Ciliate diversity and behavioural observations from the chemoautotrophic cave ecosystem of Frasassi, (Marche region, Italy).

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Chemoautotrophic cave organisms require specific adaptations to tolerate the stress of living in extreme environmental conditions, such as darkness, nutrient and energy limitations, low temperatures (12-13°C), highly variable sulphide concentrations (from 0 up to 415 μM H2S) and toxic levels of gases (H2S, CO2, CH4). To date, due to the difficulties in sampling in such harsh environment, very few studies were performed in order to describe the ciliate communities from caves; these have remained largely unexplored. Thus, the main aims of our study were to characterize for the first time, the ciliates from the sulfide-rich Frasassi cave complex (Marche region, Italy) and to observe possible behavioural differences with their non-cave-dwelling counterpart ciliate species. Four main sampling sites within Frasassi caves were selected: *Pozzo dei Cristalli*, *Lago Verde*, *Ramo Solfureo* and *Grotta Solfurea*. The ciliate diversity from the site *Pozzo dei Cristalli* was studied in greater detail for its spatio-temporal distribution, since it is highly diversified and includes several microhabitats represented by small sulfidic (H2S-rich) ponds, streams and springs as well as, deep and shallow muddy, stagnant lakes. Periodic sampling was realised from 2009 to 2011 in the form of water-sediments, picked up by scraping the surface. Classical culturing, silver staining methods and 18S rRNA gene (for some selected species) for phylogenetic analysis were employed. A total of 31 species belonging to 9 classes, 15 orders and 23 genera were identified. Fluctuation of the ciliate communities were mainly recorded at the *Pozzo dei Cristalli* sites during various sampling occasions, this could be due to changing environmental conditions (mainly H2S concentrations and water levels). Interestingly, it was observed that some species e.g. *Urocentrum turbo*, *Coleps hirtus hirtus*, *Oxytricha* sp, *Euplotes* sp, showed adaptation for the cave environment (photo-sensitivity, sulphur tolerance, feeding behaviour, morphological difference). Overall, these results provide a platform for various in-depth studies of ciliates to understand potential role in aquatic microhabitats, nature of chemical compounds secreted, dispersal pattern, and adaptations to cave environment.