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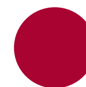

Phylogeny of Himantormia - an Antarctic genus in the Parmeliaceae (lichenized ascomycetes).

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Phylogeny of *Himantormia* – an Antarctic genus in the Parmeliaceae (lichenized ascomycetes)

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Abstract: The phylogeny of the Patagonian-Antarctic genus *Himantormia* is investigated with nuclear ITS rDNA sequences in PAUP. The monotypic genus *Nimisia* is synonymized with *Himantormia*, and the new combination *H. deusta* is proposed. The secondary chemistry was investigated using HPLC; protocetraric, stictic, succinprotocetraric and confumarprotocetraric acids were detected in *H. deusta* for the first time, and alectorialic, hypoalectorialic and decarboxyhypo-alectorialic acids are reported as new for *H. lugubris.*

Introduction

The monotypic genus *Nimisia* Kärnefelt & A. Thell was described from Tierra del Fuego based on material collected by P. L. Nimis and J. Poelt (KÄRNEFELT & THELL 1993) and the new species, *N. fuegiae* Kärnefelt & A. Thell, was distributed in Vězda's exsiccata a few years later. However, during a project to incorporate the lichen collections of Michigan State University in a data base, older samples of *Nimisia fuegiae* collected by H. Imshaug were found. The combination *Cetraria deusta* (Hook. f. & Taylor) Imshaug remained unpublished, because Imshaug was convinced that it did not belong in *Cetraria s. str.* (FRYDAY 2005). The basionym *Parmelia enteromorpha* Ach. *var. deusta* Hook. f. & Taylor, had earlier been recombined and raised to species level as *Hypogymnia deusta* (Hook f. & Taylor) C. W. Dodge (DODGE 1965). FRYDAY (2005) corrected the name to *Nimisia deusta* (Hook. f.) Fryday and presented new data on its morphology, secondary chemistry and phytogeography of this species. In particular, he found that the outer surface was not entirely black but had

pale brown patches close to the base, that fumarprotocetraric acid was present in the upper medulla, and, that the distribution range included the Cape Horn and Falkland Islands (FRYDAY 2005: 317, map).*Esslingeriana idahoensis* (Essl.) Hale & M. J. Lai and *Himantormia lugubris* (Hue) I. M. Lamb are two other species in the Parmeliaceae which have been compared to *Nimisia* because of similarities in their cortical anatomy, i.e. paraplechtenchyma composed of large dark pigmented cells with comparatively large lumina. *Himantormia lugubris* showed additional similarities with *Nimisia*, such as the pachydermatous medulla and its Antarctic distribution (KÄRNEFELT & THELL 1993). The similar distribution and the absence of lichenan in the cell-walls made *Himantormia* the strongest candidate among possibly related genera (COMMON 1991; FRIDAY 2005). Three additional species with alternative northern hemisphere distributions, *Arctocetraria nigricascens* (Nyl.) Kärnefelt & A. Thell, *Cornicularia normoerica* (Gunnerus) Du Rietz, and *Kaernefeltia merrillii* (Du Rietz) A. Thell & Goward, were discussed as possibly being related to *Nimisia*, because of similarities in overall morphology (KÄRNEFELT & THELL 1993),.

Extensive phylogenic analyses of the Parmeliaceae, based on DNA-sequences have yet to include *Nimisia,* because of the absence of fresh material, and the fact that the presumably related species mentioned above did not show close affinities with one another (THELL et al. 2004). Fortunately, fresh material of *Nimisia deusta* needed for a DNA-investigation was collected by U. Søchting and L. Sancho during an excursion to the Navarino Island, Southern Chile in 2005.

Material and methods

Selected material

The 30 samples, representing 27 species, were selected to represent a range of Parmeliaceae focussing on species presumed to be related to *Nimisia deusta*. The majority of the Parmeliaceae has been divided into four large monophyletic groups, the cetrarioid, parmotremoid, usneoid and xanthoparmelioid lichens (OHMURA 2002; THELL et al. 2002; 2004; BLANCO et al. 2004, 2005). These groups are represented in the analysis. Five species possibly related to *Nimisia* were also included (KÄRNEFELT & THELL 1993; FRYDAY 2005). Finally, a blast(n) search was performed, matching the new *Nimisia* sequence in the GenBank, to detect species with high percentage of identities. Five of the sequences are new, whereas 25 where downloaded from the GenBank at www.ncbi.nlm.nih.gov (Table 1). A blast(n) search was performed matching the new *N. deusta* sequence with sequences present in the GenBank (ALTSCHUL et al. 1997).

Two cetrarioid species, *Nephromopsis pseudocomplicata* and *N. stracheyi* were selected as the outgroup. The monophyletic group of cetrarioid lichens, investigated in several DNA-analyses, is presumed not closely related to *Nimisia*.

DNA analysis

Extraction: Employing MagAttract 96 DNA Plant Extraction Kit from Qiagen (08/2003), samples were rigorously shaken in a vortex machine in 300 ml extraction buffer incorporating one steel beed into each microtube; otherwise, the enclosed protocol for manual DNA purification was followed.

Table 1. Selected specimens with extraction numbers, sample-IDs and GenBank accession numbers.

Species	Extr	Sample-ID	GenB acc
Alectoria ochroleuca	976	AUSTRIA. Ötztal, 2001-08-11, Feuerer & Thell (HBG)	AF451735*
Arctocetraria andrejevii	1364	GREENLAND. Hansen exs. 836 (LD 1001631)	DQ004575*
Arctocetraria nigricascens	793	CANADA. Melville Isl., Westberg 1614 (LD)	AF254628*
Bryocaulon divergens	1914	SWEDEN. Härjedalen, Arup L02258 (LD)	DQ395287
Bryoria fuscescens	920	ITALY. Trentino-Alto Ad., Feuerer & Thell 62282 (HBG)	AF451736*
Bryoria sp.	1932	GREENLAND. Narsarsuaq, Thell & Feuerer (LD 1039864)	DQ395288
Coelopogon abraxas	1209	CHILE. 2001-11-11, Feuerer (HBG)	AY251414*
Cornicularia normoerica	930	ITALY. Trentino-Alto Ad., 2001, Feuerer & Thell (HBG)	AY251416*
Dactylina arctica	160	CANADA. Alberta, Miao (TDI 300)	AF115760*
Esslingeriana idahoensis	146	CANADA. B. C., Goward 961348 (UBC)	AF227513*
Himantormia deusta	1910	CHILE. Region XII Magallanes, Søchting 10257 (C)	DQ395290
Himantormia lugubris	1231	ANTARCTICA. South Shetland Islands, Søchting 7609 (C)	AF251421*
Himantormia lugubris	----	ANTARCTICA. Kim 05013	DQ219309*
Hypogymnia physodes	16	SWEDEN. Skåne, Thell 9605 (LD 1029842)	AF141368*
Kaernefeltia californica	1703	U. S. A. Oregon, McCune 27703 (LD 1045103)	DQ004571*
Kaernefeltia merrillii	190	CANADA. British Columbia, Thell 9698 (LD 1089087)	AF072230*
Kaernefeltia merrillii	1916	SPAIN. Madrid, El Berrueco, Thell et al. (LD 1044393)	DQ395291

Kaernefeltia merrillii	1918	SPAIN. Madrid, El Berrueco, Thell et al. (LD 1038537)	DQ395292
Letharielia intricata	----	SPAIN. Chanary Islands, Kroken & Taylor (2000)	AF297742*
Melanelia stygia	922	ITALY. Trentino-Alto Ad., Feuerer & Thell 64247 (HBG)	AF451775*
Menegazzia terebrata	916	GERMANY. Bayern, 2001, Feuerer & Thell (HBG)	AY251430*
Nephromopsis pseudocomplicata	907	CHINA. Sichuan, Obermayer 8276a (GZU)	AF404131*
Nephromopsis stracheyi	606	BHUTAN. Thimpu, Søchting 8095 (LD)	AF451785*
Nodobryoria abbreviata	45	CANADA. B. C., Thell & Veer 9645b (LD 1192360)	AF116177*
Parmelia saxatilis	518	CHILE. Region XII Magallanes, Feuerer 29542 (HBG)	AF410672*
Parmotrema chinense	918	GERMANY. Bavaria, 2001, Feuerer & Thell (HBG)	AF451749*
Pseudephebe minuscula	931	ITALY. Trentino-Alto Ad., 2001, Feuerer & Thell (HBG)	AY251446*
Pseudephebe pubescens	884	CHILE. Valdivia, Feuerer 29546 (HBG)	AF451738*
Pseudevernia furfuracea	781	FINLAND. Regio Ab., Feuerer & Thell (LD 1057234)	AF451768*
Usnea florida	840	SWEDEN. Skåne, Thell 0011 (TUR)	AF451739*
Xanthoparmelia pulla	935	AUSTRIA. Tyrol, Feuerer & Thell 64210 (HBG)	AF451747*

Amplification: 20 µl PCR-reactions were prepared to amplify the nuclear ITS1-5.8S-ITS2 ribosomal DNA region. The primers ITS1F (GARDES & BRUNS 1993) and ITS4 (WHITE 1990) were used . Each reaction included 5 µl water, 2 µl buffer, 8 µl nucleotides, 1 µl of the extracted DNA, and 0.1 µl of the DNA polymerase. The PCR programme described by EKMAN (2001) was employed: after a 2 min. hold at 94°C, six cycles followed with denaturation at 94° for 60 sec., annealing at 62°-56°C for 60 sec. (decreasing 1° per cycle), and extension at 72°C for 105 sec., followed by 34 cycles with denaturation at 94° for 30 sec., annealing at 56°C for 30 sec. and extension at 72°C for 105 sec.; finally, a 10 min. hold at 72°C was performed before the PCR-products were cooled to 4°C.

Purification and sequencing: The PCR-products were cleaned with PCR clean-uop NucleoFast 96 PCR Purification Kit from Macherey-Nagel as per the enclosed user manual (2002/3/Rev. 01. p.12). 50 µl TE buffer was applied to each sample prior to the standard procedure for purification of PCR-products under vacuum, which was followed, except for the second, optional, washing step, with purified water. The purified samples were collected in 50 µl water and the amount of DNA was measured in an Eppendorf BioPhotometer. The concentrations differed between 18-54 ng/µl. The required amount of DNA for sequencing, 1 ng/base pair, was dried for 1 hr at 65°C. Finally, the DNA-fragments were sent to Macrogen to be sequenced, again using the primers ITS1F and ITS4.

Phylogeny: The phylogenetic analyses of the manually aligned ITS sequences were done with PAUP version 4.0b (SWOFFORD 1998). Trees were calculated using the general heuristic search option. Gaps were treated as missing characters. Bootstrap analyses with 1000 replicates were performed using the same settings. Support values of 50 or above are marked in the consensus tree (Fig. 1).

Anatomy

Most data concerning the morphology and anatomy was obtained from earlier publications on *Himantormia* (LAMB 1964; KÄRNEFELT & THELL 1993; FRYDAY 2005), but some new anatomical sections were made to complement this data. Thalli and apothecia were sectioned (15 µm thick) using a Kryomate, Leitz freezing microtome and conserved in lactophenol cotton-blue. The sections were studied with a Zeiss Axioscope light microscope.

Secondary chemistry

Secondary compounds were detected by means of high performance liquid chromatography (ELIX et al. 2003).

Results

Blast(n) search

Performing a blast(n) search (ALTSCHUL et al. 1997) with the *Nimisia deusta* sequence in the GenBank revealed the highest percentage of matches together with two *Himantormia lugubris* sequences, DQ219309 and AF251421, showing differences at c. 20 and 21 positions (98%) resepec-tively.

Phylogeny analysis

The aligned matrix was composed of 523 characters, of which 162 were parsimony informative. The heuristic search resulted in a single most parsimonious tree, 757 steps long, CI=0.4848, RI=0.4855, RC=0.2354 (Fig. 1). The bootstrap analysis, using the same preferences as in the heuristic search, showed maximun support for a common clade for *Nimisia deusta* and *Himantormia lugubris*.

Himantormia I. M. Lamb

Brit. Antarctic Surv. Sci. Rep. **38:** 17-18 (1964). – **Typus generis:** *Himantormia lugubris* (Hue) I. M. Lamb.

Syn.: *Nimisia* Kärnefelt & A. Thell, *Lichenologist* **25:** 370–371 (1993).

Fig. 1. The phylogenetic position of *Himantormia* in the Parmeliaceae. The single most parsimonious tree length = 757, CI = 0.4848, RI = 0.4855, RC = 0.2354.Paup 4.0b. Bootstrap support values of 50 or above from 1000 replicates are indicated at the branches.

The hitherto monotypic genus *Himantormia* is now proposed to comprise two species, both of which have a Patagonian-Antarctic distribution. Thus a new combination is proposed for the sole species previously accomodated in the genus *Nimisia*. Both species have previously been described in detail (LAMB 1964, KÄRNEFELT & THELL 1993), and data presented below mainly concerns new discoveries in phytogeography and secondary chemistry.

Himantormia deusta (Hook. f.) A. Thell & Søchting, *comb. nov.* (Fig. 2)

Bas.: *Parmelia enteromorpha* var. *deusta* Hook. f., *Flora Antarctica* (*Bot. Antarct. Voyage Erebus & Terror* 1): 532 (1847). – *Hypogymnia deusta* (Hook. f.) C. W. Dodge, *Trans. Amer. Microscop. Soc.* **84:** 506 (1965). - *Cetraria deusta* (Hook. f.) Imshaug, in sched. – **Typus**: CHILE. Tierra del Fuego, near Cape Horn, c. 56°30'S, 60°W, Mt Foster, ix 1842, *J. D. Hooker*, *Voyage Erebus & Terror* (E – lectotype, Friday, *Lichenologist* **34:** 315, 2005).

Syn.: *Nimisia fuegiae* Kärnefelt & A. Thell, *Lichenologist* **25:** 370–371 (1993). – **Typus:** ARGENTINA. Isla Grande de Tierra del Fuego, Dept. Ushuaia, Sierra Alvear, just in front of Hosteria Tierra Major, on steeply inclined faces of granite rocks in the subalpine belts, 1987, *P. L. Nimis & M. Tretiach* (LD – holotype; TSB, DUKE – isotypes; Vezda: Lichenes Rariores Exsiccati, no 144; TSB 11131; leg. *P. L. Nimis, M. Codogno & M. Tretiach*).

The spermatia of *H. deusta* are almost rod-shaped but slightly thicker in the middle, hence the reason for classifying them as oblong citriform by KÄRNEFELT & THELL (1993); however, their size and shape are in fact very similar to the bacillariform spermatia of *H. lugubris* (Table 2).

Although this species was originally reported to have no chemical compounds (KÄRNEFELT & THELL 1993), FRYDAY (2005) detected fumarprotocetraric acid whereas fumarprotocetraric (major), protocetraric (minor), stictic (trace), succinprotocetraric (trace) and confumarprotocetraric (trace) acids have been detected by HPLC in the present study, none of which occur in *H. lugubris*.

Himantormia lugubris (Hue) I. M. Lamb (Fig. 3)

Brit. Antarctic Survey Sci. Rept **38:** 18–23 (1964). – **Bas.:** *Ramalina lugubris* Hue, *Exp. Antarct. Franc.*: 34-36 (1915). – **Typus:** Antarctica. Ile Petermann, peu common sur les rochers du sommet de l'île, altitude 120 mètres, XXe excursion, no 286, le 14 mars 1909, *Hue* (PC – holotype).

The excellent description of *H. lugubris* made by HUE (1915) and LAMB (1964) is completed by the detection of additional secondary compounds using modern HPLC-equipment. However, the anatomy of both *Himantormia* species was reinvestigated, the distinguishing characters of which are presented in Table 2.

LAMB (1964) reported small quantities of atranorin and barbatolic acid as an unidentified b-orcinol derivative and HUNECK & FOLLMANN (1966) detected usnic acid in addition to these two secondary compounds. Alectorialic (major), hypoalectorialic (minor), barbatolic (trace) and decarboxyhypoalectorialic (trace) acids have been detected by HPLC in the present study.

Fig. 2. *Himantormia* deusta, x 5, *US 10257* (C).

Fig. 3. *Himantormia lugubris*, x 5, *US 7609* (C).

Discussion

In a recent survey, *Himantormia lugubris* was found to belong to a section of the Parmeliaceae composed mainly of isolated genera and small groups of genera without any evident relationships with one another (THELL et al. 2002; 2004). Blast(n) searches for matching sequences with four additional two-species genera in the analysis show similar amount of matches between the species compared with *Himantormia*, where 96% matches were detected, In *Arctocetraria* Kärnefelt & A. Thell, the blast(n) search resulted in approximately the same rate of matches between the species, *A. andrejevii* and *A. nigricascens*, although the bootstrap support of 82 % was weaker (Fig. 1). Of the genera *Kaernefeltia* A. Thell & Goward and *Pseudephebe* Choisy, the latter has the highest amount of matches, 98%, when performing a blast(n) search and, in similarity with *Himantormia*, a 100% support in the bootstrap analysis. *Kaernefeltia* has 97-98% of matches. Notably, the variation between western American and Spanish populations of the disjunct species *K. merrilli* were larger than that between *K. merrillii* and the second species of the genus, *K. californica.* The genus has a moderate bootstrap support of 73% in the phylogeny analysis (Fig 1, cf. THELL et al. 2005: Figs. 1-3).

Table 2. A comparison of diverging characters of *Himantormia deusta* and *H. lugubris* (Figs. 2-3).

Character	*Himantormia deusta*	*Himantormia lugubris*
Lobes, shape and height	strap-shaped, slightly canaliculate, to 1 cm	awl-shaped to strap-shaped, to 4 cm
Apothecia, position	laminal	laminal, rarely terminal
Cortex, cell size	10–15 µm	4–7 µm
Medulla, structure	dense	lax, with dense portions
Medulla, hyphae diam.	7–9 µm	4–7 µm
Medulla, hyphae lumina diam.	0.1–0.5 µm	1–2 µm
Algae, position	below pycnida and apothecia	below cortex in light pigmented patches of areolae or verrucae
Spermatia, shape	almost bacilliform, slightly thicker in the middle	bacilliform
Spermatia, size	8–10 x 1–1.5 µm	9–10 x 0.8–1 µm
Secondary compounds	fumarprotocetraric acid (major), protocetraric acid (minor), stictic acid (trace), succinproto-cetraric acid (trace) and confumarprotocetraric acid (trace)	alectorialic acid (major), hypoalectorialic acid (minor), barbatolic acid (trace) and decarboxy-hypoalectorialc acid (trace) by HPLC. Atranorin (reported by LAMB (1964), minor).

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