

Survey for the Mariana eight spot butterfly, *Hypolimnas octocula marianensis* (Lepidoptera: Nymphalidae), in the Pagat Route 15 area of Yigo Village, Guam

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Summary

Surveys were performed for all life stages of the Mariana eight spot butterfly, *Hypolimnas octocula marianensis* Fruhstorfer, and its two documented host plant species along three transects (Rt 15 North, Rt 15 South, and Pagat Cave) in the Pagat area south of Route 15, in the southern corner of Yigo Village, Guam during the time period from July 15 to July 24, 2009. Host plants of *H. octocula marianensis* were sparse except for two areas, one on the Rt 15 North transect and one on the Rt 15 South transect, which contained large groups of both plant species. One adult *H. octocula marianensis* was seen in the large host plant area on the Rt 15 North transect. Other life stages (e.g. egg, larvae, pupae) were found on host plants in all three transects, however, without rearing these stages to the adult form they cannot be identified with complete certainty as *H. octocula marianensis*. Geographic locations are provided for all locations of *H. octocula marianensis* and host plants.

Introduction

Hypolimnas octocula marianensis Fruhstorfer, also known as the Mariana eight spot butterfly or forest flicker, is one of eight subspecies in the *Hypolimnas octocula* complex (Tennent 2006) and is currently classified as a candidate species for listing as endangered by United States Fish and Wildlife Service (FWS). It is reported to occur on the islands of Guam and Saipan (Tennent 2006); however, it may have been extirpated from Saipan (Hawley and Castro 2008, Schreiner and Nafus 1997). The status of *H. octocula marianensis* on Guam is also unclear. It was described as scarce during a 1936 Lepidoptera survey, with only one specimen collected from the Piti area (Swezey 1942). According to the Guam Agricultural Experiment Station collection, three specimens were collected at Hilaan Point in 1975, one specimen was collected from Anderson Air Force Base in 1982, and two more specimens were collected from Hilaan Point in 2001 (GDAWR 2005). Results from surveys conducted in 1996 for the FWS by Schreiner and Nafus indicated that there were 10 populations of the butterfly on Guam (Hawley and Castro 2008). The locations of these populations were as follows: Fadian Cove (1), Hilaan (2), Mangilao golf course (2), Orote (1), Pagat (2), and Tweeds Cove (2). No quantitative estimates of population sizes were provided, but it was noted that the highest number of individuals seen in one day was six (USFWS 2008). The two known host plants of *H. octocula marianensis* are *Elatostema calcareum* and *Procris pedunculata*

(Schreiner and Nafus 1997). Both host plants are from the family Urticaceae and occur in wet, native forest areas with exposed limestone karst.

The current survey was conducted in the Pagat area south of Route 15, near the Guam International Raceway in the southern corner of Yigo Village. One adult *H. octocula marianensis* was observed in this area during recent biological surveys for the Guam and Commonwealth of the Northern Mariana Islands (CNMI) Military Relocation Environmental Impact Statement (EIS) (M. Moese, personal communication, 5 Jan. 2009). The purpose of this survey was to gather more information on *H. octocula marianensis* in this area.

Methods

Two primary transects used were used to survey the butterfly and host plants. These were established by biologists from TEC Inc. and SWCA Environmental Consultants and are referred to as Route 15 North and Route 15 South. A third transect, the trail leading to Pagat Cave, was surveyed only once. Personnel participating in the surveys consisted of two entomologists from NAVFAC Pacific and one biologist from NAVFAC Marianas. All transects were surveyed during the period from 15 to 24 July, 2009. Surveys were generally conducted from late morning (~ 9:00-10:00 am) to late afternoon (~ 2:00-4:00 pm); however on 17 July the survey was conducted one hour before and after sunrise (~ 5:30 am to 7:30 am) and one hour before and after sunset (~ 7:00 pm to 9:00 pm) to determine if larvae were active during these time periods. A handheld GPS (Garmin GPSMap60Csx) was used to track all movement and record geographical locations of host plants and all observed life stages of *H. octocula marianensis*.

Transects were first surveyed over their entire length for host plants. Once the most probable areas of butterfly habitat (i.e. areas with a high density of host plants) were identified, efforts were then focused on those sites. This consisted of searching host plants for eggs, larvae, and pupae, monitoring the understory and upper forest canopy for adults, and monitoring bait pans. A digital camera (Canon 30D) was used to capture images of host plants and all butterfly life stages. Field binoculars were used to identify adult butterflies from long distances. Bait pans consisted of aluminum pie tins and were suspended approximately five to six feet from the ground. Banana and pieces of fish were used as bait. Bananas were prepared one day in advance by mashing and mixing with cane sugar and water and leaving at room temperature in a sealed bag for 24 hours. Fish pieces were obtained from a local market and placed in bait stations on the same day of purchase. Three bait pans were used in each area of butterfly habitat for a period of two days.

Results

Two areas were identified which contained numerous plants of both host plant species. These areas were near the beginning of the Route 15 North and Route

15 South transects and are shown respectively (sites N01 and S03) in Figures 1 and 2. A description of the search effort in these areas is provided in Table 1. Other host plants sighted on occurred in small isolated groups and were represented as discrete points in Figures 1, 2, and 3. All host plant locations are listed in Table 1, and images of host plants are included as appendix A.

Sightings of *H. octocula marianensis* are listed in Table 2, and displayed in Figures 1, 2, and 3. No butterflies of any species were observed at the bait pans. With the exception of the site on the Pagat Cave trail where three larvae were found, all sightings occurred within sites N01 and S03. One adult male *H. octocula marianensis* was seen and photographed within N01. The following day, an identical butterfly was seen at the same location and was presumed to be the same individual. There was a possible sighting of an adult female *H. octocula marianensis* within S03, but it passed quickly out of sight and could not be positively identified. A total of 7 *Hypolimnias* larva were found at 5 different locations on both *E. calcareum* and *P. pedunculata*. *Hypolimnias* eggs were found only on *E. calcareum*, with a total of 19 eggs at 5 different locations. One viable *Hypolimnias* chrysalis was found on *E. calcareum* within site N01, and three empty *Hypolimnias* chrysalides were found on *P. pedunculata* within site S03.

Discussion

Results from this survey and others conducted in the Pagat area of Route 15 indicate that there are at least two areas of habitat that are supporting *H. octocula marianensis*. The sighting of the adult butterfly within N01 during the current survey and the sighting of the adult butterfly in the vicinity of S03 by TEC Inc. (M. Moese, personal communication, 5 Jan 2009) are evidence that the species is present in these two areas. The site on the lower shelf down by Pagat Cave may represent a third area with *H. octocula marianensis*, but it cannot be confirmed without the presence of adults. These findings support the results from surveys conducted in 1996 by Schreiner and Nafus who reported 2 populations of *H. octocula marianensis* in the Pagat area (USFWS 2008). Whether or not the two confirmed areas support a single population or two separate populations is unclear. The habitat sites on the north and south transects are separated by approximately 1.5 kilometers. The Pagat Cave Trail site is approximately 1.5 kilometers from the south transect site and 3 kilometers from the north transect site, but it was at a much lower elevation than the other two sites. The cave trail site was on the lower island shelf at about 82 meters above sea level compared to approximately 166 m and 185 m above sea level for the north and south transect sites. If it is assumed that the larva found near Pagat cave were *H. octocula marianensis*, it would seem more likely that they would represent a separate population from the butterflies seen at the other two sites.

Unfortunately there is some uncertainty regarding the identification of immature life stages of *H. octocula marianensis*. These stages are not easily distinguishable from other *Hypolimnas* species unless they are successfully reared to the adult form. While the larva found on *E. calcareum* and *P. pedunculata* fit Schreiner and Nafus' (1997) description (black with reddish orange spines and a black head), there are two other *Hypolimnas* species, *H. anomala* and *H. bolina*, which look similar during their immature stages. Schreiner and Nafus (1997) describe *H. anomala* larva as black with black spines and greasy in appearance when they are younger, and black with orange spines when they are older. *Hypolimnas bolina* is described as similar to *H. anomala* but with a "diffuse brownish orange stripe down each side". The younger larvae also differ from *H. anomala* in that they have orange spines rather than black and they do not have a greasy appearance (Schreiner and Nafus 1997). All larva seen during the current survey were black or blackish gray with black heads and orange spines. Differentiating these from *H. anomala* is not easy since the amount of red in the orange spines is listed as the primary distinguishing factor (Schreiner and Nafus 1997) and is difficult to characterize. Based on Schreiner and Nafus' (1997) descriptions, it would seem unlikely that these larva were *H. bolina* since there was no evidence of a lateral stripe. However, they cannot be completely discounted as *H. bolina* because images of larva were also sent to Chris Samson, a lepidopterist who has worked with *H. octocula* complex (Sampson 1986), and his opinion was that some of them could be *H. bolina* or *H. anomala*, while others could be *H. octocula marianensis* (C. Samson and J. Tennent, personal communication, 21 July 2009).

Images of larvae were also sent to Ilse Schreiner, a former entomologist at the University of Guam and coauthor of Butterflies of Micronesia (Schreiner and Nafus 1997). Her comment was that while it is difficult to identify the immature stages, if they were on either of the known host plants, then they were probably *H. octocula marianensis* (I. H. Schreiner, personal communication, 17 July 2009). The only host plant listed for *H. anomala* is *Pipturus argenteus* (Wright et al. 1977, Schreiner and Nafus 1997). *Hypolimnas bolina* has also not been documented to feed on *E. calcareum* or *P. pedunculata*, but, unlike *H. anomala*, it has an extensive list of foodplants, including other species of *Elatostema* (Wright et al. 1977, Parsons 1991). It is consequently not implausible that *H. bolina* could be found on *E. calcareum*. Adult butterflies of both *H. anomala* and *H. bolina* were seen flying within the large host plant areas on the north and south transects; however, they were not common. The most common butterfly species seen flying in these areas were *Euploea eunice* (Danaiidae) and *Papilio polytes* (Papilionidae).

Eggs of *H. bolina*, *H. anomala*, and *H. octocula marianensis* are also very similar in appearance and very difficult, if not impossible, to differentiate in the field (C. Samson and J. Tennent, personal communication, 21 July 2009). It is interesting to note, however, that out of 19 *Hypolimnas* eggs found during this survey, all of them were black (Appendix B, Images 7, 10, 11, and 13) except for two, which

were green (Appendix B, image 12). Healthy, viable eggs should be green in color, and eggs which have been parasitized are black (I. H. Schreiner, personal communication, 17 July 2009). Egg parasitism of *H. bolina* and *H. anomala* on Guam was reported by Donald Nafus in 1993 (Nafus 1993); however, it was found that *H. bolina* was parasitized more frequently during the egg stage than *H. anomala*. The majority of egg parasitism on both butterfly species was carried out by three parasitoids: 1) *Telenomus* sp. 2) *Oencyrtus* sp. and 3) *Trichogramma chilonus*. This study did not include Guam's endemic nymphalid species (*Vagrans egista* (Latreille and Godart)) and subspecies (*H. octocula marianensis*), however, given that the three parasitoids listed above show a lack of host specificity, it is highly probably that the native nymphalids are also attacked.

The adult *H. octocula marianensis* that were observed on July 22 and 23 were probably the same individual. The butterflies were identical in appearance and were seen roosting in the same location on the same tree at approximately the same time. On both occasions the butterfly remained in the upper, sunlit canopy and spent the majority of its time perched. This is consistent with behavior documented for *H. octocula elsina* on New Caledonia: "*Octocula* favors well-developed rainforest, emerging from the undergrowth to sun itself on leaves, especially in the morning. It is very much commoner on the wetter, eastern side of New Caledonia (Holloway and Peters 1976)." The pattern and coloration of this butterfly alone do not provide enough information to assess the gender of the butterfly - male and female *H. octocula marianensis* are very similar in appearance, unlike other subspecies show strong sexual dimorphism (Wright et al. 1977, Schreiner and Nafus 1997). However, judging from the behavior it displayed, it was probably a male. Males are generally less active and fly about with no obvious sense of purpose while females are much more businesslike, flying from hostplant to hostplant in their quest to oviposit (I. H. Schreiner, personal communication, 17 July 2009). It also appeared to exhibit some territorialism, never flying far from its roost and chasing other butterflies which entered its air space.

In summary, there is at least one population of the Mariana eight spot butterfly in the Pagat area. There are two areas that contain relatively high numbers of both host plants for the butterfly, and which appear to be sustaining the butterfly population. Any negative impact on these areas would have a direct effect on the butterfly population. The population in these areas already appears to be under stress from parasitization, and any further pressures from habitat degradation could potentially be very damaging. Other areas of habitat for *H. octocula marianensis*, similar to the Pagat Cave trail site, may exist on the lower limestone shelf; however, these would probably support separate populations from the Route 15 area. Additional surveys would be required to identify these habitat areas at lower elevations.

References

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Table 1. *Hypolimnas octocula marianensis* host plant sites and search effort.

Site	Transect & Coordinates	Elev (ft)	Species	Date & Time Searching for <i>H. octocula</i>	Date & No. People ¹ Searching for <i>H. octocula</i>	Total Search Time (m)	Notes
N01	Rt 15 North N/A ²	545	<i>Elatostema calcareum</i> and <i>Procris pedunculata</i>	15JUL09 0948-1033 20JUL09 0900-1015 22JUL09 1030-1220 23JUL09 1000-1100	15JUL09 2 (CC, SL) 20JUL09 3 (CC, SL, MS) 22JUL09 2 (CC, MS) 23JUL09 2 (CC, SL)	290	Mostly <i>P. pedunculata</i> , some <i>E. calcareum</i> . (Appendix A, images 1-3, 5, 7-10)
N02	Rt 15 North N13 30.759 E144 53.660	563	<i>Procris pedunculata</i>	15JUL09 1100-1105	15JUL09 2 (CC, SL)	5	Small group of plants in a patch of limestone forest just after a cleared area.
N03	Rt 15 North N13 30.763 E144 53.661	570	<i>Procris pedunculata</i>	15JUL09 1130-1135	15JUL09 2 (CC, SL)	5	Small group of plants in a patch of limestone forest just after a cleared area.
N04	Rt 15 North N13 30.794 E144 53.640	576	<i>Procris pedunculata</i>	15JUL09 1150-1153	15JUL09 1 (CC)	3	Small group of plants
N05	Rt 15 North N13 30.809 E144 53.633	565	<i>Procris pedunculata</i>	15JUL09 1200-1203	15JUL09 1 (CC)	3	Small group of plants
S01	Rt15 South N13 30.144 E144 53.202	593	<i>Elatostema calcareum</i>	16JUL09 0950-0955	16JUL09 2 (CC, SL)	5	Small group of plants
S02	Rt15 South N13 30.143 E144 53.199	603	<i>Procris pedunculata</i>	16JUL09 0955-1000	16JUL09 2 (CC, SL)	5	Small group of plants
S03	Rt 15 South N/A ²	N/A ³	<i>Elatostema calcareum</i> and <i>Procris pedunculata</i>	16JUL09 1002-1138 17JUL09 0538-0745 17JUL09 1900-2034 20JUL09 1430-1545 21JUL09 1000-1200 22JUL09 1245-1315 23JUL09 1120-1220 23JUL09 1430-1600 24JUL09 1000-1115	16JUL09 2 (CC, SL) 17JUL09 2 (CC, SL) 17JUL09 2 (CC, SL) 20JUL09 2 (CC, MS) 21JUL09 2 (CC, MS) 22JUL09 2 (CC, MS) 23JUL09 2 (CC, SL) 23JUL09 2 (CC, SL) 24JUL09 2 (CC, SL)	767	Very large stands of <i>E. calcareum</i> and <i>P. pedunculata</i> . (Appendix A, images 4, 6, and 11)

Site	Transect & Coordinates	Elev (ft)	Species	Date & Time Searching for <i>H. octocula</i>	Date & No. People ¹ Searching for <i>H. octocula</i>	Total Search Time (m)	Notes
S04	Rt 15 South N13 30.123 E144 53.147	615	<i>Elatostema calcareum</i>	16JUL09 1207-1220	16JUL09 2 (CC, SL)	13	Small group of <i>E. calcareum</i> .
S05	Rt 15 South N13 30.115 E144 53.110	600	<i>Procris pedunculata</i>	16JUL09 1220-1227	16JUL09 2 (CC, SL)	7	Small group of <i>P. pedunculata</i> .
S06	Rt 15 South N13 30.095 E144 53.092	600	<i>Elatostema calcareum</i>	16JUL09 1240-1245	16JUL09 2 (CC, SL)	5	Small group of <i>E. calcareum</i> .
S07	Rt 15 South N13 30.100 E144 53.079	600	<i>Elatostema calcareum</i>	16JUL09 1247-1252	16JUL09 2 (CC, SL)	5	Small group of <i>E. calcareum</i> .
S08	Rt 15 South N13 30.106 E144 53.091	589	<i>Elatostema calcareum</i>	16JUL09 1342-1400	16JUL09 2 (CC, SL)	8	Small group of <i>E. calcareum</i> .
S09	Rt 15 South N13 30.164 E144 53.183	-	N/A ⁴	24 JUL09 1100-1130	24 JUL09 1 (CC)	30	Used binoculars to search top of canopy covering site S03.
P01	Pagat Cave Trail N13 29.524 E144 52.643	268	<i>Elatostema calcareum</i>	22JUL09 1550-1610	22JUL09 1 (CC)	20	Medium sized group of <i>E. calcareum</i> . (Appendix A, image 12)

¹CC = Cory Campora, SL = Stephan Lee, MS = Maria Santos

²This site consists of a large area and cannot be defined accurately by a single point.

³Elevation was variable within this area.

⁴This site was an observation point for looking at upper canopy.

Table 2. Observed life stages of *Hypolimnas octocula marianensis*.

Date & Time	Site	Transect & Coordinates	Elev (ft)	Life Stage (quantity)	Host Plant	Weather - Cloud Cover (%):Wind (1-3):Rain (Y/N)	Notes
15JUL09 1050	N01	Rt 15 North N13 30.819 E 144 53.651	545	Chrysalis (1)	<i>Elatostema calcareum</i>	20:1:N	Signs of feeding on leaves <i>E. calcareum</i> near the chrysalis. (Appendix B, image 1.)
16JUL09 1030	S03	Rt 15 South N13 30.157 E144 53.164	615	Larvae (1)	<i>Procris pedunculata</i>	70:2:N	Late instar, actively feeding during part of the time it was observed, large green frass pellets seen nearby. (Appendix B, images 2-4.)
16JUL09 1138	S03	Rt 15 South N13 30.132 E144 53.164	621	Larvae (1)	<i>Elatostema calcareum</i>	70:2:N	Late instar. (Appendix B, images 5 and 6.)
16JUL09 1135	S03	Rt 15 South N13 30.132 E144 53.164	621	Egg (3)	<i>Elatostema calcareum</i>	70:2:N	Located in same location as larvae, but on a separate plant. All three eggs were black. (Appendix B, image 7.)
17JUL09 1915	S03	Rt 15 South N13 30.141 E144 53.167	580	Chrysalis (2)	<i>Procris pedunculata</i>	80:0:Y	Both chrysalides were empty.
17JUL09 0630	S03	Rt 15 South N13 30.141 E144 53.167	580	Egg (4)	<i>Elatostema calcareum</i>	80:0:Y	All four eggs were black.
17JUL09 0550	S03	Rt15 South N13 30.134 E144 53.160	609	Larvae (1)	<i>Procris pedunculata</i>	80:0:Y	Very late instar, actively feeding. (Appendix B, image 8.)
17JUL09 1955	S03	Rt15 South N13 30.138 E144 53.165	589	Larvae (1)	<i>Elatostema calcareum</i>	10:0:N	Very late instar, actively feeding, large green frass pellets seen nearby. (Appendix B, image 9.)
20JUL09 0915	N01	Rt 15 North N13 30.819 E144 53.651	545	Egg (5)	<i>Elatostema calcareum</i>	80:1:N	Near the same plant we found the chrysalis 15 July. (Appendix B, images 10 and 11.)
21JUL09 1130	S03	Rt 15 South N13 30.140 E144 53.167	607	Egg (6)	<i>Elatostema calcareum</i>	70:1:N	Two eggs green, 4 eggs black. (Appendix B, images 12 and 13.)

Date & Time	Site	Transect & Coordinates	Elev (ft)	Life Stage (quantity)	Host Plant	Weather - Cloud Cover (%):Wind (1-3):Rain (Y/N)	Notes
21JUL09 1027	S03	Rt 15 South N13 30.143 E144 53.163	624	Chrysalis (1)	<i>Procris pedunculata</i>	70:1:N	Empty. (Appendix B, image 14.)
22JUL09 1130	N01	Rt 15 North N13 30.818 E144 53.653	567	Adult (1)	N/A (<i>Macaranga thompsonii</i>)	80:0:N	Was flying up in a small clearing within the canopy, but seemed to prefer resting on the leaves of the <i>M. thompsonii</i> . (Appendix B, images 15 and 16.)
22JUL09 1600	P01	Pagat Cave Trail N13 29.524 E144 52.643	268	Larvae (3)	<i>Elatostema calcareum</i>	50:1:N	One late instar, two earlier instars.
23JUL09 1145	S03	Rt 15 South N13 30.156 E144 53.174	620	Chrysalis (1)	<i>Procris pedunculata</i>	20:2:N	Empty.
23JUL09 1045	N01	Rt 15 North N13 30.818 E144 53.653	567	Adult (1)	N/A (<i>Macaranga thompsonii</i>)	30:2:N	Was in the same location as the adult butterfly seen on 22 July – appeared to be the same individual. (Appendix B, image 17.)
24JUL09 1050	S03	Rt 15 South N13 30.129 E144 53.159	606	Egg (1)	<i>Elatostema calcareum</i>	20:2:N	Egg was black.

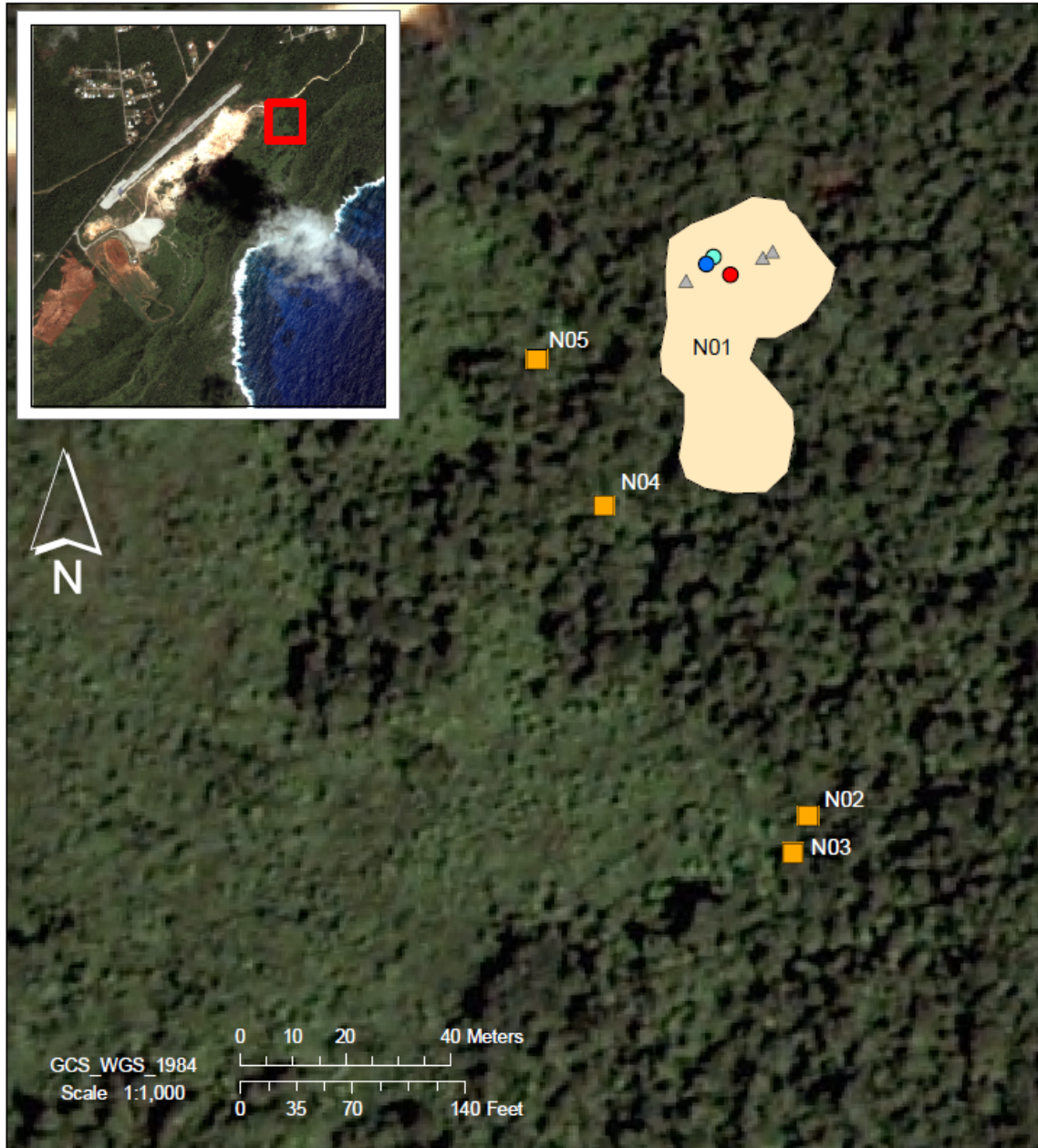


Figure 1
 Hypolimnas octocula
 and host plant sites
 RT 15 North Transect
 15 -24 July, Guam

Hypolimnas octocula

Type

- Butterfly
- Chrysalis
- Caterpillar
- Egg

▲ Bait_pans

■ Mixed host plant area

Host plant individual

Species

- *Elatostema calcareum*
- *Procris pedunculata*

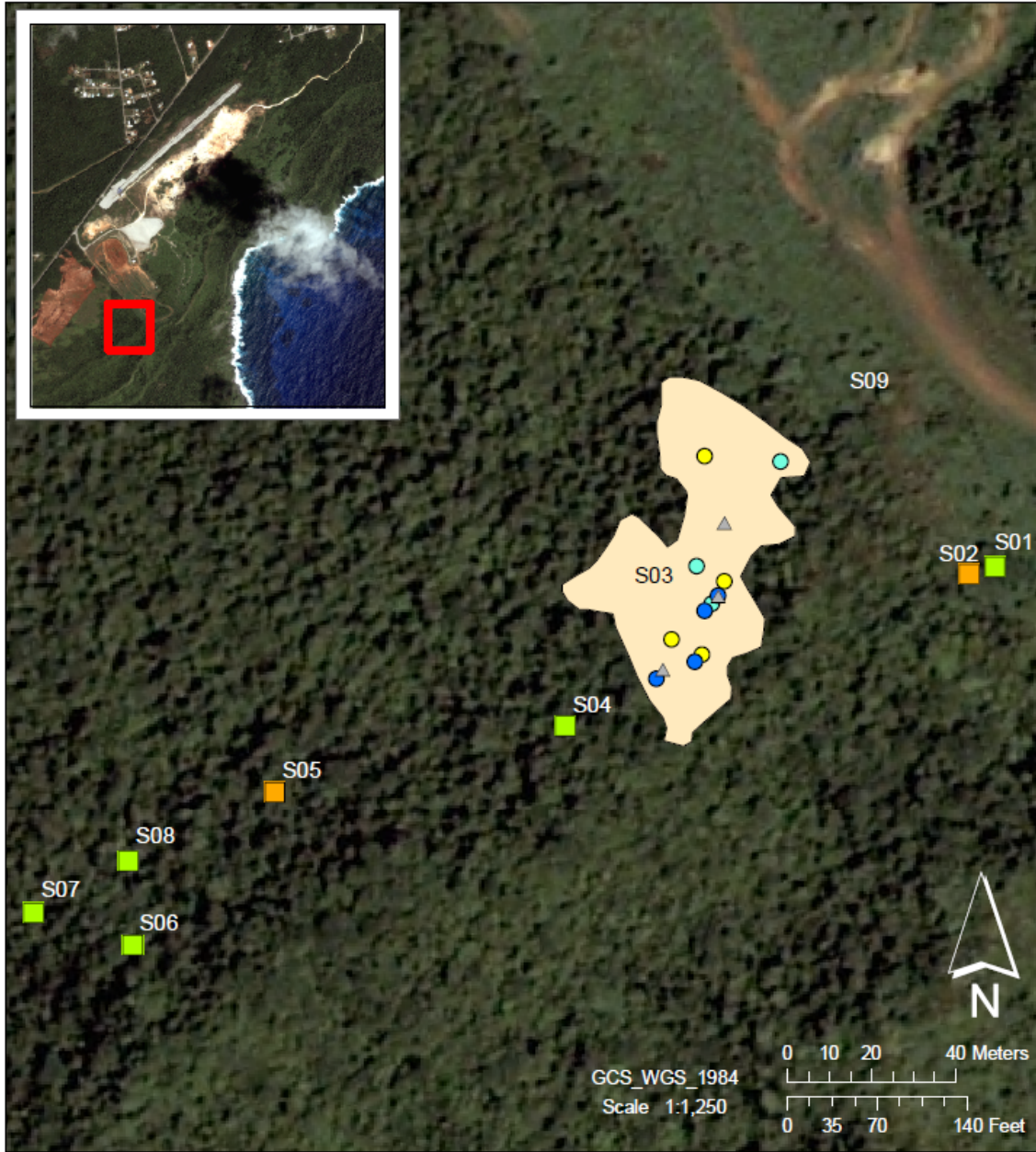


Figure 2
Hypolimnas octocula
 and host plant sites
 RT 15 South Transect
 15 -24 July, Guam

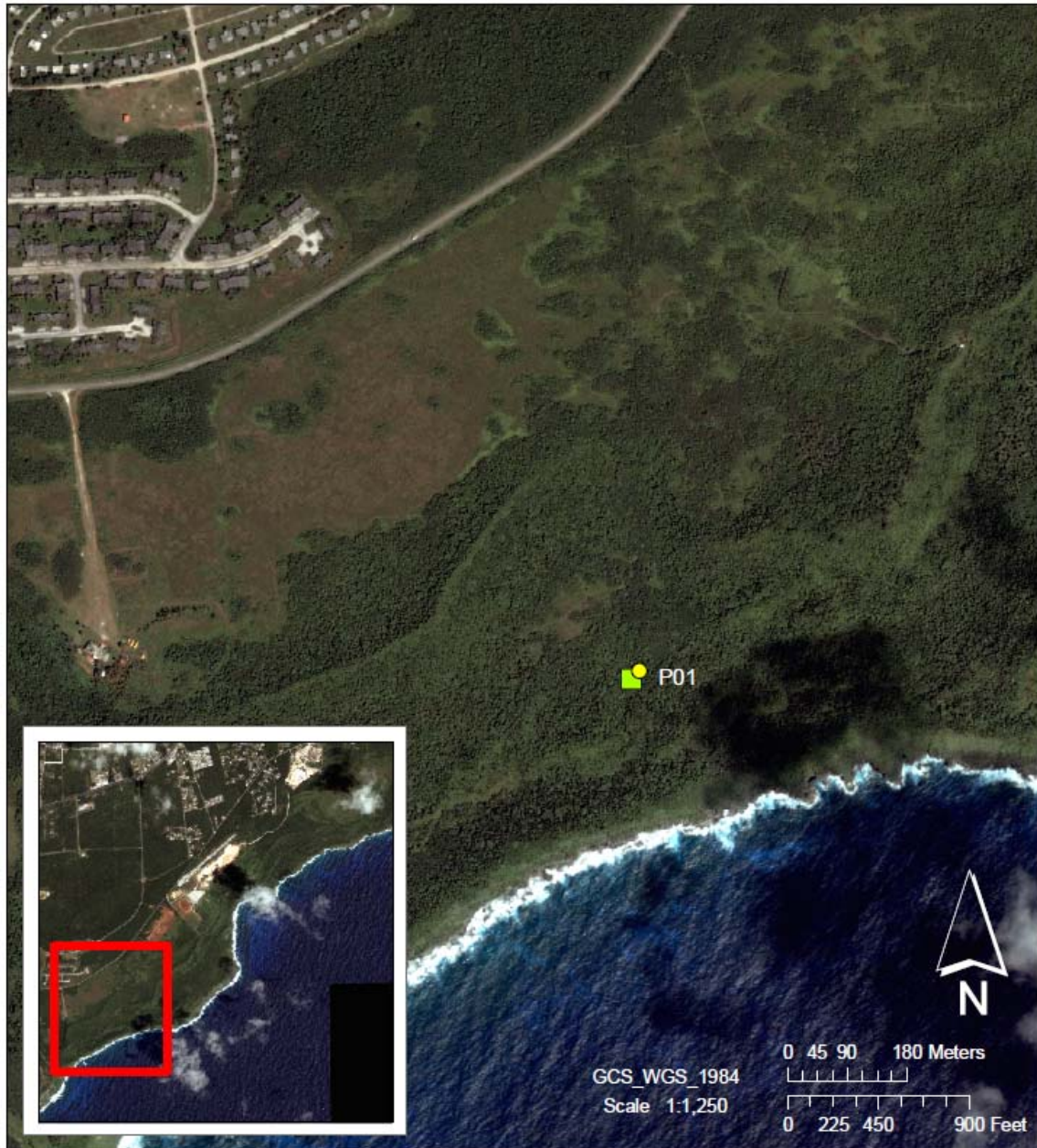
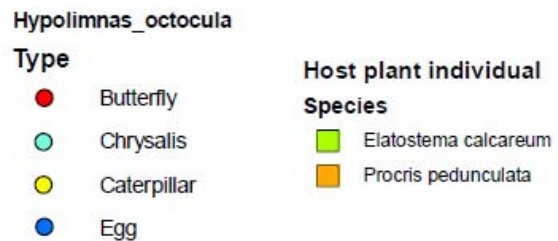


Figure 3
 Hypolimnas octocula
 and host plant sites
 RT 15 Pagat Cave Trail
 15 -24 July, Guam



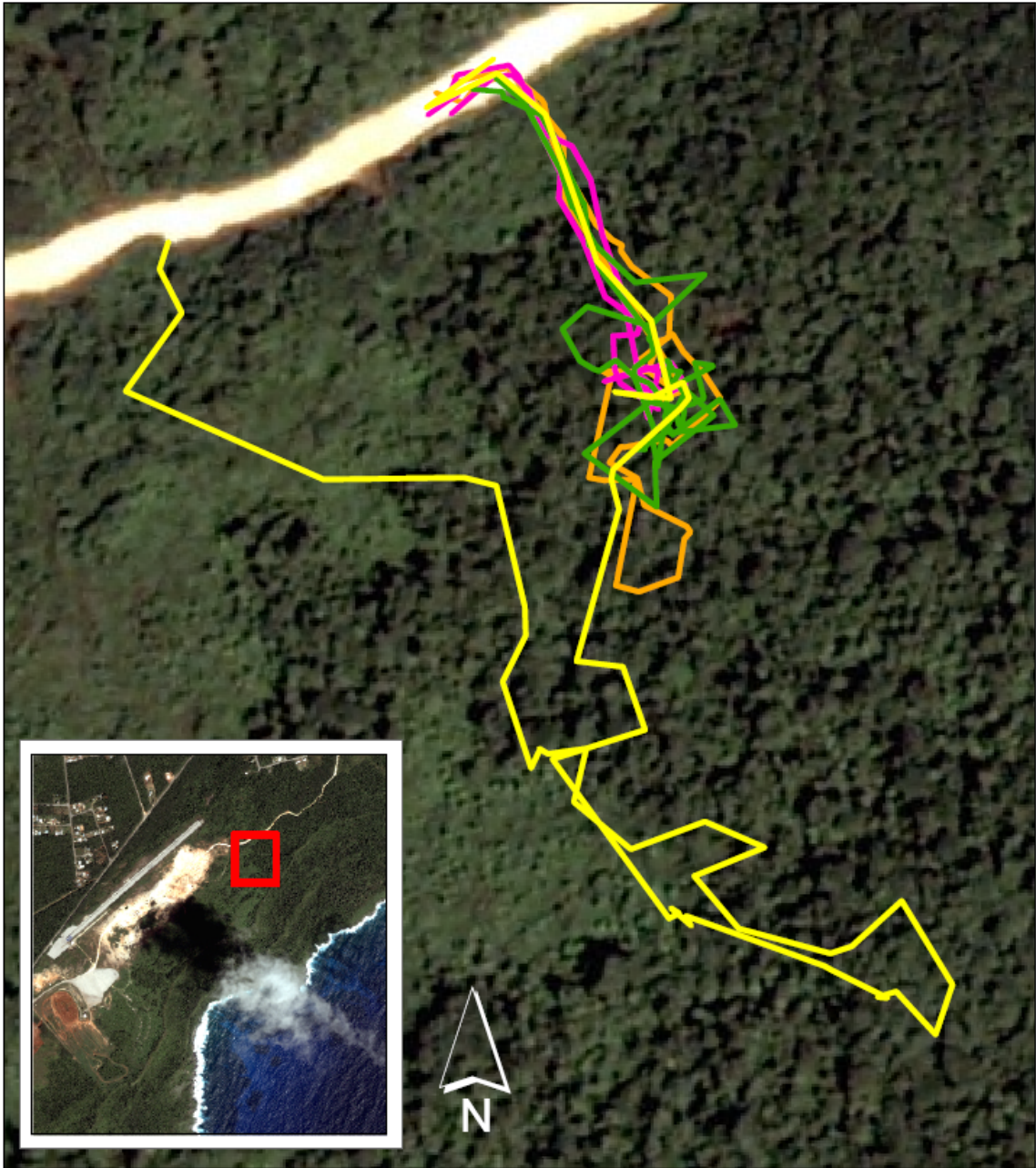
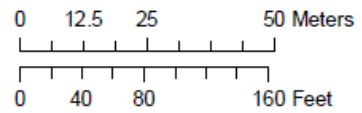


Figure 4
 RT 15 North Transect
 Survey Routes
 15 -24 July, Guam

- 15 July 2009
- 20 July 2009
- 22 July 2009
- 23 July 2009

GCS_WGS_1984
 Scale 1:1,133



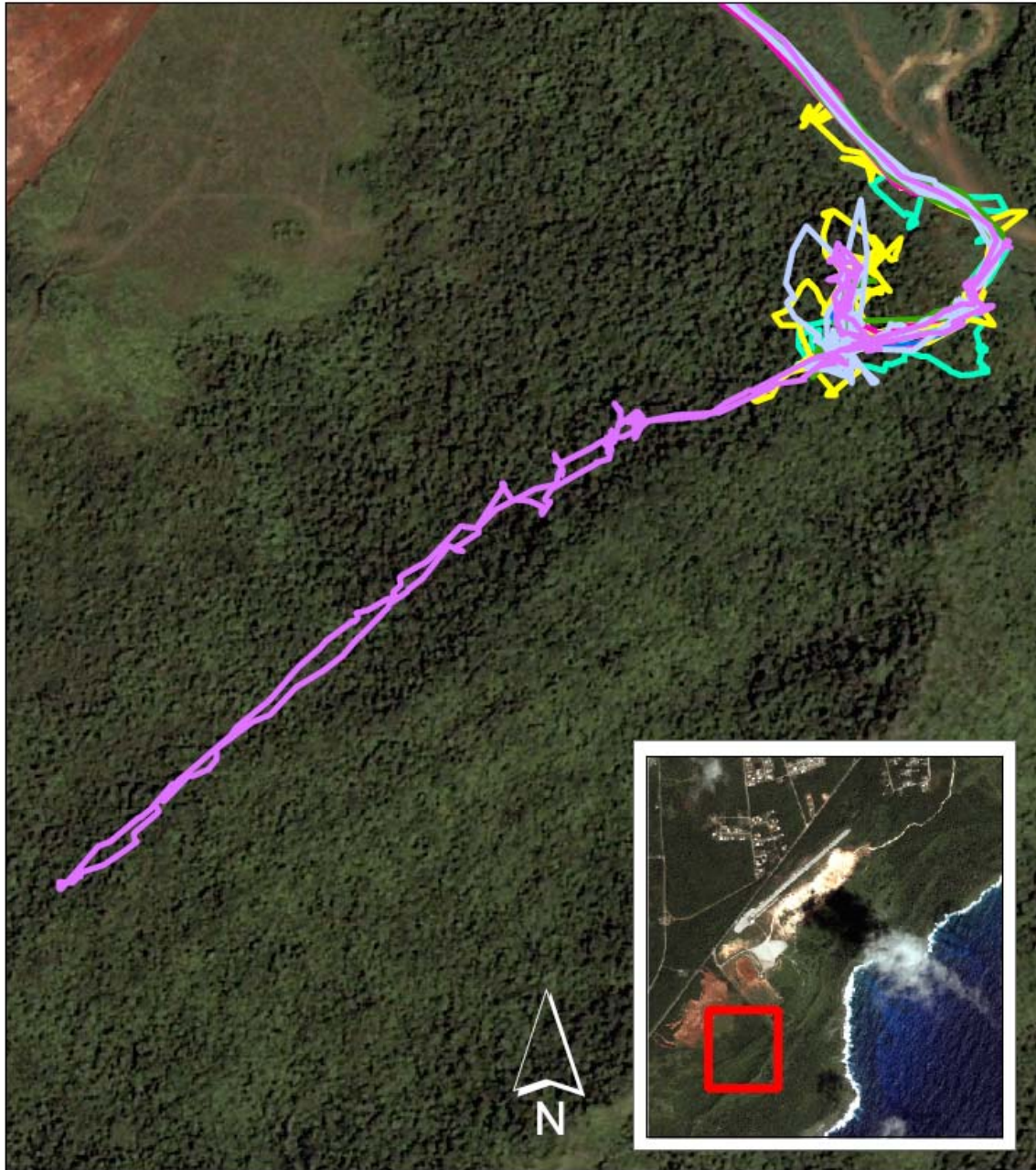


Figure 5
 RT 15 South Transect
 Survey Routes
 15 -24 July, Guam

- 16 July 2009
- 17 July 2009
- 20 July 2009
- 21 July 2009
- 22 July 2009
- 23 July 2009
- 24 July 2009

GCS_WGS_1984
 Scale 1:2,444

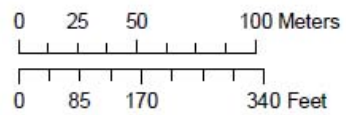




Figure 6
 RT 15 Pagat Cave Trail
 Survey Route
 15 -24 July, Guam

— 22 July 2009

GCS_WGS_1984
 Scale 1:6,200

