
New tools and methods

Spider webs, soil or leaf swabs: what are the best substrates to detect terrestrial vertebrate eDNA?

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Human activities have led to widespread population declines and extinctions among terrestrial vertebrates. In this situation, effective monitoring is more critical than ever to track species distribution and guide conservation and restoration actions. Recently, environmental DNA (eDNA) metabarcoding has emerged as a promising and cost-effective tool for the simultaneous detection of multiple taxa. However, low detection rates have hindered the widespread adoption of a single substrate for terrestrial vertebrates eDNA surveys. Substrate selection has been shown to strongly influence detection success across taxa, yet few studies have compared their effectiveness. As such, most research in this area remains at the proof-of-concept stage, lacking empirical foundation needed for broader application.

Our study aims to evaluate and compare several promising substrates—spider webs, soil and leaf swabs—that are cost-effective and easily collectable for large scale eDNA monitoring. Specifically, we examined community overlaps among substrates and their effects on vertebrate detection probabilities. We analysed 120 samples collected from the Landes Forest, a managed, species-poor temperate forest in Western France, and included additional samples from the Montpellier zoo to validate our detection capabilities. After sequence filtering, we identified 59 wild vertebrate taxa using 12SV5 primers and 43 with 16Smam primers across forest samples. Leaf swabs and spider webs consistently and significantly yielded a higher number of taxa per sample than soil, with spider webs producing the highest average detections using the 12SV5 primers.

These findings highlight eDNA metabarcoding as an efficient and cost-effective tool for monitoring terrestrial vertebrates, and advocate for spider webs and leaf swabs as optimal substrates for maximizing detection probabilities in rapid sampling contexts. Additionally, our results emphasize the need for further comparative studies on substrate efficiency to optimize terrestrial vertebrate detection through eDNA.