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Two new species of *Atla* (*Verrucariaceae*)

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Abstract: Two new species in the lichen genus *Atla*, *A. alaskana* and *A. recondita*, are described. The ITS rDNA region is used for their molecular characterization. Morphologically, *Atla alaskana* is characterized by its rather thick and well-developed whitish grey thallus, and the rather large perithecia having a thalline excipulum. The presence of a thalline excipulum renders it similar to *Sporodictyon* species; however, in *A. alaskana* a distinct zone around the ostiolum is without a thallus and covered only by a thick white pruina. *Atla recondita* has a thin olivaceous brown thallus and moderately sized, emerging perithecia. It is not possible to identify this species unequivocally as an *Atla* species only by morphology, and it might well be mistaken for a *Polyblastia*. A key to all six *Atla* species, including the two new species, is provided.

Key words: Alaska, key, lichens, Sweden, taxonomy

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Introduction

Recently, a number of genera have been described or resurrected in connection with advances, based on molecular data, in the phylogeny of *Verrucariaceae* (Savić *et al.* 2008; Gueidan *et al.* 2009). *Polyblastia* A. Massal. has been given a narrower circumscription (Savić *et al.* 2008; Savić & Tibell 2012) and some groups of *Polyblastia* (e.g. Santesson *et al.* 2004) have been included in the recently described genus *Atla* Savić & Tibell (Savić & Tibell 2008*a*), in *Henrica* B. de Lesd. (Savić & Tibell 2008*b*) and in the resurrected genus *Sporodictyon* A. Massal. (Savić & Tibell 2008*c*).

Atla has a crustose thallus, large muriform spores and the hamathecium at maturity has no hyphal elements, except for pseudoparaphyses at the ostiolum. So far, four species have been recognized. However, one *Atla* species, already molecularly characterized in a paper on *Henrica* (Savić & Tibell 2008*b*) as '*Atla* sp.', has awaited description. In addition, one new *Atla* species was recently discovered by the first author during a lichenological excursion to Alaska in 2010.

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The two new species are described below, and a key to all six *Atla* species is provided.

Materials and Methods

Material from two species tentatively identified as belonging to *Atla* was collected in Sweden and Alaska. The ITS1–5.8S–ITS2 of the nuclear encoded rDNA (nuITS) was sequenced. The sequences obtained from GenBank and a newly obtained sequence are referred to at the end of this section. For statistical estimates: N = number of samples (collections measured) investigated; n = number of observations (e.g. spores measured).

Molecular study

Total DNA was extracted from freshly collected material and/or from dried material, and kept at –20°C following the protocol of the Plant Genomic DNA extraction Kit (VIOGEN). Diluted (10^{-1}–10^{-3}) or undiluted DNA was used for PCR amplifications of the nuITS. Primers used were: ITS1F (Gardes & Bruns 1993), ITS4 and ITS5 (White *et al.* 1990). For PCR amplification of the nuITS, we used the AccuPower PCR PreMix (Bioneer, Daejeon, Korea), adding 3 µl of diluted or undiluted DNA, 1·5 µl of each primer (10 mM), 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 s at 72°C, and a final elongation for 5 min at 72°C. Amplification products were visualized on 0·5 % agarose gels stained with ethidium bromide and the PCR products were purified using Millipore plates (MultiScreen PCR, Danvers, Massachusetts, USA). Sequencing, automated reaction clean up, and visualization were carried out as described by Macrogen (www.macrogen.com).

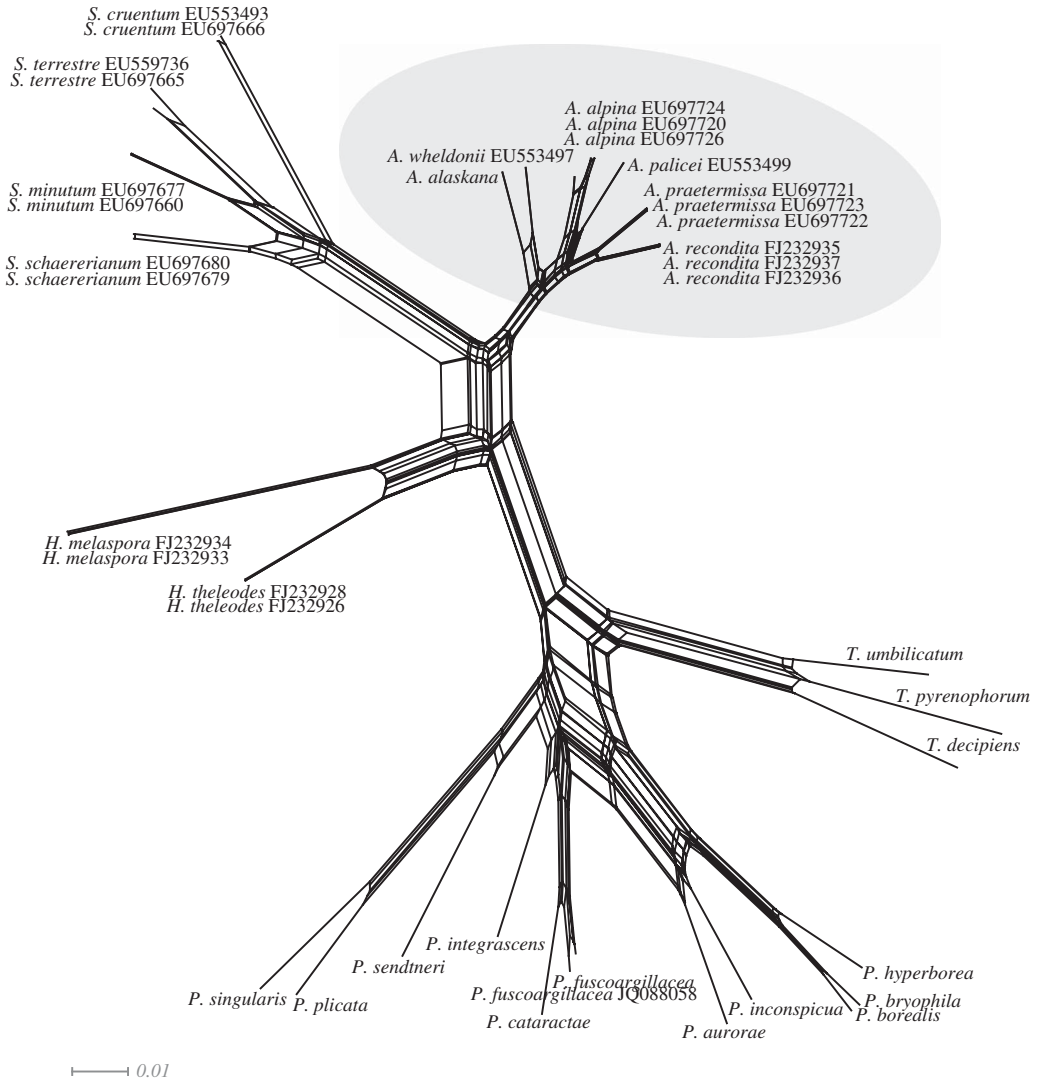

FIG. 1. Split tree representation of relationships of nuITS sequences of *Atla* and closely related genera in the '*Polyblastia* group'.

Alignments and phylogenetic analysis

The data set was aligned using MAFFT v7.182 and the L-INS-i MAFFT algorithm (Katoh *et al.* 2005) as implemented on the online server (http://mafft.cbrc.jp/alignment/software/). The alignment was generated using the default settings.

We explored region/DNA relationships using the split decomposition method; a parsimonious split tree was calculated as set out in the software SplitsTree version 4.12.6 (Bandelt & Dress 1992; Huson & Bryant 2006). This method is based on the fact that any dataset can be partitioned into sets of sequences or 'splits' (therefore, a split is the division of haplotypes into two exclusive sets), and by combining those splits successively, a split-graph can be constructed (Posada & Crandall 2001). This type of network accommodates the formation of contradictory groupings (incompatible splits), introducing a loop into the split graph (Posada & Crandall 2001), which accounts for the possible presence of alternative groupings.

Sequences used for the SplitsTree analysis; the species name is followed by GenBank accession

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number. One newly obtained sequence is in bold: ***Atla alaskana*, (GenBank KP259842)**; *A. alpina*, EU697720; *A. alpina*, EU697724; *A. alpina*, EU697726; *A. palicei*, EU553499; *A. praetermissa*, EU697721; *A. praetermissa*, EU697722; *A. praetermissa*, EU697723; *A. recondita*, FJ232935; *A. recondita*, FJ232936; *A. recondita*, FJ232937; *A. wheldonii*, EU553497; *Henrica melaspora*, FJ232933; *H. melaspora*, FJ232934; *H. theleodes*, FJ232926; *H. theleodes*, FJ232928; *Polyblastia aurorae*, JQ088063; *P. borealis*, JQ088073; *P. bryophila*, JQ088074; *P. cataractae*, JQ088061; *P. fuscoargillacea*, JQ088058; *P. fuscoargillacea*, JQ088060; *P. hyperborea*, EU553494; *P. inconspicua*, JQ088075; *P. integrascens*, JQ088051; *P. plicata*, JQ088076; *P. sendtneri*, JQ088050; *P. singularis*, JQ088077; *Sporodictyon cruentum*, EU697666; *S. cruentum*, EU553493; *S. minutum*, EU697677; *S. minutum*, EU697660; *S. schaererianum*, EU697680; *S. schaererianum*, EU697679; *S. terrestre*, EU559736; *S. terrestre*, EU697665; *Thelidium pyrenophorum*, EU553500; *T. umbilicatum*, EU559737; *T. decipiens*, EU553511.

Results

Phylogeny

In a splits analysis, nuITS sequences from two species were compared with a selection of related species in the '*Polyblastia* group' (see Savić *et al.* 2008; Gueidan *et al.* 2009). The taxon sampling included species from *Atla*, *Henrica*, *Polyblastia*, *Sporodictyon*, and *Thelidium* A. Massal. (Savić *et al.* 2008; Savić & Tibell 2008*a*, *b*, *c*). The overall topology of the generic relationships as revealed here from the nuITS is also consistent with those based on a three region analysis (Savić *et al.* 2008). The species from Alaska proved to belong to the genus *Atla* (Fig. 1), as did the '*Atla* sp.' mentioned in the introduction (Savić & Tibell 2008*b*) and were morphologically distinct from other *Atla* species. The new *Atla* species are described below.

Taxonomic treatment

Atla alaskana S. Tibell & Tibell sp. nov.

MycoBank No.: MB 811041

Thallus thick, whitish grey, granular to verrucose. Perithecia rather large, 0·6–0·8 mm diam., covered by a thalline excipulum. Spores pale yellowish brown when mature, 53–62 × 27–32 μm, with 13–18 transsepta and 5–7 longisepta.

Type: Alaska, Brooks Range, Endicott Mountains, Sukakpak Mountain, on low, exposed calciferous rock ledges facing west in open dwarf shrub community, 67°35'53"N, 149°45'34"W, alt. 776 m, 21 August 2010, *Savić* 5003 (UPS—holotype, vouch. SS555).

(Fig. 2A, C & E)

Thallus superficial, moderately thick, whitish grey, verrucose to granular, subareolate. *Perithecia* rather large, 0·58–0·75 mm ($\bar{x}$ = 0·67 mm, SD = 0·08 mm, $N = 1$, $n = 20$) diam., subspherical, sessile, black, with well-developed thalline exciple covering the lower part and a pruina-like cover in the upper part; ostiolar area depressed, black; pruina-like layer consisting of a hyphal cover without photobiont; photobiont cells arranged in columns in the thalline exciple; involucrellum dimidiate, blackish brown, thickened around the ostiolum, 110–120 μm thick in the upper part, gradually thinning and 40–45 μm thick halfway down the perithecia. *Excipulum* 0·31–0·36 mm diam., *c.* 25 μm thick at the base, brown, consisting of cells appearing narrowly rectangular in section; widening upwards, pale and merging with the involucrellum in the uppermost part. *Hamathecium* without hyphal elements except for pseudoparaphyses formed below the ostiolum; pseudoparaphyses slender, 70–170 μm long and 1·5 μm diam., septate, sparingly branching at the apices; hymenial gel I+ red, KI+ blue, except for the pseudoparaphyses. *Asci* 47–71 × 25–33 μm, ellipsoidal to clavate, 8-spored. *Ascospores* 53·3–61·5 × 27·4–31·7 μm ($\bar{x}_L$ = 57·4 μm, SD = 4·1 μm, $N = 1$, $n = 20$; $\bar{x}_W$ = 29·5 μm, SD = 2·15 μm, $N = 1$, $n = 20$), hyaline, when mature pale yellowish brown, ellipsoidal, muriform, with 13–18 transsepta reaching the periphery along one side of the spores in a median section, and 5–7 longisepta in the central part. *Photobiont* an unidentified green alga; cyanobacteria are found, partly arranged in a prothallus-like edge. They possibly have a symbiotic association with *Atla alaskana*.

Habitat and distribution. On calciferous rocks in open situations along watercourses. So far known only from Alaska.

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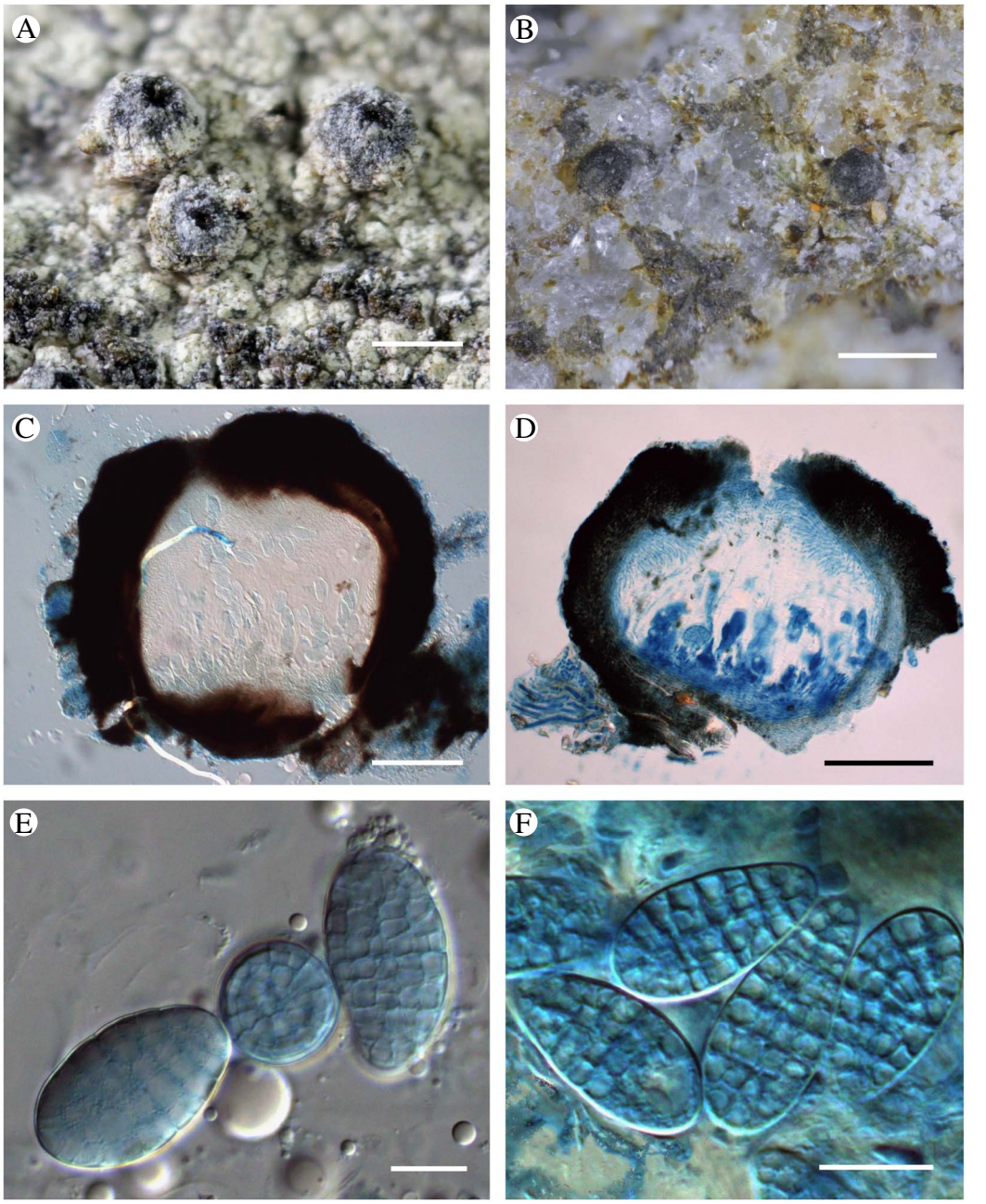

FIG. 2. A, C & E, *Atla alaskana* (holotype); A, thallus; C, section of ascoma; E, mature spores. B, D & F, *A. recondita* (holotype); B, thallus; D, section of ascoma; F, mature spores. Scales: A = 0·9 mm, B = 0·5 mm, C = 200 μm, D = 100 μm, E & F = 20 μm.

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Characterization. Recognized by the rather thick, well-developed, whitish grey, granular to verrucose thallus, the rather large perithecia covered by a thalline excipulum, and the pale brown, rather large spores with 13–18 transsepta and 5–7 longisepta. The occurrence of a thalline excipulum renders it quite similar to *Sporodictyon* species, but in *Sporodictyon* species the thallus often covers the perithecia almost to the ostiolum. In *A. alaskana*, a distinct zone around the ostiolum is without thallus and covered by a thick white pruina. In *Sporodictyon* species with a denuded zone around the ostiolum, such as *S. terrestre*, this zone is without a pruina.

Atla recondita S. Tibell & Tibell sp. nov.

MycoBank No.: MB 811042

Thallus superficial, thin, appearing fragmented, ochraceous. Perithecia medium-sized, black, 0·3–0·4 mm diam. Ascospores 41–49 × 19–23 μm, hyaline or pale yellowish, ellipsoid, muriform, with 9–15 transsepta reaching the periphery along one side of the spores in a median optical section, and with 3–4 longisepta in the central part.

Type: Sweden, Härjedalen, Ljusnedal par., Hamrafjället, 0·7 km NE of Röstavallen, on calciferous rocks in open situation, 62°33'58"N, 12°16'22"E, alt. 1075 m, 21 July 2007, *Savić* 3305 & *Tibell* (UPS—holotype, vouch. T762).

(Fig. 2B, D & F)

Thallus superficial, thin, ochraceous. *Perithecia* medium-sized, black, 0·28–0·37 mm ($\bar{x}$ = 0·32 mm, SD = 0·042 mm, $N = 3$, $n = 29$) diam., subspherical, with depressed ostiolum, sessile; involucrellum *c.* 42–80 μm thick, widened around the ostiolum, gradually thinner towards the base, blackish brown; *excipulum* indistinctly delimited from the involucrellum, pale in the upper part, pale brown at the base where it is 15–19 μm thick. *Hamathecium* without hyphal elements except for pseudoparaphyses formed below the ostiolum; pseudoparaphyses slender, 28–48 μm long and 1·5 μm diam., septate, sparingly branching at the apices; hymenial gel I+ red, KI+ blue, except for the pseudoparaphyses. *Asci* 71–127 × 33–70 μm, ellipsoidal to clavate, 8-spored. *Ascospores* 41·3–48·7 × 19·1–22·6 μm ($\bar{x}_L$ = 45·0 μm, SD = 3·66 μm, $N = 3$, $n = 29$; $\bar{x}_w$ = 20·9 μm, SD = 1·73 μm, $N = 3$, $n = 29$), hyaline or pale yellowish, ellipsoidal, muriform, with 9–15 transsepta reaching the periphery along one side of the spores in a median optical section, and with 3–4 longisepta in the central part. *Photobiont* an unidentified green alga, 8·0–9·5 μm diam.; *Stigonema* is also frequently found, like some other cyanobacteria, which possibly also have a symbiotic association with *Atla recondita*.

Habitat and distribution. On calciferous rocks in open situations along watercourses. Altitude: 610–1075 m. Seemingly a rare alpine species, so far known only from Härjedalen in the central Skandes.

Characterization. Recognized by the thin, olivaceous brown thallus, the moderately sized, emerging perithecia, the non-pigmented to pale yellowish spores with 9–15 transsepta and 3–4 longisepta. It is not possible to identify this species unequivocally as an *Atla* species by its morphology, and it might well be mistaken for a *Polyblastia*.

Additional specimens examined. **Sweden:** *Härjedalen*: Ljusnedal par., 2·0 km NE of Ljusnedal, Tevåfallet, 62°32'51"N, 12°38'11"E, 2007, *Savić* 3263b & *Tibell* (vouch. SS234, UPS); Hamrafjället, 0·7 km NE of Röstavallen, 62°33'58"N, 12°16'22"E, 2007, *Savić* 3304 & *Tibell* (vouch. T761, UPS).

Key to the species of *Atla*

1	Spores dark brown when mature	2
	Spores hyaline, yellowish or pale brown when mature	3
2(1)	Perithecia immersed, without involucrellum, on soil	**A. wheldonii**
	Perithecia sessile, with a distinct involucrellum, on rocks	**A. alpina**

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3(1) Thallus well developed, whitish grey, perithecia with thalline exciple . . . . . **A. alaskana**
Thallus thin, dark, perithecia without thalline exciple 4

4(3) Thallus scurfy, blackish brown, diffusely areolate, spores 43–51 × 23–26 μm . . . . **A. palicei**
Thallus very thin, grey to dark green, ochraceous or olivaceous brown, smooth to discontinuous, spores 41–49 × 19–23 μm 5

5(4) Thallus grey to dark green, appearing fragmented, spores 45–49 × 19–22 μm; mature spores with 8–11 transverse walls reaching the periphery on one side and with 2–4 longitudinal walls in the central part . . . . . **A. praetermissa**
Thallus ochraceous to olivaceous brown, thin, continuous, spores 41–48 × 19–23 μm; mature spores with 9–15 transverse walls reaching the periphery on one side and with 3–4 longitudinal walls in the central part . . . . . **A. recondita**

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