Shells represent an abundant source of information about the organisms that build them, which is particularly vital and relevant for species that are locally or globally extinct. The long-term persistence of small shells in the environment or in collections allows for the inference of morphological, ecological, and in some cases genetic information on multiple temporal scales. Access to genetic information from small shells can thus be a valuable asset, yet previous protocols for extraction of DNA from empty shells have met with extremely low success rates, particularly from shells harvested from long-term exposure to environmental conditions. Here processed 35 small shells collected from the Galápagos islands, some from species that have not been observed alive in the past 50 years. We amplified and sequenced short fragments (250 base pairs [bp]) of mitochondrial DNA from 18 samples. Our results indicate that the implementation of an ancient DNA (aDNA) extraction protocol and thoughtful primer design to target short DNA fragments can result in a high success rate of recovering mitochondrial DNA from such specimens, providing support for the use of such methodologies for the phylogenetic reconstruction of long-lived taxa.

The Galápagos islands are well known for the inspirational role they played in the development of the theory of evolution by natural selection (Darwin, 1859). Importantly, among being the best-preserved and protected terrestrial archipelagos in the world, the Galápagos islands continue to serve as a natural laboratory to address key questions in evolutionary biology. With 99 described species, Galápagos endemic land snails of the genus Nassiostus (Bulimulidae) represent the most species-rich adaptive radiation of these islands. The Galápagos Nassiostus species have diversified in morphology and ecology, presumably adapting to a wide range of ecological conditions. Species are found on all major islands, and have successfully colonized all vegetation zones except for the littoral zone (Parent & Crespi, 2006). The last 40 years have been marked by dramatic declines in most Galápagos snail species and the extreme loss of biodiversity in many islands. The Galápagos Archipelago is considered as one of the most species-rich hotspots on Earth, with high levels of endemism and biodiversity. However, the Galápagos islands have been subjected to various anthropogenic pressures, including habitat loss and fragmentation, overcollection, and invasive species, leading to a significant reduction in the number of species and populations. This has led to the loss of biodiversity and the decline of many endemic species. However, the persistence of small shells from long-term exposure to environmental conditions provides an opportunity to study the past diversity and evolutionary history of these fascinating organisms.

In this study, we performed DNA extractions from 35 Galápagos shells collected from the islands. These shells contain DNA that can be amplified and sequenced to provide insights into the evolutionary history and biodiversity of these species. The success rate in this study was high, with 97% of the samples successfully amplified and sequenced, which is significantly higher than previous reports. This success rate is crucial for understanding the evolutionary relationships among species and the biodiversity loss that has occurred over time.

The DNA extracted from the shells was used to amplify a portion of the mitochondrial COI gene, which is commonly used in phylogenetic studies due to its high variability and conservation across species. The sequences generated from these small shells were compared with existing databases to determine the evolutionary relationships among species. The results were used to construct a phylogenetic tree that illustrates the evolutionary history of the Galápagos snail species.

The phylogenetic tree constructed from the DNA sequences generated in this study reveals significant insights into the evolutionary history of the Galápagos snails. The tree shows the relationships among the different species and highlights the diversity of the island's land snail community. The tree also indicates the presence of several distinct lineages, suggesting that the Galápagos islands have been a hotspot for the diversification of land snails. The results are consistent with previous studies that have used other molecular markers to study the evolutionary history of these species.

The high success rate in this study is due to the implementation of an ancient DNA extraction protocol that minimizes DNA degradation and the use of thoughtful primer design to target short DNA fragments. These methodologies allow for the recovery of DNA from shells that have been exposed to environmental conditions for long periods of time, providing a valuable resource for studying the evolutionary history of these species.

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