Org Divers Evol (2016) 16:497–524
DOI 10.1007/s13127-016-0273-7

ORIGINAL ARTICLE

ORGANISMS DIVERSITY & EVOLUTION

Evaluation of traditionally circumscribed species in the lichen-forming genus *Usnea*, section *Usnea* (Parmeliaceae, Ascomycota) using a six-locus dataset

Kristiina Mark[1] · **Lauri Saag**[2] · **Steven D. Leavitt**[3] · **Susan Will-Wolf**[4] · **Matthew P. Nelsen**[5] · **Tiiu Tõrra**[6] · **Andres Saag**[1] · **Tiina Randlane**[1] · **H. Thorsten Lumbsch**[3]

Received: 22 September 2015 / Accepted: 1 February 2016 / Published online: 12 February 2016

Abstract Recent taxonomic and DNA sequence-based studies in several groups of lichen-forming fungi have revealed incongruence between the morphological and molecule-based circumscriptions of species. While the cosmopolitan genus *Usnea* is well-known and easily recognized by the yellowish beard-like thallus with central cord, delimitation of many *Usnea* species is difficult due to the high variation and complexity of diagnostic characters. In this study, we assessed the monophyly of 18 species from section *Usnea* occurring in North America and Europe, including sorediate and sexually reproducing taxa with both pendent and shrubby thalli. Six nuclear markers (ribosomal internal transcribed spacer (ITS) and intergenic spacer (IGS), and protein-coding beta-tubulin, MCM7, RPB1 and RPB2) were sequenced for 144 samples. All analyzed loci show weak genetic structure and short branch lengths in single-locus topologies, suggesting recent diversification history of the sampled taxa. Concatenated, multi-locus analyses conducted in Bayesian and maximum likelihood frameworks, as well as coalescent-based species delimitation and species tree methods, recover several distinct clades, some represent traditional morphology-based species (*Usnea cavernosa*, *U. praetervisa*, *U. silesiaca*, *U. wasmuthii*), while others form clusters of two or more species (*Usnea florida–U. subfloridana*, *U. fulvoreagens–U. glabrescens*, *U. barbata–U. chaetophora–U. dasopoga–U. diplotypus*, *U. barbata–U. intermedia–U. lapponica–U. substerilis*). We propose synonymization of *U. substerilis* under *U. lapponica*. The status of several other species within intermixed clusters requires further evaluation with more extensive sampling and the inclusion of more variable markers before taxonomic consequences can be considered. A new species, *Usnea parafloridana* is described from Wisconsin, USA.

Keywords Lichenized fungi · Rapid radiation · Species delimitation · Species trees · Taxonomy · *Usnea*

Electronic supplementary material The online version of this article (doi:10.1007/s13127-016-0273-7) contains supplementary material, which is available to authorized users.

✉ Kristiina Mark
kristiina.mark@ut.ee

[1] Institute of Botany and Ecology, University of Tartu, Tartu, Estonia

[2] Department of Evolutionary Biology, Estonian Biocentre, Tartu, Estonia

[3] Science and Education, The Field Museum, Chicago, IL, USA

[4] Estonia Department of Botany, University of Wisconsin-Madison, Madison, WI, USA

[5] Department of Geological Sciences, Stanford University, Stanford, CA, USA

[6] Estonian Marine Institute, University of Tartu, Tallinn, Estonia

Introduction

Incongruence between the morphological circumscriptions of species and phylogenetic delimitation based on DNA sequence data is known from several groups of lichen-forming fungi. In some cases, overestimation of the species diversity based on morphology and chemistry has been demonstrated (Velmala et al. 2009; Leavitt et al. 2011b; Saag et al. 2014; Velmala et al. 2014), while in others, new lineages, often representing undescribed species, have also been found (Wirtz et al. 2006; Wirtz et al. 2008; Lumbsch et al. 2011; Molina et al. 2011; Kraichak et al. 2015b; Singh et al. 2015). Finding and applying the appropriate character sets is one of the most challenging aspects in species delimitation

and can be especially difficult in groups with high morphological plasticity. Effectively integrating ecological, biogeographical, and other independent sources of data has provided more robust species delimitations than use of any single kind of data (Edwards and Knowles 2014).

Usnea Dill. ex Adans. represents an iconic example in which a lack of recognizable, diagnostic characters, and the use of homoplastic characters has led to the circumscription of many nonmonophyletic species (Clerc 1998). It is one of the largest genera in the family Parmeliaceae (Lecanorales, Ascomycota), comprising ca. 350 species (Thell et al. 2012). Its members are characterized by beard-like, finely branched pendent or erect thalli with a stiff central axis that is exposed when a branch is stretched and the cortex breaks apart. All *Usnea* species produce usnic acid in the cortex, giving the thallus a slightly yellow appearance. The monophyletic genus is well circumscribed, conspicuous, and easily recognized even by nonexperts, but the delimitation of many species in this genus is very difficult due to transitional forms and the complexity of diagnostic characters. This genus is also famous for its complicated taxonomy—more than 770 names have been published worldwide and about half of these could be considered synonyms (Clerc 1998), illustrating the necessity for taxonomic revisions in this group.

Several attempts to organize this species-rich genus into higher taxonomic units have been made based solely on morphological traits (Motyka 1936), or together with molecular characters (Ohmura 2001, 2002; Articus 2004a; Wirtz et al. 2006; Truong et al. 2013). Ohmura (2001, 2002) divided the genus *Usnea* into subgenera *Usnea*, *Dolichousnea*, and *Eumitria*. Articus (2004a) elevated these subgenera to generic rank and supported the earlier view of *Neuropogon* as a separate genus forming a monophyletic sister clade to the nonmonophyletic *Usnea* based on internal transcribed spacer (ITS) sequences. Other authors have instead retained either the three (Ohmura and Kanda 2004; Wirtz et al. 2006) or the four (Truong et al. 2013) groups at a subgeneric rank. The majority of *Usnea* species are included in the subgenus *Usnea,* which consists of sections (sect.) *Usnea*, *Ceratinea*, and according to earlier studies (Ohmura 2001, 2002; Ohmura and Kanda 2004), also *Neuropogon*. A multi-locus phylogenetic study by Truong et al. (2013) showed that *Neuropogon* forms a sister clade to subgenus *Usnea*, and the latter includes four groups, of which clades "Usnea-2" and "Usnea-4" correspond to previously defined sections *Usnea* and *Ceratinea*, respectively.

For this study, we focus on the sect. *Usnea*. It comprises a group of closely related species with a wide distribution across the Northern Hemisphere and includes the type species of the genus—*U. florida* (L.) Weber ex F.H. Wigg. This group consists of taxa with both pendulous and shrubby growth forms, and also both vegetatively (sorediate/isidiate) and sexually (apotheciate) reproducing species. The variation in lichen secondary metabolites is also high, with most species including more than one chemotype. Traditionally, thallus growth form (shrubby/subpendent/pendent), reproductive mode, ramification type, pigmentation of basal parts, presence of special structures on branches (papillae/tubercules/fibrils), morphology of soralia, presence of isidiomorphs, the morphology and thickness of the cortex, the medulla and the axis, and lichen chemistry have proven to be useful characters in delimiting taxa in *Usnea* (Clerc 2011). Many species exhibit transitional forms, and this has led to uncertainties in species boundaries and taxonomic confusion. Previous molecular studies, including species from sect. *Usnea*, have shown that some morphological species belonging in this group are monophyletic (Kelly et al. 2011; Saag et al. 2011; Truong et al. 2013); however, these analyses included only a few specimens per species. In contrast, the sequence-based study of Saag et al. (2011) evaluated the monophyly of nine shrubby species from sect. *Usnea* and found only *U. wasmuthii* Räsänen monophyletic—illustrating the extent of conflict between genetic data and traditional phenotypic species boundaries in sect. *Usnea*.

A rapid, postglacial radiation has been proposed to explain the short branch lengths and unresolved relationships between taxa in this group (Saag et al. 2011; Truong et al. 2013). Indeed, the genus *Usnea* is a rapidly evolving group, with exceptionally high speciation rates compared to many other genera in Parmeliaceae (Kraichak et al. 2015a). Estimating phylogenetic relationships and accurately delimiting species in recent rapid radiations can be difficult due to incomplete lineage sorting (ILS) and slow mutation rates in some markers relative to the speciation rate (Sistrom et al. 2013; Willis et al. 2013; Edwards and Knowles 2014; Saag et al. 2014; Velmala et al. 2014; Giarla and Esselstyn 2015). Even though coalescent-based species tree inferences account for ILS and hybridization, they typically require a priori assignment to putative species (Carstens et al. 2013). A priori assignment of species is difficult when phenotypic boundaries are unclear and/or they are in conflict with genetic data. In such cases, genetic clusters or clades, instead of phenotypic species, are used as putative species. However, this is problematic in young species complexes where low divergence of genetic markers and conflicts between loci are common (Saag et al. 2014; Velmala et al. 2014). In groups with recent and rapid diversification (Givnish 2015), vast amounts of genetic data are required to recover species (Wagner et al. 2013), but integrative approaches, including morphological, chemical, or geographical data, can also be effective in delimiting species (Edwards and Knowles 2014).

In our study, we use genetic, morphological, and chemical data to estimate phylogenetic relationships and delimit species boundaries in sect. *Usnea*. More specifically, we attempt to (1) reconstruct evolutionary relationships in sect. *Usnea* using DNA data from six markers, (2) identify evolutionarily independent lineages using multiple

coalescent-based species delimitation approaches, (3) evaluate the utility of some traditionally used characters, and (4) provide morphology-based perspectives of species in sect. *Usnea* in light of molecular data.

Material and methods

Taxon sampling and species identification

In total, over 200 samples from the genus *Usnea* were studied. In this paper, we present a dataset of 144 specimens from which sequences of the six studied markers were obtained. The dataset represents 18 phenotypically circumscribed species from section *Usnea* collected from different parts of Europe and North America, including specimens with both sorediate and sexual reproductive modes, as well as pendent and shrubby species. *Usnea ceratina* Ach., from section *Ceratinea*, was represented with two samples and was selected as an outgroup based on earlier studies (Ohmura 2001; Ohmura and Kanda 2004; Kelly et al. 2011; Truong et al. 2013). Voucher information for samples included in this study is presented in Table 1.

Morphological and anatomical characters of specimens were studied using an Olympus SZ51 stereomicroscope. Thallus chemistry was examined with standardized thin layer chromatography (TLC) or high-performance thin layer chromatography (HPTLC) using solvent system A (Orange et al. 2001). Specimen identifications of the collected material were based on morphological and chemical characters described in previous publications (Halonen et al. 1998, 1999; Halonen 2000; Clerc 2004; McCune 2005; Tõrra and Randlane 2007; Randlane et al. 2009; Clerc 2011). We followed the taxonomy of Randlane et al. (2009) for species known both in Europe and North America, and Halonen (2000) and Clerc (2011) for *U. pacificana* (Pacific North America) and *U. cylindrica* (Northern Europe), respectively, as these are not present in Randlane et al. (2009). The orthography of *Usnea dasopoga* follows Linda in Arcadia (2013). After preliminary identification, the morphology of specimens with poorly expressed diagnostic characters was rechecked in the light of phylogenetic analyses, and the determination was corrected, if reasonable. The thickness of the cortex (C) and medulla (M) was measured, and percentages of the whole width of the measured branch were calculated (CMA values; Clerc 1987) for *U. barbata* (L.) Weber ex F.H. Wigg., *U. chaetophora* Stirt., *U. cylindrica* P. Clerc, *U. dasopoga* (Ach.) Nyl., *U. diplotypus* Vain., *U. intermedia* Jatta, *U. lapponica* Vain., and *U. substerilis* Motyka. The measurements were plotted using the BoxPlotR online tool (Spitzer et al. 2014), available at http://boxplot.tyerslab.com. The degree of medullary density was evaluated visually under stereomicroscope following the categories as defined in Clerc (2011). Significant difference in structure thickness between studied morphological species and genetic clusters was tested with the nonparametric Kruskal-Wallis test using the online tool at http://www.mathcracker.com/kruskal-wallis.php.

Molecular methods

The newly collected samples were carefully examined under a stereomicroscope for the presence of lichenicolous and other contaminating fungi. About 5 mg of vegetative thallus from each specimen was ground in 2 ml microtubes. Total genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions, with an extra phase separation step using chloroform or alternatively using the Prepease DNA Isolation Kit (USB, Cleveland, Ohio, USA) following the plant leaf extraction protocol. Sequence data for six fungal nuclear markers were generated: two loci from the nuclear ribosomal cistron, the entire internal transcribed spacer (ITS) region and partial intergenic spacer (IGS); and fragments from four low-copy protein-coding genes, beta-tubulin (Bt), MCM7, and RPB1 and RPB2 (domains 6–7). Ready-To-Go PCR Beads (GE Healthcare, Pittsburgh, PA, USA) or 12.5 μl PCR Master Mix 2× (Fermentas) were used for polymerase chain reaction (PCR) amplifications, following the manufacturer's instructions. PCR cycling parameters for amplifying ITS, IGS, Bt, and RPB1 followed Wedin et al. (2009) and Schmitt et al. (2009) for MCM7. For RPB2, the cycling parameters were initial denaturation for 10 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 50 s at 50 °C, 1 min at 72 °C, and a final elongation for 10 min at 72 °C or touch-down PCR with initial denaturation for 4 min at 95 °C, followed by 6 cycles of 30 s at 95 °C, 30 s at 55–50 °C, 1 min at 72 °C, and 30 cycles of 30 s at 95 °C, 30 s at 49 °C, 1 min at 72 °C, and a final elongation for 5 min at 72 °C. Touch-down PCR following Lindblom and Ekman (2006) was also used in some cases for IGS, Bt, MCM7, RPB1 (IGS protocol of 55–40 °C) and ITS (IGS protocol of 66–56 °C). Amplification of low-copy genes MCM7, RPB1, and RPB2 usually required a nested PCR approach to obtain high-quality sequences. Using the newly generated RPB1 and RPB2 primers in the nested-PCR second step increased the PCR success of these genes to the level of other markers. Primers used in PCR amplifications and cycle sequencing temperature profiles are shown in Table 2. All PCR products were stained with ethidium bromide and visualized on a 1 % agarose gel. Successful PCR products were purified with ExoSAP-IT (USB, Ohio, USA) or via SAP/EXO treatment (Fermentas). Complementary strands of DNA were sequenced using the BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR amplifications. The products were run on ABI 3730xl DNA Analyzers (Applied

Table 1 List of studied taxa with their laboratory code, collection data, and GenBank accession numbers for the sequences from analyzed loci

Species	Laboratory code	Specimen locality	Collector and voucher information	GenBank accession number					
				ITS	IGS	Beta-tubulin	MCM7	RPB1	RPB2
U. barbata	BAR_05	Estonia	Tõrra, T. 03.07.2005 (TU32681)	KU352597	KU352443	KU352313	KU352157	KU352003	KU351853
U. barbata	BAR_06	Estonia	Tõrra, T. 15.10.2005 (TU32686)	KU352598	KU352444	KU352314	KU352158	KU352004	KU351854
U. barbata	BAR_07	Estonia	Tõrra, T. 14.10.2005 (TU32687)	KU352599	KU352445	KU352315	KU352159	KU352005	KU351855
U. barbata	BAR_11	Austria	Feuerer T. & Schultz R. 29.08.2007 (TU6824)	KU352601	KU352447	KU352317	KU352161	KU352007	KU351857
U. barbata	BAR_13	Spain	Tõrra, T. 27.09.2008 (TU)	KU352602	KU352448	KU352318	KU352162	KU352008	KU351858
U. barbata	BAR_16	Finland	Marmor, L. & Leppik, E. 15.06.2009 (TU)	KU352603	KU352449	KU352319	KU352163	KU352009	KU351859
U. barbata	BAR_17	USA, Oregon	Suija, A. 09.07.2008 (TU46335)	KU352604	KU352450	KU352320	KU352164	KU352010	KU351860
U. barbata	BAR_18	USA, California	Suija, A. 10.07.2008 (TU45118)	KU352605	KU352451	KU352321	KU352165	KU352011	KU351861
U. barbata	BAR_19	USA, Oregon	Suija, A. 10.07.2008 (TU45145)	KU352606	KU352452	KU352322	KU352166	KU352012	KU351862
U. barbata	BAR_26	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352607	KU352453	KU352323	KU352167	KU352013	KU351863
U. barbata	BAR_30	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352608	KU352454	KU352324	KU352168	KU352014	KU351864
U. barbata	BAR_31	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352609	KU352455	KU352325	KU352169	KU352015	KU351865
U. barbata	BAR_37	Italy	Tõrra, T. 09.06.2010 (TU)	KU352610	KU352456	KU352326	KU352170	KU352016	KU351866
U. barbata	CHE_09	USA, Oregon	Suija, A. 08.07.2008 (TU46320)	KU352622	KU352468	KU352338	KU352182	KU352028	KU351878
U. barbata	CHE_14	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352623	KU352469	KU352339	KU352183	KU352029	KU351879
U. barbata	CHE_16	Sweden	Tõrra, T. 11.08.2010 (TU)	KU352625	KU352471	KU352341	KU352185	KU352031	KU351881
U. barbata	diplotypus_02	Estonia	Tõrra, T. 14.10.2005 (TU32700)	JN086285	KU352475	JN086241	KU352189	KU352035	KU351885
U. barbata	diplotypus_07	Estonia	Tõrra, T. 14.05.2005 (TU33519)	JN086288	KU352478	JN086244	KU352192	KU352038	KU351888
U. barbata	FIL_37	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352632	KU352483	KU352349	KU352197	KU352043	KU351893
U. barbata/ dasopoga	diplotypus_11	Lithuania	Tõrra, T. 12.05.2006 (TU)	JN086289	KU352479	KU352345	KU352193	KU352039	KU351889
U. barbata/ dasopoga	FIL_05	Austria	Feuerer T. & Schultz R. 29.08.2007 (TU6881)	KU352629	KU352480	KU352346	KU352194	KU352040	KU351890
U. cavernosa	CAV_01	USA, Colorado	Nash III, T. H. 14.06.2005 (TU)	KU352611	KU352457	KU352327	KU352171	KU352017	KU351867
U. cavernosa	CAV_02	USA, Oregon	Suija, A. 09.07.2008 (TU45132)	KU352612	KU352458	KU352328	KU352172	KU352018	KU351868
U. cavernosa	CAV_03	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352613	KU352459	KU352329	KU352173	KU352019	KU351869
U. cavernosa	CAV_04	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352614	KU352460	KU352330	KU352174	KU352020	KU351870
U. cavernosa	CAV_05	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352615	KU352461	KU352331	KU352175	KU352021	KU351871
U. cavernosa	CAV_07	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352616	KU352462	KU352332	KU352176	KU352022	KU351872
U. cavernosa	CAV_08	USA, California	Mark, K. 14.12.2012 (TU)	KU352617	KU352463	KU352333	KU352177	KU352023	KU351873
U. ceratina	CER_02	UK	Tõrra, T. 06.10.2006 (TU66736)	KU352618	KU352464	KU352334	KU352178	KU352024	KU351874
U. ceratina	CER_05	Portugal	Tõrra, T. 30.09.2008 (TU66737)	KU352619	KU352465	KU352335	KU352179	KU352025	KU351875
U. chaetophora	CHE_07	Estonia	Tõrra, T. 18.05.2009 (TU)	KU352621	KU352467	KU352337	KU352181	KU352027	KU351877
U. chaetophora	HES_03	USA, California	Mark, K. 14.12.2012 (TU)	KU352640	KU352504	KU352362	KU352218	KU352064	KU351914
U. cf. cylindrica	CHE_15	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352624	KU352470	KU352340	KU352184	KU352030	KU351880
U. dasopoga	CHE_17	Sweden	Tõrra, T. 11.08.2010 (TU)	KU352626	KU352472	KU352342	KU352186	KU352032	KU351882

Table 1 (continued)

Species	Laboratory code	Specimen locality	Collector and voucher information	GenBank accession number					
				ITS	IGS	Beta-tubulin	MCM7	RPB1	RPB2
U. dasopoga	DIP_09	Estonia	Tõrra, T. 23.08.2006 (TU41763)	KU352627	KU352473	KU352343	KU352187	KU352033	KU351883
U. dasopoga	diplotypus_06	Estonia	Tõrra, T. 14.05.2005 (TU33520)	JN086287	KU352477	JN086243	KU352191	KU352037	KU351887
U. dasopoga	FIL_36	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352631	KU352482	KU352348	KU352196	KU352042	KU351892
U. dasopoga	FIL_40	Italy	Tõrra, T. 09.06.2010 (TU)	KU352633	KU352484	KU352350	KU352198	KU352044	KU351894
U. diplotypus	BAR_08	Canada, British Columbia	Sirp, S. 18.05.2007 (TU)	KU352600	KU352446	KU352316	KU352160	KU352006	KU351856
U. diplotypus	substerilis_06	Canada, British Columbia	Sirp, S. 18.05.2008 (TU)	JN086330	KU352537	JN086269	KU352250	KU352095	KU351943
U. florida	FLO_05	Spain	Tõrra, T. 27.09.2008 (TU)	KU352634	KU352485	KU352351	KU352199	KU352045	KU351895
U. florida	florida_01	UK	Tõrra, T. 07.10.2006 (TU)	JN086297	KU352487	KU352353	KU352201	KU352047	KU351897
U. florida	florida_02	UK	Tõrra, T. 07.10.2006 (TU)	JN086298	KU352488	JN086247	KU352202	KU352048	KU351898
U. florida	florida_03	UK	Tõrra, T. 06.10.2006 (TU)	JN086299	KU352489	JN086248	KU352203	KU352049	KU351899
U. fulvoreagens	FUL_04	Estonia	Tõrra, T. 14.10.2008 (TU)	KU352636	KU352490	KU352354	KU352204	KU352050	KU351900
U. fulvoreagens	fulvoreagens_01	Sweden	Tõrra, T. 20.07.2006 (TU)	JN086300	KU352492	JN086249	KU352206	KU352052	KU351902
U. fulvoreagens	fulvoreagens_02	UK	Tõrra, T. 06.10.2006 (TU)	JN086301	KU352493	KU352356	KU352207	KU352053	KU351903
U. fulvoreagens	fulvoreagens_05	Estonia	Tõrra, T. 09.09.2005 (TU32842)	JN086302	KU352494	JN086250	KU352208	KU352054	KU351904
U. fulvoreagens[a]	EDNA:09-02349	Ireland	Ward, S. 13.07.2009	JN943522	–	–	–	JN992570	–
U. fulvoreagens[a]	EDNA:09-02378	UK	Harrold, P. & Coppins, B.J. 02.12.2009	JN943513	–	–	–	JN992563	–
U. fulvoreagens[a]	LAP_33	USA, Oregon	Suija, A. 09.07.2008 (TU46315a)	KU352646	KU352512	KU352368	KU352226	KU352072	–
U. fulvoreagens[a]	LAP_34	USA, Oregon	Suija, A. 09.07.2008 (TU46327)	KU352647	KU352513	KU352369	KU352227	–	–
U. fulvoreagens[a]	LAP_35	USA, California	Suija, A. 11.07.2008 (TU46333)	KU352648	KU352514	KU352370	–	–	–
U. fulvoreagens[a]	Ohmura_2906	Japan	Ohmura, Y. 09.12.1996 (TNS)	AB051638	–	–	–	–	–
U. cf. fulvoreagens	USN_01	Portugal	Tõrra, T. 29.09.2007 (TU6873)	KU352664	KU352538	KU352388	KU352251	KU352096	KU351944
U. fulvoreagens[a]	USN_03	Portugal	Tõrra, T. 29.09.2007 (TU)	KU352665	–	KU352389	KU352252	–	KU351945
U. fulvoreagens[a]	WW_016	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14806)	KU352674	KU352554	KU352398	KU352268	KU352112	–
U. fulvoreagens	WW_073	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14829)	KU352691	KU352572	KU352417	KU352287	KU352131	KU351977
U. fulvoreagens	WW_079	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14835)	KU352695	KU352576	KU352421	KU352291	KU352135	KU351981
U. fulvoreagens	WW_142	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14855)	KU352706	KU352587	KU352432	KU352302	KU352146	KU351992
U. glabrescens	diplotypus_05	Estonia	Tõrra, T. 14.05.2005 (TU32698)	JN086286	KU352476	JN086242	KU352190	KU352036	KU351886
U. glabrescens[a]	EDNA:09-01569	UK	Harrold, P. 13.07.2009	JN943553	–	–	–	JN992597	–
U. glabrescens[a]	EDNA:09-02089	UK	Benfield, B. 01.08.2009	JN943543	–	–	–	JN992589	–
U. glabrescens[a]	EDNA:10-00744	UK	Yahr, R. 06.03.2010	JN943506	–	–	–	JN992556	–
U. glabrescens	glabrescens_01	Estonia	Tõrra, T. 25.08.2005 (TU32170)	JN086303	KU352497	JN086251	KU352211	KU352057	KU351907
U. glabrescens	glabrescens_02	Estonia	Tõrra, T. 02.10.2005 (TU32774)	JN086304	KU352498	KU352359	KU352212	KU352058	KU351908
U. glabrescens[a]	glabrescens_03	Estonia	Tõrra, T. 09.04.2005 (TU32775)	JN086305	KU352499	–	KU352213	KU352059	KU351909
U. glabrescens	glabrescens_14	Estonia	Tõrra, T. 30.08.2008 (TU)	JN086306	KU352500	KU352360	KU352214	KU352060	KU351910

Table 1 (continued)

Species	Laboratory code	Specimen locality	Collector and voucher information	GenBank accession number					
				ITS	IGS	Beta-tubulin	MCM7	RPB1	RPB2
U. glabrescens	glabrescens_15	UK	Tõrra, T. 07.10.2006 (TU)	JN086307	KU352501	KU352361	KU352215	KU352061	KU351911
U. glabrescens	glabrescens_16	Finland	Tõrra, T. 05.07.2007 (TU)	JN086308	KU352502	JN086252	KU352216	KU352062	KU351912
U. glabrescens	glabrescens_17	Finland	Tõrra, T. 10.07.2007 (TU)	JN086309	KU352503	JN086253	KU352217	KU352063	KU351913
U. glabrescens[a]	Ohmura_3824B	Japan	Ohmura, Y. 29.08.1997 (TNS)	AB051639	–	–	–	–	–
U. glabrescens	substerilis_01	Estonia	Tõrra, T. 01.09.2005 (TU32927)	JN086328	KU352535	JN086267	KU352248	KU352093	KU351941
U. cf. glabrescens	WAS_29	Switzerland	Tõrra, T. 27.02.2011 (TU)	KU352668	KU352541	KU352392	KU352255	KU352099	KU351948
U. intermedia	FLO_06	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352635	KU352486	KU352352	KU352200	KU352046	KU351896
U. intermedia	INT_15	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352641	KU352505	KU352363	KU352219	KU352065	KU351915
U. intermedia	INT_17	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352642	KU352506	KU352364	KU352220	KU352066	KU351916
U. intermedia	INT_20	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352643	KU352507	KU352365	KU352221	KU352067	KU351917
U. intermedia	INT_21	Italy	Tõrra, T. 09.06.2010 (TU)	KU352644	KU352508	KU352366	KU352222	KU352068	KU351918
U. intermedia	intermedia_02	Austria	Feuerer T. & Schultz R. 29.08.2007 (TU)	JN086314	KU352509	JN086256	KU352223	KU352069	KU351919
U. intermedia	intermedia_03	Austria	Feuerer T. & Schultz R. 29.08.2007 (TU)	JN086315	KU352510	JN086257	KU352224	KU352070	KU351920
U. lapponica	10_08	USA, Colorado	Leavitt, S.D. 11.08 (BRY-C)	KU352593	KU352439	KU352309	KU352153	KU351999	KU351849
U. lapponica	10_09	USA, Colorado	Leavitt, S.D. 11.08 (BRY-C)	KU352594	KU352440	KU352310	KU352154	KU352000	KU351850
U. lapponica	10_10	USA, Colorado	Leavitt, S.D. 11.08 (BRY-C)	KU352595	KU352441	KU352311	KU352155	KU352001	KU351851
U. lapponica	FUL_05	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352637	KU352491	KU352355	KU352205	KU352051	KU351901
U. lapponica	GLA_43	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352639	KU352496	KU352358	KU352210	KU352056	KU351906
U. lapponica	LAP_22	Spain	Tõrra, T. 28.09.2008 (TU6797)	KU352645	KU352511	KU352367	KU352225	KU352071	KU351921
U. lapponica	LAP_44	Italy	Tõrra, T. 09.06.2010 (TU)	KU352650	KU352516	KU352372	KU352229	KU352074	KU351923
U. lapponica	lapponica_05	Estonia	Tõrra, T. 15.10.2005 (TU32846)	JN086316	KU352517	KU352373	KU352230	KU352075	KU351924
U. lapponica	lapponica_07	Estonia	Tõrra, T. 02.10.2005 (TU32845)	JN086317	KU352518	JN086258	KU352231	KU352076	KU351925
U. lapponica	SBS_11	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352653	KU352521	KU352376	KU352234	KU352079	KU351927
U. lapponica	SBS_13	Switzerland	Tõrra, T. 27.02.2011 (TU)	KU352655	KU352523	KU352378	KU352236	KU352081	KU351929
U. lapponica	WAS_31	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352669	KU352542	KU352393	KU352256	KU352100	KU351949
U. pacificana[a]	PAC_01	USA, California	Mark, K. 14.12.2012 (TU)	KU352651	KU352519	KU352374	KU352232	KU352077	–
U. pacificana	substerilis_02	Canada, British Columbia	Sirp, S. 18.05.2007 (TU)	JN086329	KU352536	JN086268	KU352249	KU352094	KU351942
U. parafloridana	WW_002	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14801)	KU352670	KU352550	KU352394	KU352264	KU352108	KU351957
U. parafloridana[a]	WW_006	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14802)	KU352671	KU352551	KU352395	KU352265	KU352109	–
U. parafloridana	WW_013	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (F, WW14804)	KU352673	KU352553	KU352397	KU352267	KU352111	KU351959
U. parafloridana[a]	WW_018	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (TU, WW14807)	KU352675	KU352555	KU352399	KU352269	KU352113	–
U. parafloridana[a]	WW_021	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14808)	KU352676	KU352556	KU352400	KU352270	KU352114	KU351960
U. parafloridana[a]	WW_023	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14809)	–	KU352557	KU352401	KU352271	KU352115	KU351961
U. parafloridana	WW_025	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14811)	KU352678	KU352559	KU352403	KU352273	KU352117	KU351963

Table 1 (continued)

Species	Laboratory code	Specimen locality	Collector and voucher information	GenBank accession number					
				ITS	IGS	Beta-tubulin	MCM7	RPB1	RPB2
U. parafloridana	WW_030	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14812)	KU352679	KU352560	KU352404	KU352274	KU352118	KU351964
U. parafloridana[a]	WW_043	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14813)	–	–	KU352405	KU352275	KU352119	KU351965
U. parafloridana	WW_063	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14820)	KU352683	KU352564	KU352409	KU352279	KU352123	KU351969
U. parafloridana	WW_070	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14827)	KU352689	KU352570	KU352415	KU352285	KU352129	KU351975
U. parafloridana	WW_087	USA, Wisconsin	Will-Wolf, S. 28.08.2011 (WIS, WW14863)	KU352698	KU352579	KU352424	KU352294	KU352138	KU351984
U. parafloridana[a]	WW_148	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14857)	KU352707	–	KU352433	KU352303	KU352147	KU351993
U. parafloridana	WW_151	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14858)	KU352708	KU352588	KU352434	KU352304	KU352148	KU351994
U. parafloridana	WW_156	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14862)	KU352712	KU352592	KU352438	KU352308	KU352152	KU351998
U. praetervisa	DIP_15	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352628	KU352474	KU352344	KU352188	KU352034	KU351884
U. praetervisa	SUB_53	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352661	KU352529	KU352384	KU352242	KU352087	KU351935
U. praetervisa	SUB_60	Italy	Tõrra, T. 09.06.2010 (TU)	KU352663	KU352531	KU352386	KU352244	KU352089	KU351937
U. silesiaca	FIL_06	Portugal	Leis, M. 22.11.2008 (TU6829)	KU352630	KU352481	KU352347	KU352195	KU352041	KU351891
U. silesiaca	SIL_02	USA, California	Mark, K. 14.12.2012 (TU)	KU352658	KU352526	KU352381	KU352239	KU352084	KU351932
U. silesiaca	SIL_03	USA, California	Mark, K. 14.12.2012 (TU)	KU352659	KU352527	KU352382	KU352240	KU352085	KU351933
U. subfloridana	CHE_06	Spain	Tõrra, T. 28.09.2008 (TU)	KU352620	KU352466	KU352336	KU352180	KU352026	KU351876
U. subfloridana	SUB_17	Spain	Tõrra, T. 27.09.2008 (TU)	KU352660	KU352528	KU352383	KU352241	KU352086	KU351934
U. subfloridana	SUB_55	Switzerland	Tõrra, T. 05.02.2011 (TU)	KU352662	KU352530	KU352385	KU352243	KU352088	KU351936
U. subfloridana	subfloridana_01	Norway	Marmor, L. 12.07.2006 (TU)	JN102355	KU352532	KU352387	KU352245	KU352090	KU351938
U. subfloridana	subfloridana_05	Lithuania	Tõrra, T. 13.05.2006 (TU)	JN086326	KU352533	JN086265	KU352246	KU352091	KU351939
U. subfloridana	subfloridana_10	Finland	Tõrra, T. 10.07.2007 (TU)	JN086327	KU352534	JN086266	KU352247	KU352092	KU351940
U. subfloridana	WW_007	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14803)	KU352672	KU352552	KU352396	KU352266	KU352110	KU351958
U. subfloridana	WW_024	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14810)	KU352677	KU352558	KU352402	KU352272	KU352116	KU351962
U. subfloridana	WW_060	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14817)	KU352680	KU352561	KU352406	KU352276	KU352120	KU351966
U. subfloridana	WW_061	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14818)	KU352681	KU352562	KU352407	KU352277	KU352121	KU351967
U. subfloridana	WW_062	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14819)	KU352682	KU352563	KU352408	KU352278	KU352122	KU351968
U. subfloridana	WW_064	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14821)	KU352684	KU352565	KU352410	KU352280	KU352124	KU351970
U. subfloridana	WW_066	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14823)	KU352685	KU352566	KU352411	KU352281	KU352125	KU351971
U. subfloridana	WW_067	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14824)	KU352686	KU352567	KU352412	KU352282	KU352126	KU351972
U. subfloridana	WW_068	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14825)	KU352687	KU352568	KU352413	KU352283	KU352127	KU351973
U. subfloridana	WW_069	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14826)	KU352688	KU352569	KU352414	KU352284	KU352128	KU351974
U. subfloridana	WW_071	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14828)	KU352690	KU352571	KU352416	KU352286	KU352130	KU351976
U. subfloridana	WW_075	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14831)	KU352692	KU352573	KU352418	KU352288	KU352132	KU351978
U. subfloridana	WW_077	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14833)	KU352693	KU352574	KU352419	KU352289	KU352133	KU351979
U. subfloridana	WW_078	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14834)	KU352694	KU352575	KU352420	KU352290	KU352134	KU351980

Table 1 (continued)

Species	Laboratory code	Specimen locality	Collector and voucher information	GenBank accession number					
				ITS	IGS	Beta-tubulin	MCM7	RPB1	RPB2
U. subfloridana	WW_081	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14836)	KU352696	KU352577	KU352422	KU352292	KU352136	KU351982
U. subfloridana	WW_086	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14837)	KU352697	KU352578	KU352423	KU352293	KU352137	KU351983
U. subfloridana	WW_089	USA, Wisconsin	Will-Wolf, S. 28.08.2011 (WIS, WW14864)	KU352699	KU352580	KU352425	KU352295	KU352139	KU351985
U. subfloridana	WW_090	USA, Wisconsin	Will-Wolf, S. 28.08.2011 (WIS, WW14865)	KU352700	KU352581	KU352426	KU352296	KU352140	KU351986
U. subfloridana	WW_091	USA, Wisconsin	Will-Wolf, S. 28.08.2011 (WIS, WW14866)	KU352701	KU352582	KU352427	KU352297	KU352141	KU351987
U. subfloridana	WW_118	USA, Wisconsin	Will-Wolf, S. 27.08.2011 (WIS, WW14845)	KU352702	KU352583	KU352428	KU352298	KU352142	KU351988
U. subfloridana	WW_134	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14852)	KU352703	KU352584	KU352429	KU352299	KU352143	KU351989
U. subfloridana	WW_136	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14852)	KU352704	KU352585	KU352430	KU352300	KU352144	KU351990
U. subfloridana	WW_138	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14854)	KU352705	KU352586	KU352431	KU352301	KU352145	KU351991
U. subfloridana	WW_152	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14859)	KU352709	KU352589	KU352435	KU352305	KU352149	KU351995
U. subfloridana	WW_153	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14860)	KU352710	KU352590	KU352436	KU352306	KU352150	KU351996
U. subfloridana	WW_154	USA, Wisconsin	Nelsen, M.P. 27.08.2011 (WIS, WW14861)	KU352711	KU352591	KU352437	KU352307	KU352151	KU351997
U. substerilis	10_11	USA, Colorado	Leavitt, S.D. 11.08 (BRY-C)	KU352596	KU352442	KU352312	KU352156	KU352002	KU351852
U. substerilis	GLA_42	Italy	Tõrra, T. 09.06.2010 (TU)	KU352638	KU352495	KU352357	KU352209	KU352055	KU351905
U. substerilis	LAP_40	Switzerland	Tõrra, T. 26.02.2011 (TU)	KU352649	KU352515	KU352371	KU352228	KU352073	KU351922
U. substerilis	SBS_12	Switzerland	Tõrra, T. 27.02.2011 (TU)	KU352654	KU352522	KU352377	KU352235	KU352080	KU351928
U. substerilis	SBS_14	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352656	KU352524	KU352379	KU352237	KU352082	KU351930
U. substerilis	SBS_15	Switzerland	Tõrra, T. 17.05.2011 (TU)	KU352657	KU352525	KU352380	KU352238	KU352083	KU351931
U. wasmuthii	SBS_07	Spain	Tõrra, T. 28.09.2008 (TU)	KU352652	KU352520	KU352375	KU352233	KU352078	KU351926
U. wasmuthii	USN_09	Spain	Tõrra, T. 28.09.2008 (TU6842)	KU352666	KU352539	KU352390	KU352253	KU352097	KU351946
U. wasmuthii	WAS_18	Spain	Tõrra, T. 28.09.2008 (TU)	KU352667	KU352540	KU352391	KU352254	KU352098	KU351947
U. wasmuthii	wasmuthii_02	Estonia	Tõrra, T. 01.09.2005 (TU32929)	JN086331	KU352543	JN086270	KU352257	KU352101	KU351950
U. wasmuthii	wasmuthii_03	Estonia	Tõrra, T. 15.10.2005 (TU32928)	JN086332	KU352544	JN086271	KU352258	KU352102	KU351951
U. wasmuthii	wasmuthii_04	Estonia	Tõrra, T. 10.05.2005 (TU32931)	JN086333	KU352545	JN086272	KU352259	KU352103	KU351952
U. wasmuthii	wasmuthii_05	UK	Tõrra, T. 07.10.2006 (TU)	JN086334	KU352546	JN086273	KU352260	KU352104	KU351953
U. wasmuthii	wasmuthii_07	Estonia	Tõrra, T. 03.07.2005 (TU32933)	JN086335	KU352547	JN086274	KU352261	KU352105	KU351954
U. wasmuthii	wasmuthii_08	UK	Tõrra, T. 06.10.2006 (TU)	JN086336	KU352548	JN086275	KU352262	KU352106	KU351955
U. wasmuthii	wasmuthii_09	UK	Tõrra, T. 07.10.2006 (TU)	JN086337	KU352549	JN086276	KU352263	KU352107	KU351956

Sequence codes newly generated for this study are marked KU351849–KU352712

[a] Samples used in supplementary analyses only

Table 2 Primers used for PCR amplification and sequencing of the studied loci

Marker	Primer name	Direction	Primer sequence	Annealing temp. (°C)	Reference
ITS	ITS1F	F	5′-CTT GGT CAT TTA GAG GAA GTA A-3′	52–55 or 62–52 (touchdown)	Gardes and Bruns 1993
	ITS4	R	5′-TCC CCG CTT ATT GAT ATG C-3′		White et al. 1990
IGS	IGS12a	F	5′-AGT CTG TGG ATT AGT GGC CG-3′	66-56 (touchdown)	Carbone and Kohn 1999
	XIGS_R	R	5′-TAC TGG CAG AAT CAR CCA GG-3′		Leavitt et al. 2011b
	IGSf	F	5′-TAG TGG CCG WTR GCT ATC ATT-3	52–55 or 62–52 (touchdown)	Wirtz et al. 2008
	IGSr	R	5′-TGC ATG GCT TAA TCT TTG AG-3′		Wirtz et al. 2008
Bt	Bt2a	F	5′-GGT AAC CAA ATC GGT GCT GCT TTC-3′	52-55	Glass and Donaldson 1995
	Bt2b	R	5′-ACC CTC AGT GTA GTG ACC CTT GGC-3′		Glass and Donaldson 1995
MCM7	Mcm7-709for	F	5′-ACI MGI GTI TCV GAY GTH AAR CC-3′	56	Schmitt et al. 2009
	Mcm7-1348rev	R	5′-GAY TTD GCI ACI CCI GGR TCW CCC AT-3′		Schmitt et al. 2009
	X_Mcm7_F	F	5′-CGT ACA CYT GTG ATC GAT GTG-3′	56	Leavitt et al. 2011b
	X_Mcm7_R	R	5′-GTC TCC ACG TAT TCG CAT TCC-3′		Leavitt et al. 2011b
	LecMCM7f	F	5′-TAC CAN TGT GAT CGA TGY GG-3′	56	Leavitt et al. 2011a
	LecMCM7r	R	5′-GTC TCC RCG TAT TCG CAT NCC-3′		Leavitt et al. 2011a
RPB1	gRPB1-A for	F	5′-GAK TGT CCK GGW CAT TTT GG-3′	52-55	Matheny et al. 2002
	fRPB1-C rev	R	5′-CCN GCD ATN TCR TTR TCC ATR TA-3′		Matheny et al. 2002
	RP1C-uc1	R	5′-CRG CRA TRT CRT TRT CCA TRT A-3′		This study
	RPf-Usn3	F	5′-CTC GCA GTA CCY GTT TAC C-3′	55–49 (touchdown)	This study
	RPr-Usn2	R	5′-TGG CTC GAA CTC ATT SAC-3′		This study
RPB2	RPB2-6 F	F	5′-TGG GGK WTG GTY TGY CCT GC-3′	50	Liu et al. 1999
	fRPB2-7cr	R	5′-CCC ATR GCT TGY TTR CCC AT-3′		Liu et al. 1999
	RPB2-UsnF	F	5′-CTG CGG AAA CTC CTG AAG GC-3′	55–49 (touchdown)	This study
	RPB2-UsnR	R	5′-GGT AAG TRT TTC TAG GAG ACT G-3′		This study

F forward, *R* reverse

Biosystems) in the DNA Genotyping and Sequencing Core Facility of the Estonian Biocentre and Institute of Molecular and Cell Biology at the University of Tartu (Tartu, Estonia), or in the Pritzker Laboratory for Molecular Systematics at the Field Museum (Chicago, IL, USA).

Data analyses

Sequence alignment

Complementary sequence strands were viewed in 4Peaks (mekentosj.com), and assembled and edited in Sequencher v4.2 (Gene Codes Corp., Ann Arbor, Michigan, USA) and Mesquite v2.75 (Maddison and Maddison 2011). Sequence identity was checked using the "megaBLAST" function in GenBank (Madden 2002). Alignments were performed using the program MAFFT v7 (Katoh and Standley 2013) where the G-INS-i alignment algorithm (Katoh and Toh 2008) with "1PAM/K = 2" scoring matrix was implemented for all loci. For protein-coding genes, the offset value of 0.8 was applied. MEGA 5.1 (Tamura et al. 2011) was used to quantify basic parameters of genetic variability, including the number of variable and parsimony informative sites for the whole matrices and section *Usnea* only, and estimates of average evolutionary divergence within section *Usnea*. Standard error estimates were obtained by a bootstrap procedure (1000 replicates). Analyses were conducted using the Kimura 2-parameter model (Kimura 1980). The rate variation among sites was modeled with a gamma distribution (shape parameter = 6).

Single gene analyses

Single-locus alignments were analyzed using both maximum likelihood (ML) and a Bayesian approach. The ML criterion was implemented in the program RAxML v7.3.1 (Stamatakis 2006) where the evolutionary model for each single-locus dataset was set to GTRGAMMA and branch supports were assessed using 1000 "fastbootstrap" replicates (Stamatakis et al. 2008). Bayesian analyses (B/MCMC) were conducted using BEAST v1.7.2 (Drummond et al. 2012). DNA sequence evolution models for each marker were chosen in jModeltest v2.1.4 (Darriba et al. 2012) using the AIC criterion (Akaike

1974). A likelihood ratio test (LRT) was used to establish the applicability of a strict molecular clock, using clock and nonclock trees obtained in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012). In MrBayes default, priors were used except for the partition-specific rates prior that was set to "variable" (flat Dirichlet). Substitution models were set as chosen in jModeltest and are shown in Table 3. Two simultaneous analyses were run for 10 million generations, both with four chains and each starting from random trees. The LRT rejected a strict clock, and after preliminary tests with different settings, an exponential relaxed clock was applied in the BEAST analyses with a Yule model for branching events. Two independent BEAST analyses for each locus were run for 10 million generations, sampling every 1000th generation. We assessed convergence by examining likelihood plots and various statistics' effective sample sizes (ESS) values for various parameter estimates in Tracer v1.5 (Rambaut and Drummond 2007). The runs were combined in LogCombiner v1.7.4 (Rambaut and Drummond 2012a), with the first 10 % of trees discarded as burn-in. All ESS values for combined runs were over 200. Trees were summarized with TreeAnnotator v1.7.4 (Rambaut and Drummond 2012b) and visualized in FigTree v1.3.1 (Rambaut 2009).

Data congruence, recombination, and gene concatenation analyses

Gene trees do not always reflect species relationships due to gene tree estimation errors, introgression, gene paralogy, and/or deep coalescence (Maddison 1997; Edwards 2009; Knowles and Kubatko 2010). Concatenation allows better estimation of phylogenetic relationships and species boundaries (Wiens 1998; Leaché 2009) and proves especially important in cases of low variation in sequence data and low resolution of gene trees (Spinks et al. 2013; Willis et al. 2013). Before combining the datasets, agreement between single-locus trees was evaluated. First, trees were visually compared to check for topological differences between ML and B/MCMC approaches, and for strongly supported conflicts between the loci (Mason-Gamer and Kellogg 1996). A conflict was considered strong when supported by both tree construction methods (≥70 % for ML trees and ≥95 % for B/MCMC trees). Second, the recombination detection package RDP v4 (Martin et al. 2010) and the GARD method (Kosakovsky Pond et al. 2006), implemented in the Datamonkey server (Delport et al. 2010), were used to scan for possible recombination events in all matrices. The following methods in the RDP package were tested: RDP (Martin and Rybicki 2000), GENECONV (Padidam et al. 1999), Chimaera (Posada and Crandall 2001), Maxchi (Maynard Smith 1992), Bootscan (Gibbs et al. 2000; Martin et al. 2005), SiSscan (Weiller 1998; Gibbs et al. 2000), PhylPro (Weiller 1998), and 3Seq (Boni et al. 2007).

We then conducted ML and B/MCMC analyses on the concatenated six-locus dataset. In RAxML v7.3.1 (Stamatakis 2006; Stamatakis et al. 2008), each locus was treated as a separate partition with its own mutation rate and parameters. We used the same substitution models and parameters as in the single-locus analyses. Six independent B/MCMC analyses were run in BEAST v1.7.4 (Drummond et al. 2012), each for 200 million generations, sampling every 2000, and starting with a UPGMA tree. Convergence and burn-in length were assessed using Tracer v1.5. The first 10,000 trees were discarded as burn-in, and independent runs were combined in LogCombiner, resampling every 8000th tree (Rambaut and Drummond 2012a). A maximum clade credibility (MCC) tree, with posterior probabilities (PP) as branch support, was constructed in TreeAnnotator and visualized in FigTree. Alternatively, we conducted concatenated analyses for the dataset where strongly supported conflicts between gene trees (identified by visual comparison of trees) were discarded. For this, 17 samples were removed, and a 6-locus dataset of 127 specimens was analyzed in ML and B/MCMC approach. The same evolutionary models and analysis parameters as described above were used.

Table 3 Genetic variability of single locus and six-locus concatenated (combined) matrices, including number of sequences, total alignment length in basepairs (bp), number of variable and parsimony informative (PI) sites in full matrix and section *Usnea* only (given in brackets), estimates of average evolutionary divergence over sequence pairs given in substitutions per base pair with standard error (SE) within section *Usnea*, and model of evolution selected in JModelTest

Matrix	No. of seq.	Alignment (bp)	No. of variable sites (sect. *Usnea*)	No. of PI sites (sect. *Usnea*)	Mean distance in sect. *Usnea* (subst./bp ± SE)	Selected model
ITS	144	495	92 (81)	53 (49)	0.017 ± 0.003	SYM + G
IGS	144	354	97 (68)	69 (54)	0.026 ± 0.004	GTR + G
Bt	144	341	69 (53)	49 (39)	0.011 ± 0.002	K80 + G
MCM7	144	541	105 (90)	79 (71)	0.014 ± 0.002	SYM + G
RPB1	144	728	85 (61)	64 (49)	0.011 ± 0.002	SYM + I + G
RPB2	144	776	118 (86)	73 (55)	0.009 ± 0.002	GTR + G
Combined	864	3225	566 (439)	387 (317)	0.013 ± 0.001	

Species delimitation and phylogeny under the multispecies coalescent model

The multispecies coalescent model is a widely applied approach for species tree estimation, but more recently also for species delimitation (Yang and Rannala 2010; Jones et al. 2014; Yang and Rannala 2014). To estimate putative species in our study group, we implemented the package STACEY v1.0.1 (Jones 2015) in BEAST v2.2 (Bouckaert et al. 2014). The first part of this method works similarly to *BEAST (Heled and Drummond 2010), but with the difference that every individual can be treated as a potential species, and minimal split heights in species or minimal clusters tree (SMC-tree) are collapsed with a birth-death-collapse prior. This prior is controlled with CollapseHeight (ε) and CollapseWeight (ω) parameters, that were set in our analysis at 1.0E-4 and estimated $\beta(4;2)$, respectively. Substitution models and other parameters were set as in the BEAST analysis of the concatenated data as described above. Ten MCMC analyses were run for 200 million generations, sampling every 4000 generations. Parameter ESS values were above 130. The resulting SMC-trees were combined in LogCombiner, resampling every 8000 after removing 10 % as burn-in. A maximum clade credibility tree with PP support values was constructed in TreeAnnotator and visualized in FigTree. Assignment of individuals into minimal clusters was then assessed in SpeciesDelimitationAnalyser (Jones et al. 2014) where collapseHeight was set to one fraction lower than ε ($\tau = 0.001$). The output of posterior probabilities of individuals belonging in the same cluster was visualized in a similarity matrix constructed in R v2.15.1 (R Core Team 2014).

Additionally, we conducted species tree analysis implemented in BEAST v1.8.0 (*BEAST; Heled and Drummond 2010). *BEAST is widely used in constructing species trees but requires the user to assign samples a priori to putative lineages (Carstens et al. 2013). We used results from STACEY and gene concatenation analyses to delineate candidate species. Not only all strongly supported (PP ≥ 0.95) minimal clusters from STACEY but also the weakly supported lineages "glabrescens" (PP = 0.81) and "pacificana" (n.a.), with 8 and 1 accessions, respectively, were considered as candidate species. Altogether, 13 candidate species were delimited (excl. outgroup) and assigned in the *BEAST analysis. Independent evolutionary models were set for each locus (see Table 3) with an uncorrelated relaxed exponential clock (Drummond et al. 2006). We selected a Yule tree prior and "piecewise linear with constant root" as the population size model. Two independent MCMC analyses were run for 300 million generations, sampling every 5000 steps and excluding the first 90 million generations of each run as burn-in. Convergence of runs was assessed in Tracer v1.5; ESS of parameters were all above 200, and the trees from two runs were summarized using LogCombiner and TreeAnnotator. An MCC tree with PP support values was constructed in TreeAnnotator and visualized in FigTree.

To test species boundaries in the studied *Usnea* taxa, the marginal posterior probabilities of speciation events were calculated for the 13 minimal clusters or species identified in the STACEY analysis using the program BP&P v3 (Yang and Rannala 2014). The method uses reversible-jump Markov chain Monte Carlo (rjMCMC) algorithms to estimate the posterior distribution of different species delimitation models. BP&P accommodates the species phylogeny, represented by a user-specified guide tree, and accounts for lineage sorting due to ancestral polymorphism. BP&P v3 includes the nearest-neighbor interchange (NNI) algorithm that allows changes in species tree topology (Yang and Rannala 2014). The species trees from *BEAST analyses were used to generate a fully resolved guide tree. We used both algorithms 0 and 1 in BP&P. Theta (θ) and tau (τ) gamma priors were assessed using species tree analyses and were set to $G(2, 259)$ and $G(2, 57)$ corresponding to distribution means of 0.0077 and 0.0351, respectively. Preliminary analyses were run with various fine-tuning parameters to find appropriate values. Each rjMCMC analysis was run for 200,000 generations sampling every five with a burn-in of 20,000 and run at least twice to confirm consistency of results.

Results

Molecular data

We were able to generate high-quality sequences of all six loci for 144 specimens, resulting in 864 sequences; these specimens were used for the molecular analyses reported here. Six additional specimens of the new species *Usnea parafloridana* had sequences of five loci (Table 1); they are included in the reports of morphology and chemistry but not in the reported molecular analyses. Online Resources 2 and 3 include concatenated phylogenies of the *Usnea fulvoreagens* and *U. glabrescens* clade, and *U. parafloridana* clade, respectively, where additional specimens with incomplete sequence data (one to five sequenced loci) have been added (specimens included in Table 1). Sequences generated for this study have been deposited in GenBank under accession nos. KU351849–KU352712 (Table 1) and full single locus matrices and trees in TreeBASE, under study number S18645 (https://treebase.org/). The combined dataset for phylogenetic analyses included 3225 aligned nucleotides (ITS 495 bp, IGS 354 bp, Bt 341 bp, MCM7 541 bp, RPB1 728 bp, RPB2 776 bp). The summary statistics of studied loci with their genetic variability are given in Table 3. The overall genetic variability in all loci was low considering that 19 species from different locations in Europe and North America were included. The highest number of variable sites was recovered in RPB2 ($n = 118$),

although both the RPB2 and RPB1 markers gave the lowest percentage of parsimony informative (PI) sites compared to the matrix length ($n = 73$, 9.4 % and $n = 64$, 8.9 %, respectively). MCM7 had the highest number of PI sites ($n = 79$) but relative to the marker's length, IGS was the most informative from the studied loci ($n = 69$, 19.5 %). In sect. *Usnea*, the highest overall mean genetic distance was in IGS (0.026 subst./bp) and the lowest was in the protein-coding genes RPB2 (0.009 subst./bp), Bt (0.011 subst./bp), and RPB1 (0.011 subst./bp). Sect. *Usnea* with 142 sequences included 317 PI sites in the concatenated matrix, constituting 9.8 % of the full length, and the average evolutionary divergence over sequence pairs was estimated 0.013 subst./bp.

Data congruence and recombination

No credible recombination events were detected within or between loci with any tested recombination detection methods. By visual comparison, no strong conflicts were found between inference methods, but several conflicts were found between loci (see Online Resource 1 for single-locus trees). As the nodal support in individual gene topologies was generally low, most of the conflicts were only weakly supported and were often within delimited clades. Concatenated analysis where strongly supported conflicts between gene trees were discarded resulted in similar topology, but with even weaker branch supports. We concluded that data exclusion diminished tree construction power more significantly than strongly supported conflicts distort phylogeny and proceeded with the full dataset of 144 taxa.

Single-locus and concatenated gene trees

The single-locus gene trees inferred from ML analyses together with nonparametric bootstrap probability (BP) and posterior probability (PP) values from Bayesian inference are given in Online Resource 1 (Fig. S1–S6). Clades with BP ≥ 70 and PP ≥ 0.95 were considered strongly supported. In general, individual gene trees showed weak genetic structure and short branch lengths for all studied loci. Similar results with no significant topological or branch support differences were obtained with both applied inferences. Nodal supports in gene trees for significant clades from the concatenated analyses are summarized in Table 4.

The majority-rule consensus tree with posterior probabilities from the Bayesian six-locus concatenation analysis applied in BEAST, together with nonparametric ML bootstrap support from RAxML, is shown in Fig. 1. Combining loci allowed better differentiation among samples. Even though the backbone of the tree remains poorly resolved, several strongly supported groups are revealed: (1) the *Usnea cavernosa* clade (BP = 100; PP = 1); (2) the *U. silesiaca* clade (BP = 100; PP = 1); (3) a clade of specimens from Wisconsin, USA, with distinct morphology and chemistry that do not clearly fit to any of the currently accepted species and, in this paper, are described as a new shrubby *Usnea* species, *U. parafloridana* (BP = 100; PP = 1; see the "Taxonomy" section for detailed description of morphology and chemistry of this species); (4) the *U. wasmuthii* clade (BP = 94; PP = 1); (5) the fulvoreagens-glabrescens clade (BP = 100; PP = 1) that includes the species *U. fulvoreagens*, *U. glabrescens* and *U. pacificana*; (6) the florida-subfloridana clade (BP = 100; PP = 1) with *U. florida* and *U. subfloridana*; (7) the *U. praetervisa* clade (BP = 100; PP = 1); (8) the barbata-chaetophora-dasopoga-diplotypus clade that includes specimens with the morphology of *U. barbata* (in part), *U. chaetophora*, *U. cylindrica*, *U. dasopoga*, and *U. diplotypus* (PP = 0.78); and (9) the barbata-intermedia-lapponica-substerilis clade that includes mainly specimens with the morphology of *U. barbata* (in part), *U. intermedia*, *U. lapponica*, and *U. substerilis* (BP = 100; PP = 1).

In the fulvoreagens-glabrescens clade, two strongly supported subclades are present. The clade fulv-1 consists of *U. fulvoreagens* specimens from Portugal and Wisconsin (USA) and positions at the base of the clade. A second subclade includes a strongly supported group of *U. fulvoreagens* (four specimens) from northern Europe (fulv-2), *U. glabrescens* (eight specimens), and *U. pacificana* (one specimen). "Fulv-1" and "fulv-2" are both strongly supported in the concatenated tree (BP = 100; PP = 1), while the internal structure of the second subclade, and especially the monophyly of *U. glabrescens*, remains questionable. Florida-subfloridana is divided into two subclades, "subflo-1" and "florida-subloridana s.str." Subflo-1 consists of a few *U. subfloridana* specimens from North America and positions at the base of the florida-subfloridana clade. Florida-subfloridana s.str. gathers all *U. florida* and other *U. subfloridana* samples. The latter subclade is not monophyletic in any of the gene trees but forms a distinct group in the six-locus concatenated tree with high support from BEAST analysis (BP = 63; PP = 0.96). The substructure inside this clade is weakly supported and inconsistent through different analyses. The monophyletic and strongly supported *U. praetervisa* clade is positioned with low support at the base of the barbata-chaetophora-dasopoga-diplotypus clade in the concatenated tree (BP = 65; PP = 0.78). The structure inside the barbata-chaetophora-dasopoga-diplotypus clade is weakly supported and does not support separation of any included traditional species. The barbata-intermedia-lapponica-substerilis clade is sister clade to the barbata-chaetophora-dasopoga-diplotypus and praetervisa clades. This relationship is weakly supported in RPB2 (BP = 35; PP = 0.76) and in BEAST concatenated (PP = 0.62) trees. Several weakly supported subclades are present inside this clade that seem to partly correlate with morphology (clades that include taxa with irregular, excavate to concave soralia = *U. lapponica*

Table 4 Support of single locus analyses on major clades from six-locus concatenated tree (Concat) and STACEY SMC-tree (STACEY)

	Cavernosa		Silesiaca		Parafloridana		Wasmuthii		Fulvoreagens-glabrescens						Florida-subfloridana				Praetervisa		Barbata-chaetophora-dasopoga-diplotypus		Barbata-intermedia-lapponica-substerilis	
									Fulvoreagens-1		Fulvoreagens-2		Glabrescens		Subfloridana-1		Florida-subfloridana s.str.							
ITS	**99**	**1**	*39*	*0.73*	–	–	*57*	*0.91*	*69*	**1**	**99**	**1**	–	–	**99**	**1**	–	–	**83**	**0.99**	–	–	–	–
IGS	–	*0.44*	**86**	**0.98**	–	–	–	–	**100**	**1**	**99**	**1**	–	–	**99**	**1**	–	–	*65*	**0.95**	–	–	*49*	**0.95**
Bt	*42*	*0.49*	–	–	**100**	**1**	–	–	66	**0.99**	**97**	**0.99**	–	–	–	0.3	–	–	–	–	–	–	–	–
MCM7	–	–	–	–	–	–	–	–	**88**	*0.94*	*64*	**0.95**	–	–	**100**	**1**	–	–	**91**	**1**	–	–	*44*	*0.62*
RPB1	**94**	**1**	**99**	**1**	–	–	–	–	–	–	*58*	*0.33*	*67*	**0.96**	–	–	–	–	–	–	**83**	**1**	–	–
RPB2	–	*0.59*	**100**	**1**	*42*	*0.86*	–	–	–	–	*51*	*0.68*	–	–	–	0.17	–	–	–	–	–	–	–	–
Concat	**100**	**1**	**100**	**1**	**100**	**1**	**94**	**1**	**100**	**1**	**100**	**1**	–	–	**100**	**1**	*63*	**0.96**	**100**	**1**	–	*0.78*	**100**	**1**
STACEY	**1**		**1**		**1**		**1**		**1**		**1**		*0.81*		**1**		**0.99**		**1**		**0.99**		**0.99**	

Cells are given clade support from maximum likelihood (bootstrap probabilities, BP) and Bayesian (posterior probabilities, PP) analyses, in the first and second column of each clade accordingly. For STACEY, Bayesian posterior probabilities of SMC-tree clades are given. Bold cells mark monophyly with strong branch support (BP ≥ 70, PP ≥ 0.95) and italic cells monophyly with weak support (BP < 70, PP < 0.95). Cells with no emphasis indicate for nonmonophyly

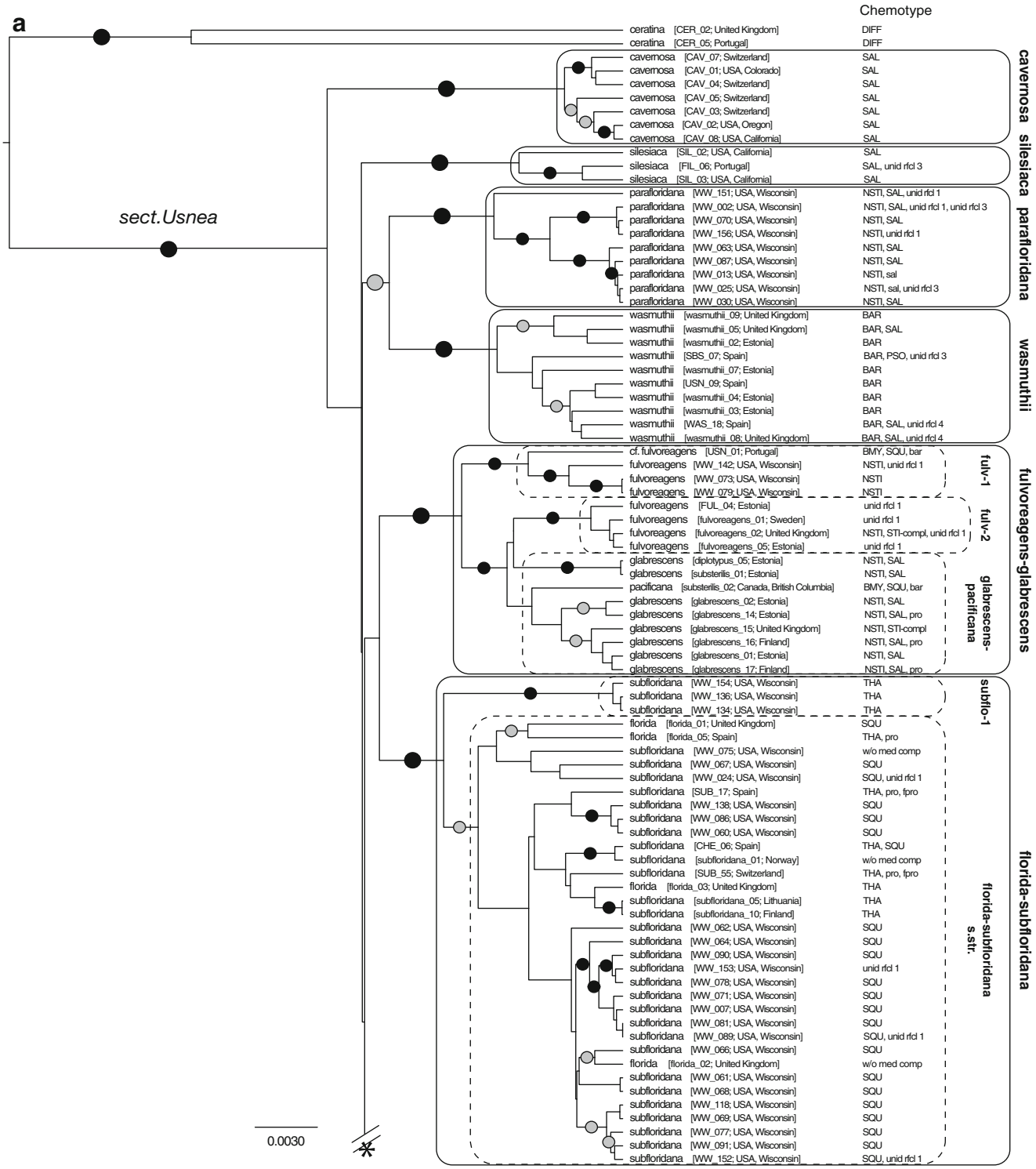

Fig. 1 Bayesian 50 % consensus tree of 18 currently accepted *Usnea* species based on six concatenated loci, inferred by BEAST. Major groups and secondary chemistry are indicated right of the tree. Branch supports are given in *circles*: *Black circles* reflect strong support from both inferences (PP ≥ 95 % for BEAST and BP ≥ 70 % for RAxML), and *gray circles* strong support from BEAST only. Location and laboratory code are given in *brackets*. *Scale bar* shows the number of substitutions per site. Secondary metabolites: *BAR* barbatic acid, *BMY* baeomycesic acid, *DIFF* diffractic acid, *FPRO/fpro* fumarprotocetraric acid, *NSTI* norstictic acid, *PAN* pannaric acid, *pan6* pannaric acid-6-methylester, *PRO/pro* protocetraric acid, *PSO* psoromic acid, *SAL* salazinic acid, *SQU* squamatic acid, *STI* stictic acid, *STI-comp* stictic acid complex with connorstictic cryptostictic acids, *THA* thamnolic acid, *w/o med comp* without medullary compounds, *unid rfcl x* unidentified substance from reference class x. *Capital letters* denotes major compounds in chemotype; *lower case* denotes accessory substances

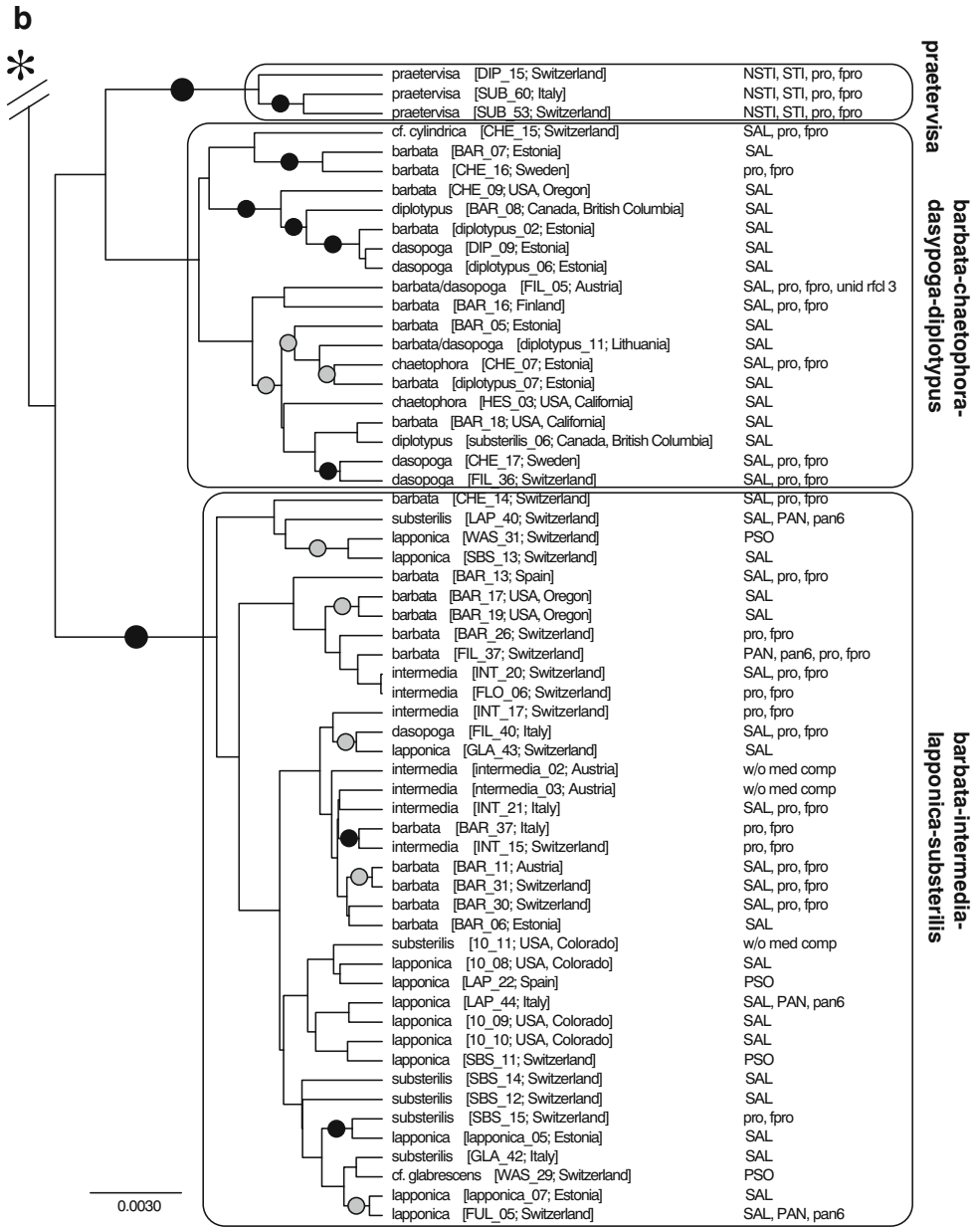

Fig. 1 continued.

and *U. substerilis*, or clades including taxa with apothecia or small punctiform soralia with isidiomorphs = *U. barbata* and *U. intermedia*).

Morphological and chemical characters

Multi-locus, concatenated ML and B/MCMC analyses clustered specimens of *Usnea barbata*, *U. chaetophora*, *U. cylindrica*, *U. dasopoga*, *U. diplotypus*, *U. intermedia*, *U. lapponica*, and *U. substerilis* into two closely related clades, where one constituted species mostly with a relatively thick cortex and a thin, compact medulla (i.e., *Usnea chaetophora*, *U. cylindrica*, *U. dasopoga*), while specimens in the other had a relatively thin cortex with a thick and more lax medulla (*U. intermedia*, *U. lapponica*, *U. substerilis*). Specimens of *U. barbata* were divided between these two clades (Fig. 1). We measured the CMA values and evaluated medullary density of specimens in these two clades and found that members of the barbata-chaetophora-dasopoga-diplotypus clade had a significantly thicker cortex ($p = 0.0001$) and thinner medulla ($p = 0.0007$), compared to barbata-intermedia-lapponica-substerilis specimens. Such significant differences were also found in *U. barbata* samples—specimens related to *U. dasopoga* had a significantly thicker cortex ($p = 0.0059$) and thinner medulla ($p = 0.0206$). The groups differed also in medulla density, where the majority

of specimens in the barbata-intermedia-lapponica-substerilis clade had a lax medulla, while those in the barbata-chaetophora-dasopoga-diplotypus clade formed a compact medulla. A similar trend was observed in *U. barbata* specimens from different clades. Box plots illustrating the differences in cortex and medulla thickness, and bar charts showing the distribution of medulla density categories between genetic groups are shown in Fig. 2. The percentages of cortex (C) and medulla (M) thickness were as follows: [(minimum) average ± standard deviation (maximum)] for barbata-dasopoga-chaetophora-diplotypus clade C = [(9.3) 13.2 ± 2.7 (19.2)], M = [(10) 15.1 ± 3.4 (24)], (n = 21); barbata-intermedia-lapponica-substerilis clade C = [(4.1) 8.3 ± 2.1 (15)], M = [(8.2) 20.1 ± 6.6 (47.6)], (n = 44); *U. barbata* related to *U. dasopoga* C = [(9.5) 13.2 ± 2.9 (19.2)], M = [(10.3) 15.6 ± 2.9 (18.9)], (n = 12); and *U. barbata* related to *U. intermedia* C = [(4.1) 8.0 ± 2.2 (12.1)], M = [(9.5) 18.6 ± 5.4 (27.4)], n = 14). Morphological species within the clades did not have significantly different CMA values and are not described here (additional information in Table 5).

Chemotypic variation was detected in most of the species studied (Table 5). We do not discuss the variation in accessory substances (e.g., protocetraric acids) or unidentified substances (ref. class 1, 3 and 4) where several new combinations were identified. We concentrate on the major compounds and their distribution over clades from multi-locus analyses. In general, chemotypic variation had some correlation with genetic clusters in sect. *Usnea* (Fig. 1, Table 5). Salazinic acid was the most dominant compound—it was present as the major compound in *U. cavernosa*, *U. silesiaca*, and *U. glabrescens*, in the barbata-chaetophora-dasopoga-diplotypus clade, and in the barbata-intermedia-lapponica-substerilis clade, and as a minor substance in *U. wasmuthii* and *U. parafloridana*. Besides salazinic acid and chemotypes with psoromic (in *U. lapponica*) and pannaric acid, usually together with pannaric acid-6-methylester and/or salazinic acid (in *U. barbata*, *U. substerilis*, and *U. lapponica*), were found in the barbata-intermedia-lapponica-substerilis clade. Norstictic acid was the major compound in *U. praetervisa* (in our data together with stictic acid) and *U. parafloridana* (often together with salazinic acid), and was dominant in the fulvoreagens-glabrescens clade. In this clade, a chemotype with squamatic (major), baeomycesic (major), and barbatic acid (minor) was present from *U. pacificana* and *U. cf. fulvoreagens*. In the florida-subfloridana clade, chemotypes with either squamatic acid or with thamnolic acid were both

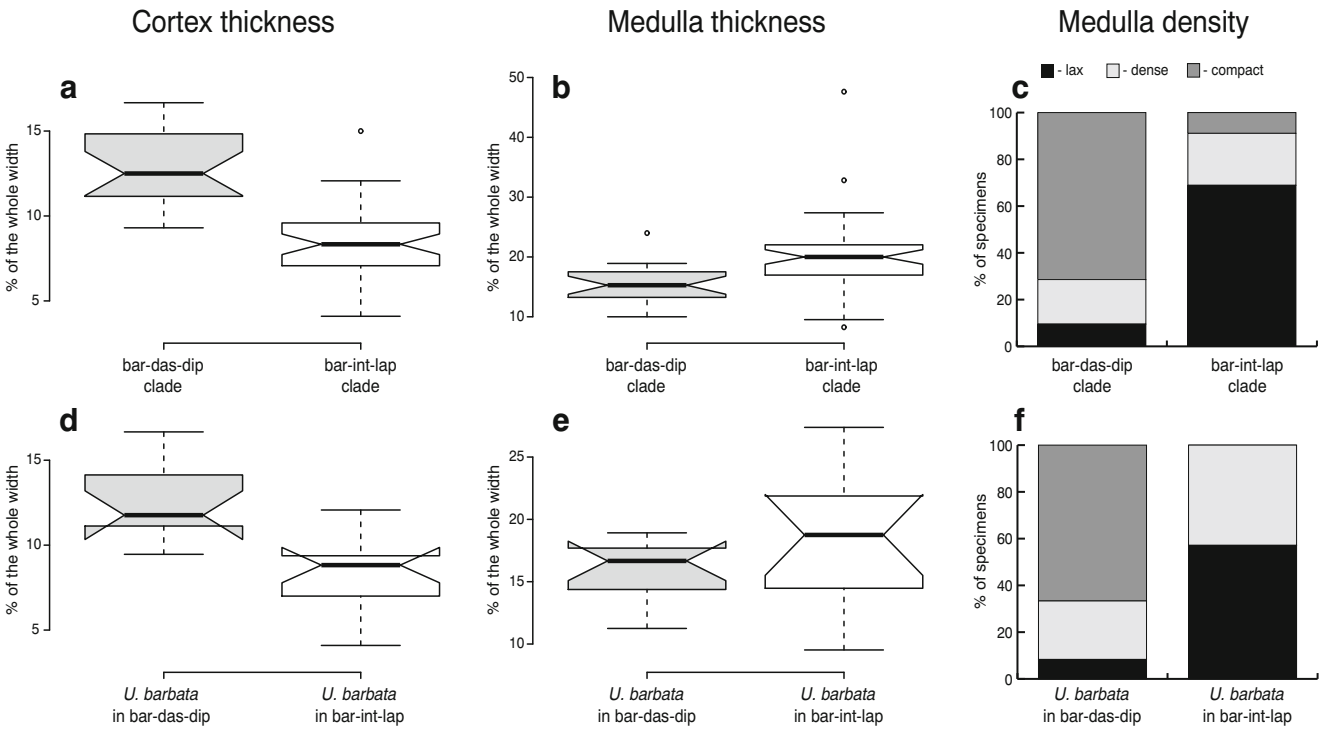

Fig. 2 *Box plots* of cortex (**a**, **d**) and medulla (**b**, **e**) thickness measurements and bar charts of medulla density categories (**c**, **f**) in clades barbata-chaetophora-dasopoga-diplotypus (bar-das-dip) and barbata-intermedia-lapponica-substerilis (bar-int-lap; first row of charts), and between *Usnea barbata* specimens from these clades (second row of charts). *Box plots* show the percentages of the whole width of the measured branch. *Center lines* are the medians, *box limits* indicate the 25th and 75th percentiles as determined by R software, *whiskers* extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; notches are defined as ±1.85*interquartile range/sqrt(n), which gives roughly 95 % confidence that two medians are different. *Bar charts* show the distribution of medulla density categories between genetic groups, given in percentage of specimens for density group. Sample points for data are, bar-das-dip clade, n = 20; bar-int-lap clade, n = 43; *U. barbata* in bar-das-dip clade, n = 11; *U. barbata* in bar-int-lap clade, n = 13

Table 5 Summary table of diagnostic characters of analyzed morphospecies in sect. *Usnea*, listed according to the major clades as seen in Figs. 1 and 3 (clades separated by a solid line)

Clade	Morphological species	Chemotypes	Thallus	Base colour	Ramification	Branches	Fibrils	Papillae	Soralia	Isidiomorphs	Cortex thickness	Medulla thickness	Medulla density
Cavernosa	*U. cavernosa* Tuck. 1850	**(1) Salazinic acid (a.)**; (2) No medullary compounds	Pendent	Pale	Isotomic-dichotomic	Foveolate and ridged	Absent to scarce	Absent	Absent	Absent	Thin	Thick	Lax to dense
Silesiaca	U. silesiaca Motyka 1930	**(1) Salazinic ± [a]unidentified substance (Rf class 3 in A)**	Shrubby to pendent	Blackened	Isotomic-dichotomic	Richly branched, interwoven	Few to numerous	Present	Transversely elliptical to irregularly rounded	Present on young soralia	Very thick (9–14 %)	Very thin (7–12 %)	Compact
Parafloridana	*U. para-floridana* sp. nov. Mark et al.	**(1) Norstictic ± salazinic, unidentified substances (Rfcl 1 & 3 in A)**	**Shrubby**	**Blackened**	**Isotomic-dichotomic**	**Few and thick branches**	**Sparse**	**Abundant**	**Punctiform to enlarged**	**Numerous and relatively long**	**Thick (9–15 %)**	**Thin (10.5–13 %)**	**Dense to compact**
Wasmuthii	*U. wasmuthii* Räsänen 1931	**(1) Barbatic ± protocetraric, 4-*O*-demethylbarbatic, psoromic, [a]unidentified substance (Rfcl 3 in A);** (2) Salazinic ± protocetraric, 4-*O*-demethylbarbatic, psoromic; **(3) Barbatic and salazinic;** (4) Thamnolic ± squamatic (possible hybrid); (5) No medullary compounds	Shrubby	Blackened	Isotomic-dichotomic	Richly branched, gradually tapering	Few to numerous	Few to numerous	Punctiform to enlarged, oblong-cylindrical, slightly excavate	Present on young soralia	Thick (8–12 %)	Thin (10–18 %)	Dense to compact
Fulvoreagens-glabrescens	*U. fulvoreagens* (Räsänen) Räsänen 1935	**(1) Norstictic ± stictic a. complex, protocetraric, diffractaic;** (2) Norstictic and salazinic ± stictic a. complex, protocetraric, diffractaic; (3) Norstictic and squamatic; **(4) Unknown substance (Rfcl 2 in A);** (5) No medullary compounds; **[a](6) Squamatic andbaeomycesic ± -barbatic**	Shrubby	Blackened	Isotomic-dichotomic	Richly branched, gradually tapering	Numerous	Abundant	Irregular, excavate when mature	Absent	Thick	Rather thin	Lax to dense
	U. glabrescens (Nyl. ex Vain.) Vain. 1925	**(1) Norstictic and salazinic ± stictic a. complex, protocetraric; (2) Norstictic ± stictic a. complex; (3) Psoromic (possible hybrid);** (4) No medullary compounds	Shrubby	Blackened	Isotomic-dichotomic	Richly branched, gradually tapering	Present at basal parts	Present on main branches	Punctiform when young, later larger, usually rounded and discrete	Present on young soralia	Thick (8–12 %)	Thin (13–23 %)	Dense to compact
	U. pacificana Halonen 2000	**(1) Squamatic and baeomycesic ± -barbatic, 4-*O*-demethylbarbatic**	Shrubby to subpendent	Blackened	Anisotomic-dichotomic	Richly branched, gradually tapering	Sparce	Present on main branches	Punctiform	Present on young soralia	Thick (11-14-19 %)	Thin (4-14-20 %)	Dense

Table 5 (continued)

Clade	Morphological species	Chemotypes	Thallus	Base colour	Ramification	Branches	Fibrils	Papillae	Soralia	Isidiomorphs	Cortex thickness	Medulla thickness	Medulla density
Florida-subfloridana	*U. florida* (L.) Weber ex F.H. Wigg. 1780	**(1) Thamnolic ± alectorialic, [a]protocetraric; (2) Squamatic;** (3) Salazinic ± norstictic (possible hybrid); [a]**(4) No medullary compounds**	Shrubby to subpendent	Blackened	Isotomic-dichotomic	Richly branched	Numerous	Abundant	Absent, apothecia frequent	Absent	Thick (9-13 %)	Thin (11-20 %)	Dense to compact
	U. subfloridana Stirt. 1882	**(1) Squamatic ± alectorialic, [a]protocetraric; (2) Thamnolic ± alectorialic, [a]unidentified substance (Rfcl 1 in A);** (3) Squamatic and thamnolic; [a]**(4) Unidentified substance (Rfcl 1 in A); [a](5) No medullary compounds**	Shrubby to subpendent	Blackened	Isotomic-dichotomic	Richly branched	Present	Abundant	Punctiform to enlarged	Numerous	Thick (8-12 %)	Thin (11-20 %)	Dense to compact
Praetervisa	*U. praetervisa* (Asahina) P. Clerc 2004	(1) Norstictic and connorstictic; [a]**(2) Norstictic and stictic ± protocetraric**	Shrubby to subpendent	Blackened	Isotomic-dichotomic	Richly branched	Present	Present	Punctiform	Numerous on young soralia	Thick (12-20 %)	Thin (6-16 %)	Variable
Barbata-chaetophora-dasopoga-diplotypus	*U. chaetophora* Stirt. 1883	**(1) Salazinic ± [a]protocetraric;** (2) No medullary compounds	Pendent	Blackened	Anisotomic-dichotomic	Divided by annular cracks into regular segments	Absent or scarce	Absent or scarce	Few, punctiform	Absent or scarce	**Thick (10-16 %)**	**Thin to moderately thick (15-26 %)**	**Dense to compact**
	U. cylindrica P. Clerc 2011	**(1) Salazinic ± protocetraric**	Pendent	Blackened	Anisotomic-dichotomic	Distinctly cylindrical and parallel, filamentose	Numerous	Absent to few	Punctiform or enlarging	Frequent	Thick (9-16 %; **13 %**)	Thin to moderately thin (10-19 %; **13 %**)	Dense to compact
	U. dasopoga (Ach.) Nyl. 1876	**(1) Salazinic ± protocetraric;** (2) No medullary compounds	Pendent	Blackened	Anisotomic-dichotomic	Richly branched, gradually tapering	Numerous	Sparse to abundant	Punctiform	Present	**Thick (13-17 %)**	**Thin (10-14 %)**	**Dense to compact**
	U. diplotypus Vain. 1925	**(1) Salazinic ± protocetraric, 4-*O*-demethylbarbatic, alectorialic**	Shrubby to subpendent	Pale or blackened	Anisotomic-dichotomic	Uneven in thickness, often with depressions and/or ridges	Present, variable	Present, variable	Punctiform	Numerous and relatively long	**Moderately thick (9-11 %)**	**Thin (14-16 %)**	**Variable**
	U. barbata (L.) Weber ex F.H. Wigg. 1780	**(1) Salazinic ± protocetraric;** (2) No medullary compounds; [a]**(3) Protocetraric**	Pendent	Pale or blackened	Anisotomic-dichotomic	Uneven in thickness, often with depressions and/or ridges	Few to numerous	Variable	Punctiform and irregular	Present	**Thick to very thick (11-17 %)**	**Thin (13-18 %)**	**Dense to compact**
Barbata-intermedia-lapponica-substerilis	*U. barbata* (L.) Weber ex F.H. Wigg. 1780	**(1) Salazinic ± protocetraric;** (2) No medullary compounds; [a]**(3) Protocetraric; [a](4)**	Pendent	Pale or blackened	Anisotomic-dichotomic	Uneven in thickness, often with depressions	Few to numerous	Variable	Punctiform and irregular	Present	**Moderately thin to moderately**	**Thin to moderately thin (13-24 %)**	**Lax to dense**

Table 5 (continued)

Clade	Morphological species	Chemotypes	Thallus	Base colour	Ramification	Branches	Fibrils	Papillae	Soralia	Isidiomorphs	Cortex thickness	Medulla thickness	Medulla density
		Pannaric ± protocetraric				and/or ridges					**thick (6-10 %)**		
	U. intermedia Jatta 1909	**(1) Salazinic ± [a]protocetraric; (2) Protocetraric; (3) No medullary compounds**	Shrubby to pendent	Pale or blackened	Anisotomic-dichotomic	Uneven in thickness, often with depressions and/or ridges	Numerous	Abundant	Absent, apothecia frequent	Absent	**Thin to moderately thin (5-8 %)**	**Moderately thin to thick (18-37 %)**	**Lax to dense**
	U. lapponica Vain. 1925	**(1) Salazinic+/− protocetraric, barbatic, caperatic; (2) Psoromic;** (3) No medullary compounds; [a]**(4) Pannaric and salazinic**	Shrubby	Pale to blackened	Anisotomic-dichotomic	Uneven in thickness, often with depressions and/or ridges	Numerous	Abundant	Large, becoming expanded and irregular or bracelet-like, flat to deeply concave	Absent	**Moderately thin to moderately thick (7-11 %)**	**Moderately thin (17-23 %)**	**Lax to dense**
	U. substerilis Motyka 1936	**(1) Salazinic ± protocetraric, barbatic, 4-*O*-demethylbarbatic;** (2) No medullary compounds; [a]**(3) Pannaric and salazinic;** [a]**(4) Protocetraric**	Shrubby	Pale to blackened	Anisotomic-dichotomic	Uneven in thickness, often with depressions and/or ridges	Numerous	Abundant	Irregular, slightly tuberculate to slightly excavate	Present on young soralia	**Moderately thin to moderately thick (7-10 %)**	**Thin to moderately thin (11-22 %)**	**Lax to dense**

Chemotypic variation includes known chemotypes from literature and from analyzed specimens. Chemotypes identified in analyzed specimens are marked in bold. The dominant character states, as described in literature (except for data of *U. parafloridana* and anatomical data for the clades "barbata-chaetophora-dasopoga-diplotypus" and "barbata-intermedia-lapponica-substerilis") are given, but variation in most characters, intermediates, and hybrids are possible. Literature data are from Halonen 2000, Randlane et al. 2009, and Clerc 2011

[a] New chemotypes or previously unlisted substances for the species

present, but they did not correlate with morphological species or subclades based on molecular data.

Species delimitation and validation analyses under the multispecies coalescent model

A maximum clade credibility SMC-tree, with PP supports from STACEY analysis together with a similarity matrix showing posterior probabilities of individuals belonging in the same cluster, is shown in Fig. 3. The structure and support of the SMC-tree are discussed in more detail under the next paragraph. Minimal distinct clusters in the STACEY analysis that formed monophyletic and strongly supported clades (except pacificana and glabrescens with weaker support) in the SMC-tree were considered as candidate species for subsequent species validation and species tree analyses. These 13 clusters are shown in Fig. 3 and include cavernosa (PP = 1), silesiaca (PP = 1), wasmuthii (PP = 1), parafloridana (PP = 1), subfloridana-1 (PP = 1), florida-subfloridana s.str. (PP = 0.99), fulvoreagens-1 (PP = 1), fulvoreagens-2 (PP = 1), pacificana (n.a.), glabrescens (PP = 0.81), praetervisa (PP = 1), barbata-chaetophora-dasopoga-diplotypus (PP = 0.99), and barbata-intermedia-lapponica-substerilis (PP = 0.99). BP&P results support the presence of 13 groups delimited with highest posterior probability (PP = 0.99204). Posterior probabilities of all delimited lineages were 1.00000 except for pacificana and glabrescens (PP = 0.99204). The 12-species scenario, where pacificana and glabrescens were clustered, was supported weakly (PP = 0.00796).

Phylogenetic relationships in species tree analyses

The STACEY SMC-tree corresponded with the *BEAST maximum clade credibility species tree, and therefore, only the SMC-tree is shown together with PP supports from STACEY (S-PP) and *BEAST (*PP) analyses (Fig. 3). In species tree analysis, section *Usnea* formed a monophyletic group on a long branch (S-PP = 0.99; *PP = 1). The backbone of the tree was not supported, and overall supports for the relationships among the putative species remained weak just as in the single-gene analyses and the concatenated multilocus phylogeny. *Usnea cavernosa* and *U. silesiaca* positioned

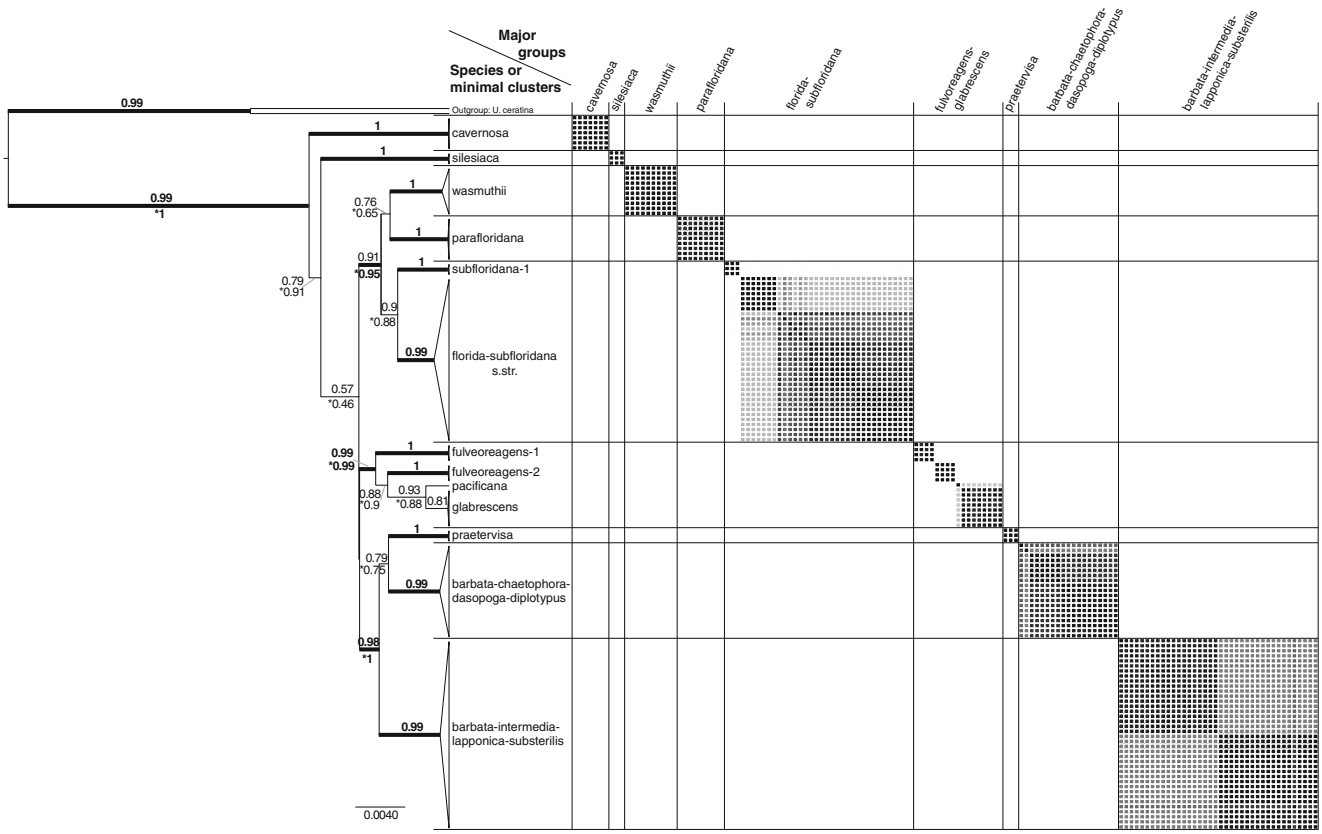

Fig. 3 STACEY maximum clade credibility SMC-tree with posterior probabilities (PP) from STACEY (*above branches*) and *BEAST (*below*) analyses together with similarity matrix for the section *Usnea* dataset. The *squares* represent posterior probabilities (*white* = 0, *black* = 1) for pairs of individuals to belong to the same cluster. The *lines* in the matrix separate major groups (named above matrix), while labels next to the cartooned clades of the SMC-tree represent the 13 species or minimal clusters delimited in STACEY analysis. Braches and probabilities scores with strong support (PP ≥ 0.95) from STACEY and/or *BEAST analysis are marked in *bold*. *Scale bar* shows the number of substitutions per site

more distantly, closer to the base of the section *Usnea* with weak support. Three strongly supported groups formed in the sect. *Usnea*. (1) The florida-subfloridana clade clustered with wasmuthii and parafloridana (S-PP = 0.91; *PP = 0.95). Inside this clade, florida-subfloridana s.str. and subfloridana-1 clustered together with low support (S-PP = 0.9; *PP = 0.88), and wasmuthii grouped with parafloridana at low support (S-PP = 0.76; *PP = 0.65). (2) The fulvoreagens-glabrescens clade was strongly supported (S-PP = 0.99; *PP = 0.99), but branch supports for candidate species inside this clade remained low. (3) The putative species barbata-intermedia-lapponica-substerilis clustered, with strong support, with barbata-chaetophora-dasopoga-diplotypus and praetervisa (S-PP = 0.98; *PP = 1), and inside, candidate species barbata-chaetophora-dasopoga-diplotypus and praetervisa clustered together with lower support (S-PP = 0.79; *PP = 0.75).

Discussion

Traditionally used diagnostic taxonomic characters and circumscribed species in light of molecular data

Here, we utilize a six-locus dataset to evaluate traditional, morphology-based species circumscription in sect. *Usnea*. The genus *Usnea* is considered one of the most taxonomically difficult macrolichen genera, as a majority of its species are highly variable in morphology and chemistry, and some traditionally used characters have proven to be homoplasious (Clerc 1998; Wirtz et al. 2012). The general appearance of specimens, e.g., whether being pendulous or shrubby and whether bearing apothecia or reproducing vegetatively, has played an important role in species identification. However, thallus length can greatly vary with age and with environmental conditions. Also, reproductive mode can vary between lineages and transitions between sexuality and asexuality, which may occur (Scherrer et al. 2005; Tehler and Irestedt 2007; Cornejo et al. 2009). Furthermore, asexual lineages have been reported as co-occurring with fertile species in the genus *Usnea* (Articus et al. 2002; Wirtz et al. 2008; Saag et al. 2011). Finally, several generally accepted species from sect. *Usnea* have been suggested as morphotypes or ecotypes (i.e., *Usnea chaetophora* as a morphotype of *U. barbata* and *U. dasopoga*; *U. diplotypus* as the epilithic form of *U. dasopoga*; *U. fulvoreagens* as the morphotype of *U. glabrescens* where most of the soralia are deeply excavate; Clerc 2011).

Eighteen morphologically circumscribed species from sect. *Usnea* were included in our study, but only four of them—*Usnea cavernosa*, *U. praetervisa*, *U. silesiaca*, and *U. wasmuthii*—were recovered as monophyletic in phylogenetic analyses, while others formed clusters of two or more species (*Usnea florida–U. subfloridana*, *U. fulvoreagens–U. glabrescens–U. pacificana*; *U. barbata–U. chaetophora–U. cylindrica–U. dasopoga–U. diplotypus; U. barbata–U. intermedia–U. lapponica–U. substerilis*). Our data suggest that using most diagnostic morphological characters together with branch anatomy and thallus chemistry are useful for delimiting of some genetic lineages in sect. *Usnea*, while other clades (e.g., barbata-chaetophora-dasopoga-diplotypus and barbata-intermedia-lapponica-substerilis) have very wide morphological variation (Table 5), and many currently accepted diagnostic morphological characters do not prove useful for delimiting these clades. Despite limited genetic variation, genotyping might be useful for separating groups in sect. *Usnea* and identifying morphological intermediates, juvenile forms, or subgroups/cryptic species within clades. At the same time, this study demonstrates that several traditional, morphology-based species are in need of reevaluation to specify their actual species boundaries.

Our study supports the view that several nominal species included in sect. *Usnea* could merely represent intraspecific phenotypes. The barbata-chaetophora-dasopoga-diplotypus clade includes several morphospecies (i.e., *U. barbata*, *U. chaetophora*, *U. diplotypus*, *U. cylindrica*) that do not separate genetically or chemically. Current data suggest conspecificity of *U. chaetophora*, *U. diplotypus*, and *U. cylindrica* to *U. dasopoga*, but better sampling of these morphotypes and reevaluation of *U. barbata* are needed for official taxonomic changes.

Usnea barbata is also present in the barbata-intermedia-lapponica-substerilis clade, together with fertile *U. intermedia*, and sorediate *U. lapponica* and *U. substerilis*. This aggregate is characterized by thin cortex, lax, and thick medulla, not clearly tapering and often foveolate branches, and salazinic acid as the most common strain (see Table 5 for reference to morphospecies). Our analyses show weak clustering of *U. intermedia* with *U. barbata* accessions and separation from *U. lapponica* and *U. substerilis*. Conspecificity of *U. intermedia* and *U. barbata* is also supported by old herbarium material where *U. barbata* is more often fertile and very much resembles contemporary collections of *U. intermedia* (Articus 2004b). *Usnea lapponica* and *U. substerilis* are considered similar and closely related in morphological studies, differing primarily in the shape of soralia and production of isidiomorphs (Halonen et al. 1998; Randlane et al. 2009; Clerc 2011). Separation of these species is often difficult as intermediate forms occur. Our study supports the close relationship between the phenotypic species. The species show no clear differentiation in anatomy or secondary chemistry, and are intermixed in a cluster in our genetic analyses. The differences in morphology could be explained with the development of soralia and erosion of isidiomorphs. These findings suggest conspecificity of *U. lapponica* and *U. substerilis*, and we propose synonymization of *U. substerilis* under *U. lapponica* (see the "Taxonomy" section below).

The analyzed species in sect. *Usnea* have wide distributional ranges, often occurring across the Northern Hemisphere, but they show a low degree of geographical structure within the phylogeny. Instead, secondary metabolites seem to mostly corroborate the phylogenetic clades recovered from our data (Fig. 1). Lichen secondary chemistry has been suggested as an important systematic character in other lichen-forming fungi (Schmitt and Lumbsch 2004; Elix et al. 2009; Spribille et al. 2011), and also in genus *Usnea*, it is considered important in species delimitation (Clerc 1998; Truong et al. 2013). However, very high chemotypic variation within morphology-based entities may indicate the need for phylogenetic reevaluation of species boundaries as was demonstrated in *Usnea cornuta* s. l. (Truong et al. 2013). Our data suggest the need for reevaluation of some chemotypes in species (1) *U. glabrescens*, (2) *U. pacificana*, and (3) *U. wasmuthii*.

Usnea glabrescens as currently circumscribed with four different chemotypes (see Table 5) does not form a monophyletic group in our analyses. A single specimen of *U.* cf. *glabrescens* with psoromic acid (WAS_29; Switzerland) clustered in the barbata-intermedia-lapponica-substerilis clade. The specimen was examined carefully to rule out an identification mistake, but the morphology, anatomy, and chemistry were identical with *U. glabrescens*. The rest of the specimens in this study have chemotypes with norstictic acid and position in the fulvoreagens-glabrescens clade. We suppose that psoromic-acid containing specimens of *U. glabrescens* could be hybrids.

Usnea pacificana is morphologically similar to *U. subfloridana* but has a unique chemistry containing baeomycesic, barbatic, and squamatic acids. In our analyses, the single specimen of this species positions in the fulvoreagens-glabrescens clade, in the vicinity of *U. glabrescens* specimens. Sequence data of five loci from an additional specimen collected from California, USA (PAC_01), support the close relationship between *U. pacificana* and *U. glabrescens* showing more than 99 % pairwise similarity over the five loci with analyzed *U. glabrescens* s. str. accessions (see Online Resource 2 for concatenated gene tree of fulvoreagens-glabrescens clade with additional samples having incomplete data). The *Usnea pacificana*-specific chemotype with baeomycesic acid may be more widespread in the fulvoreagens-glabrescens clade as it was also found in *U. cf. fulvoreagens* (USN_01) from Portugal.

Five different chemotypes have been described in *Usnea wasmuthii*, including chemotypes with barbatic acid, salazinic acid, and thamnolic acid (Table 5). The presence of barbatic acid as the major compound was consistent in our *U. wasmuthii* samples and might be a useful character in identification of the wasmuthii clade. Salazinic acid occurs widely in many other clades in sect. *Usnea*, and thamnolic acid is generally restricted to the florida-subfloridana clade. Unusual chemotypes could be the result of several factors, including introgression, hybridization, and contaminations from sample handling or misidentifications.

The phenomenon, where otherwise indistinguishable lichen species differ only in reproduction mode, is known as the species-pair concept, first developed by Poelt (1970). A species-pair includes the "primary," sexually reproducing species and the "secondary," vegetatively reproducing species. Several species-pairs have been identified in the genus *Usnea* (i.e., *Usnea florida–U. subfloridana*; *U. intermedia–U. barbata–U. lapponica*; *U. aurantiaco-atra–U. antarctica*; *U. perpusilla–U. sphacelata*; *U. trachycarpa–U. subantarctica*), but phylogenetic studies suggest that many of those should be considered conspecific (Articus et al. 2002; Articus 2004b; Seymour et al. 2007; Saag et al. 2011; Wirtz et al. 2012). Our results are in accordance with previous research and do not support independence of the studied species-pairs.

Usnea florida and *U. subfloridana* have been considered a species-pair where *U. florida* is the primary, fertile counterpart and *U. subfloridana* the sterile, secondary counterpart (Clerc 1984). Previous phylogenetic analyses have demonstrated that they do not form a species-pair but instead a clade in which they are intermixed (Articus et al. 2002; Kelly et al. 2011; Saag et al. 2011). Our analyses support synonymy of *U. florida* and *U. subfloridana*; however, we do not propose official taxonomic changes due to conservation reasons caused by ecological requirements of these entities (*U. florida* preferring old deciduous trees in areas with high humidity is threatened or near threatened in many European countries, while *U. subfloridana* is less ecologically demanding and widespread).

Halonen et al. (1998) suggested that *Usnea rigida* (Ach.) Röhl. (now synonymized under *U. intermedia*) could be regarded as the fertile and primary counterpart in the *U. rigida* aggregate that includes the sterile and only rarely fertile *U. barbata*, *U. lapponica*, and *U. substerilis*. Later, *U. intermedia* was proposed as the sexually reproducing counterpart for the sorediate *U. lapponica* (Saag et al. 2011). Our study suggests conspecificity of *U. barbata* and *U. intermedia*, but further studies in the *U. barbata* species complex are needed before taxonomic consequences could be considered (see also the discussion on this group above).

Usnea thallus anatomy has been used to delimit morphological species (Clerc 1998; Clerc 2011). The parameters of the inner structure of a branch (CMA values) were shown to be informative in identification of monophyletic lineages in genus *Usnea* (Seymour et al. 2007). This is also supported by our data where species with similar ratios for CMA tend to cluster together (clades with *Usnea barbata* and related species in Fig. 1b). *Usnea barbata* is very polymorphic; Randlane et al. (2009) have suggested that it may consist of a collection of intergrading taxa. Indeed, the species is polyphyletic in our study forming two groups different in anatomical features (Fig. 2), but at the same time, these characters are

partly overlapping and do not allow separation of intermediate forms or "hybrids" unanimously.

Several distinct lineages that do not correspond to previously described morphological taxa were detected within our data—(1) a new shrubby species *Usnea parafloridana*, (2) a potential cryptic species within *U. fulvoreagens*, and (3) a strongly supported subclade of *U. subfloridana*.

Specimens with distinct morphology and chemistry from Wisconsin (USA) form a monophyletic clade that represents an undescribed species, proposed in this paper as *Usnea parafloridana* sp. nov. This taxon is characterized by a shrubby thallus, with relatively few and thick branches. The type of soralia is most similar to *U. subfloridana*—punctiform to enlarged and bearing many short isidiomorphs. It is closely related to *U. wasmuthii* that, however, differs in its medullary chemistry and soralium morphology. For detailed species description and discussion, see the "Taxonomy" section below.

Usnea fulvoreagens is morphologically and chemically similar to *U. glabrescens*, and the two species cluster together in genetic analyses (Kelly et al. 2011; Saag et al. 2011). The first species is regarded as a morphotype of *U. glabrescens* in Clerc (2011). In our study, *U. fulvoreagens* in the current sense is polyphyletic, forming two strongly supported clades within the fulvoreagens-glabrescens clade, but being distinct from *U. glabrescens*. Both of these clades include specimens from Europe and North America (see Online Resource 2 for phylogeny with additional samples), have chemotypes with norstictic acid, and are morphologically similar. However, the two clades are genetically different enough to represent cryptic species, confirmed also in BP&P analyses.

Clade subfloridana-1 includes *U. subfloridana* specimens from North America with thamnolic acid (squamatic acid in other North American specimens); it was clearly distinct in the STACEY species delimitation analysis and was not collapsed with other florida-subfloridana in BP&P. However, more data are needed before any strong conclusions can be made.

Gene congruence and species delimitation in a young species complex

The genus *Usnea* is considered a hyper-diverse group, showing exceptionally high speciation rates compared with many other genera in the family Parmeliaceae (Kraichak et al. 2015a). The success could be explained by key innovations (Sanderson and Donoghue 1996)—such as a pendulous thallus with a central axis and the production of usnic acid in the cortex to better exploit habitats and protect the photobiont from high radiation (McEvoy et al. 2006; Trest et al. 2015). At the same time, individual groups within the genus *Usnea* are characterized by low genetic variation and unresolved relationships among species (Lumbsch and Wirtz 2011; Saag et al. 2011; Truong et al. 2013). Our data support the previous findings and show little variation and weak phylogenetic signal in the studied loci (ITS, IGS, beta-tubulin, MCM7, RPB1, RPB2) at this phylogenetic scale. Furthermore, strongly supported conflicts were found between our gene trees. Single-locus gene trees proved insufficient to delimit evolutionary groups in sect. *Usnea* and some clades (e.g., barbata-intermedia-lapponica-substerilis clade, florida-subfloridana clade) gained significant branch support only in the multilocus analyses (e.g., barbata-intermedia-lapponica-substerilis clade, florida-subfloridana clade). Even so, it is evident that more genetic markers, preferably in highly variable genome regions, are necessary to construct species trees in young diverging groups. Low support along the "backbone" of sect. *Usnea* probably reflects rapid diversification. Similar high morphological divergence with low genetic variation was demonstrated in genus *Bryoria* Brodo & D. Hawksw., section *Implexae* (Velmala et al. 2014). It is possible that both genera, *Bryoria* and *Usnea*, are undergoing the rapid diversification for similar reasons. Both have similar pendant habits in which thalli are minimally attached to the substrate and expand into unoccupied space.

Older species have had time to accumulate apomorphies and gene-tree monophyly, while young species often lack monophyly due to ILS. Few apomorphies compared to synapomorphic mutations and conflicting gene trees make inferring the species tree and delimiting species especially challenging in young species complexes (Saag et al. 2014). By visual comparison, several conflicts were found between our gene trees. However, as the nodal support in individual gene topologies was generally low, most of the conflicts were only weakly supported and were often within delimited clades. These can be explained with weak or conflicting phylogenetic signal that generates trees with short branch lengths and low support values. Considering the low divergence in our loci, it is unclear how much incongruence between genes comes from real conflicting phylogenetic signal versus gene tree estimation error due to mutational homoplasy.

In the presence of ILS and/or hybridization, using the multispecies coalescent model to infer species phylogeny is suggested (Carstens and Knowles 2007; Fujita et al. 2012; Carstens et al. 2013), but these methods require assigning samples a priori to putative lineages (Carstens et al. 2013). Species delimitation in sect. *Usnea* proved especially difficult, as commonly used models that use gene trees to estimate speciation or branching events and identify putative species based on a threshold (we tested The Generalized Mixed Yule Coalescent, GMYC of Pons et al. 2006; Poisson Tree Processes, PTP of Zhang et al. 2013; and O'Meara's heuristic method, Brownie of O'Meara et al. 2006) failed to find consensus in the number of species and grouping of specimens over replicates and inferences in our data (analyses not shown). We then applied the recently developed coalescent-based species delimitation method STACEY to estimate the species tree and identify independent evolutionary lineages in

our group. This method was more consistent over replications, and the resulting SMC-tree correlated well with the concatenated tree and species tree from *BEAST. Even then, we have remained conservative when identifying putative species for the species validation analyses in BP&P and species tree reconstruction in *BEAST, as the branch supports within clades were low in multi-locus analyses.

Taxonomy

The formal taxonomic changes are made here, as proposed in the "Discussion" section.

Usnea lapponica Vain.

Meddeland. Soc. Fauna Fl. Fenn. 48: 173 (1925) – *Usnea sorediifera* ssp. *lapponica* (Vain.) Motyka, Wydaw. Muz. Ślask. Katowicach 3: 23 (1930) – Type: Russia, Murmansk region, Lapponia Imandrae, Lovozero, 1887 *Kihlman* (H, lectotype designated by Clerc 1987: 494).

Syn. *Usnea monstruosa* Vain., Wydaw. Muz. Ślask. Katowicach 3: 25 (1930). – *Usnea arnoldii* Motyka, Lich. Gen. Usnea Monogr. 1: 288 (1936) – *Usnea fulvoreagens* auct. non (Räsänen) Räsänen

Syn. nov. *Usnea substerilis* Motyka, Lich. Gen. Usnea Monogr. 1: 257 (1936). – *Usnea sorediifera var. substerilis* (Motyka) Keissl. (1960). – Type: Italy ("Austria"), Bolzano, Gröden, St. Ulrich, above Unterkoffel, 1899 *Arnold*, Lich. Exs. no. 1538b (W, lectotype, designated by Motyka 1936: 291).

Usnea parafloridana K. Mark, Will-Wolf & Randlane sp. nov.

Type: USA, Wisconsin, Vilas Co., Trout Lake Conifer Swamp State Natural Area; 46.0135° N, −89.6586° W; 27.08.2011, Susan Will-Wolf WW14807: isolates WW_018

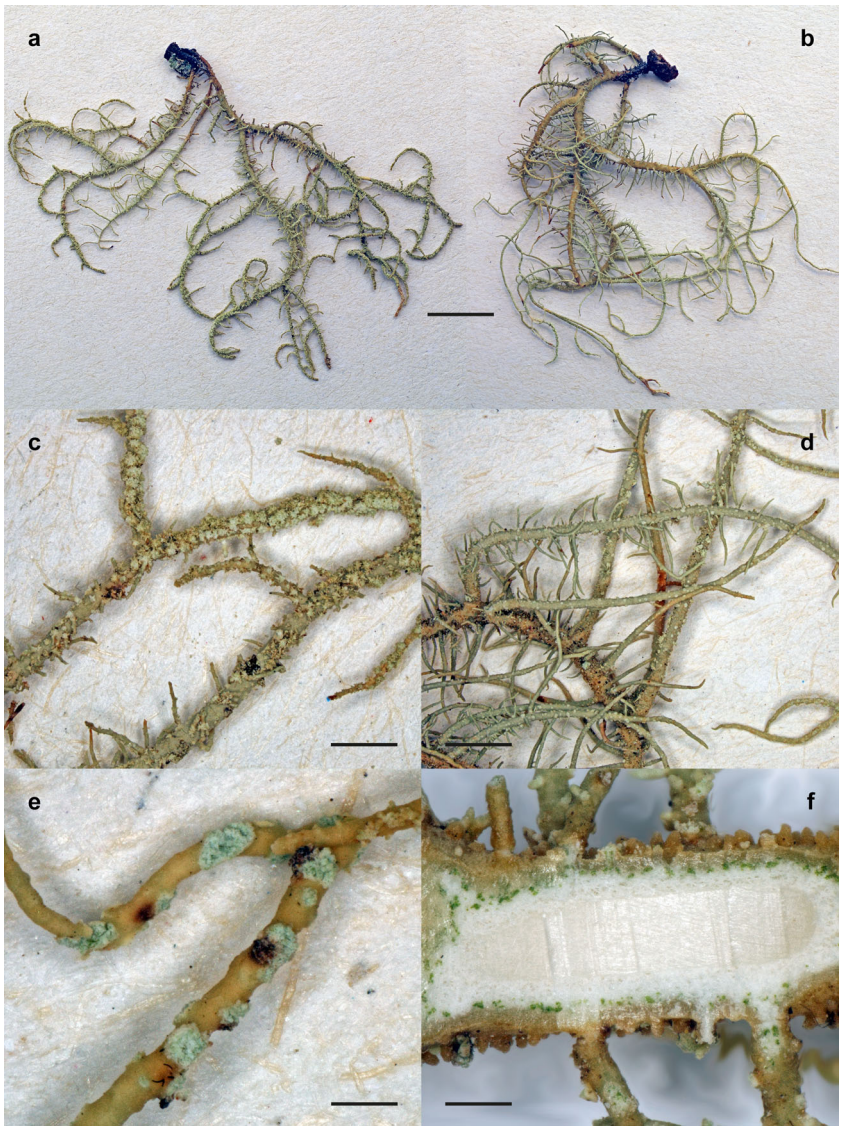

Fig. 4 *Usnea parafloridana* K. Mark, Will-Wolf & Randlane sp. nov. – view of general habit (**a**, **b**), soralia with isidiomorphs (**c**), fibrils (**d**), soralia (**e**), and branch anatomy (**f**). *Scale bars* 7 mm (**a**, **b**), 1.5 mm (**c**), 2 mm (**d**), 0.4 mm (**e**), and 0.3 mm (**f**). Photographed specimens WW14807 (holotype; **a**, **c**, **e**), WW14858 (**b**, **d**), and WW14857 (**f**)

(holotype, TU; Fig. 4a, c, e), WW_023 (isotype 1, WIS), WW_013 (isotype 2, F).

Morphology: thallus shrubby, up to 3–6 cm long, often with relatively few branches; branching mainly isotomic-dichotomous, divergent; lateral branches not narrowed at point of attachment; basal part distinctly jet black, with few annular cracks; papillae verrucose, numerous on main branches and lesser or absent on lateral branches; fibrils few to numerous; soralia small and punctiform when young, enlarging, becoming close to each other but usually staying delimited when mature, more numerous on terminal branches; isidiomorphs numerous, spinulose, relatively short and thick, both on young and mature soralia; cortex thick (9–15 %); medulla thin (10.5–13 %), dense, not pigmented; central cord thick (60–73 %) and white; apothecia not seen (Fig. 4; colour illustrations in online version). **Secondary chemistry:** usnic acid in cortex; norstictic acid as a major compound, salazinic acid as an accessory substance (present in most examined specimens) in medulla. **Ecology:** on branches of *Abies balsamea*, *Larix laricina*, *Picea mariana*, or *Pinus strobus* in cedar swamp, conifer bog and pine plantation with trees over one-hundred years old. **Distribution:** currently 15 specimens are known from four localities in Wisconsin, USA. **Etymology:** the species is morphologically somewhat similar to *Usnea subfloridana* (both taxa have similar shrubby thalli, black basal parts and delimited soralia with numerous isidiomorphs), which phylogenetically appears conspecific with *U. florida*. The same root "florida" is used in the epithet of the new taxon to underline this morphological similarity while the prefix "para-" indicates phylogenetic distinctness of the species from *U. florida* and *U. subfloridana*.

Other specimens examined: USA, Wisconsin: Trout Lake Conifer Swamp State Natural Area, 46.0135° N, −89.6586° W, 27.08.2011, Susan Will-Wolf WW14801, WW14802, WW14808, WW14811, WW14812, WW14813, Matthew P. Nelsen WW14857, WW14858, WW14862; N of Mud Creek, 45.9289° N, −89.5636° W, 27.08.2011, Susan Will-Wolf WW14820; Papoose Creek Pines State Natural Area, 46.1502° N, −89.8562° W, 27.08.2011, Susan Will-Wolf WW14827; Jyme Lake bog in Kemp Biological Station, 45.8392° N, −89.6714° W, 28.08.2011, Susan Will-Wolf WW14863.

Notes: *Usnea parafloridana* includes specimens from Wisconsin (USA) with distinct morphology and chemistry; it forms a strongly supported monophyletic clade (see Fig. 1 and Online Resource 3 for concatenated gene tree for all examined specimens). This taxon morphologically resembles most *Usnea subfloridana*, with delimited soralia bearing numerous isidiomorphs, especially on thin and terminal branches. However, it differs in chemistry; the studied specimens produce norstictic acid often together with salazinic acid (see also Online Resource 4 for TLC plate example), while the dominant depsidones in *U. subfloridana* are thamnolic and squamatic acids. The rare species *Usnea praetervisa* (in Eastern Asia, Europe, and North America; Fos and Clerc (2000)) is also morphologically similar to *U. subfloridana* and includes norstictic acid; it therefore could easily be confused with *U. parafloridana*. The development of soralia in *U. praetervisa* is different from *U. subfloridana* (as described in Clerc (2004)); a photograph of *U. praetervisa* soralia is provided in Clerc (2007)). *U. praetervisa* is usually more richly branched and chemotypes with norstictic acid together with connorstictic or stictic acids are known. It is genetically related to *U. dasopoga*, while *U. parafloridana* is closely related to *U. wasmuthii.* The latter has different medullary chemistry and shape of soralia (see Table 5 for detailed morphology and chemistry).

Acknowledgments We thank the collectors who provided the specimens used here and anonymous reviewers for useful comments. The study was financially supported by the Estonian Research Council (grants ETF9109 and PUT1017 to TR) and European Union Social Fund through the ESF Doctoral Studies and Internationalisation Programme Activity 6. The molecular work was performed in the DNA Genotyping and Sequencing Core Facility of the Estonian Biocentre and Institute of Molecular and Cell Biology at the University of Tartu (Tartu, Estonia) and in the Pritzker Laboratory for Molecular Systematics at the Field Museum (Chicago, IL, USA). Computationally demanding analyses were carried out in the High Performance Computing Center at the University of Tartu.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control, 19*(6), 716–723.

Articus, K. (2004a). *Neuropogon* and the phylogeny of *Usnea s.l.* (Parmeliaceae, Lichenized Ascomycetes). *Taxon, 53*(4), 925–934.

Articus, K. (2004b). *Phylogenetic studies in Usnea (Parmeliaceae) and allied genera* (Vol. 931, Acta Universitatis Upsaliensis. Comprehensive summaries of Uppsala Dissertations from the Faculty of Science and Technology). Uppsala: Acta Universitatis Upsaliensis.

Articus, K., Mattsson, J.-E., Tibell, L., Grube, M., & Wedin, M. (2002). Ribosomal DNA and β-tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. *Mycological Research, 106*(4), 412–418.

Boni, M. F., Posada, D., & Feldman, M. W. (2007). An exact nonparametric method for inferring mosaic structure in sequence triplets. *Genetics, 176*, 1035–1047.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., et al. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology, 10*(4), e1003537. doi: 10.1371/journal.pcbi.1003537.

Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia, 91*(3), 553–556.

Carstens, B. C., & Knowles, L. L. (2007). Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from Melanoplus grasshoppers. *Systematic Biology, 56*(3), 400–411.

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology, 22*(17), 4369–4383.

Clerc, P. (1984). Contribution à la revision de la systématique des Usnées (Ascomycotina, Usnea) d'Europe. I. *Usnea florida* (L.) Wigg. emend. Clerc. *Cryptogamie. Bryologie and Lichénologie, 5*, 333–360.

Clerc, P. (1987). Systematics of the *Usnea fragilescens* aggregate and its distribution in Scandinavia. *Nordic Journal of Botany, 7*(4), 479–495.

Clerc, P. (1998). Species concepts in the genus *Usnea* (lichenized Ascomycetes). *The Lichenologist, 30*(4–5), 321–340.

Clerc, P. (2004). Notes on the genus *Usnea* Adanson. II. *Bibliotheca Lichenologica, 88*, 79–90.

Clerc, P. (2007). Usnea. In T. H. Nash III, C. Gries, & F. Bungartz (Eds.), *Lichen flora of the Greater Sonoran Desert Region (Vol. 3)* (p. 327). Tempe: Arizona State.

Clerc, P. (2011). *Usnea*. In A. Thell, & R. Moberg (Eds.), *Nordic Lichen Flora 4*. (pp. 107–127): Museum of Evolution, Uppsala University.

R Core Team (2014). *R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. http://www.R-project.org

Cornejo, C., Chabanenko, S., & Scheidegger, C. (2009). Phylogenetic analysis indicates transitions from vegetative to sexual reproduction in the *Lobaria retigera* group (Lecanoromycetidae, Ascomycota). *The Lichenologist, 41*(03), 275–284.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods, 9*(8), 772–772.

Delport, W., Poon, A. F., Frost, S. D., & Pond, S. L. (2010). Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics, 26*, 2455–2457.

Drummond, A. J., Ho, S. Y., Phillips, M. J., & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology, 4*(5), e88. doi:10.1371/journal.pbio.0040088.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution, 29*(8), 1969–1973.

Edwards, S. V. (2009). Is a new and general theory of molecular systematics emerging? *Evolution, 63*(1), 1–19.

Edwards, D. L., & Knowles, L. L. (2014). Species detection and individual assignment in species delimitation: can integrative data increase efficacy? *Proceedings of the Royal Society B, 281*(1777), 20132765. doi:10.1098/rspb.2013.2765.

Elix, J. A., Corush, J., & Lumbsch, H. T. (2009). Triterpene chemosyndromes and subtle morphological characters characterise lineages in the *Physcia aipolia* group in Australia (Ascomycota). *Systematics and Biodiversity, 7*(4), 479–487.

Fos, S., & Clerc, P. (2000). The lichen genus *Usnea* on *Quercus suber* in Iberian cork-oak forests. *The Lichenologist, 32*(1), 67–88.

Fujita, M. K., Leaché, A. D., Burbrink, F. T., McGuire, J. A., & Moritz, C. (2012). Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution, 27*(9), 480–488.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology, 2*(2), 113–118.

Giarla, T. C., & Esselstyn, J. A. (2015). The challenges of resolving a rapid, recent radiation: empirical and simulated phylogenomics of Philippine shrews. *Systematic Biology*. doi:10.1093/sysbio/syv029.

Gibbs, M. J., Armstrong, J. S., & Gibbs, A. J. (2000). Sister-Scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics, 16*, 573–582.

Givnish, T. J. (2015). Adaptive radiation versus 'radiation' and 'explosive diversification': why conceptual distinctions are fundamental to understanding evolution. *New Phytologist, 207*(2), 297–303.

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*(4), 1323–1330.

Halonen, P. (2000). *Usnea pacificana, sp. nov.* and *U. wasmuthii* (Lichenized Ascomycetes) in Pacific North America. *The Bryologist, 103*(1), 38–43.

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.

Heled, J., & Drummond, A. J. (2010). Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution, 27*(3), 570–580.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*(8), 754–755.

Jones, G. R. (2015). *STACEY: species delimitation and phylogeny estimation under the multispecies coalescent*. doi:10.1101/010199. Preprint in biorxiv.org.

Jones, G., Zeynep, A., & Oxelman, B. (2014). DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. *Bioinformatics, 31*, 991–998.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution, 30*(4), 772–780.

Katoh, K., & Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics, 9*(4), 286–298.

Kelly, L. J., Hollingsworth, P. M., Coppins, B. J., Ellis, C. J., Harrold, P., Tosh, J., et al. (2011). DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. *New Phytologist, 191*(1), 288–300.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution, 16*, 111–120.

Knowles, L. L., & Kubatko, L. S. (2010). *Estimating species trees: practical and theoretical aspects*. Hoboken: Wiley-Blackwell.

Kosakovsky Pond, S. L., Posada, D., Gravenor, M. B., Woelk, C. H., & Frost, S. D. W. (2006). GARD: a genetic algorithm for recombination detection. *Bioinformatics, 22*(24), 3096–3098.

Kraichak, E., Divakar, P. K., Crespo, A., Leavitt, S. D., Nelsen, M. P., Lücking, R., et al. (2015a). A tale of two hyper-diversities: diversification dynamics of the two largest families of lichenized fungi. *Scientific Reports, 5*, 10028. doi:10.1038/srep10028.

Kraichak, E., Lücking, R., Aptroot, A., Beck, A., Dornes, P., John, V., et al. (2015b). Hidden diversity in the morphologically variable script lichen (*Graphis scripta*) complex (Ascomycota, Ostropales, Graphidaceae). *Organisms Diversity & Evolution, 15*(3), 447–458.

Leaché, A. D. (2009). Species tree discordance traces to phylogeographic clade boundaries in North American fence lizards (*Sceloporus*). *Systematic Biology, 58*(6), 547–559.

Leavitt, S. D., Fankhauser, J. D., Leavitt, D. H., Porter, L. D., Johnson, L. A., & St. Clair, L. L. (2011a). Complex patterns of speciation in cosmopolitan "rock posy" lichens – discovering and delimiting cryptic fungal species in the lichen-forming *Rhizoplaca*

melanophthalma species-complex (Lecanoraceae, Ascomycota). *Molecular Phylogenetics and Evolution, 59*(3), 587–602.

Leavitt, S. D., Johnson, L., & St. Clair, L. L. (2011b). Species delimitation and evolution in morphologically and chemically diverse communities of the lichen-forming genus *Xanthoparmelia* (Parmeliaceae, Ascomycota) in western North America. *American Journal of Botany, 98*(2), 175–188.

Linda in Arcadia. (2013). *Usnea dasopoga*, a name to be reinstated for *U. filipendula*, and its orthography. *Taxon, 62*(3), 604–605.

Lindblom, L., & Ekman, S. (2006). Genetic variation and population differentiation in the lichen-forming ascomycete *Xanthoria parietina* on the island Storfosna, central Norway. *Molecular Ecology, 15*(6), 1545–1559.

Liu, Y. L., Whelen, S., & Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution, 16*, 1799–1808.

Lumbsch, H. T., & Wirtz, N. (2011). Phylogenetic relationships of the neuropogonoid core group in the genus *Usnea* (Ascomycota: Parmeliaceae). *The Lichenologist, 43*(6), 553–559.

Lumbsch, H. T., Ahti, T., Altermann, S., Amo De Paz, G., Aptroot, A., Arup, U., et al. (2011). One hundred new species of lichenized fungi: a signature of undiscovered global diversity. *Phytotaxa, 18*, 1–127.

Madden, T. (2002). The BLAST sequence analysis tool. In J. McEntyre & J. Ostell (Eds.), *The NCBI handbook*. Bethesda: National Center for Biotechnology Information.

Maddison, W. P. (1997). Gene trees in species trees. *Systematic Biology, 46*(3), 523–536.

Maddison, W. P., & Maddison, D. R. (2011). *Mesquite: a modular system for evolutionary analysis. Version 2.75*. http://mesquiteproject.org.

Martin, D., & Rybicki, E. (2000). RDP: detection of recombination amongst aligned sequences. *Bioinformatics, 16*, 562–563.

Martin, D. P., Posada, D., Crandall, K. A., & Williamson, C. (2005). A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. *AIDS Research and Human Retroviruses, 21*, 98–102.

Martin, D. P., Lemey, P., Lott, M., Moulton, V., Posada, D., & Lefeuvre, P. (2010). RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics, 26*(19), 2462–2463.

Mason-Gamer, R. J., & Kellogg, E. A. (1996). Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae* (Gramineae). *Systematic Biology, 45*(4), 524–545.

Matheny, P. B., Liu, Y. J., Ammirati, J. F., & Hall, B. D. (2002). Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). *American Journal of Botany, 89*(4), 688–698.

Maynard Smith, J. (1992). Analyzing the mosaic structure of genes. *Journal of Molecular Evolution, 34*, 126–129.

McCune, B. (2005). *Usnea* in Pacific Northwest. http://people.oregonstate.edu/~mccuneb/Usnea.PDF Accessed 25 Feb 2015.

McEvoy, M., Nybakken, L., Solhaug, K. A., & Gauslaa, Y. (2006). UV triggers the synthesis of the widely distributed secondary lichen compound usnic acid. *Mycological Progress, 5*(4), 221–229.

Molina, M. C., Del-Prado, R., Divakar, P. K., Sánchez-Mata, D., & Crespo, A. (2011). Another example of cryptic diversity in lichen-forming fungi: the new species *Parmelia mayi* (Ascomycota: Parmeliaceae). *Organisms Diversity & Evolution, 11*(5), 331–342.

Motyka, J. (1936). *Lichenum generis Usnea studium monographicum*. Pars systematica (2 vol. in 1 bd.). Lublin: Editio et proprietas auctoris.

Ohmura, Y. (2001). Taxonomic study of the genus *Usnea* (lichenized Ascomycetes) in Japan and Taiwan. *Journal of 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 Hattori Botanical Laboratory, 92*, 231–243.

Ohmura, Y., & Kanda, H. (2004). Taxonomic status of section *Neuropogon* in the genus *Usnea* elucidated by morphological comparisons and ITS rDNA sequences. *The Lichenologist, 36*(3–4), 217–225.

O'Meara, B. C., Ané, C., Sanderson, M. J., & Wainwright, P. C. (2006). Testing for different rates of continuous trait evolution using likelihood. *Evolution, 60*(5), 922–933.

Orange, A., James, P. W., & White, F. J. (2001). *Microchemical methods for the identification of Lichens*. London: British Lichen Society.

Padidam, M., Sawyer, S., & Fauquet, C. M. (1999). Possible emergence of new geminiviruses by frequent recombination. *Virology, 265*, 218–225.

Poelt, J. (1970). Das Konzept der Artenpaare bei den Flechten. *Vorträge aus dem Gesamtgebiet der Botanik, Neue Folge, 4*, 187–198.

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., et al. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology, 55*(4), 595–609.

Posada, D., & Crandall, K. A. (2001). Evaluation of methods for detecting recombination from DNA sequences: computer simulations. *PNAS, 98*, 13757–13762.

Rambaut, A. (2009). *FigTree*. Version 1.3.1. http://tree.bio.ed.ac.uk/software/figtree. Edinburgh: Institute of Evolutionary Biology, University of Edinburgh.

Rambaut, A., & Drummond, A. (2007). *Tracer. Version 1.4*. http://beast.bio.ed.ac.uk.

Rambaut, A., & Drummond, A. (2012a). *LogCombiner Version 1.7.2*. http://beast.bio.ed.ac.uk.

Rambaut, A., & Drummond, A. (2012b). *TreeAnnotator Version 1.7.2*. http://beast.bio.ed.ac.uk.

Randlane, T., Tõrra, T., Saag, A., & Saag, L. (2009). Key to European *Usnea* species. *The Diversity of Lichenology: Jubilee Volume, 100*(100), 419–462.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*(3), 539–542.

Saag, L., Tõrra, T., Saag, A., Del-Prado, R., & Randlane, T. (2011). Phylogenetic relations of European shrubby taxa of the genus *Usnea. The Lichenologist, 43*(05), 427–444.

Saag, L., Mark, K., Saag, A., & Randlane, T. (2014). Species delimitation in the lichenized fungal genus *Vulpicida* (Parmeliaceae, Ascomycota) using gene concatenation and coalescent-based species tree approaches. *American Journal of Botany, 101*(12), 2169–2182.

Sanderson, M. J., & Donoghue, M. J. (1996). Reconstructing shifts in diversification rates on phylogenetic trees. *Trends in Ecology & Evolution, 11*(1), 15–20.

Scherrer, S., Zippler, U., & Honegger, R. (2005). Characterisation of the mating-type locus in the genus *Xanthoria* (lichen-forming ascomycetes, Lecanoromycetes). *Fungal Genetics and Biology, 42*(12), 976–988.

Schmitt, I., & Lumbsch, H. T. (2004). Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. *Molecular Phylogenetics and Evolution, 33*(1), 43–55.

Schmitt, I., Crespo, A., Divakar, P. K., Fankhauser, J. D., Herman-Sackett, E., Kalb, K., et al. (2009). New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia, 23*, 35–40.

Seymour, F. A., Crittenden, P. D., Wirtz, N., Øvstedal, D. O., Dyer, P. S., & Lumbsch, H. T. (2007). Phylogenetic and morphological analysis of Antarctic lichen-forming *Usnea* species in the group *Neuropogon. Antarctic Science, 19*(1), 71–82.

Singh, G., Dal Grande, F., Divakar, P. K., Otte, J., Leavitt, S. D., Szczepanska, K., et al. (2015). Coalescent-based species

delimitation approach uncovers high cryptic diversity in the cosmopolitan lichen-forming fungal genus *Protoparmelia* (Lecanorales, Ascomycota). *PLoS One, 10*(5), e0124625. doi:10.1371/journal.pone.0124625.

Sistrom, M., Donnellan, S. C., & Hutchinson, M. N. (2013). Delimiting species in recent radiations with low levels of morphological divergence: a case study in Australian *Gehyra* geckos. *Molecular Phylogenetics and Evolution, 68*(1), 135–143.

Spinks, P. Q., Thomson, R. C., Pauly, G. B., Newman, C. E., Mount, G., & Shaffer, H. B. (2013). Misleading phylogenetic inferences based on single-exemplar sampling in the turtle genus *Pseudemys*. *Molecular Phylogenetics and Evolution, 68*, 269–281.

Spitzer, M., Wildenhain, J., Rappsilber, J., & Tyers, M. (2014). BoxPlotR: a web tool for generation of box plots. *Nature Methods, 11*(2), 121–122.

Spribille, T., Klug, B., & Mayrhofer, H. (2011). A phylogenetic analysis of the boreal lichen *Mycoblastus sanguinarius* (Mycoblastaceae, lichenized Ascomycota) reveals cryptic clades correlated with fatty acid profiles. *Molecular Phylogenetics and Evolution, 59*(3), 603–614.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics, 22*(21), 2688–2690.

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology, 57*(5), 758–771.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Tehler, A., & Irestedt, M. (2007). Parallel evolution of lichen growth forms in the family Roccellaceae (Arthoniales, Ascomycota). *Cladistics, 23*(5), 432–454.

Thell, A., Crespo, A., Divakar, P. K., Kärnefelt, I., Leavitt, S. D., Lumbsch, H. T., et al. (2012). A review of lichen family Parmeliaceae – history, phylogeny and current taxonomy. *Nordic Journal of Botany, 30*, 641–664.

Tõrra, T., & Randlane, T. (2007). The lichen genus *Usnea* (lichenized Ascomycetes, Parmeliaceae) in Estonia with a key to the species in the Baltic countries. *The Lichenologist, 39*, 415–438.

Trest, M. T., Will-Wolf, S., Keuler, R., Shay, N., Hill, K., Studer, A., et al. (2015). Potential impacts of UV exposure on lichen communities: a pilot study of *Nothofagus dombeyi* trunks in southernmost Chile. *Ecosystem Health and Sustainability, 1*(4), art14. doi:10.1890/EHS15-0008R1.1.

Truong, C., Divakar, P. K., Yahr, R., Crespo, A., & Clerc, P. (2013). Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution, 68*(2), 357–372.

Velmala, S., Myllys, L., Halonen, P., Goward, T., & Ahti, T. (2009). Molecular data show that *Bryoria fremontii* and *B. tortuosa* (Parmeliaceae) are conspecific. *The Lichenologist, 41*(03), 231–242.

Velmala, S., Myllys, L., Goward, T., Holien, H., & Halonen, P. (2014). Taxonomy of *Bryoria* section *Implexae* (Parmeliaceae, Lecanoromycetes) in North America and Europe, based on chemical, morphological and molecular data. *Annales Botanici Fennici, 51*, 345–371.

Wagner, C. E., Keller, I., Wittwer, S., Selz, O. M., Mwaiko, S., Greuter, L., et al. (2013). Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Molecular Ecology, 22*(3), 787–798.

Wedin, M., Westberg, M., Crewe, A. T., Tehler, A., & Purvis, O. W. (2009). Species delimitation and evolution of metal bioaccumulation in the lichenized *Acarospora smaragdula* (Ascomycota, Fungi) complex. *Cladistics, 25*(2), 161–172.

Weiller, G. F. (1998). Phylogenetic profiles: a graphical method for detecting genetic recombinations in homologous sequences. *Molecular Biology and Evolution, 15*, 326–335.

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: a guide to methods and applications* (pp. 315–322). New York: Academic Press.

Wiens, J. J. (1998). Combining data sets with different phylogenetic histories. *Systematic Biology, 47*(4), 568–581.

Willis, S. C., Farias, I. P., & Ortí, G. (2013). Multi-locus species tree for the Amazonian peacock basses (Cichlidae: *Cichla*): emergent phylogenetic signal despite limited nuclear variation. *Molecular Phylogenetics and Evolution, 69*(3), 479–490.

Wirtz, N., Printzen, C., Sancho, L. G., & Lumbsch, H. T. (2006). The phylogeny and classification of *Neuropogon* and *Usnea* (Parmeliaceae, Ascomycota) revisited. *Taxon, 55*(2), 367–376.

Wirtz, N., Printzen, C., & Lumbsch, H. T. (2008). The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): a cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycological Research, 112*, 472–484.

Wirtz, N., Printzen, C., & Lumbsch, H. T. (2012). Using haplotype networks, estimation of gene flow and phenotypic characters to understand species delimitation in fungi of a predominantly Antarctic *Usnea* group (Ascomycota, Parmeliaceae). *Organisms Diversity & Evolution, 12*(1), 17–37.

Yang, Z., & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences, 107*(20), 9264–9269.

Yang, Z., & Rannala, B. (2014). Unguided species delimitation using DNA sequence data from multiple loci. *Molecular Biology and Evolution, 31*(12), 3125–3135.

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics, 29*(22), 2869–2876.