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Microbiome Variation in the Genus *Heterodon*

An organism's internal microbiome plays a critical role in survival, metabolism, and digestive health. While the microbiome has become a major focus in animal health and diet studies, reptiles are often underrepresented in this research. Snakes, whose body structures are shaped by prey preferences, may exhibit greater microbiome diversity among species than is seen in closely related mammals. We investigated the gut microbiomes of *Heterodon nasicus* and *Heterodon platirhinos*, two species co-occurring in a relic Sandhill prairie in southeastern Iowa. Both are typically considered bufophagic, but preliminary data suggest that adult *H. nasicus* specialize in turtle eggs. Using fecal eDNA, we sequenced samples with the Oxford Nanopore Native Barcoding Kit and analyzed data via Epi2me's metagenomics workflow with Kraken2. Statistical analysis revealed significant differences in bacterial beta diversity between species. No significant microbiome differences were observed between males and females, but juvenile *H. nasicus* differed significantly from adults, with no similar ontogenetic variation in *H. platirhinos*. Several bacterial genera were important in both species. Although beta diversity differed, alpha diversity did not, suggesting possible dietary overlap despite specialization. These results indicate a primary dietary difference between *H. nasicus* and *H. platirhinos*, and between juvenile and adult *H. nasicus*.