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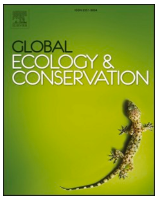

Green matters: Dietary assessment of a reptile community using DNA metabarcoding

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ABSTRACT

DNA metabarcoding is widely used for diet characterization and is becoming increasingly important for biodiversity conservation, allowing the understanding of trophic networks and community assemblies. However, to our knowledge, few studies have used this approach to investigate trophic interactions for whole communities and none for reptiles. In particular, few studies have examined the diet composition of Saudi Arabian reptiles, and all have used classical methods only. Therefore, in this work, a non-invasive approach using DNA metabarcoding of faecal pellets was implemented to investigate the diet composition of the reptile community of Wadi Ashar, in AlUla County, north-western Saudi Arabia Kingdom. In the overall diet composition of the community, arthropods were present in 90% of the samples, and plants were present in 63%, revealing the unforeseen importance of plants to this community as a secondary, but also a primary dietary item. For some species, this is the first time that plants have been reported in their diet. A significant effect of reptile body size on diet composition was also demonstrated, indicating its strong influence on prey selection and resource partitioning in the community. This study highlights the importance of community assessments and the power of combining these with non-invasive DNA metabarcoding to accurately assess biodiversity and feeding habits, revealing unknown ecological interactions of often neglected groups. This revolutionary tool for conservation and management provided rapid and holistic information at relatively low costs, allowing to inform local authorities about which elements are central to the sustainable management of the Wadi Ashar community.

1. Introduction

The traditional methods of morphological characterization of species for biodiversity assessments were revolutionized by DNA-

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based species identification. DNA barcoding is a molecular technique regularized by Hebert et al. (2003) that allows the identification of species using short standardized DNA fragments known as DNA barcodes. Metabarcoding extends DNA-based species identification to complex samples allowing a large-scale taxonomic identification (Pompanon et al., 2012), relying on high-throughput sequencing of DNA using general or group-specific primers. With the power of Next-generation sequencing (NGS) methodologies (Shendure and Ji, 2008), this multispecies identification approach is becoming faster and more cost-effective each day. Metabarcoding has been widely used to characterise species diets by extracting DNA from faeces and gut contents (Pompanon et al., 2012). This technique provides a large amount of data with less effort and time (Taberlet et al., 2012) which is particularly useful for studying the diets of animals with elusive lifestyles. In addition, compared to classical methods, this technique is less reliant on taxonomic expertise (Pompanon et al., 2012), and maximises resolution, detection of rare events, detection of soft, small and difficult-to-detect prey (Symondson, 2002) and ultimately can correct biases in ecological models. It is a particularly useful technique for studying species of conservation concern. The characterization of their dietary compositions allows the understanding of trophic interactions, which are one of the main vectors shaping natural community assemblies and ecosystem processes (Thébault and Loreau, 2005). This data is essential for assessing ecological responses to environmental change, the ecosystems' health, and ultimately the development of sustainable conservation strategies. However, few studies have used metabarcoding of diets to study trophic interactions of whole communities (Ingala et al., 2021; McShea et al., 2019), and to our best knowledge none on reptile communities.

Reptiles are the most diverse group of terrestrial vertebrates in the world, with 11,940 currently described species (Uetz et al., 2023). They are an important component of global biodiversity and are remarkable from an ecological and evolutionary perspective. As ectotherms, reptiles are strongly influenced by the thermal landscapes of their habitat, making them good indicators for studies of climate change (Sinervo et al., 2010). They are also relatively easy to capture and sample for biodiversity and phylogenetic studies, they are widely represented in museum collections worldwide (Shultz et al., 2021), and for many groups, there is abundant and detailed information on their taxonomy, distribution, and ecology (Pincheira-Donoso et al., 2013; Uetz et al., 2023). For these and more reasons, they are excellent models for evolutionary, biogeographic, ecological and conservation studies, and have been used as such for many decades (Camargo et al., 2010; Nordstrom et al., 2022). Since most of the species are adapted to particular environments, they are very good indicators of the ecosystems' health. Therefore, reptiles are a key target group in biodiversity studies, especially in arid areas where they are one of the main components of the diurnal and nocturnal vertebrate fauna, playing a relevant role in the ecosystem (Lopes et al., 2019; Miranda, 2017; Wilms et al., 2010a).

In Arabia, reptiles are the terrestrial vertebrate group that contains the highest number of endemic species and, therefore, are very important from a biodiversity point of view (Cox et al., 2012). The Arabian Peninsula started its formation around 30 million years ago

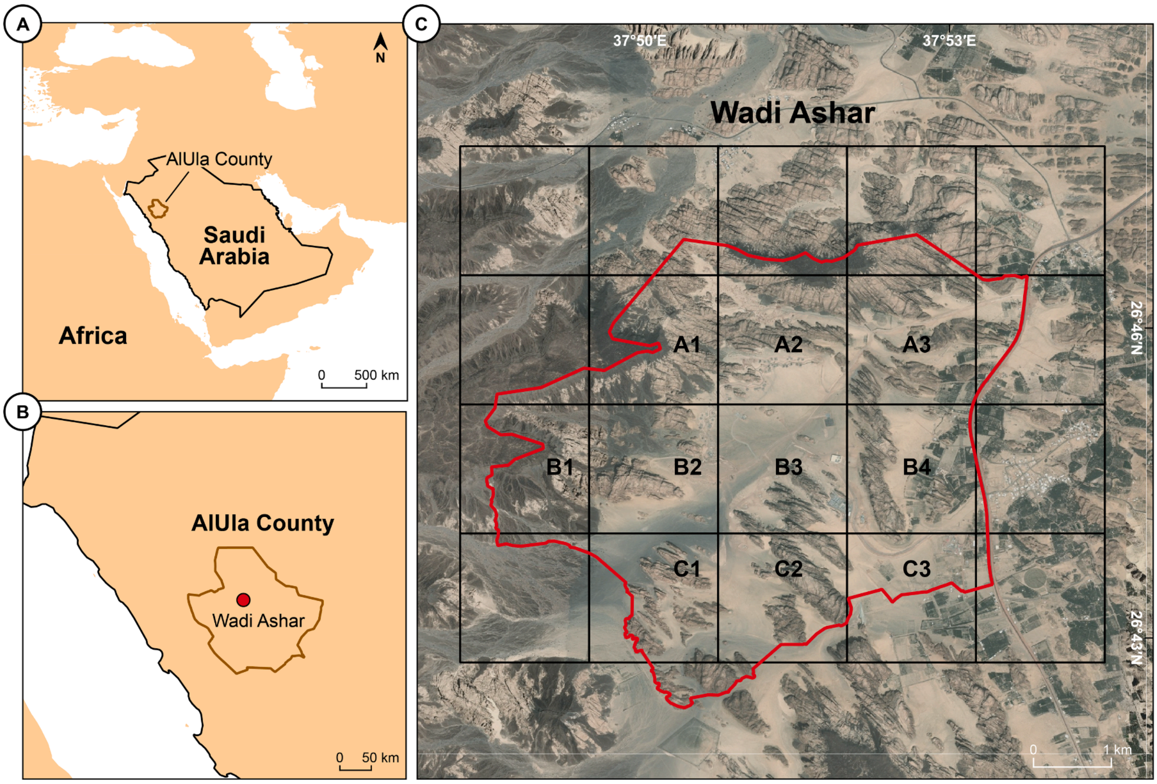

Fig. 1. Map of the study area and study site. **A)** Geographic location of the Kingdom of Saudi Arabia and **B)** the AlUla County, with the location of the study site represented by a red dot. **C)** Wadi Ashar boundary in red, and the 10 sampled squares (Geographic Coordinate System, Datum WGS84).

(Ma), driven by the separation of the Arabian and African tectonic plates (Alsharhan and Nairn, 2003; Bohannon, 1986). The split prompted the formation of the Red Sea about 25 Ma (Bosworth et al., 2005), and played a major role in shaping the geography and history of the region. Accompanying the separation was a shift to an arid climate, which together with the complex and dynamic geologic past, had major biogeographic and evolutionary implications on the unique biodiversity of the region (Kürschner, 1998; Pook et al., 2009; Šmíd et al., 2013; Tejero-Cicuéndez et al., 2021; Zinner et al., 2009). In particular, the Kingdom of Saudi Arabia, covering more than 2.15 million km^2 (Fig. 1A), encompasses a wide range of climates and terrestrial habitats, from mesic, cool, high mountains to arid deserts and hot, semi-arid coastal plains, and thus holds a very rich biodiversity (AbuZinada et al., 2004; Vincent, 2008). The terrestrial reptile fauna of Saudi Arabia includes approximately 118 species (80 lizards, 36 snakes and two freshwater turtles), 8 of which are endemic to the Kingdom (Gasperetti et al., 1993; Šmíd et al., 2017a; Šmíd et al., 2017b; Šmíd et al., 2021; Šmíd et al., 2023; Wilms et al., 2010b). However, to effectively conserve this unique reptile biodiversity, much work remains to be done to fully understand its diversity, distribution, and ecology (Aloufi et al., 2019). Conserving wildlife and maintaining healthy ecosystems in arid habitats is particularly important, as resources are typically limited, and the loss of a single resource can be catastrophic for the stability of the communities (McNeely, 2003). Thus, local authorities are giving increased attention to conservation planning in the region promoting the development of several projects to monitor and protect biodiversity (Alatawi, 2022).

Despite these efforts, few studies have investigated the diet composition of Saudi Arabian reptiles. The few ones focus mostly on the Riyadh region and all rely on the analysis of stomach contents to morphologically identify the different prey items consumed (Al-Sadoon and Al-Otaibi, 2014; Al-Sadoon and Paray, 2016; Al-Sadoon et al., 2016; Al-Sadoon et al., 2020; Ibrahim and Busais, 2016). However, the use of faecal samples is a better approach, mainly when working with threatened species, since they can be obtained with minimum or no impact on individual fitness (Ferreira et al., 2018). Consequently, this work implemented a non-invasive approach, taking advantage of DNA metabarcoding of faecal pellets to investigate the dietary composition and the possibility of resource segregation by the reptile community of Wadi Ashar located in AlUla County, north-western Saudi Arabia. Alongside, a biodiversity survey was carried out to assess the diversity of reptile species in the community. The ultimate goal was to explore ecological patterns through prey items consumed and contribute to a better understanding of the trophic networks involving plants, arthropods, and reptiles at a community level in this restricted area. This data is expected to inform local authorities about which elements are central to the sustainable management of the reptile community.

2. Methods

2.1. Study area

AlUla is a county located 1100 km north-west of Riyadh, in the Medina region of north-western Saudi Arabia (Fig. 1B). It covers a vast area of 22,561 km^2, including desert valleys, sandstone mountains and several ancient heritage sites. Wadi Ashar, located in AlUla, is a valley within the UNESCO Biosphere Reserve of Harrat Uwayrid of about 30 km^2 surrounded by sandstone mountains (Fig. 1C). It is composed of different habitat types such as dunes and sandy areas, gravel plains, rocky slopes, gullies, and agricultural

Table 1

Sampled herpetofauna of Wadi Ashar. List of taxa sampled ordinated by family and their codes, number of sampled individuals (N), of collected pellets (Np), of successfully sequenced pellets (Ns), average snout-vent length (SVL), body size category (class), activity period (Activity) and previous knowledge on diet composition (Diet) based on the literature: [1]Al-Sadoon et al. (2020); [2]Arnold (1980); [3]Arnold (1993); [4]Carranza et al. (2021); [5]Cottone and Bauer (2009); [6]Ibrahim and Busais (2016); [7]Ibrahim and El-Naggar (2013); [8]Kalboussi and Nouira (2004); [9]Ribeiro-Júnior et al. (2022); [10]Roobas and Feulner (2013); [11]Taylor et al. (2012); [12]Tsairi and Bouskila (2004); [13]Yadollahvandmiandoab et al. (2018). *Ptyodactylus* sp1 x sp3 and *Ptyodactylus* sp1 refer to two new species of the *Ptyodactylus hasselquistii* species complex in the process of description.

Family	Taxa	Code	N	Np	Ns	SVL (mm)	Class	Activity	Diet
Agamidae	*Laudakia vulgaris*	LAvu	3	3	3	-	Big	Diurnal	Arthropods, plants[7]
Atractaspididae	*Atractaspis engaddensis*	ATen	1	1	1	-	-	Nocturnal	Vertebrates[1]
Colubridae	*Spalerosophis diadema*	SPdi	1	-	-	-	-	Diurnal/ Nocturnal	Vertebrates[13]
Gekkonidae	*Bunopus tuberculatus*	BUtu	1	1	0	32.96	-	Nocturnal	Arthropods[4]
	Hemidactylus granosus	HMgr	1	-	-	45.08	-	Nocturnal	-
	Stenodactylus doriae	STdo	13	8	4	54.19	Medium	Nocturnal	Arthropods[2,4]
	Tropiocolotes yomtovi	Tryo	10	7	1	24.43	Small	Nocturnal	Arthropods[9]
Lacertidae	*Acanthodactylus boskianus*	ACbo	11	7	5	59.24	Medium	Diurnal	Arthropods[10]
Phyllodactylidae	*Ptyodactylus* sp1 x sp3	PTsp1x3	9	5	3	55.97	Medium	Nocturnal	Arthropods[6]
	Ptyodactylus sp1	PTsp1	26	12	7	59.51	Medium	Nocturnal	Arthropods[6]
Psammophiidae	*Psammophis schokari*	PSsc	1	-	-	67.40	-	Diurnal	Vertebrates[5]
Scincidae	*Chalcides ocellatus*	CHoc	5	5	4	84.94	Big	Diurnal	Arthropods, plants[8,11]
	Scincus conirostris	SCco	2	2	1	81.87	Big	Diurnal	-
	Trachylepis brevicollis	TCbr	2	1	1	119.68	Big	Diurnal	Arthropods, plants, vertebrates[4]
Sphaerodactylidae	*Pristurus guweirensis*	PRgu	6	3	2	25.48	Small	Diurnal	Arthropods[3]
Viperidae	*Echis coloratus*	ECco	1	1	0	-	-	Nocturnal	Vertebrates[12]

areas. The area is substantially influenced by overgrazing, and it holds several tourist resorts. Recent development has resulted in severe habitat destruction and fragmentation in the area.

2.2. Sampling

Sampling in Wadi Ashar took place between the 15th and 24th of March 2022. The study area was divided into 10 subareas of approximately equal size (Fig. 1C). Each subarea was surveyed both during the day and night to collect representative data on the entire herpetological community by covering the possible activity periods of the different species (Table 1). A total of 23 transects were completed by four observers in teams of two. Each transect was on average 555 m long and sampling took an average of one hour per transect. Active searches during the day included checking under stones and other possible shelters such as bushes, trees, rocks, boulders, caves, and holes. Nocturnal searches consisted mainly of visual searches for active reptiles using flashlights. In addition to the active searches, two fence trap arrays, each with four pitfalls and two funnel traps, were set at appropriate locations in each subarea throughout the study period. Other six pitfalls and three funnel traps were individually placed in appropriate habitats (dunes, shrubs, along long walls). Traps were visited at least two times a day. Individuals were captured by hand, sexed based on the absence or presence of cloacal pouches (Vasconcelos et al., 2012), measured (snout-vent length (SVL) to the nearest mm), geolocated using a GPS device, and photographed. Before releasing each animal, a sample was taken from the tip of the tail for species identification and an abdominal massage was performed to collect fresh pellets for dietary analysis. All samples were preserved in 96% ethanol.

Samples of plant leaves were also collected across the study area, in order to build a DNA reference collection of possible food items. Pictures of each plant were also taken in the field to allow for morphological taxonomic assessment by experts.

2.3. Species identification

For correct taxonomical assignment of the species identified in the field, DNA from the tissue samples was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. DNA samples were PCR-amplified and sequenced for the ribosomal 12 S rRNA (12 S) region, with primers and PCR conditions detailed in Šmíd et al. (2021). The PCR products were sequenced from both directions at Macrogen (Amsterdam, the Netherlands) and raw sequence data were edited and assembled in Geneious v.11 (Kearse et al., 2012). The identification of each specimen was confirmed by employing the BLAST algorithm available at the NCBI website *(https://blast.ncbi.nlm.nih.gov)*.

2.4. Diet analysis

2.4.1. DNA extraction, amplification, and sequencing

For the diet analysis, DNA extractions from the pellets (excluding uric acid) were performed, including one blank per sample batch, using the Stool DNA Isolation Kit (Norgen Biotek Corp., Canada), following the manufacturer's instructions. All DNA samples were amplified with two genetic markers already validated in previous studies (Pinho et al., 2022; Pinho et al., 2018). For invertebrates we used a modified version of the IN16STK-1 F/IN16STK-1R primers, targeting the mitochondrial 16 S rRNA (Kartzinel and Pringle, 2015; Pinho et al., 2018) and for plants, we used the g/h primers targeting the short P6-loop of chloroplast trnL (UAA) (Taberlet et al., 2007). For species that were expected to prey on vertebrates (e.g., *Atractaspis engaddensis* Haas, 1950; Table 1) the primers 12sv5F/12 Ssv5R, targeting the V5-loop fragment of the mitochondrial 12S gene, were also used. All PCRs were performed, including blanks, as described by Pinho et al. (2018). Libraries were prepared following the Illumina MiSeq protocol '16 S Metagenomic Sequencing Library Preparation' (Illumina, 2013). The samples were sequenced in the MiSeq sequencer (Illumina) using the 300-cycle MiSeq Reagent Kit V2 (Illumina, San Diego, CA, USA) for an expected average of 22,000 paired-end reads per sample.

The plant samples for the reference collection were amplified for the chloroplast TrnL (UAA) using two sets of primers. The primer set e/f (Taberlet et al., 1991) was used to further validate the morphological taxonomic assessment, and the primer set c/d (Taberlet et al., 2007) to allow the comparison with diet items. Both markers were sequenced using Sanger sequencing.

2.4.2. Bioinformatics filtering

The obtained DNA sequences for the diet analysis were processed at the bioinformatics level using tools incorporated in the software package OBITtools (http://metabarcoding.org/obitools). This process included the alignment of forward and reverse sequences (command illuminapairedend, –score-min=40), removal of unaligned sequences (command obigrep, mode!="joined"), assignment of reads to samples and removal of primers and adapters (command ngsfilter) and the collapsed of reads into unique sequences (command obiuniq). Unique sequences were discarded if read count was inferior to 10 and if sequence length did not correspond to expected base pair size (16S: ± 110; trnL: 10–143; 12S: 73–110). Finally, potential PCR/ sequencing errors and singletons were removed and the molecular operational taxonomic units (MOTUs) obtained (command obiclean), by removing sequences that differed by 1 bp from the most abundant ones. The MOTU tables were subsequently curated using the R package LULU (Frøslev et al., 2017) in order to remove potential remaining PCR and sequencing errors.

The diet items were taxonomically assigned by comparing the final MOTUs against the online NCBI database using the BLAST algorithm. Sequences that had less than 85% of Percent Identity value with known species were classified only to the class level, the ones with values between 85% and 90% to the order level, between 90% and 95% to the family level, and the remaining sequences with Percent Identity values above 95% to the genus or species level. Only species or genera known to occur in Saudi Arabia or in the broader region of the Middle East were considered (Al-Qthanin et al., 2020; Buttiker, 1979; POWO, 2021; Walker et al., 1987). When a

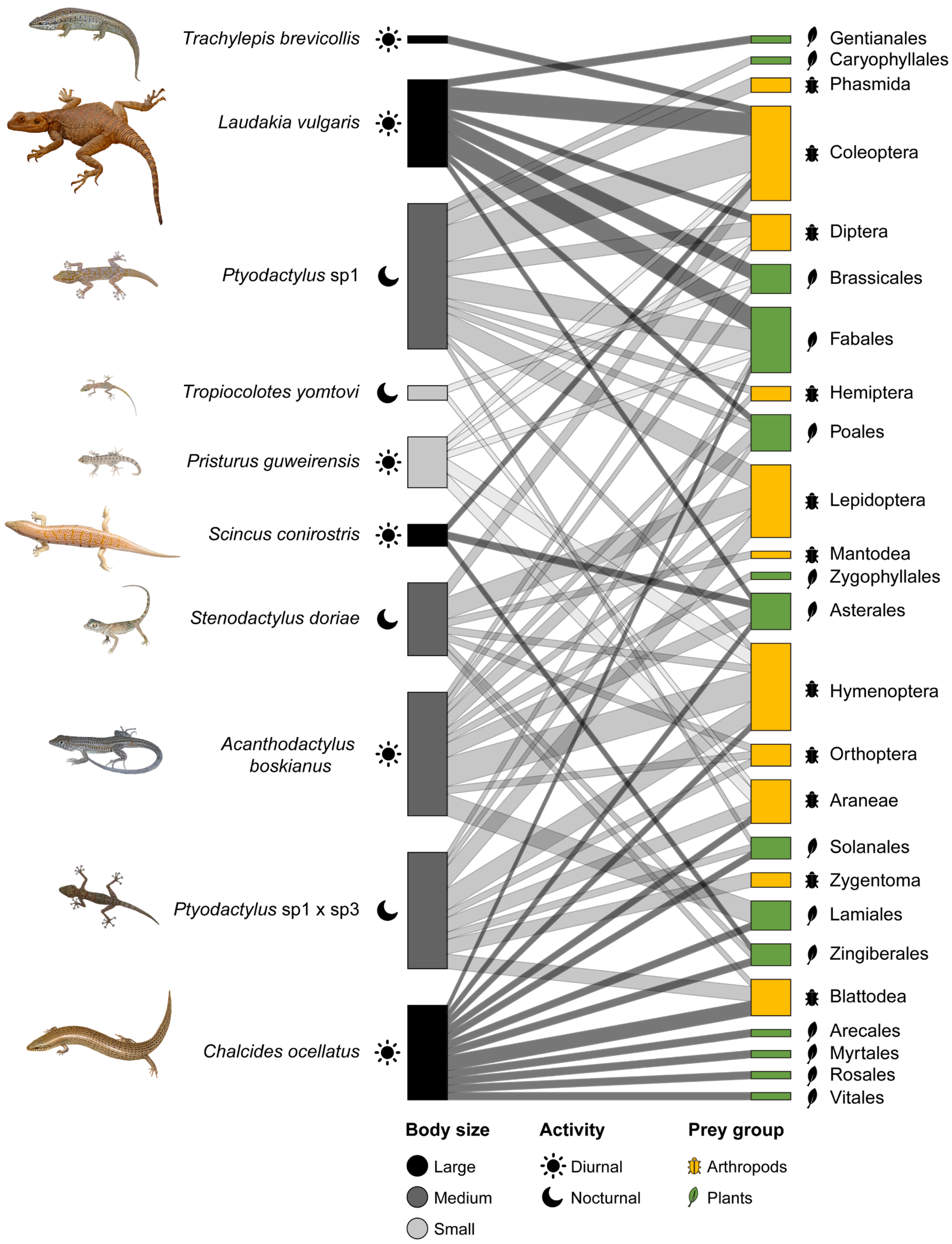

Fig. 2. Bipartite network representing associations between the reptile community of Wadi Ashar on the left and arthropod (yellow) and plant (green) prey orders on the right. The width of the connecting bars represents the frequency of occurrence of each order for each reptile species. Reptile species are represented proportionally according to their sizes, and with darker colour boxes indicating their increasing body size category. Information on reptiles' activity pattern (diurnal versus nocturnal) is also given.

MOTU matched more than one species or genus with the same identity scores or presented low query coverage (<80%), a higher taxonomic ranking would be attributed (e.g., family). If more than one MOTU corresponded to the same taxon at the same taxonomic level, a different number was attributed to each (e.g., Poaceae_1, Poaceae_2), considering genetic differences among MOTUs. The MOTUs detected in extraction blanks were identified as contaminations and removed from the corresponding batch of samples (e.g., human DNA). In the final dataset, based on PCR blanks counts, samples with less than 650 reads were removed and within kept samples, MOTUs representing less than 1% of the total number of reads of that sample were also discarded (Mata et al., 2016).

2.4.3. Data analysis

Frequencies of occurrence (FO) of plants, arthropods and vertebrates were estimated for the reptile community and for each species individually at the order, family, and MOTU level. The sample of *A. engaddensis* (CN20291) was excluded from further statistical analysis, as it was the only exclusively carnivorous species. All analysis were performed using R software version 4.2.2 (R Core Team, 2022). The package bipartite v2.17 (Dormann et al., 2008) was used to visualize the dietary networks using the function ‘plotWeb’. Adjacency plots were calculated at the order level using the function ‘metaComputeModules’ employing the "Beckett" method and visualized using ‘plotModuleWeb’. A total of 1000 null model networks were generated using ‘nullmodel’ function with the “r2dtable” method. To assess the statistical significance of the observed modularity, z-scores were calculated and *p*-values were obtained using the ‘pnorm’ function. Due to the low resolution at the MOTU level, a binary matrix (of the presence/ absence of prey) was prepared at the order and family level considering individual samples. Reptile species were divided into three body size categories following our SVL average measures per species and the literature: large > 80 mm; medium 50 – 80 mm; and small < 50 mm (Table 1). Generalized linear models (GLM) were carried out to test for diet composition differences among size categories and activity period (diurnal vs nocturnal), accounting for the unbalanced sample sizes. GLMs for multivariate binary data were fitted using the *mvabund* package (Wang et al., 2022), using the *manyglm* function. The significance of the GLMs was tested using the *anova.manyglm* function. The same function was implemented with the argument p.uni= ‘adjusted’ to perform univariate tests, identifying prey items responsible for differences among size categories. Using the *metaMDS* function from the vegan package (Oksanen et al., 2022), a non-metric multidimensional scaling (NMDS) was performed on a Jaccard distance matrix to ordinate samples according to diet dissimilarity. NMDS were calculated at the order-level and visualised in two-dimensional space.

3. Results

A total of 93 individuals of 16 reptile species, including four snakes, were observed (Table 1). From these, a total of 82 individuals were caught and a tissue sample was collected (Table S1). The most common taxon was the candidate species *Ptyodactylus* sp1 (a new species of the *Ptyodactylus hasselquistii* species complex in the process of description), with 24 observations, followed by *Stenodactylus doriae* (Blanford, 1874), *Acanthodactylus boskianus* (Daudin, 1802), and *Tropiocolotes yomtovi*, with less than 15 observations. The least common species, with only one individual recorded during the sampling effort, were *Atractaspis engaddensis*, *Bunopus tuberculatus* Blanford, 1874, *Echis coloratus* Günther, 1878, *Hemidactylus granosus* Heyden, 1827, *Psammophis schokari* (Forskål, 1775), and *Spalerosophis diadema* (Schlegel, 1837).

A total of 2.7 million reads were obtained from the reptile community faecal samples. After bioinformatic filtering, a total of

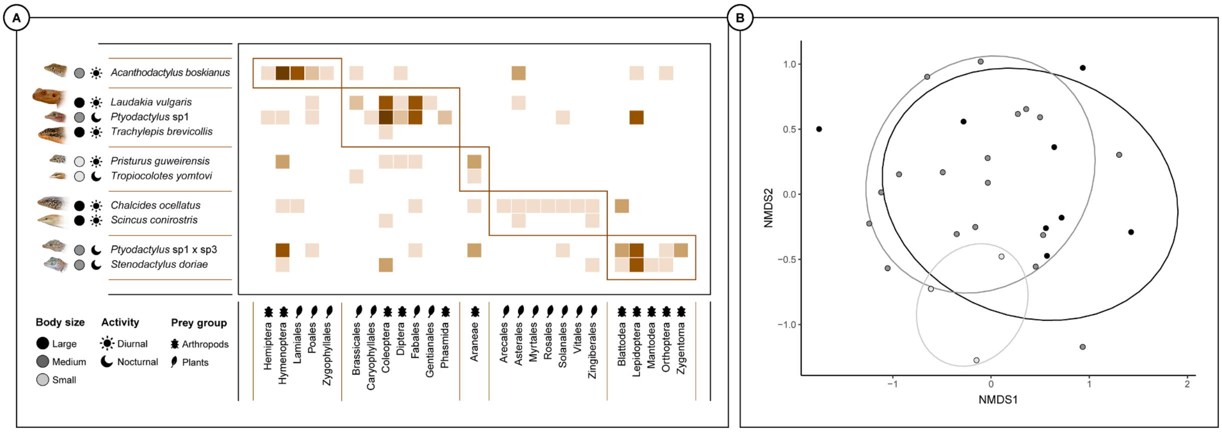

Fig. 3. Dietary composition of the reptile community of Wadi Ashar according to the body size categories. **A)** Adjacency matrix summarizing the reptile community subgroups according to their identified diet item orders. Reptile species are represented proportionally according to their sizes along the vertical axis with darker colour circles indicating their increasing body size category, and plant and arthropod orders are shown along the horizontal axis. Subgroups are delineated by brown boxes. The darker the brown squares the more frequent the association between a diet item and its predator. Information on reptiles’ activity pattern (diurnal versus nocturnal) is also given. **B)** Non-metric multidimensional scaling (NMDS) of the prey composition of the Wadi Ashar reptile community by body size categories. Points closer together correspond to individuals with a more similar diet item composition. Ellipses represent the standard error of the dietary composition centroid for each body size category at 90% confidence interval.

467,831 reads were retained, of which 11% were considered non-target (e.g., host DNA, human, bacteria), resulting in a final average of approximately 13,000 reads per sample. Of the 56 faecal samples collected, the final dataset comprised 32 samples from 11 reptile species from the Wadi Ashar community. The remaining 23 samples failed bioinformatic filtering due to low read counts or contamination (Table 1). Within these, a total of 80 MOTUs from five taxonomic classes were identified, with an average of three families and three orders per sample and seven families and six orders per reptile species (Table S2). Arthropods were distributed among two classes, 11 orders, 23 families and 44 MOTUs (Table S2). Plants were distributed among two classes, 14 orders, 18 families and 29 MOTUs (Table S2). The most frequent arthropod orders were Coleoptera (FO= 40.63%) and Hymenoptera (FO= 37.50%), both present in the diet of six reptile species of the community (Fig. 2; Table S3). In particular, *Pimelia arabica* (Klug, 1830) was the most frequent arthropod MOTU (FO= 15.63%), present in the diet of three reptile species, followed by *Monomorium pharaonis* (Linnaeus, 1758) (FO= 12.50%) present in the diet of four reptile species (Table S2). For plants, the most frequent order was Fabales (FO= 28.13%), present in the diet of five reptile species (Fig. 2; Table S3). At the MOTU level, *Vachellia_1* was the most frequent (FO= 25.00%), consumed by four reptile species (Table S2). The only vertebrate detected was *Scincus conirostris* (Blanford, 1881) in the pellet of *Atractaspis engaddensis* (Table S2). In the overall diet composition of the community, arthropods were present in 90% of the samples, whereas plants were present in 63% of them. Lepidoptera was the order with the highest diversity with 12 MOTUs, almost exclusively consumed by nocturnal reptile species (Fig. 2, Table S2).

The adjacency matrix defined a total of five modules in the community (Fig. 3A). Reptiles seem to be grouped by body size and consumption of arthropod orders (e.g., candidate species *Ptyodactylus* sp1 x sp3 – a new species of the *Ptyodactylus hasselquistii* species complex in the process of description – and *S. doriae*) or plant orders (e.g., *Chalcides ocellatus* (Forskål, 1775) and *S. conirostris*). The observed modularity was significantly different from the null model distribution ($P = 0.027$), indicating that the observed pattern deviates from what would be expected by random chance. The multivariate analysis showed a significant effect of body size in the diet composition at the order level (LRT= 70.06, $P = 0.016$), but not at the family level (LRT= 84.78, $P = 0.103$). The orders that contributed significantly for the differences among size categories were Aranae ($P = 0.043$), more frequent in small sized species, and Lepidoptera ($P = 0.035$), only present in medium sized species. The activity period also showed a significant effect at the order (LRT= 45.51, $P = 0.049$) and family level (LRT= 75.69, $P = 0.003$), however, a significant interaction between body size and activity period was also observed for both (order: LRT= 16.35, $P = 0.007$; family: LRT= 9.32, $P = 0.031$). This indicates a relationship between body size and activity period, possibly explained by big sized species being also diurnal. The NMDS plot showed three groups corresponding to the body size categories in the community at the order level ($k = 2$; stress= 0.138; Fig. 3B). Species from the three body size categories exhibit distinct dietary compositions with some overlap between medium and large species.

In terms of the reference collection, 46 samples from Wadi Ashar plants were successfully taxonomically identified and sequenced (Table S4). These samples were compared with the dietary items improving taxonomic resolution.

4. Discussion

To effectively monitor and conserve communities, the first step is to know their composition (Groves et al., 2002). Hence, this study provides, for the first time, a characterisation of the herpetofauna inhabiting Wadi Ashar. In this small valley, we found 17 reptile species of 11 different families, some with the first distribution records for the area (*Hemidactylus granosus*, *Pristurus guweirensis* Haas, 1943, *Trachylepis brevicollis*; (Aloufi et al., 2021; Badiane et al., 2014; Burriel-Carranza et al., 2023; Šmíd et al., 2023). This is one of the few studies to investigate the diet composition of Saudi Arabian reptiles and, to our best knowledge, the first to use non-invasive DNA metabarcoding techniques for this purpose in the region. The same applies to community-level studies in general, a few similar studies are using faecal metabarcoding to access the diet composition of mammal communities (Gordon et al., 2019; Ingala et al., 2021; McShea et al., 2019), but none on reptile communities. In fact, reptiles are still widely underrepresented in molecular diet studies compared to other groups (Ando et al., 2020), emphasizing the importance of not overlooking some less popular vertebrate groups.

Despite what was previously described for some species, plants show an important presence in the diets of the community. *Acanthodactylus boskianus*, *Stenodactylus doriae*, and species from the genus *Ptyodactylus*, *Tropiocolotes* and *Pristurus* were previously reported to prey exclusively on insects and other arthropods (Arnold, 1980, 1984, 1993, 2009; Bar and Haimovitch, 2011; Carranza et al., 2021; Disi et al., 2001; Ibrahim and Busais, 2016; Ribeiro-Júnior et al., 2022; Roobas and Feulner, 2013). However, this study confirms the presence of plant items in their diets with a significant frequency, occurring in half or more of the samples, except for *Stenodactylus doriae* where only one of the four samples presented plant items (Fig. 2, Table S2). Alternatively, this could be explained by secondary predation or inadvertent consumption of plants when preying on arthropods (Tercel et al., 2021), and if this is the case, the consumption of plant items may be overestimated. Nevertheless, there were samples containing only plant DNA for all the species mentioned, except for *Tropiocolotes yomtovi*, where only one sample was successfully amplified, which suggests active consumption of plant items for the remaining species. Then again, since plant DNA persists longer in the gastrointestinal tract than animal DNA (Holland et al., 2020; Pinho et al., 2022) the trophic role of plants in the diet of these reptiles should be further investigated. For *Chalcides ocellatus* and *Laudakia vulgaris* (Sonnini & Latreille, 1801) previous studies reported the presence of plant matter in their diet in other regions (Ibrahim and El-Naggar, 2013; Kalboussi and Nouira, 2004; Taylor et al., 2012). These results could support the findings for *Chalcides ocellatus* and *Laudakia vulgaris*, and substantially improve the taxonomic resolution of the plants consumed, which was not possible in previous studies, as they were based on classical morphological identification methods (Ibrahim and El-Naggar, 2013; Kalboussi and Nouira, 2004; Taylor et al., 2012). In particular, *Chalcides ocellatus* was the species that consumed a higher diversity of plant orders in the community, which can be related to all individuals being found near agricultural areas (mainly palm tree plantations), hence the presence of Arecaceae in its diet. For *Scincus conirostris* no information was available on their diet, thus the present results demonstrate for the first time its consumption of both plants and arthropods (Fig. 2, Table S2). Therefore, this study reveals the variety of plants consumed and highlights their importance for this community as a secondary and possibly also a primary diet item. It was also possible to improve the taxonomic resolution of the plant items consumed, which was leveraged by the DNA sequences obtained from the collected Wadi Ashar plants, emphasizing the importance of building a complete reference database of the potential diet items available (Pompanon et al., 2012). Even though plant material is harder to digest (Holland et al., 2020), it can be easier to access in arid ecosystems like this, where the availability of arthropods can be very low during some periods. Furthermore, there is a possibility that these reptiles can have important ecological roles as seed dispersers, which is of great importance for the structure of the plant communities in arid human-altered habitats such as this one. This pattern was already observed for other desert environments, with the Mesopotamian spiny-tailed lizard *Saara loricata* (Blanford, 1874), in human-disturbed areas of Iran (Qashqaei et al., 2023).

Through the visualisation of the adjacency matrix, it is possible to observe that the reptiles of Wadi Ashar can be broadly divided into five subgroups, associating body size categories, and in some cases activity periods, with the primary consumption of plants or arthropods (Fig. 3A). This body size association is further confirmed by the ordination of NMDS plots where points closer together correspond to individuals with more similar prey composition (Fig. 3B). This is expected since the body size and head size and shape of reptiles are strongly correlated with their bite force and therefore the hardness and size of prey items consumed (Herrel et al., 2001; Verwaijen et al., 2002). Consequently, the differences in bite force can have a strong influence on prey selection and resource partitioning in the Wadi Ashar reptile community. Likewise, activity period can be correlated with body size as larger reptiles often present higher thermoregulation capacity compared to smaller reptiles (Angilletta et al., 2002; Bogert, 1949; O'Connor et al., 2000) and can benefit from diurnal habits. The first subgroup is only composed of *Acanthodactylus boskianus* with a high frequency of Hymenoptera in the diet, in particular Formicidae, which was a strong preference previously reported for the species (Roobas and Feulner, 2013). The second subgroup, constituted by *Laudakia vulgaris*, *Ptyodactylus* sp1 and *Trachylepis brevicollis*, seems to be grouped mainly by the presence of Coleoptera. Both *Laudakia vulgaris* and *Trachylepis brevicollis* are large reptiles that may have the strength to bite harder and consume hard prey, and the first was previously reported to strongly prey on Coleoptera (Ibrahim and El-Naggar, 2013). Interestingly, although *Ptyodactylus* sp1 is a medium-sized species it does not group with the other medium-sized reptiles, in particular with *Ptyodactylus* sp1 x sp3 from the same genus, which is grouped with *Stenodactylus doriae* seemingly by the consumption of smaller and softer arthropods. This may give us some important lines of evidence, regarding their trophic or behavioural ecology, for the pending description of the unrecognized diversity within this genus. It would be important to investigate the differences in habitat use, body size, and head size and shape between the two *Ptyodactylus* that may influence these differences in resource use. The two small species, *Pristurus guweirensis* and *Tropiocolotes yomtovi*, also form a subgroup associated with the consumption of Araneae. Even though they have different activity periods (*Pristurus guweirensis* being diurnal while *Tropiocolotes yomtovi* is nocturnal), they have similar sizes and select similar refuges, as small rocks, which may lead to a similar resource selection. The large-sized and diurnal *Chalcides ocellatus* and *Scincus conirostris* are grouped by the main consumption of plants, which is expected since larger reptiles usually possess metabolic adaptations that facilitate the digestion of plant items (Dearing, 1993), facilitating their higher intake (but see Cooper and Vitt, 2002 on other factors affecting body size). This emerging pattern is consistent with the expected ecological roles and phylogenetic grouping of most species, however this hypothesis needs to be further explored at lower taxonomic levels to identify detailed patterns.

Although we only had one sample of the snake species *Atractaspis engaddensis*, this study was able to show its predation on Scincidae, similar to what was observed for its congeneric species in Nigeria, based on classical stomach content analysis (Akani et al., 2001), and previous reports on the species (Kochva, 2002). Conversely, a recent study that analysed the seasonal diet of *Atractaspis engaddensis* in the region, by examining more than 50 stomach contents, described small rodents, geckos, worm lizards, and snakes as the main prey items (Al-Sadoon et al., 2020), was not able to detect skinks. This highlights the usefulness of rapid metabarcoding sampling of inconspicuous species using non-invasive methods even with only punctual surveys, or small sample sizes (Gil et al., 2020; Neves et al., 2022). It also points to the fact that the species feeds opportunistically on locally and seasonally available prey items and does not specialize in specific groups.

This study demonstrates the potential of metabarcoding approaches associated with non-invasive sampling for conservation management. Through this method, it was possible to obtain an accurate representation of the diet of the Wadi Ashar reptile community by detecting a wider range of taxa than previous classic approaches that are unable to detect soft, small prey items (Taberlet et al., 2012). Additionally, this technique delivered rapid and holistic results on species composition, diversity, and ecological networks, among others, at relatively low costs (Taylor and Gemmell, 2016). Moreover, metabarcoding can provide a large amount of data in a short period, which is of great help to increase the success of biodiversity conservation actions by responsible institutions (Ji et al., 2013), especially in arid areas that are difficult to access and require urgent action to guide sustainable management on predicted anthropogenically highly-impacted areas, like the study area. Furthermore, accessing diet composition through faecal DNA is particularly advantageous as samples can be collected with minimal impact on individuals (De Barba et al., 2014; Pompanon et al., 2012) what is of particular importance for the Wadi Ashar community, which has already been impacted by anthropogenic activities. However, some constraints may have limited our results and need to be considered in future diet studies in arid ecosystems. Despite the potential of metabarcoding, some methodological limitations need to be taken into account. For instance, when faecal samples are used, the extracted DNA is often degraded which affects amplification and sequencing efficiency (Pompanon et al., 2012). Furthermore, variation in prey digestibility rates and primer bias can lead to over or underrepresentation of prey species (Deagle et al., 2014; Pinho et al., 2022; Pompanon et al., 2012). The lack of comprehensive and up-to-date reference databases of potential prey DNA can difficult species assignments and lead to incorrect or low taxonomic identification (Cuff et al., 2022; Nielsen et al., 2018). An attempt was made to address this shortfall by building a reference database for Wadi Ashar plants, resulting in a notable enhancement of taxonomic resolution. However, for arthropods this was not possible and needs to be considered in the future as well as a multiple primer approach to increase detection power within this group (Corse et al., 2010). Besides, adequate sample sizes are important to obtain representative diet data. From the 83 individuals that were caught by hand, we were only able to collect faecal samples from 56. This may be related to the low availability and abundance of prey items which can lead to less feeding frequency and, consequently, to low faeces production. Moreover, small reptiles, particularly *Tropiocolotes yomtovi*, are extremally difficult to sample due to their small dimensions and minute faecal pellets, which led to the successful amplification of only one sample for *Tropiocolotes yomtovi* from the seven collected. Yet, with only one sample it was possible to disclose the plant component in the diet of this species, highlighting the power of metabarcoding approaches for diet characterization. In addition, this study was carried out in only one season, so it was not possible to test for seasonal variations in the diet of the community. Besides the presented biases this study provides a basis for long-term monitoring projects of these species that should be implemented in the future.

This study shows that the reptile community at Wadi Ashar is diverse and of great ecological value but may be negatively affected by habitat degradation due to recent construction work in the area. Several plants directly related to anthropogenic activities were found in the diet of the community, such as plants from the families Arecaceae, Musaceae, Oleaceae and Vitaceae and *Festuca* sp. which is often used as a supplementary feed for camels. This emphasizes the potential impact of human disturbance on the natural diet of reptiles. It is demonstrated that anthropogenic-induced deteriorated areas can have an immense impact on the body condition of reptiles (Amo et al., 2007). Consequently, the development of sustainable conservation measures to mitigate the recent human impact in the area is crucial. This study also demonstrates the importance of plant items in the diet of the reptile community, thus, it would be also recommended to fence off some areas with different soil types, protecting vegetation from overgrazing and increasing its diversity, density, and cover (Ayyad and El-Kadi, 1982; Shaltout et al., 2021). This will create different refuges for some reptile species that depend on plants and can also benefit other vertebrate and arthropod species, as well as plant species that are mostly affected by grazers. Moreover, during fieldwork it was observed that due to the recent construction work there is a lot of rubble scattered along the area which is used as sub-optimal refuge sites by several reptiles, including venomous snakes, affecting their fitness, and increasing the risks of accidents with humans. The presence of optimal refuges, such as trees, bushes, rocks, and burrows is essential for efficient thermoregulation, protection against predators (Bauwens et al., 1996; Pereira et al., 2019) and to limit access to human settlements when searching for cover to avoid high temperatures. For that reason, we alert local authorities for the critical necessity to remove debris and place several large rocks and/or piles of smaller rocks, in areas away from tourist accommodation, to mitigate the impact on fitness and promote an increase in the abundance and diversity of reptiles. Finally, it would be beneficial for local authorities to take advantage of tourist visitors to develop educational activities to raise awareness of the links between the reptile community and other elements of fauna and flora. Thus, demonstrating their importance to the ecosystem through the control of micromammal and arthropod populations, as predators and as pollinators and seed dispersers (Miranda, 2017). These public engagement actions have been proven to increase positive attitudes towards reptiles and their conservation (Fonseca et al., 2021). Some of the Wadi Ashar resorts are very close to natural areas with cliffs and dunes and it would be easy to spot some geckos with headlights and provide visitors with unique guided walks to show Wadi Ashar's natural heritage.

In conclusion, our study highlights the importance of community assessments and the power of combining these with non-invasive DNA metabarcoding to accurately assess biodiversity and its feeding habits, revealing unknown ecological interactions of often neglected groups, such as reptiles. This information can be important for understanding the ecology of some of the more inconspicuous and understudied species, and for learning about the taxonomic groups that are most important to the diet of this community. Uncovering the trophic networks in a community can help to shift the perspective of conservation efforts from focusing solely on specific species to a more holistic approach (García and Vasconcelos, 2017), in this study highlighting that vegetation restoration could benefit not only reptiles but the entire ecosystem.

Declaration of Competing Interest

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Data availability

Datasets supporting this research can be accessed through NCBI BioProject PRJNA1018879 and GenBank accession codes OR573700–OR573791 and OR616286–OR616362.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2023.e02667.

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