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Phylogeny of the genus *Bryoria*

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Abstract: The phylogenetic relationships of the genus *Bryoria* were examined using ITS, partial glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and mitochondrial SSU rDNA sequence data in addition to 20 chemical and morphological characters. This first comprehensive molecular study to assess *Bryoria* phylogeny includes representatives from all the traditionally recognized four sections. Combined cladistic analyses of 88 *Bryoria* specimens representing at least 25 species resulted in highly resolved phylogenies. Based on the results, a new infrageneric classification for the genus is proposed. Five sections are recognized, largely corresponding to the existing classification, with the addition of section *Americanae*. The study shows that while most species with an erect growth-form are clearly monophyletic, current species status of many pendent taxa can be questioned.

Key words: *Americanae*, classification, *Parmeliaceae*, secondary chemistry, species delimitation

Introduction

Bryoria Brodo & D. Hawksw. (*Parmeliaceae*, Lecanoromycetes) is regarded as one of the taxonomically most difficult genera of macrolichens. The genus is mainly circumboreal, but occurs also in mountainous areas of Asia, Australasia and Africa (Brodo & Hawksworth 1977). *Bryoria* species, often referred to colloquially as 'horsehair lichens', consist of copiously branched, capillary, whitish grey, brown or blackish branches that vary in habit from erect to pendent or sometimes decumbent. Sexual fruiting structures are uncommon and many species are known only in the sterile condition. As a result, the diagnostic features in species delimitation include characters such as branching pattern, presence and type of soralia, presence and form of pseudocyphellae, growth form, thallus colour and secondary chemistry (see Brodo & Hawksworth 1977; Krog 1980). In some cases, the presence or absence of a single lichen substance has been used to separate taxa which are otherwise anatomically and morphologically more or less indistinguishable. Some authors have treated such taxa as mere chemotypes of one species (Holien 1989). Pendent species tend to be especially variable in morphology and chemistry, and putative intermediate forms have occasionally been reported. As a result, the infrageneric relationships and species boundaries are in some cases poorly known. The intermediates found may point to the existence of hybrids via thallus fusion or sexual reproduction, unidentified species, or morphological plasticity caused by environmental factors or photobionts (Brodo & Hawksworth 1977; Brodo 1978; Holien 1989).

Bryoria has been extensively studied by Motyka (1964), Hawksworth (1971, 1972) and, for North American taxa especially, by

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Brodo & Hawksworth (1977), who separated *Bryoria* from *Alectoria* Ach. and divided the genus into five sections on the basis of anatomical, chemical and morphological characters. Later, Common & Brodo (1995) transferred species of the section *Subdivergentes* (Mot.) Brodo & D. Hawksw. to the genus *Nodobryoria* Common & Brodo, largely on the basis of cortical structure. Jørgensen (1972, 1975), Jørgensen & Ryvarden (1970) and Jørgensen & Galloway (1983) focused on species of the section *Divaricatae* (DR.) Brodo & D. Hawksw., while Holien (1989, 1991, 1992) examined the taxonomy of North European species of sections *Bryoria* and *Implexae* (Gyeln.) Brodo & D. Hawksw. The only molecular systematic study on *Bryoria* examined the taxonomy of *Bryoria fremontii* (Tuck.) Brodo & D. Hawksw. (Velmala *et al*. 2009). Currently, *Bryoria* includes some 80 species (http://www.indexfungorum.org), although many of these 'species', viewed from the perspective of the mycobiont, may represent environmental morphotypes of a smaller number of phylospecies. According to recent phylogenetic analyses based on molecular markers *Bryoria* belongs to the *Parmeliaceae* (Thell *et al*. 2004; Crespo *et al*. 2007) and is probably related to the genus *Alectoria* (Halonen *et al*. 2009). In this paper we have reconstructed a phylogeny for *Bryoria* using three DNA regions, in addition to some chemical and morphological characters. Based on our results we propose a new infrageneric classification for the genus. We also show that while most species with an erect growth-form are clearly monophyletic, the current species status of many pendent taxa can be questioned.

Materials and Methods

Taxon selection

The analyses included 88 ingroup specimens of 25 species and seven unidentified taxa representing all four currently recognized sections of *Bryoria* (Tables 1 & 2). We have primarily followed the species concept of Brodo & Hawksworth (1977) with the exception of *B. trichodes* (Michx.) Brodo & D. Hawksw. subsp. *trichodes* and *B. trichodes* subsp. *americana* (Motyka) Brodo & D. Hawksw., which are treated as separate species, and *B. pseudofuscescens* (Gyeln.) Brodo & D. Hawksw., *B. friabilis* Brodo & D. Hawksw. and *B. vrangiana* (Gyeln.) Brodo & D. Hawksw., which are treated as chemotypes of *B. implexa* (Hoffm.) Brodo & D. Hawksw. following Holien (1989, 1994). *Bryoria tortuosa* (G. Merr.) Brodo & D. Hawksw. is not recognized as separate from *B. fremontii* based on our molecular phylogeny (Velmala *et al*. 2009; but see also Goward 2009). When available, three to six specimens for each species (or for each chemotype in case of *B. implexa*) were included in the analyses to examine the infraspecific variation and species delimitation. Furthermore, we tried to cover the geographical variation by selecting specimens from different continents.

Gowardia arctica Halonen *et al*. was used as an outgroup taxon based on the phylogenies by Halonen *et al*. (2009) and Thell *et al*. (2002, 2004). *Nodobryoria abbreviata* (Müll. Arg.) Common & Brodo, *Pseudephebe pubescens* (L.) M. Choisy and *Sulcaria isidiifera* Brodo were included in the analyses to test the monophyly of the ingroup.

Thin-layer chromatography

Secondary chemistry has played a major role in species identification, especially with regard to the pendent species. Accordingly we analyzed our material using thin-layer chromatography (TLC) for detection of secondary compounds according to the methods described by Orange *et al*. (2001). A few specimens were too small to be used for TLC and were directly subjected to DNA extraction. The acetone extracts were spotted with 75 mm / 75 µl Haematocrit glass capillary tubes (Hirschmann Laborgeräten) on 10 × 20 cm Merck silica gel 60 F-254 pre-coated glass plates and run in solvents A and B (formulae from both Culberson 1972 and Mietzsch *et al*. 1994, according to Orange *et al*. 2001, were used for solvent B). When possible, branches without soralia were used for TLC. Soralia were tested separately with Pd reagent for the presence of fumarprotocetraric acid, which is an important diagnostic character, especially in *B. fuscescens* s. lat. (Brodo & Hawksworth 1977).

Molecular techniques

Total DNA was extracted using Qiagen's DNeasy Blood & Tissue Kit following the manufacturer's instructions with the following exceptions. Thallus fragments of approximately 0·5–4 cm long were ground with mini-pestles in 40 µl of the lysis buffer, after which 140 µl of the buffer was added. Sometimes the amount of Buffer ATL, Buffer AL and ethanol used was 20 µl less and the amount of proteinase K used was 10 µl less than instructed. The extracted DNA was eluted in 120 µl of the elution buffer so that the first 60 µl of the buffer was added after which the sample was incubated for 5 min and centrifuged. The elution step was repeated once for the same microcentrifuge tube.

We used three DNA regions in our study: 1) ITS regions of the nuclear ribosomal DNA, 2) partial

TABLE 1. *List of taxa, their herbarium voucher numbers, sequence ID numbers, secondary chemistry and GenBank Accession numbers*

Taxon	Locality	Voucher specimen and sequence ID	Chemistry*	GenBank accession numbers		
				ITS	GAPDH	mtSSU
Bryoria americana	Finland, Kainuu	*Velmala* 63 (H), S69	fum	HQ402677	HQ402605	HQ402636
B. americana	Norway, Nord-Nordland	*Holien* 9433 (TRH), L294	fum	HQ402681	HQ402598	HQ402640
B. americana	Norway, Nord-Trøndelag	*Holien* 10440 (TRH), L297a	fum	HQ402680	HQ402608	HQ402639
B. americana	Canada, Newfoundland	*Ahti* 67654 (H), S329	fum	HQ402682	HQ402599	HQ402641
B. americana	Canada, B.C.	*Goward* 02-165 (UBC), L199	fum, cfum, (pro)	HQ402678	HQ402606	HQ402637
B. americana	Canada, B.C.	*Wright* 2004-77 (UBC), L234	fum, cfum, pro	HQ402679	HQ402607	HQ402638
B. bicolor	Finland, Etelä-Häme	*Kuusinen* 1063 & *Lampinen* (H), L183	—	HQ402691	HQ402612	HQ402645
B. bicolor	Finland, Koillismaa	*Velmala* 24 (H), S23	bar, pso, fum	HQ402689	HQ417113	HQ402644
B. bicolor	Sweden, Dalarna	*Hermansson* 14110 (UPS), L156	fum	HQ402692	HQ402613	HQ402646
B. bicolor	Canada, B.C.	*Björk* 14524 (UBC), S374	gyr, fum, pro, cfum	HQ402690	HQ402611	—
B. capillaris	Finland, Etelä-Häme	*Haikonen* 22228 (H), L141	bar, (ale?)	FJ668493	FJ668399	FJ668427
B. capillaris	Finland, Etelä-Savo	*Myllys* 485 (H), L211	bar, ale, fum in sor.	GQ996287	GQ996259	GQ996331
B. capillaris	Norway, Nord-Trøndelag	*Holien* 10056 (TRH), L270	bar	GQ996288	GQ996260	GQ996320
B. capillaris	Canary Is., Tenerife	*Keihäs s. n.* (OULU), S192	bar, ale, atr , (fum)	GQ996289	GQ996261	GQ996321
B. capillaris	Canada, B.C.	*Goward* 05-19 (UBC), L209	bar, ale	GQ996281	GQ996253	GQ996311
B. confusa	China, Yunnan	*Wang* 06-26974 (KUN), S292	unk. A3, B2	HQ402686	HQ417112	—
B. divergescens	China, Yunnan	*Wang* 06-26244 (KUN), S284	fum, (pro), (qua), (cfum)	HQ402705	—	HQ402654
B. fastigiata	China, Yunnan	*Wang et al.* 06-26696 (KUN), S288	fum, (pro), (cfum)	HQ402706	—	HQ402655
B. fremontii	Finland, Koillismaa	*Velmala* 13b (H), S13	vul in sor.	FJ668498	FJ668404	FJ668432
B. fremontii	Finland, Etelä-Pohjanmaa	*Myllys* 490 (H), L214	vul	FJ668507	FJ668412	FJ668440
B. fremontii	Sweden, Dalarna	*Klintberg* 11347 (UPS), L166	vul, (bar)	FJ668505	FJ668410	FJ668438
B. fremontii	Canada, B.C.	*Goward* 05-04 (UBC), L205	no subst.	FJ668503	FJ668408	FJ668436

TABLE 1. *Continued*

Taxon	Locality	Voucher specimen and sequence ID	Chemistry*	GenBank accession numbers		
				ITS	GAPDH	mtSSU
B. fremontii	Canada, B.C.	*Goward* 07-02-0025 (UBC), S236a	vul	FJ668519	FJ668424	FJ668452
B. furcellata	Finland, Etelä-Savo	*Haikonen* 22770 (H), L147	fum, pro, cfum	HQ402722	HQ402627	HQ402667
B. furcellata	Finland, Kittilän Lappi	*Haikonen* 22571 (H), L148	fum, pro, cfum	HQ402723	HQ402628	HQ402668
B. furcellata	Canada, Manitoba	*Ahti* 63217 (H), L138	fum, (pro), (cfum)	HQ402721	HQ402602	HQ402666
B. furcellata	Canada, Alberta	*Colberg & Prokopetz* s. n. (UBC), S275	no subst.	HQ402724	HQ402629	HQ402669
B. fuscescens	Finland, Åland	*Stjernberg* s. n. (H), L149	fum, pro, cfum	GQ996290	GQ996262	GQ996322
B. fuscescens	Finland, Koillismaa	*Velmala* 51 *& Halonen* (H), S56	fum, pro, cfum	GQ996291	GQ996263	GQ996332
B. fuscescens	Canada, B.C.	*Goward* 07-02-0024 (UBC), S235	fum, pro, (cfum)	GQ996292	GQ996264	GQ996334
B. glabra	Finland, Koillismaa	*Halonen* s. n. (OULU), L186	fum in sor.	FJ668494	FJ668400	FJ668428
B. glabra	Norway, Nord-Trøndelag	*Holien* 10441 (TRH), L292	fum in sor.	HQ402726	HQ402603	HQ402671
B. glabra	Norway, Nord-Trøndelag	*Holien* 10406 (TRH), L302	fum in sor.	HQ402727	HQ402631	HQ402672
B. glabra	Canada, B.C.	*Goward* 05-22 (UBC), L203	fum in sor.	HQ402725	HQ402630	HQ402670
B. glabra	Canada, B.C.	*Goward* 07-02-0033 (UBC), S244	fum in sor.	HQ402728	HQ402632	HQ402673
B. hengduanensis	China, Yunnan	*Wang et al.* 06-26692 (KUN), S287	usn, fum. (pro), (cfum)	HQ402704	—	HQ402653
B. implexa chemotype 1	Finland, Koillismaa	*Velmala et al.* 23 (H), S22	pso, (atr),	GQ996294	GQ996266	GQ996315
B. implexa chemotype 1	Finland, Koillismaa	*Velmala et al.* 37 (H), S39	pso, (gyr), fum in sor.	GQ996293	GQ996265	GQ996323
B. implexa chemotype 2	Iran, East-Azarbaijan	*Sohrabi* 4656 (H), L244a	nsti, (atr)	GQ996295	GQ996267	GQ996324
B. implexa chemotype 2	Norway, Nord-Trøndelag	*Holien* 10177 (TRH), L274	nsti	GQ996303	GQ996276	GQ996333
B. implexa chemotype 2	Canada, B.C.	*Goward* 05-31 (UBC), L240b	(nsti), fum in sor.	GQ996282	GQ996254	GQ996309

TABLE 1. *Continued*

Taxon	Locality	Voucher specimen and sequence ID	Chemistry*	GenBank accession numbers		
				ITS	GAPDH	mtSSU
B. implexa chemotype 3	Finland, Koillismaa	*Velmala et al.* 11b (H), S10	gyr	GQ996297	GQ996269	GQ996314
B. implexa chemotype 3	Finland, Koillismaa	*Velmala et al.* 31b (H), S32	gyr, (atr), fum in sor.	GQ996298	GQ996270	GQ996326
B. implexa chemotype 3	Norway, Nord-Trøndelag	*Holien 10039* (TRH), L272	gyr, fum in sor.	GQ996299	GQ996271	GQ996335
B. implexa chemotype 4	Sweden, Södermanland	*Rydberg* s. n. (UPS), L160	fum, pro, cfum, atr	GQ996300	GQ996272	GQ996327
B. implexa chemotype 4	Russia, Perm Territory	*Melekhin* 10123 (H), S166	fum, (pro), (cfum), atr	GQ996308	GQ996273	GQ996317
B. implexa chemotype 5	Finland, Koillismaa	*Velmala et al.* 43a (H), S45	fum in sor.	GQ996302	GQ996275	GQ996328
B. implexa chemotype 5	Norway, Nord-Trøndelag	*Holien* 10176 (TRH), L300	fum in sor.	GQ996301	GQ996274	GQ996330
B. implexa chemotype 5	Russia, Perm Territory	*Ateeva* 5055 (H), S164	(atr.), fum in sor.	GQ996285	GQ996257	GQ996316
B. indonesica	New Zealand, Gisborne	*Wedin* 4058 (UPS), L172	no subst.	HQ402688	—	—
B. indonesica	New Zealand, Gisborne	*Wedin* 4057 (UPS), L173	no subst.	HQ402687	—	—
B. lactinea	China, Yunnan	*Wang* 06-26966 (KUN), S279	fum, pro, (cfum)	HQ402699	—	—
B. lactinea	China, Yunnan	*Wang et al.* 06-26541 (KUN), S293	fum, (pro), (cfum),	HQ402700	—	—
B. lanestris	Denmark, Greenland	*Hansen Lich. Greenl. Exs.* 946 (H), L232	(atr.), fum in sor.	GQ996304	GQ996277	GQ996329
B. lanestris	Canada, Alberta	*Adams* 0076B & *Hall* (UBC), S256	(fum in sor.)	GQ996307	GQ996280	GQ996318
B. lanestris	Canada, Alberta	*Adams* 0037_b & *Hall* (UBC), S260b	fum, (pro), (cfum) in sor.	GQ996286	GQ996258	GQ996319
B. lanestris	Canada, Alberta	*Kamin* 016 (UBC), S274	no subst.	GQ996303	GQ996276	GQ996333

TABLE 1. *Continued*

Taxon	Locality	Voucher specimen and sequence ID	Chemistry*	GenBank accession numbers		
				ITS	GAPDH	mtSSU
B. nadvornikiana	Finland, Kainuu	*Velmala et al.* 73 (H), S79	bar, (ale), fum, (cfum), (atr)	HQ402718	HQ402624	HQ402663
B. nadvornikiana	Sweden, Dalarna	*Hermansson* 14179 (UPS), L161	bar, fum	HQ402719	HQ402625	HQ402664
B. nadvornikiana	Iran, East-Azarbaijan	*Sohrabi* 4510 (H), L245	bar	HQ402720	HQ402626	HQ402665
B. nadvornikiana	China, Yunnan	*Wang et al.* 06-26535 (KUN), S294	bar, atr, ale, sti?	HQ402715	HQ402600	HQ402660
B. nitidula	Sweden, Ångermanland	*Granbo* s. n. (UPS), L163	—	HQ402713	HQ402621	HQ402658
B. nitidula	Greenland	*Högnabba* 752 (H), S121	fum, (pro), (cfum)	HQ402711	HQ402619	HQ402656
B. nitidula	Greenland	*Högnabba* 818 (H), S123	fum, (pro), (cfum)	HQ402712	HQ402620	HQ402657
B. perspinosa	China, Yunnan	*Wang et al.* 06-26547 (KUN), S296	fum, pro, cfum, lob	HQ402698	—	—
B. poeltii	China, Yunnan	*Wang et al.* 06-26697 (KUN), S295	fum	HQ402701	HQ402617	HQ402650
B. simplicior	Finland, Koillismaa	*Velmala et al.* 30 (H), S30b	fatty acids	HQ402714	HQ402622	HQ402659
B. simplicior	Norway, Troms	*Holien* 10328 (TRH), L296	fatty acids	HQ402717	HQ402623	HQ402662
B. simplicior	Russia, Sakha Republic	*Ahti* 61399 (H), L231b	no subst.	HQ402716	HQ402601	HQ402661
B. smithii	Finland, Varsinais-Suomi	*Velmala et al.* 60 (H), S65	no subst.	HQ402684	HQ402609	HQ402642
B. smithii	Finland, Varsinais-Suomi	*Syrjänen & Vauras* 10652F (TUR), L249	(atr)	GQ379165	—	—
B. smithii	India, Uttaranchal	*Tibell* 23319 (UPS) (L174)	(unk. A3, B2)	HQ402685	HQ402610	HQ402643
B. subcana	Finland, Oulun Pohjanmaa	*Halonen* s. n. (OULU), L189	fum, (pro), (cfum)	GQ996305	GQ996278	GQ996312
B. subcana	Russia, Perm Territory	*Schajachmetova* 18.9 (H), S157	fum, pro, atr	GQ996306	GQ996279	GQ996336
B. tenuis	Finland, Kainuu	*Velmala et al.* 64 (H), S70	fum	HQ402694	HQ402615	HQ402648
B. tenuis	Sweden, Dalarna	*Hermansson* 12855d (UPS), L164	fum	HQ402695	HQ402616	HQ402649
B. trichodes	Russia, Kamchatka	*Himelbrant* K02-27 (H), S334	fum, (pro), (cfum), atr	HQ402707	—	—

TABLE 1. *Continued*

Taxon	Locality	Voucher specimen and sequence ID	Chemistry*	GenBank accession numbers		
				ITS	GAPDH	mtSSU
B. trichodes	Russia, Kamchatka	*Himelbrant* K02-43 (H), S335	fum, (pro), (atr)	HQ402709	—	—
B. trichodes	Russia, Kamchatka	*Himelbrant* EL 6 (H), S346	fum, (pro), (cfum)	HQ402708	—	—
B. trichodes	Canada, Newfoundland	*Ahti* 60134 (H), L230	fum, (cfum), (pro), atr	HQ402710	—	—
B. variabilis	China, Yunnan	*Wang* 04-23184 (KUN), S286	no subst.	HQ402683	—	—
B. sp.	Russia, Komi Republic	*Hermansson* 12625 (UPS), L168	fum	HQ402693	HQ402614	HQ402647
B. sp.	Canada, B.C.	*Goward* 05-05 (UBC), L206	no subst.	GQ996283	GQ996255	GQ996310
B. sp.	Canada, B.C.	*Goward* 07-02-0028 (UBC), S239a	fum in sor.	GQ996284	GQ996256	GQ996313
B. sp.	China, Yunnan	*Wang et al.* 06-26177 (KUN), S278	fum, (pro), (cfum), lob	HQ402697	—	—
B. sp.	China, Yunnan	*Wang* 06-26208 (KUN), S289	fum, (pro), (qua)	HQ402703	—	HQ402652
B. sp.	China, Sichuan	*Wang* 02-21518 (KUN), S290	fum	HQ402696	—	—
B. sp.	China, Yunnan	*Wang et al.* 06-26700 (KUN), S291	(fum?)	HQ402702	HQ402618	HQ402651
Gowardia arctica†	Canada, Nunavut	*Mattson* 5142 (UPS), L169	ale	EU282505	EU282521	—
Nodobryoria abbreviata	USA, California	*Knudsen* 1305 (H), L152	no subst.	HQ402675	—	HQ402634
Pseudephebe pubescens	USA, Alaska	*Ahti* 63704 (H), L221	no subst.	HQ402676	HQ402604	HQ402635
Sulcaria isidiifera	USA, California	*Riefner* 20-293 (H), L151	—	HQ402674	—	HQ402633

* ale = alectorialic acid, atr = atranorin, bar = barbatolic acid, cfum = confumarprotocetraric acid, fum = fumarprotocetraric acid, gyr = gyrophoric acid, lob = lobaric acid, nsti = norstictic acid, pro = protocetraric acid, pso = psoromic acid, qua = quaesitic acid, sti = stictic acid, usn = usnic acid, vul = vulpinic acid, unk. = unknown, in sor. = present only in soralia, ( ) = present in small amounts, - = tlc not performed.
† sequences of *Gowardia arctica* are from Halonen *et al.* (2009)

TABLE 2. *Sections of* Bryoria *according to Brodo & Hawksworth (1977) and some of their main diagnostic characters. Type species of each section is marked in bold. Species with uncertain section position are marked with question mark*

Character	Section			
	Bryoria	*Divaricatae*	*Implexae*	*Tortuosae*
Chemistry	Fumarprotocetraric acid	Fumarprotocetraric acid	β-orcinol depsidones other than fumarprotocetraric acid	Vulpinic acid
Pseudocyphellae	Present or absent	Present or absent	Present	Present or absent
Soralia	Frequent	Occasional	Occasional	Occasional
Growth form	Mostly pendent	Erect, caespitose or subpendent	Subpendent to pendent	Pendent
Lateral spinules or spinulose branches	Rare	Present, constricted basally	Rare	Absent
Taxa included in this study	*B. americana* *B. fuscescens* *B. glabra* *B. lanestris* *B. subcana* ***B. trichodes***	***B. bicolor*** *B. confusa* *B. divergescens?* *B. fastigiata* *B. furcellata* *B. indonesica* *B. lactinea* *B. nitidula* *B. perspinosa* *B. poeltii* *B. simplicior* *B. smithii* *B. tenuis* *B. variabilis*	*B. capillaris* *B. hengduanensis?* ***B. implexa*** *B. nadvornikiana*	***B. fremontii***

sequences of the small subunit of the mitochondrial ribosomal DNA (mtSSU) and 3) partial sequences from the protein-coding glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The three gene regions were amplified and sequenced from the same extraction. As many *Bryoria* species tend to grow intermixed, specimens were extracted from the same material already used for the TLC analysis.

Primers used for PCR amplification were as follows: for the ITS region, ITS1-F (Gardes & Bruns 1993) together with ITS4 (White *et al.* 1990) or ITS1-LM (Myllys *et al.* 1999) together with ITS2-KL (Lohtander *et al.* 1998); for the mtSSU region, mtSSU1-KL together with mtSSU2-KL (Lohtander *et al.* 2002); for the GAPDH region, Gpd1-LM together with Gpd2-LM (Myllys *et al.* 2002). PCR amplification was performed as described by Velmala *et al.* (2009) using PuReTaq Ready-To-Go PCR beads (GE Healthcare).

The PCR products were viewed under UV light on 1% agarose gel stained with either ethidium bromide or SYBR Safe™ DNA gel stain (Invitrogen). The PCR products were purified according to the manufacturers' protocol with either Qiagen's QIAquick PCR Purification Kit or GE Healthcare illustra's GFX tm PCR DNA and Gel Band Purification Kit, and eluted in 15–50 µl elution buffer or dH_2O.

The sequencing reactions were prepared using BigDye Terminator Cycle Sequencing Reaction Kit version 1.1 (Applied Biosystems). The above listed PCR primers were used also for sequencing reactions, except for the ITS region for which the primer ITS5 (White *et al.* 1990) was used together with ITS2-KL. Ten µl reaction samples containing 3 µl dH_2O, 2 µl BigDye, 2 µl sequencing buffer, 1 µl primer at 2·5 µm concentration and 2 µl purified PCR product were run with the following amplification parameters: initial denaturation for 1 min at 96°C or no initial denaturation, followed by 30 cycles of 30 s at 96°C, 15 s at 50°C and 4 min at 60°C.

The post-reaction purification of the samples for ABI PRISM™ DNA Sequencer (Applied Biosystems) was made following the protocol described in Högnabba (2006) and for MegaBACE 1000 DNA Analysis System (GE Healthcare) using Montage SEQ_{96} Cleanup Kit and MultiScreen SEQ_{384} Filter Plates (Millipore) according to the manufacturer's protocol. In some cases the cleaned PCR products were sequenced by Macrogen Inc., South Korea (www.macrogen.com). The DNA strands were assembled and manually corrected with SeqMan II 4.00 (DNASTAR).

Morphological and chemical characters

In addition to molecular data, 20 morphological or chemical characters were used in our phylogenetic reconstruction. Most of the selected characters have previously been used to delimit sections in *Bryoria* (see Table 2). The characters were coded primarily according to species morphology and chemistry as given in the literature (Bystrek 1969; Awasthi 1970; Jørgensen & Ryvarden 1970; Jørgensen 1972, 1975; Brodo & Hawksworth 1977; Jørgensen & Galloway 1983; Awasthi & Awasthi 1985; Holien 1989, 1991, 1992, 1994; Goward 1999; Wang & Harada 2001; Wang *et al.* 2003, 2005, 2006; Harada & Wang 2006). Given, however, the wide range of morphological and chemical variation at the infraspecific level, we carefully inspected each specimen used in the analyses. For instance, the literature indicates that pseudocyphellae (character 19) can in some species be either present or absent. For our purposes this character was coded according to the condition actually observed in the specimen. In a few cases our character coding was at variance with that given in the literature, for example one specimen of *B. bicolor* (Ehrh.) Brodo & D. Hawksw. (S23) contained not only fumarprotocetraric acid but also barbatolic and psoromic acids, these latter two substances hitherto unknown in this species. The data matrix of chemical and morphological characters is presented in the Appendix.

The characters are as follows:

1. Growth form erect to caespitose (0), subpendent (1), pendent (2)
2. Vulpinic acid present (0), only in soralia (1), absent (2)
3. β-orcinol depsidones present (0), absent (1), see characters 4–6
4. Fumarprotocetraric acid present in thallus (0), only in soralia (1), absent (2)
5. Norstictic acid present (0), absent (1)
6. Psoromic acid present (0), absent (1)
7. β-orcinol depsides present (0), absent (1), see characters 8–10
8. Atranorin present (0), absent (1)
9. Alectorialic acid present (0), absent (1)
10. Barbatolic acid present (0), absent (1)
11. Orcinol depsides present (0), absent (1), see characters 12–13
12. Gyrophoric acid present (0), absent (1)
13. Lobaric acid present (0), absent (1)
14. Usnic acid present (0), absent (1)
15. True lateral spinules present (0), absent (1). Brodo & Hawksworth (1977) defined true spinules as short branches with constricted base arising at right or slightly acute angles to the main stems. They are typical for the section *Divaricatae*.
16. Spinulose branches present (0), absent (1). Spinulose branches are not basally constricted (Brodo & Hawksworth 1977). According to our experience, however, they are sometimes difficult to distinguish from true lateral spinules. In such cases, we followed the information given in the literature.
17. Bicolorous thallus present (0), absent (1). As discussed in Brodo & Hawksworth (1977), bicolorous species are typically blackened in the basal parts and pale in the apical portions. Yet some species, for instance *B. americana* and *B. nadvornikiana* (Gyeln.) Brodo & D. Hawksw., are also bicolorous but here the blackened areas are not restricted regularly to the basal parts. This character was fairly easy to code with some exceptions: Jørgensen (1972) characterized *B. confusa* (D.D. Awasthi) Brodo & D. Hawksw. as "uniformly shining brownish," whereas our specimen was distinctly bicolorous, as already described by Awasthi (1970) and Awasthi & Awasthi (1985). Unnamed *Bryoria* specimens L168 and S291 had only slightly paler apices than basal parts but were coded as bicolorous.
18. Soralia present (0), absent (1)
19. Pseudocyphellae present (0), absent (1). For some specimens pseudocyphellae were sometimes difficult to distinguish from young soralia. In such cases the material was coded as missing data.
20. Distribution restricted to SE Asia (0), North America (1), more widely distributed (2). The distribution of each species was used as a basis when coding this character, with one exception: the North American specimens in Clade V representing putative new species were coded as separate taxa. *Bryoria indonesica* (P. M. Jørg.) Brodo & D. Hawksw. is coded as having a SE Asian distribution, although it has also been reported from New Zealand (Jørgensen & Galloway 1983).

Sequence alignment and phylogenetic analyses

We were unable to obtain GAPDH and mtSSU sequences from all of the specimens examined. Consequently, two different data matrices were analyzed to examine the effect of missing data: 1) combined ITS, GAPDH and mtSSU data, which included all 92 specimens and 2) the combined ITS, GAPDH and mtSSU data with 71 specimens successfully sequenced in all three gene regions. The first data set will be referred to in Results and Discussion as the large data set and the second as the pruned data set. In addition, we performed an analysis where the morphological and chemical characters were combined with the large data set.

Prior to analysis, the sequences were aligned with MUSCLE, v. 3.6. (Edgar 2004) located at CSC – IT Center for Science (http://www.csc.fi/english) using default parameters. The hypervariable region in the end of the mtSSU was removed from the combined analysis (characters 2328–2498 in the alignment of large data set and characters 2312–2456 in the alignment of pruned data). All the three data sets were subjected to parsimony analysis as implemented in TNT (Goloboff *et al.* 2008), using the option traditional search with the following settings: random addition sequence with 100 replicates followed by TBR branch swapping. No more than 10 trees were saved for each replicate. Gaps were treated as missing data in these analyses. Support for each node was estimated using bootstrapping (1000 repetitions; otherwise similar options as in heuristic search).

Results

The combined large data set with 92 specimens and 464 parsimony informative characters from 2637 aligned sites generated six equally parsimonious trees with a length of 1416 steps. In the highly resolved strict consensus (Fig. 1), the monophyly of the genus *Bryoria* is moderately well supported. Species are divided into five monophyletic groups (Clades I–V, Fig.1). Most of the groups receive moderately high (Clade III) to high (Clades I, II and V) bootstrap values. Only Clade IV remains unsupported.

Most *Bryoria* species form strongly supported monophyletic entities, the most notable exceptions being *B. capillaris* (Ach.) Brodo & D. Hawksw., *B. fuscescens* (Gyeln.) Brodo & D. Hawksw., *B. implexa*, *B. lanestris* (Ach.) Brodo & D. Hawksw. and *B. subcana* (Nyl. ex Stizenb.) Brodo & D. Hawksw. (Clade V, Fig. 1). These species together form a group in which none of the currently recognized species, or chemotypes in the case of *B. implexa*, appears as a monophyletic entity. Instead, the specimens are divided into two subclades based on their geographic distribution and partly on secondary chemistry. The first subclade includes only North American specimens of *B. capillaris* and *B. implexa* chemotype 2, in addition to two specimens most probably belonging to *B. fuscescens* s. lat. but not exactly conforming to known species within the complex. The second subclade consists of European and Asian specimens of *B. capillaris*, *B. fuscescens*, *B. implexa*, *B. lanestris* and *B. subcana* in addition to North American specimens of *B. fuscescens* and *B. lanestris*. Somewhat surprisingly, the morphologically quite similar *B. glabra* (Motyka) Brodo & D. Hawksw. constitutes a strongly supported sister group to the two subclades.

The large data set combined with morphological and chemical characters gave 20 trees with a length of 1549 steps. The topology of the strict consensus was almost identical to that obtained from the combined molecular data alone; in the consensus all the five major groups were present, but the relationships within the clades were slightly more resolved (Fig. 2).

The Muscle alignment of the pruned data set produced 2598 aligned sites of which 418 were parsimony informative. The TNT analysis generated three equally parsimonious trees of 904 steps, a strict consensus of which is presented in Figure 3. As the pruned data set included fewer taxa than the large data set, a direct comparison of the tree topologies obtained from different analyses is not possible. Otherwise the topologies were mostly congruent with the following exception: *B. americana* (Clade I) and *B. fremontii* (Clade II) form an unsupported group in the pruned data tree while in the tree obtained from the large data set *B. fremontii* is basal to *B. americana*. The topologies differ also in the degree of resolution: the pruned data set produced a more resolved tree with generally higher support values, especially for Clade IV.

Discussion

We detected five major groups within the genus *Bryoria* (Clades I–V, Figs 1–3). All received strong support values particularly in the pruned data tree (Fig. 3). The lower support values in Clade III and particularly in Clade IV, in trees obtained from large data sets, apparently resulted from the large amount of missing data in these clades (Figs 1 & 2). It should also be noted that in the pruned data tree *B. fremontii* and *B. americana* (i.e., lineages I and II) grouped together, but this relationship did not receive any support.

Based on these results, we propose a new infrageneric classification of the genus *Bryoria*, which we apportion in five sections, that is, section *Tortuosae* (Bystr.) Brodo & D. Hawksw., section *Americanae* Myllys & Velmala, section *Divaricatae* (DR.) Brodo & D. Hawksw., section *Bryoria* and section *Implexae* (Gyeln.) Brodo & D. Hawksw. As discussed below, our proposed sections correspond in large part with those described by Brodo & Hawksworth (1977) (see Tables 2 and 3).

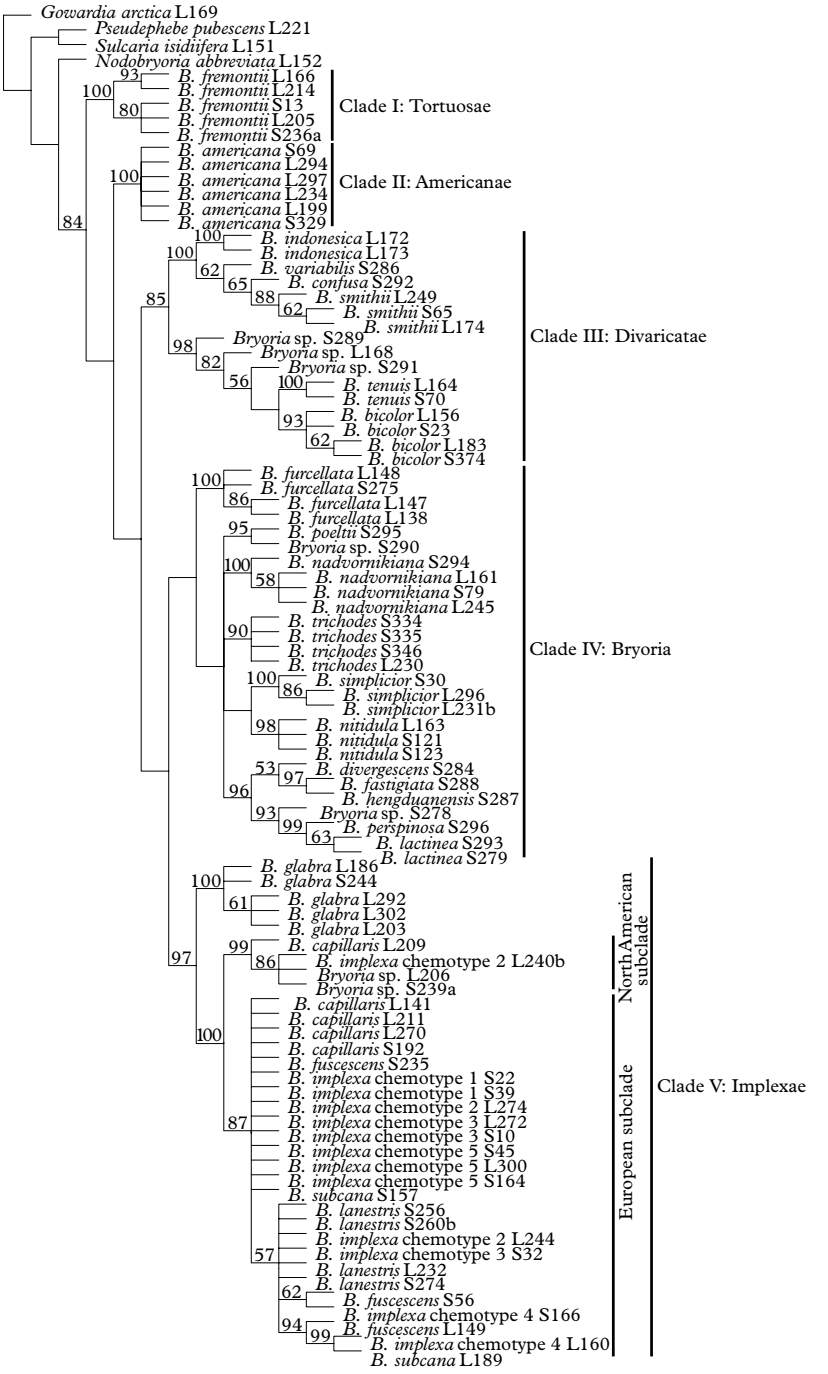

FIG. 1. Strict consensus based on the combined large data set. Bootstrap values are shown at nodes.

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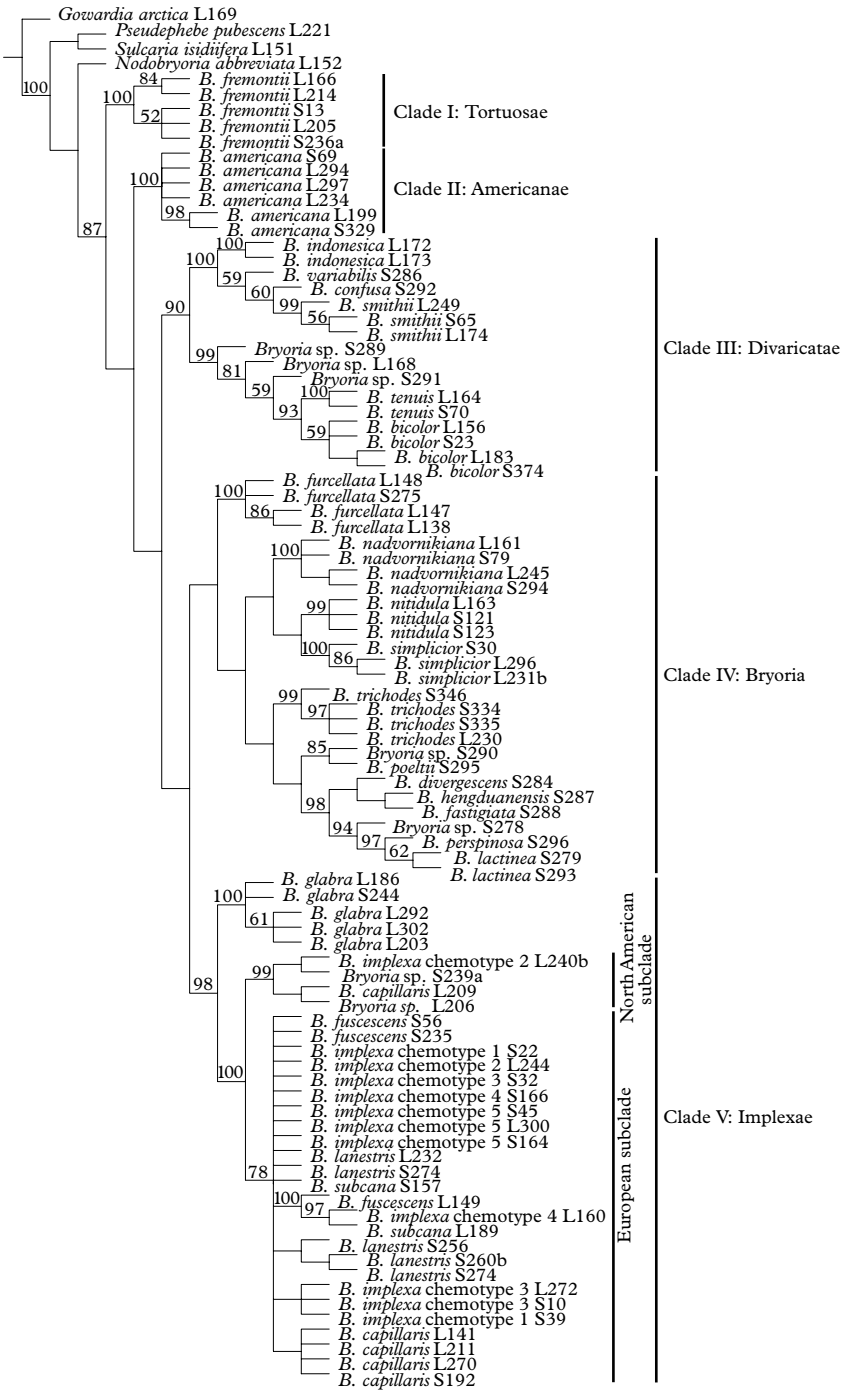

FIG. 2. Strict consensus based on the combined large data set of molecular, morphological and chemical characters. Bootstrap values are shown at nodes.

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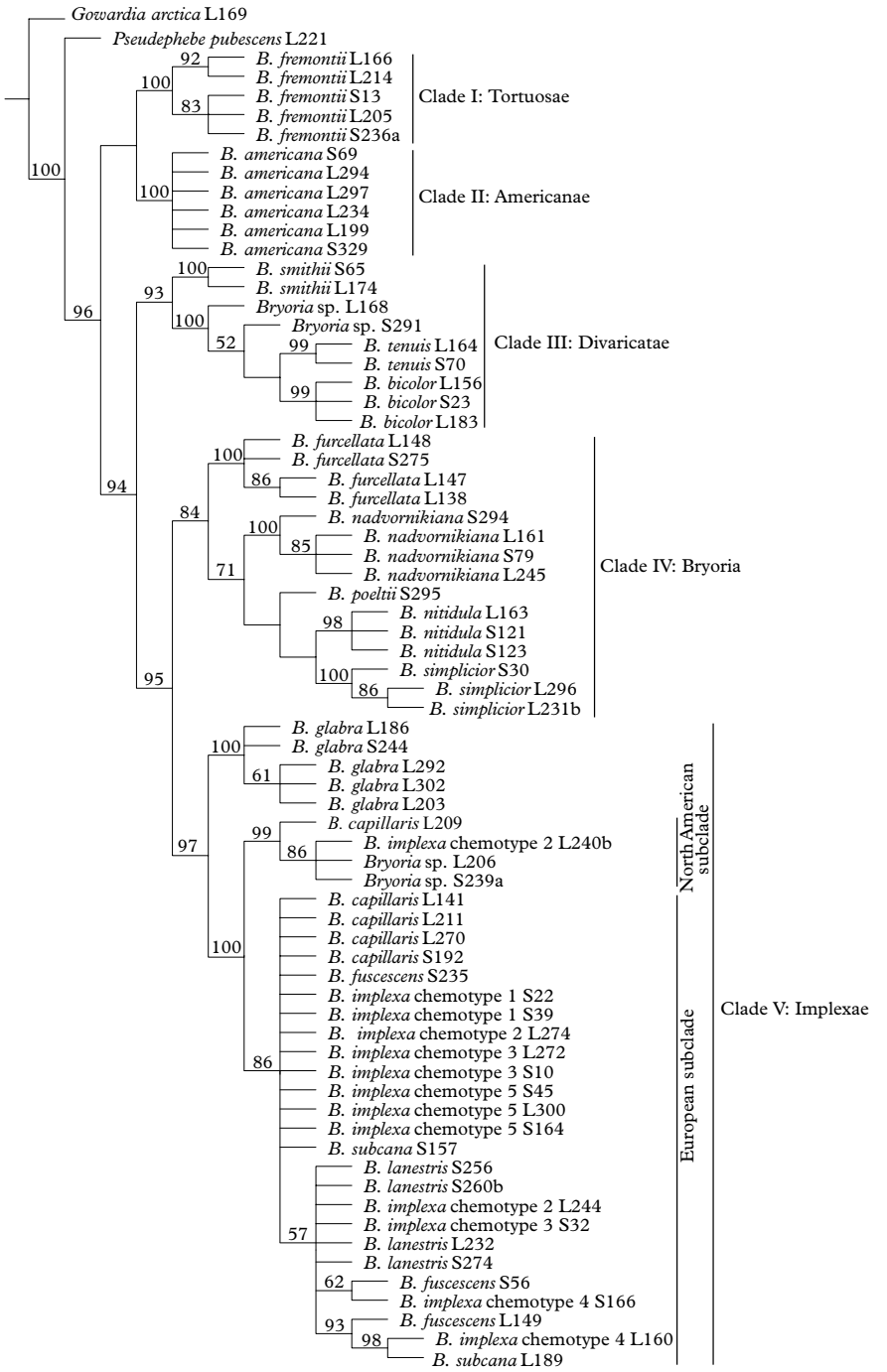

FIG. 3. Strict consensus based on the combined pruned data set. Bootstrap values are shown at nodes.

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Section *Tortuosae* corresponds to Clade I in Figures 1–3, consisting of a single species, *B. fremontii*. *Bryoria fremontii* is unique within *Bryoria* in producing a bright yellow pigment, vulpinic acid. An earlier second species within section *Tortuosae*, *B. tortuosa*, was recently synonymized under *B. fremontii* based on our DNA analyses (Velmala *et al*. 2009). Although the presence of vulpinic acid is the only synapomorphy obtained from our chemical and morphological data matrix for Clade I, *B. fremontii* is also unique within pendent species of *Bryoria* in its production of proportionately broad, twisted, foveolate and often partly flattened main branches and finer rather terete secondary branches (Fig. 4A; see also Goward 2009). The colour of the thallus is typically reddish or yellowish brown depending on the concentration of vulpinic acid. *Bryoria fremontii* occurs predominantly in western North America and northern Europe (see Velmala *et al*. 2009, Fig. 4).

Our new section *Americanae* (Clade II) also consists of a single species, *B. americana*. None of the morphological and chemical characters examined appeared as synapomorphies for this section. *Bryoria americana* may indeed be confused with other pendent taxa, especially with *B. fuscescens* and *B. implexa* s. str. from which it is distinguished by its small perpendicular side branches, its depressed pseudocyphellae, and its usually fully esorediate stems with blackened fragmentation regions (Fig. 4B). Brodo & Hawksworth (1977) treated *B. americana* and *B. trichodes* as subspecies within *B. trichodes*, owing to the presumed existence in North America of intermediate forms. Later, Holien (1994) detected *B. americana* in Europe and segregated it from the non-European *B. trichodes*. Our results confirm the latter view and show that *B. americana* is a distinct species not closely related to *B. trichodes*. The two taxa are best separated by their pseudocyphellae: fissural, depressed, dark and longer in *B. americana* versus oval, raised, white and shorter in *B. trichodes*. Interestingly, our material of *B. trichodes* from the Russian Far East had pigmented and long (up to 0·85 mm!) pseudocyphellae, but they were never depressed as in *B. americana*. True *B. americana* is an oceanic species occurring mainly in humid coastal regions of eastern and western Canada (Brodo & Hawksworth 1977) and NW Europe. In Europe the species is still probably much overlooked owing to difficulties in identification (see Brodo 1992; Holien 1994; Myllys *et al*. 2006).

Section *Divaricatae* has traditionally been considered a well-defined entity characterized by true lateral spinules and the exclusive presence of fumarprotocetraric acid (sometimes absent). Many such species, but not all, are erect or caespitose and have a characteristic bicolorous thallus with blackened basal parts and greyish brown to olive-brown apical branches and spinules (Jørgensen & Ryvarden 1970; Jørgensen 1972, 1975; Brodo & Hawskworth 1977). The centre of diversity is in East Asia but some of the species have a wider distribution. In our analyses *Divaricatae* (Clade III) is more restricted including only taxa with bicolorous thallus, an exclusive synapomorphy for this group (Fig. 4C). Other species with more or less uniformly coloured thalli traditionally included in *Divaricatae* are now nested in Clade IV.

The section *Divaricatae* includes two subclades, the first of which consists of *B. indonesica*, *B. variabilis* (Bystrek) Brodo & D. Hawksw., *B. confusa* and *B. smithii* (Du Rietz) Brodo & D. Hawksw., all characterized by the loss of fumarprotocetraric acid. Our results support Jørgensen (1972) who stated that *B. confusa* and *B. smithii* are closely related and represent a typical species pair *sensu* Poelt (1970), where *B. confusa* is fertile and *B. smithii* sterile bearing isidioid soralia. *Bryoria confusa* has a more restricted distribution found only in East Asia, while *B. smithii* is more widespread, occurring even in Fennoscandia.

The species containing fumarprotocetraric acid are restricted to the second subclade of *Divaricatae*. *Bryoria bicolor* and *B. tenuis* (E. Dahl) Brodo & D. Hawksw. have been considered closely related (Jørgensen & Ryvarden 1970; Brodo & Hawksworth 1977) and our study confirms this view. The two species are fairly easily separated, with *B.*

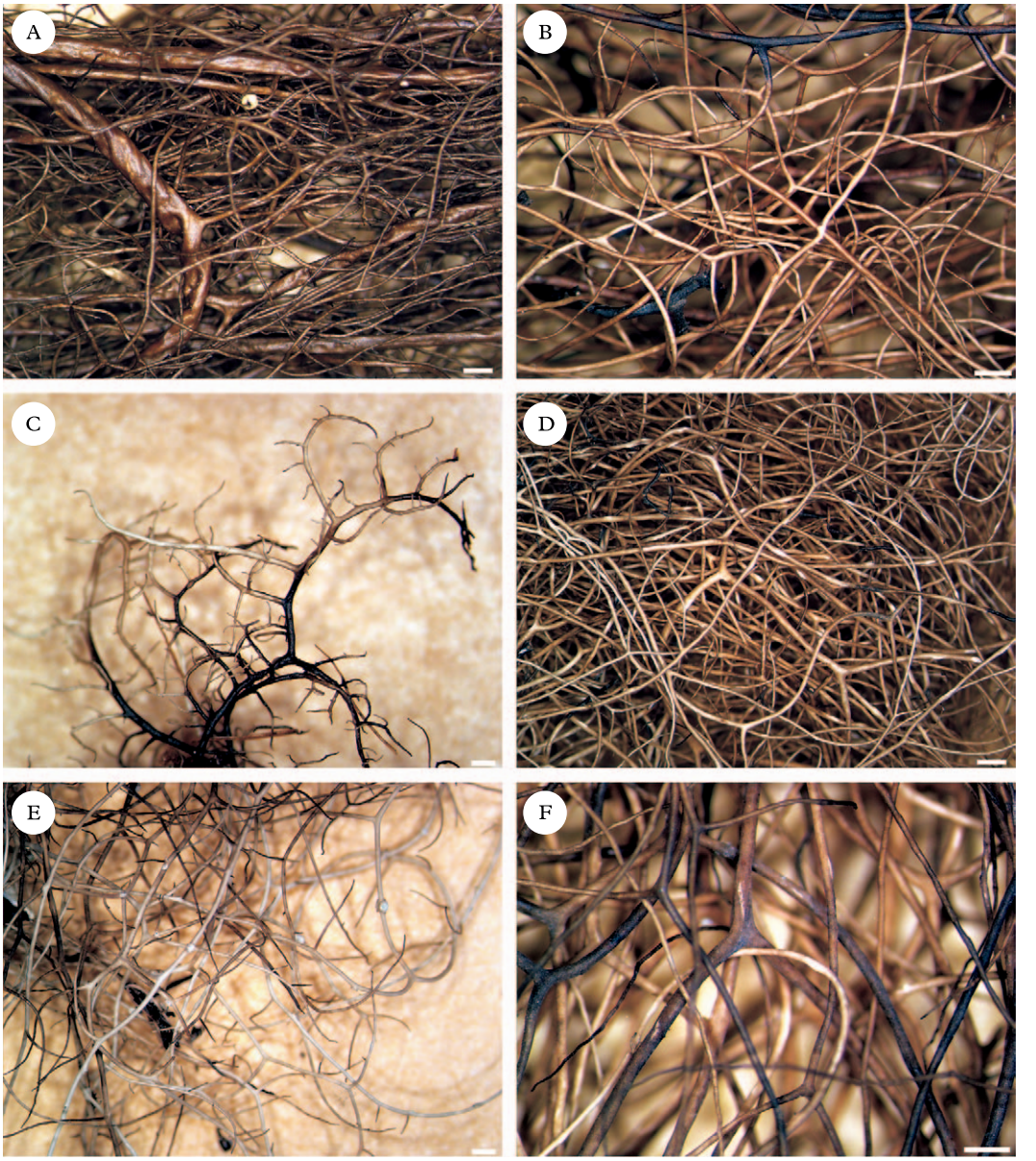

FIG. 4. *Bryoria* species representing each section of the genus. A, *Bryoria fremontii* from section *Tortuosae* (specimen S13 with yellow soralia and foveolate main branches); B, *Bryoria americana* from section *Americanae* (specimen L294 with blackened fragmentation regions); C, *Bryoria bicolor* from section *Divaricatae* (specimen L183 with bicolorous thallus and lateral spinules); D, *Bryoria trichodes* from section *Bryoria* (specimen L230 with oval and white pseudocyphellae); E, *Bryoria nadvornikiana* from section *Bryoria* (specimen S79 with spinulose side branches); F, *Bryoria implexa* from section *Implexae* (specimen L274 with pseudocyphellae and wide branch angles). Scales: A–F = 1 mm.

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tenuis having acute branch angles and lacking the tertiary branches typical for *B. bicolor*. In addition to the two species, the subclade includes three unidentified taxa. They all resemble *B. bicolor* but differ from this species in branching pattern; for instance specimen S289 collected in China has a distinct main stem and stiffer and coarser habit as compared to *B. bicolor*. Furthermore, specimens L168 and S291 were only slightly bicolorous. All three taxa probably represent new species although more specimens with similar morphology and chemistry are needed before their status can be discussed further (P. M. Jørgensen, pers. comm.).

Our circumscription of Section *Bryoria* (Clade IV) differs markedly from that of Brodo & Hawksworth (1977), with only the type of the section, *B. trichodes*, remaining from the original circumscription. All other species in Clade IV were treated within sections *Divaricatae* and *Implexae* by Brodo & Hawksworth (1977). This result, however, was not unexpected as *B. trichodes* differs from other species traditionally placed in section *Bryoria*, (e.g., *B. fuscescens*) in the production of pseudocyphellae (Fig. 4D). Pseudocyphellae, on the other hand, are lacking in many species in Clade IV, and hence are of little use in circumscribing section *Bryoria*. The same is true of growth form, which ranges from erect to caespitose, and from subpendent to pendent. Fumarprotocetraric acid is characteristic for the section, though other substances are also present, including lobaric acid [in *B. perspinosa* (Bystrek) Brodo & D. Hawksw. and *B. lactinea* (Nyl.) Brodo & D. Hawksw.], barbatolic acid (in *B. nadvornikiana*), usnic acid (in *B. hengduanensis* Li S. Wang & H. Harada), and fatty acids [in *B. simplicior* (Vain.) Brodo & D. Hawksw.]. All species in Clade IV, except *B. trichodes* have either spinulose branches or true lateral spinules (Fig. 4E). Spinulose branches appear also in Clade II and lateral spinules in Clade III but both characters are absent from Clades I and V.

Virtually all SE Asian species examined in our study belong in Clade IV, the only exceptions being *B. confusa* and *B. variabilis*, which have bicolorous thalli and belong in Clade III. Most of the remaining species (i.e., *B. divergescens* (Nyl.) Brodo & D. Hawksw., *B. fastigiata* Li S. Wang & H. Harada, *B. hengduanensis, B. lactinea*, and *B. perspinosa*) form a strongly supported subclade within section *Bryoria*. *Bryoria poeltii* (Bystrek) Brodo & D. Hawksw. also appears to belong in this subclade, especially when the molecular data are amplified with morphological and chemical characters, yet even then without support. The lobaric-acid containing species *B. lactinea* and *B. perspinosa* form a smaller group in the SE Asian subclade, although this substance was not observed in the specimens of *B. lactinea* used in our study. Also belonging in this group is an unidentified specimen (S278), which shares all the characteristic features of *B. perspinosa*, that is isidioid soralia and presence of lobaric acid, but has a distinctive ITS profile. By contrast, an unidentified specimen S290 shares an almost identical ITS sequence with *B. poeltii* but differs morphologically in having a pendent thallus (versus caespitose in *B. poeltii*), dark pseudocyphellae (absent in *B. poeltii*) and in lacking soralia (isidioid soralia present in *B. poeltii*). More thorough sampling with multiple specimens of each species is clearly needed before a reliable taxonomy for these species can be achieved. Apparently the mountains of SE Asia are an area of considerable diversity in *Bryoria*, for which they represent a centre of speciation, as discussed by Jørgensen & Galloway (1983).

With the exception of the SE Asian subclade, species relations within section *Bryoria* remain mostly unresolved. As discussed above, lack of resolution is probably due to missing data in Clade IV. Our study confirms, however, that *B. simplicior* and *B. poeltii* are closely related, as suggested by Bystrek (1969) and Jørgensen (1972). *Bryoria poeltii* resembles *B. simplicior* by having round and wide soralia but has different chemistry containing fumarprotocetraric acid.

Members of section *Implexae* (Clade V) can be characterized by their pendent growth form and the absence of spinulose branches, although neither character represents a

synapomorphy. Most *Bryoria* species included by Brodo & Hawksworth (1977) in sections *Bryoria* (except *B. americana* and *B. trichodes*) and *Implexae* (except *B. nadvornikiana*) belong here. Sections *Bryoria* and *Implexae* have traditionally been separated on the basis of chemistry and cortical characters (Brodo & Hawksworth 1977; Holien 1989). Species in section *Bryoria* typically contain fumarprotocetraric acid, at least in the soralia, while section *Implexae* is defined by the presence of *β*-orcinol depsidones other than fumarprotocetraric acid. Furthermore, species in the latter section always bear pseudocyphellae (Fig. 4F) and have a characteristic cortical structure with unusually friable branches. As shown by our analyses, however, none of these characters are useful in characterizing infrageneric groupings.

Within section *Implexae*, only *B. glabra* is unambiguously supported as a distinct species; it is usually easily distinguished by its obtuse and rounded branches and usually fissural, often regularly oval, white soralia. The systematic position of the remaining species in this section is much more problematic. Our analyses strongly suggest, for example, that *B. capillaris* (European material only), *B. fuscescens*, *B. implexa*, *B. lanestris* and *B. subcana* are all conspecific. Low resolution within the 'European subclade' (Figs 1–3) reflects a lack of information (i.e. exactly identical sequences among the specimens) rather than incongruence between different gene regions. The few monophyletic groups found within the subclade did not correlate with current taxonomic concepts, nor were corroborating morphological or chemical characters for these smaller groups found.

Unfortunately, no fresh material of *B. chalybeiformis* (L.) Brodo D. Hawksw. was available to us, though we suspect this taxon also belongs to the 'European subclade'. *Bryoria chalybeiformis* is traditionally recognized by its prostrate, stout, shiny, dark thallus and exclusive presence of fumarprotocetraric acid in the soralia (Brodo & Hawksworth 1977). All specimens originally identified as *B. chalybeiformis* and preliminarily included in our DNA analyses either contained fumarprotocetraric acid in the whole thallus or turned out to be misidentifications of other, more distantly related species such as *B. glabra*. Therefore, we suspect that the stout, dark habit may be an adaptation to exposed habitats, and that such specimens largely represent environmental modifications of *B. fuscescens* s. lat. Indeed, a parallel morphology is seen in the unrelated *Alectoria sarmentosa* subsp. *vexillifera* (Nyl.) D. Hawksw. found in similar habitats (I. Brodo, pers. comm.). When material in the European subclade is included, *B. chalybeiformis* becomes the oldest name at the species level. At this stage, however, we are reluctant to adopt this step given the extreme morphological and chemical variability found within a group. We conclude that more data, including material of *B. chalybeiformis* and additional gene loci, are needed to solve the relationships of this group.

Our analyses suggest that North American and European *B. capillaris* and *B. implexa* chemotype II, respectively, may represent taxonomically distinct entities. This result seems to be upheld by morphological differences, insofar as both *B. capillaris* and *B. implexa* are frequently sorediate in Europe, while North American specimens are esorediate in both species (Brodo & Hawksworth 1977; Holien 1989). Two unidentified specimens in the North American subclade resemble *B. fuscescens* but have pseudocyphellae (specimen L206) or lack secondary substances (specimen S239a); most probably they represent new species. However, as our study included only one North American specimen of each taxon, more data are needed to better understand species delimitation and taxonomy within Clade V.

Nomenclature

Bryoria sect. Americanae Myllys & Velmala, sect. nov.

MycoBank: 519583

Thallus pendulus, brunneus, regionibus fragmentationis nigrescentibus. Ramis principales crassi plerumque ramis brevibus perpendiculariter instructis. Soralia rara. Pseudocyphellae brunneolae, fusiformes

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TABLE 3. *Sections of* Bryoria *as accepted in this paper and some of their main diagnostic characters. Type species of each section are marked in bold*

Character	Section				
	Americanae	*Bryoria*	*Divaricatae*	*Implexae*	*Tortuosae*
Chemistry	Fumarprotocetraric acid	Usually fumarprotocetraric acid	Sometimes fumarprotocetraric acid	Usually fumarprotocetraric acid	Vulpinic acid
Pseudocyphellae	Present	Present or absent	Present or absent	Present or absent	Present or absent
Soralia	Usually absent	Present or absent	Present or absent	Present or absent	Occasional
Growth form	Pendent	Erect, caespitose, subpendent or pendent	Erect, caespitose, rarely subpendent; bicolorous	Subpendent to pendent	Pendent
Lateral spinules or spinulose branches	Present	Usually present	Present, constricted basally	Absent	Absent
Taxa included in this study	***B. americana***	*B. divergescens* *B. fastigiata* *B. furcellata* *B. hengduanensis* *B. lactinea* *B. nadvornikiana* *B. nitidula* *B. perspinosa* *B. poeltii* *B. simplicior* ***B. trichodes***	***B. bicolor*** *B. confusa* *B. indonesica* *B. smithii* *B. tenuis* *B. variabilis*	*B. capillaris* *B. fuscescens* *B. glabra* ***B. implexa*** *B. lanestris* *B. subcana*	***B. fremontii***

vel fissurales. Medulla et soralia acidum fumarprotocetraricum continens. Apothecia acidum psoromicum continens.

Type species: *Bryoria americana* (Motyka) Holien.

(Fig. 4B)

Thallus pendent. Colour brown to dark brown, often with blackened fragmentation areas. Irregularly branched, usually with wide angles, main branches often with perpendicular branches. *Soralia* absent to sparse, white, tuberculate or fissural, often causing the branch to recurve. *Pseudocyphellae* sparse, fusiform and depressed, brownish, to 1 mm long.

Ascomata locally common, to 2 mm diam., disc brown, becoming convex; ascospores ellipsoid, 5·5–7 × 4–5 µm.

Conidiomata unknown.

Chemistry. Fumarprotocetraric acid in medulla and soralia (sometimes in low concentrations). Psoromic acid in apothecia.

The section *Americanae* includes only *Bryoria americana*.

Conclusions

The phylogenetic analyses presented here identified five main groups in the genus *Bryoria* (Table 3). Although the groups, here proposed as sections, were defined by molecular characters, we also found some chemical and/or morphological characters to support them. Only two of these characters were synapomorphies: "presence of vulpinic acid" defining section *Tortuosae* and "presence of bicolorous thallus" characteristic for section *Divaricatae*. Lateral spinules characterize section *Divaricatae* and section *Bryoria* (in part), whereas spinulose branches are present in section *Americanae* and also partly characterize section *Bryoria*. Other morphological and chemical characters, including soralia, pseudocyphellae and most secondary substances seem to have little taxonomic value for infrageneric groupings, although they may be useful in delimiting closely related species.

Our study shows the importance of including multiple specimens from each species in phylogenetic studies. For instance, it is apparent that the taxonomic status of species in section *Implexae* requires further clarification. We have currently started a more comprehensive data sampling to solve the relationships within this group (S. Velmala *et al.*, unpublished). Also the taxonomic status of the unnamed specimens in sections *Divaricatae* and in *Bryoria* needs to be examined.

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Appendix. Data matrix of morphological and chemical characters (see Materials and Methods for explanation of characters). N = missing data.

Specimen	Character																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Gowardia arctica L169	0	2	1	2	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1
Nodobryoria abbreviata L152	0	2	1	2	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	2
Pseudephebe pubescens L221	0	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sulcaria isidiifera L151	2	2	1	2	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	2
Bryoria Americana L199	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1
B. americana L234	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
B. americana L294	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
B. americana L297	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
B. americana S69	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
B. americana S329	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1
B. bicolor L156	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1
B. bicolor L183	0	2	N	N	N	N	N	N	N	N	N	N	N	N	0	1	0	1	0	1
B. bicolor S23	0	2	0	0	1	0	0	1	1	0	1	1	1	1	0	1	0	1	0	1
B. bicolor S374	0	2	0	0	1	1	1	1	1	1	0	0	1	1	0	1	0	1	0	1
B. capillaris L141	2	2	1	2	1	1	0	1	0	0	1	1	1	1	1	1	1	1	0	1
B. capillaris L209	2	2	1	2	1	1	0	1	0	0	1	1	1	1	1	1	1	1	0	2
B. capillaris L211	2	2	0	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1
B. capillaris L270	2	2	N	N	1	1	0	N	N	0	1	1	1	1	1	1	1	0	1	1
B. capillaris S192	2	2	0	0	1	1	0	0	0	0	1	1	1	1	1	1	1	1	0	1
B. confusa S292	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0
B. divergescens S284	0	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0
B. fastigiata S288	0	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
B. fremontii L166	2	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
B. fremontii L205	2	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
B. fremontii L214	2	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
B. fremontii S13	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
B. fremontii S236a	2	0	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
B. furcellata L138	0	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
B. furcellata L147	0	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
B. furcellata L148	0	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
B. furcellata S275	0	2	1	2	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
B. fuscescens L149	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. fuscescens S56	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. fuscescens S235	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	N	1
B. glabra L186	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	N	1
B. glabra L203	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	N	1
B. glabra L292	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. glabra L302	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. glabra S244	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1

Appendix. ***Continued***

Specimen	Character																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
B. hengduanensis S287	2	2	0	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	0
B. implexa 1 S22	2	2	0	2	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 1 S39	2	2	0	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1
B. implexa 2 L240b	2	2	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	2
B. implexa 2 L244	2	2	0	2	0	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 2 L274	2	2	0	2	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 3 L272	2	2	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	0	1
B. implexa 3 S10	2	2	1	2	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1
B. implexa 3 S32	2	2	0	1	1	1	0	0	1	1	0	0	1	1	1	1	1	0	0	1
B. implexa 4 L160	2	2	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 4 S166	2	2	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 5 S45	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 5 L300	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
B. implexa 5 S164	2	2	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1
B. indonesica L172	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0
B. indonesica L173	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0
B. lactinea S293	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
B. lactinea S279	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
B. lanestris S256	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. lanestris S260b	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. lanestris L232	2	2	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1
B. lanestris S274	2	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B. nadvornikiana L161	1	2	0	0	1	1	0	1	1	0	1	1	1	1	1	0	1	0	0	1
B. nadvornikiana L245	1	2	1	2	1	1	0	1	1	0	1	1	1	1	1	0	1	0	0	1
B. nadvornikiana S79	1	2	0	0	1	1	0	0	0	0	1	1	1	1	1	0	1	0	0	1
B. nadvornikiana S294	1	2	1	2	1	1	0	0	0	0	1	1	1	1	1	0	1	0	1	1
B. nitidula S121	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
B. nitidula L163	0	2	N	N	N	N	N	N	N	N	N	N	N	N	0	1	1	1	0	1
B. nitidula S123	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
B. perspinosa S296	2	2	0	0	1	1	1	1	1	1	0	1	0	1	1	0	1	0	0	0
B. poeltii S295	0	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0
B. simplicior L231b	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1
B. simplicior L296	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1
B. simplicior S30b	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1
B. smithii L174	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1
B. smithii L249	0	2	1	2	1	1	0	0	1	1	1	1	1	1	0	1	0	0	1	1
B. smithii S65	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1
B. subcana L189	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
B. subcana S157	2	2	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1
B. tenuis L164	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1
B. tenuis S70	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1
B. trichodes L230	2	2	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1
B. trichodes S334	2	2	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1
B. trichodes S335	2	2	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1
B. trichodes S346	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
B. variabilis S286	0	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	0	N	0
B. sp. L168	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	N
B. sp. L206	2	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	2
B. sp. S239a	2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	2
B. sp. S278	0	2	0	0	1	1	1	1	1	1	0	1	0	1	1	0	1	0	1	0
B. sp S289	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0
B. sp. S290	2	2	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
B. sp. S291	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0

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