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Three new, seemingly-cryptic species in the lichen genus *Caloplaca* (Teloschistaceae) distinguished in two-phase phenotype evaluation

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We describe three new, seemingly-cryptic species in the lichen genus *Caloplaca* (Teloschistaceae) with black apothecia. Those species, separated in nrITS and β-tubulin DNA phylogenies, appeared to be phenotypically indistinguishable. We looked for their phenotypic differences using a two-phase method comprised of a preliminary examination in which diagnostic value of all available characters was evaluated using a small number of samples and potentially-diagnostic characters were selected, and a subsequent detailed study in which characters selected in the first phase were tested using more samples. We found 19 diagnostic characters (continuous and discrete) of which four continuous and three discrete characters could be considered "fully diagnostic", i.e. allowing for correct identification of at least one species. Hence, the three species are not cryptic, but can be distinguished phenotypically. Here, they are formally described as *Caloplaca micromarina* Frolov, Khodos. & Vondrák *sp. nova*, *C. micromontana* Frolov, Wilk & Vondrák *sp. nova* and *C. microstepposa* Frolov, Nadyeina, Khodos. & Vondrák *sp. nova*.

Introduction

A large group within Teloschistaceae, containing lichens without anthraquinones, is formed by *Caloplaca variabilis* and related species, or the genus *Pyrenodesmia sensu* Arup *et al*. (2013). Those authors stated that *Pyrenodesmia* did not seem to be monophyletic, because the *Caloplaca xerica* group (taxa with anthraquinones in apothecial disk) and *C. demissa* were placed in

Pyrenodesmia s. lato. Pending further study, we decided to use the generic name *Caloplaca* in the present paper.

Our reconstructed genealogies based on two nuclear loci (ITS and β-tubulin) showed a number of well-supported lineages within the group (Fig. 1). Some of these lineages, often distant, were morphologically very similar. We selected three phylogenetically distinct groups of specimens that at first seemed morphologically identical to test whether these supposed taxa could be phenotypically recognized using the approach described by Vondrák *et al*. (2013).

All studied specimens of the three taxa share the following features: (1) anthraquinones absent; (2) thallus small, thin to indistinct, but not distinctly endolithic; (3) apothecia small, usually less than 0.5 mm diameter; (4) apothecia immersed to adnate, very rarely sessile; (5) apothecia zeorine; (6) *Sedifolia*-grey pigment present in epihymenium, upper part of exciple and thallus; (7) ascospore septa rather thin, to 4 μm wide; (8) occasional presence of shrunken and dead cells in tips of paraphyses (Fig. 2A and B). None of the three selected taxa are conspecific with any species treated in recent works on the group (Tretiach *et al*. 2003, Tretiach & Muggia 2006, Muggia *et al*. 2008, Vondrák *et al*. 2008, Xahadin *et al*. 2010), which is also demonstrated by our phylogenetic tree (Fig. 1).

We sought forgotten or little known names that could be used for the three taxa studied here. Among a number of potential names for black apothecial *Caloplaca* taxa (many of them listed by Wunder 1974) we found only one potentially relevant name, *C. atroalba* (Tuck.) Zahlbr. Its type is superficially identical to some of our samples. However our subsequent investigation showed that *C. atroalba* is not conspecific with any of the three taxa (*see* the taxonomical note under *C. microstepposa*). As we were unable to find any previously published names for the three taxa, we describe them formally here.

Material and methods

Sampling

Lichen samples were collected mainly by the authors from various European and western Asian localities in 2004–2012 and deposited in the herbaria GZU, KHER, KRAM, KW and PRA. In this paper, we provide full sample data, including information about locality, habitat, collecting and deposition of specimens. Citations of the older herbarium samples from GZU, KRAM, KTC and W are as full as possible. The three new taxa are compared with all similar *Caloplaca* with black apothecia known to us; rich comparative lichen material is deposited in the herbarium PRA (http://botanika.prf.jcu.cz/lichenology/data.php). We also investigated material of *C. aegyptiaca* (holotype G), *C. albopruinosa* (GZU, KRAM, STU, TSB), *C. albovariegata* (lectotype UPS), *C. alociza* (GZU, STU, TSB), *C. atroalba* (lectotype FH; GZU; PRA-V; MIN), *C. aspicilioides* (type G), *C. badioreagens* (isotype GZU, TSB), *C. bullata* (lectotype G), *C. circumalbata* (neotype G), *C. diphyodes* (GZU; holotype H-NYL; KHER; PRA-V), *C. fulva* (isotype W, PRA-V), *C. lecideina* (GZU; isotype G), *C. paepalostoma* (isotype M, PRA-V), *C. paulsenii* (syntype TUR-V), *C. rhinodinoides* (type W), *C. transcaspica* (holotype H-NYL; KHER), and *C. variabilis s. lato* (GZU, H, KRAM, KTC, LE, PRA-V, TUR, W); images of the type specimens are available at http://botanika.prf.jcu.cz/lichenology/index.php?pg=5&func=cat&idx=33#photos. We used literature data (Wunder 1974, Wetmore 1994, 2009, Khodosovtsev *et al*. 2004, Tretiach & Muggia 2006, Muggia *et al*. 2008) as a secondary source of information for comparisons among taxa.

DNA extraction, amplification and sequencing

Simple NaOH extraction (Werner *et al*. 2002) was used for DNA isolation. Primers for PCR amplification of ITS were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990); PCR cycling parameters for ITS follow Ekman (2001). Primers for PCR amplification of β-tubulin were Bt3LM and Bt10LM (Myllys *et al*. 2001); PCR cycling parameters for β-tubulin were: 94 °C for 3 min, 40 × (94 °C for 30 sec, 55 °C for 60 sec, 72 °C for 60 sec), 72 °C for 10 min, hold at 15 °C. Each sequence was provided with GenBank accession number (Table 1).

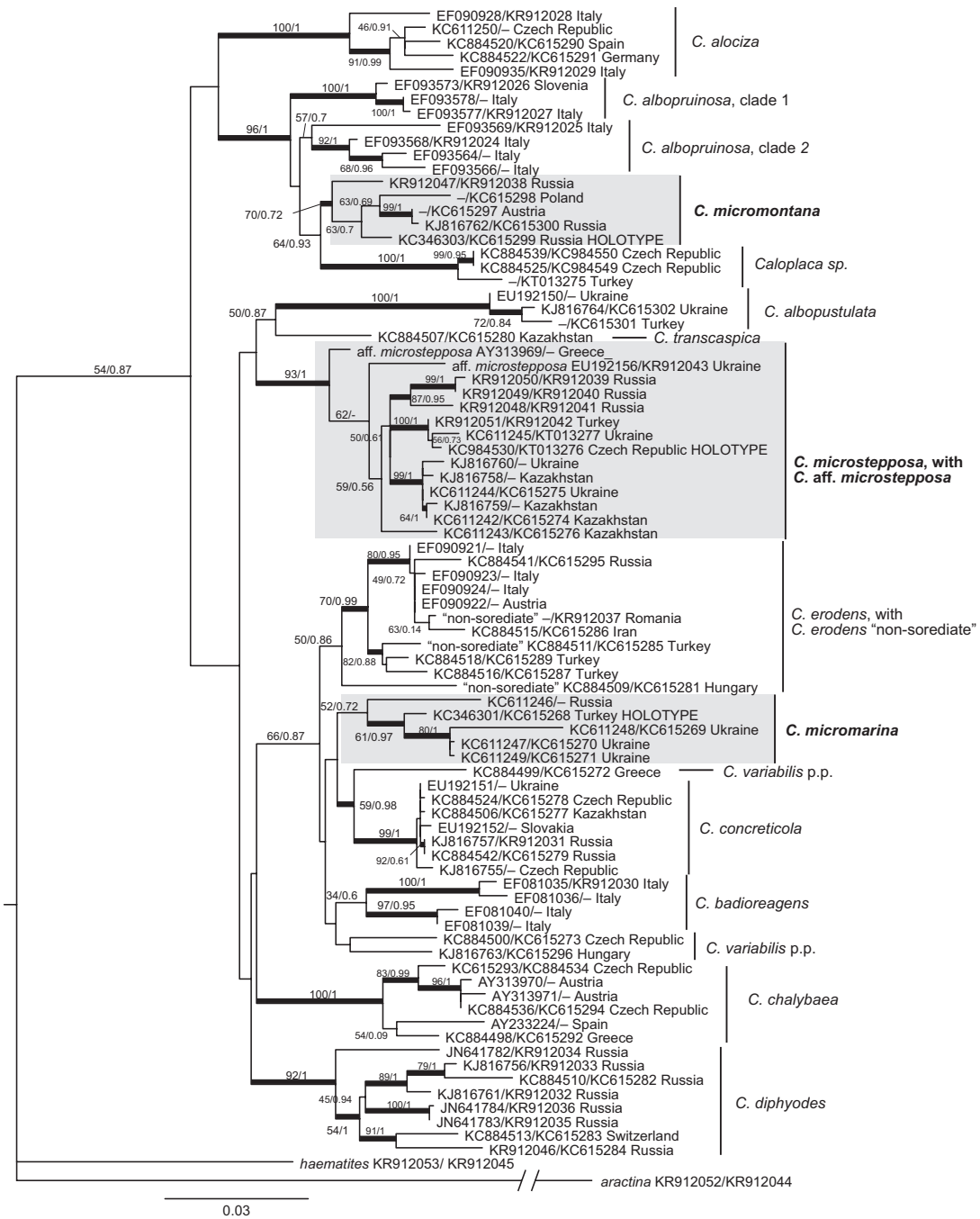

Fig. 1. Phylogenetic position of the newly proposed species of *Caloplaca* within the clade of species related to *Caloplaca variabilis*. The maximum likelihood topology based on a concatenated analysis of ITS and β-tubulin is shown and is annotated with bootstrap support and Bayesian posterior probabilities. Numbers at branches represent bootstrap values ≥ 50% (before forward slash '/') and posterior probabilities values ≥ 0.6 (after forward slash '/'). Branches with bootstrap values ≥ 70% and/or posterior probabilities ≥ 0.95 are thickened. Specimens belonging to species described in this paper are on grey background. ITS and β-tubulin accession numbers are shown before and after forward slash '/', respectively ('–' = missing data). Names at tree tips correspond to those in Table 1.

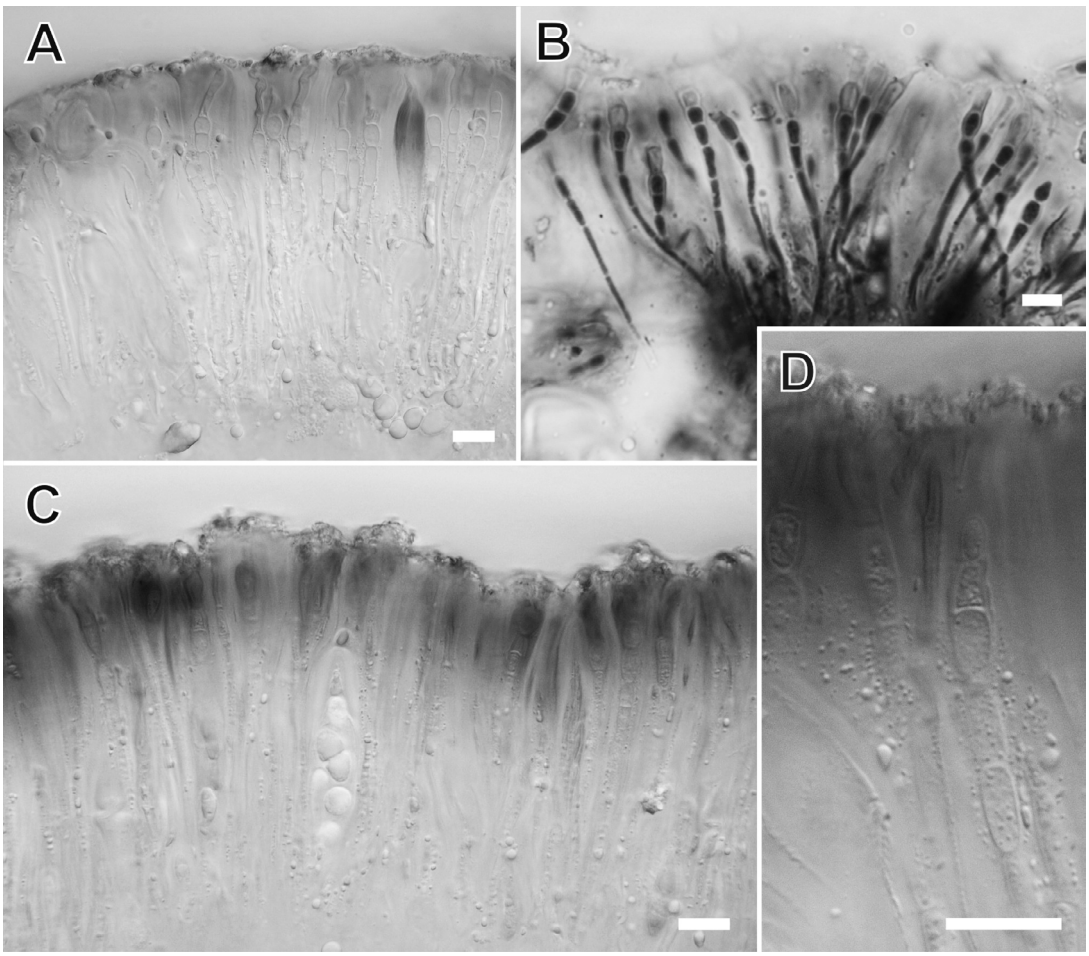

Fig. 2. *Caloplaca microstepposa* (PRA JV9454). — **A**: Paraphyses with dead, thin and shrunken upper cells. — **B**: Paraphyses stained by cotton blue; dead upper cells remained colourless. — **C** and **D**: Inspers hymenium. Bars: 10 μm.

Sequence alignment and phylogenetic reconstructions

Two independent sources of molecular data were chosen for the phylogenetic part of this study: β-tubulin (a protein-coding nuclear gene) and ITS (a non-coding nuclear locus commonly used in phylogenetic studies). The two data sets were aligned using the L-INS-i method of MAFFT ver. 6.847b (Katoh & Toh 2008). The ITS alignment showed a high proportion of unalignable positions and therefore it was subsequently cleared by the *automated1* algorithm as implemented in the *trimAl* software package (Capella-Gutierrez *et al*. 2009). To minimize the impact of computational artifacts and to get the correct reading frame for the protein-coding β-tubulin, we annotated and adjusted nucleotide and translation alignments in Geneious ver. 7.1.8 (Kearse *et al*. 2012). For characters of the alignments *see* Table 2.

Phylogenetic reconstructions were carried out both using a maximum likelihood (ML) and a Bayesian approach. Optimum partitioning of the data set and the optimum substitution models per partition were calculated with the PartitionFinder program (Lanfear *et al*. 2012, 2014). β-tubulin was strongly partitioned, separating the intronic and exonic fractions first and treating all three codon positions independently in the latter. Optimum partition schemes and substitution models were estimated separately for the ML and Bayesian analyses.

Table 1. List of sequences of *Caloplaca* used in the molecular analysis. Vouchers of the new ITS and β-tubulin sequences are set in boldface.

Species (country)	Vouchers	ITS accession	β-tubulin accession
C. albopruinosa (Italy)	Muggia *et al.* 2008	EF093564	–
C. albopruinosa (Italy)	Muggia *et al.* 2008	EF093566	–
C. albopruinosa (Italy)	Muggia *et al.* 2008; **TSB 37658**	EF093577	**KR912027**
C. albopruinosa (Italy)	Muggia *et al.* 2008	EF093578	–
C. albopruinosa (Italy)	Muggia *et al.* 2008; **TSB 37661**	EF093568	**KR912024**
C. albopruinosa (Italy)	Muggia *et al.* 2008; **TSB 37712**	EF093569	**KR912025**
C. albopruinosa (Slovenia)	Muggia *et al.* 2008; **TSB 37068**	EF093573	**KR912026**
C. albopustulata (Ukraine)	***Vondrák 7128* (PRA)**	**KJ816764**	**KC615302**
C. albopustulata (Turkey)	***Vondrák 10463* (PRA)**	–	**KC615301**
C. albopustulata (Ukraine)	Vondrák *et al.* 2008	EU192150	–
C. alociza (Czech Republic)	***Vondrák 9298* (PRA)**	**KC611250**	–
C. alociza (Germany)	***Wirth 19042* (STU)**	**KC884522**	**KC615291**
C. alociza (Spain)	***Vondrák 6272* (PRA)**	**KC884520**	**KC615290**
C. alociza (Italy)	Muggia *et al.* 2008; **TSB 37764**	EF090928	**KR912028**
C. alociza (Italy)	Muggia *et al.* 2008; **TSB 36393**	EF090935	**KR912029**
C. aractina (Greece)	***Vondrák 3806* (PRA)**	**KR912052**	**KR912044**
C. badioreagens (Italy)	Muggia *et al.* 2008; **TSB 36422**	EF081035	**KR912030**
C. badioreagens (Italy)	Muggia *et al.* 2008	EF081036	–
C. badioreagens (Italy)	Muggia *et al.* 2008	EF081039	–
C. badioreagens (Italy)	Muggia *et al.* 2008	EF081040	–
C. chalybaea (Austria)	Tretiach *et al.* 2003	AY313970	–
C. chalybaea (Austria)	Tretiach *et al.* 2003	AY313971	–
C. chalybaea (Spain)	Gaya *et al.* 2003 (as *C. variabilis*)	AY233224	–
C. chalybaea (Greece)	***Vondrák 4059* (PRA)**	**KC884498**	**KC615292**
C. chalybaea (Czech Republic)	***Vondrák 9684* (PRA)**	**KC884536**	**KC615294**
C. chalybaea (Czech Republic)	***Vondrák 9686* (PRA)**	**KC884534**	**KC615293**
C. concreticola (Czech Republic)	***Vondrák 9348* (PRA)**	**KJ816755**	–
C. concreticola (Czech Republic)	***Vondrák 9676* (PRA)**	**KC884524**	**KC615278**
C. concreticola (Kazakhstan)	***Vondrák 9443* (PRA)**	**KC884506**	**KC615277**
C. concreticola (Russia)	***Vondrák 9392* (PRA)**	**KC884542**	**KC615279**
C. concreticola (Russia)	***Vondrák 10465* (PRA)**	**KJ816757**	**KR912031**
C. concreticola (Ukraine)	Vondrák *et al.* 2008	EU192151	–
C. concreticola Slovakia	Vondrák *et al.* 2008	EU192152	–
C. diphyodes (Russia)	***Vondrák 9391* (PRA)**	**KR912046**	**KC615284**
C. diphyodes (Russia)	***Vondrák 8236/1* grey thallus (PRA)**	**KJ816761**	**KR912032**
C. diphyodes (Russia)	***Vondrák 8236/2* pale ochre thallus (PRA)**	**KC884510**	**KC615282**
C. diphyodes (Russia)	***Frolov 50* (Herb. I. Frolov)**	**KJ816756**	**KR912033**
C. diphyodes (Switzerland)	**1984, *J. Poelt* (GZU114-84)**	**KC884513**	**KC615283**
C. diphyodes (Russia)	Vondrák *et al.* 2012; ***Vondrák 8326* (PRA)**	JN641782	**KR912034**
C. diphyodes (Russia)	Vondrák *et al.* 2012; ***Vondrák 8179* (PRA)**	JN641783	**KR912035**
C. diphyodes (Russia)	Vondrák *et al.* 2012; ***Vondrák 8182* (PRA)**	JN641784	**KR912036**
C. erodens (Italy)	Muggia *et al.* 2008	EF090921	–
C. erodens (Austria)	Muggia *et al.* 2008	EF090922	–
C. erodens (Italy)	Muggia *et al.* 2008	EF090923	–
C. erodens (Italy)	Muggia *et al.* 2008	EF090924	–
C. erodens (Iran)	***Vondrák 5579* (PRA)**	**KC884515**	**KC615286**
C. erodens (Russia)	***Vondrák 9387* (PRA)**	**KC884541**	**KC615295**
C. erodens (Turkey)	***Vondrák 8574* (PRA)**	**KC884518**	**KC615289**
C. erodens (Turkey)	***Vondrák 5362* (PRA)**	**KC884516**	**KC615287**

continued

Table 1. Continued.

Species (country)	Vouchers	ITS accession	β-tubulin accession
C. erodens "non-sorediate" (Hungary)	***Vondrák 6380* (PRA)**	**KC884509**	**KC615281**
C. erodens "non-sorediate" (Romania)	***Vondrák 6606* (PRA)**	–	**KR912037**
C. erodens "non-sorediate" (Turkey)	***Vondrák 6659* (PRA)**	**KC884511**	**KC615285**
C. haematites (Spain)	***Vondrák 6259* (PRA)**	**KR912053**	**KR912045**
C. micromarina (Ukraine)	***Vondrák 7236/1* non-pruinose apothecia (PRA)**	**KC611248**	**KC615269**
C. micromarina (Ukraine)	***Vondrák 7236/2* pruinose apothecia (PRA)**	**KC611247**	**KC615270**
C. micromarina (Ukraine)	***Vondrák 6420* (PRA)**	**KC611249**	**KC615271**
C. micromarina (Russia)	***Vondrák 6537* (PRA)**	**KC611246**	–
C. micromarina (Turkey) holotype	***Vondrák 8199* (PRA)**	**KC346301**	**KC615268**
C. micromontana (Austria)	**1994, *J. Poelt* (GZU 52-94)**	–	**KC615297**
C. micromontana (Poland)	***L. Śliwa 3118* (KRAM)**	–	**KC615298**
C. micromontana (Russia) holotype	***Vondrák 9467* (PRA)**	**KC346303**	**KC615299**
C. micromontana (Russia)	***Vondrák 9523* (PRA)**	KJ816762	**KC615300**
C. micromontana (Russia)	***Vondrák 11083* (PRA)**	**KR912047**	**KR912038**
C. microstepposa (Czech Republic) holotype	***Vondrák 9141* (PRA)**	**KC984530**	**KT013276**
C. microstepposa (Kazakhstan)	***Vondrák 9135* (PRA)**	**KC611242**	**KC615274**
C. microstepposa (Kazakhstan)	***Vondrák 9448* (PRA)**	**KJ816758**	–
C. microstepposa (Kazakhstan)	***Vondrák 9452* (PRA)**	**KJ816759**	–
C. microstepposa (Kazakhstan)	***Vondrák 9454* (PRA)**	**KC611243**	**KC615276**
C. microstepposa (Russia)	***Vondrák 10436* (PRA)**	**KR912048**	**KR912041**
C. microstepposa (Russia)	***Vondrák 11071* (PRA)**	**KR912049**	**KR912040**
C. microstepposa (Russia)	***Vondrák 11107* (PRA)**	**KR912050**	**KR912039**
C. microstepposa (Turkey)	***Vondrák 12732* (PRA)**	**KR912051**	**KR912042**
C. microstepposa (Ukraine)	***O. Nadyeina 111* (KW)**	**KC611245**	**KT013277**
C. microstepposa (Ukraine)	***Vondrák 6943* (PRA)**	**KC611244**	**KC615275**
C. microstepposa (Ukraine)	***O. Nadyeina 5-14* (KW)**	**KJ816760**	–
C. aff. *microstepposa* (Greece)	Tretiach *et al.* 2003	AY313969	–
C. aff. *microstepposa* (Ukraine)	Vondrák *et al.* 2008; ***Vondrák 5466* (PRA)**	EU192156	**KR912043**
C. transcaspica (Kazakhstan)	***Vondrák 9432* (PRA)**	**KC884507**	**KC615280**
C. variabilis p.p. (Greece)	***Vondrák 4219* (PRA)**	**KC884499**	**KC615272**
C. variabilis p.p. (Czech Republic)	***Vondrák 5114* (PRA)**	**KC884500**	**KC615273**
C. variabilis p.p. Hungary	***Vondrák 6357* (PRA)**	**KJ816763**	**KC615296**
C. sp. (Czech Republic)	***Vondrák 9140* (PRA)**	**KC884539**	**KC984550**
C. sp. (Czech Republic)	***Vondrák 9673* (PRA)**	**KC884525**	**KC984549**
C. sp. (Turkey)	***Vondrák 9814* (PRA)**	–	**KT013275**

Table 2. Summary of phylogenetic analyses.

Alignment	Number of sequences	Length of alignment	Informative positions (all/ingroup only)
β-tubulin	61	783	186/168
ITS	79	537	188/179
Concatenated	84	1320	487/451

Maximum likelihood reconstructions were carried out in *RAxML* (Stamatakis *et al*. 2005) through the *RAxMLGUI* interface (Silvestro & Michalak 2012). The analysis included three partitions: ITS with the GTR + I + G substitution model, first and second codon positions of

β-tubulin exon also with the GTR + I + G model, and the third codon position of β-tubulin and the intronic fraction together with the GTR + G model. Bootstrap support was calculated on 1000 bootstrap replicates using thorough bootstrapping.

Bayesian reconstructions were carried out in MrBayes 3.2.5. (Ronquist & Huelsenbeck 2003) using the five-partition scheme in which the GTR + G model was used for ITS, K80 for the β-tubulin intron while the exon was partitioned in three codon positions with the SYM + I, JC and GTR + G substitution models used for first, second and third codon positions respectively. Four independent runs with four incrementally heated chains each were run for 80 000 000 sampling every 4000 tree to avoid autocorrelation. All sampled topologies were summarized in a strict consensus tree. In addition the nodal support for the ML topology in the Bayesian sample was calculated using function SumTrees from the Dendropy package (Sukumaran & Holder 2010).

Phenotype evaluation

We employed a two-phase approach consisting of (1) a preliminary study in which diagnostic value of all available characters is evaluated using a small number of samples and potentially-diagnostic characters are selected, and (2) a subsequent detailed study in which characters selected in the first phase are tested using more samples. Three samples, previously used in the phylogenetic analysis, were selected for each seemingly-cryptic taxon for the preliminary study: *C. micromarina* (Ukraine, PRA JV6420; Ukraine, PRA JV7236; Turkey, PRA JV8199); *C. micromontana* (Russia, PRA JV9467; Russia, PRA JV9523; Poland, *L. Śliwa 3118*, KRAM); *C. microstepposa* (Czech Republic, PRA JV9141; Kazakhstan, PRA JV9454; Ukraine, *O. Nadyeina 5-14*, KW). First, in each of those samples we evaluated 29 continuous and about 60 discrete characters (data available from the first author; for the list of the characters and methods of their investigation *see* Vondrák *et al*. 2013). Discrete characters constant in all samples of any one species but different in all other samples were selected for the next phase. Most powerful continuous characters were selected with the help of linear discriminant analysis (*see* below). Second, characters selected in the preliminary study were tested using 10 specimens of *C. micromarina*, 11 of *C. micromontana* and 11 of *C. microstepposa*. As a result, we found some characters that could be considered "partly diagnostic" and some "fully diagnostic" (i.e. allowing correct identification of nearly 100% of samples). We considered a continuous character fully diagnostic if the interval between the smallest and the greatest means among the sample means of one species did not overlap with the corresponding interval in at least one other species. A discrete character is considered fully diagnostic if its value in all samples of any one species is different from its value in all other samples.

All morphological observations were done on hand-cut sections in water, without any chemical treatment. Measurement accuracies were 0.5 μm for cells, 1 or 10 μm for larger structures. All measurements of cells include their walls, with exception of tissues with glutinized cell-walls. In each species sample, each measurable character was measured on 10 objects (5–9 if objects were scarce) and the mean values for the sample were calculated. Subsequently, a mean value using all measurements of a given character from all samples of a species was calculated. In the results, the measurements are given in the following format: (minimum)x_1–x_2–x_3(maximum), where minimum and maximum are extreme values in all samples of one species, x_1 is the smallest mean among the sample means of one species, x_2 is the mean calculated using all the values of all samples of one species, x_3 is the greatest mean among the sample means of one species. The total number of measurements from all samples of one species (N), the number of species samples (n), and standard deviation of x_2 (SD) are given for each measured character in brackets [N, n, SD]. Morphological terminology follows Smith *et al*. (2009) and Vondrák *et al*. (2013).

Chemistry

Spot tests with KOH (K), sodium hypochlorite (C), and paraphenylenediamine (P) as well as examination under UV light were performed

in each new species; tissues were also tested on cross sections for amyloidity in the reaction with Lugol's solution (I). Pigments insoluble in acetone were evaluated on cross sections in reactions with K and N (50% nitric acid) following Meyer and Printzen (2000). Extracellular crystals were examined in the reaction with H_2SO_4 for detection of calcium salts (recrystalization into needles of calcium sulphate). Presence of acetone-soluble compounds was tested by HPLC after Feige *et al*. (1993) on a LichroCART 250-4 RP18-e (5 μm) column using an Agilent 1100 Series Chromatograph.

Discriminant analysis

Linear discriminant analysis (LDA) is a simple probabilistic classification technique which searches for a linear combination of variables that best separates two or more categories. It is applied for classifying groups of specimens (e.g. Casale *et al*. 2015) and sometimes for a feature selection, to identify which characters best discriminate groups of objects (e.g. Marcysiak *et al*. 2007).

We employed LDA (in STATISTICA: StatSoft Inc., Tulsa, USA) to evaluate the diagnostic value (discrimination power) of 29 continuous characters measured in the preliminary study (Table 3). Characters were evaluated for the three species together (Table 3, second column) and for each species pair (Table 3, columns 3–5). We arranged the characters in Table 3 according to their diagnostic value, from the best to the worst character, and chose first ten characters

Table 3. Results from linear discriminant analysis (LDA). Ranking of the most explicative continuous characters for the data set comprising the three studied taxa and the discrimination success in distinction between pairs of taxa in the preliminary study (shown only for the most powerful characters).

Character	Rank/ discrimination success	*microstepposa*/ *micromontana*	*micromontana*/ *micromarina*	*microstepposa*/ *micromarina*
Ascospore-septum width	1/0.644	0.7	0.9	0.767
Septum-width/ascospore-length ratio	2/0.644	0.683	0.917	0.783
Width of cells in uppermost true exciple	3/0.567	0.867		0.783
Thallus width	4/0.533	0.666	0.783	0.633
Length of cells in uppermost true exciple	5/0.489	0.717		0.783
Width of widest cell of paraphysis	6/0.489	0.717	0.767	
Ascospore width	7/0.478			0.7
Lower-/upper-paraphysis-width ratio	8/0.467	0.7	0.683	
Width of alveolate cortex plus epinecral layer	9/0.463		0.867	0.717
Apothecium diameter	10/0.444	0.683		0.666
Ascospore length/width ratio	11/0.444			0.733
Width of paraphysis bellow tip	12/0.433		0.65	0.666
Apothecium height	13/0.422			
Algal-layer width	14/0.413	0.683	0.633	
Hypothecium width	15/0.411			
Ascus height	16/0.411			
Ascus width	17/0.400			
Areola width	18/0.400	0.683	0.683	
Areola length	19/0.400		0.683	
Ascospore length	20/0.389			
Thalline-exciple width	21/0.378			
Hypothecium-cell length	22/0.378			
Length of cells in lower true exciple	23/0.376			
Width of cells in lower true exciple	24/0.376			
Thalline-exciple/true-exciple ratio	25/0.367			
Hymenium width	26/0.367			
Hypothecium-cell width	27/0.367			
Algal-cell diameter	28/0.367			
True-exciple width	29/0.344			

for the detailed study (except for "lower-/upper-paraphysis-width ratio" and "conidium length"; *see* the section "Phenotype diagnostics").

Results

Phylogenetic analyses

Single-gene trees (available from the first author) as well as the concatenated two-locus tree (Fig. 1) showed positions of species treated in recent works on the group. *Caloplaca albopustulata*, *C. alociza*, *C. chalybaea*, C. *concreticola* and *C. diphyodes* form well-supported monophyletic groups. *Caloplaca badioreagens* appears monophyletic but is not supported. *Caloplaca transcaspica* is represented by only one terminal, which is not close to any other taxon. The sorediate *C. erodens* forms a clade together with non-sorediate species (called here *C. erodens* "non-sorediate") very similar to *C. albopruinosa*. *Caloplaca variabilis* is a heterogeneous taxon; we included only three samples into analyses (*C. variabilis p.p.*). Three previously unnamed taxa, "*C. micromarina*", "*C. micromontana*" and "*C. microstepposa*", which are revealed as three distinct phylogenetic species (Fig. 1), were subjected to phenotypic investigation.

Phenotype diagnostics

On the basis of the preliminary study and the subsequent LDA analysis (Table 3), we selected eleven continuous and eight discrete, potentially diagnostic characters for the detailed study (Table 4). Of these 19 features four continuous and three discrete characters were considered "fully diagnostic" after the detailed study (with asterisks in Table 4). The character "lower-/upper-paraphysis-width ratio" selected by LDA was not evaluated in the detailed study, because it was strongly correlated with "width of widest cell of paraphysis" (Pearson's correlation coefficient $r = 0.7380$, $p < 0.001$) and its discriminative power was lower. "Conidium length" was not used in the preliminary study, because conidia were observed only in a limited number of samples due to common absence of pycnidia. However, conidial length measured in all available specimens appears to distinguish *C. micromontana* from the other two species (Table 4).

Species descriptions

Caloplaca micromarina Frolov, Khodos. & Vondrák, *sp. nova* (Fig. 3A and B)

MB 803398; sequences of the holotype: KC615268 (β-tubulin), KC346301 (ITS).

TYPE: Turkey. Sea of Marmara coast. Tekirdağ, in valley of small brook near Gaziköy, alt. 20–40 m a.s.l., 40°45´21´´N, 27°20´04´´E, on stones and pebbles of calcareous sandstone, 11 April 2007, *J. Vondrák* (holotype PRA JV8199; isotypes GZU, LD). — PARATYPES: *See* Appendix.

DIAGNOSTIC CHARACTERS: (1) anthraquinones absent; (2) thallus epilithic, but usually less than 200 μm thick, without distinct cortex, with ± identifiable *Sedifolia*-grey; (3) mature apothecia usually less than 0.5 mm diameter, with black disc and true exciple, zeorine; (4) cells in uppermost true exciple 4–8 μm wide; (5) hymenium without extracellular oil drops, sometimes with stacks of crystals; (6) epihymenium and outer part of true exciple grey, with *Sedifolia*-grey, K+ violet; (7) width of widest cell of paraphysis 3–6 μm; (8) ascospores with rather wide septa, 2–4.5 μm.

DETAILED DESCRIPTION: Thallus epilithic, ochre, grey or with white spots on ochre/grey thallus (thallus colour often varible even within one sample or single thallus); forming small irregular spots to several cm wide or sometimes roundish spots to about 1 cm diameter; of tightly arranged, angular to rounded, flat areoles, (0.22)0.34–0.41–0.46(0.70) × (0.20)0.28–0.31–0.33(0.55) mm [30, 3, 0.12 & 0.08]. Thickness of thallus (75)125–161–206(375) μm [90, 9, 61]. Medulla inconspicuous, to about 50 μm thick; cells hardly observable due to presence of extracellular crystals only partly dissolved and recrystallized into needles in H_2SO_4. Algal layer (25)50–55–61(100) μm thick [30, 3, 17]; algal cells globose, (8.0)12.9–14.3–16.4(20.0) μm diameter [30, 3, 3.1]. Cortex not developed; alveolate cortex usually present. Epinecral layer often present, but its boundary with alveolate cortex indistinct. Thickness of alveolate cortex

Table 4. Characters evaluated in the detailed study; eleven continuous and eight discrete characters. Continuous characters are ordered as in Table 3. The character "lower-/upper-paraphysis-width ratio" selected for the detailed study was not considered, because it is strongly correlated (*see* the section "Phenotype diagnostics") with "width of widest cell of paraphysis" hence its discriminative power is lower. Length of conidia appears to be a useful character, but conidia were observed only in limited number of samples due to common absence of pycnidia. Asterisks indicate values or features considered "fully diagnostic", i.e. allowing for correct identification of at least one taxon. The measurements are given in the following format: (minimum)x_1–x_2–x_3(maximum), where minimum and maximum are extreme values in all samples of one species, x_1 is the smallest mean among the sample means of one species, x_2 is the mean calculated using all the values of all samples of one species, x_3 is the greatest mean among the sample means of one species. The total number of measurements from all samples of one species (N), the number of species samples (n), and standard deviation of x_2 (SD) are given for each measured character in brackets.

Characters	*C. micromarina*	*C. micromontana*	*C. microstepposa*
Ascospore septum width (μm)	(2.0)2.6–2.9–3.4(4.5) [90, 9, 0.6]*	(1.0)1.5–1.8–2.1(2.5) [95, 10, 0.4]	(1.0)1.6–1.9–2.4(3.5) [93, 10, 0.4]
Septum-width/ascospore-length ratio	(0.13)0.17–0.20–0.23(0.30) [90, 9, 0.03]*	(0.07)0.09–0.12–0.14(0.18) [95, 10, 0.02]	(0.06)0.10–0.12–0.16(0.23) [93, 10, 0.03]
Width of cells in uppermost true exciple (μm)	(3.0)3.9–5.0–6.2(8.5) [100, 10, 1.1]	(3.5)4.1–5.3–7.0(7.5) [103, 11, 1.0]	(2.0)2.6–3.5–4.5(7.0) [110, 11, 0.9]*
Thallus width (μm)	(75)125–160–206(375) [90, 9, 60.8]	(0)19–87–123(175) [30, 3, 57.9]	(75)86–157–369(500) [102, 11, 89.3]
Length of cells in uppermost true exciple (μm)	(4.5)6.3–7.7–8.9(11.5) [100, 10, 1.6]	(5.0)7.1–7.7–10.1(10.5) [103, 11, 1.2]	(4.0)5.9–6.6–7.3(10.0) [110, 11, 1.2]
Width of widest cell of paraphysis (μm)	(3.0)3.7–4.0–4.5(6.0) [100, 10, 0.7]	(4.0)4.9–5.3–6.1(7.5) [110, 11, 0.9]*	(3.0)3.7–4.2–4.7(6.0) [110, 11, 0.7]
Ascospore width (μm)	(5.5)6.5–7.5–8.5(10.5) [90, 9, 1.0]	(5.5)7.2–7.9–8.6(10.0) [95, 10, 1.0]	(4.5)6.0–6.6–7.9(9.5) [93, 10, 0.9]
Width of alveolate cortex plus epinecral layer (μm)	(7.5)14.8–28.0–36.3(50.0) [90, 9, 10.4]	(12.5)20.5–21.2–22.0(25.0) [20, 2, 3.9]	(7.5)16.0–23.6–38.5(62.5) [102, 11, 9.2]
Apotecium diameter (mm)	(0.20)0.28–0.34–0.40(0.55) [100, 10, 0.07]	(0.22)0.27–0.32–0.42(0.44) [90, 9, 0.06]	(0.24)0.32–0.39–0.49(0.66) [110, 11, 0.09]
Ascospore length/width ratio	(1.44)1.79–1.95–2.30(2.67) [90, 9, 0.24]	(1.45)1.72–1.94–2.09(2.71) [95, 10, 0.22]	(1.51)1.93–2.34–2.93(3.33) [93, 10, 0.45]
Conidium length (μm)	(2.5)2.8–3.1–3.3(4.0) [70, 7, 0.3]	(3.0)3.6–3.7–3.8(4.5) [30, 3, 0.4]	(3.0)3.4–3.4–3.5(4.0) [30, 3, 0.3]
Attachment of mature apothecia to thallus	usually half immersed	sessile to immersed	sessile
Color reaction of epithecium and true exciple with K	violet	violet	brown-violet*
Epithecium and true exciple color	grey	grey	brown*
Habitat	close to sea	mountains, from low steppe foothills to alpine belt	usually inland arid and semi-arid regions
Inspers hymenium	absent	absent	present*
Pruina on apothecial disc and true exciple	sometimes present	sometimes present	absent
Reaction of thallus alveolate cortex with K	usually violet (90% samples), rarely K–	sometimes violet (50% samples), or K–	usually K– (80% samples), rarely violet
Stacks of crystalls in hymenium	present in some apothecia	absent	rarely present

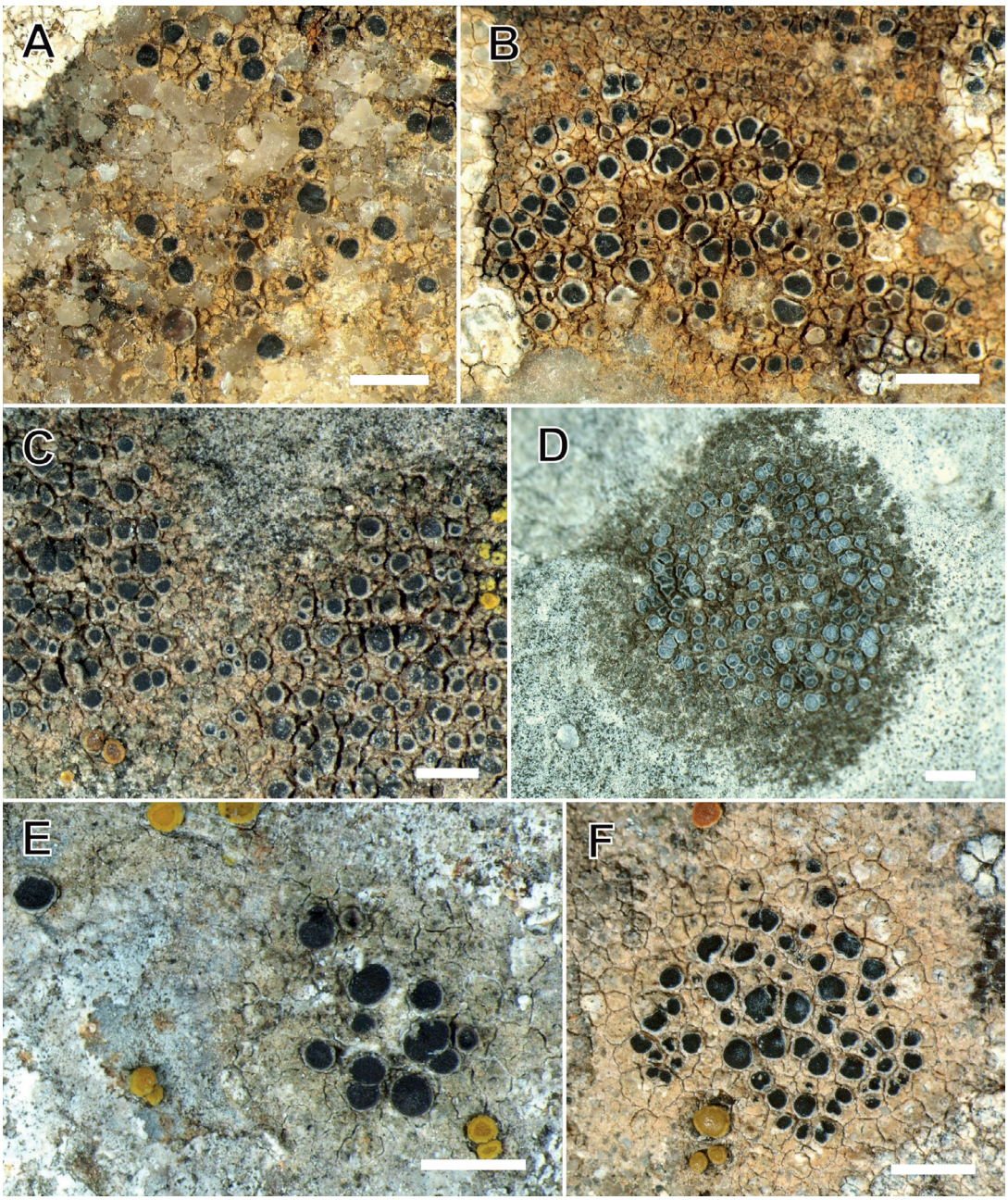

Fig. 3. — **A** and **B**: *Caloplaca micromarina* (holotype). — **C**: *Caloplaca micromontana* (holotype). — **D**: *Caloplaca micromontana* (PRA JV9523). — **E**: *Caloplaca microstepposa* (holotype). — **F**: *Caloplaca microstepposa* (PRA JV9454). Bars: 1 mm.

together with the epinecral layer (8)15–28–36(50) μm [90, 9, 10]. Alveolate cortex cells ± spherical, (4.5)6.0–6.4–7.0(8.0) μm diameter [30, 3, 0.9], cell-wall thickness about 1.5 μm. Vegetative diaspores absent. Extracellular crystals of calcium salts present in medulla and also forming pruina. Pruina sometimes present (white spots on thalli, often surrounding apothecia or apothecial primordia). Prothallus indistinct or distinct, ochre (paler than thallus) or grey.

Apothecia (0.20)0.28–0.34–0.40(0.55) mm diameter [100, 10, 0.07]; zeorine or rarely biatorine; mature apothecia suppressed to adnate, rarely immersed. Disc black; true exciple black; thalline exciple of same colour as thallus; white pruina often present on disc and exciples (apothecia with and without pruina usually present in one sample). Hymenium (75)89–90–93(100) μm high [30, 3, 8], colourless, not glutinized, without extracellular oil drops, but sometimes with stacks of extracellular, often rectangular crystals (insoluble in K, recrystalized into needles in H_2SO_4); epihymenium grey. Hypothecium colourless, underlain by algal layer, with extracellular oil drops, without extracellular crystals; with a central conical extension downward, (75)89–97–93(150) μm high [30, 3, 16]; formed of thin-walled cells variable in shape. Exciple about 0–75 μm wide, formed of true exciple, (0)14–24–29(50) μm wide [30, 3, 12], and thalline exciple, (0)5–8–12(38) μm wide [30, 3, 12]. Upper part of true exciple of thin-walled cells (4.5)6.3–7.7–8.9(11.5) × (3.0)3.9–5.0–6.2(8.5) μm [100, 10, 1.6 & 1.1]. Lower part of palisade prosoplectenchyma of thin-walled cells (6.0)9.3–10.2–10.9(14.0) × (2.0)2.3–2.4–2.5(3.5) μm [30, 3, 2.1 & 0.4]. Thalline exciple without cortex or with indistinct alveolate cortex with numerous extracellular crystals (in H_2SO_4 partly dissolved and recrystalized into needles). Paraphyses (2.0)2.1–2.1–2.2(2.5) μm wide [30, 3, 0.2] in lower part, but widening gradually to (3.0)3.7–4.0–4.5(6.0) μm [100, 10, 0.7] in upper part; rarely branched and anastomosed; uppermost paraphyses cells usually dead (not stained by cotton blue), thin and shrunken (Fig. 2A and B). Asci clavate, (45)55–59–63(75) × (10)15–16–19(23) μm [30, 3, 7 & 3]. Ascospores 8 per ascus, colourless, polarilocular, (10.5)13.2–14.3–15.5(18.0) × (5.5)6.5–7.5–8.5(10.5) μm [90, 9, 1.5 & 1.0], with rounded ends. Septa (2.0)2.6–2.9–3.4(4.5) μm [90, 9, 0.6], cytoplasmatic channel within septum broad or thin. Ascospore length/width ratio: (1.44)1.79–1.95–2.30(2.67) [90, 9, 0.24]; septum width/ascospore length ratio: (0.13)0.17–0.20–0.23(0.30) [90, 9, 0.03].

Pycnidia common, about 90–130 μm wide, usually with a single chamber, distinguished by their darker grey tops on thallus surface. Conidiophores of spherical, rectangular or triangular, ± isodiametric cells, about 2–5 μm diameter. Conidia ellipsoid to broadly ellipsoid, (2.5)2.8–3.1–3.3(4.0) × (1.0)1.6–1.8–2.1 (2.5) μm [70, 7, 0.3 & 0.3].

Chemistry: Spot tests: thallus and apothecia K–, C–, P–. Thallus and apothecia UV–. In section, true exciple non-amyloid (I–); hymenium and hypothecium amyloid (I+). Uppermost cells in alveolate cortex of thallus with *Sedifolia*-grey (pale grey in water, K+ violet; the reaction not always observable). Concentration of *Sedifolia*-grey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple also with *Sedifolia*-grey. No substances detected by HPLC in apothecia and thallus (done in samples PRA JV6420, JV7236 and JV8199).

Similar taxa: *Caloplaca albopruinosa* (thallus distinctly endolitihic; apothecia usually pruinose, thalline exciple indistinct; montane species), *C. alociza* (hymenium with numerous extracellular oil drops, thalline exciple indistinct; thallus distinctly endolithic), *C. atroalba* & *C. microstepposa* (hymenium with numerous extracellular oil drops, epihymenium brown, K+ brown-violet, spore septa thinner, cells in uppermost true exciple thinner; Table 4), *C. badioreagens* (endolithic thallus; with different apothecial chemistry), *C. circumalbata* (apothecia and thallus larger, thallus usually white), *C. diphyodes* (apothecia and thallus larger; apothecia sessile; spore septa wider), *C. micromontana* (spores septum thinner, upper cells in paraphyses wider, hymenium always without stacks of crystals; Table 4), *C. transcaspica* (apothecia and thallus larger, thallus usually white, apothecia sessile, hymenium sometimes with extracellular oil drops).

Phylogeny: *Caloplaca micromarina* forms a monophyletic group (Fig. 1), but its support is low in both ML and Bayesian analyses. The internal clade without one Russian specimen is well supported in the Bayesian analysis.

Distribution and ecology: *Caloplaca micromarina* is a maritime species distributed in Eastern Mediterranean. It is known from Russia, Turkey and Ukraine along the coasts of the Black Sea and the Sea of Marmara, where it grows on coastal rocks and at some distance (up to several kilometres) from the shoreline.

The species occurs on calcareous conglomerates, schist and sandstone outcrops, stones or pebbles and rarely on concrete in sites with well-lit Mediterranean scrub vegetation. Co-occurring taxa are e.g. *Aspicilia contorta*, *Caloplaca conversa*, *C. crenulatella s. lato*, *C. ferrarii s. lato*, *C. neotaurica*, *Candelariella aurella*, *Diplotomma* sp. and *Lecanora dispersa s. lato*. In semi-arid conditions of the Crimean Peninsula, *C. micromarina* grows together with *C. microstepposa*, but it never occurs with *C. micromontana*.

Caloplaca micromontana Frolov, Wilk & Vondrák, *sp. nova* (Fig. 3C and D)

MB 803399; sequences of the holotype: KC615299 (β-tubulin), KC346303 (ITS).

Type: Russia. Orenburg region: Sakmara district, village Grebeni (about 12 km NE of Orenburg), shrubby steppe on SE slope of the hill Grebeni, W of village, alt. 120–160 m a.s.l., 51°56´28´´N, 55°16´48´´E, on lime-rich schist and sandstone boulders and pebbles in scree, 7 June 2011, *I. Frolov & J. Vondrák* (holotype PRA JV9467). — Paratypes: *See* Appendix.

Diagnostic characters: (1) anthraquinones absent; (2) thallus epilithic or strongly reduced but not endolithic, usually < 150 μm thick, without distinct cortex, with *Sedifolia*-grey; (3) mature apothecia usually less than 0.4 mm diameter; (4) cells in the uppermost true exciple 3.5–7.5 μm wide; (5) hymenium without extracellular oil drops; (6) epihymenium and outer part of true exciple grey, rarely with a weak brown tinge, with *Sedifolia*-grey (K+ violet); (7) width of widest cell of paraphysis 4–7.5 μm; (8) ascospores with thin septa, 1–2.5 μm.

Detailed description: Thallus epilithic, ochre or grey, forming small roundish spots to about 1 cm diameter or irregular spots to several cm wide; of tightly arranged, angular to rounded, ± flat areoles, (0.15)0.29–0.31–0.33(0.44) × (0.15)0.24–0.25–0.27(0.35) mm [20, 2, 0.16 & 0.13]; sometimes thallus diminishing and present only below apothecia or as only a few areoles. Thickness of thallus (0)95–118–123(175) μm [22, 3, 58]. Medulla inconspicuous, to about 50 μm thick; cells hardly observable due to presence of extracellular crystals only partly dissolved and recrystallized into needles in H_2SO_4. Algal layer (38)56–62–68(100) μm [20, 2, 32] thick; algal cells globose, (8.0)11.3–13.5–15.0(19.5) μm diameter [30, 3, 2.9]. Cortex not developed; usually alveolate cortex present. Epinecral layer often present, but its boundary with alveolate cortex indistinct. Total thickness of alveolate cortex and epinecral layer (13)21–21–22(25) μm [20, 2, 4]. Alveolate cortex cells ± spherical, (4.0)6.1–6.3–6.4(8.8) μm diameter [20, 2, 1.2], thickness of cell wall about 1.5 μm. Vegetative diaspores absent. Extracellular crystals of calcium salts present only in medulla. Pruina absent. Prothallus indistinct.

Apothecia (0.22)0.27–0.32–0.42(0.44) mm diameter [90, 9, 0.06]; zeorine or rarely biatorine; mature apothecia immersed to adnate, rarely sessile. Disc and true exciple brown to black; thalline exciple same colour as thallus; white pruina on disc and exciples often present (observed in seven out of eleven evaluated samples). Hymenium colourless without extracellular oil drops, rarely with crystals, (63)81–89–98(100) μm high [30, 3, 10]; epihymenium grey, rarely with weak brown tinge. Hypothecium colourless, underlain by algal layer, with extracellular oil drops, with a central conical extension downward, (55)78–85–101(125) μm high [30, 3, 21], formed of cells variable in shape. Exciple about 15–75 μm wide, formed of true exciple, (8)16–23–31(50) μm wide [30, 3, 11], and thalline exciple, (0)9–10–11(30) μm wide [30, 3, 9]. Upper part of true exciple of thin-walled cells (5.0)7.1–7.7–10.1(10.5) × (3.5)4.1–5.3–7.0(7.5) μm [103, 11, 1.2 & 1.0]. Lower part of palisade prosoplectenchyma of thin-walled cells (7.0)9.9–11.2–12.4(17.0) × (1.5)2.1–2.5–2.7(3.5) μm [25, 3, 2.3 & 0.5]. Thalline exciple without cortex or with indistinct cortex, to 15 μm thick, with extracellular crystals (in H_2SO_4 partly dissolved and recrystalized into needles). Paraphyses (1.5)2.3–2.3–2.4(3.0) μm wide [30, 3, 0.4] in lower part, but widening gradually to (4.0)4.9–5.3–6.1(7.5) μm [110, 11, 0.9] in upper part; rarely branched and anastomosed; uppermost paraphyses cells usually dead (not stained by cotton blue), thin and shrunken (Fig. 2A and B). Asci clavate, (45)53–58–65(75) × (10)15–17–18(25) μm [30, 3, 8 & 3]. Ascospores 8 per ascus, colourless, polarilocular, (11.0)14.3–14.7–15.6(19.0) × (5.5)7.2–7.9–8.6(10.0) μm

[95, 10, 1.3 & 1.0], with rounded ends. Septa (1.0)1.5–1.8–2.1(2.5) μm [95, 10, 0.4], cytoplasmatic channel within septum always rather broad. Ascospore length/width ratio: (1.45)1.72–1.94–2.09(2.71) [95, 10, 0.22]; septum width/ascospore length ratio: (0.07)0.09–0.12–0.14(0.18) [95, 10, 0.02]. Extracellular crystals of calcium salts not seen in apothecia, but possibly forming pruina, which is rarely present.

Pycnidia observed in three samples with well developed thalli, about 60–100 μm wide, distinguished by their darker grey tops on thallus surface. Conidiophore cells not studied. Conidia ellipsoid, (3.0)3.6–3.7–3.8(4.5) × (1.5)1.8–1.9–2.0 (2.0) μm [30, 3, 0.4 & 0.2].

CHEMISTRY: Spot tests: thallus and apothecia K– (but true exciple slightly violet in apothecia with pruina), C–, P–. Thallus and apothecia UV–. In section, true exciple non-amyloid (I–); hymenium and hypothecium amyloid (I+). Uppermost cells in alveolate cortex of thallus and in cortex of thalline exciple ± with *Sedifolia*-grey (pale grey in water, K+ violet; the reaction not always observable). Concentration of *Sedifolia*-grey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple also with *Sedifolia*-grey. No substances in apothecia and thallus detected by HPLC (done in samples PRA JV9467, *L. Śliwa 3118* KRAM).

SIMILAR TAXA: *Caloplaca albopruinosa* (ascospore septum wider, thalline exciple indistinct, thallus distinctly endolitihic, grey or white; perhaps restricted to Europe), *C. alociza* (hymenium with numerous extracellular oil drops, thalline exciple indistinct, ascospore septum wider, thallus distinctly endolithic), *C. atroalba* & *C. microstepposa* (hymenium with numerous extracellular oil drops, epihymenium brown, K+ brown-violet, upper paraphyses cells and cells in uppermost true exciple thinner; Table 4), *C. badioreagens* (endolithic thallus; larger apothecia; with different apothecial chemistry), *C. circumalbata* (apothecia and thallus larger, thallus usually white), *C. diphyodes* (apothecia and thallus larger, ascospore septa wider, hymenium sometimes with extracellular oil drops), *C. micromarina* (spore septum wider, upper cells in paraphyses thinner, thallus thicker; Table 4), *C. transcaspica* (apothecia and thallus larger, thallus white or pale grey, apothecia always sessile, hymenium sometimes with extracellular oil drops).

PHYLOGENY: *C. micromontana* forms a monophyletic group supported by ML analysis (Fig. 1). It is sister to an undescribed blastidiate taxon (*Caloplaca* sp. in Fig. 1) and close to the paraphyletic *C. albopruinosa*. *Caloplaca albopruinosa* is a closely related taxon, but it is clearly different in its ITS characters. Our three ITS sequences of *C. micromontana* are almost identical (variability in less than 1% of base pairs), but all ITS sequences of *C. albopruinosa sensu* Muggia *et al.* (2008) differ in more than 4% of base pairs from our ITS sequences of *C. micromontana*.

DISTRIBUTION AND ECOLOGY: *Caloplaca micromontana* is known from inland territories of Europe and Asia. It is always recorded from mountains where it occurs in different altitudes. In the Alps (Austria) *C. micromontana* is known at altitudes between 1700 and 2100 m, in the Carpathians (Poland and Slovakia) it grows between 550 and 1500 m (in Poland the taxon has been known under the name *C. atroalba*; e.g. Wilk 2011, 2012). Pakistani locality is at 4100 m. Russian records are from 150–400 m (Ural Mts.) and 2200 m (Sayan Mts.). It is commonly observed that lichens of high montane to alpine habitats in central and western Europe may grow at much lower altitudes in continental southern Russia (e.g. our observations on *C. diphyodes*, *C. epithallina* and *C. percrocata*). *Caloplaca micromontana* occurs on outcrops, stones and pebbles of limestone, lime-rich schist and sandstone. Co-occurring taxa are *Acarospora moenium*, *Caloplaca crenulatella s. lato*, *C. variabilis s. lato*, *Lecanora dispersa s. lato*, *Lecidella carpathica*, *Sarcogyne regularis*, *Verrucaria* sp. *Caloplaca micromontana* can sometimes grow with *C. microstepposa*, but it never reaches coastal areas harbouring *C. micromarina*.

Caloplaca microstepposa Frolov, Nadyeina, Khodos. & Vondrák, *sp. nova* (Figs. 2A–D, 3E and F)

MB 803400; sequences of the holotype: KT013276 (β-tubulin), KC984530 (ITS).

TYPE: Czech Republic. Bohemian karst. Praha, Radotín, Kosoř, protected area Černá rokle, E of village, alt. about 250–300 m a.s.l., 49°59´21´´N, 14°20´8´´E, on pebbles in sun-exposed limestone scree below SE-exposed limestone outcrop in steppe with shrubs, 3 Aug. 2011, *Z. Palice & J. Vondrák* (holotype PRA JV9141). — PARATYPES: *See* Appendix.

DIAGNOSTIC CHARACTERS: (1) anthraquinones absent; (2) thallus epilithic, usually less than 300 μm thick, without cortex (but alveolate cortex often developed), *Sedifolia*-grey usually absent; (3) mature apothecia up to 0.7 mm diameter, with ± brown disc and true exciple; (4) cells in uppermost true exciple narrow, 2–7 μm wide; (5) hymenium inspers, without crystals; (6) epihymenium and outer part of the true exciple brown or rarely brown-grey, containing a brown pigment and *Sedifolia*-grey, K+ brown-violet; (7) width of widest cell of paraphysis 3–6 μm; (8) ascospores with 1–3.5 μm wide septa.

DETAILED DESCRIPTION: Thallus epilithic, in shades of ochre, grey or grey-white, forming small roundish spots to about 1 cm diameter or irregular spots to several cm wide, sometimes mixed with thalli of other lichens; of tightly arranged, angular to rounded, flat areoles, (0.18)0.26–0.39–0.53(0.66) × (0.15)0.22–0.29–0.39(0.44) mm [30, 3, 0.14 & 0.08]. Thickness of thallus (75)86–157–369(500) μm [102, 11, 89]. Specimens from desert of western Kazakhstan have thicker thallus than lichens from Turkey, Ukraine, Czech Republic and France. Medulla inconspicuous, to about 50 μm thick; cells hardly observable due to presence of extracellular crystals only partly dissolved and recrystallized into needles in H_2SO_4. Algal layer (25)54–67–90(115) μm thick [30, 3, 23]; algal cells globose, about (9.0)13.5–16.0–20.6(26.0) μm diameter [30, 3, 4.6]. Real cortex not developed; alveolate cortex usually present. Epinecral layer often present, but its boundary with alveolate cortex indistinct. Alveolate cortex with epinecral layer (7)16–24–39(63) μm thick [102, 11, 9.2]. Alveolate cortex cells ± spherical, (5.0)6.2–6.4–6.6(8.0) μm diameter [20, 2, 0.9], thickness of cell walls about 1.5 μm. Vegetative diaspores absent. Extracellular crystals of calcium salts not observed in thallus. Pruina inconspicuous or absent from thallus surface. Prothallus usually absent or poorly developed, ochre.

Apothecia (0.24)0.32–0.39–0.49(0.66) mm diameter [110, 11, 0.09]; zeorine or rarely biatorine; mature apothecia suppressed to adnate, rarely immersed or sessile. Disc brown to black; true exciple same colour as disc; thalline exciple same colour as thallus; pruina absent from apothecia. Hymenium (75)83–87–90(100) μm high [30, 3, 7.9], colourless, not glutinose, inspers (with numerous extracellular oil drops), about 0.5–5.0 μm diameter; epihymenium usually brown but rarely brown-grey (observed in four samples). Hypothecium colourless, underlain by algal layer, with extracellular oil drops, with a central conical extension downward, (75)82–93–100(125) μm high [30, 3, 19], formed of cells variable in shape. Exciple about 10–90 μm wide, formed of true exciple, (7)17–21–24(38) μm wide [30, 3, 9.1], and thalline exciple, (0)6–10–15(55) μm wide [30, 3, 13.7]. Upper part of true exciple of thin-walled cells (4.0)5.9–6.6–7.3(10.0) × (2.0)2.6–3.5–4.5(7.0) μm [110, 11, 1.2 & 0.9]. Lower part of palisade prosoplectenchyma of thin-walled cells (5.5)8.9–9.6–10.3(14.0) × (1.5)2.1–2.3–2.5(3.0) μm [30, 3, 0.4 & 2.3]. Thalline exciple without cortex or with indistinct alveolate cortex, with extracellular crystals (in H_2SO_4 partly dissolved and recrystalized into needles). Paraphyses (1.5)2.2–2.3–2.5(3.0) μm wide [30, 3, 0.4] in lower part, but widening gradually to (3.0)3.7–4.2–4.7(6.0) μm [110, 11, 0.7] in upper part; rarely branched and anastomosed; uppermost paraphyses cells usually dead (not stained by cotton blue), thin and shrunken (Fig. 2A and B). Asci clavate, 8-spored, (45)56–60–65(75) × (13)17–17–19(22) μm [30, 3, 7 & 3]. Ascospores 8 per ascus, colourless, polarilocular, (12.0)13.6–15.1–18.4(21.0) × (4.5)6.0–6.6–7.9(9.5) μm [93, 10, 2.0 & 0.9], with rounded ends. Septa (1.0)1.6–1.9–2.4(3.5) μm wide [93, 10, 0.4], cytoplasmatic channel within septum always rather broad. Ascospore length/width ratio: (1.51)1.93–2.34–2.93(3.33) [93, 10, 0.45]; septum width/ascospore length ratio: (0.06)0.10–0.12–0.17(0.23) [93, 10, 0.03]. Extracellular crystals of calcium salts absent from all apothecial parts.

Pycnidia not common (observed only in three samples), about 100–150 μm wide, distinguished by their darker grey tops on thallus surface.

Conidiophore cells not evaluated. Conidia narrowly ellipsoid to broadly ellipsoid, (3.0)3.4–3.4–3.5(4.0) × (1.0)1.4–1.7–2.0 (2.5) μm [30, 3, 0.4 & 0.3].

Chemistry: Spot tests: thallus and apothecia K–, C–, P–. Thallus and apothecia UV–. In section, true exciple non-amyloid (I–); hymenium and hypothecium amyloid (I+, but hypothecium only weakly I+). Upper cells in alveolate cortex of thallus and thalline exciple with low concentration of *Sedifolia*-grey (colourless or very pale grey in water, K+); K+ violet reaction observable only in two samples. Concentration of *Sedifolia*-grey is higher in pycnidial tops. Epihymenium and outer cells in the true exciple with a brown pigment together with the *Sedifolia*-grey (usually brown or grey-brown in water, K+ brown-violet). No substances detected in apothecia and thallus by HPLC (done in samples PRA JV9344, JV9448, *O. Nadyeina 111* KW).

Similar taxa: *Caloplaca albopruinosa* (thallus endolithic, grey or white; apothecia usually white pruinose; epihymenium grey, K+ violet; ascospore septum wider, thalline exciple indistinct), *C. alociza* (thallus endolithic; margin and disc of apothecia often white pruinose; thalline exciple indistinct; ascospore septum wider), *C. atroalba* (see the taxonomic note), *C. badioreagens* (endolithic thallus; different apothecial chemistry), *C. circumalbata* (apothecia and thallus larger, thallus usually white), *C. diphyodes* (apothecia and thallus larger; ascospore septa distinctly wider), *C. micromarina* (hymenium without extracellular oil drops; epihymenium grey, K+ violet; cells in uppermost true exciple wider; ascospore septa wider; Table 4), *C. micromontana* (hymenium without extracellular oil drops; epihymenium grey, K+ violet; upper cells in paraphyses and cells in uppermost true exciple wider; Table 4), *C. transcaspica* (apothecia and thallus larger; thallus white, grey, usually pruinose; epihymenium grey).

Phylogeny: *C. microstepposa* forms a well-supported monophyletic group together with two sequences of lichens macroscopically similar to *C. transcaspica*, with white large thalli and large apothecia (Fig. 1). These two terminals are called "*C.* aff. *microstepposa*". In our unpublished single-locus phylogenies of MCM7 and RPB2, samples of *C.* aff. *microstepposa* (e.g. Vondrák 5466) do not group with *C. microstepposa*, but form a supported clade with *C. transcaspica*.

Distribution and ecology: *Caloplaca microstepposa* is known from inland arid and semi-arid regions of Asia and from dry inland localities throughout Europe in altitudes up to 1000 m. It is common in deserts of the Mangystau region in western Kazakhstan, where it grows on soft limestone outcrops. In the steppe and forest-steppe zone of Russia and Ukraine, the lichen occurs on pebbles or outcrops of calcareous schist, calcareous sandstone and limestone. The taxon has been known from Ukrainian Donetsk Upland as *C. transcaspica* (Nadyeina 2009). In northern Turkey, it was collected from calcareous sandstone pebbles and limestone outcrops in continental forest-steppe, open sub-Mediterranean bush, but also from sunny habitats in the zone of montane forests. In central and southern Europe (Austria, Bulgaria, Czech Republic, France, Germany, Italy, Poland, Serbia, Spain) *C. microstepposa* grows usually on calcareous pebbles and stones, rarely on limestone outcrops or concrete, often in sunny, S-exposed screes and in rocky steppes, up to 1000 m alt. Co-occurring taxa are *Aspicilia calcarea*, *A. contorta*, *Caloplaca concreticola*, *C. crenulatella s. lato*, *C. decipiens*, *C. ferrarii s. lato*, *C. interfulgens*, *C. teicholyta*, *C. variabilis s. lato*, *Candelariella aurella*, *Diplotomma* sp., *Lecanora dispersa s. lato*, *Leptogium plicatile*, *Rinodina bischoffii*, *Verrucaria muralis* and *V. nigrescens s. lato*. *Caloplaca microstepposa* reaches coastal areas in the Crimean Peninsula where it can grow together with *C. micromarina*. It also rarely occurs with *C. micromontana*, for instance in steppe foothills of Asian mountains.

Taxonomical note: We investigated the type specimen of *C. atroalba* (FH, lectotype) and some other samples identified as this taxon described from North America (samples identified/revised by C. Wetmore in GZU, MIN and several samples from herbaria T. Spribille and T. Wheeler). *Caloplaca atroalba sensu* Wetmore (1994) is a heterogeneous taxon containing *C. atroalba s. stricto* and *C. diphyodes* (our unpubl. data) and possibly other taxa. Our evaluation of the type specimen did not show any phenotypic differences from *C. microstepposa*; our observa-

tions are available inside the specimen envelope. Some recently collected samples named "*C. atroalba*" are phenotypically identical with the type of *C. atroalba* (USA. Montana, 2010, *T. Spribille s.n.*; USA. Montana, 2010, *T. Wheeler 3152*), but their β-tubulin, MCM7, RPB2 and ITS sequences do not group with *C. microstepposa* (data not shown). *Caloplaca atroalba* and *C. microstepposa* are probably true cryptic species. Revision of "*C. atroalba*" specimens will be the subject of a separate paper.

Key to the Eurasian species related to *Caloplaca variabilis* and without anthraquinones (genus *Pyrenodesmia sensu* Arup *et al.* 2013)

Epiphytic and some epilithic Teloschistaceae crusts without anthraquinons that are unrelated to *C. variabilis* are not included in the key (e.g. *Caloplaca demissa*, *C. obscurella* and *C. servitiana*).

1. Thallus with soredia, minute granules or pustulate outgrowths 2
1. Thallus without soredia, minute granules or outgrowths 5
2. Thallus with pustulate outgrowths, areolate, well-developed, sordid-grey to white-greyish ..... *Caloplaca albopustulata*
2. Thallus with soredia or minute granules 3
3. Thallus endolithic with obscurely sublobed prothallus, thalli often form shallow bowl-shaped depressions in limestone, soredia or soredia-like minute granules often completely covering central part of thallus ..... *Caloplaca erodens*
3. Thallus epilithic, well-developed 4
4. Thallus with well-developed fungal and algal stacks (*sensu* Vondrák & Kubásek 2013), upper surface with ridges derived from epinecral layer ..... *Caloplaca molariformis*
4. Thallus without fungal or algal stacks and ridges on upper surface ..... *Caloplaca concreticola* (be aware of other taxonomically unclear sorediate/blastidiate species with similar appearance to *C. concreticola*, as e.g. *Caloplaca* sp. in Fig. 1)
5. Thallus distinctly endolithic 6
5. Thallus epilithic 9
6. Epihymenium reddish-brown in KOH ..... *Caloplaca badioreagens*
6. Epihymenium violet in KOH 7
7. Thalline exciple well-developed ..... *Caloplaca erodens* "non-sorediate" (*see* comments in the text)
7. Thalline exciple indistinct 8
8. Hymenium with numerous extracellular oil drops ..... *Caloplaca alociza* (apprised isotype of *C. lecideina* (*Callopisma variabile* var. *lecideina*, G 00290968) belongs to *C. diphyodes*)
8. Hymenium without extracellular oil drops ..... *Caloplaca albopruinosa*
9. Thallus small and thin, often forms roundish spots to about 1 cm diameter, apothecia usually less than 0.5 mm diameter 10
9. Thallus and apothecia distinctly larger 12
10. Hymenium with extracellular oil drops, epihymenium brown or rarely brown-grey, width of cells in uppermost true exciple usually 2.5–4.5 μm, rarely 2–7 μm ..... *Caloplaca microstepposa*
10. Hymenium without extracellular oil drops, epihymenium grey, width of cells in uppermost true exciple usually 4–7 μm, rarely 3–8.5 μm 11
11. Spores septa usually 1.5–2.1 rarely up to 2.5 μm, widest cell of paraphyses usually 4.9–6.1 rarely 4–7.5 μm; in mountains, never close to seashore ..... *Caloplaca micromontana*
11. Spores septa usually 2.6–3.4 rarely 2–4.5 μm, widest cell of paraphyses usually 3.7–4.5 rarely 3–6 μm; close to seashore ..... *Caloplaca micromarina*
12. Thallus bullate; arid regions of Asia ... *Caloplaca bullata*
12. Thallus not bullate; in different regions of Eurasia ... 13
13. Apothecia immersed to adnate 14
13. Apothecia sessile 15
14. Thallus thick, consisting of flat, very tightly arranged areoles, hypothecium containing vertical rows of small round paraplectenchymatous cells; mainly in temperate and Mediterranean regions, also in high mountains, throughout Europe, the Near East, rarely in northern Africa and southern Siberia ..... *Caloplaca chalybaea*
14. Thallus thick, areoles not flat, often convex, not tightly arranged, hypotecium without vertical rows of small round paraplectenchymatous cells; in arid regions of northern Africa and Near East, very rarely in southern Europe ..... *Caloplaca circumalbata*
15. Spores septa thin (1–3 μm); in arid regions of eastern Europe and Asia, very rarely in southern Europe 16
15. Spores septa wide (more than 3 μm), thallus of variable colors; in temperate, arctic and alpine regions, rarely in arid regions 17
16. Thallus consists of peltate areoles, yellowish-brown ..... *Caloplaca tianshanensis*
16. Areoles not peltate, thallus white to white-greyish ..... *Caloplaca transcaspica s. lato* (we accomodate *C. ayachina* here until its taxonomy is not resolved)
17. Apothecial margin and sometimes also disk white pruinose, thallus of variable colors; always on calcareous substrate, avoiding humid conditions close to water; mainly in temperate and Mediterranean regions of Europe, but also in the Near East, northern Africa and southern Siberia ..... *Caloplaca variabilis s. lato* (taxon with variable morphotypes; we accommodate all of them here until the relationships between them are resolved)
17. Apothecia without pruina, thallus grey; often on siliceous substrates, often near water; mainly in montane to alpine belts in European mountains, in Arctic and conti-

nental parts of Asia also at low altitudes .... *Caloplaca diphyodes*

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Appendix

Paratype specimens. — *Caloplaca micromarina*: **Russia**. Black Sea coast: Tuapse, coastal rocks S of Gryaznova, 44°11´13´´N, 38°53´16´´E, on schist, 2007 *J. Vondrák* (PRA JV7470); Gelendzhik, coastal rocks W of Krinitsa (near Betta), 44°23´34´´N, 38°19´22´´E, on calcareous conglomerate, 2007 *J. Vondrák* (PRA JV6537, JV6662). **Ukraine**. Crimean Peninsula: Sudak, Kurortnoe, in slopes of Karadag Mts., alt. 100–200 m a.s.l., on lime-rich schist, 2008 *J. Vondrák* (PRA JV7230); Sudak, Kurortnoe, Mt. Eczkedag SW of village, alt. about 300 m a.s.l., on lime-rich schist, 2008 *J. Vondrák* (PRA JV6412, JV6414, JV6420, JV6422); Sudak, Dachnoe, alt. about 100 m a.s.l., on lime-rich schist, 2008 *J. Vondrák* (PRA JV7229); Alushta, Mt. Ayudag, alt. about 200 m a.s.l., on base-rich volcanic rock, 2008 *J. Vondrák* (PRA JV7236). — *Caloplaca micromontana*: **Austria**. Kärnten (Carinthia): Karnische Alps, [Lubenhochwald], alt. 2000 m a.s.l., 46°34´N, 13°19´E, on limestone, 1994 *J. Poelt* (GZU 52-94); Karnische Alps, Oisternig SW from Feistritz im Gailtal, SE slope near Feistritzer Alm, alt. 1850 m a.s.l., on limestone, 1987 *J. Hafellner 17279* (GZU 45-87); Steiermark (Styria): Hochschwabgruppe, Griesmauer above Vordernberg, ridge near peak 2019, alt. 2000–2118 m a.s.l., on limestone, 1986 *J. Poelt & Cl. Roux* (GZU 97-86); Niedere Tauern, Wölzer Tauern, Kasofen, 2 km N from Pusterwald, W-exposed wall directly below peak "Stinkmarmor", alt. 1860–1890 m a.s.l., on quite dry marble rocks, 1993 *A. Wilfling & M. Möslinger 540* (GZU 43-98); Bruck an der Mur, Hochschwabgruppe, Hochstein, N of Aflenz, Gipfelschrofen, alt. 1730–1740 m a.s.l., on limestone, 1993 *J. Poelt* (GZU 1-93); Nördliche Kalkalpen, Dachsteingruppe, Ramsau [am Dachstein], between Dachsteinsüdwandhütte [cabin] and Hunerscharte, below Scheiblingsteins, alt. 1900–2000 m a.s.l., on marl, 1993 *J. Poelt & M. Grube* (GZU 73-93). **Pakistan**. Baltistan: Haramosh range, "Alm" Pakora SE of pass Ganto La, pasture and rocks around alm, rocky slopes, alt. about 4100 m a.s.l., 35°41´N, 75°21´E, on limestone, 1991 *J. Poelt* (GZU 109-91). **Poland**. Western Carpathians: West Tatra Mts., Dolina Chochołowska, Polana Chochołowska, alt. 1105 m a.s.l., 49°14´16´´N, 19°47´47´´E, on limestone, 2004 *L. Śliwa 3118* (KRAM); Western Carpathians: Pieniny Właścive Mts., Pieniny National Park, limestone outcrops at Czorsztyn Castle, alt. 560 m a.s.l., 49°26´11´´N, 20°18´48´´E, on limestone on sunny S-exposed slope, 2005 *K. Wilk 3470b* (KRAM). **Russia**. Sverdlovsk Region: Rezh, 2 km SW of village Samocvet, rocky steppe on small limestone and siliceous outcrops above left bank of river Rezh, alt. 165 m a.s.l., 57°35´33´´N, 61°44´28´´E, on limestone cliff in extrazonal steppe, 2013 *J. Vondrák* (PRA JV11081 & 11088); Rezh, Aramashevo, limestone cliffs at village above left bank of river Rezh, alt. 150–200 m a.s.l., 57°36´31´´N, 61°44´11´´E, on limestone cliff in extrazonal steppe, 2013 *J. Vondrák* (PRA JV11087; distributed in Vondrák: sel. exs. of *Caloplaca*, fasc. 4); Voronezh Region: Khokholsky District, near village Kostyonki, protected area "Kostyonki-Borschyovo´", alt. about 200 m a.s.l., on limestone in steppe, 2003 *I. Bogdanova* (KRAM-L-65593). Republic of Bashkortostan: Sterlitamak, about 8 km S of town, Mt. Shikhan Toratau, alt. 300–400 m a.s.l., 53°33´10´´N, 56°5´53´´E, on limestone in steppe, 2011 *O. Vondráková & J. Vondrák* (PRA JV9523); Republic of Tyva: West Sayan Mts., Ak-Dovurak, Ak-Sug, Enge-Beldir, glacier cirque in S-slope from pass "Sayanskiy pereval; 2200 m" by the road A161, close to border with Khakasia, alt. 2150–2200 m a.s.l., 51°42´0´´N, 89°53´14´´E, on base-rich schist, below S-exposed overhanging outcrop, in alpine zone, 2013 *J. Vondrák & I. Frolov* (PRA11083). **Slovakia**. Tatra Mts.: Feigsblösse [Faixová in Belianské Tatry], on limestone, 1868 *(W.) H. Lojka* (W 2013-02600). — *Caloplaca microstepposa*: **Austria**. Tirolia: Ötztaler Alpen, Landeck District, SW of Fließ, above Neuer Zoll [hotel], W-exposed dry overhang, alt. about 1000 m a.s.l., on schist, 1989 *J. Poelt* (GZU 65-89). **Bulgaria**. Eastern Rodopi Mts.: Kardzhali, Momchilgrad, Starovo, about

2 km E of village, alt. 390 m a.s.l., 41°28´N, 25°25´E, on concrete, 2004 *J. Vondrák* (PRA JV2160). **Czech Republic**. Bohemian karst: Praha, Kosoř, SW-exposed rocks in valley of Radounský potok, about 1.5 km NW of village, alt. about 250 m a.s.l., 49°59´41´´N, 14°18´26´´E, on limestone outcrops and pebbles in steppe, 2012 *I. Frolov & J. Vondrák* (PRA JV9678, JV9679, JV9675); Praha, Třebotov, rock at SW slope of Kulivá hora, about 1 km SW of village, alt. 330 m a.s.l., on vertical sun-exposed limestone outcrop, 49°57´51´´N, 14°17´10´´E, 2012 *I. Frolov & J. Vondrák* (PRA JV9671, JV9724). **France**. Maritime Alps: Menton, Breil-sur-Roya, La Brigue, rocks on S-slope above valley of brook La Levensa, alt. about 1000 m a.s.l., 44°04´02´´N, 7°38´17´´E, on sun-exposed limestone, 2012 *I. Frolov, J. Malíček, J. Vondrák* (PRA JV10221). **Germany**. Bavaria: Würzburg, near Sugenheim, [alt. about 400 m a.s.l.], on marl, 1865 *H. Rehm* (W 1906-10939, 1990-00463, 2013-02598, 2013-02599). **Italy**. South Tyrol (Südtirol, Alto Adige): [Bolzano], Margola near Predazzo, S-exposed slope, [alt. about 1000 m a.s.l.], on limestone, 1883 *F. C. G. Arnold* (W 1900-9904, 1913-5686, 2013-02601). **Kazakhstan**. Mangistau Region: Mangistau District, by the road between villages Shetpe and Say-Utes, about 30 km SW of Say-Utes, alt. 260 m a.s.l., 44°09´20´´N, 52°39´10´´E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9136, JV9452); Mangistau District, west chink (slope) of Ustyurt plateau, Manashy, by the road between villages Say-Utes and Shetpe, alt. 290 m a.s.l., 44°06´12´´N, 53°12´40´´E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9428); Mangistau District, East Karatau ridge, rocks by the road between Zhatybay and Shetpe, about 30 km SW of Shetpe, alt. 180 m a.s.l., 43°57´00´´N, 52°05´52´´E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9448, JV9470, JV9462); Mangistau District, village Shetpe, West Karatau ridge, about 10 km N of village, alt. 130 m a.s.l., 44°12´48´´N, 52°03´58´´E, on soft limestone, 2009 *J. Vondrák & A. Khodosovtsev* (PRA JV9450, JV9454). **Poland**. Góry Świętokrzyskie: village Łukowa, hill without forest, SW of village, on limestone pebbles scattered on ground, 1976 *K. Toborowicz* (KTC-4705); Kielce County: Wesoła above Wierna Rzeka, hill without forest near railway, eastern slope, alt. about 200–300 m a.s.l., on limestone, 1976 *K. Toborowicz* (KTC-6332, as *Caloplaca atroalba* in Wilk 2011); Opatów District, about 15 km WSW of Ostrowiec Świętokrzyski, Grzegorzewice near village Waśniów, alt. about 300 m a.s.l., on dry limestone outcrops, 1976 *J. Nowak* (KRAM-L-22963); Western Carpathians: Pogórze Przemyskie, Rzeszów, about 12 km SW of Przemyśl, village Koniusza, alt. about 400 m a.s.l., on sun-exposed calcareous schist outcrops, 1981 *J. Kiszka, J. Piórecki* (KRAM-L-56053). **Russia**. Orenburg Region: Tanalik, 5 km N of village Chapaevka, W-exposed limestone outcrops in steppe, above water reservoir Iriklinskoe, alt. 330 m a.s.l., 52°05´44´´N, 58°49´36´´E, on limestone outcropes in steppe, 2013 *J. Vondrák* (PRA JV11079); Republic of Altay: Kosh-Agach District, SE part of Kuray Ridge, NE of village Chagan-Uzun, alt. 2000–3000 m a.s.l., on lime-enriched siliceous outcrop in alpine steppe, 2012 *J. Vondrák & Frolov* (PRA JV10436); Republic of Khakassia: 20 km N of town Shira, quartzite, limestone and schist outcrops in short-grass steppe, alt. 410 m a.s.l., 54°39´48´´N, 89°50´35´´E, on calcareous sandstone in steppe, 2013 *J. Vondrák* (PRA JV11107); Tashtip, steppes on hills between villages Nizhnaya Teya and Poltakov, with sandstone and schist outcrops, alt. 480 m a.s.l., 52°56´14´´N, 90°05´29´´E, on calcareous sandstone in steppe, 2013 *J. Vondrák* (PRA JV11111); Republic of Tyva: Sarig-Sep, Buren-Bay-Khaak, concrete gutter in steppe at village, alt. 840 m a.s.l., 51°11´57´´N, 95°31´33´´E, on horizontal, sun-exposed face of concrete, 2013 *J. Vondrák* (PRA JV11071). **Serbia**. Đerdap National Park: SW exposed limestone road cut along road from Mosna to Tekija, alt. about 160 m a.s.l., 44°38´18.8´´N 22°18´28.0´´E, on relatively fresh limestone outcrops, 2014 *I. Frolov* (herbarium I. Frolov 878). **Spain**. Catalonia: Barcelona, outscirts of Santa Maria de Montserrat Abbey, on way to Mt. Miranda de Sant Jeroni, conglomerate outcrops, alt. about 1000 m a.s.l., 41°36´18.3´´N, 1°48´41.1´´E, on xerothermic conglomerate outcrops, 2015 *I. Frolov* (herbarium I. Frolov 1002). **Turkey**. Çorum Region: Çorum, Dodurga, Yeniköy, alt. 970 m a.s.l., 40°49´29´´N, 34°44´02´´E, on soft limestone in bottom of dry gorge in forest-steppe, 2012 *J. Vondrák* (PRA JV9779); Isparta Region: near Hadschi Bey, on shore of lake Egerdin, [alt. about 1000 m a.s.l.], 1931 *V. Pietschmann* (W 1959-6621); Sinop Region: Sinop, Boyabat, by the road Kastamonu–Sinop, in valley of brook at village Şeyhli Köyü, alt. 700 m a.s.l., 41°42´41´´N, 34°55´26´´E, on limestone pebbles, 2012 *J. Vondrák* (PRA JV9811); Tokat Region: Tokat, Merkez, Geyras Mahallesi, small gorge above highway, alt. 800 m a.s.l., 40°14´54´´N, 36°32´47´´E, on calcareous stones in open sub-Mediterranean bush, 2012 *J. Vondrák* (PRA JV9757). **Ukraine**. Kherson Region: Nikolskoe, slope above left bank of Ingulec river, alt. 10–20 m a.s.l., on S-exposed limestone outcrop, 2009 *A. Naumovich, A. Khodosovtsev, J. Vondrák* (PRA JV6943; KHER 4999); Antonovka, slope above right bank of Dniepr river, on limestone, 1992 *A. Khodosovtsev* (KHER 2594); Kairy, Dniepr river, on limestone, 2010 *A. Khodosovtsev & L. Gavrylenko* (KHER 5000). Lugansk Region, Donetsk Upland: Sverdlovsk District, near village Provallya, alt. about 200 m a.s.l., steppe slopes with sandstone outcrops and isolated trees, 2005 *O. Nadyeina 63* (KW; PRA JV6952); same locality in "Provalskaya step" Reserve, 2005 *O. Nadyeina 53* (KW); Sverdlovsk District, at village Medvezhanka, in "Medvezhanskyi´" Botanical Reserve, alt. about 150 m a.s.l., on calcareous schist outcrops in steppe, 2006 *O. Nadyeina* (PRA JV6953); same locality, 2006 *O. Nadyeina 111* (KW); Sverdlovsk District, Dar'ino-Yermakovo, by the road Kharkiv–Rostov, alt. about 150 m a.s.l., on calcareous sandstone, 2006 *O. Nadyeina 109* (KW); Lutuhyno District, near village Verkhnia Orikhivka, Pershozvanivsy water-reservoir, 0.5 km N of village, alt. 200–250 m a.s.l., on calcareous stone, 2005 *O. Nadyeina 5-14* (KW); Khmelnitsk' Region: National Park "Podilskyi Tovtry", 15 km SE of Kamianets Podilskyi, Kitaihorod, canyon of river Tarnava, alt. 140 m a.s.l., 48°38´25´´N, 26°46´58´´E, on calcareous sandstone on SW slope, 2003 *P. Czarnota* (KRAM-L-48695; as *Caloplaca atroalba* in Wilk 2011); National Park "Podilskyi Tovtry", junction of rivers Smotrich and Dniestr, near village Ustia, alt. 136 m a.s.l., 48°33´54´´N, 26°39´24´´E, on limestone outcrops, 2003 *M. Kukwa* (KRAM-L-48831).