

2014 Project Report for Palawan sampling: *Collaboration on the Inventory and DNA Barcoding of Commercial Fishes of the Philippines for Food Safety and Biodiversity*

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Background:

The Division of Fishes of the National Museum of Natural History, Smithsonian Institution, has an ongoing program studying the fishes of the western Pacific with historical projects dating from the 1800's and more recent projects dating from 1966 to the present, during which time they have organized collaborative BFAR fisheries projects in every major island group in the area.

The fish fauna of the Philippines is one of the most diverse in the world and this produces a wide diversity of fish species represented in the commercial markets of the Philippines. In fact, there have been numerous reports of recent discoveries of new species of marine organisms in the country. Because of this, there is a need to conduct and establish a comprehensive inventory of food fishes in the Philippines. In addition, correct taxonomic identification of filets and partial specimens is often difficult and misidentification can lead to potential food safety problems. It is particularly important to develop methods to identify regulated species and/or potentially toxic species, such as pufferfishes (tetrodotoxin), in markets. A DNA (using the mitochondrial CO1 gene) barcoding reference database of commercial and similar species will allow ready identification of mislabeled species. Advances in the technologies applying these results to use in commercial markets should provide inexpensive and portable equipment to confirm identification of market items.

This project builds on the results obtained during a 2011-2013 collaborative project between BFAR-NFRDI and NMNH/SI. The 2011-2013 project collected 1,177 vouchered samples representing 862 commercial species from Philippine fish markets. These samples provide DNA barcodes for about one-third of the estimated 1,500 fish species sold in Philippine fish markets.

Objectives:

- a. To generate information on the inventory of commercial fishes gained to contribute to food safety efforts in the Philippines using morphological and genetic methods.
- b. To involve and/or train Philippine scientists in scientific techniques and reporting of results to ensure that the information gained will be readily available to Philippine institutions.

Methodology and Results:

The primary scientists will be Dr. Jeffrey T. Williams (NMNH/SI) and Dr. Kent E. Carpenter (NMNH/SI Research Collaborator), in collaboration with Dr. Mudjekeewis Santos (BFAR-NFRDI). The Project sampling was conducted at Palawan, Philippines from April 2-11, 2014. As this is a baseline inventory study, commercial fish markets and fish landings at a variety of locations around the island of Palawan were visited and fish samples were purchased from local vendors and fishermen.

The Project plans to inventory and collect representative specimens of commercial and similar species from Philippine fish markets and landings from throughout the Philippines to obtain vouchered samples of as many species as possible. Each specimen obtained was photographed to capture the fresh color pattern, a tissue sample was taken and preserved, the voucher specimen was tagged with a unique identifying number, and the specimens were then preserved in formalin to

subsequently be archived as reference vouchers at the NMNH/SI with a unique link to the CO1 DNA barcode for food safety and biodiversity. All specimens were explicitly labelled with place of origin/collection site and collection date.

Tissue samples were collected in duplicate for each specimen where one set will be sent to Laboratories of Analytical Biology (LAB) at NMNH/SI for genetic barcoding and the other set will remain at BFAR-NFRDI. Briefly, initial processing as needed may be done at the BFAR-NFRDI Genetic Fingerprinting Laboratory (GFL) of Dr. Santos at Quezon City and at the Laboratories of Analytical Biology (LAB), NMNH/SI, at the Museum Support Center in Suitland, Maryland, USA. Total DNA will be extracted at the LAB, aliquots of the extracted DNA will be processed at the LAB (NMNH/SI), including PCR amplification and sequencing of the CO1 gene. A complete set of tissues will be maintained at NFRDI, where they will be stored as backup, or used for other purposes at the discretion of BFAR-NFRDI. Analysis of the specimens and corresponding sequences will be jointly done by the collaborators. After analysis, the CO1 sequences will be uploaded to the Barcode of Life Database (BOLD) and other appropriate international databases to maximize their use for Philippine and international food safety, biotechnology and biodiversity.

Discussion:

The sampling conducted in Palawan resulted in collections from 34 different stations. A total of 144 vouchered samples of commercial fish species were taken in the markets and at fish landings on Palawan. Once the CO1 sequences are obtained and checked for accuracy from these samples, the sequences will be added to the DNA barcode reference database. The list of species sampled at Palawan is provided in Table 1.

In only eight days of sampling at Palawan, we were able to take vouchered samples representing 109 different species. Even after four years of sampling at fish markets throughout the Philippines, we found specimens representing 66 species we collected nowhere else in the Philippines. In many cases this is simply due to limited sampling time during each sampling period, but some of the species sampled during this sampling period are found within the Philippines only at Palawan. The Freckled tilefish, *Branchiostegus sawakinensis*, was commonly seen in the markets in Puerto Princessa, but we have not found it in other parts of the Philippines. This tilefish has a disjunct distribution, known only from the Red Sea, South Africa, Indonesia to Western and Northern Australia and at Palawan, Philippines. Differences in color pattern in different parts of its range suggest that this might possibly be a species complex and needs additional taxonomic study. The Hookfin cardinalfish, *Ostorhinchus griffini*, was also found only in Palawan in the Philippines, but it occurs elsewhere at Brunei, Malaysia and Indonesia. Additional sampling in the Palawan region would undoubtedly reveal other species only occurring at Palawan in the Philippines.

The goal of obtaining DNA barcodes for as many additional commercial species as possible will contribute to advances in biotechnology, biodiversity and food safety in the Philippines. Surveys of the fishes found at fish markets and fish landings in the Philippines have reveals new species, species complexes and many new records for the Philippines. We hope these surveys will continue in the future to add to our knowledge of the commercial fishes of the Philippines.

Acknowledgements:

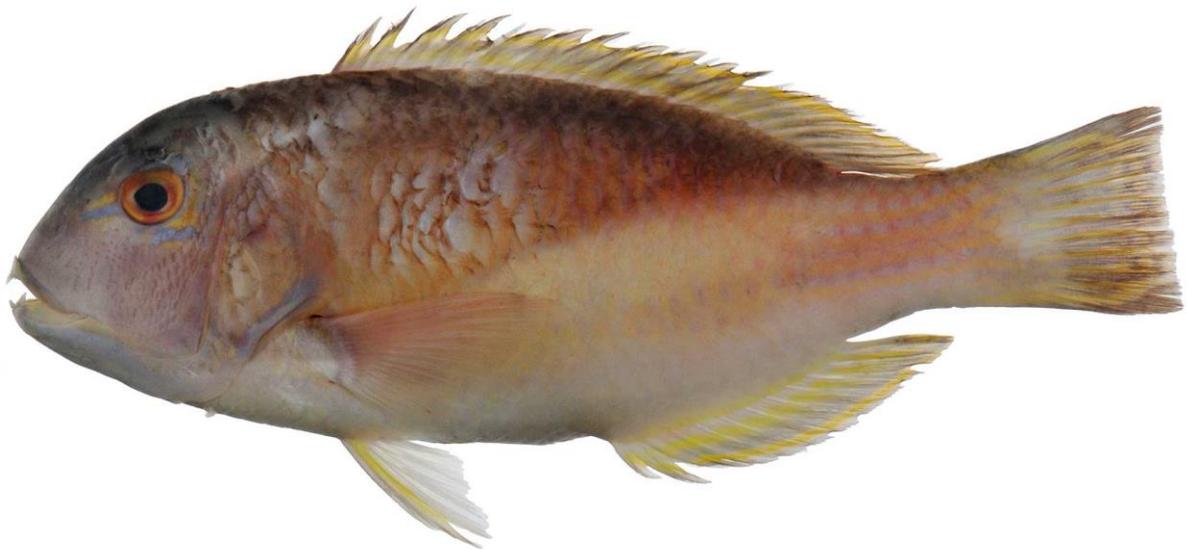
We are grateful to everyone who made this project possible. We would particularly like to thank Glenda Cadigal for assisting with permits for the project. The entire BFAR staff on Palawan went out of their way to make the project a success. Myrna Candelario was extremely supportive throughout the project and made sure that the appropriate BFAR enumerators were available at fish landings and fish markets to assist with the project. Elmar Villaflor provided help and guidance in locating appropriate sampling sites around Palawan. BFAR enumerators, Dexter Sabay and Elena Palope, provided assistance in locating specimens at the Baheli fish landing and Donald Cajolo and Reymart Degaraga provided assistance in southern Palawan . Elmer Candelario was always ready to assist with the project and showed up at a critical time when we needed assistance finalizing a shipment from the airport. We are particularly grateful to our research assistant, Apollo Marco de Lizano, who worked tirelessly and without complaint during the long days and nights working on the project to make it a success.

Table 1. Fish species sampled at Palawan fish markets and fish landings, April 2-11, 2014.
 (* indicates that this is the first sample taken of the species during this 4-year project)

<i>Abudefduf sexfasciatus*</i>	<i>Decapterus russelli</i>	<i>Parupeneus indicus</i>
<i>Acanthochromis polyacanthus*</i>	<i>Dendrophysa russelli*</i>	<i>Pentapodus species*</i>
<i>Acanthurus bariene*</i>	<i>Diagramma melanacrum*</i>	<i>Photopectoralis bindus</i>
<i>Acanthurus olivaceus</i>	<i>Dischistodus prosopotaenia*</i>	<i>Plectorhinchus vittatus</i>
<i>Alectis indica*</i>	<i>Elapsopsis species*</i>	<i>Plectorhynchus lessonii</i>
<i>Alepes vari*</i>	<i>Epibulus brevis*</i>	<i>Plectropomus areolatus*</i>
<i>Anampses caeruleopunctatus</i>	<i>Epinephelus caeruleopunctatus*</i>	<i>Plotosus sp</i>
<i>Aphareus furca*</i>	<i>Epinephelus coiodes*</i>	<i>Pomacanthus sexfasciatus*</i>
<i>Atherinomorus lineatus*</i>	<i>Epinephelus corallicola</i>	<i>Pomacanthus xanthometopon*</i>
<i>Aurigequula fasciata</i>	<i>Epinephelus cyanopodus*</i>	<i>Priacanthus sagittarius*</i>
<i>Aurigequula longispina*</i>	<i>Epinephelus fuscoguttatus*</i>	<i>Pristipomoides filamentosus*</i>
<i>Branchiostegus sawakinensis*</i>	<i>Epinephelus lanceolatus*</i>	<i>Pristipomoides seiboldii</i>
<i>Carangoides armatus*</i>	<i>Epinephelus maculatus*</i>	<i>Pseudorhombus cinnamoneus*</i>
<i>Carangoides chrysophrys*</i>	<i>Epinephelus malabaricus*</i>	<i>Pterocaesio chrysozona*</i>
<i>Carangoides fulvoguttatus*</i>	<i>Epinephelus melanostigma*</i>	<i>Pterois russellii*</i>
<i>Carangoides gymnotethus*</i>	<i>Epinephelus quoyanus</i>	<i>Sardinella fimbriata</i>
<i>Carangoides malabaricus*</i>	<i>Epinephelus undulosus*</i>	<i>Sargocentron praslin*</i>
<i>Caranx melampygus*</i>	<i>Equulites stercorarius*</i>	<i>Saurida micropectoralis*</i>
<i>Caranx tille*</i>	<i>Eubleekeria jonesi*</i>	<i>Scolopsis monogramma</i>
<i>Carcharhinus dussumieri</i>	<i>Eubleekeria splendens</i>	<i>Scolopsis margaritifer</i>
<i>Cephalopholis caeruleopunctatus</i>	<i>Gnathanodon speciosus</i>	<i>Scomberoides lysan*</i>
<i>Cephalopholis cyanostigma*</i>	<i>Hemirhamphus far</i>	<i>Scomberomorus commerson</i>
<i>Cephalopholis formosa*</i>	<i>Jaydia smithi</i>	<i>Selar boops*</i>
<i>Cetoscarus ocellatus</i>	<i>Lactarius lacatarius*</i>	<i>Selaroides leptolepis</i>
<i>Cheilinus fasciatus</i>	<i>Lagocephalus guentheri*</i>	<i>Siganus punctatissimus*</i>
<i>Cheilinus trilobatum</i>	<i>Lethrinus erythropterus*</i>	<i>Siganus vermiculatus*</i>
<i>Cheilodipterus artus*</i>	<i>Lethrinus rubrioperculatus</i>	<i>Strongylura incisa*</i>
<i>Cheilopogon astriginis</i>	<i>Lutjanus malabaricus*</i>	<i>Symphorus nematophorus</i>
<i>Cheilopogon cyanopterus</i>	<i>Lutjanus russelli*</i>	<i>Synodus sageneus*</i>
<i>Cheilopogon intermedius</i>	<i>Monotaxis grandoculis</i>	<i>Thryssa mystax*</i>
<i>Cheilopogon katoptron</i>	<i>Myripristis amaena</i>	<i>Toxotes jaculatrix*</i>
<i>Cheilopogon species</i>	<i>Nemipterus tambuloides</i>	<i>Ulua mentalis*</i>
<i>Cheilopogon spilopterus</i>	<i>Nemipterus zysron*</i>	<i>Upeneus vittatus</i>
<i>Chlorurus microrhinos</i>	<i>Ostorhinchus griffini*</i>	<i>Wattsia mossambica*</i>
<i>Choerodon robustus*</i>	<i>Otolithes ruber*</i>	<i>Zebrasoma vellifer</i>
<i>Cromileptes altivelis</i>	<i>Pardachirus pavoninus*</i>	<i>Zoramia gilberti</i>
<i>Cypselurus poecilopterus</i>		



Branchiostegus sawakinensis



Choerodon robustus



Diagramma melanacrum



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Lactarius lactarius