Caloplaca citrina and *C. lactea* are incorrectly understood in the British Isles

Introduction

In the British Isles the genus *Caloplaca* is rich in species and new taxa are still being described from there (e.g. Arup, 2006b). One would think that the genus would be rather well known in this country; nevertheless we have found, with little effort, two species not recorded from the territory before.

The recent publication of a paper about the *Caloplaca crenulatella* species complex (Vondrák *et al.*, 2011) raised some interesting issues for the first author of the current paper who sent some material tentatively identified as *C. crenulatella* and *C. lactea* to the second author for his appraisal. Specimens of this English material have been analysed by the second author. Now we have data that may be surprising for British lichenologists.

We have gone further and investigated two specimens of the *Caloplaca citrina* group from an English church and have similarly surprising results.

Methods

Molecular bar-coding and phylogenetic analyses

Identification of the samples was supported by molecular data – ITS nrDNA sequences. Direct PCR was used for PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA following Arup (2006a). Primers for amplification were ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990). PCR cycling parameters followed Ekman (2001). The most similar known sequences were found using the BLAST search in the GenBank.

New sequences were aligned along with similar GenBank sequences using BioEdit; unaligned regions and unaligned sequence ends were manually excluded. Final alignment of 14 sequences for analysis of sequences from the *C. citrina* group contains 527 positions; 41 positions are variable. Final alignment of 35 sequences for analysis of sequences from the *C. crenulatella* group contains 512 positions; 120 positions are variable. Bayesian phylogeny shows relations of the newly generated sequences from the *Caloplaca crenulatella* group and the *C. citrina* group to the known clades (Figs 1 & 2). For Bayesian inference, the likelihood model was set to general time reversible model (Rodríguez *et al.*, 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G). Two runs with 600 000 generations (in both analyses) starting with a random tree and employing 4 simultaneous chains each (one hot, three cold) were executed. The temperature of a hot chain was set to 0.2 and every 100th tree was saved. After finishing the analysis, the average standard deviation of split frequencies among the runs dropped below 0.01. The first 1500 trees (25%) were discarded as the burn-in phase, and remaining 4500 trees were used for construction of the 50% majority consensus tree.

Phenotypic examination

Phenotype characters of two specimens of British "*C. lactea*" were appraised in detail to generate data for distinguishing between known characters of *C. lactea* (A. Massal.) Zahlbr. vs. *C. marmorata* (Bagl.) Jatta. Measurements of ascospore sizes and widths of ascospore septa are given here as (min–) X±SD (–max), where X = mean value and SD = standard deviation. Total numbers of measurements in each sample was 10. Investigated lichens were photographed to show their morphology and images are available on *http://botanika.bf.jcu.cz/lichenology/index.php?pg=5*.

Results & discussion

Molecular analysis of the *Caloplaca crenulatella* group together with three sequences from the British material (Fig. 2) shows that specimens called "*C. lactea*" belong to the clade of *C. marmorata*; the specimen of "*C. crenulatella*" does not belong to any of the known clades within the group but possess characters of *C. crenulatella* (Nyl.) H. Olivier s. lat. (sensu Vondrák *et al*. 2011). Morphological investigation of the two samples of *C. marmorata* (called *C. lactea* before) shows that (1) apothecia are (pale) orange to yellow, (2) apothecia are 0.15–0.4 mm in diam., (3) ascospores are (13.0–) 15.35±1.4 (–17.25) × (5.25–) 6.62±0.7 (–7.5) µm in the sample *Powell* 1944 and (12.5–) 14.18±0.9 (–15.25) × (5.25) 6.25±0.9 (–8.25) µm in the sample *Powell* 1445, (4) ascospore septa are (1.25–) 2.0±0.4 (–3.0) µm wide in the sample *Powell* 1944 and (1.5–) 2.15±0.4 (–2.75) µm wide in the sample *Powell* 1445.

According to Navarro-Rosinés & Hladun (1996) and also according to the new British "Flora" (Fletcher & Laundon, 2009), *Caloplaca lactea* has smaller and broader ascospores than the observed samples and *C. marmorata* (*sensu* Navarro-Rosinés & Hladun, 1996) should have ascospores that are longer. However, Fig. 1 shows that ascospore lengths of the observed samples are closer to *C. marmorata*; as well as the widths (not depicted). Colour of apothecia in the observed samples is paler than usual in *C. marmorata* but this can be understood by the geographic position of the records; apothecia in specimens from northern parts of the area of distribution may be paler, in this case resembling *C. lactea*. These British records are the northernmost localities known for *C. marmorata*.

Molecular analysis of the two specimens of "*Caloplaca citrina*" from the British Isles (Fig. 3) shows placement of the first one (*Powell* 1958) into *C. limonia*. It is not surprising to us, because the specimen matches morphologically *C. limonia* collected in Central and South Europe. *C. limonia* is usually recognizable even in the field by its pale yellow coarse soredia/blastidia (Vondrák *et al*., 2007). The second sample is more problematic, falling into an unknown clade (*C.* aff. *austrocitrina*) along with specimens from Greece. This taxon must be studied further.

Caloplaca limonia is probably common on English churches along with other members of *C. citrina* group (*C. arcis* (Poelt & Vězda) Arup, *C. dichroa* Arup and *C. flavocitrina* (Nyl.) H. Olivier). The English record of *C. limonia* is the northernmost known for this species. We still do not know whether *C. citrina* (Hoffm.) Th. Fr. occurs in the British Isles.

Our results are based on insufficient specimens to allow broad conclusions about the distribution of these new British species. It will be interesting to analyse specimens of

"*C. lactea*" from natural habitats, perhaps from south-facing slopes of the Mendip Hills or Portland, to find out whether or not these too are *C. marmorata*. Further research is also necessary to ascertain whether *C. limonia* and *C.* aff. *austrocitrina* are common on churches and other saxicolous substrata across the country and whether there are further taxa of the *C. citrina* group present in Britain.

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Table 1. Newly generated ITS sequences from British *Caloplaca* samples with their closest relatives (found by Blast search).

Species	Herbarium number	Closest BLAST (% max. identity / % max. coverage)	GenBank accession nrs
Caloplaca aff. *austrocitrina*	P1959; dupl. in CBFS JV9058	*C.* sp. EU563414; 99/100	JN806220
Caloplaca crenulatella s.lat.	P1877; dupl. in CBFS JV9055	*Caloplaca ferrarii* HQ699657; 98/97	JN806217
Caloplaca limonia	P1958; dupl. in CBFS JV9056	*C. limonia* EU563422; 99/98	JN806219
Caloplaca marmorata	P1445; dupl. in CBFS JV9057	*Caloplaca marmorata* EU639621; 99/96	JN806216
Caloplaca marmorata	P1944; dupl. in CBFS JV9059	*Caloplaca marmorata* EU639621; 99/94	JN806218

Note: ITS sequences of both investigated samples of *C. marmorata* differ only in one nucleotide (in alignment length = 512bp)

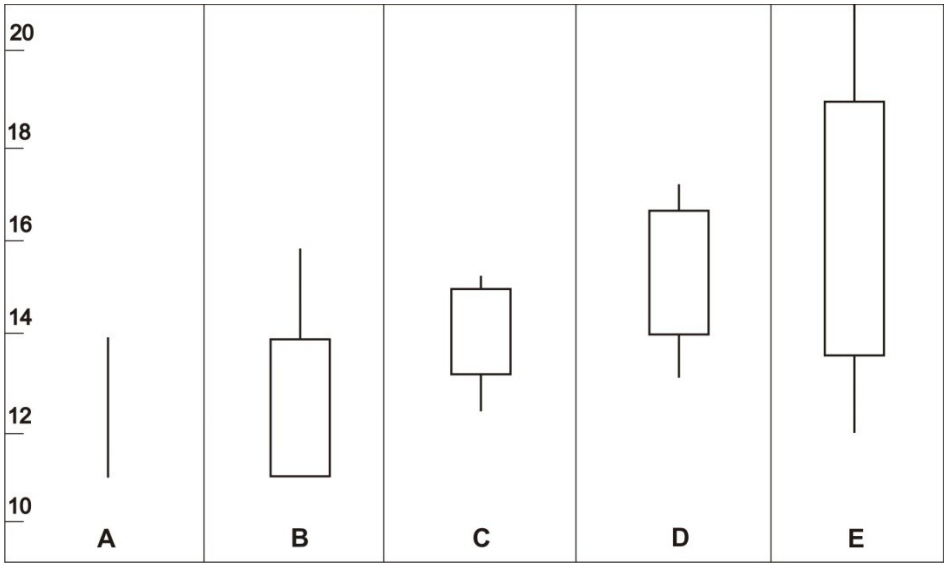

Fig. 1. Lengths of ascospores (µm), depicted as box and whisker plots (when possible), where boxes are intervals between mean + standard deviation and mean – standard deviation; ends of whiskers are the extremes: A, *Caloplaca lactea* according to Fletcher & Laundon (2009); B, *C. lactea* according to Navarro-Rosinés & Hladun (1996); C, *C. marmorata*, *Powell* 1445; D, *C. marmorata, Powell* 1944; E, C. *marmorata* according to Navarro-Rosinés & Hladun (1996).

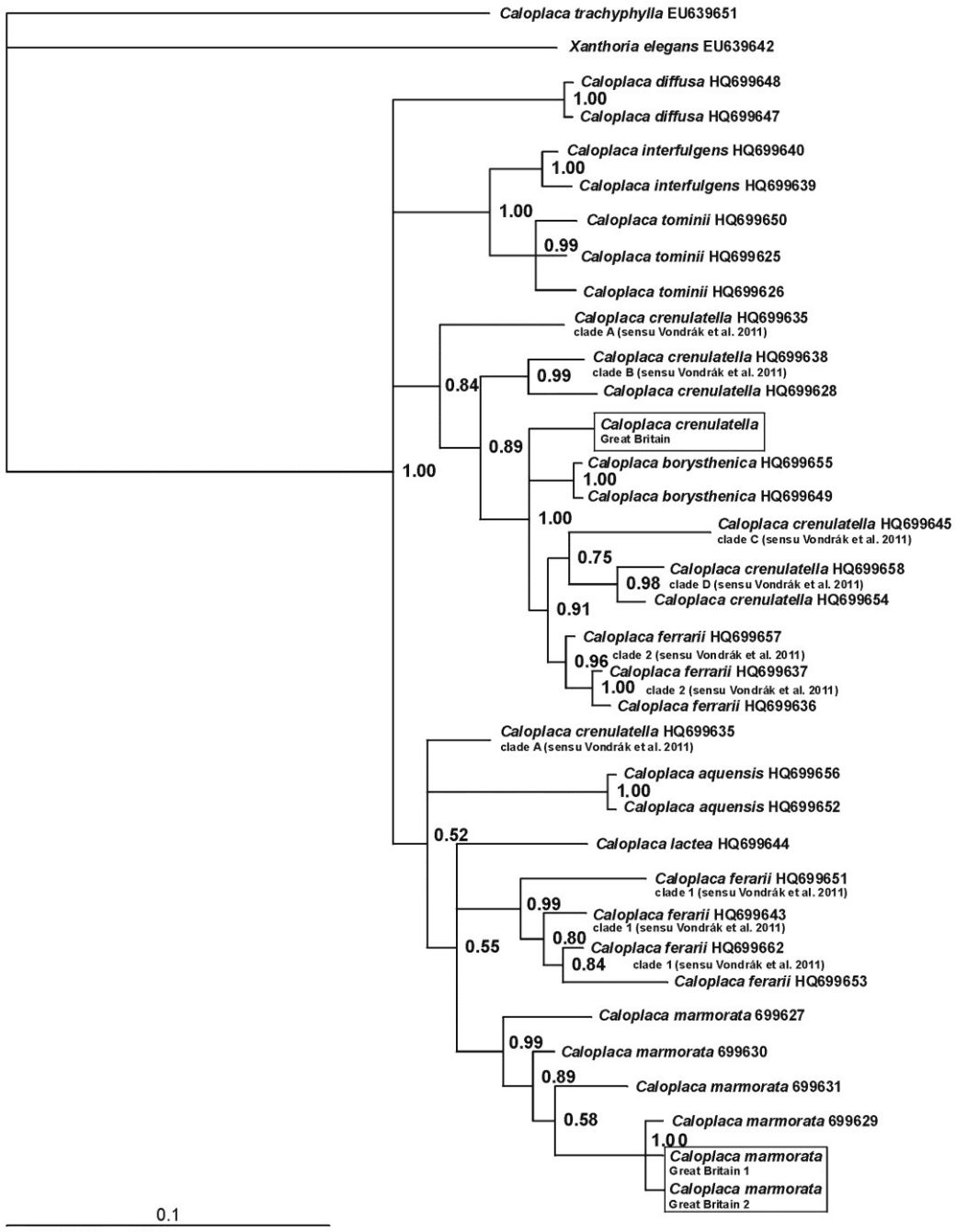

Fig. 2. Bayesian phylogeny of the *Caloplaca crenulatella* group showing placements of the British lichens

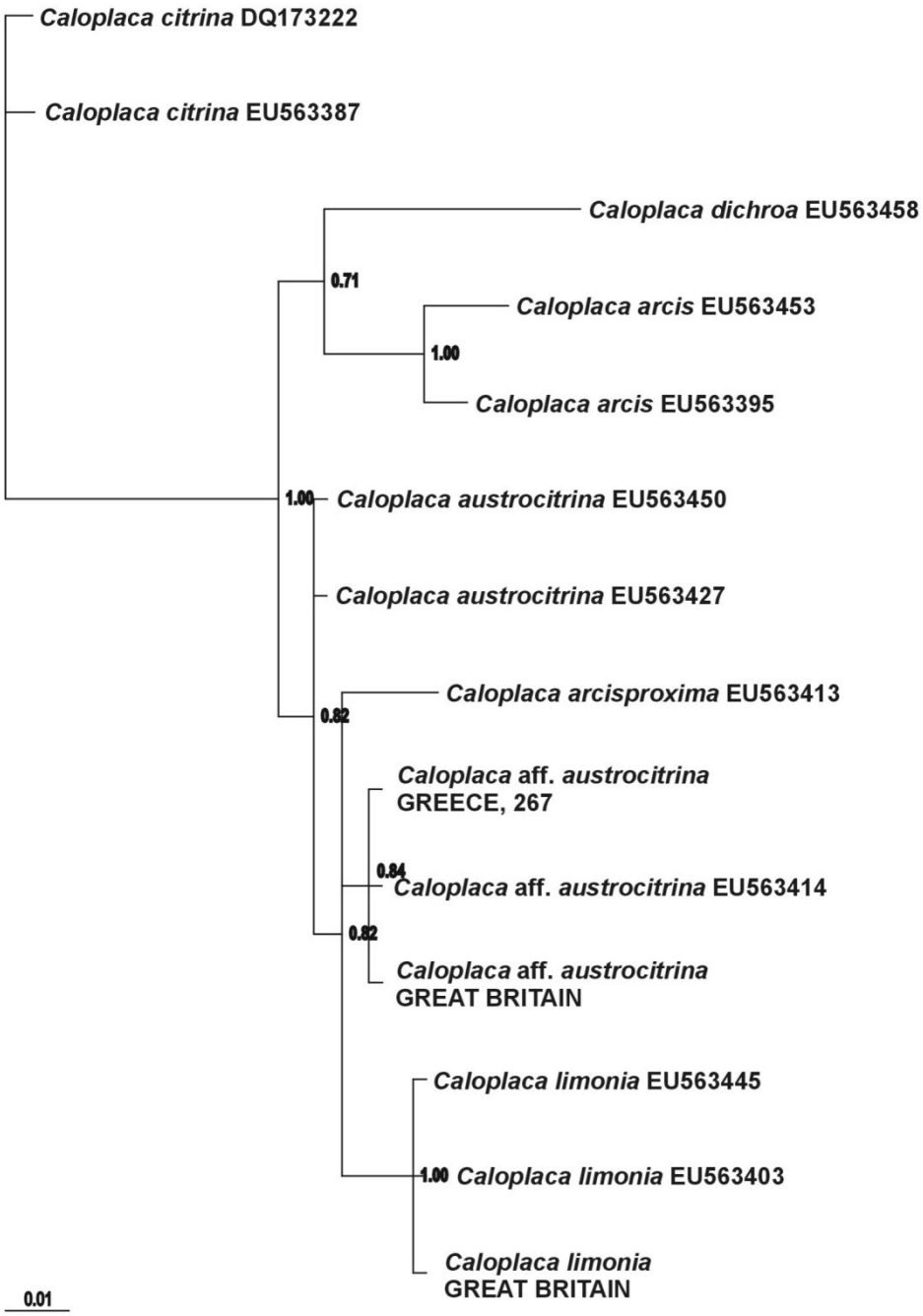

Fig. 3. Bayesian phylogeny of the *Caloplaca citrina* group showing placements of the British lichens