Molecular analysis of *Oryctes rhinoceros* collected from Philippines (January 2017)

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CONTENTS

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1. EXECUTIVE SUMMARY 4
2. BACKGROUND 5
3. METHODS 6
   3.1 Collection and DNA extraction of CRB tissue 6
   3.2 PCR-RFLP detection of CRB-G haplotype 6
   3.3 PCR detection of OrNV infection 7
   3.3 Histopathology detection of OrNV infection 7
4. RESULTS AND DISCUSSION 7
5. RECOMMENDATIONS 9
6. ACKNOWLEDGEMENTS 9
7. REFERENCES 9
8. APPENDIX 1 10

1. EXECUTIVE SUMMARY

Prior to initial detection of *Oryctes rhinoceros* (coconut rhinoceros beetle; CRB) on Guam in 2007, *Oryctes rhinoceros* nudivirus (OrNV) was effective in suppressing and maintaining CRB populations at low levels on Pacific islands for over 40 years. However, to date the CRB population that has invaded Guam (CRB-G haplotype) has so far proven recalcitrant to currently available OrNV isolates that are able to cause disease in other CRB populations. OrNV is known to be widespread across the Philippines, where CRB is considered as native, and the CRB-G haplotype has been identified from here. Native habitats provide a good opportunity to identify candidate OrNV isolates (and other potential biocontrol agents).

The objective of this project was to begin searching for new OrNV isolates that could be effective against the CRB-G haplotype. Identification of an OrNV isolate with good efficacy against CRB-G would assist in preventing further mortality and damage to coconut palms on CRB-G infested Pacific islands (such as Guam), and slow or halt its spread into other areas of the Pacific region.

CRB tissue samples were obtained from live CRB adults collected using both pheromone trapping and hand collection from the Philippines. Gross observation and molecular DNA analyses were used to determine the OrNV infection status and haplotype of individual specimens. The pheromone trapping results were disappointing, with only a single CRB adult caught; however, hand collection enabled a number of adults and larvae to be collected. From the CRB specimens collected in the Philippines, one OrNV infected CRB adult was identified. This specimen was confirmed to be of the CRB-G haplotype, and OrNV from the infected tissue was isolated to prepare inoculum for testing efficacy against CRB-G.

Recommendations:

- Begin testing the new isolate as potential biocontrol agent for CRB-G.
- It is still important to continue searching for OrNV isolates (and other potential biocontrol agents) in regions known to harbor CRB-G populations, particularly where CRB caused palm damage is not reported as an issue.
- As the current CRB lure does not appear to be efficient at attracting CRB-G adults, new lures that can attract CRB-G adults need to be developed.

2. BACKGROUND

The coconut rhinoceros beetle (CRB),*Oryctes rhinoceros*, is a major pest of coconut palm, oil palm and other palm species. Palms are damaged when adult beetles bore into the crowns of palms to feed on sap. Tree mortality occurs when beetles destroy the growing tip (meristem). Immature beetles (larvae) do no damage; they feed on dead, decaying vegetation in breeding sites. Preferred breeding sites are dead, standing coconut stems, and piles of decaying vegetation such those left behind by typhoons or after replanting of oil palm plantations. If a CRB population is not suppressed, it is possible for a positive feedback cycle to initiate whereby adult beetles kill massive numbers of palms, thereby generating more food for even more grubs that turn into adults which kill even more palms. An outbreak following this scenario occurred in the Palau Islands during the late 1940s resulting in about 50% of the coconut palms being killed by CRB throughout the archipelago and 100% mortality on some of the smaller islands (Gressitt, 1953).

Following 40 years of no geographical range expansion, CRB is on the move in the Pacific. CRB has been detected for the first time at several Pacific Island locations including Saipan (2006), Guam (2007), Port Moresby, Papua New Guinea (2010), Oahu, Hawaii (2013), and Honiara, Solomon Islands (2015). Eradication of CRB is extremely difficult, having been achieved only once, on Niuatoputapu (Keppel) Island, an island with an area of only 16 km^2 belonging to the Kingdom of Tonga (Catley, 1969). Failing eradication, the usual response to CRB infestations during the second half of the 20th century was introduction of OrNV, the biological control agent of choice for this pest. OrNV infects *Oryctes rhinoceros*, typically reducing CRB damage by up to 90% with population suppression lasting indefinitely (Bedford, 2013). OrNV is auto disseminated, meaning the pathogen is carried between breeding sites by CRB adults. Like many biocontrol agents, OrNV is density dependent, working best at high population densities of CRB. Highly pathogenic OrNV isolates suppress population growth to levels, which result in only minor damage to palm species.

Current invasions of Pacific Islands by CRB involve a new invasive biotype (CRB-G) that has escaped from biological control by OrNV (Marshall et al.). Discovery of *Oryctes rhinoceros* nudivirus in the 1960s enabled the successful management of populations in Pacific Island Countries. Augmentative release of OrNV continues to be an important mechanism for CRB management in both coconut and oil palm growing regions. For 40 years after adoption of this biocontrol strategy, no new outbreaks of CRB were reported from uninfested palm growing islands in the Pacific ensuring continuity of palm based village economies. However, the situation has recently changed. For the first time in 40 years, CRB invasion into completely new areas has been reported. Additionally, Pacific areas with established CRB populations (e.g. Palau) have reported increased severity and frequency of CRB damage. Common to all these areas is the high incidence of severe palm damage not seen since the introduction of OrNV. Initial attempts to introduce OrNV into the Guam CRB population (CRB-G) were unexpectedly unsuccessful, raising the possibility that the CRB-G population that invaded Guam become tolerant or resistant to the commonly applied OrNV isolates. Analysis of several CRB populations has demonstrated that, in addition to Guam, the CRB-G biotype is also found in Hawaii, Palau, and most recently in Port Moresby (PNG) and Honiara (Solomon Islands). Within the native range of CRB, the CRB-G biotype has been detected in Taiwan, Indonesia, Malaysia, and Philippines (Marshall et al., 2017; Reil et al., 2016).

Uncontrolled infestations of CRB may kill most palms within a few years, as is currently being observed. A worse case scenario can be triggered by a massive outbreak of adult CRB emerging from abundant breeding sites caused by large amounts of decaying vegetation left in the wake of a typhoon. This is the situation in Guam, which was visited by Typhoon Dolphin in May, 2015. Very high feeding activity will kill mature coconut palms, leaving standing dead coconut trunks that are ideal breeding sites for subsequent generations of beetles. During a CRB outbreak, there will be an increased risk of further spread to uninfested islands throughout the Pacific through transportation networks. Palms are important on Pacific Islands for various reasons: as a cash crop for nuts, oil and lumber, as an ornamental tree appreciated by residents and tourists. On some of

the smaller, more traditional islands the coconut palm is referred to as the tree of life. Here, this species is an essential natural resource providing income, housing, food, oil, soap, clothing, mats, baskets, and other containers. The smaller, poorer Pacific islands will suffer the most if the spread of CRB-G cannot be controlled.

The objective of this project was to begin searching for new OrNV isolates that could be effective against the CRB-G haplotype. Native habitats provide a good opportunity to identify candidate OrNV isolates (and other potential biocontrol agents). OrNV is known to be widespread across the Philippines, where CRB is considered as native, and the CRB-G haplotype has been identified from here. Identification of an OrNV isolate with good efficacy against CRB-G would assist in preventing further mortality and damage to coconut palms on CRB-G infested Pacific islands (such as Guam), and slow or halt its spread into other areas of the Pacific region.

3. METHODS

3.1 Collection and DNA extraction of CRB tissue

CRB specimens were collected using both hand collection (larvae and adults) and 'DeFence Traps' (hanging Tekken fish gill netting) fitted with the CRB attractant, Oryctalure (www.chemtica.com). Gut tissue samples from live CRB specimens were dissected and used for DNA extraction because decaying tissue is often of limited value in terms of DNA quality. To ensure DNA quality was maintained, a 0.5 - 1 cm piece of the midgut tissue was submerged in monopropylene glycol (PPG), and stored at -20°C.

DNA was extracted from CRB tissue using a ZR Genomic DNA Tissue MiniPrep (Zymo Research) kits. DNA elution was carried out using 100 µl of elution buffer from the appropriate kit, and aliquots of eluted DNA samples were subsequently used for further analyses.

3.2 PCR-RFLP detection of CRB-G haplotype

The primers used to amplify a 523 base pair (bp) fragment of the CRB COI gene were C1-J-1718Oryctes (5'- GGAGGTTTCGGAAATTGACTTGTTCC -3') and C1-N-2191Oryctes (5'-CCAGGTAGAATTAAAATRTATACCTC -3') (Marshall et al., 2017). Each 25 µl PCR reaction constituted 0.125 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10x PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (10 mM), 0.5 µl C1-J-1718Oryctes (10 µM), 0.5 µl C1-N-2191Oryctes (10 µM), 2 µl diluted (1 in 50) DNA template, and 18.75 µl water. PCR amplifications were performed in a C2100 (BioRad) thermocycler with a cycling profile of 35 cycles of 94°C denaturation (30 s), 50°C annealing (45 s), 72°C extension (1 min) with an initial denaturation of 3 min at 94°C and a final extension of 5 min at 72°C. A 5 µl aliquot of each PCR reaction was checked by agarose gel electrophoresis (1%, 0.5xTBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVIdoc HD2 gel doc (UVItech). For RFLP analysis (Marshall et al., 2017), successfully amplified COI PCR products (8 µl) were each combined with 0.2 µl Mse1 (10U/µl; New England BioLabs, NEB), 1 µl 10x NEB Buffer#4, 0.1 µl 100x NEB BSA and 10.7 µl water, and incubated at 37°C for 3 h. Digested samples (10 µl) were mixed with DNA loading dye, loaded onto on a 2% agarose gel in 0.5xTBE buffer. The gel was electrophoresed using 60 V for 1.5 h, stained with RedSafe and DNA fluorescence detected over UV light. Photographs were taken using an UVIdoc HD2 gel doc.

3.3 PCR detection of OrNV infection

The PCR protocol for detection of OrNV was based on that described in Richards et al. (1999), and has been subsequently modified to distinguish infection from mere presence (e.g. incidental contact contamination). The primer pairs used to amplify a 945 base pair (bp) fragment of the OrNV genome were OrNV15a (5'-ATTACGTCGTAGAGGCAATC-3') and OrNV15b (5'-ATGATCGATTCGTCTATGG-3')(Richards et al., 1999). Each 25 µl PCR reaction contained 0.2 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10x PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (10 mM), 0.5 µl OrNV15a (10 µM), 0.5 µl OrNV15b (10 µM), 1 µl diluted DNA (paired reactions of 1 in 100 and 1 in 5000), and 19.8 µl water. PCR amplifications were performed in a C2100 (BioRad) thermocycler with a cycling profile of 35 cycles of 94°C denaturation (30 s), 50°C annealing (45 s), 72°C extension (1 min) with an initial denaturation of 3 min at 94°C and a final extension of 5 min at 72°C. An 8 µl aliquot of each PCR reaction was separated by agarose gel electrophoresis (1%, 0.5xTBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVIdoc HD2 gel doc (UVItech). Detection of OrNV PCR product in the 1 to 5000 dilution was considered indicative of OrNV infection (unpublished data; validated in combination with gross visual inspection and histological analysis based on pathology description (Huger, 2005)).

3.3 Histopathology detection of OrNV infection

Histopathology studies were performed on tissue displaying visual symptoms of OrNV infection. Dissected midgut tissue was fixed in FAA fixative (5% formaldehyde, 2.5% acetic acid, 50% ethanol as an aqueous solution) for at least 48 h. Standard paraffin embedding, serial sectioning and hematoxylin and eosin staining methods were carried out on samples by Gribbles Veterinary Services (Christchurch, New Zealand). Stained slides of the alimentary tract were examined under brightfield and differential interference contrast (DIC) optics using an Olympus BX50 upright microscope and photographed with an Olympus DP-72 digital camera.

4. RESULTS AND DISCUSSION

A total of 214 CRB larvae, pupae, and adults were collected from within the Dumagate-Dauin region of Negros Island, Philippines. All larvae were collected by hand from breeding sites. Adults were collected by three methods: by hand from breeding sites (n=39), attracted to household lighting (n=3), in pheromone traps (n=1). The single OrNV-infected beetle was caught by hand after it appeared at the illuminated house window. Pheromone traps were ineffective. Only a single beetle was caught in six traps deployed for a total of 31 trap-nights. Trap catch rate was 0.03 beetles per trap-night. One trap, which caught nothing, was deployed at a very active breeding site at a coconut sawmill.

Despite a high CRB population in the Dumaguete-Dauin region, as indicated by easily detected breeding sites and V-shaped cuts to between 20% and 40% of coconut palms, damage was very light. We saw no coconut palm mortality.

Tissue from 94 individuals was preserved for further analysis (see Appendix 1 for specimen details). Only one specimen (a hand caught adult female; Dug-42) displayed the symptoms of OrNV infection (thick, milky coloured gut; see Huger, 2005). From this collection of preserved tissue specimens, 16 representative samples (including Dug-42) were selected for molecular analysis to determine haplotype and OrNV infection status. A summary of the results is presented in Table 1.

Amplification of the partial COI gene failed for specimens Dug-55, -56, and -57; therefore, the CRB-G haplotype status could not be determined from these specimens. As COI is present in all host cells, this meant that the OrNV infection status could also not be resolved, despite the observation of OrNV DNA in specimens Dug-55 and -56. For the remaining 13 specimens, all were of the CRB-G haplotype, while only Dug-42 was positive for OrNV infection. Histopathology examination of Dug-42 gut tissue revealed diagnostic indicators of OrNV infection as described in Huger (Huger, 2005).

Since three separate methods (visual, DNA, histology) indicated OrNV presence, we conclude that the Dug-42 tissue was infected with OrNV. Isolation of OrNV from Dug-42 tissue has been undertaken, and early results from testing this material on the OrNV permissive cell line DSIR-Ha-1179 have shown the expected cytopathic effects (data not shown). If the early cell culture results are confirmed, the next step will be to conduct a pathogen challenge assay using the Dug-42 OrNV isolate against CRB-G.

Table 1: Summary of haplotype and OrNV infection status for CRB collected from Negros Island, Philippines.

Specimen[1]	COI PCR	OrNV PCR	Virus present[2]	Haplotype
Dug-1	+	-	No	CRB-G
Dug-2	+	-	No	CRB-G
Dug-16	+	-	No	CRB-G
Dug-17	+	-	No	CRB-G
Dug-18	+	-	No	CRB-G
Dug-19	+	-	No	CRB-G
Dug-42	+	+	Yes	CRB-G
Dug-43	+	-	No	CRB-G
Dug-44	+	-	No	CRB-G
Dug-45	+	-	No	CRB-G
Dug-55	-	+	Undetermined	Undetermined
Dug-56	-	+	Undetermined	Undetermined
Dug-57	-	-	Undetermined	Undetermined
Dug-62	+	-	No	CRB-G
Dug-63	+	-	No	CRB-G
Dug-69	+	-	No	CRB-G

[1] Further specimen details are listed in Appendix 1.
[2] Based on DNA analysis.

5. RECOMMENDATIONS

- Begin testing the new isolate as potential biocontrol agent for CRB-G.
- It is still important to continue searching for OrNV isolates (and other potential biocontrol agents) in regions known to harbor CRB-G populations, particularly where CRB caused palm damage is not reported as an issue.
- As the current CRB lure does not appear to be efficient at attracting CRB-G adults, new lures that can attract CRB-G adults need to be developed.

6. ACKNOWLEDGEMENTS

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8. APPENDIX 1

Details of CRB tissue specimens collected from Philippines in January 2017.

Date	Specimen ID	Site	Collection Method	Stage/Gender	Condition	PPG[1]	FAA[2]	Alive[3]
26/01/2017	Dug1	Farm log2	Fallen log By hand	L3 -large	slow, reddish colour	1	1	1
26/01/2017	Dug2	Farm log2	Fallen log By hand	L3 -large	slow, red all over (almost purple)	1	1	1
26/01/2017	Dug3	Farm log2	Fallen log By hand	L3 -large	slow, reddish colour, stripped, a little purple	1	1	1
26/01/2017	Dug4	Farm log2	Fallen log By hand	L3 -large	slow, reddish colour	1	1	1
26/01/2017	Dug5	Farm log2	Fallen log By hand	L3 -small	slow, reddish colour	1	1	1
26/01/2017	Dug6	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug7	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug8	Farm log2	Fallen log By hand	L2	normal	1	0	0
26/01/2017	Dug9	Farm log2	Fallen log By hand	L2	normal	1	0	0
26/01/2017	Dug10	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug11	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug12	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug13	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug14	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug15	Farm log2	Fallen log By hand	L3 -small	normal	1	0	0
26/01/2017	Dug16	Lumber yard, Sibulan	Sawdust By hand	Male	vigorous, active, fatty, brown medium gut	1	1	1
26/01/2017	Dug17	Lumber yard, Sibulan	Sawdust By hand	Male	reasonabley active, not fatty, thin light brown gut	1	1	1
26/01/2017	Dugg18	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg19	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg20	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg21	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg22	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg23	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg24	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg25	Farm log1	Fallen log By hand	L3	normal	1	0	0
26/01/2017	Dugg26	Lumber yard, Sibulan	Sawdust By hand	L3 -small	flacid	1	1	1

26/01/2017	Dug27	Lumber yard, Sibulan	Sawdust By hand	L3 -small	flacid	1	1	1
26/01/2017	Dug28	Lumber yard, Sibulan	Sawdust By hand	prepupa	flacid, very fatty, lots liquid	1	1	1
26/01/2017	Dug29	Lumber yard, Sibulan	Sawdust By hand	prepupa	very fatty	1	1	1
26/01/2017	Dug30	Lumber yard, Sibulan	Sawdust By hand	L3 -large	soft flacid posterior, firm anterior (white tissue)	1	1	1
26/01/2017	Dug31	Lumber yard, Sibulan	Sawdust By hand	prepupa	very fatty	1	1	1
26/01/2017	Dug32	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	1	1
26/01/2017	Dug33	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	1	1
26/01/2017	Dug34	Lumber yard, Sibulan	Sawdust By hand	L3 -small	normal	1	0	0
26/01/2017	Dug35	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Dug36	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Dug37	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Dug38	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Dug39	Lumber yard, Sibulan	Sawdust By hand	L3 -small	normal	1	0	0
26/01/2017	Dug40	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
26/01/2017	Dug41	Lumber yard, Sibulan	Sawdust By hand	L3 -large	normal	1	0	0
28/01/2017	Dug42	Grandma home	window by hand	Female	live, fatty, thick white gut	1	1	1
28/01/2017	Dug43	Grandma home	window by hand	Male	live, thin brown gut (kept in same bottle as #42 for 2 days)	1	1	1
28/01/2017	Dug44	Bascofadco coop, Bacong	By hand	Male	live, thin brown gut	1	1	1
28/01/2017	Dug45	Bascofadco coop, Bacong	By hand	Male	live, thin brown gut	1	1	1
28/01/2017	Dug46	Bascofadco coop, Bacong	By hand	Male	live, thin brown gut	1	1	1
30/01/2017	Dug47	Ton home, Dauin	stumps/logs By hand	Male	live, fatty, thick brown gut	1	1	1
30/01/2017	Dug48	Ton home, Dauin	stumps/logs By hand	Teneral Male	live, fatty, thick brown gut	1	1	1
30/01/2017	Dug49	Ton home, Dauin	stumps/logs By hand	Male	live, thick brown gut	1	1	1
30/01/2017	Dug50	Ton home, Dauin	stumps/logs By hand	Teneral Male	live, fatty, thick brown gut	1	0	1

30/01/2017	Dug51	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	0	1
30/01/2017	Dug52	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	0	1
30/01/2017	Dug53	Ton home, Dauin	stumps/logs By hand	Male	live, thick brown gut	1	0	1
30/01/2017	Dug54	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	0	1
30/01/2017	Dug55	Ton home, Dauin	stumps/logs By hand	L3	found dead in breeding site log	1	0	1
30/01/2017	Dug56	Ton home, Dauin	stumps/logs By hand	L2 (x6)	composite sample	1	0	1
30/01/2017	Dug57	Ton home, Dauin	stumps/logs By hand	L3	found dead in log	1	0	1
28/01/2017	Dug58	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
28/01/2017	Dug59	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
28/01/2017	Dug60	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
28/01/2017	Dug61	Bascofadco coop, Bacong	Fallen log by hand	L3	Healthy looking	1	0	0
1/02/2017	Dug62	Aubrey's, Lunga	balcony light	Female	live, thin brown gut	1	1	1
1/02/2017	Dug63	Aubrey's, Lunga	Pheromone trap	Male	live, thick brown gut	1	1	1
31/01/2017	Dug64	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Dug65	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Dug66	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut, very fatty	1	1	1
31/01/2017	Dug67	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Dug68	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Dug69	palm plantation, San Jose	stumps/logs By hand	Female	live, thin light brown gut ,	1	1	1
31/01/2017	Dug70	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut, fatty	1	1	1
31/01/2017	Dug71	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Dug72	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Dug73	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Dug74	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Dug75	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Dug76	palm plantation, San Jose	stumps/logs By hand	Male	live, medium brown gut	1	1	1

31/01/2017	Dug77	palm plantation, San Jose	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
31/01/2017	Dug78	palm plantation, San Jose	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
31/01/2017	Dug79	palm plantation, San Jose	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
31/01/2017	Dug80	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Dug81	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut	1	1	1
31/01/2017	Dug82	palm plantation, San Jose	stumps/logs By hand	Female	live, thin brown gut	1	1	1
31/01/2017	Dug83	palm plantation, San Jose	stumps/logs By hand	Male	live, thin brown gut , fatty	1	1	1
1/02/2017	Dug84	Ton home, Dauin	stumps/logs By hand	Male	live, thin dark brown gut	1	1	1
1/02/2017	Dug85	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	1	1
1/02/2017	Dug86	Ton home, Dauin	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
1/02/2017	Dug87	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut	1	1	1
1/02/2017	Dug88	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	1	1
1/02/2017	Dug89	Ton home, Dauin	stumps/logs By hand	Female	live, thin dark brown gut	1	1	1
1/02/2017	Dug90	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	1	1
1/02/2017	Dug91	Ton home, Dauin	stumps/logs By hand	Female	live, thin brown gut , fatty	1	1	1
1/02/2017	Dug92	Ton home, Dauin	stumps/logs By hand	Male	live, thin brown gut	1	1	1
1/02/2017	Dug93	Ton home, Dauin	stumps/logs By hand	Female	live, thin light brown gut	1	1	1
1/02/2017	Dug94	Ton home, Dauin	stumps/logs By hand	pupa (male)	all white (messy inards)	1	1	1

[1] 1=Tissue preserved using monopropylene glycol (PPG) for subsequent DNA analysis; 0= no PPG preservation of tissue.

[2] 1=Tissue preserved using FAA fixative (5% formaldehyde, 2.5% acetic acid, 50% ethanol as an aqueous solution) for subsequent histological analysis; 0= no FAA preservation of tissue.

[3] 1=Specimen was alive immediately prior to dissection; 0=specimen was already dead prior to dissection.