The Lichenologist (2023), **55**, 325–334
doi:10.1017/S0024282923000464

Standard Paper

A distinctive new species of *Biatora* (*Ramalinaceae, Lecanorales*) described from native European forests

Zdeněk Palice[1], Jiří Malíček[1], Jan Vondrák[1,2] and Christian Printzen[3]

[1]Institute of Botany of the Czech Academy of Sciences, CZ-252 43 Průhonice, Czech Republic; [2]Faculty of Biological Sciences, University of South Bohemia, CZ-370 05 České Budějovice, Czech Republic and [3]Department of Botany and Molecular Evolution, Senckenberg Research Institute and Natural History Museum Frankfurt, D-60325 Frankfurt am Main, Germany

Abstract

A unique crustose lichen species was recently documented from various types of preserved forests across boreal and temperate Europe (Norway, Ukraine, the Czech Republic) and the Caucasus (Russia). It is formally described here as the new species *Biatora amylacea*. A phylogeny based on ITS and mtSSU sequences demonstrates that it belongs to an isolated group within the core of *Biatora* s. lat., together with the recently described *B. radicicola*. It is a distinctive taxon within the genus on account of its amyloid exciple, otherwise known only from members of the *Biatora rufidula* group. The new species is also characterized by amyloid thalline hyphae and the production of soredia with a blue-green pigment. This microlichen may serve as a bioindicator species of old-growth forests.

Keywords: bioindicators; lichen pigments; old-growth forests; taxonomy

(Accepted 18 June 2023)

Introduction

The genus *Biatora* Fr. is a quite diverse group of predominantly epiphytic crustose lichens with biatorine apothecia and narrowly ellipsoid to bacilliform pale ascospores. In his revision, Printzen (1995) used the ontogeny of apothecia as a useful criterion to delimit *Biatora* from similar looking taxa, such as *Mycobilimbia* Rehm. Almost two decades later, Printzen (2014) performed a phylogenetic revision of the group (using three molecular markers: the internal transcribed spacer region of the nuclear rDNA (ITS), RNA polymerase II gene (*RPB*2) and the small subunit of the mitochondrial rDNA (mtSSU)) and the genus *Biatora* was emended by the inclusion of several species formerly classified in other genera. The lumping trend has continued by including *Ivanpisutia oxneri* S. Y. Kondr. *et al.* and the polysporic *Myrionora albidula* (Willey) R. C. Harris into *Biatora* (Kistenich *et al.* 2018).

In its current circumscription, the genus is almost impossible to define by morphological characters alone. Even some features formerly believed to be characteristic for *Biatora*, such as the *Biatora*-type of asci, were recently rejected as phylogenetically useful synapomorphic characters based on molecular evidence. For example, the species *Biatora ligni-mollis* T. Sprib. & Printzen has asci approaching the *Micarea*-type (Spribille *et al.* 2009). One of the best features to characterize the genus is the heavily gelatinized chondroid exciple in which individual hyphae are hardly discernible, a character that may be connected with the specific mode of apothecial ontogeny (Printzen 1995).

Printzen (2014) mentioned 42 species for the genus *Biatora*, among which several undescribed taxa were noted, and this number was followed in the last generic classification of lichens by Lücking *et al.* (2017). Further additions were recently published by Printzen *et al.* (2016), Kistenich *et al.* (2018), Ekman & Tønsberg (2019) and Spribille *et al.* (2020). However, the exact number of species is hard to determine because some authors have recently started to simultaneously split off new genera from, and combine taxa from, outside *Biatora* into the genus based on largely unsupported phylogenies (Kondratyuk *et al.* 2019). It is clear, however, that numerous taxa still await descriptions. One of these so far unrecognized species is described here. It has been known to the authors since 2015, and was listed as '*Biatora amylacea* ined.' in two previous studies (Vondrák *et al.* 2018; Urbanavichus *et al.* 2020). In the former work, the most distinctive and diagnostic features of this taxon were summarized in the supplementary material of the paper. Mitochondrial SSU and nuclear ITS sequences were generated (in GenBank under *Biatora* sp.) in the latter publication for a Caucasian specimen of this species.

Material and Methods

Microscopic examinations were made on hand-cut sections and squash preparations mounted in water, Lugol's solution or lactophenol cotton blue (LCB) using a Zeiss Axioskop 2 equipped with a Zeiss Axiocam 305 for imaging, or using an Olympus CX41 and SZ12, the latter equipped with an Olympus DP70 camera for imaging. External features were studied through a Zeiss Stemi 11 and an Olympus BX60 dissecting microscopes. Habit images

Corresponding author: Zdeněk Palice; Email: zdenek.palice@ibot.cas.cz

Cite this article: Palice Z, Malíček J, Vondrák J and Printzen C (2023) A distinctive new species of *Biatora* (*Ramalinaceae, Lecanorales*) described from native European forests. *Lichenologist* 55, 325–334. https://doi.org/10.1017/S0024282923000464

were made with a Zeiss Axiozoom V16 or using the Olympus DP70 camera's extended depth of field module, Deep Focus. Vouchers are deposited in BG, FR, PRA and the private herbarium of J. Malíček. Colour reactions of acetone-insoluble pigments in apothecia or prothalline hyphae were observed after application of a 10% aqueous solution of potassium hydroxide (K), a 50% aqueous solution of nitric acid (N) and a 10% aqueous solution of hydrochloric acid (HCl). Ascospore measurements are given as (smallest single measurement–) smallest mean-largest mean (–largest single measurement), ($n =$ number of measurements). The values have been rounded to the nearest 0.5 µm. Thin-layer chromatography (TLC) was used for detection of secondary lichen metabolites, using solvents A, B′ and C (Orange *et al.* 2010).

To check whether the new species belonged to *Biatora* and find its closest relatives within the genus, a phylogenetic analysis was carried out using five samples, the data set used by Printzen (2014) with the exception of *Cliostomum* and three species (*Biatora chrysantha* (Zahlbr.) Printzen, *B. ementiens* (Nyl.) Printzen and *B. printzenii* Tønsberg) responsible for conflicts among data sets, and a small number of additional sequences of species that were formerly not available for analysis (Table 1). DNA was extracted using either the Invisorb Spin Plant Mini Kit (Invitek) or a Chelex protocol (Ferencová *et al.* 2017). The following primers were used for amplification of the internal transcribed spacer region of the nuclear ribosomal DNA and the small subunit of the mitochondrial ribosomal DNA: ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), mrSSU1, mrSSU2R, mrSSU3R (Zoller *et al.* 1999) and MSU7 (Zhou & Stanosz 2001). PCR conditions followed protocols described in Printzen (2014) and Malíček *et al.* (2017). Newly generated DNA sequences were submitted to GenBank (Table 1).

Alignments of the single-locus data sets were created using an online application of MAFFT v. 7 (Katoh *et al.* 2019; https://mafft.cbrc.jp/alignment/server/) with default settings ('Auto' strategy, aligning gappy regions and the default guide tree). Based on these alignments, regions of uncertain alignment were identified using the GUIDANCE2 server (http://guidance.tau.ac.il; Landan & Graur 2008; Penn *et al.* 2010; Sela *et al.* 2015). Regions with GUIDANCE scores < 0.93 were removed from the analyses. Single-locus maximum likelihood (ML) trees were reconstructed using the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/; Trifinopoulos *et al.* 2016) with default search parameters (perturbation strength 0.5, stopping the analysis if no better tree was found after 100 random perturbations). The optimal substitution models and partitioning schemes for these data sets were inferred by IQ-TREE based on the Bayesian information criterion. For ITS, we suggested separate character sets for ITS1, 5.8S rRNA and ITS2. Branch support values were calculated using the ultrafast bootstrap algorithm with 1000 replications. Single-locus trees were checked for well-supported topological incongruencies. Since none were found, data sets were concatenated and an ML bootstrap tree reconstructed, this time suggesting four separate character sets (the above mentioned plus mtSSU; see Table 2). Branch support values for the concatenated data set were calculated using the standard bootstrap algorithm with 100 replications, the maximum possible on the web server.

A Bayesian phylogeny was reconstructed using MrBayes v. 3.2.7 (Ronquist *et al.* 2012). We used the partitioning scheme inferred by IQ-TREE and, because the optimal models were not implemented in MrBayes, simplified substitution models as outlined in Table 2. Model parameters were unlinked between partitions. The mean of the branch length prior was inferred as outlined in Printzen (2014), based on branch lengths in the standard bootstrap ML tree. MrBayes was set to sample every 200th tree out of 40 M generations using two independent runs, each with four chains that were incrementally heated by a factor of 0.15. To infer convergence of the Markov chains, the average standard deviation of bipartition frequencies among runs was calculated every 100 000th generation, discarding the first 50% of the sampled trees as burn-in and including only those bipartitions with a frequency of at least 10%. The analysis was stopped after 3.5 M generations when the standard deviation had dropped below 0.01.

Results

Phylogeny

The concatenated alignment comprised 1277 bp (ITS: 433, mtSSU: 844). Overall support for the phylogenetic tree is low, probably due to the low number of gene loci. The genus *Biatora* is well supported as a monophyletic group (Fig. 1). Within *Biatora*, of the groups previously defined by Printzen (2014), the *beckhausii*-, *meiocarpa*-, *rufidula*- and *hertelii*-groups (including *B. mendax* Anzi) are recovered, some of them appearing unsupported (Fig. 1). The *vernalis*-group is distributed over two clades, with the *meiocarpa*-group in between. However, these relationships were not statistically supported. Close relationships were supported between *B. flavopunctata* (Tønsberg) Hinter. & Printzen and *B. vacciniicola* (Tønsberg) Printzen (BP = 100, PP = 1.0), *B. hemipolia* (Nyl.) S. Ekman & Printzen and *B. globulosa* (Flörke) Fr. (BP = 85, PP = 1.0), *B. hypophaea* Printzen & Tønsberg, *B. ocelliformis* (Nyl.) Arnold and *B. oxneri* (S. Y. Kondr. *et al.*) Printzen & Kistenich (BP = 98, PP = 1.0), five taxa of the *meiocarpa*-group (BP = 91, PP = 1.0), six species from the *vernalis*-group 1 (BP = 89, PP = 1.0), as well as *B. radicicola* Printzen *et al.* and five specimens of the hitherto undescribed *Biatora* (BP = 97, PP = 1.0). These latter form a strongly supported monophyletic clade (BP = 100, PP = 1.0) which supports their status as a separate, new species.

Taxonomy

Biatora amylacea Palice, Malíček, Vondrák & Printzen sp. nov.

MycoBank No.: MB 849563

Recognizable within the genus *Biatora* by the combination of an immersed blue-green tinted or episubstratal creamy white to pale grey or pale ochre thallus, possessing minute dark green to blue-grey pigmented soralia, sparsely present dark grey-bluish, pale-rimmed apothecia and an absence of secondary lichen metabolites. The characteristic feature is a distinct dark violaceous reaction of the apothecial exciple and thalline hyphae with Lugol's solution.

Type: Norway, Sogn og Fjordane, Gloppen, Våtedalen valley, forest with *Betula*, *Alnus* and *Sorbus* on W-facing slope just above the road E39, 61°40′42.6″N, 6°31′16.8″E, alt. 140 m, on bark of *Sorbus aucuparia*, 8 September 2015, *Z. Palice* 19999 & *T. Tønsberg* (BG—holotype!). GenBank Accession no.: OQ682881 (as *Biatora* sp.).

(Figs 2A–G, 3A & B)

Table 1. Voucher information and GenBank Accession numbers for collections used for phylogenetic analyses. Accession numbers in bold indicate newly generated sequences.

Species	Origin	ITS	mrSSU
Bilimbia sabuletorum	Norway, *S. Ekman* 3091 (BG)	AM292670	AY567721
Lecania croatica	Turkey, Prov. Trabzon, *C. Printzen* 5946 (BG)	KF650949	KF662397
L. cyrtella	Sweden, *S. Ekman* 3017 (BG)	AF282067	AY567720
'*Lecidea*' *albohyalina* 1	Sweden, Hälsingland, *F. Jonsson* 6:29 (hb. Mellansel)	KF650950	KF662398
'*Lecidea*' *albohyalina* 2	Czech Rep., Southern Bohemia, Šumava Mts, *Z. Palice* 839 (FR)	KF650951	KF662399
'*Lecidea*' *sphaerella*	Czech Rep., Southern Bohemia, Šumava Mts, *Z. Palice* 4621 (FR)	KF650952	KF662400
Mycobilimbia epixanthoides	Finland, Prov. Uusima, *C. Printzen* & *M. Kuusinen* s. n. (FR)	KF650953	KF662401
M. sphaeroides	Norway, Lindås, *T. Tønsberg* 39665 (BG)	KF650954	KF662402
M. tetramera	Finland, Prov. Uusima, *C. Printzen* & *M. Kuusinen* s. n. (FR)	KF650955	KF662403
Biatora aegrefaciens	USA, Alaska, Mitkof Isl. W, *T. Tønsberg* 30212 (BG)	KF650956	n/a
B. alaskana 1	USA, Alaska, Borough of Sitka, *C. Printzen* 5229 (FR)	KF650957	KF662404
B. alaskana 2	Japan, Hokkaido, Kitami Prov., *G. Thor* 24732 (UPS)	KF650958	KF662405
B. amylacea 1	Ukraine, Eastern Carpathians, Velyka Uhol'ka, *Z. Palice* 19170 (PRA)	OQ717329	OQ682880
***B. amylacea* 2**	**Ukraine, Eastern Carpathians, Velyka Uhol'ka, *Z. Palice* 19363 (PRA)**	**OR122524**	**OR124846**
B. amylacea 3	Norway, Sogn og Fjordane, Gloppen, *Z. Palice* 19999 & *T. Tønsberg* (holotype—BG)	n/a	OQ682881
B. amylacea 4	Russia, Caucasus, Adygea, Lagonaki, *J. Malíček* 11048 (hb. Malíček)	MK778585	MK778516
B. amylacea 5	Russia, Caucasus, Kabardino-Balkaria, Elbrus, *J. Vondrák* 22719 (PRA)	n/a	OQ682879
B. appalachensis	USA, North Carolina, Graham Co., *C. Printzen* 6661 (FR)	KF650959	n/a
B. bacidioides 1	Turkey, Prov. Rize, *B. Kanz* & *C. Printzen* s. n. (FR)	n/a	KF662406
B. bacidioides 2	Ukraine, Eastern Carpathians, Velyka Uhol'ka, *Z. Palice* 19685 (PRA)	MG773664	MG773674
B. bacidioides 3	Ukraine, Eastern Carpathians, Velyka Uhol'ka, *Z. Palice* 19221 (PRA)	MG773663	MG773673
B. beckhausii	Norway, *H. Holien* 6744 (TRH)	AF282071	KF662407
B. britannica	UK, Wales, *A. Orange* 11003 (NMW)	AY032897	n/a
B. chrysanthoides	USA, Washington, Clallam Co., *C. Printzen* 5318 (FR)	KF650960	KF662409
B. cuprea	Sweden, Torne Lappmark, par. Jukkasjärvi, *B. Kanz* & *C. Printzen* 5437 (BG)	KF650961	KF662410
B. efflorescens	Czech Rep., Southern Bohemia, Šumava Mts, *Z. Palice* s. n. (FR)	AJ247555	n/a
B. fallax	Czech Rep., Southern Bohemia, Šumava Mts, *Z. Palice* s. n. (FR)	AJ247548	KF662412
B. flavopunctata	USA, Washington, Clallam Co., *C. Printzen* 5327 (FR)	KF650963	KF662413
B. globulosa	Sweden, *S. Ekman* 3142 (BG)	AF282073	KF662414
B. helvola	Finland, Etelä-Savo, *M. Kuusinen* s. n. (BG)	KF650964	n/a
B. aff. *helvola* 1	Finland, *M. Kuusinen* (H)	AJ247570	n/a
B. aff. *helvola* 2	Japan, Hokkaido, Kitami Prov., *G. Thor* 24259 (UPS)	KF650965	KF662415
B. hemipolia	Czech Rep., Southern Bohemia, Šumava Mts, *J. Vondrák* 25080 (PRA)	OQ717332	OQ682885
B. hertelii	Madeira, Rabaçal, *B. Kanz* & *C. Printzen* s. n. (FR)	AJ247536	KF662416
B. hypophaea	USA, Oregon, Linn Co., *C. Printzen* s. n. (BG)	KF650966	n/a
B. kodiakensis	USA, Alaska, Kodiak Island Borough, *T. Tønsberg* 29371 (BG)	KF650967	KF662417
B. ligni-mollis 1	Czech Rep., Southern Bohemia, Novohradské hory Mts, *J. Malíček* & *Z. Palice* 14609 (FR)	KF650968	KF662418
B. ligni-mollis 2	Canada, British Columbia, Incomappleux River, *T. Spribille* 12692 (FR)	EU669178	n/a
B. longispora	USA, Massachusetts, Berkshire Co., *P. May* 5409 (hb. May)	KF650969	KF662419
B. marmorea nom. illeg.	USA, Alaska, Glacier Bay National Park, *T. Spribille* 38009 (MSC)	MN483107	MN508265
B. meiocarpa	Norway, Hordaland, Granvin, *T. Tønsberg* 28317a (BG)	AM292667	AM292710
B. meiocarpa var. *tacomensis*	USA, Washington, Lewis Co., *C. Printzen* 5015 (FR)	n/a	KF662420

(*Continued*)

Table 1. (*Continued*)

Species	Origin	ITS	mrSSU
***B. mendax* 1**	**Russia, Caucasus, Guzeripl', *Z. Palice* 21231a (PRA)**	**OR125936**	**OR135361**
***B. mendax* 2**	**Ukraine, Eastern Carpathians, Velyka Uhol'ka, *Z. Palice* 19292 (PRA)**	**OR125937**	**n/a**
B. nobilis	USA, Washington, *T. Tønsberg* 29057 (BG)	KF650970	KF662421
B. ocelliformis 1	Germany, Bavaria, Niederbayern, *C. Printzen* s. n. (FR)	KF650972	n/a
***B. ocelliformis* 2**	**Russia, Kamchatka, Mil'kovo, *I. Stepanchikova* NIK-17-2016 (LE)**	**OR125938**	**n/a**
***B. ocelliformis* 3**	**Czech Rep., Southern Bohemia, Šumava Mts, *Z. Palice* 20524 (PRA)**	**OR125939**	**n/a**
B. oligocarpa	USA, Alaska, Kodiak Island Borough, *T. Tønsberg* 29571 (BG)	KF650973	KF662423
B. orientalis ined.	Japan, Hokkaido, Kitami Prov., *G. Thor* 23714 (UPS)	KF650974	KF662424
B. oxneri	South Korea, *E. Davydov* 12006 (ALTB)	MG925973	n/a
B. pallens	Sweden, Lule Lappmark, Jokkmokk par., *U. Nordin* 2161 (BG)	KF650975	KF662425
B. pausiaca	USA, Washington, Clallam Co., *T. Tønsberg* 28017 & *C. Printzen* (BG)	KF650976	KF662426
B. pontica 1	Ukraine, Eastern Carpathians, Velyka Uhol'ka, *J. Malíček* 8269 (hb. Malíček)	MG773666	MG773677
B. pontica 2	Turkey, Prov. Trabzon, *C. Printzen* 6114 (BG)	KF650977	KF662427
B. pycnidiata	Canada, Newfoundland, Ferryland District, *C. Printzen* 5497 (BG)	KF650979	KF662429
B. radicicola 1	Ukraine, Eastern Carpathians, Velyka Uhol'ka, *Z. Palice* 19970 (PRA)	KX389588	KX389607
B. radicicola 2	Ukraine, Eastern Carpathians, Velyka Uhol'ka, *J. Vondrák* 14388 (PRA)	KX389586	KX389606
B. rufidula	USA, Washington, Pierce Co., *C. Printzen* 5055 (FR)	KF650981	KF662430
B. sphaeroidiza	Sweden, Uppland, Alsike par., *Z. Palice* s. n. (FR)	KF650982	n/a
B. subduplex	Sweden, Torne Lappmark, par. Jukkasjärvi, *B. Kanz* & *C. Printzen* 5436 (FR)	KF650983	KF662431
B. cf. *subduplex*	Italy, *C. Printzen* 2683 (FR)	AJ247540	n/a
B. terrae-novae	Canada, Newfoundland, Fortune Bay-Hermitage District, *C. Printzen* 5758 (BG)	KF650971	KF662422
B. toensbergii	USA, Washington, Pierce Co., *C. Printzen* 5053 (FR)	KF650984	KF662432
B. vacciniicola	USA, Alaska, City and Borough of Juneau, *T. Tønsberg* 27486 (BG)	KF650985	KF662433
B. vernalis	Norway, Hordaland, Bergen, *T. Tønsberg* 23757 (BG)	AF282070	AM292711
B. veteranorum	Czech Rep., Southern Bohemia, Novohradské hory Mts, *J. Malíček* & *Z. Palice* 14753 (FR)	KF650986	KF662434
Biatora sp. (Norway)	Norway, Nord-Trøndelag, Steinkjer, *H. Holien* 8595e (hb. Holien)	KF650987	KF662435

Thallus immersed (endosubstratal) to distinctly superficial, continuous to ±rimose-areolate, sometimes cracked into irregularly delimited, strongly convex, minute areoles, surface creamy white to pale grey or pale ochre, matt, becoming scurfy with age; pigments in immersed parts of thalli staining the substratum blue-green. *Soredia* 12–35(–40) µm, corticate, outside with a dark greenish to bluish grey pigment (in more exposed parts) or pale, whitish-greenish or dull yellowish, covered by a continuous and uneven one-layered cortex composed of intricately interwoven hyphae, 2–3 µm thick. *Soralia* delimited, small, usually up to 0.2 mm, rarely exceeding 0.3 mm diam., rounded or irregular, occasionally prolonged, excavate or tuberculate, rarely confluenting, usually containing soredia in the order of tens, sometimes as tiny aggregations of several soredia. *Hypothallus* not clearly developed, usually seen as a prothallus composed of blue-green patches of a loose fine net of pigmented hyphae, 2–3 µm thick. *Cortex* absent or indistinct, up to *c.* 10 µm high, usually not distinguishable from a variably thick epinecral, largely

Table 2. Partitioning scheme and substitution models used in the maximum likelihood (ML) and Markov chain Monte Carlo (MCMC) analyses.

Partition	Positions	ML (standard bootstrap concatenated)	ML (ultrafast bootstrap ITS)	ML (ultrafast bootstrap mtSSU)	MCMC
ITS1	1–153	TIM2e + G4	TIM2 + F + G4	-	GTR + G4
5.8S rRNA	154–312	TIM2e + G4	TIM2e + G4	-	SYM + G4
ITS2	313–433	TN + F + G4	TN + F + G4	-	HKY + G4
mtSSU	434–1277	HKY + F + I + G4	-	TPM2u + F + I + G4	HKY + I + G4

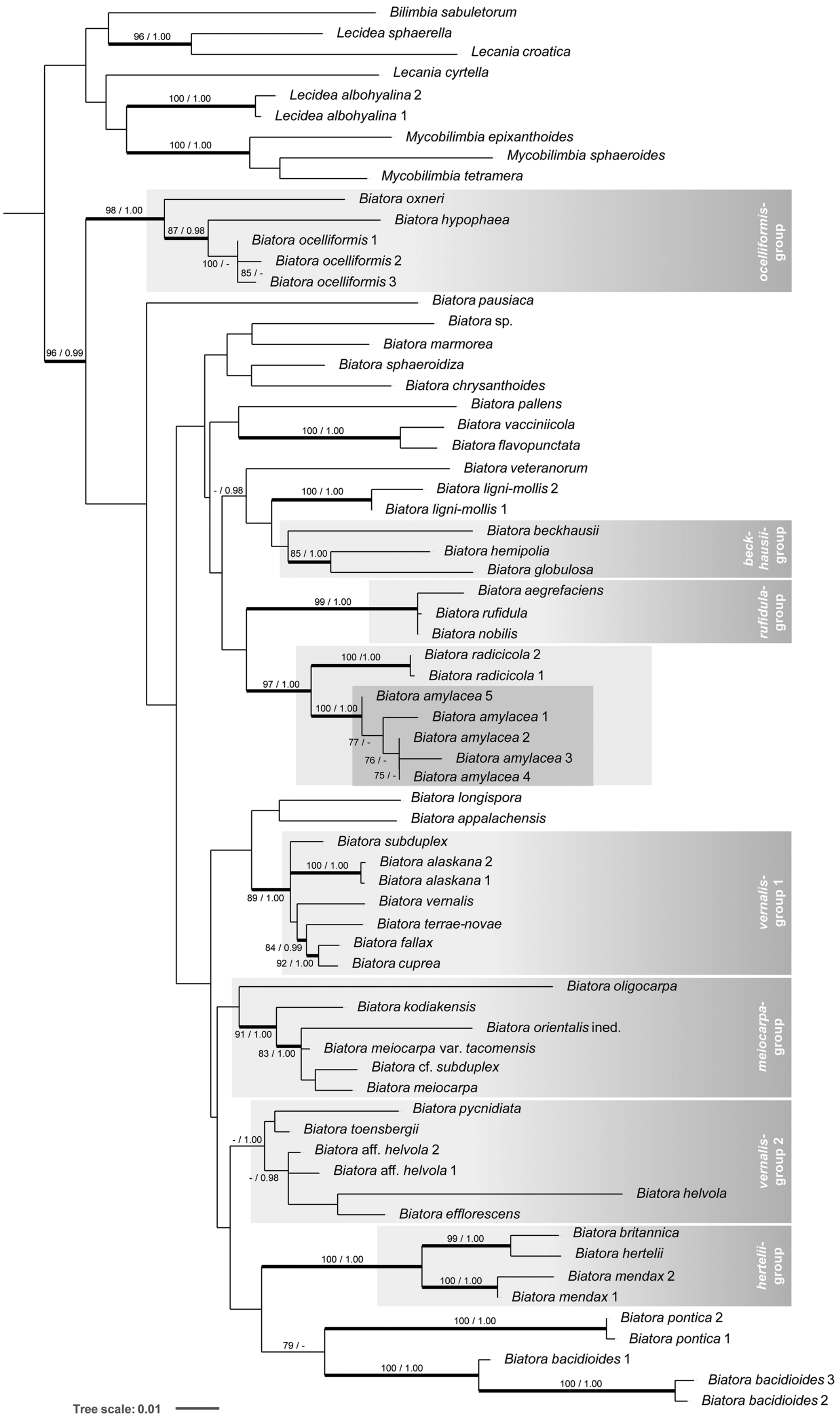

Figure 1. Maximum likelihood (ML) tree based on the concatenated data set of ITS and mtSSU sequences of *Biatora* species and related taxa. Standard non-parametric bootstrap support values (BP) from the ML analysis and posterior probabilities (PP) are given below or above branches. Branches with BP > 70 and PP > 0.95 are in bold. Infrageneric groups previously identified by Printzen (2014) are highlighted. The close relationship between *Biatora amylacea* (in bold) and *B. radicicola* is well supported in both analyses.

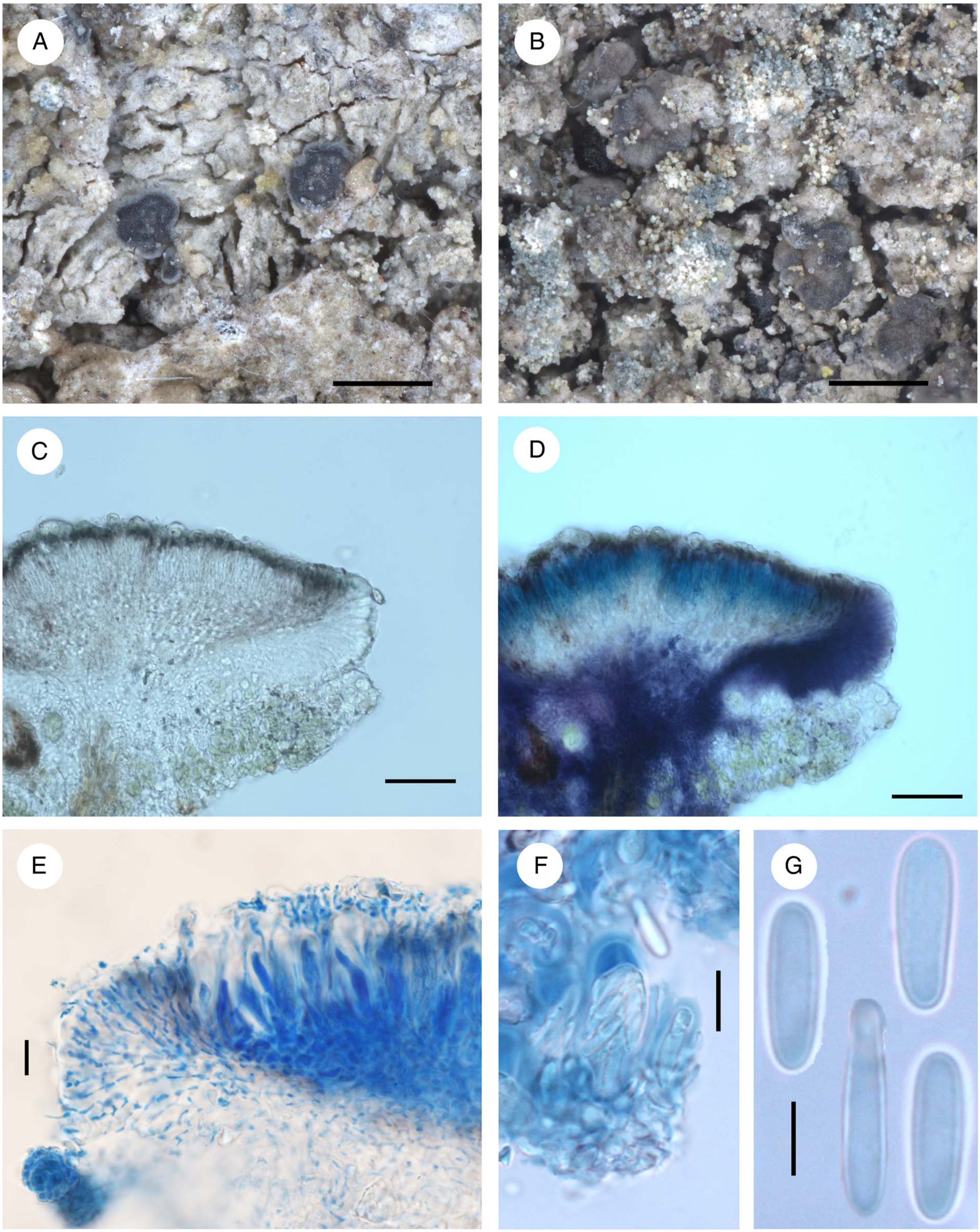

Figure 2. Habit (A & B) and microscopical characters (C–G) of generative structures of *Biatora amylacea*. A, flat apothecia with pale margin (*Palice* 19363). B, irregularly deformed, convex apothecia without margin (*Palice* 19170). C, apothecial section in water (*Palice* 19363). D, same section stained with Lugol's solution; the hymenium stains dark blue, the exciple, hypothecium and parts of the interalgal hyphae dark violaceous. E, irregularly branching excipular hyphae and paraphyses in lactophenol cotton blue (*Palice* 19999 & *Tønsberg*). F & G, *Biatora*-type ascus and ascospores in Lugol's solution (*Palice* 19999 & *Tønsberg*). Scales: A & B = 0.5 mm; C & D = 50 μm; E & F = 10 μm; G = 5 μm. In colour online.

amorphous layer, which may exceed 60 μm in height in extreme cases (aged thalli). *Algal layer* 60–90 μm high (when well developed), often discontinuous, in smaller patches or colonies, disrupted by the host tissue and/or bundles of medullary hyphae, medullary/interalgal hyphae 2–3 μm thick, largely or patchily I_{Lugol}+ dark violaceous (Fig. 2D). *Photobiont* chlorococcoid, cells

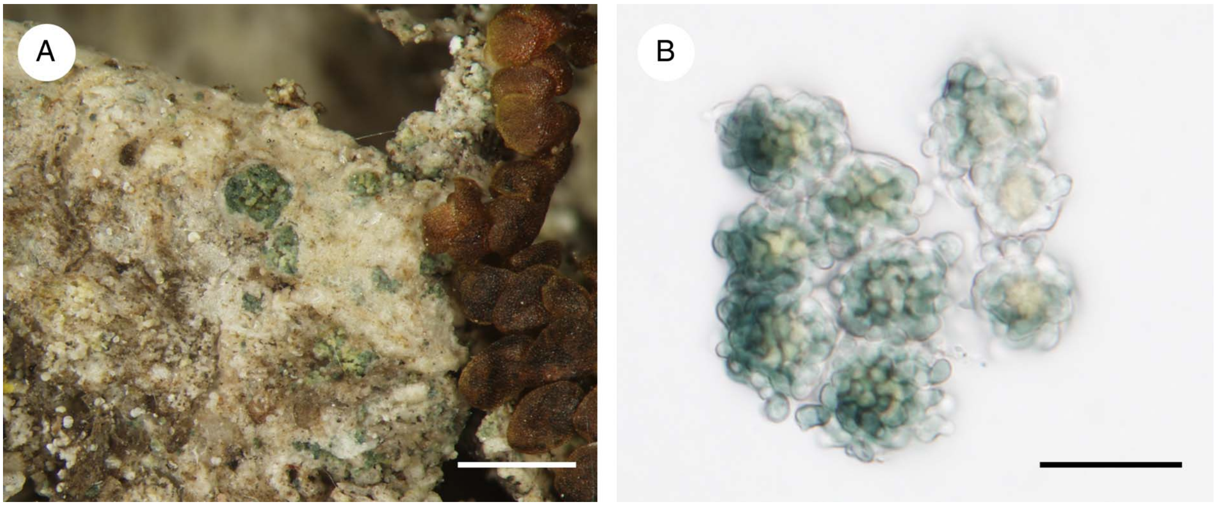

Figure 3. Habit (A) and microscopical characters (B) of vegetative structures of *Biatora amylacea*. A, detail of several excavate soralia with blue-green pigmented external soredia (*Vondrák* 22719). B, squash preparations of corticate soredia with uneven surface in water; note the distinct Cinereorufa-green pigment in part of their cortex (*Malíček* 13765). Scales: A = 0.5 mm; B = 20 µm. In colour online.

broadly ellipsoid to globose, 5–14 µm diam. *Medulla* lacking or 35–40 µm high.

Apothecia (Fig. 2A & B) few, single or in groups of up to three, 0.15–0.60 (mean 0.3–0.4) mm diam., rounded or deformed, sessile with a slightly constricted base. *Disc* flat to moderately convex, surface sometimes irregular, different shades of bluish grey (Fig. 2A), sometimes partly beige, epruinose, matt. *Margin* level with disc when young, rarely becoming slightly prominent or excluded (Fig. 2B), white to pale grey, matt or slightly glossy. *Proper exciple* strongly gelatinized, laterally 30–40 µm, basally 30–50 µm wide, mostly colourless, but sometimes pale turquoise or purplish near hymenium and subhymenium, I_{Lugol}+ dark violaceous (Fig. 2D), composed of radiating, apically branched hyphae (Fig. 2E), lumina 1–2 µm (apically up to 3 µm) wide. *Hypothecium* 25–70 µm high, colourless or pale yellowish. *Subhymenium* 10–30(–50) µm high, pale greenish grey to brown with a faint pink to violaceous hue. *Hymenium* 30–40 µm high, colourless or pale grey to brown with a faint violaceous hue, I_{Lugol}+ medium to dark blue. *Epihymenium c.* 5 µm high, colourless or greyish/greenish black (Fig. 2C) with a pinkish hue, K+ violaceous, KC+ green, in parts also dark green or purplish and K+ intensifying, pigment amorphous, rarely as patchy granules around the ends of paraphyses or slightly spreading as vertical streaks. *Paraphyses* mostly simple, but apically branched, lumina 1.0–1.5 µm (apically 1.0–2.0 µm) wide, mostly colourless, but some with pigmented apical cells. *Asci* of *Biatora*-type (Fig. 2F), 8-spored. *Ascospores* (Fig. 2G) colourless, usually simple or rarely 1–3-septate, narrowly ellipsoid, straight, (9–)12.0–16.0(–20) × (2.5–)3.5–5.0 (–6.0) µm ($n = 50$).

Pycnidia not seen.

Chemistry. Thalli K−, C−, KC−, Pd−, UV−. No lichen metabolites detected by TLC. Apothecial pigments refer to Laurocerasi-brown and Cinereorufa-green as described by Meyer & Printzen (2000). In addition, Cinereorufa-green is present in protothalline and cortical hyphae of external soredia.

Etymology. The specific epithet refers to the typical violaceous colour reaction of the exciple and thalline hyphae with Lugol's iodine, resembling that of starch.

Ecology and distribution. *Biatora amylacea* is widely distributed and so far known from montane temperate and boreal Europe and the Caucasus. It occurs in preserved woodlands and old-growth forest habitats at elevations between 140 m a. s. l. (type locality in Norway) and 1870 m (Caucasus). It is an inhabitant of smooth to slightly roughened, mildly acidic bark of deciduous trees. In a Caucasian specimen (*Vondrák* 22719), the thallus was also found to spread to neighbouring epiphytic liverworts of the genus *Frullania*. The species has been recorded from *Carpinus betulus*, *Fagus orientalis*, *F. sylvatica* and *Sorbus aucuparia*. Virtually all specimens were found in well-preserved unmanaged forests in meso-/microclimatically stable and humid areas.

Additional specimens examined (paratypes). **Ukraine:** *Zakarpatska Oblast Region*: Eastern Carpathians, Khust, Velyka Uhol'ka, E–ESE descending limestone ridge, mixed deciduous forest on steep SSW–S-facing slope 0.9 km WNW of the rock Molochnyi kamen, 48°15′22″N, 23°39′40″E, alt. 820 m, on bark of *Fagus sylvatica* and *Carpinus betulus*, 2015, *Z. Palice* 19170 (PRA), 19363 (FR).—**Russia:** *Republic of Adygea*: Caucasus Mts, Caucasian Biosphere Reserve, Mezmay, KPP Lagonaki, mixed primeval forest (*Abies*, *Acer*, *Sorbus*, *Ulmus*) on limestone bedrock, 44°04′40″N, 40°00′50″E, alt. 1830 m, on bark of *Sorbus aucuparia*, 2016, *J. Malíček* 11048, *Z. Palice*, *J. Vondrák* & *G. Urbanavichus* (hb. Malíček). *Republic of Kabardino-Balkaria*: Caucasus, Baksan, Elbrus, mixed forest on left slope above River Adyl-Su, 43.23549, 42.64539, alt. 1870 m, on mossy bark of *Sorbus aucuparia*, 2018, *J. Vondrák* 22719 (PRA, sterile). *Krasnodar Territory*: Caucasus, Sochi, Krasnaya Polyana, Estosadok, 4 km SSW of Mt Pik Geomorfologov [2665], well-lit hornbeam/beech/oak forest on W-descending crest, WNW of the point 1106,6, between the streams of Achipse and Assara, 43°43′19″N, 40°15′40″E, alt. 820–850 m, on bark of young *Fagus orientalis*, 2019, *Z. Palice* 35475, *S. Svoboda*, *G. Urbanavichus*, *I. Urbanavichene* & *J. Vondrák* (PRA, sterile).—**Czech Republic:** *Southern Bohemia*: Novohradské hory Mts, Horní Stropnice, Hojná Voda National Nature Monument, fragment of primeval forest predominated by beech above road, 48°42′27″N, 14°45′05″E, alt. 830–880 m, on bark of *Fagus sylvatica*, 2020, *J.*

Malíček 13765 (hb. Malíček); *ibid*., below road, 48°42′21″N, 14°45′09.9″E, alt. 857 m, *Z. Palice* 28991 (PRA, sterile). *Western Bohemia*: Šumava Mts, Prášily: Mt Ždanidla, SW–SSW-facing slope, remnant of montane mixed forest, 49°06′02.5″N, 13°20′41.4″E, alt. 1198 m, on bark of old hollow *Fagus sylvatica*, 2021, *Z. Palice* 32822 (PRA, FR, sterile).

Specimens of other species examined. Bacidia caesiovirens. **Norway:** *Nordland*: Sømna, Kvaløya Island, forest on steep NE–NNE-facing slope, along a small stream, 0.4–0.5km SW–WSW from Vennesund, 65°12′50.1″N, 12°02′00.5″E, alt. 90 m, on bark of *Sorbus aucuparia*, 2016, *Z. Palice* 31265 (PRA).

Caloplaca ahtii. **Russia:** *Orenburg Region*: Saraktash, village of Andreevka, protected area 'Andreevskie Shishki hills' at village, 51°56′49″N, 56°39′12″E, alt. *c*. 250–350 m, on bark of *Ulmus laevis*, 2011, *J. Vondrák* 13014 (PRA).

Caloplaca turkuensis. **Austria:** *Salzburg*: Hohe Tauern, Bucheben, Hüttwinkltal valley, a small alder wood among pastures on W-facing slope, 47°07′29.3″N, 12°59′11.2″E, alt. 1210 m, on bark of *Alnus incana*, 2016, *F. Bouda, Z. Palice* 18571 & *O. Peksa* (PRA).

Parvoplaca nigroblastidiata. **Russia:** *Krasnodar Territory*: Caucasus, Sochi, Krasnaya Polyana, Estosadok, 4 km SSW of Mt Pik Geomorfologov [2665], well-lit hornbeam/beech/oak forest on W-descending crest, WNW of the point 1106,6, between the streams of Achipse and Assara, 43°43′19″N, 40°15′40″E, alt. 820–850 m, on bark of *Fagus orientalis*, 2019, *Z. Palice* 27012, *S. Svoboda, G. Urbanavichus, I. Urbanavichene* & *J. Vondrák* (PRA).

Discussion

The description of this so far unrecognized, morphologically distinctive and identifiable species (without the help of molecular data) highlights the fact that even lichenologically relatively well-studied areas of temperate and boreal Europe are still under-explored. The new species is quite well recognizable within the genus *Biatora*, when richly developed and fertile. It is therefore unlikely to be a frequent species but is rather a rare forest lichen and niche specialist with high bioindication potential for well-preserved old-growth forests. The first three authors have undertaken numerous, very detailed lichen surveys in a number of valuable old forest reserves, mainly across Central Europe, and *Biatora amylacea* was detected only rarely. It was never prominent in epiphytic crustose lichen communities, and usually only scanty specimens were found.

Phylogenetically, the closest relative of *B. amylacea* is *B. radicicola*, another rare specialist species preferentially growing in places subjected to water (on roots of trees at river banks) or to snow (bases of trees in montane forests with a long snow cover; Printzen *et al.* 2016). The latter species has recently also been found saxicolous on humid rocks in Sweden (Ekman *et al.* 2019).

Other *Biatora* species with bluish grey apothecia and lacking secondary lichen metabolites, such as *B. beckhausii*, *B. globulosa*, *B. hemipolia* and *B. radicicola*, have a non-amyloid exciple and cannot be confused with *B. amylacea*. The problem is rather that *B. amylacea* is apparently only rarely fully developed and richly fertile, and therefore easily missed by lichenologists. The characteristic amyloid exciple, visible as a dark violaceous reaction after adding iodine solution, is well known from *Biatora aegrefaciens* Printzen and *B. rufidula* (Graewe) S. Ekman & Printzen (Printzen 1995, 2014; Printzen & Tønsberg 2000). These taxa are distinguishable macroscopically by the orange to reddish brown colour of their apothecia, and microscopically by the broader, usually 3-septate ascospores. The amyloid exciple is also typical for the poorly known and phylogenetically unrelated taxon *Lecidea betulicola* f. *endamylea* (Hedl.) Hinter., which also has dark grey apothecia but produces more than 8 spores in the asci and has a more pronounced exciple, formed by radiating and anastomosing hyphae with thin lumina (see Printzen & Tønsberg 2000).

Biatora amylacea regularly produces small, often blue-green-grey pigmented vegetative propagules containing one or more photobiont cells enveloped by a single-layered cortex with a bulging surface (Fig. 3B), frequently originating in small delimited crater-like areas developing from ruptures in the thin outermost layer of the thallus (Fig. 3A). Similar vegetative structures have been called soredia (arising in soralia) in descriptions of taxa such as *Gyalideopsis helvetica* van den Boom & Vězda (van den Boom & Vězda 2000) or *Caloplaca ahtii* Søchting (Søchting 1994). Both these microlichens may resemble our species in the sterile state. Interestingly, some subsequent authors referred to the same propagules as goniocysts (produced in goniocystangia) in the former species (e.g. Spribille & Björk 2008) or as blastidia (Arup *et al.* 2015) in the latter species, apparently based on presumed differences in the ontogeny of these propagules. More recently, Ekman (2023) used the more universal term 'granule' for the fine soredia-like propagules in some members of the genus *Bacidina*, interpreting the tiny particles as the result of gradual splitting of the thallus. Earlier, Printzen (1995: 24) had explained the development of vegetative, soredia-like propagules in *Biatora fallax* Hepp in a similar way. In *B. amylacea*, establishment of propagules has not been studied in much detail because it is beyond the scope of this primarily taxonomic contribution. However, on the hand-cut sections of thalli and adjacent bark it was evident that the propagules were being formed before they were released. In one specimen (*Malíček* 13765), the soralia were observed in ±endophloedic parts of thalli where pigmented hyphae of the mycobiont predominated (prothallus). Propagule formation may therefore be likely to occur in early stages of development, after the first interactions of the fungal hyphae with the photobiont. This implies that the propagules in *B. amylacea* are not referrable to the granules formed secondarily by some representatives of the genus *Bacidina* (Ekman 2023) or to those of *Biatora fallax* (Printzen 1995). Hence, we prefer to call the propagules in the new species soredia in a broad sense (*sensu* Tønsberg 1992), although the pigmented soredia formed in tiny, often excavate soralia (Fig. 3A) of the new species differ somewhat from the soredia produced by most representatives of the genus *Biatora*.

When considering sterile specimens, species similar to *Biatora amylacea* (lacking lichen substances and possessing blue-green to blue-grey vegetative propagules in delimited soralia) include several taxa of *Teloschistaceae*, such as *Caloplaca ahtii*, *C. turkuensis* (Vain.) Zahlbr. or *Parvoplaca nigroblastidiata* Arup *et al.* (Søchting 1994; Šoun *et al.* 2011; Arup *et al.* 2015). All these species contain a blue-grey pigment which is, however, referable to Sedifolia-grey (K+ purple). A habitually similar lichen also exists among *Ramalinaceae*. The Cinereorufa-green pigment in the propagules and hypothallus is shared by the predominantly north-western European *Bacidia caesiovirens* S. Ekman & Holien. This taxon can be easily distinguished by the larger isidioid granules, usually exceeding 40 µm in diameter, occasionally showing projecting hyphae, and sometimes containing traces of atranorin (Ekman & Holien 1995).

The presence of the amyloid reaction in thalline hyphae of the newly described species is a good character for the identification of sterile specimens of *Biatora amylacea* in combination with the content of the Cinereorufa-green pigment, absence of secondary metabolites and the general character of the thallus. Among the epiphytic crustose lichens that occur in the boreal and temperate zones of Europe, we know of only one lecideoid species with an I+ dark violaceous medulla, *Lecidea roseotincta* Coppins & Tønsberg (Coppins & Tønsberg 1988). An amyloid reaction of thalline hyphae was seen by us in all examined specimens of *B. amylacea*. This reaction was particularly conspicuous in the mycelium surrounding the algal layer or between photobiont colonies, and less obvious in pigmented hyphae of the prothallus and cortex of soredia, where the violaceous colour was visible in larger aggregations of soredia or hyphae. Conversely, the amyloid reaction was not observed in any of the examined samples of the potentially confusable lichens mentioned above (see 'Specimens of other species examined').

Acknowledgements. This paper is dedicated to Pier Luigi Nimis who has contributed so much to the various aspects of lichenology, in particular to the documentation of lichen diversity in Southern Europe. ZP is grateful to Tor Tønsberg for organizing a field trip to Norway and bringing him to the type locality of *Lecanora flavoleprosa*, where coincidentally also the holotype of *Biatora amylacea* was collected. ZP, JM and JV acknowledge the long-term research development project RVO 67985939. Jiří Machač helped photograph vegetative structures. Måns Svensson is thanked for his valuable comments on the manuscript.

Author ORCIDs. Zdeněk Palice, 0000-0003-4984-8654; Jiří Malíček, 0000-0002-3119-8967; Jan Vondrák, 0000-0001-7568-6711; Christian Printzen, 0000-0002-0871-0803.

Competing Interests. The authors declare none.

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