**T01: Reintroduced giant tortoises exhibit effectiveness and topological shifts but maintain structural roles in Seychelles’ mutualistic and antagonistic individual ecological networks.**

**T02: Differential Ecological Roles of Keystone Individuals of Reintroduced Giant Tortoises Across Mutualistic and Antagonistic Interaction Networks**

**T03: The neglected role of keystone individuals of giant tortoises as drivers of mutualistic and antagonistic processes in interaction networks**

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**Abstract (247 out of 250).**

Giant tortoises are critical to restoration projects aimed at reviving extinct ecological interactions on defaunated tropical islands. In some Seychelles islands, small populations of *Aldabrachelys gigantea* mean that key ecosystem services such as seed dispersal and browsing-grazing depend on only a few individuals and individual dietary variability may lead to differences in their effectiveness across mutualistic and antagonistic processes. However, the role and contributions of individual tortoises to different ecological processes remain underexplored. To address this gap, we combined observational and genetic methods to study ecological interactions of ten reintroduced Aldabra giant tortoises on Aride Island, assessing their ecological roles based on their effectiveness in dispersing native plants, controlling exotic flora, and their topological and structural roles within individual-based networks. DNA metabarcoding of tortoise scats revealed 98 taxa, with native species such as *Ficus reflexa* and *Pisonia grandis* being the most frequently consumed in browsing and grazing. In seed dispersal, native plants accounted for nearly 90% of seeds dispersed, with *Morinda citrifolia* showing the highest interaction frequency. Individual tortoises exhibited varying levels of effectiveness in both antagonistic and mutualistic processes. Topological roles showed no correlation between mutualistic and antagonistic networks, but within-module connectivity values were significantly positively correlated, suggesting consistent modular roles across antagonistic and mutualistic processes. Our study demonstrates the rapid re-establishment of lost ecological interactions post-reintroduction and highlights the importance of individual-scale variability, as the persistence of key ecosystem functions in insular ecosystems may depend heavily on a few individuals performing distinct ecological roles.

**Keywords:** *Aldabrachelys gigantea*, Aride Island, browsing, diet, seed dispersal, DNA metabarcoding, refaunation, trophic rewiring**.**

**Introduction.**

Biodiversity is currently undergoing an unprecedented crisis worldwide, with myriads of ecosystems facing the loss of unique and irreplaceable interactions due to the rapid and increasing extinction of species (Kaiser-Bunbury et al., 2017, 2010). Islands, recognised as isolated and fragile ecological systems, are particularly sensitive to species extinction events and their cascading effects derived from human overexploitation (Dirzo et al., 2014; Ferreiro-Arias et al., 2024; Hansen and Galetti, 2009). The loss of key vertebrate species in these systems can lead to disproportionate ecological disruption compared to mainland ecosystems, where functional redundancy within vertebrate assemblages (i.e., several species performing similar ecological roles) is more prevalent (Hansen and Galetti, 2009; Kemp, 2023).

For example, insular large vertebrates, while being highly susceptible to anthropogenic-induced extinction, often provide key non-redundant functional roles in seed dispersal processes (Dirzo et al., 2014; Vidal et al., 2013). A clear example are the islands inhabited by giant tortoises in Seychelles and Galapagos archipelagos where they stand out as iconic embodiments of island gigantism, having independently developed large body sizes from diverse ancestors due to diminished ecological pressures (Jaffe et al., 2011; Le et al., 2006; Vargas, 2014). In such insular systems, there is a scarcity of large-bodied sized herbivores aside from giant tortoises, which occupy unique ecological niches as both antagonists (i.e., via browsing and grazing) and mutualists (e.g., via primary seed dispersers) of many plant species (Blake et al., 2012; Nogales et al., 2017).

Given their unparalleled significance as ecosystems engineers, giant tortoises gained relevance in restoration projects aimed at recovering extinct ecological interactions in defaunated insular ecosystems (Griffiths et al., 2011, 2010; Polak and Saltz, 2011). This importance is exemplified by the reintroduction of Aldabra giant tortoises (*Aldabrachelys gigantea*) whose widespread distribution in the western Indian Ocean has been historically dwindled due to the overexplotation of the species by humans (Gerlach et al., 2013). The population of Aldabra giant tortoises remained in the wild in the Aldabra atoll within Seychelles archipelago, prompting restoration initiatives involving refaunation onto other Seychelles islands such as Curieuse, Frégate, Ile du Nord, Grand Soeur, Moyenne, Cousin, Cousine and Silhouette (Gerlach et al., 2013). Intriguingly, this species has been also used as a taxon substitute for giant tortoises of the genus *Cylindraspis sp.*, a genus of five extinct species distributed across the Mascarene Islands of the Indian Ocean (Austin and Arnold, 2001). For instance, Mauritius islands were inhabited by *Cylindrapsis sp*., which were the largest native fruit-eaters, weighing around 100 kg. However, after its human-driven extinction, the Mauritian fruit bat (*Pteropus niger*), which weight only 0.54 kg, was the largest frugivore standing on the island (Heinen et al., 2023). This drastic extinction, representing a loss in body size of the largest frugivore of about 185 times, has prompted a significant ecological imbalance (Heinen et al., 2023; Kehlmaier et al., 2019) and raised the question of whether it is feasible to restore the benefits of historical tortoise-plant interactions lost due to human-driven extinctions (Hansen, 2015a; Hansen et al., 2010).

Nonetheless, two potential drawbacks must be considered. On one hand, the large size and generalist diet of these species could make tortoises significant dispersers of introduced plants, potentially threatening native species in sensitive ecosystems (Waibel et al., 2013). On the other hand, a concern associated with these reintroductions is the potential for habitat degradation, counteracting the conservation objectives of these programs (Waibel et al., 2013). Hence, assessing the current conditions of reintroductions is imperative to evaluate conservation efforts that may effectively restore ecosystems processes by rewiring historical interactions. Exploring the dietary behaviours of key species like giant tortoises is fundamental in understanding their ecological roles within insular ecosystems, particularly in the context of conservation and restoration efforts of ecological interactions. For example, the feeding behaviour of Aldabra giant tortoises is known to affect plant composition through selective browsing and grazing (Griffiths et al., 2013). It has been suggested that the herbivory defences in Mauritian native flora as evolutionary adaptation to tortoise grazing may turn non-native plants more palatable due to the lack of such defences (e.g., spines or pubescences), ultimately leading to the control of such exotic plants (Griffiths et al., 2013).

In recent decades, assessing the diet of introduced giant tortoises in Seychelles has been hampered by small sample sizes and challenges in accurately discerning dietary items from faecal samples (Moorhouse-Gann, 2017). Recent advances in DNA metabarcoding techniques provided an invaluable opportunity to delve deeper in the feeding ecology of Aldabra giant tortoises (Moorhouse-Gann, 2017), but these investigations have predominantly focused on browsing and grazing behaviours and neglected the exploration of ecosystem services associated with seed dispersal. Aldabra giant tortoises are able to disperse large-seeded plant species and their prolonged gut retention times may compensate for their limited movement rates (Falcón et al., 2021, 2020; Griffiths et al., 2011), providing to the ingested seeds an opportunity to escape Jazen-Connell effects (Falcón et al., 2021; Hansen et al., 2008). Nevertheless, on some islands within Seychelles archipelago, Aldabra giant tortoise populations are small (Gerlach et al., 2013), meaning that key ecosystem services, such as seed dispersal and browsing, are sustained by only few individuals. Moreover, Aldabra giant tortoises showed variability in diet among individuals which have been related to habitat preferences and body size (Moorhouse-Gann, 2017; Pemberton and Gilchrist, 2009). These differences in individual preferences may ultimately lead to fluctuations in their effectiveness as seed dispersers and/or browsers-grazers, impacting both mutualistic and antagonistic ecological processes. Nevertheless, there is a gap in the scientific literature regarding the evaluation of the contribution and importance (i.e., ecological role) of different individuals to mutualistic and antagonistic ecological services.

To address this research gap, we used a multifaceted approach combining both observational and genetic methodologies in Aride Island, where ten individuals of Aldabra giant tortoises were reintroduced in 2018. The historical backdrop of commercial exploitation and coppicing with introduced species on Aride Island highlights the urgent need for the role of Aldabra giant tortoises in restoring ecosystem services through the reestablishment of lost ecological interactions with both native and introduced plants. Despite Aride’s designation as a Special Reserve in 1975, remnants of these introduced plants persist, offering a unique opportunity to observe the initial stages of vegetation response to tortoise reintroduction, including the dispersal dynamics of both native and non-native species. By integrating field observations, faeces examination, and DNA metabarcoding, this study aims to better inform evidence-based conservation and restoration strategies by: 1) characterising the novel antagonistic and mutualistic interaction networks between the Aride Island flora and reintroduced tortoises; 2) exploring the ecological role of individual tortoises in antagonistic interaction networks through browsing and grazing; and 3) describing the ecological role as seed dispersers of both native and non-native plant species.

**Material and Methods.**

*Study area.*

Ten Aldabra giant tortoises (*Aldabrachelys gigantea*) were translocated from the close-by Fregate Island to Aride Island in 2018. Aride Island (4°12'48"S, 55°39'57"E), situated as the northernmost granitic island of the Seychelles archipelago, spans approximately 0.71 km2, reaching 135 meters above sea level (Fig. 1A). Along its southern shoreline lies a 0.07 km2 flat, low-lying plateau. In contrast, the northern section of the island rises abruptly beyond the plateau, with hills ascending to towering cliffs, accentuating a pronounced difference in topography among sections. Up to 154 flora species have been historically recorded at least once on Aride Island, including both introduced and native plants (Table S1) (Castle and Mileto, 1994; Hansen and Laboudallon, 2016). On the basis of the historical records, introduced plant species may represent approximately 48% of the species richness on Aride Island, while native species may represent nearly the 52%, including two endemisms from the Seychelles archipelago and Aride Island respectively: *Pandanus balfourii* and *Rothmannia annae*.

*Plant reference collection.*

We delineated convex polygons using GPS locations from focal observations of individual tortoises as vertices, spanning feeding areas (Fig. S1). To estimate the plant species available to the tortoises, we conducted collection surveys along these individual feeding areas. To build a reference collection, we collected identified tissue samples from each recorded species, storing them in bags with silica gel to keep them dry. For each plant species recorded, we gathered information on their distribution range from the Plants of the World Online Database (POWO, 2024), classifying each species as either native or introduced to Aride Island. DNA extraction from all plant species found within tortoise feeding areas was performed following the protocol for the DNeasy Plant Mini Kit (Qiagen, Manchester, UK). We performed a Polymerase Chain Reaction (hereafter, PCR) to amplify the second internal transcribed spacer (ITS-2, 160-320 bp) of nuclear ribosomal DNA, which has been proposed as a useful DNA barcoding region for identifying plant species in dietary studies (Chen et al., 2010; Hollingsworth, 2011; Moorhouse-Gann, 2017; Yao et al., 2010).

In particular, primers UniPlantF (5′-TGTGAATTGCARRATYCMG-3′) and UniplantR (5′-CCCGHYTGAYYTGRGGTCDC-3′) were used for ITS-2 sequencing (Moorhouse-Gann *et al.,* 2018). PCR conditions were as follow: 24 μl of total reaction volume which included 2,5 μl 10X Buffer, 1,4 μl of MgCl2 50mM, 1,6 μl of dNTP’S 2,5mM, 0,5 μl of each primer, 0,3 μl of Taq DNA Polymerase 5U/μl, 1 μl BSA 1mg/μl and 16,2 μl of Milli-Q purified water plus 1 μl of extracted DNA product from each sample. PCR cycling conditions were: initial denaturation at 94ºC for 2 minutes, 40 cycles of 94ºC for 1 minute, 56ºC and 72ºC for 1 minute, a final extension of 72ºC for 10 minutes and an ending base temperature of 4ºC. The results were verified in a 1% agarose gel electrophoresis with SYBR Safe Gel stain for visualization of DNA. For samples that did not amplify, the PCR was repeated testing temperature ranges between 48ºC to 56ºC, including 2 μl of extracted DNA product and removing 1 μl from the 24 μl mix. Verified PCR products were sequenced in both directions by Macrogen (MACROGEN Inc., Madrid, Spain). ITS-2 sequences from the species collected in the field were edited with Geneious (Geneious Prime) and subsequently contrasted with the GenBank reference database using a BLASTn search (Altschul et al., 1990) in order to determine nucleotide differences within each species. Subsequently, a multiple alignment was performed for our sequences and those of the same species deposited in GenBank.

*Interaction data collection.*

Six months after the release, all tortoises were monitored daily throughout a six-week period, from February to March, 2019. During this period, we carried out focal observations of each individual tortoise for 30 minutes, contingent upon the feasibility of locating them. In each focal observation, we recorded the GPS location, activity, and feeding behaviour of each individual, including the identification of plant species and the specific parts consumed (e.g., leaves, fruits…), thus characterising each interaction as antagonistic (i.e., browsing and grazing) or mutualistic (i.e., frugivory).  When available during focal observations, we collected faeces from the tortoises, safely identifying each scat with the corresponding individual. A total of three dung piles per individual were retrieved, with each scat stored in an individual zip-lock bag duly labelled. Scats collected were placed on aluminium baking trays and undigested macroscopic material such as fruits and large visible seeds were manually extracted. Seeds were counted using a magnifier and identified visually at species level using local flora field guides (Castle and Mileto, 1994; Hansen and Laboudallon, 2016). All the seeds extracted from faeces were separated in order to count the total number of seeds per plant species.

The remaining organic matter corresponding to leaves and grasses that could not be identified *in-situ* was dried and stored in a zip-lock bag with silica gel for posterior analysis in the laboratory. In order to avoid exchange of plant material and, thus, cross-contamination between samples, all the instruments were washed, rinsed and dried. Once completely dry, each sample was ground, mixed, and homogenised using a Moulinex Multimoulinette chopper. To extract DNA for metabarcoding analyses, 180 mg of each sample was used following the QIAmp Fast DNA Stool Mini Kit protocol (Qiagen, Manchester, UK). We took three replicates per sample, resulting in a total of 90 replicates from which DNA was extracted and sequenced. Subsequently, we carried out an agarose gel electrophoresis to verify that the resulting extractions had enough DNA content. DNA concentration was measured using a Qubit Flourometer (Mardis & McCombie, 2017). DNA metabacording analyses from faecal samples were performed by the Exeter Sequencing Service (Exeter, United Kingdom), where 90 Amplicon libraries (ITS-2) and a unique MID-tag combination were created for each sample, allowing us tracing back not only DNA sequences, but also individual faecal samples. To ensure that samples were suitable for sequencing and to minimise errors during the sequencing process, a pool quality control (QC) was performed. Each library was then sequenced separately using 300 bp paired end reads on an Illumina HIseq 2500 rapid run (Illumina, San Diego, CA). To this end, we used the UniPlantF and UniplantR primers previously described (Moorhouse-Gann et al., 2018). All ITS-2 sequences were trimmed using Cutadapt (Martin, 2011) to remove low quality sequences, and a second round of trimming removed PCR primers (for amplifying ITS-2). A total of 215,792 sequences containing the ITS-2 spacer from *Streptophyta* were downloaded from the NCBI Nucleotide Database and used to create a library for use in the QIIME2 platform (Bolyen et al., 2019). Paired-end sequences were imported into QIIME2 and denoised using the DADA2 plugin (Callahan et al., 2016). Samples with fewer than 500 assembled reads after denoising were excluded from these analyses.

*Data analysis.*

We assessed interaction sampling completeness by building up accumulation curves of distinct pairwise interaction in relation to the number of tortoises sampled as well as the sampling effort in hours (Jordano, 2016). We constructed two individual-based interaction matrices to represent two distinct ecological processes mediated by tortoises: an antagonistic one based on interactions derived from browsing and grazing, and a mutualistic one based on interactions derived from frugivory and seed dispersal. For the antagonistic interaction matrix, we used focal observation data on feeding events involving browsing and grazing, supplemented by DNA metabarcoding data from faecal samples containing grass and leaf remains. For the mutualistic interaction matrix, we used focal observation data on foraging events involving fruit ingestion, along with counts of seeds and fruits found in fresh tortoise faeces. To build each ecological interaction matrix, we applied a data-merging approach that integrates these methods. Merging data from multiple sampling techniques has been shown to yield a net increase in detected interactions and provide a more comprehensive characterisation of ecological networks, resulting in more robust network descriptors (Quintero et al., 2022). Accordingly, we applied a quantitative combination using sampling effort standardisation (SES) by controlling for the sampling effort of each individual tortoise (Quintero et al., 2022). For the antagonistic matrix, we standardised by sampling effort the relative read abundance (hereafter, RRA) from the metabarcoding analysis and the count of feeding events obtained during focal observations. The RRA is based on the average sequence count of an item (i.e., plant species) across the three replicates obtained from an individual scat (Deagle et al., 2018) and has been shown to provide a reliable quantitative proxy for herbivore consumption rates (Kartzinel et al., 2015). For the mutualistic matrix, we standardised by sampling effort the count of feeding events from focal observations and the number of seeds found in the faeces of each individual tortoise.

Once the matrices were constructed, we assessed the importance of different plant species in the diet of individual tortoises using centrality metrics. Plant importance was defined based on three metrics: degree (the total number of unique interactions a plant species has with individual tortoises), interaction strength (the frequency of interactions between a plant species and the tortoises), and weighted closeness, which quantifies how accessible or central a plant species is to all individual tortoises in the network, considering the interaction weights (Delmas et al., 2019). Subsequently, we used our plant reference collection (see *Plant reference collection* section) to classify each plant species as native or introduced, generating a ranking for both antagonistic and mutualistic ecological processes.

To define the role of tortoises in both antagonistic and mutualistic ecological processes, we applied three complementary approaches. First, we assessed their effectiveness as browsers-grazers and seed dispersers by adapting the seed dispersal effectiveness framework (Schupp et al., 2010). The quantitative component for seed dispersal was characterised by the standardised number of seeds dispersed and for browsing and grazing effectiveness, we used the standardised abundance of reads from DNA metabarcoding, while the qualitative component for both processes was evaluated based on the proportion of native flora. From a reintroduction perspective, optimal effectiveness would be achieved by tortoises that disperse a high number of native seeds (i.e., high values for both quantitative and qualitative components) while predominantly browsing and grazing on exotic flora (i.e., high quantitative values but lower qualitative values). For the topological role of individual tortoises, we used centrality metrics analogous to how we assessed the importance of different plant species in the tortoises' diets (i.e., degree, interaction strength and weighted closeness) (Delmas et al., 2019). Once these individual-level metrics were obtained, we calculated Pearson correlation coefficients (*r*) of each individual’s metric values in browsing and grazing processes with the corresponding values in frugivory and seed dispersal. A high and statistically significant correlation would suggest that a given individual tends to retain its topological role across both antagonistic and mutualistic networks, and thus in both ecological processes.

The structural role was evaluated through a modularity analysis of the ecological networks (Delmas et al., 2019). Modularity (Q) in networks is defined as a measure of the extent to which a network is divided into distinct modules or communities, where nodes (i.e., individual tortoises and plant species) within a module have denser connections to each other than to nodes in other modules (Olesen et al., 2007). We separately estimated modularity values for the antagonistic and mutualistic networks and tested whether these deviated from expected by chance using 1000 randomizations using null models with the *Vaznull* algorithm and then calculating z-scores (Vázquez et al., 2007). After estimating modularity, we defined the structural roles of individual tortoises by calculating their within-module connectivity (z) and among-module connectivity (c) values (Olesen et al., 2007). High z values indicate tortoises that are well-connected within their module, acting as central hubs within a specific subgroup of plant interactions. High c values, on the other hand, identify tortoises that connect across multiple modules, linking different plant groups and acting as "connectors" within the broader network. Using the 95th percentiles of connectivity values from randomised networks as thresholds, we classified tortoises into structural roles: peripheral (low z and c values); network hub (high z and c values); module hub (high z, low c); and network connector (high c, low z) (Olesen et al., 2007). To assess whether individual tortoises retained their structural roles across antagonistic and mutualistic networks, we evaluated the correlation between each tortoise’s within-module connectivity values in the antagonistic and mutualistic networks, as well as the correlation between their among-module connectivity values across both networks using Pearson correlation coefficients (*r*). High and statistically significant correlations would indicate that individuals tend to maintain their structural roles in distinct ecological processes, while low correlations would suggest differing structural roles in different ecological processes. Additionally, we calculated the proportion of tortoises classified as peripheral, module hubs, network hubs, or network connectors in the antagonistic network that received different structural role classifications in the mutualistic network. All network analyses and visualization were carried out in R Statistical Software v. 4.2.2 (R Core Team, 2023)  using “*bipartite*” (Dormann et al., 2008) and “*ggplot2*” packages (Dormann et al., 2008), respectively,

**Results.**

*Plant reference collection*

A total of 51 different plant species were collected on tortoises feeding areas (Table S1). Of them, 53% (n=27) correspond to introduced plant species, while 47% (n=24) correspond to native specimens, including two endemisms: *Pandanus balfourii* and *Rothmannia annae*. Among the 51 identified species, sequencing of the ITS-2 region was successfully accomplished for 31 species (ca. 61%). Sequences extracted from GenBank database yielded 44 ITS-2 sequences, while sequences for the remaining seven species were not found in GenBank database neither successfully sequenced (Table S1). The ITS-2 sequences obtained from plant species occurring within the feeding tortoise area matched those ITS-2 sequences obtained from GenBank, thus indicating that species were correctly identified (Table S1).

*Browsing and Grazing.*

For the identification of plant species using DNA metabarcoding of scats, 90 DNA extractions (i.e., 3 replicates per scat) were performed, of which only 85 had sufficient quality for sequencing, and six did not yield results after analysis. As a result, DNA metabarcoding unveiled 98 distinct taxa, encompassing a cumulative count of 8,992,243 sequencing reads across all samples. Among these taxa, 76 were identified at the species level, including 45 introduced plant species, 14 native species, 16 human food leftovers (which were excluded from subsequent analyses), and one bryophyte species (*Macrothamnium macrocarpum*). Additionally, among the taxa identified at the genus level (7,548,053 reads), the vast majority (97%) corresponded to the genus *Ficus* (7,317,486 reads), representing 81.4% of the total sequencing reads.

By merging focal observation data and DNA metabarcoding results, the resulting accumulation curve of distinct pairwise interactions relative to browsing and grazing displayed a growing trend further from the asymptote, while still approaching it in both number of individual sampled and sampling effort in hours (Figure S2). Based on node centrality metrics, *Ficus* sp. has been the only plant species consumed by all 10 tortoise individuals, followed by *Pisonia grandis* and *Ficus reflexa*, both native species. However, interaction strength and weighted closeness indicate that *Pisonia grandis*, *Ficus* *sp*., and *Commelina benghalensis* are the plants sustaining the highest interaction frequency, all of which are native to Aride Island. Other plants exhibited relatively high degree values, interacting with approximately half of the individuals, yet displayed very low interaction strength and weighted closeness (e.g., *Euphorbia* *sp*.).

*Frugivory and Seed Dispersal.*

A total of 11,248 seeds (mean ± SD per scat: 375 ± 609, min: 0; max=2599) were found in 20 tortoise scats, while the 10 remaining had no seeds. The mean (± SD) percentage of species richness of native and introduced plants consumed by tortoises was 73.3% and 26.7% (± 37), respectively. However, in terms of the total number of seeds consumed, introduced species accounted for only 10.5% (± 31.5), whereas native species comprised approximately 90% (± 31.5) of the seeds consumed. *Morinda citrifolia* was by far the species with the highest number of seeds recorded on tortoise feaces (9063, nscats=11), followed by *Ficus reflexa* (1732, nscats= 5) and *Megathyrsus maximus* (249, nscats= 3), all native species.

The accumulation curves of interactions nearly reach an asymptote in the processes of frugivory and seed dispersal, both in the number of individuals sampled and in the number of hours of sampling (Figure S2). Based on node centrality metrics, *Morinda citrifolia* exhibited the highest values for degree and closeness, as well as the second highest for interaction strength. *Ficus reflexa* shared the highest degree value but showed greater interaction strength despite lower levels of weighted closeness compared to *Morinda citrifolia*. Several introduced plant species (e.g., *Terminalia catappa, Ananas comosus, Ficus benghalensis*) displayed a degree of less than 2, indicating interactions with only 2 individuals or fewer, alongside low levels of interaction strength and/or weighted closeness.

*Ecological role of individual tortoises.*

Based on the effectiveness approach, we found that the majority of tortoises maintain high values in the qualitative component of seed dispersal effectiveness. However, only three individuals demonstrated high effectiveness in both quantitative and qualitative terms, meaning they disperse a large number of seeds, almost all of which are native plant species (Fig. 2). Similarly, tortoises also proved to be efficient as browsers and grazers, with a low proportion of native species being subjected to antagonistic interactions (Fig. 2). However, only two individuals showed high effectiveness in both quantitative and qualitative aspects for browsing and grazing. Furthermore, we found that effectiveness roles vary among individuals (Fig. 2). Notably, individuals with high effectiveness as seed dispersers (e.g., T01, T08, and T02) displayed low effectiveness as browsers and grazers, while those with high effectiveness in browsing and grazing (e.g., T06 and T07) had low effectiveness as seed dispersers (Fig. 2).

Regarding the topological role, tortoises displayed higher degree and interaction strength in the antagonistic networks compared to the mutualistic ones, where the maximum degree was 4 links in the mutualistic network and 27 in the antagonistic network (Fig. 4A and 4B). Each tortoise’s degree values showed a low but positive and statistically non-significant correlation between the networks (*r* = 0.2; *p-value* = 0.582) (Fig. 4C). Interaction strength values showed a moderate positive correlation, though not statistically significant (*r* = 0.46; *p-value* = 0.184) (Fig. 4C). Weighted closeness values were similar between both processes but individual values showed a low, positive, and statistically non-significant correlation (*r* = 0.23; *p-value* = 0.522) (Fig. 4C).

The antagonistic individual interaction network showed lower modularity than expected by chance (Q observed: 0.32, Q expected: 0.42, Z-score: -13.8), forming two modules: one comprising two tortoises (T02 and T08) primarily interacting with *Morinda citrifolia*, *Terminalia catappa*, *Commelina benghalensis*, and *Artocarpus altilis*; and another module of eight tortoises (T01, T03, T05, T06, T07, T10, T11, T12) mainly interacting with *Ficus sp.* and *Pisonia grandis*. Conversely, the mutualistic individual interaction network exhibited a more modular structure which do not deviate from random (Q observed: 0.582, Q expected: 0.515, Z-score: 1.16), with five modules: one composed of T03 and *Ananas comosus*, another of T05, T10, and T12 with *Ficus reflexa*, a third of T06 and T07 with *Megathyrsus maximus*, a fourth module comprising T02, T08, and T11 with *Cordia subcordata*, *Ficus benghalensis*, *Morinda citrifolia*, and *Terminalia catappa*, and a fifth module of T01 with *Phyllanthus amarus* and *Syzygium cumini*. Tortoises generally showed low among-module connectivity (c) in both ecological processes, with higher values recorded in frugivory interactions, particularly for T02 and T01 (Fig. 5A). Within-module connectivity (z) was highest for T12, T08, and T06 in both networks. Overall, 90% of individuals retained their peripheral role in both the antagonistic and mutualistic networks, with only one individual maintaining a module hub role in the antagonistic network and a close approximation in the mutualistic network (Fig. 5A). The among-module connectivity values showed a moderate positive but non-significant correlation between processes (*r* = 0.44, *p-value* = 0.205), while within-module connectivity values had a high, statistically significant positive correlation (*r* =0.78, p*-value* = 0.008), suggesting that individuals forming modules in the antagonistic network also do so in the mutualistic network (Fig. 5B).

**Discussion.**

Our study highlights the pivotal importance of restoring lost ecological interactions through the reintroduction of a keystone species, specifically Aldabrachelys gigantea, into the insular ecosystem of the Seychelles archipelago. Based on data collected from ten individuals, representing the entire Aldabra giant tortoise population on Aride Island, this reintroduction facilitated the rapid reestablishment of ecological interactions absent since their extinction around 1840 (Gerlach et al., 2013). Using a multifaceted approach that combines observational and genetic methodologies, alongside an individual-based network analysis within two different and contrasting ecological processes, we found that different tortoise individuals exhibit varying roles and performance as antagonists or mutualists, a variation that has direct implications for the seed dispersal of native plants and the control of exotic flora.

Our methodology, which integrates complementary sampling techniques for ecological interactions (i.e, focal observations, faecal examination, and DNA metabarcoding), has led to a net increase in detected interactions that would otherwise have remained unrecorded (Jordano, 2016; Quintero et al., 2022). Although our study is constrained by the limited number of reintroduced individuals and the sampling period, the interaction accumulation curves showed a stable trend approaching an asymptote indicating a relative complete sampling of ecological interactions (Jordano, 2016). Notably, our zoo-centric data collection approach helped us to mitigate some biases that may arise from a combined zoo-centric and phytocentric data gathering strategy of ecological interactions (Quintero et al., 2022). Nonetheless, we must acknowledge several methodological considerations, particularly regarding DNA metabarcoding. For antagonistic interactions, we used DNA metabarcoding of faecal material as a proxy of consumption. The use of metabarcoding for quantifying interactions has sparked debate, as using sequence counts as proxies for consumption rates is contentious (Cuff et al., 2022; Deagle et al., 2018). For example, accurately obtaining a biomass signature from sequence counts is fraught with technical and biological biases that influence barcode marker recovery rates across different taxa. Deagle et al. (2018) suggested that relative read abundance (RRA) could offer a weighting of food present in a sample based on biomass, but the differential recovery of markers —especially from dominant food taxa— may impact data interpretations. However, studies employing RRA based on metabarcoding in herbivores have shown a strong correlation between grass RRA and isotopic estimates of grass consumption, indicating that RRA conveys reliable quantitative information about herbivory dietary intake (Kartzinel et al., 2015). Additionally, the completeness of reference databases is a significant challenge in metabarcoding and, more broadly, in DNA-based studies that aim for taxonomic resolution (Cuff et al., 2022). Taxonomic resolution can profoundly influence network structure, with even minor changes leading to alterations in network metrics and reducing inter-network comparability. The assembly of comprehensive reference databases, particularly in regions with poorly characterised or hyper-diverse flora and fauna, is often a laborious and costly endeavour (Gonzalez et al., 2009), which can result in inadequate characterisation of the taxa consumed by local species. However, the established of a plant reference collection from individual tortoise feeding areas helped us to ensure that plant species detected through DNA metabarcoding were indeed available for tortoises.

Based on our results, we emphasise the role of Aldabra giant tortoises as a keystone species within insular ecosystems, highlighting their multitasking function as both browsers and grazers, as well as seed dispersers. Large chelonians, such as *Aldabrachelys gigantea*, significantly influence seed dispersal processes, particularly on small islands like Aride Island (Falcón et al., 2020). The seeds of two plant species most frequently identified in tortoise faecal samples were the common trees *Morinda citrifolia* and *Ficus reflexa*. From a plant's perspective, tortoise dung piles may provide ingested seeds with a favourable microhabitat, offering the moisture and nutrients necessary for seedling establishment far away from maternal plants (Falcón et al., 2021, 2020; Hansen et al., 2008). Our results indicated that the tortoises disperse a large number of native seeds compared to exotic seeds, while interacting antagonistically with a wide variety of exotic species, adding evidence that the restoration of ecological interactions is a more cost-effective approach to controlling non-native vegetation than other methods involving mechanical removal (Griffiths et al., 2013). Furthermore, the dual roles of Aldabra giant tortoises as mutualistic agents for certain plants and antagonists for others can yield indirect benefits for other vertebrate species. For example, extremely sticky fruit resin of the gran devil´s claws tree (*Pisonia grandis*) adheres strongly to birds’ feathers, making seabirds long-distance seed dispersal vectors (Burger, 2005). However, the multi-seeded infructescences often entangle birds, limiting their ability to fly and sometimes causing bird mortality (Burger, 2005). On Aride Island, the trees of gran devil´s claws are commonly consumed by all tortoises, which suggest that *Aldabrachelys gigantea* may play an important role in controlling *Pisonia grandis* abundance - potentially plantlets - and thus decreasing the number of its sticky fruits harmful to seabirds.

The role of Aldabra giant tortoises as keystone herbivores highlights their ecological significance even if their population size is small. For the first time, our study addresses the inherent variability among individuals in performing different ecological roles. Our findings illustrate that, although these species are crucial as browsers, grazers, and seed dispersers, it is essential to consider how individual differences in diet may influence their effectiveness in these roles. As keystone species can perform multiple ecological functions (Timóteo et al., 2023), one might also ask: do individuals exhibit multitasking capabilities? Our findings suggest that the contributions of tortoises to browsing-grazing and seed dispersal effectiveness can vary significantly from individual to individual, particularly when viewed through the lens of rewiring ecological interactions and managing exotic and invasive species in insular ecosystems. The reintroduction of diverse individuals into a given habitat can yield equally beneficial outcomes concerning the control of exotic species through antagonistic interactions and the dispersal of native plants via mutualistic relationships (Falcón et al., 2020; Hansen, 2015b; Waibel et al., 2013). However, our results showed a trend in which the majority of reintroduced individuals performed more effectively as seed dispersers for native plants than as browser-grazers of exotic plants. Notably, only one individual excelled as a browser-grazer of exotic species, demonstrating the complexities inherent in these multitasking roles.

These variations are also intrinsically linked to the topological roles of individual tortoises as assessed by centrality metrics in antagonistic and mutualistic individual ecological networks. Topological roles within mutualistic ecological networks were closely linked to species' contributions to seed dispersal, indicating that those most significant from a topological perspective were also key contributors to seed dispersal processes (Acevedo-Quintero et al., 2020). The lack of correlation observed between degree, interaction strength, and weighted closeness suggests individual topological differences across mutualistic and antagonistic ecological networks, meaning that different individuals of tortoises may have different relative importance in contrasting ecological processes, underscoring the potential for some individuals to contribute disproportionately to certain functions while being less effective in others. The fact that the topological importance of different individuals varies across ecological processes may have implications for the robustness of ecological networks (Domínguez-García and Kéfi, 2024; Ramos-Jiliberto et al., 2012), as the disappearance of an individual could significantly impact one ecological process while having little effect on another.

Nevertheless, despite this switch in topological roles, our analysis of the structural roles of individuals within both browsing and seed dispersal networks indicates a tendency for individual tortoises to maintain their structural roles across different ecological processes. This stability is pivotal, as the modularity of ecological networks is associated with increased robustness to external perturbations (Delmas et al., 2019; Olesen et al., 2007). By limiting the propagation of disturbances —such as species extinction or, in our case study, the removal of a single individual— modular networks help maintain the integrity of ecological processes. Moreover, the robustness of mutualistic-antagonistic tripartite networks has been intrinsically related to the robustness of the two bipartite networks composing them (Domínguez-García and Kéfi, 2024). The observation that a single individual may fulfil similar structural roles in different ecological processes may carry significant ecological implications, especially for those processes governed by keystone species with small population sizes such as Aldabra giant tortoises in Aride Island. For instance, individuals with relevant structural roles (such as module hubs, network connectors, or network hubs) which share same structural roles within different ecological processes, may hold disproportionate ecological importance, as their loss can trigger the propagation of perturbations across both antagonistic and mutualistic networks.

Hence, these findings highlight the importance of individual-scale ecological processes, which, when examined at the community level (i.e., species-based networks), reveal structural nuances that would otherwise be overlooked (Guimarães, 2020). Ultimately, the persistence of key ecological functions on islands may rely heavily on a select few individuals (i.e., keystone individuals) (Modlmeier et al., 2014), underscoring the critical need for conservation and research efforts to focus on the roles of individual organisms in maintaining the balance of ecological processes. Given the variability in the relative importance of different individuals when examining ecosystem services and in an analogous way to the concept of effective population size for maintaining genetic viability (Lande and Barrowclough, 1987), ecologists should start to conceptualise the effective number of individuals necessary to sustain ecological processes. To ensure the integrity of ecological processes, the effective number should reflect individual variability in ecological roles and help establish a minimum population size that accounts for the diverse contributions of each individual. Thus, understanding the distribution and identity of these key individuals within populations may be crucial for effective conservation strategies aimed at restoring ecological integrity, such as reintroduction programs of Aldabra giant tortoises across various islands in the Seychelles archipelago. Yet, future studies involving a greater number of individuals, extended sampling periods accounting for plant phenology, and a multilayer network approach could yield further robust insights into the ecological roles of individual tortoises in larger populations and different ecological process, as well as the underlying drivers of ecological role shifts.

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**Authors’ contributions.**

SGP and PVG conceived the original idea of studying the diet of reintroduced tortoises on Aride Island and IFA conceived the idea of differential contribution of individuals to antagonistic and mutualistic processes. PVG contributed with financial support. CKB supported the methodology and logistics of this study in Seychelles. SGP conducted the field observations, compiled observational data, and performed DNA extraction on the samples. CKB and PVG coordinated the study's design. IFA led data analysis and figure design. IFA and SGP led the manuscript writing. All authors contributed to manuscript writing and review.

**Competing interests.**

The authors declare no conflict of interest.

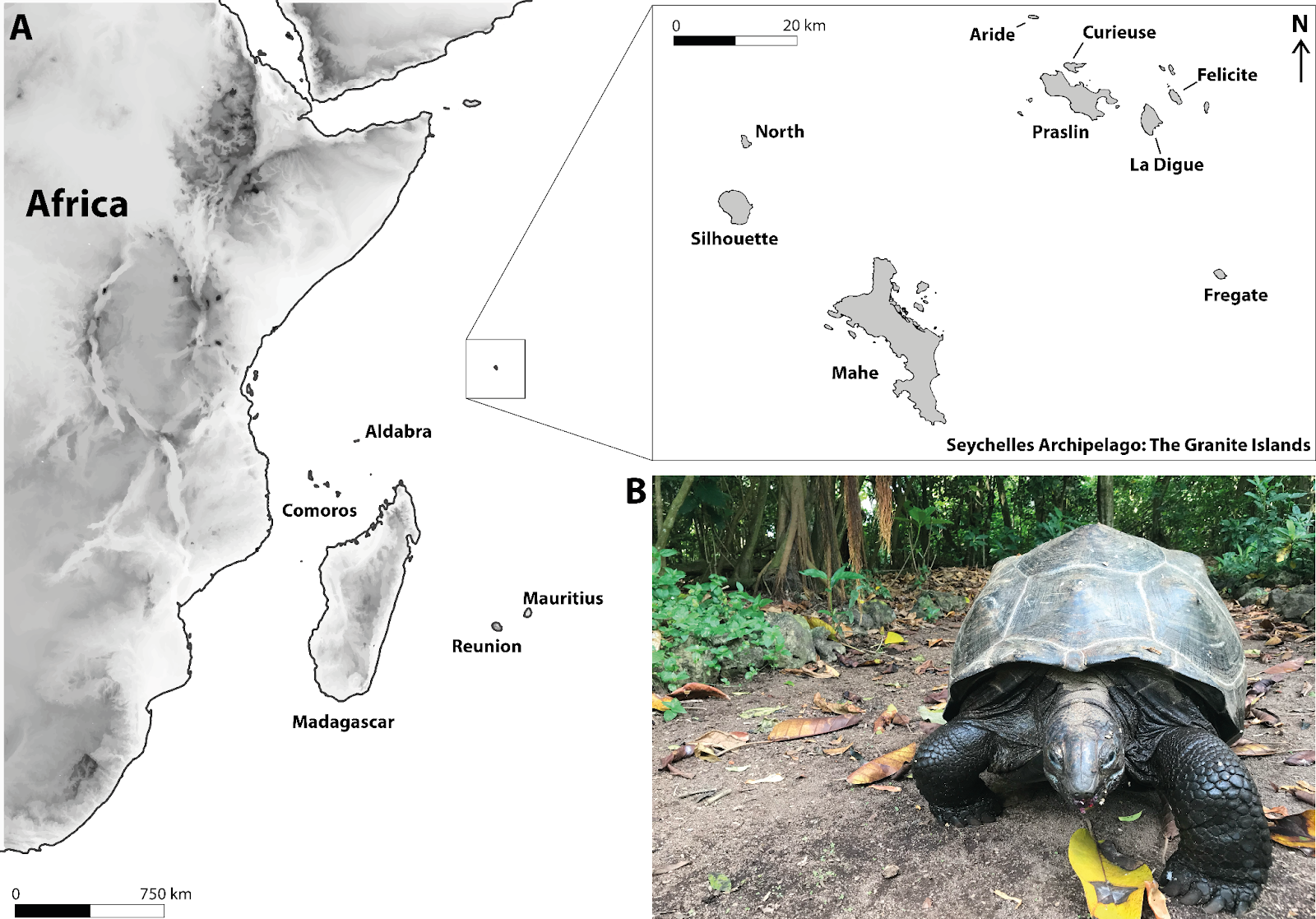
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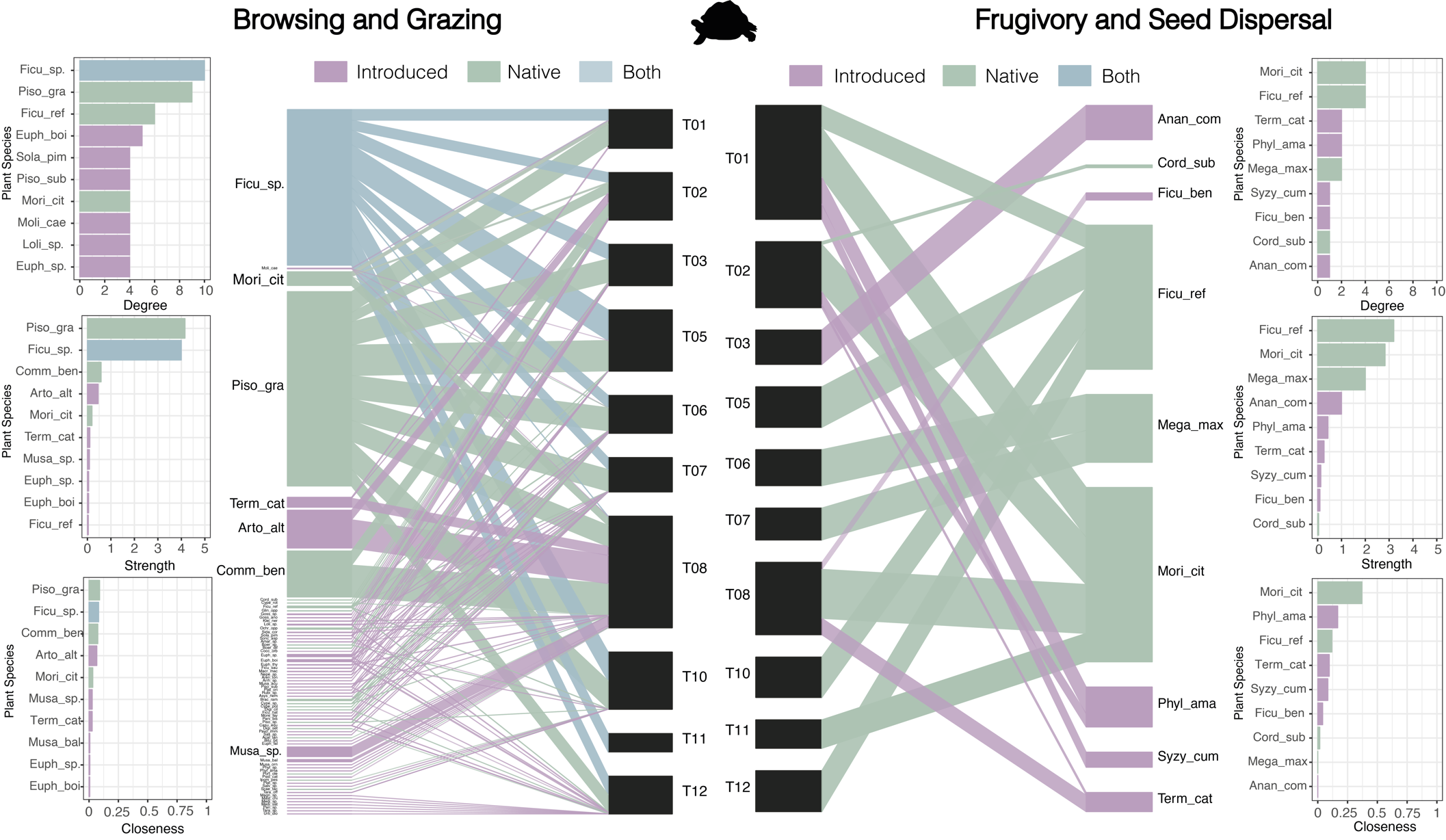
**Funding.**

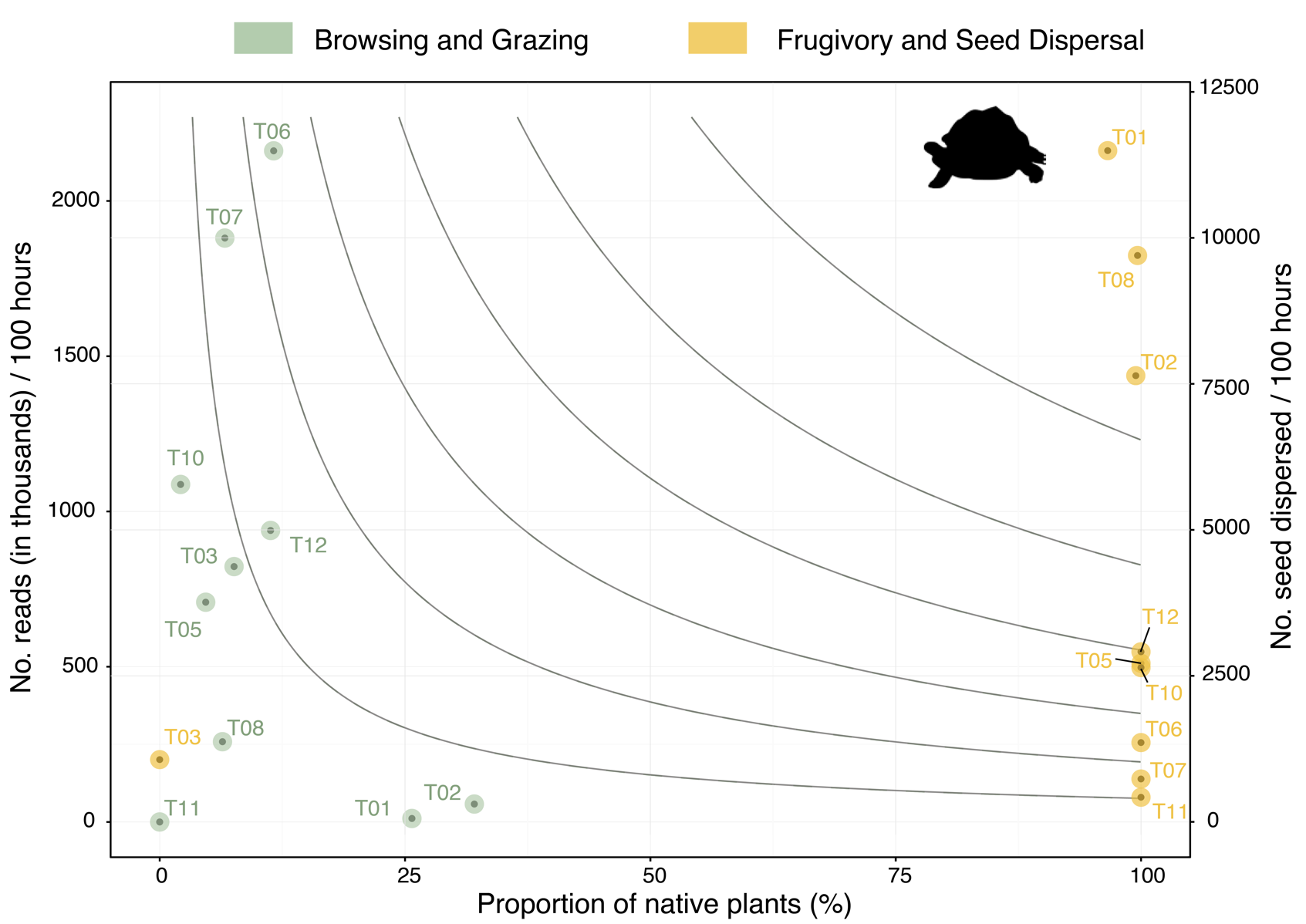
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**Figures**

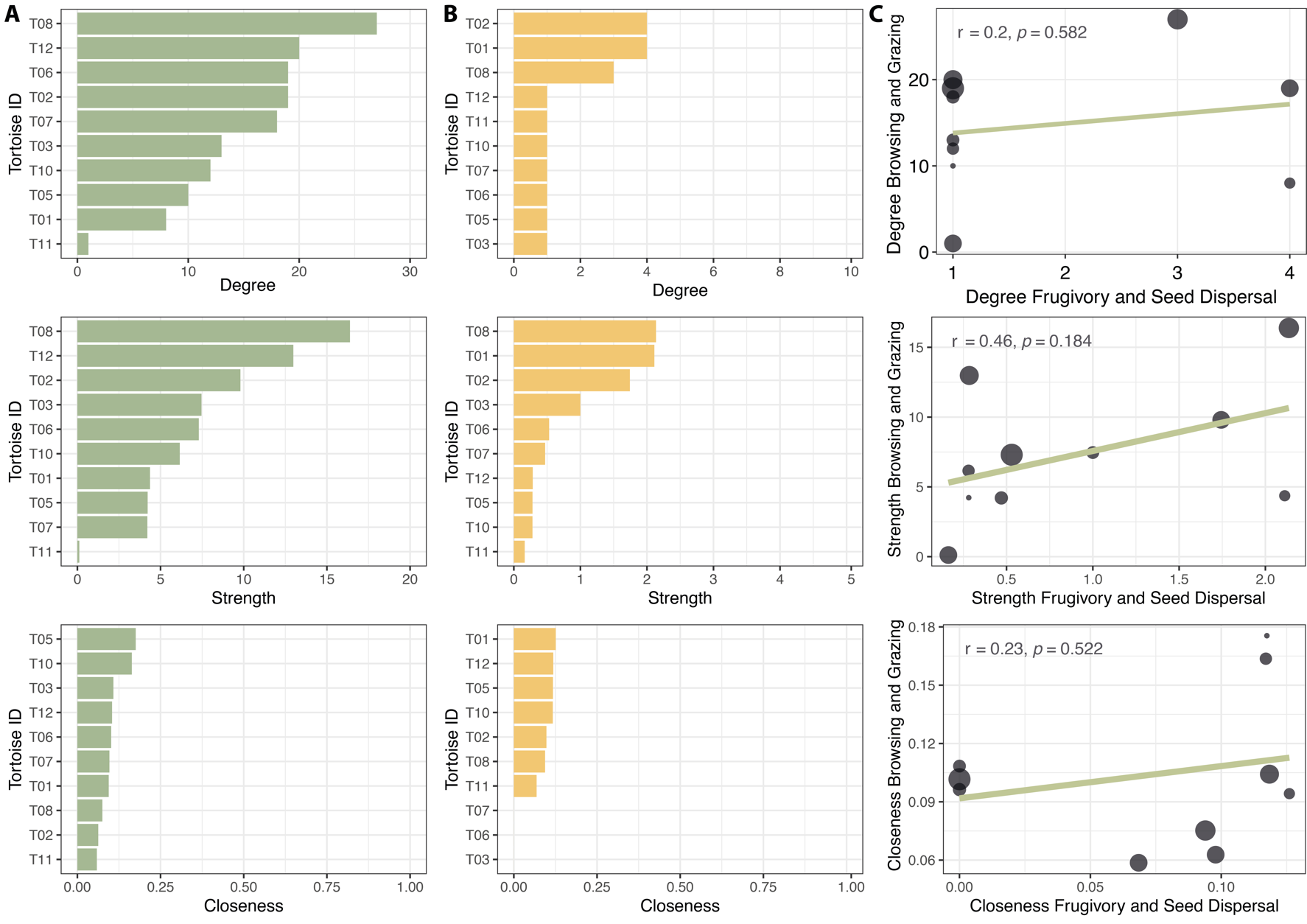
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**Fig. 1. (A)** Map showing the location of Seychelles archipelago on the Western Indian Ocean and its main granitic islands, including Aride Island. (B) One of the ten reintroduced individuals of Aldabra giant tortoises (*Aldabrachelys gigantea)* on Aride Island.

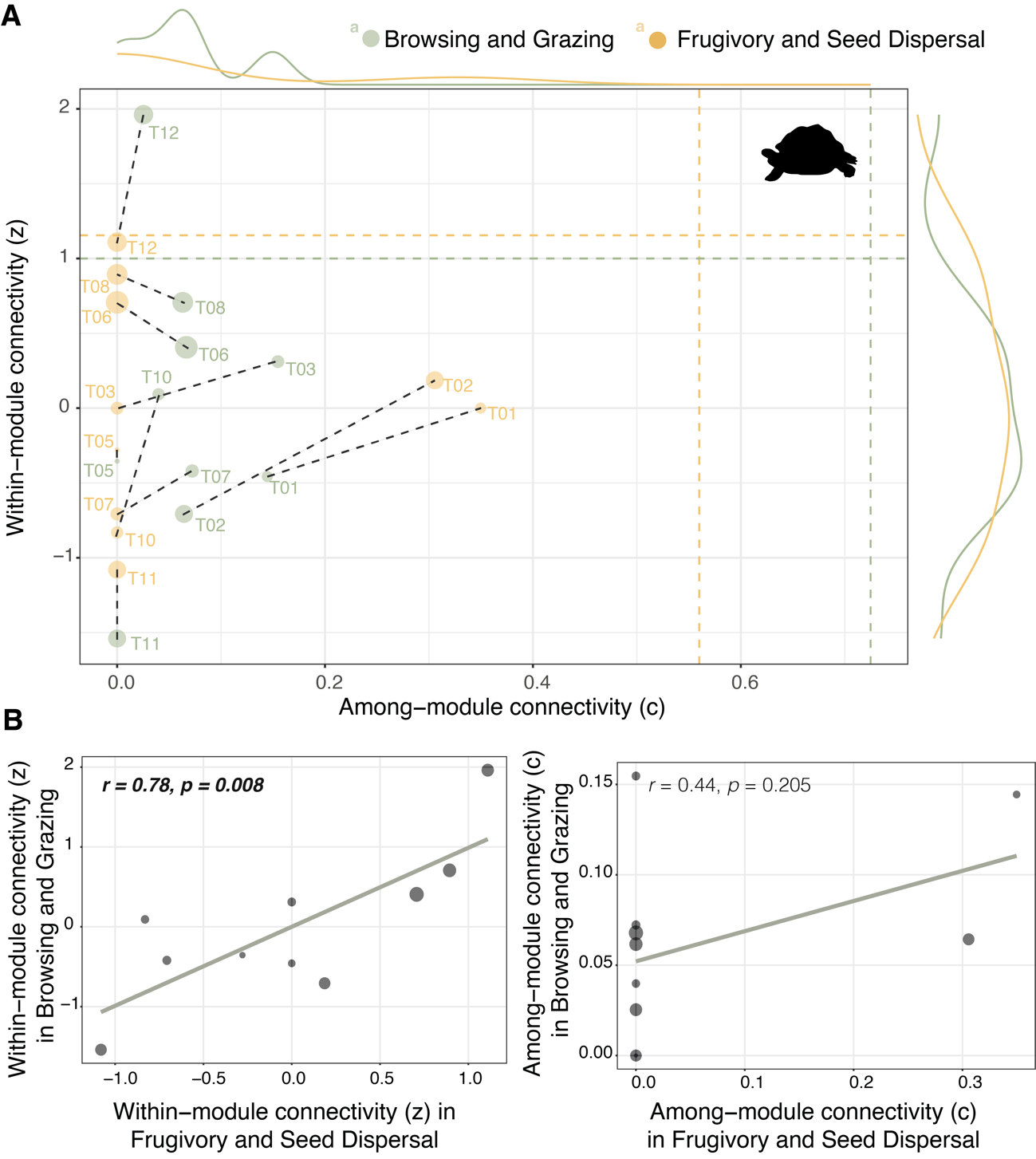
**Fig. 2.** Bipartite individual-based network depicting antagonistic (browsing and grazing; left panel) and mutualistic (frugivory and seed dispersal; right panel) interactions. Black rectangles represent individual tortoises. Green rectangles represent native flora from Aride Island, purple rectangles represent introduced taxa, and blue rectangles represent genera with species that may be either native or introduced (e.g., *Ficus* spp.). Links between nodes represent interaction frequency. The lateral bar plots rank plant species by importance based on centrality metrics (i.e., degree, interaction strength, and weighted closeness) within the two distinct ecological processes mediated by Aldabra’s giant tortoises.



**Fig 3.**  Browsing-grazing and seed dispersal effectiveness of individual tortoises. The X-axis represents the proportion of native plants involved, reflecting the qualitative aspect of antagonistic and mutualistic interactions. The Y-axis shows the number of reads in thousands, standardised by sampling effort (antagonistic, left axis) and the number of seeds dispersed, also standardised by sampling effort (mutualistic, right axis). Isoclines connect values of quantity and quality aspect yielding the same effectiveness for both antagonistic and mutualistic processes. Green dots represent individual tortoise effectiveness in browsing and grazing, while yellow dots indicate individual tortoise effectiveness in seed dispersal.



**Fig. 4.** Topological roles of individual giant tortoises based on centrality metrics. Columns show the ranking of individual tortoises based on degree, interaction strength, and weighted closeness in (A) antagonistic interactions (browsing and grazing) and (B) mutualistic interactions (frugivory and seed dispersal). Column C shows the correlation between each centrality metric for individual tortoises across both ecological processes.

**Fig. 5**. Structural roles of individual tortoises in antagonistic and mutualistic ecological networks. (A) Among-module (c) and within-module (z) connectivity for each individual tortoise in antagonistic (browsing and grazing, green dots) and mutualistic (frugivory and seed dispersal, blue dots) networks. Black dashed lines indicate distances between points in the two types of networks. Yellow and green dashed lines (vertical and horizontal) represent the 95% quantile thresholds used to define structural roles within mutualistic and antagonistic networks, respectively. (B) Correlation of within-module connectivity (z, left panel) and among-module connectivity (c, right panel) values for each tortoise across antagonistic and mutualistic networks.