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Atla, a new genus in the *Verrucariaceae* (*Verrucariales*)

Sanja SAVIĆ and Leif TIBELL

Abstract: The new genus *Atla* forms a well-supported clade in a molecular phylogeny based on the ITS1-5.8S-ITS2 and LSU regions of the nuclear ribosomal DNA. The genus has a crustose thallus, a hamathecium at maturity without hyphal elements except for pseudoparaphyses remaining at the ostiolum, and large, muriform spores. *Atla wheldonii* was previously referred to *Polyblastia*. Three new species, *A. alpina* (the type of the new genus), *A. palicei* and *A. praetermissa*, are included in the genus and described here as new to science. They were found on calcareous rocks and soil in Northern Scandinavia; *A. alpina* also occurs in Central Europe, and *A. wheldonii* likewise in Central Europe, the Pyrenées and in the British Isles. An identification key to the species and a revision of the genus are also provided.

Key words: Ascomycetes, lichens, molecular phylogeny, morphology, revision

Introduction

The traditional morphology-based classification in *Verrucariaceae* has long been recognised as artificial, and in our opinion the only way ahead in this family is to recognize genera well supported by DNA data (C. Gueidan *et al.*, unpublished). In phylogenetic studies based on molecular data, *Polyblastia* has recently been shown to be polyphyletic (Gueidan *et al.* 2007; Savić *et al.* 2008). A well-supported monophyletic group, *Polyblastia* s. str., was recognized, containing the type *P. cupularis*; *Sporodictyon* A. Massal., a morphologically well-characterized group with synapomorphic indels, both in the ITS1 and ITS2 region, was resurrected (S. Savić & L.Tibell, unpublished). The sister-group of *Sporodictyon* was shown by Savić *et al.* (2008) to contain *'Polyblastia' wheldonii* Travis and an unnamed species. This group was strongly supported and clearly distinct both in the nuclear rDNA ITS-region and LSU, and also in *RPB*1. *'Polyblastia' wheldonii* was described from England (Travis 1947; Swinscow 1971), and, although included by Clauzade & Roux (1985) and Purvis & James (1992), who reported it to be 'scattered throughout British Isles. Endemic', it remained a very poorly known species. It has since been reported only from a few collections from the Pyrenées (Sérusiaux *et al.* 1999) and Austria (Berger & Priemetzhofer 2005).

During field studies of *Polyblastia* and related lichens in Scandinavia we found *P. wheldonii* on several occasions and also three, closely related, undescribed species. This paper describes *P. wheldonii* and three new species and proposes a new genus to accommodate them.

S. Savić and L. Tibell: Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, S-75236 Uppsala, Sweden. Email: sanja.savic@ebc.uu.se

Material and Methods

Material from the following herbaria has been investigated in this study: BM, GB, M, O and UPS. Collecting during field trips was carried out in Norway and Sweden in 2004–2007. In addition, material of one species was provided by Z. Palice.

Morphology

Longitudinal sections 12–18 µm thick of thalli and perithecia were cut using a freezing microtome. The samples were first hydrated with de-ionized water, and then mounted in diluted Gum Arabic and frozen at

− 20 °C. The sections were examined and measured in water, and then stained with lactophenol/Cotton Blue. Asci and ascospores were studied in squash preparations of perithecia mounted in water. A minimum of 14, and a maximum of 53 measurements were taken for perithecium and spore sizes, and arithmetic means and standard deviations were calculated. The range of ascoma and spore sizes is indicated as the arithmetic mean minus one standard deviation − arithmetic mean plus one standard deviation, followed by a parenthesis (x=arithmetic mean, SD=standard deviation, N= specimens sampled, n=total number of measurements).

DNA extraction, PCR and sequencing

Total DNA was extracted either from freshly collected material or from dried material kept at − 20 °C for a few months (up to two years), as well as from a few herbarium specimens (no older then four years) using the Plant Genomic DNA Extraction Miniprep System (Viogene, Umeå, Sweden), following the the manufacturer's instructions. Perithecia were carefully cleaned from adjacent material to avoid contaminants. Diluted (10^{-1} up to 10^{-3}) or undiluted DNA was used for PCR amplifications. Primers used were: ITS1f (Gardes & Bruns 1993), ITS4, ITS5 (White *et al.* 1990), and LR1n (Tibell 2006). For PCR-amplification of the nuITS and nuLSU we used the AccuPower® PCR PreMix (Bioneer), adding 3 µl diluted or undiluted DNA, 1·5 µl of each primer (10 µM), and water to a total volume of 20 µl. The PCR thermal cycling parameters were: initial denaturation for 4 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 54 °C, 45 sec at 72 °C, and final elongation for 5 min at 72 °C. Amplification products were visualized on 0·5% agarose gels stained with ethidium bromide and the PCR product was purified using Millipore plates (MultiScreen™ PCR). Sequencing, automated reaction clean up and visualization were carried out as described by Macrogen Inc. (www.macrogen.com).

Alignments and phylogenetic analyses

Sequences were assembled and edited using BioEdit (Hall 1999) version 7.0.9.0 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The alignments were obtained with Clustal W (Thompson *et al.* 1994), using the default options and without manual adjustments. Based on previous results (Savić *et al.* 2008), the species belonging to 'clade E' (op. cit.) plus additional sequences of *Atla* were aligned. *Polyblastia hyperborea* in the sistergroup of 'clade E' was selected as outgroup. Sequences downloaded from GenBank and newly obtained sequences are listed in Table 1. Phylogenies based on nuITS and nuLSU were investigated separately, and since no incongruence between them was detected the datasets were concatenated. The maximum parsimony (MP) analyses as well as MP bootstrapping (MPbs) were conducted using PAUP* 4.0b10 (Swofford 2002). The most parsimonious trees were obtained from analyses using a heuristic search with 1000 random addition sequence replicates, TBR branch swapping algorithm, save multiple trees, and gaps treated as a fifth character state. A bootstrap analysis of 10 000 replicates with five RAS per replicate, TBR branch swapping was then conducted.

Additional support values were provided by a Bayesian inference analysis of the combined dataset. Using the Akaike Information Criterion (AIC) implemented in Modeltest 3.7 (Posada & Crandall 1998), the Bayesian analysis employed the GTR+I+G model for both partitions. Using MrBayes 3.1.1 (http://mrbayes.csit.fsu.edu/index.php) two analyses of two parallel runs were carried out for 5 000 000 generations. Each run included four chains, and trees were sampled every 500 generations. Every 500th tree was saved, resulting in 10 000 trees for each data set; a burn in sample of 2000 trees was discarded from each run. Bayesian posterior probabilities (PP) ≥95% (Alfaro *et al.* 2003), MPbs ≥70% were considered to be significant.

Results and Discussion

Seven nuITS and nuLSU sequences, respectively, were newly obtained for this study (Table 1). A total of 28 concatenated sequences were included in the phylogenetic analyses, and the combined matrix consisted of 1330 characters; 1031 characters were constant and 219 parsimony informative. The heuristic search resulted in four trees 628 steps long with a consistency index (CI) of 0·64 and a retention index (RI) of 0·82. One of the MP trees is shown in Fig. 1. In a MCMC analysis using *Polyblastia hyperborea* as outgroup, all *Atla* species were monophyletic. The clade obtained strong support in the MCMC analysis and also in the bootstrapping of a maximum parsimony analysis. *Sporodictyon* formed the strongly supported sister group of *Atla*, and in turn *Henrica* de Lesd. (S. Savić *et al.* unpublished), *Verrucaria rupestris* and *Thelidium* sp. formed the sistergroup of *Atla*/*Sporodictyon*, although only with low support (Fig. 1). *Atla alpina* and *A. praetermissa* were strongly supported, whereas the single sequences of *A. palicei* and *A. wheldonii* were clearly distinct. Two haplotypes were found in *A. praetermissa*, and also in *A. alpina*. Although *Atla* is not easily characterized morphologically, none of the species should be mistaken for a *Sporodictyon*, even in the field. The most obvious cases for mistaken identification might be *S. schaererianum* A. Massal. and *A. alpina*, both of which have large and very

TABLE 1. *Voucher and GenBank accession numbers for material used in the phylogenetic analyses. Accession numbers in bold represent new sequences produced in this study*

Taxon	Isolation number (isn)	Locality	Voucher (herbarium)	nuITS	nuLSU
Atla alpina	SS189	Sweden, Torne Lappmark	*Savić* 3139a (UPS)	**EU697724**	**EU697731**
A. alpina	SS193	Sweden, Härjedalen	*Savić* 3127 (UPS)	**EU697725**	**EU697732**
A. alpina	SS206	Sweden, Härjedalen	*Savić* 3129 (UPS)	**EU697726**	**EU697733**
A. alpina	SS220	Sweden, Torne Lappmark	*Savić* 3139b (UPS)	**EU697720**	**EU697727**
A. palicei	SS027	Sweden, Torne Lappmark	*Palice* 7182 (UPS)	EU553499	EU598726
A. praetermissa	SS107	Sweden, Härjedalen	*Savić* 3284(UPS)	**EU697721**	**EU697728**
A. praetermissa	SS152	Sweden, Härjedalen	*Savić* 3285(UPS)	**EU697722**	**EU697729**
A. praetermissa	SS165	Norway, Troms	*Savić* 3283(UPS)	**EU697723**	**EU697730**
A. wheldonii	SS016	Sweden, Jämtland	*Savić* 3070 (UPS)	EU553497	EU598728
Henrica theleodes	SS122	Norway, Troms	*Savić* 3159 (UPS)	EU559733	EU598703
H. melaspora	SS006	Sweden, Jämtland	*Savić* 3060 (UPS)	EU553495	EU598730
H. melaspora	SS044	Sweden, Jämtland	*Tibell* 23547 (UPS)	EU553502	EU598723
Polyblastia hyperborea	SS005	Sweden, Jämtland	*Savić* 3059 (UPS)	EU553494	EU598731
Sporodictyon cruentum	SS146	Sweden, Jämtland	*Savić* 3286(UPS)	EU697648	EU697693
S. cruentum	T634	Sweden, Jämtland	*Savić* 3032 (UPS)	EU697637	EU697682
S. minutum	SS154	Sweden, Härjedalen	*Savić* 3287(UPS)	EU697652	EU697697
S. minutum	SS174	Norway, Troms	*Savić* 3288(UPS)	EU697656	EU697701
S. minutum	SS184	Sweden, Jämtland	*Savić* 3111 (UPS)	EU697660	EU697705
S. terrestre	SS089	Sweden, Jämtland	*Savić* 3097 (UPS)	EU553515	EU598710
S. terrestre	SS091	Greenland, Narsarsuaq	*Savić* 3110 (UPS)	EU553516	EU598709
S. schaererianum	SS047	Sweden, Jämtland	*Tibell* 23545 (UPS)	EU553504	EU598721
S. schaererianum	SS069	Sweden, Lycksele Lappmark	*Svensson* 182 (UPS)	EU553508	EU598717
S. schaererianum	SS185	Sweden, Härjedalen	*Savić* 3131 (UPS)	EU697661	EU697706
Thelidium sp.	SS115	Norway, Troms	*Savić* 3157 (UPS)	EU559732	EU598704
Trimmatothele perquisita	T560	Sweden, Torne Lappmark	*Savić* 3160 (UPS)	EU559742	EU598695
Verrucaria sp.	–	Estonia	*Gueidan* 742 (DUKE)	–	EF689867
V. rupestris	SS043	Estonia	*Suija* 652 (TU)	EU553501	EU598724
V. rupestris	–	France	*Gueidan* 578 (DUKE)	EU643803	EF689878

dark brown, muriform spores, but the former always has a very well-developed, thick thallus. At the molecular level *Sporodictyon* is strongly supported (Fig. 1) and characterized by numerous synapomorphies including very characteristic indels in the nuITS region (Savić *et al.* 2008). *Atla* has also been shown to be the sister group of *Sporodictyon* with strong support in an analysis based on three genes (S. Savić unpublished); but in that study *Atla* was represented only by *A. wheldonii* and *A. palicei* (as '*Polyblastia* sp.' isn. SS027).

Morphologically *Atla* is only rather vaguely characterized. The thallus is thin, minutely verrucose or semi-immersed, and often found among cyanobacterial colonies. The perithecia are variable; immersed and without an involucrellum in *A. wheldonii*, but in the epilithic species there is a well-developed involucrellum. The spores are muriform, dark brown and very large in *A. alpina* and *A. wheldonii*, but more moderately sized and non-pigmented in *A. palicei* and *A. praetermissa*. In order to stabilize usage of names we find it useful to include sequence information in the descriptions of the species, and we think that it is particularly important that whenever possible such information should be provided when new species are being described.

Atla species were encountered in wet and humid localities, the epilithic species mainly on rocks along riverbeds and streams. *Atla wheldonii* occurs on damp basic, unstable soil colonized by cyanobacteria and mosses. In

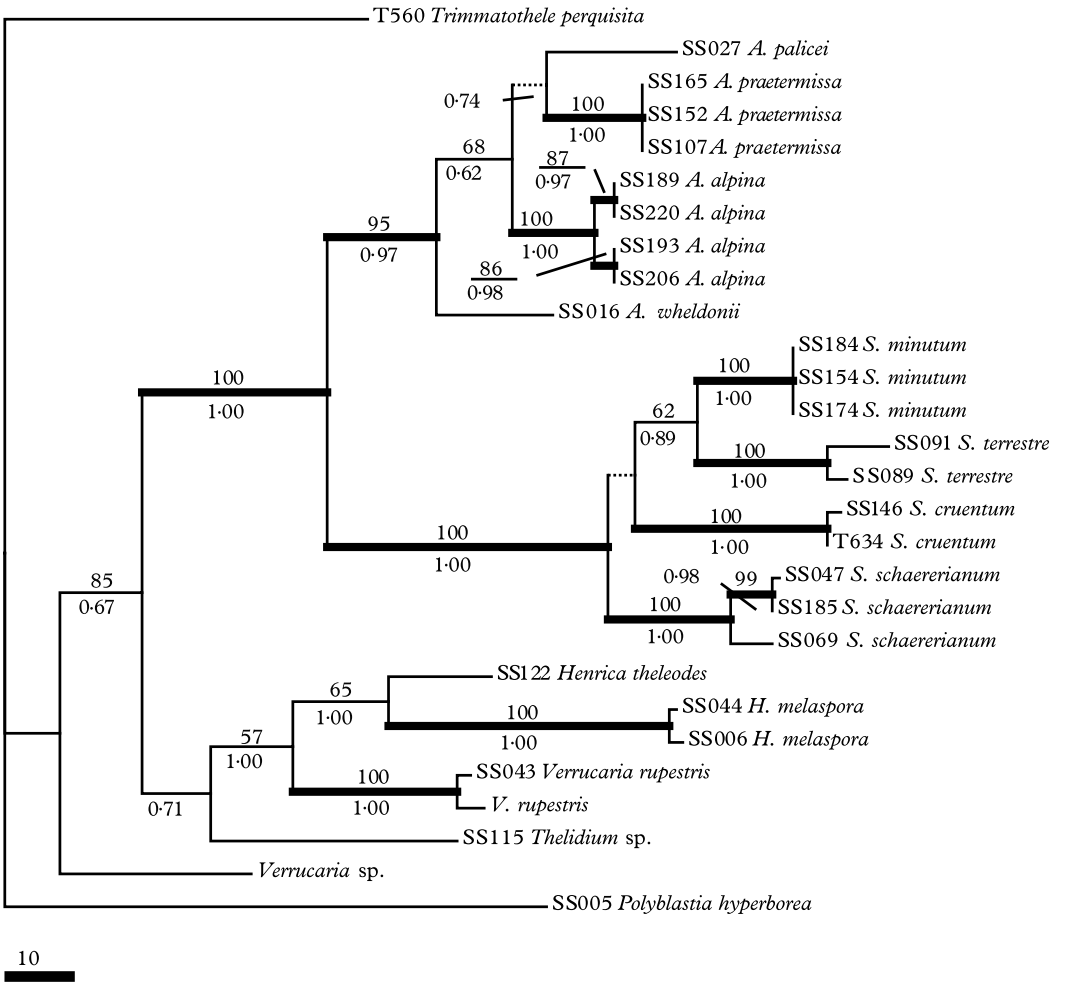

FIG. 1. One of the four most parsimonious trees from the analysis of the nuITS and nuLSU sequences. Tree length = 628 steps. Numbers above branches indicate bootstrap support (>50%), and numbers below branches represent posterior probabilities obtained from Bayesian analysis. Dotted lines indicate branches which collapse in the strict consensus tree. Branches in bold indicate a support of a MLbs ≥ 70% and PP ≥ 95%. For abbreviations of species see Table 1.

Scandinavia it is often associated with *Thelocarpon impressellum* and *Solorina spongiosa*, and occurs along ditches and road-sides of fairly new road-cuts, on unstable waste areas close to rivers and on abandoned dirt roads and deserted tracks, but always in quite open situations.

Only *A. alpina* is easily identified in the field, so that it is suprising it has not been previously described at species level. It is characterized by having large perithecia with a verrucose perithecium wall around the ostiolum, and a very poorly developed thallus. It has been collected now and again in the past, and some specimens have been found in the herbaria investigated, but it has been confused with *Sporodictyon schaererianum* (e.g. by Lynge 1928). *Atla wheldonii* is difficult to find in the field since its perithecia are almost completely immersed in the soil, and it was described as late as 1947. *Atla palicei* and *A. praetermissa* are also

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inconspicuous, both having rather small perithecia and a poorly developed thallus. Consequently these three latter species have been overlooked.

Taxonomy

Atla S. Savić & Tibell gen. nov.

Thallus crustosus, atrocinereus vel nigrescens, verrucosus ad indistincte areolatus vel debiliter evolutus vel immersus. Perithecia 0·3–0·9 mm diametro, sessilia vel immersa. Involucrellum bene evolutum vel destitutum. Asci apicalites non incrassati, ellipsoidei ad clavati, 8-spori, 113–306 × 39–112 µm. Ascosporae muriformes, 43–87 × 19–49 µm, ellipsoideae, ubi maturae hyalinae ad fuscae. Photobions ad Chlorophyta et/vel Cyanobacteria pertinens.

Typus: *Atla alpina* S. Savić & Tibell.

Thallus crustose, blackish, verrucose to diffusely areolate or immersed. *Photobiont* a green alga and/or cyanobacterium.

Perithecia medium-sized to large, 0·3–0·9 mm diam., sessile or immersed. *Excipulum* spherical, black to dark brown. *Involucrellum* well developed, in the upper part fused with the excipulum or missing. *Asci* when mature without apical thickening, ellipsoidal to clavate, 8-spored, 113–306 × 39–112 µm. *Hamathecium* at maturity without hyphal elements except pseudoparaphyses formed below the ostiolum. *Ascospores* 43–87 × 19–49 µm, ellipsoidal, when mature hyaline to dark brown, muriform, with 7–15 transverse and 2–5 longitudinal walls.

Etymology. Atla is the name of a Norse water goddess who sometimes accompanied us in the same habitats as the species named after her. She is the daughter of Aegir and Ran, and one of the 'Billow Maidens' all beautiful maidens and personifications of waves (Atla being the Fury), according to the the Poetic Edda, Hyndluljóð.

Ecology. The species grow on calcareous rocks and soil.

Key to the species of *Atla*

1 Spores dark brown when mature, 70–87 × 33–49 µm 2
Spores hyaline or yellowish when mature, 43–50 × 19–26 µm 3

2(1) Perithecia immersed, without involucrellum, on soil . . . . . **A. wheldonii**
Perithecia not immersed, almost spherical, with thick involucrellum, on rocks . . . . . **A. alpina**

3(1) Thallus scurfy-looking, continuous, diffusely areolate, blackish brown to black, perithecia 0·4–0·5 mm diam.; ascospores ellipsoidal, 43–51 × 23–26 µm . . . . . **A. palicei**
Thallus very thin, fragmented mesh-like to confluent, grey to dark green or brownish, perithecia 0·3–0·4 mm diam.; ascospores narrowly ellipsoidal, 45–49 × 19–22 µm . . . . . **A. praetermissa**

The Species

Atla alpina S. Savić & Tibell sp. nov.

Thallus tenuis, minute areolatus ad granulosus, interdum fere omnino immersus, cinereus vel viridifuligineus. Perithecia satis magna, 0·7–0·9 mm diametro, subsphaerica, thallo non tecta. Involucrellum crassum, valde carbonaceum. Hamathecium praeter periphysoidea sine hyphis interascalibus. Asci apicaliter non incrassati, 153–178 × 70–112 µm, late ellipsoidei vel clavati, 8-spori. Ascosporae 70–83 × 39–49, ellipsoideae ad late ellipsoideae, fuliginosae, muriformes. Photobions ad *Chlorophyta* pertinens sed non determinatur.

Typus: Sweden, Härjedalen, Ljusnedal par., Mittåkläppen, 1·8 km NNW of Djupdalsvallen, 62°43′56″N 12°27′11″E, 1170 m altitude, on vertical slate rocks facing SW, 2006, *Savić* 3129 (UPS—holotypus; isn: SS206; GenBank EU697726, EU697733).

Polyblastia theleodes (Sommerf.) Th. Fr. v. *inundata* Nyl. ex Th. Fr., *Reg. Soc. Sci. Upsal.* **1877**: 11 (1877); type: '*Polyblastia* Jtl. Handöl 1868 *S. Almqvist*' (UPS—lectotype, designated here).

(Figs 2A–C, 3A & B, 4A)

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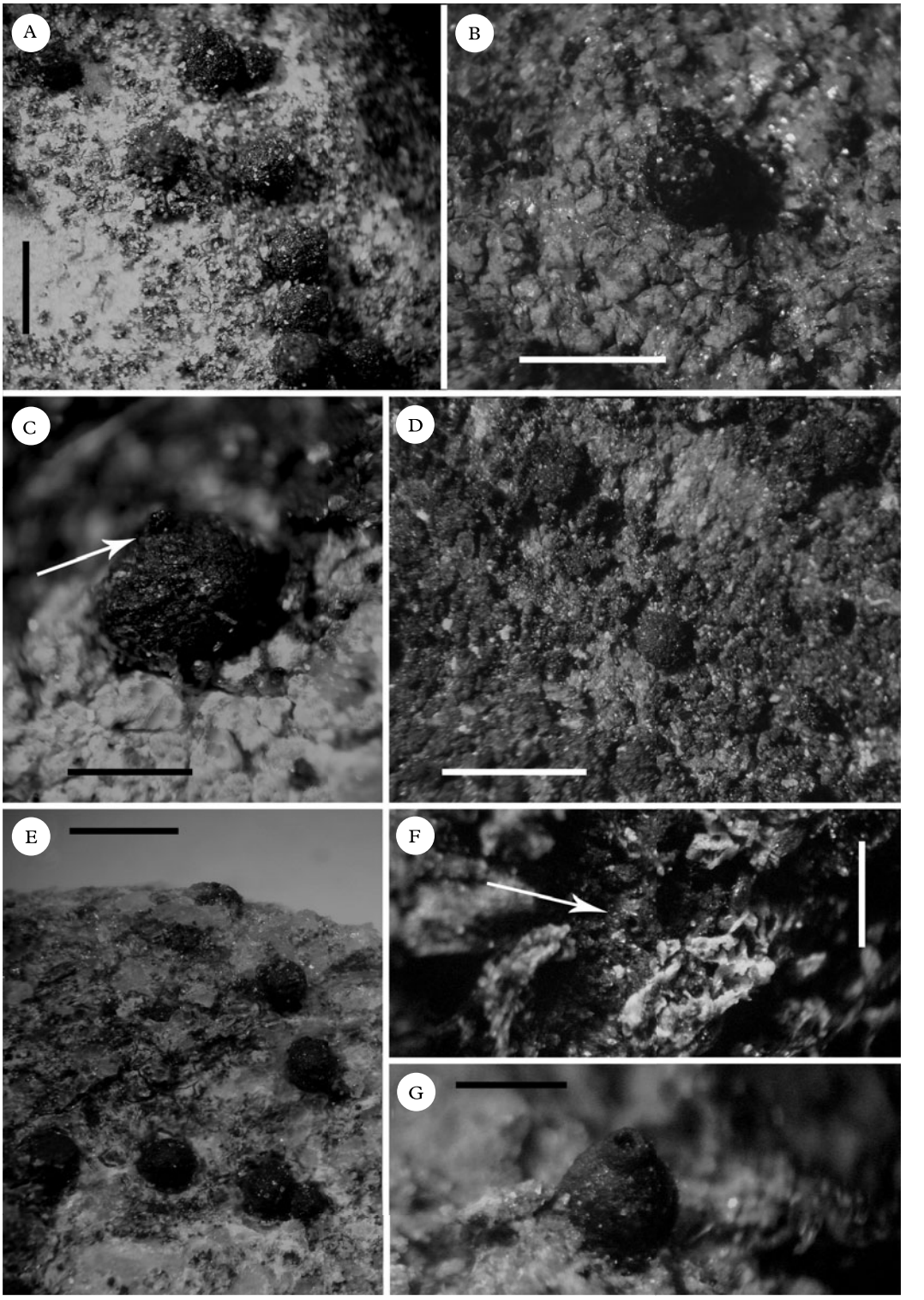

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Thallus crustose, when well-developed thin, minutely areolate, areoles 0·4–0·6 mm wide, irregular and with slightly uneven surface, grey to dark greenish grey; sometimes minutely granular, thin, matt or partly immersed and only emerging as blackish green patches; sometimes almost completely immersed. *Photobiont*: the areoles of well-developed thalli contain an unidentified green alga; small, almost black, verrucose colonies of the cyanobacterium *Nostoc* are frequently associated with the thallus containing green algae and may also participate in a symbiotic relationship.

Perithecia rather large, 0·71–0·87 mm diam. (x=0·79 mm, SD=0·081 mm, N=9, n=42), shining black, almost spherical, adnate, broadly attached at the base, without a thalline cover, when old with verrucose surface in the upper part and impressed ostiolum. *Involucrellum* thick, thickened around the ostiolum, *c*. 80–120 µm thick, in the outermost part forming a continuous, strongly carbonized, black layer with irregular extensions reaching far into the less pigmented, mottled interior of the involucrellum; in the inner and upper part the involucrellum is fused with the excipulum; the middle and inner part the involucrellum formed by a pseudoparenchyma of angular to rounded, slightly elongated cells. *Excipulum* 15–25 µm thick, brown, in section consisting of narrow, concentrically arranged cells, pale in the lowermost part. *Hamathecium* without hyphal elements except for pseudoparaphyses formed below the ostiolum; periphysoids 55–98 µm long, numerous, slender, 1·5–2·5 µm wide, branching at wide angles; gel I+ red, KI+ blue. *Asci* when mature without apical thickening, 153–178 × 70–112 µm, broadly ellipsoidal to clavate, 8-spored. *Ascospores* 70·1–83·0 × 39·1–49·0 µm (x=76·6 µm, SD=6·5 µm, N=9, n=53; x=44·1 µm, SD=5·0 µm, N=9, n=53), ellipsoidal to broadly ellipsoidal, when mature very dark brown, muriform; 7–8 transverse walls reaching the periphery along one side of the spores in a median optical section, and with 3–4 longitudinal walls in the central part; peripheral walls, however, are often difficult to observe since the mature spores are very dark.

Habitat and distribution. On calcareous rocks in humid situations, often on steep rocks or by water. Altitudinal range: 345–1170 m (2700 m in Central Europe). Known from Scandinavia, Spitsbergen, Novaya Zemlya, Germany and Austria.

Characterization. Recognized by the large, sessile, epilithic perithecia with thick involucrellum but without thalline cover, the thin, areolate or poorly developed thallus, and the large, very dark brown, broad spores. DNA sequences of the ITS and LSU regions have been deposited in GenBank.

Taxonomic note. Being a rather conspicuous species, *A. alpina* has been collected and noted by lichenologists for a long time, but its status as a distinct species has not been understood. It was published as '*Polyblastia*' from Novaya Zemlya (Lynge 1928: 32). Lynge commented on the variation in spore size in '*P. theleodes*'. However he included in '*P. theleodes*' not only *Sporodictyon schaererianum*, but also *Atla alpina* and *Sporodictyon arcticum* Savić & Tibell (S. Savić & L. Tibell, unpublished). The record of '*S. theleodes*' from the island of Vega in Northern Norway (Degelius 1982: 102, appropriately noting '± without thallus'), belongs to *A. alpina*. Specimens of *A. alpina* from continental Europe, from Württemberg, were identified

FIG. 2. Thalli and perithecia in *Atla*. A, *A. alpina*, perithecia and poorly-developed, granular thallus (holotype); B, *A. alpina*, perithecium and well-developed, verrucose to areolate thallus (holotype); C, *A. alpina*, perithecium with uneven outer surface and dark ascospores (arrow) emerging at the ostiolum (holotype); D, *A. palicei*, perithecia and scurfy to subareolate thallus (holotype); E, *A. praetermissa*, perithecia and very thin, scattered thallus (holotype); F, *A. wheldonii*, inconspicuous thallus among mosses, only ostiolum of a perithecium (arrow) emerging (Jämtland, Tännforsen, 15 vii 2005, *Savić*, UPS); G, *A. wheldonii*, exposed perithecium visible after the thallus has been eroded away. Scales: A & B; D=1 mm; C, E–G=0·5 mm.

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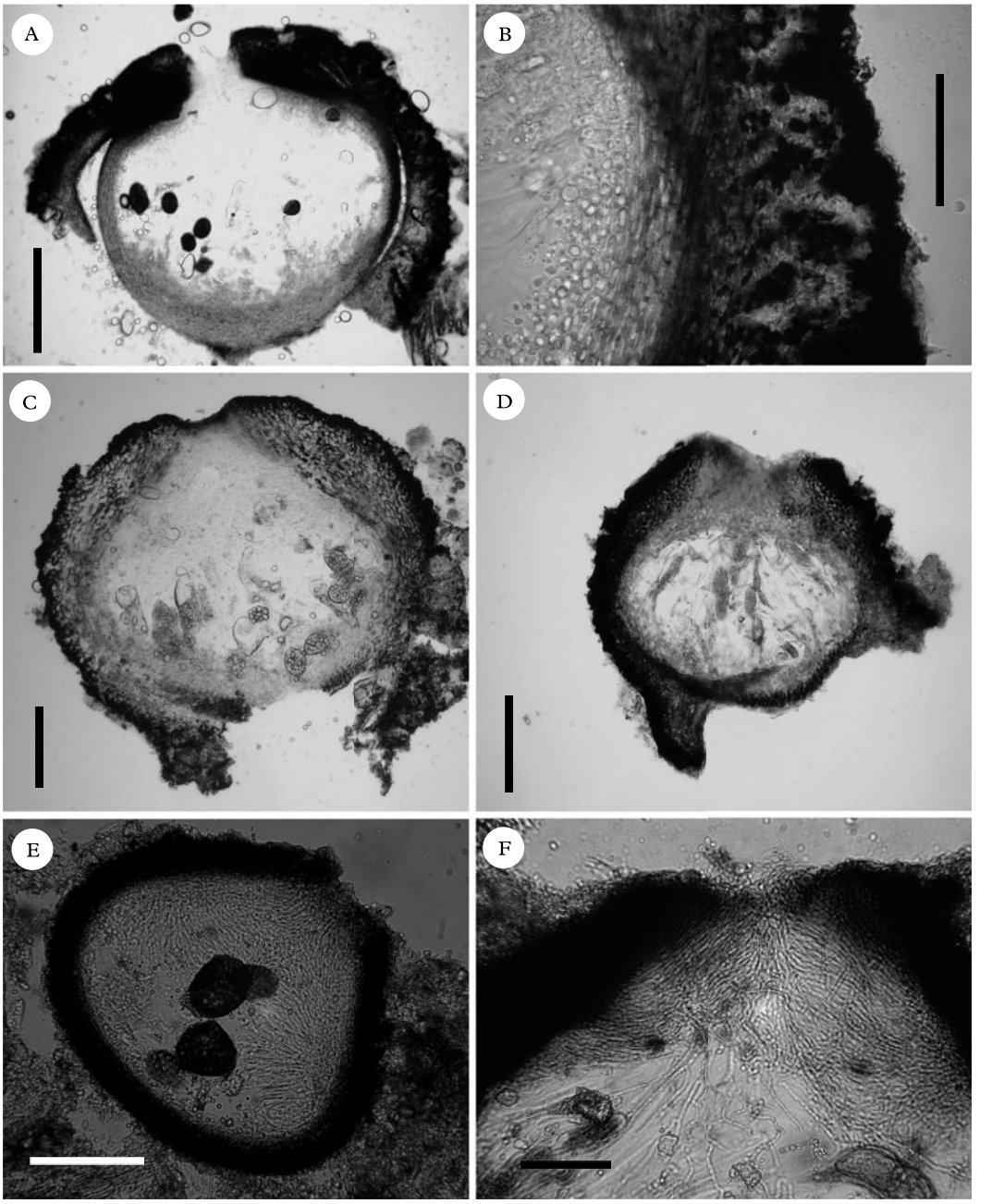

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as '*Polyblastia theleodes* Fr. f. *crusta obsoleta*' or 'f. *inundata*' by Rieber, and distributed in three different exsiccata! *Atla alpina* has thus been confused with *S. schaererianum*, but the latter species differs in having a well-developed, moderately thick to thick, greyish, verrucose or sometimes rimose thallus which forms lobe-like extensions partially enclosing the perithecia. It also usually harbours distinctive, dark, granular to verrucose cephalodia; it has smaller and more numerous cells in the spores which are both shorter and narrower than in *A. alpina*. *Atla wheldonii* differs from *A. alpina* in having much smaller, immersed perithecia without an involucrellum, and by growing on soil. Both *A. palicei* and *A. praetermissa* have smaller perithecia and non-pigmented, smaller spores.

Nomenclatural note. Th. Fries (1877: 11), when discussing *Polyblastia theleodes sensu auct.* (=*Sporodictyon schaererianum*), referred to *A. alpina* as follows: 'Crusta. neque desunt (v.c. in Jemtlandia) formae crusta obsoleta (f. *inundata* Nyl. in CROMB. Lich. Brit. p. 110).' This provided the description of *Polyblastia theleodes* 'f. *inundata* Nyl.' ex Th. Fr., and was most likely based on material collected by Almquist, some of it having earlier been named '*P. theleodes* c. var. *acrustacea*' (Almqvist 1869: 446), a nomen nudum.

Additional specimens investigated. **Sweden:** *Härjedalen*: Ljusnedal par., Mittåkläppen, 1·6 km NNW of Djupdalsvallen, 62°43′43″N, 12°27′06E. 2006, *Savić* 3127 (isn: SS193, UPS). *Jämtland*: Handöl, 1868, *S. Almquist* (UPS). *Torne Lappmark*: Jukkasjärvi par., Abisko, Abiskojokk Canyon, 68°21′47″N, 18°47′28″E, 2005, *Savić* 3139 (isn: SS189, UPS), *Savić* (isn: SS220, UPS).—**Norway:** *Nordland*: Vega hd., Vega Island, Naustnes, exposed calcareous rock, on pebbles, 1978, *Degelius* V-2115 (UPS).—**Spitsbergen:** Kings Bay, 17 viii 1868, *Th. M. Fries* (O).—**Novaya Zemlya:** Sydsidan av Blaafjell Basin, Mashigin, 1921, *Lynge* (O).—**Germany:** *Württemberg*: Wendelthal bi Heidenheim, 1893, *Rieber* ('f. *crusta obsoleta*', Arnold, *Lich. exs*. 1572, M, UPS; 'f. *inundata*', *Krypt. exs. vind*. 579, M; 'f. *crusta obsoleta*', Rehm, *Ascomyceten* 1440, M); Wendelthal bei Heidenheim, 1896, *Rieber* (GB).—**Austria:** *Tirol*: Tuxer Alpen, Westhang des Bentelsteins, 1961, *Steiner* 4836 (M); Silvretta, Ritzenjoch, 1963, *Steiner* (M); Stubaier Alpen, Serlesgruppe, Wesenwand, 1963, *Besterman & Hertel* 519 (M).

Atla palicei S. Savić & Tibell sp. nov.

Thallus superficialis, furfuraceus, tenuis, diffuse areolatus, fuliginosus vel ater. Perithecia satis parva, 0·4–0·5 mm diametro, hemisphaerica, e parvis areolis emergentia. Involucrellum apicale bene evolutum. Asci ubi maturi apicaliter non incrassati, 139–146 × 40–44 µm, ellipsoidei ad clavati, 8-spori. Ascosporae 43–51 × 23–26 µm, ellipsoideae ad anguste ellipsoideae, ubi maturae hyalinae, muriformes. Photobions ad *Chlorophyta* et/vel *Cyanobacteria* pertinens.

Typus: Sweden, Torne Lappmark, Jukkasjärvi par., Låktatjåkka, Kärkevagge valley, 68°24′37″N, 18°18′27″E, on W-facing side of large, calcareous mica-schistose boulder near stream, 2002, *Palice* 7182 (UPS—holotypus; isn: SS027, GenBank EU553499, EU598726).

(Figs 2D, 3C, 4B)

Thallus superficial, scurfy-looking, thin, blackish brown to black, diffusely areolate. *Photobiont*: the thalline areolae in the type specimen consistently contain a mixture of an unidentified green alga, and unnamed species of cyanobacterial *Nostoc* and *Chroococcus*. Which of these potential photobionts actually enter a symbiotic relationship with *A. palicei* is presently not known.

Perithecia rather small, 0·43–0·49 (x=0.46 mm, SD=0·031 mm, N=2,

FIG. 3. Anatomy of perithecia in *Atla*. A, *A. alpina*, section of perithecium through the ostiolum showing distinct excipulum separated from the involucrellum in the lower part, but in the area around the ostiolum excipulum and involucrellum are merged and thickened (holotype); B, *A. alpina*, perithecium wall with outer involucrellum mottled from melanized granules and without visible cell structure and consisting of less pigmented, concentrically arranged cylindrical cells to the interior of the excipulum (holotype); C, *A. palicei*, section of perithecium through the ostiolum showing excipulum merged with the involucrellum, which is mottled from melanized granules (holotype); D, *A. praetermissa*, section of perithecium through the ostiolum with excipulum merged with the involucrellum, which is mottled from melanized granules (holotype); E, *A. wheldonii*, section of immersed perithecium through the ostiolum; showing the excipulum dark and of even thickness throughout, no involucrellum (*Savić* 3070, UPS); F, *A. wheldonii*, numerous pseudoparaphyses in the upper part of the perithecium (*Savić* 3070, UPS). Scales: A=200 µm; B=50 µm, C–E=100 µm, F=25 µm.

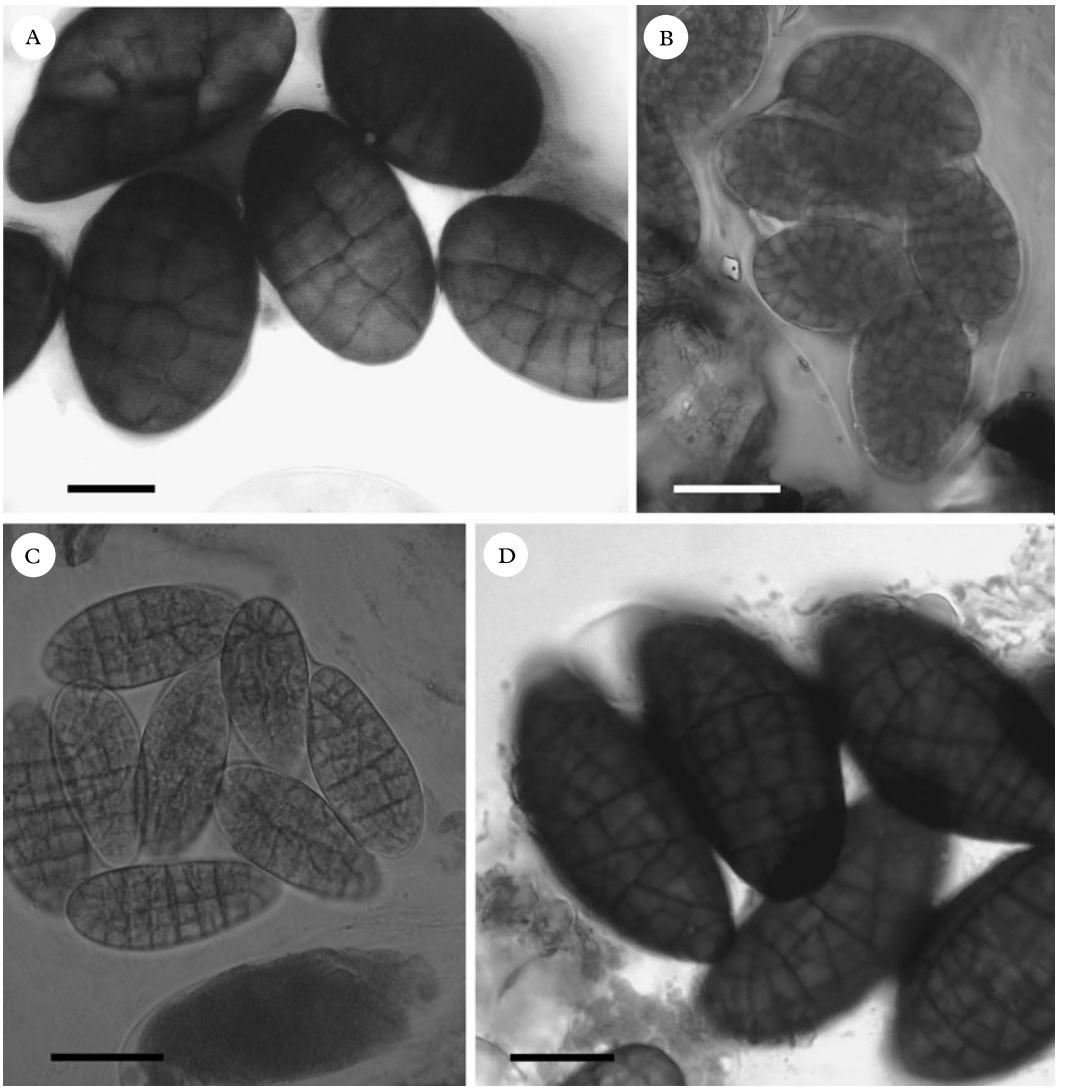

FIG. 4. Spore structure in *Atla*. A, *A. alpina*, very dark brown, often broadly ovoid, mature spores (*Savić* 3127); B, *A. palicei*, mature, hyaline, ellipsoidal spores retained in the ascus (holotype); C, *A. praetermissa*, mature, hyaline, narrowly ellipsoidal spores retained in an ascus (*Savić* 3283); D, *A. wheldonii* dark brown, often ellipsoidal, mature spores (*Tibell* 23626, UPS). Scales: A–D = 25 μm.

n=25) mm diam., hemispherical, emerging from small areolae surrounding the perithecium like a cuff, and immersed only at the base, without a thalline cover. *Involucrellum* well developed apically, *c.* 90–110 μm thick, dark brown in section, strongly carbonized in its outer part and fused with the excipulum. In the central part it is formed by a pseudoparenchyma of rounded to slightly elongated cells. *Excipulum* greenish brown, consisting of narrow, concentrically arranged hyphae. *Hamathecium* without hyphal elements except for pseudoparaphyses formed below the ostiolum; pseudoparaphyses 75–110 μm long, very slender and numerous; gel I+ red, KI+ blue. *Asci* when mature without apical thickening, 139–146 × 40–44 μm, ellipsoidal to

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clavate, 8-spored. *Ascospores* 43·7–51·1 × 22·9–26·0 µm (x=47·4 µm, SD=3·7 µm, N=2, n=25; x=24·4 µm, SD=1·6 µm, N=2, n=25), ellipsoidal, when mature hyaline, muriform, with 12–15 transverse walls reaching the periphery along one side of the spores in a median optical section, and with 4–5 longitudinal walls in the central part.

Habitat and distribution. On calcareous rocks close to streams. Altitude 410–650 m. Known only from Torne Lappmark in Sweden, but probably overlooked elsewhere.

Characterization. Recognized by the blackish, areolate thallus, the rather small perithecia without thalline cover, and the hyaline, rather large spores. Like *A. praetermissa* it is a species with rather small perithecia, an inconspicuous thallus, and rather large, non-pigmented spores, but *A. praetermissa* differs in having a thinner, mesh-like, dark green thallus, smaller perithecia, and narrower spores with fewer septa.

The holotype was included in a study by Savić *et al.* (2008) as '*Polyblastia* sp. isn: SS027', and DNA sequences of the ITS and LSU of the nuclear encoded ribosomal DNA, and *RPB*1 have been deposited in GenBank.

Additional specimens investigated. **Sweden:** *Torne Lappmark*: Jukkasjärvi par., Abisko National Park, Rihtunjira brook, 2002, *Palice* 7613 (hb. Palice); 3 km W of Abisko, Marmorbrottet, 68°20′35″N, 18°46′08″E, 2005, *Savić* (UPS).

Atla praetermissa S. Savić & Tibell sp. nov.

Thallus superficialis, tenuissimus, reticulatus, circa perithecia plusminusve confluens, cinereus ad atroviridis vel fuscescens. Perithecia satis parva, 0·3–0·4 mm diametro, subsphaerica, thallo non tecta. Involucrellum bene evolutum. Asci ubi maturi apicaliter non incrassati, 113–133 × 39–47 µm, ellipsoidei ad clavati, 8-spori. Ascosporae 45–49 × 19–22, ellipsoideae ad anguste ellipsoideae, saepe irregulares extremo uno leviter dilatato, ubi maturae hyalinae, muriformes. Photobions ad *Chlorophyta* pertinens sed non determinatur.

Typus: Sweden, Härjedalen, Funäsdalen par., 1·5 km NW of Hamra, Andersjöåforsen, 62°35′04″N, 12°12′35″E, 2006, *Savić* 3284 (UPS—holotypus; isn: SS107, GenBank EU697721, EU697728).

(Figs 2E, 3D, 4C)

Thallus superficial, crustose, very thin, mesh-like to more or less confluent around the perithecia, grey to dark green or with a brownish hue. *Photobiont* an unidentified green alga.

Perithecia rather small, 0·31–0·41 (x=0·36 mm, SD=0·050 mm, N=3, n=30) mm diam., almost spherical, adnate at the base, without thalline cover. *Involucrellum* well developed, *c*. 35–55 µm thick, almost reaching the base of the perithecium, where it diverges from the excipulum. Outermost layer 18–25 µm thick, black; interior of the involucrellum dark brown with irregularly intertwined, heavily sclerotized cells. *Excipulum* brown throughout, consisting of narrow, concentrically arranged cells 11–15 × 2–4 µm. *Hamathecium* without hyphal elements except for pseudoparaphyses formed below the ostiolum; pseudoparaphyses slender, 1·5–2 µm diam., septate, branching at wide angles; gel I+ red, KI+ blue. *Asci* when mature without apical thickening, 113–133 × 39–55 µm, ellipsoidal to clavate, 8-spored. *Ascospores* 44·7–49·2 × 19·0–22·4 µm (x=46·9 µm, SD=2·3 µm, N=3, n=27; x=20·7 µm, SD=1·7 µm, N=3, n=27), narrowly ellipsoidal, often asymmetrical with one end slightly widened, when mature hyaline, muriform, with 8–11 transverse walls reaching the periphery along one side of the spores in a median optical section, and with 2–4 longitudinal walls in the central part.

Habitat and distribution. On calcareous rocks close to streams and in the mist of waterfalls. Altitude 165–955 m. Known only from Scandinavia, but probably overlooked elsewhere.

Characterization. Recognized by the very thin, often mesh-like, dark green thallus, the small perithecia without thalline cover, and the hyaline, rather large spores. Both *A. praetermissa* and *A. palicei* are species with rather small perithecia (<0·5 mm diam.) and large spores (>40 µm long). Compared to *A. praetermissa*, however, *A. palicei* has a

thicker and darker thallus, larger perithecia and wider spores. DNA sequences of the ITS-region and LSU of the nuclear encoded ribosomal DNA, have been deposited in GenBank.

Additional specimens investigated. **Sweden:** *Härjedalen*: Ljusnedal par., Mittåkläppen, 1·1 km NNW of Djupdalsvallen, 62°43′30″N, 12°27′30″E, 2006, *Savić* 3285 (isn: SS152, UPS).—**Norway:** *Troms*: Målselv, Rostadalen, N of Tverrelvmo, below the falls of Tverrelven, 68°58′10″N, 19°45′32″E, 2006, *Savić* 3283 (isn: SS165, UPS).

Atla wheldonii (Travis) S. Savić & Tibell comb. nov.

Polyblastia wheldonii Travis, *North Western Naturalist* **23:** 240 (1947; as '*Polyblastia Wheldoni*'), 'Anglia, terricola'. Typus: England, '*Polyblastia* On sand dunes, Hightown, Lancashire. Nov. 1924', leg. Travis (BM!—lectotype, Swinscow in *Lichenologist* **5:** 102).

(Figs 2F & G, 3E & F, 4D)

Thallus crustose, thin, minutely verrucose, slightly glossy and often only observable close to the perithecia, gelatinous when wet, dark green. Photobiont a green alga, but cyanobacterial colonies often occur in close connection with the perithecia and thallus.

Perithecia rather small, 0·35–0·43 mm diam. (x=0·39 mm, SD=0·043 mm, N=6, n=14), spherical, or with somewhat extended ostiolum, almost fully immersed; sometimes one third exposed and then with a thalline cover around the base; only in decaying thalli almost sessile; without involucrellum. *Excipulum* 35–60 µm thick, brown, consisting of narrow, concentrically arranged cells, paler in the innermost part and in surface view isodiametric, polygonal. *Hamathecium* without hyphal elements except for pseudoparaphyses formed below the ostiolum; pseudoparaphyses 75–95 µm long, numerous, slender, 1·0–1·5 µm wide, branching at wide angles; gel I+ faintly red, KI+ blue. *Asci* when mature without apical thickening, very variable in size, 159–306 × 69–87 µm, from broadly to narrowly ellipsoidal or clavate, containing 8 spores, but sometimes the number of developing spores is reduced to 6 or 4, larger than when eight spores are formed; occasionally some of the spores are extraordinarily large (up to 150 × 80 µm). *Ascospores* in eight-spored asci 70·1–87·2 × 33·3–45·2 µm (x=78·7 µm, SD=8·5 µm, N=9, n=53; x=39·3 µm, SD=6·0 µm, N=9, n=53), ellipsoid to broadly ellipsoid or slightly asymmetrical with one end being thicker, soon becoming dark brown and strongly muriform; transverse walls reaching the periphery along one side of the spores in a median optical section 10–15, and with 4–5 longitudinal walls in the central part.

Habitat and distribution. On damp basic, unstable soil colonized by cyanobacteria and mosses, often associated with *Thelocarpon impressellum* and *Solorina spongiosa* and occasionally with *Polyblastia helvetica* Th. Fr. *sensu* Swinscow (1971). It was found in ditches and along road-sides of fairly new road-cuts, on unstable waste areas close to rivers, on abandoned dirt roads and deserted tracks, but always in quite open situations. Altitudinal range 130–495 m. In Sweden it has been recorded from Västergötland in the south to Torne Lappmark in the far north. In Norway it is known only from the northern part.

Characterization. Recognized by the moderately sized, immersed perithecia occurring on soil, the lack of an involucrellum, the thin, poorly developed thallus, and by the large, dark brown, broad spores. DNA sequences (ITS, LSU, and *RPB*1) have been deposited in GenBank.

Notes. *Atla wheldonii* was originally described from the British Isles, and it was depicted by Travis (1947) and Swinscow (1971). The spore size reported by Swinscow (1971) and also indicated in the original description (Travis 1947) is larger than in the Scandinavian material we have studied, and this is also true for material from Austria studied by Berger & Priemetzhofer (2005). Otherwise, the material from the type locality agrees well morphologically with the material from Scandinavia, and the habitats also seem to be similar. A more thorough investigation,

particularly including molecular data, is needed to clarify the situation, but it cannot be excluded that the Scandinavian material belongs to a different species. The species has recently been discovered in the Spanish Pyrenées (Sérusiaux *et al.* 1999) and Austria (Berger & Priemetzhofer 2005). It is reported here for the first time from Northern Europe. *Atla wheldonii* was found in several locations during our field work in Sweden (Jämtland, Torne Lappmark) and Norway (Nordland and Troms) when suitable habitats were investigated. It is easily overlooked so it may have a wider distribution in Scandinavia. The occurrence of *P. wheldonii* may often be first located by the observation of the large, strongly shining spores that often occur on the soil in the vicinity of the perithecia and also accumulating around the ostiolum.

Nomenclatural notes. The Travis material we studied contains several perithecia and has earlier been investigated in detail by Alois Wilfling, who supplied 21 spore measurements. The spores of the Travis material are both longer and wider than those from Scandinavian material; a statistically highly significant difference. The thallus in the BM material is also grey rather than dark green to brownish as observed by us in the fresh material studied. In other morphological details, however, the British and Scandinavian materials seem to agree well. Molecular data from the British population are desirable.

Additional specimens investigated. **Sweden:** *Västergötland*: Varnhem par., Ulunda, on moist earth on calcareous ground, 1938, Magnusson 16266 (UPS). *Härjedalen*: Ljusnedal par., 2 km NE of Ljusnedal, Tevafällen, 62°32′51″N, 12°38′11″, 2007, Savić 3260 (UPS). *Jämtland*: Åre par., Bodsjöedet, 63°26′45″N, 12°41′48″, 2004, Savić 3070 (isn: SS193, UPS), 3073 (UPS) and Tibell 23626 (UPS); Tännforsen, 63°27′N, 12°45′E, 2005, Savić (UPS); Undersåker par., Handöl, 1868, S. Almquist (UPS); Just S of Undersåker, by the river Indalsälven, 63°18′N, 13°14′E, 2005, Savić 3105 (UPS); 3 km NE of Trillevallen, along the road, 0·8 km SSE of Brännvinsvallen, 63°17′N, 13°16′E, 2005, *Savić* 3103 (UPS); N shore of Ristafallet, 63°19′N, 13°21′, 2005, *Savić* (UPS). *Torne Lappmark*: Jukkasjärvi par., Abiskojokk, 68°12′N, 18°48′E, 2005, *Savić* 3151 (UPS); 1·5 km SSW of Björkliden, Kåppasjåkka, 68°23′53″N, 18°41′08″E, 2005, *Savić* 3150 (UPS).—**Norway:** *Troms*: Lenvik hd, 7·5 km NNW of Silsand, Lakselva, close to the mouth of the river, 69°18′09″N, 17°54′04″E, 2006, *Savić* (UPS); Målselv par., Dividalen, along the road, 69°44′21″N, 19°45′00″E, 2006, *Savić* 3114 (UPS); Nordland. Rana par., close to Grönligrotten, 66°25′13″N, 14°15′29″E, 2006, *Savić* 3116 (UPS).

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