

## Abstract

Life radiated in aquatic environments worldwide. Brackish waters, however, seemingly hinder diversification as their physiologically demanding environmental stochasticity favours transient and impoverished communities assembled from widespread generalist species. Yet, the Ponto-Caspian basin (Aral, Azov, Black and Caspian seas) defies this rule, its rich endemic biota representing the only brackish biodiversity hotspot on Earth. Nevertheless, its origin, age and biogeographic history remain contentious. Here, we reconstruct the evolutionary timescale and diversification dynamics of gammaroidean amphipods, the most diverse Ponto-Caspian faunal group. We show that amphipods started radiating 11 Myr ago when the Paratethys Sea became isolated from the global ocean and stable brackish conditions ensued. Diversification proceeded steadily despite the subsequent water level fluctuations that persisted towards the end of the Miocene. The final Paratethyan Pliocene dissolution into the isolated Black and Caspian seas, followed by their brief intermittent connections during the mid-late Pleistocene, sparked further diversification via dispersal-vicariance pulses. Lastly, a five times faster mitochondrial substitution rate was estimated relative to the typical arthropod rate. Despite the significant geo-environmental upheaval, amphipods show a remarkably continuous evolutionary history shaped by a unique interplay between marine and inland biogeographical processes, highlighting that brackish water can support diverse and enduring evolutionary radiations.