

DNA barcoding indicates the presence of unrecognized species and phylogenetic diversity within the *Biatora vernalis*- and *B. meiocarpa*-groups

Christian Printzen^{1*}, Håkon Holien^{2,3}, Annina Kantelinen⁴, Leena Myllys⁴, Felicia Ratschow^{5,1}, Irina Stepanchikova⁶, Lilith Weber⁴ & Einar Timdal⁷

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Abstract. The *Biatora vernalis*- and *meiocarpa*-groups comprise species with pale beige to reddish brown apothecia and 0-, rarely also 1(–3)-septate ascospores. A DNA barcoding approach based on ITS sequences shows that these two groups comprise more species and phylogenetic diversity than previously known. Specimens identified as *B. vernalis*, the type species of the genus, appear to be paraphyletic with regard to *B. chrysantha*. In addition, there is a morphologically similar species belonging to the *B. meiocarpa*-group and tentatively named “*B. orientalis*” in previous publications. *Biatora subduplex* has for some time been known to comprise specimens from the *B. vernalis*-, as well as the *B. meiocarpa*-group. Similar to the situation in *B. vernalis*, samples from the *meiocarpa*-clade form several subclades close to *B. meiocarpa*. Anatomical studies reveal subtle, but recognizable morphological differences between *B. subduplex* s.str. and the species in the *meiocarpa*-clade, but not between the subclades. Here, we describe *Biatora orientalis* as new to science, raise *B. meiocarpa* var. *tacomensis* to species rank and provide revised identification keys for the *B. vernalis*- and *B. meiocarpa*-groups.

Key words: crustose lichens, cryptic species, DNA barcoding, East Asia, Europe

Introduction

The genus *Biatora* Fr. comprises mostly inconspicuous crustose lichens with a trebouxoid photobiont, biatorine apothecia (i.e., without photobionts in their non-carbonized margins) and *Biatora*-type asci (Hafellner 1984) usually containing eight hyaline 0–3(–7)-septate spores. Its most distinctive feature is the strong gelatinization of apothecial tissues. Paraphyses, hypothecial and excipular hyphae are embedded in a chondroid matrix, in which individual hyphal walls can rarely be distinguished. Argopsin and gyrophoric acid are the most common secondary

compounds, but many species do not produce substances detectable by thin layer chromatography (TLC). Species of *Biatora* inhabit organic substrata (bark, wood, bryophytes and plant debris), mostly in temperate and boreal forests as well as arctic regions of the northern hemisphere. Three species are known to occur in South America (Rodríguez-Flakus & Printzen 2014; Printzen et al. 2016). *Biatora* belongs in the family *Ramalinaceae*, with the predominantly tropical *Phyllopsora* Müll. Arg. as its closest relative (Kistenich et al. 2018). Currently more than 50 species are distinguished, but the number is constantly increasing, mainly due to the detection of hitherto unrecognized species (e.g., Printzen et al. 2016; Kistenich et al. 2018), less often because species were transferred to *Biatora* from other genera (Kistenich et al. 2018).

As a result of phylogenetic studies, Printzen (2014) distinguished six more or less well supported species groups within *Biatora*. The core or “crown group” of the genus is formed by the *vernal*-group, named after the generic type, *B. vernalis* (L.) Fr., and the *meiocarpa*-group. These groups comprise species with pale beige to reddish brown apothecia and 0-, rarely also 1(–3)-septate ascospores. Many of the species are phenotypically similar and difficult to distinguish. This does not only apply to closely related species. The recently described

¹ Senckenberg Research Institute and Natural History Museum Frankfurt, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany (Printzen, ORCID: 0000-0002-0871-0803)

² Faculty of Biosciences and Aquaculture, Nord University, N-7729 Steinkjer, Norway

³ NTNU University Museum, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

⁴ Finnish Museum of Natural History, P.O. Box 7, FI-00014 University of Helsinki, Finland

⁵ Johannes Gutenberg University Mainz, Saarstr. 21, 55122 Mainz, Germany

⁶ Department of Botany, St Petersburg State University, Universitetskaya emb. 7–9, 199034 St. Petersburg, Russia

⁷ Natural History Museum, University of Oslo, Blindern, N-0318 Oslo, Norway

* Corresponding author e-mail: christian.printzen@senckenberg.de

B. terrae-novae and *B. pycnidiata* are good examples of species that are rather distantly related, but may be difficult to distinguish. *Biatora* “*orientalis*” was mentioned by Printzen (2014) as a member of the *meiocarpa*-group almost indistinguishable from *B. vernalis*. And *B. subduplex* turned out to comprise at least two different taxa, one of which belongs to the *vernalis*-group, the other to the *meiocarpa*-group. These (semi-)cryptic species have not been formally described to date.

It is commonplace to say that lichens are difficult to distinguish and identify. In particular, small crustose lichens may lack clear distinguishing features. Molecular data often indicate the presence of more taxa than can be distinguished by morphology alone, a situation that has been described as the “cryptic species trap” (Schneider et al. 2016). Cryptic species are commonly defined as well-supported phylogenetic lineages that are “at least superficially morphologically indistinguishable” (Bickford et al. 2007). While one could argue that “superficially”, the majority of lichens are indistinguishable, the increased use of molecular genetic data by taxonomists has indeed led to the detection of many cryptic lichen species (e. g. Leavitt et al. 2011, 2016; Zhao et al. 2017; Zakeri et al. 2019). “DNA barcoding” (Hebert et al. 2003) has been advocated as a tool for safe identification of such cryptic lineages and has successfully been used in some lichen genera (e. g., Divakar et al. 2016). These data can then be used to map phenotypic traits on lineages in order to find distinguishing characters between previously indistinguishable lineages (Lücking et al. 2020). However, the differences between these lineages are sometimes so subtle that they have been named “seemingly-cryptic”, “semi-cryptic” or have been treated at the infraspecific level (Hodkinson & Lendemer 2011; Altermann et al. 2014; Frolov et al. 2016; Coca et al. 2018; Ossowska et al. 2018; Lutsak et al. 2020).

In this study, we use a DNA barcoding approach based on DNA sequences of the internal transcribed spacer region (ITS) of the ribosomal RNA, the proposed universal barcoding marker for fungi (Schoch et al. 2012), to shed more light on the phylogenetic diversity within the genus *Biatora* focusing on the *vernalis*- and *meiocarpa*-groups. Most of the sequences were generated within the project “OLICH: A reference library of DNA barcodes for Nordic lichens” (Marthinsen et al. 2019) to which we tried to add as many sequences from as many different localities as were currently available to us. In addition to documenting the phylogenetic diversity, we attempted to find phenotypic differences between genetically distinguishable clades, in order to circumscribe and key out new species. We dedicate this contribution to our good friend and colleague Tor Tønsberg, who over the years contributed substantially to the taxonomy of *Biatora* (Printzen & Tønsberg 1999, 2003, 2004; Tønsberg 2002; Printzen et al. 2016; Tønsberg & Printzen 2018).

Materials and methods

This study is based on collections from Canada, the Czech Republic, Finland, Germany, Greenland, Italy, Norway,

Russia, South Korea, Sweden, Switzerland and Turkey. Further DNA sequences retrieved from GenBank were generated from material collected in Austria, Japan, Slovakia, Svalbard, and Ukraine. Voucher specimens are deposited in BG, CANL, FR, H, MIN, O, OSC, PRA, TNS, TRH, UPS and the private herbaria of S. Bayerová, J. Malíček, P. May, P. van den Boom and M. Vust (Table 1).

Measurements of ascomatal structures were performed on hand sections or microtome sections (Zeiss Hyrax S-30 microtome with KS 34 cryostat) in lactophenol cotton blue (LCB). Spore measurements were made on sections or squash preparations mounted in water. Spore measurements are given as (smallest single measurement–) smallest mean–largest mean (–largest single measurement). Boxplots were produced in Excel to illustrate the distribution of numerical values in *B. subduplex* and *B. aff. meiocarpa* for the following characters: density of paraphyses and excipular hyphae, epihymenium, hymenium, subhymenium and hypothecium height. Lichen substances were identified by thin-layer chromatography (TLC, Culberson & Kristinsson 1970) with later modifications (Culberson 1972; Culberson & Ammann 1979).

Methods of DNA extraction, PCR, DNA sequencing and editing followed Printzen (2014). We performed NCBI BLAST searches (Johnson et al. 2008) to check the identity of our sequences in Genbank. Additional sequences of species belonging to the *B. vernalis*- and *B. meiocarpa*-group were downloaded from Genbank. *Biatora flavopunctata* and *B. vacciniicola* were used as outgroup to root the tree. Sequences used in the analyses are listed in Table 1. Sequences were aligned with an online application of MAFFT version 7 (Katoh et al. 2019, URL: <https://mafft.cbrc.jp/alignment/server/>) with default settings (“Auto” strategy, aligning gappy regions and the default guide tree). Regions of uncertain alignment (GUIDANCE score below 0.93) were removed from this alignment using the GUIDANCE2 server (URL: <http://guidance.tau.ac.il/ver2/>, Landan & Graur 2008, Sela et al. 2015). A maximum likelihood (ML) tree was reconstructed with IQ-TREE version 2.2.2.7 (Nguyen et al. 2015) on the CIPRES Science Gateway (<https://www.phylo.org/>) using 1,000 bootstrap replicates and the ultrafast bootstrap option with the nearest neighbor interchange option to reduce the impact of possible model violations on support values. The partitioning scheme and substitution models were simultaneously optimized using the Bayesian information criterion, initially suggesting three separate partitions for the ITS1, 5.8S, ITS2 regions. We reconstructed a Bayesian phylogeny using the Markov Chain Monte Carlo (MCMC) approach implemented in MrBayes, version 3.2.7 (Ronquist et al. 2012), the partitioning scheme and substitution models inferred by IQ-Tree and the settings outlined in Palice et al. (2023). The analysis was stopped after 1.9 million generations when the standard deviation had dropped below 0.01. As support values, we report results of the Shimodaira-Hasegawa approximate likelihood ratio test and bootstrap percentages for the ML tree and posterior probabilities for the MCMC. Newly generated DNA sequences were submitted to GenBank (Table 1).

Table 1. Collections used for DNA sequencing and Genbank accession numbers. Accession numbers in bold indicate newly generated sequences.

Sample	Origin	Accession number
<i>Biatora alaskana</i> 1	Japan, Haugan & Timdal 16661 (O)	MH481915
<i>Biatora alaskana</i> 2	Japan, Haugan 170705 (O)	MH481922
<i>Biatora alaskana</i> 3	USA, Alaska, Printzen 5229 (FR)	KF650957
<i>Biatora alaskana</i> 4	Japan, Thor 24732 (UPS)	KF650958
<i>Biatora alaskana</i> 5	Japan, Thor 32399 (UPS)	KX389591
<i>Biatora alaskana</i> 6	Japan, Sugimoto MSu112 (TNS)	LC742596
<i>Biatora alaskana</i> 7	Japan, Ohmura YO10700 (TNS)	LC742597
<i>Biatora alaskana</i> 8	Japan, Ohmura YO8211 (TNS)	LC742598
<i>Biatora alaskana</i> 9	USA, Alaska, McCune 36047 (OSC)	MN906267
<i>Biatora chrysantha</i> 1	Germany, Printzen s.n. (FR)	AJ247569
<i>Biatora chrysantha</i> 2	Russia, Republic of Adygea, Vondrák 16141 (PRA)	MK778587
<i>Biatora chrysantha</i> 3	Norway, Holien 11525 (TRH)	OR775079
<i>Biatora chrysantha</i> 4	Czech Republic, Malíček 14572 (hb. Malíček)	ON226893
<i>Biatora chrysantha</i> 5	Norway, Frisch N12-2-Pa11-1 (TRH)	OR775012
<i>Biatora chrysantha</i> 6	Norway, Holien 11525 (TRH)	OR775034
<i>Biatora cuprea</i> 1	Sweden, Kanz & Printzen 5437 (BG)	KF650961
<i>Biatora cuprea</i> 2	Norway, Timdal 11076 (O)	OR775011
<i>Biatora cuprea</i> 3	Norway, Timdal 12044 (O)	OR775033
<i>Biatora cuprea</i> 4	Norway, Haugan 8482 (O)	OR775036
<i>Biatora cuprea</i> 5	Norway, Timdal 9552 (O)	OR775008
<i>Biatora cuprea</i> 6	Norway, Timdal 9599 (O)	OR775047
<i>Biatora cuprea</i> 7	Norway, Haugan 10575 (O)	OR775046
<i>Biatora efflorescens</i> 1	Germany, Printzen s.n. (FR)	AJ247554
<i>Biatora efflorescens</i> 2	Czech Republic, Palice s.n. (FR)	AJ247555
<i>Biatora efflorescens</i> 3	Ukraine, Palice 19334 (PRA)	MG773665
<i>Biatora efflorescens</i> 4	Russia, Republic of Adygea, Vondrák 16449 (PRA)	MK778586
<i>Biatora efflorescens</i> 5	Norway, Klepsland JK09-L619 (O)	OR775058
<i>Biatora efflorescens</i> 6	Norway, Klepsland JK13-L240 (O)	OR775045
<i>Biatora efflorescens</i> 7	Norway, Klepsland JK14-L102 (O)	OR775065
<i>Biatora efflorescens</i> 8	Norway, Frisch S19-1-Pp1-6 (TRH)	OR775009
<i>Biatora efflorescens</i> 9	Norway, Frisch N11-2-Pa1-4 (TRH)	OR775050
<i>Biatora efflorescens</i> 10	Norway, Frisch N8-1-Ai1-5 (TRH)	OR775044
<i>Biatora</i> cf. <i>efflorescens</i>	Norway, Frisch N10-2-Pa3-3 (TRH)	OR775020
<i>Biatora fallax</i> 1	Czech Republic, Palice s.n. (FR)	AJ247526
<i>Biatora fallax</i> 2	Czech Republic, Palice s.n. (FR)	AJ247527
<i>Biatora fallax</i> 3	Czech Republic, Palice s.n. (FR)	AJ247548
<i>Biatora fallax</i> 4	Czech Republic, Malíček & Palice 19135 (FR)	KX389592
<i>Biatora flavopunctata</i> 1	Norway, Klepsland JK11-L119 (O)	OR775037
<i>Biatora flavopunctata</i> 2	Norway, Tønsberg 40383 (BG)	OR775019
<i>Biatora flavopunctata</i> 3	Norway, Tønsberg 40605 (BG)	OR775024
<i>Biatora flavopunctata</i> 4	Norway, Tønsberg 43131 (BG)	OR775016
<i>Biatora flavopunctata</i> 5	Norway, Holien 12407 (TRH)	OR775055
<i>Biatora flavopunctata</i> 6	USA, Washington, Printzen 5327 (FR)	KF650963
<i>Biatora helvola</i> 1	Czech Republic, Palice & Printzen s.n. (FR)	AJ247556
<i>Biatora helvola</i> 2	Czech Republic, Palice & Printzen s.n. (FR)	AJ247557
<i>Biatora helvola</i> 3	Czech Republic, Halda et al. s.n. (FR)	AJ247549
<i>Biatora helvola</i> 4	Czech Republic, Palice & Printzen s.n. (FR)	AJ247550
<i>Biatora helvola</i> 5	Austria, van den Boom et al. 16213 (hb. van den Boom)	AJ247541
<i>Biatora helvola</i> 6	Slovakia, Palice s.n. (FR)	AJ247542
<i>Biatora helvola</i> 7	Slovakia, Bayerová s.n. (hb. Bayerová)	AJ247543
<i>Biatora helvola</i> 8	Slovakia, Guttová et al. s.n. (FR)	AJ247544
<i>Biatora helvola</i> 9	Finland, Kuusinen s.n. (FR)	AJ247545
<i>Biatora helvola</i> 10	Finland, Palice s.n. (FR)	AJ247546
<i>Biatora helvola</i> 11	Finland, Palice s.n. (FR)	AJ247547
<i>Biatora helvola</i> 12	Austria, Türk 18217 (FR)	AJ247514
<i>Biatora helvola</i> 13	Austria, Türk 18217 (FR)	AJ247515
<i>Biatora helvola</i> 14	Czech Republic, Palice & Printzen s.n. (FR)	AJ247516
<i>Biatora helvola</i> 15	Ukraine, Palice 262 (hb. Palice)	AJ247517
<i>Biatora helvola</i> 16	Finland, Kuusinen s.n. (FR)	AJ247518
<i>Biatora helvola</i> 17	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Kor-Galin-27-2018 (FR)	OR770000

Table 1. Continued.

Sample	Origin	Accession number
<i>Biatora helvola</i> 18	Finland, Kuusinen s.n. (BG)	KF650964
<i>Biatora helvola</i> 19	Norway, Haugan 10673 (O)	OR775043
<i>Biatora helvola</i> 20	Norway, Haugan 10722 (O)	OR775051
<i>Biatora kodiakensis</i> 1	USA, Alaska, Tønsberg 29371 (BG)	KF650967
<i>Biatora kodiakensis</i> 2	Norway, Klepsland JK13-L382 (O)	OR775066
<i>Biatora kodiakensis</i> 3	Norway, Frisch 18/No212 (TRH)	OR775028
<i>Biatora longispora</i> 1	USA, Massachusetts, May 5409 (hb. May)	OR770001
<i>Biatora longispora</i> 2	Turkey, Kanz & Printzen T37 (FR)	OR770002
<i>Biatora longispora</i> 3	USA, New Hampshire, May 5956A (hb. May)	OR770003
<i>Biatora longispora</i> 4	USA, Vermont, May 5981 (hb. May)	OR770004
<i>Biatora longispora</i> 5	USA, New York, May 6002 (hb. May)	OR770005
<i>Biatora longispora</i> 6	Turkey, Printzen et al. 6539 (BG)	OR770006
<i>Biatora longispora</i> 7	Turkey, Kanz & Printzen 6045 (BG)	OR770007
<i>Biatora longispora</i> 8	USA, Pennsylvania, May 6025 (hb. May)	OR770008
<i>Biatora longispora</i> 9	USA, Maine, May 5967A (hb. May)	OR770009
<i>Biatora longispora</i> 10	Turkey, Palice & Printzen 6122 (BG)	OR770010
<i>Biatora longispora</i> 11	USA, Tennessee, Printzen 6875 (BG)	OR770011
<i>Biatora longispora</i> 12	USA, Virginia, Printzen 6746 (BG)	OR770012
<i>Biatora longispora</i> 13	USA, North Carolina, Printzen 6818 (BG)	OR770013
<i>Biatora longispora</i> 14	USA, Tennessee, Printzen 6781 (BG)	OR770014
<i>Biatora longispora</i> 15	USA, Georgia, Printzen 6638 (BG)	OR770015
<i>Biatora longispora</i> 16	South Korea, Thor 17003 (UPS)	OR770016
<i>Biatora longispora</i> 17	South Korea, Thor 17673 (UPS)	OR770017
<i>Biatora longispora</i> 18	USA, Massachusetts, May 5409 (hb. May)	KF650969
<i>Biatora longispora</i> 19	Ukraine, Palice 19307 (hb. Palice)	MG773667
<i>Biatora meiocarpa</i> 1	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Kor-Galin-27-2018 (FR)	OR770018
<i>Biatora meiocarpa</i> 2	Norway, Tønsberg 28317a (BG)	AM292667
<i>Biatora</i> aff. <i>meiocarpa</i> 1	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Kor-Galin-25-2018 (FR)	OR770019
<i>Biatora</i> aff. <i>meiocarpa</i> 2	Italy, Printzen 2683 (FR)	AJ247540
<i>Biatora</i> aff. <i>meiocarpa</i> 3	Switzerland, Vust 6826 (hb. Vust)	OR770020
<i>Biatora</i> aff. <i>meiocarpa</i> 4	Switzerland, Vust 6860 (hb. Vust)	OR770021
<i>Biatora</i> aff. <i>meiocarpa</i> 5	Finland, Weber MAL-089-9 (H)	OR770037
<i>Biatora</i> aff. <i>meiocarpa</i> 6	Norway, Klepsland JK09-L109 (O)	OR775030
<i>Biatora</i> aff. <i>meiocarpa</i> 7	Norway, Holien 13019 (TRH)	OR775062
<i>Biatora</i> aff. <i>meiocarpa</i> 8	Norway, Holien 11875 (TRH)	OR775060
<i>Biatora</i> aff. <i>meiocarpa</i> 9	Norway, Klepsland JK15-L744 (O)	OR775041
<i>Biatora</i> aff. <i>meiocarpa</i> 10	Norway, Klepsland JK10-L351 (O)	OR775039
<i>Biatora</i> aff. <i>meiocarpa</i> 11	Norway, Bendiksby et al. 10396 (O)	OR775073
<i>Biatora</i> aff. <i>meiocarpa</i> 12	Norway, Holien 11756 (TRH)	OR775076
<i>Biatora</i> aff. <i>meiocarpa</i> 13	Norway, Holien 9155 (TRH)	OR775007
<i>Biatora</i> aff. <i>meiocarpa</i> 14	Norway, Holien 13235 (TRH)	OR775005
<i>Biatora</i> aff. <i>meiocarpa</i> 15	Norway, Holien 12745 (TRH)	OR775054
<i>Biatora</i> aff. <i>meiocarpa</i> 16	Norway, Holien 11297 (TRH)	OR775026
<i>Biatora</i> aff. <i>meiocarpa</i> 17	Norway, Klepsland JK09-L094 (O)	OR775049
<i>Biatora</i> aff. <i>meiocarpa</i> 18	Finland, Weber Kevo-12 (H)	OR770038
<i>Biatora</i> aff. <i>meiocarpa</i> 19	Finland, Weber MAL-089-13 (H)	OR770039
<i>Biatora</i> aff. <i>meiocarpa</i> 20	Finland, Weber 27-2021 (H)	OR770040
<i>Biatora</i> aff. <i>meiocarpa</i> 21	Finland, Weber MAL-020-4 (H)	OR770041
<i>Biatora</i> aff. <i>meiocarpa</i> 22	Finland, Weber MAL-30-9 (H)	OR770042
<i>Biatora</i> aff. <i>meiocarpa</i> 23	Svalbard, Zhang 2013049	KP314332
<i>Biatora</i> aff. <i>meiocarpa</i> 24	Svalbard, Zhang 2013109	KP314369
<i>Biatora oligocarpa</i>	USA, Alaska, Tønsberg 29057 (BG)	KF650973
<i>Biatora orientalis</i> 1	Japan, Thor 23714 (UPS)	KF650974
<i>Biatora orientalis</i> 2	Japan, Haugan & Timdal 16666a (O)	MH481924
<i>Biatora orientalis</i> 3	Norway, Frisch S5-2-Fe1-6 (TRH)	OR775027
<i>Biatora orientalis</i> 4	Norway, Holien 12504 (TRH)	OR775006
<i>Biatora orientalis</i> 5	Norway, Holien 9602 (TRH – holotype!)	OR775053
<i>Biatora pycnidia</i> 1	Canada, Newfoundland, Printzen 5497 (BG)	KF650979
<i>Biatora pycnidia</i> 2	Canada, Newfoundland, Printzen 5540 (BG)	OR770022
<i>Biatora pycnidia</i> 3	Canada, Newfoundland, Printzen 5583 (BG)	OR770023
<i>Biatora pycnidia</i> 4	Canada, Newfoundland, Printzen 5642 (BG)	OR770024
<i>Biatora pycnidia</i> 5	Canada, Newfoundland, Printzen 5691 (BG)	OR770025

Table 1. Continued.

Sample	Origin	Accession number
<i>Biatora pycnidiata</i> 6	Canada, Newfoundland, Printzen 5703 (BG)	OR770026
<i>Biatora pycnidiata</i> 7	Canada, Newfoundland, Printzen 5729 (BG)	OR770027
<i>Biatora pycnidiata</i> 8	Canada, Newfoundland, Printzen 5733 (BG)	OR770028
<i>Biatora pycnidiata</i> 9	Canada, Newfoundland, Printzen 5775 (BG)	OR770029
<i>Biatora pycnidiata</i> 10	Canada, Newfoundland, Printzen 5665 (BG)	OR770030
<i>Biatora subduplex</i> 1	Sweden, Kanz & Printzen 5436 (FR)	KF650983
<i>Biatora subduplex</i> 2	Russia, Kamchatka Kray, Himelbrant Kor-Am-18-2016 (FR)	OR770031
<i>Biatora subduplex</i> 3	Greenland, McMullin 17769 (CANL)	OR770032
<i>Biatora subduplex</i> 4	Norway, Klepsland JK11-L461 (O)	OR775013
<i>Biatora subduplex</i> 5	Norway, Haugan 9880 (O)	OR775025
<i>Biatora subduplex</i> 6	Norway, Klepsland JK11-L474 (O)	OR775035
<i>Biatora subduplex</i> 7	Norway, Holien 6707 (TRH)	OR775068
<i>Biatora subduplex</i> 8	Norway, Holien 9570 (TRH)	OR775032
<i>Biatora subduplex</i> 9	Norway, Klepsland JK16-176 (O)	OR775061
<i>Biatora subduplex</i> 10	Norway, Klepsland JK15-L037 (O)	OR775022
<i>Biatora subduplex</i> 11	Norway, Brosø & Jonsson s.n. (TROM)	OR775042
<i>Biatora subduplex</i> 12	Finland, Weber RA-47-11 (H)	OR770043
<i>Biatora tacomensis</i> 1	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Kor-Galin-15add-2018 (FR)	OR770033
<i>Biatora tacomensis</i> 2	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Com-Medny-22-2019 (FR)	OR770034
<i>Biatora tacomensis</i> 3	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Com-Medny-25-2019 (FR)	OR770035
<i>Biatora tacomensis</i> 4	Russia, Kamchatka Kray, Himelbrant & Stepanchikova Com-Medny-25-2019 (FR)	OR770036
<i>Biatora tacomensis</i> 5	Norway, Holien 11452 (TRH)	OR775070
<i>Biatora</i> cf. <i>tacomensis</i>	Norway, Klepsland JK11-L119 (O)	MG925957
<i>Biatora terraenovae</i> 1	Canada, Newfoundland, Printzen 5758 (BG)	KF650971
<i>Biatora terraenovae</i> 2	Canada, Newfoundland, McCarthy 2361 (FR – holotype!)	KX389589
<i>Biatora toensbergii</i> 1	USA, Washington, Tønsberg 25131 (BG)	AJ247519
<i>Biatora toensbergii</i> 2	USA, Washington, Printzen 5053 (FR)	KF650984
<i>Biatora toensbergii</i> 3	Norway, Klepsland JK09-L083 (O)	OR775077
<i>Biatora toensbergii</i> 4	Norway, Holien 12881 (TRH)	OR775002
<i>Biatora toensbergii</i> 5	Norway, Tønsberg 41930 (BG)	OR775059
<i>Biatora toensbergii</i> 6	Norway, Holien 13833 (TRH)	OR775072
<i>Biatora toensbergii</i> 7	Norway, Frisch N2-3-Pa2-4 (TRH)	OR775003
<i>Biatora toensbergii</i> 8	Norway, Frisch N6-3-Ai1-2 (TRH)	OR775014
<i>Biatora toensbergii</i> 9	Norway, Frisch N4-3-Bp1-1 (TRH)	OR775069
<i>Biatora toensbergii</i> 10	Norway, Frisch N3-3-Pa1-3 (TRH)	OR775031
<i>Biatora toensbergii</i> 11	Norway, Frisch N4-3-Pa2-1 (TRH)	OR775048
<i>Biatora toensbergii</i> 12	Norway, Frisch N10-3-Pa4-1 (TRH)	OR775056
<i>Biatora toensbergii</i> 13	Norway, Frisch N10-1-Pa3-2 (TRH)	OR775063
<i>Biatora toensbergii</i> 14	Norway, Frisch N6-2-Pa1-6 (TRH)	OR775029
<i>Biatora toensbergii</i> 15	Norway, Frisch N10-3-Pa5-1 (TRH)	OR775010
<i>Biatora toensbergii</i> 16	Norway, Klepsland JK14-L151 (O)	OR775018
<i>Biatora vacciniicola</i> 1	USA, Alaska, Tønsberg 27486 (BG)	KF650985
<i>Biatora vacciniicola</i> 2	Norway, Klepsland JK11-L121 (O)	OR775064
<i>Biatora vacciniicola</i> 3	Norway, Haugan 10764 (O)	OR775057
<i>Biatora vacciniicola</i> 4	Norway, Klepsland JK13-L330 (O)	MG925960
<i>Biatora vacciniicola</i> 5	Czech Republic, Malíček 8520 (hb. Malíček)	MH174255
<i>Biatora vacciniicola</i> 6	Norway, Tønsberg 43233 (BG)	OR775004
<i>Biatora</i> cf. <i>vernalis</i>	Norway, Klepsland JK09-L616 (O)	KF360369
<i>Biatora vernalis</i> 1	Norway, Tønsberg 23757 (BG)	AF282070
<i>Biatora vernalis</i> 2	Norway, Holien 12297 (TRH)	OR775067
<i>Biatora vernalis</i> 3	Norway, Frisch S20-2-Bp6-3 (TRH)	OR775015
<i>Biatora vernalis</i> 4	Norway, Klepsland JK09-L098 (O)	OR775071
<i>Biatora vernalis</i> 5	Norway, Holien 12444 (TRH)	OR775074
<i>Biatora vernalis</i> 6	Norway, Holien 6578a (TRH)	OR775038
<i>Biatora vernalis</i> 7	Norway, Klepsland JK13-L204 (O)	OR775078
<i>Biatora vernalis</i> 8	Norway, Frisch S14-1-Ag3-2 (TRH)	OR775023
<i>Biatora vernalis</i> 9	Norway, Frisch 18/No17 (TRH)	OR775040
<i>Biatora vernalis</i> 10	Norway, Holien 8140 (TRH)	OR775021
<i>Biatora vernalis</i> 11	Norway, Holien 6376 (TRH)	OR775075
<i>Biatora vernalis</i> 12	Norway, Holien 16359 (TRH)	OR775052
<i>Biatora vernalis</i> 13	Norway, Klepsland JK09-L249 (O)	OR775017

Results and discussion

The final alignment after removal of uncertain positions consisted of 183 terminal nodes and was 478 bp long. The *B. vernalis*- and *B. meiocarpa*-groups already postulated by Printzen (2014) are also reconstructed in this analysis, but support is low, likely because only ITS sequences were used in the present analysis. Only two internal nodes are highly supported. The outgroup species *B. flavopunctata* and *B. vacciniicola* appear as sister taxa and a close relationship between *B. alaskana*, *B. chrysantha*, *B. cuprea*, *B. fallax*, *B. subduplex*, *B. terrae-novae* and *B. vernalis* is also supported. The other higher level and most infraspecific nodes of the resulting tree lack statistical support, but support values for nodes combining sequences from the same taxon are, with few exceptions, very high (Fig. 1). These exceptions concern *B. efflorescens*, *B. helvola* and *B. vernalis*. Sequences from *B. helvola* appear monophyletic, but the relevant node is unsupported. Instead, an internal node combining 13 out of the 20 sequences is highly supported. The seven samples basal to this node are evidence for the high genetic variability of *B. helvola* that seems in accordance with its wide distribution in boreal forests of Eurasia and Alaska. *Biatora helvola* is morphologically and chemically well defined by the presence of amorphous gyrophoric acid around the paraphyses and excipular hyphae (Printzen 1995). Ten out of eleven individuals of *B. efflorescens* form a well-supported clade sister to *B. helvola*, but one sequence (here called “*B. cf. efflorescens*”) appears at the base of the node combining these two species. *Biatora efflorescens* is well delimited from *B. helvola* by the presence of soralia and the production of argopsin instead of gyrophoric acid. A similar paraphyly is found in *B. vernalis*, which appears in two groups and a single sequence (“*B. cf. vernalis*”) at the base of a clade that also includes a monophyletic *B. chrysantha*. Only one of the subclades of *B. vernalis* is supported. All of these sequences, as well as the two major clades of *B. vernalis*, together with *B. chrysantha*, form well-supported monophylies, but the internal relationships between the sub-clades remain unresolved in our analysis. Similar to *B. efflorescens* and *B. helvola*, *B. chrysantha* is strongly supported as a distinct species by morphology and chemistry. It produces soralia and gyrophoric acid, while *B. vernalis* is esorediate and lacks secondary compounds identifiable by TLC. Whether the two clades of *B. vernalis* merit recognition as separate species, is up to discussion. So far, we have been unable to find any phenotypic differences between members of the two clades, which basically constitute two ITS haplotypes differing in 8 base pairs. In spite of these paraphylies and the lack of support for the monophyly of any of the four species, we currently see no reason to lump species or to assume the presence of cryptic species. In our opinion, it is more likely that a barcoding approach solely based on ITS fails to clearly separate these sister species due to a lack of resolution, as has recently been reported from, e.g., the genus *Usnea* (Lagostina et al. 2018).

On the other hand, other parts of the tree indeed show evidence for the existence of so far undescribed

species-level lineages. Within the *B. meiocarpa*-group, five sequences of “*B. orientalis*”, a lineage and name already mentioned by Printzen (2014), cluster close to the single sequence of *B. oligocarpa*. The samples, from which these sequences were generated, are extremely similar to *B. vernalis*, but differ by slightly narrower and more elongate ascospores (see notes below). Sequences of *B. tacomensis* (so far named *B. meiocarpa* var. *tacomensis*) appear separate from those of *B. meiocarpa* and next to *B. kodiakensis*. The taxon also differs from *B. meiocarpa* by the production of xanthonenes. In both cases, the sister group relationships lack statistical support. Samples and sequences previously named *B. subduplex* merit special attention, because one part of them belongs to the *B. vernalis*-group (“*B. subduplex* s.str.”) while the larger part ends up close to *B. meiocarpa* (“*B. aff. meiocarpa*”). The *B. subduplex* clade sister to *B. vernalis* and *B. chrysantha* includes the sequence from Sweden cited by Printzen (2014), while the sequence called “*Biatora* cf. *subduplex*” in Printzen (2014, *B. meiocarpa* 2 in this study) belongs to “*Biatora* aff. *meiocarpa* clade 2”. This clade, “*B. aff. meiocarpa* clade 1” and *B. meiocarpa* form an evolutionary grade comprising 23 sequences from samples previously identified as either *B. meiocarpa*, *B. subduplex* or *B. vernalis*.

The previously recognized polyphyly of what has traditionally been called “*B. subduplex*” (Printzen 1995, 2014) encouraged us to look for distinguishing morphological features between members of the three clades (Fig. 2). Most characters did not allow assignment of samples to any of these clades, but the density of paraphyses in the hymenium and excipular hyphae differed strongly between samples in the *B. vernalis* clade and those in the *B. meiocarpa* clade, with samples of “*B. aff. meiocarpa*” consistently containing less and those from the *B. vernalis* clade more than 20 hyphae along 50 µm of hymenium or exciple (Figs 2A–B, 3C–F). According to the density of excipular hyphae and paraphyses, the type of *B. subduplex* belongs to the *B. vernalis* clade, but the types of *Lecidea apochroeiza* Nyl. (= *B. apochroeiza* (Nyl.) Cumm.) and *L. internectens* Nyl., considered to be synonyms of *B. subduplex* by Printzen (1995), belong to the *B. meiocarpa*-group. Samples from the two groups also differ in the height of the subhymenium (Fig. 2C), hypothecium (Fig. 2D) and hymenium (Fig. 2E), but the ranges overlap, particularly for hypothecium and hymenium height, so that these traits allow no safe assignment of collections. The tips of the paraphyses tend to be wider in samples from the *B. meiocarpa*-clade (Fig. 3B, D) than in *B. subduplex* s.str. (Fig. 3F), but this trait is not always well discernible.

We were unable to find any phenotypic characters that allow the distinction of the two clades of “*B. aff. meiocarpa*”. Since *B. meiocarpa* appears sister to one of the two clades and can be distinguished on account of its wider excipular hyphae (Fig. 3A), it would make sense to assume that the two clades represent cryptic species. However, our phylogenetic tree is only based on a single barcoding marker. Although nearly all non-cryptic species-level lineages (apart from *B. helvola*) form highly supported clades, while infraspecific branches of these

taxa are unsupported without exception, it is possible that a wider geographic sampling or the inclusion of more genetic loci could break up the high support values of the two clades of *B. aff. meiocarpa* or render them monophyletic with regard to *B. meiocarpa*. The current taxonomical uncertainty is matched by a nomenclatural difficulty. The

epithets *apochroeiza* and *internectens* would be available for the two lineages (see above), but cannot be assigned with certainty to any of them. Once the taxonomical question is resolved by deeper sampling, conservation of both names with conserved types or epitypification with samples for which molecular data is available, might

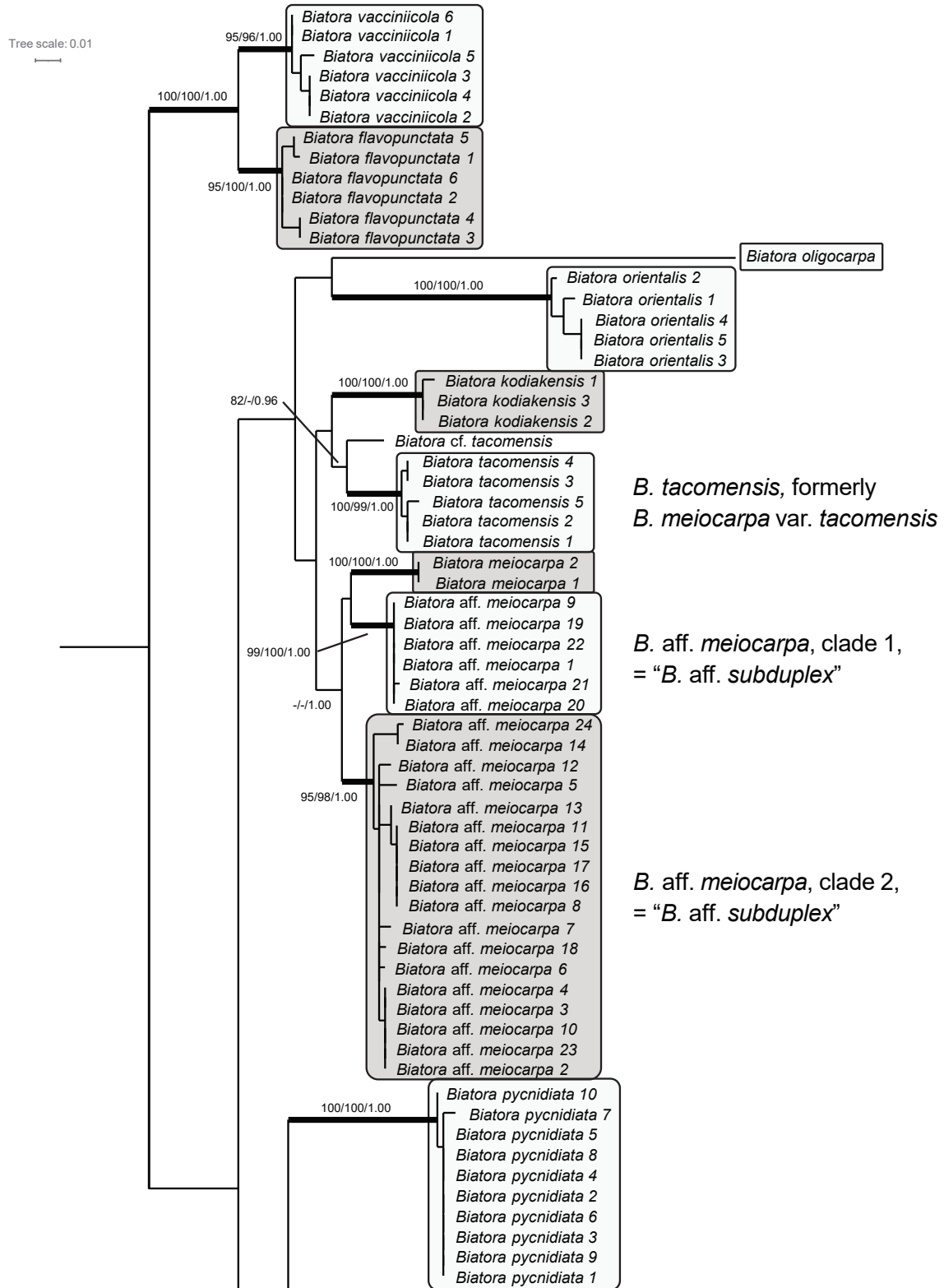


Figure 1. Maximum likelihood tree of the *B. vernalis*- and *B. meiocarpa*-groups with *B. flavopunctata* and *B. vacciniicola* used as outgroup. Support values (SH-aLRT \geq 80%, ML BP \geq 95%, MCMC PP \geq 0.95) above, below or left of branches. Branches without any numbers have no support above these thresholds.

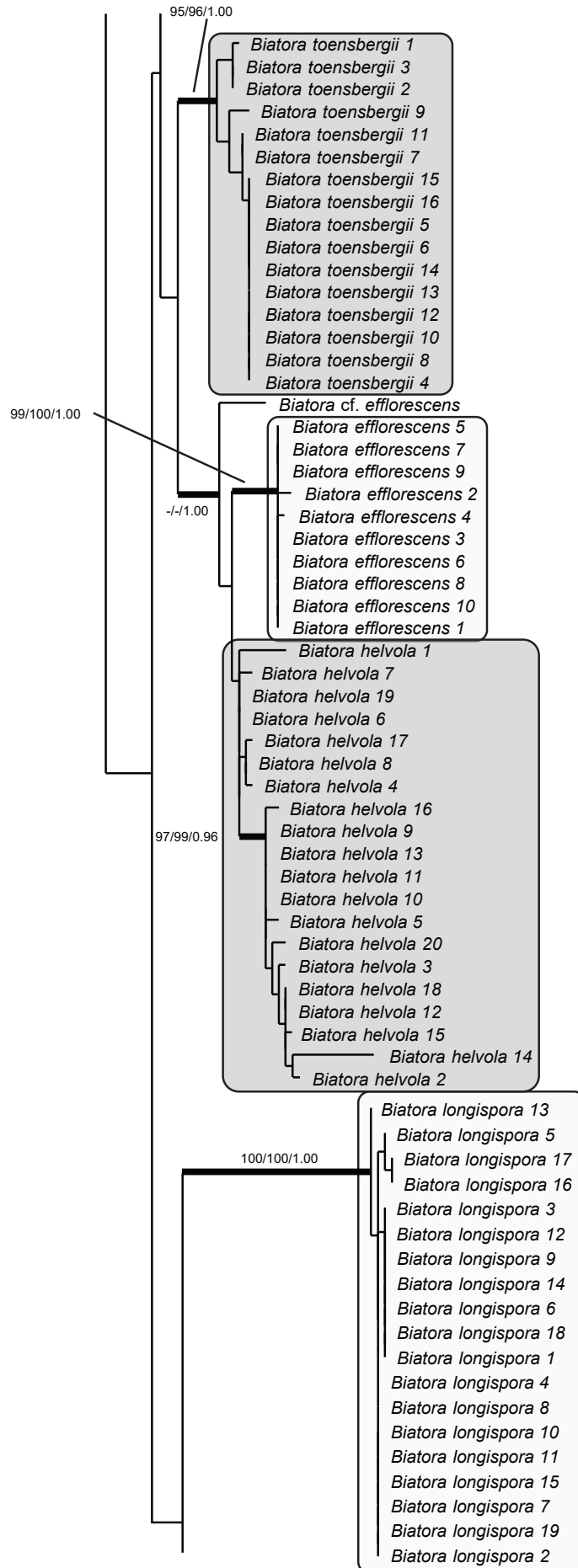


Figure 1. Continued.

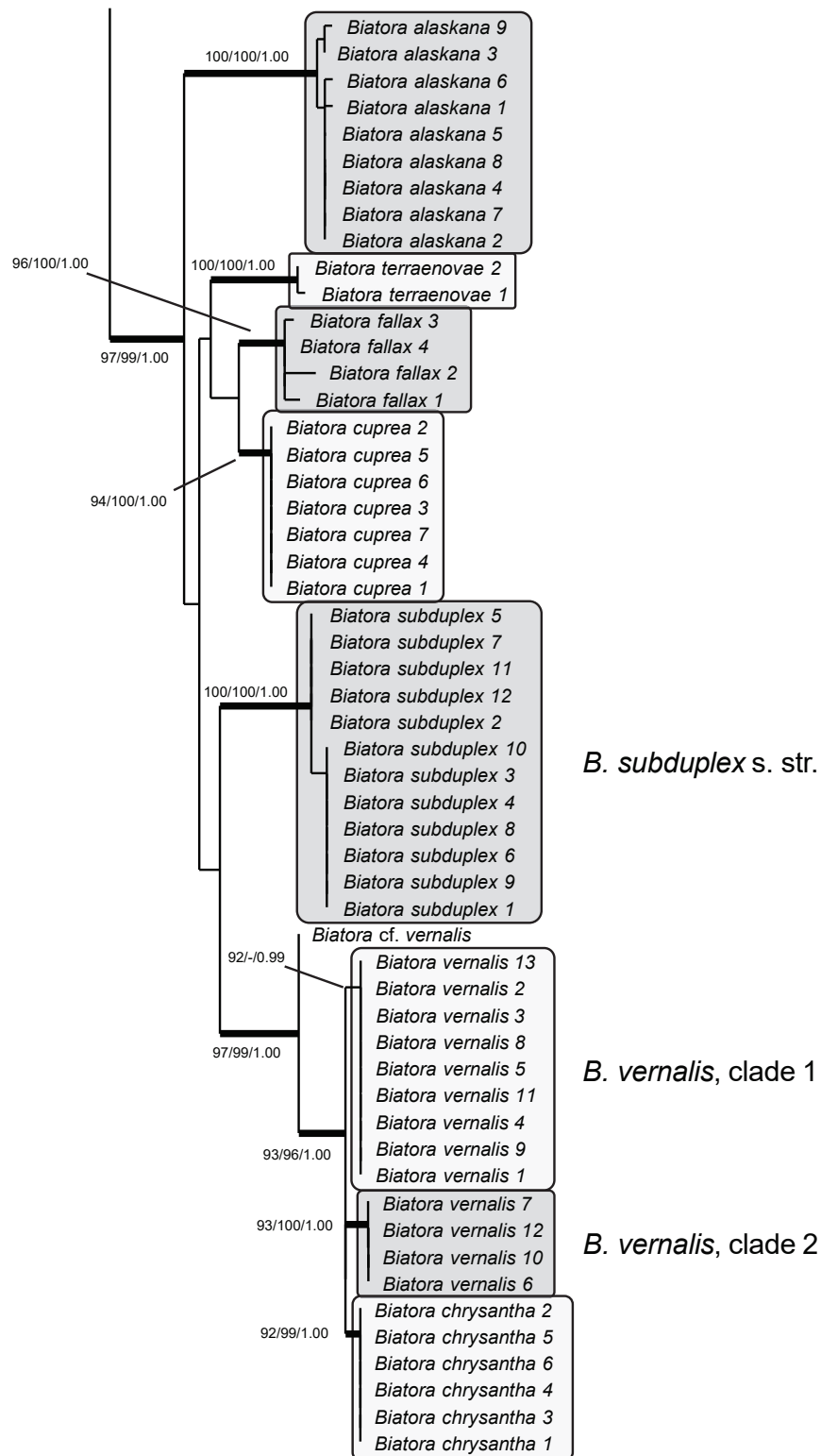


Figure 1. Continued.

eventually resolve this unsatisfying nomenclatural situation. Because the two names are based on collections from the Bering Strait region, any such attempt would have to involve material from that region. Unsatisfying as it may be, we currently refrain from any nomenclatural acts and suggest to treat material of “*B. cf. subduplex*” sensu Printzen (2014) under the more correct provisional name “*B. aff. meiocarpa*”.

In the following, we describe the new species *Biatora orientalis* and raise *B. meiocarpa* var. *tacomensis* to species rank. As outlined above, the samples belonging to these two taxa form separate, well-supported clades within the *B. meiocarpa*-group. *Biatora orientalis* is morphologically similar to *B. vernalis*, but distinguishable on account of its narrower ascospores, while *B. tacomensis* differs from *B. meiocarpa* by its UV+ orange thallus due to the production of the xanthones 2,5,7-trichloro-3-0-

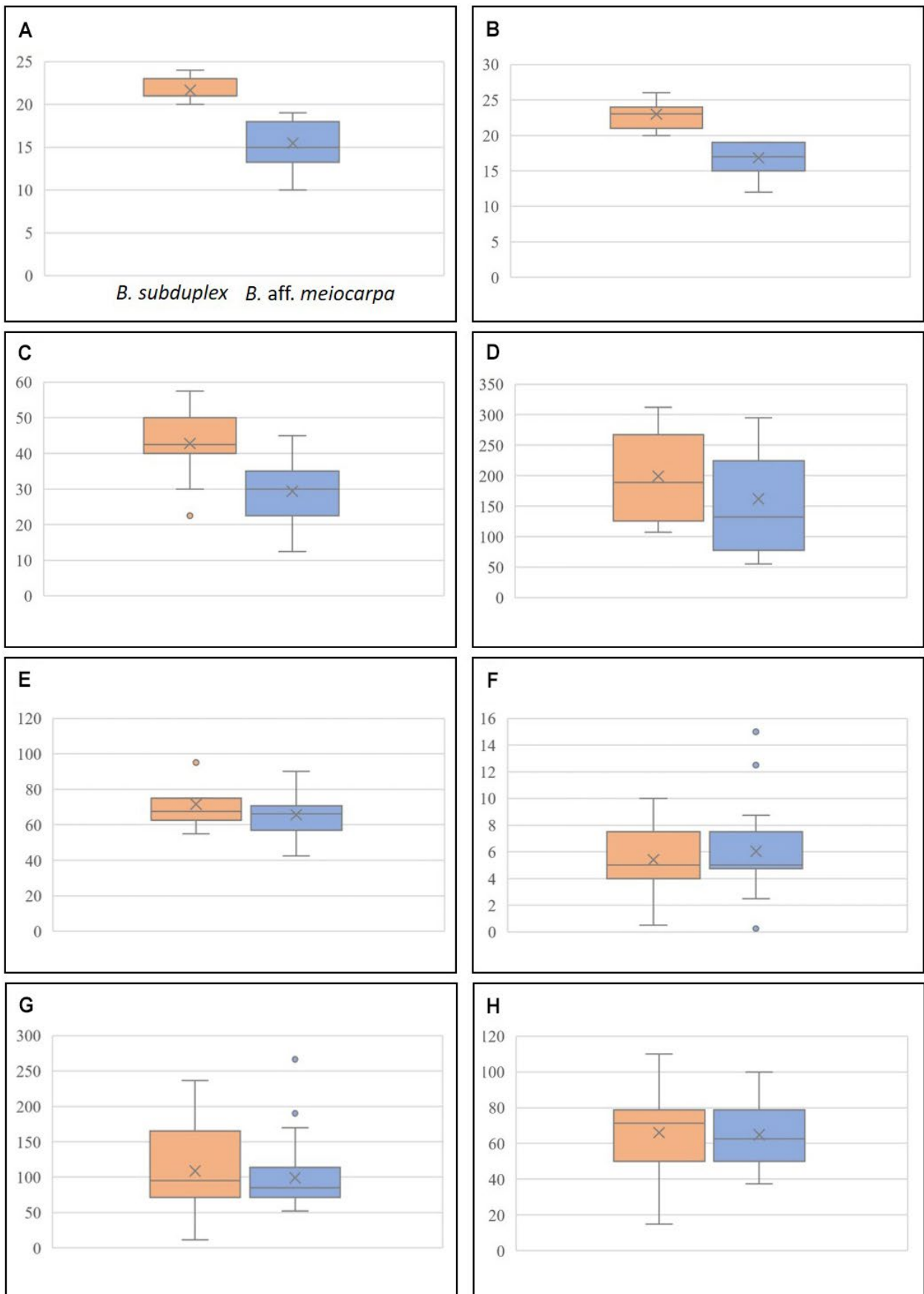


Figure 2. Comparison of morphometric traits in *B. subduplex* (orange) and *B. aff. meiocarpa* (blue). A – number of paraphyses in 50 μm of hymenium; B – number of excipular hyphae in 50 μm of exciple; C – subhymenium height (μm); D – hypothecium height (μm); E – hymenium height (μm); F – epihymenium height (μm); G – basal width of exciple (μm); H – lateral width of exciple (μm).

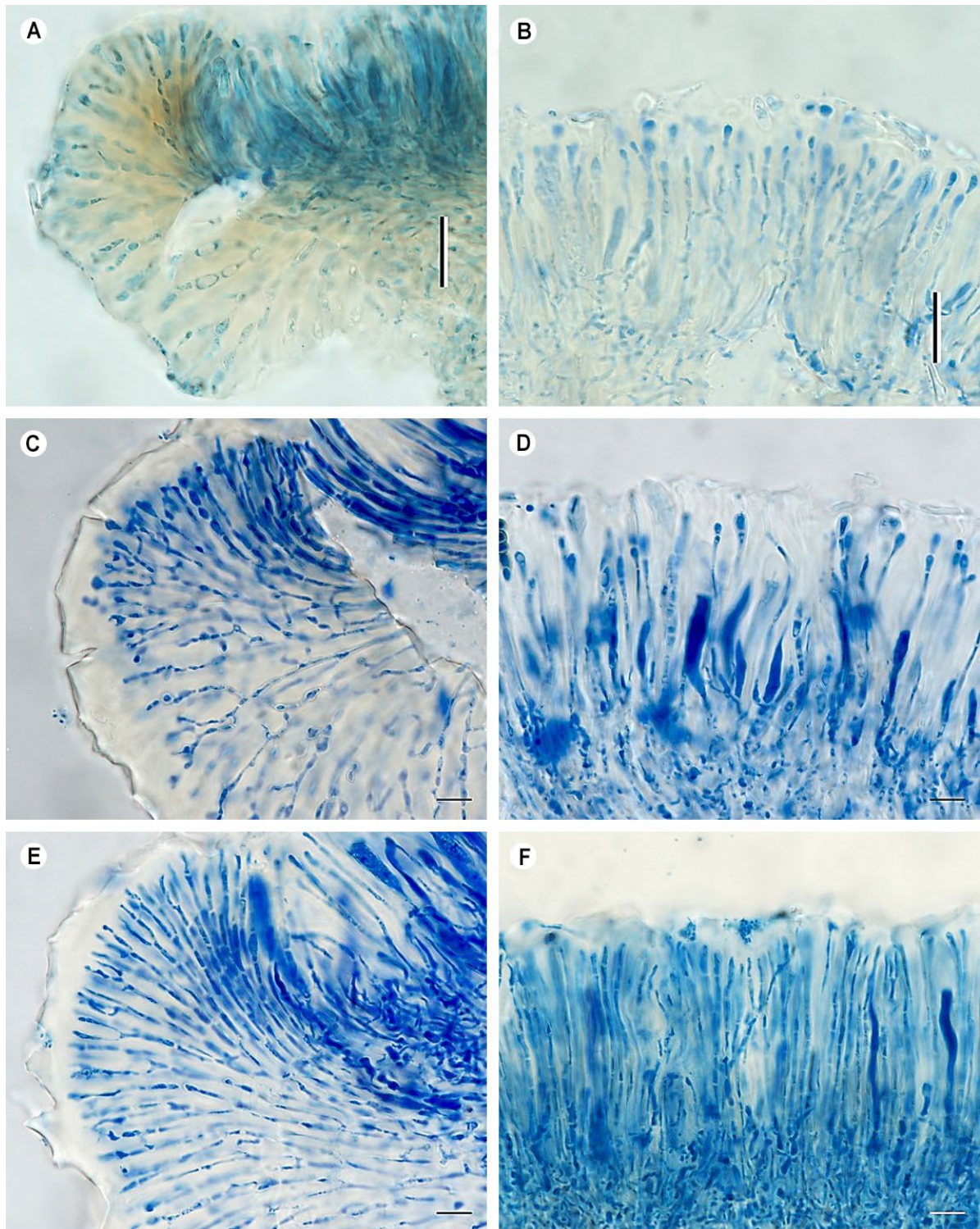


Figure 3. *Biatora meiocarpa* (A–B), *Biatora* aff. *meiocarpa* (C–D) and *B. subduplex* (E–F), apothecial anatomy in LCB. A, C, E – excipula, note thickened hyphal lumina in *B. meiocarpa* and densely packed hyphae in *B. subduplex*; B, D, F – hymenia, note thickened paraphysis tips in *B. meiocarpa* and *B. aff. meiocarpa* and densely packed paraphyses in *B. subduplex*. A–B – Arup L90217 (LD); C – Klepsland JK10-L351 (O); D – Weber MAL-089-9 (H); E – Holien 6707 (TRH); F – McMullin 17769 (CANL). Scales: A–B = 20 μ m; C–F = 10 μ m.

methylnorlichexanthone, 5,7-dichloro-3-O-methylnorlichexanthone, 3-O-methylasemone and 5,7-dichloronorlichexanthone (Printzen & Tønsberg 1999). The distributional range of *B. tacomensis* is restricted to the north Pacific region (Northwestern North America, Kamchatka) and Central Norway, while *B. meiocarpa* is widely distributed in polar and high mountain regions of the Northern Hemisphere.

Taxonomy

Biatora orientalis Printzen, Holien & Timdal, sp. nov.
(Figs 4–5)

Mycobank MB 850917

Type: Norway, Troms, Storfjord, Skibotndalen, between the road and Gustavsvingen, 69.2771°N, 20.4872°E, ~100–180 m, pine forest, muscicolous on decaying wood, 7 August 2003,

H. Holien 9602 (TRH-L-14482 – holotype!, ITS sequence: OR775053).

Description. Thallus (greyish-)white to dull green, endosubstratal or effuse (45–100 µm high), or of minute (0.1 mm) to subsquamulose (0.15–0.35 mm) warts; soredia and isidia absent; hypothallus not clearly developed; cortex 10–20 µm high, medulla 30–85 µm high; photobiont trebouxoid. Apothecia single or in groups of up to three, rarely more, (0.25–)0.40–0.65(–1.10) mm in diameter, rounded or slightly irregular in outline, sessile with a strongly constricted base; disc moderately to strongly convex, sometimes subglobose, pale beige to orange brown, but often sallow, buff, ochre or dull brown, epuriose, matte; margin often lacking from the beginning, otherwise barely prominent in young apothecia and soon excluded, slightly paler or concolorous with disc; proper exciple strongly gelatinized, laterally 45–130 µm, basally 70–200 µm wide, colorless or pale (orange-)brown near hymenium or within, composed of radiating hyphae with 1.0–3.5 µm, apically 1.5–4.5 µm wide lumina, many anastomoses, even in (sub-)apical cells; hypothecium 50–140 µm high, colorless or faint ochre to pale yellowish or orange brown, of strongly gelatinized hyphae with 1–3 µm wide lumina; subhymenium 35–100 µm high, pale (orange-)brown; hymenium 50–70 µm high, colorless or very pale orange-brown; epihymenium not distinct from hymenium; paraphyses simple to branched and rarely anastomosing, lumina 1.0–2.0 µm, apically 1.0–2.5(–3.3) µm wide, colorless; asci of *Biatora*-type, 8-spored; ascospores colorless, simple, rarely 1-septate, narrowly ellipsoid, sometimes slightly curved, (9.5–)12.6–20.0(–25.0) × (3.0–)3.4–4.8(–5.5) µm, length/width index: (2.8–)3.4–5.4(–6.5) (n = 181). Pycnidia not seen.

Chemistry. No substances detected by TLC, all standard spot tests negative.

Etymology. The specific epithet refers to its occurrence in eastern North America, East Asia and the eastern Black Sea region, where it was first detected.

Habitat and distribution. The occurrence of *B. orientalis* in East Asia, Eastern North America and the Eastern Black Sea region has been known to us for several years. Here, it is also reported from Norway. The species has been found in lowland to subalpine deciduous and mixed coniferous-deciduous forests from 10–2,100 m. It usually overgrows bryophytes on trunks of, e.g., *Carpinus*, *Fagus*, *Fraxinus*, *Picea*, *Quercus* or *Tilia*, shaded mossy boulders or decaying wood, but is often also found directly on bark.

Comments. In the field, *B. orientalis* is indistinguishable from *B. vernalis*. However, when mean values of at least ten ascospores are measured, the width and length/width ratio of the spores differ between the two species. *Biatora orientalis* has narrowly elongate spores of up to 4.8 µm mean width and 3.4–5.4 times longer than wide. In *B. vernalis* the mean width of the spores ranges from 4.8–6.3 µm and they are on average 2.7–3.5 µm longer than wide. The combination of both values usually allows to safely identify collections. We have, however, seen material from

Kamchatka with a mean spore width of less than 4.6 µm and a mean length/width ratio of less than 3.4. These collections may represent another semi-cryptic species of *Biatora*. In the field, *B. orientalis* can also be confused with *B. alaskana*, which has 1–3-septate ascospores of (16.5–)20.9–27.7(–33.5) × (4.0–)4.8–5.4(–6.0) µm. *Biatora longispora* has apothecia with a slightly glossy margin that rarely exceed 0.5 mm in diameter and ascospores with a mean width of 3–4 µm. *Biatora subduplex* occurs on detritus and basal stems of shrubs in (sub)arctic or (sub)alpine environments. The other species from the *B. vernalis*-group are easily distinguished by their chemistry: argopsin in *B. cuprea* and *B. terrae-novae*, gyrophoric acid in *B. chrysantha* (which is also sorediate), and both in *B. fallax*. The superficially similar *Mycobilimbia carneoalbida* and *M. tetramera* have 3-septate spores.

Additional specimens examined. JAPAN. Hokkaido, Kitami Prov., Rishiri-to Island, Rishiri-gun, Rishirifuji-cho, Oniwaki area, along Oniwaki mountain trail from the parking area 3 km NW Oniwaki city and 1 km to the W, 45°09'N, 141°17'E, 280–420 m, old growth, open, mixed deciduous/coniferous forest dominated by *Abies sachalinensis*, on bark of *Abies*, 2 June 1995, T. Tønberg 22505 (BG); Shari-gun, Shari-cho, Shiretoko National Park, 9 km NE Utoro village, along the trail around Shiretoko-goko Lakes, 44°07'N, 145°05'E, 260 m, old growth, mixed deciduous/coniferous forest, *Abies sachalinensis*, 6 June 1995, T. Tønberg 22824 (BG); Shiretoko Nat. Park, NW slope of Shiretoko Peninsula, ~10 km NE Utoro town, along the trail from Iwaobetsu hot-spring hotel (Onsen) to Mt Rausu-dake, 44.05447°N, 145.06740°E, 840 m, old growth subalpine forest dominated by *Betula ermannii* but also with e.g., *Acer* spp., *Alnus maximowiczii*, *Pinus pumila*, *Prunus nipponica* and *Sorbus commixta*, on *Weigela middendorffiana* at the trail, 14 July 2010, G. Thor 24794 (UPS); Ibid., 44.10017°N, 145.05687°E, 73 m, old growth lowland forest with e.g., *Abies sachalinensis*, *Acer mono*, *Betula ermannii*, *Cercidiphyllum japonicum*, *Fraxinus mandshurica*, *Kalopanax pictus*, *Magnolia kobus*, *M. obovata*, *Padus ssiiori*, *Picea glehnii*, *Quercus crispula* and *Tilia maximowicziana*, on *Picea glehnii*, 18 July 2010, G. Thor 25619 (UPS); Shiretoko Nat. Park, NW slope of Shiretoko Peninsula ~7 km NE of Utoro village, N of small road to Iwaobetsu hot-spring hotel (Onsen), 44.11203°N, 145.08436°E, 247 m, old growth lowland forest dominated by *Abies sachalinensis* and *Quercus crispula*, but also with e.g., *Acer mono*, *Betula ermannii*, *Kalopanax pictus*, *Tilia maximowicziana*, *Sorbus commixta* and *Taxus cuspidata*, on *Quercus crispula*, 15 July 2010, G. Thor 25062 (UPS); Honshu, Gunma Prefecture, along trail from Sugenuma to Mt Shirane, 36.8158°N, 139.3788°E, 1800 m, mixed forest, [bryophytes on] steep face of boulder, 30 September 2017, R. Haugan & E. Tindal 16666a (O-L-209816); Tochigi Prefecture (Shimotsuke Province), Nikko City administrative region, Nikko National Park, 1.3 km SSE of Yumoto village (Yumoto onsen), along the trail W of Yukawa stream from Yutaki waterfall to Senjogahara marsh, 36°47.686'N, 139°25.662'E, ~1,420 m, nemoral forest of *Acer*, *Abies*, *Ulmus* and *Tilia* along creek below waterfall, on base of cf. *Tilia*, 28 September 2019, C. Printzen 15213 (FR); Nikko National Park, 5.3 km ESE of Yumoto village (Yumoto Onsen), the Utsunomiya University forest on the south slope of Mt. Taro, NE of where the dirt road ends, 36°47.946'N, 139°28.982'E, 1,640 m, humid old growth forest with *Tsuga*, *Betula*, *Acer*, *Phellodendron*, *Aria* etc., on bryophytes on rocks, 29 September 2019, C. Printzen 15353, 15382 (FR); Ibid., on trunk of old deciduous tree, 29 September 2019, C. Printzen 15396 (FR). NORWAY. Nordland, Alstahaug,

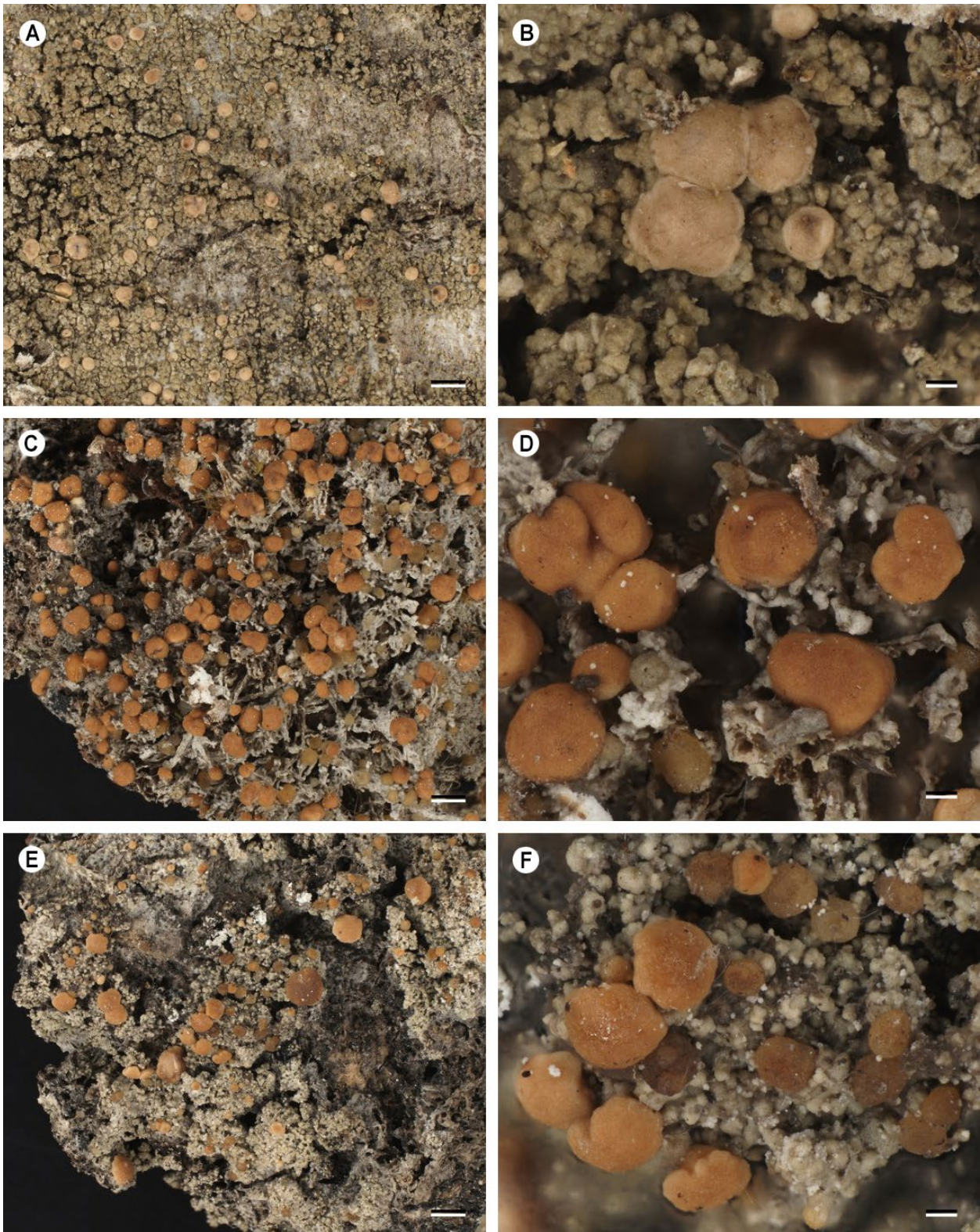


Figure 4. *Biatora orientalis* and *B. vernalis*, thallus and apothecia at different magnifications. A–D – *B. orientalis*; E–F – *B. vernalis*. A–B – Printzen 14060 (FR): corticolous thallus with marginate pale ochre apothecia; C–D – Holien 9602 (TRH): muscicolous thallus with immarginate, reddish brown apothecia; E–F – Klepsland JK09-L616 (O): corticolous, areolate thallus with slightly glossy apothecia. Scales: A, C, E = 1.0 mm; B, D, F = 0.2 mm.

Skeilia nature reserve, 65.9038°N, 12.4968°E, ~25 m, on mossy trunk of *Ulmus glabra*, 15 September 2009, H. Holien 12504 (TRH-L-13508); Rogaland, Strand, Tysdalsvatnet, Strandabrynnane east, 59.0759°N, 6.0931°E, 100–150 m, boreonemoral rain-forest with domineering ash, elm, birch and rowan, on *Fraxinus excelsior*, 17 July 2017, A. Frisch S5-2-Fe1-6 (TRH-L-37190). RUSSIA. Sakhalin province (Сахалинская область), Dolinsky

district (Долинский район), river Firsovka (Фирсовка) 25 km S of village Vzmorye (Взморье), 47°38'46.4"N, 142°34'18.9"E, old growth forest of *Picea jezoensis* and *Abies sachalinensis*, 10–20 m, on trunk of young *Abies sachalinensis*, 29 July 2004, C. Printzen 9469 (FR); Dolinsky district (Долинский район), ~8 km SE of village Starodubskoye (Стародубское), 47°22'24.0"N, 142°54'12.5"E, *Acer maximoviczii*-forest, 20 m,

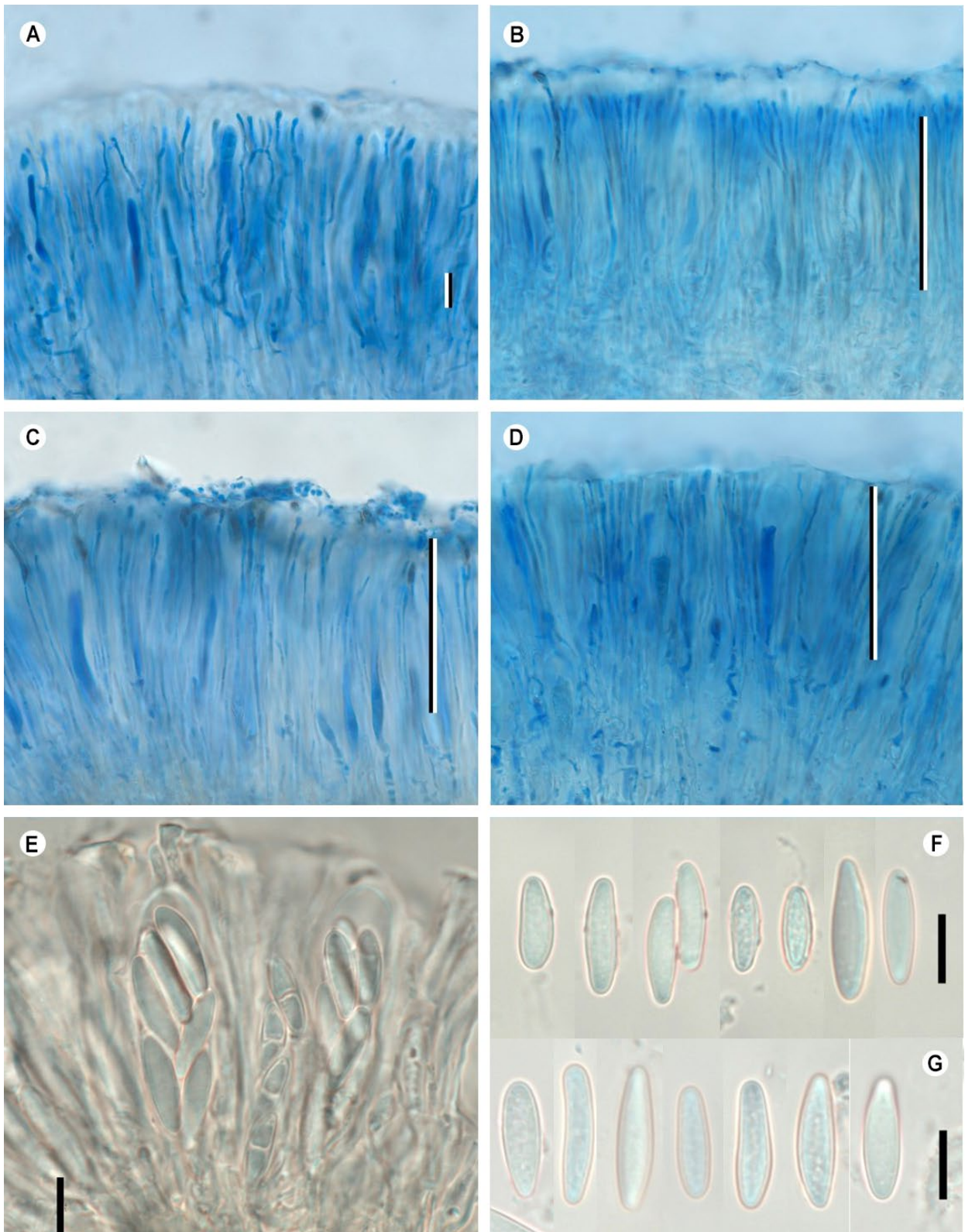


Figure 5. *Biatora orientalis* and *B. vernalis*, hymenial anatomy (in LCB) and ascospores (in water). A, C, E–F – *B. orientalis*; B, D, G – *B. vernalis*. A – Holien 9602 (TRH); B – Frisch S20-2-Bp6-3 (TRH); C – Haugan & Tindal 16666a (O); D – Klepsland JK09-L249 (O); E – Holien 12504 (TRH); F – Holien 9602 (TRH); G – Holien 8140 (TRH). Scales: A, E–G = 10 µm; B–D = 50 µm.

on trunk of *Acer pictum*, 30 July 2004, C. Printzen 9482 (FR); Tумовский district (Тымовский район), Tshatginsky-pass (Чатгинский) close to village Palevo (Палево), 50°45'33.5"N, 143°17'17.1"E, subalpine stand of *Betula ermanii* in steep valley, ~800 m, on trunk of *Alnus* sp., 27 July 2004, C. Printzen 9400 (FR). SOUTH KOREA. Jeju Island, Namcheju-gun, Namwon-up, along the Songpanak trail on the E slope of Mt.

Halla, from the Songpanak Nat. Park Office to the Azalea Field Shelter, 33°23'N, 126°37'E, 750–1500 m, forest dominated by *Acer*, *Carpinus* and *Quercus*, on mossy shaded rock, 28 May 2001, G. Thor 17468 (UPS); Jeju city, Ohra 2 dong, N trail of Mt. Halla (Gwanumsa trail), en route from Gwanumsa campsite to summit, 33°24.206'N, 126°32.446'E, 830 m, lowland forest dominated by *Carpinus*, *Acer* and *Quercus serrata*, on *Carpinus*,

27 September 2016, C. Printzen 14060 (FR). TURKEY. Rize vilayet, village Ayder 16 km SSE of Çamlıhemşin, S slope of Mt. Huser (2548 m), 40°57'50.7"N, 41°07'23.6"E, ~2100 m, near timberline, on *Fagus orientalis*, 01 July 2001, A. Guttová, P. Halda, Z. Palice & C. Printzen 6296 (BG); S slope of Mt. Huser (2548 m) along small creek S of summit, 40°57'36.5"N, 41°07'08.5"E, ~1750 m, at forest edge, 01 July 2001, A. Guttová, P. Halda, Z. Palice & C. Printzen 6216 (BG). USA. North Carolina, Swain Co., Great Smoky Mountains Nat. Park, along Kephart Prong Trail, 2 km W of Richland Mt., 35°36'03"N, 83°22'02"W, ~990 m, hardwood forest along creek, on base of cf. *Quercus rubra*, 21 March 2002, C. Printzen 6828 (BG); Vermont, Bennington Co., Lye Brook Wilderness, Green Mt. Nat. For., 4 miles S of Manchester Center, 43°10'37"N, 73°03'26"W, 1,700 ft., in deep gully on N facing slope and ridge with yellow birch and some hemlock and maple, on maple, 31 July 1993, C.M. Wetmore 72616 (MIN).

Biatora tacomensis (Printzen & Tønsberg) Printzen, Holien & Timdal, comb. nov.

Mycobank MB 850918

Basionym: *Lecidea meiocarpa* var. *tacomensis* Printzen & Tønsberg, The Bryologist 102: 711. 1999.

≡ *Biatora meiocarpa* var. *tacomensis* (Printzen & Tønsberg) Printzen & Tønsberg, Symbolae Botanicae Upsalienses 34: 352. 2004.

Type: USA. Washington. Lewis Co., Mt. Rainier Nat. Park, E of Stevens Ridge, at Box Canyon, S of road at picnic area, 46°46'N, 121°39'W, 940 m, *Alnus rubra*, 22 September 1994, T. Tønsberg 20337 (BG! – holotype).

Comments. Although the relationships within the *B. meiocarpa*-group lack statistical support, Fig. 1 shows that the 5(–6) samples of *B. tacomensis* represent a lineage distinct from *B. meiocarpa* var. *meiocarpa* apparently related to *B. kodiakensis*. *Biatora meiocarpa* s.str., on the other hand, is related to two cryptic lineages, for which the names *B. apochroeiza* (Nyl.) Cumm. and *Lecidea internectens* Nyl. are available (see above). This relationship received strong support in the Bayesian, but not in the ML analysis. We conclude that *B. meiocarpa* var. *tacomensis* deserves species rank, because subsuming the morphologically and chemically clearly distinct *B. kodiakensis* as a variety under *B. meiocarpa* seems entirely unjustified.

Revised key to species of *Biatora* with pale to reddish-brown apothecia lacking bluish or greyish pigments

- 1 Thallus sorediate 2
- Thallus esorediate 15
- 2(1) Thallus of minute, densely crowded squamules, mostly vivid to dark green in fresh collections; soredia, if present, scattered and poorly delimited ***B. fallax***
- Thallus not as above, thin and not minutely squamulose, with punctiform soralia or sorediate in most parts ... 3
- 3(2) Thallus and soralia P+ red, containing argopsin 4
- Thallus and soralia P–, argopsin absent 5
- 4(3) Soralia flat, containing only argopsin, apothecia orange-

- to red-brown, width of spores (mean of 10) 3.4–4.3 µm ***B. efflorescens***
- Soralia strongly convex, containing gyrophoric acid in addition to argopsin, apothecia greyish ochre, width of spores (mean of 10) 2.8–3.3 µm ***B. printzenii***
- 5(3) Thallus C+ red, containing gyrophoric acid 6
- Thallus C–, without gyrophoric acid 11
- 6(5) Soralia punctiform, often strongly convex 7
- Soralia soon confluent, not strongly convex, or thallus leprose 8
- 7(6) Soralia yellowish white, recalling those in species of *Pertusaria* or *Ochrolechia*, apothecial sections C–, cells of excipular hyphae irregularly rounded ***B. kodiakensis***
- Soralia pale green (becoming straw yellowish in the herbarium), apothecial sections C+ red, cells of excipular hyphae cylindrical ***B. appalachensis***
- 8(6) Thallus leprose, containing 5-O-methylhiassic acid as major compound, apothecia unknown ... ***B. aureolepra***
- Soralia frequently confluent, but thallus not leprose, containing gyrophoric acid as major compound 9
- 9(8) Apothecia up to 0.85(–1.1) mm in diameter, in section C–, ascospores (mean of 10) 3.8–5.9 µm wide ***B. chrysantha***
- Apothecia up to 0.45 mm in diameter, in section C+ red, ascospores (mean of 10) 2.8–3.3 µm wide 10
- 10(9) Apothecia with a distinct epipsamma of gyrophoric acid, mostly on thin branches of shrubs in (sub)arctic to (sub) alpine environments of the Northern Hemisphere ***B. vacciniicola***
- Without a distinct epipsamma, mostly on conifer trunks in Norway and the Pacific Northwest of North America ***B. chrysanthoides***
- 11(5) Soralia UV+ white (divaricatic acid), on decaying wood ***B. troendelagica***
- Soralia UV–, on bark 12
- 12(11) Thallus of globose to isidioid granules that break into soredia, no clearly delimited soralia present, ascospores (mean of 10) more than 5 µm wide, South America ... ***B. hafellneri***
- Thallus with clearly delimited soralia that may become confluent, ascospores (mean of 10) less than 4.5 µm wide, Northern Hemisphere 13
- 13(12) Thallus and soralia K–, without secondary substances, apothecial sections without granules, excipular hyphae with irregularly rounded cell lumina 1.5–4.0 µm wide, on tree trunks in lowland environments ***B. oligocarpa***
- Thallus and soralia K+ yellowish to distinctly yellow, containing atranorin, excipular hyphae with cylindrical cell lumina 0.7–2.0 µm wide 14
- 14(13) Soralia arising from convex pustules, K+ yellow due to large amounts of atranorin, soredia ellipsoid, 20–80 × 20–45 µm, apothecia with a distinct margin, ascospores 3(–7)-septate, on *Alnus* in the Pacific Northwest of North America ***B. alnetorum***
- Soralia flat to convex, but not arising from convex pustules, K+ weakly yellow containing atranorin, usnic, isousnic acid, ± stictic acid, soredia globose, 10–25 µm

- in diam., apothecia without a distinct margin, ascospores 0(–1)-septate, on twigs of shrubs in (sub)arctic and (sub)alpine environments of the Northern Hemisphere *B. flavopunctata*
- 15(1) Ascospores 3-septate 16
Ascospores 0–1-septate, rarely 3-septate spores intermixed 20
- 16(15) Exciple $I_{Lugol}+$ violaceous 17
Exciple $I_{Lugol}-$ 18
- 17(16) Apothecia in section C+ pink, with a distinct epipsamma of gyrophoric acid *B. aegrefaciens*
Apothecia in section C–, without an epipsamma *B. rufidula*
- 18(16) Apothecia reddish brown, to 1.5 mm in diam., ascospores 5.0–8.5 μm wide *B. nobilis*
Apothecia white to pale ochre or pale pinkish brown, to 0.6 mm, ascospores 2.5–5.5 μm wide 19
- 19(18) Apothecia to 0.4 mm in diam., strongly convex, disc slightly pruinose, ascospores 2.5–3.5(–4) μm wide, generally rare but widely distributed in boreal forests of the Northern Hemisphere *B. pallens*
Apothecia to 0.6 mm in diam., weakly to moderately convex, epruinose, ascospores 3.3–5.5 μm wide, only known from the southwest Balkans and the Black Sea region *B. epirotica*
- 20(15) Conspicuous, protruding pycnidia present 21
Pycnidia not protruding, (semi-)immersed in thallus or absent 24
- 21(20) Pycnidia stalked, white, numerous (more frequent than apothecia), apothecia pale pinkish brown, white pruinose, conidia 2.8–3.5(–3.8) \times 0.8–1.0(–1.2) μm *B. veteranorum*
Pycnidia adnate, sessile or immersed in thallus, beige to reddish brown, apothecia reddish brown, epruinose, conidia bacilliform or filiform, longer than 4 μm 22
- 22(21) Thallus P+ red, containing argopsin, ascospores 3.0–5.5 μm wide, pycnidia conspicuous (semi-)immersed with a wide ostiole, conidia 6–8(–10) \times 1 μm , Eastern North America *B. pycnidiata*
Thallus P–, ascospores 2–3 μm wide, pycnidia sessile, conidia either shorter (3.5–5.5 μm) or much longer (24–40 μm), widespread but rare 23
- 23(22) Thallus UV–, pycnidia apically with a white blob of protruding conidia, conidia 24–50 \times 1.5–2 μm *Lecidea albohyalina*
Thallus UV+ white, containing lobaric acid, pycnidia without a white blob of protruding conidia, conidia 3.5–5.5 \times 1.0 μm *B. ligni-mollis*
- 24(20) On shrubs, bryophytes, soil or detritus in (sub-)arctic and (sub-)alpine environments 25
On bark of trees and bryophytes in forests and lowland environments 30
- 25(24) Thallus P+ red, containing argopsin, apothecia dark red-brown to almost black, thallus forming extremely firm crusts on soil in arctic environments *B. cuprea*
Thallus P–, apothecia pale beige to reddish brown, on shrubs and detritus, not restricted to the Arctic 26
- 26(25) Excipular hyphae with irregularly rounded, 1.5–4.5 (apically 2–6) μm wide lumina, lumina of paraphysis tips up to 6 μm wide 27
Excipular hyphae with cylindrical, 1.0–2.5 (apically 1.5–3) μm wide lumina, lumina of paraphysis tips up to 3 (rarely 4) μm wide 28
- 27(26) Thallus UV–, no substances detected by TLC, widespread *B. meiocarpa*
Thallus UV+ orange, containing xanthones, Norway and Western North America *B. tacomensis*
- 28(26) Apothecia in section with an epipsamma of yellowish brown granules streaking into the hymenium, ascospores (mean of 10) 4–5 times longer than wide, thallus containing usnic and isousnic acid, on *Rhododendron* in the Eastern Alps *B. subgilva*
Apothecia in section without granules, ascospores (mean of 10) 2.5–4 times longer than wide, thallus without substances detectable by TLC, widely distributed in the Northern Hemisphere 29
- 29(28) Excipular hyphae and paraphyses densely packed, 20 or more within 50 μm of exciple or hymenium *B. subduplex*
Less than 20 excipular hyphae or paraphyses within 50 μm of exciple or hymenium *B. aff. meiocarpa*
- 30(24) Thallus P+ red, containing argopsin 31
Thallus P–, without argopsin 34
- 31(30) Thallus of minute, densely crowded squamules, mostly higher than 100 μm , vivid to dark green in fresh collections; apothecia usually strongly convex 32
Thallus rimose, up to 100 μm thick, usually some shade of white or grey; apothecia weakly to moderately convex 33
- 32(31) Thallus C+ pink (reaction can be very faint), with gyrophoric acid, uncommon but widespread, on rotten bark and wood in *Picea abies* forests *B. fallax*
Thallus C–, gyrophoric acid absent; so far only known from Newfoundland; on healthy bark, sometimes overgrowing bryophytes *B. terrae-novae*
- 33(31) Thallus usually with numerous, conspicuous semi-immersed pycnidia; conidia 6–8(–10) μm long; eastern North America *B. pycnidiata*
Thallus usually without pycnidia; conidia, if present 7.5–10 μm long; western North America and Norway *B. toensbergii*
- 34(30) Apothecial sections C+ pink, containing gyrophoric acid *B. helvola*
Apothecial sections C– 35
- 35(34) Ascospores 3.3–7 times as long as broad 36
Ascospores 2–4.5 times as long as broad 38
- 36(35) Exciple and/or epihymenium with small granules, apothecial sections KC+ violet, probably lobaric acid, on rotten bark, South America *B. australis*
Without granules in exciple or epihymenium, apothecial sections KC–, lobaric acid absent, on living bark of trees in the Northern Hemisphere 37
- 37(36) Apothecia strongly convex or tuberculate, margin lacking, ascospores 11.0–14.5 \times 2.0–3.5 μm *B. alborufidula*

- Apothecia flat to weakly (rarely strongly) convex, margin not or weakly prominent, slightly more glossy than the disc, ascospores $12.5\text{--}26.0 \times 3.0\text{--}5.0 \mu\text{m}$
 ***B. longispora***
- 38(35) Ascospores (mean of 10) $3.4\text{--}4.8 \mu\text{m}$ wide 39
 Ascospores (mean of 10) $4.8\text{--}6.3 \mu\text{m}$ wide 41
- 39(38) Hymenium $50\text{--}70 \mu\text{m}$ high, ascospores (mean of 10) $12.6\text{--}20.0 \mu\text{m}$ long, on tree trunks, either directly on bark or overgrowing bryophytes ***B. orientalis***
 Hymenium $40\text{--}50(\text{--}55) \mu\text{m}$ high, ascospores (mean of 10) $9.8\text{--}14.7 \mu\text{m}$ long, on shrubs, very rarely on trunks of trees 40
- 40(39) Thallus UV–, no substances detected by TLC, wide-spread ***B. meiocarpa***
 Thallus UV+ orange, containing xanthenes, Norway and Western North America ***B. tucomensis***
- 41(38) Ascospores mostly simple, but 1–3-septate ones frequently intermixed, on average (mean of 10) $21\text{--}25 \mu\text{m}$ long and 4.4–5.1 times as long as wide ***B. alaskana***
 Ascospores simple or rarely 1-septate, on average (mean of 10) $13\text{--}19 \mu\text{m}$ long and 2.7–3.5 times as long as wide ***B. vernalis***

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