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Caloplaca ruderum new to Sweden and new combinations in *Flavoplaca* (Ascomycota, Teloschistales)

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Arup, U., Söchting, U. & Lorentzon, J. 2024. *Caloplaca ruderum* new to Sweden and new combinations in *Flavoplaca* (Ascomycota, Teloschistales). *Graphis Scripta* **36** (6): 113–122. Oslo. ISSN 2002-4495.

Caloplaca ruderum is reported as new to Sweden from three localities. Based on a phylogenetic analysis it is transferred to the genus *Flavoplaca*, where the closest relatives are *F. communis*, *F. maritima* and *F. havaasii*. In addition, *Caloplaca sol* and *C. itiana* are transferred to *Flavoplaca*. The identities of *C. itiana*, *C. calcitrapa*, and *C. dalmatica* in the sense of British treatments are discussed.

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Introduction

Caloplaca ruderum (Malbr.) J.R. Laundon is a crustose lichen species in the family *Teloschistaceae*. The thallus is uneven, composed of strongly convex to verruculose or almost globose areoles, sometimes almost as squamules, and large zeorine apothecia. It was described from Normandie in France by Malbranche (1873) as *Lecanora aurantiaca* var. *rudera*, but when Laundon (1976) reported it as new to the British Isles he transferred it to *Caloplaca* and elevated it to species level as *C. ruderum*. The species is known from western Europe, from Spain (Llimona & Hladun 2001) in southwest Europe and north up to Denmark (Aptroot 2000, Svampeatlas, Danish Mycological Society 2024). We now report it from three localities in Skåne, southernmost Sweden. On the basis of morphology and phylogenetic analyses of ITS data it is, together with *Caloplaca itiana* Cl.Roux, M. Boulanger & Malle and *Caloplaca sol* Orange, transferred to the genus *Flavoplaca*, one of the largest genera in the family *Teloschistaceae* and one of the most widespread and common ones in Europe.

Material

New DNA sequences were obtained from recent material collected in Sweden and Denmark deposited in LD and C, and material from United Kingdom kindly supplied by C. Hitch and M. Powell. A further data set of DNA sequences was used from previous studies by us or other authors deposited in GenBank.

Table 1. Sequences used in the analysis, newly produced in bold and others downloaded from Genbank. Names within brackets are those appearing in Genbank but not corresponding to the current study.

Species	Country, collector, collection nr, herbarium	nrITS
<i>Athallia holocarpa</i>	Sweden, Arup L04019, LD	FJ346558
<i>Calogaya decipiens</i>	Denmark, Søchting, 1995, C	KC179344
<i>Flavoplaca arcis</i>	Austria, Arup L97514, LD	DQ173213
<i>Flavoplaca arcisproxima</i>	Greece, Vondrák JV4125, PRA	EU563413
<i>Flavoplaca austrocitrina</i>	Argentina, Traversa et al., LD	GQ338422
<i>Flavoplaca calcitrapa</i> 1	Greece, Vondrák 8375, PRA	JN813409
<i>Flavoplaca calcitrapa</i> 2 (<i>navasiana</i>)	Greece, Vondrák 8871, PRA	JN813402
<i>Flavoplaca calcitrapa</i> 3	Turkey, Vondrák 6100, PRA	EU563444
<i>Flavoplaca calcitrapa</i> 4 (<i>navasiana</i>)	Greece, Vondrák 5939, PRA	JN813430
<i>Flavoplaca calcitrapa</i> 5	Greece, Vondrák 8729, PRA	JN813413
<i>Flavoplaca calcitrapa</i> 6	Greece, Sipman & Raus 63790, B	MN989242
<i>Flavoplaca calcitrapa</i> 7 (<i>itiana</i>)	France, Orange 23700, NMW	MF595950
<i>Flavoplaca calcitrapa</i> 8 (<i>itiana</i>)	France, Orange 23694, NMW, claimed topotype of <i>F. itiana</i>	MF595944
<i>Flavoplaca calcitrapa</i> 9	Spain, Navarro-Rosines et al. 13387, BCN isotype	DQ173227
<i>Flavoplaca citrina</i>	Sweden, Arup L03013, LD	DQ173224
<i>Flavoplaca communis</i> 1	Turkey, Vondrák JV3471, PRA	EU563409
<i>Flavoplaca communis</i> 2	Greece, Gavalas & Sipman, B	MN512250
<i>Flavoplaca communis</i> 4	Greece, Sipman & Raus 63377, B	MN989248
<i>Flavoplaca communis</i> 4	Greece, Sipman & Raus 63402, B	MN172432
<i>Flavoplaca communis</i> 5	Bulgaria, Vondrák JV6113, PRA	EU563447
<i>Flavoplaca communis</i> 6	Russia, Vondrák JV6104, PRA	EU563446
<i>Flavoplaca communis</i> 7	Russia, Vondrák JV14419, PRA	KU926992
<i>Flavoplaca communis</i> 8	Turkey, Vondrák JV3763, PRA	EU563410
<i>Flavoplaca communis</i> 9	Turkey, Vondrák JV6119, PRA	EU563448
<i>Flavoplaca communis</i> 10	Turkey, Vondrák JV3042, PRA	EU563397
<i>Flavoplaca communis</i> 11	Turkey, Vondrák JV3367, PRA	EU563399
<i>Flavoplaca communis</i> 12	Greece, Frödén 1889, LD	PQ481158
<i>Flavoplaca confusa</i>	Portugal, Berger 15742, Berger priv. hb.	EU563468
<i>Flavoplaca coronata</i>	Austria, Arup L00038, LD	DQ173239
<i>Flavoplaca cranfieldii</i>	Australia, Kondratyuk 20423, LD	KC179365
<i>Flavoplaca dichroa</i>	Sweden, Arup L04005, LD	DQ173228

<i>Flavoplaca flavocitrina</i>	Sweden, Arup L04024, LD	DQ173216
<i>Flavoplaca geleverjæ</i>	Ukraine, Vondrák JV5415, PRA	EU563423
<i>Flavoplaca granulosa</i>	Spain?, Gaya 223 & Llimona, BCN 13702	EU639630
<i>Flavoplaca havaasii</i> 1	Norway, Tønsberg 33940, LD	DQ647649
<i>Flavoplaca havaasii</i> 2	Norway, Klepsland JK09-L676, O	PQ481159
<i>Flavoplaca havaasii</i> 3	Norway, Klepsland JK12-L195, herb. Klepsland	PQ481160
<i>Flavoplaca havaasii</i> 4	Norway, Klepsland LK14-L539, herb. Klepsland	PQ481161
<i>Flavoplaca havaasii</i> 5	Russia, Frolov 1174, herb. Frolov	KX022976
<i>Flavoplaca itiana</i> 1 (<i>calcitrapa</i>)	Bulgaria, Vondrák JV5846, PRA	EU563431
<i>Flavoplaca itiana</i> 2	France, Orange 23697, NMW	MF595947
<i>Flavoplaca itiana</i> 3	France, Roux, Boulanger & Malle s.n., C isotype	PQ481162
<i>Flavoplaca itiana</i> 4 (<i>dalmatica</i>)	U.K., Wales, Orange 21053, NMW	MF595917
<i>Flavoplaca itiana</i> 5 (<i>dalmatica</i>)	U.K., England, Orange 21067, NMW	MF595922
<i>Flavoplaca itiana</i> 6 (<i>dalmatica</i>)	U.K., England, Orange 21065, NMW	MF595921
<i>Flavoplaca itiana</i> 7 (<i>dalmatica</i>)	U.K., Wales, Orange 21050, NMW	MF595914
<i>Flavoplaca itiana</i> 8	U.K., England, Arup L92145, LD	PQ481163
<i>Flavoplaca itiana</i> 9	U.K., Wales, Arup L92139, LD	PQ481164
<i>Flavoplaca itiana</i> 10	France, Søchting 12125, C	PQ481165
<i>Flavoplaca itiana</i> 11	France, Arup L19450, LD	PQ481166
<i>Flavoplaca itiana</i> 12 (<i>dalmatica</i>)	Greece, Sipman & Raus 63219, B	MN172434
<i>Flavoplaca kantvilasii</i>	Australia, Kondratyuk 20418a, LD	KC179366
<i>Flavoplaca limonia</i>	Turkey, Vondrák JV6101, PRA	EU563445
<i>Flavoplaca marina</i> ssp. <i>marina</i>	U.K., England, Arup L92106, LD	AF353946
<i>Flavoplaca marina</i> ssp. <i>americana</i>	Canada, British Columbia, Arup L89635, LD	AF353947
<i>Flavoplaca maritima</i> 1	U.K., Wales, Arup L92092, LD	AF353948
<i>Flavoplaca maritima</i> 2	U.K., Wales, Vondrák JV4943, PRA	EU563462
<i>Flavoplaca maritima</i> 3	U.K. England, Orange 21072, NMW	MF595925
<i>Flavoplaca maritima</i> 4	U.K. Wales, Orange 21903, NMW	MF595938
<i>Flavoplaca maritima</i> 5	U.K. Wales, Orange 21047, NMW	MF595913
<i>Flavoplaca maritima</i> 6	The Netherlands, Vondrák JV4987, PRA	EU563451
<i>Flavoplaca maritima</i> 7	Spain?, Gaya 248b & Llimona, BCN	EU639627
<i>Flavoplaca mereschkowiana</i>	Australia, WA, Kärnefelt 20041503, LD	KC179367
<i>Flavoplaca microthallina</i>	Sweden, Søchting 7480, C	KC179368
<i>Flavoplaca navasiana</i> 1	France, Arup L19417, LD	PQ481167
<i>Flavoplaca navasiana</i> 2	France, Arup L19447, LD	PQ481168
<i>Flavoplaca navasiana</i> 3	Greece, Sipman & Raus 63135, B	MN172439

<i>Flavoplaca navasiana</i> 4	Italy, Poelt 1992, LD	KC179369
<i>Flavoplaca nigromarina</i>	Turkey, Vondrák JV3035, PRA	EU563394
<i>Flavoplaca oasis</i>	Sweden, Arup L03017, LD	FJ346546
<i>Flavoplaca ora</i>	France, Lambinon, Rondon & Vezda, W-15494	HQ234602
<i>Flavoplaca polycarpa</i>	Italy, 1996, Nimis & Tretiach s.n., S	FJ346551
<i>Flavoplaca ruderum</i> 1	U.K. England, Powell 3058, LD	PQ481169
<i>Flavoplaca ruderum</i> 2	U.K., England, Hitch 31028, herb. Hitch	PQ481170
<i>Flavoplaca ruderum</i> 3	Sweden, Lorentzon, LD	PQ481171
<i>Flavoplaca ruderum</i> 4	U.K. England, Powell, LD	PQ481172
<i>Flavoplaca ruderum</i> 5	Denmark, Søchting 12207, C	PQ481173
<i>Flavoplaca ruderum</i> 6	Sweden, Arup & Ekman L1993, LD	PQ481174
<i>Flavoplaca ruderum</i> 7	Sweden, Arup & Ekman L1994, LD	PQ481175
<i>Flavoplaca sol</i> 1	U.K., Wales, Orange 21848, NMW	MF595933
<i>Flavoplaca sol</i> 2	U.K., England, Orange 21068, NMW	MF595923
<i>Flavoplaca sol</i> 3	U.K., Wales, Orange 21052, NMW	MF595916
<i>Flavoplaca sol</i> 4	U.K., Wales, Orange 21909, NMW	MF595940
<i>Flavoplaca sol</i> 5	U.K., England, Orange 21062, NMW	MF595920
<i>Flavoplaca tavaresiana</i>	Spain, Llimona, Navarro-Rosiné & Roux s.n., 1992, BCN	KC179371

DNA extraction

New sequences for this study (Table 1) were produced using direct PCR according to Arup et al. (2015). Amplifications were made of the internal transcribed spacer regions (nrITS). Primers for amplification were ITS1F (Gardes & Bruns 1993), ITS4 (White et al. 1990). The PCR parameters included an initial hold at 95°C for 5 min, then denaturing at 95°C for 1 min, annealing at 56°C for 1 min, decreasing 1°C per cycle for the first 6 of the 39 cycles (touchdown), and extension at 72°C for 3 min. The sequencing was done by MacroGen Inc., South Korea, using the same primers as for the PCR. The two resulting strands were assembled using Geneious 11.1.5. Subsequent alignments were done in the same program and adjusted manually. Sequences have been submitted to GenBank as indicated in Table 1.

A BLAST search in GenBank (<https://blast.ncbi.nlm.nih.gov>) revealed a close relationship of *Caloplaca ruderum* with species of the genus *Flavoplaca* Arup, Søchting & Frödén. An alignment was thus constructed using sequences according to Table 1 using *Calogaya decipiens* as outgroup. Data were analyzed using the program MrBayes 3.2.4 (Ronquist et al. 2012). A suitable model of molecular evolution was selected using the Bayesian Information Criterion (BIC) as implemented in jModeltest ver. 2.1.4 (Guindon & Gascuel 2003; Darriba et al. 2012), evaluating only the 24 models available in MrBayes 3.2.0 (Ronquist et al. 2012). For the analysis the GTR+G model was found to be optimal.

Results

We generated 18 new sequences for this study. The alignment of the nrITS data consisted of the outgroup and 57 ingroup sequences with 548 aligned nucleotide sites, of which 128 were parsimony informative. The Bayesian ITS analysis halted after 4 100 000 generations, and a 50% majority-rule tree is shown in Fig. 1.

In the phylogenetic tree *C. ruderum* is nested within the genus *Flavoplaca* demonstrating a close relationship with especially *F. communis* (Vondrák, Říha, Arup & Søchting) Arup, Søchting & Frödén, *F. havaasii* (H. Magn.) Arup, Frödén & Søchting and *F. maritima* (B. de Lesd.) Arup, Frödén & Søchting. These are all located on a central clade in the genus with *F. citrina* (Hoffm.) Arup, Frödén & Søchting as sister species. *Flavoplaca havaasii* appears at the base of the clade including four Norwegian sequences with full support. The remaining three clades basically consist of *F. maritima* with high support, *C. ruderum* almost with high support (PP = 0.922) and *C. communis* without support. The last species also has some scattered sequences in the tree at the bases of the *F. maritima* and the *C. ruderum* clades.

Caloplaca sol and *C. itiana* are located on two different branches with *F. navasiana* inbetween in two different clades. These three species together form a sister clade to most other *Flavoplaca* species on a fully supported node, but clearly inside *F. mereschowskiana* and *F. oasis* in one clade, and *F. coronata*, *F. granulosa*, and *F. polycarpa* in another one.

Discussion

The results show that *C. ruderum* belongs to the genus *Flavoplaca* and the species is accordingly transferred to this genus. It is also clear that ITS sequences alone are not enough to fully separate the closely related species and more genes will probably help in this regard. The four species in the *F. ruderum* clade are very similar in morphology and anatomy, but with well-separated distribution and ecological demands. *Flavoplaca havaasii* is so far known only from Norway and from the Murmansk region of Russia (Frolov & Konoreva 2016) where it grows on slightly calcareous rocks, slate or sandstone, mainly under overhangs, in the mountains but at modest elevations, 115–580 m, and not occurring close to the seashore. *Flavoplaca ruderum* on the other hand grows on soft calcareous walls and mortar, in Britain often on old church walls facing south, more rarely on seashore rocks (Fletcher & Laundon 2009). *Flavoplaca communis* and *F. maritima* on the contrary are more or less confined to seashore rocks or rocks close to the coast but where the salinity and/or the exposure to salt water is high. Morphs of *F. communis* with a more compact thallus and a less distinct granular surface resembles *F. maritima*, but their known geographical distributions do not overlap; the former is confined to Southeastern Mediterranean, the Black and the Caspian Sea, and the latter is a western European species.

In Sweden *F. ruderum* was found on a concrete wall 550 m from the seashore, on a wall of a house 350 m from the coastline on the island of Hallands Väderö and on a concrete roof of a water reservoir and a concrete pole, also on Hallands Väderö. In none of these sites are the lichens directly affected by salt spray or splashing waves. The first finding of *F. ruderum* in Sweden was actually done on the island of Hallands Väderö already in 1990, but identified as a deviating form of *Flavoplaca marina*. This finding was made on a concrete roof of an underground water reservoir,

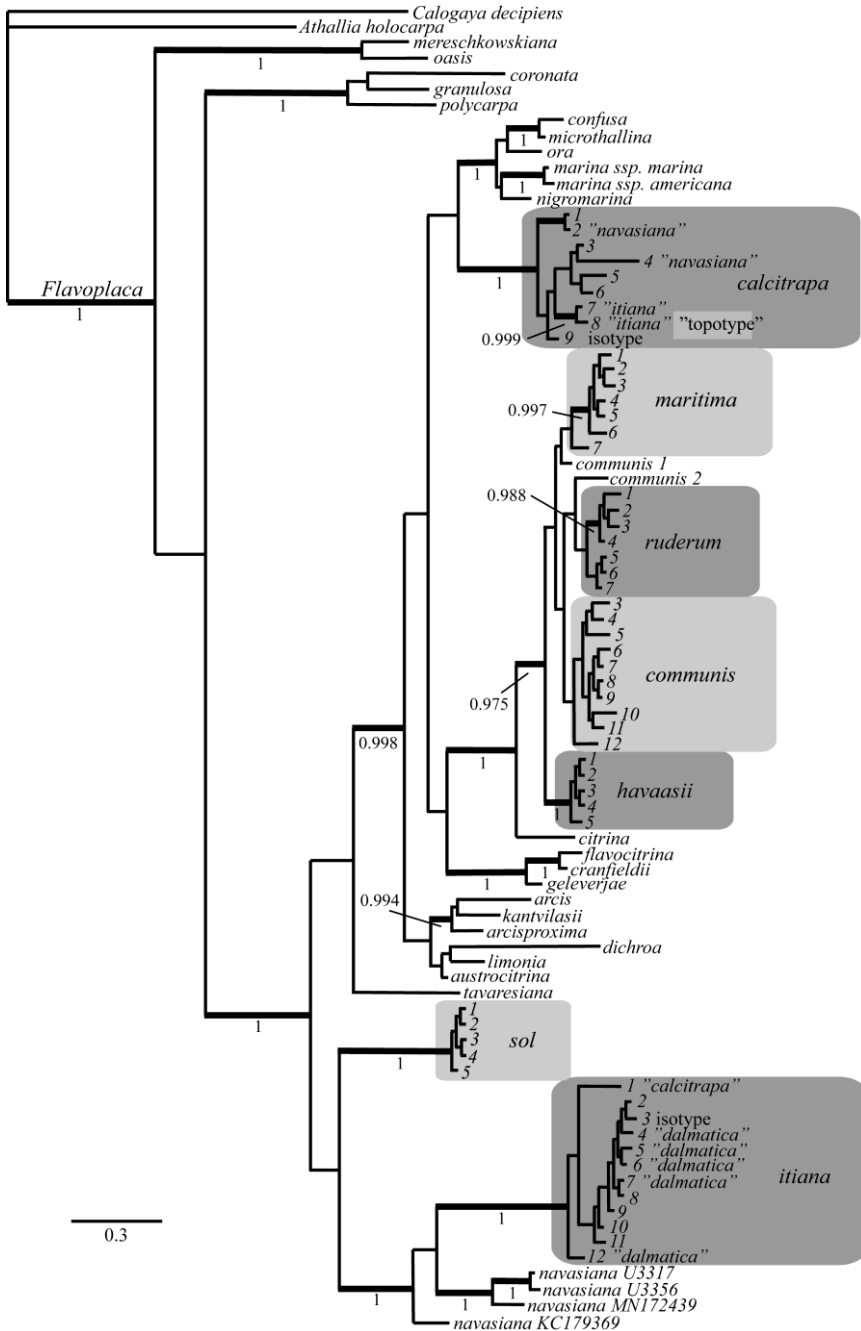


Figure 1. Majority-rule consensus tree based on a Bayesian MCMC analysis of the nrITS gene showing the position of *Flavoplaca ruderum* and its closest relatives. Names within quotes indicate the name submitted to GenBank by the authors of these sequences. Branches with posterior probabilities higher or equal to 0.95 are shown in bold.

almost at the same locality as the concrete pole mentioned above. In all localities the substrate is calcareous and fairly soft. All Swedish records have been made in well-lit places, either on vertical or horizontal surfaces. The Swedish records thus fit well with the ecology of the British records (Laundon 1976, Fletcher & Laundon 2009). The Swedish specimens agree well with the typical morphology of the species except for one thing, they are not as pruinose as they seem to be in most British specimens.

Caloplaca sol and *C. itiana* were included by Orange (2018) in a paper about *Flavoplaca*, where he described *C. sol* as new to science. Despite concluding that all species treated in his analysis belonged to the genus *Flavoplaca*, he did not describe *C. sol* in this genus or transferred *C. itiana* to it. The latter was later transferred to *Athallia* by Roux (2022), but without any comments or analysis of DNA data. Based on our molecular results there is no doubt that both these species belong to *Flavoplaca* and we make the formal combinations below.

There seems to be a lot of taxonomic confusion about *F. itiana* and its relationship to *F. calcitrapa* (Nav.-Ros., Gaya & Cl. Roux) Arup, Søchting & Frödén and *Caloplaca dalmatica* (A. Massal.) H. Olivier. As can be seen in the tree (Fig. 1), the clade marked *F. itiana* consists of GenBank accessions named *Caloplaca dalmatica*, *F. calcitrapa*, *F. navasiana* and our own sequence of *F. itiana* number three representing an isotype. Orange (2018) argued that the British material traditionally called *C. dalmatica* was represented by this clade, but according to our results it corresponds to *F. itiana*. The true *C. dalmatica* differs morphologically and as there are large morphological similarities to several species of *Variospora* Arup, Søchting and Frödén it may belong to that genus, but this is yet to be shown by molecular data. The combination *V. dalmatica* (A. Massal.) Cl. Roux has, however, already been made by Roux (2022), without any comments or supporting data.

Additionally, the *F. calcitrapa* clade is problematic and consists of GenBank submissions of true *F. calcitrapa*, among them an isotype sequence, *F. navasiana*, and *F. itiana* sequences claimed by Orange (2018) to represent this species because the material was collected at the type locality (topotype). Orange noticed the mixture of sequences of *F. calcitrapa* and what he interpreted as *F. itiana* and called it the *F. calcitrapa* complex. Furthermore, sequences claimed to belong to *F. navasiana* appear on three different branches, among them one inside what we believe is *F. calcitrapa*. Morphologically, all these species can be very similar when the thallus is poorly developed or even endolithic, and possibly incorrectly identified specimens used for molecular studies have added to the misunderstanding of the species. In addition, Vondrák et al. (2009) stated that the typical sand-clock type of spores of *F. calcitrapa* are often not found, which can be another reason for the confusion. In our experience, the immature and young spores of *F. calcitrapa* normally possess the typical thick walls and at least in well developed spores also the sand-clock shape, but older spores may become similar to normal “*Caloplaca*” spores with thinner wall and traditional shape of the lumina reminding of those in *F. navasiana* and *F. itiana*. The thallus in *F. calcitrapa* may be thin, but we have not yet seen any material with endolithic thallus.

Our concepts of *F. itiana*, *F. calcitrapa* and *Caloplaca dalmatica* are clearly different from those of Orange (2018), but we base our conclusions on sequences of type material of *F. itiana* and *F. calcitrapa* and believe that our tree shows the correct relationships, even though it is a little hard to understand why Orange would have sequenced *F. calcitrapa* from the type locality of *F. itiana*. *Flavoplaca navasiana* still appears on two branches in our tree, but the differences in the sequences data are not large and an extended study of this species may clarify the relationship.



Figure 2. Habitus photo of *F. ruderum* from house wall on Hallands Väderö, Skåne. Scale bar = 1 mm.

Taxonomy

***Flavoplaca itiana* (Cl. Roux, M. Boulanger & Malle) Arup & Søchting, comb. nov.**

Mycobank No.: MB 856137.

Basionym: *Caloplaca itiana* Cl. Roux, M. Boulanger & Malle, *Bull. Ass. Fr. Lichénologie* 34: 4 (2009). – *Athallia itiana* (Cl. Roux, M. Boulanger & Malle) Cl. Roux, *Bull. Ass. Fr. Lichénologie* 47: 30 (2022).

Type: France, Pas-de-Calais, Boulonnais, commune de Audinghen, cap Griz-Nez, le banc des Épaulards, sur une surface de calcaire gréseux très cohérent, inclinée de 30° vers le nord-ouest, immédiatement au-dessus de la zone à *Hydropunctaria maura*, alt. d'environ 5–10 m. 2008/05/01, C. Roux, M. Boulanger & N. Malle (MARSSJ–holotype; BNC-lich., C–isotypes).

***Flavoplaca ruderum* (Malbr.) Arup & Søchting, comb. nov.**

Figs 2–3

Mycobank No.: MB 856136.

Basionym: *Lecanora aurantiaca* var. *rudera* Malbr., *Lich. Norm.*: no. 322 (1873). – *Caloplaca ruderum* (Malbr.) Laundon, *Lichenologist* 8: 139 (1976).



Figure 3. Habitus photo of *F. ruderum* on concrete wall at Skanör, Skåne. Scale bar = 3 mm.

Type: France. Seine-Inférieure, Rouen, Quevilly, on mortar of calcareous walls, Malbranche, Lich. Norm. exs. No 322 (UPS–lectotype!).

Specimens examined: **Denmark.** *Jutland:* Ferring Church, soil on church dike, 30 Oct. 2014, Søchting 12207 (C). **Great Britain.** *England:* Bedfordshire, V.C. 30, Shillington church, on sloping limestone of windowsill, TL 123/339, 19 April 2013, M. Powell (LD-2205524); Suffolk, V.C. 25, Monewden church, on sloping limestone of windowsill on south wall of church, TM 2358, 18 April 2015, C. Hitch & K. Carr-Tansley 31028 (herb. Hitch); Suffolk, V.C. 25, wall of Orford church, TM 422.499, 2 June 2013, M. Powell 3058 (LD-2206292). **Sweden.** *Skåne:* Skanör/Falsterbo par., Skanör, W of Dykerigatans W end, 55.418508°N 12.845510°E, on concrete wall, J. Lorentzon s.n. (LD-2206228). Torekov par., Hallands Väderö, old water reservoir at the lighthouse, 56°26'N 12°34'E, Sect. 1, on mortar, 4 May 1990, Arup & Ekman L1270 (LD-1019423), 30 m ESE of the lighthouse, on concrete pole lying on the ground, elev. 9 m., 56.45055°N 12.54292°E, 30 Aug. 2023, Arup & Ekman L1993 (LD-2206164, Skogvaktarbostället (Youth Hostel), on plaster on house wall, elev. 6 m., sect. 8., 56.43445°N 12.56513°E, 31 Aug. 2023, Arup & Ekman L1994 (LD-2206100).

***Flavoplaca sol* (Orange) Arup & Søchting, comb. nov.**

Mycobank No.: MB 856138.

Basionym: *Caloplaca sol* Orange, *Lichenologist* 50: 414 (2018).

Type: Great Britain, Wales, Anglesey, V.C. 52, Benllech, SH 52468237, 53.317545°N, 04.216396°W, on limestone on vertical cliff at top of seashore, 18 April 2012, Alan Orange 21052 (NMW–C.2015.005.37–holotype; MARSSJ–isotype; GenBank Accession MF595916).

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