

# Survey of Three Federally-Listed Tree Species on Guam National Wildlife Refuge

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## Final Report

**Introduction:** A systematic visual survey of the Guam National Wildlife Refuge – Ritidian Unit was conducted from June to July 2017. Two main objectives of this survey were first, to locate existing populations of three species of trees – *Heritiera longipetiolata*, *Psychotria malaspinae*, and *Tabernaemontana rotensis* – and second, assess the habitats throughout the refuge where these three were thought to exist. 55 hectares of land (550,000m<sup>2</sup>) on the East side of the refuge was selected as a study site, and approximately 1/5<sup>th</sup> of the total area was surveyed. Total of 9 *T. rotensis* individuals were found in two different sites. 5 individuals of *H. longipetiolata* were found on one site, and no individual *P. malaspinae* was found. During this survey, 993 individuals of *Cycas micronesica* were located and relevant data collected. Four major pests that threaten *C. micronesica* to the verge of ecological extirpation is described in detail here, as evidenced by the pictures taken on site. Biological control agent *Rhyzobius lophanthae* was not seen during this study period. Collected data may be useful for future conservation effort of *C. micronesica*, as the plant has been experiencing a serious degree of depredation in number in the recent years.

## *Cycas micronesica*

According to an extensive forest inventory conducted in 2002, *Cycas micronesica* was the most abundant tree on Guam<sup>4,6,7</sup>. As the species that once dominated the island ubiquitously, *C. micronesica* served a very important ecological role not only as a provider of fundamental niche spaces for several native species of insects and birds, but more importantly, as the only dominant native tree on Guam that associates with nitrogen-fixing endosymbionts with a tripartite relationship with cyanobacteria<sup>4,7,8</sup>. Exhibiting densities greater than 12,000 plants per hectare, it most likely served as a foundation species, terraforming the island soil by depositing a substantial amount of nitrogen into the forest ecosystem<sup>4,7</sup>. In the event that *C. micronesica* becomes ecologically extirpated, many unforeseen and far-reaching consequences may follow, including the risk of co-extinction of numerous species that previously depended on *C. micronesica*'s role as a foundation species<sup>4</sup>.

In 2003, armored cycas aulacaspis scale (Hemiptera: Sternorrhyncha: Diaspididae - hitherto abbreviated as CAS), a native of Thailand and Vietnam, was detected on an ornamental king sago *Cycas revoluta* for the first time in Tumon Bay, Guam<sup>1,3,4,8,9,14</sup>. CAS is a pest insect that directly feeds on the leaf tissues of its host cycad plant, eventually causing the tree to die due to its saliva toxicity; when not controlled, CAS is highly destructive and often lethal to its host plants<sup>5,9,14</sup>.

Within 2 years, CAS had spread throughout the island and consequently the *C. micronesica* populations have experienced dramatic decline; in fact, the magnitude of the damage to the *C. micronesica* population was so immense that by 2006 – only 3 years after the invasion began – *C. micronesica* was red-listed as Endangered on International Union for Conservation of Nature and Natural Resources (IUCN)<sup>3,4,6,8,9,13,20</sup>. As of January 2013, 92% of the known population on Guam had died, all seedlings in observed areas perished, and the ecological extirpation of *C. micronesica* was projected by 2019, should no further mediation efforts be employed<sup>3,13,20</sup>.

In response to this catastrophic event, University of Guam initiated a classical biological control of CAS early as November of 2004, releasing a population of predatory lady-beetle (*Rhyzobius lophanthae*: Coleoptera, Coccinellidae) known to control scale infestations<sup>1,6,14</sup>. Despite the predator's permanent establishment on Guam and some measure of success that ensued, the predator's size, preference towards taller cycad trees, and behavioral avoidance away from the ground failed to completely eradicate CAS hiding under ground, or protect *C. micronesica* juveniles and seedlings from dying<sup>3,6,13</sup>.

There are three invasive arthropod pests and one native pest that utilize *C. micronesica* as their hosts for all or some part of their life-cycle; the diagnostic symptoms of infestation by each of these pests are unique and easily distinguishable, although some specimens warrant thorough and laborious inspections due to various fungal coverage that discolor the leaves and other *in situ* challenges often present in a thick limestone forest<sup>5,6,8,9,13</sup>. One plant may be infested with any of the four major pests concomitantly or sequentially in varying degree of severity at any point of its life stage; however, visible observations of these symptoms aren't - by any means - obligate

components of infestation, and thus, any serious investigator should inspect every leaf, every surface, the entire circumference of the stem and roots, as well as the stem tips where overlapping cataphylls and trichomes create a perfect refuge for these pests to hide<sup>5,9,13,15</sup>.

**Cycad Aulacspis Scale: *Aulacspis yasumatsui* Takagi (Hemiptera: Sternorrhyncha: Diaspididae).**

Since its introduction to Guam in 2003, cycad aulacspis scale (CAS) has been the greatest biotic threat to *Cycas micronesica* because the scale attacks every part of the leaf, fruits, and trunk; shortly after the infestation, the entire petiole, rachis, and abaxial leaflet surfaces become entirely white, causing the leaves to collapse or senesce early, forcing the females to abort the fruits, and impeding the male reproductive megastrobili to develop properly<sup>1,4,6,8</sup>. CAS also penetrates small sites on the trunk as well as the roots of the plant, effectively becoming invisible by the human eyes, and unreachable by the predatory beetle *Rhyzobius lophanthae*<sup>5,9,12</sup>. Despite the ongoing predation by the *R. lophanthae*, long term infestations of this armored scale lead to sequential defoliation events with each successive flush of leaves generating fewer and smaller leaves, a process that ultimately results in plant death<sup>6,9,12</sup>.

CAS most readily disperses through wind in its first instar-crawler stage and is not discriminatory in its host choice<sup>14</sup>. No primary pathogens are known to cause disease in the CAS, as the mouthparts of the insect do not allow ingestion of bacteria or viruses that commonly attack mobile, chewing insects; also, the ‘armor’ produced by the female serves as a barrier to spores of fungi, effectually making the CAS immune to any known form of entomopathogenic biocontrol so far<sup>1</sup>.

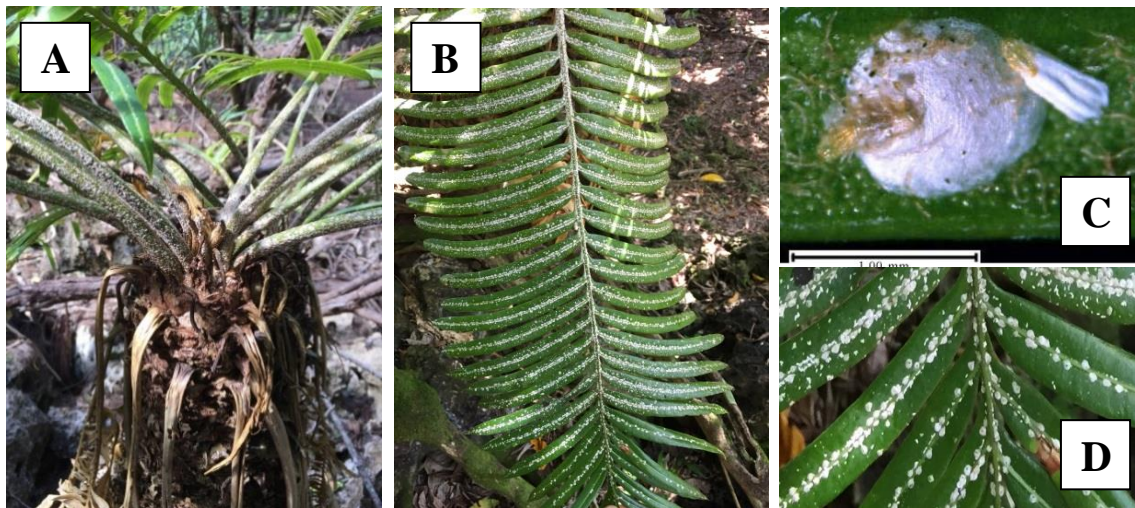


Figure 1. *Aulacspis yasumatsui* is a scale insect that infests every part of the cycad tree (A). The scale positions itself along the veins and near the base of the leaves (B), directly consuming the leaf tissues. Once the whole plant is covered with *A. yasumatsui*, growth, reproduction, and new leaf generations are all severely impaired. Females form an armor around their body as a barrier to spore or fungi (C,D).

**Cycad Leaf Miner: *Erechthias sp.* (Lepidoptera: Tineidae).**

The cycad leaf miner *Erechthias sp.* was first reported in the southern habitats of the island in 2003<sup>9,13</sup>. Caterpillars of this microlepidopteran insect tunnel into leaflets of hardened mature *Cycas* leaves and cause distinctive blotching<sup>9,13</sup>. Older leaves are preferentially targeted by this moth species for some reason; consequently, cycad individuals infested with this moth species show orange-yellow blotched leaves exclusively on the lower fronds, leaving the newer top fronds green<sup>9,13</sup>. Direct damage to photosynthetic tissues ultimately create significant net-carbon deficit for the host plant, causing the infested leaf to senesce early and die<sup>9,13</sup>.

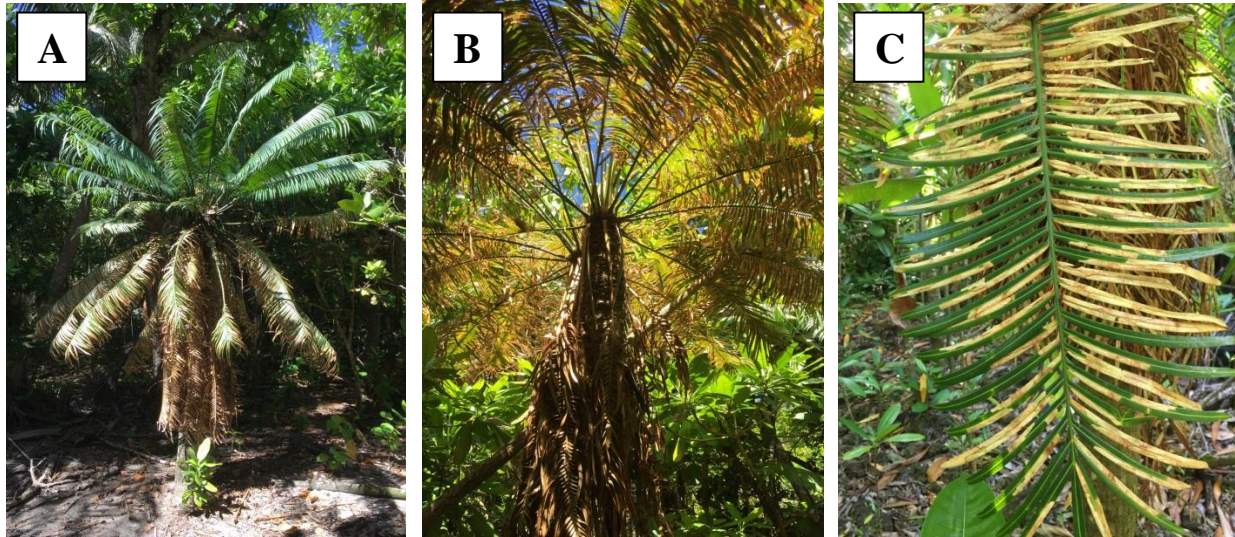


Figure 2. *Erechthias sp.* is a leaf miner moth whose larvae mine into *Cycas micronesica* adult leaflet. The moth preferentially infests the lower fronds (A) and creates a distinctive yellow blotching (B) which is caused by the loss of photosynthetic tissues. At high densities, the leaves collapse downward, unable to maintain the integrity (C).

**Cycad Blue Butterfly: *Chilades pandava* (Lepidoptera: Lycaenidae).**

Cycad blue butterfly was first found in northern Guam in 2005<sup>3,13</sup>. *C. pandava* is a specialist butterfly, whose caterpillar stage requires soft, expanding *Cycas* leaf tissue as food<sup>13</sup>. The female adult butterfly oviposit her eggs directly into *Cycas* young leaflet flush, eventually consuming the young leaflets at the center of the tree<sup>13</sup>.

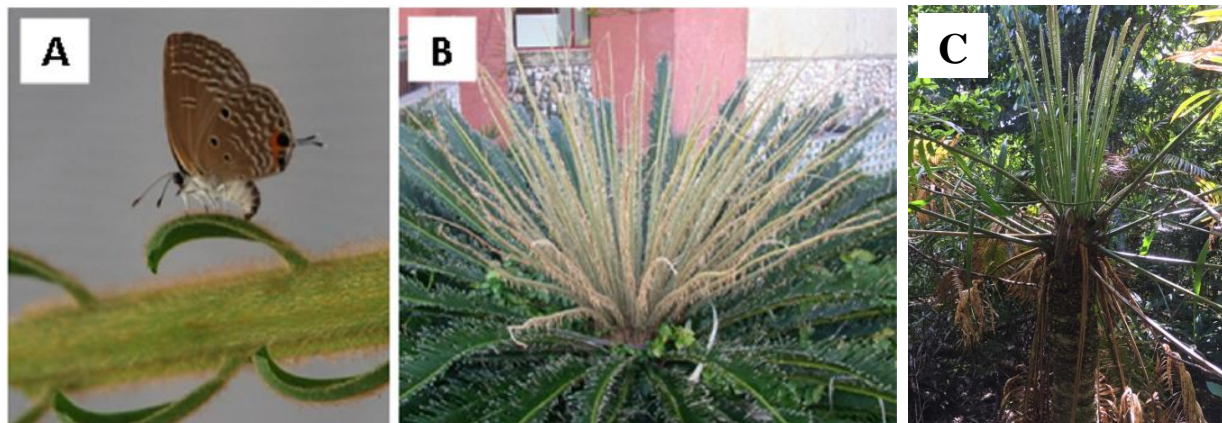


Figure 3. *Chilades pandava* is a specialist butterfly that oviposit its larvae onto a soft, expanding *Cycas* leaves (A). Subsequent infestation results in deaths of the young *Cycas* leaves at the center of the tree (B). Young shoots appear most readily during the rainy season in July (C).

**Stem Borer: *Dihammus marianarum* (Coleoptera: Cerambycidae).**

This longhorn beetle is a native insect that has recently garnered the conservationists' attention as a major pest to Guam's cycad population<sup>9,13</sup>. Primarily found in dead branches of many trees, *D. marianarum* was not considered to be a major pest to the *Cycas* trees prior to the invasion of *Aulacaspis yasumatsui*, *Erechthias sp.*, and *Chilades pandava*<sup>13</sup>. Stem borer species only attack weakened trees and the introduction of other invasive pests really set the stage for *D. marianarum* to begin its infestation as the overall vigor and health of *Cycas* trees declined<sup>13</sup>. Marler (2013) discovered that the stem borer infestation was less than 5% before 2003, whereas by 2008 – 5 years after the introduction of *A. yasumatsui* – approximately 90% of all cycads were infested with *D. marianarum*<sup>9</sup>. This beetle lays a single egg in cavities gnawed through the surface of the stem around 1m above the ground; in the next year or so, the grub of this beetle bores into the live cycad stem tissue<sup>9</sup>. As the grub further tunnels into the stem tissue, larva plugs the tunnel with frass, fibrous chips, and mucilaginous secretion<sup>9</sup>. Eventually the stem above the tunneling site becomes necrotic, and causes synchronous and rapid yellowing of the entire leaves<sup>9</sup>. However, the stem below the cavity remains healthy and the infested plants may initiate new stem growth near the base<sup>9</sup>.

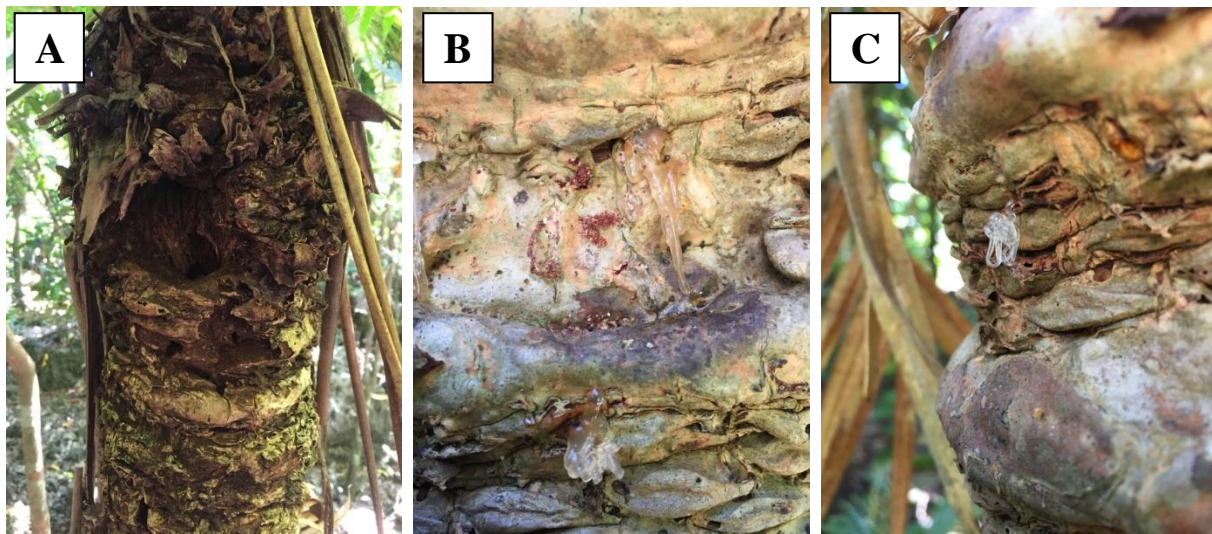


Figure 4. *Dihammus marianarum* is a native beetle that only attack weekend or dead trees (A). This longhorn beetle bores directly into the cycad stem tissue, plugging the tunnel with fibrous frass and thick mucilage (B,C). The grub stage may last up to two years.

**Prognosis:**

During the transect survey from June 2017 to July 2017, the author was able to inspect 993 individuals of *Cycas micronesica* located in the East side of the Guam National Wildlife

Refuge [13° 39.924' N 144°52. 262' E]. Nine hundred ninety two of the 993 individuals were infested with one or more of the abovementioned pests, and the most prominent of them all was *Aulacaspis yasumatsui*. Four hundred and ten individuals were heavily infested with *A. yasumatsui*, and 250 individuals were moderately infested. Not one juvenile or sapling was discovered. These findings were congruent with the literature reviews. The author was never able to first handedly witness *Rhizobius lophanthae* on site or detect any sign of its predation on *Aulacaspis yasumatsui*.

Should an out-planting of *Cycas micronesica* occur in the near future, special care of new saplings will be required because the biocontrolling agent *Rhizobius lophanthae* was proven to be ineffective in protecting cycad saplings. Any prophylactic approach should include close examination and complete eradication of all and any *A. yasumatsui* that may be hidden in a container, space in-between the overlapping cataphylls, and stem grooves. Shortly after the move, frequent site visits and immediate treatment of cycad scales are paramount. Because the *A. yasumatsui* is most readily dispersed through mechanical movement of infested plants as well as through wind, scale-free *C. micronesica* seedlings should be planted far away from any nearby cycad trees.

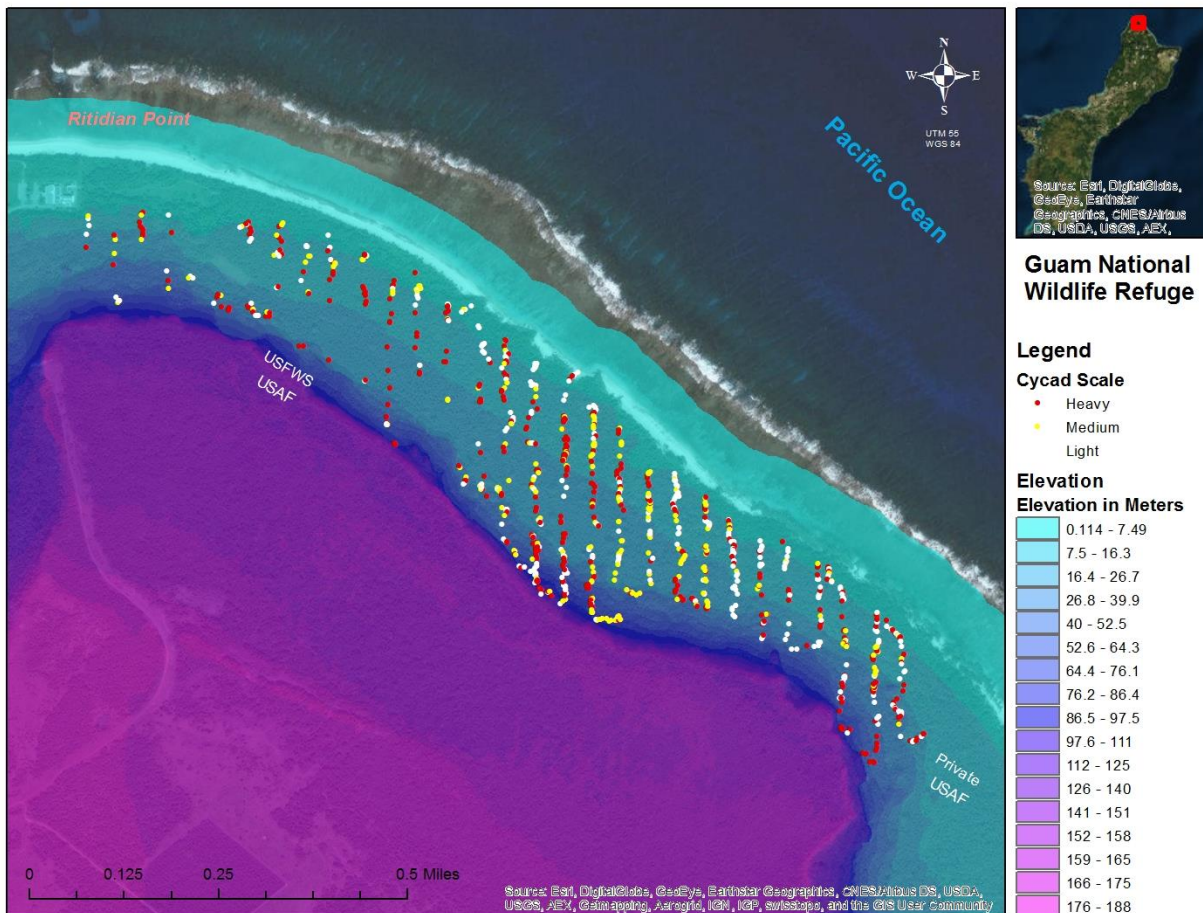


Figure 5. *Cycas micronesica* distribution pattern is mapped against the elevation layer. During the study, 993 *C. micronesica* individuals were diagnosed for *Aulacaspis yasumatsui*.

## *Tabernaemontana rotensis*

Native to the limestone forests of Guam, *Tabernaemontana rotensis* most readily co-occurs with *Cycas micronesica*. *T. rotensis* first caught the eyes of scientists by its conspicuous orange fruits with red pulp that appeared en masse unexpectedly in 1998 as a direct result of Typhoon Paka on December 1997<sup>18</sup>. *T. rotensis* behaves like a pioneer species in that it responds with a mass fruiting event immediately after a typhoon, and its germination potential is maximized in full sun conditions<sup>11,18</sup>.

According to the systematic survey of *T. rotensis* on Andersen Air Force Base (AAFB) in 2007, more than 21,000 *T. rotensis* individuals were found throughout AAFB. Individuals occurred in an aggregated spatial distribution pattern, showing a population structure that represented healthy recruitments from reproductive mature individuals of diverse canopy size<sup>18</sup>.

Its clumped distribution pattern may reveal the excellent health of these individuals - indicating that self-recruitment potential is high; however, it also reveals that seed dispersal is extremely limited, if at all<sup>18,21</sup>. Despite the increased number of known individuals of *T. rotensis*, the tree population that exists on Guam and Rota continue to exist in a clumped aggregation pattern<sup>18,20</sup>. Its limited habitat size, combined with the lack of avian-mediated dispersal, puts the species in particular danger towards habitat fragmentation, habitat destruction, direct herbivory of the saplings by feral ungulates, and most importantly its close proximity of occurrences to an area that is likely to be developed into military base expansions<sup>18,20</sup>.

### Prognosis:

During the transect survey from June 2017 to July 2017, the author was able to locate 9 individuals of *Tabernaemontana rotensis* located on two different sites within the east side of the Guam National Wildlife Refuge [13° 39.924' N 144°52. 262' E]. Eight of the 9 trees occurred in tight aggregation within 10m of each other, whereas the 1 *T. rotensis* stood alone among the thickets of the limestone forest. The lone *T. rotensis* individual was separated from other known *T. rotensis* by a considerable distance (600 m), and judging from its extremely tall stature and large girth (17.4 cm, diameter at breast height), the tree was most likely dispersed by an avian carrier more than 20 years ago when the native bird species were still existent on Guam.

The author does not recommend any outplanting of *T. rotensis* on the east side of Guam National Wildlife Refuge, as two feral ungulate species - *Sus scrofa* and *Cervus mariannus* - presence are still very great. There were times when the author, during the survey, could not find a place to sit because of the sheer amount and dispersion of deer scat across varying terrain, not to mention regular pig and deer sightings in the forest. Direct herbivory of *T. rotensis* by these two ungulates is a well-documented phenomenon. For this reason, it is to the author's opinion that any *T. rotensis* outplanting efforts be carried on inside the main gate common area, until the ungulate populations are completely depredated out of the ungulate exclusion fence.

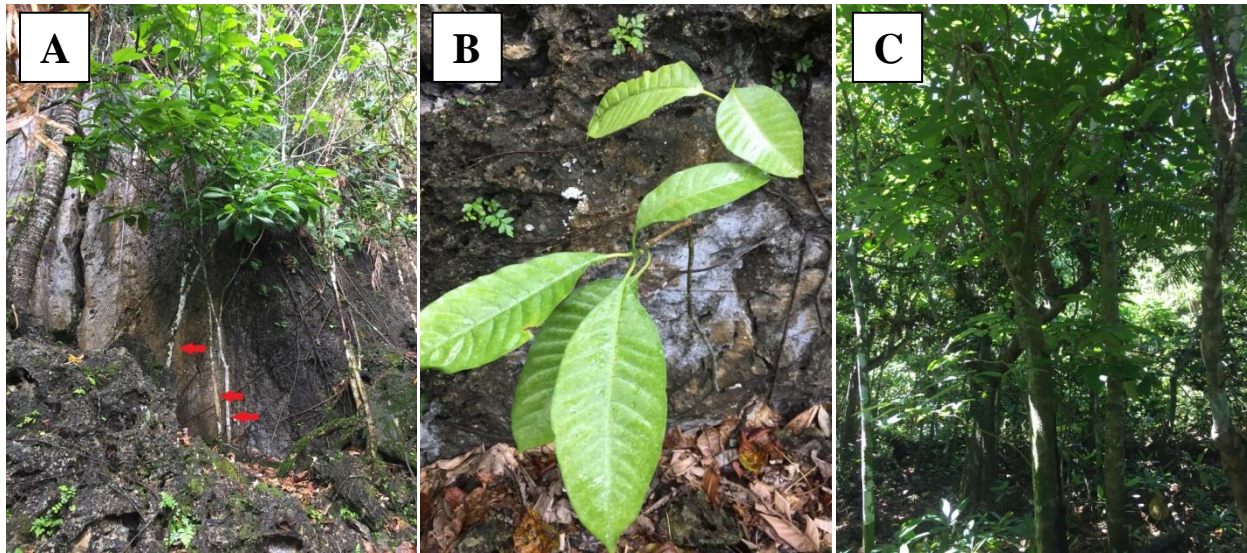


Figure 6. Standing against the big boulder, 8 *Tabernaemontana rotensis* individuals were found in aggregated spatial pattern (only three is showing) (A). Underneath the aggregated *T. rotensis* was one seedling, indicating a modicum amount of self-recruitment (B). Six-hundred meters away from the nearest *T. rotensis* was one very large individual (17.4 cm, dbh) that showed no sign of fruiting or flowering (C).

### *Heritiera longipetiolata*

Also known as Ufa Halom Tano, there were about 1,000 identified individuals on Guam in 1997; as of 2013, *Heritiera longipetiolata* is known from 10 occurrences, totaling approximately 200 individuals on Guam, Saipan, Tinian, and Rota combined<sup>20</sup>. *H. longipetiolata* is most readily found in mature and second growth limestone forests, primarily near coastal cliffs and fringes where limestone formations have created sharp crevasses on dissected slopes<sup>2,16,19,21</sup>. Although several hundred seedlings were detected beneath the known 53 individuals on Saipan, there seems to be strong evidence that seedlings and seeds of *H. longipetiolata* on Guam are being eaten by ungulates and crabs<sup>20</sup>.

#### Prognosis:

During the transect survey from June 2017 to July 2017, the author was able to locate 5 individuals of *Heritiera longipetiola* within the east side of the Guam National Wildlife Refuge [13° 39.924' N 144°52. 262' E]. The five individuals varied greatly in size. Four individuals were large enough to be the most dominant canopy in that area, whereas one was a juvenile. There were 6 seedlings distributed around the area directly beneath the four mature *H. longipetiolata* individuals. Surprisingly, these individuals were found less than 100m from the northern coast line. No flowering or fruiting events were occurring at the time of their inspection – August 4<sup>th</sup>, 2017. Thankfully, the refuge botanist Ryan deRegnier was able to collect several seeds from the site, and the seeds have since been planted in nursery pots.



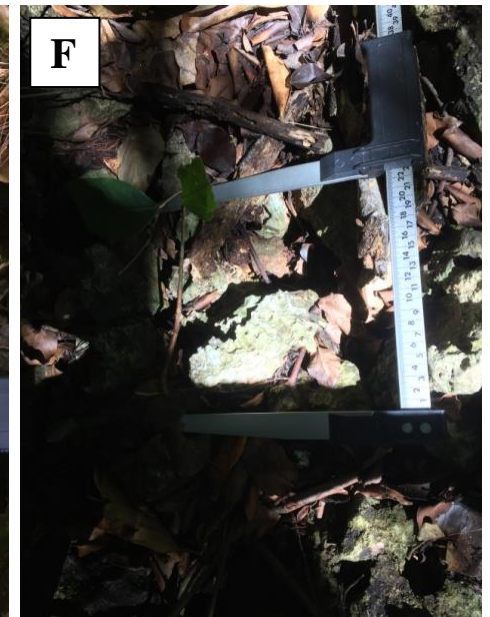
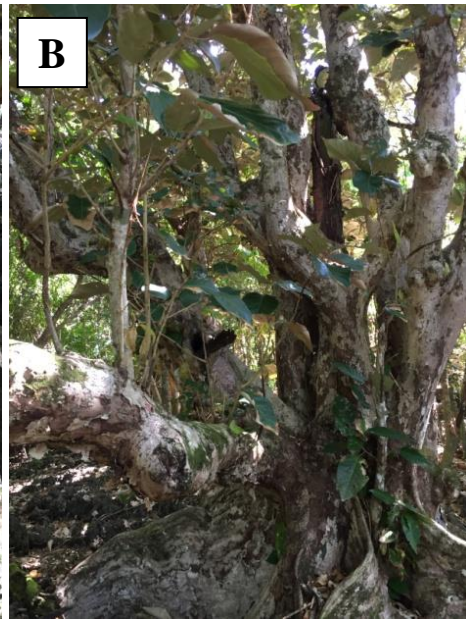


Figure 7. *Heritiera longipetiola* individuals are located on transect site code AA1-AA2. All mature individuals formed characteristic buttress roots (A,B) that covered the edges of the limestone crevasses (C) on which they grow. *H. longipetiola* seedlings are located directly underneath 4 mature trees. Height measurements from top left to clockwise direction (D - I): 34.5cm, 16.5cm, 23cm, 33cm, 23.7cm, 33.7cm.

### Methods:



Figure 8.

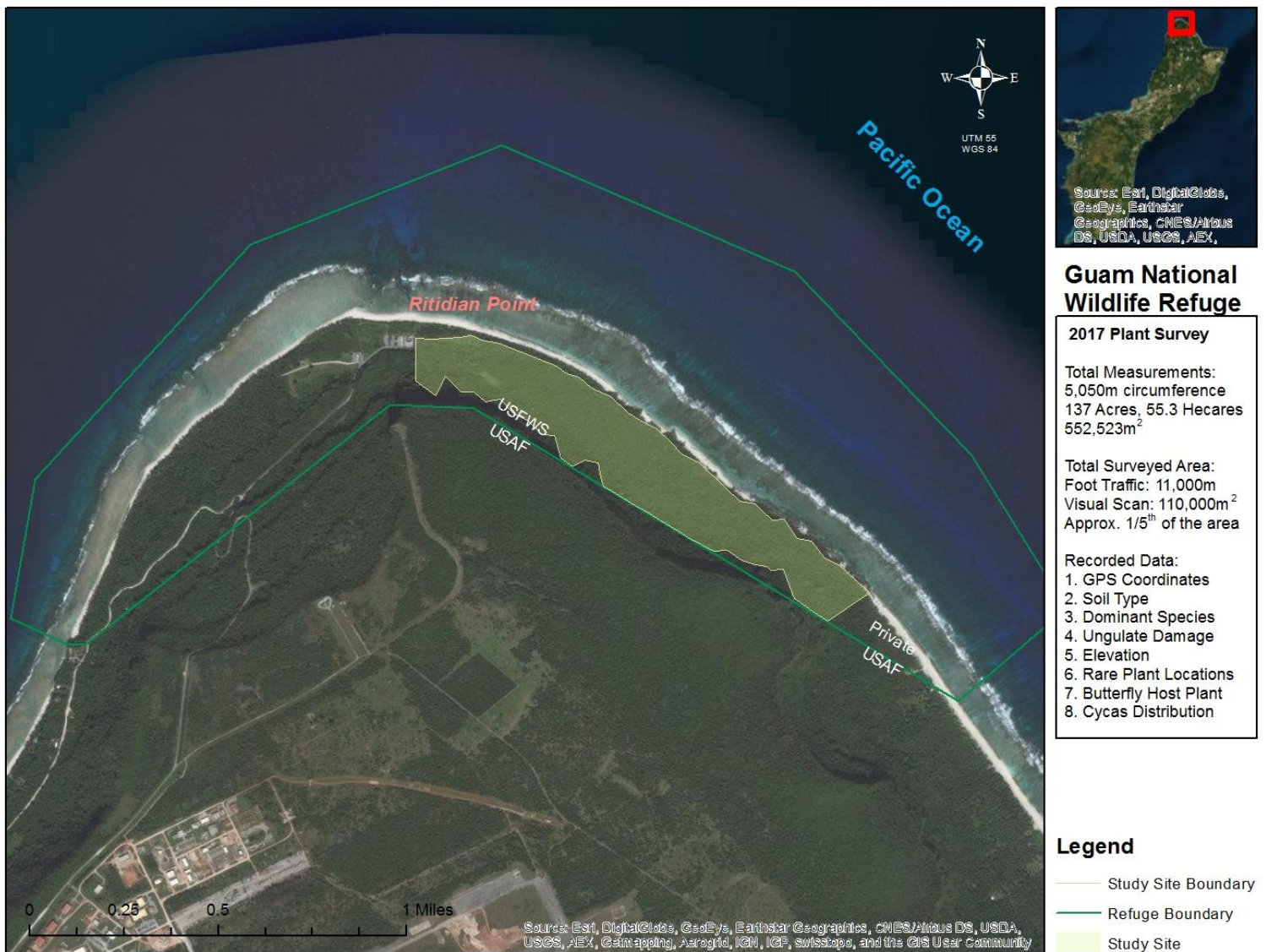


Figure 9: Study Site: Guam National Wildlife Refuge - Ritidian unit is located on the northern tip of Guam. The western half of the refuge is available for the public, whereas the eastern half of is closed. This study was conducted exclusively on the eastern side of the refuge. The field survey covered approximately 550,000 m<sup>2</sup> area of land that stretched from the northern coast to the southern cliff-base that marks the refuge boundary. The survey site was systematically ground-truthed using transect belt coordinates that overlaid the entire study area. Beginning of each day, GPS coordinates were manually inputted into the hand-held GPS unit to ensure a correct pathing through the jungle.

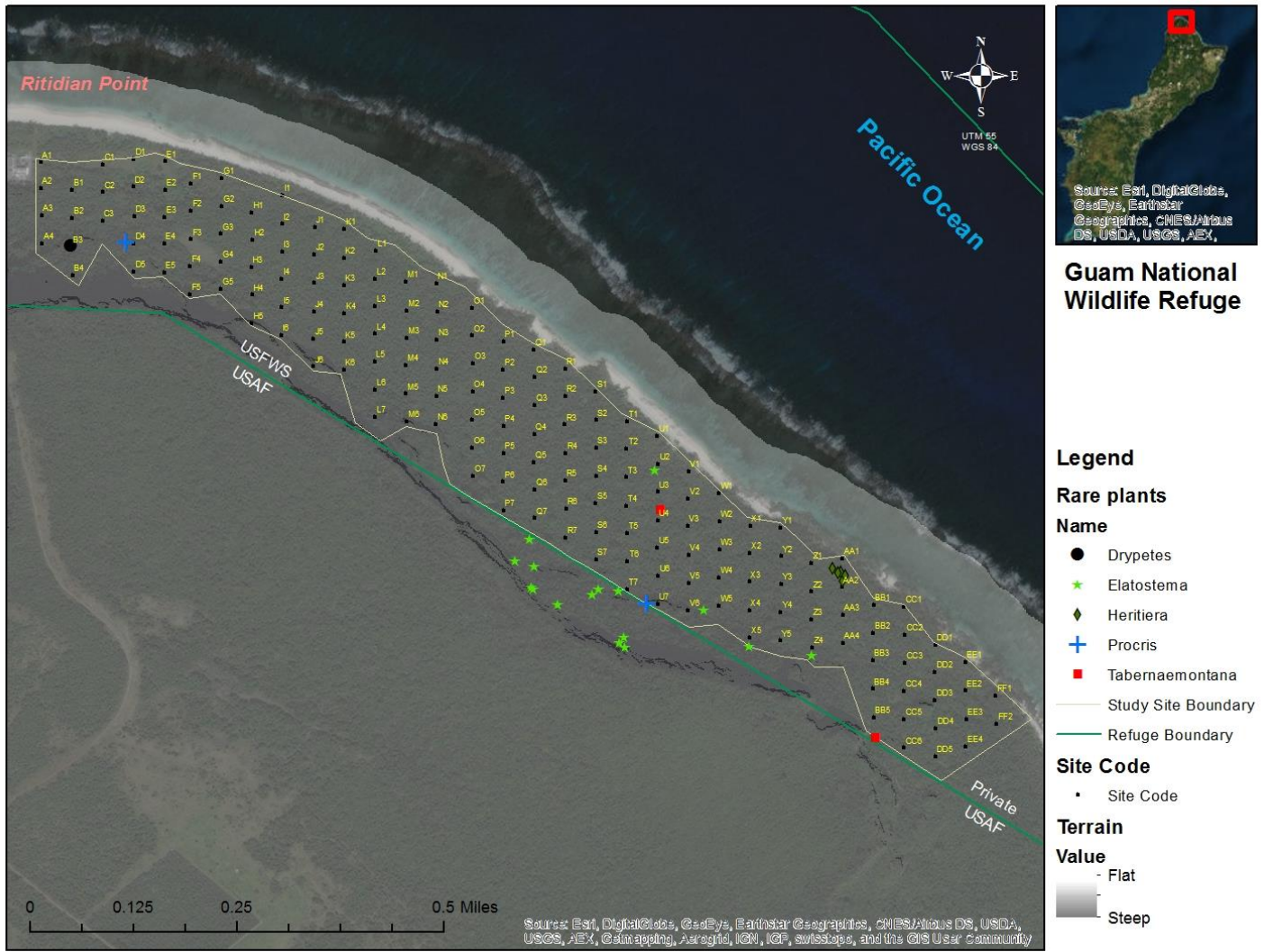


Figure 10.

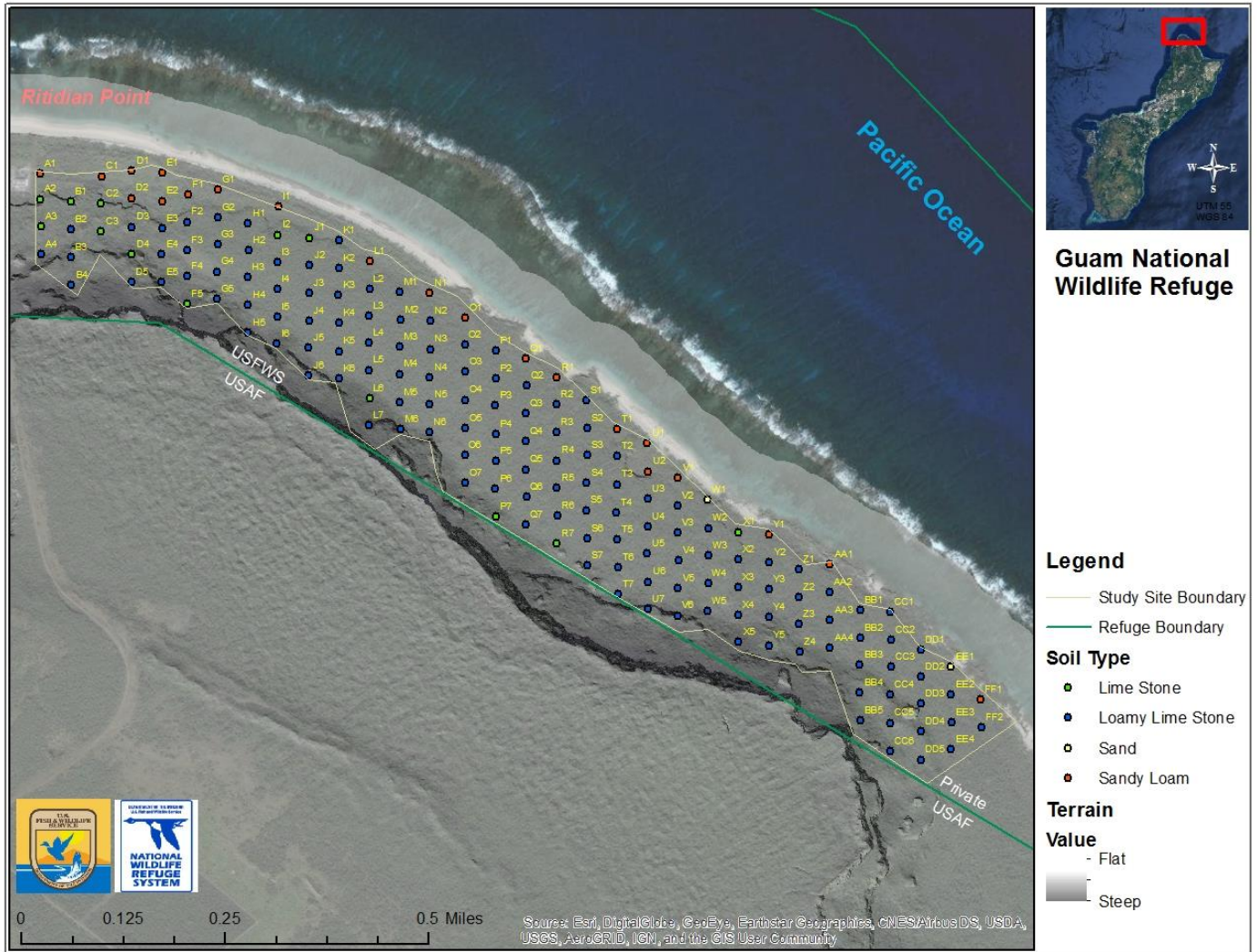


Figure 11.

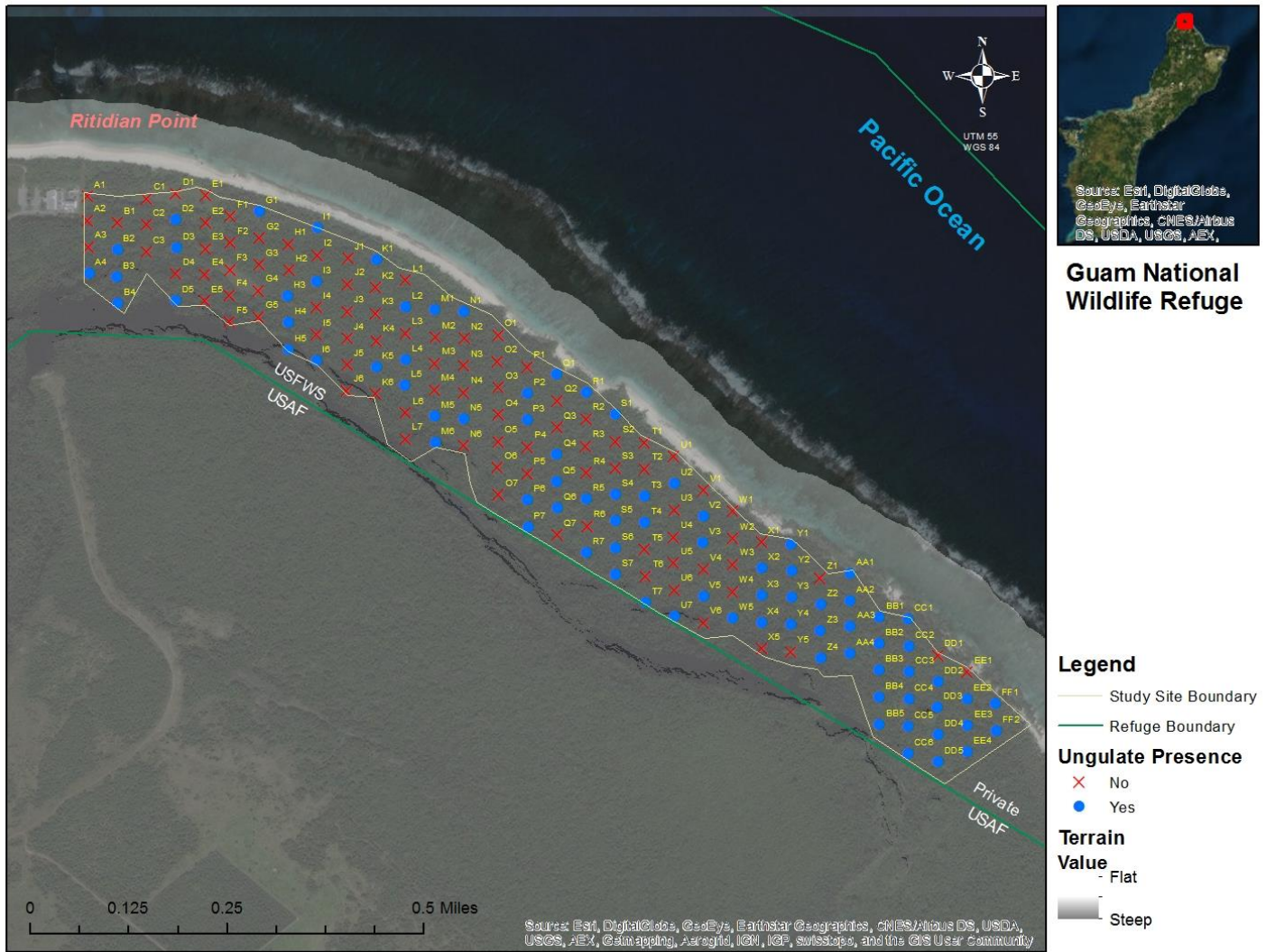


Figure 12.

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