

Neuropogon and the phylogeny of *Usnea* s.l. (Parmeliaceae, Lichenized Ascomycetes)

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Molecular phylogenies based on the ITS-LSU nrDNA and part of the β -tubulin region were used to investigate the position of *Neuropogon* in *Usnea* s. lat. Bayesian inference and maximum parsimony strongly supported the monophyly of *Neuropogon*. Subgenus *Usnea* and *Neuropogon* form a strongly supported group with subg. *Eumitria* and subg. *Dolichousnea* as consecutive monophyletic sister groups. The following generic classification is proposed: *Usnea* (subgenus *Usnea* only), *Neuropogon*, *Eumitria*, and *Dolichousnea*. *Dolichousnea* is elevated to generic rank. *Usnea* as presently conceived is clearly non-monophyletic but with the present circumscription it may be monophyletic, or not; this requires further analysis. The genera are well characterised morphologically, and pending further investigations this classification is in agreement with current practice and minimises the need for nomenclatural changes. The following new combinations are made: *Dolichousnea* (Y. Ohmura) Articus, *Dolichousnea longissima* (Ach.) Articus, *D. trichodeoides* (Vain.) Articus, *D. diffracta* (Vain.) Articus, and *Eumitria pectinata* (Taylor) Articus.

KEYWORDS: ITS-LSU, β -tubulin, molecular phylogeny, *Usnea*, *Eumitria*, *Dolichousnea*, *Neuropogon*, usneoid, Bayesian inference.

INTRODUCTION

The lichen genus *Usnea* contains fruticose lichens with a central axis consisting of a cartilaginous strand of longitudinal hyphae. The delimitation of *Usnea* has been discussed by several authors (e.g., Motyka, 1936–1938; Krog, 1976, 1982; Walker, 1985; Ohmura, 2001, 2002), and the concept of the genus has changed over time. Several subgenera have been recognised. *Neuropogon*, described by Nees & Flotow (1835), shares many morphological features with *Usnea* and has by some authors been placed as a subgenus of *Usnea* (e.g. Motyka, 1936–1938; Walker, 1985). Other authors stress the morphological autapomorphies of *Neuropogon*, its unusual ecology, and distinctive distribution and prefer a generic position (e.g., Krog, 1976, 1982; Galloway, 1983).

Motyka (1936–1938) proposed a classification in which he included all fruticose lichens with an inner, cartilaginous central cord in *Usnea* and recognised six subgenera: *Euusnea*, *Protousnea*, *Lethariella*, *Chlorea*, *Neuropogon*, and *Eumitria*.

The position of *Neuropogon* as a subgenus to *Usnea* was accepted by several authors (Lamb, 1964; Henssen & Jahns, 1974). Krog (1976) raised *Protousnea* and *Lethariella* to generic rank. In a later paper (1982) she proposed a hypothetical relationship among *Usnea*, *Protousnea*, *Lethariella*, *Neuropogon*, *Letharia*, and *Evernia*. In this hypothesis *Neuropogon* forms the sister

group to *Usnea*. Krog showed *Protousnea*, together with *Evernia*, forming the sister group to *Usnea* and *Neuropogon*, while *Letharia* and *Lethariella* were grouped together and formed the sister group to all other usneoid genera. *Neuropogon* was accepted as a genus by Rogers (1981) and Galloway (1983). Walker (1985) in a revision of *Neuropogon* argued for a subgeneric position of *Neuropogon*. Walker was critical towards the recognition of *Usnea* and *Neuropogon* as separate genera, stressing that some species have an intermediate morphology (Walker, 1985). She further considered characteristics such as habitat, thallus colour, and pigmentation insufficient for recognising genera.

Despite this view, the acceptance of *Neuropogon* as a genus was evident, especially in checklists and lichenological literature of the southern hemisphere (Galloway, 1985, 1992; Malcolm & Galloway, 1997; Stevens, 1999). The first molecular study of the subgenera of *Usnea* was undertaken by Ohmura (2002), who presented a phylogeny of 21 *Usnea* species. In his study, the subgenera *Usnea* and *Eumitria* formed monophyletic groups, and he recognised a new subgenus, *Dolichousnea*, containing *U. longissima*, *U. diffracta* and *U. trichodeoides*. Ohmura (2001, 2002) characterised the subgenera morphologically, and emphasised differences in the cortex structure. However, no *Neuropogon* material was included in these studies.

At present three subgenera are recognised within

Usnea: *Usnea*, *Eumitria*, and *Dolichousnea* (Ohmura, 2001, 2002). The position of *Neuropogon* is still controversial.

In this study molecular data are for the first time used to elucidate the relationship of *Neuropogon* and *Usnea* s. lat. The aim is to investigate if *Neuropogon* is monophyletic, and if so to explore the consequences regarding classification and ranking in *Usnea* s. lat.

MATERIALS AND METHODS

Sampling. — New sequences were obtained for specimens of *Neuropogon* and *Dolichousnea*. GenBank data have been included of the species *U. baileyi* and *U. pectinata* from the subgenus *Eumitria* (Ohmura, 2002). All species included in this study are listed in the Appendix.

Anatomical studies. — The cortex structures of *Neuropogon antarcticus*, *N. trachycarpus*, *N. taylorii*, as well as from *Usnea hirta*, *U. articulata*, and *U. baileyi* were studied with scanning electron microscopy (SEM) and light microscopy. Cross and longitudinal sections analysed in the light microscope were cut with the freezing microtome (15–25 μ m) and coloured with lactic blue. For the SEM the lichens were frozen in liquid nitrogen and then broken manually. The samples were plated with gold in a vacuum at 20 Am for 105 sec.

Extractions and PCR amplifications. — Total DNA from lichen specimens was extracted using the Qiagen DNeasy Plant Mini Kit. Two gene loci have been investigated: partial nuclear β -tubulin and continuous sequences of the ITS and LSU regions of the nuclear ribosomal DNA. Polymerase chain reaction (PCR) amplification and automated sequencing of the ITS and LSU genes were conducted using the settings utilised by Döring & al. (2000). The PCR amplifications for β -tubulin were produced with the following settings: 95°C (4 min), 30 cycles of 95°C (45 sec), 52°C (40 sec) and 72°C (1 min 30 sec). The following PCR primer pairs were used: β -tubulin 2a - β -tubulin 2b (Glass & Donaldson, 1995), ITS1F - LR3, nu-LSU-155-5' - LR5 and nu-LSU-155-5' - LR6 (Gardes & Bruns, 1993; Döring & al., 2000; Vilgalys, 2001). Sequencing primers used were: β -tubulin 2a, β -tubulin 2b, ITS1F, ITS4, ITS2, nu-LSU-155-5', nu-LSU-362-5', LR1, LR3, LR5, LR0R, LR3R (White & al., 1990; Gardes & Bruns, 1993; Vilgalys, 2001; Döring & al., 2000). The settings for the sequencing reactions were: 30 cycles of 95°C (20 sec), 50°C (15 sec), and 60°C (3 min).

Sequence alignment. — The sequences were automatically aligned using the ClustalW algorithm as implemented in BioEdit (Hall, 2001) and then adjusted manually. Two members of the family Parmeliaceae,

Platismatia glauca and *Alectoria sarmentosa*, were chosen as outgroup.

Bayesian analysis. — The dataset was analysed by Bayesian inference with the program MrBayes version 3.0 beta4 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Bayesian inference of phylogeny aims at estimating the posterior probabilities of trees and other parameters of the evolutionary model. There are two main components, the likelihood function (specified by the data and a model of character substitution) and the prior probability on trees and other parameters of the substitution model (specified by the researcher). The priors for the tree and other parameters of the model were used as implemented as default in MrBayes version 3.0 beta 4. Markov chain Monte Carlo (MCMC) is used for approximating the posterior probabilities and heated chains are run so that the analysis is not stuck on a local optimum (Huelsenbeck & Ronquist, 2001). The models for nucleotide substitutions were selected for each gene individually prior to the MCMC using the Akaike information criterion (AIC; Akaike, 1973). The model of evolution was chosen with MrModeltest Version 1.1b (Nylander, 2002) in conjunction with PAUP* (Swofford, 2002). The Akaike Information criterion 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). The Markov chain was run for 2,000,000 generations and every 100th tree was sampled. The tree samples of the burn-in phase were discarded. Groups with posterior probabilities above 60 are presented.

Parsimony analysis. — The maximum parsimony analysis was carried out in PAUP* 4.0 beta 10 (Swofford, 2002). The heuristic search was performed with 1000 random addition sequence replicates, the TBR branch swapping option and MulTrees option were ON. Insertion/deletion events (indels) in DNA sequences were expressed as gaps in the alignment and were coded as missing data. Uninformative characters and ambiguous regions were excluded from the analyses. Jackknifing for rapid identification of well-supported monophyletic groups (Farris & al., 1997) was performed by using PAUP*. The jackknife settings were 10000 jackknife replicates with JAC-emulation, nominal deletion of characters 36.79%, retaining groups with frequency >60%, 10 random replicates and MulTrees OFF.

RESULTS

Eumitria is not represented in the dataset based on sequences of the nrDNA ITS-LSU and β -tubulin region, since *Eumitria* sequences could not be obtained for all these regions studied here. A more comprehensive

dataset, including *Eumitria* species, was obtained for ITS and for this locus an analysis including more taxa could be accomplished.

Analyses based on the ITS-LSU and β -tubulin dataset. — The data matrix of the combined dataset of ITS-LSU and β -tubulin contains 27 taxa and 1854 aligned sites. 1419 aligned sites belong to the ITS-LSU region and 435 to the β -tubulin region. In total 222 sites are parsimony-informative, 132 in the ITS-LSU and 90 in the β -tubulin region. Thirteen characters were removed since they belong to ambiguous regions.

Bayesian analysis based on the ITS-LSU and β -tubulin dataset. — The tree resulting from the Bayesian inference is shown in Fig. 1. Posterior probability values (PP) are indicated above the branches. A well-supported clade comprises all usneoid lichens included in this study (A: PP = 1.00). This clade has two highly supported sub-clades, one with *Dolichousnea* as a monophyletic group (B: PP = 1.00) and the other showing a close relationship of subgenus *Usnea* and *Neuropogon* (C: PP = 1.00). The *Neuropogon* specimens form a highly supported monophyletic group (D: PP = 1.00), while the specimens of subgenus *Usnea* group in three clades. One clade (G) contains *Usnea florida*, *U. wasmuthii*, *U. chaetophora*, *U. filipendula*, *U. barbata*, and *U. rigida* (PP = 1.00). This clade has been discussed in detail by Articus & al. (2002) and corresponds to section *Usnea* identified by Ohmura (2001, 2002). Another clade (F: PP = 1.00) comprises *U. hirta*, *U. fragileszens*, and *U. articulata*. The specimens of *Usnea ceratina* group in a third clade (E: PP = 1.00). In the *Neuropogon* clade, *N. trachycarpus* and *N. sphacelatus* (PP = 0.99) constitute the sister group to *N. antarcticus* (PP = 1.00). In the *Dolichousnea* clade, *U. diffracta* forms the sister group to *U. longissima* and *U. trichodeoides* (PP = 1.00).

Parsimony analysis based on the ITS-LSU and β -tubulin dataset. — The parsimony analysis generated 50 shortest trees, 439 steps long (CI = 0.64, RI = 0.82). The strict consensus tree has a similar topology as the tree obtained from Bayesian inference, and the support values of the jackknife analysis are indicated below the branches in Fig. 1. The same main clades are well-supported in both analyses.

Analyses based on the ITS dataset. — The matrix based on the ITS region (ITS1, 5.8S and ITS2) contains 31 taxa and 536 aligned sites, of which 119 are parsimony-informative. Sixteen characters were removed since they belong to ambiguous regions.

Bayesian analysis based on the ITS dataset. — The tree resulting from the Bayesian inference is shown in Fig. 2. Posterior probability values (PP) are indicated above the branches. All usneoid lichens included are comprised in a well-supported clade (A: PP =

1.00). *Dolichousnea* forms a monophyletic group (B: PP = 1.00) and *Eumitria* likewise obtained strong support (H: PP = 1.00). Together they form a clade (I: PP = 0.69) which is the sister group to a clade comprising *Neuropogon* and subgenus *Usnea* (C: PP = 1.00). The *Neuropogon* specimens form a highly supported monophyletic group (D: PP = 1.00), and subgenus *Usnea* is represented by three clades. Section *Usnea* (clade G, PP = 1.00) with *Usnea florida*, *U. wasmuthii*, *U. chaetophora*, *U. filipendula*, *U. barbata*, and *U. rigida*, has a sister group relation with *Neuropogon* (J: PP = 0.89). *Usnea fragileszens*, *U. articulata*, and *U. hirta* are part of a polytomy together with the clade comprising *Neuropogon* and section *Usnea*. *Usnea ceratina* (PP = 1.00) is the sister to *Neuropogon* and the other members of subgenus *Usnea* (PP = 1.00).

Parsimony analysis based on the ITS dataset. — The parsimony analysis generated 76 shortest trees, 244 steps long (CI = 0.57, RI = 0.78). The strict consensus tree (not shown) differs little in topology from the tree obtained by Bayesian inference. Jackknife values are indicated below the branches in Fig. 2. *Dolichousnea*, *Eumitria*, and *Neuropogon* each form well-supported monophyletic groups. The relationship of *Dolichousnea* (j = 97) and *Eumitria* (j = 100) is unresolved. Together they form the sister to a clade comprising *Neuropogon* and subgenus *Usnea* (j = 99). *Usnea ceratina* (j = 100) forms the sister (j = 62) to a polytomy including *U. hirta*, a clade comprising *U. fragileszens* and *U. articulata* (j = 91), and a clade containing *Neuropogon* and section *Usnea* (j = 67).

Introns in *Neuropogon*. — An intron of ca. 400 base pairs at the end of the SSU region was found in four of the five *Neuropogon* specimens studied. The introns of the two *N. antarcticus* specimens (KN 85 and KN 86) are very similar. *Neuropogon sphacelatus*, collected in the northern hemisphere, has a similar intron to the *N. antarcticus* specimens, while the intron in *N. trachycarpus* (KN 91) differs from *N. antarcticus* and *N. sphacelatus* in length.

Cortical structures. — The cortical structure of *Neuropogon* was investigated by SEM and light microscopy and the terminology used by Ohmura (2001) was followed. The hyphae of the cortex are pachydermatous (thick-walled) and the cell walls are strongly conglutinated. The orientation of the hyphae is mainly anticlinal, but irregularly oriented and periclinal hyphae also occur (Figs. 3, 4).

DISCUSSION

This study is based on comparisons of sequences from the nuclear rDNA (the ITS-LSU regions) and the

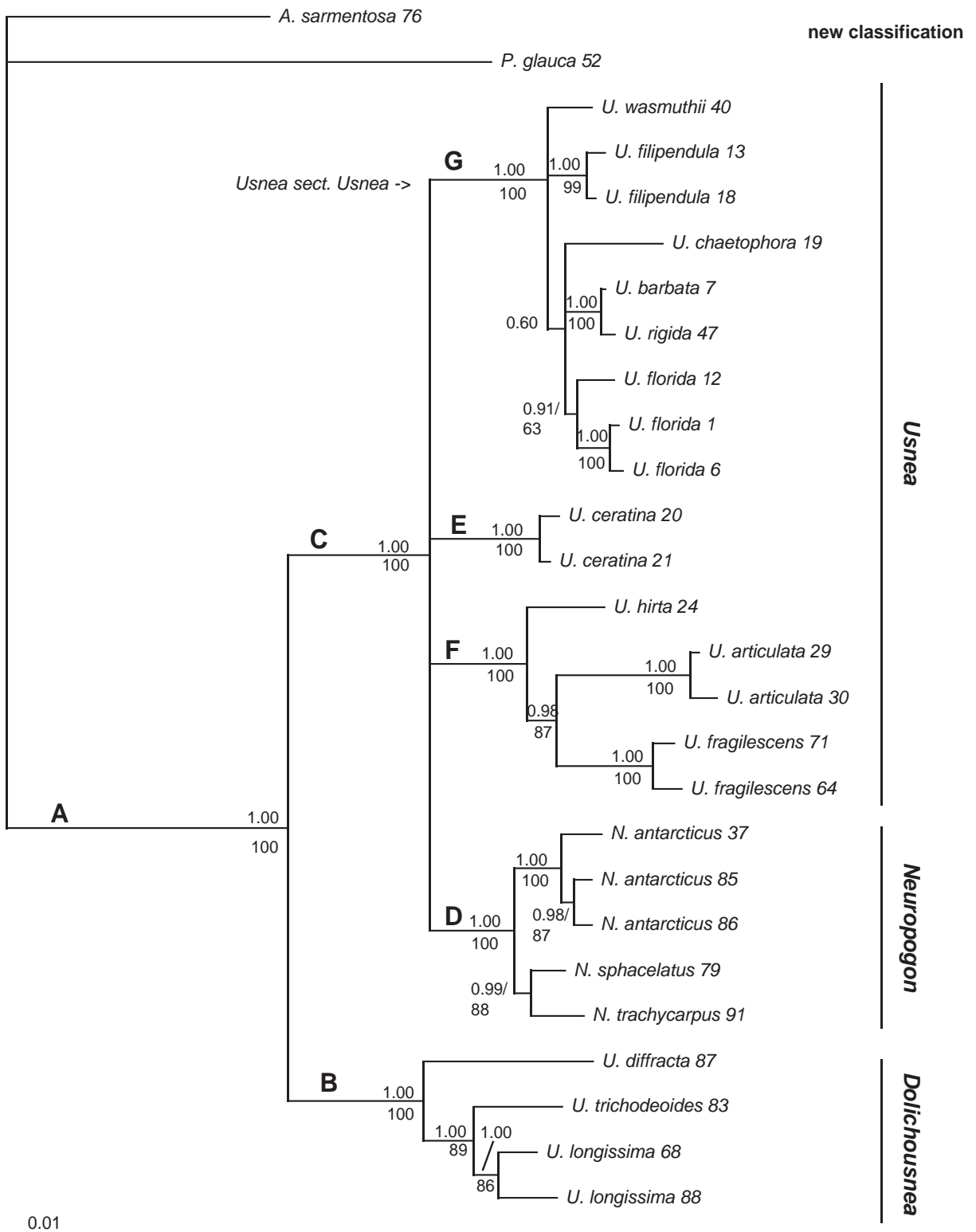


Fig. 1. Phylogeny of the ITS-LSU and β -tubulin dataset obtained by Bayesian inference. Posterior probabilities indicated above branches, jackknife values below.

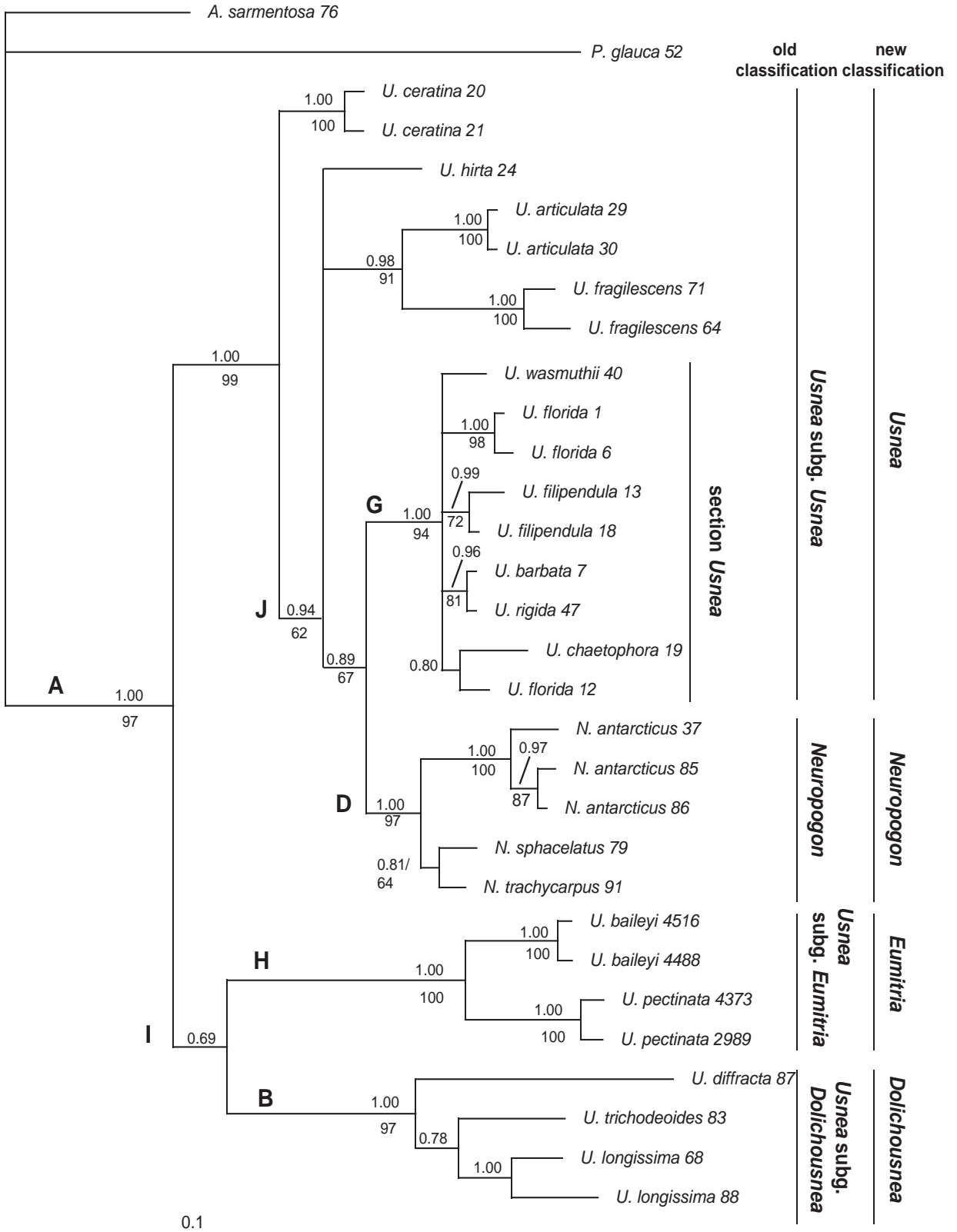


Fig. 2. Phylogeny of the ITS data matrix, obtained by Bayesian inference (PP values above branches, jackknife values below). The old and new classifications of *Usnea* s. lat. are indicated.

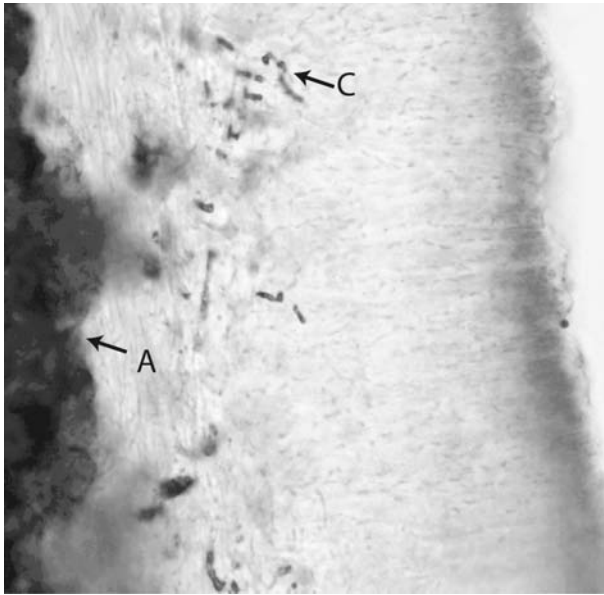


Fig. 3. Cortex structure in *Neuropogon trachycarpus* (longitudinal section, light microscope image). The cortex layer consists of periclinal, irregular, and predominantly anticlinally orientated hyphae, with tightly conglutinated cell walls. A, algae; C, crystals.

gene coding for β -tubulin. Bayesian inference and analyses using maximum parsimony resulted in phylogenies which showed *Neuropogon* to form a highly supported monophyletic group (Figs. 1, 2). In the ITS-LSU/ β -tubulin analyses *Neuropogon* is part of a polytomy also including species from *Usnea* subg. *Usnea*. *Dolichousnea* is a highly supported monophyletic group. Subgenus *Usnea*, however, did not form a monophyletic group. It was also revealed that *Dolichousnea* is more distantly related to subgenus *Usnea* than *Neuropogon*. *Neuropogon* and subgenus *Usnea* form a well-supported clade, but the relationships among the clades of *Usnea* subg. *Usnea* and *Neuropogon* were not clarified in this study.

In the analysis based on ITS data only, *Neuropogon* forms a group together with subg. *Usnea* section *Usnea* (PP = 0.89). This relationship has only weak support in the parsimony analysis ($j = 67$) and no support in the phylogeny based on the combined dataset. Therefore this relationship is considered uncertain here, and no taxonomic changes concerning the delimitation of subgenus *Usnea* are made. *Eumitria* and *Dolichousnea* form a clade which is sister to the clade comprising *Neuropogon* and subgenus *Usnea*. The resolution and topology of the phylogenies obtained by the two datasets differ in minor details. Even though the ITS phylogeny is more resolved concerning the relationship of *Neuropogon* and subg. *Usnea*, it is based on fewer data than the ITS-LSU and β -tubulin phylogeny and therefore not discussed in detail.

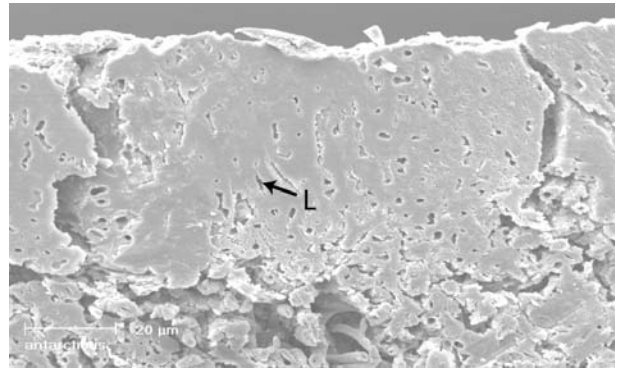


Fig. 4. Cortex structures in *Neuropogon antarcticus* (transverse section, SEM image). The hyphae in the cortex layer are pachydermatous with tightly conglutinated cell walls. The orientation of the hyphae is irregular to anticlinal. L, cell lumen.

According to Ohmura (2001) the cortex structure is an important character for the recognition of the subgenera in *Usnea* s. lat. He observed several cortex types, but did not study the cortex structure of *Neuropogon*. The cortex of *Neuropogon* consists of pachydermatous (thick-walled), tightly conglutinated hyphae which are mainly anticlinal (Figs. 3 and 4). This structure resembles the *merrillii*-type described by Ohmura, which also occurs in *Dolichousnea* and in several species of subgenus *Usnea*. *Eumitria* also has pachydermatous hyphae, but they are irregularly oriented. *Usnea* section *Usnea* is, according to Ohmura, the only group within *Usnea* s. lat. that has leptodermatous (thin-walled) hyphae in the cortex, which thus seems to be an autapomorphy of section *Usnea*. The methods of Ohmura were followed to complete the cortex information even for *Neuropogon*. However, the cortex structure is used here as an additional information only and not as a main criteria. Therefore, no further cortex studies have been made using other methods as for example described by Tavares (1987) and Tavares & al. (1998).

The monophyly of *Neuropogon* has been questioned by some authors. Walker (1985) and Du Rietz (1926) indicated some *Neuropogon* species to be morphologically intermediate between *Neuropogon* and subgenus *Usnea*. One of these species, *Neuropogon trachycarpus*, was investigated here, and in the molecular phylogeny it was part of the well-supported *Neuropogon*. *Neuropogon trachycarpus* was regarded to be morphological intermediate since the apothecia discs are light brown in colour (not pale as in *Usnea* or black as in other *Neuropogon* species). The thallus, otherwise, shows the typical black and patchy pigmentation of *Neuropogon* species.

Walker mentions several other species to be morphological intermediate (*U. acanthella*, *N. durietzii*, *U. neuropogonoides*, and *N. patagonicus*). These species

are similar to *Neuropogon* in the thallus colour; apothecia have not been observed. According to Walker (1985) these species may show affinities to *Usnea* or may be difficult to classify since they grow on different substrates, not on rocks only (*Usnea acanthella*), have a non-*Neuropogon*-like texture after herbaria storage (*Neuropogon durietzii*), vary in the amount of black pigmentation (*N. patagonicus*), or exist from few collections only (*U. neuropogonoides*). Since no material was available for DNA extraction, none of these species was included in the study. However, Walker included all these species in *Neuropogon*, and there is no morphological character indicating that they should not belong to *Neuropogon*.

Subgenus *Usnea* in the rDNA/ β -tubulin analyses was represented by three clades, one of them corresponding to section *Usnea* as mentioned by Ohmura (2001, 2002). Section *Usnea* is well-supported; in this study *Usnea barbata*, *U. chaetophora*, *U. filipendula*, *U. florida*, *U. rigida*, and *U. wasmuthii* are included. Section *Usnea* and *Neuropogon*, as indicated in the ITS analysis, might be closely related. Another highly supported clade in the rDNA/ β -tubulin analyses includes *U. articulata*, *U. fragilesceus*, and *U. hirta*. They do not, however, form a monophyletic group in the ITS analysis. These species differ considerably in morphology, and further taxa need to be included in an analysis to ensure that the few sampled here really represent one strongly supported clade. The only unifying character found here is the thin cortex. *Usnea ceratina* is part of a polytomy including the species of subgenus *Usnea* and *Neuropogon*. Ohmura recognised a section *Ceratinae*, but here only one species of *Ceratinae* was included and an evaluation of its status is not possible. A denser taxon sampling in subgenus *Usnea* may reveal several monophyletic entities, which may be candidates for recognition at the generic level.

Ranking. — The phylogeny obtained in this investigation offers different options for the taxonomic treatment of the groups involved. *Neuropogon* could be included as a subgenus of *Usnea* s. lat. In this option the synapomorphies of *Usnea* s. lat., such as cartilaginous central axis and usnic acid in the cortex are emphasised. This, however, means that *Usnea* will include several morphologically distinctive and quite different monophyletic groups, as subgenus *Usnea*, subg. *Neuropogon*, subg. *Eumitria*, and subg. *Dolichousnea*. *Usnea* s. lat. would in this case be monophyletic, but information about the relationships among subgenera would not be expressed. Subgenus *Usnea* is in this solution non monophyletic. Another possibility is to raise *Dolichousnea* and *Eumitria* to generic rank and to include *Neuropogon* as a subgenus of *Usnea* together with subgenus *Usnea*. This solution clarifies the position of *Dolichousnea* and *Eumitria* as more distantly related to subgenus *Usnea*

than *Neuropogon*, and the close relationship of subgenus *Usnea* and *Neuropogon* is emphasised.

Since the relationships between *Neuropogon* and some strongly supported clades in subgenus *Usnea* were not clarified, I prefer to treat *Neuropogon* as a genus. *Neuropogon* forms a well-supported monophyletic group and is morphologically easily recognised, and it is therefore reasonable to keep it as a genus. The discovery that *Usnea* s. str. might not be monophyletic is one of the major results. More research is needed to clarify if *Usnea* should be divided into different entities. *Eumitria* and *Dolichousnea*, as a consequence of the treatment of *Neuropogon* as a genus, have to be raised to generic rank. Thus the recognition of four genera is suggested. *Neuropogon*, *Dolichousnea*, and *Eumitria* are morphologically well characterised and monophyletic, whereas *Usnea* may or may not be monophyletic. This solution is an improvement of the classification of the involved genera, even though more research on *Usnea* is needed.

Morphological characterisation of the genera. — *Neuropogon* species have a solid central axis, a black patchy pigmentation in the cortex, and a brown to black apothecium disc. The thallus is erect with a mainly anisotomic-dichotomous branching pattern. The cortex consists of pachydermatous, tightly conglutinated hyphae which mainly have an anticlinal orientation. *Neuropogon* grows on rocks in the Antarctic and Arctic regions, and in high altitudes in south America, and Australasia. *Neuropogon* contains ca. 15 species (Walker, 1985).

Usnea species are recognised by the solid central axis of the branches, and the pale apothecium disc. The thallus is erect to pendent and the branching pattern is anisotomic- or isotomic-dichotomous. The thallus colour is green. Section *Usnea* has leptodermatous hyphae, which are anticlinal and loosely conglutinated (*florida*-type), while the other *Usnea* species have the *merrillii*- or *ceratina*-cortex type, with pachydermatous hyphae, which are tightly conglutinated and anticlinal. *Usnea* has a world-wide distribution. *Usnea* contains ca. 600 species (Hawksworth & al., 1995).

Dolichousnea species are characterised by having annular-pseudocyphellae between the segments (Ohmura, 2001), pale apothecium discs, and a solid central axis. The central axis has a positive iodine reaction, and the hypothecium is thicker than in the other subgenera (Ohmura, 2001). The thallus is pendent with an isotomic-dichotomous branching pattern, a holdfast may be missing, and the branches may be partly decorticated (e.g., *U. longissima*). The cortex structure is of the *merrillii*-type, with tightly conglutinated, pachydermatous hyphae which are anticlinal. *Dolichousnea* occurs in the northern hemisphere (*U. longissima*, *U. trichodeoides*), in East Asia (*U. diffracta*), and in Africa and Australia

(*U. trichodeoides*). So far three species are recognised within *Dolichousnea*.

Eumitria species are recognised by a tubular axis, which may appear fistulose or is traversed by loosely or densely arranged hyphae (Swinscow & Krog, 1974), and the pale apothecium disc. The thallus is shrubby to pendent and the branching is anisotomic- and isotomic-dichotomous. The axis is not fistulose in all species, making this character unreliable. The inclusion of some species in *Eumitria* (e.g., *E. pectinata*) is based on molecular support (Ohmura, 2002). The cortex is of the *eumitria*-type with pachydermatous, well conglutinated, and irregularly orientated hyphae. *Eumitria* species occur in temperate and tropical regions. *Eumitria* comprises about 13 species.

Nomenclature changes and accepted names.

Dolichousnea (Y. Ohmura) Articus **stat. nov.** Basionym: *Usnea* subgenus *Dolichousnea* Y. Ohmura, J. Hattori Bot. Lab. No. 90: 80, 2001. – Type: *Dolichousnea longissima* (Ach.) Articus.

Dolichousnea longissima (Ach.) Articus **comb. nov.** Basionym: *Usnea longissima* Acharius, Lich. univ.: 626, 1810. – Holotype: Lusatia, *Mosig 14-I* (H-ACH 1893).

Dolichousnea diffracta (Vain.) Articus **comb. nov.** Basionym: *Usnea diffracta* Vainio, Bot. Mag. (Tokyo): 45, 1921. – Holotype: Japan, Hokkaido, Pro. Kushiro, 19 August 1918, *A. Yasuda 231* (TUR-V 478).

Dolichousnea trichodeoides (Vain.) Articus **comb. nov.** Basionym: *Usnea trichodeoides* Vainio, Ann. Acad. Sci. Fenn. 7: 8, 1915. – Lectotype (selected by Swinscow & Krog, Norweg. J. Bot. 23: 239, 1978): South Africa, Capite Bonae Spei, mis. *Persoon 27* (H-ACH-1882).

Eumitria Stirt. Stirton, Scott. Naturalist (Edinburgh) 6: 100, 1881. – Type: *Eumitria baileyi* Stirt.

Eumitria baileyi Stirt. Stirton, Scott. Naturalist (Edinburgh) 6: 100, 1881. *Usnea baileyi* (Stirt.) Zahlbruckner, Denkschr. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl. 83: 191, 1909. – Lectotype (selected by Rogers & Stevens, Australian Systematic Botany 1: 357, 1988): Australia, Queensland, near Brisbane, *F. M. Bailey 164* (BM).

Eumitria pectinata (Taylor) Articus **comb. nov.** Basionym: *Usnea pectinata* Taylor, in Hooker,

London J. Bot. 6: 191, 1847. – Holotype: Bangladesh, Sylhet, *Wallich s.n.* (FH).

Neuropogon Nees & Flotow. Nees v. Esenbeck & v. Flotow, Linnaea 9: 496, 1835. – Lectotype (see Walker, 1985: 4): *Neuropogon antennarius* Nees & Flotow.

Neuropogon antarcticus (Du Rietz) Lamb. Lamb, J. Linn. Soc., Bot. 52: 210, 1939. Basionym: *Usnea antarctica* Du Rietz, Svensk Bot. Tidskr. 20: 93, 1926. – Holotype: Regio Antarctica, South Victoria Land, Admiralty Range, 2000' s.m., 1900, *C. R. Borchgrevink* (UPS!).

Neuropogon sphacelatus (R. Br.) D.J. Galloway. Galloway DSIR Land Resources Scientific Report (Christchurch) 26: 7, 1992. Basionym: *Usnea sphacelata* R. Br., Chloris melvilliana: 49, 1823 [1821]. – Holotype: Melville I., *J. Ross 114* (BM).

Neuropogon trachycarpus Stirt. Stirton, Scott. Naturalist (Edinburgh) 6: 105, 1881. – Holotype: Kerguelen's Land (So. Antarctic) *Challenger Exped.* 6 (BM)

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Appendix. Specimens included in this study, accession number, collector, and locality.

Taxon	Source localities and voucher information	Lab code	GenBank Acc. No.	
			ITS-LSU	β -tubulin
<i>Alectoria sarmentosa</i>	Canada: British Columbia, <i>Articus 707</i> (UPS)	KA 76	AJ748111	AJ748097
<i>Dolichousnea diffracta</i>	Japan: Hokkaido, <i>Bergsten 25/08/2000</i> (UPS)	KDIF 87	AJ748107	AJ748093
<i>D. longissima</i>	India: Uttaranchal, <i>Tibell 21980</i> (UPS)	KL 88	AJ748108	AJ748095
<i>D. longissima</i>	Canada: British Columbia, <i>Articus 728</i> (UPS)	KL 68	AJ748109	AJ748094
<i>D. trichodeoides</i>	Japan: Hokkaido, <i>Bergsten 24/08/2000</i> (UPS)	KTRI 83	AJ748110	AJ748096
<i>Neuropogon antarcticus</i>	TAAF: Kerguelen, <i>Poulsen 1143</i> (UPS)	KN 37	AJ748101	AJ748087
<i>N. antarcticus</i>	Antarctica: Deception Island, <i>Schroeter 20/02/02</i> (UPS)	KN 85	AJ748099	AJ748085
<i>N. antarcticus</i>	Antarctica: Livingston island, <i>Lumbsch 19013b</i> (UPS)	KN 86	AJ748100	AJ748086
<i>N. sphacelatus</i>	Iceland: Austur-Island, <i>Heidmarsson A02</i> (UPS)	KN 79	AJ748102	AJ748088
<i>N. trachycarpus</i>	Chile: Patagonia, <i>Frøden 1572</i> (LD)	KN 91	AJ748103	AJ748089
<i>Platismatia glauca</i>	Sweden: Uppland, <i>Articus 673</i> (UPS)	KPG 52	–	AF502271
<i>P. glauca</i>	Sweden: Uppland, <i>Mattsson 4007</i> (UPS)	–	AF058035	–
<i>Usnea articulata</i>	England: Devon, <i>Articus 617</i> (UPS)	KAR 29	AJ457139	AF502258
<i>U. articulata</i>	England: Somerset, <i>Articus 615</i> (UPS)	KAR 30	AJ457140	AF502259
<i>U. baileyi</i>	Japan: Honshu, <i>Ohmura 4488</i> (TNS)	–	AB051050	–
<i>U. baileyi</i>	U.S.A.: Hawaii, <i>Ohmura 4516</i> (TNS)	–	AB051051	–
<i>U. barbata</i>	Sweden: Västmanland, <i>Uliiska L-9261</i> (UPS)	KA 7	AJ457138	AF502257
<i>U. ceratina</i>	England: Devon, <i>Articus 606</i> (UPS)	KC 20	AJ457141	AF502260
<i>U. ceratina</i>	England: Somerset, <i>Articus 607</i> (UPS)	KC 21	AJ457142	AF502261
<i>U. chaetophora</i>	Sweden: Dalarna, <i>Thor 523</i> (Herb. G. Thor)	KCH 19	AJ748106	AJ748092
<i>U. filipendula</i>	Sweden: Uppland, <i>Articus 502</i> (UPS)	KFP 13	AJ457149	AF502268
<i>U. filipendula</i>	Scotland: East Lothian, <i>Coppins 519</i> (UPS)	KFP 18	AJ457150	AF502269
<i>U. florida</i>	Sweden: Östergötland, <i>Articus 428</i> (UPS)	KF 1	AJ457143	AF502262
<i>U. florida</i>	Sweden: Östergötland, <i>Articus 512</i> (UPS)	KS 6	AJ457156	AF502275
<i>U. florida</i>	Sweden: Östergötland, <i>Articus 423</i> (UPS)	KS 12	AJ457153	AF502276
<i>U. fragilescens</i>	Canada: British Columbia, <i>Articus 748</i> (UPS)	K 64	AJ748105	AJ748091
<i>U. fragilescens</i>	Canada: British Columbia, <i>Articus 740</i> (UPS)	K 71	AJ748104	AJ748090
<i>U. hirta</i>	Scotland: East Lothian, <i>Coppins 521</i> (UPS)	KH 24	AJ457151	AF502270
<i>U. pectinata</i>	Indonesia: Sumatra, <i>Ohmura 4373</i> (TNS)	–	AB051656	–
<i>U. pectinata</i>	Japan: Kyushu, <i>Ohmura 2989</i> (TNS)	–	AB051655	–
<i>U. rigida</i>	Austria: Steiermark, <i>de los Rios & Grube</i> (GZU)	KRI 47	AJ457152	AF502272
<i>U. wasmuthii</i>	England: Somerset, <i>Articus 652</i> (UPS)	KW 40	AJ457158	AF502277