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Research Article

Substrate switches, phenotypic innovations and allopatric speciation formed taxonomic diversity within the lichen genus *Blastenia*

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Abstract *Blastenia* is a widely distributed lichen genus in Teloschistaceae. We reconstructed its phylogeny in order to test species delimitation and to find evolutionary drivers forming recent *Blastenia* diversity. The origin of *Blastenia* is dated to the early Tertiary period, but later diversification events are distinctly younger. We recognized 24 species (plus 2 subspecies) within 6 infrageneric groups. Each species strongly prefers a single type of substrate (17 species occur on organic substrates, 7 on siliceous rock), and most infrageneric groups also show a clear substrate preference. All infrageneric groups tend to have the Mediterranean and Macaronesian distribution, but some epiphytic species have much larger geographic ranges and some evolved after a long-distance dispersal outside the region. Chlorinated and nonchlorinated anthraquinone chemosyndromes co-occur in apothecia of most species, but the chemotype has been secondarily reduced in some lineages. One infrageneric group has a marked reduction in apothecial size, associated with a substrate shift to twigs. Only seven species have vegetative diaspores; they also produce apothecia but have smaller ascospores. Genome sizes (22–35 Mb in *Blastenia*) are significantly higher in epilithic species. Within-species genetic variation is low in widely distributed species but high in some epilithic species with small geographical ranges. New taxa are: *B. afroalpina*, *B. anatolica*, *B. caucasica*, *B. gennargentuae*, *B. herbidella* subsp. *acidophila*, *B. lauri*, *B. monticola*, *B. palmae*, *B. psychrophila*, *B. purpurea*, *B. relicta*, *B. remota*, *B. xerothermica*, and *B. xerothermica* subsp. *macaronesica*. New combinations are: *B. festivella* and *B. subathallina*; both names and *B. catalinae* are lectotypified.

Key words: anthraquinones, genome size, long-distance dispersal, Mediterranean–Macaronesian diversity hot-spot, Teloschistaceae, vegetative diaspores.

1 Introduction

The genus *Blastenia* (Ascomycota, Lecanoromycetidae, Teloschistaceae) was introduced by Massalongo (1852a, 1852b) for a group of crustose species with "blasteniospores" and with a reddish tinge to the apothecial disc. Massalongo's term blasteniospore refers to what we now call polarilocular ascospores. He coined the term, with more learning than judgment, from the Greek noun βλαστός, "a shoot of a plant"; presumably, he regarded the two locules as sprouting from the center of the ascospore. Massalongo included seven species. Later authors were less restrained and over 360 names have been published within *Blastenia*, many of them for taxa not closely related to Massalongo's concept of the genus and some for taxa that do not even belong in Teloschistaceae.

Massalongo did not designate a type for *Blastenia*, but Clements & Shear (1931) designated *B. ferruginea* as a type. In the decades following 1931, most authors treated that species within *Caloplaca*, as *C. ferruginea*, and consequently *Blastenia* fell into disuse, being regarded as a synonym of *Caloplaca*. Arup et al. (2013) resurrected the name *Blastenia* and gave the genus a more precise circumscription, mainly on the basis of three-loci phylogeny. In their sense, it is a genus close to *Gyalolechia* in the subfamily Caloplacoideae, with nine species. Taxonomic literature dealing with *Blastenia* in its recent sense is sparse; the main sources are Magnusson (1944a, 1944b); Wetmore (1996, 2004); Arup et al. (2007); Søchting et al. (2008); Arup & Åkelius (2009); Kondratyuk et al. (2009a); and Vondrák et al. (2013b).

While studying Turkish Teloschistaceae, it became clear that numerous species belonging to *Blastenia* were undescribed and

this led us to make a taxonomic study of the genus using DNA sequence data and including putative members of the genus from other parts of the world. Our original intention was merely to prepare a clear taxonomic summary of the genus, but while doing that, we were led to consider the question of what has driven diversification in this genus, and we discuss that topic here too.

Using three DNA loci, we first set the following three goals.

1. Determine species richness and describe the diversity within the genus.
2. Reconstruct the evolutionary history and development of (i) geographical ranges; (ii) ecology; and (iii) selected morphological traits.
3. Determine the genome size (GS) of all species.

We then formulated the following seven hypotheses:

1. Ecology: Each species of *Blastenia* is restricted to either organic or inorganic substrates.
2. Geography: Evolution of *Blastenia* has occurred mostly in the Mediterranean basin and Macaronesia.
3. Within-species genetic variation: The greatest within-species genetic variation occurs in epilithic species with Mediterranean-Macaronesian distribution.
4. Secondary metabolites: The ancestral chemotype was complex, and reductions have led to the several chemotypes observed today.
5. Morphology: In those species that have shifted to twigs: (i) apothecial size has reduced, and (ii) it has done so because of the substrate shift.
6. Morphology: Vegetative diaspores are a derived character in *Blastenia* and are linked to the reduction of the ascospore size.
7. Genome size: Genome sizes are higher in epilithic species.

We present evidence in support of each of these hypotheses.

2 Material and Methods

2.1 Sampling

We searched for *Blastenia* in numerous regions in all continents and surveyed more than a thousand specimens from the western Palearctic, mainly Mediterranean regions and Macaronesia. There are few specimens from the eastern Palearctic and other continents. (As discussed below, we consider that this difference reflects the true distribution of the genus and that the lesser amount of research in those other regions is not materially biasing our study.) Specimens were mainly collected by the authors and are deposited in PRA (Vondrák), LD (Arup), ERC (Halıcı), C (Søchting), and in Frolov's and Malíček's personal herbaria. A significant number of specimens were collected by Evgeny Davydov (ALTB), Josef Hafellner (GZU), Zdeněk Palice (PRA), Irina Urbanavichene (PRA), and Gennadii Urbanavichus (PRA). Other collectors are acknowledged below. Although we studied more than one thousand specimens, DNA sequence data were only generated for 350 specimens (Table S1).

2.2 Molecular protocols

DNA was extracted with a cetyltrimethylammonium bromide (CTAB)-based protocol (Aras & Cansaran, 2006). Three DNA loci were amplified: beta-tubulin nuclear gene, large subunite mitochondrial ribosomal gene (mtLSU in further text), and internal transcribed spacer (ITS) region of nuclear ribosomal DNA (ITS in further text). Polymerase chain reactions were performed in a reaction mixture containing 2.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 0.3 μmol/L of each primer, 0.5 U Taq polymerase (Top-Bio, Praha, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 10 μL. The primers and the cycling conditions are summarized in Table 1. Successful amplifications were sent for Sanger sequencing (GATC Biotech, Konstanz, Germany). The amplification primers were used as the sequencing primers.

2.3 Alignments, phylogenetic analyses, and genotype variability assessment

Sequences were edited in BioEdit 7.2.5 (Hall, 1999) and aligned by MAFFT version 7 (Katoh & Standley, 2013; available online at http://mafft.cbrc.jp/alignment/server/) with the L-INS-i method (Katoh et al., 2005). Gaps were coded as binary data in SeqState by simple coding (Simmons & Ochoterena, 2000). For the concatenated dataset analysis, we used specimens with sequence data from at least two of the three loci (172 specimens). Single-gene analyses included 336 sequences of ITS, 145 sequences of beta-tubulin, and 131 sequences of mtLSU. Further information on alignments is in Table 2. Alignments are available at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S21434).

Table 1 Details to sequenced loci

Locus	Reference	Primers	PCR settings
ITS	Gardes & Bruns (1993)	ITS1F (forward): CTTGGTCATTTAGAGGAAGTAA; ITS4 (reverse): TCCTCCGCTTATTGATATGC	94 °C - 3 min; 7×: 94 °C - 30 s, 62 °C - 30 s (temperature was decreased by 1 °C in each subsequent cycle), 72 °C - 60 s; 38×: 94 °C - 30 s, 56 °C - 30 s, 72 °C - 60 s; 72 °C - 10 min
Beta-tubulin	Designed for this study	TubCf1 (forward): ATATGTTCCCCGTGCTGT; TubCr1 (reverse): ATCATGTTCTTTGGGTCGAA	94 °C - 10 min; 40×: 94 °C - 30 s, 53 °C - 30 s, 72 °C - 60 s; 72 °C - 10 min
mtLSU	Designed for this study	mLSU Cf (forward): GGGGGTCGTGAAGATTCTAT; mLSU Cr (reverse): CCAGAACACTTATCACTTTTACACA	94 °C - 10 min; 40×: 94 °C - 30 s, 56 °C - 30 s, 72 °C - 60 s; 72 °C - 10 min

Phylogenetic reconstructions were carried out using maximum likelihood and Bayesian inference. Models of nucleotide substitutions (Table 2) were selected using the Akaike information criterion implemented in jModelTest v.0.1.1 (Posada, 2008). Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). It was employed for the single-gene alignments and concatenated alignment (Figs. 1 and 2). Analyses were performed using two independent runs with four Markov chain Monte Carlo (MCMC) chains. Trees were sampled after every 500th generation. The analyses were stopped when the average standard deviation (SD) of the split frequencies between the simultaneous runs dropped below 0.01. The first 25% of trees were discarded as the burn-in phase, and the remaining trees were used for the construction of a 50% majority consensus tree.

We expressed the within-species genotype variability by counting polymorphic sites in single-loci alignments (Table 3). Each indel position was considered one character. We also divided the number of polymorphic sites in all loci by the number of all generated sequences to make the data more objective.

2.4 *BEAST: Species tree with dated nodes and with ancestral state mapping

*BEAST as implemented in BEAST v.2.4.5 (Drummond & Rambaut, 2007) was run on the same sequence dataset as employed for Bayesian inference. We used the Site model GTR+G4, Strict Clock model, the Yule model, constant population function and default values for the remaining priors. Two independent MCMC analyses were performed for a total of 100 million generations, sampling every 5000 steps. The convergence of the two runs and the adequacy of sampling were assessed with Tracer v.1.6 (Rambaut et al., 2014). After removing the first 20% of the samples as burn-in, the runs were combined to generate posterior probabilities of nodes from the sampled trees using TreeAnnotator v.2.4.5 (Rambaut & Drummond, 2009).

Dating of nodes was calibrated by two events adopted from Gaya et al. (2015): the divergence time of *B. catalinae* from *B. crenularia* (10.5 ± 4.5 million year ago (Mya)) and the divergence time of *B. ammiospila* from those two species (22.5 ± 7.5 Mya). We are aware of the approximate character of priors and we used the dated tree mostly for relative estimation of taxa ages.

Ancestral state reconstruction was performed for three phenotype characters: types of secondary chemistry, presence/absence of vegetative diaspores, and substrate ecology. We mapped the characters as binary data to species tree terminals and ran the *BEAST (in the version 2.3.2 enabling this analysis) with the same settings as described above. The input data and results of the analysis are depicted in Fig. 3.

2.5 BP&P: Species delimitation

Bayesian phylogeny based on the concatenated data-set of ITS, beta-tubulin, and mtLSU sequences served as a phylogenetic hypothesis for testing species delimitations. Twenty-six groups resolved in the concatenated tree or in some single-locus trees were tested for species delimitation (see below).

The putative taxa were evaluated using Bayesian MCMC analysis for multi-loci data under the multispecies coalescent model (Rannala & Yang, 2003; Yang & Rannala, 2010). The joint analysis of species delimitation and species-tree estimation (Yang & Rannala, 2014) was conducted using the program BP&P v.3.1 (Yang, 2015). This method accommodates uncertainty in the species phylogeny as well as lineage sorting due to ancestral polymorphism. The species tree inferred by *BEAST was used as a starting tree. The rjMCMC algorithm 1 ($\alpha = 2$, $m = 1$) was used to change the species delimitation model and the NNI/SPR move was used to change the species tree topology. Species model prior was set to equal probabilities for rooted trees. A gamma prior G (1, 15), with mean $1/20 = 0.05$ (one difference per 15 bp), was used on the population size parameters. The age of the root in the species tree was assigned the gamma prior G(2, 2000), which means 0.1% of sequence divergence, while the other divergence time parameters were assigned the Dirichlet prior (Yang & Rannala, 2010: equation 2). The mutation rate among loci was specified using a random-rates model ($\alpha = 20$). The first 8000 MCMC iterations were set as burn-in. A total of 200 000 post-burn-in iterations were carried out, and MCMC samples were taken in each iteration. The analysis was run three times to confirm consistency between runs. The species or subspecies with posterior probability consistently exceeding a threshold of 0.95 were accepted as distinct taxa.

2.6 Morphological descriptions

All detailed studies on morphology were carried out after analyzing DNA sequence data when the species boundaries had been settled. First, we conducted a pilot study where we studied a couple of specimens from hardly distinguishable species: (Group 1) epilithic species without vegetative

Table 2 Basic information on alignments

Alignment	Number of sequences	Lenght of alignment/Number of indel codes	Variable characters (all/ingroup only)	Parsimony informative characters (all/ingroup only)	Nucleotide substitution model
ITS	336	577/122	433/396	317/295	GTR+G
beta-tubuline	145	677/8	290/267	235/230	HKY+I+G
mtLSU	131	764/39	292/228	218/146	HKY+G
Concatenated (ITS/beta-tubuline/mtLSU)	172	2005/134	928/827	717/611	GTR+G/HKY+I+G/HKY+G

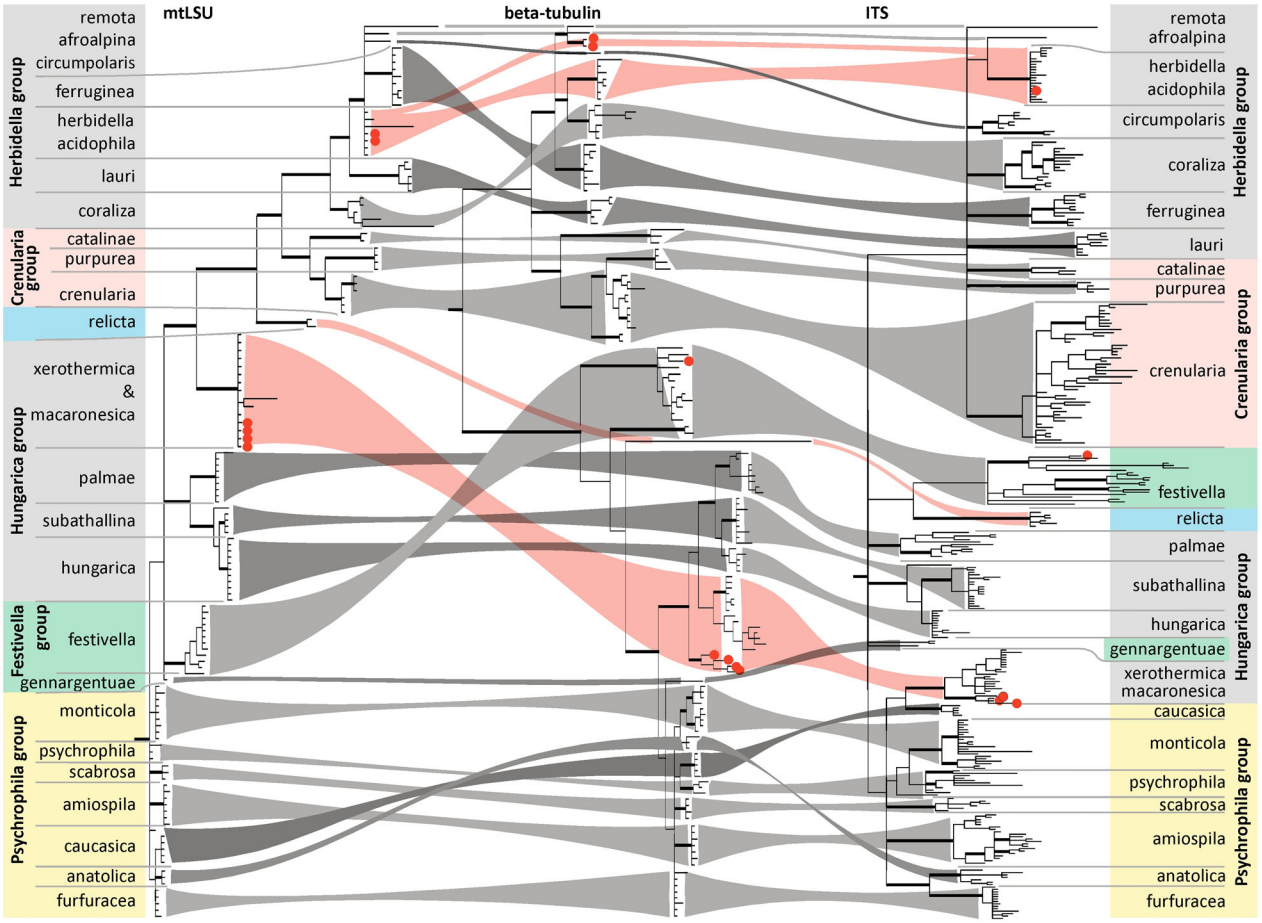

Fig. 1. Bayesian single gene phylogeny reconstructions. Supported clades (pp > 0.95) indicated by thick branches. Clades representing species are connected by grey or red links. Red links indicate taxa with incongruent topologies between single gene phylogenies. Six infrageneric groups are indicated at the right and left margins. The red dots indicate positions of *Blastenia herbidella* subsp. *acidophila*, *B. xerothermica* subsp. *macaronesica* and the epiphytic population of *B. festivella*.

diaspores and (Group 2) epiphytic species without vegetative diaspores. We realized that variability in numerous morphological and anatomical characters is substantial, but most characters are strongly variable within species and do not show differences between species (see the genus description in the taxonomic part). For that reason, we have reduced the morphological descriptions of species in this paper so that they include only diagnostic characters, mainly morphology of vegetative diaspores (if present), thallus thickness, apothecial size, ascospore length and the color of thallus, apothecia, and pycnidia.

The methods for morphological evaluation follow Vondrák et al. (2013a). All observations were done on hand-cut sections in water, without any chemical treatments. Measurements are accurate to 0.5 μm for ascospore size, the width of ascospore septa and width of paraphyses, 1 μm for sizes of vegetative cells and width of asci or 10 μm for larger scales. All measurements of cells include their walls, except for tissues with glutinized cell walls. Following Ekman (1996), the results of ascospore length measurements are given as (min.–) X1–X2–X3 (–max.), where X1 is the lowest specimen arithmetic mean observed, X2 is the arithmetic mean of all observations, and X3 is the highest specimen arithmetic mean observed. SD, the total number of measurements (*N*), and number of investigated samples (*n*) measured in each species are given in square parenthesis [*SD*; *N*; *n*]. Morphological terminology follows Smith et al. (2009) and Vondrák et al. (2013a).

2.7 Identification of secondary metabolites

Most lichen substances present in *Blastenia* are anthraquinones (yellow to red pigments) and are known from previous studies (e.g., Søchting, 1997, 2001). Their characteristics are available in Elix (2014), including their thin layer chromatography (TLC) response factor (RF) values. With the help of these published data, we were able to identify the dominant substances of all *Blastenia* species by TLC (solvents B', C). We used the purple spot reaction with hypochlorite ion ("C" reagent) for detection of chlorinated anthraquinones and also for their spatial distribution within apothecia (see Vondrák & Wirth, 2013; Vondrák et al., 2013a for details). We specifically noted the presence/absence of chlorinated anthraquinones in epihymenium (apothecial disc) and exciple.

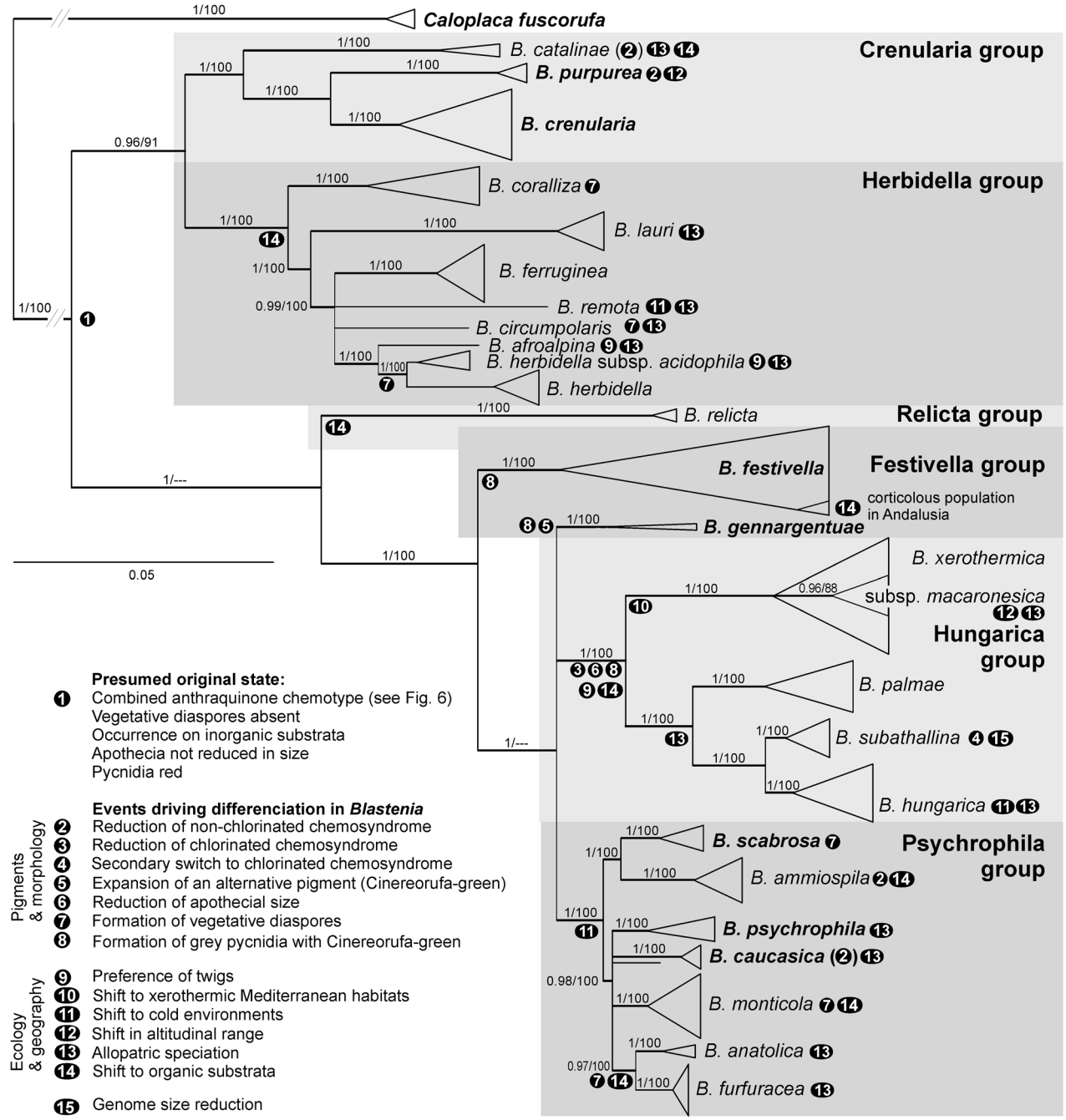

Fig. 2. Bayesian phylogeny of the concatenated dataset of beta-tubulin, ITS and mtLSU loci. Bayesian posterior probabilities and bootstrap supports from the maximum likelihood analysis (after slashes) are shown above branches. Branches with posterior probability >0.95 are thick. Six infrageneric groups are indicated by shading. Names in bold indicate epilithic taxa. Clades recognized at species level are displayed as triangles. Length of triangle (horizontal dimension) reflects genotype diversity within clades. Height of triangle reflects sampling size. Character states are listed in the bottom left corner and mapped onto the tree.

For authentication of TLC identifications, eight samples were subsequently analyzed by LC-MS (ultra performance liquid chromatography and mass spectrometry): 3x *Blastenia ammiospila*, *B. ferruginea*, *B. monticola*, *B. palmae*, *B. purpurea* and *B. subathallina*. Apothecia together with adjacent thallus were extracted in methanol using an ultrasonic device. The LC-MS methods followed Valný et al. (2016) with a few modifications: the analyses were performed under a linear gradient program (min/%B) 0/5, 1.5/5, 12.5/58 followed by a 1.5-minute column clean-up (100% B) and 1.5-minute equilibration (5% B); the total analysis time was 20 minutes. The mass spectrometer was operated in the W mode. Individual

Table 3 Within species variability in the three loci expressed by number of variable nucleotide positions. Species of *Blastenia* ordered according to the ratio in the last column. Numbers of available sequences are given in brackets at each species in order: ITS, mtLSU and beta-tubulin. Saxicolous species in bold; species with vegetative diaspores underlined; questionmarks indicate unknown variability in nucleotide positions

	Variable nucleotide positions				
Species	ITS	mtLSU	Beta-tubulin	All loci	Variable positions in all loci/number of all sequences
***B. gennargentuae* (3,1,1)**	12	?	?	12	4.00
***B. festivella* (21,12,7)**	85	11	63	159	3.98
B. catalinae (5,2,3)	16	?	11	27	3.38
***B. psychrophila* (10,4,3)**	23	7	3	33	1.94
B. circumpolaris (8,1,1)	15	?	?	15	1.50
***B. crenularia* (43,6,11)**	56	5	25	86	1.43
B. palmae (15,9,8)	34	1	9	44	1.38
***B. caucasica* (7,5,6)**	16	0	7	23	1.28
B. coralliza (17,5,6)	20	6	7	33	1.18
B. lauri (9,5,5)	10	4	7	21	1.11
B. ferruginea (11,9,8)	17	2	9	28	1.00
B. relicta (5,2,1)	5	?	?	5	1.00
B. anatolica (7,3,3)	8	0	3	11	0.85
B. xerothermica (29,15,18)	21	0	28	49	0.79
subsp. *xerothermica* (25,11,13)	16	0	19	35	0.71
subsp. *macaronesica* (4,4,5)	7	0	8	15	1.15
B. scabrosa (6,3,5)	4	2	5	11	0.79
B. monticola (20,9,8)	20	2	6	28	0.76
***B. purpurea* (4,4,4)**	5	0	4	9	0.75
B. herbidella (24,7,9)	4	2	23	29	0.73
subsp. *herbidella* (22,5,7)	4	2	3	9	0.26
subsp. *acidophila* (2,2,2)	?	?	?	?	?
B. subathallina (19,5,5)	12	6	1	19	0.65
B. ammiospila (21,7,7)	18	2	1	21	0.60
B. furfuracea (14,5,7)	6	1	0	7	0.27
B. hungarica (12,11,9)	5	1	1	7	0.22

metabolites were identified in UV (DAD detector) and in the negative mode of electrospray ionization, which worked with higher efficiency compared to the positive mode.

Cinereorufa-green (an accessory green-black pigment) is not extractable by acetone and not detectable by TLC, but it was detected in sections of tissue by the negative reaction with KOH and the violet reaction with nitric acid.

2.8 Flow cytometry: Genome size assessment

Flow cytometry was carried out on isolated nuclei by the method described in Veselská et al. (2014) with a few modifications, using *Aspergillus fumigatus* CEA10 with a GS of 29.2 Mb (Fedorova et al., 2008) as an external standard for GS calculations. Lichen apothecia or sterile mycelium of *A. fumigatus* were fixed in methanol: acetic acid (3: 1 v/v), 10% DMSO, 0.1% Triton X-100 for one hour at 4 °C and then washed with 0.1% Triton X-100. The samples were then chopped using a razor blade in Tris-$MgCl_2$ buffer supplemented with RNAse A (0.1 mg/mL). The suspension containing released nuclei was filtered through a 20 µm nylon filter to remove large debris and incubated at 37 °C for 15 min. Samples were measured immediately after Propidium iodide (Fluka, Glossop, England) —final concentration of 50 µg/mL—was added on the LSRII machine (Becton Dickinson, NJ, USA) with FACSDiva 6 Software (Becton Dickinson) at the Service Centre for Cytometry and Microscopy of the Institute of Microbiology, ASCR, Czech Republic. All fluorescent events were recorded. The measurement was stopped when 10 000 events were captured within the area responding to the signals of labeled nuclei. The output was processed in FlowJo 7.6.1 (Tree Star, Ashland, TN, USA). The relationships between lichens GS and their ascospore length, apothecia size, substrate preference, and abiotic conditions were tested with the program PAST using a linear RMA model or Mann–Whitney test.

The GS was assessed for herbarium specimens with a broad range of age (1990–2015), but 25 of 35 measured specimens were collected after 2009. Old specimens had apparently lower GS and higher coefficient of variation (CV value) than younger ones. Therefore, we decided to test the effect of specimen age on estimated GS. We chose *B. herbidella* (epiphytic) and *B. crenularia* (epilithic) as model species. In *B. herbidella*, we found stable GS, about 30.9 Mb, in specimens collected between 2013 and 2016, but specimens from 2004–2009 had smaller GS, between 23.4 Mb and 26.1 Mb. Ten specimens collected in 1951–1997 produced no histograms. The data obtained from *B. crenularia* revealed less age-induced genomic change. Genome sizes for this species remain stable over the period 2007–2016. Only a single specimen from 1990 had markedly lower GS,

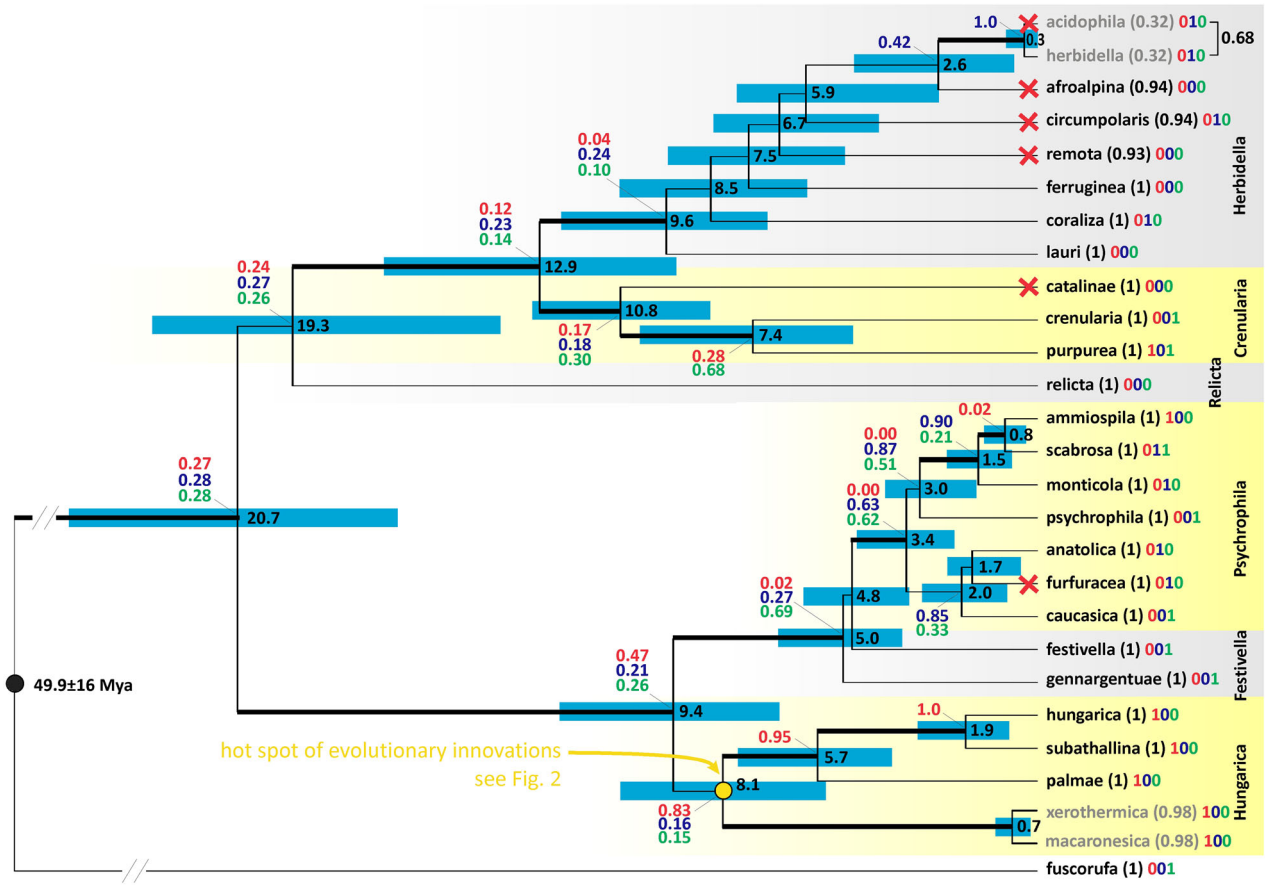

Fig. 3. Species tree reconstructed by *BEAST with estimated divergence time for the nodes. Terminal names in black are recognized as species; grey names are considered subspecies. Infrageneric groups are indicated on the right. Branches supported by posterior probabilities (PP ≥ 0.95) are thick. Nodes are dated in millions of years, with the 95% credibility intervals (blue boxes). Average posterior probabilities from three BP&P runs are indicated for each taxon and some groupings (numbers in brackets after names of taxa). Red numbers are probabilities of the reduced anthraquinone chemotypes (see Fig. 6). Blue numbers are probabilities of vegetative diaspore presence. Green numbers are probabilities of occurrence on inorganic subsrata. Taxa distributed only outside the Mediterranean-Macaronesian diversity hotspot are indicated by red crosses.

22.6 Mb (compared to 29–35 Mb in other samples). Based on these tests, we only included data from specimens more recent than 2009 (Table S2) in tracing characteristics linked with the GS.

3 Results

3.1 *Blastenia* includes 6 infrageneric groups, 24 species, and 2 subspecies

A single-locus ITS analysis of Caloplacoideae revealed a group of taxa around *Caloplaca fuscorufa* that is close to *Blastenia*. In all our unrooted trees (single-loci and concatenation), the *C. fuscorufa* clade has a distinctly longer branch than any *Blastenia* clade. We regard it as being outside *Blastenia* and we employ it as an outgroup for rooting our analyses (Figs. 1 and 2). The coalescent-based species tree (Fig. 3) also implied that *C. fuscorufa* is outside *Blastenia*.

Single-gene topologies are generally congruent, with only a few exceptions indicated by red links in Fig. 1. Although the backbones of single-locus trees are unresolved or only poorly resolved, the concatenated tree (Fig. 2) and the *BEAST species tree (Fig. 3) have a well-resolved backbone structure and allow division of *Blastenia* into several infrageneric groups. As few as four or as many as seven such groups could reasonably be recognized, but we recognize six (Fig. 2), as this seems most consistent with the data on geographical ranges (Fig. 4), ecological preferences (Table 4), and morphology (see the Taxonomy part). For the convenience of discussion, we merged *B. festivella* and *B. gennargentuae* into a single group even though they do not form a monophyletic group in any analysis. The two species are close, and both have a sister relationship to the Psychrophila group (Fig. 3). They also share most phenotypic characters but are restricted in their altitudinal range.

The groups were further divided into 26 taxa that we tested for species delimitation by BP&P (Fig. 3). The delimitation of 19 taxa was clearly supported (PP = 1.00). Support for *B. afroalpina*, *B. circumpolaris*, and *B. remota* was slightly lower (PP = 0.90–0.99), probably because

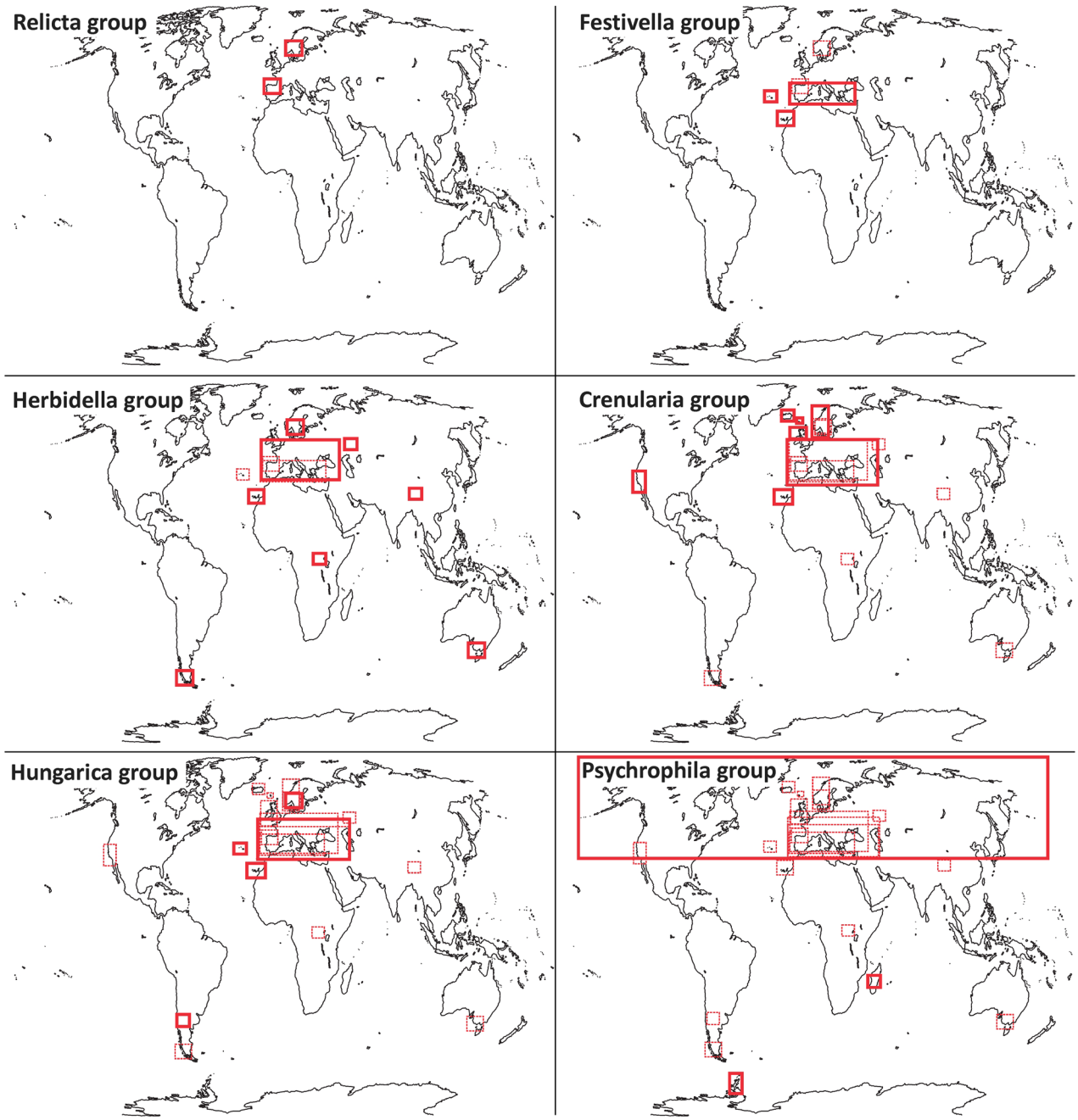

Fig. 4. World distribution of the six infrageneric *Blastenia* groups. Distribution maps are ordered according to increasing size of geographical ranges. Thick lines delimit approximate ranges of the groups; thin dotted lines indicate nestedness of all groups in the Mediterranean and adjacent regions.

phylogenetic information is scarce, as only one specimen with a full three-locus dataset is involved for each taxon here. The putative taxa "*xerothermica*" and "*macaronesica*" received PP = 0.98 (Fig. 3), but their grouping, when both taxa were merged, received PP = 1; we regard them as subspecies within *B. xerothermica*, because they are sufficiently resolved only in the beta-tubulin single-gene phylogeny (more details in the taxonomic section). The putative taxa "*herbidella*" and "*acidophila*" were poorly supported (PP = 0.32 for both), but their grouping received higher support (PP = 0.68) and received PP = 1 when the two taxa were merged into a single putative species (data not shown). We regard them as subspecies within *B. herbidella*, as they are geographically and ecologically distinct, but are not resolved in ITS and mtLSU phylogenies (they are polyphyletic in beta-tubulin; see red dots in Fig. 1). Altogether, we recognized 24 taxa at the rank of species and 2 at the rank of subspecies.

Table 4 Substrate preferences of species and infrageneric groups within *Blastenia*. Predominant substrates are in bold

		Organic substrates								Inorganic substrates		
Infrageneric groups	species (No. of specimens)	plant debris, mosses	alpine shrubs	wood	coniferous trees (not twigs)	deciduous trees (not twigs)	coniferous trees (twigs)	deciduous trees (twigs)	Mediterranean & coastal shrubs	siliceous rocks in arctic / (sub) alpine zone	siliceous inland non-alpine rock	siliceous coastal rock
Crenularia group	*B. catalinae* (3)							2	1			
	B. crenularia (118)			3							**71**	**44**
	B. purpurea (5)										**5**	
	total per group (126)			3				2	1		**76**	**44**
Herbidella group	*B. afroalpina* (1)								1			
	B. circumpolaris (8)				**7**						1	
	B. coralliza (72)				**27**	**38**	5		2			
	B. ferruginea (52)				4	**44**		2	2			
	B. herbidella (140)			1	**28**	**106**	5					
	B. herbidella subsp. *acidophila* (4)					1	3					
	B. lauri (25)					**14**	3	8				
	B. remota (7)							**7**				
	total per group (309)			1	**66**	**203**	16	17	5		1	
Relicta group	*B. relicta* (18)					**18**						
Festivella group	*B. festivella* (53)					1			1		**21**	**30**
	B.gennargentuae (3)									2	1	
	total per group (56)					1			1	2	**22**	**30**
Hungarica group	*B. hungarica* (88)			7	4	**24**	**18**	**35**				
	B. palmae (52)			1		**9**	**15**	2	**25**			
	B. subathallina (37)				4	6	**12**	**7**	**8**			
	B. xerothermica (90)			1	6	**12**	**14**	**17**	**41**			
	total per group (264)			9	14	**51**	**59**	**61**	**74**			
Psychrophila group	*B. ammiospila* (102)	**57**	**25**	5		5	6	5		1		
	B. anatolica (5)			3	2							

Continued

Table 4 Continued

Infrageneric groups	species (No. of specimens)	Organic substrates								Inorganic substrates		
		plant debris, mosses	alpine shrubs	wood	coniferous trees (not twigs)	deciduous trees (not twigs)	coniferous trees (twigs)	deciduous trees (twigs)	Mediterranean & coastal shrubs	siliceous rocks in arctic / (sub) alpine zone	siliceous inland non-alpine rock	siliceous coastal rock
	B. caucasica (8)									**8**		
	B. furfuracea (11)			**6**		**4**	1					
	B. monticola (52)		8	**18**	**20**	4	2					
	B. psychrophila (23)									**23**		
	B. scabrosa (12)									**12**		
	total per group (214)	**57**	**33**	**32**	22	13	9	5		**44**		

3.2 *Blastenia* history since its early tertiary origin

We dated the origin of *Blastenia* to the period 66–34 Mya, i.e., somewhere within the first half of the Tertiary period when *Blastenia* separated from the *Caloplaca fuscorufa* group (Fig. 3). All infrageneric groups are much younger; the clade including the Festivella, Hungarica and Psychrophila groups separated from the clade of the Crenularia, Relicta, and Herbidella groups within the period 26–16 Mya, i.e., late Oligocene to early Miocene. The Relicta group, which includes only a single contemporary species, is possibly the oldest extant group; it had separated by the early Miocene. Separation of the Crenularia and Herbidella groups is dated to 16–9 Mya, i.e., Miocene, separation of the Hungarica group from the Psychrophila and Festivella groups is younger, dated to 12–6 Mya. Ages of the infrageneric groups are 13–9 Mya for the Crenularia group, 12–7 Mya for the Herbidella group, 11–5 Mya for the Hungarica group, 7–3.5 Mya for the Festivella group, and 4.5–2 Mya for the Psychrophila group. The range of ages for particular species is about 10.8–0.8 Mya (Fig. 3), but that estimate could be distorted by imperfect species sampling and the absence of extinct lineages. Separations of subspecies within *B. herbidella* and *B. xerothermica* are probably more recent than 1 Mya (Fig. 3). Recent speciation is mainly in the Psychrophila group; some recognized species probably separated after 2 Mya, i.e., in the Pleistocene.

3.3 *Blastenia* species are restricted to either organic or inorganic substrates

Each species of *Blastenia* is restricted or almost restricted, to either an organic or an inorganic substrate (Table 4). Fifteen species are restricted to organic substrates, usually, bark, and two other species (*B. ammiospila* and *B. circumpolaris*) occur only rarely on inorganic substrates. We use the broad term epiphytic for these species in this paper, and do not generally distinguish between species that are epiphloedal, epixylic or occur on plant debris and bryophytes. Seven species are epilithic (reports of one of them on bark may represent an incipient young species not resolved in the molecular analysis: see discussion of *B. festivella* below). Epilithic species are restricted to siliceous, mostly base-rich rocks; they avoid calcareous substrates like limestone. Substrates were mapped on the species tree to reconstruct the ancestral states. The results imply that most groups (but not Festivella and Psychrophila) originated from epiphytic ancestors (Fig. 3). However, we discuss below the alternative hypothesis that epiphytic lineages are generally derived from epilithic ancestors (see Discussion).

The six larger infraspecific groups also display strong substrate preferences. The Crenularia and Festivella groups prefer inorganic substrates. The Herbidella and Relicta groups are restricted to organic substrates with a preference for tree trunks. The Hungarica group is also restricted to organic substrates, but it prefers twigs of trees and shrubs. All these groups avoid subalpine and alpine habitats. In contrast, the Psychrophila group is variable in substrates but is restricted to cold environments in boreal-montane to arctic-alpine habitats.

3.4 Tethys basin and Macaronesia are plausible evolutionary centers for *Blastenia*

Blastenia is predominantly a genus of the Northern Hemisphere, and the distribution patterns of all six infrageneric groups (Fig. 4) suggest that it always has been. The place of origin of the genus and all its infraspecific groups and a center of their further diversification appears to be the Tethys basin, recently with 6 groups and 17 species in its western remnant, i.e., the Mediterranean basin. Another evolutionary center could be Macaronesia, mainly the Canary Islands and Madeira, with four recent groups and eight species. Two taxa are presumably endemic to Macaronesia (*B. purpurea* and *B. xerothermica* subsp. *macaronesica*) and *B. palmae*, common in Azores, Canary Islands and Madeira, has only a small range outside Macaronesia in coastal areas in the south-western Iberian Peninsula.

High diversity is recorded also in non-Mediterranean Western Eurasia (5 groups and 14 species), but most species have principally a Mediterranean distribution with some occurrences in more northern territories (e.g., *B. coralliza* and *B. ferruginea*). *Blastenia lauri* has principally a Macaronesian distribution, but also has numerous occurrences in the north-western British Isles. The eastern part of Eurasia has four species from only two groups and North America has only three species of two groups (according to present knowledge).

All groups generally avoid the tropics where the exceptional records are restricted to high altitudes (Fig. 5). No confirmed records from the Northern Hemisphere are known south of 28° latitude, except for the specimen from Mt. Elgon, Uganda from 4100 m altitude. In the Southern Hemisphere, records are scarce: the latitudinal range of confirmed records is 67.5°–36.5° plus one record from Madagascar at high altitude (1800 m). All records outside the Mediterranean-Macaronesian diversity hot-spot, and especially those in more distant regions, are fairly scattered, and long-distance dispersal is the most probable origin of distant populations; most of these populations became distinct species (see the Taxonomy part and Fig. 3).

3.5 Species with large geographic ranges and species occurring far from the Mediterranean-Macaronesian center are epiphytic

There are very clear differences in distributions between epilithic and epiphytic species. All epilithic species are centered on Macaronesia and the Mediterranean Basin, though the ranges of a few extend further north, up to the European Arctic, or to the east in an arc from the Baltic Sea coast, through the Carpathians, Crimea, and the Caucasus, to western Iran. All species recorded outside this region (Fig. 4) are epiphytic. The geographical ranges of epiphytic species (e.g., *B. ammiospila*, *B. furfuracea*, and *B. monticola*) are considerably larger than those of even broadly distributed epilithic species (*B. crenularia* and *B. scabrosa*). Epilithic species occurring in alpine or high montane zones of the Mediterranean mountains (*B. caucasica*, *B. gennargentuae*, and *B. psychrophila*) have especially small ranges and do not reach ecologically suitable habitats in the Arctic or in mountains east of the Caucasus.

3.6 Within-species genetic variation is low in widely distributed species but high in epilithic species with Mediterranean-Macaronesian distribution

The number of polymorphic nucleotide positions within species is 0–11 in mtLSU, 0–63 in beta-tubulin, 4–85 in ITS, and 5–159 in the whole dataset (Table 3). Species with large a geographical range (especially the epiphytic *B. ammiospila*, *B. furfuracea*, *B. monticola* and epilithic *B. scabrosa*) have rather low genetic variation. On the contrary, *B. gennargentuae*

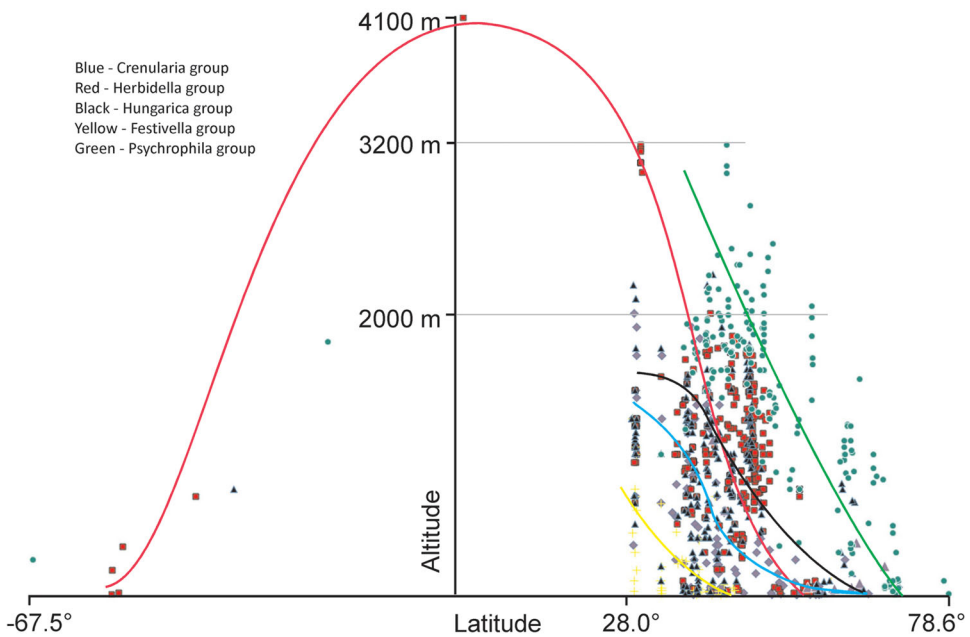

Fig. 5. World latitude / altitude range of the infrageneric *Blastenia* groups (legend in the picture). Trend curves interposed by hand.

sampled in a very small part of the Mediterranean region has surprisingly high genetic diversity; ITS sequences obtained from three co-occurring thalli varied among each other in 12 nucleotide positions. Another epilithic species with Mediterranean-Macaronesian distribution, *B. festivella*, has distinctly higher overall genetic variation (159 polymorphic sites) than the other species (5–86). High variation in beta-tubulin within *B. herbidella* and *B. xerothermica* is caused by the diversity between the two infraspecific taxa in both species (Table 3). High variation within the few available sequences of *B. catalinae* and *B. circumpolaris* is probably caused by the existence of two putative species within each (see the ITS tree in Fig. 1 and details for *B. catalinae* in the Taxonomy part).

3.7 Chemical differentiation in apothecia: Ancestral chlorination of anthraquinones and secondary reductions

Three chemotypes occur in *Blastenia*. The first and most common chemotype contains nonchlorinated parietin and a small proportion of its oxidation products teloschistin, fallacinal and parietinic acid, and emodin in the hymenium (chemosyndrome A of Søchting, 1997), whereas the excipulum is dominated by chlorination products of emodin and its oxidation products: 7-chloroemodin, 7-chlorocitreorosein, 7-chloroemodinal, and 7-chloroemodic acid (chemosyndrome C of Søchting, 2001). Small amounts of fragilin are occasionally detected. This chemotype is also found in the phylogenetic outgroup *Caloplaca fuscorufa*. Accordingly, this chemotype can be regarded as basal in *Blastenia* (Fig. 3).

In the second chemotype, the nonchlorinated parietin persists in all apothecial parts, but the ability to chlorinate other anthraquinones is secondarily reduced (Fig. 6). This chemotype evolved in the Hungarica group and is always present in *B. hungarica*, *B. palmae*, and *B. xerothermica*.

The third chemotype is characterized by chlorinated anthraquinones in all apothecial parts and usually by reduced production of parietin (Fig. 6). This evolved independently in the Crenularia, Hungarica and Psychrophila groups. It is always present in *B. ammiospila*, *B. purpurea* and *B. subathallina* and it is occasional in *B. catalinae* and *B. caucasica*. Parietin is not always reduced; it is absent to present in abundance in *B. ammiospila*.

These three chemotypes can be detected using UPLC chromatograms in UV light and TLC (Fig. 6). However, mass spectrometry (a more sensitive method) also detected most of the anthraquinones reported by Søchting (2001) in all analyzed samples (see Table S3). In specimens with the nonchlorinated anthraquinone chemotype, chlorinated anthraquinones were detected (though usually only in small amounts) and vice-versa. This means that lichens with reduced accumulation of chlorinated or nonchlorinated anthraquinones did not entirely lose these substances.

3.8 Reduction in apothecial size is connected with substrate shift to twigs

Most *Blastenia* species have rather large apothecia (compared to other microlichens), ca. 0.7–1.2 mm in diameter. Some species, however, have distinctly smaller apothecia, consistently small in all sampled specimens. The Hungarica group only includes lichens with small apothecia, ca. 0.3–0.7 mm in diameter. Reduction of apothecial size in the group reflects the preference for growing on twigs of trees and shrubs where 180 of 248 specimens were recorded (more in Table 4). Small apothecial sizes also occur in the other 68 specimens growing on the bark of trunks and on wood. This implies that smaller apothecia evolved as an adaptation to limited space on twigs, but the character is fixed even in specimens on tree trunks. Epiphytic *B. afroalpina*, *B. catalinae*, and *B. herbidella* subsp. *acidophila* also have small apothecia and are mostly known from twigs. The other 11 epiphytic species have distinctly larger apothecia and occur mainly on the bark of tree trunks (Table 4).

3.9 Vegetative diaspores are a derived character in *Blastenia* linked to the reduction of ascospore size

All 24 *Blastenia* species produce apothecia, and 7 of them also produce vegetative diaspores. Ancestral state mapping supports the hypothesis that vegetative diaspores formed as a secondary character in *Blastenia* (Fig. 3). According to the available data, we suggest at least five independent origins of vegetative reproduction during the diversification of *Blastenia*. Vegetative diaspores, mostly isidia or blastidia (soralia are present only in *B. circumpolaris*), are present only in the Herbidella and Psychrophila groups, but their occurrence in these groups is substantial. Within the Herbidella group, three of six species have vegetative diaspores. These species produce vegetative diaspores in most observed specimens, but a few thalli were completely or mainly without them (*B. coralliza* Malíček 5561; Andalusia). In the Psychrophila group, four of eight species have vegetative diaspores (observed in all specimens studied).

There is a clear tendency for ascospore size to be smaller in species with vegetative diaspores. The mean ascospore length in species without vegetative diaspores is 14.0 μm, but only 12.9 μm in species with them ($n = 590/n = 263$). The difference is even more significant within the groups, such as 15.1/12.8 μm ($n = 110/63$) in the Herbidella group and 15.5/12.9 μm ($n = 106/200$) in the Psychrophila group. The volume of acospores in species with vegetative diaspores is reduced by about 40% in both groups.

3.10 Genome sizes are higher in epilithic species

Measurements of GS were attempted in all species (Fig. S1; Table S2), but measurements failed in *B. remota* and measurements in *B. afroalpina* are not reliable (see the Methods for age-induced genomic changes). The measured GSs ranged between ca. 22–35 Mb. Genome size variations within the infrageneric groups were slightly smaller (Fig. 7). We evaluated infraspecies variability in GS in two species, *B. crenularia* (in 10 specimens) and *B. festivella* (7) and we found variability in both species that slightly exceeded measurement error: 28.6–35.7 Mb in *B. crenularia* and 30.1–34.5 Mb in *B. festivella*.

Our data revealed the reduction in the genome linked with occurrence on organic substrates ($P < 0.001$; tested by Mann–Whitney). This trend is even stronger within particular groups. For instance, in the Crenularia group, epiphytic *B. catalinae* has smaller GS (28.9 Mb) than its epilithic relatives (32.7–35.7 Mb), or in the Psychrophila group, epiphytic *B. ammiospila* (27 Mb) has smaller genome than its sister

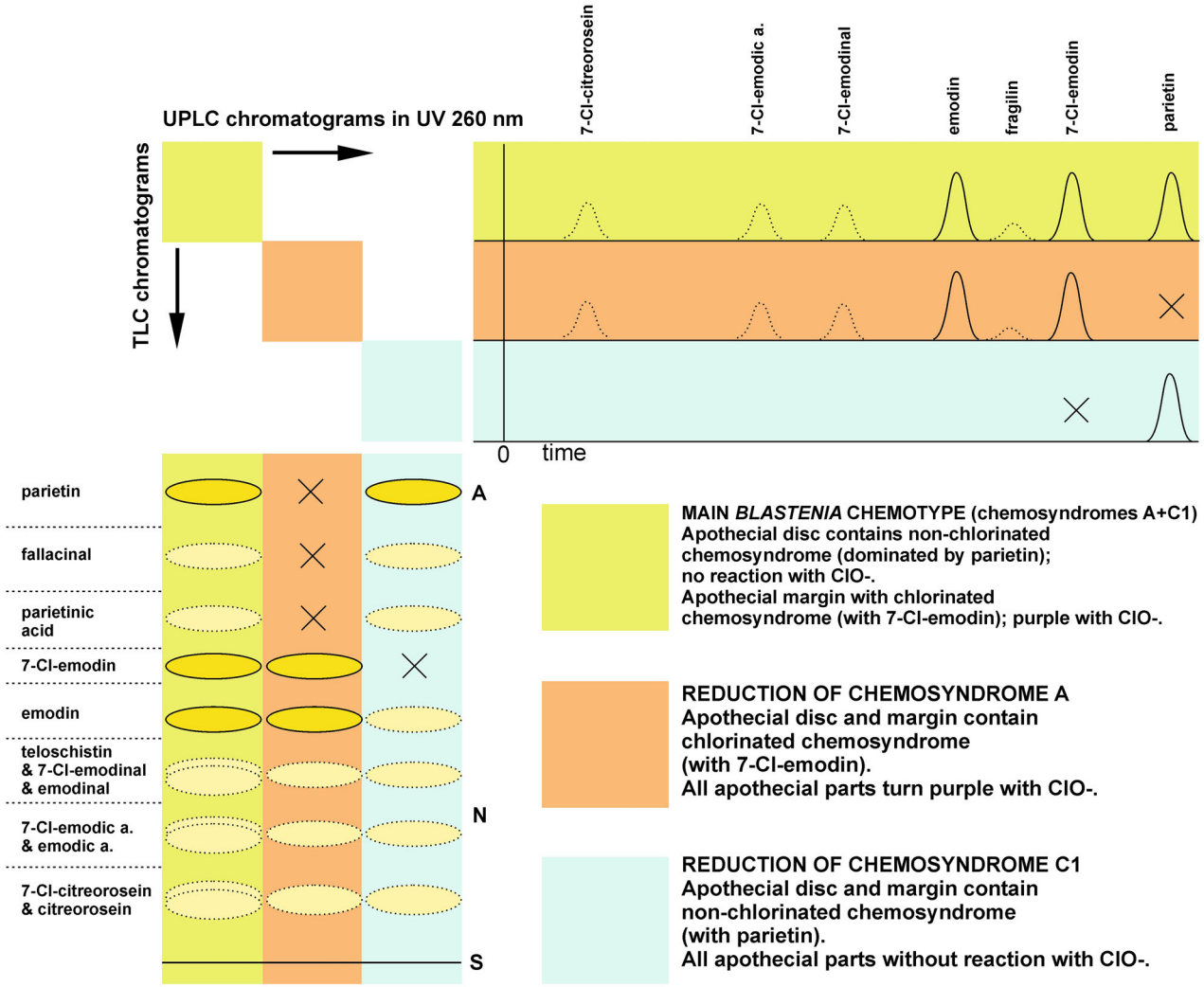

Fig. 6. Chemistry of apothecia in *Blastenia*. Differences among three known chemotypes are demonstrated. On the left: schematic TLC chromatograms (solvent system C; abbreviations: A, atranorin; N, norstictic acid; S, starting line). On top right: UPLC chromatograms in UV 260 nm. Major substances indicated by full line, minor substances by dotted line. Black crosses indicate diagnostic absences of the substances. Examples of the three *Blastenia* chemotypes represented by distinct colours. ClO- is a hypochlorine ion present in chlorine detergents routinely used for identification of some lichen substances.

epilithic species, *B. scabrosa* (33 Mb). Nevertheless, we found some deviation from this rule; the epiphytic *B. xerothermica* has the large GS typical of epilithic species and the epilithic *B. psychrophila* has an unexpectedly small genome.

We did not find any significant correlations between GS and the following morphological traits: ascospore length, apothecium diameter and vegetative reproduction. Specimens from xerothermic conditions tend to have a larger genome than those from cold and humid conditions, but the trend is not significant.

4 Discussion

4.1 Effect of limited sampling

The collections available to us are heavily biased towards Europe and North-western Asia, and those from other regions have a rather random character. We strongly suspect that there remain undiscovered species, especially in those other regions. In addition to several species recognized in the Southern hemisphere, we examined a specimen close to *Blastenia monticola* from Madagascar (herb. Halda 0968) and a specimen similar to *B. hungarica* from Chile (herb. Etayo 24477b). Both specimens probably represent well delimited *Blastenia* species, but we did not obtain a full three-loci DNA dataset for them and we prefer not to describe them as new here. We also expect one species in the Caribbean; see the comment on Wetmore (1996) in the taxonomy section below *B. crenularia*. There may be additional species even in well-surveyed areas of Europe and North-western Asia. For example, we recently sequenced a specimen collected by Roman Türk in Austria that is similar to *B. ferruginea*, but its ITS barcode sequence placed the specimen in an uncertain position within *Blastenia*.

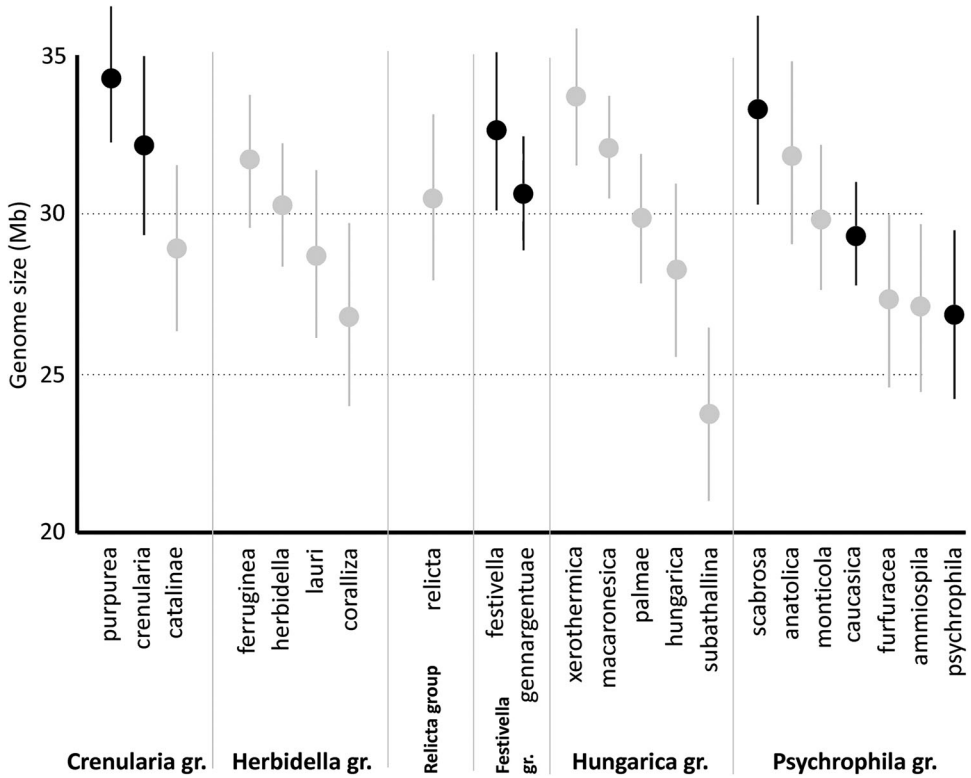

Fig. 7. Genome sizes in *Blastenia*; mean+/−CV. Black symbols represent epilithic lichens; grey symbols are epiphytic. Taxa are divided into the six infrageneric groups. Within each group, taxa are arranged from highest to lowest genome size. Fluorescence intensity histograms are available in Fig. S1.

It does appear that some regions outside Europe and North-western Asia are genuinely poor in *Blastenia*. We expect few (if any) overlooked species in the North American coastal areas surveyed by Arup or in the southern part of South America and Subantarctic regions surveyed by Søchting. Recently we made field trips to sample Teloschistaceae, including *Blastenia* to southern Siberia, eastern China, USA and Chile. Those regions proved to be poor in *Blastenia*. Specimens collected from southern Africa, Australia and New Zealand that resemble *Blastenia* morphologically turned out to belong to the genus *Eilifdahlia* according to ITS sequence data. Our failure to find large numbers of *Blastenia* in areas outside western Eurasia implies that our conclusion that all infrageneric groups are geographically centered in the Mediterranean basin and Macaronesia is robust. It cannot be dismissed as an artifact of inadequate sampling.

4.2 Ecological and geographical constraints

In comparison to some large genera of Teloschistaceae that are mostly restricted to inorganic substrates (e.g., *Flavoplaca*, *Pyrenodesmia*, *Rufoplaca*, *Xanthocarpia*), *Blastenia* occupies a large spectrum of niches. Its species occur in various epiphytic and epilithic communities in cold to warm regions of the temperate zone. However, there are some gaps, and no *Blastenia* species occur on calcareous substrates, even though those substrates are usually rich in Teloschistaceae. *Blastenia* is also absent from dry continental regions which support other genera of Teloschistaceae (e.g., *Calogaya*, *Xanthocarpia*). These two constraints suggest that *Blastenia* originated in an area without limestone rocks and with a rather oceanic climate. The Canarian and Madeira archipelagos are a contemporary instance of the kind of region in which we consider *Blastenia* to have originated, though the actual region of origin was probably further east in the Tethys basin, as those archipelagos are considered to be younger than *Blastenia*.

We are astonished by the limited distribution ranges of epilithic species: they are almost confined to Mediterranean areas and Macaronesia. Only a few species reached more northern inland sites. *Blastenia crenularia* also reached coastal areas in Scandinavia and Iceland and the blastidiate *B. scabrosa* also reached some arctic areas. The limited dispersal abilities in epilithic populations may be caused in part by the low availability of suitable substrates (mostly base-rich siliceous rocks), but in addition to that most species are rare and have specialised requirements, e.g., spots with basic siliceous rocks in the humid alpine zone (Psychrophila group), or coastal rocks in a mild oceanic climate (*B. festivella*). For such species, the large continental areas of Eurasia would have presented a major obstacle to dispersion.

On the other hand, some epiphytic species form large populations in forested areas (e.g., *B. coralliza* and *B. herbidella*) and represent a significant source of diaspores for short and long-distance dispersal. As a result, at least three epiphytic species occur in and presumably originated in very distant regions, even in the Southern Hemisphere. An abundance of suitable habitats also allowed *B. furfuracea* and *B. monticola* to spread widely in boreal-montane forests

of the Northern Hemisphere. *Blastenia ammiospila*, which occurs on various organic substrates, does even better: it is circumpolar in the Northern Hemisphere and also occurs in the Antarctic.

Our conclusions about epilithic species in *Blastenia* having more restricted geographical ranges cannot be generalized to all lichens. It is valid for some genera (e.g., Tehler et al., 2013), but numerous epilithic lichens have large geographic ranges and some are considered cosmopolitan (e.g., Quilhot et al., 2007). It is probably not even true for all of Teloschistaceae; for example, the epilithic *Flavoplaca flavocitrina* may be cosmopolitan (Vondrák et al., 2016), and *Xanthomendoza borealis* is bipolar (Lindblom & Søchting, 2008).

4.3 Repeated switches from inorganic to organic substrates

If Gaya et al. (2015) are correct in suggesting an ancient shift from organic substrates to inorganic in the early evolution of Teloschistaceae, then the shift back to organic substrates had to occur repeatedly, because both epilithic and epiphytic species are present in most larger genera of Teloschistaceae including *Blastenia*. Ancestral substrate state mapping supported the scenario that *Blastenia* had an epiphytic ancestor (only 28% probability for the epilithic state, Fig. 3) and few subsequent switches to inorganic substrates. There are however good reasons for supposing an epilithic ancestor and repeated switches to organic substrates, as follows: (i) Most genera of Teloschistaceae close to *Blastenia* are exclusively or predominantly, epilithic. The only exception is the small genus *Bryoplaca* (Arup et al., 2013). (ii) Six epiphytic lineages, but no epilithic lineages, originated in regions distant from the Mediterranean-Macaronesian diversification center (red crosses in Fig. 3). (iii) All species of the epiphytic Hungarica group of *Blastenia* share a specific chemotype not found elsewhere in *Blastenia* (Figs. 3, 6), reduced apothecia and a strong preference for occurrence on twigs. This Hungarica phenotype is not found elsewhere in *Blastenia* and appears to be an apomorphic evolutionary innovation associated with a major evolutionary event, probably a substrate switch from an epilithic ancestor (see the results). (iv) We observed a local epiphytic population within the large epilithic population of *B. festivella*. Although not recognized within the molecular analysis, this population may be an incipient species following a substrate switch (see *B. festivella* below). We thus have contemporary evidence that epilithic to epiphytic switches can occur in *Blastenia*, but none for switches in the opposite direction. (v) Epilithic species of *Blastenia* tend to have greater genetic variation than the epiphytic ones, suggesting that they are older. The two species with the largest within-species genetic variation in *Blastenia*, *B. festivella* and *B. gennargentuae* are both epilithic (Table 3).

The topic of substrate switches in lichens has been little studied, but Otálora et al. (2013), on the basis of ancestral state mapping, suggested that the ancestral state of Collemataceae was epilithic, although today the family has numerous epiphytic species. Lücking et al. (2013) concluded that the ancestor of Redonographoideae (a subfamily of Graphidaceae) was epilithic, though today there are species on both organic and inorganic substrata. For Graphidaceae itself, they suggested an epiphytic ancestor. This parallels our own situation: *Blastenia* (epilithic ancestor) within Teloschistaceae (epiphytic ancestor).

4.4 Reduced genome in epiphytic species

The smaller GS in epiphytic *Blastenia* is probably caused by secondary reduction. Mohanta & Bae (2015) report the average GS in Ascomycota to be 36.91 Mb which is greater than most measurements in *Blastenia*. Within Teloschistales, only *Xanthoria parietina* with 31.9 and 40 Mb is included in the Fungal genome size database (Kullman et al., 2005). Most measurements of epilithic *Blastenia* are also within this range, but numerous epiphytic *Blastenia* species have GS below 30 Mb (Fig. 7). Although evolution most commonly increases GS, examples of reduction are also known and its mechanisms have been described (Yuen et al., 2003; Gregory, 2005). Correlations between GS and a variety of physiological, morphological, and ecological traits are well established in a broad range of organisms. In fungi, genomic changes connected with ecological transitions have been revealed by genome sequencing (Ma et al., 2010; Spanu et al., 2010) and thus a change in GS could be an adaptation to ecological switch.

In *Blastenia*, the usual pattern is a small genome in epiphytic species and a larger one in epilithic species, but there are exceptions: the epiphytic *B. xerothermica* has a large genome and the epilithic *B. psychrophil*a a small genome. Several abiotic factors are known to influence GS in plants (Wakamiya et al., 1993; Knight & Ackerly, 2002) which could indicate that other selection pressures played a role in GS evolution of *Blastenia*. For example, we found that species living in xerothermic condition tend to have larger GS (mean 33.5 Mb) than species living in a cold environment (mean 29.1 Mb) or a humid one (mean 30 Mb).

4.5 Vegetative diaspores are secondary in *Blastenia*

Purely asexual lineages are rare in Teloschistaceae (Vondrák et al., 2016) and are absent from *Blastenia*. However 29% of *Blastenia* species (7 out of 24; 6 epiphytic, 1 epilithic) form vegetative diaspores. Based on ancestral character state mapping in numerous lichen phylogenies, Tripp (2016) concluded that lineages forming vegetative diaspores sometimes represent a source for evolutionary innovation. According to our data, this is not the case in *Blastenia*. Mapping of ancestral character states in *Blastenia* indicated only a low probability of vegetative diaspores (<30%) in most nodes (Fig. 3). Furthermore, these lineages were found only in two of the six infrageneric groups. Secondary losses of vegetative diaspores are only possible in the Psychrophila group (Fig. 3). However, the species with vegetative diaspores (apart from *B. monticola*) appear to be younger than the other species in the Psychrophila group, because their variability in genotype is distinctly lower (Table 3).

5 Taxonomy

5.1 Notes

1. We propose a hierarchic taxonomy employing three levels. (i) Infrageneric groups are taxa recognized in the backbone structure of the phylogenetic trees that have their own phenotype characteristics. We prefer not to

give them formal taxonomic rank because *Blastenia* is a small genus and morphologically rather uniform. (ii) Species are recognized as clades resolved in the concatenated tree that are supported by the species delimitation test (BP&P), and that form phenotypically circumscribed groups. (iii) Subspecies are used for taxa that are resolved in only one or two single-loci phylogenies and that are semicryptic (sensu Vondrák et al., 2009), meaning not morphologically recognizable, but with distinct ecology or distribution.

2. The generic description below is intentionally long and we describe there all the characters that are either invariable within the genus or variable but the variability pattern is not diagnostic for any species. Descriptions of species are deliberately short because most species in *Blastenia* differ little in morphology. Geographical ranges, ecology or chemistry are usually more important for species identifications.
3. Presence/absence of chlorinated anthraquinones in particular apothecial tissues is a valuable character in *Blastenia* taxonomy. The spot reaction with hypochlorite ion ("C") is a helpful character reflecting the presence (C+ purple) or absence (C–) of chlorinated anthraquinones (Vondrák et al., 2013a). When using C-reaction, care must be taken to use the correct concentration. Chlorinated detergents bought in drugstores are often strongly concentrated and cause a C+ red spot reaction even on samples without chlorinated anthraquinones. Therefore, we strongly recommend testing the negative reaction on apothecia of the common *Xanthoria* or *Rusavskia* species, which never have chlorinated anthraquinones. The concentration of the C-solution must be reduced until it does not cause a red reaction on the apothecial discs of *Xanthoria* or *Rusavskia*.
4. Only type specimens are provided with the details in the text. Other investigated specimens are listed in Table S1.

5.2 Genus description

Morphology: Thallus crustose, areolate, variable in size and shape, round or irregular, sometimes several centimeters in diameter, but sometimes reduced to small areas around apothecia or almost disappearing; varying within each species. The thallus is usually without anthraquinones and color ranges from white to dark grey (grey tinge caused by the pigment Cinereorufa-green; details in Meyer & Printzen, 2000). Some species (mostly those with vegetative diaspores) with partly or completely yellow thallus contain anthraquinones; the presence and amount of anthraquinones in thallus is variable and varies among specimens within each species. Prothallus may be present in all species (most pronounced in the contact zone with surrounding lichen thalli), black, formed by hyphae melanized by Cinereorufa-green; its extent is very variable within most species. In some species, the prothallus is also visible among dispersed areoles, and forms a black hypothallus in *B. gennargentuae*. Thallus areoles are usually flat, but older areoles may be convex or with an uneven upper surface, giving thalli a scabrose appearance. Thallus thickness is variable, but thalli are generally thin, up to 150 μm, only crusts with dense vegetative diaspores appear to be thicker owing to heights of the diaspores. Epiphytic species forming endophloedal or thin epiphloedal thallus are usually less than 100 μm thick; epilithic species may have slightly thicker thallus, exceeding 100 μm in older areoles. Some species have vegetative diaspores (soredia, blastidia, isidia); their presence, shape and size are often species specific. Cortex is not developed (except for cortex of thalline exciple, see below); alveolate cortex sometimes developed, but usually inconspicuous. Epinecral layer often present, but very thin (usually <10 μm), without clear borderline with alveolate cortex. Algal layer continuous (mostly <100 μm thick) or discontinuous, forming irregular cushions (ca. 50–100 μm diam.) surrounded by a loose fungal tissue defined as either algonecral medulla (in the lower part of thallus) or alveolated cortex (in the upper part). Medulla absent or thin, observable in thick epilithic thalli.

Apothecia usually large (often >1 mm diam.), but in some species, apothecia are consistently small, not exceeding 0.5 mm. Young apothecia are slightly concave to flat, later remaining flat or becoming slightly convex. Mature apothecia are sessile, sometimes with constricted base. Colour of apothecia varies from pale orange to dark red; paler apothecia are in species without chlorinated anthraquinones. Apothecial margin (true exciple) has the same colour or is paler than the disc. Old or injured apothecia sometimes turn black (anthraquinones are replaced by Cinereorufa-green). The apothecial disc is usually roughened by anthraquinone crystals (epipsamma). Apothecia biatorine or zeorine, both types are present in most species, sometimes in a single specimen. Apothecial margin consists of a true exciple and (in zeorine apothecia) also a thalline exciple. True exciple is usually thin (up to 100 μm), the same color as the disc, or darker. It is prosoplectenchymatous, often clearly divided into an upper part (fan-shaped true exciple) and a lower part (initial cortex; see below). Fan-shaped true exciple is formed of thin-walled radiating hyphae becoming shortened and broadened towards the surface, superficial cells up to 5 μm wide. Cortex part formed of palisade prosenchyma of ±equally wide (ca. 3–5 μm) inner and outer cells, but hyphae in this tissue have sometimes very thin lumina and glutinized walls (both glutinized and non-glutinized tissues are found within some species). Clusters of algal cells are sometimes located between the fan-shaped exciple and the initial cortex.These clusters are small and occasional in young apothecia, but they sometimes expand and turn into thalline exciple in old apothecia (This is demonstrated for *B. herbidella* by Poelt & Wunder 1967: fig. 2). In some old apothecia, the lower part of the true exciple (initial cortex) changes into the cortex (usually up to ca. 30 μm thick) at the lower part of the thalline exciple. Hypothecium (together with subhymenium) is prosoplectenchymatous, up to ca. 150 μm thick in the axial part of apothecia. Subhymenium containing ascogenous and paraphysogenous hyphae is sometimes distinct from the hypothecium by irregularly thickened cells and by the amyloid, I+ blue reaction (lower hypothecium and true exciple are nonamyloid, I–). Hypothecium and inner true exciple are often partly yellowish or brownish, K-, N+ orange. Hypothecium is usually at least slightly inspersed and ca. 60–120 μm high. Hymenium 70–100 μm tall, not inspersed in most species, but sometimes inspersed in *B. crenularia*. Paraphyses are 1.5–2 μm wide in the lower part, widened to 2.5–5.5 μm in tips; sometimes branched and anastomosed; glutinized, partly glutinized or

not glutinized (variable within species). Asci usually 50–70 × 12–22 μm; their size varies with the development stage, and number and size of ascospores inside. Ascospores polarilocular, usually ellipsoid (rarely narrowly ellipsoid or subspherical or indistinctly rhomboid), ca. 10–20 × 5–10 μm, length/width ratio ranges 1.3–2.3; with broad equatorial thickenings of the wall (4–8 μm); usually 8 spores in asci, but 4 or 6 spores occasionally observed in mature asci in most species.

Pycnidia frequent or rare (depending on species), usually forming low projections on thallus, but sometimes fully immersed; their size is very variable, even in a single specimen (ca. 50–200 μm diam.). Pycnidial tops are usually red or orange, with chlorinated anthraquinoes (C+ purple); less frequently, dark-grey or blackish, containing Cinereorufa-green. Well developed pycnidia multi-chambered (*Xanthoria*-type sensu Vobis, 1980). Conidia bacilliform, rarely narrowly ellipsoid, ca. 3–5 × 1–1.5 μm; differences among species not observed.

Chemistry: Two anthraquinone chemosyndromes may be present: (i) Nonchlorinated chemosyndrome with parietin (dominant), emodin, fallacinal, parietinic acid and teloschistin (chemosyndrome A of Søchting, 1997); (ii) chlorinated chemosyndrome with 7-Cl- emodin (dominant), emodin, 7-Cl-citreorosein and 7-Cl-emodinal (chemosyndrome C1 of Søchting, 2001). Some species specifically contain only one of the two syndromes, either chlorinated or nonchlorinated (Fig. 6), while others have a combination of the two (referring to chemosyndromes C3 and C4 of Søchting, 2001). In the latter group, chlorinated anthraquinones predominate in apothecial margins, while non-chlorinated in apothecial discs (Fig. 6). Cinereorufa-green (green-grey pigment; K−, N+ violet in section) is present in all species, but hardly detectable in some specimens. Sedifolia-grey, contained in some species similar to *Blastenia*, is absent.

Ecology & Geography: See Table 4; Figs. 4, 5.

Key diagnostic characters: Thallus crustose, without marginal lobes, usually in some shade of grey, rarely yellow. Apothecia orange to rusty red. Ascospores ellipsoid with thick equatorial thickenings of the wall. Pycnidia mostly red, with anthraquinones. Conidia bacilliform to narowly ellipsoid. Chlorinated anthraquinones (C+ purple) usually restricted to the apothecial margin. Cinereorufa-green (K−) in darkened parts of thallus and apothecia. Table 5 shows differences from similar genera or species of Teloschistaceae.

Images: Photographs of all recognized species and subspecies are at http://botanika.prf.jcu.cz/lichenology/index.php?pg=5&func=cat&idx=32#photos.

5.3 Infrageneric groups

5.3.1 Crenularia group

Species: *B. catalinae*, *B. crenularia*, *B. purpurea*.

Morphology: Vegetative diaspores absent; thallus grey, up to 100 μm thick in epiphytic *B. catalinae*, but occasionally thicker in older convex areoles of epilithic species; apothecia orange-red to dark red, on average 0.8–1 mm diam., but smaller in the epiphytic species; hymenium not inspersed (like in other groups within *Blastenia*) or inspersed (sometimes in *B. crenularia*); pycnidia red, with anthraquinones.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple (*B. crenularia* and part of *B. catalinae*) or chlorinated anthraquinones in whole apothecia (*B. purpurea* and part of *B. catalinae*). Thallus with Cinereorufa-green, but only traces may be detectable in the epiphytic species. Anthraquinones not detected in the thallus.

Ecology: epilithic or epiphytic (only *B. catalinae*); preferring warm temperate climate.

Geography: Centred in the Mediterranean basin and Macaronesia (*B. crenularia* and *B. purpurea*) and western North America (*B. catalinae*) (Fig. 4).

Genome size: Variable; 28.9–35.7 Mb (Fig. 7).

Phylogeny: The group is strongly supported in the analysis of the concatenated dataset, with the Herbidella group as a sister clade (Figs. 2, 3); it is monophyletic also in Beta-tubulin and mtLSU phylogenies, but it is unresolved in ITS (Fig. 2).

5.3.2 Festivella group

Species: *B. festivella*, *B. gennargentuae*.

Morphology: Vegetative diaspores absent; thallus grey, never yellow; young areoles flat, up to 150 μm thick, but old areoles becoming convex to bullate or with uneven upper surface, up to 800 μm thick; black prothallus distinct, forming lines delimiting thalli; apothecia pale to dark red, on average 0.7–1.1 mm diam.; hymenium not inspersed; pycnidia black, with Cinereorufa-green, but red pycnidia with anthraquinones are sometimes present in *B. festivella*.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple. Cinereorufa-green forms black color of distinct prothallus and hypothallus and sometimes it causes blackening of apothecia. Anthraquinones not detected in the thallus.

Ecology: Epilithic on siliceous rocks.

Geography: Mediterranean-Macaronesian distribution.

Genome size: 30.6–35.2 Mb (Fig. 7).

Phylogeny: Two species included in this group do not have any close relatives (Figs. 2, 3). They do not form a common monophyletic group in any analysis, but it is convenient to put them together for purposes of discussion, because of their similar phenotype.

5.3.3 Herbidella group

Species: *B. afroalpina*, *B. circumpolaris*, *B. coralliza*, *B. ferruginea*, *B. lauri*, *B. herbidella*, *B. remota*.

Morphology: Vegetative diaspores (isidia, blastidia and rarely soralia) present in three of seven species; thallus usually pale to medium grey, but occasionally yellow to orange, thin, mostly <100 μm thick; apothecia orange-red to dark red, in average 0.7–1 mm diam., but smaller in some species (e.g., *B. afroalpina*); hymenium not inspersed; pycnidia red, with anthraquinones.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple (all species). Cinereorufa-green occasionally in thallus, but only in traces, mostly in prothallus, sometimes in tips of blastidia. Anthraquinones sometimes present in thallus (occasionally observed in five of eight species).

Ecology: Always epiphytic; usually on tree trunks, some species specialized to twigs; preferring forests in a humid temperate climate.

Geography: Centred in the Mediterranean basin (three species) and Macaronesia (two species) with scattered

Table 5 Differences between *Blastenia* and the most similar Teloschistaceae

Taxon	Differences from *Blastenia*	References
Bryoplaca sinapisperma	Resembling *B. ammiospila* in its occurrence on bryophytes and plant debris and by chlorinated anthraquinones in whole apothecial surface. Differing in convex apothecia of brownish tinge and by substantial amount of atranorin in thallus.	Søchting et al., 2008; Arup et al., 2013
Eilifdahlia	Distributed in Southern Hemisphere. No marked phenotypic differences from *Blastenia*.	Kondratyuk et al., 2009b, 2014, 2017
Gyalolechia	Melanisation by Cinereorufa-green not observed. Thallus usually yellow, containing substantial amount of fragilin.	Arup et al., 2013; Vondrák et al., 2016
Huneckia	Whole apothecial surface C+ purple, i.e., chlorinated anthraquinones not restricted to only apothecial margin. Main anthraquinones: chrysophanol, chrysophanal and rhein.	Kondratyuk et al., 2014
Rufoplaca	Distinguished by narow ascospores with narow septa. Chlorinated anthraquinones absent. Melanisation by Sedifolia-grey (K+ violet). Cinereorufa-green absent.	Arup et al., 2013
Caloplaca xerica group (including e.g., *C. fuscoatroides*, *C. erythrocarpa,* and *C. neotaurica*)	Whole apothecial surface C+ purple, i.e., chlorinated anthraquinones not restricted to only apothecial margin. Melanisation by Sedifolia-grey (K+ violet). Cinereorufa-green absent.	Vondrák et al., 2012
Caloplaca caesiorufella/*C. spitsbergensis*	Morphology and ecology similar to *B. ammiospila.* Slight differences in apothecia and spores reported, but not always sufficient for reliable identification.	Søchting et al., 2008
Caloplaca fuscorufa	Hardly distinguished from morphologically and ecologically similar *B. psychrophila*, but often more melanized in apothecia. No marked differences from *Blastenia*.	Arup et al., 2007
Caloplaca leptocheila	Morphologically and ecologically similar to *B. psychrophila*, but thallus hardly developed and chlorinated anthraquinones in whole apothecial surface.	Magnusson, 1944b

records northwards up to Northern Scandinavia; *B. circumpolaris* is broadly distributed in temperate zone of Southern Hemisphere; other taxa are restricted to small areas in Ural Mts. (*B. herbidella* subsp. *acidophila*), Himalayas (*B. remota*), and mountains in tropical Africa (*B. afroalpina*).

Genome size: Variable; 26.8–32.7 Mb.

Phylogeny: The group is supported in the concatenated tree and the *BEAST tree, with the Crenularia group as a sister clade (Figs. 2, 3); it is monophyletic in mtLSU single-gene phylogeny, but unresolved by Beta-tubulin and ITS (Fig. 1).

5.3.4 Hungarica group

Species: *B. hungarica*, *B. palmae*, *B. subathallina*, *B. xerothermica*.

Morphology: Vegetative diaspores absent; thallus grey, thin, mostly <100 µm thick; apothecia orange-red (usually paler than in other groups), reduced in size, on average 0.3–0.7 mm diam.; hymenium not inspersed; pycnidia grey with Cinereorufa-green, inconspicuous.

Chemistry: Apothecial disc and exciple with predominated nonchlorinated anthraquinones (three species) or chlorinated anthraquinones (*B. subathallina*). Cinereorufa-green present in thallus (and in injured apothecia), but sometimes only in traces. Anthraquinones absent in thallus.

Ecology: Always epiphytic; usually on twigs, but sometimes on tree trunks; in warm temperate to boreal-montane forests or in Mediterranean scrublands.

Geography: Mediterranean basin and Macaronesia with occurrences northwards up to Northern Scandinavia; one undescribed species related to *B. hungarica* occurs in Chile.

Genome size: Variable; 21.6–34.1 Mb (Fig. 7).

Phylogeny: The group is strongly supported in the concatenated tree (Fig. 2) and the *BEAST species tree (Fig. 3) and is related to Festivella and Psychrophila groups. It is monophyletic in Beta-tubulin single-gene phylogeny, but it is divided into two supported clades in mtLSU where *B. xerothermica* does not group with the rest of species. The group is unresolved in ITS (Fig. 1).

5.3.5 Psychrophila group

Species: *B. ammiospila*, *B. anatolica*, *B. caucasica*, *B. furfuracea*, *B. monticola*, *B. psychrophila*, *B. scabrosa*.

Morphology: Vegetative diaspores (isidia, blastidia) are present in four of eight species; thallus grey, or sometimes yellow (more frequently in epiphytic species); young areoles

flat, up to 150 µm thick, but old areoles of epilithic species becoming convex to bullate or with uneven upper surface, up to 500 µm thick; apothecia pale to dark red, on average 0.7–1.2 mm diam.; hymenium not inspersed; pycnidia red with anthraquinones.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple (most species) or chlorinated anthraquinones in both disc and apothecial exciple (*B. ammiospila* and rarely in *B. caucasica*). Cinereorufa-green present in thallus (and in some old and injured apothecia), but sometimes only in traces. Anthraquinones occasionally present in the thallus of epiphytic species, but very rarely in epilithic species.

Ecology: Epilithic (three species) or on organic substrata (four species); psychrophilous; preferring boreal-montane to arctic-alpine habitats.

Geography: In mountains of temperate zone and in arctic and boreal zone of Northern Hemisphere (Fig. 4); diversity is concentrated in mountains in Mediterranean regions (seven of eight species); absent from Macaronesia; scattered in Southern Hemisphere: in Antarctica (*B. ammiospila*) and an undescribed species in mountains of Madagascar.

Genome size: Variable; 26.9–33.3 Mb (Fig. 7).

Phylogeny: The group is supported in the concatenated tree (Fig. 2) and the*BEAST species tree (Fig. 3) and is related to Festivella and Hungarica groups. It is poorly resolved in the single-gene trees, i.e., it forms a group with low support (Fig. 1). It is the youngest group within *Blastenia*, dated to 4.5–2 Mya.

5.3.6 Relicta group

Species: only *B. relicta* (described below).

Phylogeny: The group is supported in the concatenated tree and also in single-gene trees (Figs. 1, 2). Its position in *Blastenia* phylogeny is unsettled; whereas it is related to Crenularia and Herbidella groups in the mtLSU phylogeny (Fig. 1) and in the *BEAST species tree (Fig. 3), it is placed within the clade together with Festivella, Hungarica and Psychrophila groups in the concatenated tree (Fig. 2). Its position is unresolved in the beta-tubulin and ITS phylogenies (Fig. 1). It is the oldest recognized group, separated some 14–23 Mya (Fig. 3).

5.3.7 Key to the groups

1a. Arctic-alpine or boreal-montane……..…… **Psychrophila group**

1b. More thermophilous; absent from boreal and arctic zones; up to sub-alpine belt in temperate zone……2

2a. Apothecia of reduced size, usually <0.7 mm diam., without or with negligible amounts of chlorinated anthraquinones (not recognized by the spot test with C reagent); pycnidia with Cinereorufa-green and without anthraquinones; without vegetative diaspores; epiphytic, often on twigs …….. **Hungarica group**

2b. Apothecia mostly not reduced in size, mostly 0.5–1.2 mm diam., with chlorinated anthraquinones; pycnidia with anthraquinones (except the Festivella group); vegetative diaspores present or absent; epilithic or epiphytic (rarely on twigs) ……..3

3a. Melanisation by Cinereorufa-green reduced (often in prothallus only) or absent; thallus less than 150 µm thick, grey or occasionally yellow with anthraquinones; vegetative diaspores present or absent; pycnidia red; epiphytic, mostly on the bark of trunks (rarely on twigs)…….. 4

3b. Parts of thallus (sometimes also parts of apothecia) melanized by Cinereorufa-green; thallus more than 150 µm thick in old areoles, always without anthraquinones, not yellow; vegetative diaspores absent; pycnidia red or dark grey; mostly on siliceous rocks (except *B. catalinae*)……..5

4a. Chlorinated anthraquinones often reduced to the outer part of apothecial margin; vegetative diaspores absent; a single recent species, in southern Scandinavia, Spain ……**Relicta group**

4b. Chlorinated anthraquinones in the most surface of apothecial margin; vegetative diaspores present or absent; seven recent species; broadly distributed…….... **Herbidella group**

5a. With distinct black prothallus/hypothallus; pycnidia usually dark grey, with Cinereorufa-green, rarely red with anthraquinones; hymenium not inspersed; restricted to Mediterranean regions and Macaronesia…….....**Festivella group**

5b. Black prothallus present, but often inconspicuous; pycnidia always red; hymenium inspersed (except *B. catalinae*); broadly distributed…….. **Crenularia group**

5.4 Species and infraspecific taxa

5.4.1 **Blastenia afroalpina** Vondrák, **sp. nov.**

MycoBank: MB 822478; Fig. 8A

Etymology: Known from an alpine habitat in Central Africa.

Type: Uganda. Mt. Elgon, alt. 4100 m, 1.13333° N, 34.51666° E, on twigs of *Erica trimera*, 30 January 1997, *G. & S. Miehe U09-10701* (holotype, GZU).

Type sequences: MF114602 (ITS); MF114864 (mtLSU); MF114997 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey (or yellowish in patches), less than 100 µm thick, scabrose; vegetative diaspores absent; apothecia red, 0.4–0.7 mm diam.; ascospore length (12.5–)14.1(–16.0) µm [1.24; 1; 10]; pycnidia red, common in the type specimen. Other related species from the Herbidella group have larger apothecia, often >0.7 mm diam. Species from the Hungarica group have similar size of apothecia, but different anthraquinone chemistry.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones; Cinereorufa-green in thallus.

Ecology: Epiphytic, on shrub twigs in subalpine zone in tropics.

Geography: Central Africa. Known only from the type specimen.

Genome size: 21.3 Mb (CV = 11.7); not reliable; see the Methods for age-induced genomic changes.

Phylogeny: According to all analyses (Figs. 1–3), *B. afroalpina* belongs to the Herbidella group, and its closest relationship to *B. herbidella* is supported in the concatenated

tree (Fig. 2), but not supported in the *BEAST species tree (Fig. 3). BP&P supported *B. afroalpina* as a delimited species (PP = 0.94).

5.4.2 **Blastenia ammiospila** (Wahlenberg) Arup, Søchting & Frödén

Lecidea ammiospila Wahlenb., in Acharius, Methodus (Supplementum) 13-14. 1803.

Type: Norway. Kuatokeino, [ca. 69.155690° N, 23.766820° E], on wood, 22 April 1802, *G. Wahlenberg* (holotype, UPS, L-097792; isotype, S, L1903!).

Description: *Morphology:* Thallus crustose, grey, less than 100 µm thick; vegetative diaspores absent; apothecia red, 0.7–1.0 mm diam.; acospore length (12.5–) 14.5–15.0–15.5(–18.0) µm [1.18; 4; 35]; pycnidia rarely present, red, with anthraquinones.

Chemistry: Chlorinated anthraquinones in whole apothecia; nonchlorinated chemosyndrome with predominated parietin absent or present (see Table S3); thallus without anthraquinones; Cinereorufa-green only in traces.

Ecology: Epiphytic, on bryophytes, plant debris or wood, alpine shrubs (*Juniperus*, *Rhododendron*, *Salix*, etc.), rarely on tree bark (e.g., *Populus tremula*); see Table 4 for details. Mostly arctic-alpine, but also recorded in boreal forests. The species has been exceptionally recorded on (seemingly) inorganic substrates (e.g., Vondrák 13638; Hrubý Jeseník Mts.), but in these cases, inconspicuous deposits of organic material were present below the thalli and other *B. ammiospila* thalli were present nearby on organic substrates.

Geography: Circumpolar in arctic to temperate zones of the Northern Hemisphere and also known to be widespread in Antarctica (Søchting et al., 2004). Only ITS sequences are available for four Antarctic specimens and they are in 99% identical with European sequences, suggesting recent long-distance dispersal to Antarctica.

Genome size: 27.1 Mb (CV = 9.8), measured in sample Urbanavichus PAZ150801.

Phylogeny: According to all analyses (Figs. 1–3), *B. ammiospila* belongs to the Psychrophila group and is related to *B. scabrosa* (Figs. 2, 3). BP&P supported *B. ammiospila* as a delimited species.

5.4.3 *Blastenia anatolica* Halıcı, Arup & Vondrák, **sp. nov.** MycoBank: MB 822479; Fig. 8B

Etymology: Named after the region where it was first recorded, Anatolia in Turkey.

Type: Turkey. Kayseri, Talas, Ali Dağı, alt. 1680 m, 38.6582° N, 35.5546° E, on bark of *Pinus nigra* subsp. *pallasiana*, 2008, *Gökhan Halıcı CL82* (holotype, PRA).

Type sequences: MF114794 (ITS); MF114983 (mtLSU); MF115122 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey or yellow, less than 100 µm thick; vegetative diaspores present, granular isidia, (50–)80–108–130(–220) µm diam. [37; 7; 65]; isidia, when dense, give the thallus a thicker appearance (up to 300 µm); apothecia red, 0.6–1.0 mm diam.; ascospore length (11.0–)11.8–12.8–13.8(–17.0) µm [1.40; 5; 69]; pycnidia red with anthraquinones. We consider the new species morphologically indistinguishable from *B. monticola*. *Blastenia herbidella* is also similar, but has smaller isidia, often at least partly coralloid.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple; thallus with or without anthraquinones (yellow thalli observed in Caucasian localities); traces of Cinereorufa-green in thallus.

Ecology: Epiphytic, on bark or wood in upper montane forests (on *Abies nordmanniana*, *Pinus nigra*) in altitudinal range 1500–2000 m.

Geography: Known from Caucasus Mts. (Russia, Abkhasia) and from several Turkish mountains in provinces Bursa, Kayseri and Konya.

Genome size: 31.8 Mb (CV = 9.7), measured in sample Frolov 675.

Phylogeny: According to all analyses (Figs. 1–3), *B. anatolica* belongs to the Psychrophila group. It is closely related to *B. furfuracea* in the ITS tree (Fig. 1) and in the concatenated tree (Fig. 2), but *BEAST did not resolved its closer relationships within the group (Fig. 3). BP&P supported *B. anatolica* as delimited species (PP = 1).

Note*:* Whereas *B. anatolica* has a grey thallus in all Turkish localities, in the Caucasus Mts. it also has a variant with a yellow thallus with anthraquinones.

5.4.4 *Blastenia catalinae* (H. Magnusson) E.D. Rudolph, in Kondratyuk, Kim, Yu, Jeong, Jang, Kondratiuk, Zarei-Darki & Hur

Caloplaca catalinae H. Magnusson, Botaniska Notiser 1944: 71–72. 1944.

Blastenia catalinae (H. Magnusson) E.D. Rudolph, Revisionary studies in the lichen family Blasteniaceae in North America north of Mexico. - Diss. Abst. 15(8): 100–101. 1955; Nomen invalidum (not effectively published; in Ph.D. thesis; Article 30.8 of ICBN).

Type: USA. California, Santa Catalina Island, Avalon, on bark of *Quercus*, 14 March 1904, coll. C.F. Baker, C.F. Baker: Pacific slope lichens 4028 (lectotype, S, L2615; lectotype selected here as a part of the specimen, the lichen with chlorinated anthraquinones in both the margin and the disc, MBT386428).

Description: *Morphology:* Thallus crustose, grey, less than 100 µm thick; vegetative diaspores absent; apothecia orange to dark red, 0.4–0.9 mm diam. (smaller than in the related *B. crenularia* and *B. purpurea*); ascospore length (13.0–) 15.1(–18.0) µm [1.91; 1; 10]; pycnidia red, with anthraquinones.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple (Frolov 1237, 1238, Klepsand JK14-1130A) or chlorinated anthraquinones in both disc and exciple (type specimen, Vondrák 7488) or chlorinated anthraquinones completely reduced (Klepsand JK14-1130B). Thallus without anthraquinones; traces of Cinereorufa-green in the thallus.

Ecology: Epiphytic, known from shrub and deciduous tree twigs in maritime habitats or inland in altitudes up to 600 m.

Geography: Western North America; DNA data from California only.

Genome size: 28.9 Mb (CV = 10.2), measured in sample Frolov 1238.

Phylogeny: According to the analysis of the concatenated dataset, *B. catalinae* belongs to the Crenularia group and is related to *B. crenularia* and *B. purpurea* (Fig. 1); this relationship is also supported in Beta-tubulin and mtLSU

phylogenies, but it is not resolved by ITS (Fig. 2). BP&P supported *B. catalinae* as a delimited species (PP = 1).

Note: The three different chemotypes may represent three closely related species within *B. catalinae*. This hypothesis is supported by the ITS tree where the specimens without chlorinated anthraquinones form a group distinct from the two genotypes representing the other two chemotypes. Wider sampling and additional mtLSU and beta-tubulin sequence data are necessary to test the three-species hypothesis.

Nomenclature: In the original description, Magnusson (1944a) designated the type as the Baker's exsiccate "Pacific slope lichens 4028" placed in the Lund herbarium (LD). This was the only reference he gave, not citing any other herbaria. It is documented that he had material on loan from Lund in 1942 at the time of preparing the publication on *Caloplaca* in North America (Magnusson, 1944a). No material of the Baker's exsiccate is currently available in Lund, but a fragment of the exsiccate, possibly taken from Lund, is now deposited in Magnusson's own herbarium in Uppsala (UPS). Another specimen of Baker's exsiccate 4028, deposited in Stockholm (S), is marked by Magnusson "*Calopl. catalinae* H. Magn. n. sp. Typus! Det. A. H. Magnusson 1942". It is unclear whether the specimen in Stockholm is the original type and Magnusson incorrectly stated that it was in Lund or whether the Lund material has ended up in S after passing through the herbarium of E. P. Vrang. We consider both specimens to be part of the original material (syntypes) and we typify the name with the material in S since it is richer than the material in Uppsala. This material contains two of the chemotypes described above and we select the one that contains chlorinated anthraquinones in both the margin and the disc as lectotype.

5.4.5 **Blastenia caucasica** I.V.Frolov & Vondrák, **sp. nov.** MycoBank: MB 822480; Figs. 8C, 8D

Etymology: Named after the Caucasus Mts.

Type: Abkhazia. Caucasus Mts, Ritsinski National Park, pass Pyv about 3 km SE of hospital Auadkhara, alt. 1990 m, 43.48333° N, 40.68333° E, on the vertical face of base-rich siliceous outcrops just above timberline, 2 July 2014, *Ivan Frolov 763b* (holotype, PRA). The holotype specimen is only a part of the specimen 763 that includes three phenotypes (see Fig. 8D): with C- pale apothecia and dark thallus (Frolov 763a), with C- dark apothecia and pale thallus (Frolov 763b) and with dark apothecia and with C+ purple apothecial discs (Frolov 763c).

Type sequences: MF114691 (ITS); MF114927 (mtLSU); MF115055 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey, usually up to 150 µm thick, but thicker in old thalli with convex areoles; vegetative diaspores usually absent, but rough isidia present in some specimens (e.g., Frolov 676); apothecia red, 0.8–1.2 mm diam.; ascospore length (12.5–)14.6(–16.0) µm [1.25; 1; 10]; pycnidia red with anthraquinones. Specimens without vegetative diaspores are morphologically indistinguishable from *B. psychrophila*, specimens with rough isidia are indistinguishable from *B. scabrosa*.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; in specimen Frolov 763, we observed also a thallus with chlorinated anthraquinones in the whole apothecial surface; thallus without anthraquinones, with Cinereorufa-green.

Ecology: Epilithic, on vertical and overhanging base-rich siliceous rocks in the subalpine/alpine zone, at altitudes around 2000 m.

Geography: Known only from six localities in western Caucasus Mts. (Abkhazia).

Genome size: 29.3 Mb (CV = 5.5), measured in specimen Frolov 670.

Phylogeny: According to all analyses (Figs. 1–3), *B. caucasica* belongs to the Psychrophila group, but its relationships within the group are unresolved. BP&P supported *B. caucasica* as a delimited species (PP = 1).

5.4.6 **Blastenia circumpolaris** Søchting, Frödén & Arup

Type: Australia. Victoria, Mt. Macedon, on tree bark, April 1886, *F.R.M. Wilson 716* (holotype, NSW 732248-1).

Caloplaca wilsonii S.Y. Kondr. & Kärnefelt in Kondratyuk, Kärnefelt, Elix & Thell, Bibliotheca Lichenologica 100: 271. 2009.

Description: *Morphology:* Thallus crustose, grey or yellow, less than 100 µm thick; vegetative diaspores present, soredia, ca. 10–35 µm diam., soralia ± concave, yellow–brownish orange; apothecia orange to rusty red, disc partly green or blackened (see fig. 27 in Kondratyuk et al., 2009a), 0.3–0.7 mm diam.; ascospore length not examined, but (7–)10–13(–16) µm long according to Kondratyuk et al. (2009a); pycnidia not seen.

Chemistry: Nonchlorinated anthraquinones in apothecial disc and exciple, chlorinated anthraquinones in exciple (7-chloroemodine reported by Kondratyuk et al., 2009a: 272), but in a low amount (hypochlorite reaction indistinct); thallus without or with traces of anthraquinones, but anthraquinones present in the yellow soralia; Cinereorufa-green distinct in prothallus and often in apothecial discs.

Ecology: Epiphytic, on bark of tree trunks (e.g., *Acacia*, *Eucalyptus*, *Nothofagus*) at low altitudes, up to 700 m. Once recorded epilithic, on stone in forest floor (Søgaard 69, Chile).

Geography: Known from Australia, Tasmania (Kondratyuk et al., 2009a) and Chile (Arup et al., 2013).

Genome size: Not measured (scarcity of material).

Phylogeny: According to all analyses (Figs. 1–3), *B. circumpolaris* belongs to the Herbidella group. It is related to *B. afroalpina*, *B. ferruginea*, *B. herbidella* and *B. remota* in the concatenated tree (Fig. 2), but its position is unresolved within the group in the *BEAST species tree (Fig. 3). BP&P supported *B. circumpolaris* as a delimited species (PP = 0.94).

5.4.7 **Blastenia coralliza** (Arup & Åkelius) Arup, Søchting & Frödén

Caloplaca coralliza Arup & Åkelius, Lichenologist 41: 471. 2009.

Type: Sweden. Skåne: Kågeröd par., Knutstorp, ca. 200 m N of the castle. On old *Quercus* in wooded meadow, alt. ca. 90 m, *Ulf Arup 06075* (holotype, LD; isotypes, C, MIN).

Description: *Morphology:* Thallus crustose, grey or beige or yellow to orange, <100 µm thick; vegetative diaspores present, coralloid blastidia, 50–120 µm wide and up to 800 µm tall, exceptionally vegetative diaspores absent (Malíček 5561, Andalusia); blastidia, when dense, give the thallus a thicker appearance (up to 900 µm; Arup & Åkelius

2009); apothecia absent or rare (Scandinavia, Canary Islands) or frequent, orange to pale red, 0.7–1.1 mm diam.; ascospore length (9.0–)10.7–12.8–14.8(–16.0) µm [1.97; 3; 29]; pycnidia red with anthraquinones, but rarely present. Further data in Arup & Åkelius (2009).

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones or with anthraquinones; Cinereorufa-green only in traces (in prothallus and tips of blastidia).

Ecology: Epiphytic, on bark or wood of numerous tree species (seeTable S1), in low altitudes in Scandinavia (up to 100 m), but with a high altitudinal range in the Mediterranean basin and Macaronesia (reaching 1600 m; Fig. 11). According to our data, *B. coralliza* is more common in the Mediterranean region and more thermophilous than the similar *B. herbidella* (Fig. 11).

Geography: Known in the Mediterranean basin from Albania, Croatia, France, Greece, Italy, Slovenia, Spain, Syria, Tunisia and Turkey. Also present in Canary Islands (La Palma). In the north, reaches oceanic western Europe (France, Germany) and southern Scandinavia (See fig 5 in Arup & Åkelius, 2009). It is absent from most of Central Europe and in more eastern regions.

Genome size: 26.8 Mb (CV = 11.1), measured in sample Vondrák 10876.

Phylogeny: According to all analyses (Figs. 1–3), *B. coralliza* belongs to the Herbidella group, but its position differs slightly among trees. BP&P supported *B. coralliza* as a delimited species (PP = 1).

5.4.8 **Blastenia crenularia** (Withering) Arup, Søchting & Frödén

Lichen crenularius Withering, Bot. arr. veg. Gr. Brit. (London) 2: 709. 1776.

Type: United Kingdom. Isle of Wight, May 1794, *Withering* (lectotype, BM; selected by Laundon 1984, p. 231).

Description: *Morphology:* Thallus crustose, grey; young thalli with flat areoles up to 150 µm thick, but old thalli with uneven upper surface of areoles may be thicker (up to 500 µm); thallus may also be indistinct, especially when growing on sandstone; vegetative diaspores absent; apothecia red, 0.7–1.1 mm diam.; hymenium frequently inspersed (unlike in other *Blastenia* species); ascospore length (11.5–)14.0–14.8–15.8(–17.5) µm [1.27; 7; 63]; pycnidia red with anthraquinones.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones; Cinereorufa-green usually present in thallus and sometimes in apothecia (blackened parts).

Ecology: Epilithic, on various types of coastal or inland siliceous rocks (occasionally on dust-impregnated wood). On seashores, it usually avoids supralittoral zone and occurs in places sheltered from salt spray. It is restricted to regions with a mild climate; for instance in Central Europe, it occurs only on xerothermic volcanic rocks, mostly in river valleys at low altitudes. It reaches higher altitudes only in Iran, Mediterranean mountains and in Macaronesia (up to 2000 m). In Macaronesia, it grows at a higher altitude than the similar *B. festivella*. In Western Europe and in the Mediterranean basin, it descends to seashore rocks, so it has a broad altitudinal range (about 0–1900 m). Its upper altitudinal limit decreases to some 200 m in more northern territories, e.g., in Great Britain and in Scandinavia (Fig. 10).

Geography: Widely distributed in the whole Mediterranean basin, from Caspian Sea coasts to Spain. In oceanic northern Europe, it reaches Iceland (65.83°N) and the coast of Northern Scandinavia (70.63°N), but in the more continental parts of Europe, it only reaches Germany, the Czech Republic and Slovakia. Its easternmost limits are Crimea, SW coasts of the Caspian See and NW Iran. It also occurs in Madeira and the Canary Islands.

Genome size: Ranges between 28.6 and 35.7 Mb (CV = 6.4–9.8; ten samples measured).

Phylogeny: According to all analyses, *Blastenia crenularia* is a part of the Crenularia group and is closely related to *B. purpurea* (Figs. 1–3). BP&P supported *B. crenularia* as a delimited species (PP = 1).

Note: Wetmore (1996) reported *B. crenularia* from the Caribbean islands. His description of the Caribbean population fits *Blastenia* well, but we have not seen his material. If it belongs to *Blastenia*, it is probably a distinct species, more thermophilous than any known epilithic *Blastenia*.

5.4.9 **Blastenia ferruginea** (Hudson) A. Massal.

Lichen ferrugineus Hudson, Fl. Angl.: 444. 1762.

Type: France. Alpes-de-Haute-Provence, Gorges Du Verdon, SW-S from La Palud-sur-Verdon, alt. 850 m, 43.76294° N, 6.31700° E, 9 May 2015, *Ivan Frolov 966* (conserved type, PRA; isotypes, BM, GZU, herb. Frolov). Conserved type proposed by Arcadia & Vondrák (2017). (The type was eventually placed in PRM, contrary to the intention stated in Arcadia & Vondrák, 2017.)

Description: *Morphology:* Thallus crustose, white to grey, usually less than 100 µm thick; vegetative diaspores absent; apothecia red; 0.7–1.0 mm diam.; ascospore length (11.0–)13.2–13.9–14.5(–17.0) µm [1.5; 4; 40]; pycnidia red with anthraquinones.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc; chlorinated anthraquinones in exciple; thallus without or rarely with traces of anthraquinones; Cinereorufa-green in prothallus.

Ecology: Epiphytic, on the bark of tree trunks; 43 records on various deciduous trees (e.g., *Quercus* spp., *Acer* spp., more in Table S1), but only four records on conifers. Only two records on twigs and two on Mediterranean shrubs; not recorded on wood. It occurs at altitudes 10-1200 m in the Mediterranean, but only lowland records are known outside the Mediterranean region (London and New Forest in Great Britain).

Geography: Widely distributed in the northern half of the Mediterranean region. Known from Crimea, Croatia, Cyprus, France, Greece, Italy, Slovenia, Spain and Turkey. It is probably very sparse in non-Mediterranean Europe, known only from two localities in southern Great Britain. Historical specimens from Germany, called "*Caloplaca ferruginea*" (e.g., Lübeck, Erichsen 6.6.1903; Schwarzwald, Poelt 12437), probably belong to *B. ferruginea*, but are not confirmed by DNA sequences.

Genome size: 31.7 Mb (CV = 8.0); measured in specimen Ivan Frolov 966 (Verdon, France).

Phylogeny: Blastenia ferruginea belongs to the Herbidella group (Figs. 1–3). It is related to *B. afroalpina*, *B. circumpolaris*, *B. herbidella* and *B. remota* in the concatenated tree (Fig. 2), but its position is unresolved within the group in the *BEAST species tree (Fig. 3). BP&P supported *B. ferruginea* as a delimited species (PP = 1).

Nomenclature: Historically, the name has been applied to what we here recognize as three species (*Blastenia ferruginea*, *B. lauri* and *B. relicta*) that are hardly distinguishable morphologically. They differ in geographical range, and only one of them occurs in southern England, the type locality for *B. ferruginea*. All sequences provided by Arup et al. (2013) under the name *B. ferruginea*, KC179416 (ITS), KC179163 (nrLSU), KC179493 (mtSSU), belong to the newly described *B. relicta*.

5.4.10 **Blastenia festivella** (Nylander) Vondrák, **comb. nov.**
MycoBank: MB 822481

Lecanora ferruginea var. *festivella* Nylander, Flora, Regensburg 56: 197. 1873.

Type: France. Pyrenees-Orientales, Collioure, Port Vendres [on maritime schist rocks], 4 July 1872, *William Nylander* (lectotype, H-NYL 30260; isolectotype, H-NYL 30259; lectotype selected here, MBT386430).

Blastenia subochracea sensu Arup et al. (2013), not *Caloplaca subochracea* (Wedd.) Werner (see nomenclature note below).

?*Caloplaca limitosa* (Nyl.) H. Olivier (see nomenclature note below); Basionym: *Lecanora limitosa* Nyl. in Flora 63: 387. 1880; Type: Porto in Portugal, ad saxa argilaceo-schistosa [on schist rock], *Newton* (not located).

Description: *Morphology:* Thallus crustose, grey; young thalli up to 150 µm thick, but old thalli with convex to bullate areoles may be thicker (up to 800 µm); black prothallus usually distinct, surrounding thallus margin; vegetative diaspores absent; apothecia red, 0.7–1.1 mm diam., margin sometimes blackened; ascospore length (11.5–) 11.0–11.7–12.7(–17.5) µm [1.34; 9; 89]; pycnidia dark grey with Cinereorufa-green or rarely red with anthraquinones. From *B. crenularia*, it differs by more distinct black prothallus line surrounding thalli, not inspersed hymenium and shorter ascospores, but some specimens of *B. crenularia* are hardly distinguishable.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple (chlorinated anthraquinones in the disc and exciple in similar *B. purpurea*); thallus without anthraquinones; Cinereorufa-green usually present in thallus and sometimes in apothecia (blackened parts).

Ecology: On maritime siliceous rocks. In Portugal and in Macaronesia, also on inland siliceous rocks at altitudes to 1250 m (Fig. 10). Once found on dust impregnated bark of Euphorbiaceae shrub in Madeira. A single truly epiphytic population was recorded in Spanish Andalusia close to Tarifa (36.085533° N, 5.716849° W) on the bark of *Eucalyptus*, *Quercus ilex*, *Q. suber* and *Olea*. In our opinion, the epiphytic population represents a young taxon already delimited from epilithic *B. festivella*, however, it is not separated by the GS and by beta-tubulin and ITS sequences (mtLSU not available) from the saxicolous *B. festivella* (see red dots in Fig. 1).

Geography: Very common in Macaronesia and in the western Mediterranean. Rarely recorded also from the eastern Mediterranean (Greece and Turkey).

Genome size: Ranges between 30.1 and 34.5 Mb (CV = 6.4–8.6; seven specimens measured). *Phylogeny: Blastenia festivella* does not belong to any of the large groups and forms a group of its own (Figs. 1–3). Its position in *Blastenia* is not clear; either it is sister to the Psychrophila group (Fig. 3), or to both Psychrophila and Hungarica groups (Fig. 2). BP&P supported *B. festivella* as a delimited species (PP = 1).

Nomenclature: We adopted the name *Caloplaca festivella* for this taxon because its syntypes reflect our concept of the species: dark prothallus delimiting the thallus, ascospores 10–14 × 5–7 µm, dark grey pycnidia, darkening of apothecial margin by Cinereorufa-green, etc. The syntypes have small (up to 0.7 mm diam.) and partly blackened apothecia which was a reason for describing them as a separate taxon from *L. ferruginea* (at that time in a wide sense). However, the small size of apothecia is caused partly by their youth and partly by poor development. Both syntypes (H-NYL 30259 called *Lecanora festivella*, and H-NYL 30260 called *L. ferruginea* * *festivella*) represent the same species collected by W. Nylander from schist (very probably maritime rock) in the same place and at the same date [Port Vendres, 4 July 1872]. We designated the latter as lectotype here because its name reflects exactly the name in the protologue (Nylander, 1873) and it consists of richer material.

For this taxon, Arup et al. (2013) made the new combination *Blastenia subochracea* (Wedd.) Arup, Søchting & Frödén from *Lecanora aurantiaca* var. *subochracea* Wedd. (Weddell, 1873: 363; type not located). The sequenced material was collected on basalt in the Azores close to the sea whereas the type of *Lecanora aurantiaca* var. *subochracea* was collected on shaded walls of limestone at Parc de Bollac, Poitiers, France. It must belong to a different species that is not *Blastenia* (*Blastenia* avoids limestone). In addition, the type description of *Lecanora aurantiaca* var. *subochracea* indicates that the thallus is pale yellow and K+ purple, unlike *B. festivella*.

Another name, *Caloplaca limitosa* (Nyl.) H. Olivier, has been currently used for this species by some Mediterranean authors (e.g., Nimis, 2016). Although we have not seen its type (not located in H-NYL), it may be conspecific with *Blastenia festivella*. Nylander (1880: 387–388), in his protologue, mentioned an important character, the black prothallus line delimiting individual thalli. This and all other characters in the protologue fit *Blastenia*. Nylander indicated one locality, Porto (Portugal), on schist rock. That is consistent with the ecology and distribution of *Blastenia festivella*, which is common in Portugal and can grow on non-calcareous schist. If the synonymy could be confirmed, the correct name for this species would be *Blastenia limitosa*.

5.4.11 **Blastenia furfuracea** (H. Magnusson) Arup, Søchting & Frödén

Caloplaca furfuracea H. Magnusson, Göteborgs Kungl. Vetensk. Samhälles Handl., Ser. B, Math. Naturvensk. Skr. 3: 33. 1944.

Type: Sweden. Jämtland, Undersåker Hålland, *G. O. Malme, Lich. Suec. Exs. 763*

(lectotype, GB, selected by Wetmore 2004 (as holotypus); isotypes, H, LD, S).

Description: *Morphology:* Thallus crustose, grey to almost black or rarely yellow, <100 µm thick; vegetative diaspores present, granular blastidia, 40–70 µm diam.; blastidia, when dense, give the thallus a thicker appearance (up to 340 µm; Arup & Åkelius, 2009); apothecia pale to dark red, 0.7–1.2 mm diam.; ascospore length (11.0–)12.9–13.0–13.2(–15.0) µm [1.04; 4; 40]; pycnidia red, but sparse or absent. Further data in Arup & Åkelius (2009).

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones or rarely yellow, with anthraquinones (Davydov 10713, Altai Mts.); Cinereorufa-green usually present in the thallus, especially in tips of blastidia.

Ecology: Epiphytic on tree bark or weathered wood of snags and stumps. Usually associated with boreal tree species (e.g., *Betula*, *Chosenia*, *Pinus*, *Pseudotsuga*), but also on *Quercus* in southern Ural Mts. Known from a broad range of altitudes (300–2200 m).

Geography: Circumpolar in the boreal zone of the Northern Hemisphere. Known from Scandinavia, the Alps and North America (Arup & Åkelius, 2009; Wetmore, 2004). We further recorded this species from a broad range of longitude in Eurasia: the Ural Mts. (57.5° E), Altai Mts. (83.0° E & 85.6° E), and from the Kodar ridge in the Zabaikalsky Krai (117.3° E).

Genome size: 27.3 Mb (CV = 9.8); measured in sample Davydov 10713 (Altai Mts.).

Phylogeny: According to all analyses (Figs. 1–3), *B. furfuracea* belongs to the Psychrophila group. It is closely related to *B. anatolica* in the ITS phylogeny, but its position within the group is unresolved in the mtLSU and beta-tubulin trees (Fig. 1). Whereas the concatenated tree supported a sister relationship of *B. anatolica* and *B.* furfuracea, the *BEAST species tree did not resolve their relationship. BP&P supported *B. furfuracea* as a delimited species (PP = 1).

Note: Arup & Åkelius (2009) characterized *B. furfuracea* by a grey to black thallus without anthraquinones, which may be true for Europe, but in the Altai Mts there is also a variant with a yellow thallus with anthraquinones.

5.4.12 **Blastenia gennargentuae** Vondrák, **sp. nov.**
MycoBank: MB 822482; Fig. 8E

Etymology: Named after the type locality in the area Gennargentu.

Type: Italy. Sardinia: Gennargentu National Park, Fonni, N slope of Mt. Monte Spada, alt. 1450 m, 40.06666° N, 9.28333° E, on the vertical face of a granite outcrop in the montane pasture, 1 May 2012, *Jan Vondrák 9609* (holotype, PRA).

Type sequences: MF114665, MF114686, MF114763 (ITS); MF114923 (mtLSU); MF115051 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey; young thalli up to 150 µm thick, but old thalli with convex to bullate areoles may be thicker (up to 800 µm); black prothallus is not restricted to thallus margin, but is usually distinct among areoles and also forms a black layer in medulla (hypothallus); vegetative diaspores absent; apothecia red, 0.7–1.0 mm diam., apothecial margin often blackened; ascospore length (10.0–)12.2–12.9–13.9(–15.0) µm [1.24; 3; 31]; pycnidia dark grey with Cinereorufa-green. Black hypothallus is characteristic for the species but may be absent. Blackening of apothecial margin is typical, but is occasionally observed in other species (e.g., *Blastenia festivella*, *Caloplaca fuscorufa*). Small ascospores and grey (not red) pycnidia are diagnostic against *B. crenularia*, *B. caucasica* and *B. psychrophila*. Strongly melanized specimens of *B. festivella* with grey pycnidia are hardly distinguishable morphologically.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones; Cinereorufa-green forms black patches in thallus, apothecia and pycnidia and often expands into medullar tissue, forming black hypothallus.

Ecology: Epilithic species known from siliceous rocks in Mediterranean mountains at altitudes ca. 1400–1800 m.

Geography: Rare in the Mediterranean mountains. Known only from Calabria and Sardinia.

Genome size: 30.6 Mb (CV = 6.9); holotype measured.

Phylogeny: The position of *B. gennargentuae* is ambiguous; it is possibly related to the Psychrophila group (Fig. 3) or to *B. festivella*. Although it may form a group of its own, we formally included it in the Festivella group (see notes on the group above). BP&P supported *B. gennargentuae* as a delimited species (PP = 1).

5.4.13 **Blastenia herbidella** (Hue) Servít subsp. **herbidella**
Lecidea caesiorufa f. *herbidella* Hue, Nouv. Arch. Mus., Series V, 3: 151. 1911.

Type: Hungary. Arva, supra corticem *Abietis pectinatae* in alpe Chocs comit, [Slovakia. Orava: Mt. Choč, on bark of *Abies alba*], 21 August 1880, *Lojka: Lich. Reg. Hung. Exs. 31* (holotype, PC!; isotypes, B, LD, M, S, UPS!).

Description: *Morphology:* Thallus crustose, grey or rarely yellow, usually less than 100 µm thick; vegetative diaspores present, coralloid or granular blastidia/isidia, 60–160 µm wide and up to 600 µm tall; blastidia, when dense, give the thallus a thicker appearance (up to 700 µm; Arup & Åkelius, 2009); apothecia pale to dark red, 0.7–1.1 mm diam.; ascospore length (9.5–)10.8–12.7–15.0(–17.0) µm [2.00; 4; 33]; one specimen from Turkey (Halıcı, CL226) has large ascospores (mean length 15.0 µm) whereas Arup & Åkelius (2009) reported ascospore length in the range 10.5–13.0 µm; pycnidia red, usually frequent. Further data in Arup & Åkelius (2009).

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones or rarely yellow with anthraquinones; Cinereorufa-green usually absent or in traces, but often detectable in tips of isidia/blastidia.

Ecology: Epiphytic on tree trunks (134 records) or rarely on twigs (five records) and only once found on wood. Associated with a number of tree species; Arup & Åkelius (2009) reported its occurrence on forty different tree species. In our dataset, deciduous trees predominate over conifers in ratio 106:28. The preferred substrate is *Acer pseudoplatanus*. It occurs at 25–1800 m altitude (Fig. 11), but records from low altitudes (below 500 m) are mostly from Scandinavia. In the Mediterranean basin, it is restricted to altitudes above 1000 m, but it does not occur above the timberline. Arup & Åkelius (2009) reported it in the alpine zone and on *Rhododendron* shrubs, but those reports refer to *Blastenia monticola* (=*B. herbidella* p.p. sensu Arup et al., 2013).

Geography: Restricted to Europe and adjacent Mediterranean regions (fig. 7 in Arup & Åkelius, 2009). It reaches Southern Scandinavia in the north and Eastern Carpathians and Caucasus Mts. in the east. Records from northern Norway by Arup & Åkelius (2009) belong to *B. monticola*. Its occurrence on Canary Islands (Arup & Åkelius, 2009) is possible but is without DNA confirmation. Various reports from other continents are doubtful and without DNA confirmation.

Genome size: 30.2 Mb (CV = 7.9); measured in sample Vondrák 11335 (Slovakia).

Phylogeny: According to all analyses (Figs. 1–3), it belongs to the Herbidella group. In the beta-tubulin tree, it is closely related to *B. coralliza*, but in the mtLSU tree, it forms a supported subgroup together with *B. afroalpina*, *B. circumpolaris*, *B. ferruginea*, and *B. remota*; in the ITS tree, its position is unresolved (Fig. 1). The concatenated tree supports its closest relationship with *B. afroalpina* (Fig. 2), but the *BEAST tree did not resolve its relationships within the Herbidella groups (Fig. 3). See the Results part for its BP&P support.

5.4.14 **Blastenia herbidella** subsp. **acidophila** Urbanavichene & Vondrák, **subsp. nov.**

MycoBank: MB 822483; Fig. 8F

Type: Russia. Chelyabinsk region, Zyuratkul' National Park, at the coast of the lake Zyuratkul, on bark of *Picea obovata*, 26 May 2009, *Irina Urbanavichene s.n. [Vondrák 17838]* (holotype, PRA).

Type sequences: MF114751 (ITS); MF114964 (mtLSU); MF115059 (beta-tubulin).

Etymology: Named after its strong preference for acid bark.

Diagnosis: *Morphology:* Thallus crustose, grey, <100 µm thick; vegetative diaspores present, coralloid or granular blastidia/isidia, 50–160 µm wide; apothecia pale to dark red, 0.5–0.8 mm diam.; ascospore length (12.0–)13.7(–17.0) µm [1.55; 1; 10]; pycnidia red, but not common. Morphologically similar the subsp. *herbidella*, but has slightly smaller apothecia.

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones, yellow thalli with anthraquinones not observed; Cinereorufa-green sometimes present in the thallus, mostly in tips of blastidia.

Ecology: Epiphytic, on twigs (rarely on trunks) of boreal trees, such as *Betula* and *Picea obovata,* with rather acidic bark.

Geography: Known from three localities in the north-eastern part of South Ural Mts.

Genome size: Measurements not reliable; old material (Table S2).

Phylogeny: In the mtLSU and ITS trees, subsp. *acidophila* is unresolved from subsp. *herbidella*, but in the beta-tubulin tree, it is distinct from subsp. *herbidella* and more closely related to *B. afroalpina* and *B. remota* (Fig. 1, red dots). In two of three specimens sequenced for beta-tubulin, we revealed also a secondary sequence signal (low peaks) belonging to subsp. *herbidella*. While ancestral within-specimen polymorphism in beta-tubulin is present in subsp. *acidophila*, it was not observed in subsp. *herbidella*.

5.4.15 **Blastenia hungarica** (H. Magnusson) Arup, Søchting & Frödén

Caloplaca hungarica H. Magnusson, Göteborgs Kungl. Vetensk. Samhälles Handl., Ser. B, Math. Naturvensk. Skr. no. 1: 228. 1944.

Type: Hungary. Veszprem, about Juhaszhaz near village Szent Ivan, on bark of *Abies*, 1 March 1917, *Fóriss* (holotype, S).

Description: *Morphology:* Thallus crustose, grey, <100 µm thick; vegetative diaspores absent; apothecia orange to pale red, 0.3–0.8 mm diam.; ascospore length (11.5–) 12.8–13.7–14.3(–16.0) µm [1.39; 3; 29]; pycnidia dark grey with Cinereorufa-green, but usually sparse or absent.

Chemistry: Nonchlorinated anthraquinones in apothecia; chlorinated anthraquinones absent; thallus without anthraquinones; Cinereorufa-green usually hardly detectable, but present around pycnidial ostioles and sometimes accumulated in injured apothecia.

Ecology: Epiphytic on tree trunks (28 records) or twigs (53 records); seven specimens are from wood. Associated with a number of deciduous and coniferous tree species (Table S1), but more frequent on deciduous trees (Table 4). Occurring from lowlands to high altitudes (up to 2000 m), but occurrences below 400 m are mostly restricted to Scandinavia. In the Mediterranean basin, it is restricted to altitudes above 800 m, generally above the altitudinal range of *B. xerothermica* (Fig. 9).

Geography: Restricted to Europe and adjacent Mediterranean regions (Turkey and Caucasus). It reaches Southern Scandinavia in the north (65.1° N) and eastern Caucasus Mts. in the east (46.9° E). The westernmost record is from the French foothills of the Alps (6.32° E). It is probably absent from the Iberian Peninsula, because all eleven sequenced specimens from Spain that resembled *B. hungarica* were identified as *B. xerothermica*, even specimens from high altitudes (up to 1900 m). In more eastern Mediterranean regions, *B. hungarica* is mostly restricted to high altitudes, but with a few records in low altitudinal sub-Mediterranean habitats (e.g., Utrish reserve in the western Caucasus). Records from Mediterranean habitats and from Macaronesia published under *B. hungarica* mostly belong either to *B. xerothermica* or *B. palmae*.

Genome size: 28.2 Mb (CV = 8.8); measured in sample Palice 18699 (Austria).

Phylogeny: According to all analyses, it belongs to the Hungarica group and it is closely related to *B. subathallina* (Figs. 1–3). BP&P supported *B. hungarica* as a delimited species (PP = 1).

5.4.16 **Blastenia lauri** Vondrák, **sp. nov.**

MycoBank: MB 822484; Fig. 8G

Etymology: Our first records came from laurel forests of La Palma.

Type: United Kingdom. Scotland, Oban, Balvicar, alt. 40 m, 56.2700° N, 5.6152° W, on twigs of *Ulmus*, 22 August 2014, *Jan Vondrák 12566* (holotype, PRA).

Type sequences: MF114676 (ITS); MF114916 (mtLSU); MF115040 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey, <100 µm thick; vegetative diaspores absent; apothecia red, 0.7–1.2 mm diam.; ascospore length (13.5–)14.8–15.8–17.3(–19.0) µm [1.55;

4; 41]; pycnidia red with anthraquinones. *Blastenia ferruginea* and *B. relicta* are very similar, but have slightly smaller ascospores.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; Cinereorufa-green only in prothallus.

Ecology: Epiphytic on trunks and twigs of *Alnus*, *Corylus*, *Ilex*, *Salix* and *Ulmus* in the British Isles, and on *Castanea sativa*, *Ficus carica*, *Lauraceae* spp. and *Pinus canariensis* in Macaronesia. On solitary trees or in forests in a humid climate.

Geography: Known from humid regions of British Isles (mainly western Scotland) and Ireland, and from Canary Islands (data from La Palma and Tenerife) and Madeira.

Genome size: 28.7 Mb (CV = 8.2); measured in sample Vondrák 13109 (Madeira).

Phylogeny: According to all analyses (Figs. 1–3), *B. lauri* belongs to the Herbidella group, but its closer relationships are not resolved. BP&P supported *B. lauri* as a delimited species (PP = 1).

5.4.17 **Blastenia monticola** Arup & Vondrák, **sp. nov.**
MycoBank: MB 822485; Fig. 8H

Etymology: The Latin term means "inhabitant of the mountains", and it reflects the montane occurrence of the species.

Type: Russia. Chelyabinsk' region, Mountain ridge "Bolshaya Suka", at main road Chelyabinsk – Ufa, about 8 km SE of town Bakal, alt. 750–800 m, 54.9166° N, 58.9000° E, on bark of *Picea obovata* in wetland spruce forest, 24 June 2011, *Jan Vondrák 11337* (holotype, PRA).

Type sequences: MF114607 (ITS); MF114867 (mtLSU); MF114999 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey or rarely yellow, usually up to 100 µm thick; apothecia red, 0.8–1.2 mm diam.; vegetative diaspores present, granular blastidia/isidia, 50–200 µm diam.; isidia, when dense, give the thallus a thicker appearance (up to 300 µm); ascospore length (8.0–) 9.6–11.8–14.7(–17.0) µm [1.96; 7; 72]; pycnidia red. Morphologically indistinguishable from *B. anatolica*. *Blastenia herbidella* is also similar, but has smaller isidia, often at least partly coralloid.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones or rarely with traces of anthraquinones; Cinereorufa-green usually in traces in the thallus, but accumulated in tips of isidia/blastidia.

Ecology: On bark and wood of subalpine/subarctic trees (e.g., *Abies nordmanniana*, *Cedrus libanii*, *Larix decidua*, *Picea obovata* and *Pinus heldreichii*) and on twigs of arctic/alpine shrubs (e.g., *Juniperus sibirica*, *Rhododendron ferrugineum*).

Geography: Most records are from mountains surrounding the Mediterranean basin including the Alps, Apennines, Pyrenees, and mountains in Balkans and Turkey. Known from Albania, Austria, France, Greece, Italy, Macedonia, Montenegro, Serbia, Spain, Switzerland and Turkey. Also recorded in Caucasus (Abkhazia, Russia), Ural Mts, northern Scandinavia (Sweden and Norway), Russian Arctic (Kola Peninsula) and from southern Siberia (Altai Mts.).

Genome size: 29.8 Mb (CV = 8.5); measured in sample Urbanavichus LK01 (Caucasus Mts.).

Phylogeny: According to all analyses (Figs. 1–3), *B. monticola* belongs to the Psychrophila group, but its closer relationship is not resolved in any of the single-locus trees (Fig. 1) or in the concatenated tree (Fig. 2). The *BEAST tree supported its relationship with *B. scabrosa* and *B. ammiospila* (Fig. 3). BP&P supported *B. monticola* as a delimited species (PP = 1).

5.4.18 **Blastenia palmae** Vondrák, **sp. nov.**
MycoBank: MB 822486; Fig. 8I

Etymology: Our first records came from La Palma.

Type: Portugal. Estremadura, Lisbon, Malveira da Serra, Biscaia, coastal granite cliffs SW of village, alt. 50 m, 38.7538° N, 9.4761° W, on twigs of *Rosmarinum*, 9 October 2014, *Jan Vondrák 12572* (holotype, PRA).

Type sequences: MF114674 (ITS); MF114914 (mtLSU); MF115038 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey, <100 µm thick; vegetative diaspores absent; apothecia orange to pale red, 0.4–0.6 mm diam.; ascospore length (10.0–) 11.9–12.6–13.6(–15.0) µm [1.35; 3; 30]; pycnidia dark grey with Cinereorufa-green, but usually sparse or absent. Morphologically indistinguishable from *B. hungarica* and *B. xerothermica* (but both species have different ecology and distribution).

Chemistry: Nonchlorinated anthraquinones in apothecia; chlorinated anthraquinones absent; thallus without anthraquinones; Cinereorufa-green usually hardly detectable, but present around pycnidial ostioles and rarely present in injured apothecia.

Ecology: Epiphytic on tree trunks (9 records) or more frequent on tree twigs (17 records) and on shrub twigs (25 records); only once recorded on wood. Associated with a number of deciduous and coniferous tree and shrub species (Table S1). Occurring from lowlands to high altitudes (up to 1450 m) in Macaronesia, but only in coastal areas in Atlantic Spain and Portugal (up to 300 m; Fig. 9).

Geography: Restricted to Macaronesia (Azores, Canary Islands, Madeira) and to coastal areas of the westernmost Europe (SW Spain, S Portugal).

Genome size: 29.9 Mb (CV = 7.1); measured in sample Frolov 1007 (Spain, Andalusia).

Phylogeny: According to all analyses, it belongs to the Hungarica group and it forms a sister group to *B. hungarica* and *B. subathallina* (Figs. 1–3). BP&P supported *B. palmae* as a delimited species (PP = 1).

5.4.19 **Blastenia psychrophila** Halıcı & Vondrák, **sp. nov.**
MycoBank: MB 822487; Fig. 8J

Etymology: The epithet reflects the strong preference of the species for cold environments.

Type: Turkey. Bursa region, Uludağ Mts., near hotel village, alt. 1960 m, 40.1000° N, 29.1275° E, on siliceous rock, 24 May 2012, *Gökhan Halıcı CL355* & *E. Kılıç* (holotype, PRA).

Type sequences: MF114784 (ITS); MF114976 (mtLSU); MF115112 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey, but exceptionally with yellow tinge (Fig. 8K), partly exceeding 100 µm thickness; vegetative diaspores usually absent, but rough isidia-like outgrowths rarely present; apothecia red,

0.7–1.1 mm diam.; ascospore length (10.0–)13.6–15.8–19.1 (–25.0) µm [2.14; 7; 70]; pycnidia red.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones; Cinereorufa-green in the thallus.

Ecology: Epilithic, on vertical and overhanging, but also on rain exposed, base-rich siliceous rocks in subalpine/alpine zone. It occurs at altitudes 1500–2500 m in Mediterranean mountains, but at 1300–2000 m in mountains north of the Mediterranean (Fig. 10).

Geography: Occurring in Mediterranean and Balkan Mountains, the Alps, Carpathinas and Sudetes. Known from Bulgaria (Rila Mts.), France (Massif Central), Greece (Mt. Smolikas), Italy (southern Alps, Apennine Mts.), Kosovo and Macedonia (Šar Planina), Serbia (Stara Planina Mts.), Turkey (seven mountain areas) and Ukraine (Carpathians). The northernmost occurrence is in Poland, Krkonoše Mts. (50.8° N), the easternmost in the Kars province of Turkey (42.7° E).

Genome size: 26.9 Mb (CV = 10.5), measured in specimen Vondrák 11852 (Krkonoše Mts.).

Phylogeny: According to all analyses (Figs. 1–3), *B. psychrophila* belongs to the Psychrophila group, but its relationships within the group are unresolved. BP&P supported *B. psychrophila* as a delimited species (PP = 1).

5.4.20 **Blastenia purpurea** Vondrák, **sp. nov.**

MycoBank: MB 822488; Fig. 8K

Etymology: Named after the C+ purple spot reaction in the whole apothecial surface.

Type: Portugal. Madeira, Funchal, Curral das Freiras, at hill Pico do Gato, alt. 1600-1800 m, 32.739043° N, 16.933149° W, on base-rich volcanic rock, 6 March 2015, *Jan Vondrák 13101* (holotype, PRA).

Type sequences: MF114720 (ITS); MF114943 (mtLSU); MF115076 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey; young thalli with flat areoles up to 150 µm thick, but old thalli with an uneven upper surface of areoles may be thicker (up to 500 µm); vegetative diaspores absent; apothecia usually dark red, 0.7–1.2 mm diam.; ascospore length (13.0–)15.9(–18.0) µm [1.43; 1; 10]; pycnidia red. *Blastenia crenularia* and *B. festivella* may occur together with *B. purpurea*, but the latter species has a usually deeper red tinge of apothecia and a specific chemistry.

Chemistry: Chlorinated anthraquinones in the apothecial disc and exciple (in closely related *B. crenularia*, chlorinated anthraquineones only in exciple); nonchlorinated anthraquinone chemosyndrome absent; thallus without anthraquinones; Cinereorufa-green in the thallus.

Ecology: Epilithic, on hard volcanic rocks (often with *B. festivella*); known at altitudes 650–1700 m (Fig. 10).

Geography: Known only from the Canary Islands (La Palma, four localities) and Madeira (two localities).

Genome size: 34.2 Mb (CV = 7.8), measured in specimen Vondrák 12629 (La Palma).

Phylogeny: According to all analyses (Figs. 1–3), *B. purpurea* belongs to the Crenularia group and is closely related to *B. crenularia*. BP&P supported *B. purpurea* as a delimited species (PP = 1).

5.4.21 **Blastenia relicta** Arup & Vondrák, **sp. nov.**

MycoBank: MB 822489; Fig. 8L

Type: Sweden. Östergötland, Boxholm, Ö Trehörningen about 10 km NW of Melaxander, alt. 55 m, 58.103393° N, 15.176726° E, on the bark of *Fraxinus excelsior* trunk, 12 May 2012, *Ulrika Nordin FU7663* (holotype, LD).

Type sequences: MF114667 (ITS); MF114911 (mtLSU); MF115034 (beta-tubulin).

Etymology: The epithet reflects the relict character of the species.

Diagnosis: *Morphology:* Thallus crustose, white to grey, <150 µm thick; vegetative diaspores absent; apothecia red, 0.6–1.2 mm diam.; ascospore length (10.5–) 12.3–13.6–15.1(–18.0) µm [1.84; 4; 40]; pycnidia rarely present, red, with anthraquinones. Hardly distinguishable from *B. ferruginea* (but the two species are geographically distinct).

Chemistry: Nonchlorinated anthraquinones in the apothecial disc and in excipulum parts adjacent to disc; chlorinated anthraquinones in exciple, but often reduced to outer excipular ring (in the similar *B. ferruginea*, chlorinated anthraquinones in the whole surface of true exciple); thallus without anthraquinones or rarely with traces; Cinereorufa-green only in prothallus.

Ecology: Epiphytic, on bark of tree trunks (*Fraxinus*, *Quercus*, *Populus*, *Salix*, etc.) at low altitudes in Scandinavia (up to 350 m), but up to 1230 m in Spain.

Geography: Known from southern Scandinavia (Norway, Sweden), with the northernmost record confirmed by DNA data at 63.15° N, and from Spain (Asturias, Castilla y León, Castilla La Mancha, Galicia, La Rioja).

Genome size: 30.5 Mb (CV = 7.5); measured in sample Vondrák 17886 (Spain).

Phylogeny: Blastenia relicta forms a group by itself (Figs. 1–3). It forms a sister group to a clade with the Festivella, Hungarica and Psychrophila groups in the concatenated tree (Fig. 2), but its relationship to other groups is unresolved in the *BEAST species tree (Fig. 3). Its position in single-locus trees is incongruent (Fig. 1); whereas in the mtLSU tree, it is sister to the Crenularia and Herbidella groups, in the beta-tubulin tree, it belongs to the clade with the Limitosa, Hungarica and Psychrophila groups. BP&P supported *B. relicta* as a delimited species (PP = 1).

Note: *Blastenia ferruginea* auct., as understood by Arup et al. (2013), refers to *Blastenia relicta* (see also the note below *B. ferruginea*).

5.4.22 **Blastenia remota** Obermayer & Vondrák, **sp. nov.**

MycoBank: MB 822490; Fig. 8M

Etymology: The epithet reflects the geographic isolation of the species from the Mediterranean-Macaronesian hot-spot of *Blastenia* diversity.

Type: China. Sichuan, Daxue Shan, 57 km S of Kangding, Gongga Shan, Hailougou glacier and forest park, alt. 3180-3240, 29.56666° N, 101.96666° E, on *Rhododenron* twigs, 28 July 2000, *Walter Obermayer 9054* (holotype, GZU).

Type sequences: MF114600 (ITS); MF114862 (mtLSU); MF114995 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey, <100 µm thick; vegetative diaspores absent; apothecia usually dark red, 0.4–0.9 mm diam.; ascospore length (12.0–)14.0–16.1–17.3(–19.5)

μm [2.17; 3; 30]; pycnidia red, but sparse or absent. Morphologically similar to European species of the Herbidella group, but geographically and ecologically distinct.

Chemistry: Nonchlorinated anthraquinones in the apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones; Cinereorufa-green in traces (in the thallus, rarely in apothecia).

Ecology: On bark and twigs of trees and shrubs (*Rhododendron*, *Rosa*, *Salix*) in humid montane forests at an altitude about 3000 m.

Geography: Known from two sites, not far apart, in Sichuan, China. All specimens were collected close to the Hailougou glacier (see Table S1 for details).

Genome size: Measurement failed (old material).

Phylogeny: According to all analyses (Figs. 1–3), *B. remota* belongs to the Herbidella group, but its closer relationships are not resolved. BP&P supported *B. remota* as a delimited species (PP = 0.93).

5.4.23 **Blastenia scabrosa** (Søchting, Lorentsen & Arup) S.Y. Kondratyuk, I. Kärnefelt, J.A. Elix, A. Thell, J. Kim, A.S. Kondr. & J.-S. Hur

Caloplaca scabrosa Søchting, Lorentsen & Arup, Nova Hedwigia 87: 89. 2008.

Type: Norway. Svalbard, Nordenskiöld Land, Reindalen N of Sørhytta, alt. 100 m, 77.994450° N, 15.869419° E, on and under overhanging sandstone, 4 August 1986, *Søchting 5513* (holotype, C!; isotypes, BG, LD, PRA!).

Type sequence: KX022975 (ITS).

Description: *Morphology:* Thallus crustose, grey, thalli usually less than 100 μm thick; vegetative diaspores (tiny blastidia to large knobbly isidia) present, granular or with an irregular shape, 40–130 μm diam.; isidia/blastidia, when dense, give the thallus a thicker appearance (up to 400 μm); apothecia red, 0.8–1.2 mm diam.; ascospore length (12.0–)13.5–14.0–14.3(–17.0) μm [1.05; 3; 30]; pycnidia red, sparse or absent; more information in Søchting et al. (2008); Vondrák et al. (2013); Frolov & Konoreva (2016).

Chemistry: Nonchlorinated anthraquinones in apothecial disc, chlorinated anthraquinones in exciple; thallus without anthraquinones; Cinereorufa-green in thallus, more concentrated in tips of isidia; atranorin in thallus (reported by Søchting et al., 2008 and Vondrák et al., 2013b) was not confirmed by our TLC results.

Ecology: Epilithic, on vertical and overhanging, base-rich siliceous rocks in the arctic and subalpine/alpine zone in European mountains. It occurs at low altitude in the Arctic but is restricted to high altitudes in more southern mountains (1250–2500 m).

Geography: Although described from a single locality in Svalbard (Søchting et al., 2008), it has since been found in Hrubý Jeseník Mountains in the Czech Republic (Vondrák et al., 2013; Vondrák & Malíček, 2015), Tatra Mountains in Poland (Wilk, 2015), Caucasus Mountains in Abkhasia, Murmansk region in Russia, Torne Lappmark in Sweden (Frolov & Konoreva, 2016) and here it is newly reported from Sierra Nevada in Spain.

Genome size: 33.3 Mb (CV = 11.1), measured in specimen Vondrák 13628 (Hrubý Jeseník Mts.).

Phylogeny: According to all analyses (Figs. 1–3), *B. scabrosa* belongs to the Psychrophila group and is closely related to *B. ammiospila* (Figs. 2 and 3). BP&P supported *B. scabrosa* as a delimited species (PP = 1).

5.4.24 **Blastenia subathallina** (H. Magnusson) Arup & Vondrák, **comb. nov.**

MycoBank: MB 822491

Caloplaca subathallina H. Magnusson, Botaniska Notiser 1951 (1): 82. 1951.

Type: Sweden. Gotland, Östergarn, Grogarnsberget, corticolous, August 1871, *Wilhelm Molér* (lectotype, S; lectotype selected here, MBT386429).

Caloplaca depauperata H. Magnusson, K. Vet. O. Vitterh. Samh. Handl., f. 6. ser. B. 3(1): 29–30 (1944b); nomen illegitimum (later homonym).

Description: *Morphology:* Thallus crustose, grey, <100 μm thick; vegetative diaspores absent; apothecia dark red (rarely pale red), 0.3–0.5 mm diam.; ascospore length (12.0–)13.3–13.5–13.8(–15.0) μm [0.83; 3; 31]; pycnidia dark grey with Cinereorufa-green, but usually sparse or absent. Distinct from other species of the Hungarica group with small apothecia by red (not orange) apothecia with chlorinated anthraquinones in the whole surface.

Chemistry: Chlorinated anthraquinones in apothecia; nonchlorinated anthraquinone chemosyndrome with parietin reduced or absent; thallus without anthraquinones; Cinereorufa-green hardly detectable.

Ecology: Usually on twigs of trees (19 records) and shrubs (8 records); more rarely on tree trunks (10 records); not recorded on wood. Associated with a number of deciduous and coniferous tree and shrub species (Table S1). Occurring only at lower altitudes in southern Scandinavia (up to 100 m), in a broad altitudinal range in the Mediterranean basin (0–1500 m) and in the range 1000–1500 m in Madeira and the Canary Islands (Fig. 9). *Geography:* Throughout the Mediterranean region. Known from Bosnia and Herzegovina, France, Greece, Italy, Russia (western Caucasus), Spain and Turkey. In Macaronesia, it is known from La Palma and Madeira. North of the Mediterranean basin, known only in southern Scandinavia (Sweden; up to 58.9° N).

Genome size: 21.6 and 25.9 Mb (CV = 11.8 & 9.0); measured in samples Vondrák 12105 (La Palma) and 13107 (Madeira).

Phylogeny: According to all analyses, it belongs to the Hungarica group and is closely related to *B. hungarica* (Figs. 1–3). BP&P supported *B. subathallina* as a delimited species (PP = 1).

Note: Distinguished from all other species of the Hungarica group by its chemistry. Other species contain nonchlorinated anthraquinone chemosyndrome, but *B. subathallina* only has the chlorinated chemosyndrome (Fig. 6).

5.4.25 **Blastenia xerothermica** Vondrák, Arup & I.V.Frolov, **sp. nov.** subsp. xerothermica

MycoBank: MB 822492; Fig. 8N

Etymology: The epithet reflects the occurrence of the species in dry, warm (i.e., xerothermic) habitats.

Type: France. Alpes-de-Haute-Provence, Gorges du Verdon, SW-S from La Palud-sur-Verdon, alt. ca. 850 m, 43.762933° N, 6.317004° E, on twigs of *Pinus halepensis* in the submediterranean sparse forest on limestone on SE slope, 9 May 2015, *Ivan Frolov 1033* (holotype, PRA; isotype, herb. Frolov).

Type sequences: MF114743 (ITS); MF114955 (mtLSU); MF115091 (beta-tubulin).

Diagnosis: *Morphology:* Thallus crustose, grey, <100 µm thick; vegetative diaspores absent; apothecia orange to pale red, 0.3–0.7 mm diam.; ascospore length (10.0–) 12.7–13.5–15.0(–16.0) µm [1.28; 7; 61]; pycnidia dark grey with Cinereorufa-green, but usually sparse or absent. Morphologically indistinguishable from *B. hungarica* (which prefers upper altitudes and is distributed also outside the Mediterranean) and *B. palmae* (distinct in geographical range).

Chemistry: Nonchlorinated anthraquinones in apothecia; chlorinated anthraquinones strongly reduced or absent; thallus without anthraquinones; Cinereorufa-green usually hardly detectable, but present around pycnidial ostioles and also present in injured apothecia.

Ecology: Epiphytic on trunks of trees (18 records), twigs of trees (31 records) or twigs of shrubs (41 records). Associated with a number of deciduous and coniferous tree and shrub species (Table S1). Occurring mostly at lower altitudes in the Mediterranean regions, but reaching 1550 m in Spain (Fig. 9). The subsp. *macaronesica* has a different ecology (see below).

Geography: Restricted to the Mediterranean basin: known from Albania, southern France, Greece, Italy, Spain and Turkey. The Macaronesian population is separated as a subspecies (see below). Ranges of the subspecies probably do not overlap.

Genome size: 34.1 Mb (CV = 8.9); measured in holotype.

Phylogeny: According to all analyses, *B. xerothermica* belongs to the Hungarica group and it forms a sister group to *B. hungarica*, *B. palmae* and *B. subathallina* (Figs. 2 and 3). Its position in single-loci phylogenies is incongruent: it is a part of the Hungarica group in the beta-tubulin tree, but it is related to the Herbidella and Crenularia groups in the mtLSU tree; its position is unresolved in the ITS tree (Fig. 1). *Blastenia xerothermica* is clearly divided into two clades in the beta-tubulin phylogeny (Fig. 1); they are treated here as geographically separated subspecies. The substantial within-species genotype variability is due to differences between the subspecies (Table 3). Both subspecies in *B. xerothermica* are supported by BP&P as delimited taxa (PP = 0.98).

Note: We did not find any morphological characters separating *B. hungarica*, *B. palmae* and *B. xerothermica*. Nevertheless, *B. xerothermica* occupies a different niche than the other two species. In the Mediterranean basin, it occurs at lower altitudes than *B. hungarica* (Fig. 9), but both species co-occur in some regions (e.g., both species occur in the area of Gorges du Verdon in France). *Blastenia xerothermica* is absent from coastal areas in the south-western part of the Iberian Peninsula where *B. palmae* is common. In Macaronesia, *B. xerothermica* (subsp. *macaronesica*) occurs in "subalpine" habitats above the altitudinal range of *B. palmae* (Fig. 9).

5.4.26 **Blastenia xerothermica** subsp. **macaronesica** Vondrák, **subsp. nov.**

MycoBank: MB 822493; Fig. 8O

Etymology: The epithet reflects the geographical range of the subspecies, parts of Macaronesia.

Type: Portugal. Madeira, Funchal, Curral das Freiras, at hill Pico do Gato, alt. 1750 m, 32.739043° N, 16.933149° W, on dead twigs of *Sarothamnus* shrubs, 6 March 2015, *Jan Vondrák 13103* (holotype, PRA).

Type sequences: MF114722 (ITS); MF114945 (mtLSU); MF115077 (beta-tubulin).

Diagnosis: *Morphology & Chemistry:* As in *B. xerothermica* subsp. *xerothermica* (see above).

Ecology: Epiphytic on trunks, twigs and wood of *Pinus canariensis* (three records) or on alpine shrub twigs (two records) at 1750–2200 m altitudes, above the zone of morphologically identical *B. palmae* (Fig. 9).

Geography: Macaronesia, known from La Palma, Tenerife and Madeira.

Genome size: 32.1 (CV = 6.5); measured in sample Vondrák 12111 (La Palma).

Phylogeny: In the beta-tubulin phylogeny, it forms a clade within the Hungarica group separated from the subsp. *xerothermica*; in ITS, it forms a supported group within the *B. xerothermica* clade, and in mtLSU, it is unresolved from the other subspecies (Fig. 1). It forms a supported lineage inside the subsp. *xerothermica* in the concatenated tree (Fig. 2). BP&P supported this taxon as a delimited species (PP = 0.98), recently separated from the subspecies *xerothermica* (Fig. 3).

Note: Although the taxon is supported as a delimited species by BP&P, we prefer to be conservative and describe it at the rank of subspecies. It is sufficiently resolved only in the beta-tubulin single-gene phylogeny. In the concatenated and other single-gene phylogenies, it is not resolved from *B. xerothermica* subsp. *xerothermica*.

5.5 Key to *Blastenia* species in western Eurasia and Macaronesia

For correct identifications of specimens from regions that are rich in species (especially the Mediterranean), we recommend confirmation by the ITS barcode. It is helpful for distinguishing the following species: (i) *Blastenia hungarica* and *B. xerothermica*; (ii) *B. coralliza* and *B. herbidella*; (iii) *B. ferruginea*, *B. lauri* and *B. relicta*. The key is supplemented by Notes 1–9 (see below).

1a. Apothecia orange to pale red; chlorinated anthraquinones absent or reduced in apothecia (negative spot reaction with diluted hypochlorite solution; C-); apothecia rarely exceeding 0.7 mm diam.; pycnidia dark grey, with Cinereorufa-green; most commonly on the bark of trunk or twigs or on wood……2

1b. Apothecia pale to dark red or rusty red; chlorinated anthraquinones present, at least in outer part of apothecial margin (strong spot reaction with hypochlorite; C+ purple); apothecia of various size; pycnidia red or dark grey; substrates various……..5

2a. In Azores, Canary Islands, Madeira or Atlantic coast of Spain and Portugal……..3

2b. In another region……..4

3a. Above 1500 m, in subalpine and upper *Pinus canariensis* belt (known in Tenerife, La Palma and Madeira)…… ……..... **B. xerothermica** subsp. **macaronesica**

3b. Below 1500 m in Macaronesia; in Spain and Portugal (continent), restricted to coastal areas…… …….. **B. palmae**

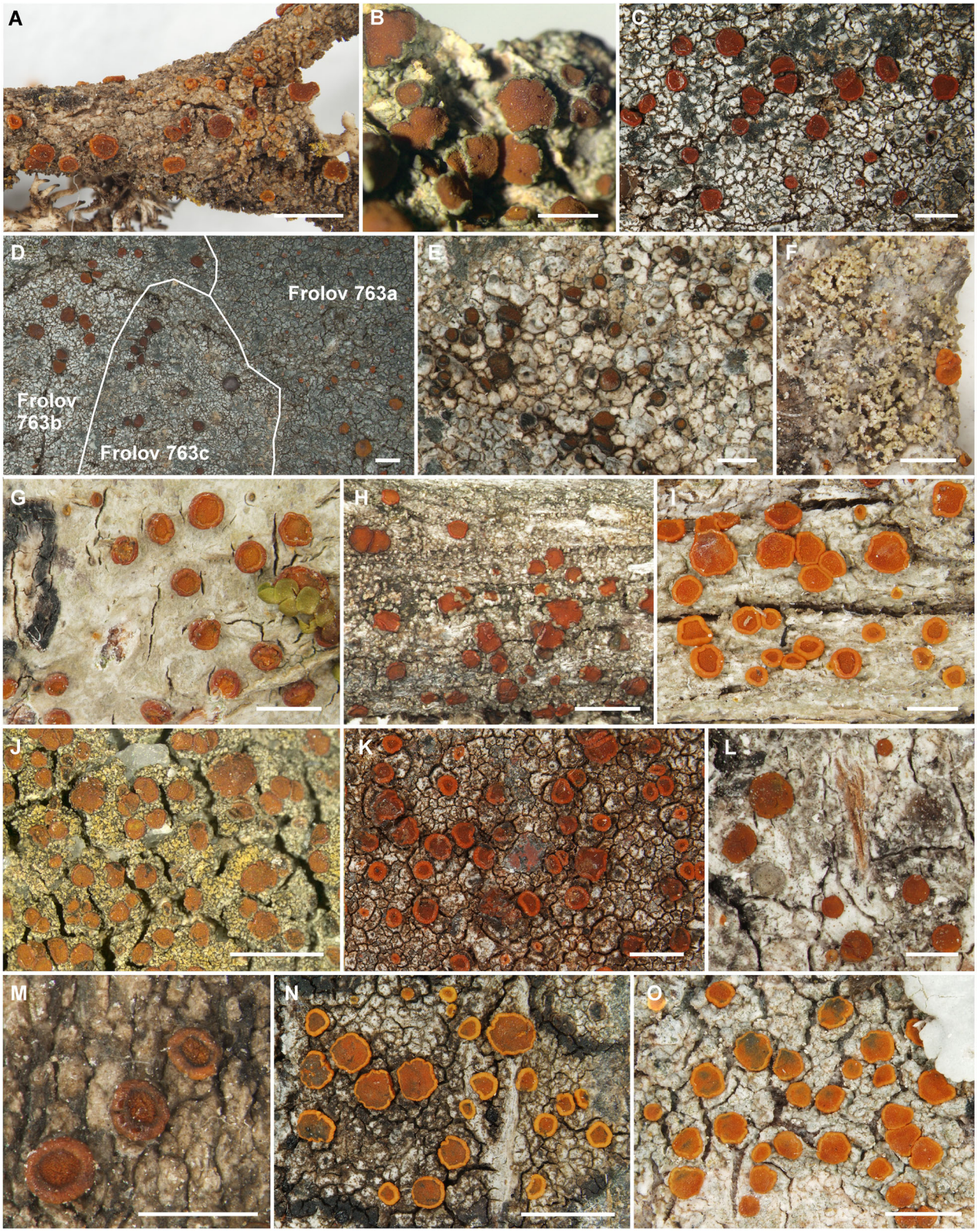

Fig. 8. Holotypes of the new species and subspecies. **A,** *Blastenia afroalpina*. **B,** *B. anatolica*. **C, D,** *B. caucasica*. **E,** *B. gennargentuae*. **F,** *B. herbidella* subsp. *acidophila*. **G,** *B. lauri*. **H,** *B. monticola*. **I,** *B. palmae*. **J,** *B. psychrophila*. **K,** *B. purpurea*. **L,** *B. relicta*. **M,** *B. remota*. **N,** *B. xerothermica* subsp. *xerothermica*. **O,** *B. xerothermica* subsp. *macaronesica*. All scales: 1 mm.

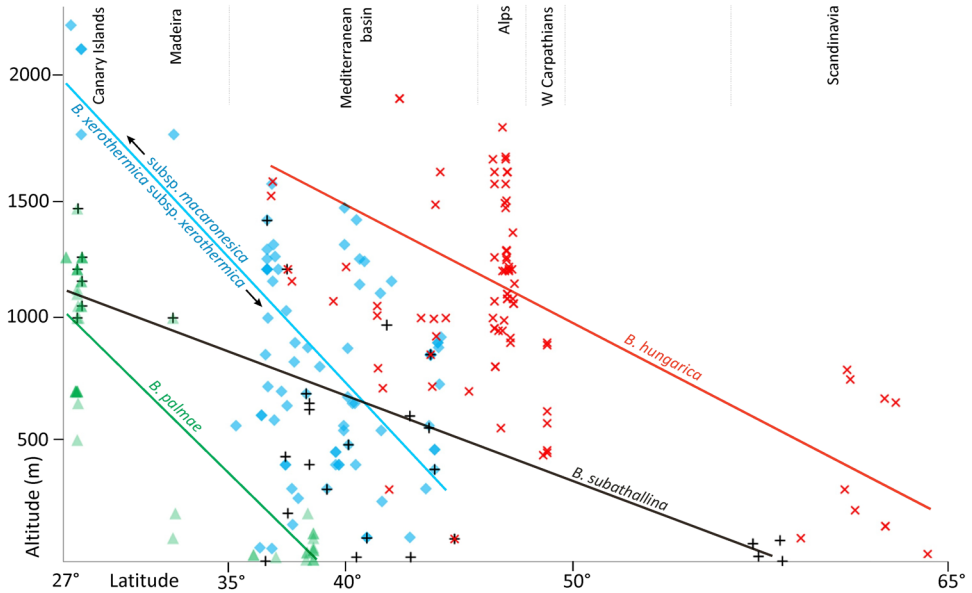

Fig. 9. Latitude/altitude range of species within the Hungarica group: *B. hungarica* (red inclined crosses), *B. palmae* (green triangles), *B. subathallina* (black crosses) and *B. xerothermica* (blue inclined squares). Trend curves interposed by hand.

4a. In Mediterranean lowlands; common in maquis shrublands…… **B. xerothermica** subsp. **xerothermica**

4b. North of the Mediterranean regions……**B. hungarica**

4c. In colder Mediterranean areas; mostly in mountains above 1000 ……**B. xerothermica** subsp. **xerothermica** or **B. hungarica** (identification requires ITS DNA barcode)

5a. Chlorinated anthraquinones (recognized by C+ purple spot test; see methods for details) in apothecial disc and margin……6

5b. Chlorinated anthraquinones restricted to apothecial margin……8

6a. Apothecia small, usually below 0.5 mm diam. (mean of our measurements 0.36 mm); ascospores 12–15 µm long, Cinereorufa-green hardly detected; on twigs (preferred) or trunk bark……**B. subathallina** (see note 1)

6b. Apothecia larger, usually above 0.5 mm diam. (means 0.86 and 0.89 mm); ascospores 13–18 µm long; Cinereorufa-green present or not; epiphytic or epilithic……7

7a. Mostly arctic-alpine; on organic substrates (bryophytes, plant debris, shrub twigs, wood, rarely bark); atranorin absent from thallus or in traces (not detected by KOH spot reaction); Cinereorufa-green usually not detectable in sections; apothecia flat, rarely slightly convex……**B. ammiospila** (see note 2)

7b. Restricted to Macaronesia; on volcanic rocks; atranorin absent; Cinereorufa-green detectable in sections; apothecia usually flat……**B. purpurea** (see note 3)

8a. Vegetative diaspores absent……9

8b. Vegetative diaspores present……16

9a. On inorganic substrates; thallus white to dark grey, epilithic; old areoles often convex or with uneven surface and more than 150 µm thick……10

9b. On organic substrates (usually bark of tree trunks); thallus white to pale grey, endophloedal or thinly epiphloedal; areoles flat to slightly convex, usually with even surface (up to 150 µm thick)……14 (see note 4)

10a. In mountains (mostly in the alpine zone); not in Macaronesia……11

10b. Below alpine zone; some species present in Macaronesia……13

11a. Pycnidia dark-grey to black, with Cinereorufa-green; apothecial margin often blackened, with Cinereorufa-green; medulla often black accumulating Cinereorufa-green; ascospores small, mostly less than 15 µm long; in dry Mediterranean mountains (known from Calabria and Sardinia)……**B. gennargentuae** (see note 5)

11b. Pycnidia red, with anthraquinones; blackened apothecia with Cinereorufa-green rare; medulla, when present, without Cinereorufa-green; ascospore size variable; in humid mountains in western Eurasia……12

12a. In Caucasus Mts……**B. caucasica**

12b. In other regions……**B. psychrophila**

13a. Thallus usually distinctly delimited by black prothallus line (forming black lines surrounding thalli); pycnidia black (with Cinereorufa-green) or red (with anthraquinones); hymenium not inspersed; ascospores usually less than 15 µm long; restricted to coastal areas; distributed in the Mediterranean (Spain to Turkey) and Atlantic coast of Europe (Spain and Portugal); common in Macaronesia, mostly at altitudes up to 1000 m……**B. festivella**

13b. Black prothallus marginal rings usually indistinct; pycnidia red with anthraquinones; hymenium often inspersed; ascospores often more than 15 µm long; in coastal areas and inland; in Macaronesia at altitudes above 1000 m (above the zone of B. festivella)……**B. crenularia**

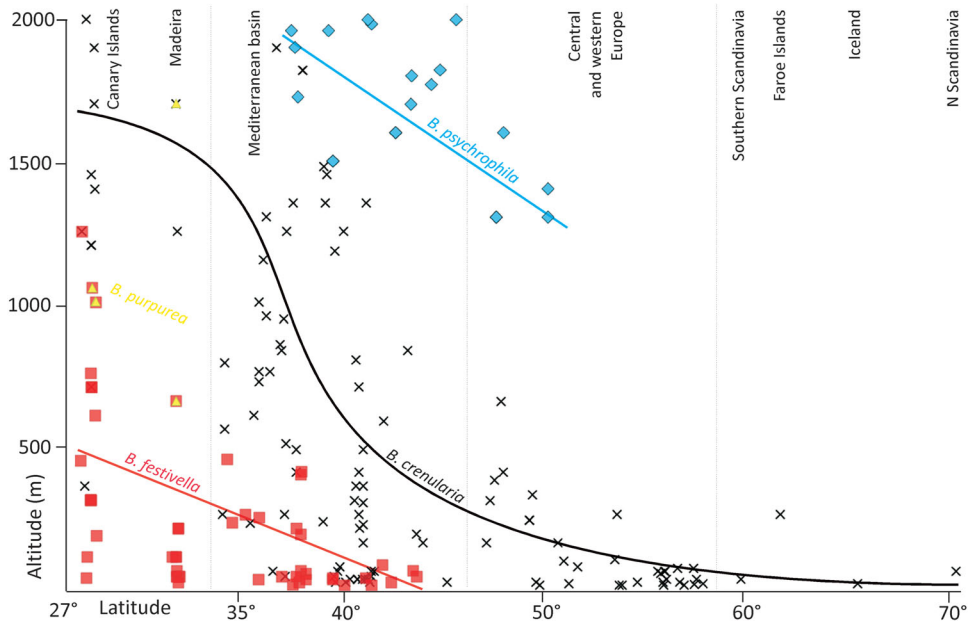

Fig. 10. Latitude/altitude range of phenotypically similar epilithic *Blastenia* species without vegetative diaspores: *B. festivella* (red squares), *B. purpurea* (yellow triangles), *B. crenularia* (black crosses) and *B. psychrophila* (blue inclined squares). Trend curves interposed by hand.

14a. In Macaronesia and in eu-oceanic Europe (known in Scotland, Ireland)……**B. lauri**

14b. In other regions……15

15a. In Mediterranean regions; outside the Mediterranean known only in southern Great Britain; chlorinated anthraquinones in the surface of whole apothecial margin……**B. ferruginea**

15b. Known from Scandinavia and Spain; chlorinated anthraquinones restricted to a thin ring of the outer part of apothecial margin……**B. relicta**

16a. Epilithic, arctic-alpine……
……**B. scabrosa** (see note 6)

16b. Epiphytic, ecology and distribution various……
……17 (see note 7)

17a. Boreal-montane to arctic-alpine; mostly in acidophilous lichen communities in open coniferous forests or tundra-like habitats; with globose or coralloid blastidia/isidia……18

17b. Not boreal and not arctic-alpine; in various forest types; in species-rich, slightly nitrophilous and basiphilous lichen communities; typically with coralloid blastidia/isidia……21

18a. With granular or coralloid vegetative diaspores not exceeding 100 µm diam……19

18b. With granular vegetative diaspores, commonly exceeding 100 µm diam……20

19a. With tiny granular blastidia, 30–70 µm diam.; thallus usually grey, but rarely yellow (known from Altai Mts.); on wood (preferred) and bark; boreal……
……**B. furfuracea**

19b. With granular and coralloid blastidia/isidia, ca. 50–100 µm wide; only grey thallus seen; on twigs (preferred) and trunk bark; in Ural Mts……
……**B. herbidella** subsp. **acidophila**

20a. In Turkey or in Caucasus Mts……
……**B. anatolica** or **B. monticola** (see note 8)

20b. In other regions……**B. monticola**

21a. In Canary Islands……**B. coralliza**

21b. In Central Europe north of the Alps……
……**B. herbidella**

21c. In other regions……22

22a. Pycnidia red, usually present and sometimes abundant; isidia coralloid or granular, 60–170 µm wide, grey (rarely yellow); rare and restricted to mountain forests in Mediterranean regions……**B. herbidella** (see note 9)

22b. Pycnidia red, but rarely present (if present, then usually sparse); isidia coralloid or rarely granular, 50–120 µm wide, yellow or grey; common throughout the Mediterranean……**B. coralliza** (see note 9)

Note 1: *Huneckia pollinii* is very variable and, when growing on twigs, it may also have small apothecia and is hardly distinguishable from *B. subathallina*, but Cinereorufa-green frequently causes blackenings of apothecia in *H. pollinii*. Both species are also distinct in anthraquinones. 7-chloroemodin is the major substance in *B. subathallina*, but chrysophanol, chrysophanal and rhein are major in apothecia of *Huneckia pollinii* (Kondratyuk et al., 2014). Employing TLC on *H. pollinii*, we detected only chrysophanol (yellow spot in the parietin height) and a distinct orange spot in RF 60–70 (solvent C); 7-chloroemodin was not detected. Another similar twig

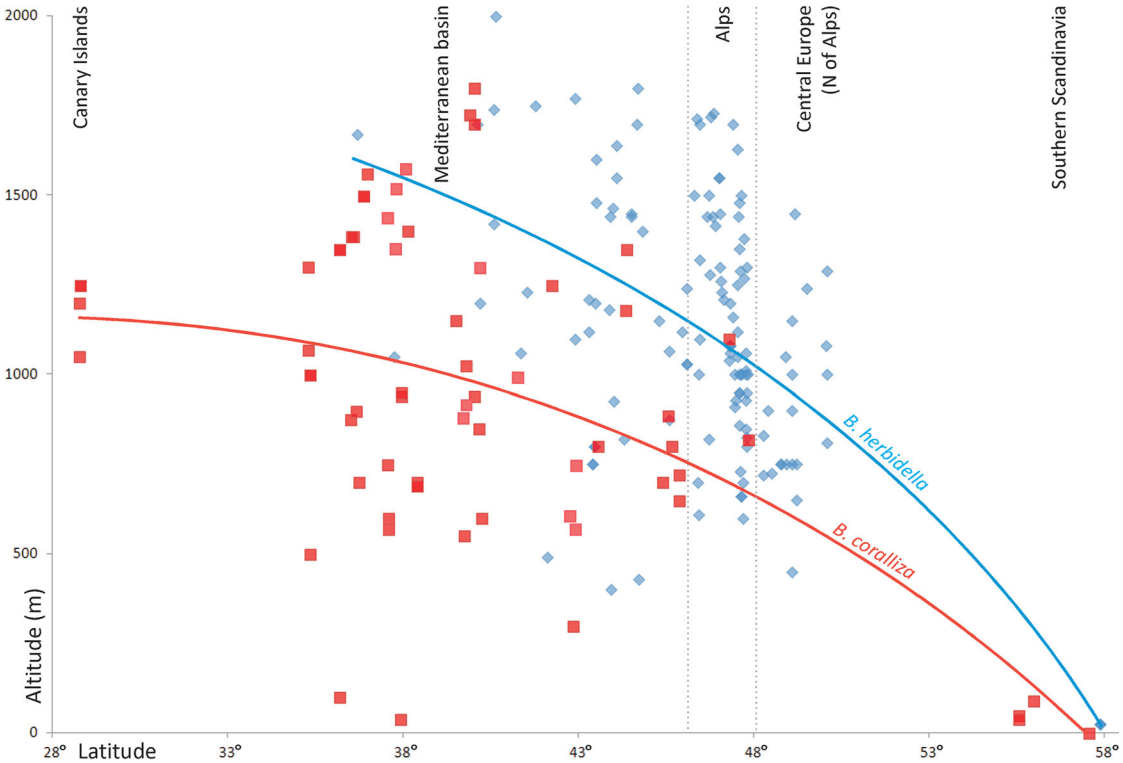

Fig. 11. Latitude/altitude range of *Blastenia herbidella* (red squares) and *B. coralliza* (blue inclined squares). Trend curves interposed by hand.

dwelling species is *Caloplaca asserigena*; it has also small apothecia, but typically dark brown-red with a rusty tinge, and with negative C-reaction, without 7-chloroemodin (Søchting & Fröberg, 2003).

Note 2: Similar species in arctic-alpine habitats, on organic substrates and with chlorinated anthraquinones in apothecia: *Bryoplaca sinapisperma* (atranorin in thallus detected by spot reaction with KOH, more convex apothecia commonly with brown tinge); *Caloplaca caesiorufella* (apothecia usually below 0.5 mm diam. and ascospores 12–14.5 µm long) and *C. spitsbergensis* (similar to *C. caesiorufella*; see Søchting et al., 2008).

Note 3: Chemotypes with positive hypochlorite reaction in whole apothecial surface may be found also in other epilithic species; we observed it in one specimen of *B. caucasica*.

Note 4: Epiphytic species without vegetative diaspores are very similar to each other. Identification of these specimens is especially complicated in the Iberian Peninsula, where *B. ferruginea* and *B. relicta* are present and *B. lauri* is expected. In addition, in Andalusia we recognized epiphytic population of *B. festivella* (see the taxonomic part) and found specimens of *B. coralliza* without vegetative diaspores (Malíček 5561). We recommend the ITS barcode for recognition of specimens from the Iberian Peninsula.

Note 5: Arctic-alpine *Caloplaca fuscorufa* is similar in common expansion of Cinereorufa-green (sometimes also to apothecial discs), but it has larger ascospores, often more than 15 µm long, and pycnidia are unknown (more about *C. fuscorufa* in Arup et al., 2007).

Note 6: *Blastenia psychrophila* with similar ecology may exceptionally have poorly developed coarse isidia, but it is not distinctly blastidiate/isidiate as *B. scabrosa*. Our newest research in the Caucasus Mts revealed blastidiate populations related to *B. caucasica* (unpublished). We consider this population not morphologically separable from *B. scabrosa*.

Note 7: Generally difficult group; for instance in the Caucasus Mts., several species meet and some species show abnormal phenotype variability. Some specimens cannot be unambiguously recognized and ITS sequencing is recommended.

Note 8: *Gyalolechia epiphyta* is similar to yellow-thallus morphotypes of both *B. anatolica* and *B. monticola* and may grow in the same habitats (Vondrák et al., 2016): it has yellow (or rarely grey) blastidiate thallus, red pycnidia containing chlorinated anthraquinones and apothecia with chlorinated anthraquinones accumulated in exciple. It differs in the usual presence of chlorinated anthraquinones also in the disc and it contains fragilin as a dominant anthraquinone (hardly observed on TLC plates behind the parietin spot; HPLC in need).

Note 9: Arup & Åkelius (2009) distinguished these species by some other characters. They considered *B. coralliza* to be rarely fertile with usually yellow-orange thallus (isidia). This may be true in Scandinavia and the Canary Islands, but numerous Mediterranean specimens are richly fertile and have a grey thallus. For instance, in Sierra de las Nieves Mts. (Spain), where *B. coralliza* is very common in upper montane coniferous forests, the species is rich in apothecia and yellow thalli are only exceptional.

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12503/suppinfo:

Fig. S1. Examples of fluorescence intensity histograms for all measured Blastenia species. Minor peaks represent G2 phase of cell cycle. GS, mean genome size [Mb]; CV, mean coefficient of variation of fluorescent intensity histograms [%].

Table S1. List of studied specimens ordered by species names.

Table S2. Results of flow cytometry measurements.

Table S3. Secondary metabolites revealed by mass spectrometry in the negative electrospray ionization (ESI).