

DNA metabarcoding of environmental samples provides an effective and efficient method to assess biodiversity but has lagged in parasite biodiversity assessments; one reason being the high taxonomic diversity and genetic divergence of parasites, which precludes the development of universal parasite primers. Our goal was to implement environmental DNA metabarcoding to determine parasite diversity in sediment and water from four physically connected habitats (wetland, freshwater pond, brackish impoundment, and tidal creek) in coastal South Carolina, USA, as part of a parasite BioBlitz in April 2023. Sediment was collected using a syringe corer, and water samples were collected using two methods (active filtration and passive collection via the deployment and recovery of water filters). Six amplicon libraries were produced using metabarcoding primers targeting platyhelminths (two COI mtDNA libraries), nematodes, myxozoans, microsporidians, and a final library targeting all eukaryotes to capture parasite taxa with no available metabarcoding primers (four 18S rDNA libraries). There was variable specificity among the primers targeting specific parasite taxa: e.g., the microsporidian primers exhibited high target fidelity, whereas the nematode and myxozoan primers were less specific. Nontargeted parasite taxa detected included apicomplexans, dinoflagellates, ichthyosporeans, and perkinsids. Results reflect the expected differences in parasite community composition across habitats and demonstrate the potential of using metabarcoding to assess parasite diversity. Results also highlight the need for more comprehensive reference databases, as well as the development of more specific and novel primers for those parasite taxa for which there are no available genetic markers.