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Extensive yellow crusts below limestone overhangs: a new taxon close to a minute epiphytic lichen

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A conspicuous yellow crust forming extensive covers on some dry and shaded limestone rocks in Europe is described here as *Caloplaca substerilis* subsp. *orbicularis* M. Haji Moniri, Vondrák & Malíček subsp. nov. Based on nuITS rDNA, 28S nuLSU rDNA and mtSSU rDNA sequence data, the new taxon is closely related to *Caloplaca substerilis* and *C. ulcerosa*. The three taxa form a supported clade in the subfamily Xanthorioideae (Teloschistaceae), but none of the recently seggregated genera are suitable for them. In the ITS phylogeny, the new taxon forms a monophylum nested within *C. substerilis*. However, its extensive yellow thalli and absence of vegetative diaspores clearly distinguish it from *Caloplaca substerilis* (subsp. *substerilis*). Indeed, if it had not been for the molecular evidence, we would have described it at the rank of species. We suggest that the substrate switch and accompanying processes are responsible for the striking phenotypic difference between *Caloplaca substerilis* subsp. *substerilis* and *C. substerilis* subsp. *orbicularis*.

Several generations of central European lichenologists have probably noticed extensive yellow-white, non-sorediate lichen crusts growing in shade below limestone overhangs in the Moravian Karst or Pálava hills (Czech Republic, southern Moravia). These crusts were, however, very seldom collected and have probably been considered as shade forms of the bright yellow Teloschistaceae crusts growing on sun-exposed rocks just above the overhangs (e.g. *Variospora dolomiticola*, *V. velana*). For several years we have suspected that these crusts may be a distinct species and we recently decided to obtain its nuITS rDNA (ITS in following text) sequence. Our first results suggested that it is indeed distinct. We were surprised when they also indicated that it is close to the species *Caloplaca substerilis* and *C. ulcerosa*. The two latter species are morphologically and ecologically similar to each other (Vondrák et al. 2013b), but have little in common with the crust from below overhangs. They are almost always epiphytic, and have a much reduced, sorediate thallus without anthraquinones (except at the tops of pycnidia, which are yellow). Further ITS sequences, and also data from 28S nuLSU rDNA (further nuLSU) and mtSSU rDNA (further mtSSU), loci confirmed our initial results and supported a close relationship of the two epiphytic species with the crust from below overhangs. We have not found any previously published name for this crust and we decided to describe it as a new taxon.

Methods

Sequencing and phylogenetic reconstructions

DNA was extracted with a CTAB-based protocol (Aras and Cansaran 2006). Primers for ITS DNA sequence amplification were ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The PCR parameters included an initial step hold at 94°C for 5 min, and then 45 cycles with denaturating at 94°C (30 s), annealing at 62°C with the touch-down to 56°C during the first 7 cycles (30 s), and extension at 72°C (60 s). Primers for mtSSU were mrSSU1 (Zoller et al. 1999) and mrSSU7 (Zhou and Stanosz 2001). The PCR parameters were 94°C for 5 min, 45 × (94°C for 30 s, 54°C – 30 s, 72°C – 60 s), 72°C – 10 min, hold 15°C. Primers for partial nrLSU were nuLSU 155-5 (Döring et al. 2000) and LR5 (Vilgalys and Hester 1990); PCR parameters were as for mtSSU. Successfully sequenced specimens are listed in Table 1. ITS sequences were obtained from all specimens (except with TUN83), but only four samples (JV6368, JV12562, ZP13441 and TUN83) were selected for mrSSU and nrLSU.

Sequences were edited in the BioEdit 7.2.5 free software (Hall 1999) and then aligned by the on-line application MAFFT ver. 7 (Katoh and Standley 2013) with the L-INS-i method (Katoh et al. 2005). Two alignments were

Table 1. New *Caloplaca* sequences with voucher information and GenBank accession numbers.

Taxon	Voucher information	ITS	mtSSU	nrLSU
C. substerilis subsp. *orbicularis*	Romania, Apuseni Mts, Turda; herb. Malíček JM5631	KU554429	–	–
C. substerilis subsp. *orbicularis*	Czech Rep., Moravian karst, Sloup; herb. Malíček JM7999	KU554431	–	–
C. substerilis subsp. *orbicularis*	Hungary, Bükk Mts, Eger; PRA JV4319	KU554428	–	–
C. substerilis subsp. *orbicularis*	Hungary, Bükk Mts, Varbó; PRA JV6368	KU554430	KU554435	KU554437
C. substerilis subsp. *orbicularis*	Czech Rep., Pálavské vrchy hills, Klentnice; PRA JV12562	KU554427	KU554436	–
C. substerilis subsp. *orbicularis*	Czech Rep., Moravian karst, Vilémovice; PRA JV13627	KU554432	–	–
C. substerilis subsp. *substerilis*	Slovakia, Muránská planina Mts, Mt Cigánka; PRA ZP13441	KC416110 (Vondrák et al. 2013b)	KU554433	KU554438
C. ulcerosa	Tunisia, Cap Bon, Chot Ezzorhou; PRA (Guttová TUN83)	–	KU554434	KU554439

prepared, both focused on Xanthorioideae phylogeny and with sequences from Caloplacoideae and Teloschistoideae as an outgroup. 1) nrITS alignment with 593 positions and 44 sequences; it includes seven sequences of the new taxon, sequences of closely related taxa published in Vondrák et al. (2009, 2013b) and sequences of ten Xanthorioideae genera. 2) Concatenated alignment including 38 combined sequences of ITS (593 positions), mtSSU (716 positions) and nrLSU (765 positions); it includes one sequence of the new taxon (JV6368), *Caloplaca substerilis* (ZP13441), *C. ulcerosa* (TUN83; ITS failed, compensated by consensus ITS sequence of all *C. ulcerosa* specimens) and sequences of nineteen Xanthorioideae genera. Gaps were coded in SeqState by simple coding (Simmons and Ochoterena 2000). Molecular phylogenies were reconstructed by Bayesian inference incorporated in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). The general time reversible model using a gamma shaped distribution and proportion of invariant sites (GTR + G + I) was proposed by the program Modeltest (Posada and Crandall 1998) as the best DNA substitution model for ITS and for all three partitions of the concatenated alignment. Analyses of both alignments were performed using two independent runs with four MCMC chains. Trees were sampled after every 500th generation. The analyses were stopped when the average standard deviation of the split frequencies between the simultaneous runs were below 0.01. To eliminate trees sampled before reaching apparent stationarity, the first 25% of entries were discarded as burn-in and the rest were used to compute a majority-rule consensus, where the relative occurrences of nodes are identified as Bayesian posterior probabilities (Fig. 1–2).

Alternative topological hypotheses were evaluated using Bayesian inference performed in MrBayes ver. 3.2.1 (Ronquist et al. 2012). The dataset was re-analysed using the same settings as described above, except that models were constrained to monophyly/polyphyly of a particular group. The marginal model likelihoods of constrained trees were estimated using stepping-stone analysis (Ronquist et al. 2012). Differences in log likelihoods >3 log units were considered as significant (Kass and Raftery 1995).

Phenotype description

Methods for phenotype description 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 cells, 1 or 10 μm for larger structures. All measurements of cells include their walls, except for tissues with glutinized cell walls. In each sample, ten or more measurements were done for each measurable character (rarely fewer, when measurable objects were scarce). Following Ekman (1996), results of the measurements are given as (min.–) X1–X2–X3 (–max.), where min/max are extremes from all measurements, 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. Total number of measurements (N), number of investigated samples (n), and standard deviation from all measurements (SD) are given for each measured character in square parenthesis [N; n; SD]. Morphological terminology follows Smith et al. (2009) and Vondrák et al. (2013a).

Thin-layer chromatography

Thin-layer chromatography (TLC) follows the methods of Orange et al. (2010), with a few minor modifications. Lichen compounds were applied on two glass plates and placed into A and C solvents. The distance between starting and finishing line was ca 10 cm. One or two drops of acetone were added to the tube depending on the quantity of the material. Thallus tissues and apothecial tissues of the sample JM7999 (herb. J. Malíček) were run separately; samples of selected Teloschistaceae (both thallus and apothecia of *Bryoplaca sinapisperma*, *Caloplaca xerica*, *Gyalolechia flavovirescens* and *Xanthoria parietina*) were run aside as a reference. Rf values in Elix (2014) were our baseline for identification of substances, but our Rf values differed slightly. Therefore the TLC results were compared to HPLC data presented by Søchting (1997, 2001) who mentioned relative concentrations of particular anthraquinones in numerous Teloschistaceae samples.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.rp13h> (Vondrák et al. 2016).

Results

Phylogenetic analyses

For simplicity, in this section and in the discussion we use the name *Caloplaca substerilis* in its original sense, i.e. exclusive of the new subsp. *orbicularis*.

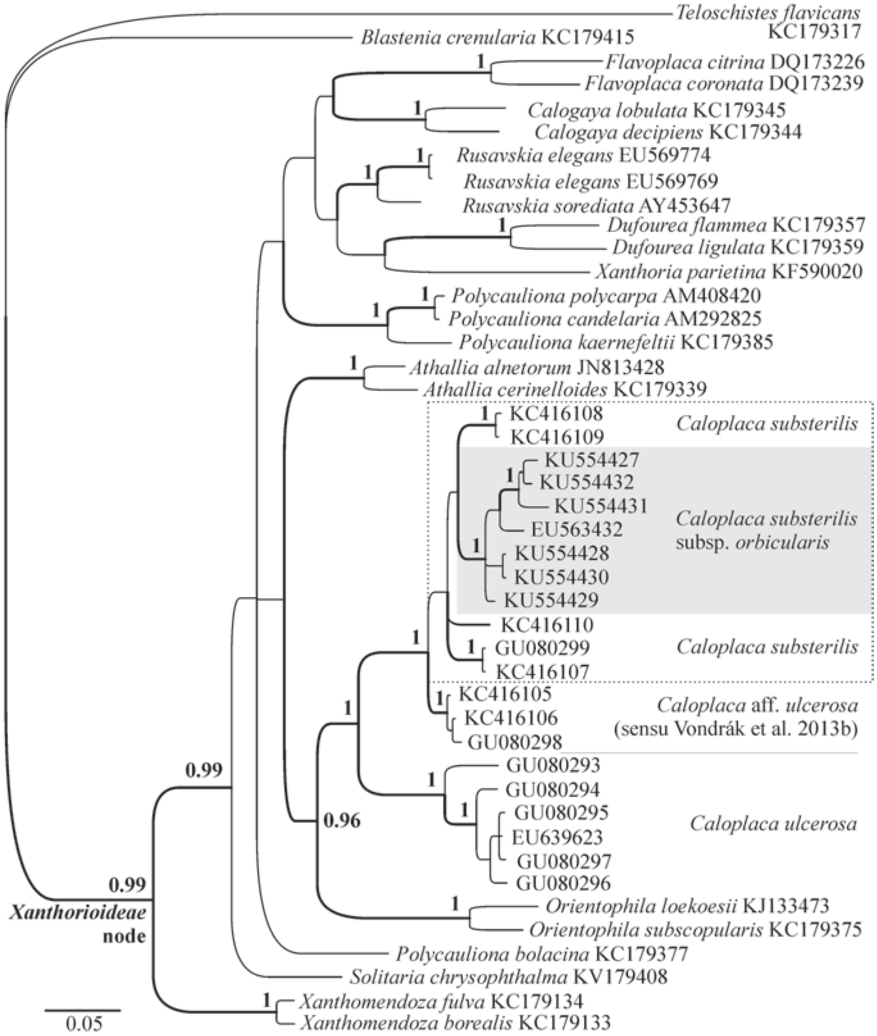

Figure 1. Bayesian ITS phylogeny of Xanthorioideae. *Caloplaca substerilis* subsp. *orbicularis* (in grey) is nested within *C. substerilis* subsp. *substerilis* (within dotted line). Bayesian posterior probabilities > 0.95 are shown.

The new taxon is placed in Xanthorioideae in the nrITS tree (Fig. 1) as well as in the tree based on analyses of combined ITS, mtSSU and nrLSU (Fig. 2). Both trees also show a close relationship between the new taxon and *C. substerilis*, *C. ulcerosa* and the North American *C.* aff. *ulcerosa* (*C. "ulcerosa"* sensu Vondrák et al. 2013b). The closest species is *C. substerilis* (Fig. 1, 2); in the ITS tree, the new taxon forms a supported subclade nested within the clade formed by *C. substerilis* (Fig. 1). Although *C. substerilis* is paraphyletic in the ITS tree, the topology of the clade involving *C. substerilis* and the well-supported clade of the new taxon is otherwise poorly resolved. The alternative topology with constrained monophyly of five *C. substerilis* sequences was tested against a negative constraint. The topology with non-monophyletic *C. substerilis* (marginal likelihood −6190.61) has about 10 log units worse score than the tested monophyly (marginal likelihood −6180.82), suggesting no reliable evidence for the paraphyly of *C. substerilis*.

In the ITS clade involving 21 sequences of the new taxon, *C. substerilis*, *C. ulcerosa* and *C.* aff. *ulcerosa*, we counted 100 variable positions, which is fewer than in various genera within Teloschistaceae. The new taxon (7 available sequences) contains 30 variable positions, *C. substerilis* (5 sequences) contains 34 variable positions, *C. ulcerosa* (6 sequences) contains 38 variable positions.

A further source of variability is in sequences of the North American *C.* aff. *ulcerosa* (Fig. 1). While the new taxon differs from *C. substerilis* in only eight nucleotide positions in ITS, *C. ulcerosa* is much less closely related to both (different in 33, respectively 34 positions). This result does not correspond with phenotype similarities because phenotypically *C. substerilis* and *C. ulcerosa* are more similar to each other than to the new taxon (cf. Vondrák et al. 2013b).

The relationships of the clade containing the new taxon, *C. substerilis*, *C. ulcerosa* and *C.* aff. *ulcerosa* to close genera of Xanthorioideae are not resolved. In the ITS tree, the closest genus is *Orientophila* (PP = 0.96), but the relationship to the other seven genera is not clear (Fig. 1). The tree based on analyses of the concatenated alignment only shows a close relationship to nine genera (Fig. 2).

Caloplaca substerilis subsp. *orbicularis* M. Haji Moniri, Vondrák & Malíček subsp. nov.

MycoBank no. MB 815803; GenBank: KU554427 (nrITS), KU554436 (mtSSU).

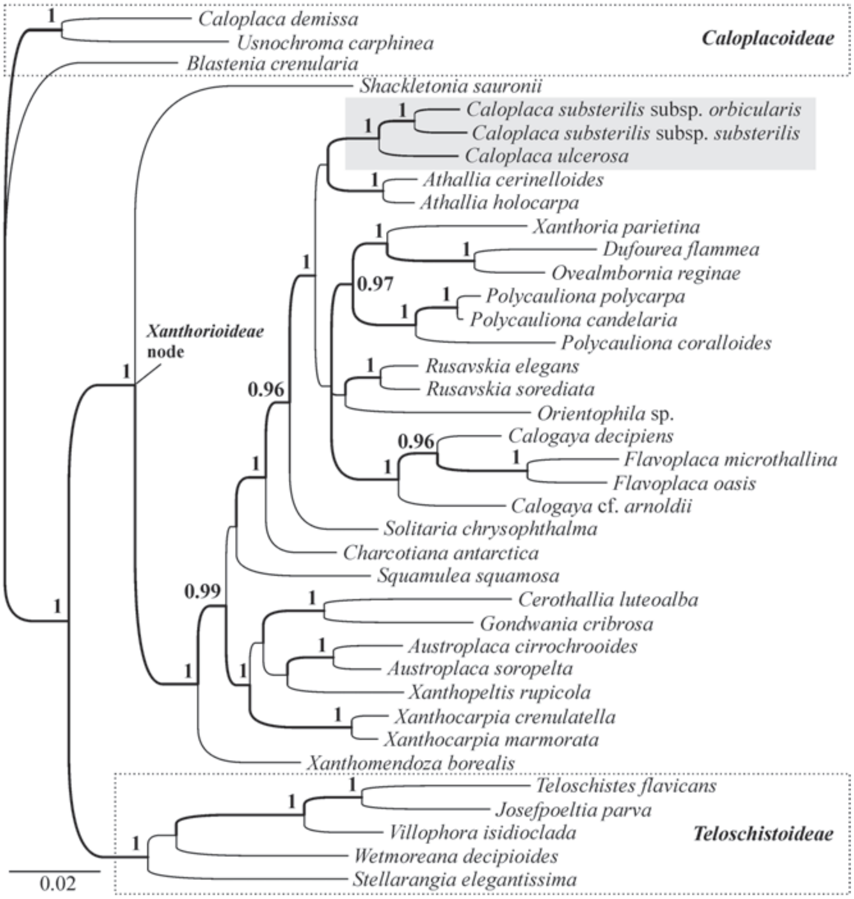

Figure 2. Bayesian phylogeny of Xanthorioideae based on the combined dataset of ITS, mtSSU and nrLSU. *Caloplaca substerilis* subsp. *orbicularis* and its closest relatives are in grey. Bayesian posterior probabilities > 0.95 are shown.

Characterized by the circular outline of the thallus which is about 1–3 cm in diameter, variable in colour from white to orange depending on the concentration of anthraquinones, usually brighter coloured in the 1.0–1.5 mm wide marginal zone. Vegetative propagules absent; orange zeorine apothecia abundant. Growing on limestone overhangs and vertical rocks, often forming extensive crusts of merged individual thalli.

Type: Czech Republic. Mikulov, Klentnice, protected area Tabulová, southwest slope of Stolová hora with limestone cliffs, 400 m a.s.l., 48.8394N, 16.6358E, on shaded limestone outcrop, below overhang, 28 Nov 2013, J. Malíček and J. Vondrák (holotype: PRA JV12562, isotype: herb. Malíček JM6306, further isotypes will be distributed in Sel. Exs. of *Caloplaca*, fasc. 4).

Etymology

The epithet *orbicularis* means ‘forming small circles’ and it reflects the typically rounded yellow spots formed by *C. substerilis* subsp. *orbicularis* thalli.

Description

Thallus crustose, areolate, bright orange-yellow, lemon-yellow (Fig. 3A), ochre-yellow, orange, white-yellow, or rarely white without yellow tinge (Fig. 3B). Thallus forming circular patches, (5–) 13–14–18 (–37) mm in diameter [24; 6; 8.2], often merging to form conglomerate thalli covering large areas (Fig. 3C). A brighter yellow zone is often developed at the thallus margin, ca 1.0–1.5 mm wide, often rimmed by a thin white to light orange fibrillate prothallus, (0.10–) 0.13–0.19–0.32 (–0.50) mm wide [36; 6; 0.09] (Fig. 3D). Areoles angular or rounded, (0.1–) 0.2–0.3–0.6 (–1.0) mm in diameter [60; 6; 0.26], flat to convex, but often of granular character at the thallus margin (Fig. 3E); granules (0.04–) 0.08–0.15–0.22 (–0.35) mm wide [53; 6; 0.08]. Height of areoles (0.11–) 0.25–0.49–0.74 (–1.10) mm [24; 6; 0.25]. Areoles forming raised tufts in older parts of thallus center (0.4–) 0.8–1.0–1.8 (–3.0) mm high [43; 6; 0.5]; the thickness of tufts caused by a widened decaying and gapped medullary tissue (0.25–) 0.40–0.59–0.63 (–1.50) mm height [37; 6; 0.29]. Vegetative diaspores absent.

Cortex conspicuous, even, often with gaps resulting from dead algal cells (“alveolate cortex” sensu Vondrák et al. 2013a), (10–) 20–23–26 (–42) μm high [49; 6; 7]; formed of 3–7-cellular layers of thin-walled isodiametric cells, (3.0–) 3.9–4.2–4.5 (–6.0) μm in diameter [58; 6; 1.0], usually including large, ± isodiametric crystals of calcium salts (4–) 7.7–8.5–9.9 (–17) μm in diameter [55; 6; 3.4]. Cortex mostly covered with an yellow-brown epinecral layer (3–) 6–9–13 (–30) μm thick [41; 6; 6.4], and sometimes with epilichenic algal cells/colonies. Algal layer not continuous, but forming most of the thallus interior, (23–) 57–82–130 (–250) μm thick [50; 6; 43.7]; Algal cells (1.5–)

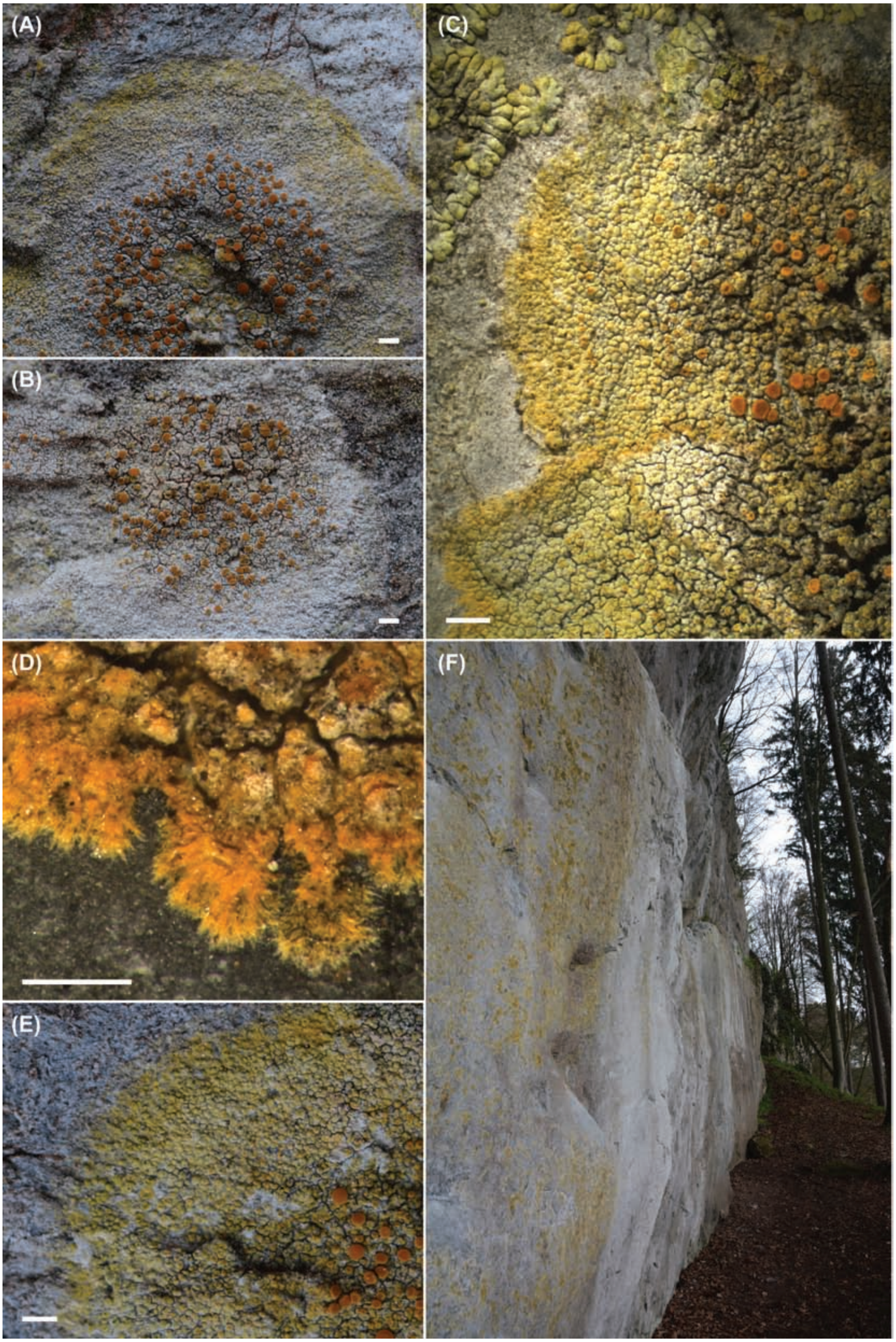

Figure 3. *Caloplaca substerilis* subsp. *orbicularis*. (A) thallus with yellow marginal zone; (B) white thallus without anthraquinones; (C) thalli merged into extensive crust; (D) detail of the tiny prothallus zone; (E) detail of the marginal part of thallus without apothecia, but with numerous pycnidia; (F) habitat: a dry and shaded limestone cliff in the locality Holštejn, Czech Republic (see the sample JV13783).

9.5–11.3–15.0 (–17.5) µm in diameter [60; 6; 3.4]. Medulla usually conspicuous (10–) 17–20–28 (–38) µm thick [41; 6; 5.8], formed by a prosoplectenchymatous tissue of thin-walled hyphae, (1.5–) 2.9–3.2–3.9 (–5.0) µm thick [53; 6; 0.8], with minute, ± isodiametric crystals of calcium salts, (1.0–) 2.3–2.8–3.4 (–5.0) µm in size [52; 6; 0.9].

Apothecia often abundant, zeorine, at first immersed, becoming sessile to adnate, (0.33–) 0.48–0.62–0.66 (–1.16) mm in diameter [90; 6; 0.13] and (0.06–) 0.11–0.25–0.48 (–1.48) mm tall [90; 6; 0.30]. Disc orange, flat to convex, even, not pruinose. Thickness of medulla under the largest discs (0.10–) 0.15–0.21–0.23 (–0.38) mm [38; 4; 0.06]. Thalline exciple yellow to orange, always paler than the disc, raised above the disc in young apothecia, reduced and lowered with age, but not disappearing, (14–) 40–51–54 (–120) µm thick [75; 6; 22]. Cortex/alveolate cortex of thalline exciple covering most of the surface, formed of isodiametric to elongated thin-walled cells, (2.5–) 2.8–6.3–9.1 (–12.0) µm long [76; 6; 2.11] and (1.7–) 2.6–3.5–3.6 (–7.5) µm wide [65; 5; 1.4], but the cells are generally thinner and longer toward the lower part of the thalline exciple. True exciple visible as an orange ring between the disc and the thalline exciple, (16–) 27–38–47 (–75) µm thick [75; 4; 6], colourless in its inner part, formed by thin-walled, almost isodiametric cells in the uppermost part, (3.0–) 3.3–5.6–7.0 (–11.0) µm long [75; 6; 2.0] and (1.5–) 2.3–3.2–3.9 (–5.5) µm wide [75; 6; 1.1], lower part of palisade prosoplectenchyma of thin-walled cells, (4.0–) 4.6–7.6–9.3 (–12.5) µm long [75; 6; 2.1], and (1.0–) 2.0–2.8–2.9 (–5.0) µm wide [75; 6; 2.8]. Hymenium (50–) 76–87–96 (–150) µm high [84; 6; 19.4], colourless, partly glutinized, with extra-cellular oil drops; the uppermost part of hymenium yellow-brown from anthraquinones and covered with colourless granular extracellular crystals. Hypothecium (62–) 94–103–146 (–187) µm high [83; 6; 25] in central part, usually bowl-shaped, but sometimes extended downwards through the subhypothecial algal layer in the center, colourless, somewhat glutinized, or formed by thin-walled cells of various shapes, (3.0–) 6.0–6.9–9.1 (–12.5) µm long [52; 4; 2.3], and (1.5–) 2.5–3.7–3.8 (–8.5) µm wide [54; 4; 1.4]. Asci of the *Teloschistes* type, clavate to cylindrical, 8-spored, (40–) 50–69–77 (–112) µm high [88; 6; 1.3] and (7.5–) 13.9–14.6–18.2 (–30.0) µm wide [88; 6; 3.1]. Ascospores polarilocular, (7.5–) 10.2–11.2–13.3 (–16.0) × (2.5–) 5.1–5.9–6.7 (–10.0) µm [90; 6; 1.8; 1.2]; length/width ratio (1.19–) 1.53–1.96–2.07 (–2.30) [90; 6; 0.37]. Ascospore septa (2.5–) 4.4–4.9–5.9 (–7.5) µm wide [90; 6; 1.2]; ratio of septum width/ascospore length (0.19–) 0.37–0.42–0.47 (–0.67) [90; 6; 0.11]. In addition to typical elliptic ascospores, rhomboid ascospores were occasionally observed in most of the samples and a few citriform ascospores were observed in one sample. Paraphyses usually branched, containing oil droplets, (1.5–) 1.7–2.2–2.6 (–4.5) µm wide in the lower part [90; 6; 0.6], widening to (3.5–) 5.5–6.4–8.1 (–10.0) µm in the upper part [90; 6; 1.4].

Pycnidia common, present in a 0.5 cm wide marginal thallus zone, usually forming brighter yellow-orange spots on the thallus surface (Fig. 3E), (50–) 88–115–135 (–180) µm wide in section through the middle part [26; 6; 33.89]. Conidiophores usually forming a packed tissue, variable in length, formed of obtuse rectangular or triangular cells, ca 2–5 × 3–7 µm diam. Conidia usually ellipsoid, straight or seldom slightly curved, or tear shaped, rarely bacilliform, (2.0–) 3.1–3.3–3.4 (–5.0) × (1.0–) 1.7–1.9–2.2 (–3.0) µm [60; 6; 0.7; 0.5].

Phylogeny

Based on ITS, mtSSU and nrLSU sequence data, *Caloplaca substerilis* subsp. *orbicularis* clearly belongs in Teloschistaceae, subfamily Xanthorioideae (Fig. 1, 2), but none of the recently segregated genera is suitable for it. It is closely related to *C. substerilis* and *C. ulcerosa*. The former species is closer; it is paraphyletic with *C. substerilis* subsp. *orbicularis* in the ITS phylogeny (Fig. 1).

Ecology and distribution

While most Teloschistaceae crusts on European limestone cliffs prefer sunny and rain-exposed sites, *Caloplaca substerilis* subsp. *orbicularis* prefers vertical to overhanging, rather shaded but dry limestone faces, where it grows with only a few other species of Teloschistaceae, e.g. *Flavoplaca flavocitrina*, *Leproplaca chrysodeta* and *L. xantholyta*. In suitable habitats, *Caloplaca substerilis* subsp. *orbicularis* forms extensive crusts composed of numerous individual thalli, which may cover substantial parts of rock surfaces (Fig. 3F). Our records came from several localities in the Czech Republic (Pálava Hills, Moravian Karst), Hungary and Romania; the species is very abundant in most places where it has been recorded.

Chemistry

Anthraquinones of chemosyndrome A (sensu Søchting 1997) are present in thallus and apothecia; all yellow to orange parts K+ purple, C–. TLC revealed parietin as the main compound, with traces of emodin, fallacinal, parietinic acid and teloschistin (samples 10 and 11 in Fig. 4A–D). Although the minor compounds were indistinct on plates under daylight (Fig. 4A, C), their spots were visible under short wavelength UV light (Fig. 4B, D). Brighter anthraquinone spots were observed in analyses of apothecia (sample 10) than when parts of the thallus were sampled (sample 11), suggesting lower anthraquinone concentrations in the thallus. Extracellular crystals in all thalline and apothecial parts are visible in polarized light and have a positive reaction with sulphuric acid (forming needle-like crystals).

Similar species

The two related taxa, i.e. *C. substerilis* subsp. *substerilis* and *C. ulcerosa*, differ substantially in having a sorediate thallus that is not yellow (and which is often poorly developed) and in being usually epiphytic. The differences from *C. substerilis* subsp. *substerilis* are shown in Table 2. Instead, *Caloplaca substerilis* subsp. *orbicularis* resembles some unrelated epilithic Teloschistaceae with yellow(ish) areolate thalli. *Gyalolechia flavovirescens* is perhaps the most similar, but it has usually brighter (and more reddish) apothecia and usually occurs on base-rich siliceous rocks, rarely on pure limestone; it also has a different chemistry and contains fragilin as one of its major compounds (Søchting 2001). Similar lichens of the *Variospora velana* complex usually have brighter yellow or orange areoles; their thalli usually form irregular, not circular, patches, and they generally avoid shaded rock faces. Some Mediterranean or Macaronesian species (e.g. *Flavoplaca calcitrapa* s.l. and *Haloplaca* spp.) may have similar circular thalli with a brighter yellow outer zone (as in Fig. 3A), but they are ± maritime species, absent from central Europe. *Caloplaca inconnexa* may be somewhat similar when forming

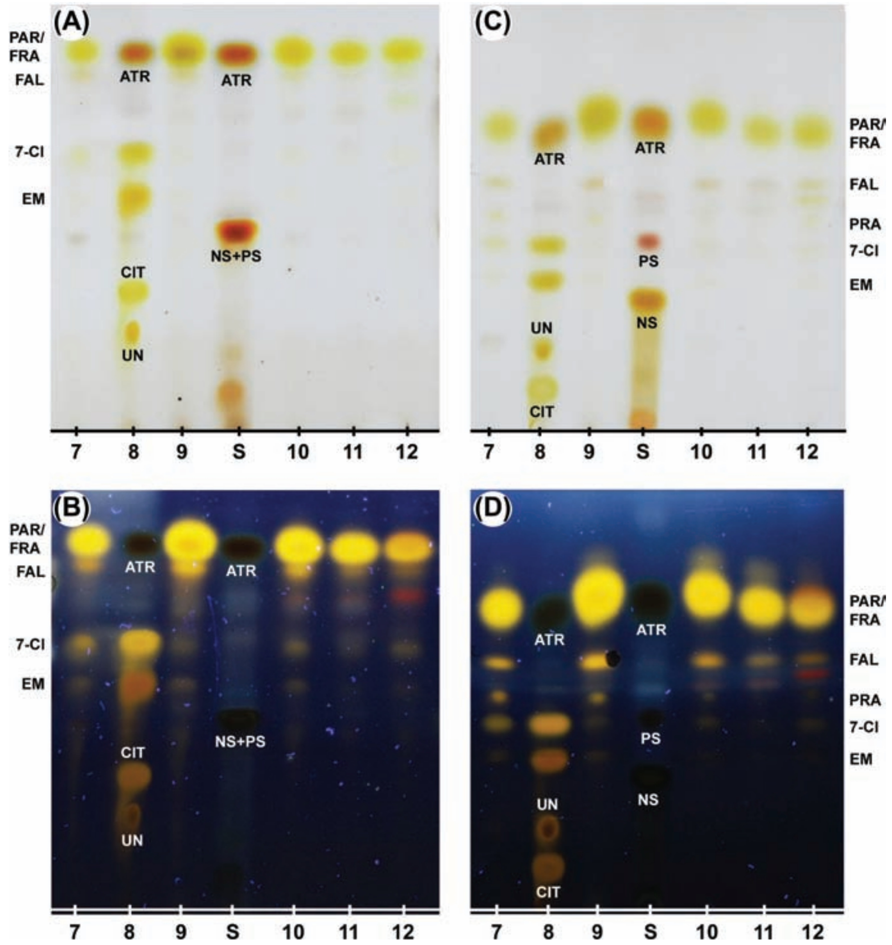

Figure 4. TLC results. (A) plate developed in solvent A photographed in daylight; (B) the same plate under short wavelength UV light (254 nm) after sulphuric acid application; (C) plate in solvent C in daylight; (D) the same plate under short wavelength UV light after sulphuric acid application. Samples: 7 = *Caloplaca xerica*, 8 = *Bryoplaca sinapisperma*, 9 = *Xanthoria parietina*, S = standard (*Cladonia symphycarpia*), 10 = *Caloplaca substerilis* subsp. *orbicularis*, apothecia, 11 = *Caloplaca substerilis* subsp. *orbicularis*, thallus, 12 = *Gyalolechia flavovirescens*. Chemical compounds: 7-Cl = 7-Cl-emodin, ATR = atranorin, CIT = citreorosein, EM = emodin, FAL = fallacinal, FRA = fragilin (visible at sample 12 in pictures B and D as an orange-pink spot behind yellow parietin), NS = norstictic acid, PAR = parietin, PRA = parietinic acid, PS = psoromic acid, TEL = teloschistin and UN = unknown anthraquinone.

deviating non-lichenicolous forms in shade, but this species is usually lichenicolous with scattered to grouped areoles on host crusts in sun-lit sites.

Additional specimens examined (paratypes)

Czech Republic: Moravian Karst, Blansko, Holštejn, 380 m a.s.l., 49°40′75.30″N, 16°77′44.84″E, on shaded limestone rock, 18 Apr 2015, J. Vondrák (PRA JV13783); Moravian Karst, Blansko, Rudice, protected area Rudické propadání, 460 m a.s.l., 49°33′41.594″N, 16°73′41.825″E, on shaded limestone wall, 16 Apr 2015, J. Kocourková (PRA JV13762); Moravian Karst, Blansko, Vilémovice, ruin of Blansek, 450 m a.s.l., 49°37′13.03″N, 16°72′25.09″E, shaded over-hanged limestone rock in forest, 17 Apr 2015, J. Vondrák (PRA JV13627); ibid: 49°37′10.90″N, 6°72′19.27″E, 4 Apr 2014, Z. Fačkovcová and Z. Palice (PRA ZP17952); Moravský kras, Sloup, Sloupsko-šošůvské jeskyně Nature Reserve, Hřebenáč rock close to entry to caves, 49°41′02.51″N, 16°73′77.86″E, 470 m a.s.l., on vertical limestone rock, 17 Apr 2015, J. Malíček (herb. Malíček JM7999); Pálava, Horní Věstonice, northwest-facing limestone rocks at forest edge under Děvín Hill, 48°86′81.40″N, 16°64′54.19″E, 470–480 m a.s.l., on vertical limestone rock, 29 Nov 2013, J. Malíček and J. Vondrák (herb. Malíček JM6373). Hungary: Bükk Mts, Borsod-Abaúj-Zemplén county, Varbó,Örvény-kő, 48°12′94.53″N, 20°53′95.02″E, 750 m a.s.l., on shaded limestone outcrop, 2 Jun 2008, J. Vondrák and A. Khodosovtsev (PRA JV6368); Bükk Mts, Eger, Repáshuta, 3 km northeast east of village, rocks on south-slope of Mt Három-kő, ca 900 m a.s.l., 48°06′00.63″N, 20°47′87.47″E, on shaded vertical limestone rock, 12 May 2006, J. Vondrák and J. Šoun (PRA JV4319). Romania: Dobrogea, Târguşor, Baba valley at village Cheia, on partly shaded limestone rock, 1974, A. Vězda (PRA (s.n.), duplicates distributed in Sel. Exs. of *Caloplaca*, fasc. 4); Transylvania, Cluj County, Apuseni Mts, Turda, Cheila, protected area Cheile Turzii, deep limestone canyon of the Hășdate (Raul Belareca) River, 46°56′30.57″N, 23°67′97.18″E, 550–600 m a.s.l., on limestone overhang, 27 Jun 2012, J. Malíček (herb. Malíček JM5631).

Table 2. Characters differenciating between the subspecies of *Caloplaca substerilis*. Characters for subsp. *substerilis* adopted from Vondrák et al. (2013b).

Character set	Character	*C. substerilis* subsp. *orbicularis*	*C. substerilis* subsp. *substerilis*
Ecology	substrate	on vertical sides of calcareous rocks or below overhangs	on nutrient-rich bark or on twigs
	response to rain	on sites shaltered to rain	on sites exposed to rain
	response to sun light	prefering shaded sites	prefering light sites
Chemistry	anthraquinones	present in thallus and all apothecial parts	absent in thallus, except in the tops of pycnidia; present in apothecia
	extracellular crystals	present in all thalline and apothecial parts	absent
Morphology	thallus colour/structure	white to orange / areolate	white to grey / forming tiny squamules raising from ± endophloedal thallus
	thallus shape	± circular	irregular
	prothallus	white fibrilate prothallus often present	indistinct
	thallus size	thalli of several cm diam., but often merging into large conglomerations	often forming thalli of only few mm diam., but sometimes covering large areas of bark
	vegetative diaspores	absent	soralia formed at margins of squamules
	apothecia	usually numerous	usually absent (rarely scattered)
Anatomy	thallus cortex	true or alveolate cortex present (about 15–30 µm thick)	true cortex absent, alveolate cortex sometimes present (up to 20 µm thick)
	thallus medulla	present, about 15–30 µm thick	indistinct
	hymenium	with extracellular oil drops	without extracellular oil drops
	hypothecium	up to 180 µm tall	up to 100 µm tall
	asci	clavate to cylindrical, 40–112 × 7.5–30 µm	clavate, 50–60 × 10–16 µm
	paraphyses tips	about 5–8 µm wide	about 4–5 µm wide
	pycnidia	formed of a single chamber, 50–180 µm wide	formed of several chambers, 50–100 µm wide
	conidia	ellipsoid, about 3–3.5 × 1.5–2.5 µm	bacilliform, about 3–3.5 × 1–1.5 µm

Discussion

The relationship between the new taxon and *Caloplaca substerilis*

We originally intended to describe the new taxon at the rank of species, as on morphological and ecological grounds it appears clearly distinct from all previously described species of *Caloplaca* (s.l.). However, the analysis of our ITS sequence dataset showed that the new 'species' is nested within *C. substerilis* and it may indicate a problem with non-monophyly of *C. substerilis* at the organismal level. This paraphyletic character of *C. substerilis* (in the ITS region) was obtained by maximum parsimony, maximum likelihood and the Bayesian analyses; results of the latter analysis are shown in Fig. 1. Anyway, the paraphyly of *C. substerilis* is rather hypothetical, as it received poor support in phylogenetic reconstructions, and the test of topological constraints could not reject the hypothesis that all sequences of *C. substerilis* form a monophyletic group.

Paraphyly is a natural transitional stage in the evolution of taxa (Hudson and Coyne 2002, De Queiroz 2007). When a new monophyletic taxon arises, it may coexist for some time with its paraphyletic stem group. We suggest that this is the case with *C. substerilis* subsp. *orbicularis*. We speculate that it originated by a substrate switch from bark to limestone rock. Substrate switches are probably very common in lichens, most species of which are substrate specific (e.g. strictly epiphytic or strictly epilithic). In our opinion, such switches are possible by deviating diaspores carrying a genotype enabling growth on some other substrate type, but not viable on the original substrate type. An example of a deviating genotype is a strikingly modified genome size influencing gene doses (sequences of most DNA loci may stay unchanged). The thallus morphology of *C. substerilis* subsp. *orbicularis*, which is very different from *C. substerilis* subsp. *substerilis* (Table 2), may represent the phenotypic expression of the new genotype.

Generic position of the new taxon

Before 2013, we would undoubtedly have placed the new crust in the genus *Caloplaca*, but Arup et al. (2013) have subdivided *Caloplaca* into numerous genera within three subfamilies. *Caloplaca* (s.s.) now includes only a couple of taxa related to *C. cerina* and *C. chlorina*. None of the recent segregates from *Caloplaca* s. lat. appeared to be suitable for the new taxon and the related *C. substerilis* and *C. ulcerosa*. Therefore we must either introduce a new genus for them, or describe the new taxon in *Caloplaca* (s.l.), knowing that someone may eventually transfer the group elsewhere. We choose the latter option here because we are aware of some difficulties with the new classification of Teloschistaceae. Its main problem is that some genera are difficult to circumscribe on phenotypic traits alone, and our potential new genus would exhibit the same problem.

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