Standard Paper

Caloplaca tswaluensis (*Teloschistales*, *Teloschistaceae*): a new species from South Africa with plurilocular ascospores

Alan Fryday¹ (b), Stanislav Svoboda² (b), Jan Vondrák^{2,3} (b), Danielle A. Ward⁴ (b) and Madeleen Struwig⁵ (b)

¹Herbarium, Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA; ²Institute of Botany of the Czech Academy of Sciences, CZ-25243 Průhonice, Czech Republic; ³Department of Botany, Faculty of Science, University of South Bohemia, CZ-37005 České Budějovice, Czech Republic; ⁴Department of Integrative Biology, University of California, Berkeley, CA 94720, USA and ⁵Unit for Environmental Sciences and Management, North-West University, Mafikeng Campus, Mmabatho 2745, South Africa

Abstract

The new species *Caloplaca tswaluensis* is described from Tswalu Kalahari Reserve, Northern Cape Province, South Africa. *Caloplaca tswaluensis* occurs on the trunks of *Vachellia erioloba* (camelthorn) trees and is characterized by its 3-septate to quadrilocular ascospores. Molecular data indicate that the new species is placed in the subfamily *Teloschistoideae* but cannot be assigned to any existing genus and, because its systematic position is unclear, we choose to describe it in *Caloplaca* s. lat. *Caloplaca tswaluensis* is compared with other crustose *Teloschistaceae* species with plurilocular ascospores.

Keywords: arid habitats; corticolous lichens; disjunct distributions; Teloschistoideae; Tswalu Kalahari Reserve

(Accepted 13 May 2024)

Introduction

Lichens are often overlooked in ecological studies and biodiversity inventories and, consequently, they are poorly known or understood in many parts of the world. This is particularly true of crustose lichens that typically constitute around two thirds or more of all lichen biodiversity in an area (Lendemer *et al.* 2013; Spribille *et al.* 2020; Manzitto-Tripp *et al.* 2022; Vondrák *et al.* 2022).

The lichen biodiversity of South Africa is reasonably well documented but, at the same time, poorly understood. The most recent checklist (Fryday 2015) listed 1748 taxa for mainland South Africa (i.e. excluding the Prince Edward Islands). The first supplement (Ahti *et al.* 2016) made numerous additions and deletions for a net gain of three taxa. A subsequent publication (Medeiros & Lutzoni 2022) and a second supplement currently in preparation (I. D. Medeiros & A. M. Fryday, unpublished data) add a further 25, making a total of 1776 taxa. Estimates for the potential total number of taxa present in the country range from 2000 (Crous *et al.* 2006) to 3000 (Fryday 2015), which is well below the more than 21 000 vascular plants reported for the country (South African National Biodiversity Institute 2023). It is also comparatively low when compared with the 1838 lichen taxa reported from Great Britain and Ireland

Corresponding author: Alan Fryday; Email: fryday@msu.edu

Cite this article: Fryday A, Svoboda S, Vondrák J, Ward DA and Struwig M (2024) Caloplaca tswaluensis (Teloschistales, Teloschistaceae): a new species from South Africa with plurilocular ascospores. Lichenologist 1–9. https://doi.org/10.1017/ S0024282924000185 (Smith *et al.* 2009) or *c.* 2000 from Germany (Wirth *et al.* 2018); these are smaller countries (0.25 and 0.3 the size of South Africa, respectively) with a temperate climate, which is arguably less conducive to a diverse lichen biota than the subtropical-Mediterranean climate of South Africa.

Many South African lichens were first described in the late 19th and early 20th centuries and are frequently known only from their type collections, which have often not been studied since the species were described. Key among these are the taxa described by Ernestus Stizenberger in his Lichenaea Africana (Stizenberger 1890, 1891), but Nylander ((1869) and in Crombie (1876)), Vainio (1926) and Zahlbruckner (1926, 1932, 1936) also described many new species from the country. Unfortunately, as is the case for most descriptions from this period, they are mostly inadequate for assessing which species they represent under current taxonomic concepts. This can usually be done only by direct comparison with the type specimens, which are often poorly developed and invariably housed in European herbaria. This is a major impediment to the taxonomy and systematic study of lichens in South Africa and needs to be urgently addressed.

In the second half of the 20th and early 21st centuries, there was intermittent research on the lichen biota of South Africa, mainly by visiting European lichenologists (e.g. Almborn 1966, 1987; Schultz *et al.* 2009) but also by lichenologists resident in South Africa. The most notable of these was Franklin Brusse who, between 1985 and 1994, published over 30 papers describing new genera and species from the country and often included new reports of other species. However, all these researchers usually

© The Author(s), 2024. Published by Cambridge University Press on behalf of The British Lichen Society. This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike licence (http://creativecommons.org/licenses/by-nc-sa/4.0), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the same Creative Commons licence is used to distribute the re-used or adapted article and the original article is properly cited. The written permission of Cambridge University Press must be obtained prior to any commercial use.



restricted themselves to describing new species and have not explored the systematic position or relationships of their discoveries (but see Kondratyuk *et al.* 2015; Leavitt *et al.* 2018*a*, *b*; Aptroot *et al.* 2019). A fuller history of lichenology in South Africa is provided by Fryday (2015).

The first author of the current contribution, along with co-workers, recently described a new genus of crustose lichens in the *Caliciaceae* from Mpumalanga, South Africa (Fryday *et al.* 2020). Subsequent fieldwork in other regions of South Africa by two of the current authors (AF, DAW) has revealed the presence of new species of crustose lichen that are known only from South Africa. This is the group of lichens that suffers from the greatest lack of attention within the South African lichen biota (Fryday 2015) and, although most require further study to clarify their systematic position, molecular data have been obtained for two of them. One occupies an isolated position within the *Lichinomycetes* and will be described elsewhere (A. Fryday *et al.*, unpublished data), whereas the other occupies an unclear position within the *Teloschistaceae* and is described below as a new species.

Materials and Methods

This study is based upon specimens of crustose lichens collected by the first and fourth authors (AF, DAW) from the Tswalu Kalahari Reserve, Northern Cape, South Africa in April 2023.

Site description

Tswalu Kalahari Reserve (TKR; 27.20°S, 22.47°E, 102 000 ha) is located in the southern Kalahari Desert between the towns of Hotazel and Van Zylsrus in Northern Cape Province (Fig. 1) and has been developed into a high-end game lodge, nature reserve, and conservation-focused research facility. It ranges in altitude from 1020 m on the plains to 1586 m on the highest mountain peak and has a typically hot and arid climate, receiving an average of 286–318 mm of rain per year, 76% of which falls from mid- to late summer (December–April). The landscape is geomorphologically and ecologically heterogeneous, comprising plains, and fields of parallel dunes with the quartzitic Korannaberg Mountains extending from north to south through the eastern half of the reserve. Tswalu is centred within the

Borstrall Runcies Chiefen Constration Cons

Figure 1. Map showing location of Tswalu Kalahari Reserve in South Africa (insert) and sites where Caloplaca tswaluensis was collected (white dots). In colour online.

Eastern Kalahari Bioregion, but the northern part extends into an outlier patch of the Kalahari Dunefield Bioregion. The included vegetation units comprise the northern tip of the Koranna Langeberg Mountain Bushveld, with Gordonia Duneveld to the north, Gordonia Plains Shrubveld to the west, Olifantshoek Plains Thornveld close to the mountains both to the east and west, and Kathu Bushveld to the east (Davis *et al.* 2010; Tokura *et al.* 2018).

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 water. Hamathecial filaments are referred to as 'paraphyses' regardless of their origin. Ascospore measurements are given as (minimum value–) mean \pm standard deviation (–maximum value), n = number of measurements. Chlorinated anthraquinones were tested for in *Teloschistaceae* using a weak bleach solution (C) as described by Vondrák *et al.* (2013).

Molecular methods

DNA was extracted using a cetyltrimethylammonium bromide (CTAB)-based protocol (Aras & Cansaran 2006). Two DNA loci were amplified: mtSSU and ITS. Polymerase chain reactions were performed in a reaction mixture containing 2.5 mmol/l MgCl₂, 0.2 mmol/l of each dNTP, 0.3 µmol/l of each primer, 0.5 U Taq polymerase (TOP-Bio, Praha, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 10 µl. Details of sequenced loci, primers and PCR settings are given in Table 1. PCR products were enzymatically purified and sent for Sanger sequencing (GATC Biotech, Konstanz, Germany). Sequences were checked and edited using BioEdit v. 7.0.9.0 (Hall 1999), and compared for similarity with the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Datasets for mtSSU and ITS with relevant sequences were aligned using ClustalW multiple alignment in BioEdit, and manually adjusted. Both datasets were saved in nexus format and concatenated in SeaView v. 4.3.5 (Gouy et al. 2010).

Phylogenetic analyses

A BLAST search with the ITS region of our new species showed it to belong to the subfamily *Teloschistoideae*. In order to reveal its systematic position, we used specimens of *Teloschistoideae* that had been sequenced for both ITS and mtSSU to produce a 3

reasonable concatenated phylogenetic reconstruction covering most known genera in that subfamily of Teloschistaceae. The outgroup was selected from Caloplacoideae to be outside the target subfamily, but within the Teloschistaceae. Using the Smart Model Selection program (http://www.atgc-montpellier.fr/sms/), we selected $GTR + \Gamma + I$ as the best-fitting model for subsequent analyses. Phylogenetic analyses were carried out in MrBayes v. 3.2.7a (Huelsenbeck & Ronquist 2001) for 10 million generations. Average standard deviations of split frequencies were 0.048859. Maximum likelihood analysis was performed in PhyML v. 2.4.3 (Guindon & Gascuel 2003), with 1000 nonparametric bootstrap replicates. Final trees were visualized using TreeView v. 1.6.6 (Page 1996), and graphically adjusted in Inkscape v. 0.92 (https://www.inkscape.org). Lichen voucher specimens, and existing and new accession numbers from these vouchers in NCBI databases, are given in Supplementary Material Table S1 (available online).

Results

Microscopic examination of the unidentified specimens of crustose lichens collected from Tswalu Kalahari Reserve revealed one to have quadrilocular ascospores and anthraquinones in both thallus and apothecia. It clearly belonged to Teloschistaceae but did not correspond to the descriptions of any previously described crustose Teloschistaceae with plurilocular ascospores (Hafellner & Poelt 1979; Wetmore 1999). Consequently, DNA sequences of the ITS and mtSSU regions were obtained from the specimen and its placement in Teloschistaceae subfamily Teloschistoideae was confirmed by a concatenated dataset of ITS and mtSSU sequences (Fig. 2). The closest sequenced specimen available in GenBank was an epiphytic lichen from Mexico (ITS: KT291446, mtSSU: KT291484; Gaya et al. 2015) identified as Solitaria chrysophthalma (Degel.) Arup et al. The Mexican specimen, presumably assigned to Solitaria chrysophthalma on the basis of morphological similarity, is fairly close to the Tswalu collection (94% identical in ITS and 99.5% in mtSSU) and the specimens can be considered congeneric. However, because Gaya et al. (2015) recovered the Mexican collection in Teloschistoideae, it is almost certainly misidentified. The type specimen of the basionym of S. chrysophthalma (Caloplaca chrysophthalma Degel.) is from Europe (Switzerland), and Arup et al. (2013) sequenced a European collection of S. chrysophthalma (Sweden) and showed that it belonged in the subfamily Xanthorioideae. As demonstrated in the phylogenetic tree, the likely congeneric South African and Mexican specimens do not belong to any known genus in Teloschistoideae; sequences of related genera (e.g. Catenarina, Cinnabaria, Scutaria and Wetmoreana) are up to 89% identical in ITS and 98% in mtSSU. Unfortunately, the Mexican collection was not available for us to study at this time.

Table 1. Details of loci sequenced in this study including primers, PCR settings and references

Locus	Reference	Primers	PCR settings
ITS	White <i>et al.</i> (1990)	ITS1F (forward):	94 °C for 3 min, 30× (94 °C for 30 s, 56 °C for 30 s, 7 72 °C for 2 min), 72 °C for 7 min; 4 °C for $\infty.$
	Gardes & Bruns (1993)	ITS4 (reverse):	
mtSSU	Zoller <i>et al.</i> (1999)	mrSSU1 (forward):	94 °C for 10 min, 40× (94 °C for 30 s, 52 °C for 30 s, $^{-72}$ °C for 2 min), 72 °C for 7 min, 4 °C for ∞.
	Zhou & Stanosz (2001)	MSU7 (reverse):	



Figure 2. Phylogenetic relationships of the *Teloschistoideae* inferred by the maximum likelihood (ML) analysis of the concatenated datasets of ITS and mtSSU sequences. Numbers at the nodes show bootstrap values derived from ML analysis/posterior probabilities under the Bayesian inference (BI) analysis. When branching calculated in the ML analysis was not present in the BI analysis, the node was marked with a dash (-). Each original sample code consists of the species name and the GenBank Accession number (ITS/mtSSU).

Taxonomy

Caloplaca tswaluensis Fryday, S. Svoboda & D. A. Ward sp. nov.

MycoBank No.: MB 853830

Separated from all other crustose *Teloschistaceae* species by the quadrilocular ascospores, corticolous habit, presence of soredia and by its molecular sequence data.

Type: South Africa, Northern Cape, Tswalu Kalahari Reserve, 3.25 km south of Dedeben, 27.31084°S, 22.50910°E, 1224 m, on trunk of *Vachellia erioloba* (*Fabaceae*) in *Acacia* thicket (Olifantshoek Plains Thornveld) beside District Road, 24 April 2023, *A. M. Fryday* (11785), *D. A. Ward & D. Smith* (PRE—holotype; MSC—isotype). GenBank nos: PP254020 (ITS), OR982361 (mtSSU).

(Figs 3 & 4)

Thallus effuse, in small patches *c*. 0.3–1.0 cm wide, yellow, areolate, areoles flat to convex, 0.2–0.4 mm wide, becoming irregularly sorediate; *soralia* laminal, rarely marginal on areoles, initially ±orbicular (0.1–)0.15–0.2(–0.3) mm diam., but becoming confluent and irregular in outline; *soredia* granular, (25–)35–40(–50) μ m diam.; in section areoles 0.12–0.14 mm thick; *upper cortex* alveolate, *c*. 20 μ m thick, of two layers, upper layer composed of 3–5 layers of horizontally aligned hyphae 1.5–2 μ m wide, with yellow pigment (K+ crimson), lower layer (and rest of the thallus) composed of hyaline ±spherical cells, 5–10 μ m diam.; *photobiont layer* 40–70 μ m thick, in columns mostly 20–30 μ m (but occasionally up to 100 μ m) wide, interrupted with columns of medulla cells 20–30 μ m wide; *medulla* hyaline, 15–30 μ m deep. *Photobiont* trebouxioid, cells ±spherical, 10–20 μ m diam.

Apothecia 0.2–0.45 mm diam.; disc orange, flat with a rough surface; proper margin persistent, concolorous, 0.025 mm wide, slightly raised above the level of the disc; thalline margin usually



Figure 3. Caloplaca tswaluensis (A–D, Fryday 11785—holotype; E, Fryday 11762). A, the type locality, in Tswalu Kalahari Reserve. B, Vachellia erioloba (camelthorn) tree at the type locality. C, habit of Caloplaca tswaluensis among foliose Physciaceae. D, C. tswaluensis (fertile). E, C. tswaluensis (sorediate). Scales: C = 5 mm; D & E = 0.5 mm. In colour online.

visible at the base of the proper margin, especially in young apothecia, yellow, not corticate, clumps of algal cells usually visible at the periphery of the apothecia in surface view (especially when wet) but these appear to be different from those in the thallus and exciple. In section, proper exciple narrow at base, widening to 30 µm at the surface, composed of narrow vertical hyphae, algal layer up to 70 μ m thick, with a narrow (10 μ m) orange-brown cortex (K+ crimson) containing small crystals (POL+), photobiont layer ±continuous beneath the apothecia. Hymenium c. 70 µm tall, KI+ blue; paraphyses narrow, not branched or anastomosing except near the apex, upper 3-4 cells moniliform and widening to 4-5 µm; epihymenium 10-15 µm with numerous minute, golden brown crystals (POL+, K+ crimson). Ascus Teloschistes-type, cylindrical becoming somewhat clavate, $40-55 \times 10-15 \mu m$; ascospores 3- (rarely 4-) septate or quadrilocular (1-septate when immature), $(13-)16.5 \pm 1.9(-20) \times (5-)5.4 \pm$ 0.4(-6) μ m; l/w ratio (2.5-)3.0 \pm 0.2(-3.4), n = 20; cells lensshaped when quadrilocular, especially in KOH. Hypothecium 70-80 µm tall at the centre of the section, narrowing to nothing at the exciple, hyphae predominately vertically aligned above and horizontal below.

Conidiomata not observed.

Chemistry. Upper cortex of thallus and epihymenium of apothecia K+ crimson, C-.

Distribution and ecology. The new species was collected at two localities within Tswalu Kalahari Reserve. At both locations it occurred on the trunks of Vachellia erioloba (E. Mey.) P. J. H. Hurter (camelthorn) trees in a corticolous lichen community dominated by Physciaceae species, with Phaeophyscia orbicularis (Neck.) Moberg as the dominant species. Corticolous communities were investigated at only three localities in Tswalu and C. tswaluensis was present at two of these, indicating that the species is probably frequent and widespread in the area. The trees in the localities where C. tswaluensis was collected were quite close together, whereas the locality where it was not observed consisted of large, isolated trees, suggesting that some shade was required for the lichen to be present. The disjunct distribution of C. tswaluensis and its closest relative from a similar habitat in Mexico suggests that this lineage has a transatlantic subtropical distribution in arid, partially wooded areas.

Remarks. Ascospores observed in water were 3-septate, without swelling of the septa or spore walls to form locules and with oil droplets visible inside the cells. However, on the addition of KOH the spores became quadrilocular and the oil droplets were no longer apparent, but were otherwise unchanged in shape or size. Further flushing of the section with water did not return the cells to their previous 3-septate state, indicating that the change was permanent.



Figure 4. *Caloplaca tswaluensis (Fryday* 11785—holotype). A, section of thallus in K showing K+ purple cortex and interrupted photobiont layer. B, section of exciple showing photobiont cells in thalline exciple. C, section of apothecium in K showing paraphyses with swollen tips. D, section of exciple showing radiating hyphae in proper exciple. E–G, ascospores; mounted in KOH (E) and water (F & G). H, exciple and epihymenium under normal light. I, exciple and epihymenium under polarized light showing location of crystals. Scales: $A = 20 \mu m$; B, H & $I = 50 \mu m$; C & $E-G = 5 \mu m$; D = 10 μm . In colour online.

Additional collections. South Africa: Northern Cape: Tswalu Kalahari Reserve, North Gosberg, 27.2501°S, 22.4657°E, 1267 m, on trunk of Vachellia erioloba in acacia thicket on south-facing slope, 2023, A. M. Fryday (11762), D. A. Ward & D. Smith (MSC, UNWH); *ibid.*, 3.25 km south of Dedeben, 27.31084°S, 22.50910°E, 1224 m, acacia thicket beside District Road, on trunk of Vachellia erioloba, 2023, D. A. Ward (2076) & D. Rossouw (MSC, PRA—topotype).

Discussion

There are over 1000 species of crustose *Teloschistaceae* (Arup *et al.* 2013), the vast majority of which have polarilocular ascospores, that is ascospores with a cell lumen constricted into two locules situated at opposing poles of the cell and connected by a narrow isthmus. The remainder have ascospores with either no septa or locules (simple), one septum but not polarilocular (1-septate), or more than two locules (plurilocular). We differentiate locular spores from septate spores by the latter lacking any wall thickenings. The group of species with plurilocular spores was revised by Hafellner & Poelt (1979), who recognized 17 species and one subspecies. Since then, *Caloplaca lagunensis* Wetmore has been described from Mexico (Wetmore 1999) but we are not aware of any additional *Teloschistaceae* species with plurilocular ascospores.

Hafellner & Poelt (1979) showed that immature plurilocular ascospores were initially non-septate with unthickened, threelayered walls and that the locules were formed by a gelatinous substance expanding between the inner two walls that ultimately dissipates, leaving the ascospores resembling regular septate spores. In *C. tswaluensis*, however, at least one septum develops before the formation of locules because immature spores lacking wall thickening are clearly 1-septate. In addition, although overmature spores are 3-septate in water, the same spores become plurilocular in 10% KOH.

Hafellner & Poelt (1979) were of the opinion that the species with plurilocular ascospores did not form a monophyletic group and that the character had evolved in the Teloschistaceae on at least three separate occasions. Previously, molecular data were available for only three Teloschistaceae species with plurilocular ascospores. Molecular data for Xanthocarpia ochracea (Schaer.) A. Massal. & De Not. was first provided by Søchting et al. (2007; as Caloplaca ochracea (Schaer.) Flagey), with additional sequences provided by Arup et al. (2013) and Gaya et al. (2012, 2015). Arup et al. (2013) showed that X. ochracea belonged in the subfamily Xanthorioideae and that several other species with regular polarilocular ascospores were congeneric, indicating that plurilocular ascospores were not a genus-level character. Subsequently, Wilk et al. (2021) provided molecular data for Caloplaca brebissonii (Fée) Zahlbr. and C. quadrilocularis (Nyl.) Zahlbr., with both species included in the subfamily Caloplacoideae, and with C. brebissonii occupying an isolated position. Caloplaca tswaluensis, newly described here, occupies a position in the subfamily Teloschistoideae, distant from both C. brebissonii and C. quadrilocularis in the Caloplacoideae as well as X. ochracea in the Xanthorioideae. This reaffirms Hafellner & Poelt's opinion that species with plurilocular ascospores do not form a monophyletic group and have evolved in the Teloschistaceae on at least three separate occasions.

Of the species treated by Hafellner & Poelt (1979), most have only three locules, unlike the current species, which has four. Of the quadrilocular species treated by Hafellner & Poelt (loc. cit.), *Caloplaca tetramera* (Müll. Arg.) Imshaug ex Hafellner & Poelt and *Xanthocarpia* ochracea are both saxicolous and occur only in Costa Rica and the Europe/Mediterranean region respectively; *Caloplaca oahuensis* Hafellner, *C. quadrilocularis* and *C. spadicea* (Tuck.) Zahlbr. are corticolous but all have brown apothecia and are known only from the Americas (including Hawai'i). In addition, *C. quadrilocularis* and *C. spadicea* have a cream-coloured to grey thallus and *C. spadicea* is also isidiate. *Caloplaca lagunensis* from Mexico (Wetmore 1999) was described as having ascospores with only three locules but otherwise resembles *C. spadicea* in having a grey isidiate thallus.

Previously described South African species

We considered the possibility that the species described here had been included among the numerous taxa described from South Africa by previous lichenologists (e.g. Stizenberger 1890, 1891; Vainio 1926; Zahlbruckner 1926, 1932, 1936). Many of these are known only from their type species and a short Latin description but the plurilocular ascospores of *C. tswaluensis* are a distinctive and uncommon character that make searching protologues for similar species much easier. We therefore searched the protologues of all the species assigned to *Caloplaca* and its segregate genus *Blastenia* (*sensu* A. Massal. *non* Arup *et al.*) listed by Fryday (2015) but failed to find any that mentioned this character. It is reasonable to conclude, therefore, that *C. tswaluensis* has not been described previously.

Systematic position of Caloplaca tswaluensis

The Teloschistaceae is a large, cosmopolitan family of lichenized fungi that is frequent in most habitats worldwide. Until recently, the family was divided into three main genera based on growth form: Teloschistes (fruticose), Xanthoria (foliose) and Caloplaca (crustose). The crustose species, with more than 1000 described species, are particularly frequent and widespread. However, investigation of the genetics of the family using molecular methods has shown this simplistic arrangement to be untenable (Arup et. al. 2013). Although the three main groups were largely supported, and recognized as the subfamilies Teloschistoideae, Xanthorioideae and Caloplacoideae, there was a considerable variation of thallus type within each subfamily and, consequently, the family was divided into 39 separate genera, either resurrected from synonymy or described as new (Arup et al. 2013). Since then, the process of separating new genera has continued by further dividing existing genera and recognizing newly revealed lineages as new genera (e.g. Søchting et al. 2014; Frolov et al. 2021) with the result that over 100 genera have now been erected (Wikispecies 2023a, b, c, d). Although some of these have been reduced to synonymy and many more are not widely accepted (Wilk et al. 2021; Consortium for Classification of Fungi and Fungus-like Taxa 2023), over 50 genera are now recognized in the Teloschistaceae by most lichenologists.

Unfortunately, most molecular work on the *Teloschistaceae* has been carried out on Northern Hemisphere species, with work on Southern Hemisphere species being largely restricted to Antarctic, Australian and South American taxa. Sixty seven species of crustose *Teloschistaceae* have been reported from South Africa (Fryday 2015), 54 in *Caloplaca* and 13 in the segregate genus *Blastenia*. However, to the best of our knowledge molecular sequence data have not been obtained from any South African collection. It is no surprise, therefore, that the only sequenced collection that was at all close to *C. tswaluensis* was from Mexico and that even less closely related collections were also all from Central and South America (Bolivia, Peru, Mexico). The current arrangement of the *Teloschistaceae*, coupled with the relative genetic isolation of our new species, meant we had two alternatives: erect a new genus for the species or describe it in a widely circumscribed *Caloplaca* s. lat. It is probable that a molecular analysis of other southern African crustose *Teloschistaceae* would show that at least some are congeneric with *C. tswaluensis*, which would reduce its isolation in the phylogeny of the family and justify erecting a new genus to accommodate them. However, because the systematic position of the new species is unclear, and monotypic genera serve little practical purpose, and also because the last thing lichen systematics needs right now is another new genus of crustose *Teloschistaceae*, we choose to describe the species in a widely circumscribed *Caloplaca*. This is despite it clearly not being congeneric with the type species of that genus (*C. cerina* (Hedw.) Th. Fr.).

Distribution of species with plurilocular ascospores

The crustose Teloschistaceae with plurilocular spores appear to have a mainly tropical/subtropical distribution with only Xanthocarpia ochracea, the distribution of which extends into NW Europe, and Caloplaca homologa (Nyl.) Hellb. from New Zealand laying outside this range. Six species have previously been reported from Africa, all corticolous, and only one from South Africa. The African species are Caloplaca auratopruinosa Sambo (Ethiopia), C. crocea (Kremp.) Hafellner & Poelt subsp. crocea (South Africa and Madagascar), C. erythroleuca (Nyl.) Zahlbr. (Eritrea and Guinea), C. erythroleucoides (Nyl.) Zahlbr. (Tanzania), C. vainioi Hafellner & Poelt (Angola) and C. zavattarii Sambo (East Africa). The South African record of C. crocea was collected from Howick in KwaZulu-Natal by van der Bijl [Byl] in 1920 (Hafellner & Poelt 1979). In addition, Stizenberger (1890) described the new taxon Lecanora ochracea var. parvula Stizenb., with ascospores reported as $8-10 \times$ 5-6 µm, from a collection on basaltic rocks near Mashishing (Lydenburg), Mpumalanga by Friedrich Wilms. However, the typical variety of Xanthocarpia ochracea is known only from the Mediterranean region and Europe so it is unlikely that this Mashishing collection is related to that species. Its true identity can be determined only by examination of the type collection, which is probably in a European herbarium but could not be found in either Herbarium Berolinense, Berlin (B) where Wilms was employed on his return from South Africa, or Herbarium der Eidgenössische Technische Hochschule Zürich (ZT), which houses Stizenberger's herbarium.

Conclusion

The new species, described here, along with the new genera described by the first author and co-workers (Fryday *et al.* 2020; A. Fryday *et al.*, unpublished data) and the nine new genera described by Brusse between 1985 to 1994 (Brusse 1985*a, b,* 1987*a, b, c,* 1988*a, b,* 1994; Brusse & Kärnefelt 1991) are symptomatic of the poorly understood nature of the lichenized fungi of southern Africa. Add to this the numerous new species described by Brusse (cf. Fryday 2015) and the putative undescribed taxa collected during the fieldwork that resulted in the discovery of the new species described here, and the case for a significant undiscovered biodiversity of lichenized fungi in southern Africa is irrefutable. This is in need of serious and concerted study that can be achieved only by a well-funded, joint field- and laboratory-based research programme.

Acknowledgements. We thank the Explorers Club for an Exploration Fund Grant to DAW that permitted DAW and AF to travel to South Africa, and the Tswalu Foundation, Oppenheimer Generations Research and Conservation 'OGRC' and the Tswalu Kalahari Reserve for funding the fieldwork and providing logistical support. AF and DAW are particularly grateful to Dylan Smith, Executive Dedeben Research Centre at Tswalu Kalahari Reserve for initiating our visit and for sharing his time and knowledge of, and enthusiasm for, the reserve and all its biodiversity. We also thank Dr Nishanta Rajakaruna for organizing the project and assisting with fieldwork, and the curators and staff of the herbaria of Herbarium Berolinense, Berlin (B), Duke University, Durham, North Carolina, USA (DUKE) and Eidgenössische Technische Hochschule Zürich (ZT) for searching their collections for relevant material. Molecular work was supported by a long-term research development grant RVO (grant number 67985939) to JV and SS.

Author ORCIDs. D Alan Fryday, 0000-0002-5310-9232; Stanislav Svoboda, 0000-0001-9797-4984; Jan Vondrák, 0000-0001-7568-6711; Danielle A. Ward, 0009-0000-0595-643X; Madeleen Struwig, 0000-0001-9626-4796.

Competing Interests. The authors declare no competing interests.

Supplementary Material. The Supplementary Material for this article can be found at https://doi.org/10.1017/S0024282924000185.

References

- Ahti T, Mayrhofer H, Schultz M, Tehler A and Fryday AM (2016) Additions, deletions and other changes to the lichen checklist of South Africa. *Bothalia* 46, a2065.
- Almborn O (1966) Revision of some lichen genera in southern Africa I. Botaniska Notiser 119, 70–112.
- Almborn O (1987) Lichens at high altitudes in southern Africa. Bibliotheca Lichenologica 25, 401–417.
- Aptroot A, Maphangwa K, Zedda L, Tekere M, Alvarado P and Sipman H (2019) The phylogenetic position of *Culbersonia* is in the *Caliciaceae* (lichenized ascomycetes). *Lichenologist* 51, 187–191.
- Aras S and Cansaran D (2006) Isolation of DNA for sequence analysis from herbarium material of some lichen specimens. *Turkish Journal of Botany* 30, 449–453.
- Arup U, Søchting U and Frödén P (2013) A new taxonomy of the family Teloschistaceae. Nordic Journal of Botany 31, 16–83.
- Brusse F (1985a) Corynecystis, a new lichen genus from the Karoo, South Africa. Bothalia 15, 552–553.
- **Brusse F** (1985b) *Glyphopeltis (Lecideaceae)*, a new lichen genus from southern Africa. *Lichenologist* **17**, 267–268.
- Brusse F (1987a) Psathyrophlyctis, a new lichen genus from southern Africa. Bothalia 17, 182–184.
- Brusse FA (1987b) Echidnocymbium (Biatoraceae), a new lichen genus from southern Africa. Mycotaxon 29, 173–176.
- Brusse FA (1987c) Schistoplaca (Lichenes, Biatoraceae), a new lichen genus. Mycotaxon 29, 245–249.
- Brusse F (1988a) Lithoglypha, a new lichen genus from Clarens Sandstone. Bothalia 18, 89–96.
- Brusse F (1988b) Schizodiscus, a new porpidioid lichen genus from Drakensberg. Bothalia 18, 94–96.
- Brusse FA (1994) A remarkable new lichen genus Catarrhospora (Ascomycotina, Porpidiaceae), from Cape Floral Kingdom, South Africa. Mycotaxon 52, 501–512.
- Brusse FA and Kärnefelt I (1991) The new southern hemisphere lichen genus Coelopogon (Lecanorales, Ascomycotina), with a new species from southern Africa. Mycotaxon 42, 35–41.
- Consortium for Classification of Fungi and Fungus-like Taxa (2023) Outline of Fungi [WWW resource] URL https://outlineoffungi.org/outline. php. [Accessed 24 February 2024].
- Crombie JM (1876) Lichenes capenses: an enumeration of the lichens collected at the Cape of Good Hope by the Rev. A. E. Eaton during the Venus-Transit Expedition in 1874. *Journal of the Linnean Society, Botany* 15, 165–180.
- Crous PW, Rong IH, Wood A, Lee S, Glen H, Botha W, Slippers B, de Beer WZ, Wingfield MJ and Hawksworth DL (2006) How many species of fungi are there at the tip of Africa? *Studies in Mycology* 55, 13–33.

- Davis A, Scholtz CH, Kryger U, Deschodt CM and Strümpher WP (2010) Dung beetle assemblage structure in Tswalu Kalahari Reserve: responses to a mosaic of landscape types, vegetation communities, and dung types. *Environmental Entomology* **39**, 811–820.
- Frolov I, Vondrák J, Košnar J and Arup U (2021) Phylogenetic relationships within *Pyrenodesmia* sensu lato and the role of pigments in its taxonomic interpretation. *Journal of Systematics and Evolution* **59**, 454–474.
- Fryday AM (2015) A new checklist of lichenised lichenicolous and allied fungi reported from South Africa. *Bothalia* 45, 59–122.
- Fryday AM, Medeiros ID, Siebert SJ, Pope N and Rajakaruna N (2020) Burrowsia, a new genus of lichenized fungi (*Caliciaceae*), plus the new species B. cataractae and Scoliciosporum fabisporum, from Mpumalanga, South Africa. South African Journal of Botany 132, 471–481.
- Gardes M and Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**, 113–118.
- Gaya E, Högnabba F, Holguin Á, Molnar K, Fernandez-Brime S, Stenroos S, Arup U, Søchting U, van den Boom P, Lucking R, et al. (2012) Implementing a cumulative supermatrix approach for a comprehensive phylogenetic study of the *Teloschistales (Pezizomycotina, Ascomycota)*. Molecular Phylogenetics and Evolution 63, 374–387.
- Gaya E, Fernandez-Brime S, Vargas R, Lachlan RF, Gueidan C, Ramirez-Mejia M and Lutzoni F (2015) The adaptive radiation of lichenforming *Teloschistaceae* is associated with sunscreening pigments and a bark-to-rock substrate shift. *Proceedings of the National Academy of Sciences of the United States of America* 112, 11600–11605.
- Gouy M, Guindon S and Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27, 221–224.
- **Guindon S and Gascuel O** (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704.
- Hafellner J and Poelt J (1979) Die Arten der Gattung Caloplaca mit plurilocularen Sporen (*Meroplacis, Triophthalmidium, Xanthocarpia*). Journal of the Hattori Botanical Laboratory **46**, 1–41.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95–98.
- Huelsenbeck JP and Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Kondratyuk SY, Kärnefelt I, Thell A, Elix JA, Kim JA, Kondratiuk AS and Hur J-S (2015) Ovealmbornia reginae (Teloschistaceae, Ascomycetes), a new xanthorioid lichen from South Africa. Herzogia 28, 465–472.
- Leavitt SD, Kirika PM, Amo de Paz G, Huang J-P, Hur J-S, Elix JA, Grewe F, Divakar PK and Lumbsch HT (2018a) Assessing phylogeny and historical biogeography of the largest genus of lichen-forming fungi, *Xanthoparmelia* (*Parmeliaceae*, Ascomycota). Lichenologist 50, 299–312.
- Leavitt SD, Westberg M, Nelsen MP, Elix JA, Timdal E, Sohrabi M, St Clair LL, Williams L, Wedin M and Lumbsch HT (2018b) Multiple, distinct intercontinental lineages but isolation of Australian populations in a cosmopolitan lichen-forming fungal taxon, *Psora decipiens (Psoraceae, Ascomycota)*. Frontiers in Microbiology 9, 283.
- Lendemer JC, Harris RC and Tripp EA (2013) The lichens and allied fungi of Great Smoky Mountains National Park. *Memoires of the New York Botanical Garden* 104, 1–152.
- Manzitto-Tripp EA, Lendemer JC and McCain CM (2022) Most lichens are rare, and degree of rarity is mediated by lichen traits and biotic partners. *Diversity and Distributions* 28, 1810–1819.
- **Medeiros ID and Lutzoni F** (2022) Contribution to a modern treatment of *Graphidaceae* biodiversity in South Africa: genera of tribe *Graphideae* with hyaline ascospores. *Lichenologist* **54**, 253–270.
- Nylander W (1869) Note sur les lichens de Port-Natal. Bulletin de la Société Linnéenne de Normandie 3, 4–15.
- Page RDM (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12, 357–358.
- Schultz M, Zedda L and Rambold G (2009) New records of lichen taxa from Namibia and South Africa. *Bibliotheca Lichenologica* **99**, 315–334.

- Smith CW, Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW and Wolseley P (eds) (2009) The Lichens of Great Britain and Ireland, 2nd Edn. London: British Lichen Society.
- Søchting U, Huneck S and Etayo J (2007) Caloplaca epigaea sp. nova from arid soil in Spain and dead grass in Germany. Bibliotheca Lichenologica 96, 279–286.
- Søchting U, Garrido-Benavent I, Seppelt R, Castello M, Pérez-Ortega S, de los Rios, Murillo A, Frödén P and Arup U (2014) Charcotiana and Amundsenia, two new genera in Teloschistaceae (lichenized Ascomycota, subfamily Xanthorioideae) hosting two new species from continental Antarctica, and Austroplaca frigida, a new name for a continental Antarctic species. Lichenologist 46, 763–782.
- South African National Biodiversity Institute (2023) *e-Flora of South Africa, v.1.42.* [WWW resource] URL http://ipt.sanbi.org.za/iptsanbi/resource? r=flora_descriptions&v=1.42
- Spribille T, Fryday AM, Pérez-Ortega S, Svensson M, Tønsberg T, Ekman S, Holien H, Resl P, Schneider K, Stabentheiner E, et al. (2020) Lichens and associated fungi from Glacier Bay National Park, Alaska. Lichenologist 52, 61–181.
- Stizenberger E (1890) Lichenaea Africana. Bericht über die Thätigkeit der St. Gallischen Naturwissenschaftliche Gesellschaft 1888–89, 105–249.
- Stizenberger E (1891) Lichenaea Africana. Bericht über die Thätigkeit der St. Gallischen Naturwissenschaftliche Gesellschaft 1889–90, 133–268.
- Tokura W, Jack S, Anderson T and Hoffman MT (2018) Long-term variability in vegetation productivity in relation to rainfall, herbivory and fire in Tswalu Kalahari Reserve. *KOEDOE: African Protected Area Conservation and Science* **60**, a1473.
- Vainio E (1926) Lichenes Africani novi. Annales Universitatis Fennicae Aboënsis, Series A 2(3), 1–33.
- Vondrák J, Frolov I, Arup U and Khodosovtsev A (2013) Methods for phenotypic evaluation of crustose lichens with emphasis on *Teloschistaceae. Chernomorsky Botanichnii Zhurnal* 9, 382–405.
- Vondrák J, Svoboda S, Malíček J, Palice Z, Kocourková J, Knudsen K, Mayrhofer H, Thüs H, Schultz M, Košnar J, et al. (2022) From Cinderella to Princess: an exceptional hotspot of lichen diversity in a longinhabited central-European landscape. Preslia 94, 143–181.
- Wetmore CM (1999) Four new species of Caloplaca from Mexico. Bryologist 102, 99–103.
- White TJ, Bruns TD, Lee SB and Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds), PCR Protocols: a Guide to Methods and Applications. New York: Academic Press, pp. 315–322.
- Wikispecies (2023a) Brownlielloideae. [WWW document] URL https://species.wikimedia.org/wiki/Brownlielloideae. [Accessed 24 February 2024].
- Wikispecies (2023b) Caloplacoideae, [WWW document] URL https://species. wikimedia.org/wiki/Caloplacoideae. [Accessed 24 February 2024].
- Wikispecies (2023c) Teloschistoideae. [WWW document] URL https://species. wikimedia.org/wiki/Teloschistoideae. [Accessed 24 February 2024].
- Wikispecies (2023d) Xanthorioideae. [WWW document] URL https://species. wikimedia.org/wiki/Xanthorioideae. [Accessed 24 February 2024].
- Wilk K, Pabijan M, Saługa M, Gaya E and Lücking R (2021) Phylogenetic revision of South American *Teloschistaceae* (lichenized Ascomycota, *Teloschistales*) reveals three new genera and species. Mycologia 113, 278–299.
- Wirth V, Schiefelbein U and Litterski B (2018) The lichen flora of Germany regional differences and biogeographical aspects. In Blanz P (ed.), *Biodiversity* and Ecology of Fungi, Lichens, and Mosses. Biosystematics and Ecology Series No. 34. Vienna: Austrian Academy of Sciences, pp. 565–588.
- Zahlbruckner A (1926) Afrikanische Flechten (Lichenes). Englers Botanische Jahrbücher 60, 468–552.
- Zahlbruckner A (1932) Lichenes in Africa lecti. Annales de Cryptogamie Exotique 5, 198–275.
- Zahlbruckner A (1936) Neue Flechten, XII. Annales Mycologici 34, 159-179.
- Zhou S and Stanosz G (2001) Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* 105, 1033–1044.
- Zoller S, Scheidegger C and Sperisen C (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* 31, 511–516.