

Project Title	Using genetic techniques to determine the unknown diversity and possible alien origin of sponges present in Hawaii
Agency, Division	University of Hawaii, Hawaii Institute of Marine Biology
Total Amount Requested	\$114,200
Amount Awarded	\$49,145
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Project Start Date	1-Oct-18
Estimated Project End Date	31-May-20

Efforts to detect and prevent alien introductions depend on understanding which species are already present<sup>1-3</sup>. This is particularly important when working with taxonomically challenging groups like marine sponges (phylum Porifera), where morphological characters are highly limited, and misidentifications are common<sup>4</sup>. Although sponges are a major component of the fouling community, they remain highly understudied because they are so difficult to identify<sup>4</sup>. The Keyhole Sponge is already present in Hawai'i<sup>5,6</sup>, but others like *Terpios hoshinota*, which is invading many locations across the Pacific<sup>7,8</sup>, kills corals and turns the entire reefscape into a gray carpet that would be devastating to Hawai'i tourism if introduced here. However, many gray sponges look alike, and it is only through the combined use of morphological and genetic characters that most sponges can be identified reliably<sup>4</sup>.

To date, there have been very few taxonomic assessments of sponges in Hawai'i<sup>9-14</sup>, and only the most recent of these has included any DNA barcodes in an effort to confirm the visual identifications<sup>15</sup>. Most of the early studies did not provide museum specimens or even detailed descriptions about how the species were identified, and the few vouchers that exist from these studies were dried which precludes DNA comparisons. These early studies also describe very few morphological characters to justify their identification, and none of them, including the most recent<sup>15</sup> compared their identifications to the original holotype specimens. Thus, it is difficult to use these previous studies as a baseline dataset against which to determine which species might be native, and which have already been introduced without anyone realizing.

To address this essential knowledge gap, our project sought to provide a baseline of the unknown diversity and try to infer the likely origin of sponges present in Hawai'i, with particular focus on adding samples from Honolulu Harbor. This work adds to an ongoing survey of Kāne'ohe Bay, a location that has been intensively surveyed by previous efforts, to determine which species of sponges were missed by previous studies, which are cryptogenic (of unknown origin), and which may have been introduced by human activities. Based on this new detailed survey of the sponges, we hoped to begin to develop an identification guide and accompanying genetic database against which to compare sponge samples from throughout Hawai'i. The resulting collection of sponges with vouchered specimens, DNA barcodes, morphological and histological characters is the most complete to date in the state and provides a valuable starting point as database from which future early detection tools (such as eDNA from harbors, ballast seawater samples or hull scrapings around O'ahu) might be developed.

The funding for this project awarded 9 months of post-doctoral fellowship (plus associated costs for sample processing) for Jan Vicente to make progress on the collection and vouchering of 116 sponges from Kewalo Marina, Ke'ehi Harbor, and Kapālama pier in collaboration with the Aquatic Invasive Species team from the Division of Aquatic Resources. All sponges were added to his growing collection from Kāne'ōhe Bay and deposited for permanent archiving and preservation at the Florida Museum of Natural History. Taxonomic assessment to date from these sponges has confirmed about 50 species, many of which are new records for Hawai'i. Collectively, the species richness from this 9 month survey of fouling communities in Honolulu Harbor already equals that of the entire State and when added to those collected as part of the on-going survey in Kāne'ōhe Bay, brings the total species count to over 200 – a 4-fold increase in the number of sponge species reported in Hawai'i previously.

Samples have been photographed, examined for morphological differences and vouchered. Barcoding (i.e., sequencing a consistent fragment of the 28S rRNA gene used to ID the sponge species) of specimens have mostly been completed but are on-going. Using the barcode sequences to generate a phylogenetic tree has revealed the presence of many cryptic species. Cryptic species are those that lack any morphological diagnostic characters or visible means to tell them apart. Thus, it is almost impossible to measure the species diversity of taxonomically challenging groups like sponges, or identify potential invaders from visually similar species already present, without the use molecular techniques. However, molecular techniques alone cannot identify the species, and it is only through a combination of techniques such as outlined here that we can make progress on understanding which sponge species exist in Hawai'i and sort out which might be introduced. Unfortunately for field identification efforts, cryptic species are commonly encountered among the sponge community in Hawai'i, and there are many sponge orders within the phylum that are frequently difficult or impossible to tell apart without genetic evaluation.

One example of cryptic diversity encountered in this project belong to those in the family Halichondriidae from the order Suberitida. Previous surveys have recorded only a single species from this genus *Hymeniacion chloris*. However, our survey including a barcoding approach has revealed at least six species belonging to *Hymeniacion* which are morphologically indistinguishable from *H. chloris*. The same is true for sponges belonging to the genus *Oscarella* within the class Homoscleromorpha. There has only been a single *Oscarella* sp. previously reported for the entire Hawaiian archipelago, whereas we have discovered that there are actually seven different *Oscarella* spp. The identity and origin of these previously unknown sponges remains to be discovered.

Support from this project has contributed to Jan identifying roughly 150 more sponge species than were previously known in Hawai'i. This project roughly triples the current species richness of sponges from Kāne'ōhe Bay and quadruples the current species richness of sponges in the State. This increased number of recorded species includes primarily sponges belonging to the classes Demospongiae (>60 new records), followed by Calcarea (27 new records), and Homoscleromorpha (9 new records). These new records are sponges for which we cannot currently determine the species name. At this point it is difficult to determine whether these species are entirely new to science or simply new records for Hawai'i, because this determination would require an integrative taxonomic approach by comparing with conspecific type specimens deposited in museums around the world. Such an integrative approach would be a particularly exhaustive undertaking for species belonging to the class Demospongiae, which currently encompasses 7,532 species worldwide, and less complicated

for conspecific type specimens that belong to the class Homoscleromorpha, which consist of only 120 species worldwide. We are therefore beginning the formal description process for these 9 new Homoscleromorph species records. Our identification guide will be provided to HISC upon request, or as soon as it is in a form that facilitates sharing.

Among collections to date, the sponge community associated with Autonomous Reef Monitoring Structures (ARMS<sup>16</sup>) in particular has revealed an astonishingly high biodiversity of new records. We compare the ARMS to the harbor collections, because the ARMS samples are those with the greatest potential of being native and possibly even endemic to Hawai'i. Most of the sponges identified from the ARMS are new records that we believe are not introductions but have been previously overlooked, likely due to the difficulties in accessing these habitats which are deeply embedded within the interstices of the reef. However, most sequence data of sponges from ARMS shows a very distinct community from those collected by visual census on adjacent reef habitats of Kāne'ohe Bay. Thus, it remains to be seen whether each of the 60 or so species of sponges confined to ARMS to date represent native species difficult to sample from within the reef matrix or species that excel on disturbed habitats and human-made substrates (which is typical of invasive species<sup>2</sup>).

This project has begun a baseline of the sponge diversity throughout the Hawaiian Archipelago, which has proven to be much richer than expected and in need of further characterization. Sponges play very important ecological roles, especially those that are found beneath the reef framework. Sponges living in these cryptic communities can transform inaccessible forms of carbon nutrients to particulate form which fuels lower trophic level organisms, promotes tight nutrient cycling, which further sustains biodiversity of organisms in higher trophic levels<sup>17</sup>. Although sponges are relatively easy to overlook on healthy coral reefs, their functional role cannot be understated, and they may be far more abundant than appreciated<sup>17</sup>. A new study based on eDNA as a tool to measure coral cover<sup>18</sup> has shown that sponges can make up ~40% of the total DNA in seawater adjacent to coral reefs within Kāne'ohe Bay, further supporting the significance of sponges in coral reef communities.

We intend to continue this effort to characterize the entire sponge fauna of the Hawaiian Archipelago. The development of a vouchered and DNA-barcoded identification guide will assist future development of early detection tools and facilitate research into the importance of sponges on coral reefs throughout Hawai'i. For example, nearly all of the sponges detected in the eDNA study referenced above<sup>18</sup> remain unidentified until we complete this project and make barcodes for these species available. Our future efforts will assist in matching eDNA data to species names to provide measures of biodiversity through molecular methods and with an ID guide to assist ecological and monitoring work to have the best possible identifications for sponges in Hawai'i. If we are able to continue this work, it will facilitate the use of eDNA and metabarcoding as early detection tools in the future. We are also looking forward to finding funds that would help with the completion of objectives that were unfunded from our initial proposal for the development of future monitoring of the detection and spread of alien invasive species through vectors such as hull fouling and ballast water from incoming ships. In particular, we hope to find future support for our remaining unfunded objectives: 1) perform a proof-of-concept comparison of eDNA sampled from the Kāne'ohe Bay location that has been intensively surveyed to determine the reliability and detection limits of eDNA now that a reference database exists; and 2) developing an early detection and monitoring tool that would allow incoming intruders to be identified from eDNA samples extracted from ballast seawater samples or hull scrapings in harbors around O'ahu.

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