Biodiversity and Evolution of Halophilic Protozoa.

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Most research on microorganisms adapted to hypersaline habitats has focused on Archaea and Bacteria, with eukaryotes receiving much less attention. Nonetheless, accounts of phagotrophic microbial eukaryotes, i.e. protozoa, living at very high salinities stretch back more than a century, and our knowledge of these organisms has improved markedly over the last 15 years, through combinations of microscopy, molecular phylogenetics, environmental sequencing, transcriptomics and growth experiments. Parts of this work of evolutionary relevance will be reviewed: High salinity waters from salterns, other landlocked water masses and deep hypersaline anoxic basins contain unique and diverse halophilic protozoan assemblages. They represent many separate phylogenetic lineages; species of Heterolobosea, Bicosoecida, and Ciliophora have been most intensively characterized, with several proven to be extreme (or borderline extreme) halophiles. Transcriptomic examinations of the bicosoecid *Halocafeteria* (and the heteroloboseid *Pharyngomonas*) indicate that high-salt adaptation is associated with a subtle shift in protein amino acid composition, and involves the differential expression of genes participating in ion homeostasis, signal transduction, stress management, and lipid remodeling. Instances of gene duplication and lateral transfer possibly conferring

adaptation have been documented. Indirect evidence suggests that these protozoa use 'salt-out' osmoadaptive strategies (as reported also in more direct examinations of less extremely halophilic protozoa). A surprisingly high proportion of the morphotaxa reported from high salt environments in the traditional literature has now been cultivated, however ongoing observations make clear that many distinct groups await characterization. Conversely, uncertainty still surrounds repeated instances where familiar marine morphotaxa (mainly of heterotrophic flagellates) have been reported at very high salinities.