## Aspicilia berntii, a new name for a poorly known species

#### Anders NORDIN, Leif TIBELL and Björn OWE-LARSSON

**Abstract:** The new name *Aspicilia berntii* is proposed to accommodate *Lecanora mastoidea* Lynge in *Aspicilia* and a lectotype is designated. Additional characters are added to the species description and a comparison is made with similar species. The species is reported as new to Scandinavia with localities in northern Norway. An assessment of the phylogenetic relationships, based on a split network analysis of ITS sequences, places *A. berntii* close to *A. verrucigera* in the *A. cinerea* group.

Key words: Aspicilia, Greenland, ITS, Lecanora mastoidea, Norway, SplitsTree4

### Introduction

During work on a revision of Scandinavian Aspicilia species (Nordin et al. 2007), we investigated recently collected material of a dark Aspicilia species from Greenland and Norway, which proved to agree with the description and the type material of Lecanora mastoidea Lynge (Lynge 1937). Unlike the material seen by Lynge, however, this material contained both well-developed ascospores and pycnidia, and the variation in the appearance of the thallus was greater than what was indicated in Lynge's description. A closer examination of the nomenclature made us realize that the combinations into Aspicilia proposed, first by Creveld (1981) and later by Thomson (Egan 1987), are invalid because of the older name Aspicilia mastoidea (Wedd.) Maheu & A. Gillet (Maheu & A. Gillet 1926). This name is based on a different type specimen, identical with or closely related to Aspicilia cinerea (L.) Körb. Hence the new name Aspicilia berntii A. Nordin, Tibell & Owe-Larss. is proposed here and a species description provided, together with a comparison with similar

species. Further, the phylogenetic position of the species is assessed and visualized by use of a split network analysis of molecular data.

#### **Materials and Methods**

Fresh material from Norway and Greenland was studied together with herbarium specimens from UPS and S. The material was examined using dissection and compound microscopes. Sections, mainly cut by hand, were studied in water, 10% KOH (K) (tips of paraphyses) and Lactic Blue. Lugol's solution (I) was used for the detection of amyloid reactions, and c. 50% HNO<sub>3</sub> (N) for epihymenial pigments. Spore and conidia measurements are given as (min.-)M-SD-[M]-M+SD(-max.), rounded to the nearest  $0.1 \,\mu\text{m}$ , where 'min.' and 'max.' are the extreme values recorded, M the arithmetric mean and SD the corresponding standard deviation. Measurements of other characters represent extreme values. HPTLC was performed according to standard methods (Arup et al. 1993; Orange et al. 2001). Photographs were taken with a Nikon Coolpix 4500, adapted to the dissection microscope.

Nuclear rDNA ITS1-5.8S-ITS2 sequences from a number of *Aspicilia* spp. (Table 1) were assembled and processed using the phylogenetic network program SplitsTree4 (Huson & Bryant 2006). Of the three identical new sequences of *A. berntii* produced, one was included in the analysis (Table 1). Extractions of DNA, amplifications and alignment were carried out according to methods described in Nordin *et al.* (2007). In the program SplitsTree4 the neighbour-net option (Bryant & Moulton 2004) with default settings was used for network construction. A bootstrap analysis, as implemented in the program, of 1000 replicates was also carried out.

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Species	Origin	Voucher*	Genbank Accession Number
A. aquatica Körb.	Sweden	Hermansson 11467	EU057896
A. berntii A. Nordin, Tibell & Owe-Larss.	Norway	Nordin 6392	EU502747
A. caesiocinerea (Nyl. ex. Malbr.) Arnold	Sweden	Tibell 22612	EU057897
A. calcarea (L.) Mudd	Sweden	Nordin 5888	EU057898
A. cinerea (L.) Körb.	Sweden	Hermansson 13275	EU057899
A. contorta (Hoffm.) Kremp.	Sweden	Nordin 5895	EU057900
A. coronata (A. Massal.) Anzi	Sweden	Lättman 13 v 2006	EU057901
A. cuprea Owe-Larss. & A. Nordin	USA	Owe-Larsson 9112	EU057902
A. cupreogrisea (Th. Fr.) Hue	Sweden	Nordin 6046	EU057903
A. cyanescens Owe-Larss. & A. Nordin	USA	Owe-Larsson 9151	EU057904
A. desertorum (Kremp.) Mereschk.	USA	Owe-Larsson 8770	EU057905
A. dudinensis (H. Magn.) Oxner	Sweden	Nordin 6036	EU057906
A. epiglypta (Norrl. ex Nyl.) Hue	Sweden	Nordin 6303	EU057907
A. gibbosa (Ach.) Körb.	Sweden	Nordin 5878	EU057908
A. indissimilis (H. Magn.) Räsänen	Sweden	Nordin 5943	EU057909
A. laevata (Ach.) Arnold	Sweden	Tibell 23659	EU057910
A. leprosescens (Sandst.) Hav.	Sweden	Nordin 5906	EU057911
A. mashiginensis (Zahlbr.) Oxner	Sweden	Nordin 5790	EU057912
A. mastrucata (Wahlenb.) Th. Fr.	Norway	Nordin 5708	EU057913
A. rivulicola (H. Magn.) Räsänen	Sweden	Nordin 5957	EU057922
A. permutata (Zahlbr.) Clauzade & Rondon	Sweden	Nordin 5980	EU057930
A. sp.	Sweden	Nordin 5952	EU057933
A. supertegens Arnold	Sweden	Svensson 190	EU057934
A. verrucigera Hue	Sweden	Tibell 22669	EU057939
A. verruculosa Kremp.	Norway	Owe-Larsson 9007	EU057940
A. zonata (Ach.) R. Sant.	Sweden	Nordin 5486	EU057944
Thamnolia subuliformis (Ehrh.) W. L. Culb.	New Zealand	<i>OTA</i> -59464	AY961605

TABLE 1. Sequence and voucher information for Aspicilia species used in phylogenetic analysis. New sequence in bold

\*All voucher specimens except *Thamnolia 'subuliformis'* [= *Thamnolia vermicularis* var. *subuliformis* (Ehrh.) Schaer] are deposited in UPS.

#### **Results and Discussion**

The network analysis resulted in 87 splits, in the graph displayed as parallel edges separating the fractions (Fig. 1), where the length of the edges is proportional to the weight of the splits. Aspicilia berntii appears as closely related to A. verrucigera Hue (Bootstrap Support value 100%). Both species belong to the group containing the type species of Aspicilia, A. cinerea (L.) Körb. (BS 70). This group is separated from the A. aquatica Körb.-A. leprosescens (Sandst.) Hav. group (BS 100) and the A. rivulicola (H. Magn.) Räsänen-A. simoënsis Räsänen group (BS 61). The split uniting A. berntii with the A. rivulicola-A. simoënsis group has low support (BS 24). Well-supported subgroups are A. rivulicola-A. sp. 1 (BS 94), A. leprosescens-A. calcarea (L.) Mudd (BS 85) and A.

*rivulicola–A. permutata* (Zahlbr.) Clauzade & Rondon (BS 81). In the alignment the ITS sequence of *A. berntii* differed from the other species by an indel (CTAGC) at the position 112–116. It differed from *A. verrucigera* in a further 19 bases.

The three major groups agree with those of the parsimony analysis of data set 1 in Nordin *et al.* (2007), but in the present analysis the *A. aquatica–A. leprosescens* group is strongly supported, contrary to the low support in the parsimony analysis. With the outgroup species used in the parsimony analysis (*Thamnolia vermicularis* var. *subuliformis*) added to the analysis (graph not shown), the support for the groups was lower, but the split separating the *A. aquatica–A. leprosescens* group and the outgroup from the other species had strong

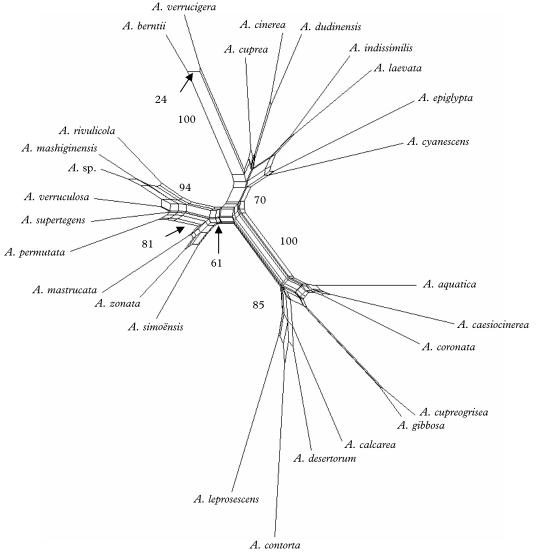


FIG. 1. Graph resulting from a neighbour-net analysis implemented in the program SplitsTree4. The splits are displayed as parallel edges. The length of the edges is proportional to the weight of the splits. Bootstrap support values (BS) are indicated at edges mentioned in the discussion. The bootstrap values for the other edges are low.

support (BS 95.9). Otherwise the relationships between the groups and between the species in the groups remained the same. The position of *A. berntii* in the *A. cinerea*group and the close relationship with *A. verrucigera* was also confirmed by a parsimony analysis corresponding to that of Nordin *et al.* (2007). Thus it can be concluded that, as judged from ITS-data, *A.*  berntii belongs to the core-group of Aspicilia and is most closely related to A. verrucigera. These two species are also morphologically similar (Table 2). Material of A. berntii from Norway was, for instance, mistaken for A. verrucigera by A. H. Magnusson, although he included both species (sub Lecanora verrucigera and L. mastoidea) in his study of the 'Aspicilia gibbosa group' (Magnusson 1939).

	A. berntii	A. verrucigera	A. dudinensis
Thallus colour	blackish grey	dark grey to brownish	grey to brownish grey
Thallus shape	verrucose	cracked-areolate	areolate
Apothecia	substipitate	$\pm$ immersed	$\pm$ sessile
Thalline exciple	elevated	$\pm$ level with disc	$\pm$ level with disc
Disc	epruinose	sometimes pruinose	sometimes pruinose
Spore length* ( µm)	18.1-[21.9]-28.3	14.7-[18.5]-21.5	12.4-[16.5]-20.3
Spore width* ( µm)	10.2-[13.1]-15.8	7.9-[10.6]-13.6	6.9-[9.6]-12.4
Conidium length <sup>+</sup> (µm)	14.7-22.6	11.3-19.2	$18 \cdot 1 - 24 \cdot 8$

TABLE 2. Aspicilia berntii compared with A. verrucigera and A. dudinensis

\*min.-[arithmetric mean]-max.; †min.-max.

#### The Species

# Aspicilia berntii A. Nordin, Tibell & Owe-Larss. nom. nov.

Lecanora mastoidea Lynge, Meddel. Grönland 118(8): 130 (1937).—Aspicilia mastoidea (Lynge) Creveld, Biblioth. Lichenol. 17: 270 (1981), nom. illeg., non Aspicilia mastoidea (Wedd.) Maheu & A. Gillet, Lich. Est. Corse: 50 (1926).—Aspicilia mastoidea (Lynge) J. W. Thomson in Egan, Bryologist 90: 163 (1987), nom. illeg., non Aspicilia mastoidea (Wedd.) Maheu & A. Gillet, op. cit. (1926); type: 'Grönland, Godthaab, 11–12.6.1871, Th. M. Fries' (S-L4633—lectotypus, designated here, UPS—isolectotypus).

(Fig. 2A)

*Thallus* almost black to dark or medium grey, sometimes with a brownish tinge, shiny or matt, verrucose-areolate, forming extensive patches. *Areoles* widely dispersed to almost contiguous, sometimes aggregated, convex, usually constricted at the base and often substipitate, sometimes slightly flattened laterally, up to 0.9 mm diam. *Cortex* paraplectenchymatous, 14–35  $\mu$ m thick, upper part dark brown, lower part pale, cells irregularly rounded, 3–7  $\mu$ m diam., epinecral layer absent or thin, up to 6  $\mu$ m thick. *Hypothallus* well-developed, black to dark brown, usually fimbriate at the margin. *Photobiont* trebouxoid.

Apothecia when young immersed in small verruciform areoles, later sessile to substipitate, scattered to densly crowded, 0·3– 1·1 mm diam. *Thalline exciple* distinctly elevated, rarely level with disc, usually

smooth and regularly rounded but sometimes slightly crenulate and/or lobed, concolorous with thallus, up to 0.2 mm thick. Disc black, smooth, concave to plane, epruinose. Proper exciple well-developed, in upper part to 80 µm broad (but usually thinner) and with brown,  $\pm$  globose cells. Epihymenium blackish green, N+ green, K+ brown. Hymenium hyaline, 115-145 µm tall. Paraphyses sparingly branched and anastomosing, predominantly moniliform, with  $3-5 \pm$  globose apical cells, up to 5 µm wide (in K). Asci clavate, with distinctly thickened apex, I - , 8-spored,  $87-130 \times 17-35 \,\mu m$ . Ascospores hyaline, broadly ellipsoid,  $(18 \cdot 1 -)$  $20.0 - [21.9] - 23.8(-28.3) \times (10.2 - )11.8 [13\cdot1]-14\cdot3(-15\cdot8) \ \mu m \ (n=100)$ . Subhymenial layers to 100 µm, indistinctly delimited from cellular medulla rich in oil droplets below the apothecia.

*Pycnidia* globose to slightly elongated or pyriform, 0.1-0.2 mm diam., occurring singly or a few together. *Walls* pigmented around the ostiole, pale in lower part. *Conidiophores* simple or branched. *Conidiogenous cells* to  $7 \times 1.5 \,\mu\text{m}$ , tapering at apices. *Conidia* filiform, straight to slightly and irregularly bent, (14.7-)15.9-[17.6]-19.2 $(-22.6) \times 0.8 \,\mu\text{m}$  (n=56).

Chemistry. Thallus K+ yellow, KC – , C – , Pd+ orange, containing stictic acid (major) and  $\pm$  norstictic acid (trace). Caesiocinerea-green (Meyer & Printzen 2000) present in epihymenium.

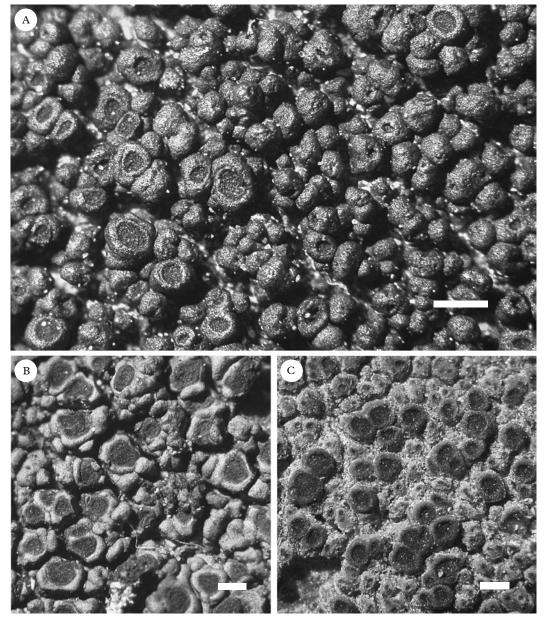


FIG. 2. Aspicilia species, habitus. A, A. berntii (Owe-Larsson 9553, UPS); B, A. verucigera (Degelius 10.VI.1941, UPS); C, A. dudinensis (Nordin 5963, UPS). Scales: A-C=0.5 mm.

*Etymology.* The new epithet refers to Lynge's first name Bernt. His second name has earlier been used in *Lecanora (Aspicilia) lyngei* Zahlbr., a name with uncertain application.

Distribution and ecology. Originally Aspicilia berntii was described from Nuuk ('Godthaab') in south-western Greenland but has also been reported (as A. mastoidea) from the north-eastern (Alstrup et al. 2000), southern (Hansen 2006) and eastern (Hansen 2002) parts. The material studied by us originates from southern Greenland. Apparently the species is widespread and probably rather common in Greenland. According to Thomson (1997) it also occurs in north-eastern Canada and Siberia, and in O there is a collection from Svalbard (not seen by us). Here it is also reported from Nordland in Norway. It has not been possible to confirm an earlier report from Norway of a specimen lacking apothecia (Creveld 1981).

According to the reports of Alstrup *et al.* (2000) and Hansen (2002, 2006) *Aspicilia berntii* grows on basalt and sandstone as well as on charnokitic, gneissic rocks and pure quartz. At the localities in southern Greenland it was collected on basalt, sandstone and metamorphic siliceous rocks and grew on both vertical rock walls and more or less horizontal surfaces on exposed rock outcrops, boulders and loose stones on the ground. At the locality at Ofotenfjorden in Norway it was found growing abundantly on steep to slightly sloping siliceous rocks on the shore.

Notes. Aspicilia berntii is primarily recognized by its dark, mainly verrucose thallus and the substipitate apothecia, that have a distinctly elevated thalline margin and an epruinose disc. The presence of stictic acid distinguishes it from the bulk of Aspicilia species which lack secondary substances. Other species in the A. cinerea group with stictic acid that might be mistaken for A. berntii are A. verrucigera and A. dudinensis. Aspicilia verrucigera has a dark grey to brownish, but generally lighter, crackedareolate thallus and mainly immersed apothecia with a less prominent thalline margin and often pruinose disc. Further the spores are smaller and the conidia slightly shorter. In A. dudinensis the thallus is lighter in colour, often with a brownish tinge, and the apothecia usually sessile with the thalline margin level with the disc, and the disc is often pruinose. The spores are smaller but the conidia distinctly longer (Table 2).

Additional specimens examined (all in UPS). Greenland: Narsarsuaq, rock outcrops just SE of the hostel close to the airport, alt. 45 m, 61°09.901'N, 45°24.335'W, 2005, Owe-Larsson 9525; N part of Qagssiarsuk, c. 4 km W of Narsarsuaq, alt. 40-50 m, 61°09.396'N, 45°31.014'W, 2005, Owe-Larsson 9546; c. 1 km N of Qagssiarsuk, c. 4 km W of Narsarsuaq, alt. 60-100 m, 61°09.521'N, 45°31.241'W, 2005, Owe-Larsson 9553, 9555; Blomsterdalen, c. 3.5 km NE of Narsarsuaq airport, alt. 80 m, 61°11.219'N, 45°21.554'W, 2005, Owe-Larsson 9564; Mt. Sutuluaqqap Qaqqaa, upper part of the NW slope, c. 1 km SE of Narsarsuaq, alt. 280-290 m, 61°09.426'N, 45°23.868'W, 2005, Owe-Larsson 9623, 9624; Mt. Sutuluaqqap Qaqqaa, northern part of the top plateau, c. 1.5 km SE of Narsarsuaq, alt. 368 m, 61°09.329'N, 45°23.958'W, 2005, Owe-Larsson 9637.-Norway: Nordland: Lofoten, Lødingen, 1919, Magnusson 3578; Lofoten, Moskensøy, Sørvagen, 1 viii 1937, Degelius; Lofoten, Svolvær, 3 viii 1937; Shore of Ofotenfjorden 2 km S of Bjerkvik, alt. 5 m, 68°31'35.9"N 17°34'08.7" E (RT90), 2007, Nordin 6392, 6394.

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