The peculiar chemoautotrophic subterranean groundwater ecosystem of the Frasassi cave represents a core of biodiversity that is currently being studied and that has been explored with particular reference to microbial eukaryotes such as ciliated protists. Through comprehensive exploration, 33 taxa of ciliates, along with single species of flagellate, heliozoans, and naked amoebae, were identified across four primary sampling sites. Notably, the "Pozzo dei Cristalli" location comprises diverse microhabitats/ponds with distinct chemical-physical and biological parameters, including sulfur and nutrient concentrations, with the presence of bacterial biofilm. This site revealed distinctive adaptations in cave-dwelling ciliates not observed in their surface counterparts, such as the remarkable photosensitivity of *Urocentrum turbo*, the cannibalistic behavior of *Coleps hirtus*, and the variable thorn counts in *Aspidisca* species as a defense mechanism against predation. Furthermore, extensive investigation delved into the chemical offensive mechanism employed by the predatory ciliate, *Coleps hirtus*. 