

Comprehensive Summaries of Uppsala Dissertations  
from the Faculty of Science and Technology 931



# Phylogenetic Studies in *Usnea* (Parmeliaceae) and Allied Genera

BY

KRISTINA ARTICUS



ACTA UNIVERSITATIS UPSALIENSIS  
UPPSALA 2004

Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, EBC, Uppsala, Friday, February 27, 2004 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

#### **Abstract**

Articus, K. 2004. Phylogenetic Studies in *Usnea* (Parmeliaceae) and Allied Genera. Acta Universitatis Upsaliensis. *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 931. 120 pp. Uppsala. ISBN 91-554-5860-2

This thesis deals with the phylogeny of the lichen genus *Usnea* (Parmeliaceae, Ascomycetes). The relationships and the morphological variation among *Usnea* species has been studied, as well as the relationship of *Usnea* to allied genera.

Two species, *U. florida* and *U. subfloridana*, which earlier were regarded to form two separate species have been synonymized. In an analysis based on sequence data these two taxa formed a monophyletic group of intermixed specimens. *Usnea florida* and *U. subfloridana* have earlier been regarded to form a species pair, but the species pairs concept cannot be applied in this case.

The morphological characters traditionally used for species recognition of a number of European *Usnea* species have been analyzed regarding their reliability. The evolution and distribution of the morphological characters was studied in relation to a phylogeny based on sequence data. Most characters proved to be homoplastic in relation to the phylogeny. Few characters were consistent in a clade, and the same character could be inconsistent in another clade. Therefore a combination of several characters is recommended for species recognition.

The relationship of *Neuropogon* to *Usnea* was investigated based on sequence data. *Neuropogon* showed to be closely related to *Usnea* subg. *Usnea*. The subgenera *Eumitria* and *Dolichousnea* formed the sister group to the clade comprising subg. *Usnea* and *Neuropogon*. *Usnea* is paraphyletic in this investigation. *Eumitria* is treated as a genus and the subgenus *Dolichousnea* is elevated to generic rank.

The position of *Usnea*, *Neuropogon*, *Eumitria*, and *Dolichousnea* in the family Parmeliaceae was investigated based on a phylogeny obtained by sequence data. *Protousnea* probably forms the sister group to the clade of *Usnea*, *Neuropogon*, *Eumitria*, and *Dolichousnea*. Several monophyletic groups in the family Parmeliaceae were identified.

*Keywords:* *Usnea*, *Neuropogon*, *Dolichousnea*, *Eumitria*, *Protousnea*, Parmeliaceae, phylogeny, taxonomy, species pairs, morphology, ITS-LSU,  $\beta$ -tubulin, mtSSU

*Kristina Articus, Department of Evolutionary Biology, Department of Systematic Botany, Norbyv. 18D, Uppsala University, SE-75236 Uppsala, Sweden*

© Kristina Articus 2004

ISSN 1104-232X

ISBN 91-554-5860-2

urn:nbn:se:uu:diva-3974 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-3974>)

## Papers included in this thesis

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Kristina Articus, Jan-Eric Mattsson, Leif Tibell, Martin Grube and Mats Wedin (2002) Ribosomal DNA and  $\beta$ -tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. *Mycological research* 4: 412-418.
- II Kristina Articus, Jan-Eric Mattsson, Mats Wedin and Leif Tibell (submitted) Morphology and sequence data - conflict and concordance in a phylogeny of some European *Usnea* species.
- III Kristina Articus (submitted) *Neuropogon* and the phylogeny of *Usnea* s. lat. (*Parmeliaceae*, Ascomycetes).
- IV Jan-Eric Mattsson, Kristina Articus, Elisabeth Wiklund & Mats Wedin (manuscript) The monophyletic groups in the *Parmeliaceae*.

Published and accepted papers are reproduced with the publishers' kind permission.

In **Paper I** and **II** KA is responsible for the morphological and molecular data, the phylogenetic analyses and had the main responsibility for the writing. In **Paper IV** KA is responsible for some of the sequences, the Bayesian inference analysis, and contributed to the writing.

**Important note:** **Paper III** of this thesis is a manuscript that contains new combinations. This paper has been submitted for publication elsewhere, and in order to make clear that the names of these new combinations are not validly published in this thesis the basionyms necessary according to the International Code of Botanical Nomenclature are omitted.

# Contents

Introduction.....	1
Lichens .....	2
Systematics.....	2
The species pair concept .....	3
<i>Usnea</i> .....	3
Aims of the thesis .....	4
Comments on Material and Methods.....	5
Molecular studies .....	5
Ribosomal rDNA .....	5
$\beta$ -tubulin.....	6
Mitochondrial SSU .....	6
Analyses .....	7
Parsimony .....	7
Bayesian inference.....	7
Are there species pairs in <i>Usnea</i> ? .....	9
<i>Usnea florida</i> / <i>U. subfloridana</i> .....	9
<i>Usnea rigida</i> / <i>U. barbata</i> .....	9
Molecular data do not support a species pair relationship .....	10
Evaluation of morphological characters in some European <i>Usnea</i> species ..	13
Background .....	13
Morphology in relation to a molecular phylogeny .....	14
Conclusions .....	14
The relationship of <i>Usnea</i> to other usneoid lichens in the family	
Parmeliaceae .....	17
Historical outline .....	17
<i>Usnea</i> , <i>Neuropogon</i> , and <i>Protousnea</i> .....	18
<i>Evernia</i> , <i>Letharia</i> , and <i>Lethariella</i> .....	18
Conclusions .....	18
Svensk sammanfattning (Swedish summary) .....	22
Acknowledgements – tack!.....	24
References.....	27

# Introduction

The lichen genus *Usnea* (Parmeliaceae) has a world-wide distribution and contains about 600 species (Hawksworth et al. 1995). *Usnea* is by most lichenologists regarded to be taxonomically difficult (Clerc 1998) and is in need of revision. The species are morphologically extremely variable and ecophenotypes of the same species may look radically different. This has led to the description of phenotypes as species and to a chaotic situation in taxonomy. Significant progress in the understanding of *Usnea* has been made during the past few years. Polymorphic species have been accepted and a polythetic concept, where a species is recognized based on several characters, has been applied more often (Clerc 1998).

There are many taxonomic uncertainties at the species level, and also higher taxonomic relationships are unclear. Several subgenera have been recognized within *Usnea* and different delimitations have been discussed (e.g. Motyka 1936-38, Ohmura 2001, 2002). The relationship of *Usnea* to other, closely related genera has been debated for decades (e.g. Motyka 1936-38, Krog 1976, 1982, Walker 1985). The relationship to *Neuropogon*, a morphologically similar group of Arctic and Antarctic lichens, has been especially unclear.

*Usnea* was earlier placed in the family Usneaceae (Eschweiler 1824) together with other lichen genera with a similar appearance. Nowadays *Usnea* is thought to belong to one of the largest lichen families, Parmeliaceae, because of similarities in the fruiting bodies (Henssen & Jahns 1974). The position of *Usnea* in this family has been discussed and a hypothesis of the phylogenetic relationship among *Usnea* and allied genera based on morphological data has been proposed (e.g. Krog 1976, 1982).

This thesis is the first to investigate morphological variation within and among *Usnea* species, the generic circumscription of *Usnea*, and its relationships to other genera in Parmeliaceae, in relation to phylogenies based on molecular sequence data.

## Lichens

Lichens are symbiotic systems consisting of a fungus (mycobiont) and algae and/or cyanobacteria (photobiont) which together form a tightly integrated structure (thallus). Fungi need external sources of carbohydrates, which in the lichens are provided by the photobiont. If cyanobacteria are part of the symbiosis the lichen also has an internal supply of nitrogen. The photobiont is believed to profit from the relationship by being protected against dehydration, UV radiation, and mechanical destruction.

The fungal partner reproduces sexually by the production of spores and is propagated asexually in many different ways, often together with the photobiont. The photobiont does not reproduce sexually within the lichen symbiosis. Both components can disperse together by fragmentation or by small packages, called soredia and isidia, containing both fungal hyphae and algae.

The lichen symbiosis has proven to be an extremely successful life form. Lichens manage to survive in different kinds of climates and grow on a large variety of substrates (bark, rocks, soil, but also on metal, concrete, and other artificial materials). Lichenization is common and it is estimated that more than 20% of all fungal species are lichenized (Hawksworth et al. 1995).

## Systematics

Systematics is the study of the identification, taxonomy, and nomenclature of organisms, including the classification of living things with regard to their natural relationships and the study of variation and the evolution of the taxa (Lawrence 1995). The aim of taxonomy is to recognize and name organisms and taxa. Systematics is a basic discipline and forms the platform for most other areas of biology.

Phylogenetic studies attempt to reconstruct the evolutionary history, which can be represented as phylogenetic trees. Natural groups share a common ancestor and derived characters; they form monophyletic groups. The closest relatives in a phylogeny are called sister taxa.

Lichens, as mentioned above, are symbiotic systems consisting of at least two organisms. Their names refer to that of the mycobiont. Lichenized and non-lichenized fungi are found in closely related groups, particularly among the ascomycetes. Lichenization has evolved several times independently and lichenized fungi do not form a natural group (Gargas et al. 1995).

## The species pair concept

The species pair concept is commonly used in lichenology. It explains the relationship of morphologically similar species, which differ in their dispersal strategy. One partner in the species pair reproduces sexually, while the other reproduces asexually (via soredia). The fertile taxa are referred to as primary species with a connected sterile, secondary species. Poelt (1970) regarded the sterile taxa to be descendants of fertile taxa. The species pair concept in lichenology has been extensively debated, and has been criticized, by e.g. Tehler (1982) and Myllys et al. (2001).

## *Usnea*

*Usnea* (Parmeliaceae) was first named by Dillenius in *Historia muscorum* (1742). Members of *Usnea* (including the subgenera *Usnea*, *Dolichousnea*, and *Eumitria*), are recognized by their fruticose (“hair-like”) thallus having a radial organization. In the center of the branches we find an axis consisting of a cartilaginous strand of longitudinal hyphae. The central strand is surrounded by a medulla and an outer cortex. The fruiting bodies (apothecia) are disc-shaped and pale green, producing unicellular spores. The cortex contains usnic acid, which is responsible for the yellowish greenish color. The associated photobionts are unicellular green algae of the genus *Trebouxia*. Most *Usnea* species grow on trees or wood, but some occur on rocks. With thalli up to several decimeters in length *Usnea* belongs to the largest lichens.

*Usnea* species have attracted recent attention as bioindicators, since they are vulnerable to air pollution and environmental changes. In Europe several *Usnea* species have been ‘red-listed’, since their existence is regarded as threatened (Clerc, Scheidegger & Ammann 1992; Arup et al. 1997; Thor & Arvidsson 1999; Türk & Hafellner 1999). *Usnea* is an important food source for deer and reindeer. The chemical compound usnic acid has been used for its antibiotic effect. In Asia *Usnea* is harvested on a large scale for commercial production of skin medicine (Brodo et al. 2001). In traditional medicine *Usnea* extracted in vodka may be used against a sore throat. *Usnea* has traditionally been used by Indians in northern America in handicraft (Brodo et al. 2001). In Europe the typical Christmas decoration “tinsel” probably is inspired by the long and pendant *Dolichousnea longissima*. Westring (1804), in his investigation on lichen dyes, experimented with *Usnea* and obtained many pretty colors. His drawings and color samples are illustrated on the cover of this thesis.

## Aims of the thesis

This thesis is based on morphological and molecular sequence data. It aims at clarifying the diagnostic reliability and evolution of morphological features in *Usnea* (**Paper I and II**), to analyze the relationship of *Usnea* s. lat. and *Neuropogon* (**Paper III**), and to investigate the position of *Usnea* in the family Parmeliaceae (**Paper IV**). These aspects have here for the first time been investigated in relation to phylogenies based on sequence data.



# Comments on Material and Methods

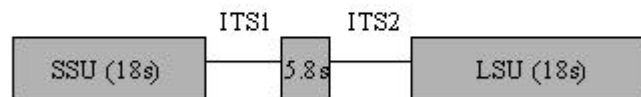
## Molecular studies

It is easiest to obtain sequences from fresh *Usnea* material (not older than five years). DNA from the central axis has been extracted to minimize the risk of contamination with lichen parasites.

Three different gene regions have been analyzed in this thesis. Since the mutation rate of different genes varies it is important to choose a gene appropriate for the study purpose. A more variable gene region should be studied when comparing closely related taxa and a less variable region for studies of less closely related taxa.

## Ribosomal rDNA

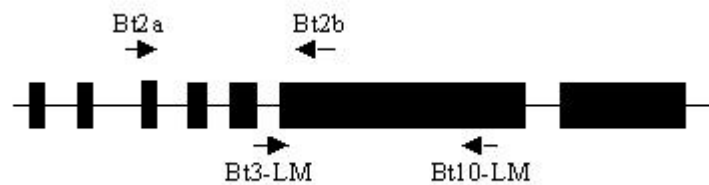
The ribosomal DNA is a region in the nuclear DNA coding for the ribosomes. It is the most commonly studied gene region in lichen phylogenetics. Ribosomes are cell organelles that are responsible for protein synthesis and they consist of a small and a large subunit. The gene encoding the small subunit (SSU), the large subunit (LSU), and the 5.8S region form a cluster (Fig. 1). The genes are interrupted by two internal transcribed spacers (ITS1 and ITS2), which are not translated into proteins and are more variable. The ribosomal DNA consists of numerous tandem copies. ITS1, ITS2, 5.8S, and part of the LSU regions have been studied in all four papers.



*Figure 1* The structure of the nuclear ribosomal DNA repeat. Boxes indicate genes, lines internal spacers.

## $\beta$ -tubulin

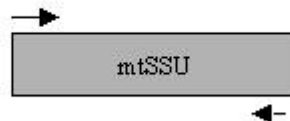
Microtubules, consisting of  $\alpha$ - and  $\beta$ -tubulin molecules, are involved in movements, e.g. the chromosomes movements during mitosis and meiosis.  $\beta$ -tubulin is highly conserved, but the introns within the gene are variable and useful for phylogenetic studies. Here,  $\beta$ -tubulin has been sequenced partially and two different regions were studied. In **Paper I, II, and III** the region between Bt2a and Bt2b (Glass & Donaldsson 1995) and in **Paper IV** the region between Bt3-LM and Bt10-LM (Myllys et al. 2001) have been studied.



*Figure 2* The structure of  $\beta$ -tubulin. Boxes indicate exons, lines introns. Primer sites are marked with arrows.

## Mitochondrial SSU

Mitochondria are cell organelles which play an important role in respiration. They have their own DNA, coding for some of the mitochondrial structural proteins and enzymes involved in respiration. Mitochondria also have ribosomes (Deacon 1984). The genes coding for these are located in the mitochondrial genome. The genes for the small subunit (SSU) and the large subunit (LSU) do not form a cluster as in the nuclear ribosomal DNA, and they exist in few copies only. In **Paper IV** the region of the small subunit of the mitochondrial ribosomes (MtSSU) has been studied.



*Figure 3* Structure of the mitochondrial ribosomal small subunit. Arrows indicate primer sites.

## Analyses

There are different approaches to reconstruct phylogenetic trees from the observed data. Since the “real history” of evolution is unknown and we only can sample a part of the total diversity, the reconstructions can only provide us with estimates of the phylogenetic history. In this thesis two approaches to phylogenetic inference have been applied, parsimony analysis and Bayesian inference.

### Parsimony

In maximum parsimony the number of changes in different evolutionary scenarios is counted and the solution with minimal numbers of changes is preferred (**Paper I, III, and IV**). There might be conflicts in the data, e.g., a character might have evolved twice independently or might have been lost in some taxa. These changes count each one step. There are several methods to test the support for different groups in the phylogeny, in this thesis jackknifing was applied (Farris et al. 1997) as implemented in the program PAUP\* 4.0 beta 10 (Swofford 2002). The parsimony settings used in this thesis were: steepest descent off, collapse branches if minimum length is 0, and gaps were treated as missing data. Heuristic search settings were: 1000 random addition sequence replicates with start from random trees, TBR branch swap, multiple trees were saved, and uninformative characters were excluded from the analysis.

### Bayesian inference

Bayesian inference is a statistical approach for inferring phylogenies which requires an explicit choice of a model for evolutionary change (e.g. Rannala & Yang 1996, Huelsenbeck et al. 2001, Huelsenbeck & Ronquist 2001). Posterior probabilities for phylogenies are calculated based on a priori assumptions of parameters (branch length, substitution model, etc.) and a given data set (an alignment of sequence data). MrBayes (Huelsenbeck & Ronquist 2003) uses Markov chain Monte Carlo (MCMC) to approximate the posterior probabilities. If a tree is sampled often by the Markov chain it has a high posterior probability. To prevent that the analysis gets stuck on a local optimum parallel chains investigate the tree landscape at the same time and may exchange information. Trees from the initial phase of the chain, the burn-in period, are removed and the trees sampled from the phase where the parameter values have stabilized are used to calculate the posterior probabilities. The Bayesian inference approach gives us the possibility to vary freely assumptions about the evolutionary process.

The priors for the tree and other parameters of the model were used as implemented by default in MrBayes version 3.0 beta 4 (Huelsenbeck &

Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The models for nucleotide substitutions were selected prior to the MCMC. The model of evolution was chosen in accordance with an analysis by MrModeltest Version 1.1b (Nylander, 2002) in conjunction with PAUP\* (Swofford, 2002). The Akaike Information criterion (AIC; Akaike, 1973) suggested the usage of the general time reversal model of evolution (GTR) with a gamma distribution of substitution rates (G) and a proportion of invariant sites (I) (**Paper II, III, and IV**). The Markov chain was run for 2,000,000 generations and every 100<sup>th</sup> tree was sampled. The tree samples of the burn-in phase (200 trees) were discarded. Groups with posterior probabilities above 0.60 are presented.

## Are there species pairs in *Usnea*?

### *Usnea florida*/*U. subfloridana*

In *Usnea* only a few cases of species pairs have been suggested. Poelt (1970) considered the richly fertile and shrubby species *Usnea florida* to be a primary species without mentioning a connected secondary species. Later (Clerc 1984, 1987b, 1998, Purvis et al. 1992) *Usnea subfloridana* was suggested to be a secondary species derived from *U. florida*. *Usnea florida* and *U. subfloridana* share many similarities, e.g. a shrubby to subpendent thallus with a black base and with many cylindrical papillae. *Usnea florida* traditionally is richly fertile and lacks soralia, while *U. subfloridana* in the traditional sense has soralia and sometimes also apothecia. *U. florida* is a much rarer species and occurs only in rather special localities, e.g. old deciduous trees in areas with high atmospheric humidity (Clerc 1998). *Usnea subfloridana* is much more common and can be found on a wide variety of substrates in many different habitats. The occurrence of apothecia and soralia has been considered to be of great taxonomic importance and *U. florida* and *U. subfloridana* have for a long time been treated as distinct species. Specimens of *U. subfloridana* developing both soralia and apothecia have been thought to be rare. However, during field work in Sweden I found several richly fertile specimens, which also produce soralia.

### *Usnea rigida*/*U. barbata*

*Usnea barbata* and *U. rigida* have also been considered to form a species pair (Halonen et al. 1998). However, their status as species pair is less widely accepted than in the case of *U. florida* and *U. subfloridana*. *Usnea rigida* is richly fertile and does not produce soralia, while *U. barbata* mainly disperses by soredia and only occasionally produces apothecia. The morphology of *U. barbata* is quite variable and some specimens, although being sorediate, are quite similar to *U. rigida*. *U. rigida* occurs in the central part of Europe, *U. barbata* in northern and central Europe. The study of old herbarium material of *U. barbata* in Scandinavia showed that *U. barbata* was more often fertile in the 19<sup>th</sup> century than it is today. Those fertile specimens very much resemble contemporary collections of *U. rigida* from central Europe.

## Molecular data do not support a species pair relationship

The field observations of *U. florida* and *U. subfloridana* and the studies of herbarium material of *U. barbata* and *U. rigida* made me wonder, if features associated with the different dispersal strategies in *Usnea* species really could be used in species delimitation. The molecular approach opened new possibilities for investigating these putative species pairs.

*U. florida* and *U. subfloridana* were studied in **Paper I**. Specimens with different dispersal strategies (apothecia only, soralia only, and specimens with both apothecia and soralia) were investigated. The phylogeny of the combined data set of the rDNA and  $\beta$ -tubulin data (Fig. 4) showed that *U. florida* and *U. subfloridana* formed one monophyletic group of intermixed specimens. They also formed a monophyletic group of intermixed specimens in the phylogenies of the separate data sets. The data of **Paper I** do not support a species pair relation of *U. florida* and *U. subfloridana*.

*Usnea barbata* and *U. rigida* were investigated in **Paper II**. The phylogeny showed that *U. rigida* and *U. barbata* form a monophyletic group of intermixed specimens (Fig. 5). Only a few fresh specimens were available for sequencing, however, and the conclusions need further corroboration.

From the data now available, it seems that Poelt's ideas about species pairs are not applicable for *Usnea florida/U. subfloridana* and *U. rigida/U. barbata*. One possible explanation for the occurrence of clades including both fertile and sorediate specimens might be that the individuals have an optional ability to express these features. However, which features that actually are expressed might depend on climatic or other environmental conditions. This hypothesis has to be further investigated in a different type of study involving many more individuals.

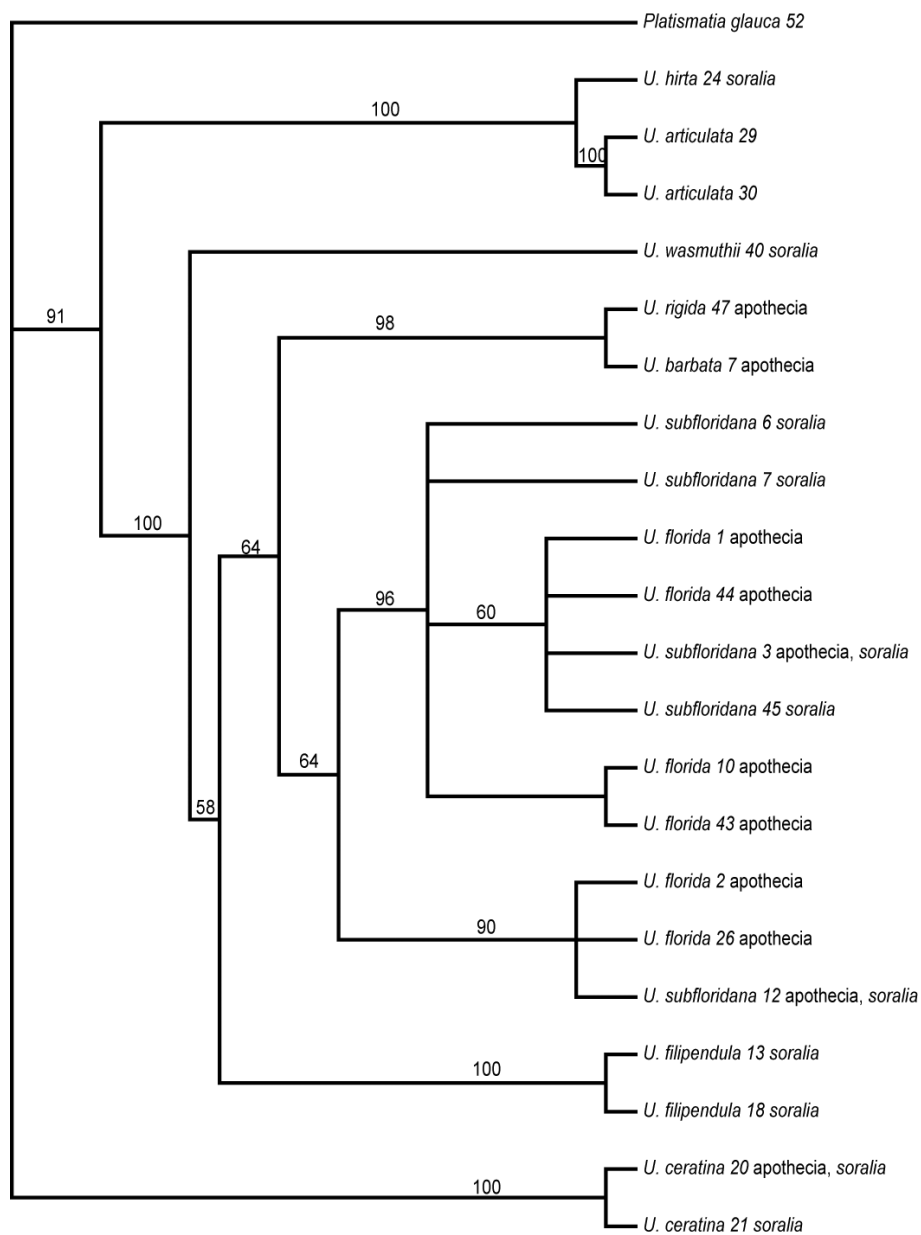


Figure 4 The strict consensus tree of the combined  $\beta$  tubulin and ITS-LSU data matrix for analyzing the relationship of *U. florida* and *U. subfloridana*. Jack-knife values are indicated above branches. The occurrence of apothecia and soralia is marked.

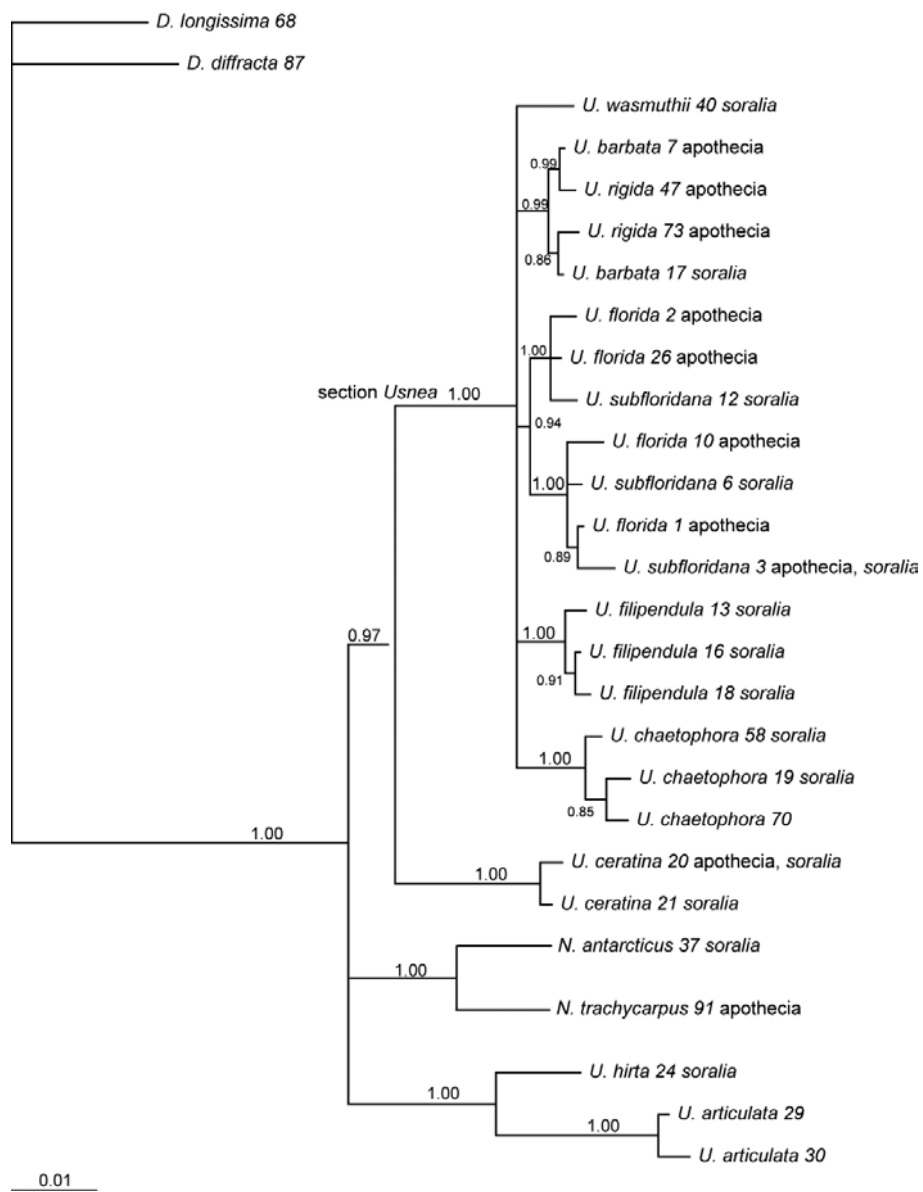


Figure 5 Phylogeny of the combined  $\beta$  tubulin and ITS-LSU data matrix of various *Usnea* species, obtained by Bayesian inference (PP values indicated). The occurrence of apothecia and soralia is indicated.



# Evaluation of morphological characters in some European *Usnea* species

## Background

The species recognition in *Usnea* is considered to be difficult due to the plasticity of the species. This has led to a situation where many lichenologists avoid collecting and identifying *Usnea* material. Since many *Usnea* species in Europe are regarded as threatened, a correct identification of the species and an improvement of the understanding of *Usnea* is required. Progress has been made in recent years due to careful taxonomic studies (e.g. Clerc 1984, 1987a and b, 1992; Halonen et al. 1998; Halonen et al. 1999; Clerc & Herrera-Campos 1997; Herrera-Campos, Clerc & Nash 1998; Articus 1998, 2000; Articus et al. 2002; Ohmura 2001).

In **Paper II** morphological characters which are thought to be less prone to ecophenotypic variation (Clerc 1998) were investigated. A phylogeny of some commonly accepted *Usnea* species of the temperate areas of the Northern hemisphere was obtained by using sequence data. The species investigated were: *Usnea articulata*, *U. barbata*, *U. ceratina*, *U. chaetophora*, *U. filipendula*, *U. florida* (including *U. subfloridana*), *U. hirta*, *U. rigida*, and *U. wasmuthii*. Some of these species are used as indicators for forest continuity. Some *Neuropogon* species were included, since a recent study (**Paper III**) showed *Neuropogon* and *Usnea* (the former subg. *Usnea*) to be closely related and their relationship has not yet been resolved. The morphological characters important for the recognition of these species were mapped onto the phylogeny. The purpose was to study the distribution of these morphological characters in the phylogeny (Fig. 5), to see if these characters are consistent within clades, and to search for synapomorphies for supraspecific clades.

## Morphology in relation to a molecular phylogeny

The morphology and the occurrence of soralia is the feature which has most frequently been used for species recognition in *Usnea* (Clerc 1998). Many species in this analysis have soralia, and different soralia types occur (Fig. 6). These types have evolved several times independently and different types may be represented within a clade. Soralia proved to be highly homoplastic in *Usnea* and *Neuropogon*.

The occurrence of isidiomorphs is also a commonly used character. Isidiomorphs are a plesiomorphic feature in the ingroup, and has been lost several times. The occurrence is consistent in *U. articulata*, *U. ceratina*, and *U. filipendula*, but may vary in other species.

Fibrils are a plesiomorphic character in relation to the phylogeny, and they have subsequently been lost and modified. Fibrils attached at a right angle are characteristic for *U. filipendula*, *U. ceratina*, and the clade containing *U. florida* and *U. subfloridana*,

Papillae are a characteristic feature for section *Usnea* and have an independent origin in *Neuropogon trachycarpus*. However, several losses occurred in sect. *Usnea*.

The pigmentation of the base is a relatively stable character in this phylogeny, although it could not be assessed for some species. A black base is a characteristic for section *Usnea* and its sister group *U. ceratina*. A pale base developed twice.

The relative size of cortex, medulla, and axis is a character commonly applied in recent studies (Clerc 1998). A thin cortex is a plesiomorphic character state in the ingroup. A thin medulla is a plesiomorphic character state which several times has developed into a thick medulla. A relatively thick axis is a plesiomorphic character state in the phylogeny.

The occurrence of apothecia is in relation to the phylogeny characteristic for the clades of *U. barbata/U. rigida* and *U. subfloridana/U. florida*. However, losses occurred in these clades.

The anisotomic-dichotomous branching pattern is a plesiomorphic character in *Usnea* and *Neuropogon*. The branching pattern varies in some species.

## Conclusions

This study illustrates the complicated situation in *Usnea*, where character expressions vary within a species and features may be reliable for species recognition in one species and unreliable in another. From these results it can be concluded that only a combination of characters can be used for identifying a species and that different characters have to be used for circumscribing different species. The character optimizations showed that

most features were homoplastic in relation to the phylogeny. Few species had autapomorphies and several characters varied within a species (or a clade of closely related species). The high amount of homoplasy may indicate that the expression of a character might be effected by ecological conditions and that a species has the ability to optionally express several different character states. Nevertheless some features characterize some of the clades. Papillae seem to be a synapomorphy for sect. *Usnea*, although they have been lost in some specimens. A black base is a synapomorphy for a clade comprising sect. *Usnea* and *U. ceratina*, although also here with some reversals. An anisotomic-dichotomous branching is a synapomorphy for all the studied species except for *Dolichousnea*, although a parallel evolution of isotomic branching has occurred in some clades.

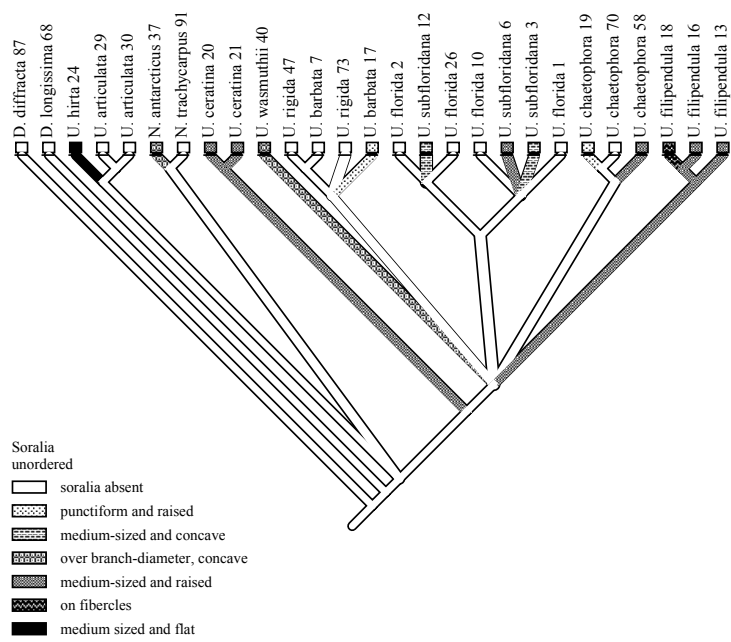


Figure 6 The character optimization for soralia occurrence and shape

# The relationship of *Usnea* to other usneoid lichens in the family Parmeliaceae

## Historical outline

Hypotheses about the delimitation of *Usnea* and its relationship to other genera have varied over time. Eschweiler (1824) described the family Usneaceae and included mainly fruticose lichens (*Usnea*, *Alectoria*, *Evernia*, *Cetraria*, *Ramalina*). Later, *Usnea* was regarded as belonging to Parmeliaceae (Henssen & Jahns 1974) because of similarities in the cupular exipulum of apothecia. Hale (1983) argued for a separate family Usneaceae consisting of several fruticose lichens (*Usnea*, *Dactylina*, *Evernia*, *Letharia*, and *Neuropogon*). In 1986 Eriksson & Hawksworth proposed a classification in which Usneaceae Eschw. together with other former families were included in the Parmeliaceae. Nowadays the latter classification is widely accepted.

Motyka (1936-38) suggested a classification of the genus *Usnea*, in which all fruticose lichens with an inner, cartilaginous tissue are included. He recognized six subgenera: *Euusnea*, *Protousnea*, *Lethariella*, *Chlorea*, *Neuropogon*, and *Eumitria*. Later *Protousnea* and *Lethariella* (including *Chlorea*) were raised to generic rank (Krog 1976). A recent study based on ITS sequence data (Ohmura 2002) supported the subgenera *Eumitria* and *Usnea*, and revealed a new subgenus, *Dolichousnea*. Ohmura concluded that *Usnea* contains at least three taxa at subgeneric level, *Usnea*, *Eumitria*, and *Dolichousnea*. *Neuropogon* was not included in this study and the position remained unclear. It has been heavily debated if *Neuropogon* should be classified as a subgenus of *Usnea* (e.g. Motyka 1936-38; Lamb 1964; Walker 1985) or if it deserves the rank of a genus (Nees & Flotow 1835; Krog 1976, 1982; Galloway 1985, 1992; Malcolm & Galloway 1997; Stevens 1999).

Krog (1982) proposed a hypothesis of the relationship of the 'usneoid' genera (genera similar to *Usnea* in their thallus structure). In this hypothesis (Fig. 7) *Neuropogon* and *Usnea* are sister groups. *Protousnea* and *Evernia* form together the sister to the clade comprising *Neuropogon* and *Usnea*. Finally, *Letharia* and *Lethariella* form the sister group to the other usneoid genera.

## *Usnea*, *Neuropogon*, and *Protousnea*

The relationship of *Usnea* (comprising *Usnea*, *Eumitria*, and *Dolichousnea*) to the genera *Neuropogon*, *Protousnea*, *Lethariella*, *Letharia*, and *Evernia* was investigated in relation to a phylogeny based on sequence data (**Paper III** and **IV**). *Neuropogon* formed a well-supported monophyletic group nested within *Usnea* (Fig. 8). *Usnea* subg. *Usnea*, represented by three clades, formed a polytomy together with *Neuropogon*. *Dolichousnea* and *Eumitria* were the sister taxa to the clade comprising *Usnea* and *Neuropogon*. *Protousnea* formed the sister group to the clade containing *Neuropogon*, *Usnea*, *Eumitria*, and *Dolichousnea* (Fig. 9). Here the group consisting of *Protousnea*, *Dolichousnea*, *Eumitria*, *Neuropogon*, and *Usnea* is considered as usneoid.

## *Evernia*, *Letharia*, and *Lethariella*

In contrast to Krog's hypothesis (1982) *Evernia* and *Protousnea* did not group together in the molecular investigation. *Evernia* appeared to be paraphyletic in the parsimony analysis (not shown), where *Evernia mesomorpha* and *E. divaricata* formed a clade together with *Allantoparmelia alpicola*. *Evernia prunastri* grouped together with *Letharia* and *Lethariella*. In the Bayesian analysis *Evernia* is monophyletic, however, a relationship to neither *Protousnea* nor *Lethariella* and *Letharia* is supported. *Lethariella* and *Letharia* formed a sister group as expected by Krog, but they did not come out as sister to the usneoid lichens.

## Conclusions

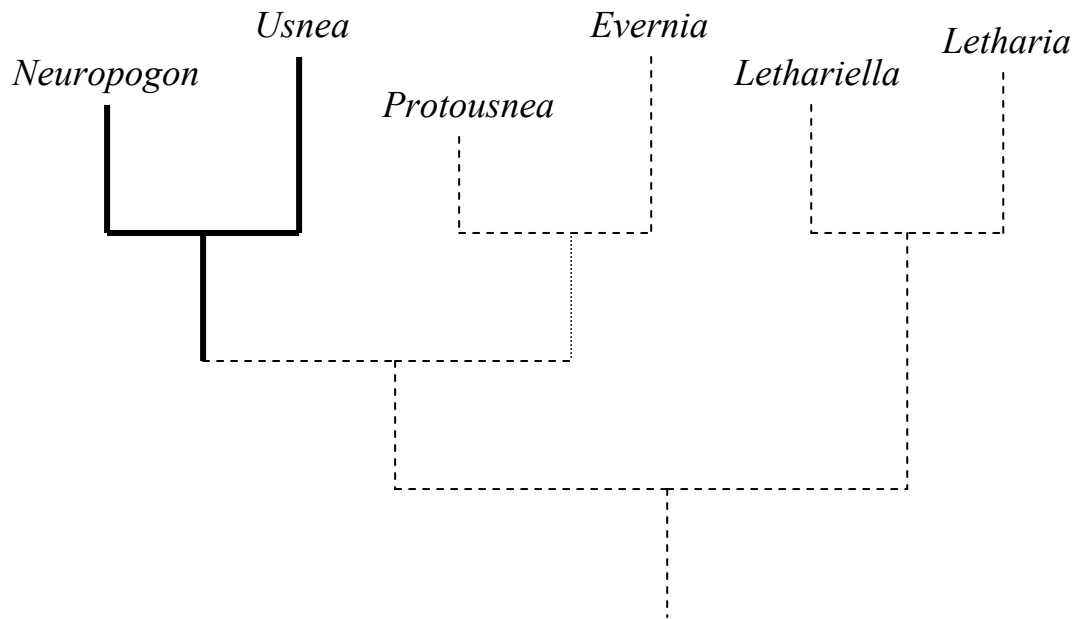
The investigations confirmed a close relationship of *Usnea*, *Neuropogon*, *Eumitria*, *Dolichousnea*, and *Protousnea*. A relation to other fruticose genera as for example *Evernia*, *Lethariella*, and *Letharia* was not supported.

*Neuropogon* formed a well-supported monophyletic group, which is morphologically easily recognizable. Therefore the generic rank is preferred here. As a consequence *Eumitria* is recognized as a genus and *Dolichousnea* is elevated to generic rank. *Usnea* s. strict. is unresolved in the combined data set ( $\beta$  tubulin, ITS-LSU) and paraphyletic in the ITS data set.

Since the Parmeliaceae is monophyletic here, with poorly resolved basal nodes, a delimitation into smaller families, e.g. Usneaceae, is not preferred.

The usneoid genera are recognized by the following features: *Usnea* has a solid central axis and pale apothecial disc, *Eumitria* has a hollow axis and pale apothecial disc, *Dolichousnea* has a solid axis, annular pseudocyphellae, and pale apothecial disc, while *Neuropogon* has a solid

axis, a patchy black pigmentation, and dark and matt apothecial disc. *Protousnea* is characterised by dark and glossy apothecial disc and black pycnidia.



*Figure 7* The relationship of the usneoid genera according to Krog (1982). Black lines indicate most likely, dots more hypothetical relationships.

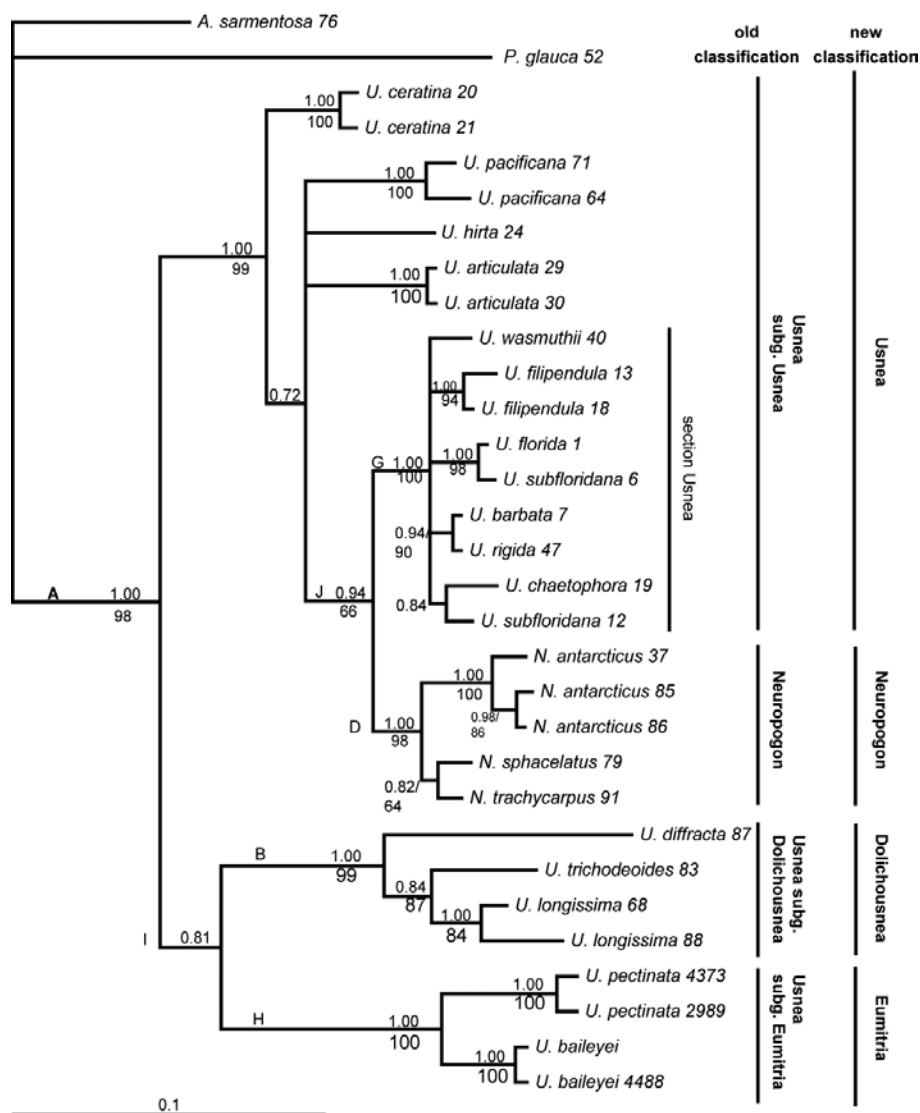


Figure 8 Phylogeny of the ITS data matrix of *Usnea* s. lat., obtained by Bayesian inference (PP above, j-values below branches). The old and new classifications of *Usnea* s. lat. are indicated on the side.



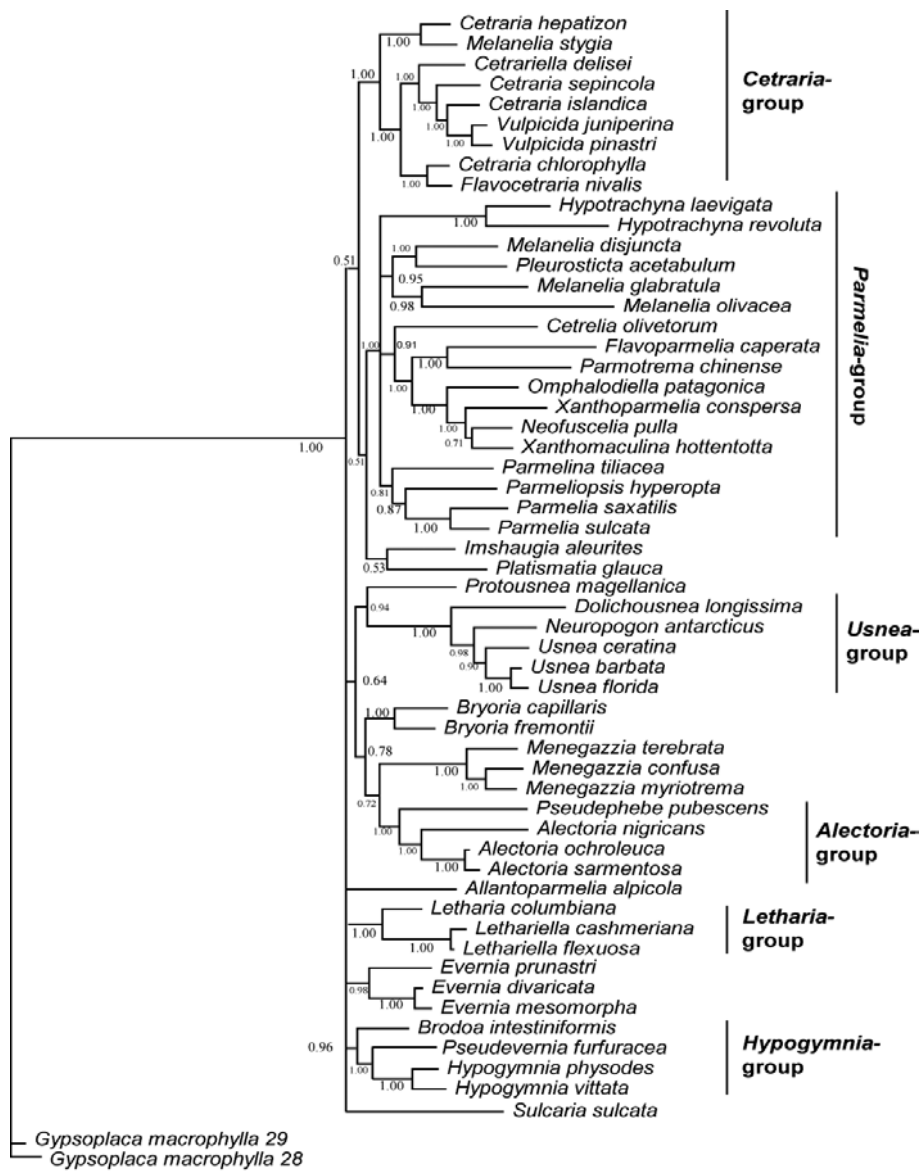


Figure 9 Phylogeny obtained by Bayesian inference of the combined  $\beta$  tubulin, mitochondrial SSU, and ITS-LSU data matrix of representatives of the Parmeliaceae (PP values indicated).

## Svensk sammanfattning (Swedish summary)

Denna avhandling handlar om skägglavssläktet *Usnea* (Parmeliaceae) och dess släktskapsförhållanden. Skägglavarna är utbredda över hela världen och det finns uppskattningsvis 600 arter i släktet (Hawksworth et al. 1995). Artavgränsningen anses problematisk, eftersom lavens utseende påverkas av den miljö den växer i. Detta har lett till stor förvirring kring artavgränsningen.

I **Bidrag I** och **II** försöker jag att utreda vilka morfologiska karaktärer som är pålitliga. Några karaktärer har ansetts vara viktiga och inte så lättpåverkade av miljön (Clerc 1998). Dessa karaktärer testade jag mot en fylogeni (släktträd) som bygger på molekylära data. Fördelningen av karaktärerna visade att de ofta är homoplastiska, d. v. s. de har uppkommit flera gånger oberoende av varandra. Några karaktärer förekommer konsekvent inom en grupp, men samma karaktär kan vara mer instabil inom en annan grupp. Slutsatsen är bland annat, att man alltid måste använda sig av en kombination av flera karaktärer för att identifiera en *Usnea*-art och olika karaktärsuppsättningar måste tillämpas för olika arter.

Karaktärer som har med spridning att göra, som soral (asexuell förökning) och fruktkroppar (sexuell förökning), har ansetts som viktiga för artavgränsningen. Detta har lett till en hypotes om artpar, där man antar att asexuella arters ursprung kan härledas till morfologiskt snarlika arter som sprider sig sexuellt. *Usnea florida*-*U. subfloridana* och *U. rigida*-*U. barbata* har betraktats som sådana artpar. Mina sekvensdata visar att dessa arter inte bildar artpar i klassisk bemärkelse. Skulle de vara artpar, så skulle varje art för sig bilda en egen grupp, men analyserna visar att arterna i de förmodade artparen bildar blandade grupper. Slutsatsen är att de undersökta arterna inte bildar artpar enligt artparhypotesen. Vidare finner jag det sannolikt att produktionen av soral eller fruktkroppar kan vara miljöbetingad och att samma art kan ha olika spridningsorgan.

I **Bidrag III** och **IV** analyseras släktskapsförhållanden mellan undersläkten av *Usnea* i vid bemärkelse och närstående släkten. *Usnea* innehåller undersläktena *Usnea*, *Eumitria* och *Dolichousnea*. Dessa undersläkten skiljer sig i vissa karaktärer. De flesta europeiska *Usnea*-arterna tillhör undersläktet *Usnea*. *Usnea longissima* (långskägg), som förekommer i Europa, tillhör dock *Dolichousnea*. *Eumitria* innehåller mest tropiska arter. Andra släkten liknar *Usnea* genom att ha en hårliknande bål och en mäg med en gummiaktig mittsträng. Ett av dessa släkten är

*Neuropogon*, som förekommer i arktiska och antarktiska områden och skiljer sig från *Usnea* i strikt bemärkelse genom en svart pigmentering. Man har misstänkt att *Neuropogon* och *Usnea* är mycket närstående.

I en analys av släktskapen mellan *Usnea* och *Neuropogon*, baserad på molekylära data, kunde jag visa att *Neuropogon* är mycket nära släkt med undersläktet *Usnea*. De bildar en grupp tillsammans och deras närmaste släktingar är *Eumitria* och *Dolichousnea*. Detta resultat ledde till en uppdelning av *Usnea* i vid bemärkelse i fyra släkten: *Usnea*, *Neuropogon*, *Eumitria* och *Dolichousnea*.

Ett annat släkte som har misstänkts vara nära släkt med *Usnea* är *Protousnea*. *Protousnea* förekommer i Sydamerika och skiljer sig genom en annorlunda färg på fruktkroppsskivan och förekomsten av svarta pyknid. I en stor molekylär analys där flera släkten av familjen Parmeliaceae ingår undersöktes bland annat om *Usnea*, *Eumitria*, *Dolichousnea* och *Neuropogon* är besläktade med *Protousnea*. Resultatet av analysen visade att *Protousnea* utgör systergruppen till de andra *Usnea*-liknande släktena. Inga andra släkten verkade stå nära de *Usnea*-liknande lavarna. Resultatet visade vidare att det finns stöd för uppdelningen av *Usnea* s. lat. i *Neuropogon*, *Eumitria*, och *Dolichousnea*.

## Acknowledgements – tack!

### **Nog finns det mål och mening i vår färd- men det är vägen, som är mödan värd (Karin Boye)**

Min tid vid Avdelningen för systematisk botanik och här i Sverige har varit väldigt fin och lärorik och jag vill tacka alla som har bidragit till detta!

Främst vill jag tackar min handledare Leif Tibell för att ge mig chansen till denna avhandlingen och mina biträdande handledare Mats Wedin och Jan-Eric Mattsson för era insatser. Alla mina handledare har visat stor engagemang i olika faser av min avhandling och jag tackar er för råd och otaliga synpunkter på manuskripten, handledning och stöd. Speciellt vill jag tackar Leif för att du öppnade dörren till lavvärlden för mig, Jan-Eric för allting jag lärde mig redan under exjobbet och Mats för den fina tiden på Natural History Museum i London.

Lavgruppen i Uppsala med Rolf Santesson, Roland Moberg, Leif Tibell, Anders Nordin, Sanja Savic, Rikke Reese Naesborg, och Christina Wedin, vill jag tackar för erat intresse för mitt arbete och synpunkter på manuskript.

På institutionen vill jag tackar lektorerna Kåre Bremer, Birgitta Bremer, Mats Thulin och Bengt Oxelman för allt jag lärde mig under kurser, exkursioner och seminarier och för den uppskattning jag har fått. Tack också för de synpunkter jag fick på avhandlingen.

Nahid Heidari - hjärtligt tack för hjälp på labb och med sekvenseringar, men främst för Ditt personliga engagemang. Ulla Hedenquist räddade mig många gånger från den tyska och svenska byråkratin och jag är mycket tacksamt för alla välstämplade intyg. Agneta Brandtberg-Falkman tackar jag för uppmuntran i min foto karriär!

En särskilt tack går till alla mina glada före detta och nuvarande doktorand kollegor och exjobbare! Det var underbart att uppleva en sådan bra gemenskap och en glad vardag är väl det bästa man kan få. Våra exkursioner, det glada livet på alg- och moss, doktorandfester, nattliga exkursioner på orangeriets tak, fikaraster, pubkvällar, diskussioner och samtal vägde upp allt slit. Speciella tack går till Annika för att vi kämpade oss genom det här så bra gemensamt; Dick för alla goda skratt; Elisabeth för alla bra samtal om livet; Hege för den fina vänskapen och allt roligt, speciellt under sambotiden; Henrik för den trevliga rumskompistiden; Isabel för

sambotid och alla trevliga historier om Galicien; Jesper - min vän från första stund på Systbot; Johan för alla goda råd angående MrBayes; Magnus för att du är rolig och glad; Maria för glada lunchdiskussioner; Per E. för Din glädje och min favorit grimas; Per K. för bra samtal; Rikke min nuvarande tålamodiga rumskompis; Sylvain for friendship, and Yonas for your happy smile. Tack till den doktorand som gjorde bordsplaceringen på alg- och moss festen och gav mig en underbar, belgisk bordskavaljer!

Från fytoteket vill jag särskilt tackar Barbro Lidfeldt-Rahm för alla trevliga samtal och Svengunnar Ryman för den givande gemensamma undervisningen. Roland Moberg tackar jag hjärtligt för allt stöd och uppmuntran under alla år och min tid som herbarieassistent.

Från Botaniska trädgården tackar jag Karin Martinsson för att ge mig den fina chansen att göra en utställning.

During my time as Ph.D. student I had the chance to work with different research groups. I would like to thank the lichen group in London at the Natural History Museum, with Peter James, William Purvis, Amanda Waterfield, and Pat Wolseley, for the wonderful time in 1999. The discussions and excursions were very valuable for me. I also would like to thank Christina James and Sally Henderson from the Botany Department for their friendship! Mats Wedins' family is warmly thanked for making my stay so pleasant.

Special memories are also connected with the lichen group in Graz, where I worked in 2000. Martin Grube is thanked for inviting me and his creative input into my work. Elisabeth Baloch and Asuncion Rios are thanked for taking care of me so well. A definitive highlight of my stay was the night in the Bermuda triangle!

Several visits in Umeå were very important for my work. I would like to thank the Umeå lichen group with Ove Eriksson, Mats Wedin, Katarina Winka, Elisabeth Wiklund, Åsa Nyberg, Anna Crewe, Heidi Döring, and Per Ihlen for a pleasant time and for their interest in my work. I have good memories from our Hemavan excursion with local guidance by Katarina! Elisabeth Wiklund is thanked for co-authorship.

I would like to thank the Swedish and International lichenologists who supplied me with *Usnea* collections and followed my work with interest. Several excursions with the Nordic Lichen Society, as well as international congresses belong to my favorite memories and they influenced my work very positively. I am very happy for all new friends.

Special thanks for numerous loans of material go to Jan-Olof Hermansson, Göran Thor, Liselott Skarp, and Johan Bergsten.

My work was sponsored by several scholarships. The following funds made fieldwork, laboratory work, conference visits, and my work in Uppsala possible: Helge Ax:son Johnson stiftelse, Kungliga Vetenskapsakademi (on several occasions), Regnell, Bjärka-Säby stiftelse, Svenska Institutet, Eliassons Fond, Gunnar Bergs stiftelse, Gertrud Thelins resestipendium, Large scale facility scholarship from the NHM in London, Anna Maria Freds Scholarship from Smålands nation, Karin och Axel Binning fond.

Förutom den vetenskapliga världen bidrog mina vänner i Uppsala till att jag trivdes så bra. Ett hjärtligt tack till Klemens & Lies, Carolin & Dave, Alex & Pilar, Lina & Andy, Lisa och Sandra. Tack för er vänskap!

Finska nationen stod de senaste åren för de flesta av mina fritidsaktiviteter och jag tackar er alla. En särskilt tack går till nationens hjärtat: Jörg, Therese, Matthis och Anne, för er otrolig gästvänlighet.

Under alla åren har jag haft så otroligt kul med världens kanske bästa, och definitivt roligaste studentorkester- Glasblåsarna! Tack så mycket för denna tid och alla fantastiska spelningar, t.ex. vår jättesuccé i Prag, och när vi fick Holland att hoppa!

Alexandro, Paula och Beatrice från Canyenge tango gruppen tackar jag för allt ni lärde oss om tango!

Meine lieben Freundinnen Maike, Erin, Ute, Ingrid, and Vera! Ohne Euch wäre Schul- und Studienzeit nicht das Gleiche gewesen!

Meinen Eltern und Geschwistern danke ich für Eure wunderbare Unterstützung in Form von unzähligen Briefen, Päckchen und emails! Vielen Dank für Eure Liebe.

Olivier, en stor tack till dig! Utan dig skulle jag inte hade klarat mig genom Sveriges kyliga sidor och all avhandlingsstress!

## References

- Akaike, H. 1973. *Information theory as an extension of the maximum likelihood principle*. In Second International Symposium on Information theory (Petrov, B. N., Csaki F., eds.) 267–281. Akademiai Kiado, Budapest.
- Articus, K. 1998. *Bartflechten (Usnea) in Südwestschweden. Morphologie, Taxonomie und Ökologie*. Julius-Maximilians-Universität Würzburg. Diplomarbeit.
- Articus, K. 2000. Artbestämning av långa hängande skägglavar (släktet *Usnea*) i Sverige [The long and pendant *Usnea* species in Sweden]. *Svensk Botanisk Tidskrift* 94: 81–97.
- Articus, K., Mattsson, J-E., Tibell, L., Grube, M. & Wedin, M. 2002. Ribosomal DNA and  $\beta$ -tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. *Mycological research* 106 (4):412–418.
- Arup, U., Ekman, S., Kärnefelt, I. & Mattsson, J-E. 1997. *Skyddsvärda Lavar i Sydvästra Sverige* [Red-listed lichens and changes in the lichen flora of South-western Sweden]. SBF-Förlaget. Lund.
- Brodo, I. M., Duran Sharnoff, S. & Sharnoff, S. 2001. *Lichens of North America*. Yale University Press. New Haven & London
- Clerc, P. 1987a. On the morphology of soralia in the genus *Usnea*. In *progress and problems in lichenology in the eighties* (E. Peveling, ed.) Bibliotheca Lichenologica 25: 99–102.
- Clerc, P. 1987b. Systematics of the *Usnea fragilesceus* aggregate and its distribution in Scandinavia. *Nordic Journal of Botany* 7: 479–495.
- Clerc, P. 1984. Contribution à la revision de la systématique des Usnées (*Ascomycotina*, *Usnea*) d'Europe. 1.– *Usnea florida* (L.) Wigg. emend. Clerc. *Cryptogamie, Bryologie et Lichénologie* 5: 333–360.
- Clerc, P. 1992. Some new or interesting species of the genus *Usnea* (lichenised Ascomycetes) in the British Isles. *Candollea* 47: 513–526.
- Clerc, P. 1998. Species concepts in the genus *Usnea* (lichenized ascomycetes). *Lichenologist* 30: 321–340.
- Clerc, P. & Herrera-Campos, M. A. 1997. Saxicolous species of *Usnea* subgenus *Usnea* (lichenized ascomycetes) in North America. *Bryologist* 100: 281–301.
- Clerc, P., Scheidegger, C. & Ammann, K. 1992. The red data list of Swiss macrolichens. *Botanica Helvetica* 102: 71–83.
- Deacon, J. W. 1980. *Introduction to Modern Mycology*. Blackwell Scientific Publications. Oxford.
- Dillenius, J. J. 1742. *Historia muscorum*. Oxford.
- Eriksson O.E. & Hawksworth, D.L. 1986. Outline of the ascomycetes – 1986. *Systema Ascomycetum* 5: 185–324.
- Eschweiler, F G 1824. *Systema lichenum, genera exhibens rite distincta, pluribus novis adaucta. Norimbergiae*

- Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D. & Kluge, A. G. 1997. Parsimony jackknifing outperforms neighbour-joining. *Cladistics* 12: 99–124.
- Galloway, D. J. 1985. *Flora of New Zealand Lichens*. P. D. Hasselberg, Government Printer. Wellington.
- Galloway, D. J. 1992. *Checklist of New Zealand Lichens*. DSIR Land Resources Scientific Report, No. 26, DSIR Land Resources. Christchurch.
- Gargas, A., DePriest, P. T., Grube, M. & Tehler, A. 1995. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. *Science* 268: 1492–1495.
- Glass, N. L. & Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323–1330.
- Hale, M. E., Jr. 1983. *The Biology of Lichens*. Third Edition. Edward Arnold. London.
- Halonen, P., Clerc, P., Goward, T., Brodo, I. M. & Wulff, K. 1998. Synopsis of the genus *Usnea* (lichenized ascomycetes) in British Columbia, Canada. *Bryologist* 101: 36–60.
- Halonen, P., Myllys, L., Ahti, T. & Petrova, O. V. 1999. The lichen genus *Usnea* in East Fennoscandia. III. The shrubby species. *Annales Botanici Fennici* 36: 235–256.
- Hawksworth, D. L., Kirk, P. M., Sutton, B. C. & Pegler, D. N. 1995. *Ainsworth & Bisby's Dictionary of the Fungi*. CAB International, Cambridge University Press. Wallingford.
- Henssen, A. & Jahns, H. M. 1974. *Lichenes*. Georg Thieme Verlag. Stuttgart.
- Herrera-Campos, M. A., Clerc, P. & Nash, T. H. 1998. Pendulous species of *Usnea* from the temperate forests in Mexico. *Bryologist* 101: 303–329.
- Huelsenbeck, J. P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- Huelsenbeck, J. P. & Ronquist, F. 2003. *MrBayes* 3.0 beta 4. bayesian analysis of phylogeny. Computer program available at <http://morphbank.ebc.uu.se/mrbayes/>.
- Huelsenbeck, J. P., Ronquist, F., Nielsen, R. and Bollback, J. P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294 2310–2314.
- Krog, H. 1976. *Lethariella* and *Protousnea*, two new lichen genera in the Parmeliaceae. *Norwegian Journal of Botany* 23: 83–106.
- Krog, H. 1982. Evolutionary trends in foliose and fruticose lichens of the Parmeliaceae. *Journal of the Hattori Botanical Laboratory*. 52: 303–311.
- Lamb, I. M. 1964. Antarctic lichens I. The genera *Usnea*, *Ramalina*, *Himantormia*, *Alectoria*, *Cornicularia*. *British Antarctic Survey, Scientific Report* 38: 1–34.
- Lawrence, E. (ed) 1995. *Henderssons dictionary of biological terms*. 11<sup>th</sup> edition. Longman Singapore Publishers (Pte) Ltd. Singapore.
- Malcolm, W. M. & Galloway, D. J. 1997. *New Zealand Lichens: Checklist, Key, and Glossary*. Museum of New Zealand Te Papa Tongarewa. Wellington.
- Motyka, J. 1936–1938. *Lichenum generis Usnea studium monographicum. Pars systematica, volumen primum*. Leopoli.
- Myllys, L., Lohtander, K. and Tehler, A. 2001.  $\beta$ -tubulin, ITS and group I intron sequences challenge the species pair concept in *Physcia aipolia* and *P. caesia*. *Mycologia* 93 (2): 335–343.
- Nees v. Esenbeck, C. D. & Flotow v. 1835. Einige neue Flechtenarten. *Linnaea* 9: 495–502.



- Nylander, J. A. A. 2002. *MrModeltest v1.0b*. Program distributed by the author. Department of Systematic Zoology, Uppsala University. <http://www.ebc.uu.se/systzoo/staff/nylander.html>.
- Ohmura, Y. 2001. Taxonomic study of the genus *Usnea* (lichenized ascomycetes) in Japan and Taiwan. *Journal of the Hattori Botanical Laboratory* 90: 1–96.
- Ohmura, Y. 2002. Phylogenetic evaluation of infrageneric groups of the genus *Usnea* based on ITS regions in rDNA. *Journal of the Hattori Botanical Laboratory*. 91: 231–243.
- Poelt, J. 1970. Das Konzept der Artenpaare bei den Flechten. *Deutsche Botanische Gesellschaft Neue Folge* 4:187–198.
- Purvis, O. W., Coppins, B. J., Hawksworth, D. L., James, P. W. & Moore, D. M. 1992. *The Lichen Flora of Great Britain and Ireland*. Natural History Museum. London.
- Rannala, B., Yang, Z 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular evolution* 43: 304–311.
- Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Stevens, G. N. 1999. A revision of the lichen family *Usneaceae* in Australia. *Bibliotheca Lichenologica*. 72: 1–128. J. Cramer, Berlin. Stuttgart.
- Swofford, D. L. 2002. *PAUP\**. *Phylogenetic Analysis Using Parsimony (\*and Other Methods)*. Version 4. Sinauer Associate, Sunderland. Massachusetts.
- Tehler, A. 1982. The species pair concept in lichenology. *Taxon* 31: 708–717.
- Thor, G. & Arvidsson, L. 1999. *Rödlistade lavar i Sverige – Artfakta*. ArtDatabanken, SLU. Uppsala.
- Türk, R. & Hafellner, J. 1999. Rote Liste gefährdeter Flechten (Lichenes) Österreichs. 2. Fassung. In *Grüne Reihe des Bundesministeriums für Umwelt, Jugend und Familie* (H. Niklfeld, ed) 10: 187–228.
- Walker, F. J. 1985. The lichen genus *Usnea* subgenus *Neuropogon*. *Bull. Brit. Mus. <Natural History> Bot.* 13: 1–130.
- Westring, J. P. 1805-09. *Svenska lafvarnas färghistoria*. Stockholm.

# Acta Universitatis Upsaliensis

*Comprehensive Summaries of Uppsala Dissertations  
from the Faculty of Science and Technology*

Editor: The Dean of the Faculty of Science and Technology

---

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology*. (Prior to October, 1993, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science”.)

## Distribution:

Uppsala University Library  
Box 510, SE-751 20 Uppsala, Sweden  
[www.uu.se](http://www.uu.se), [acta@ub.uu.se](mailto:acta@ub.uu.se)

ISSN 1104-232X  
ISBN 91-554-5860-2