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Short Communication

The identity of *Calicium corynellum* (Ach.) Ach.

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Recently, Yahr (2015) studied British populations of *Calicium corynellum* (Ach.) Ach. to test whether these were distinct from *C. viride* Pers. As *C. corynellum* had the highest conservation priority in Britain, and was apparently declining rather dramatically, it was important to clarify its taxonomic status. Yahr (2015) used both genetic (ITS rDNA) and morphological data from two British *Calicium* aff. *corynellum* populations and could not find differences with *C. viride*, suggesting that the British material represented saxicolous populations of the otherwise epiphytic or lignicolous *C. viride*. Although this study focused on British material, it introduced serious doubts about the identity and relationships of these two species in other parts of the distribution area of *C. corynellum*. Interestingly, the British material that Yahr (2015) investigated was morphologically very similar to *C. viride*, but the latter was described as differing rather substantially from *C. corynellum* in other parts of its distribution area. Thus, *C. corynellum* differs from *C. viride* in its short-stalked, greyish white pruinose ascomata (*C. viride* has long stalks and a brown pruina), distinctly narrower spores compared with *C. viride*, and the leprose thallus (Fig. 1) which is granular to verrucose in *C. viride* (Tibell 1999).

Calicium viride is a common and widely distributed species in temperate areas of the Northern Hemisphere and southern South America (Tibell 1999; GBIF 2019). *Calicium corynellum* is, however, not as well understood, with scattered occurrences in Europe and North America (Sarrión *et al.* 1999; Tibell 1999; Pérez-Ortega 2007; GBIF 2019). Additionally, *C. corynellum* is currently being evaluated for the Red List of lichens of Spain and Portugal (Atienza *et al.* 2019), which makes it essential to assess the taxonomic status of the species.

As *C. corynellum* is such a poorly known species and the results obtained by Yahr *et al.* (2015) may be interpreted to suggest that *C. corynellum* could be better treated as a synonym of *C. viride*, the status of *C. corynellum* should be studied further. Our aim here is to study the delimitation of these two taxa based on a larger sample, covering the whole known distribution range of *C. corynellum*. For that purpose, we have conducted a phylogenetic analysis based on sequences of the ITS region of the nuclear rDNA.

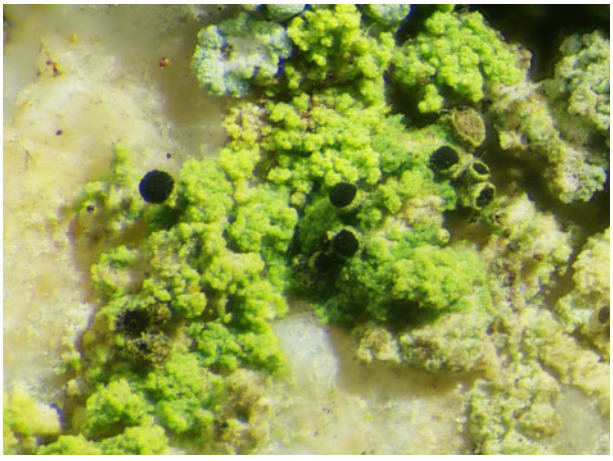

Fig. 1. *Calicium corynellum* habitus (*M. Prieto* C4 (ARAN-Fungi 8454)). Scale = 1 mm. In colour online.

We sampled our own recently collected material, several herbarium specimens and a selection of samples from GenBank (Table 1). *Calicium salicinum* Pers. was included as outgroup based on Prieto & Wedin (2017). DNA was extracted and amplified (nrITS region) following Prieto & Wedin (2017). Sequences were assembled and edited using Sequencher v. 4.10.1 (Genes Codes Corporation, Ann Arbor, MI, USA) and deposited in GenBank (Table 1). Subsequently, sequences were aligned manually using MacClade 4.01 (Maddison & Maddison 2001). No ambiguous regions and introns were found. A phylogenetic analysis was carried out using maximum likelihood-based inference (ML) as implemented in RAxML v. 8.2.10 (Stamatakis 2014) run on the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2010). The analysis was performed with a GTRGAMMA model for tree inference and GTRCAT model and 1000 replicates for bootstrapping.

Thin-layer chromatography was carried out following standard methods (Orange *et al.* 2001) and using solvent systems B and C. As data given in the literature (i.e. Tibell 1999) refer to the thallus and not to the ascomata, we sampled thallus parts.

A total of seven sequences were newly generated for this study (Table 1). The data set consisted of 20 taxa and 486 unambiguously aligned sites. The ML tree with bootstrap values is shown in Fig. 2 and demonstrates that samples classified as *Calicium corynellum* and *C. viride* based on morphology form two distinct

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Table 1. *Calicium* specimens used in this study, with GenBank Accession numbers. Entries for newly obtained sequences and new chemical analyses are in bold. Specimen data are given for newly produced sequences with collection number and location of voucher. Chemistry: R = rhizocarpic acid, U = usnic acid, E = epanorin, C = calycin. Codes enclosed by parentheses indicate trace amounts. Data from specimens not analyzed here come from Yahr (2015).

Species	Code/Voucher	Locality	GenBank Acc. no.	Chemistry
Calicium corynellum 1	**_S. Pérez-Ortega_ S103**	**Madrid, Spain**	**MT068633**	**not tested**
C. corynellum 2	**_Thor_ 23134 (UPS)**	**Södermanland, Sweden**	**MT068630**	**R**
C. corynellum 3	**_M. Prieto_ C5 (ARAN-Fungi 8329)**	**Madrid, Spain**	**MT068634**	**R, U**
C. corynellum 4	**_Svensson_ 940 (UPS)**	**Småland, Sweden**	**MT068635**	**R**
C. corynellum 5	**_M. Prieto_ C4 (ARAN-Fungi 8454)**	**Madrid, Spain**	**MT068632**	**R, (C?)**
C. corynellum 6	*M. Prieto* CC1 (S)	Cabañeros, Spain	KX512908	
C. corynellum 7	**_Spribille_ 11865 (UPS)**	**Montana, USA**	**MT068631**	**R**
C. viride 1 (as *C.* aff. *corynellum* in Yahr 2015)	*Yahr* 5398	Whitfield, England	KF991222	R
C. viride 2	*Yahr* 5404	Whitfield, England	KF991224	R
C. viride 3 (as *C.* aff. *corynellum* in Yahr 2015)	*Yahr* 5400	Whitfield, England	KF991227	R, (U), (E)
C. viride 4	-	England	FR799144	-
C. viride 5	-	England	FR799143	-
C. viride 6	-	England	FR799142	-
C. viride 7	AFTOL348	-	HQ650703	-
C. viride 8	**_M. Wedin_ 8283 (S)**	**Södermanland, Sweden**	**MT068629**	**R**
C. viride 9	-	Whitfield, England	KF991223	-
C. viride 10	*Wedin* 24/4 2000	-	AY143393	-
C. viride 11	*Tibell* 17553 (UPS)	Argentina	DQ812142	-
C. salicinum 1	*Tibell* 23270 (UPS)	India	DQ812133	-
C. salicinum 2	*Tibell* 23193 (UPS)	India	DQ812131	-

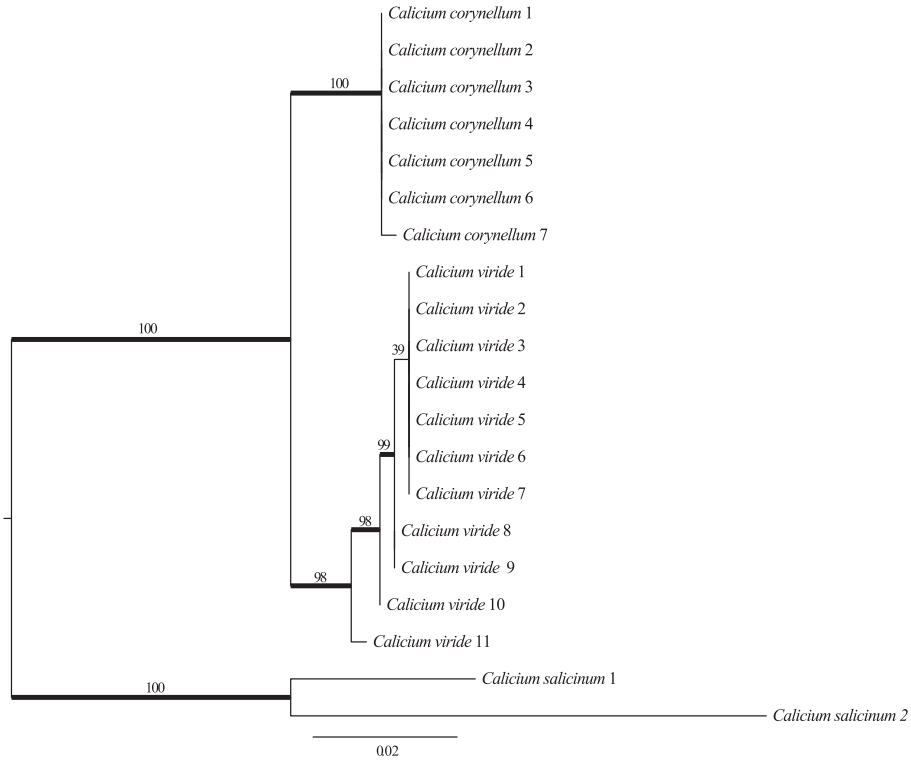

Fig. 2. Phylogenetic tree (best maximum likelihood (ML) tree) of *Calicium corynellum* and *Calicium viride* resulting from RAxML analysis of nrITS sequences. Bootstrap supports (ML-BS) are shown above branches and supported clades (ML-BS ≥ 70) are marked with thicker black branches. *Calicium salicinum* is used to root the tree.

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monophyletic groups, which we interpret here as different species. While there is some genetic variation within *Calicium viride*, *C. corynellum* has very little (Fig. 2).

Our results suggest that *Calicium corynellum* is a distinct species which can be distinguished from *C. viride* by both morphological and genetic characters. Our results also support that the two British populations studied by Yahr (2015) correspond to specimens of *C. viride* growing on rocks. However, this does not exclude the possibility that the real *C. corynellum* might also exist in Britain.

The two species also apparently differ chemically, as *C. viride* contains rhizocarpic acid and epanorin, and *C. corynellum* contains rhizocarpic and usnic acids (Tibell 1999; Yahr 2015). This was not evident in the study by Yahr (2015). Of the samples collected by Yahr (*C.* aff. *corynellum*) that grew on rock, only three out of seven chemically tested specimens contained usnic acid. From the five herbarium specimens chemically studied by Yahr (probably representing the real *C. corynellum*), one did not have usnic acid. The *C. corynellum* specimens sampled here contained only rhizocarpic acid, except one sample that also contained usnic acid (Table 1). Similar to Yahr, we did not detect epanorin in the *C. viride* specimen studied. Our results further support that the amounts of usnic acid in *C. corynellum* and epanorin in *C. viride* are at least very variable (and that usnic acid may also occur in saxicolous *C. viride*) and difficult to use to identify these species, just as Yahr (2015) suggested.

The studied specimens of *Calicium corynellum* were not lichenicolous and produced well-developed thalli (Fig. 1). The Spanish specimens were all found in a very typical but easily overlooked habitat: overhanging siliceous rocks in shaded and humid situations. The Spanish distribution is mainly along the Central Mountains but, as we have recently found a number of new localities, we believe that the species is probably very overlooked in these areas, as in the rest of the world.

We can conclude that *Calicium corynellum* is a species distinct from *C. viride* and thus should be treated separately in future conservation activities and Red List assessments.

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