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Genetics, survival, and demographic decline of *Cycas micronesica* due to invasive insect species on an oceanic island

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Abstract

This is the first study in cycads to measure genetics, demography, and survival of plant populations due to an invasive insect. The armored scale *Aulacaspis yasumatsui* invaded the island of Rota in 2007 posing a new threat to the native *Cycas micronesica* population. The pre-invasion genetic structure of the host plant populations revealed considerable genetic variation and restricted gene flow among habitats and identified genetic clusters representing the genetic variation of the species in Rota. Prior to localized *A. yasumatsui* infestations, 28 permanent plots were established among seven locations in 2008. Data were collected to document survival and demography until 2023. There were 11,254 stems per ha in 2008, 9.2% of which were adult trees. The seedlings rapidly reached 100% mortality, and no seedlings were observed in the plots from 2014 until 2023. There were 149 stems per ha in 2023, 100% of which were adult trees. This invasion behaved similarly to the invasions of other islands, with the native cycad population reaching 98.6% mortality of the entire population and 85.6% mortality of the adult tree population within a 15-year span. Mean stem height was only 30 cm in 2008 because of the majority of the population was seedlings and juveniles. Mean stem height gradually increased with each successive year of data collection due to selective mortality of the small plants, and was 245 cm in 2023. Mean leaf number per adult tree was 83 in 2008, abruptly declined to a low of 8 in 2013, then gradually increased to 20 in 2023. Incidence of the herbivore rapidly increased to a peak in 2011 before stabilizing to about 40% to 50% of the soft organ surfaces. The invasion of this insular cycad population by *A. yasumatsui* caused selective mortality of the smallest plants, and the population-level mortality was more rapid than the prior invasion of nearby Guam. These combined results provide a genetic baseline and population structure benchmark for future conservation protocols during species recovery efforts. The geographically separated and genetically isolated populations indicate this cycad species remains highly vulnerable to localized extirpations, as the unique alleles from extirpated habitats are at risk of being lost forever.

Keywords *Aulacaspis yasumatsui*, Demography, Conservation genetics, Cycad, *Luthrodes pandava*, Invasive species

1 Introduction

Species extinctions continue to drive the global biodiversity crisis [1, 2]. Whereas historical extinctions were largely caused by climate change, contemporary losses are primarily driven by anthropogenic factors such as land-use change and biological invasions [3]. Invasive species can disrupt ecosystems and devastate native populations, sometimes through indirect ecological effect, and in other cases, through direct damage to host organisms. Documenting their arrival, impact and associated mortality is therefore critical to designing targeted interventions that prevent species extinction [3]. Ideally, early involvement of species experts, long-term population monitoring, and sharing of results through global consortia are also essential to effective conservation outcomes [4].

Cycads (Cycadales), an ancient lineage of gymnosperms, represents the most threatened plant group globally [5–7], and their risk status has worsened since global threat assessments began [8]. Among their major threats are invasive arthropods [9]. The genus *Cycas*, the most species-rich cycad genus [10], is particularly affected by two invasive herbivores: the armored scale *Aulacaspis yasumatsui* Takagi [11, 12] and the Lycaenidae butterfly *Luthrodes pandava* Horsfield (formerly *Chilades pandava*) [13, 14]. In their native ranges, both species coevolved with local *Cycas* hosts and exert minimal damage. However, beginning in 1994 [15], *L. pandava* and subsequently *A. yasumatsui* [12] began invading new territories containing various cycad species. New country invasions continue to be reported, with *A. yasumatsui* reaching Okinawa 2023 [16], and *L. pandava* detected in Australia in 2025 [17]. Where both herbivores coexist on non-native cycad hosts, they appear to compete directly [18], exposing native species in these newly invaded regions to unprecedented threats.

Cycas micronesica K.D. Hill is an arborescent cycad indigenous to a range extending from the southern islands of the Palau archipelago to Guam and Rota in the southern Mariana islands [19]. This species thrived in the benign environments of Micronesia until the arrival of *A. yasumatsui*, first recorded in cycad habitats on Guam in 2003, Rota in 2007, and Palau in 2008 [12]. The butterfly *L. pandava* was first reported in Saipan in 1996 [20] invaded Guam in 2005 [21], and was already widespread on nearby Rota in 2007 when *A. yasumatsui* arrived (T.E.M., personal observations). Plant mortality of the host cycad population was severe during the initial years following invasion on Guam and Rota [22], reaching 96% mortality on Guam by 2020 [23].

Small oceanic islands provide unique natural laboratories for studying invasion dynamics due to their isolation and discrete boundaries [24, 25], and demographic and genetic data can be useful even as an invasion is taking place. The response of *C. micronesica* on Rota may differ from that on Guam, as the effects of invasive species can be highly context dependent. The long-term consequences of the Rota invasion on the native cycad population have not been reported. Establishing permanent plots and revisiting them over time yields reliable long-term data [26] that can inform conservation policy, a need that is especially critical for island ecosystems [27]. For example, when population-level changes occur over time and new threats emerge, intervention decisions may be uninformed if the decision-makers do not understand the status of the population prior the initiation of those changes.

This study was initiated in 2007 on the island of Rota, the southernmost island of the Commonwealth of the Mariana Islands. Rota is a small oceanic island of 85 km^2 and is located about 60 km north-northeast of Guam in the southern limits of the Mariana archipelago. The tropical climate (Köppen Classification: Af) is characterized by a mean temperature of 28.3 °C. The annual rainfall of 3,548 mm serves as the primary driver of seasonal patterns, with the wettest months occurring from July to October and the driest in February and March.

Tropical cyclones occur periodically, and the intense damage caused over a few hours can alter forest structure for years. During this study, the most influential events on Rota were Typhoon Dolphin (May 2015), Typhoon Yutu (October 2018), and Typhoon Mawar (May 2023).

In this study, we analyzed long-term demographic and genetic data collected from permanent plots established throughout the island of Rota in 2008 and monitored until 2023. Prior to the *A. yasumatsui* invasion, the genetic diversity of populations in these habitats were characterized using population-level markers. The objectives in this study were to (1) assess the demographic responses of Rota's cycad population to the *A. yasumatsui* invasion, (2) compare these responses with the well-documented mortality responses in Guam, and (3) identify populations that represent the genetic variation of this species on the island to guide conservation strategies.

Notable ecological contrasts between the islands include Guam's greater land area and geographic separation of habitats, Rota's higher cycad density, the presence of *L. pandava* on Rota prior to *A. yasumatsui*'s arrival, and a broader array of damaging herbivores and omnivores on Guam. The integration of genetic variation and long-term demographic data provides an important foundation for informing future policy and conservation action. To our knowledge, this is the first study to integrate population genetics with long-term demographic monitoring in cycads, and among the few such investigations of oceanic island plants following an invasive species introduction.

2 Materials and methods

2.1 Study area

Rota represents the northern limit of the indigenous range of *C. micronesica* [19]. Following the May 2007 invasion of *A. yasumatsui*, the entire island was surveyed to assess the areas of occupancy of native *C. micronesica*. The founder outbreak site was located in the northeast littoral forests, at the eastern margin of a large coastal subpopulation extending approximately 9.5 km along the shoreline. By July 2009, *A. yasumatsui* had dispersed across and infested the full extent of this cycad subpopulation. A second, disjunct outbreak was observed along the southeastern coast one year after the invasion, infesting a *C. micronesica* subpopulation that extended about 5.5 km along the southern coastal forests. This entire subpopulation was infested with *A. yasumatsui* by December 2009. A third independent *A. yasumatsui* invasion front was detected in April 2010 on a disjunct *C. micronesica* subpopulation located on a peninsula at the western limits of Rota, growing on uplifted karst benches and extending about 1 km along the coast. This event marked the final infestation of any *C. micronesica* subpopulation on either Guam or Rota. In addition, scattered *C. micronesica* and *Cycas revoluta* Thunb. plants in urban forests served as stepping stones for island-wide dispersal.

The study sites encompassed the full geographic range of the cycad subpopulations present on the island. Alphanumeric designations were used, with letters identifying field sites included in the genetic analyses and numerals identifying those used for survival analysis. Site 1 was positioned immediately west of the founder invasion location before the *A. yasumatsui* population dispersed (Fig. 1). Sites 2, 3 and 4 were positioned within the north-coast subpopulation, with Site 4 occupying its western limit. Infestations of *A. yasumatsui* were first observed in January 2008 at Site 1, May 2008 at Site 2, April 2009 at Site 3, and July 2009 at Site 4. All four sites consisted primarily of sandy substrates with small isolated karst outcrops, and all were situated less than 10 m above sea level (masl). Site C corresponded to Sites 1 and 2, Site A corresponded to Site 3, and Site H corresponded to Site 4.

Site 5 was positioned near the eastern limit of the south-coast subpopulation at an elevation of 190 masl, where *A. yasumatsui* arrived in June 2008 (Fig. 1). Site 6 was situated near the western limit of the same subpopulation at 135 masl, and the scale dispersed into this area in August 2009. Site 7 comprised a small subpopulation on the isolated western peninsula, with plants growing at 80 masl. The *A. yasumatsui* population reached this forest fragment in April 2010. Sites 5, 6, and 7 were characterized by tectonically uplifted calcareous rock-outcrop substrates. Sites D and E corresponded to Site 5, Site F corresponded to Site 6, and Site G corresponded to Site 7.

2.2 Leaflet collection for population genetic methods

Leaflet tissue for population genetic analyses was collected following methods previously established for Guam [28]. After surveying the entire island to develop an understanding of the locations and sizes of the cycad subpopulations, leaflet tissue was collected from seven localities in December 2007. Sampling was conducted along 100–300 m transects, with at least one plant sampled every 10–30 m within each locality. A total of 116 individuals were sampled across the seven localities, representing the full gradient of environments and soil types and capturing the breadth of *Cycas micronesica* distribution on

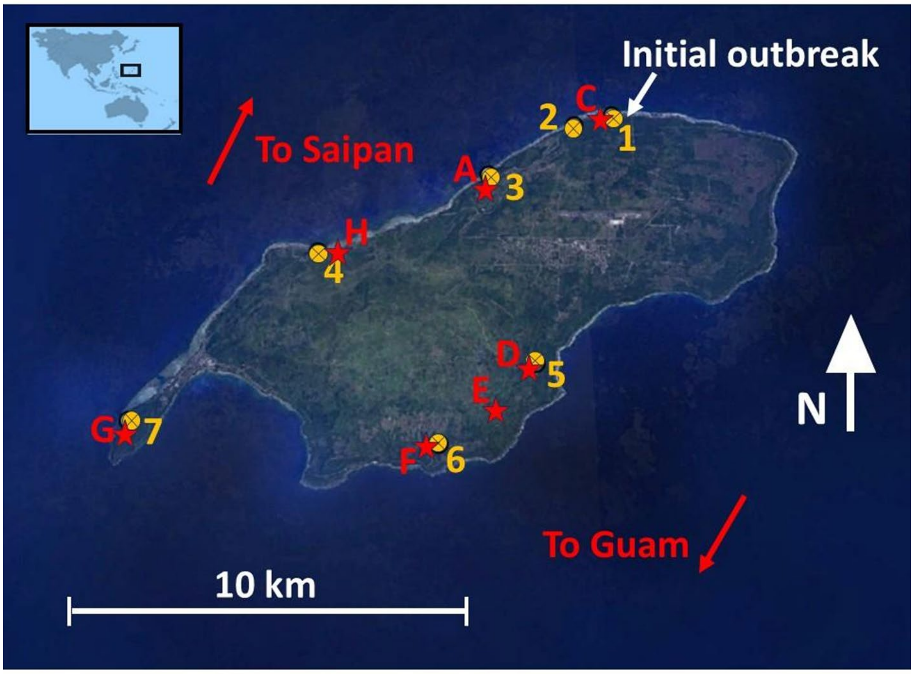

Fig. 1 Map of Rota showing the location of the initial outbreak site and the positions of the sites used to document the genetic structure and population mortality of *Cycas micronesica* following the 2007 invasion of *Aulacaspis yasumatsui*. Letter designations (with stars) indicate sites used for genetic analyses, and numeral designations indicate sites used for survival monitoring

the island. At the time of leaflet collection, the scale *Aulacaspis yasumatsui* was present at Site C but had not yet invaded the other sites. A similar sampling strategy was used for the leaflet tissue collected for population genetic analyses.

2.3 Survival and plant data collection methods

A longitudinal demographic survey was conducted to monitor plant survival and mortality, establishing a quantitative baseline in 2008 and concluding with final mortality counts in 2023. Survival and plant data collection measurement followed methods previously established for Guam [23]. Permanent plots were established and monumented across the seven study sites in January 2008, with each site containing two 10 × 10 square plots and two 2 × 50 m belt transects. The 28 plots were designed to encompass approximately 100 m^2 each, but slight variation in corner placement resulted in minor differences in total area. Consequently, all plant density data were standardized to a per-hectare (ha^{-1}) basis.

On each census date, all living plants were counted, stem height was measured, and the number of leaves was recorded for each individual. The relative intensity of *A. yasumatsui* infestation was estimated for each plant by evaluating the proportion of organ surfaces colonized by the armored scale. The plant organs assessed included cataphyll, petiole, rachis, and leaflet surfaces. If a strobilus was present, the sporophyll and seed surfaces were also examined. Although ephemeral deep cracks in stem bark may be colonized by *A. yasumatsui*, these heterogeneous surface locations were not included in the ranking. Each plant was assigned an infestation score of 0 to 10, where 1 indicated approximately 10% of total surface area infested and 10 indicated complete (100%) infestation.

2.4 Statistics - population genetics

QIAGEN DNeasy Mini kits (Valencia, CA, USA) were used to isolate genomic DNA from leaflet tissue. We used 10 EST-microsatellites previously developed to measure genetic variation [29]. PCR reactions used 12.5 μl of Lucigen EconoTaq™ Plus Green 2X Master Mix, 1 pmol/μl of each M13 reverse and FAM-M13(21) primer, 0.5 1 pmol/μl of forward primer, 1 μl DNA. Amplification was also successful following Schuelke [30] with 28 and then 8 PCR cycles, and various annealing temperatures as described in Cibrián-Jaramillo et al. [29]. We used an ABI 3730xl for genotyping and the Genemapper 3.5 software (Applied Biosystems) for fragment analysis [29].

Microsatellite genotypes were imported into R as an adegenet::genind object [31]. Characteristics of the 10 microsatellite loci used in this study, including allele size ranges and levels of polymorphism, are provided in Supplementary File 1. Alleles at each locus were represented by their repeat sizes. Integrity checks (sample and locus identifiers, ploidy) were performed. Allelic diversity was estimated per locus. Exact/Monte-Carlo Hardy–Weinberg tests were run per locus using pegas::hw.test with 100,000 replicates [32], and false-discovery rate (Benjamini–Hochberg) was computed for p-values. Heterozygosity was measured as the unbiased expected heterozygosity (*H*E), and observed heterozygosity (*Ho*) corrected for sample size [33] per population from the genind object and summarized alongside sample size and allelic richness.

We estimated pairwise *F*st among populations using Weir & Cockerham's estimator (hierfstat::pairwise.WCfst) [34]. Because microsatellite distances are not Euclidean in allele size space, we performed principal coordinates analysis (PCoA) on Nei's 1972 genetic distance (adegenet::dist.genpop) to visualize among-population structure in a low-dimensional space. We inferred genetic clusters using Discriminant Analysis of Principal Components (DAPC) implemented in R (adegenet). Genotypes were encoded as diploid, codominant genind object from microsatellite loci. To obtain reproducible group priors for the discriminant step, we applied find.clusters to the centered allele matrix in PC space with the number of clusters fixed at $K = 4$; up to 100 principal components (bounded by data rank) were allowed to provide a high-signal, low-noise feature space for k-means initialization. We then fitted dapc retaining up to 100 PCs for the discriminant step and specified n.da = 3 (maximum $K - 1$) to summarize among-group separation. Posterior membership probabilities (Q) were extracted for each individual, and samples lacking a dominant assignment (e.g., max $Q \leq 0.90$) were flagged as uncertain/potentially admixed. Visualization included discriminant scatter plots (first two functions with 95% inertia ellipses) and membership barplots ("compoplots").

2.5 Statistics – plant metrics

Plants were assigned to three demographic categories based on leaf number and stem height. Individuals with one to three leaves were classified as seedlings; those with four or more leaves and stems up to 100 cm in height were categorized as juveniles; and those with stems greater than 100 cm were ranked as adults.

Changes in plant and population variables were analyzed using a three-way factorial Analysis of Variance (ANOVA) with year, site, and plot type as factors. This approach allowed us to test the individual importance of each factor while also examining their interactions. This framework also treated year as a repeated measure, accounting for data collected from the same plots over time. Consequently, comparisons across years were statistically controlled for any significant effects coming from site or plot type.

Calendar year was retained as a categorical variable in the primary models to capture the simultaneous impact of island-wide environmental events, such as typhoons, across all sites. However, to account for the staggered arrival of *A. yasumatsui*, an additional continuous-time Generalized Linear Mixed Model (GLMM) using a standardized zero-start time for invasion arrival is provided in Supplementary File 2.

Response variables were separated into two categories for analysis: (1) population demographic variables and (2) plant health metrics. Because variables within each group were likely correlated, we first used a Multivariate Analysis of Variance (MANOVA) for each group to test for overall significant changes while reducing the risk of false-positive results. When a MANOVA indicated significant effects, individual univariate ANOVAs were performed to identify which factors or interactions contributed to the observed changes. Significant effects involving the year factor were further explored with a post-hoc test to compare specific years. Due to the large number of years in the study, a Bonferroni correction was applied to adjust p-values for pairwise comparisons between years.

Several data handling procedures were necessary. In 2023, extensive missing values for scale infestation, stem height, and adult leaf number prevented estimation of marginal means. The majority of these were not due to a failure to collect data, but were 'structural zeros' resulting from the catastrophic 100% mortality of seedlings and juveniles; without living plant tissue, infestation counts could not be made. However, to address instances of true missing data among the surviving adult plants, we applied multiple imputation and data jittering to enable the generation of marginal mean estimates.

All survival and health analyses were conducted in Stata Statistical Software Release 19 (StataCorp LLC, College Station, TX, U.S.A.) using the mixed module to execute the ANOVA models and contrasts. Statistical significance was assessed at $\alpha = 0.05$ for all tests.

3 Results

3.1 Genetics

A total of 10 SSR loci were successfully genotyped, all of which were polymorphic both at the island-wide scale and within individual populations (Supplementary File 3). The most informative marker was X250, with 32 alleles, whereas loci such as X270 (8 alleles) and X278 (5 alleles) exhibited comparatively low levels of variation (Supplementary File 4). Across loci, 75% of alleles were rare (frequency $\leq 5\%$), while 25% were common ($> 5\%$), indicating a substantial reservoir of low-frequency alleles contributing to overall genetic diversity. Such rare alleles may be important for adaptive potential but are particularly vulnerable to stochastic loss through genetic drift.

Observed heterozygosity (HO_OO) was consistently lower than expected heterozygosity (HE_EE) across most sampling sites, resulting in heterozygote deficits (Fig. 2a). These deficits are consistent with population substructure, where individuals are drawn from genetically differentiated demes rather than from a single panmictic population. Per-population comparisons of HO_OO and HE_EE revealed broadly similar patterns among sites, although the magnitude of the heterozygote deficit varied spatially.

Pairwise genetic differentiation estimated using Weir & Cockerham's FST_{ST}ST revealed moderate to strong differentiation among sites, indicating generally limited gene flow across the island (Fig. 2b). Sites A and D showed relatively lower levels of differentiation, suggesting greater connectivity or shared ancestry, whereas sites F, G, and H exhibited higher FST_{ST}ST values, reflecting stronger genetic isolation.

Ordination of populations using principal coordinates analysis (PCoA) based on Nei's genetic distance was congruent with the FST_{ST}ST results (Fig. 2c). Populations that were genetically similar in the pairwise FST_{ST}ST matrix, such as Sites A and D (with some affinity to Site E), clustered closely in ordination space, while more differentiated populations, including Site C, were separated along the primary axes of genetic variation.

Discriminant Analysis of Principal Components (DAPC) identified four genetic clusters across Rota ($K = 4$; Fig. 2d). Most individuals showed strong posterior assignment to a single cluster, indicating well-defined genetic structure, while a smaller proportion of individuals exhibited mixed cluster membership (Fig. 2e). Grouping individuals by sampling site revealed that clusters were unevenly distributed among sites, highlighting localized genetic differentiation and limited admixture.

To evaluate whether geographic distance explained patterns of genetic differentiation, we tested for isolation by distance using a Mantel test between pairwise FST_{ST}ST /(1 – FST_{ST}ST) and log-transformed geographic distance. The correlation was weak and nonsignificant (Pearson's $r = -0.048$, $p = 0.335$; Fig. 2f), indicating no clear isolation-by-distance pattern across the island. The absence of isolation by distance, together with the discrete genetic clusters identified by DAPC, suggests that factors other than simple geographic separation—such as historical fragmentation, demographic processes, or

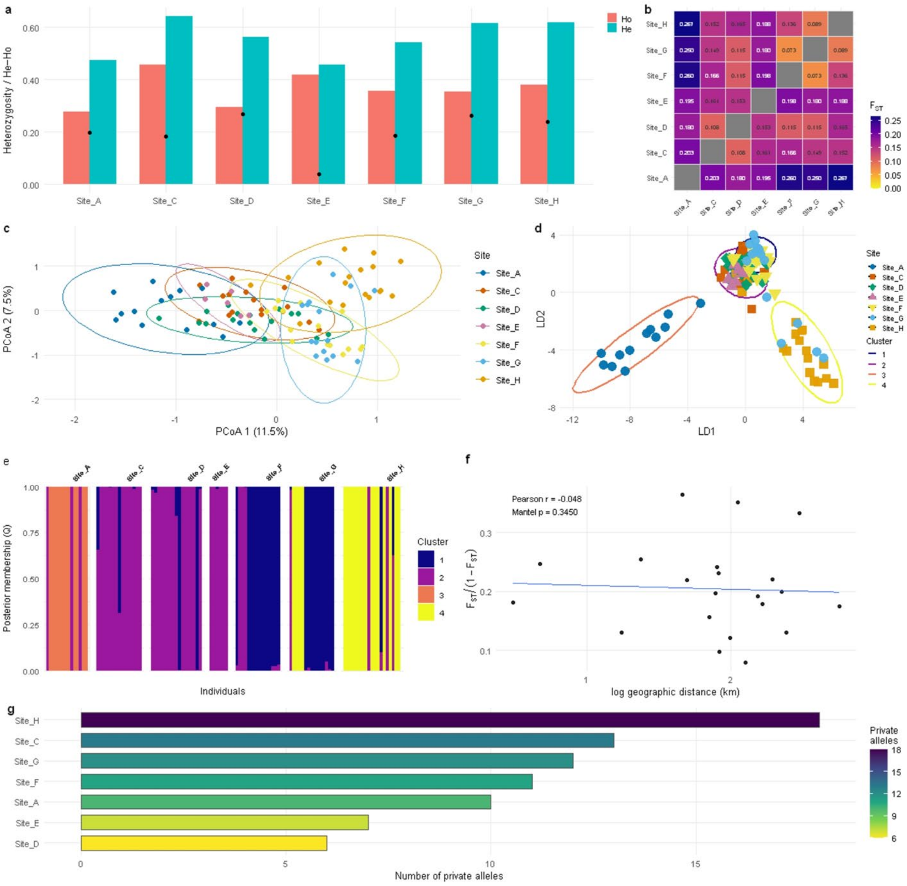

Fig. 2 Population genetic structure of Rota cycad sites. **a** Per-population heterozygosity showing observed (H ~ O~, pink) and expected (H ~ E~, teal) values per site; overlaid black points indicate the heterozygote deficit (H ~ E ~ – H ~ O~) per site. **b** Pairwise Weir & Cockerham F ~ ST~ heatmap with numeric cell values; NA cells indicate population pairs with insufficient data for estimation. **c** Principal coordinates analysis (PCoA) on individual-level Euclidean distances in allele-frequency space (axes 1–2); each point represents one individual colored by sampling site, with 95% confidence ellipses and percentage of variance explained per axis. **d** Discriminant Analysis of Principal Components (DAPC) scatter on the first two discriminant functions (K = 4 inferred clusters); each point represents one individual colored and shaped by sampling site, allowing identification of which sites contribute to each cluster; ellipses represent 95% confidence regions for each inferred genetic cluster. **e** DAPC membership barplot (compoplot) showing per-individual posterior cluster-assignment probabilities (Q) grouped by sampling site; colors correspond to the four inferred clusters. **f** Isolation-by-distance relationship: pairwise F ~ ST~/(1 – F ~ ST~) plotted against log geographic distance (km) with linear fit, Pearson's r, and Mantel test result, indicating no significant isolation by distance among sites. **g** Number of private alleles detected per population, highlighting site-specific contributions to overall geneticdiversity

localized disturbance—have played a major role in shaping the genetic structure of *C. micronesica* on Rota.

Finally, the distribution of private alleles across sites (Fig. 2g) revealed marked heterogeneity in site-specific genetic contributions, with some populations harboring a disproportionate number of unique alleles. This pattern underscores the importance of conserving multiple sites to preserve the full spectrum of genetic diversity present on the island.

3.2 Plant metrics

The *Cycas micronesica* population on the island of Rota was thriving in 2007 prior to the expansion of *A. yasumatsui* throughout the island (Fig. 3a). The host tree's response to

Fig. 3 General appearance of a *Cycas micronesica* habitat on the island of Rota. **a** A 2007 photograph showing robust mature trees with numerous, large compound leaves surrounded by numerous juvenile and seedling plants. **b** A 2012 photograph from the same location showing no cycad seedlings or juveniles and mostly dead stems of mature plants

the *A. yasumatsui* damage was immediate, and after several years of herbivory the habitats were dramatically altered compared to pre-invasion conditions (Fig. 3b).

Before examining temporal changes, we first assessed the possible influence of the spatial factors, specifically site and plot type (full ANOVA results shown in Supplementary File 5). Plot type had no significant effect on any of the primary demographic metrics ($F_{7,112}$ = 1.03, 0.16, 1.03, and 0.68 for seedling, juvenile, adult, and total stem counts; all $P > 0.40$), allowing these two designs to be pooled for the primary analysis of temporal changes. In contrast, significant differences across sites were present in the early years when some sites were invaded while others were not. For seedlings (2008–2009) and total stems (2008–2009), densities were significantly higher in Sites 5, 6, and 7, while juveniles showed higher densities in these same sites from 2008 to 2013. For adults, significant site differences persisted from 2008 to 2014, with higher densities in Sites 4 and 5.

Nevertheless, our factorial design inherently controlled for these baseline site differences by using contrasts calculated on means adjusted for site effect, ensuring that the assessment of temporal changes was not confounded by spatial variability. Notably, site differences became non-significant in the later years of the study, likely due to the overwhelming pest pressure, which caused rapid mortality and led to a uniformly low number of surviving plants across the island. Thus, the following results focus on the impact of the invasion as assessed by the main effect of year. The seedling population responded rapidly to the *A. yasumatsui* invasion ($F_{7,112}$ = 499.14; $P < 0.001$). Seedling mortality was immediate (Fig. 4a), with the entire seedling population succumbing to herbivory within one year of *A. yasumatsui*'s arrival at each site. By the 2009 census (representing one to two years post-invasion across the different sites), the seedling survival had significantly declined, reaching near-zero levels by 2011 (two to four years post-invasion). *i* By the 2009 census, the seedling survival had significantly declined, reaching near-zero levels by 2011. Although newly emerging seedlings were killed rapidly until 2013, no regeneration was observed for duration of the study.

The death of juvenile plants occurred more slowly than seedlings, but was similarly significant ($F_{7,112}$ = 182.56; $P < 0.001$). By the 2011 census, more than 70% of the juvenile plants had died (Fig. 4b). The juvenile survival curve depicted a gradual decline after 2011, reaching 100% mortality by the 2023 census.

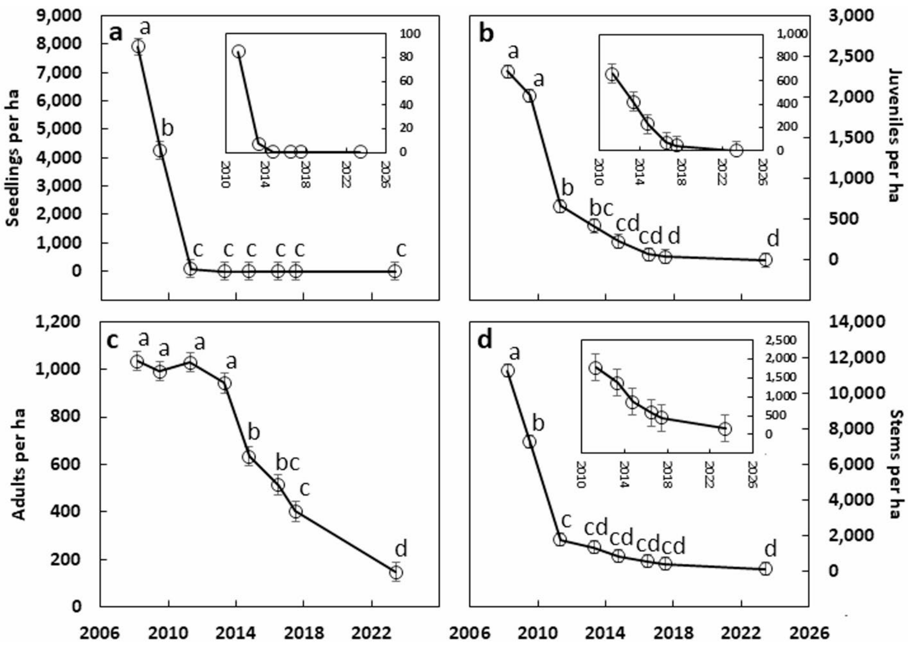

Fig. 4 The number of *Cycas micronesica* plants throughout the island of Rota following the 2007 invasion of *Aulacaspis yasumatsui*. **a** Seedling plants. **b** Juvenile plants. **c** Adult plants. **d** Total plant population. Inset figures in panels a, b, and d restricted to the years following 2011. Markers are mean ± *SE*, $n = 28$ plots. Letters represent statistical groupings from a post-hoc multiple comparison test; means for years that do not share a letter are significantly different ($P < 0.05$)

The adult tree population exhibited more resilience to *A. yasumatsui* herbivory, with significant decline ($F_{7,112} = 71.20$; $P < 0.001$) in living stems delayed until the 2014 census (representing four to seven years post-invasion) (Fig. 4c). From 2013 to 2023, an average of 80 trees per ha were lost each year, culminating in an 86% mortality by the final 2023 census.

Overall, the total plant population experienced a highly significant decline ($F_{7,112} = 640.78$; $P < 0.001$). The selective culling of seedlings and juveniles in the early years of the *A. yasumatsui* invasion led to an 84% decline in the total plant population by 2011 (Fig. 4d). Mortality rates declined abruptly after 2013, when the relative size of remaining adult trees began to influence mortality patterns. Between 2013 and 2017, an average of 231 stems per ha per year were lost, while mortality slowed to an average of 50 stems per ha per year from 2017 to 2023, (representing 7 to 16 years post-invasion across the various sites).

We also assessed the influence of spatial factors on plant health metrics. Plot type differences were largely non-significant, though occasional differences in plant height and scale infestation were observed. Site effects were more nuanced, with significant variation in leaf counts during the early years, while mean stem height remained consistent. However, these patterns changed during the latter years. The presence of these sporadic yet significant effects further underscore the importance of our analytical approach, which controlled for other sources of spatial variation. Because the contrasts for the year effect were calculated on means adjusted for these spatial factors, the temporal trends in plant health were not confounded by site-related variation. As year was the dominant driver of change for all plant health metrics, the following results focus on this temporal trend.

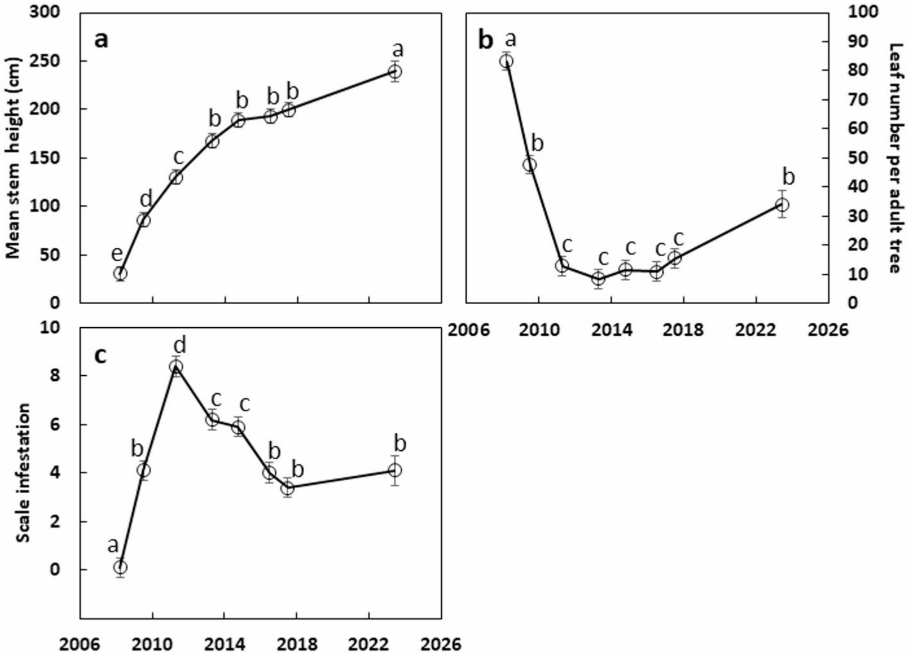

Fig. 5 Influential response variables of Rota's *Cycas micronesica* population following the 2007 invasion of *Aulacaspis yasumatsui*. **a** The height of every living stem. **b** The number of leaves on living adult trees. **c** The relative incidence of *A. yasumatsui* scale with 0 indicating no scale and 10 indicating 100% infestation of soft organ surfaces. Markers are mean ± *SE*, $n = 28$ plots. Letters represent statistical groupings from a post-hoc multiple comparison test; means for years that do not share a letter are significantly different ($P < 0.05$)

The population-level changes in mean stem height were dramatic following the *A. yasumatsui* invasion ($F_{7,101}$ = 114.42; $P < 0.001$). The rapid selective culling of the smallest plants drove these changes (Fig. 5a). In 2008, the cycad population on Rota had a mean plant height of 30 cm, reflecting the widespread presence of seedlings and juveniles in this thriving arborescent cycad's island community. The substantial increase in mean plant height following the *A. yasumatsui* invasion did not result from overall plant growth, but rather from the survival of the largest initial individuals as smaller plants succumbed to herbivory. This caused a severe left-truncation of the population's size distribution. As demonstrated by the mean stem height (Fig. 5a), this size-asymmetric, selective mortality continued within the broad adult category (> 100 cm). The rate of increase in mean plant height was greatest between 2008 and 2014. By 2023, the mean plant height was eight times greater than the pre-invasion value, with the 2008 mean representing only 12% of the 2023 mean. Because stem height serves as a proxy for the volume of parenchymatous tissue and stored non-structural carbohydrates, these data suggest that surviving prolonged herbivory required copious physiological buffering that smaller adults lacked. Furthermore, since the complete mortality of younger classes resulted in structural zeros later, mean stem height provides a reasonable and practical metric to track the shifting dynamics of the surviving population. Ultimately, it quantitatively demonstrates the selective loss of the smallest, youngest, and most promising members of the population for future survival, signifying a severe and irreversible reduction in the population's future survival potential.

The population-level responses of adult tree leaf number were also dramatic ($F_{7,101}$ = 96.16; $P < 0.001$). The *A. yasumatsui* herbivory killed all standing leaves shortly after the invasion and rapidly caused the death of new leaves as they were sequentially produced.

In 2008, the healthy cycad population exhibited trees with more than 80 leaves per tree, and some individuals supported over 150 leaves each (Fig. 5b). As the trees began producing fewer leaves for each primary growth event and the successive new leaves exhibited rapid *A. yasumatsui* mortality, these adult trees exhibited only 8 leaves per tree by 2013. The living trees were clearly in survival mode from this time through the 2016 census when leaf counts remained critically suppressed. However, a slow recovery began in 2017 (at least 7 years post-invasion across the various sites), and by 2023 (at least 13 years post-invasion across the various sites), the surviving mature trees were supported by an average of 20 leaves.

The relative infestation levels of *A. yasumatsui* exhibited similar patterns within each transect or plot, with significant differences observed among years ($F_{7,101}$ = 312.24; $P < 0.001$). By one to two years after the armored scale's migration into each site, infestation scores of 10 were common across all plots and transects within a site. The mean infestation score increased significantly to about 4 by the 2009 census, driven by sites with scores of 10 and others with scores of 0 (Fig. 5c). By the 2011 census, 100% of Rota's cycad habitats had become infested and the infestation score reached a peak of 8.4 with the most recently invaded sites exhibiting the greatest infestation scores. As both host and herbivore populations adjusted to their new insular relationships, infestation scores began to decline slightly starting 2013 and stabilized at scores of 3 to 4 from 2016 until 2023.

4 Discussion

The armored scale *A. yasumatsui* began invading various countries in 1994 [12] and first invaded the *C. micronesica* native range in 2003 [11, 12]. The 2007 Rota invasion occurred after four years of diligent observations of *C. micronesica* responses following the initial invasion of Guam. What can the invasion of Rota by this lethal arthropod herbivore teach the cycad conservation community?

The establishment of pre-invasion genetic baselines and the long-term demographic monitoring provides a comprehensive view of how pre-existing population structure influenced species collapse. Although genetic erosion was not measured directly, establishing the genetic structure before the invasion allowed the demographic results to be interpreted within an evolutionary framework. Few studies of threatened plants combine these two dimensions, and this approach offers a strategy for understanding how population history and genetic structure can shape vulnerability and recovery potential in other long-lived species.

When we compared site-level genetic diversity with demographic outcomes, we found no indication that higher expected heterozygosity (H_E) translated into greater survival following invasion by *A. yasumatsui*, although this site-level comparison is limited by the small number of sites (see Supplementary File 6). From Figs. 2 and 4 we find that seedling and juvenile mortality reached 100% across all sites, and adult mortality was extreme even at sites with relatively high diversity (e.g. Site C, H_E = 0.63; Site G, H_E = 0.61). Together, these patterns suggest that intense pest pressure may overwhelm the natural buffering effect of genetic variation that might otherwise be detectable at this scale. With recruitment failure across sites, our pre-invasion genetic data provide a critical baseline for tracking the severe genetic bottleneck and localized extirpations these populations are currently undergoing.

4.1 Island comparisons

Rota is the second island within the *C. micronesica* indigenous range to be characterized genetically and for plant survival following the invasion of the armored scale *A. yasumatsui*. We found the Rota population exhibited high genetic variation primarily within populations, congruent with strong genetic structure among sites. Similar genetic patterns for *C. micronesica* were previously reported for Guam [28], the largest island in Micronesia with a land area of about 540 km^2. That Rota exhibited similar microsatellite results was somewhat unexpected, given its land area is only 16% of Guam's, and thus more gene flow among sites was expected. Moreover, the maximum distance separating our Rota sampling sites was only 16 km, whereas the limits of our Guam sampling sites were separated by as much as 45 km.

The highly structured genetic patterns observed on both islands suggest limited seed dispersal. In recent years, this may partly reflect the decline of populations of the bat *Pteropus mariannus* Desmarest and congener species [35]. Flying foxes and *P. mariannus* in particular eat the outer integuments of mature cycad seeds [36]. Historically, these bats were conserved as part of the local CHamoru culture as part of cycad-bat-human interactions [37], but their populations have declined due to introduced diseases, habitat loss and illegal hunting [38]. *Cycas micronesica* belongs to a complex of species capable of oceanic seed dispersal via flotation on ocean currents, a process called thalassochory [19], which could have led to various founding events of Rota from other islands. Some of the genetic structure and isolation we observed, in combination with the high within-population genetic diversity, may also result from past human movement of seeds within islands, given their historical use as a starch source in the local diet. Distinct sites, such as Site G and Site F on Rota, could reflect unique founder events resulting from seed introductions from other islands within the species' native range.

The genetic structure on Guam and Rota suggests limited pollen flow distances. Restricted gene flow and strong genetic variation among sites is congruent with cycad pollinators that are limited in their geographic range such as small moths (*Anatrachyntis* spp) and beetles (*Carpophilus* spp), and perhaps a reduction in their populations. Although *C. micronesica* strobili attract several insect species as visitors, only *Anatrachyntis* exhibits the pollination mutualism defined by exploiting male cone tissues as a brood site [39]. The behaviors of this native pollinator may explain the genetic mixing that we observed within habitats with large cycad populations. The lack of connectivity among habitats that are separated geographically also suggests this small moth does not travel long distances while vectoring pollen or may have declined in recent years prior to the invasion. These possible causal factors (seed dispersal and pollination) remain to be tested explicitly, but our findings provide a baseline for understanding genetic connectivity among the cycad habitats, inform future conservation strategies, and associate these patterns to the knowledge and direct use of cycads by humans.

The high genetic variation observed across all sites previous to the scale invasion indicates that seeds preserved *ex situ* are now critical for restoring locally extinct populations, especially at Site 7. This forest fragment was isolated geographically (Fig. 1) and genetically (Fig. 2). The habitat was the last habitat on Guam and Rota to become infested with *A. yasumatsui* in 2010. However, it was our only site that reached 100% extirpation prior to 2023. The most rapid mortality of Guam's population also occurred in small, isolated cycad habitats [23]. We have shown that unique alleles occur for *C.*

micronesica across the island habitats. The loss of this small isolated habitat reveals how localized extirpations may demand the use of *ex situ* germplasm for reintroductions as the only approach for sustaining genetic structure.

Overall, the population responses to *A. yasumatsui* were remarkably similar for Guam and Rota, with minor differences explained by pre-invasion plant population density and demography. One key difference between the two islands was the greater plant density and greater proportion of seedlings in Rota, resulting in a more rapid initial population-level mortality. After 15 years of *A. yasumatsui* herbivory, total plant mortality was 96% on Guam [23] and 99% on Rota (Fig. 4d). However, when analyzed by the three demographic categories, the patterns were similar: seedlings and juveniles both reached 100% mortality within similar time frames, and adult mortality was 87% on Guam [23] and 86% mortality on Rota (Fig. 4c) during the 15 years following each invasion.

A second difference between the two islands involved timing of the *L. pandava* butterfly invasion in relation to the *A. yasumatsui* invasion. A pre-existing *L. pandava* population was thriving in Rota prior to the *A. yasumatsui* invasion, but the *A. yasumatsui* invasion of Guam preceded the *L. pandava* invasion by two years [22]. Priority effects explain how the arrival of one species influences community assembly in a manner that changes the impacts of a second species that arrives at a later date [40].

Ultimate community assembly may be influenced by the species that arrive early through niche preemption or niche modification [41]. Therefore, when ecosystems are invaded by multiple non-native species, a greater understanding of the temporal dependencies as defined by the timing of arrivals should improve our abilities to manage the impacts of invasive species. The lepidopteran *L. pandava* and the hemipteran *A. yasumatsui* appear to be in direct competition [18], predicting that the timing of which species arrives first and the lag time between the two invasions may generate a profound influence on the host plant community. But plant mortality during the initial three years following the two island *A. yasumatsui* invasions did not support this prediction [22], and our results herein indicate that the timing of the *A. yasumatsui* invasion is the single factor controlling plant mortality regardless of when the *L. pandava* invasion occurs.

A third difference between the two islands involves the suite of damaging herbivores and omnivores present on each island. On Guam, *C. micronesica* is threatened by *A. yasumatsui*, *L. pandava*, the leaf miner *Erechthias* sp. (since 2003), the native stem borer *Acalolepta marianarum* Aurivillius, and feral pigs (*Sus scrofa* L.) that consume stem tissue [42]. In contrast, Rota's known threats are limited to *A. yasumatsui* and *L. pandava*. Despite these differences in the number of herbivore and omnivore consumers, the *C. micronesica* populations exhibited similar responses following each of the *A. yasumatsui* invasions [22]. The Rota *L. pandava* population was established decades before the *A. yasumatsui* invasion. The Guam stem borer and feral pig populations were established centuries before the *A. yasumatsui* invasion. These two island communities are a testimony to this tree's ability to survive in the presence of multiple native and non-native consumers until the arrival of *A. yasumatsui*. They also indicate that the host plant's survival responses following the *A. yasumatsui* invasion do not appear to be substantially influenced by other pre-occurring consumers.

Despite these ecological contrasts, the overall pattern remains clear. The comparative results from Guam and Rota show that similar demographic outcomes can occur even under very different ecological histories and community contexts. The consistency of population collapse on both islands demonstrates that *A. yasumatsui* is the dominant driver of decline, rather than local environmental variation. At the same time, the strong genetic structuring found on both islands, and the high proportion of rare alleles on Rota, indicate that each surviving population represented a distinct source of evolutionary potential. Such rare alleles may be important for adaptation but are particularly vulnerable to stochastic loss through genetic drift. These findings suggest that any future conservation or restoration work should recognize this fine scale genetic differentiation when selecting material for translocation, reintroduction or off site propagation.

4.2 Benchmarking for plant conservation

The conservation community cannot devise effective protocols for species recovery in the absence of an understanding of where an imperiled species stood prior to the onset of the primary threats. Documenting responses to invasion may best be met with long-term time series of data collection which sequentially document the history of responses to the invasion [43].

This study establishes such a dataset, providing a rare record of the abrupt decline of an insular cycad population and illuminating pre-invasion benchmarks that can guide future conservation outcomes. For example, the surviving *C. micronesica* plants today appear to be in greater health than only 10 years ago, but the cause of the recent decline in *A. yasumatsui* damage remains unidentified. Conservationists must therefore avoid complacency; assuming recovery is underway may be premature.

Comparisons between the 2008 and 2023 censuses illustrate this concern. In 2008, the Rota habitats contained more than 10,000 cycad plants less than 100 cm in height per ha, an indicator of vigorous regeneration and recruitment that connotes flourishing regeneration and recruitment, and signaling a prosperous future for the species. Despite the healthier appearance of the living adult trees in 2023 when compared to the previous decade, these same habitats contained no cycad plants less than 100 cm in height. There is no reason to believe that the regeneration and recruitment that characterized the 2008 population will reappear passively. Instead, active conservation interventions may be required to foster the return of regeneration of seedlings and recruitment into the juvenile category. Similarly, the mean leaf number of adult trees was 20 in 2023, which represented a 2.5-fold increase in leaf number compared with 2013. While this improvement is encouraging, the 2023 leaf count was only 24% of the 2008 leaf count. Clearly, the surviving Rota cycad population faces a long trajectory of recovery to reach the pre-invasion benchmarks, and expert-guided management informed by knowledge of pre-invasion habitat dynamics will likely be necessary to enable that recovery.

The description of genetic variation among the pre-invasion island cycad populations also serves as a benchmark for future conservation geneticists. This island cycad has been conserved in several *ex situ* germplasm garden locations. Our results in Fig. 2 provide the historical point of reference for determining how much genetic variation has been captured in the off-site botanical gardens, but also how much genetic variation has been lost in conjunction with the 99% loss of the plant population since 2008. As we did in nearby Guam, the description of genetic variation and gene flow patterns provide a valuable tool for decision-making, for example in the selection of *ex situ* seeds that could adapt locally to the island conditions after reintroduction. Their genetic pedigree can be assessed with the EST-microsatellite markers presented in this study. Similar protocols of relatively-low cost and effort and high individual and population-level resolution, can be applied to other cycad species on islands but also inland where habitat loss and fragmentation is increasing.

4.3 The future

Efforts to conserve *Cycas micronesica* have involved the establishment of an *ex situ* conservation program in Tinian [44], collections in botanic gardens, rescue and transplant operations [45], and to a lesser extent, the introduction of biocontrol agents such as *R. lophanthae* [21]. Nevertheless, more than 96% of the cycad population has been lost from these island ecosystems over the past 15 years, indicating that existing measures have not halted population collapse. The *C. micronesica* experience highlights the need to complement localized protection and rescue efforts with broader, process-based conservation strategies that account for species interactions, ecosystem dynamics, and long-term recovery processes [46]. *In situ* and landscape-level priorities include strengthened biosecurity and quarantine to reduce *A. yasumatsui* spread among islands and sites, restricting reliance on biologists with appropriate knowledge of the primary threats, sustained on-the-ground monitoring to support early detection and rapid response, and protection of remaining habitat and high-value stands to preserve recruitment potential while longer-term control strategies are developed. Among these, the establishment of effective biological control programs represents a particularly critical process-based approach because it directly addresses the primary ecological driver of mortality. Although limited introductions of biocontrol agents such as *R. lophanthae* have occurred [21], effective biological control of *A. yasumatsui* through formally funded programs has yet to be achieved. International cycad experts first urged the development of biological control programs following the 2003 invasion of Guam [11, 21], and members of the IUCN Species Survival Commission's Cycad Specialist Group have reiterated these appeals for Guam and Rota repeatedly over the past two decades [45, 47, 48]. This ongoing gap underscores the emergency need for research and implementation of sustainable control strategies.

Dominant tree species often exert disproportionate influences on ecosystem structure and function, and are therefore considered foundation species. When such a species provides unique ecosystem services, its selective removal from a biodiverse community can have profound and cascading ecological consequences. For example, the native *Acalolepta* stem borer and the native *Anatrachyntis* cone borer rely on mature *C. micronesica* trees for recruitment and population survival [39, 49]. The *A. yasumatsui* invasion resulted in a decline of mature tree densities in Guam from 1,238 per ha in 2005 to only 157 per ha in 2020 [23], while in Rota they declined from 1035 per ha in 2008 to only 149 per ha in 2023 (Fig. 4c). The loss of *C. micronesica* has implications for local biocultural knowledge, as this species has been a component of traditional food sources [37]. Clearly, the impending changes to ecosystem health due to this selective culling of a foundation species deserve the attention of the ecology and conservation research communities. Future modeling approaches [50] could be employed to anticipate these shifts and to guide the design of more effective conservation strategies that also involve local communities and their traditional knowledge.

5 Conclusion

The armored scale *A. yasumatsui* invaded the island of Rota in 2007, and subsequent *C. micronesica* responses in plant survival and health were documented using permanent monitoring plots. Consistent with patterns observed during the earlier Guam invasion, the seedling population in Rota reached 100% mortality within a short period, followed by the juvenile cohort. The seedling: juvenile: adult relationships were 70:21:9 in 2008, and were 0:0:100 in 2023. Overall, the native cycad population experienced 98.6% mortality within 15 years. The invasion of this second island within the indigenous range of *C. micronesica* has revealed striking parallels with the previous invasion of Guam, with the few differences largely explained by the relative proportions of seedlings and juvenile plants present before each invasion. Genetic analyses underscored the importance of maintaining local population structure with most variation found within populations, and provided guidance for future translocations and reintroductions using *ex situ* seed sources. All reintroduction efforts should maintain provenance integrity to conserve Rota's fine scale structure and rare alleles identified here. Collectively, our findings underscore the need for cycad conservationists to adopt a triage ethos which focuses on the primary threat. In this case, establishing effective biological control of *A. yasumatsui* remains the single most critical conservation intervention.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

Supplementary Material 6.

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Author contributions

TEM—Writing—draft; writing—revision; validation; methodology and analysis; funding acquisition. MC—Writing—revision; methodology. PYRL—Writing—draft; writing—revision; methodology; analysis. RNL—writing—revision; formal analysis. ACJ—Writing—draft; writing—revision; validation; methodology and analysis.

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Data availability

Data available from the corresponding author upon request.

Code availability

Not applicable.

Declarations

Ethics approval and consent to participate

Field observations and leaflet collections of *Cycas micronesica* were conducted on Rota Island, Commonwealth of the Northern Mariana Islands in 2007. These activities complied with all applicable local and national regulations. No collections occurred on federal or protected lands, and no specific permits were required at the time of collection. The species was not listed under the U.S. Endangered Species Act until 2015, so no E.S.A. authorization was needed at the time of collection. All collections remained within U.S. territorial borders; therefore, CITES documentation was not required. Herbarium vouchers to accompany Rota habitats are deposited at the University of Guam herbarium.

Collectors were Lynn Raulerson, voucher Raulerson13316, Agnes Rinehart, voucher Rinehart19202, and Agnes Rinehart, voucher RinehartLR19202. The microsatellite data are also publicly available, any bona fide researcher may contact author A.C.J. to obtain these data. The plants were identified as *Cycas micronesica* K.D. Hill by Ken D. Hill, who first described and validly named the species in 1994 and included all known cycad populations occurring on Rota in his species circumscription. The study species is the only cycad species in Micronesia, and is the only gymnosperm on Rota. Identification of our study species is unmistakable. Our goal was demography, survival following an invasive species threat, and population genetics. We did not include any aspect of systematics.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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