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Review

Crossiella, a Rare *Actinomycetota* Genus, Abundant in the Environment

Tamara Martin-Pozas [1,†], Jose Luis Gonzalez-Pimentel [2,†], Valme Jurado [3], Leonila Laiz [3], Juan Carlos Cañaveras [4], Angel Fernandez-Cortes [5], Soledad Cuezva [6], Sergio Sanchez-Moral [1] and Cesareo Saiz-Jimenez [3,*]

[1] Museo Nacional de Ciencias Naturales, MNCN-CSIC, 28006 Madrid, Spain
[2] Centro Andaluz de Biologia del Desarrollo (CABD, UPO-CSIC-JA), Facultad de Ciencias Experimentales, Departamento de Genetica, Universidad Pablo de Olavide, 41013 Sevilla, Spain
[3] Instituto de Recursos Naturales y Agrobiologia, IRNAS-CSIC, 41012 Sevilla, Spain
[4] Departamento de Ciencias de la Tierra y del Medio Ambiente, Universidad de Alicante, 03080 Alicante, Spain
[5] Departamento de Biologia y Geologia, Universidad de Almeria, 04120 Almeria, Spain; acortes@ual.es
[6] Departamento de Geologia, Geografia y Medio Ambiente, Universidad de Alcala, 28802 Alcala de Henares, Spain; soledad.cuezva@uah.es
* Correspondence: saiz@irnase.csic.es
† These authors contributed equally to this work.

Abstract: The genus *Crossiella* contains two species, *C. equi*, causing nocardioform placentitis in horses, and *C. cryophila*, an environmental bacterium. Apart from *C. equi*, which is not discussed here, environmental *Crossiella* is rarely reported in the literature; thus, it has not been included among "rare actinobacteria", whose isolation frequency is very low. After *C. cryophila*, only five reports cover the isolation of *Crossiella* strains. However, the frequency of published papers on environmental *Crossiella* has increased significantly in recent years due to the extensive use of next-generation sequencing (NGS) and a huge cascade of data that has improved our understanding of how bacteria occur in the environment. In the last five years, *Crossiella* has been found in different environments (caves, soils, plant rhizospheres, building stones, etc.). The high abundance of *Crossiella* in cave moonmilk indicates that this genus may have an active role in moonmilk formation, as evidenced by the precipitation of calcite, witherite, and struvite in different culture media. This review provides an overview of environmental *Crossiella*, particularly in caves, and discusses its role in biomineralization processes and bioactive compound production.

Keywords: *Crossiella*; biofilms; caves; moonmilk; biomineralization; rhizosphere; soils; bioactive compounds

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1. Introduction

The first strain included in the genus *Crossiella* has a long history of transfers and amendments. Takahashi et al. [1] studied the soil isolate *Nocardiopsis mutabilis*, capable of producing novel antibiotics, and classified it as a new subspecies: *Nocardiopsis mutabilis* subsp. *Cryophilis* based on its growth at low temperatures (8–33 °C). This strain was subsequently transferred by Labeda and Lechevalier [2] to the genus *Saccharothrix* as *Saccharothrix cryophilis* because its morphological and chemotaxonomical properties were more typical of the genus *Saccharothrix* than *Nocardiopsis*. In another study, Labeda [3] erected the genus *Crossiella* to accommodate the species *Saccharothrix cryophilis*, which was misplaced within the genus *Saccharothrix*. The genus only contained the species *Crossiella cryophila*, which was soon accompanied by *Crossiella equi*, responsible for causing abortion cases in horses with equine nocardioform placentitis in Kentucky [4].

In the last 20 years, no other species of *Crossiella* have been described, with scarce reports on the isolation of *Crossiella* strains in the literature. Sánchez-Moral [5] isolated a few strains of *Crossiella* from Altamira Cave, Spain. Adeyemo and Onilude [6] described a strain of *Crossiella* isolated from Nigerian soil with a broad spectrum of antimicrobial activity. Cimermanova et al. [7] isolated nine actinobacteria collected from different heavy metal-contaminated soils and found that one strain could represent a new species within the genus *Crossiella*; however, they did not provide any characterization or description other than its position in the phylogenetic tree and that the strain differed from *Crossiella cryophila* in several biochemical properties. González-Riancho [8] isolated three *Crossiella* strains from white and grey biofilms from Altamira Cave. Finally, Gonzalez-Pimentel et al. [9] studied the genomes of two *Crossiella* strains selected from 13 strains previously isolated from Altamira Cave (unpublished report).

The isolation of *Crossiella* strains on only five occasions in the last 10 years included this genus within the so-called "rare actinobacteria". Oren and Garrity [10] considered *Actinobacteria* a synonym of *Actinomycetota.* They presented the names and formal descriptions of 42 phyla to effect valid publication in their names based on genera as nomenclatural types. However, in this paper, we maintained the original names, as previously published by each author, to avoid mistakes.

"Rare actinobacteria" are non-*Streptomyces* actinobacteria whose isolation frequency is much lower than *Streptomyces* strains, commonly isolated by conventional methods [11,12]. Tiwari and Gupta [13,14] reported 120 new genera of "rare actinobacteria" in the first decade of the 21st century. A total of 40 out of 120 genera were isolated from soils with comparatively lower percentages from other environments: marine and freshwater sediments, marine animals, plants, buildings, etc. A few reports included the rare genera *Actinomadura*, *Nonomuraea*, *Micromonospora*, *Streptosporangium*, *Nocardiopsis*, and *Pseudonocardia* as most frequent in diverse environments [15–20]. It is noteworthy that an abundance of "rare actinobacteria" is in extreme environments, as exemplified in Atacama [18] and other deserts [21–24]. However, *Crossiella* has not been included among "rare actinobacteria" thus far. We have found that *Crossiella* is an abundant genus in most studied Spanish caves, whether they are gypsum, karstic, or volcanic [9,25–27], and in other terrestrial and aquatic environments.

In this paper, we review the occurrence of environmental *Crossiella*, its relative abundance in Spanish caves and other subterranean environments, as well as its involvement in caves' mineral precipitation. The interest in *Crossiella* lies in its role in biomineralization and potential use in biotechnological processes (stone bioconsolidation, enzyme sources, bioactive compounds, etc.).

2. The Genus *Crossiella* in Caves

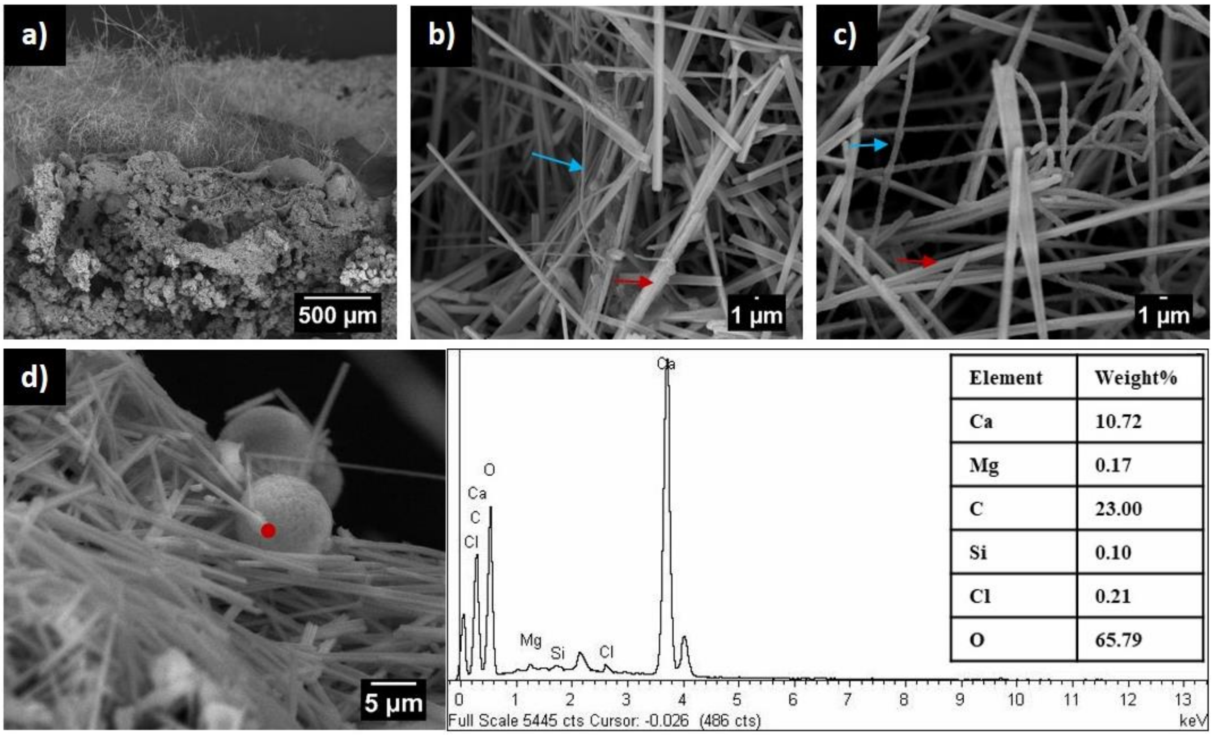
Table 1 shows the occurrence of *Crossiella* in different Spanish caves. The high relative abundance of this genus is in moonmilk (Figure 1), either from karstic (Pindal) or volcanic (Fuente de la Canaria and Bucara II) caves, as well as in coloured biofilms (Pindal, Altamira, Castañar, Covadura) is remarkable [27–31]. The relative humidity is near 100% in these caves. In addition, other mineral/biological formations, such as a pink formation in Bucara II, exhibit high relative abundance (38.9%). Similarly, formations such as mucous formations or brown deposits also reach relatively high abundances (6.7–12.8%) [29]. Interestingly, low percentages of *Crossiella* found in the sediments under the moonmilk indicate an aerobic behaviour for this genus [3]. *Crossiella* was also found in phototrophic biofilms from Nerja Cave [30].

Table 1. Occurrence and relative abundance (>1%) of *Crossiella* in Spanish karstic environments, as reported in NGS studies.

Cave	Relative Abundance	Genus	Type of Sample	References
Pindal	16.0–27.1	*Crossiella*	Moonmilk	[26,28,31]
	1.4–1.7	*Crossiella*	Sediment under moonmilk	
	11.3–11.7	*Crossiella*	Top-layer sediments	

Table 1. *Cont.*

Cave	Relative Abundance	Genus	Type of Sample	References
	6.0–9.0	*Crossiella*	Sediments	
	5.3–7.9	*Crossiella*	Yellow biofilm	
	2.0–8.0	*Crossiella*	Grey biofilms	
	7.0–8.0	*Crossiella*	Pink biofilms	
Fuente de la Canaria	12.6–12.8	*Crossiella*	Mucous formations	[29]
	12.3	*Crossiella*	Moonmilk	
	6.7	*Crossiella*	Brown and yellow deposits	
Bucara II	38.9	*Crossiella*	Pink deposit	[29]
	24.9	*Crossiella*	Moonmilk	
Nerja	0.1–1.5	*Crossiella*	Phototrophic biofilms	[30]
Castañar	15.0	*Crossiella*	Grey biofilm	[31]
Altamira	>20.0	*Crossiella*	Grey biofilms	[30]
	27.0	*Crossiella*	White biofilms	
	38.0	*Crossiella*	Yellow biofilms	
Covadura	26.4–54.1	*Crossiella*	White biofilm	Unpublished data
	21.8–51.9	*Crossiella*	Yellow biofilm	
	4.5–19.7	*Crossiella*	Sediments	
Yeso	1.3–13.3	*Crossiella*	Sediments	Unpublished data
Thyssen Museum basement	16.6	*Crossiella*	White biofilm	[32]
	64.2	*Crossiella*	Grey biofilm	
	2.8–7.4	*Crossiella*	Sediment	



Element	Weight%
Ca	10.72
Mg	0.17
C	23.00
Si	0.10
Cl	0.21
O	65.79

Figure 1. Scanning electron micrographs of moonmilk deposits in Pindal Cave, Spain. (**a**) Longitudinal view of sediment covered by moonmilk. (**b**,**c**) Crystalline calcite fibres (red arrow) and *Actinomycetota* filaments (blue arrow). (**d**) Scanning electron micrographs and EDX spectra of crystalline calcite fibres. Note the swelling of filaments in (**c**), similar to those reported for *Crossiella cryophila* [3].

Crossiella, at a relative abundance of 15.0%, was found in grey biofilms from Castañar Cave [31]. Similar grey biofilms were observed in Altamira Cave [8] and the Thyssen Museum, reaching a relative abundance of 64.2% [32]. Data from a geomicrobiological study of a Roman nymphaeum located in the archaeological basement of the Thyssen Museum in Malaga, Spain, were also included in Table 1 due to its interest.

The environmental conditions of this archaeological basement are special because they mix the characteristics of an environment heavily influenced by the natural underlying karst system with those of an enclosure located in an urban building. Apart from caves, it was remarkable that a subterranean environment, the Roman mortar pavement in the archaeological basement, was colonized by grey biofilms with a high relative abundance of *Crossiella*. This environment is characterized by permanent darkness, the absence of visits, and high relative humidity.

Table 1 shows the occurrence of *Crossiella* in moonmilk, grey, yellow, pink and white biofilms, and sediments from different caves and subterranean environments. *Crossiella* is abundant in different types of rocks, either in volcanic (Fuente de la Canaria, Bucara II), karstic (Pindal, Nerja, Castañar, Altamira) or gypsum (Covadura, Yeso) caves.

In addition to the studies in Table 1, authors have reported the occurrence of *Crossiella* using methodological approaches other than NGS. Stomeo et al. [33] found metabolically active *Crossiella* in white biofilms from Ardales Cave, Malaga, Spain. Portillo and Gonzalez [34] identified *Crossiella* as a major metabolically active bacterium in the black crust of a shelter located in Aragon, Spain, and Sanchez-Moral [5] reported *Crossiella* in Altamira Cave.

Table 2 shows the widespread occurrence of *Crossiella* in caves in the USA, France and China. Less frequent records were found in caves in Italy, Pakistan, Portugal, Serbia, and Thailand, among other countries [35–57].

Table 2. Occurrence and relative abundance (>1%) of *Crossiella* in caves all over the world.

Karstic Caves	Relative Abundance%	Genus	Type of Sample (Method)	References
Heshang	n.d.	*Crossiella*	Weathered rocks (NGS)	[35]
Laugerie-Haute	4.0	*Crossiella*	Salt efflorescences (clones)	[36]
Sorcerers	30.0	*Crossiella*	Salt efflorescences (NGS)	[37]
Pillier	n.d.	*Crossiella*	Wall rock (NGS)	[38]
Yixing Shanjuan	3.9	*Crossiella*	Speleothem (NGS)	[39]
Shuanghe	9.5	*Crossiella*	Rock (NGS)	[40]
Manao-Pee	4.1	*Crossiella*	Soil (NGS)	[41]
KN14	27.1–52.3	*Crossiella*	Rock/Clay (NGS)	[42]
RN5	1.0–17.9	*Crossiella*	Rock/Clay/Mud (NGS)	[42]
Maijishan Grottoes	n.d.	*Crossiella*	Walls paintings (NGS)	[43]
Heshang	n.d.	*Crossiella*	Weathered rocks (NGS)	[44]
Kashmir and Tiser	11.9–36.6	*Crossiella*	Soil (NGS)	[45]
Zhijin	4.1	*Crossiella*	Wall rock (NGS)	[46]
Rouffignac	~70.0	*Crossiella*	Wall rock (NGS)	[47]
Stiffe	9.9	*Crossiella*	Biofilms (NGS)	[48]
Heshang	n.d.	*Crossiella*	Weathered rocks (NGS)	[49]
Cave Church	0.1–4.9	*Crossiella*	Fresco (NGS)	[50]
Volcanic Caves				
Azorean caves	18.6	*Crossiella*	Biofilms (clones)	[51]

Table 2. *Cont.*

Karstic Caves	Relative Abundance%	Genus	Type of Sample (Method)	References
Hawaiian caves	n.d.	*Crossiella*	Biofilms (NGS)	[52]
Californian caves	n.d.	*Crossiella*	Biofilms (NGS)	[53]
Idahoan caves	n.d.	*Crossiella*	Biofilms (NGS)	[54]
Sicilian caves	62.5–77.6	*Crossiella*	Biofilms (NGS)	[55]
Other Cave Types				
Carlsbad Cavern	n.d.	*Crossiella*	Rocks (clones)	[56]
Imawarì Yeuta	n.d.	*Crossiella*	Patina/Speleothems (NGS)	[57]

n.d. Not determined.

Apart from the high abundance in Spanish caves, the high relative abundance of Crossiella in Italian caves is also remarkable. In this regard, Nicolosi et al. [55] recorded high relative abundances in four Etna volcano caves. One of them ranged from 62.5 to 77.6%. Other notable abundances were found in the salt efflorescences of a French shelter [37] and in caves in the USA [42], France [47], Pakistan [45], and the Azores, Portugal [51].

Crossiella has been identified as one of the dominant bacterial phylotypes, with an increasing prevalence when global humidity conditions rise, in a research covering 1050 cave microbiomes worldwide (manuscript in preparation).

3. *Crossiella* in the Environment

Supplementary Table S1 lists papers in the literature that use the keyword "*Crossiella*", including *Crossiella* misspelling [58–146]. The occurrence of the genus *Crossiella* in different environments is significant. Papers reporting *Crossiella equi* and its involvement in animal diseases [4] were excluded.

The papers listed in Supplementary Table S1 rely on molecular methods, except for five articles describing the properties of isolated strains [5–9]. The genus *Crossiella* shows a ubiquitous and extensive geographical distribution on all continents, including Antarctica, but not in Australia/Oceania, likely due to a lack of relevant studies.

Fewer reports locate *Crossiella* in mines and reclaimed mine soils [7,120–124]. However, the number of studies on its presence in soils and the rhizospheres of diverse plants is considerable. Several *Crossiella* findings in stones and building stones were also confirmed [125–138]. Finally, a few records in sea sediments and freshwaters were significant [139–146] because they included aquatic environments among *Crossiella* habitats.

From Supplementary Table S1, we can conclude that *Crossiella*, in addition to caves, is relatively abundant in diverse environments, namely soils, plant rhizospheres, mines, building stones, and other occasional habitats, but is rarely isolated.

Considering the abundance of reports on *Crossiella* in soils [58–96] and plant rhizospheres [97–119], the presence of this genus in caves and other subterranean environments could be attributed to its transport to the subsurface via percolation waters. In this regard, *Crossiella* in percentages <1% have been found in drip waters from Pindal Cave [28]. It may be possible that once transported to the caves, the environmental conditions favour and increase the colonization and growth of *Crossiella* on different mineral substrata.

4. *Crossiella* Isolates

Only five reports shed light on *Crossiella* isolates. A screening of Nigerian soils resulted in the isolation of *Crossiella* sp. strain EK18. The 16S rRNA nucleotide sequence showed 98% similarity to *C. equi*. This strain grew well in different culture media and exhibited broad-spectrum antimicrobial activity [6]. The authors studied the effects of pH, temperature, carbon and nitrogen sources, sodium chloride concentration, and incubation time on antimicrobial activity. In addition, they reported a list of 12 so-called antimicrobial metabolites, including alkanes, alkenes, commonly synthesized by bacteria, and phthalates, which are contaminants from materials and impurities from products used in culture media. Therefore, no conclusive data on the real bioactive compounds produced by the *Crossiella* strain can be derived from this study.

Cimermanova et al. [7] isolated *Crossiella* sp., strain S2, from mining wastes, with a 16rRNA gene sequence similarity of 99.1% to *C. cryophila*. The authors suggested that it may represent a novel, never described species, based on its location in the phylogenetic tree. The strain also exhibited high heavy metal resistance.

González-Riancho [8] found relative abundances of *Crossiella* >20% in white, yellow, and grey biofilms from Altamira Cave. She isolated two strains from white and one strain from grey biofilms with similarities of 99.0–100.0 to *C. cryophila* using the medium Actinomycete Isolation Agar (AIA).

Gonzalez-Pimentel et al. [9] studied two of the thirteen *Crossiella* strains previously isolated from grey biofilms colonizing Altamira Cave, Spain (unpublished report). In vitro and in silico analyses showed the inhibition of pathogenic bacteria and fungi. The exclusive combination of gene clusters involved in the synthesis of lanthipeptides, lasso peptides, nonribosomal peptides and polyketides indicates that these two strains represent a source of new bioactive compounds. The taxonomical distance of both strains from their closest relative, *C. cryophila*, suggests that they represent a new species of *Crossiella*, which will be described in future works.

So far, the low number of isolated *Crossiella* strains indicates that most of the culture media used are inadequate to reproduce their growth in the laboratory. The environmental conditions of their ecological niche should also be considered when designing specific culture media, which are superior to conventional ones.

5. Biomineralization in Caves Induced by *Crossiella*

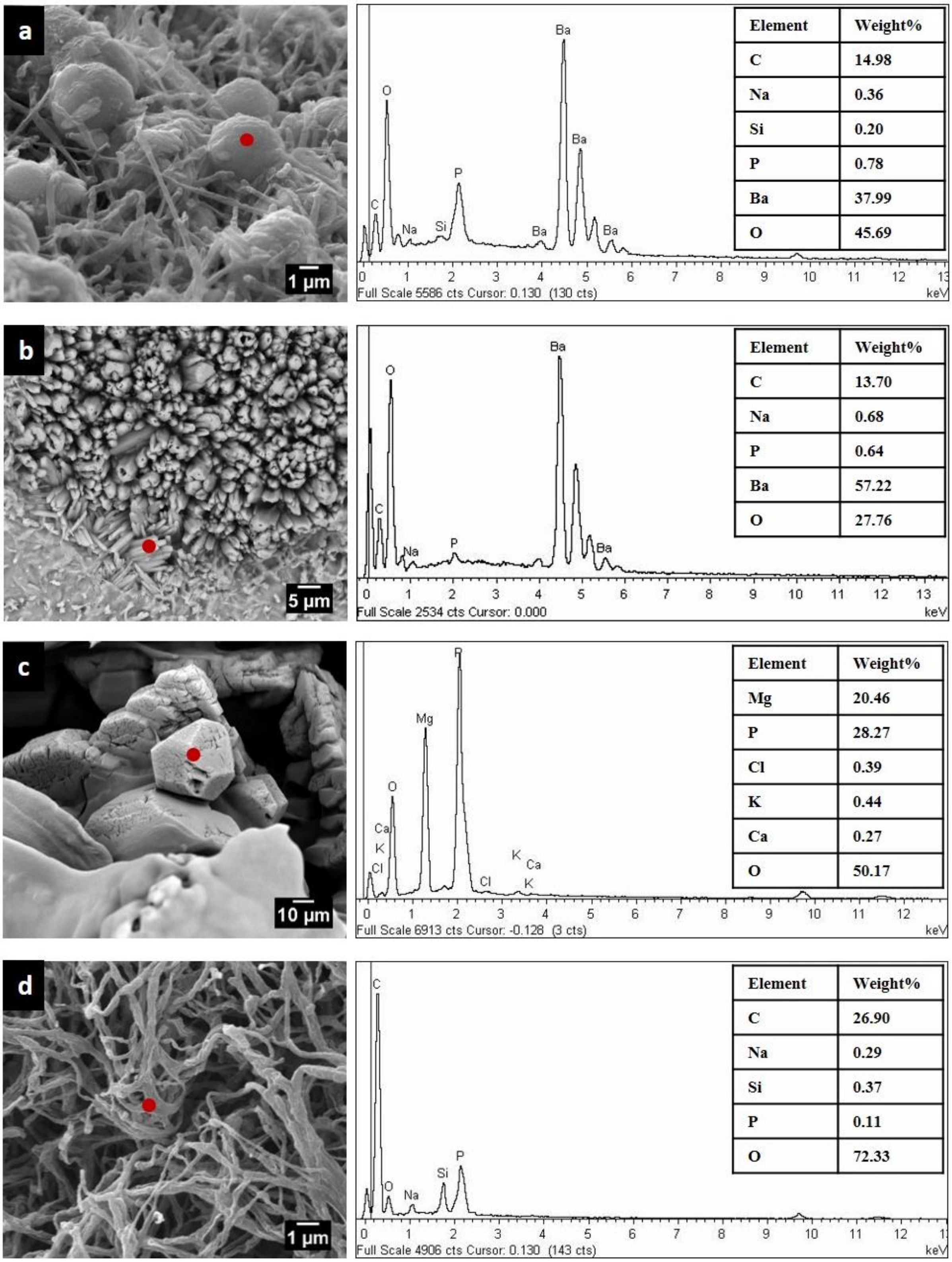
Biomineralization or crystal formation is a general phenomenon caused by soil bacteria, as reported by Boquet et al. [147]. These authors isolated 210 bacteria that could form calcite crystals in a medium with calcium acetate and stated that their occurrence depended on the composition of the medium used.

The role of bacteria in speleogenesis has been discussed for decades. Barton and Northup [148] stated that in the 1960s, a few authors proposed that microbes played a role in forming cave deposits. Banks et al. [149] confirmed the link between calcium metabolism in bacteria and calcification using cave isolates. They suggested that the toxicity of Ca^{2+} ions to bacteria promoted the need to remove Ca^{2+} ions from the cell via calcification as a detoxification mechanism.

Further evidence of biomineralization has been reported in recent decades that sheds light on microbially induced mineral precipitation [150–153]. This precipitation has been attributed to several causes: the modulation of environmental pH, nucleation sites on cell surfaces, or enzymatically driven processes involving carbonic anhydrase, urease, etc. [154].

Grey biofilms from Altamira Cave were studied, and scanning electron microscopy (SEM) revealed an abundance of bioinduced calcite crystals in addition to moonmilk [152]. The biofilms mainly comprised *Actinomycetota* filaments promoting carbon dioxide uptake and formation of calcite deposits. A model for bioinduced calcite formation, supported by scanning and transmission electron microscopy data, was proposed by Cuezva et al. [152].

Apart from the precipitation of calcite by *Crossiella*, another experiment (Figure 2) with two strains of *Crossiella* isolated from Altamira Cave [9] revealed that both strains induced the formation of different crystals when incubated in a culture medium with barium acetate, yeast extract, and agar (Ba1). Two crystal types were identified on the plates: witherite (barium carbonate) and struvite (magnesium ammonium phosphate), with distinct abundances that were higher for witherite and scarcer for struvite. Witherite precipitation is due to an abundance of barium in the medium. Occasional struvite crystals can be derived from the amino acids and minor amounts of phosphorus and magnesium in the yeast extract [155].



Element	Weight%
C	14.98
Na	0.36
Si	0.20
P	0.78
Ba	37.99
O	45.69

Element	Weight%
C	13.70
Na	0.68
P	0.64
Ba	57.22
O	27.76

Element	Weight%
Mg	20.46
P	28.27
Cl	0.39
K	0.44
Ca	0.27
O	50.17

Element	Weight%
C	26.90
Na	0.29
Si	0.37
P	0.11
O	72.33

Figure 2. Scanning electron micrographs and EDX spectra of barium carbonate and phosphate crystals from two strains of *Crossiella* sp. (Cross-1 and Cross-2) [7]. (**a**) Witherite (barium carbonate) crystals and *Crossiella* filaments in culture medium Ba1 (Cross-1). (**b**) Witherite crystals in culture medium Ba1 (Cross-2). (**c**) Struvite (magnesium ammonium phosphate) crystals in culture medium Ba1 (Cross-1). (**d**) *Crossiella* filaments without crystal formation in tryptic soy agar medium (Cross-2).

Baryte (barium sulphate) precipitation by bacteria was previously reported by Joubert et al. [156]. Sanchez-Moral et al. [157] found that baryte was associated with filamentous bacteria in altered volcanic rocks. *Agromyces* spp., *Bacillus* spp., *Lysobacter* spp., *Ralstonia* sp., *Stenotrophomonas maltophilia,* and *Streptomyces* sp. were isolated from the volcanic rocks and precipitated witherite or calcite/vaterite in media with barium or calcium acetate, respectively. The occurrence of baryte, but not witherite, in volcanic rocks was due to the presence of sulphate ions that easily transform witherite into barite. This transformation was not produced on the *Crossiella* plates without sulphate ions.

Struvite precipitation is not as widespread as calcite or witherite in bacteria. Sanchez-Moral et al. [158] tested eight bacteria isolated from the Roman catacombs of St. Callixtus and Domitilla and reported that *Agromyces ramosus* precipitated calcite, magnesium calcite, witherite, and struvite, depending on the media composition. Other bacteria precipitating struvite were *Bacillus* sp. and *Ralstonia metallidurans*.

Rivadeneyra et al. [159] found that only 20.8% of the tested bacterial isolates precipitated struvite and that calcium acetate appeared to inhibit struvite precipitation in culture media, whereas ammonium ions triggered it [160]. Manzoor et al. [161] stated that urease-producing bacteria play a key role in struvite precipitation, controlling nucleation, and modulating crystalline phases and crystal shapes. Urease is present in the strain type of *Crossiella*, *C. cryophila* [3], and urease genes have been identified in the genomes of the two *Crossiella* strains from Altamira Cave [9].

Sánchez-Román et al. [162] reported that carbon and phosphorus cycles are interrelated during biomineralization. They also demonstrated the co-precipitation of carbonate and struvite, which we also found in *Crossiella* strains.

The data reported show that biomineralization by *Crossiella* is an active process in the presence of different ions, confirming its role in moonmilk formation. *Crossiella* strains' ability to induce carbonate precipitation, which is used to consolidate cultural heritage stones and buildings, should be explored.

6. Moonmilk Formation

Moonmilk formation has been discussed in the literature for a long time [150,163–168]. The question: Is moonmilk an abiotic process driven physicochemically, or is it biotic, mediated by microorganisms? A biological origin currently prevails; even a combination of physicochemical and biogenic processes is being considered [166]. Cañaveras et al. [150,165] indicated that bacteria influenced the physicochemistry of calcite precipitation. They observed that cave moonmilk comprises a network of calcite crystals and active filamentous bacteria. They also found that hydromagnesite and needle-fibre aragonite deposits were associated with bacteria in Altamira Cave, predominantly *Streptomyces,* for which they demonstrated their ability to precipitate calcite/vaterite in the laboratory. The association between bacteria and mineral crystals was reported in other papers and described using SEM [152,153,165,168–170]. In addition, different bacterial genera such as *Agromyces*, *Amycolatopsis*, *Brachybacterium*, *Nocardioides*, *Nocardiopsis*, *Paenibacillus,* and *Rothia* precipitated vaterite/calcite and Mg-calcite [171].

Maciejewska et al. [153] found that all the *Streptomyces* strains tested could promote calcification and biomineralization. The metabolic activities involved in the precipitation were amino acids ammonification and ureolysis, which increased environmental pH. Sanchez-Moral et al. [169] stated that microbial activity induces carbonate precipitation in the early stages of deposition. However, as carbonate accumulates, a progressive decline in microbial activity occurs, as deduced from the RNA/DNA ratio, which is used as a marker of metabolic activity. The decreased metabolic activity is due to the progressive accumulation of carbonate and bacterial entrapment in mineral deposits.

The high relative abundance of *Crossiella* in moonmilk from different caves indicates that this genus is active in moonmilk formation (Table 1). Enzymatic processes induce this mineralization, and several enzymes have been linked to *Crossiella* activity in moonmilk. Martin-Pozas et al. [26] suggested that moonmilk formation is related to syntrophic relationships between *Crossiella* and nitrifying bacteria, and Cuezva et al. [27] associated *Crossiella* with the ability to capture CO_2 from the atmosphere and precipitate calcium carbonate as a by-product of carbonic anhydrase action, as observed in cave moonmilk.

7. Is Moonmilk a Source of Bioactive Compounds?

Caves and moonmilk are colonized by complex bacterial communities. Maciejewska et al. [153] reported that *Proteobacteria* was the dominant phylum of moonmilk from a Belgian cave, followed by *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, *Gemmatimonadetes*, and *Planctomycetes*. These seven phyla accounted for 85.8–90.2% of the total community. Martin-Pozas et al. [26] investigated the moonmilk composition from Pindal Cave in Spain. They found that *Proteobacteria* and *Actinobacteria* dominated the community with over 30% of relative abundance for each phyla, followed by *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, *Gemmatimonadetes,* and *Nitrospirae*. These phyla accounted for 93.1–93.9% of the total community. The similarities between the phyla compositions of moonmilk from two different caves are remarkable. Moonmilk from a geographically distant cave [172] and another subterranean environment [173] also showed relatively similar phyla compositions.

Moonmilk has been a promising reservoir for novel bacteria producing bioactive compounds, and a few novel species have been isolated, namely *Streptomyces lunaelactis* [174], *Pseudomonas karstica,* and *Pseudomonas spelaei* [175]. Several studies have stressed the great diversity of unknown bacteria inhabiting moonmilk and the isolates' production of bioactive compounds [176–178].

The high abundance of *Actinomycetota* (=*Actinobacteria*) and *Pseudomonadota* (=*Proteobacteria*) in moonmilk has prompted researchers to test a series of strategies to isolate hard-to-culture "rare actinobacteria" and discover novel bioactive compounds [13,14]. Adam et al. [176] obtained 40 isolates represented by *Agromyces*, *Amycolatopsis*, *Kocuria*, *Micrococcus*, *Micromonospora*, *Nocardia*, *Streptomyces,* and *Rhodococcus* species. The *Streptomyces* isolates displayed strong inhibitory activities against Gram-positive and Gram-negative bacteria and fungi [179]. Genome mining of *Streptomyces lunaelactis* revealed 42 biosynthetic gene clusters [180] and the production of the antibiotics bagremycins and lunaemycins [180,181]. The genome of *Crossiella*, abundant in moonmilk [26], showed the presence of a combination of gene clusters involved in synthesising different bioactive compounds [9]. The data suggest the possibility of finding other moonmilk bacteria involved in synthesising bioactive compounds.

8. Conclusions

The genus *Crossiella* is widely distributed in all environments, reaching a relative abundance of up to 78% in a Sicilian cave. Its occurrence in soils, plant rhizospheres and caves is especially important. The last case is probably due to its transport to the subsurface by percolating waters. Despite this abundance, the strains isolated were scarce.

The data suggest that more environmental *Crossiella* species are waiting to be described, apart from *Crossiella cryophila* and *Crossiella equi*. The increasing number of metagenomic sequence data from all environments offers clear opportunities to guide the isolation and cultivation of *Crossiella*. Therefore, further efforts are required to design suitable isolation culture media. They should consider the environmental conditions of the niches where *Crossiella* thrives, namely alkaline pH and high mineral concentrations.

Crossiella has an important role in carbon sequestration in subterranean environments. Metagenomic studies and isolating more *Crossiella* strains and/or species are the only way to advance knowledge of *Crossiella* functions in different ecosystems. Furthermore, its role in biomineralization and moonmilk formation is also apparent.

Finally, *Crossiella* appears to be a promising source of active compounds, and the isolated strains deserve more attention regarding their potential use in biotechnological processes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applbiosci2020014/s1, Table S1: Occurrence of the genus *Crossiella* in different environments.

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