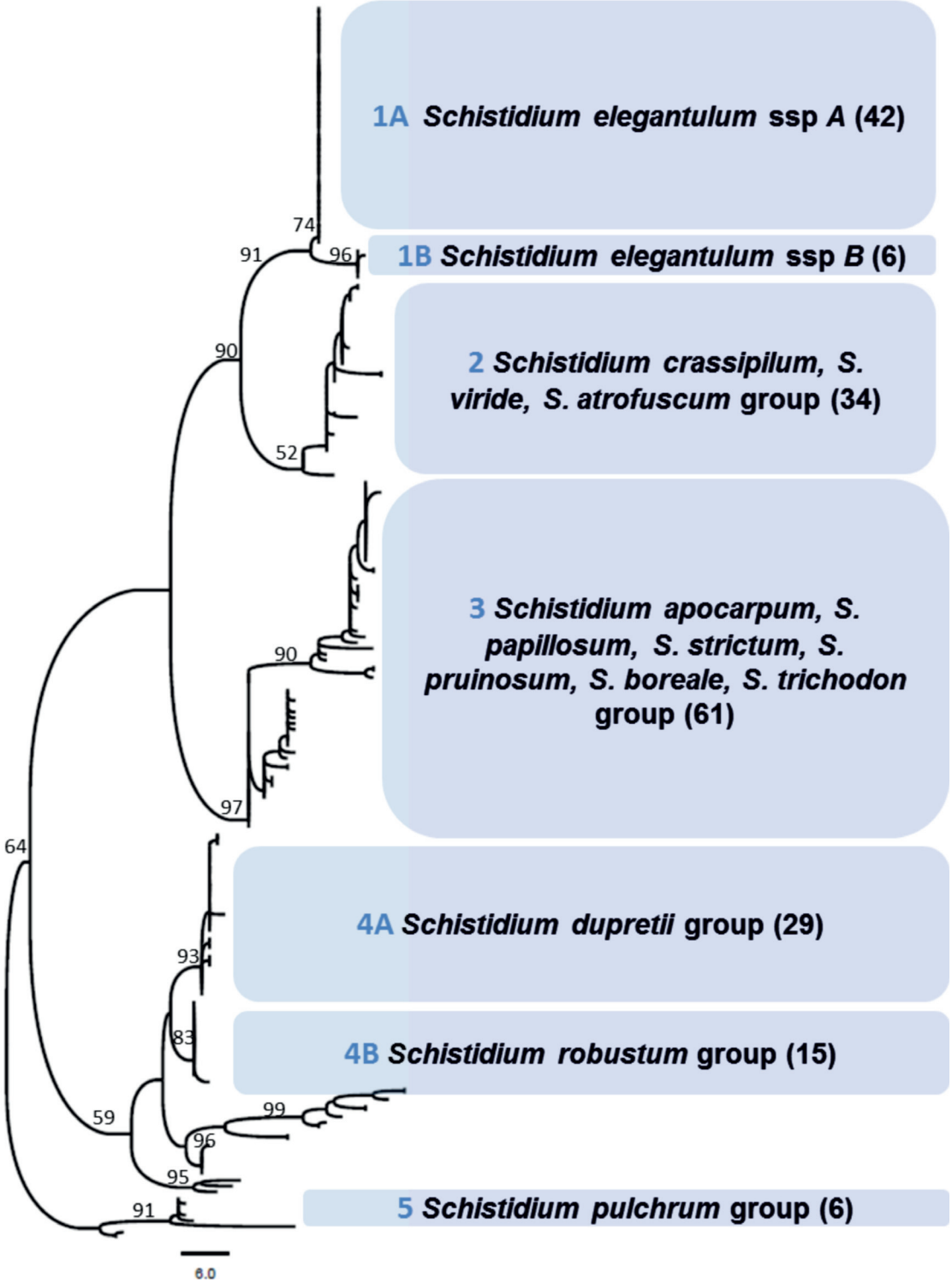

FIGURE 2. Maximum parsimony tree for 214 *Schistidium* samples, including 140 newly sequenced accessions (scale bar and parsimony bootstrap support values are given), for the nuclear internal transcribed spacer region ITS2, showing the groups into which newly sequenced accessions fall.

Given that the different analyses of the ITS2 matrix produced congruent groupings, and that parsimony branch lengths are simplest to interpret, only maximum parsimony results are presented. A maximum parsimony tree generated using DNA sequences from the nuclear ITS2 region for 214 *Schistidium* accessions (Figure 2) shows five major groups within our sample set, labelled here as Groups 1–5.

Group 1 is split into two subgroups (Figure 3).

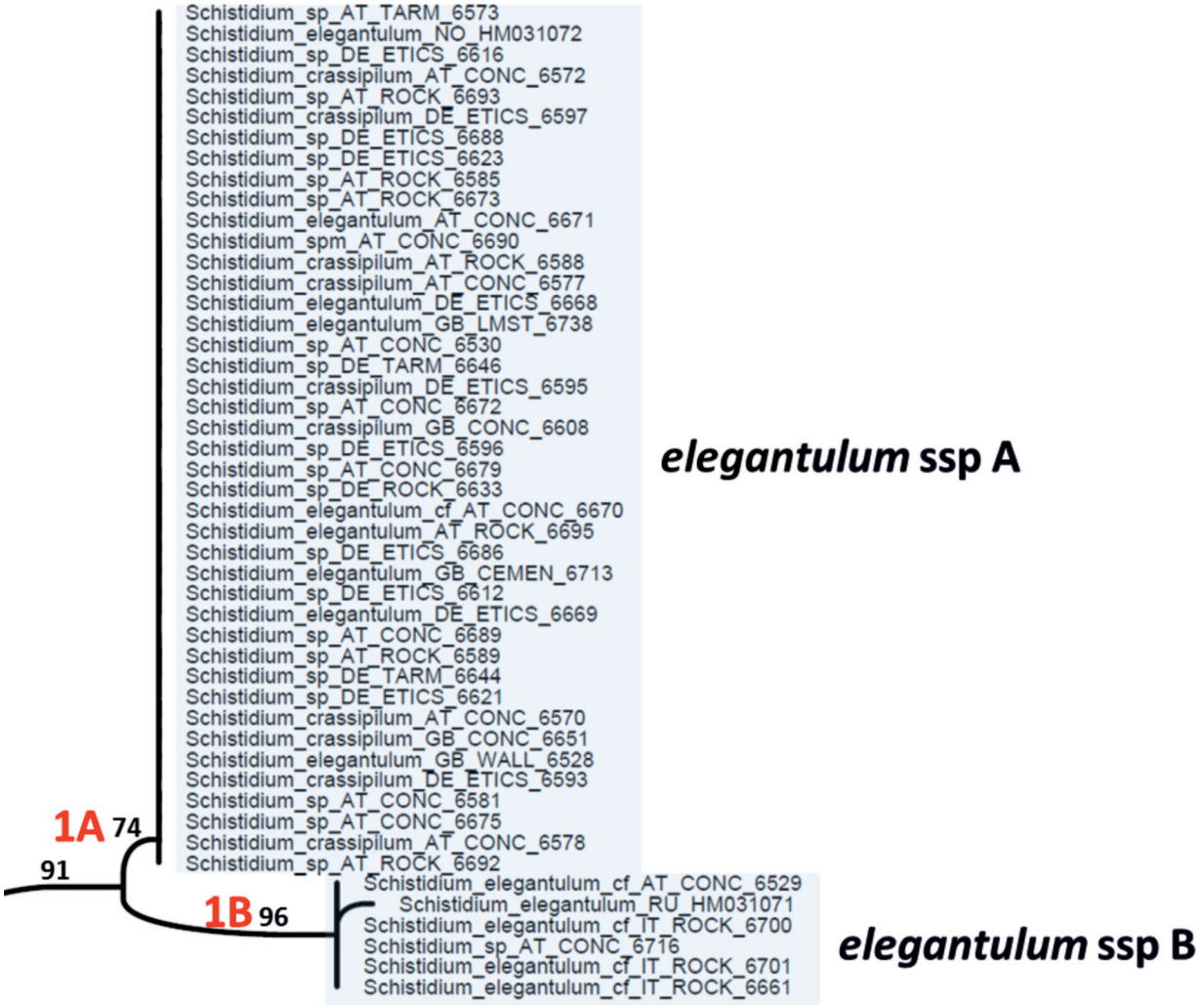

FIGURE 3. Morphological identifications of samples resolved in *Schistidium* Group 1. Samples which could not be identified to species level using morphology are marked 'sp'. DNA accession number, the plant's substrate, and a two-letter country code are given for each sample, where possible.

Group 1A comprises 42 accessions in total, with one GenBank sample, ***Schistidium elegantulum*** (Norway HM031072) that is an exact match to accessions from Germany (15), Austria (21) and Britain (5), growing on rock (8), limestone (1), stone walls (1), concrete (15), cement (1), tarmac (3) and ETICS (12). This genetically uniform lineage may comprise a single species. Most samples that possess capsules have "egg-shaped" ones; combined with other morphological characters this group is a good match to *S. elegantulum*.

Group 1B comprises 6 accessions in total, with one GenBank sample, ***Schistidium elegantulum*** (Russia HM031071) (possibly representing *Schistidium elegantulum* subsp. *wilsonii* H.H. Blom [1996: 239]) that differs by a single base pair from the newly sampled accessions from Austria (2) and Italy (3), growing on concrete (2) and rock (3).This lineage may comprise a single species or subspecies.

Group 2 comprises 34 accessions in total (Figure 4). This genetically diverse group contains five accessions from GenBank (***Schistidium crassipilum*** Russia HM031070, ***Schistidium crassipilum*** Poland HM031073, and ***Schistidium atrofuscum*** [Schimper {1876: 240}] Limpricht [1889: 713] Russia HM053887, as well as two ***Schistidium viride*** H.H. Blom & Darigo [2009: 273] accessions from the US). Our samples in this lineage comprise accessions from Austria (9), Germany (6) and Britain (14), growing on rock (4), concrete (14), buildings (1), walls (2), brick (1), roofing (1), cement (1), tarmac (1) and ETICS (2). This clade is likely to comprise several distinct species, including the true *S. crassipilum*.

Group 3 comprises 61 accessions in total (Figure 5). This clade contains GenBank accessions that have been identified as belonging to several different species, ***Schistidium pruinosum*** (2 accessions, from Russia), ***Schistidium papillosum*** (6 accessions, from Sweden and Russia), ***Schistidium holmenianum*** Steere & Brassard (1976: 208) (1 accession, from Canada), ***Schistidium strictum*** (1 accession, from Norway), ***Schistidium andreaeopsis*** (C. Müller [1883: 126]) Lazarenko (1940: 71) (2 accessions, Canada and Russia), ***Schistidium apocarpum*** (9 accessions, from Britain, Russia and USA), ***Schistidium lancifolium*** (Kindberg [1897: 234]) H.H Blom (1996: 55) (7 accessions, from Ireland, Russia and USA), ***Schistidium boreale*** Poelt (1953: 256) (5 accessions, from Russia and Sweden) and ***Schistidium trichodon*** (2 accessions, from Russia and Sweden). Our samples in this lineage comprise accessions from Austria (7), Germany (5), Italy (5) and Britain (8), growing on rock (14), limestone (3), ETICS (1), walls (1), buildings (1), concrete (3) and tarmac (1).

FIGURE 4. Morphological identifications of samples resolved in *Schistidium* Group 2. Samples which could not be identified to species level using morphology are marked 'sp'. DNA accession number, the plant's substrate, and a two-letter country code are given for each sample, where possible.

This clade is likely to include several distinct species.

Group 4 (Figure 6) is poorly supported in the analyses, but can be subdivided into several supported lineages, including two nested clades that contain some of our barcoding accessions.

Group 4A comprises 29 accessions. This group contains three GenBank accessions, ***S. dupretii*** (2 accessions, from Russia and Austria) and ***S.* sp "*lingulatum*"** (1 accession). Our samples in this lineage comprise accessions from Austria (4 accessions), Germany (21 accessions) and Britain (1 accession), growing on rock (6), concrete (7) and ETICS (12). This clade may comprise a single species.

Group 4B comprises 15 accessions. This group contains one GenBank accession, ***Schistidium robustum*** (Nees & Hornschuch [1827: 123]) H.H. Blom (1996: 149) (from Sweden), which is sister to our samples. Our sampling comprises accessions from Austria (5 accessions) and Germany (9 accessions) growing on rock (7), concrete (3), tarmac (1) and ETICS (3). The sister lineage to *S. robustum* is genetically uniform and may comprise a single species.

Group 5 comprises 6 accessions (Figure 7). This group contains 2 GenBank accessions of ***Schistidium grandirete*** from Russia, 5 GenBank accessions of ***Schistidium pulchrum*** from Russia, as well as our single sample, from Austria, which was growing on a natural substrate (rock). Our sample is genetically quite distinct from the Russian material, and may represent a species that has not yet been included in GenBank.

Morphological re-evaluation following the first round of genetic analysis allowed some of the unidentified samples to be retrospectively attributed to known species, matching groups that were recovered in the analyses, but others still could not be conclusively deliminated morphologically, because of poorly developed or missing characters. If the recurvation on the leaf margins is incompletely expressed, and if urns are not fully developed or are damaged, specimens from Group 2 (Figure 4) with more or less well-developed hairpoints can be misinterpreted as *S. crassipilum*. With suboptimal material, *S. crassipilum, S. elegantulum, S. dupretii, S. robustum* and *S. pulchrum* are very hard to distinguish.

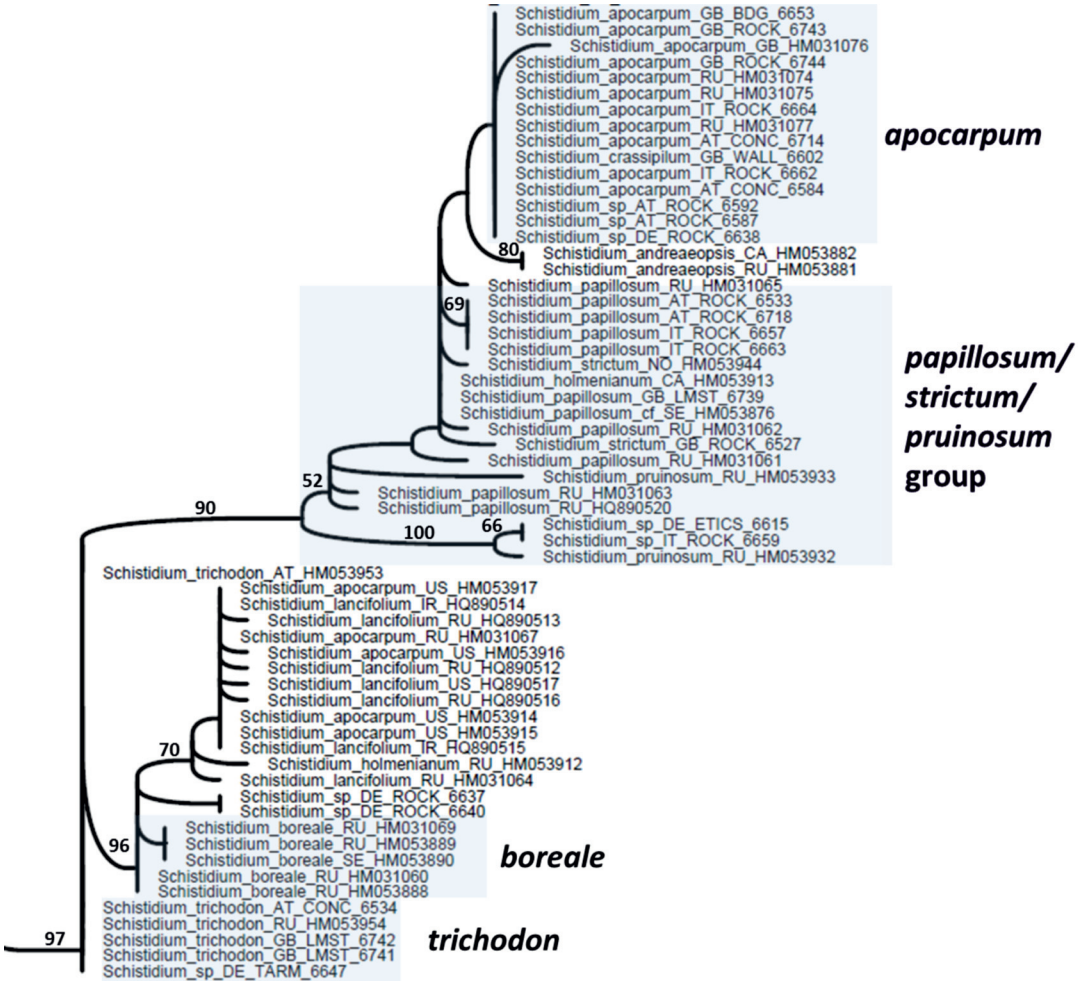

FIGURE 5. Morphological identifications of samples resolved in *Schistidium* Group 3. Samples which could not be identified to species level using morphology are marked 'sp'. DNA accession number, the plant's substrate, and a two-letter country code are given for each sample, where possible.

Discussion

The ITS2 DNA locus amplifies readily and sequences well across *Schistidium*, and there is a wealth of existing information freely available from GenBank that can be used to help identify lineages. However, the high levels of sequence variability in the locus are problematic, in that a robust sequence alignment could not be achieved across the whole genus. In our study, many GenBank samples that were not genetically similar to our own samples were excluded, and the alignment subdivided into blocks of similar sequences. It is possible that not all characters are homologous between these blocks across the alignment. Thus this analysis is not, and should not be treated as, a phylogeny for the genus, but is purely intended as a tool for clustering accessions into groups with similar or the same DNA sequences,

for identification purposes. The plastid marker *mat*K, although less variable than ITS2, has no alignment ambiguities, and may therefore be useful for examining phylogenetic relationships within *Schistidium* (Table 4).

Although several species are mentioned in literature as growing on old man-made surfaces (Blom 1996, Ignatova *et al.* 2009, Bosanquet in Atherton *et al.* 2010, Milyutina *et al*. 2010), Thickpoint Grimmia (*Schistidium crassipilum*) is considered prevalent on concrete and similar substrates. These substrates are, however, often rather poorly characterized, meaning that fine scale differences in taxon composition may have been overlooked. However, the scale of the genetic differences between the clades, and lack of clear geographic signal, suggest the presence of more than one species (see also Hofbauer *et al.* 2014). Morphological differences between the samples from the different groups include such features as the configuration of the leaf tip and the glass hair. Further work is underway to enhance our understanding of these different genetic groupings.

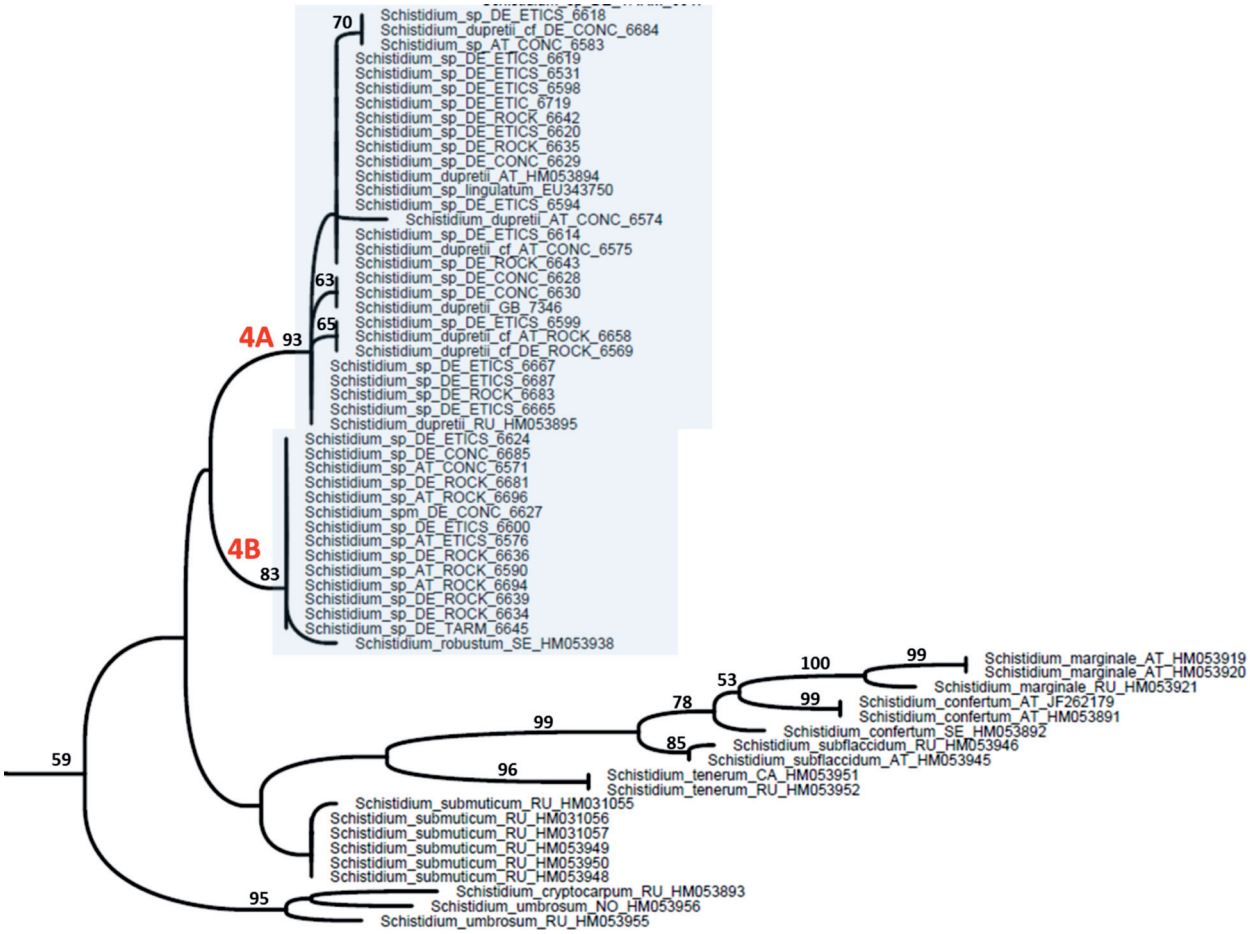

FIGURE 6. Morphological identifications of samples resolved in *Schistidium* Group 4. Samples which could not be identified to species level using morphology are marked 'sp'. DNA accession number, the plant's substrate, and a two-letter country code are given for each sample, where possible.

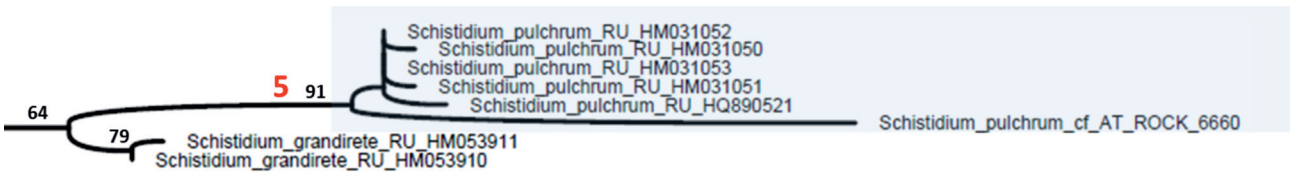

FIGURE 7. Morphological identifications of samples resolved in *Schistidium* Group 5. DNA accession number, the plant's substrate, and a two-letter country code are given for each sample, where possible.

The four main groups (Groups 1–4) of *Schistidium* that we identified all contain accessions that had been collected from man-made surfaces. Taking account of genetic variability within some of the groups, these may represent ca. eight species occurring on man-made surfaces, with a subset of these, five species, found on 4–6 year old external thermal insulation compound systems (ETICS) (Table 5).

The many different systems, designs and materials used in modern construction may impact on the different species/subspecies of *Schistidium* that are part of the primary colonization. Resolving the taxonomy of these colonising species offers a number of potential benefits: this research may lead to better control of moss growth on building surfaces, not only by tailor-made chemical or physical measures, but also by exclusive biocontrol of dominant species. Conversely, such knowledge can also be utilized to allow deliberate induction of moss growth on masonry, which can be beneficial for insulation, aesthetics and carbon sequestration; it has already been shown that moss growth can bind and break down particulate matter and pollution from the air (Frahm 2008). Further outputs of our wider project will include: 1) a reference library of DNA barcodes; 2) a review of genetic characters for herbarium and recent collections of *Schistidium* that were previously classified by morphological characters, and 3) further application of DNA barcoding to identify otherwise indistinguishable samples. Future work could include the cultivation of *Schistidium* accessions identified by DNA barcoding, for deliberate establishment on building surfaces. Combining the existing experience of bryophyte cultivation at both RBGE and IBP, on varied surfaces, and RBGE's expertise in growing a broad range of organisms could prove fruitful.

TABLE 5. *Schistidium* taxa sampled from manmade habitats

Clade	Any manmade habitat	ETICS	Habitat description (Bosanquet in Atherton *et al.* 2010)
1A	*Schistidium elegantulum*	*Schistidium elegantulum*	limestone rocks and rock faces, roadside walls and bridges, churchyards
1B	*Schistidium elegantulum*	-	limestone rocks and rock faces, roadside walls and bridges, churchyards
2	*Schistidium crassipilum*	*Schistidium crassipilum*	calcarious walls, mad-made habitats, tarmac, limestone and sandstone blocks
3A	*Schistidium apocarpum*	-	shaded masonry, gravestones, gutters, rocks and bridge piles, base rich siliceous rocks
3A	*Schistidium pruinosum cf*	*Schistidium pruinosum cf*	exposed rocks, basalt (*Schistidium pruinosum*)
3	*Schistidium trichodon*	-	calcicole - limestones, schist, volcanic tuff, outcrops and boulders
4A	*Schistidium dupretii*	*Schistidium dupretii*	dry montane
4B	*Schistidium robustum cf*	*Schistidium robustum cf*	calcicole - limestone blocks, schist, basalt (*Schistidium robustum*)

At a wider geographical scale, moss samples recently collected by WKH from old concrete surfaces in Navarino, Chile (January 2015) are morphologically referable to *Grimmia anodon* Bruch & Schimper in Bruch, Schimper & Gümbel (1845: 110) and *Schistidium andinum* (Mitten [1868: 97]) Herzog (1916: 53). *Grimmia anodon* has an almost cosmopolitan distribution and is already known from old manmade structures (Greven 1992). Although not the first record of the species from southern Patagonia, the species is usually quite rare locally (cf. Greven 1995, Müller 2009, Buck & Goffinet 2010). For *Schistidium,* on the other hand, there may be a quite different pattern in the southern hemisphere than that which we have described from Europe. *Schistidium andinum* seems to replace other *Schistidium* species on concrete structures in Patagonia. *Schistidium crassipilum* is recorded from manmade structures in North America, but is not known from southern South America to date. Molecular examination of this pattern also offers a promising future research area and may provide new insights into the biogeography of the genus.

Conclusion

Our project revealed that samples of *Schistidium* from anthropogenic surfaces belong to several genetically distinct groups which appear to be widespread on manmade surfaces in Europe. However, sampling is still poor for some of the lineages, and samples from more geographic regions should be considered. Furthermore, additional morphological characterization of the samples is required, in order to make sense of their genetic placements. Therefore we propose future investigations, including sampling from a wider range of places, which could not only help resolve the complicated taxonomy of *Schistidium* growing on anthropogenic surfaces, possibly provide better management of unwanted growth, but also help achieve deliberate cultivation of attractive growth forms.

Acknowledgments

WKH gratefully received support from the SYNTHESYS project (http://www.synthesys.info) for a short term working visit to RBGE, application number: GB-TAF-3881. WKH further wishes to thank the staff of RBGE for hosting this visit. Special thanks are due to David Long for providing valuable material. WKH wishes to thank his home institution (IBP) for support, while LLF, PMH and MLH acknowledge funding from the Scottish Government's Rural and Environment Science and Analytical Services Division. Many thanks also to Hans Blom for valuable discussions and promising to check material and Ryszard Ochyra for help answering critical questions and for providing literature.

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