



Nota breve | Short note

Caught in the web: spider diets as a window into arthropod diversity in remote areas

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Insects are the most diverse group of animals, hence many taxa remain poorly studied, especially in remote areas (Eggleton 2020). Spiders are among the most abundant insectivores, important for regulating insect populations (Michalko *et al.* 2019). Thus, understanding their diet is essential for indirectly assessing insect diversity within poorly studied habitats.

This study uses DNA metabarcoding to analyse the diet of *Argiope sector* (Forsskål, 1776) spiders collected in 2021 at three sites on the uninhabited island of Santa Luzia, Cabo Verde (Fig. 1), as detailed in Jowers & Caut (2021), to test whether it can indirectly assess insect diversity in this remote area. DNA from

nine different opisthosomas were extracted using a standard high-salt protocol (Sambrook *et al.* 1989) and host DNA was removed using AMPureXP beads following Krehenwinkel *et al.* (2017). A two-marker approach was used to maximise prey identification and compare primer effectiveness targeting 16S rRNA (IN16STK-1F-mod/IN16STK-1R-mod, Pinho *et al.* 2018), and cytochrome c oxidase subunit I (COI) (ZBJ-ArtF1c/ ZBJ-ArtR2c, Zeale *et al.* 2011). PCR conditions followed Pinho *et al.* (2018) for the 16S and Krehenwinkel *et al.* (2017) for the COI. Libraries were prepared according to Illumina MiSeq protocols (Illumina 2013) and sequenced using a 300-cycle MiSeq Reagent Kit V2. DNA sequences

were processed bioinformatically using the OBITools package (Boyer *et al.* 2016), following Pinho *et al.* (2023). Contigs were discarded if read counts were <10 and sequence lengths differed from expected base pair (bp) sizes (16S: 110 bp; COI: 210 bp). Potential PCR/ sequencing errors, singletons

and contaminations were removed, and molecular operational taxonomic units (MOTUs) were obtained and compared against sequences in NCBI Nucleotide Database and our reference collection (Pinho *et al.* 2018), using the BLAST+ software (Camacho *et al.* 2009).

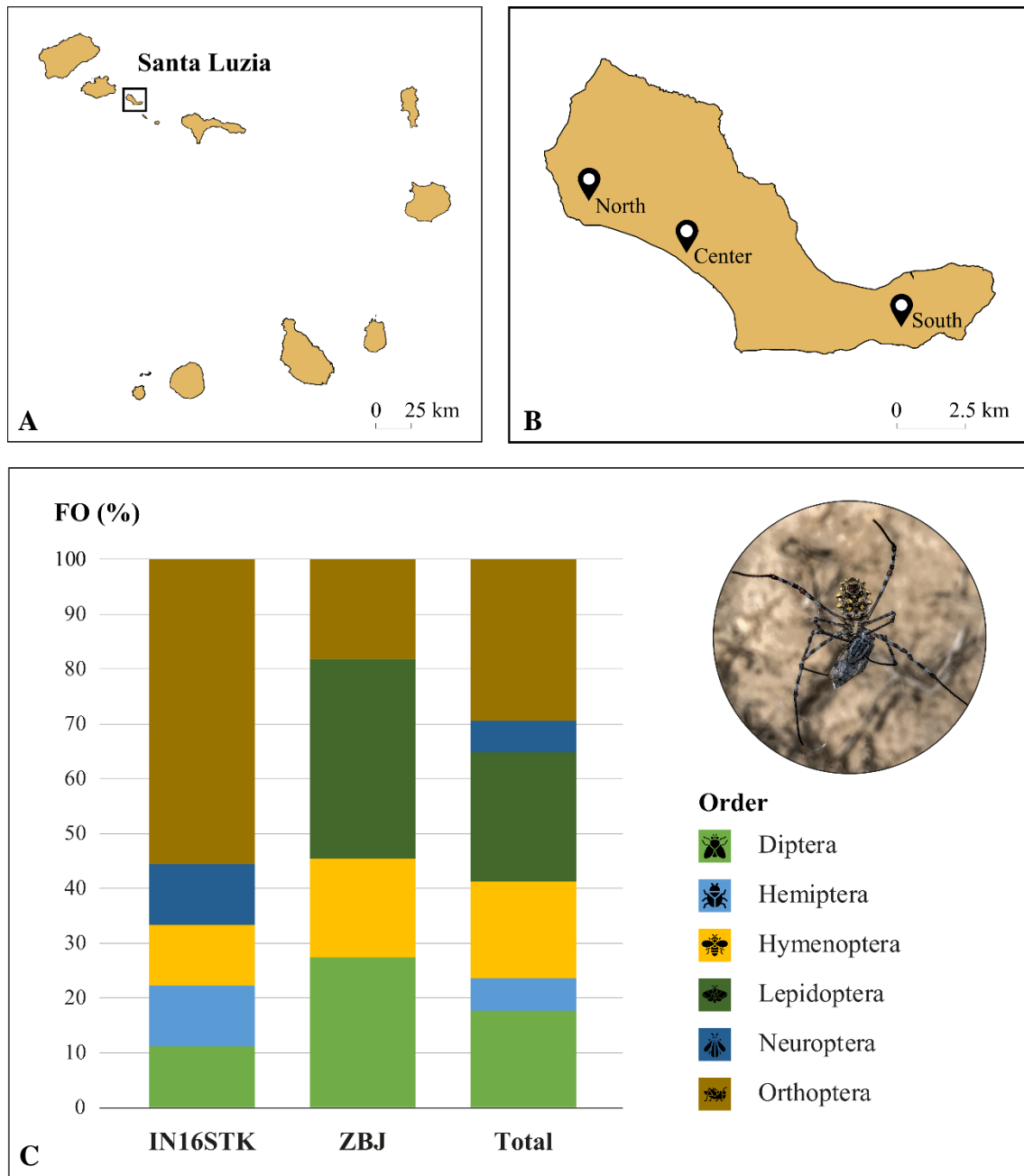


Fig. 1. Study area and results of the diet of *Argiope sector*. **A)** Map of Cabo Verde showing the geographic location of Santa Luzia Island. **B)** Map of Santa Luzia depicting the three sampling points. **C)** Stacked column chart showing the relative percentages (FO) of each insect order retrieved from DNA metabarcoding analyses using each mitochondrial marker (IN16STK and ZBJ) and considering the total of the two. In the top right corner, an orb spider is depicted preying a fly (photo by S. Caut).

Six orders (Fig. 1C), 11 families (Anthomyiidae, Psychodidae, Nabidae, Braconidae, Diapriidae, Eulophidae, Crambidae, Gelechiidae, Tortricidae, Myrmeleontidae, and Acrididae), and 16 MOTUs were identified, eight of which were identified at the genus or species level (*Apantales*, *Ochrodia*, *Scrobipalpa*, *Creoleon*, *Acrotylus*, *Sphingonotus*, *Nomophila noctuella*, and *Nabis capsiformis*).

Given the limited sample size, the diet of these individuals revealed a noteworthy diversity of insects, some particularly challenging to sample and unrecorded on the island (e.g., *N. noctuella*). It was not possible to amplify Hemiptera or Neuroptera with ZBJ

primers or Lepidoptera with IN16STK (Fig. 1C). Similar results were observed by da Silva *et al.* (2019), with ZBJ showing clear biases, especially by overestimating Lepidoptera.

This study highlights the importance of using a multiple marker approach in dietary studies (Cuff *et al.* 2022), and of studying the diet of top predators with DNA metabarcoding for biodiversity assessments in remote areas (Santos *et al.* 2022). Furthermore, it emphasises the need for comprehensive surveys of arthropod biodiversity on Santa Luzia (e.g., Fortes 2021) to preserve unique species that may be declining before they even have been described.

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