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Sulfidic Habitats in the Frasassi Caves, Italy: A Hotspot of Subterranean Biodiversity †

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Abstract: Caves in the Frasassi Gorge (Marche, Italy) contain numerous sulfidic habitats that represent hotspots of subterranean biodiversity. They host unusually rich and diverse invertebrate communities that display specific adaptations to cave life. As in other sulfidic subterranean ecosystems discovered worldwide, primary production in Frasassi's sulfidic sections is fueled by chemoautotrophic sulfur-oxidizing microorganisms. Conversely, fewer animals are present in cave sections where trophic resources depend on surface input. Numerous aquatic and terrestrial species of invertebrates have been identified in Frasassi's sulfidic and non-sulfidic habitats, including 12 endemic species. Specific adaptations to extreme environmental conditions have been identified in some species.

Keywords: cave adaptations; sulfidic habitats; chemoautotrophy; sulfur-oxidizing microorganisms; endemic species

1. Introduction

Unusually abundant aquatic and terrestrial subterranean biological communities were discovered in the sulfidic sections of the Frasassi caves in Italy in 1996. Subsequent microbiological investigations showed that similarly to deep-sea vent ecosystems and to the Movile Cave ecosystem, in situ chemoautotrophic food sources sustain a rich and diverse aquatic and terrestrial subterranean fauna in Frasassi. Since then, biologists have observed, investigated, and described several cave-adapted invertebrate species that are endemic to the Frasassi caves and/or new for science, and extensive microbiological

investigations have documented the role of sulfur-oxidizing microorganisms in securing the food base for the subterranean ecosystem.

The aim of this study is to provide a complete inventory of species that were identified so far and described from the Frasassi caves, in both sulfidic and non-sulfidic cave habitats. The Frasassi caves represent a biodiversity hotspot located in fragile environments, and its rare and special fauna has to be known and protected. Studying these cave-adapted invertebrates, together with the microorganisms present in their habitat, and their interrelationships with the sulfidic environment, can generate knowledge on life in extreme environments in general, life in absence of solar-derived energy in particular.

2. The Frasassi Caves

The Frasassi cave system (Figure 1) is located in the Frasassi Gorge (12°57′ E, 43°24′ N), a 500 m deep canyon cut by the Sentino River through a small limestone ridge in the Northeastern Apennines.

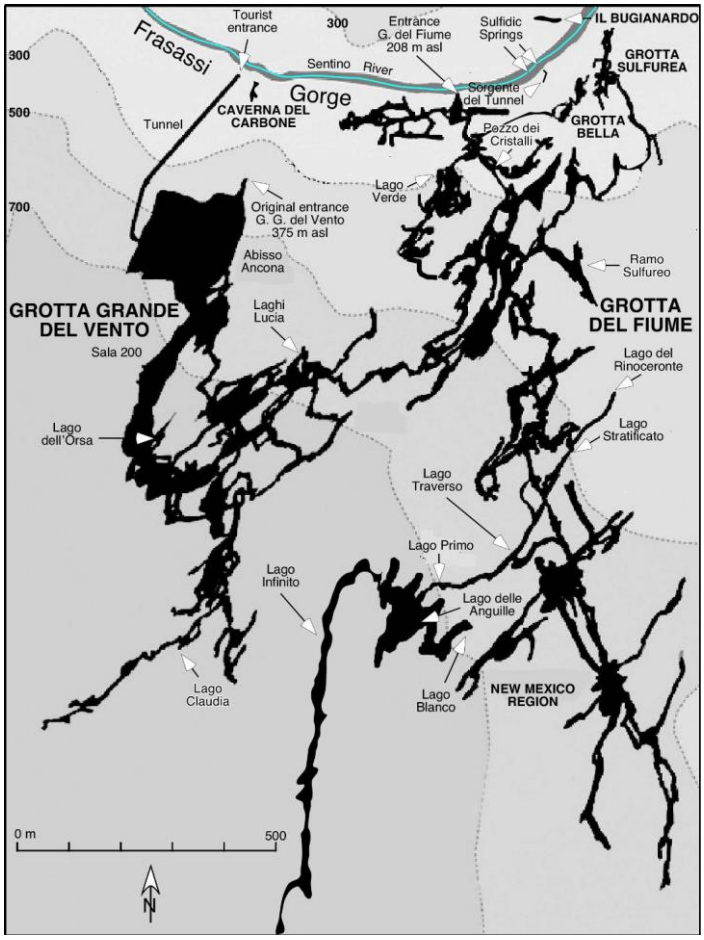

Figure 1. Map of the caves in the Frasassi Gorge (Gola di Frasassi), Marche, Italy.

The caves consist of mainly sub-horizontal passages, organized in superimposed and interconnected levels. The largest cave system comprising Grotta del Fiume, Grotta Grande del Vento, Grotta Sulfurea and Grotta Bella extends mainly close to the present local base level, at 200 m elevation, while Bucco Cattivo and Grotta del'Infinito are known at higher elevation, between 360 and 500 m a.s.l. In the present condition, the sulfidic groundwater can be reached from the lower cave levels at the end of descending passages or directly at the bottom of shafts. In most cave pools the water is stagnant, and a surface layer of less saline, non-sulfidic water lies on top of the sulfidic layer. Most pools are

isolated from each other at the surface but connected by a well-developed underwater draining system. The north-eastern cave section is the only area where an evident flow of sulfidic water occurs into pools and short streams.

3. Chemoautotrophy as Food Base for the Subterranean Fauna

Much like deep-sea vent ecosystems, which are very rich and diverse in contrast with most of the deep-sea environments that lack a rich source of autochthonous food, sulfidic subterranean habitats contain abundant food produced in situ by chemoautotrophic microorganisms that represent the food base for complex subterranean food-webs. Unusually large numbers of species are present in these cave environments, some of which reach very high densities up to hundreds of specimens per m^2. Stable isotope studies performed in the Frasassi caves have shown that organic carbon and nitrogen from the abundant cave fauna inhabiting the deep sulfidic sections of these caves are isotopically lighter than allochthonous organic material near skylights and other cave entrances. These distinctive isotopic values indicate that the food base of the subterranean ecosystem is produced locally from the sulfidic aquifer and it does not originate in the epikarst (as is the case in non-sulfidic caves). More recent and thorough stable isotope studies by Galdenzi, Macalady, and Sarbu (unpublished) confirm these conclusions.

In sulfidic cave streams, chemolithoautotrophic sulfide-oxidizing microorganisms form distinctive white mats that have substantial biomass for consumers. The primary producers and biofilm architects in these areas include filamentous sulfur-oxidizing bacteria of the genera *Beggiatoa*, *Thiothrix*, *Thiovulum*, and unnamed Epsilonproteobacteria, the relative abundance of which varies based on dissolved sulfide and oxygen concentrations as well as on stream flow velocity. These biofilms support other diverse microbial taxa, including other sulfur-oxidizing populations and many heterotrophs. In streams, most sulfide is oxidized to elemental sulfur, giving the microbial mats their distinctive white color and leading to sulfur accumulation in the sediments. These robust, sulfide-oxidizing autotrophic microorganisms provide a rich food source for consumers, both above and below the water table, that supports the diverse aquatic and terrestrial fauna within the cave.

As in other sulfidic caves investigated in this regard (namely Movile cave in Romania and Melissotrypa Cave in Greece), the sulfidic environments in Frasassi caves are host to a unique freshwater ectosymbiosis between sulfide-oxidizing bacteria (*Thiothrix*) and cave amphipods. In Frasassi caves, three strains of *Thiothrix* bacteria (dubbed T1, T2 and T3) have been documented to occur on three species of *Niphargus* amphipods: T1 and T3 on *N. frasassianus*, T2 and *N. ictus*, and T2 and T3 on *N. montanarius*. The ecological and evolutionary significance of this highly specific ectosymbiosis remains unclear, but it is strongly reminiscent of similar ectosymbioses between sulfide-oxidizing bacteria and crustaceans at deep-sea vents.

4. Faunal Composition of the Aquatic and Terrestrial Communities Found in Sulfidic Portions of Frasassi Caves

* Species considered endemic to the Frasassi caves.

*Dendrocoelum leporii** Stocchino & Sluys, 2017 (Platyhelminthes, Tricladida, Dendrocoeliidae) was observed in several sulfidic and non-sulfidic locations in the Frasassi caves: Sorgente del Tunnel, Pozzo dei Cristali, Grotta Sulfurea, Ramo Sulfureo, and Lago Claudia. This planarian is white and blind and it displays no specific adaptations to the sulfidic environment where it thrives.

Potamothrix cf. *heuscheri** (Bretscher, 1900) (Annelida, Clitellata, Naididae). Specimens belonging to a putative new species of *Potamothrix* collected in Grotta Sulfurea, and Sorgente del Tunnel are currently studied by Christer Erséus, Stockholm, Sweden.

Guestphalinus cf. *wiardi** (Michaelsen, 1933) (Annelida, Clitellata, Lumbriculidae). A dense population of small sized specimens belonging to this species was observed in the

Ramo Sulfureo section of the Frasassi caves. Much larger specimens were also observed swimming in the water column and crawling on the sediments in sulfidic lakes in Pozzo dei Cristalli, Ramo Sulfureo, and Grotta Bella.

Rhynchelmis sp. (Annelida, Clitellata, Lumbriculidae). A small population of annelid worms discovered on the wet surface of cave walls in the Ramo Sulfureo passage, as well as in the deep sulfidic section of Grotta Sulfurea, tentatively identified as a new species of *Rhynchelmis*.

Islamia sulfurea Bodon & Cianfanelli, 2012 (Mollusca, Gastropoda, Hydrobiidae). Eyed, pigmented specimens thrive around the surface in sulfidic springs in the gorge, while troglobiont populations inhabit the sulfidic in the deep recesses of the Frasassi caves (Lago delle Anguille, Lago Verde, Pozzo dei Cristalli, Lago Stratificato, Lago Blanco, Lago Infinito, and Lago Claudia).

Eudiaptomus intermedius (Steuer, 1897) (Crustacea, Copepoda, Calanoida). This troglomorphic population of a surface-water species, likely an incipient troglobiont species, is endemic to the Frasassi caves. The species has been collected from the saturated sulfidic karst, as a planktonic element, in Lago Verde, Lago Claudia, and Pozzo dei Cristalli.

Diacyclops cosanus Stella & Salvadori, 1954 (Crustacea, Copepoda, Cyclopoida) (syn. *Diacyclops antrincola* Kiefer, 1967) was found in Lago Verde, Lago Claudia, and Pozzo dei Cristalli as well as in non-sulfidic dripping pools. It is a planktonic and hyperbenthic species that swims also freely close to the benthic sediments, with its highest abundances in the sulfidic karst.

Speocyclops sp.* (Crustacea, Copepoda, Cyclopoida). Eight specimens of *Speocyclops* sp. were collected in Pozzo dei Cristalli, predominantly in the deep sulfidic level of the subterranean lake. This species is new to science, awaiting formal description, and appears to be endemic to the Frasassi caves.

Maraenobiotus sp.* (Crustacea, Copepoda, Harpacticoida). Specimens belonging to a new, yet undescribed species of *Maraenobiotus* have been collected from both sulfidic and non-sulfidic waters in the Frasassi caves. It is rare and at present it is to be considered endemic to the Frasassi cave system.

Nitocrella stammeri Chappuis, 1938 (Crustacea, Copepoda, Harpacticoida) has only been found in Pozzo dei Cristalli. This species is widely distributed in coastal groundwaters in Italy and it is also found in caves from central and southern Italy, with a Mediterranean distribution.

Nitocrella psammophila Chappuis, 1954 (Crustacea, Copepoda, Harpacticoida) has only been found in Pozzo dei Cristalli. It is known from several localities in Italy. It thrives in the interstitial zone of streams and rivers, in aquifers in unconsolidated sediments, in the saturated karst, and in the epikarst.

Mixtacandona sp.* (Crustacea, Ostracoda, Candonidae). An endemic and undescribed species of *Mixtacandona* was found in sulfidic waters of Pozzo dei Cristalli and Lago Claudia, where it forms abundant populations.

Pseudolimnocythere sp.* (Crustacea, Ostracoda, Loxoconchidae) is a troglobiont ostracod found in the sulfidic sections of Frasassi caves. It has been collected from Lago Claudia, Lago Stratificato, and Lago Infinito but also from the freshwater habitats of Lago Traverso, Lago delle Anguille and Lago Blanco.

Androniscus dentiger Verhoeff, 1908 (Crustacea, Isopoda, Trichoniscidae) has been reported from several sites and caves of the Frasassi area, such as Grotta Grande del Vento, Grotta Bella, Grotta Fiume, or Grotta dell'Infinito.

Niphargus ictus Karaman, 1985 (Crustacea, Amphipoda, Niphargidae) was collected in Il Bugianardo, Lago Verde, Pozzo dei Cristalli, Ramo Sulfureo, Lago del Rinoceronte, Lago Stratificato, Lago Primo and Lago Claudia. *N. ictus* reaches densities of up to 200 specimens per m^2 in some of the cave lakes such as Lago Verde. It occasionally swims upside-down in the cave lakes, an unusual behavior for most species in this genus.

*Niphargus frasassianus** Karaman, Borowsky & Dattagupta, 2010 (Crustacea, Amphipoda, Niphargidae) was only found in the Frasassi cave system, where its distribution is linked to the sulfidic habitats (Sorgente del Tunnel, Grotta Sulfurea, Grotta Bella, Pozzo dei Cristalli, Ramo Sulfureo,). Similarly to *N. ictus*, *N. frassasianus* can reach high densities (100 to 200 specimens per m^2). It is a strictly benthic species.

*Niphargus montanarius** Karaman, Borowsky & Dattagupta, 2010 (Crustacea, Amphipoda, Niphargidae) was described and it is still only known from freshwater pools of Il Bugianardo Cave located on the left side of the Sentino River in the Frasassi Gorge.

Niphargus frontalis Karaman, 2016 (Crustacea, Amphipoda, Niphargidae) was found only in Lago Primo, at which time it remained unidentified. A recent recollection of *Niphargus frontalis* from its type locality near Coldigioco (Marche region) made it possible to ascertain the conspecificity of the two populations.

Labidostomma cornuta (Canestrini & Fanzago, 1877) (Arachnida, Acari, Labidostommatidae) was found on the floor of the cave passages in Ramo Sulfureo and Pozzo dei Cristalli sulfidic sections of the Frasassi caves. They form stable populations in the deep sections of the caves.

*Ephippiochthonius sulphureus** (Gardini 2013) (Arachnida, Pseudoscorpiones, Chthoniidae) is a troglomorphic chthoniid that lives in the sulfidic sections of the Frasassi caves: Ramo Sulfureo and Grotta Sulfurea and it is considered endemic to the Frasassi cave system.

Palliduphantes pallidus (O. Pickard-Cambridge, 1871) (Arachnida, Araneae, Linyphiidae) has been found in dense populations in several sulfidic sites in the Frasassi caves, albeit only in areas located rather close to the surface: Grotta Sulfurea, Pozzo dei Cristalli, and Lago Verde

*Porrhomma frasassianum** Weiss & Sarbu, 2021 (Arachnida, Araneae, Linyphiidae) has been collected in the deep sulfidic sections of the Frasassi caves: Ramo Sulfureo and Lago del Rinoceronte. They display troglomorphic characteristics: they are blind, they lack pigmentation, and they have very long legs.

Kryptonesticus eremita (Simon, 1880) (Arachnida, Araneae, Nesticidae) is an eyed and pigmented spider present in the Guano Room, Grotta Sulfurea, Lago Verde, Pozzo dei Cristali, Ramo Sulfureo, Grotta Bella. Specimens from the deep sulfidic sections display reduced eyes, depigmentation, and elongated legs.

Neelus murinus Folsom, 1896 (Collembola, Neelidae) was found in large numbers in the deep sulfidic recesses of the Frasassi cave system, where it appears form stable, although it is considered a trogloxene.

*Deuteraphorura frasassii** (Fanciulli, 1999) (Collembola, Onychiuridae) was often observed on the water surface in gours in the lower sulfidic cave sections (Ramo Sulfureo, Grotta Bella, Grotta Sulfurea), as well as in the non-sulfidic upper passages of Grotta del Fiume—Grotta Grande del Vento complex.

Dolichopoda laetitiae Menozzi, 1920 is an Italian endemic species with a distribution limited to the central Apennines; eutroglophile species found in dense population not far from the several entrances of the complex, sometimes deeply inside.

Duvalius lombardii Straneo, 1939 (Insecta, Coleoptera, Carabidae) is a troglobiont beetle originally known only from the caves of the karst complex of Frasassi, but it has also been discovered recently in the Monte Cucco Cave located less than 20 km from the Frasassi caves.

Tychobythinus gladiator gladiator (Reitter, 1884) (Insecta, Coleoptera, Staphylinidae, Pselaphinae), an Italian endemic subspecies living in the Apennines, was found in the Ramo Sulfureo section of Grotta del Fiume.

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