

Neo-functionalization of lineage-specific cement protein paralogs underpin the evolution of barnacle bioadhesives in larval and adult life stages

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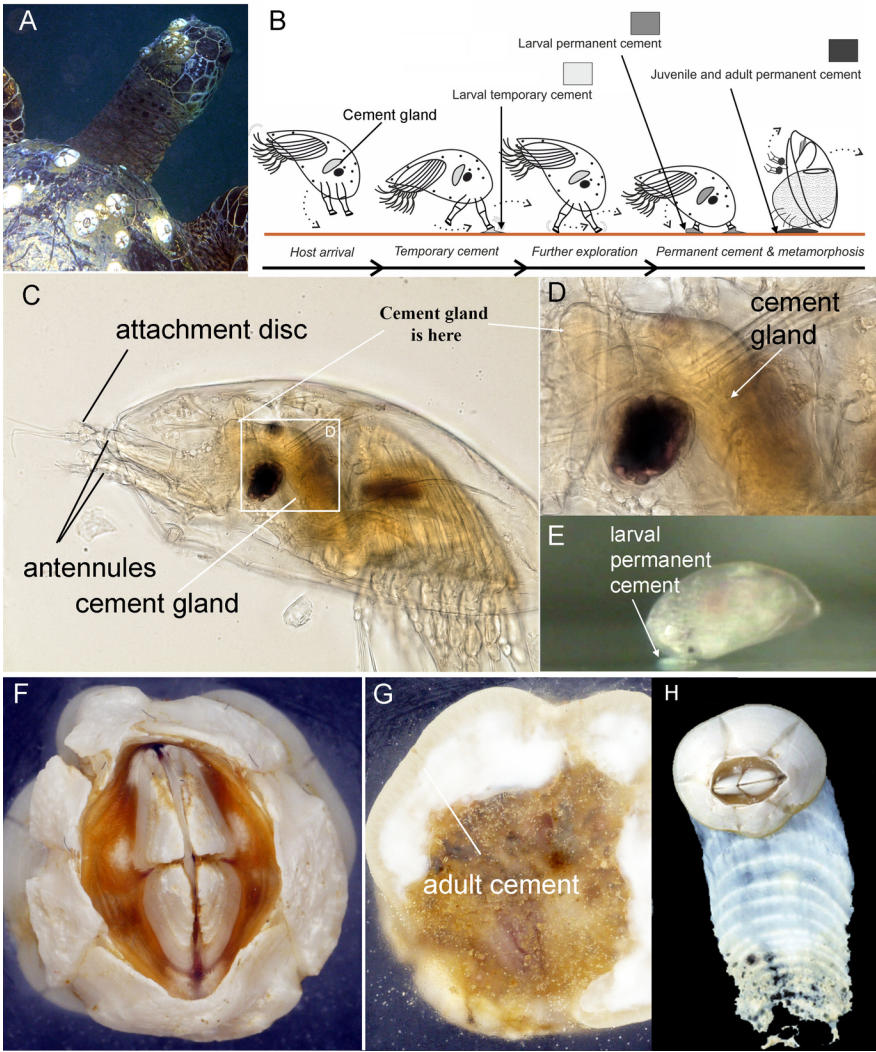
April 4, 2023

Abstract

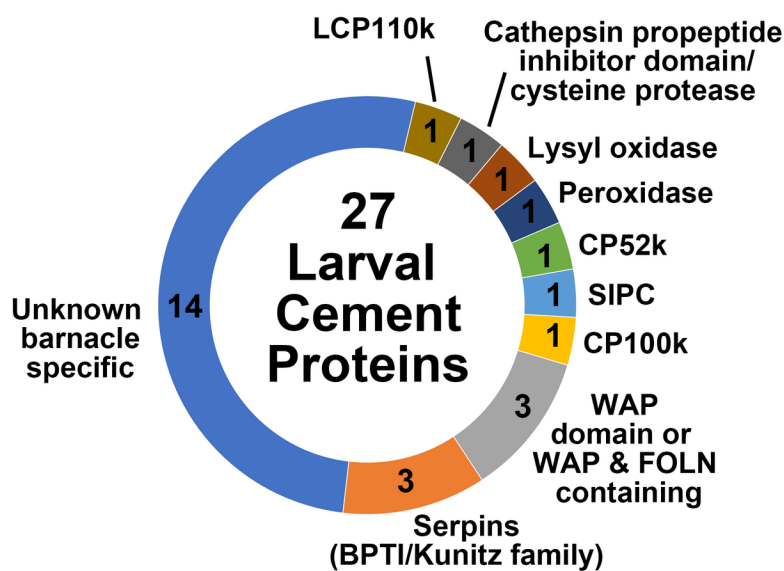
Acquisition of new genes often results in the emergence of novel functions and is a key step in lineage-specific adaptation. As the only group of sessile crustaceans, barnacles establish permanent attachment through initial cement secretion at the larval phase followed by continuous cement secretion in juveniles and adults. However, the origins and evolution of barnacle larval and adult cement proteins remain poorly understood. By performing microdissection of larval cement glands, transcriptome and shotgun proteomics and immunohistochemistry validation, we identified 30 larval and 27 adult cement proteins of the epibiotic turtle barnacle *Chelonibia testudinaria*, of which the majority are stage- and barnacle-specific. While only two proteins, SIPC and CP100K, were expressed in both larvae and adults, detection of protease inhibitors and the cross-linking enzyme lysyl oxidase paralogs in larvae and adult cement suggested functional convergence. Other barnacle specific cement proteins such as CP100k and CP52k likely share a common origin dating back at least to the divergent of Rhizocephala and Thoracica. Different CP52k paralogs could be detected in larval and adult cement, suggesting stage-specific cement proteins may arise from duplication followed by changes in expression timing of the duplicates. Interestingly, the biochemical properties of larval- and adult-specific CP52k paralogs exhibited remarkable differences, reflecting the composition of cement in different life stages of turtle barnacle might be chemically different. We conclude that de novo gene formation and duplicate neofunctionalization are pivotal to the evolution of lineage-specific cement toolkits in barnacles, which may explain how barnacles can inhabit diverse marine substrata.

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