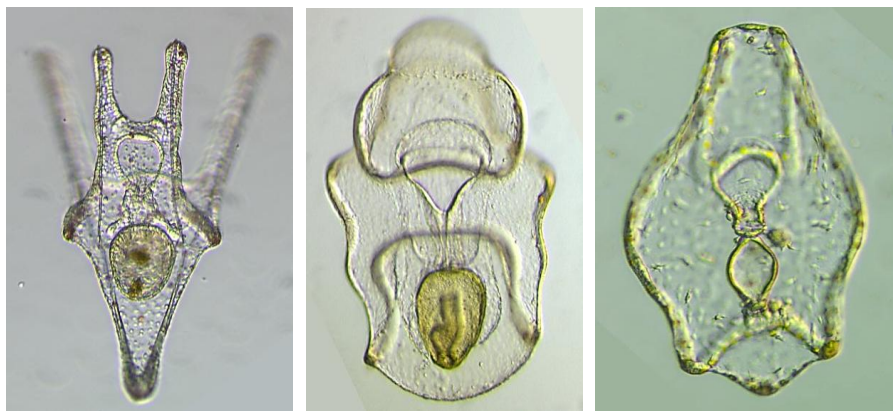




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# The molecular basis of circadian rhythms in echinoderm larvae



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# ABSTRACT

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Most organisms inhabiting land and sea have evolved endogenous circadian clocks to synchronize their physiology with daily light-dark cycles. While extensively studied in terrestrial species, the molecular basis of circadian rhythms in marine invertebrates, particularly echinoderms, remain poorly understood.

To address this gap, I analysed genomic, transcriptomic and proteomic databases from 14 Ambulacraria species, identifying nearly the complete set of canonical clock genes, with the notable exception of *Per*, a crucial component of the negative loop in both protostome and deuterostome oscillators. Although this gene loss was previously reported in four Ambulacraria species, here I expanded the analysis using a dedicated HMM-based approach, confirming the absence of *Per* across all surveyed species and highlighting a divergent circadian clock architecture within Ambulacraria.

I next explored the transcriptome profiling of the sea urchin *Paracentrotus lividus* larvae exposed to 12L:12D cycles over 48 hours, revealing 939 genes exhibiting robust transcriptional oscillations aligned with the light-dark cycle. The rhythmic transcripts were predominantly associated with metabolic processes, transcriptional regulation, signal transduction, transmembrane transport and redox activity. Notably, intracellular ROS levels exhibited a pronounced nocturnal peak, this marking the first evidence of a diel redox rhythm in sea urchin larvae.

HCR and FISH experiments were then performed in the sea urchin *P. lividus*, the sea star *Patiria miniata* and the sea cucumber *Holothuria tubulosa* to explore the spatial expression of conserved clock genes, revealing that different larval territories across species, including the apical organ and the ciliary band, are employed for circadian regulation.

Finally, active regulatory regions analysis of the most rhythmic genes in *P. lividus* larvae revealed strong enrichment of canonical E-box motifs, supporting CLOCK/BMAL-mediated transcriptional control.

Elucidating the transcriptional dynamics of circadian rhythms in non-chordate deuterostomes, this study provides valuable insights into the evolution and diversification of salient timekeeping mechanisms in non-model marine organisms.