**Methods**

*DNA extraction, PCR amplification, library preparation, sequencing, and denoising*

We individually measured the length of each predator (mm) and separated the thorax, opisthosoma, or trunk (depending on predator species, Krehenwinkel, Macias Herndandez) for DNA extraction following a modified CTAB extraction protocol (Fulton et al., 1995). Following methods in Krehenwinkel et al. (2017), we standardized concentrations of 40uL of each sample to 20ng/ul and used Ampure XP (Agencourt, Beverly, MA, USA) beads to remove higher molecular weight predator DNA prior to PCR steps. We then amplified the CO1 gene, which is well-represented in online databases (Porter and Hajibabaei 2018) with general metazoan primers (mlCOIintF/Fol-degen-rev; Krehenwinkel et al. 2017, Leray et al. 2013, Yu et al. 2012). We ran total reaction volumes per sample of 25μL, with 9μL nuclease free water, 12.5μL GoTaq Green Master Mix (Promega Corp., Madison, WI, USA), 1.25μL of each primer (at 10mM), and 1μL of DNA template (at 10ng/μL) and ran a duplicate for each sample. We followed a PCR protocol as followed: 3 minutes at 95ºC, 35 cycles of: 95ºC for 30 seconds, 46ºC for 30 seconds, 72ºC for one minute; ending with 72ºC for five minutes. We removed reaction dimer with Ampure XP beads at 0.8x bead-to-DNA ratio. We then attached Illumina index primers (Nextera XT Index Kit v2) with 5μL of PCR product per reaction and the recommended PCR protocol for these primers (Illumina). We combined and cleaned successfully amplified duplicate samples using Ampure XP beads (0.7x beads-to-DNA) and diluted each sample to 5nM in 10mM TRIS, using 1uL of each sample for sequencing.

Because of the sample size and the need for a large number of sequences per predator in order to detect rarer prey DNA (Krehenwinkel, miller-ter Kuile, SI Figure 2), we ran samples for this study across four separate sequencing runs (SI Table 1). All individuals within a predator species were sequenced on the same run and each run contained one to five predator species. We ran 19 samples of one predator species (*H. venatoria*) across all runs to quantify run-to-run variation in sequencing (SI Figure 3). For each run, we multiplexed all samples along with one negative control and two PCR4-TOPO TA vectors (Invitrogen, Carlsbad, CA, USA) containing the internal transcribed spacer 1 region from two fungal species as positive controls (GenBank accession numbers: MG840195 and MG840196; Apigo & Oono, 2018; Clark et al., 2016; Toju et al., 2012). We submitted multiplexed samples for sequencing at the University of California, Santa Barbara Biological Nanostructures Laboratory Genetics Core. Samples were run on an Illumina MiSeq platform (v2 chemistry, 500 cycles, paired-end reads) with a 15% spike-in of PhiX. Following sequencing, samples were demultiplexed using Illumina’s bcl2fastq conversion software (v2.20) at the Core facility. Our full protocol from DNA extraction through submission for Illumina sequencing can be found in Miller-ter Kuile et al..

We merged, filtered (max ee = 1.0), and denoised (clustered) our sequences around amplicon sequence variants (ASVs) using the DADA2 algorithm in R (dada2 package version 1.1.14.0; Callahan et al., 2016). Prior to denoising with DADA2, we used cutadapt (version 1.18, Martin, 2011) to remove primers from each sequence. We compared results to a similar protocol using the UNOISE3 algorithm (unoise3 function in unoise CITE EDGAR), but found that DADA2 gave more high-read abundance ASVs (SI Figure 4). We ran DADA2 on sequences from all sequencing runs combined but verified that this was appropriate by first ensuring that error rates per run were similar, following recommendations from the algorithm developers (SI Figure 5). We removed samples from analysis that had not been sequenced to sufficient depth using iNEXT (CITE) and a lower quantile cutoff (SI Figures 6 & 7). We rarefied remaining samples (McKnight et al., 2019) based on the sample with the lowest sequencing depth which had been sequenced with 95%+ sampling completeness based on iNEXT (version 2.0.20) interpolation and extrapolation methods (Hsieh & Chao, 2017). We rarefied using the rrarefy() function in the vegan (version 2.5.6) package in R to 15,954 reads per sample.

*ASV taxonomic assignment with BLAST and BOLD*

From the output of the DADA2 algorithm, we created a list of unique ASVs which we matched to taxonomies both in the GenBank and BOLD databases. For GenBank, we used BLAST (version 2.7.1) with the blastn command for taxonomic assignment of each ASV using the computing cluster at UC Santa Barbara, comparing against the GenBank nucleotide database with an evalue of 0.01 (downloaded on November 20, 2019). We visualized and exported taxonomic alignment using MEGAN Community Edition (version 6.18.0, Huson et al., 2016), using default settings and selecting the subtree with all possible diet items for this species (Kingdom: Animalia, Clade: Bilateria). For BOLD taxonomic assignment, we used the BOLD IDEngine of the CO1 gene with Species Level Barcode Records (accessed May 21, 2020; 4,070,029 Sequences, 225,114 Species, and 104,607 Interim Species in database) to match each ASV list to taxonomies. We combined taxonomic assignments from both programs and discarded taxonomic assignments that were mismatched at the family level or higher (Elbrecht et al., 2017). Most (\_\_%) ASVs received a family-level taxonomic assignment, so we chose to combine prey taxonomies at the family level, similar to diet resolution in both metabarcoding and histological methods in this field (e.g. Brose et al. 2019, Kartzinel – find other metabarcoding ones) by summing the cumulative read abundances across the ASVs that corresponded to each diet family in each sample. All DNA matching any predator family present on an individual sequencing run was also removed as a conservative method to account for potential sequence jumping within sequencing runs which could alter prey identity or diversity in favor of predator species on a shared run (CITE SEQUENCE JUMPING).

**Results**

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Of a total of 280 samples, we successfully extracted DNA from 99% of samples (n = 278 of 280, SI Table 1). Amplification success across all samples was 80%, with 222 of 278 initially extracted samples successfully amplified and sequenced. The Illumina MiSeq runs yielded 3.9, 3.8, 3.8, and 3.3 x 107 unpaired reads that passed filtering and had Q30 quality scores of 87.82, 85.46, 79.34, and 78.02%, respectively. After quality filtering and denoising with DADA2, 3.02 x 107 paired-end reads corresponded to 1,738 ASVs. Following filtering and clustering, we determined that 40 samples had too-low sequencing depths (less than 11,211 reads total) and so they were removed from further analyses.Of the total individual predator samples, 182 remained after removal of poorly-sequenced samples.

*ASV taxonomic assignment with BLAST and BOLD*

Sixty-seven percent (1,167 of 1,738) of ASVs matched to a taxonomic assignment at Class level or lower. Thirty percent (n = 524) of these taxonomic assignments corresponded to prey items at the family level or lower and so were used in analyses. There were two conflicting taxonomic assignments at the family level or higher between the BOLD and BLAST assignments which were removed from the final total above.