



Burrowsia, a new genus of lichenized fungi (Caliciaceae), plus the new species *B. cataractae* and *Scoliciosporum fabisporum*, from Mpumalanga, South Africa

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ABSTRACT

The new genus *Burrowsia* (Caliciaceae) is proposed to accommodate the new species *B. cataractae*, which is known from only a single locality in Mpumalanga, South Africa. *Burrowsia* is characterized by its pigmented, submuriform ascospores and ascus with an apical tube structure, and also by its DNA sequence data that place it outside related buellioid genera. We also describe the new species *Scoliciosporum fabisporum*, also known from a single locality in Mpumalanga, which differs from all other species of that genus in having distinctive kidney-shaped, 0–1-septate ascospores. It is most closely related to the Northern Hemisphere species *S. intrusum*, which is here confirmed by molecular data as belonging to this genus in a well-supported Scoliciosporaceae. The potential of the region to yield additional novel lichen taxa is explored.

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1. Introduction

Lichens are an integral part of most terrestrial ecosystems, from arid polar deserts to tropical rainforests, from maritime rocks to alpine summits. They provide important ecosystem services such as nutrient recycling, food and shelter for many species of animals (e.g., birds, snails, ants and mites), regulate soil moisture and produce nitrogen for other species (e.g., Asplund and Wardle 2013, 2017; Elbert et al., 2012; Knops et al., 1996; Slack 1988). Unfortunately, they are often overlooked and their biodiversity and ecology are poorly known or understood in many parts of the world. This is particularly true of crustose lichens that typically constitute around two-thirds of all lichen biodiversity in an area (Lendemer et al., 2013; Spribille et al., 2020).

The lichen biota of South Africa is reasonably well documented. The current checklist (Fryday, 2015 and 2016; Ahti et al., 2016) lists 1 751 taxa, and a second supplement currently in preparation (Medeiros and Fryday, in prep.) adds a further 15 taxa, making a total

of 1 766. Estimates for the potential total number of taxa present in the country range from 2 000 (Crous et al., 2006) to 3 000 (Fryday, 2015), which is well below the over 21 000 vascular plants reported for the country (Schnitzler et al., 2011) and low when compared with the 1 838 taxa reported from Great Britain (Smith et al., 2009), a country which is only one-fifth the size. At the same time, the lichen biodiversity is poorly understood – especially the microlichen biota (crustose species). Many of these were described from South Africa in the late 19th century and are known only from their type collections, which have often not been studied since the species were described. Chief among these are the taxa described by Ernestus Stizenberger in his *Lichenaea Africana* (Stizenberger, 1890 and 1891), but Nylander (1869 and in Crombie, 1876), Vainio (1926) and Zahlbruckner (1906, 1926, 1932 and 1936) also described many new species from the country. Since that time, there has been intermittent research on the lichen biota of South Africa, mainly by visiting European lichenologists (e.g., Almborn, 1966 and 1987; Schultz et al., 2009) but also by researchers resident in South Africa, most notably Franklin Brusse. Between 1985 and 1994, Brusse published over 30 papers describing new species from the country and often included new reports of other species (see Fryday, 2015 for references and a

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fuller history of lichenology in South Africa). However, these researchers have usually restricted themselves to describing new species and have not explored the systematic position or relationships of their discoveries (but see Aptroot et al., 2019; Kondratyuk et al., 2015; Leavitt et al., 2018a and b). Here we describe two new species of saxicolous, crustose lichens – the group of lichens that suffers from the greatest lack of attention within the South African lichen biota (Fryday, 2015) – that are known only from South Africa. One of these species is shown to occupy an isolated position within the Caliciaceae on the basis of both molecular and morphological data and so we erect a new genus to accommodate it. The other species is readily accommodated within the genus *Scoliciosporum* A. Massal.

2. Materials and methods

This study is based upon specimens of crustose, lecideoid lichens collected by the first author from Mpumalanga, South Africa, in 2015 and 2016.

2.1. Morphological methods

Apothecial characteristics were examined by light microscopy on hand-cut sections mounted in water, 10% KOH (K), 50% HNO₃ (N) or Lugol's reagent (0.15% aqueous IKI). Thallus sections were investigated in water, K and lactophenol cotton-blue. The ascus structure was studied in IKI, both without prior treatment and after pretreatment with K. Measurements of ascospores and paraphyses were made in K. Ascospore dimensions are given as (smallest measured–) *arithmetic mean* ± standard deviation (–largest measured). Hamathecial filaments are referred to as 'paraphyses' regardless of their origin. Thin layer chromatography (TLC) follows the methods of Orange et al., 2001. Nomenclature for apothecial pigments follows Meyer and Printzen, 2000.

2.2. Molecular methods

Approximately 10 apothecia from each of the two holotype specimens were excised from the thallus, frozen with liquid nitrogen, and ground to a powder with a plastic pestle. Pulverized samples were then lysed overnight in a 2% sodium dodecyl sulfate (SDS) extraction buffer. DNA extraction followed a standard phenol:chloroform protocol. Extracted DNA was precipitated with the addition of isopropyl alcohol and centrifugation before being cleaned with 80% ethanol and a final centrifugation. The DNA pellet was air-dried before being rehydrated in sterile water and stored at –20 °C.

We attempted to amplify the following regions from the mycobiont genome with polymerase chain reaction (PCR): the mitochondrial small subunit (mtSSU) using the primer pair mrSSU1 and mrSSU3R (Zoller et al., 1999); the internal transcribed spacer (ITS); including ITS1, 5.8S, and ITS2) using the primer pair ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) or LR3 (Vilgalys and Hester, 1990); the nuclear large subunit (nrLSU) using the primer pair LROR (Rehner and Samuels, 1994) and LR7 (Vilgalys and Hester, 1990); the nuclear small subunit (nrSSU) using the primer pair NSSU131 (Kauff and Lutzoni, 2002) and NS24 (Gargas and Taylor, 1992); the RNA polymerase II largest subunit (*RPB1*) using the primer pair *RPB1*-aFasc (Hofstetter et al., 2007) and *RPB1*-cR (Matheny et al., 2002); and the RNA polymerase II second-largest subunit (*RPB2*) using the primer pair *fRPB2*-7cF and *fRPB2*-11aR (Liu et al., 1999). To identify the photobiont, we also amplified the algal ITS region with primers ITS1T (Kroken and Taylor, 2000) and either ITS4 or LR3. PCR products were viewed on 1% agarose gels and subjected to an enzymatic cleanup step with exonuclease and shrimp alkaline phosphatase. Sanger sequencing was performed by Eurofins Genomics (Research Triangle Park, North Carolina, USA) using PCR primers, except that ITS1 (White et al., 1990) was substituted for ITS1F. Forward and reverse reads were assembled in Sequencher 5.4.6 (Gene Codes Corporation, Ann

Arbor, MI, USA) or Geneious Prime 2020.0.3 (www.geneious.com) and the assembled contigs were checked by eye for base call errors.

We used the online T-BAS portal (Carbone et al., 2016 and 2019; Miller et al., 2015) to obtain preliminary phylogenetic placements for the two species. T-BAS uses the RAxML evolutionary placement algorithm (Berger et al., 2011) to infer approximate phylogenetic placements for input sequences. We placed the newly obtained sequences in a phylogeny of Pezizomycotina to confirm their position in Lecanoromycetes, then re-ran the placement with a phylogeny of Lecanoromycetes (Miadlikowska et al., 2014) to determine a family-level placement. Sequences from related species were obtained from GenBank, and additional new sequence data were generated for a specimen of *Pseudothelomma occidentale* (Herre) M. Prieto & Wedin (Alaska, Fryday 10 069, MSC). ITS and mtSSU sequences were aligned with MAFFT (Katoh et al., 2017; Kuraku et al., 2013) using the G-INS-1 method; alignment errors were corrected and ambiguously aligned regions were excluded by eye in Mesquite. Nuclear rDNA regions nrLSU and nrSSU were aligned by eye in Mesquite according to known intron positions. Protein-coding sequences (*RPB1*, *RPB2*, beta-tubulin and *MCM7*) were aligned in Mesquite using the amino acid translation. Intron sequences were excluded from the analysis. Single-locus trees were inferred with RAxML (Stamatakis, 2014); finding no well-supported conflicts in the single locus trees for either of our species, we performed subsequent analyses on concatenated datasets. PartitionFinder2 (Lanfear et al., 2016) was used to delimit partitions in the concatenated alignment of all loci and determine the best evolutionary model for the data. We ran Partitionfinder2 with the greedy search algorithm, allowing independent branch lengths per partition, and used the second-order Akaike Information Criterion (AICc) to determine the best-performing substitution model and partition scheme. The partitioned datasets were subjected to maximum likelihood analysis in RAxML 8.2.12 using the GTR+I+Γ model; RAxML was allowed to automatically halt bootstrapping with the autoMRE stopping criterion (Pattengale et al., 2009). PartitionFinder2 and RAxML were run on the CIPRES server (Miller et al., 2010). Alignment files and trees have been uploaded to TreeBASE as accession S26468. To identify photobiont genus, we used BLAST (Zhang et al., 2000) to compare the algal ITS sequences against the NCBI Nucleotide database.

3. Results

3.1. Molecular results

Morphological studies indicated that the two saxicolous specimens represent two species new to science. The preliminary analysis in T-BAS showed that sequences from the two specimens were consistently associated with Caliciaceae and *Scoliciosporum intrusum* (Th. Fr.) Hafellner (Lecanorales incertae sedis), respectively. Using sequences from Miadlikowska et al. (2014) as a starting point, we assembled sequence datasets for Caliciaceae and Lecanorales, the latter focusing on clades outside suborder Lecanorineae (Miadlikowska et al., 2014). The five-locus dataset for Lecanorales (ITS, mtSSU, nrSSU, nrLSU, and *RPB1*; Table 1) includes 4520 characters and is dominated by sequences from Andersen and Ekman (2005), with additions from other studies. The eight-locus dataset for Caliciaceae (ITS, mtSSU, nrSSU, nrLSU, *RPB1*, *RPB2*, beta-tubulin and *MCM7*; Table 2) includes 9726 characters and contains many sequences from Prieto and Wedin (2017). The maximum likelihood analysis for Caliciaceae (Fig. 1) confirmed the placement of one of the new species in this family as a long branch among several buellioid genera, whereas the analysis of Lecanorales (Fig. 2) confirmed the relationship of the other new species with *Scoliciosporum intrusum* within a well-supported Scoliciosporaceae. Based on the morphological and molecular results, one new genus and two new species are described below.

Table 1

GenBank accession numbers for specimens used in the phylogenetic analysis of Caliciaceae. Physciaceae (*Heterodermia vulgaris* + *Physcia aipolia*) was used as the outgroup. Newly generated sequences are in bold. The final row shows the number of sites for each locus present in the concatenated alignment.

Species	ITS	mtSSU	nrSSU	nrLSU	<i>RPB1</i>	<i>RPB2</i>	beta-tubulin	<i>MCM7</i>
<i>Physcia aipolia</i>	DQ782836	DQ912290	DQ782876	DQ782904	DQ782820	DQ782862	KX529021	KX529052
<i>Heterodermia vulgaris</i>	HQ650704	DQ912288	DQ883789	DQ883798	DQ883741	DQ883754	–	–
<i>Acolium inquinans</i>	AY450583	AY143404	U86695	AY453639	–	–	KX529023	–
<i>Acolium karelicum</i>	KX512897	–	–	KX512879	–	–	–	KX529045
<i>Acrosocyphus sphaerophoroides</i>	KX512898	KX512984	–	–	–	–	–	KX529029
<i>Alloalcalium adaequatum</i>	KX512906	KX512986	–	KX512859	–	–	KX528996	–
<i>Amandinea coniois</i>	KJ607904	KX512978	–	KX512865	–	–	KX528998	–
<i>Amandinea punctata</i>	HQ650627	DQ986874	DQ986721	DQ986756	KJ766906	DQ992435	–	KX529025
<i>Baculifera remensa</i>	–	KX512962	–	KX512881	–	–	–	–
<i>Buellia disciformis</i>	AY143392	AY143401	AF241543	AY340537	–	–	–	–
<i>Buellia elegans</i>	AY143411	AY143417	–	–	–	–	KX528988	–
<i>Buellia erubescens</i>	KX512902	KX512969	–	KX512874	–	–	KX529004	–
<i>Buellia fimbriata</i>	–	–	DQ973010	DQ973034	DQ973057	DQ973097	–	–
<i>Buellia frigida</i>	HQ650628	DQ986903	DQ883699	DQ883695	DQ883724	DQ883712	–	–
<i>Buellia stillingiana</i>	–	DQ912287	DQ912319	–	DQ912368	DQ912391	–	–
<i>Buellia tesserata</i>	KX512904	–	–	KX512885	–	–	–	KX529050
<i>Burrowsia cataractae</i>	MT622501	–	–	MT622322	MT610731	MT610733	–	–
<i>Calicium pinicola</i>	KX512917	KX512991	–	KX512887	–	–	KX529014	KX529066
<i>Calicium tigillare</i>	AY452498	JQ301534	JQ301646	JQ301593	–	–	KX529002	JX000162
<i>Calicium viride</i>	HQ650703	AY584696	AF356669	AF356670	–	AY641031	KX529013	–
<i>Culbersonia nubila</i>	MH121318	–	–	MH121320	–	–	–	–
<i>Dimelaena radiata</i>	JQ301693	–	JQ301647	JQ301594	JQ301736	JQ301787	–	KX529049
<i>Diploicia canescens</i>	AF250793	AY464084	AJ421684	–	–	–	–	–
<i>Diplotomma alboatrum</i>	KX512924	KX512966	–	KX512877	–	–	KX529007	KX529043
<i>Diplotomma epipolium</i>	AF540509	JQ301535	AJ506969	JQ301595	–	–	–	–
<i>Pseudothelomma occidentale</i>	MT622500	–	MT611985	MT611534	MT610734	MT610735	–	–
<i>Pseudothelomma ocellatum</i>	KF020559	JQ301540	–	JQ301599	–	–	KX529020	KX529063
<i>Pyxine sorediata</i>	JQ301697	DQ972984	DQ973012	DQ973036	–	DQ973071	KX529001	KX529039
<i>Pyxine subcinerea</i>	HQ650705	DQ912292	DQ883793	DQ883802	DQ883745	DQ883758	–	–
<i>Tetramelas chloroleucus</i>	KX512938	–	–	KX512875	–	–	KX529006	–
<i>Texosporium sancti-jacobi</i>	KX512941	KX512981	–	KX512863	–	–	KX528994	KX529031
<i>Thelomma mammosum</i>	KX512943	KX512953	–	KX512851	–	–	KX529017	KX529067
<i>Thelomma santessonii</i>	KX512944	KX512951	–	KX512889	–	–	–	KX529064
<i>Tholurna dissimilis</i>	AY452499	DQ972974	DQ973002	–	–	–	KX528992	KX529053
Alignment sites	264	690	1582	1304	2760	1881	687	558

3.2. The taxa

Burrowsia Fryday and I. Medeiros gen. nov.

Mycobank No.: MB 835878

The new genus is characterized by its pigmented, submuriform ascospores and asci with an apical tube-like structure. It is further distinguished from all other genera by its DNA sequence data.

Type species: Burrowsia cataractae Fryday and I. Medeiros

Etymology: The name honours John and Sandra (Sandie) Burrows, the managers of Buffelskloof Nature Reserve from where the type species was collected, for their outstanding, life-long contribution to conservation and biodiversity research in South Africa.

Because the genus is monotypic, a separate description of the genus would be superfluous.

Burrowsia cataractae Fryday and I. Medeiros sp. nov.

Mycobank No.: MB 835879

Separated from all other species in the Caliciaceae by its submuriform ascospores, ascus structure and DNA sequence data. Superficially similar to *Rhizocarpon lavatum* but with a different ascus structure, smaller pigmented submuriform ascospores and a thallus containing norstictic acid and two unidentified substances.

Typus: South Africa, Mpumalanga, Ehlanzeni District, Thaba Chweu Municipality, Mashishing (Lydenburg), Buffelskloof Nature Reserve, Calodendrum Falls, 25° 17.839'S, 30° 30.656'E, 1500 m, damp closed forest, saxicolous boulders in splash zone of waterfall, 11 February 2016, A.M. Fryday (11 591), I. Medeiros, J. Burrows and A. Frisby (PRE–holo!) (Fig. 3).

Thallus widespreading, grey–brown (greenish–brown when wet), rimose to cracked–areolate, margin obscurely lobed with a thin black prothallus, 0.3–0.4 mm thick; *upper surface layer* ±hyaline,

cells with dilutely brown pigmented cap buried within an epinecral layer c. 10 µm deep; *photobiont layer* continuous, 20–70 µm deep; *photobiont Trebouxia*; cells chlorococcoid ±orbicular 6–7 µm diam. to elongate 10–12 × 5–6 µm; *lower surface layer*, c. 250 µm deep, brown pigmented, composed of ±vertically aligned paraplectenchyma, cells 10–15 × 5–6 µm.

Apothecia frequent, lecideine, orbicular, occasionally flexuose when mature, dark brown to black, semi–immersed to adnate with a broad base, becoming sessile, (0.4–)0.6–0.8 mm diam. *Proper margin* c. 0.1 mm broad, slightly raised and persistent, paler than the disk; in section cupular, initially brown but clearing and producing red, acicular crystals with K, in thin section with a brown, poorly defined cortex and ±hyaline medulla; composed of rows of radiating hyphae, 6–10 × 4–5 µm. *Hymenium* 110–130 µm, I+ blue; *paraphyses* ±simple, rarely branched, septate, becoming ±moniliform above, 1.5–2.5 µm thick, widening to 3–4 µm at apex with a thin brown cap. *Ascus* cylindrical, c. 70 × (15–)20 µm, walls 2–3 µm thick; tholus IKI+ pale blue with a central, darker–staining tube-like structure narrowing towards the apex; *ascospores* 4–6(–8)/ascus, pigmented, submuriform, non–halonate, (17–)23.61 ± 4.37(–32) × (7–)11.74 ± 5.66 (–15) µm, l/b ratio (1.267–)2.05 ± 0.39(–2.46), n = 23, ellipsoid, sometimes slightly curved. *Hypothecium* dark brown, c. 150 µm thick, composed of ±randomly aligned hyphae.

Conidiomata pycnidia, immersed in thallus with just dark ostiole visible, flask–shaped with pale brown wall; *conidia* long bacilliform, 10–12 × 1 µm.

Chemistry K+ red, C–, Pd–; norstictic acid and two unidentified substances by TLC. Both unidentified substances gave UVC++ pinkish–cream spots; a large one at Rf 7.0 and a smaller one at Rf 5.5 in solvent C.

Table 2

GenBank accession numbers for specimens used in the phylogenetic analysis of Lecanorales. *Rusavskia elegans* was used as the outgroup. Newly generated sequences are in bold. The final row shows the number of sites for each locus present in the concatenated alignment.

Species	ITS	mtSSU	nrSSU	nrLSU	RPB1
<i>Rusavskia elegans</i>	EF423390	DQ912304	DQ912329	DQ912352	DQ973068
<i>Aquacidia antricola</i>	—	MH817969	—	—	—
<i>Bapalmia palmularis</i>	AY756457	AY567781	—	—	—
<i>Bilimbia sabuletorum</i>	—	KJ766361	KJ766694	KJ766534	KJ766839
<i>Brianaria sylvicola</i>	JX983583	JX983587	—	—	AY756392
<i>Byssoloma leucoblepharum</i>	AY756459	AY567778	AF455135	AY756317	AY756380
<i>Byssoloma subdiscordans</i>	AY756461	AY567779	KJ766696	KJ766538	KJ766841
<i>Calopadia foliicola</i>	AY756462	AY567782	—	AY756318	AY756381
<i>Calopadia phylogena</i>	—	KJ766365	—	KJ766539	—
<i>Calycidium cuneatum</i>	JX000114	JX000117	—	JX000083	JX000134
<i>Fellhanera boutellei</i>	AF414858	KJ766392	KJ766716	KJ766559	—
<i>Fellhanera subtilis</i>	AY756464	AY567786	—	AY756321	—
<i>Fellhaneropsis vezdae</i>	—	AY567744	—	—	—
<i>Lasioloma arachnoideum</i>	AY756467	AY567783	—	—	—
<i>Lecanora hybocarpa</i>	DQ782849	DQ912273	DQ782883	DQ782910	DQ782829
<i>Leimonia erratica</i>	AY756475	AY567737	—	AY756328	AY756390
<i>Micarea adnata</i>	AY756468	AY567751	AF455134	AY756326	AY756388
<i>Micarea alabastrites</i>	AY756469	AY567764	—	AY756327	AY756389
<i>Micarea byssacea</i>	AY756485	AY567749	—	AY756330	—
<i>Micarea cinerea</i>	AY756472	AY567763	—	—	—
<i>Micarea denigrata</i>	—	KJ766437	KJ766750	KJ766598	KJ766873
<i>Micarea doliformis</i>	HQ650654	HQ660557	—	HQ660534	—
<i>Micarea lapillicola</i>	AY756479	AY567735	—	—	—
<i>Micarea lithinella</i>	AY756482	AY567734	—	—	—
<i>Micarea misella</i>	AY756486	AY567752	—	—	—
<i>Micarea myriocarpa</i>	AY756487	AY567736	—	—	—
<i>Micarea paratropa</i>	AY756490	AY567740	—	—	—
<i>Micarea synotheoides</i>	AY756493	AY567756	—	—	—
<i>Protoblastenia calva</i>	HQ650618	DQ98690	JQ301653	JQ301601	DQ986830
<i>Protoblastenia rupestris</i>	—	—	KJ766771	KJ766631	KJ766880
<i>Psilolechia leprosa</i>	AY756496	AY567730	—	AY756333	AY756395
<i>Psilolechia lucida</i>	—	KJ766472	KJ766777	KJ766639	KJ766884
<i>Szczawinskia tsugae</i>	AY756499	—	—	—	—
<i>Scoliciosporum chlorococcum</i>	—	AY567768	—	—	—
<i>Scoliciosporum fabisporum</i>	—	MT611934	—	MT611533	MT610732
<i>Scoliciosporum intrusum</i>	—	AY567767	—	AY756329	—
<i>Scoliciosporum umbrinum</i>	—	AY300911	AF091587	AY300861	AY756397
<i>Septotrapelia usnicum</i>	—	AY300894	—	AY300843	DQ870952
<i>Sphaerophorus fragilis</i>	HQ650600	DQ986789	DQ983487	DQ986805	—
<i>Sphaerophorus globosus</i>	HQ650622	AY584723	DQ986712	DQ986767	DQ986836
<i>Sporopodium antoninianum</i>	AY756498	AY567785	—	—	—
<i>Tephromela atra</i>	HQ650608	DQ986879	DQ986724	DQ986766	DQ986835
Alignment sites	258	741	1601	1323	597

Etymology: The specific epithet references the habitat of the only known collection, which is at the base of a waterfall: *cataractae* = of the waterfall (Latin: genitive, singular).

Distribution and Ecology: The new species is known only from the type collection, which is at the base of a waterfall in a deep ravine (Fig. 4). The falls are on the Dwaalheuvell Quartzites and the new species grows on moist upper surfaces of flat, quartzite rocks around rock pools where it would have been permanently damp, if not periodically inundated.

The vegetation of the ravine is classified as Eastern Dry Afrotropical Forest (Lötter et al., 2014). This forest type receives mean annual precipitation of 1 084 mm and has a mean annual temperature of 16.7°C, but this is of little relevance to the highly humid microhabitat in which the new species occurred.

Remarks:

The phylogenetic tree (Fig. 1) clearly places the new species in the Caliciaceae, albeit in an isolated position. It is included in a clade that also includes *Buellia disciformis* (Fr.) Mudd, which is the type species of *Buellia*, but with a very long branch length. The genus-level phylogeny and taxonomy of the buellioid Caliciaceae has not yet been settled, despite recent work on the family (Marbach, 2000; Prieto and

Wedin, 2017). Species of *Buellia* typically have pigmented, 1-septate ascospores (rarely 3-septate) and a different ascus structure (*Bacidia* or *Biatora*-type; Fig. 5). This type of ascus has a tholus (the area at the ascus apex between the inner and outer walls) that stains dark blue with an unstained central cone, either with (*Biatora*-type) or without (*Bacidia*-type) a darker staining wall (Rambold et al., 1994; Bungartz et al., 2007). Our new species would, therefore, be anomalous if placed in that genus. Submuriform ascospores are otherwise known in the Caliciaceae only in the genus *Diplotomma* Flot., which is well-separated from our new species in our phylogeny.

The gross morphology of the new species shows a remarkable similarity to *Rhizocarpon lavatum* (Ach.) Hazsl., which occurs in similar situations (damp, semi-inundated siliceous rocks) in the Northern Hemisphere (Fletcher et al., 2009) and has also been reported from New Zealand (Fryday, 2004), but not South Africa. However, the new species is readily separated from that species microscopically by the presence of pigmented ascospores and it also differs in its ascus structure and chemistry (*Rhizocarpon*-type and no substances in *R. lavatum*).

We experienced some difficulty obtaining DNA sequence data from this species due to the frequent co-amplification of an

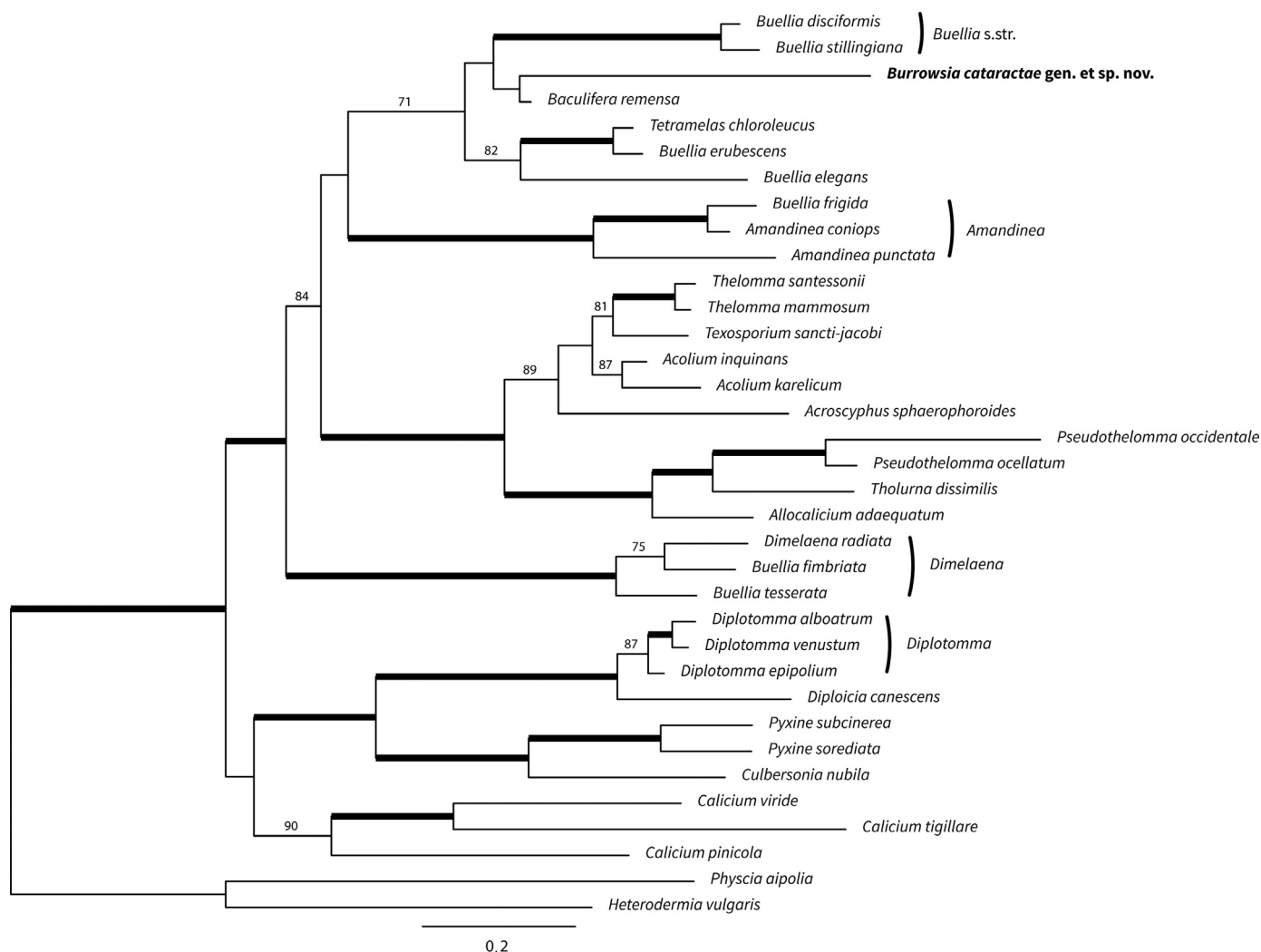


Fig. 1. Phylogeny of Caliciaceae based on maximum likelihood analysis of concatenated ITS, nrLSU, nrSSU, mtSSU, RPB1, RPB2, beta-tubulin and MCM7. Branches in bold have bootstrap support values ≥ 95 ; bootstrap values < 70 are not displayed. Scale indicates substitutions per site. The new species is indicated with bold text.

endolichenic fungus in Chaetothyriales (Eurotiomycetes). Sequences of this fungus have been submitted to GenBank (“uncultured Chaetothyriales”; nrLSU, MT611532; nrSSU, MT611986). Species of Chaetothyriales have recently been shown to be common endolichenic inhabitants of saxicolous lichens (Muggia et al., 2016) in addition to their previously documented role as extremophile rock-inhabiting fungi (Gueidan et al., 2008).

The ITS and nrLSU sequences for the *Trebouxia* photobiont are available on GenBank as accessions MT622498 and MT611535, respectively.

***Scoliosporum fabisporum* Fryday and I. Medeiros sp. nov.**

Mycobank No.: MB 835880

Separated from all other species of *Scoliosporum* by the curved, kidney-shaped ascospores. Also distinguished by its \pm flat apothecia with a thick, slightly raised margin and its DNA sequence data.

Typus: South Africa, Mpumalanga, Ehlanzeni District, Umjindi Municipality, Barberton, eMenzana (Badplaas) Rd, c. 1.0 km northeast of Nelshoogte, 25.8443S, 30.7795E, 1505 m., weathered ultramafic (serpentine) rock outcrop in grassland by roadside, 30 March 2015, Fryday (11 123) and S. Siebert (PRE–holo!) (Fig. 6).

Thallus effuse, > 2 cm across, grey-brown, areolate; areoles 0.5–1.0 mm across with an irregular surface, edges often raised from the substrate and sometimes becoming \pm umbilicate, 0.2–0.3 mm thick; **cortex** absent; **medulla** 1–. **Photobiont** *Symbiochloris*, cells 9–12 μ m diam.

Apothecia black, lecideine, orbicular, 0.2–0.3(–0.4) mm diam., disc, initially convex, becoming plane to slightly concave. **Proper margin** barely apparent in young apothecia becoming more prominent in mature apothecia, poorly differentiated and barely raised, 0.05–0.10 mm wide; in section cupular, 90–100 μ m wide, hyaline becoming dilute brown in the outer 12–20 μ m, composed of conglutinate, radiating branched and anastomosing hyphae, 1–2 μ m wide, not widening at the surface, readily separating in K. **Hymenium** 60–70 μ m tall, hyaline at the base becoming increasingly dilute aeruginose towards the epihymenium, at least the upper 25 μ m aeruginose, sometimes the whole hymenium pigmented, pigment N+ magenta (cinereorufa-green); **paraphyses** simple, thin (c. 1 μ m wide) not septate, readily separating in K, not capitate; **epihymenium** aeruginose. **Ascus** *Lecanora*-type; initially cylindrical becoming slightly clavate, 40–45 \times 10–15 μ m; **ascospores** (0–)1-septate, fabiform, rarely straight, (10–)12.0 \pm 1.51(–15) \times (4–)4.6 \pm 0.50(–5) μ m, $n = 15$, broadly ellipsoid with rounded apices. **Hypothecium** hyaline, c. 70 μ m thick, composed of thick (4–5 μ m wide) randomly arranged, contorted hyphae, appearing almost granular.

Conidiomata not observed.

Chemistry. No substances by TLC.

Etymology: The specific epithet refers to the bean-shaped ascospores.

Distribution and Ecology: The new species is known only from the type collection where it occurred on exposed, magnesium-rich,

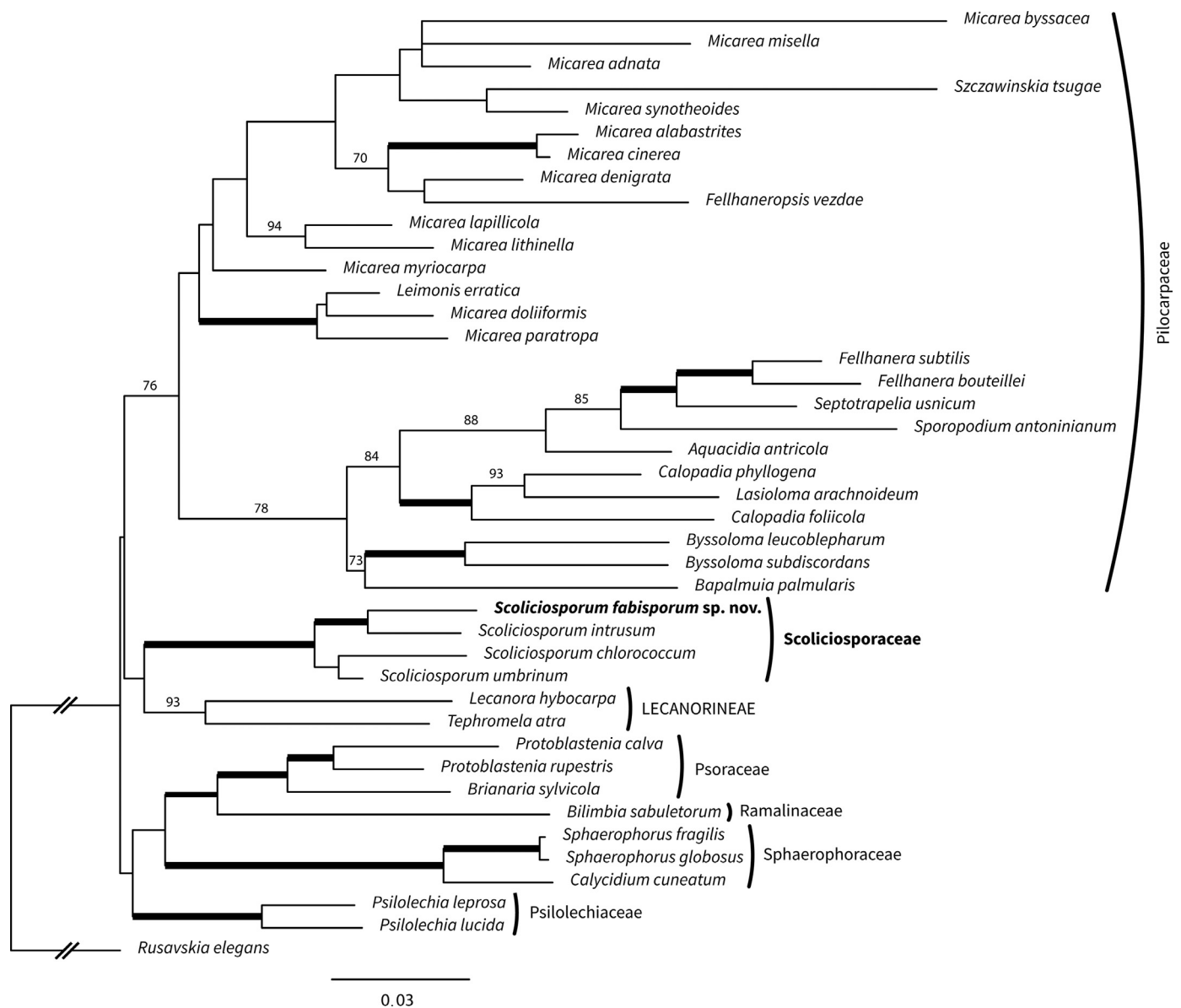


Fig. 2. Phylogeny of Lecanorales based on maximum likelihood analysis of concatenated ITS, nrLSU, nrSSU, mtSSU and *RPB1*. Branches in bold have bootstrap support values ≥ 95 ; bootstrap values < 70 are not displayed. Scale indicates substitutions per site. The new species is indicated with bold text.

ultramafic (serpentinite; Venter et al., 2018) rocks by a road-cut (Anhaeusser, 2001) in short grassland and rocky shrubland of the Barberton Montane Grassland of the Mesic Highveld Grassland Bioregion (Mucina and Rutherford, 2006). This grassland receives mean annual precipitation of 1 194 mm and has a mean annual temperature of 16.7°C. Analyzed rock samples had a Mg:Ca quotient of 24.9 and total Ni concentrations of 2 200 ppm.

In 2016, we spent three weeks sampling the lichen biota of ultramafic rocks in Mpumalanga and did not encounter the species at any other site. If it is an ultramafic specialist, then it would appear to be an extremely rare one. However, experience in other areas suggests that, unlike vascular plants, very few, if any, lichen taxa are ultramafic specialists (Rajakaruna et al., 2012; Favero-Longo et al., 2018). It appears more likely, therefore, that this species occurs on a wider range of rock types and we only discovered it on ultramafic rocks because that was the rock type that we were sampling.

Remarks:

The ITS sequence for the *Symbiochloris* photobiont of *Scoliciosporum fabisporum* is available on GenBank as accession MT622499.

Our phylogenetic tree (Fig. 2) shows the new species as sister to the Northern Hemisphere species *S. intrusum* (Fig. 7) in a well-supported Scoliciosporaceae. The systematic position of *S. intrusum* has previously been unclear. Because of its lecideoid apothecia, it was initially described by Th. Fries in the widely circumscribed genus *Lecidea* Ach. (Fries, 1867) but he soon transferred it to *Catillaria* A. Massal. (Fries, 1874) because of its 1-septate ascospores. Coppins and Kiliias (Coppins, 1983) included it in *Micarea* Th. Fr., but with some hesitation and noted similarities with *Scoliciosporum*. Rambold and Triebel (Hertel, 1991; Aptroot et al., 1997) transferred the species to *Carbonea* (Hertel) Hertel, before Hafellner (2004) took up Coppins and Kiliias' suggestion and made the combination into *Scoliciosporum*. All these taxonomic changes were made on the basis of morphological characters alone but an initial phylogenetic analysis by Andersen and Ekman (2005) with mtSSU confirmed the monophyly of *Scoliciosporum* including *S. intrusum* (as *Micarea intrusa*). However, subsequent molecular research has suggested that *S. intrusum* is not con-generic with *S. umbrinum* (Ach.) Arnold (the type species of *Scoliciosporum*). In an extensive, multi gene phylogeny, Miałlikowska et al. (2014),

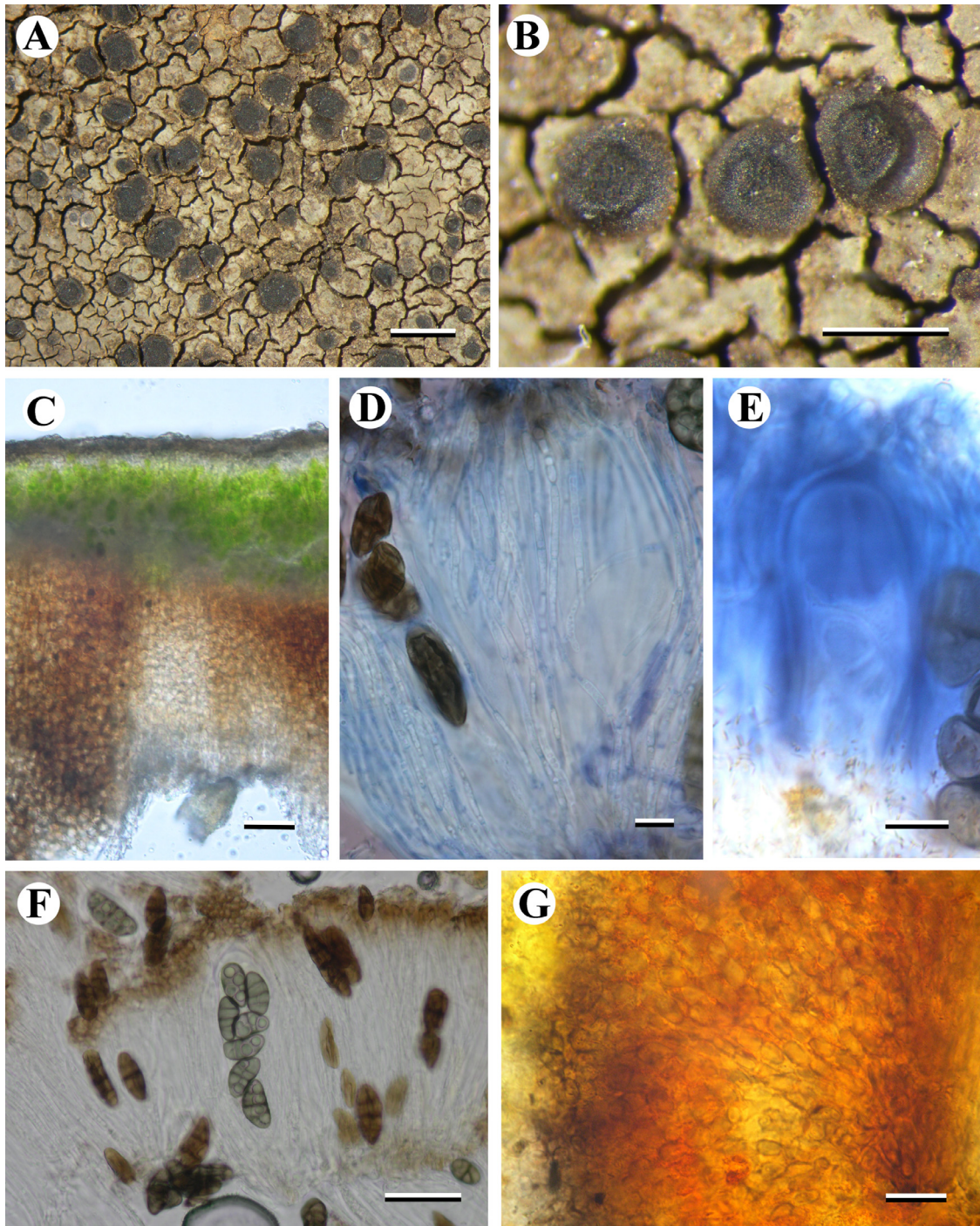


Fig. 3. *Burrowsia cataractae* (holotype). A: Thallus and apothecia, B: Immature apothecia, C: Thallus section, D: Paraphyses (in ink, pretreated with vinegar), E: Ascus (in IKI), F: Ascospores, G: Exciple (in K). Scale bars: A & B = 1 mm; C, F & G = 20 μ m; D & E = 10 μ m.

maintained an independent Scoliciosporaceae but placed *S. intrusum* in the Pilocarpaceae, although they did not, for the most part, find well-supported relationships between families in this portion of the Lecanoromycetes phylogeny and the placement of *S. intrusum* in the Pilocarpaceae was supported by some but not all of their analyses. More recently, Kraichak et al. (2018) placed *S. umbrinum* in the Sphaerophoraceae while retaining *S. intrusum* in an independent Scoliciosporaceae. However, our phylogenetic analysis places *S. umbrinum*, *S. chlorococcum*, *S. intrusum*, and *S. fabisporum* in a well-supported Scoliciosporaceae distinct from both Pilocarpaceae and

Sphaerophoraceae. The relationships between these three families, Psilolechiaceae, Ramalinaceae, and the families of suborder Lecanoriineae are still unresolved. These results will be discussed in more detail elsewhere.

The placement of our new species in *Scoliciosporum* is confirmed by its morphological similarity to *S. intrusum*. Both species have a dark dull olivaceous-brown thallus with small, black apothecia, hyaline, 0–1-septate ascospores (see Coppins, 1983, Fig.17A) and a photobiont with large (to 20 μ m diam.), thick walled cells. See Coppins (1983, as *Micarea intrusa*) and Edwards et al. (2009) for full



Fig. 4. A: *Calodendrum* George on Buffelskloof Nature Reserve, B: *Calodendrum* Falls. *Burrowsia cataractae* was collected from rocks at the base of the falls (photographs by John Burrows).

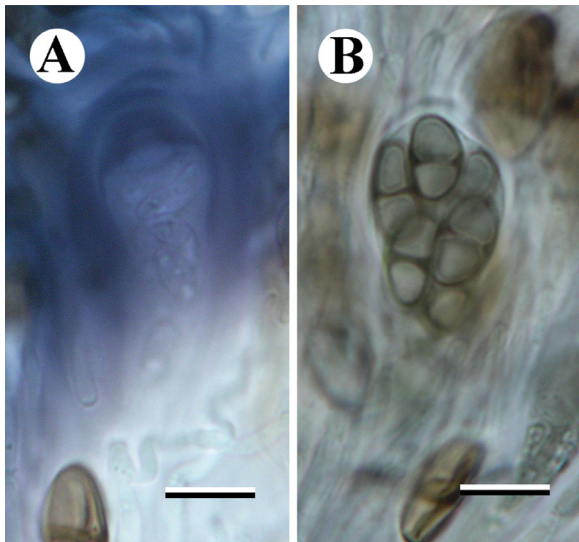


Fig. 5. Characters of typical buelluoid genera (*B. gypsensis* Fryday). A, Ascus in IKI after pretreatment with K; B, ascospores in water. Scale bars = 10 μm .

descriptions of *S. intrusum*. However, *S. fabisporum* differs from *S. intrusum* in having flat to \pm concave apothecia with a well-developed margin (convex and immarginate in *S. intrusum*), curved ascospores and also in the apparent lack of haustoria, which are significant character of *S. intrusum*.

Additional specimens examined:

Scoliciosporum intrusum

Canada, NEWFOUNDLAND AND LABRADOR, Newfoundland, Avalon Peninsula, Route 13, Witless Bay Line, about 1 km south of Witless Bay Line, south of Whale's Pond, 47.33325 –52.9537, 189 m., Siliceous rock, soft sedimentary outcrop in ombrotrophic *Scirpus*, *ericaceous*, *Sphagnum*, *Cladonia* bog next to mature *Abies balsamea*, *Picea mariana*, *Larix laricina*, *Betula papyrifera*, feathermoss, *Sphagnum* stand, 22 June 2012, J. W. McCarthy 2264 (MSC0154938).

Finland, In TRAVASTIA, Evo, supra latus saxi subumbrosum, 1984, J. P. Norrlin s.n., (MSC01323640: *Herbarium Lichenum Fenniae: Fasciculus IV* (1875) #182 – as. *Lecidea pelidna* Ach.).

4. Discussion

At the end of the 19th century, Stizenberger and most other lichenologists had a very broad concept of the genus *Lecidea*,

including in it all crustose species with apothecia lacking algae in the margin (Schmull et al., 2011). As such, they would have included the two species described here in that genus. We have checked all the descriptions of South African species currently included in *Lecidea* but none resemble our new taxa.

The current South African lichen checklist (Fryday, 2016) includes 71 taxa in the genus *Lecidea*, of which 56 are based on collections from South Africa: 20 described by Stizenberger and 36 by other authors (e.g., Nylander, Vainio, Zahlbruckner; see introduction). The previous checklist (DoIDGE, 1950) listed 105 species (plus 15 varieties) of *Lecidea*, but 34 of these taxa have subsequently been transferred to other genera. There is no doubt that the majority of the South African species currently included in *Lecidea* belong in other genera because the genus as currently circumscribed includes only saxicolous species with simple hyaline ascospores and a *Lecidea*-type ascus structure (Aptroot et al., 2009). As such, all corticolous and terricolous species, along with many saxicolous species, will need to be transferred to other genera, but the descriptions provided in the protologues are inadequate for determining the correct systematic placements for these species. This can only be achieved by examination of the type collections, which are housed in European herbaria, and even then it is probable that many will be hard to place without DNA sequence data that will be difficult, if not impossible, to obtain from the 100-plus year-old type collections.

Lichens are an important component of most terrestrial ecosystems (see Introduction) and are also important as model systems for the study of coevolutionary processes (O'Brien et al., 2013) and understanding the nature of symbiosis (Spribille et al., 2016). However, their contribution to ecological processes are often overlooked or undervalued. In particular, cyanolichens – those species that contain a cyanobacterium as the photosynthetic partner rather than a green chlorococcoid alga – are major contributors to nitrogen fixation and cycling (Elbert et al., 2012).

The larger part of Mpumalanga forms part of the Great Escarpment of southern Africa, which runs all the way from Angola on the western seaboard around the subcontinent and back north to Zimbabwe/Mozambique. The diversity of species and habitats along the escarpment makes it an important frontier for biodiversity exploration (Clark et al., 2011). These new lichen taxa were described from two centres of plant endemism, namely the Barberton and Lydenburg Centres (Schmidt et al., 2002), which are well known for the discovery of edaphic specialist and high-altitude restricted range species (Balkwill et al., 2011; Hankey and Edwards, 2008). The taxa described here are indicative of the undescribed lichen biodiversity of the region. During our visit to Mpumalanga we collected many other lichen specimens that we

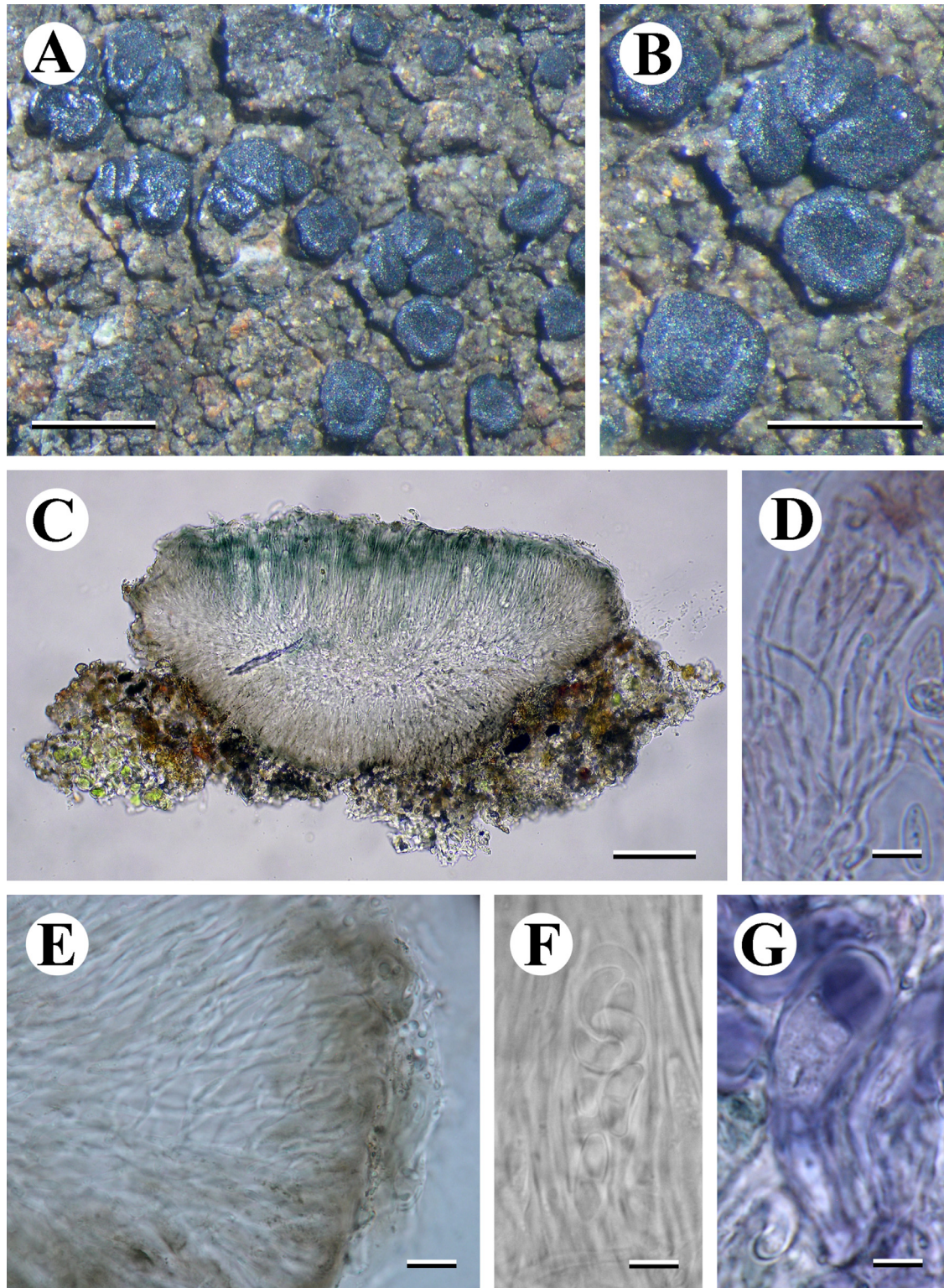


Fig. 6. *Scoliciosporum fabisporum* (holotype). A: Thallus and apothecia, B: Apothecia, C: Section of apothecium, D: Paraphyses (in N), E: Exciple (in K), F: Ascospores in ascus (in K), G: Ascus (in IKI). Scale bars: A = 1.0 mm; B = 0.5 mm; C = 50 μm ; D – G = 10 μm .

were unable to assign to described taxa. Work is continuing on these and, although some are small and will have to remain as “known unknowns” (Spribille et al., 2020) until better collections become available, further species new to science are confidently predicted. For example, we identified at least seven distinct species of the genus *Trapelia* M. Choisy among our collections whereas

only three appear on the current checklist of South African lichens (Fryday, 2016), and one of those is a northern hemisphere species that probably does not occur in South Africa. We hope that this contribution will prompt further investigation of the largely unexplored, rich lichen biodiversity of the area as well as southern Africa in general.

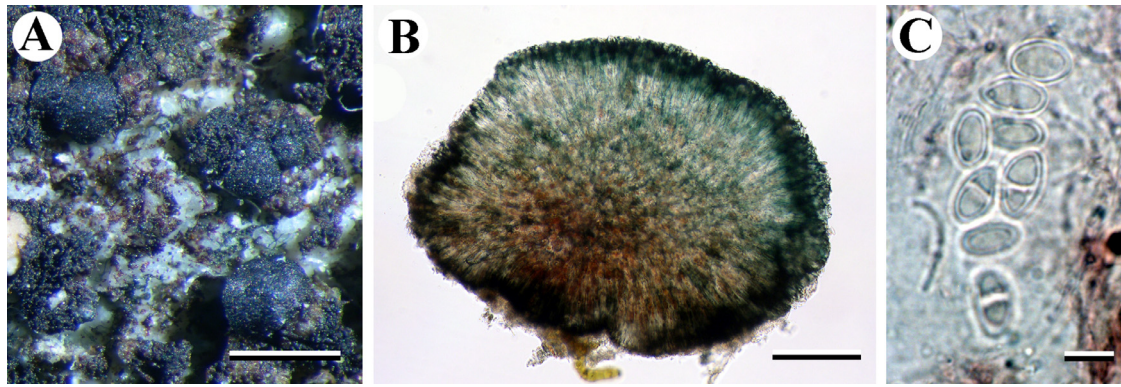


Fig. 7. *Scoliciosporum intrusum* (A –Norrlin s.n.; B & C – McCarthy 2264; both specimens in MSC). A: Thallus with apothecia, B: Section of apothecium, C: Ascospores in ascus (in N). Scale bars: A = 0.5 mm; B = 50 μ m; C = 10 μ m.

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