Identity and distribution of invasive land snails infected with Rat Lungworm in Hawaii: Surveys, identification guides, and taxonomic workshops (FINAL REPORT)

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Budget: $58,415

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Overview:

Hawaii has more recorded, established non-indigenous land snails (NILS; which includes slugs and semi-slugs) than any other island chain or archipelago in the Pacific (Cowie 2001). Many are agricultural and/or horticultural pests and pose a threat to natural ecosystems through competition, predation, and herbivory (Joe and Daehler 2008, Curry et al. 2016). They are also a public health risk and threaten food security as vectors of disease-causing parasites, including Rat Lung Worm (RLW), *Angiostrongylus cantonensis*, (Kim et al. 2014, 2018). The aim of the current project was to determine which species of non-indigenous land snails (NILS) are established in horticultural/agricultural facilities Hawaii and assess their distributions via five objectives:

1) Complete 18 surveys for NILS in horticultural/agricultural facilities across four Hawaiian Islands (Kauai, Oahu, Molokai, Big Island)

2) Compare these survey data with historical records to identify newly established species and identify NILS that pose the most serious threats to agriculture, native ecosystems, and public health

3) Develop an online guide with information about each of the established NILS in the Hawaiian Islands, including distribution, updated taxonomy, digital images, threats, and ecological data.

4) Organize and teach a taxonomic workshop on the NILS of Hawaii

5) Screen invasive snails for Rat Lungworm (RLW) and identify hotspots of likely disease transmission based on modeling by Kim et al 2018.

Background:

From 2004-2010 Hayes, Yeung, Kim, and Cowie, with students and collaborators, recorded 29 NILS from 237 sites across the 6 largest Hawaiian Islands, including 7 new state records and 31 new island records (Cowie et al 2008, Hayes et al 2012). Since 2010 no systematic efforts were funded to surveys for NILS broadly in the islands, although chance encounters have recorded additional NILS as have other research efforts not focused exclusively on NILS. As of 2015 there were 43 established NILS recorded, but this number was certainly an underestimate, as new species are surely

introduced and spread unabated without the efforts of continued monitoring and quarantine. Efforts to identify new introductions at ports of entry or prior to their widespread establishment are hampered by lack of up-to-date information on established NILS and their pathways. Without good guides and adequate training on the identification of Hawaii's NILS, most new introductions and their spread will continue to go unrecorded by inspectors, pest managers, import-export personnel, horticulturalists and resource managers, until a severe outbreak, or it is too late to control them without enormous financial investment. Taxonomic workshops coupled with the proposed online guide to NILS of Hawaii will fill a critical resource need for quarantine personnel and conservation and resource managers.

Rat lungworm (*Angiostrongylus cantonensis*), the cause of eosinophilic meningitis, is carried by at least 14 NILS found in Hawaii, most first recorded in in horticultural or agricultural sites. Sixty cases of the disease (2001-12) in Hawaii were attributed to consumption of produce contaminated with infected molluscs, and the number of cases continues to increase with reports of the disease on Big Island and Maui more recently. Increases may be related to the continued spread of infected NILS, and lack of knowledge regarding potential as vectors of the disease. For example, *Parmarion martensi* was only recorded on Oahu and Big Island during our 2004-2010 surveys. However, a lack of funding for continued surveys and monitoring, allowed this species, which is an effective host of RLW, to go undetected as it established on Maui (Cowie et al. 2018). Several cases of RLW on Maui have been attributed to *P. martensi*, which along with several other species serves as a vector for the continued spread of the disease. In addition to public health, food safety concerns, and economic impacts, RLW may be infecting already threatened native snail fauna with unknown impacts.

There is a critical need for information on identity, distributions, habitats and potential impacts (including RLW infection) of NILS in Hawaii. These data will facilitate effective management strategies, reducing spread and potential impacts, including the inadvertent transport of NILS among islands and into native habitats.

Methods:

For this project, we proposed to survey at least 18 horticultural/agricultural facilities across the islands of Kauai, Oahu, Molokai, and Hawaii, including facilities involved with import-export and outplanting native flora for restoration projects. Surveys consisted of one-person hour searches at all facilities by experienced snail researchers. All potential snail habitats (e.g. under mats and pots; in pots; along sidewalk crevices; perimeter of greenhouses) were searched, and we stopped searching when no additional new species were found after a period of 20 minutes. All live animals and empty shells were collected and returned to the laboratory for sorting and identification. Identifications were completed using both morphological and molecular approaches (i.e. DNA Barcodes). Using data from our previous research we developed a DNA barcode library for all known NILS in Hawaii, along with some of the most common NILS globally. This

genetic library was used to verify identification of representative species and assist with identification of cryptic and/or juvenile specimens, which can be particularly difficult to identify using traditional taxonomic characters. All specimens were vouchered at the Bishop Museum.

All species were screened for RLW following the protocol of Kim et al. (2014, 2018). When five or more individuals of a species were found at a site, up to five individuals/species/site were screened for RLW, and when fewer than five individuals of a species where found at a site, we screened all individuals. Species distributions and positive/negative results of RLW screenings were mapped using QGIS (2009).

Survey Results

We surveyed 24 sites (Fig. 1A), with five on Kauai, six on Oahu, eight on Molokai, and five on Hawaii island. A total of 1,017 specimens were collected representing 40 species. Three species represent new island records for Molokai (i.e. *Zonitoides arboreus, Hawaiia miniscula, Liardetia doliolum;* see supplemental table). The recorded number of individuals per species ranged from 1 for several species, to a maximum of 116 *Cyclotropis* sp. The second and third most abundant species were *Paropeas achatinaceum* and *Lissachatina fulica*, respectively. More than 30 individuals of each species were found in multiple sites. These three species along with six others have increased their ranges throughout the islands between funded surveys in 2004-10 and 2016-18 (Fig. 2).

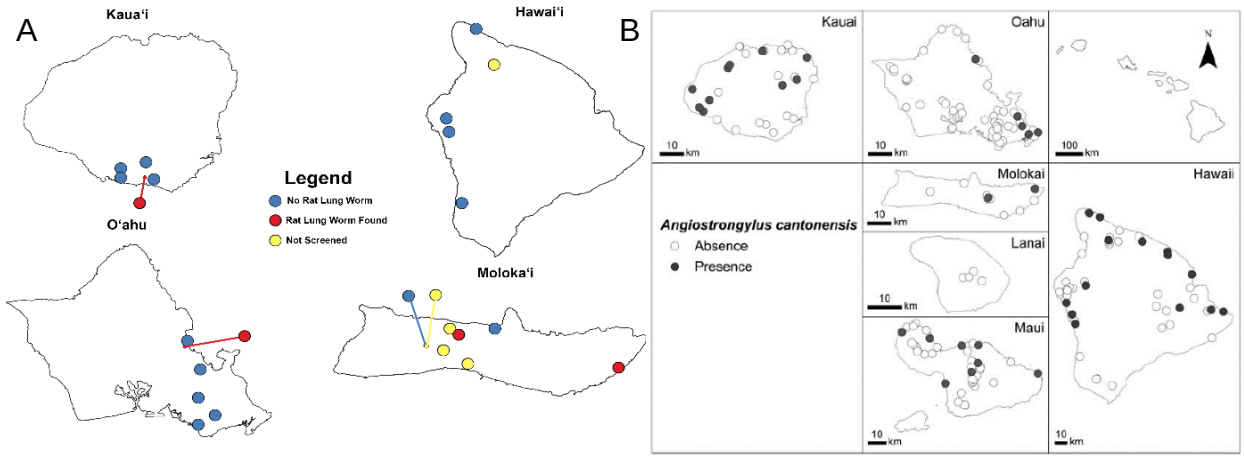

Figure 1A: The absence (blue, 15 sites) and presence (red circles, 4 sites) of *Angiostrongylus cantonensis* (RLW) at the survey sites. Sites in yellow indicated survey sites with unscreened NILS. 2B: Presence and absence of RLW across the Hawaiian Islands from Kim et al. (2019).

RLW Screening Results:

A total of 348 of the 1017 NILS we collected were screened for RLW. Seven individuals from 4 sites tested positive (Fig. 1A), three of which had not previously tested positive for RLW (Fig. 1A, B). The three most abundant species are known

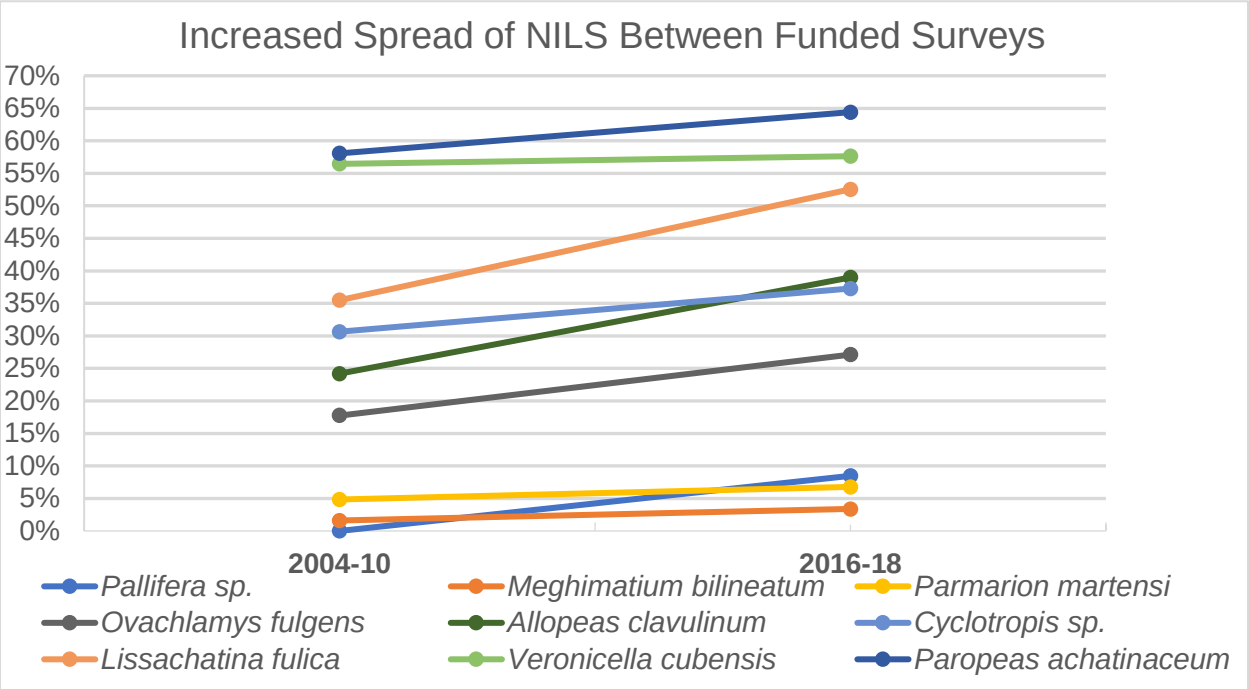

Figure 2. Graph showing the increase in nine NILS between funded survey periods.

carriers of the RLW parasite (Kim et al. 2014) and were found positive for carrying RLW in this study (see supplemental table).

Taxonomic Workshop Results

In June 2018, we organized a taxonomic workshop for 28 participants from various NGO and government agencies such as Hawaii Department of Agriculture (Plant Quarantine Branch, Plant Pest Control Branch), Hawaii Department of Land and Natural Resources (DOFAW, SEPP, NEPM), United States Department of Agriculture, Oahu Army Natural Resources Program, RCUH, Bishop Museum, Hawaii Invasive Species Council, US Fish and Wildlife Service, and Puu Kukui Watershed. This 7-hour intensive workshop provided hands on training in 1) invasive land snail species of global quarantine importance, 2) invasive land snails in Hawaii, 3) land snail dissections, 4) land snail identification based on external and internal morphology, and 5) how to survey for land snails.

Online guide to Hawaii's NILS

We purchased the domain hawaiisnails.org to host the online guide to land snails of Hawaii and have begun the development of the pages providing information on invasive land snails established in the state. The website is being developed so that it can be used from a computer and on mobile phones and tablets, making the information widely available, even in field conditions. To date we have developed the structure for the website, which will eventually host information for both native and non-native snails and have begun populating the pages with a list of non-native species found in Hawaii with images of shells and live specimens for most species. This alphabetical list will serve as

a jumping off point for those looking for information on species that they may encounter in Hawaii and give them a way to begin narrowing down the identity from among the more than 50 invasive snails established in the islands. We have compiled all the necessary information and have nearly completed all the taxonomic identifications and mapping to build species pages for all NILS currently established in Hawaii. Once these are complete, they will be added to the website and provide detailed profiles for each species, including native range, impacts, whether they are a vector of Rat Lung Worm, and taxonomic data. We will notify HISC when this website is available to the public

Lastly, we will eventually be developing a guide to the native land snails in Hawaii (pending funding support) to compliment the data available on the site. This will allow conservationists and other researchers comparative data for native and non-native species of land snails that they might encounter in their field work. Eventually we hope to expand this online guide to a detailed hard copy published version, and a mobile app for use by researchers and resource managers.

Discussion:

Current and accurate information of NILS distributions and spread in Hawaii facilitates the development of strategies aimed at reducing the likelihood of pests being inadvertently spread across the islands and can help prevent new introductions to Hawaii. In the absence of such monitoring, the numbers of introduced snails are likely to increase, and impact public health, agricultural, and tourism with greater intensity and frequency. Below we provide two examples of continued spread of NILS and the parasites they carry that were likely facilitated by a break in ongoing monitoring. While we are not certain that monitoring will prevent the spread or introduction of species, ongoing efforts to detect such events early can surely have a substantial impact on control efforts and prevent species from establishing.

In 2017, we were able to acquire funds via MISC to survey for non-native snails on the island of Maui, with the aim of screening for RLW. The last systematic surveys for NILS on Maui were done by us more than a decade ago. We conducted 18 surveys and found new records of NILS on Maui, confirming the unabated spread that is allowed to continue without support for ongoing monitoring. Additionally, several taxa such as *Veronicella cubensis, Lissachatina fulica, Cyclotropis* sp., *Pallifera* sp., *Parmarion martensi*, and four others have increased their ranges within and among islands (Fig. 2). All of these species, with the exception of *Pallifera* sp. are known vectors of RLW (Kim et al. 2014). All of these species are known to have significant negative impacts to our agricultural/horticultural trade and in restoration areas within our forest reserves. Additionally, our data found six additional areas on Maui (Yeung et al. 2018), which were not previously recorded as harboring RLW (Kim et al. 2014), with snails that tested positive for the parasite. These are in addition to the four new sites with RLW in the current study (Fig 1A).

In January 2019 we conducted surveys on Lanai (funding support from USDA). As with the previous example, the last known systematic surveys for NILS on Lanai were conducted by us more than a decade ago. In the recent surveys we recorded the presence of live *Euglandina* spp. (Rosy Wolf Snail) in Lanai City and quickly assessed the distribution of this species. Prior to this, no live *Euglandina* spp. had ever been found on Lanai. This snail has been implicated in the extinction of many of our native land snails (Hadfield 1986, Gerlach 2001) and its establishment on Lanai is a serious concern for the conservation of our remaining native land snails on the island, including the recent ESA listed *Partulina variabilis* and *P. semicarinata*. Because we were able to detect the establishment of this species on Lanai relatively early, we were able to determine that this species may not have spread into the native forests, yet. As such, it is highly likely that given the limited distribution in a small area of the island that it may be contained and eradicated before it spreads to critical areas. However, without such efforts, it will surely spread, ultimately impacting the few remaining Lanai endemic species.

These two examples are a small sampling of the potential implications of the continued, unmonitored spread of NILS, and the importance for support to maintain a minimal threshold of monitoring to keep some of the most dangerous NILS in check. From this recent study, we documented three species new to Molokai (i.e. *Zonitoides arboreus, Hawaiia miniscula, Liardetia doliolum*). *Zonitoides arboreus* and *Liardetia doliolum* are agricultural pests and recorded hosts of RLW. We also recorded *Cyclotopis* sp. being most abundant across the islands with *Paropeas achatinaceum* and *Lissachatina fulica* also found in high abundance in multiple sites. All three of these species were previously recorded as carrying RLW. *Cyclotropis* sp. can be easily missed by inexperienced snail surveyors as the shell is brown and less than 3 mm in shell height. Both *Paropeas achatinaceum* and *Lissachatina fulica* were found to be positive for RLW in our screening.

There is a critical need for updated information on identity, distributions, habitats and potential impacts of NILS in Hawaii, and not just for now, but as an ongoing process. These data will facilitate effective management strategies, reducing spread and potential impacts, including the inadvertent transport of NILS among islands and into native habitats.

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