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 H_2S) and toxic levels of gases (H_2S, CO_2, CH_4). 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 (H_2S-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 H_2S 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.