BAYESIAN ANALYSES OF GENETIC VARIATION AND POPULATION DIFFERENTIATION IN PACIFIC SWORDFISH (*Xiphias gladius* L.) AND THE DEVELOPMENT OF HIGH RESOLUTION MELTING ASSAYS FOR SPECIES IDENTIFICATION AND POTENTIAL SEX-LINKED MARKER SURVEY IN ISTIOPHORID BILLFISH

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2014

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

Swordfish (*Xiphias gladius* L.) and istiophorid billfish fisheries in all ocean basins are important commercially and recreationally. Proper assessments of these fisheries are hampered by species misidentification, unknown sex ratios, and unclarified population structure. This dissertation focuses on: 1) genetic assays to identify Pacific billfishes, 2) the characterization of molecular markers potentially linked to gender determination in swordfish and billfishes, and 3) the characterization of the genetic population structure of Pacific swordfish.

Unambigous identification of black marlin (*Istiompax indica*), blue marlin (*Makaira nigricans*), striped marlin (*Kajikia audax*) and sailfish (*Istiophorus platypterus*) was accomplished with two variants of high resolution melting (HRM), including HRM of a 491 bp segment, and melting profiles of a 48 bp unlabeled probe. Both HRM assays target variation in the mitochondrial DNA NADH dehydrogenase subunit 2 (ND2) gene and represent fast and robust alternatives to identify Pacific billfish.

Surveys to identify gender-linked molecular markers were conducted using gender-validated samples. The characterization of Randomly Amplified Polymorphic DNA (RAPD) primers suggests a XY chromosomal system in blue marlin, and a ZW chromosomal system in sailfish, and possibly swordfish. Nucleotide sequence analyses of 12 loci known linked to gender determination in other teleosts showed no linkage in blue marlin, sailfish and swordfish.

The genetic population structure of Pacific swordfish was surveyed using 16 samples (n=891) with an ample geographic coverage and that included early stages (n=150) and adults (n=741). Bayesian analyses of 20 single nucleotide polymorphisms (SNPs) contained in 10 nuclear loci indicate statistically significant genetic heterogeneity of tropical samples relative to temperate samples, but also with respect to other tropical samples, but no differences among temperate samples. The observed patterns are discussed in light of differences among regions in oceanographic conditions, adult and larval distributions, and tagging experiments.

DEDICATION

This dissertation is dedicated to my parents and brother, who have always supported in me everything throughout my entire doctoral study. Although we are in different time zones and parted by long geographic distances, you make me feel there is no distance and no loneliness with your kindness, patience and love. No matter what kind of difficulties I have, you always believe in and encourage me to face and solve them with no doubts. With your love and trust, I am confident to enjoy and finish this adventure. This dissertation is the fruit of both the good times and the bad times of this adventure. Thank you for making me brave and strong enough to achieve this goal in my life. To my friends, I am very thankful for you and understand how lucky I am to having all your company. This dissertation, I would like to share with all of you. No matter what will happen in the future, we can overcome the difficulties and enjoy the happiness together.

ACKNOWLEDGEMENTS

The first person I would like to thank is my advisor and committee chair Dr. Jaime R. Alvarado-Bremer. Without his strong support, patience, and wisely guidance throughout my graduate studies, I won't be able to complete my research. Additional thanks to my other committee members: Dr. Patrick Louchouarn, Dr. Mariana Mateos, and Dr. Jay R. Rooker, whose encourages and suggestions helped improve this dissertation. Special thanks to my colleagues, especially Dr. Brad L. Smith, who assisted in the development of nuclear markers and also thanks to fellow graduate student, Janelle Espinoza who helped for editing the manuscripts. Great thanks to for collecting and providing swordfish tissue samples for completing this research including Craig Bambling, Seinen Chow, Shu-Ying Chen, Wei-Chuan Chiang, Robert Dollar, Miguel Donoso, Edward Everett, Peter Grewe, Michael Hinton, Bob Humphreys, Sofía Ortega-García, Oscar Sosa-Nishisaki, Nan-Jay Su, Chi-Lu Sun, Sheng-Ping Wang, Robert Ward, Shean-Ya Yeh, EMBRC of FRI,and IATTC. Thanks to Department of Marine Biology of TAMUG, Graduate Research Office of TAMUG, Erma Lee and Luke Mooney Foundation, McDaniel Charitable Foundation, The late Mr. John William (Bill) Lyons, Texas Institute Oceanography (TIO). Finally, I would like to thank my family and friends who always be my side and have supported me eternally.

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CHAPTER I

GENERAL INTRODUCTION

Swordfish and istiophorid billfishes (marlins, spearfishes and sailfish) are targeted by commercial and recreational fisheries in the Pacific, Atlantic, Indian Oceans, and the Mediterranean Sea. Because of their highly migratory behavior, it is very difficult to fully characterize pertinent biological parameters and the interactions of these with environmental factors and human activities. In order to provide reasonable assessments to ensure the sustainable exploitation of marine resources, it is essential to establish a framework based on pertinent biological and ecological information. The negative impacts of human activities on marine resources underscore the need for responsible and well-informed management efforts (Worm *et al.* 2009).

Overfishing is the leading environmental and socioeconomic problem in the marine realm and has reduced biodiversity, reduced the spawning biomass of the majority of targeted fish populations and modified ecosystem functioning (Jackson *et al.* 2001; Worm *et al.* 2006). Between 1950 and 1996, the world's marine fisheries production steadily increased five-fold, from 16.8 to 86.4 million tons. After that period, catches have averaged about 80 million tons, never exceeding the record set in 1996 (FAO yearbook 2012). In spite of the reduction in average exploitation rate that is now at or below the rate predicted to achieve maximum sustainable yield for seven systems, 63% of assessed fish stocks worldwide still require rebuilding (Worm *et al.* 2009). Over 50% of the world's ocean catches (about 45 million tons) correspond to fisheries

operating in the Pacific Ocean on highly migratory species, such as tunas, with billfishes and swordfish representing an important component of these catches. Despite the economic and ecological importance of billfishes and swordfish as apex predators, many relevant aspects of their biology remain unknown, and management decisions are based primarily on catch statistics. This void is particularly pronounced for billfishes, which are not directly targeted, except by sport fisheries that account for a minor proportion of the total catches. Instead, these species are considered bycatch of other fisheries, and for statistical purposes, are collectively reported as 'billfish'. In recent years, however, there has been an increased emphasis on collecting information for each species of billfish separately. Such information, together with pertinent biological data, is necessary to conduct separate stock assessments for each species of Pacific billfish, with the goal of improving management practices.

Fisheries biologists conduct stock assessments to estimate the size of stock and pertinent population dynamics parameters, such as age structure, sex ratio, fishing (F) and natural (M) mortalities, along with other relevant biological data that may affect recruitment to make projections and thus guide management decisions. Important biological information often used for stock assessments includes age at first spawning, fecundity, growth rates, spawning behavior, essential habitats, migratory habits, diet and feeding behavior. However, estimates of the size of the stock would not be possible without determining first whether a stock consists of a single population, a portion of a population, or multiple populations (mixed stock), and whether the biological information recorded characterize the entire stock. In addition, billfishes and swordfish

display pronounced sexual dimorphism in size, with females attaining considerably larger sizes than males, and also differing in age at maturity, growth rate and longevity (Strasburg, 1970, Berkeley and Houde, 1983; Ehrhardt *et al.*, 1996; Sun *et al.*, 2002; Chiang *et al.*, 2004, Hoolihan 2006, DeMartini *et al.*, 2007, Kopf *et al.* 2010). Differences in the sexes of these species may have important implications towards stock assessment; Wang *et al.* (2005 and 2007) demonstrated that sexual dimorphism could be a factor biasing the stock assessment for swordfish in the North Pacific Ocean. Without this information, stock assessments may be confounded, particularly if there is a strong bias in gender-specific catches.

Further, a fish stock, in contrast to a fish population in the biological sense, is defined as much by management concerns – such as jurisdictional boundaries or harvesting location – as by biology. For instance, the stock of Pacific swordfish may consist of multiple populations differing substantially in size, and if capture levels are estimated for a single large population, the smaller populations may be overharvested. Therefore, accurate biological information could provide basic knowledge to reduce the risk of inaccurate stock assessments and improve fisheries management (Caddy and Mahon, 1995; Rosenberg and Restrepo, 1994; Heino *et al.* 2013). There are several potential sources of uncertainty, including variability in morphological and meristic characters, and of demographic parameters. In order to reduce uncertainty, fisheries biologists require sound knowledge of the biological aspects of marine resources, including accurate species identification, biological parameters (i.e., gender identification, growth, and age), spatial distribution, migration, habitat requirements

including feeding ecology, reproductive biology, and population dynamics. All this knowledge about biology would not be useful without properly characterizing the genetic population structure of the species in question.

While multiple molecular methods were employed in this study, it is important to underline high resolution melting analysis (HRMA) as a novel genotyping technology. HRMA is a highly sensitive, single closed-tube, and high throughput molecular method for mutation scanning and genotyping that until recently was reserved to clinical and diagnostic studies (Smith *et al.* 2010; 2013; Reed *et al.* 2007; Vandersteen *et al.* 2007; Zhou *et al.* 2005). HRMA detects single nucleotide polymorphisms (SNPs) in the target fragment of amplified DNA by comparing fluorescence as a function of temperature. HRMA relies on characterizing SNPs as molecular markers that have been used to differentiate marine populations (Morin *et al.* 2004; Smith, 2012; Smith *et al.* 2010; Seeb *et al.* 2011; Wetten *et al.* 2012) and for species identification (Bréchon *et al.* 2013; McGlauflin *et al.* 2010). Here, HRMA are utilized for the four Pacific billfish species identification, gender identification in swordfish and istiophorid billfishes and population structure in Pacific swordfish.

The goal of this research is to close the information gap in several of this areas for swordfish and istiophorid billfishes using molecular methods (DNA-based), in order to provide more refined data aimed to the conservation and management of these marine resources. Because the main emphasis of this dissertation centers on the study of Pacific swordfish, a review of pertinent biological information of this species follows.

Biological background of Pacific swordfish

Swordfish (*Xiphias gladius* Linnaeus, 1758), is the monotypic member of the family Xiphiidae, that can be distinguished from istiophorid billfishes (sailfish, marlins, and spearfishes) by the absence of pelvic fins, a long-flatted bill, and a single keel present on each side of the caudal peduncle (Nakamura, 1985). Swordfish are widely distributed in oceanic tropical and temperate waters from 50˚N to 50˚S (Nakamura, 1985), displaying a remarkable adaptations that allows them to inhabit waters with sea surface temperatures (SST) ranging from 10℃ to 28℃, tolerate temperatures <4℃ to 28℃, and endure daily shifts over 15℃ (Abascal *et al.* 2010). Such high tolerance to diel and seasonal changes in seawater temperatures is possible due to physiological and morphological adaptations that enable brain, eye and coronary circulation function in low temperature waters (Block, *et al.* 1993; Carey, 1982; Carey and Robison 1981; Dewar *et al.* 2011; Dickson and Graham 2004; Galli, *et al.* 2009).

Several studies have been conducted on the reproductive biology of Pacific swordfish including descriptions gonadal stages, the distribution of larval and juvenile stages and the characterization of spawning areas. In general, swordfish larvae range widely from 35˚N to 28˚S latitude in the western and central Pacific Ocean. Spawning behavior has been observed between March and July in the central and western North Pacific, between September to March, with intense activity also from December through February, in the western South Pacific, and all-year round in equatorial Pacific waters (Grall, *et al.* 1983; Nakamura, 1985; Nishikawa, *et al.* 1985; Palko, *et al.* 1981; Young, *et al.* 2003). Although most reproductive activities occur in the western and central

Pacific Ocean, it has been suggested that potential spawning areas for the Pacific swordfish could be relatively broader and farther east with sporadic-seasonal or moderate reproductive behaviors occurring in the other regions of the Pacific Ocean (Mejuto, *et al.* 2008). Although swordfish display sexual dimorphism in size – large specimens exceeding 180 cm lower jaw fork length (LJFL) are mostly (>90%) females – there are no obvious sexual dimorphism in shape. Gonadal inspections are also difficult for immature and fish with resting gonads and gender is rarely recorded in the field by observers, and cannot be determined at port, since gonads and other viscera are removed from the fish (dressed) at sea. Accordingly, the development of molecular assays to determine gender would prove extremely valuable to better correct for gender bias in stock assessment.

Several studies aiming to characterize horizontal and vertical displacements of Pacific swordfish have been conducted with different types of tags including conventional and electronic tag such as pop-up satellite archival tags (PSATs) in different areas of the Pacific Ocean. Although the information available on swordfish movements within and among regions of the Pacific Ocean was limited, due to the limited number of tagged specimens, the short duration of deployments, and low tag return rates, we now have a better understanding of horizontal and vertical displacements using PSATs informative data of this species (Abascal *et al.*, 2010; Carey and Robison, 1981; Dewar, *et al.*, 2011; Holdsworth and Saul, 2011; Ito and Coan, 2005; Takahashi, *et al.*, 2003). For instance, diel incursions into deep sound scattering layer have been with foraging behavior during the day (Abecassis, *et al.* 2012; Dewar, *et al.* 2011), with

fish staying in the mixed layer at night (Abascal *et al.* 2010; Evans *et al.* 2014). Several PSATs studies indicate that swordfish reach a maximum mean daytime depth of 760 m, indicative of a high tolerance to extremely low oxygen conditions, although deeper dives have been recorded. PSATs deployed in the South Pacific (Evans, *et al.* 2014), suggest that swordfish do not conduct long-distance trans-Pacific and trans-equatorial movements, and instead display differences in horizontal and vertical movements, some of which may be indicative of potential population sub-structure in the south Pacific.

Purposes and organization of chapters

This dissertation consists of three parts: the first corresponds to a general introduction (Chapter I), the second includes three chapters (II-IV) in the format of individual manuscripts, and the third part (Chapter V) summarizes the major findings of this study. The purpose of Chapter II is to develop molecular assays based on HRMA to identify the four istiophorid billfishes found in the Pacific Ocean. It is difficult to identify billfish species correctly particularly when the diagnostic morphological and meristic characters are missing, and it is particularly important when the only material available is tissue. Accurate identification is particularly relevant to validate capture data for a particular species, and when studying early-life stages. Several molecular methods with multiple genetic markers have been applied to species identification of billfish, including restriction fragment length polymorphisms (RFLP), mitochondrial DNA (mtDNA), and nuclear DNA (Chow, 1994; Collette *et al.* 2006; Hanner *et al.* 2011; Hsieh *et al.* 2007; Innes *et al.* 1998; McDowell and Graves, 2002; Shivji *et al.*

2006). All these methods are both labor intensive, prone to cross-contamination, and may take several days to complete. This study focuses on developing HRMA assays, which is highly sensitive, inexpensive and extremely fast (hours as opposed to days), and thus is particularly well suited for the characterization of large sample sizes, while reducing the possibility of cross-contamination as both amplification and genotyping are conducted in the same close tube (Reed *et al.* 2007; Smith *et al.* 2010; Vossen *et al.* 2009;) Two HRMA-genotyping assays were developed for identifying Pacific billfishes, including black marlin (*Istiompax indica*), blue marlin (*Makaira nigricans*), striped marlin (*Kajikia audax*) and sailfish (*Istiophorus platypterus*), based on the SNPs of mtDNA ND2 gene.

Chapter III aims to identify loci linked to gender-determination. The ultimate goal is to develop molecular assays to identify gender from tissue samples of billfish and swordfish. It should be noted that there is no universal mechanism of sex determination in fishes, and in some species, sex determination results from the complex interaction of genetic, environmental, and physiological factors. In other fishes, a genetic basis for sex determination exists. Here, two approaches were applied because their proven success in identifying gender-linked markers in other fishes (Matsuda *et al.* 2002, Nanda *et al.* 2002, Takehana *et al.* 2007, Hattori *et al.* 2012) and in avian species (Griffith *et al.* 1998). The first consists of random characterization of polymorphisms across the genome with randomly amplified polymorphic DNA (RAPD) primers. The second consists of determining the sequence of specific genes known either to operate as sex-

determining genes, or to be linked to sex determination in other fishes. Both approaches were employed in swordfish, blue marlin, and sailfish.

Chapter IV focuses on clarifying the genetic population structure of swordfish in the Pacific Ocean. Currently, there is no consensus on Pacific swordfish population structure, and alternative views based upon catch statistic data have been advanced, varying in number from a single panmictic unit to up to four populations. Although the null hypothesis of no differentiation (panmixia) has been tested with different genetic markers and molecular approaches, including restriction fragment polymorphisms (RFLPs) of total mitochondrial DNA (mtDNA), allozyme electrophoresis, sequence analyses of the mtDNA Control Region I (CR-I), microsatellite DNA, and single copy nuclear (scn) DNA, the results have been inconclusive and no consensus can be reached (Alvarado-Bremer *et al.*, 2006; Chow *et al.* 1997; Grijalva-Chon *et al.*1994; 1996; Kasapidis *et al.*, 2008; Lu *et al.* 2006; Reeb *et al.*, 2000; Rosel and Block, 1996; Ward *et al.*, 2001). This study focuses on using multi-locus analyses of nuclear single nucleotide polymorphisms (SNPs) and employs HRMA as a genotyping technology for studying the population structure of Pacific swordfish. This approach has been shown to have sufficient resolution to clarify the population structure swordfish in the Atlantic Ocean (Smith 2012; Smith *et al.* 2010; 2013), and is applied here to characterize Pacific swordfish.

CHAPTER II

GENETIC IDENTIFICATION OF PACIFIC ISTIOPHORID BILLFISH BY HIGH-RESOLUTION MELTING ANALYSIS (HRMA)

Introduction

Istiophorid billfishes are targeted by both recreational and commercial fisheries of many countries using a variety of gears. The vast majority of these catches, however, are classified as bycatch of commercial longline fisheries targeting tunas. Over the past five decades the total catch of billfishes (including swordfish) at a global scale increased steadily from 31,882 tons in 1950 to 224,065 tons in 2012, with roughly 59% of these catches occurring in the Pacific Ocean (FAO yearbook 2012). In spite of this dramatic increase in landings, species names are rarely reported, mainly because of species identification problems, and thus these catches are catalogued for statistical purposes collectively as "billfish". The removal at sea of fins, head and other diagnostic morphological characters (i.e. dressed fish) further complicates the identification of most landing (Innes *et al.* 1998). The ability to identify billfishes from carcasses, from minute, easily obtained samples, equips fisheries managers with diagnostic tools to improve istiophorid catch statistics (Shepard and Hartman 1996). Similarly, the identification of billfish early life stages (ELS) based solely on morphology is difficult, unreliable, and time consuming (Hyde *et al.* 2005; McDowell and Graves 2002). For all these reasons, molecular genetic methods have been advocated as an alternative approach to unambiguously identify billfish (Graves and McDowell 1994; Chow 1994).

Several laboratory methods have been used to identify billfish. Shepard and Hartman (1996) developed monoclonal antibodies highly specific to sailfish (*Istiophorus platypterus*) albumin serum, allowing them to positively identify sailfish from minute samples obtained from carcasses, yet no success was achieved in developing similar assays for other billfishes. Alternatively, several molecular (i.e., DNA-based) methods have been carried to identify istiophorids, including restriction fragment length polymorphisms (RFLPs) of the entire mitochondrial DNA (mtDNA) molecule (Alvarado-Bremer, 1994), RFLPs of polymerase chain reaction (PCR) amplified segments (PCR-RFLP) of both mtDNA and nuclear DNA (nDNA) markers (Chow, 1994; Hsieh *et al.* 2007; Innes *et al.* 1998; McDowell and Graves, 2002). Alternatively, multiplex PCR of mtDNA loci and direct sequencing of PCR amplified mtDNA and nDNA loci have been used to derive phylogenies and identify billfish (Alvarado-Bremer, 1994; Collette *et al.* 2006; McDowell and Graves, 2002; Shivji *et al.* 2006; Hanner *et al.* 2011). All these molecular methods, particularly when intended solely for the purpose of species identification, are not well suited for high throughput and potential automation. For instance, RFLP-based methods to identify billfish require multiple restriction treatments after PCR, and while these can be circumvented with multiplex PCR (e.g., Hyde *et al.* 2005), gel electrophoresis after PCR amplification is a universal requirement to score variants.

Genotyping via high resolution melting analysis (HRMA) has been advocated as a fast and highly sensitive genotyping alternative with several advantages over gel-based genotyping methods. Both PCR and genotyping are conducted in the same closed-tube

which lowers the risk of cross-contamination, minimizes gel loading and scoring errors, thus faster, cheaper and amenable to high throughput when large sample sizes are needed (Reed *et al.* 2007; Smith *et al.* 2010; Vossen *et al.* 2009). HRMA relies on characterizing single nucleotide polymorphisms (SNPs) as genetic markers that been used to differentiate marine populations (Morin *et al.* 2004; Smith *et al.* 2010; Seeb *et al.* 2011; Wetten *et al.* 2012) and for species identification (Bréchon *et al.* 2013; McGlauflin *et al.* 2010). SNPs are visualized as changes in fluorescence due to differences in the melting temperature (T_m) of the amplicons. McGlauflin *et al.* (2010) used HRMA to diagnose 11 SNPs to identify rainbow and cutthroat trout and its hybrids. Similarly, Bréchon *et al.* (2013) used HRMA to identify four clupeid species in the western English Channel, including anchovy (*Engraulis encrasicolus*), herring (*Clupea harengus*), pilchard (*Sardina pilchardus*) and sprat (*Sprattus sprattus*) using larval and adult samples, including some formalin-preserved specimens. Here, we employ HRM to characterize SNPs contained in the NADH dehydrogenase subunit 2 (ND2) mitochondrial gene to distinguish four species of Pacific billfishes belonging to four genera, namely black marlin (*Istiompax indica*), blue marlin (*Makaira nigricans*), striped marlin (*Kajikia audax*) and sailfish (*Istiophorus platypterus*). Unambiguous species identifiction was achieved with two HRMA-based genotyping assays. Because these species are frequent bycatch of the longline commercial fisheries targeting tunas, the availability of high throughput assays for species identification represents a potentially valuable tool to improve fishery management practices of these billfishes.

Materials and methods

Sampling and DNA isolation of Pacific billfish

Tissue samples were collected by both commercial and recreational fisheries targeting billfish in the Pacific Ocean, as follows: 1) black marlin, 2) blue marlin, 3) striped marlin and 4) sailfish (**Table 1**). Genomic DNA was isolated from a small amount (≈4µg) of muscle tissue from each individual with a Proteinase K digestion followed by EtOH precipitation without organic extractions (Greig, 2000). Primer designs of the two HRMA-genotyping assays were based on the multiple sequence alignment of 20 sequences from GenBank that included a representation of all the billfish species known to date (**Table 2**).

HRMA-genotyping assays design

HRMA-genotyping assays were designed and optimized as described in Smith *et al.* (2013). Initially, we used mtDNA CR-I multiple sequence alignments to design an HRMA assay for billfish identification. However, excessive intraspecific variation in melting profiles was observed and thus this assay was deemed unsuitable (not shown). Instead, we focused on 388 variable sites contained within a 995 bp segment of mtDNA NADH dehydrogenase subunit 2 (ND2) that are highly informative to solve phylogenetic relationships among billfish (Collette *et al.* 2006).

Table 1. Details of sampling information of the specimens used in this study. With the noted exceptions, all other specimens were used as standards to diagnose unknown individuals.

Common Name	Species Name	Location	Sample Size	Latitude	Longitude	Sampling Year	Tissue / Preservation
Blue marlin	*Makaira nigricans*	Eastern Pacific[1]	52	23˚N~19˚S	75˚W~157˚W	2004~2007	Muscle/Ethanol
		Cabo San Lucas, Mexico[2]	22			2003,2004	Muscle/Ethanol
		Taiwan[2]	44			2012	Muscle/Ethanol
		Kona, Hawaii*	2			1988	Muscle/Ethanol
							Muscle/Ethanol
Black marlin	*Istiompax indica*	Eastern Pacific[1*]	3	6˚N~11˚S	78˚W~102˚W	2006	Muscle/Ethanol
		Cabo San Lucas, Mexico[2]	2			2003,2005	Muscle/Ethanol
							Muscle/Ethanol
Striped marlin	*Kajikia audax*	Cabo San Lucas, Mexico*	2	20˚N	105˚W	1989	Muscle/Ethanol
							Muscle/Ethanol
Sailfish	*Istiophorus platypterus*	Taiwan*	2			2010	Muscle/Ethanol
Total			**129**				

[1]: Sample tested by HRMA of a 491 bp fragment; [2]: Sample tested by UP-HRMA; *: Standard specimens

Table 2. The mtDNA ND2 sequences database from GenBank of 10 billfish species and primer set details of the two newly designed assays used in this study (Gray blocks show the primers position in the sequences database; sequence length was shown by number in the end of each line).

Species_GenBank No.	IsticND2-F1 →								IsticND2-F2 →				IsticND2-probe1 >>					165
Ipla_DQ854669.1	CACTGGCTCC	TAGCATGAAT	GGGGTTAGAA	ATCAACACCC	TCGCCATCCT	CCCCCTTATG	GCTCAACATC	ACCACCCTCG	AGCAGTTGAA	GCAACCACCA	AATATTTTCT	CACTCAGGCT	ACCGCAGCTG	CCATACTGTT	ATTTGCTAGC	ACAACCAATG	CTTGA	
Ipla_DQ854670.1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	.....	
Mnig_DQ854671.1	....	....	....	..T..T....	....	T........A	.....G....	.......C..	G.........	....	.......C..	....	....	....G...C.	...C......	.....T..C.	.....	
Mnig_DQ854672.1	....	....	....	..T..T....	....	T........A	.....G....	.......C..	G.........	....	.......C..	....	....	....G...C.	...C......	.....TN.C.	.....	
Iind_DQ854673.1	....	....	....	..T..T....	....	T.........	....	.T.....C..	....	....	.......C..	....	........C.	....	...C......	.....T..C.	.....	
Iind_DQ854674.1	....	....	....	..T..T....	....	T.........	....	.T.....C..	....	....	.......C..	....	........C.	....	...C......	.....T..C.	.....	
Talbi_DQ854675.1	....	....	....	..T.......	....	T.........	....	.......C..	....	....	.G.....C..	....	........C.	.......A..	....	........C.	.....	
Talbi_DQ854676.1	....	....	A.........	..T.......	....	T.........	....	.......C..	....	....	.G.....C..	....	........C.	.......A..	....	........C.	.....	
Kaud_DQ854677.1	....	....	....	..T.......	....	T.........	....	.......C..	....	....	.G.....C..	....	........C.	.......A..	....	........C.	.....	
Kaud_DQ854678.1	....	....	....	..T.......	....	T.........	....	.......C..	....	....	.G.....C..	....	........C.	.......A..	....	........C.	.....	
Tang_DQ854679.1	....	....	...A......	..T..T....	.......T..	T........A	....	.T.....C..	....	....	.......C..	....	........C.	.......AC.	...C......	........C.	.....	
Tang_DQ854680.1	....	....	...A......	..T..T....	.......T..	T........A	....	.T.....C..	....	....	.......C..	....	........C.	.......AC.	...C......	........C.	.....	
Tpfl_DQ854681.1	....	....	...A......	..T..T....	.......T..	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	...C......	........C.	.....	
Tpfl_DQ854682.1	....	....	...A......	..T..T....	.......T..	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	...C......	........C.	.....	
Tbel_DQ854683.1	....	....	...A......	..T..T....	.......T..	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	....	........C.	.....	
Tbel_DQ854684.1	....	....	...A......	..T..T....	.......T..	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	....	........C.	.....	
Tgeo_DQ854685.1	....	....	...A......	..T..T....	....	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	G..C......	........C.	.....	
Tgeo_DQ854686.1	....	....	...A......	..T..T....	....	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	G..C......	........C.	.....	
T.sp.JRM_DQ854687.1	....	....	...A..G...	..T..T....	....	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	G..C......	........C.	.....	
T.sp.JRM_DQ854688.1	....	....	...A......	..T..T....	....	T........A	....	.......C..	....	....	.......C..	....	........C.	.......AC.	G..C......	........C.	.....	

	→																	330
Ipla_DQ854669.1	CTTACCGGCC	AATGAGATAT	CCTCAACATA	TCCCACCCCC	TCCCTACAAC	AATAATTACT	CTTGCTTTAG	CCCTAAAAAT	TGGGCTGGCT	CCAACACATA	CCTGACTTCC	CGAAGTACTC	CAAGGCCTAG	ACCTAACCAC	AGGACTCATT	TTATCCACCT	GACAA	
Ipla_DQ854670.1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	.....	
Mnig_DQ854671.1	....	.G.....C..	T...C.....	....	....	.....C....	....	....	...A......	........C.	....	....	....	....	....	.....T....	.....	
Mnig_DQ854672.1	....	.G.....C..	T...C.....	....	....	.....C....	....	....	...A......	........C.	....	....	....	....	....	.....T....	.....	
Iind_DQ854673.1	..C.......	....	T...C.....	....	....	......C..C	....	....	...A......	....	....G.....	.........T	....	....	...G......	....	.....	
Iind_DQ854674.1	..C.......	....	T...C.....	....	....	......C..C	....	....	...A......	....	....G.....	.........T	....	....	...G......	....	.....	
Talbi_DQ854675.1	..C.......	....	T...C.....	....	....	C	....	....	.....A....	....	.A........	.........T	....	....G.....	....	.....T....	.....	
Talbi_DQ854676.1	..C.......	....	T...C.....	....	....	C	....	....	.....A..C.	....	.A........	.........T	....	....G.....	....	.....T....	.....	
Kaud_DQ854677.1	..C.......	....	T...C.....	....	....	C	....	....	.....A....	....	.A........	.........T	....	....G.....	....	.....T....	.....	
Kaud_DQ854678.1	..C.......	....	T..TC.....	....	....	C	....	....	.....A....	....	.A........	.........T	....	....G.....	C..G......	.....T....	.....	
Tang_DQ854679.1	..C.......	.......C..	T..TC.....	....	....	C	....	....	...A.A....	....	.......C..	.........T	.......T..	....	G.........	....	.....	
Tang_DQ854680.1	..C.......	.......C..	T..TC.....	....	....	C	....	....	...A.A....	....	.......C..	.........T	.......T..	....	G.........	....	.....	
Tpfl_DQ854681.1	..C.......	.......C..	T..TC.....	....	....	C	....	....	...A.A....	....	.......C..	.........T	.......T..	....	G.........	....	.....	
Tpfl_DQ854682.1	..C.......	.......C..	T..TC.....	....	....	C	....	....	...A.A....	....	....G..C..	.........T	.......T..	....	G.........	....	.....	
Tbel_DQ854683.1	..C.......	.......C..	T..TC.....	....	....	G........C	....	....	...A.A....	....	.......C..	.........T	.......T..	....	G.........	....	.....	
Tbel_DQ854684.1	..C.......	.......C..	T..TC.....	....	....	G........C	....	....	...A.A....	....	.......C..	.........T	.......T..	....	G.........	....	.....	
Tgeo_DQ854685.1	..C.......	.......C..	T..TC.....	....	....	......C..C	....	....	...A.A....	....	....G.....	.........T	....	....	G.........	..G..T....	.....	
Tgeo_DQ854686.1	..C.......	.......C..	T..TC.....	....	....	......C..C	....	....	...A.A....	....	....G.....	.........T	....	....	G.........	..G..T....	.....	
T.sp.JRM_DQ854687.1	..C.......	.......C..	T..TC.....	....	....	C	....	....	...A.A....	....	....G.....	.........T	....	....	G.........	..G..T....	.....	
T.sp.JRM_DQ854688.1	..C.......	.......C..	T..TC.....	....	....	......C..C	....	....	...A.A....	....	....G.....	.........T	....	....	G.........	..G..T....	.....	

														← IsticND2-R2				491
Ipla_DQ854669.1	AAGCTAGCAC	CCTTCGCCCT	CCTTCTACAA	ATCCAAACAA	CTAACCCTTC	GATCCTTATT	CTGCTCGGAG	TCCTATCAAC	ACTCGTAGGC	GGCTGAGGTG	GTTTAAACCA	AACCCAGTTA	CGAAAAATTC	TTGCTTATTC	CTCAATCGCA	CACCTAGGCT	G	
Ipla_DQ854670.1	....	....	....	....	....	....	.........A	....	....	....	....	....	....	....	....	....	.	
Mnig_DQ854671.1	..A.......	....	....	.........G	.......CA.	C	....	....	....	........A.	.C........	G....A....	....	....	....	....	.	
Mnig_DQ854672.1	..A.......	....	....	.........G	.......CA.	C	....	....	....	........A.	.C........	G....A....	....	....	....	....	.	
Iind_DQ854673.1	..A.......	....	...C......	..T.......	........A.	.G........	....	....	.........T	........A.	.CC.......	.........G	........C.	.C........	....	....	.	
Iind_DQ854674.1	..A.......	....	...C......	..T.......	........A.	.G........	....	....	.........T	........A.	.CC.......	.........G	........C.	.C........	....	....	.	
Talbi_DQ854675.1	..A.......	....	....	..T.......	........A.	A.........	....	....	...T......	........A.	.C........	......A...	..G.......	....	....	....	.	
Talbi_DQ854676.1	..A.......	....	....	..T.......	........A.	A.........	....	....	...T......	........A.	.C........	......A...	..G.......	....	....	....	.	
Kaud_DQ854677.1	..A.......	....	....	..T.......	........A.	A.........	....	....	...T......	........A.	.C........	......A...	..G.......	....	....	....	.	
Kaud_DQ854678.1	..A.......	....	....	..T.......	........A.	A.........	....	....	...T......	........A.	.C........	......A...	..G.......	....	....	....	.	
Tang_DQ854679.1	..A.......	....	....	..T.......	.C......A.	....	....	....	....	........A.	.C........	......A..G	....	....	....	....	.	
Tang_DQ854680.1	..A.......	....	....	..T.......	.C......A.	....	....	....	....	........A.	.C........	......A..G	....	....	....	....	.	
Tpfl_DQ854681.1	..A.......	....	....	..T.......	.C......A.	C.........	....	....	....	........A.	.C........	......A..G	....	....	....	....	.	
Tpfl_DQ854682.1	..A.......	....	....	..T.......	.C......A.	C.........	C	....	....	........A.	.C........	......A..G	....	....	....	....	.	
Tbel_DQ854683.1	..A.......	....	....	..T.......	.C......A.	C.........	....	....	....	........A.	.C........	......A..G	....	....	....	....	.	
Tbel_DQ854684.1	..A.......	....	....	..T.......	.C......A.	C.........	....	....	....	........A.	.C........	......A..G	....	....	....	....	.	
Tgeo_DQ854685.1	..A.......	....	....	..T.......	........A.	....	....	....	....	........G.	.C........	......A..G	....	....	....	....	.	
Tgeo_DQ854686.1	..A.......	....	....	..T.......	........A.	....	....	....	....	........A.	.C........	......A..G	....	....	....	....	.	
T.sp.JRM_DQ854687.1	..A.......	....	....	..T.......	........A.	....	....	....	....	........A.	.C........	......A..G	..G.......	....	....	....	.	
T.sp.JRM_DQ854688.1	..A.......	....	....	..T.......	........A.	....	....	....	....	........G.	.C........	......A..G	....	....	....	....	.	

Accordingly, multiple sequence alignments of the mtDNA ND2 gene (GenBank ID: DQ854669~DQ854688) of ten billfish species (two sequences per species; Collette *et al.* 2006) were carried in Geneious v.6.1 (Kearse *et al.* 2012) to identify segments containing diagnostic SNPs (i.e. fixed differences) for the design of HRMA-genotyping assays. The consensus alignment of ND2 revealed several potential conserved segments for primer placement flanking regions containing SNPs that could be diagnostic for species identification. Primers were selected with the aid of Primer3 (Rozen and Skaletsky, 1999). Two variants of HRM targeting ND2 were designed for Pacific billfish identification. The first corresponded to a HRMA (Reed *et al.* 2007) based on the PCR amplification of a 491 bp ND2 segment whose multiple sequence alignment contained 67 variable sites (**Table 2**) using primers IstioND2_F1 (5'- CAC TGG CTC CTA GCA TG -3') and IstioND2_R2 (5'- CAG CCT AGG TGT GCG ATT GAG GA -3'). The second assay corresponded to an unlabeled probe (UP) HRMA (Liew *et al.* 2007; Poulson and Wittwer 2007) based on the asymmetric PCR amplification of a 410 bp ND2 segment using primer IstioND2_F2 (5'- GCA GTT GAA GCA ACC ACC -3') and IstioND2_R2 to generate single-stranded DNA in excess for the hybridization of a 48 bp long probe IstioND2_Probe1 (5'- AGC TGC CAT ACT GTT ATT TGC TAG CAC AAC CAA TGC TTG ACT TAC CGG/Phos/–3') that matches sailfish ND2 sequence. This unlabeled probe would act as a reporter of distinct melting temperatures defined by the character states of nine SNPs (**Table 2**).

PCR of the HRMA assays were conducted in total 10 µl volume including 200µM dNTPs, 1U Platinum® Taq (Invitrogen), 1X PCR Buffer, 3.0 mM $MgCl_2$, 250

µg/mL Bovine serum albumin (BSA), 0.2 M Trehalose, 1X LCGreen® Plus^{+} Melting Dye (Biofire Diagnostics, Inc.), 20 ng DNA template with 0.5 µM of primers IstioND2_F1 and IstioND2_R2, and ddH2O to adjust to volume. Reactions were overlaid 15 µl mineral oil to prevent evaporative losses that may affect the melting profiles. PCR reactions were performed in a RapidCycler II (Idaho Technology), with the following thermocycling parameters: an initial denaturing step at 94℃ for 1 minute, followed by 25 cycles of denaturation at 94℃ for 0 seconds, annealing at 56℃ for 0 seconds and extension at 72℃ for 20 seconds. This was followed by 20 more cycles of denaturation at 94℃ for 0 second, annealing at 44℃ for 0 second and extension at 72℃ for 20 seconds. Additional cycles at lower annealing temperature resulted in stronger amplification signals (data not shown). The melting profiles were collected and analyzed in HR-1^{TM} instrument (Idaho Technology) by the control and data analysis software with the following parameters used to collect data: Acquisition start temperature: 78℃ , cool temperature: 70℃ , final temperature: 90℃ and ramp rate:0.3℃.

For UP-HRMA assays, the asymmetrical PCR were conducted in 10 µl reaction volumes consisting of 200 µM dNTPs, 1U FastStart Taq (Roche Diagnostics), 1X PCR Buffer (with 20mM $MgCl_2$), 1X LCGreen® Plus^{+} Melting Dye (Biofire Diagnostics, Inc.), 10 ng DNA template with 0.5 µM of each primers IstioND2_F2 diluted 1:20 and IstioND2_R2, 0.5 µM unlabeled probe IstioND2_Probe1 and ddH_2O to adjust volume. Thermocycling parameters included an initial denaturing step at 95℃ for 10 minutes, followed by 75 cycles of denaturation at 95℃ for 10 seconds, annealing at 56℃ for 30 seconds and extension at 72℃ for 10 seconds. The melting profiles were collected from

65 to 90℃ with a melting ramp rate of 0.03℃ per sec using LightCycler® 480 Real-Time PCR System (Roche Diagnostics), followed by genotyping using LightCycler® 480 Gene Scanning Software v. 1.5.0 SP1 (Roche Diagnostic) to identify the Pacific billfish species. Positive controls were used in all reactions using individuals whose species identification had been validated by examination of morphological and meristic characters (Nakamura 1985), and by direct sequencing of PCR-amplified Control Region I (CR-I). The PCR products were sequenced in both directions with the BigDye™ Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) for the cycle sequencing reaction using Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA), followed by multiple sequence alignment against billfish CR-I sequences.

Sensitivity, repeatability, and species assignment testing

HRMA-genotyping assays were tested for repeatability and sensitivity with additional specimens using the protocols described above. The assessment of repeatability involved the ability to duplicate a given melting curve in separate experiments or in reference to a validated specimen (positive control) of the species in question. Sensitivity was assessed by the ability of the assay to unambiguously discriminate species by their melting profiles. Two samples were employed to test the HRMA and the UP-HRMA independently. The first sample, employed in testing the HRMA (no probe) assay, consisted of a subset of 37 individuals obtained from a larger sample (n=57) that had been identified as black marlin by observers of the Inter-

American Tropical Tuna Commission (IATTC) while on board of commercial fishing vessels, but which were thought to include misidentified blue marlin. A total of 37 individuals were characterized with HRMA. The mtDNA CR-I of the rest of the individuals (n=20) in that sample was sequenced as described in Alvarado-Bremer *et al.* (2005). A second sample was utilized to test the UP-HRMA assay and consisted of a subset of 24 individuals selected from a large sample of billfish characterized by Ortega-García *et al.* (2006). Tissue samples were obtained from billfish landed by three recreational fleets operating off Cabo San Lucas, Mexico, and all samples were provided by Sofía Ortega-García, Centro Interdisciplinario de Ciencias Marinas–Instituto Politécnico Nacional, La Paz, Mexico. The subsample included two individuals positively identified black marlin and the rest as blue marlin. Species identification was based on meristic and morphological characters (Ortega-García, Pers. Comm.). Positive controls were included in all HRM reactions.

Results

HRMA assays without probe

The melting profiles of a 491 bp fragment of ND were diagnostic for the identification of four Pacific billfish species, either by their unique T_m or the shape of the melting curves, or both (**Figure 1**). Although the amplicon melts for the four species took place between 83.0˚C to 88.0˚C, the differences in melting peaks among species occurred between 86.0˚C to 87.0˚C, and in spite of such narrow temperature range, each species displayed a very distinct and diagnostic melting curve (**Figure 1**).

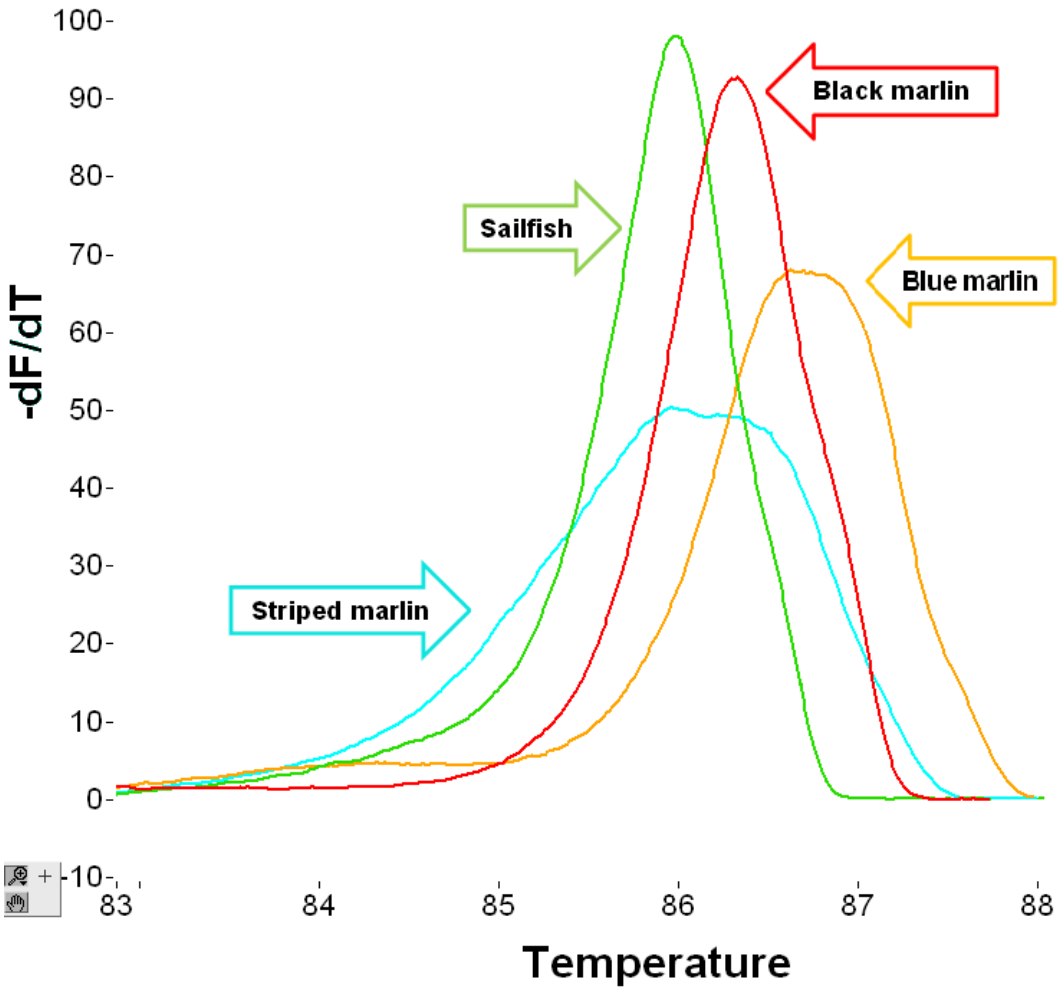


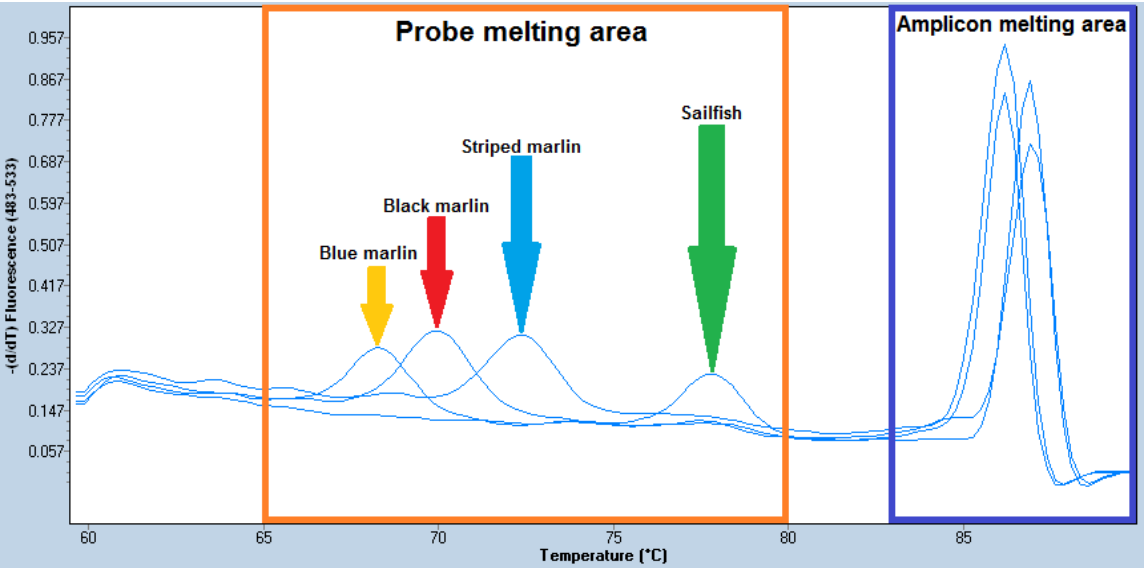
Figure 1. Normalized derivative plot of fluorescence with respect to temperature for a 491 bp fragment of the mtDNA ND2 gene. Each melting curve corresponds to one of four species of Pacific billfish, namely black marlin, blue marlin, striped marlin and sailfish.

The melting curve derivatives for sailfish and black marlin were markedly unimodal and leptokurtic with the maximum melting rate peaking at 86.0˚C and 86.5˚C, respectively. Conversely, the melting temperature of striped marlin and blue marlin differed by one degree, 86.0˚C and 87.0˚C, respectively, but their curves were broader lacking the well-defined peaks displayed by sailfish and black marlin melts. The curve

for striped marlin was the broadest and to some extent bimodal with melting temperatures peaks at about 86˚C and 86.5˚C, which overlap with the melting peaks of sailfish and black marlin. However, the position and shape of the curve of striped marlin precludes species misidentification. Finally, blue marlin melted at the highest temperature (87.0˚C) among the four billfish species, and thus it easily differentiated from the rest. Accordingly, all four species of Pacific billfish possess HRM profiles that are diagnostic due to the shape of their curves or the peak melting temperature of their amplicons.

Unlabeled probe: UP-HRMA assays

We targeted the variation contained within a 410 bp fragment using an UL probe for Pacific billfish identification. Melting temperatures against the 48 nt-long probe ranged from 68˚C to 78˚C, with each species displaying discrete melting temperatures (**Figure 2**). Since the probe matched sailfish ND2 sequence, the T_m for this species was, as expected, the highest (78˚C). A total of eight SNPs were identified along the probed segment among the representative haplotypes of the four species of Pacific billfish targeted in this study. Striped marlin differs by four SNPs against the sailfish-specific probe equivalent to a 5.5˚C (T_m=72.5 ˚C) difference in T_m. These SNPs, all transitions, include one G/A (SNP site: 138) and three T/C (SNP sites: 129,159, and 168) changes. Black marlin and blue marlin both differ by five SNPs from the sailfish-specific probe, but their corresponding melting temperatures are 70˚C and 68˚C, respectively. All five differences between black marlin and sailfish are pyrimidine transitions and include one



Species Common Name	SNP site								SNP variance					
	129	*135*	*138*	*139*	*144*	*156*	*159*	*168*	*V*	*A/G*	*T/C*	*C/T*	*G/A*	*Tm(°C)*
Sailfish	T	A	G	T	T	C	T	T	0					78.0
Striped marlin	C	A	A	T	T	C	C	C	4	0	3	0	1	72.5
Black marlin	C	A	G	T	C	T	C	C	5	0	4	1	0	70.0
Blue marlin	T	G	G	C	C	T	C	T	5	1	3	1	0	68.0

Figure 2. Derivative plot of fluorescence with respect to temperature with unlabeled probe for a 410 bp fragment of the mtDNA ND2 gene. Each melting curve corresponds to one of four species of Pacific billfish, namely black marlin, blue marlin, striped marlin and sailfish.

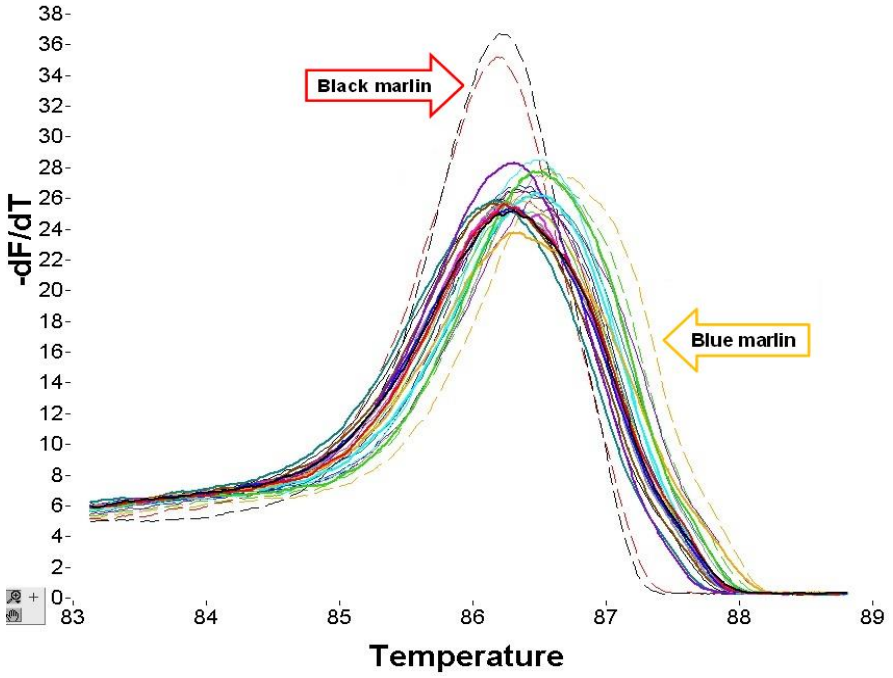
C/T (SNP site: 156) and four T/C (SNP sites: 129,144,159, and 168) changes with a corresponding 8˚C temperature difference (T_m=70˚C). The differences of blue marlin with respect to the probe are also transition changes but include one A/G (SNP site: 135), one C/T (SNP site: 156) and three T/C (SNP sites: 139, 144, and 159) corresponding to a 10˚C temperature difference (T_m=68˚C) relative to sailfish. The details of the probe SNP sites and melting temperatures across four target billfish species are shown in Figure 2. In sum, all four Pacific billfish species were easily distinguished

by UP-HRMA of ND2 using their respective melting peaks relative to the probed segment.

Repeatability and sensitivity testing

The repeatability and sensitivity test of the HRMA assay using individuals that had tentatively been identified as black marlin generated two very distinct curves (**Figure 3A**) that matched those of blue marlin and black marlin, respectively. In total, only three individuals were identified as black marlin, 32 as blue marlin, and two undetermined because of failed HRMA reactions after several amplification attempts. Normalized derivative plots of HRMA also revealed very little variation in the shape of the melting curves and the corresponding T_m for the two species (**Figure 3A**), confirming the repeatability of this assay. Although a slight shift (0.2°C) in T_m compared to the original HRMA experiment (see **Figure 1**) was observed, it did not affect the diagnostic power of the assay because of the inclusion of positive controls. It is relevant to remember that the entire IATTC sample (n=57) had been identified as black marlin. DNA sequencing of the mtDNA CR-I identified the remaining specimens (n=20) as blue marlin, and confirmed the identification obtained with HRMA for the three black marlin and for six arbitrarily chosen blue marlin. The CR-I of the two individuals that could not be characterized with HRMA also failed. In sum, the IATTC sample consisted of three black marlin, 52 blue marlin, and two undetermined.

A) HRMA assay



B) UP-HRMA assay

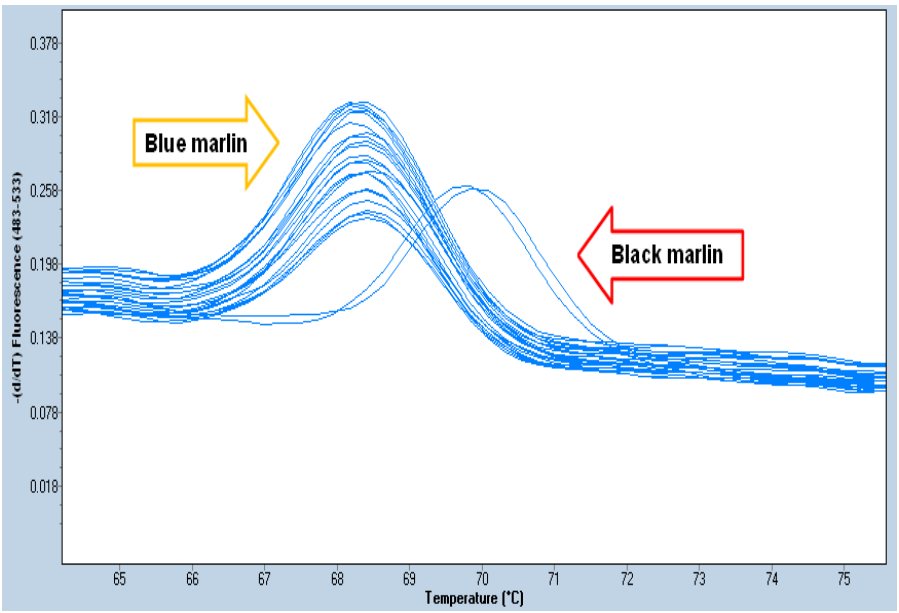


Figure 3. Assay sensitivity and repeatability for both HRMA-genotyping assay using unknown samples. A) HRMA assay and B) UP-HRMA assay.

The UP-HRMA assay was used to characterize a sample of 24 individuals whose species identification had been validated by experts specialized in billfish reproductive biology. UP-HRMA corroborated the identity of the two black marlin and the 22 blue marlin specimens. Figure 3B displays the UP-HRMA profiles corresponding to the distinct melting temperatures against the probe for the two species, respectively at 70˚C (black marlin) and 68˚C (blue marlin). Although the melting curves display variability in fluorescence signal (-dF/dT), there is little variation in the corresponding T_m peak for the two species, and thus in the resolving power of the assay.

Discussion

Two novel assays based on HRMA were designed to identify four species of Pacific billfishes. We focused on characterizing the variation contained in the mtDNA ND2 because this gene contains a robust signal to resolve the phylogenetic relationships among billfish (Collette *et al.* 2006). The HRMA assay required the design of two new primers to amplify a 491bp segment of ND2. Conversely, the UP-HRMA assay required the design of an additional internal primer in order to amplify a 410bp segment of ND2, and also the selection of a segment for the placement of an unlabeled probe whose differential fluorescence during DNA melting (-dF/dT) would act as a reporter for species identification. Although the segments characterized in this study were substantially shorter than the ND2 segment (995 bp) characterized by Collette *et al.* (2006), the SNPs contained within the 491 bp segment of the HRMA assay, and along the 48 bp probe characterized with UP-HRMA, were sufficiently robust to yield

unambiguous species identification of black marlin, blue marlin, striped marlin and sailfish, and also the potential to identify shortbill spearfish and swordfish (see below).

HRMA is generally used to detect nucleotide differences of relatively small fragments (60-200 bp) because higher temperatures differences (ΔT_m) between alleles are obtained targeting relatively short segments. Such higher sensitivity lead to the development of short-amplicon (SA) HRMA (Gundry *et al.* 2008), that has the advantage of minimizing 'genotype masking' (Smith *et al.* 2013) visualized as undistinguishable melting profiles due to multiple or base-pair neutral variants. In here, HRMA generated diagnostic curves for striped marlin, black marlin, blue marlin and sailfish when characterizing a large fragment (491 bp) of ND2. Although the difference in T_m among the four species was very small, only about one degree Celsius, the shape of each curve was diagnostic for species identification. This differentiation was accomplished even when differences in peak T_m were small (≤0.5°C), as among sailfish, black marlin and blue marlin, or overlapping, as with striped marlin relative to sailfish and black marlin (**Figure 2**). The bimodal shape of the curve for striped marlin, most likely associated to the presence of two melting domains within the amplicon, facilitated species identification in spite of overlapping T_m.

While testing the HRMA assay for sensitivity and repeatability with a larger sample, a slight shift in melting temperature among samples (about 0.2 ˚C) was observed. This shift, however, did not affect the species assignation because the positive controls were included, but also because blue marlin and black marlin, the potential two candidate species present in that sample, could be easily identified by the corresponding

melting curve shapes. In those instances where curve shapes are not diagnostic, shifts in melting temperature could result in severe genotyping errors, particularly if positive controls are not included. Salt concentration, that may be associated with the method of DNA isolation employed, and PCR reagent chemistry may cause shifts in T_m (Vossen, *et al.* 2009). Accordingly, when conducting HRM-based assays for species identification it is very important to maintain the same chemistry throughout the experiments, and include a minimum of one reference standard per allele diagnosed to minimize potential scoring errors.

The results obtained with UP-HRMA based on ΔT_m of amplicon against a 48 nt long probe generated diagnostic melting curves and temperatures for each of the four species of Pacific billfishes assayed as well. The ΔT_m due to the number of SNPs relative to the sailfish-specific probe were as expected, yielding the highest T_m for sailfish (78.0℃), followed by striped marlin (72.0℃), black marlin (70.0℃) and blue marlin (68.0℃). Repeatability and sensitivity experiments using larger sample sizes revealed no additional SNPs, or additional melting profiles among the validated sample of blue marlin and black marlin. Contrary to what was found with the HRMA assay, no shifts in T_m were observed with UP-HRMA. However, the melting curves display variability in the intensity of fluorescence signal (-dF/dT), due to differences in amplification efficiency among samples, without affecting haplotype scoring.

Previous studies aimed at Pacific billfishes identification have characterized variation at different loci using a variety molecular techniques. Both, Chow (1994) and Hsieh *et al.* (2007) used PCR-RFLP analysis of the mtDNA cyt *b* gene for billfish

species ID. Chow (1994) digested a 350 bp cyt *b* with three restriction endonucleases (*Alu I*, *Bsa JI*, and *Taq I*) to distinguish the same Indo-Pacific billfish species assayed here, although the same restriction assays would also identify shortbill spearfish (*Tetrapterus angustirostris*) and swordfish. Similarly, Hsieh *et al.* (2007), cut a 348 bp cyt *b* fragment with three enzymes (*Bsa JI*, *Cac 8I*, and *Hpa II*) to identify the four Pacific billfish species characterized in here and also swordfish. Conversely, Innes *et al.* (1998) digested the hypervariable mtDNA CR with four enzymes (*Hinf I*, *Rsa I*, *Sau 3AI*, and *Taq I*) to identify Pacific billfishes. A revision of their results reveal that the restriction patterns generated with *Hinf I* could potentially identify the four species characterized in here, with an additional cut with *Sau 3AI* needed to separate blue marlin from swordfish (Innes *et al.* 1998). The interpretation of that assay could be complicated due to within-species variability resulting from the hyper-variability of the CR. The PCR-RFLP assays of McDowell and Graves (2002) also required a minimum of two digests to identify the four billfishes characterized here whether cutting mtDNA ND4 fragment with *Hae III* and *Ban I*, or the nuclear MN32-2 locus with *Dra I* and *Dde I*. Using either serial digest, the identification of the four billfishes included in this study plus swordfish and shortbill spearfish is possible. Seeking to expedite the identification of Pacific billfishes, Hyde *et al.* (2005) designed a multiplex PCR assay targeting cyt *b* that employed species-specific primers to generate fragment size polymorphisms to identify all five Pacific species of istiophorid billfishes and swordfish, and in addition, wahoo (*Acanthocybium solandri*), common dolphinfish (*Coryphaena hippurus*), and pompano dolphinfish (*C. equiselis*). Much shorter times were required using multiplex

PCR than any of the PCR-RFLP billfish identification assays, which would typically require at least two days to complete yielding the identification a few dozens of individuals. By contrast, Hyde *et al.* (2005) were able to conduct shipboard species identification from eggs or larvae within three hours of sample acquisition when the multiplex assay was combined with a fast DNA isolation method and precast gels (E-Gel, Invitrogen). Accordingly, multiplex PCR represents an important advance towards expediting billfish species identification. The HRMA-based assays presented here, although taking about the same time to complete than multiplex PCR, have a much higher throughput, as gel electrophoresis is not needed. Both PCR and scoring are conducted in the same closed-tube, and when performed in the LC-480 instrument, 96 or 384 specimens, depending on the configuration of the thermocycler block, can be characterized simultaneously within 2 h 15 m. Recently, Alvarado-Bremer *et al.* (2014) introduced Shake and Stew, a non-destructive DNA isolation protocol that streamlines DNA isolation from fish larvae and other small samples. DNA isolation from 96 larvae that can be completed in less than 2 h. Using Shake and Stew in combination with an UP-HRMA assay designed to identify tunas, nearly 1,000 tuna larvae (*Thunnus spp.*) per week can be processed by a single technician (Alvarado-Bremer *et al.* 2014.). A disadvantage of high throughput HRMA is the need of real time (RT) PCR instruments to acquire melting data. RT-PCR platforms, however, are becoming standard tools of most modern molecular genetics laboratories, so the use of these technologies for species identification is on the rise (e.g., McGlauflin *et al.* 2010; Bréchon *et al.* 2013).

Alternative molecular techniques based on DNA hybridization to identify fishes are available, including TaqMan probes, lab-on-a-chip, and microarray chips (reviewed in Rasmussen and Morrissey 2008), but these have failed to gain general acceptance and have not been expressly developed for billfishes identification. The development of any of these hybridization-based assays requires prior knowledge of the location of diagnostic sites (i.e., fixed differences), and advances in next generation sequencing (NGS) may facilitate this search. Currently, the identification of all nine recognized species of billfish based on sequence data is only possible through the analysis of 3787 bp of concatenated sequence that included three mtDNA genes (CR, ND2, 12S), and one nuclear locus (MN32-2) (Collette *et al.* 2006). That analysis suggested that nine nominal billfish species belonged to five genera: blue marlin (*Makaira*), sailfish (*Istiophorus*), black marlin (*Istiompax*), striped and white marlin (*Kajikia*), and four spearfishes (*Tetrapturus*). Further, the monophyletic origin of eight billfishes, namely blue marlin, black marlin, sailfish, shortbill spearfish, Mediterranean spearfish, longbill spearfish and roundscale spearfish, and white marlin is supported. Because striped marlin mtDNA contains a group of lineages paraphyletic to the white marlin mtDNA clade, the validity of two species needs to be investigated further (Collette *et al.* 2006; Hanner *et al.* 2011). However, the separation of white marlin (Atlantic) from striped marlin (Pacific) lineages using mtDNA CR sequences is clear, and thus the identification of these to their corresponding basin is not problematic (Collette *et al.* 2006; Hanner *et al.* 2011), regardless of their taxonomic status. Similarly, CR sequence can be used to separate Mediterranean spearfish (Northeast Atlantic and Mediterranean) from longbill

spearfish (Atlantic), and also to differentiate roundscale spearfish (*T. georgeii*) from the morphologically similar white marlin and from other spearfishes (Collette *et al.*, 2006), with the separation of this latter pair also possible by characterizing 1268 bp of sequence cyt *b* and ND4L-ND4 genes (Shivji *et al.* 2006). By contrast, attempts to establish DNA barcodes to identify billfishes using mtDNA COI and rhodopsin (Rho) sequences were less successful. COI sequences can only distinguish four of nine billfishes, namely blue marlin, sailfish, black marlin and roundscale spearfish (Hanner *et al.* 2011). The remaining five species clustered together into two groups: one formed by white marlin and striped marlin, and the other by Mediterranean spearfish, longbill spearfish and shortbill spearfish. The HRMA assays developed here based on ND2 SNPs have different levels of diagnostic power to distinguish additional billfishes than those reported in this study. The HRMA assay is capable of distinguishing longbill spearfish and roundscale spearfish from white marlin, blue marlin and sailfish in the Atlantic (Alvarado-Bremer, Pers. Comm.). Conversely, the UP-HRMA is capable of distinguishing longbill spearfish from roundscale spearfish and potentially from Mediterranean spearfish, but not from shortbill spearfish. The UP-HRMA can also distinguish swordfish from all billfishes (Alvarado-Bremer, Pers. Comm.).

In conclusion, two HRMA-genotyping assays were developed based on the characterization of variation contained in the mtDNA ND2 gene capable to unambiguously identify four Pacific billfish species, namely striped marlin, blue marlin, black marlin and sailfish, and the potential to identify shortbill spearfish. Both HRM-based assays developed here could be particularly useful to identify billfishes from a

variety of samples, notably 1) similarly-shaped early life stages, 2) specimens lacking diagnostic morphological characters such as dressed fish, or processed products, and 3) when large sample sizes are needed, and 4) when unequivocal species identification is required, such as when conducting population genetics studies, or when conducting linkage studies (e.g., gender determination). In all these instances, species misidentification would lead to major errors. HRMA-based assays have several advantages over previous methods used to identify billfishes, including higher sensitivity, efficient, low cost per assay, diagnostic easy to interpret profiles and suitability for high throughput. Since these species are frequent bycatch of longline commercial fisheries targeting tunas, the availability of high throughput assays for species identification represents a potentially valuable tool to improve fishery management practices of these billfishes in the Pacific Ocean.

CHAPTER III

A SURVEY OF POTENTIAL SEX-LINKED MARKERS IN SWORDFISH (*Xiphias gladius*) AND ISTIOPHORID BILLFISH

Introduction

Proper management of marine pelagic fisheries relies on obtaining accurate estimates of abundance that, together with other demographic and biological parameters, can be used to estimate potential recruitment (Myers *et al.* 1997; Schnute and Richards 2002). Abundance estimates in fisheries can be derived from two sources: historical catch data, and direct scientific surveys, which in the case of pelagic fisheries, typically rely on placing trained observers on board commercial fishing vessels to document catch. Catch data is then extrapolated to the entire fishery; however, contrary to common perception, direct surveys are rarely used to estimate the size of pelagic fish stocks. Instead, abundance trends are derived from historical series of catch-at-age data from commercial fisheries (Gudmundsson 1994; Myers *et al.* 1997) using the modeling technique known as Virtual Population Analysis (VPA) (Gulland 1965; Murphy 1965). Errors in the catch-at-age (or weight-at-age) data matrices can be large if the species of interest displays sexual dimorphism in size and growth rate. Such bias can be particularly large if the gender that reaches the largest size grows faster. This condition is common among many pelagic fishes, including swordfish (*Xiphias gladius*), blue marlin (*Makaira nigricans*), black marlin (*Istiompax indica*), and several species of tuna. To correct for this bias, gender-specific growth-curves are commonly fitted allowing the

inclusion of sex-at-size ratios into VPA models. Unfortunately, with exception of the sexual dimorphism in size, swordfish and billfishes lack obvious secondary sexual characters that would enable fishery biologists to distinguish gender. This situation is compounded by the practice of commercial pelagic fisheries to dress fish at sea. Consequently, gonads are not available for gender determination at port. New unpublished research indicates that gender determination of billfishes should be possible by careful inspection of the gonadal groove of reproductively active mature individuals (Alvarado-Bremer, Pers. Comm.), but the reliability and practicality of this approach in field situations has not been tested.

The development molecular assays to determine the gender from tissue samples could represent an extremely valuable tool to improving abundance estimates of males and females from VPA modeling. Additionally, molecular sex-determination assays could be valuable in eco-physiological and tagging programs aimed to reconstruct patterns of dispersal, diving behaviors and habitat using satellite-tracking devices (e.g., archival tags and Pop-up Tags or PSATs), as gender-specific diving and migration patterns may be revealed. Tissue samples (e.g., fin clips) obtained at the time of tagging could be used as DNA sources to determine gender, adding a new dimension to the interpretation of observed movement patterns. Finally, due to logistical limitations, population genetics studies of pelagic fishes often rely on commercial fishing operations to provide samples, and in only exceptional cases, when trained observers are involved, that information of gender is included. Even in those instances, the reliability of such information cannot be guaranteed because of the difficulties of determining gender by

inspecting the gonads of immature or resting individuals. Because males and females can differ in their respective patterns of gene flow, there is the potential for them to carry distinct patterns of differentiation which may account for reported differences in genetic signal between mtDNA and microsatellite data (Qiu *et al.* 2013). Accordingly, a hierarchical arrangement by sex is recommended to determine the relative proportion of males and females to the total genetic variation in the population, and also to determine the magnitude of potential bias in genetic signal as a result of gender-biased dispersal. Thus, the availability of molecular gender-determination assays would increase the refinement of genetic population structure analyses and provide a better understanding of the evolutionary forces that contribute to the observed patterns of genetic variation of populations.

There is no universal mechanism of gender determination in bony fishes, and gender determination may be more complex than in other superclass of vertebrates (Ezaz *et al.* 2006), and include genetic, environmental, behavioral and physiological factors, or the result of interactions of these factors (Devlin and Nagahama 2002; Mank *et al.* 2006). However, there are fishes where genetic systems of gender determination exist, although these are also varied. In some species, multiple-gene interactions appear to operate (Volff *et al.* 2003; Volff 2005), while in others, the presence of sex chromosomes analogous to those in mammals (heterogametic males XY) or most birds (heterogametic females ZW) has been documented (Artoni and Bertollo 2002; Peichel *et al.* 2004). Sex-linked genes have been identified in several fish species, including the DM-domain gene on the Y chromosome (DMY) of different strains of the medaka and

Japanese rice fish (*Oryzias latipes*) (Matsuda *et al.* 2002, Nanda *et al.*2002 and Takehana *et al.* 2007), and the Anti-Müllerian Hormone (AMH) gene of the Patagonian pejerrey (*Odentesthes hatcheri*) (Hattori *et al.* 2012).

The cytological characterization of white marlin (*Tetrapterus albidus*) suggests the presence of XY-chromosomal complement in males (Durán-González and Laguardia-Figueras 1992), and it is possible that homologous systems are present in other billfish species. If sex chromosomes exist, the chances of identifying sex-determining genes analogous to the mammalian *SRY* gene or regions linked to sex-determination would increase. This could lead to the development of simple gender determination assays based on the Polymerase Chain Reaction (PCR) using DNA samples isolated from a wide variety of tissue sources (e.g, muscle, fin clips, scales, etc.) and preservation methods (see Griffiths and Tiwari 1993). Such methods are available for mammals, birds, and several fish species (e.g., Griffiths *et al.* 2000; Zhang 2004).

Different molecular approaches have been used to search for sex-linked markers, including amplified fragment length polymorphisms (AFLPs), microsatellite markers, randomly amplified polymorphic DNA (RAPDs), and restriction fragment length polymorphism (RFLP) (Devlin and Nagahama 2002). The objective of this study is to develop molecular sex determination assays for the swordfish (*Xiphias gladius*), and for two billfish species: blue marlin: (*Makaira nigricans*) and sailfish (*Istiophorus platypterus*) based on PCR technologies. Two very different strategies to maximize the potential of identifying markers linked to gender-determination in billfish were employed. The first approach was to use RAPDs to screen randomly the genome to

detect sex-specific loci (Griffiths and Tiwari 1993). Kovács *et al.* (2001), searched for sex-specific DNA polymorphisms in the male and female African catfish (*Clarias gariepinus*) by comparative RAPD assays performed on pooled DNA samples, and identified two sex-linked RAPD markers from the male DNA pool. Their results were confirmed on individual samples, showing good agreement with phenotypic sex, and lead to the development of a PCR-based assay after the characterization of sex-link associated sequences derived from RAPDs.

The characterization of genetic variation with RAPDs has several advantages over other molecular techniques because universal primer sets can be employed, without preliminary work such as probe isolation, filter preparation, or nucleotide sequencing (Ali *et al.* 2004). However, several limitations (e.g. dominance, homology inferences, artifact fragments, and thus reproducibility) have restricted practical application of RAPD analysis. For instance, the use of RAPDs in population genetics studies is not appropriate because they appear to be dominant markers, scored in a gel as presence or absence of an amplicon (i.e., band). Dominance is not a concern in linkage studies to identify markers for gender determination (Griffiths and Tiware 1993; Griffiths *et al.* 2000). However, several studies have reported poor RAPD reproducibility (Weeden *et al.*, 1992; Penner *et al.*, 1993; Skroch and Nienhuis, 1995). However, Bagley *et al.* (2001) compared both the level of polymorphism and reproducibility of RAPDs and AFLPs in pedigreed populations of rainbow trout (*Oncorhynchus mykiss*). By excluding bands that comprised less than 1% of total intensity, and the largest and smallest 10% of

the bands, nearly 100% reproducibility of AFLP fingerprints was achieved with RAPDs. Similar performance of AFLPs and RAPDs was reported by Renganayaki *et al.* (2001).

The second approach was to characterize genes (e.g., zinc finger gene (*Znf*), DMY, AMH, etc.) known to be linked to the Y-chromosome in other fishes (Devlin *et al.* 2001; Griffiths *et al.* 2000; Nanda *et al.* 2002; Naruse *et al.* 2004; Peichel *et al.* 2001, 2004; Takehana *et al.* 2007; Hattori et al 2012), with the expectation that some of these may also determine gender in billfishes. In any case, if sex-linked markers were to be identified, a High Resolution Melting (HRM) based assay would be developed to provide a rapid, single closed tube means of genotyping for gender determination, similar to the approach used to determine gender in birds (Chapman 2012), and to provide gender-related genetic information for improving the resolution of assessments of the three target species.

Materials and methods

Sample and DNA extraction

All fish tissues samples were obtained from recreational and commercial fisheries operating in in Australia, Chile, Mediterranean Sea and North Atlantic for swordfish, the Gulf of Mexico, Hawaii and Taiwan for blue marlin, and in Ixtapa, Zihuatanejo, and Cancun, Mexico and Taiwan for sailfish. The majority of tissue used in this study was muscle; only sailfish samples from Mexico consisted of both fin clip and muscle. For the three target species, we collected a total of 94 swordfish (43 females and 51 males), 64 blue marlin (32 females and 32 males), and 77 sailfish (36

females and 41 males). Sample details are shown in **Table 3**. Gender for each individual was identified by visual inspection of the gonads. For each of the species characterized, a sample of ten specimens, including five females and five males, were initially screened. After the initial screening, twenty specimens, including ten females and ten males of each species, were used for further validation of the gender-related markers in this study. The DNA isolation of all samples was followed by EtOH precipitation without phenol chloroform extraction (Greig 2000). DNA concentration was measured with a NanoDrop 1000 instrument (NanoDrop Technologies Inc.), and all samples were diluted to the same standardized concentration (10 ng/μl) for use in PCR experiments as template.

Developing the molecular assay for gender determination

RAPDs

Experiments were conducted to test the effect of *Taq* DNA polymerase formulation and tissue type on RAPD reproducibility using five males and six females of sailfish, with two types of tissue (i.e., fin clip and muscle tissue), and four Taq formulations: 1) AccuStart™ TaqDNA Polymerase HiFi (Quanta Biosciences); 2) Platinum® Taq DNA Polymerase (Life Technologies Corporation); 3) AccuStart™ PCR SuperMix (Quanta Biosciences); and 4) AccuStart™ Taq DNA Polymerase (Quanta Biosciences).

Table 3. Details of sampling information of the specimens collected in this study.

Species Name (Common Name)	Location	Year	Latitude	Longitude	Female	Male	Total
Makaira nigricans	Northern Gulf of Mexico[1]	2009	n/a	n/a	5		
(Blue marlin)	Taiwan[1]	2012	n/a	n/a	23	21	
	Hawaii[1,2]	1988	n/a	n/a	4	11	64
Istiophorus platypterus	Ixtapa and Zihuatanejo, Mexico[1]	2009	17.5˚N	101.5˚W	10	16	
(Sailfish)	Taiwan[1]	2010	22˚N~23˚N	121˚E~122˚E	20	20	
	Cancun, Mexico[2]	1989	20˚N	85 ˚W	6	5	77
Xiphias gladius	Australia[1,2]	2006	32˚S	166˚E	27	32	
(Swordfish)	Chile[2]	1998	31˚S	81˚W	2	2	
	Mediterranean Sea[2]	2003	n/a	n/a	4	4	
	North Atlantic[2]	1993	20˚N~46˚N	43˚W~95˚W	10	13	94

[1]: specimen collected for the RAPDs experiments. [2]: specimen collected for the gender-linked gene markers experiments.

The RAPDs reactions (rxns) were performed in a total volume of 12.5 µl, including 10 ng of template DNA, 0.2mM dNTPs, 1 µM OPA-01 RAPD primer, 0.5 U *Taq* DNA polymerase, optimized PCR components with various *Taq* DNA polymerase brands (listed in **Table 4**), and ddH_2O to adjust to the final volume. PCR was carried with the following thermocycling parameters: an initial denaturing step at 95℃ for 5 minutes, followed by 45 cycles of denaturation at 95℃ for 1 minute, annealing at 30℃ for 1 minute and extension at 72℃ for 2 minutes, with a final extension step at 72℃ for 10 minutes. The specifics of the four *Taq* DNA polymerase formulations are listed in **Table 4**. *Taq* performance was evaluated using the following criteria: product yield (low or high), definition of RAPD bands (sharp versus diffuse bands), uniformity (equal amplification success of differently sized amplicons) and whether difference in success was observed with tissue type. Based on the results of initial tests (see Results), we selected 1) AccuStart™ TaqDNA Polymerase HiFi (Quanta Biosciences) to conduct RAPD tests because of high and even yields of small and large products, and no discernable difference between different tissue types as DNA source.

A total of 100 RAPD primers (10 nt) were purchased from Eurofins MWG Operon (Huntsville, Alabama, USA) (Appendix Table A1). The RAPD reactions were performed in a total volume of 12.5 µl, including 10 ng of template DNA, 0.2mM dNTPs, 3mM $MgCl_2$, 0.5 U AccuStart™ TaqDNA Polymerase HiFi (Quanta Biosciences), 1X Buffer, 1 µM RAPD primer and adjusted ddH_2O volume, using the thermocycling parameters described above. The PCR products were visualized via gel electrophoresis using 2% agarose gels in 1X TA buffer for 2 hours at 100-volts. The gels

were stained with 0.5 mg/mL ethidium bromide (EtBr) and the band patterns were recorded using the Gel Doc™ XR+ System (Bio-Rad Laboratories, Inc. USA). The resulting RAPD patterns were scored visually for the presence or absence of DNA fragments.

Table 4. The comparisons of PCR component variables among four Taq DNA polymerase formulations for Taq selection testing.

Taq DNA Polymerase	PCR component variable and final concentration		
	Magnesium Sulfate ($MgSO_4$)	Magnesium Chloride ($MgCl_2$)	Buffer
1. AccuStart™ Taq DNA Polymerase HiFi	3 mM	n/a	60mM Tris-SO_4(pH 8.9), 18mM $(NH_4)_2SO_4$)
2. Platinum® Taq DNA Polymerase	n/a	3 mM	
3. AccuStart™ PCR SuperMix	n/a	1.5 mM	
4. AccuStart™ Taq DNA Polymerase	n/a	3 mM	20mM Tris-HCl(pH 8.4), 50mM KCl

Sex-linked gene markers

The second PCR-based approach to identify genetic markers in billfishes was to target gene markers known to be linked to gender determination in other fish species. A total of twelve markers were characterized, including Anti-Müllerian Hormone (AMH), Acidic ribosomal phosphoprotein P0 (ARP), Dosage-sensitive sex reversal (DSS) - Adrenal hypoplasia congenital (AHC) critical region on the X chromosome, gene 1(Dax-1), DM-related transcription factor 1 (Autosomal DMRT1), DM-domain gene on the Y chromosome (DMY), Growth Hormone (GH), sex-linked Growth Hormone (GH-Y),

NADP-dependent isocitrate dehydrogenase (*Idh*), Y-Chromosomal sex-determination in Chinook Salmon (OtY1), SRY-related genes containing a high mobility group (HMG) box (*Sox*), zinc finger protein (*Znf*), and sex-linked DNA markers using expressed sequence tags (ESTs) (**Table 5**). PCR was carried at the lowest-annealing stringency conditions possible to maximize the chance of amplifying the intended target, with adjustments in annealing temperature according to observed specificity. Each primer set was PCR-amplified using the following thermocycling parameters: an initial denaturing step of 2 minutes at 94℃, followed by 35 cycles of denaturation at 94℃ for 1 minute, loci-specific annealing at target temperature (Appendix Table A2, and references therein) for 1 minute and extension at 72℃ for 1 minute, with a final extension step at 72℃ for 10 minutes. Annealing temperatures were optimized for each primer pair based on preliminary results using a Epperndorf Mastercycler gradient instrument. PCR reactions consisted of 12.5 µl volumes, containing 10 ng of template DNA, 0.5µM of each primer, 1X of EconoTaq® PLUS GREEN 2X Master Mixes (Lucigen) and final volume adjusted with ddH_2O. The quality and quantity of PCR products were verified by running 3 µl of the amplicon in a 1% Tris-acetate (TA) agarose gel pre-stained with EtBr (0.1µg/mL). Female and male templates were run side by side in the same gel to facilitate the comparison of sex-specific fragment size polymorphisms. Cycle sequencing reactions of 'single-band' PCR products were performed using the BigDye Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) in both directions, and sequences were analyzed using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Table 5. Sex-linked markers from other fishes assayed in billfish and swordfish.

Sex-linked marker	*Species*	Reference research
AMH Anti-Müllerian Hormone	**European sea bass** *Dicentrachus labrax*	Halm et al 2007;
	Patagonian pejerrey *Odontesthes hatcheri*	Hattori et al 2012;
	Rainbow trout *Oncorhynchus mykiss*	Affaqh et al 2009;
	Tiger Pufferfish Fugu *Takifugu rubripes*	Kamiya et al 2012;
	Tilapia *Oreochromis niloticus* *Oreochromis aureus*	Poonlaphdecha et al 2011
ARP Acidic ribosomal phosphoprotein P0	**Medaka** *Oryzias dancena* *Oryzias latipes* *Oryzias hubbsi*	Naruse et al 2004; Takehana et al. 2007
Dax-1 Dosage-sensitive sex reversal(DSS)-Adrenal hypoplasia congenital(AHC) critical region on the X chromosome, gene 1	**Rainbow trout** *Oncorhynchus mykiss*	Affaqh et al 2009
Autosomal	**Medaka**	Nanda et al 2002;
DMRT1 DM-related transcription factor 1	*Oryzias latipes* *Oryzias hubbsi*	Kondo et al 2004;
	Rainbow trout *Oncorhynchus mykiss*	Affaqh et al 2009
DMY DM-domain gene on the Y chromosome	**Medaka** *Oryzias latipes*	Nanda et al 2002
GH Growth Hormone **GH-Y** sex-linked Growth Hormone	**Salmonids** chinook, *O. tshawytscha* chum, *O. keta* coho, *O. kisutch* pink, *O. gorbuscha*	Devlin et al 2001
Idh NADP-dependent isocitrate dehydrogenase	**Threespine Sticklebacks** *Gasterosteus aculeatus*	Pelchel et al 2001
OtY1 Y-Chromosomal sex-determination in Chinook Salmon	**Chinook** *Oncorhynchus tshawytscha*	Devlin et al 1994
Sox *SRY*-related genes containing a high mobility group (HMG) box	**European sea bass** *Dicentrarchus labrax*	Galay-Burgos et al 2004;
	Rainbow trout *Oncorhynchus mykiss*	Affaqh et al 2009
Znf zinc finger protein	**Channel catfish** *Ictalurus punctatus*	Tiersch et al 1992;
	Threespine Sticklebacks *Gasterosteus wheatlandi*	Pelchel et al 2004
ESTs: sex-linked DNA markers using expressed sequence tags(ESTs)established	**Medaka** *Oryzias hubbsi*	Takehana et al 2007
	Threespine Sticklebacks *Gasterosteus aculeatus* *Gasterosteus wheatlandi*	Griffiths et al 2000; Naruse et al 2004;

The sequences were aligned using CLUSTAL W algorithm (Thompson *et al.* 1994) implemented in Geneious v6.16 (created by Biomatters), and submitted to GenBank using BLAST to validate identity. Multiple sequence alignments obtained from validated males and females (positive controls) were used for initial screening of potential gender-linked polymorphisms (e.g., SNPs or indels). After potential polymorphisms were identified, HRM assays were developed as describe in Smith *et al.* (2013) and used as a rapid and highly sensitive alternative to sequencing to screen variation in gender validated samples (positive controls) for each of the three species targeted. HRM reactions were performed in the LightCycler® 480 Real-Time PCR System (Roche Applied Science, USA) in 10µl rxn volumes consisting 10 ng template DNA, 1X EconoTaq® PLUS 2X Master Mix (Lucigen), 0.5µl LCGreen® Plus+ Melting Dye (BioFire Diagnostics, Inc), 0.5 µM of each sex-linked primer and ddH_2O to adjust to the final volume. Melting curves were scored with the LightCycler® 480 Software (Service Pack 4). Details for each primer used to amplify piscine gender-linked markers, including oligonucleotide sequences and sources, as well as new primers designed to increase the specificity and reliability of each assay (this study) are listed in Appendix Table A2.

Results

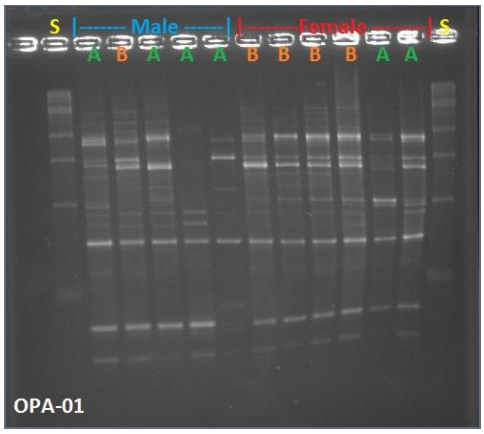
The effect of Taq DNA polymerase formulation and tissue types

We conducted experiments with four different *Taq* DNA polymerase formulations and two types of sailfish tissues to choose the appropriate *Taq* for the

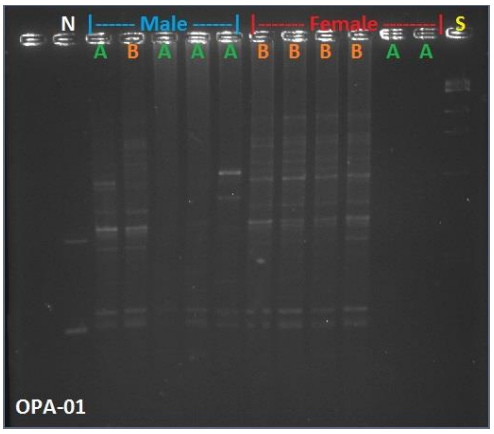
characterization of RAPDs. The evaluation of performance of four Taq formulations indicated that the best performance, based on yield amount and uniformity (i.e., not favoring small or large amplicons), and band sharpness was achieved with AccuStart™ *Taq*DNA Polymerase HiFi (**Figure 4**). Inefficient PCR amplification, characterized by low yields with products lacking definition and uniformity, was obtained using AccuStart™ PCR SuperMix. Second, AccuStart™ *Taq*DNA Polymerase HiFi was the only formulation tested that generated strong, clear banding patterns for both fin-clip and muscle specimens. The others three Taqs produce high yields only when using DNA isolated from muscle tissue. Accordingly, only AccuStart™ *Taq* DNA Polymerase HiFi among the four formulations tested was found suitable for RAPD assay development.

A total of 100 different RAPD assays using a single primer each were performed, and the banding patterns of the three target species were scored by visual inspection. Details of the results of the RAPD assays are listed in Appendix Table A1. Approximately 13, 12, and 26 percent of the assays failed to amplify using swordfish, blue marlin, and sailfish template, respectively. Searches of gender-specific bands for RAPD primer consisted on screening images for the presence or absence of bands in males and females in each species. Gel image for those RAPD primers potentially linked to gender in the three target species are presented in Appendix Figure A1.-A3.

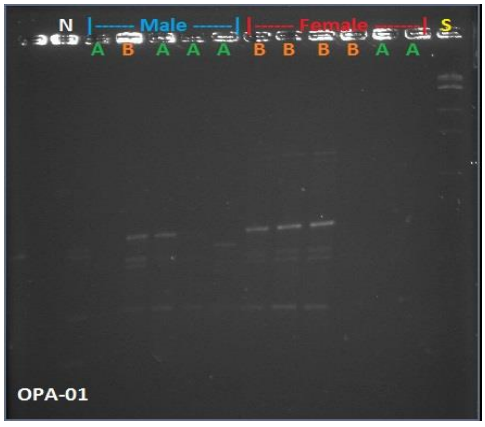
1) AccuStartTM TaqDNA Polymerase HiFi



2) Platinum®Taq DNA Polymerase



3) AccuStartTM PCR SuperMix



4) AccuStartTM TaqDNA Polymerase

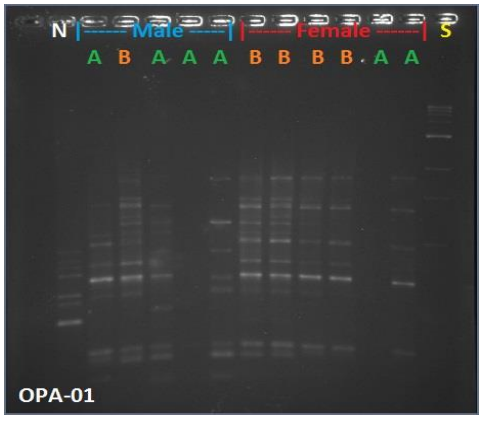


Figure 4. TA agarose gels (2.0%) presenting taq DNA polymerase selection and tissue preference using different taq DNA polymerases in sailfish (*Istiophorus platypterus*). Size markers (S) correspond to a 1 kb DNA Ladder (New England BioLabs) with the following fragment sizes (10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, 0.5 kb) from top to bottom. Each gel was loaded with five males and six females including fin-clip (A) and muscle (B) tissue types, plus a negative control (N).

Development of gender determination RAPD assays

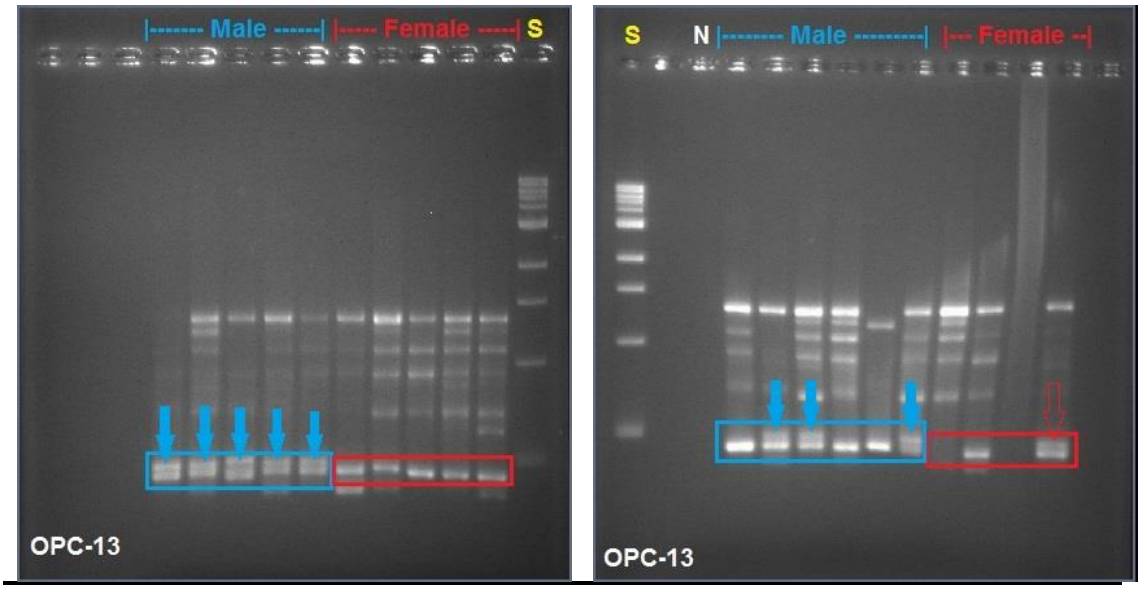
Swordfish

Two RAPD primers generated patterns that suggest certain level of linkage among observed polymorphisms and gender determination. However, no single primer

among the 100 different RAPD primers characterized could be identified as potentially diagnostic for gender determination in swordfish. Although there is no significant sex-specific band for identifying gender in swordfish, RAPD OPE-10 (Figure A1.B) primer generated banding patterns in females that was absent or barely amplified in males. While this marker displayed the greatest potential linkage for gender differentiation in swordfish, there is the risk to generate false positives (females that fail to amplify and are assigned as males).

Blue marlin

Six out of 100 RAPD primers showed potential sex-linked bands (Figure A2). Primer OPC-13 revealed the most distinguishable pattern among the six RAPD primers potentially gender-linked in blue marlin (**Figure 5**). Comparison between female (n=11) and male (n=11) templates showed that male DNA produced a strong extra band around 500 bp next to another band that was also amplified in females. Chi-square test were significant (χ^2= 6.74, p=0.009**) suggesting that the extra band generated this RAPD in males is sex-linked. Additional five RAPDs revealed potential gender-linked differences between female and male blue marlin. However, the banding patterns were weak and difficult to replicate. Primer OPA-01 (Figure A2.A) generated a strong band about 1.8 kb long in both males and females, but also a second, smaller distinct band in some males and two extra smaller bands in some females. RAPD OPA-11 (Figure A2.B) and RAPD OPB-01 (Figure A2.C) generated extra bands in most males but also in some females. Consequently, these RAPDs were not reliable.



Gender	*Sex-specific band score*										
Female	0	0	0	0	0	1	0	0			
Male	1	1	1	1	1	1	0	0	1	1	0

χ^2= 6.74, p=0.009**

Figure 5. TA agarose (2%) gels displaying potential sex-specific band (arrow) in blue marlin (*Makaira nigricans*) with RAPD OPC-13. RAPD marker associated identified the gender by the criterion of the sex-specific band present or absent. Presence of the sex-specific band was scored as 1, and absence of the sex-specific band was scored as 0. Confirmation of the sex-specific band generated with this RAPD primer is associated with gender difference using the Chi square test.

Yields were also low, and several attempts to optimize the PCR reaction failed using these primers. Similarly, RAPD OPB-20 (Figure A2.D) generated very clear extra bands in some males and not in females. RAPD OPC-11 (Figure A2.E) presented polymorphisms in the banding patterns. However, the frequency of differences of potentially diagnostic bands for gender differences was not fixed. The results of these RAPD primers are difficult to reproduce with additional samples, suggesting that other

factors related to DNA template quality and quantity are relevant. Attempts to optimize RAPDs using several additional *Taq* DNA polymerase formulations and buffer systems generated banding patterns that were not diagnostic (not shown). Based on the presence and absence of randomly amplified polymorphisms for loci OPC-12, OPA-01, OPA-11, OPB-01 and OPB-20, where in the majority of the cases an extra band was generated in males (Fig. S2), suggest a chromosomal XY system of gender determination in blue marlin.

Sailfish

A total of eight RAPD primers generated banding patterns that potentially linked to gender differentiation, with the majority of primers producing extra bands in females not observed in males (Figure A3.B-H). Only RAPD OPA-01 primer generated bands in males that were absent, or barely amplified, in females (Figure A3.A). However, the banding patterns are not well defined, and in general, in spite of attempts to optimize the PCR reaction, no additional yield of some of the polymorphisms of interest was obtained. Such differential amplification was particularly pronounced with OPA-20 (Figure A3.C), where males failed to produce any PCR product. In addition, OPC-08 (Figure A3.E) and OPD-03 (Figure A3.H) produced strong amplifications in both males and females, with sailfish females clearly displaying a band absent in males. However, larger sample sizes of gender-validated sailfish are needed to determine whether these bands are diagnostic. Based on the results of polymorphic patterns, where RAPD amplifies certain bands in females but not in males, suggests a ZW system of gender

determination in sailfish, with a chromosomal complement ZZ for males and ZW for females.

Screening of sex-linked genetic markers

A total of 12 loci that have been linked to gender determination in other teleosts were targeted in billfish as potential gender identification markers (**Table 5**), and a total of 103 primers consisting of original gene markers and newly designed primers were used to target and amplify genes linked to gender determination (Appendix Table A2). Most loci were successfully amplified using various primer combinations available in the references or by designing conserved primers based on the alignment of the sequences of the candidate species against the target gene sequences of medaka, fugu, seabass, stickleback, tilapia, and zebra fish, such as the AMH gene available in GenBank.

However, not all of the PCR amplifications generated diagnostic characters to be potential markers. A total of 48 primer-sets generated two kinds of PCR amplification products, either multiple bands or single bands for different loci in the three species (Appendix Table A3). The patterns displayed by multiple bands were not diagnostic to distinguish males from females in the three species. Conversely, seven loci produced sufficient PCR yields of specific products (single-banded) that it was possible to generate nucleotide sequences of adequate quality to identify potential polymorphisms (SNPs or indels) that could be linked to gender-determination. Five genetic loci, including AMH, ARP, DMRT1, OtY1, and *Znf* were successfully sequenced and aligned

for the three species. The aligned sequences are presented in the Appendix figures (Figure A4.-S6.). However, the quality of the sequence for loci GH-Y and *Idh* was low even after multiple sequencing attempts and were discarded. Multiple sequence alignments of other loci were used to screen for potential polymorphisms linked to gender. Once those polymorphisms were identified new primer sets were designed for HRMA genotyping based on multiple sequence alignments for the three species.

Swordfish

A total of six loci were sequenced, including AMH, ARP, *Znf*, DMRT1, DMRT1 exon3, and OtY1 in swordfish. Compared to the blue marlin and sailfish, there were more single band patterns generated in swordfish, and also an increased number of polymorphic sites discovered. The details of variation in each locus are shown in **Table 6** and described as follows: AMH. Approximately 681 bp of the AMH gene were aligned for three specimens (GENBANK Accession # pending). A mutation at nucleotide site 331 was identified as heterozygous in a female, whereas two males were homozygous for adenine at this position. To determine whether this SNP is gender-linked in swordfish, two forward and two reverse primers were designed (listed in Appendix Table A2) and were used in pairs in all four possible combinations in HRM experiments, and also to perform additional sequencing experiments. HRMA experiments were conducted on 10 females and 13 males swordfish from the North Atlantic (**Table 3**). HRMA experiments with primer sets XglaAMH 371F/450R and XglaAMH 371F/457R yielded a single melting curve, and thus was not diagnostic. Primer set XglaAMH390F/457R produced multiple melting curves that were not linked

to gender. Finally, primer combination XglaAMH390F/450R produced curves that were diagnostic of two homozygous alleles and a heteroduplex curve for heterozygotes, and it was determined that the variable site at 331 is not linked to gender in swordfish. ARP. About 369 bp of the ARP sequence were obtained for swordfish, and no gender-linked polymorphisms were identified (GENBANK Accession # pending). There were only four variable sites, indicating that ARP is highly conserved in swordfish. One SNP was useful in population structure studies of Atlantic and Pacific swordfish (Smith *et al.* 2010; 2012; see Chapter IV.). *Znf*. A total of 420 bp of sequence of the *Znf* gene were obtained from gender-validated swordfish specimens (GENBANK Accession # pending). There was much reduced variation and no evidence of gender-linked polymorphisms. DMRT1. About 336 bp of sequence of the DMRT1 gene were characterized for fourteen specimens, seven females and seven males (GENBANK Accession # pending). Multiple polymorphic sites were discovered in this segment of sequence, but none of these sites corresponded to differences linked to the gender. DMRT1 exon3. A segment of about 217 bp was characterized from eight swordfish, five females and three males (GENBANK Accession # pending). This segment was highly conserved, and no gender-linked polymorphisms were detected. OtY1 (Golgi pH regulator: GpHR). About 372 bp of sequence of the OtY1 gene were characterized for 15 swordfish, nine females and six males (GENBANK Accession # pending). Similar to the other gene loci, the swordfish sequences of the OtY1 gene were highly conserved with only six variable sites, and no gender-linked differences found. Four polymorphic sites were targeted to design new primers for studying the population structure in

swordfish (Smith *et al.* 2010). However, because this locus had been linked to gender determination in other fish, and to discard the possibility that its use in population genetic studies would introduce bias because of linkage to gender, additional testing with larger sample sizes was conducted. Swordfish specimens collected from two locations in the South Pacific Ocean whose gender was validated by gonadal inspection in the field were used for testing whether loci ARP and OtY1 loci were linked to gender determination.

A total 105 swordfish were employed including 27 females and 32 males from Australia, and 30 females and 16 males from Chile. After genotyping all these specimens alternative Analysis of Molecular Variance (AMOVA, Excoffier *et al.* 1992) tests were performed. None of locus by locus AMOVA was significant, indicating that the polymorphisms characterized in these two loci are not gender-linked (Appendix Table A4). Although none of the polymorphisms discovered (SNPs or indels) were linked to gender-determination, multiple SNPs targeted in ARP and GpHR were useful in the development of short amplicon (SA) HRMA for ARP, and unlabeled-probe (UP) HRMA for GpHR, and then applied in the study of swordfish population structure in the Atlantic and Pacific Oceans (Smith *et al.* 2010, 2013; Smith 2012, see Chapter IV.).

Blue marlin

Sequences of high quality were obtained in blue marlin for three loci allowing for screening of potential SNPs linked to sex differentiation. None of the loci contained SNPs diagnostic for sex differentiation.

Table 6. Variable sites (contains at least two types of nucleotides) of six loci A) anti-Müllerian (AMH) gene; B) Acidic ribosomal phosphoprotein P0 (ARP); C) Zinc finger (*Znf*); D) DM related transcription factor 1 (DMRT1); E) DMRT1 gene Exon3; F) OtY1 gene (Golgi pH regulator: GpHR), for swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (K=G or T; M=A or C; R=A or G; S=C or G; W=A or T; Y=C or T;), and identical (.) relative to the reference sequence. Number above reference sequence shows the position in the sequence. Different color boxes are presented as potential gender differentiation sites (red), polymorphic sites for swordfish population structure study (green), and potential polymorphic sites for studying population structure (grey).

A) AMH

	3345 73710 31386
Xgla1072F	CMCGG
Xgla1073M	MAT..
Xgla1074M	.AYRR

B) ARP

	112 8351 0791
Xgla0034M	TGGR
Xgla0656M	.RSA
Xgla5804M	G..G

C) Znf

	12233 5600605 0336728
Xgla0108F	MCACTCC
Xgla0113F	C.W....
Xgla0655F	C.....
Xgla1153F	C.....
Xgla1171F	C..Y...
Xgla5757F	C.....
Xgla5795F	C.....
Xgla5803F	C.....
Xgla5811F	C.....
Xgla5814F	C.....
Xgla0033M	C...YS.
Xgla0034M	C.....
Xgla0656M	C.....
Xgla1160M	C.....
Xgla1166M	C.....
Xgla5755M	CS....S
Xgla5797M	C.....
Xgla5804M	C.....
Xgla5805M	C.....

D) DMRT1

	11111 1112222222 3445702233 4770014 49 2178551502 6140812 38
Xgla0108F	AAGAGYWAMT TACSASCGG
Xgla0113F	R.R...TRCW ...C.....
Xgla1488F	G....TTRC. ..Y..C.R.
Xgla1490F	G...RTTRC. ..Y..C...
Xgla1492F	G....TT.C. .M.....
Xgla5706F	G....TT.C. .C.....
Xgla5709F	R....CT.C. ...C.G..R
Xgla1160M	...W.CT.C. ...CRG...
Xgla1482M	.....CT.C. ...C.G.R.
Xgla5707M	R.....T.C. ..Y.....
Xgla5708M	.....CT.C. W..C.G...
Xgla5711M	.....T.C. ..Y.....
Xgla5755M	RR...CT.C. WM.C.G...
Xgla5804M	G....CTR.. W..G.CY..

E) DMRT1 Exon3

	11112 911361 9918142
Xgla0108F	CYTAACC
Xgla0113F	.C.....
Xgla5504F	YC....S
Xgla5641F	.C..R..
Xgla5795F	.C.....
Xgla0034M	.C.....
Xgla0656M	.C.M...
Xgla1160M	.CK..S.

F) OtY1(GpHR)

	111133 667905 164631
Xgla0113F	TYCCGT
Xgla1153F	WCM...
Xgla1171F	.C....
Xgla5756F	.C....
Xgla5757F	.C....
Xgla5795F	.....
Xgla5803F	.C....
Xgla5811F	.....
Xgla5814F	.C....
Xgla0033M	WCMY.Y
Xgla0034M	.C..SK
Xgla0656M	.T....
Xgla5797M	.....
Xgla5804M	.T....
Xgla5805M	.C....

First, approximately 423 bp of DNA sequence of the AMH gene for 12 specimens (six each male and female) were aligned revealing seven variable sites. Second, 285 bp of the ARP gene for six specimens (two females and four males) were aligned, and four variable sites were detected. Finally, 420 bp of sequence of the *Znf* gene were aligned for seven gender-validated specimens of blue marlin, revealing 14 variable sites. None of these SNPs were linked to gender. The polymorphic sites found in blue marlin in loci AMH, ARP and *Znf* (**Table 7**), might be suitable for population structure studies of blue marlin with further testing using larger sample sizes and sampling coverage.

Sailfish

Three loci were efficiently sequenced in sailfish to discover potential SNPs linked to sex differentiation. The sequences were remarkably conserved in these three loci and none of them contained SNPs related to sex differentiation in sailfish. First, approximately 335 bp of the AMH gene in six specimens (three each male and female) were aligned and revealed two variable sites. Second, only one female specimen generated 401 bp of good quality sequence of the ARP gene, and thus no variable sites between female and male could be identified. Third, approximately 420 bp of the *Znf* gene for six specimens including four females and two males were aligned and five variable sites were identified. Similar to blue marlin, no gender-linked SNPs or indels, were found. Again, no variable sites were linked to gender determination, although these may be useful for studying population structure of sailfish (**Table 8**).

Table 7. Variable sites of three loci A) anti-Müllerian (AMH) gene; B) Acidic ribosomal phosphoprotein P0 (ARP); C) Zinc finger (*Znf*) for blue marlin (*Makaira nigricans*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (M=A or C; R=A or G; S=C or G; K=G or T; W=A or T; Y=C or T;), and identical (.) relative to the reference sequence. Number above reference sequence shows the position in the sequence. Highlighted boxes represent polymorphic sites.

A) AMH

	11113
	55078 90
	89132 59
Mnig201F	GCGGGGC
Mnig277F	..KR..Y
Mnig278F	..... .
Mnig281F	....RRY
Mnig284F	KY.R..Y
Mnig285F	..K....
Mnig211M	...R..Y
Mnig279M	..KR..Y
Mnig280M	...R..Y
Mnig282M	...R..Y
Mnig283M	..K....
Mnig287M	.....Y

B) ARP

	2222
	2346
	0781
Mnig201F	CCAY
Mnig203F	...C
Mnig204M	.YWC
Mnig202M	....
Mnig207M	Y..T
Mnig211M	....

C) *Znf*

	1112222 2233
	2533484466 7905
	3049022689 3350
Mnig201F	ACGYRCCACA WCTC
Mnig203F	.Y..A....R ...Y
Mnig212F	MY.CA..... ....
Mnig202M	.Y.C.YM... T...
Mnig204M	MYSCA...Y. ....
Mnig207M	.T.....W.R TS..
Mnig211M	.... T.C.

Table 8. Variable sites of two loci A) anti-Müllerian (AMH) gene; B) Zinc finger (*Znf*) for sailfish (*Istiophorus platypterus*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (M=A or C; R=A or G; S=C or G; W=A or T; Y=C or T;), and identical (.) relative to the reference sequence. Number above reference sequence shows the position in the sequence. Highlighted boxes represent polymorphic sites.

A) AMH

	1
	26
	57
Ipla0031F	GY
Ipla304F	.C
Ipla305F	.C
Ipla0041M	..
Ipla324M	RC
Ipla325M	.C

B) *Znf*

	1222
	24777
	30367
Ipla0002F	MAWSM
Ipla0031F	ARTGC
Ipla0038F	ARTG.
Ipla0039F	AGT..
Ipla0028M	ARTGC
Ipla0041M	ARTG.

Discussion

In this study, we applied two molecular approaches to search for potential genetic markers related gender determination in swordfish, blue marlin, and sailfish. RAPD reproducibility was affected by multiple factors, including quality of DNA; concentration of template DNA, primer sequence, and magnesium chloride concentration, and *Taq* formulation (Meunier and Grimont., 1993; Fraga *et al.*, 2005). Therefore, we used the same thermocycling parameters and fixed the concentrations of template DNA tissue type, and RAPD primer to conduct the selection experiments of various *Taq* DNA polymerases. We found that RAPD reproducibility is highly sensitive to the formulation of the DNA *Taq* polymerase, and to the source of tissue used to extract DNA employed in the experiments. However, High-Fidelity (Hi-Fi) *Taq* polymerase was able to produce repeatable banding patterns independent of tissue source and this formulation was used in experiments aimed to characterize differences between males and females of swordfish, sailfish and blue marlin.

RAPDs have been used to identify sex-determining DNA markers in several fishes, including rainbow trout (Iturra *et al.* 1998); Nile tilapia (Bardakci 2000), African catfish (Kovács *et al.* 2001), Asian arowana (Yue *et al.* 2003), common carp (Chen *et al.* 2009), and turbot (Casas *et al.* 2011). In this study, potential gender-linked differences were found in blue marlin and sailfish, leading to suggest chromosomally based systems of sex determination. RAPD banding patterns suggest an XY chromosomal system in blue marlin based on the patters of six out of 100 RAPD tested, with the remaining 94 RAPDs either failing to amplify or failing to produce distinct bands. Tests conducted

with OPC-13 in blue marlin strongly suggest that this RAPD is linked to gender determination. It would be desirable to clone and characterize the sequence of this segment to determine its identity and or develop an assay based on HRMA.

Eight RAPDs primers generated amplification patterns potentially linked to gender determination in sailfish. Certain bands amplified solely in females suggesting that a ZW system of gender determination operates in sailfish. In swordfish, only two RAPDs produced banding patterns that show some level of linkage to gender-determination. RAPD OPE-10 amplified multiple bands in females, but generated no bands in males. Unfortunately, since failed PCR reactions would translate into falsely identifying females as males, this RAPD is not reliable. However, there is a potential for further development of this marker, perhaps through the inclusion of another primer that always amplifies in males and females, such that the absence of bands generated by OPE-10 would confirm those specimens as male swordfish.

None of the polymorphisms (SNPs and indels) characterized in 12 loci linked to gender-determination in other fish species appear to operate in swordfish, blue marlin, or sailfish. However, several of the SNPs identified in this study have proven useful as markers for population structure studies of swordfish (Smith *et al.* 2010,2013; Smith 2012; see Chapter IV.), and others have the potential for conducting population studies of sailfish and blue marlin using SNPs.

Sex determination in fishes can be complex (Piferrer and Guiguen 2008) in some cases involving genetic factors (single gene or multiple genes), environmental factors, or both with different levels of impact in teleost fishes (Devlin and Nagahama 2002;

Ospina-Á lvarez and Piferrer, 2008; Penman and Piferrer, 2008; Kikuchi and Hamaguchi 2013). In many laboratory model fish species these mechanisms remain unknown. This study focused on identifying gender in pelagic, non-model species, which increases the challenge in identifying the genetic basis of sex-determination as is not possible to conduct controlled experiments on different life stages, or carrying experimental crosses. Here, we provided a preliminary screening of genetic markers that may stimulate further investigation of sex-determination in pelagic fish species. Recent advances in next generation sequencing (NGS) have been applied in non-model species, such as the ninespine stickleback and the European eel, to achieve wide coverage of the genome for population, ecological, and association studies (Bruneaux *et al.* 2013; Ghani *et al.* 2013; Pujolar *et al.* 2013). Molecular approaches such as the targeting of candidate genes related to fish sex differentiation could prove useful for discovering other important genes associated with the processes of sex-determination and differentiation (Piferrer and Guiguen 2008).

Understanding the mechanisms of sex determination and sex differentiation are essential information for estimating the sex ratio of a population. Sex ratio is an important parameter related the population reproductive capacity, size variation and growth patterns in different life stages, such as before and after sexual maturation (Penman and Piferrer, 2008; Piferrer and Guiguen, 2008). Therefore, for further study, we suggest the inclusion of other molecular methods involving NGS, such as Double Digest RADtag to screen large portions of the genome of samples of males and females of swordfish and billfishes aimed to identify candidate genes for sex-determination. The

development of assays based on this information could help improve the estimation of sex ratio and in the population assessment of billfishes and swordfish.

CHAPTER IV

GENETIC POPULATION STRUCTURE OF PACIFIC SWORDFISH (*Xiphias gladius*) INFERRED BY MULTILOCUS BAYESIAN ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) CHARACTERIZED WITH HIGH RESOLUTION MELTING (HRM)

Introduction

Swordfish, *Xiphias gladius* (Linnaeus, 1758,) is a highly migratory teleost of commercial and ecological importance found in all ocean basins with a wide distribution extending from 45° N to 45° S (Carey and Robinson 1981). In the Pacific Ocean, swordfish is primarily targeted by the longline fishery and the capture production reached about 53,517 t in 2011, corresponding to about 49% of global swordfish catch (109,716 t) (FAO yearbook. 2012). Currently, there is no consensus on Pacific swordfish population structure and alternative views based upon catch statistic data have been advanced, varying in number from a single panmictic unit to up to five populations (**Figure 6**). These hypotheses include a couple of two-stock hypotheses that separate the North Pacific and the South Pacific swordfish populations but that differ in placement of the latitudinal boundary (Nakano 1998, Ichinokawa and Brodziak 2008). Conversely, there are four alternative hypothesis of a three-stock separation that vary substantially in the placement of boundaries (Sakagawa and Bell 1980, Bartoo and Coan 1988, Nakano 1998, Ichinokawa and Brodziak 2008). Finally, there are two four-stock models that coincide in the regional subdivision of the Pacific Ocean into northwest, southwest,

northeast, and southeast subpopulations, but that differ in the placement of the regional boundaries (Sosa-Nishizaki 1990, Sosa-Nishizaki and Shimizu 1991, Hinton and Deriso 1998, and Hinton 2003).

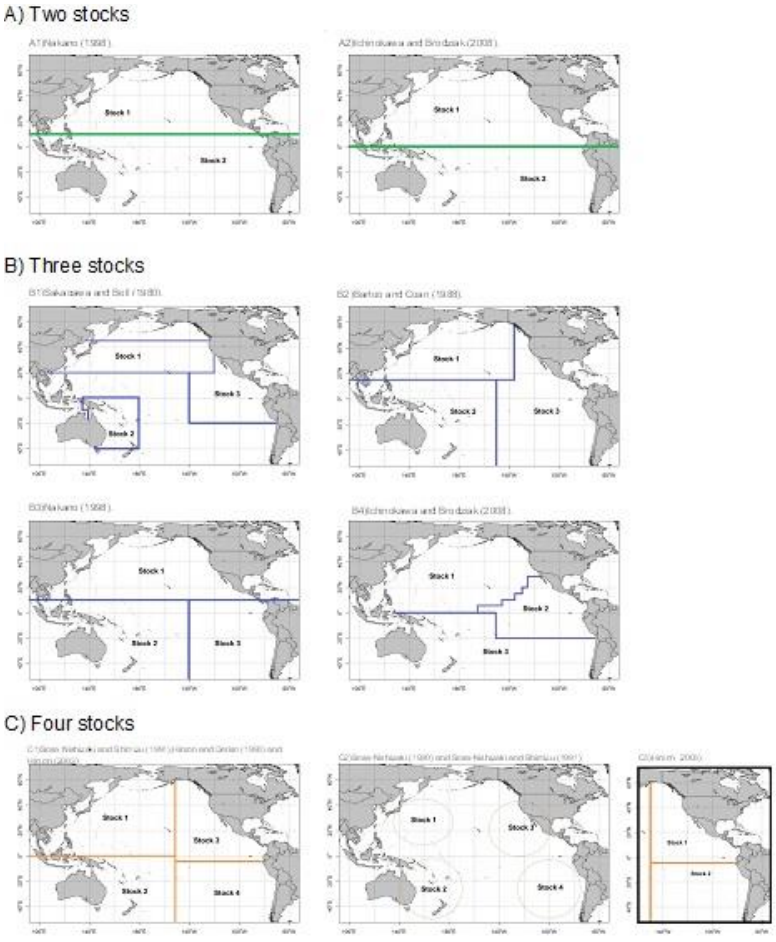


Figure 6. Alternative stock structure hypotheses proposed for Pacific swordfish based on fisheries data. A): Two-stocks hypotheses separating the North Pacific and the South Pacific stocks differing in the latitudinal position of the boundary (Ichinokawa and Brodziak, 2008; Nakano, 1998). B): Three-stock hypotheses that differ substantially in the placement of boundaries (Bartoo and Coan, 1989; Ichinokawa and Brodziak, 2008; Nakano, 1998; Sakagawa and Bell, 1980). C): Four-stock hypotheses models that coincide in the regional subdivision of the Pacific Ocean into northwest, southwest, northeast, and southeast subpopulations, but that differ in the placement of the regional boundaries (Sosa-Nishizaki , 1990; Sosa-Nishizaki and Shimizu, 1991; Hinton and Deriso, 1998; Hinton, 2003).

An understanding of the population structure of Pacific swordfish is intended to improve management practices of this fishery, and several genetic studies have been conducted with that goal (**Table 9**). Most of these previous studies failed to find significant statistical support in part because of small sample sizes, lack of resolution of the markers employed, insufficient, sampling coverage, or a combination of all these (Grijalva-Chon *et al.*1994, Rosel and Block 1996, Chow *et al.* 1997, Chow and Takeyama 2000, Reeb *et al.* 2000, Lu *et al.* 2006, Alvarado-Bremer *et al.* 2006, Kasapidis *et al.* 2008). However, regardless of the shortcomings these studies revealed significant differentiation among Pacific swordfish, including heterogeneity with mtDNA between Hawaii versus Mexico (Grijalva-Chon *et al.* 1996), and Japan versus Australia (Reeb *et al.* 2000). Further, based on mtDNA control region data a ⊃-shape pattern of genetic connectivity conforming to isolation by distance (IBD) connecting the northwest Pacific and southwest Pacific swordfish via the eastern Pacific was proposed (Reeb *et al.* 2000). The matrilineal mode of inheritance of mtDNA, together with the segregation of males from female swordfish, raises questions whether mtDNA alone represents the genetic signature of the entire swordfish population (Muths *et al.* 2009). Accordingly, the assessment of genetic variability of nuclear DNA (nDNA) markers was prioritized and the characterization of single nucleotide polymorphisms contained in the introns of nuclear genes was conducted (Greig 2000; Alvarado-Bremer *et al.* 2006; Smith *et al.* 2010), as well as that of microsatellite markers (Kasapidis *et al.* 2008). However, different conclusions of the population structures of Pacific swordfish were reached by these studies. The initial objective of this study was to develop informative

nuclear markers to clarify the genetic population structure of swordfish in the Pacific, and these results were published in Smith *et al.* (2010). In here, a genetic population analysis of Pacific swordfish using those ten nuclear markers is presented with an unprecedented geographic sampling coverage that includes larvae, juveniles, and adults. Genotyping was conducted for the majority of these loci with High Resolution Melting Analysis (HRMA) and the description of that approach has been published elsewhere (Smith *et al.* 2013). In addition, three markers were scored as size polymorphisms, two as Simple Sequence Repeat (SSR), and the third as a Restriction Fragment Length Polymorphism (RFLP) (see Materials and Methods). The genetic population structure was tested using Bayesian statistics for individual assignment and with analyses of molecular variance under different hierarchical arrangements. The results indicate that Pacific swordfish does not conform to a single panmictic unit. Instead, the genetic pattern of population structure is complex and not adhering to the phylogeographic associations documented within the Atlantic or the isolation by distance reported with mtDNA in the Pacific and along the Mediterranean.

Table 9. Summary of previous genetic research on Pacific swordfish.

Reference	Data type	Regions compared	Interpretation
Grijalva-Chon *et al.*1994	mtDNA (RFLP)	North Central Pacific (Hawaii) and EPO (Mexico), Western (Japan and China Sea)	No differences
Rosel and Block 1996	mtDNA (Seq)	EPO (Mexico, Chile), North Central Pacific, (Hawaii), NW Pacific (Japan, Taiwan)	No differences
Chow *et al.* 1997	mtDNA (RFLP)	NW Pacific (Japan), C. Pac. (Hawaii), EPO (Mexico, Ecuador and Peru), SW Pacific (New Zealand)	No differences
Chow and Takeyama 2000	mtDNA ; nDNA (RFLP, Seq)	NW Pacific (Japan) and EPO (Peru)	No differences
Lu *et al.* 2006	mtDNA (Seq)	PWest (Northwest Pacific) and PCen (North Central Pacific)	No differences
Kasapidis *et al.* 2008	Microsatellite	Southwest Pacific (SWPA), South Pacific (SPA), Southeast Pacific (SEPA), Central eastern Pacific (MEPA), Central Pacific (MPA), Northwest Pacific (NWPA).	Not statistically significant.
Grijalva-Chon *et al.*1996	Allozymes	North Central Pacific (Hawaii) and EPO (Mexico)	Significant differences
Reeb *et al.* 2000	mtDNA (Seq)	NW Pacific (Japan), C. Pacific (Hawaii), C.Equator, EPO(California/Mexico), and SW Pacific (Australia)	⊃-shaped corridor
Alvarado-Bremer *et al.* 2006	mtDNA ; nDNA(Seq)	Hawaii, Mexico, Peru, Ecuador, Australia	D-loop data/Significant ldh-A incipient differentiation between EPO and 3 Hawaiian samples

Table 10. Sampling details for 891 Pacific swordfish used in this study.

No.	Pop Name	Region	n	Latitude	Longitude	Sampling Date
Adult						
1	HAW99	ANC	106	17-29 N	159-174 W	1999 Apr.-May
2	HAWNE	ANC	112	30-33 N	135-157 W	1993 Nov.-Dec.,1998 Feb.,1999 Feb.
3	NMCA	ANE	96	29-35 N	118-125 W	1997 Oct.,1999 Jan.-Feb.
4	Ecuador	TSE	80	3-5 S	85-91 W	1998 Aug.-Oct.,1999 Sep.-Oct.
5	Chile97	ASE	67	26-33 S	80-81 W	1997 Sep., Nov.-Dec.
6	Chile99	ASE	53	30-33 S	74-81 W	1999 Apr.-May
7	CenNPac	TNC	31	8 N	140 W	1997 Sep.
8	Australia	ASW	65	31-33 S	166-168 E	2006 Mar.-Apr.
9	W.Aus	TSW	30	16 S	118 E	1995 Feb.
10	Taiwan	TNW	63	21-23 N	119-122 E	2010 May
11	Japan	ANW	38	24-35 N	144-154 E	1991 Nov.-Dec., 1992 Feb.
Larvae and Juvenile						
12	HawLa	TNC	42	19 N	156 W	2000 Apr.-Sep.,2001 May-Aug.,2002 Apr.-May
13	CenNJu	TNC	46	2-7 N	159-165 W	1998 Mar.,2001 Jun.-Oct.,2002 Jan.-Mar.
14	CenSJu	TSC	36	9-18 S	141-153 W	1999 Sep.,2000 Jan.-Nov.
15	AusJu	ASW	14	26-31 S	156-162 E	1999 Feb.,Oct.-Dec.,2000 Jan.-Feb.
16	GuamJu	TNW	12	13 N	145 E	1999 Oct.-Dec.
	Total:		**891**			

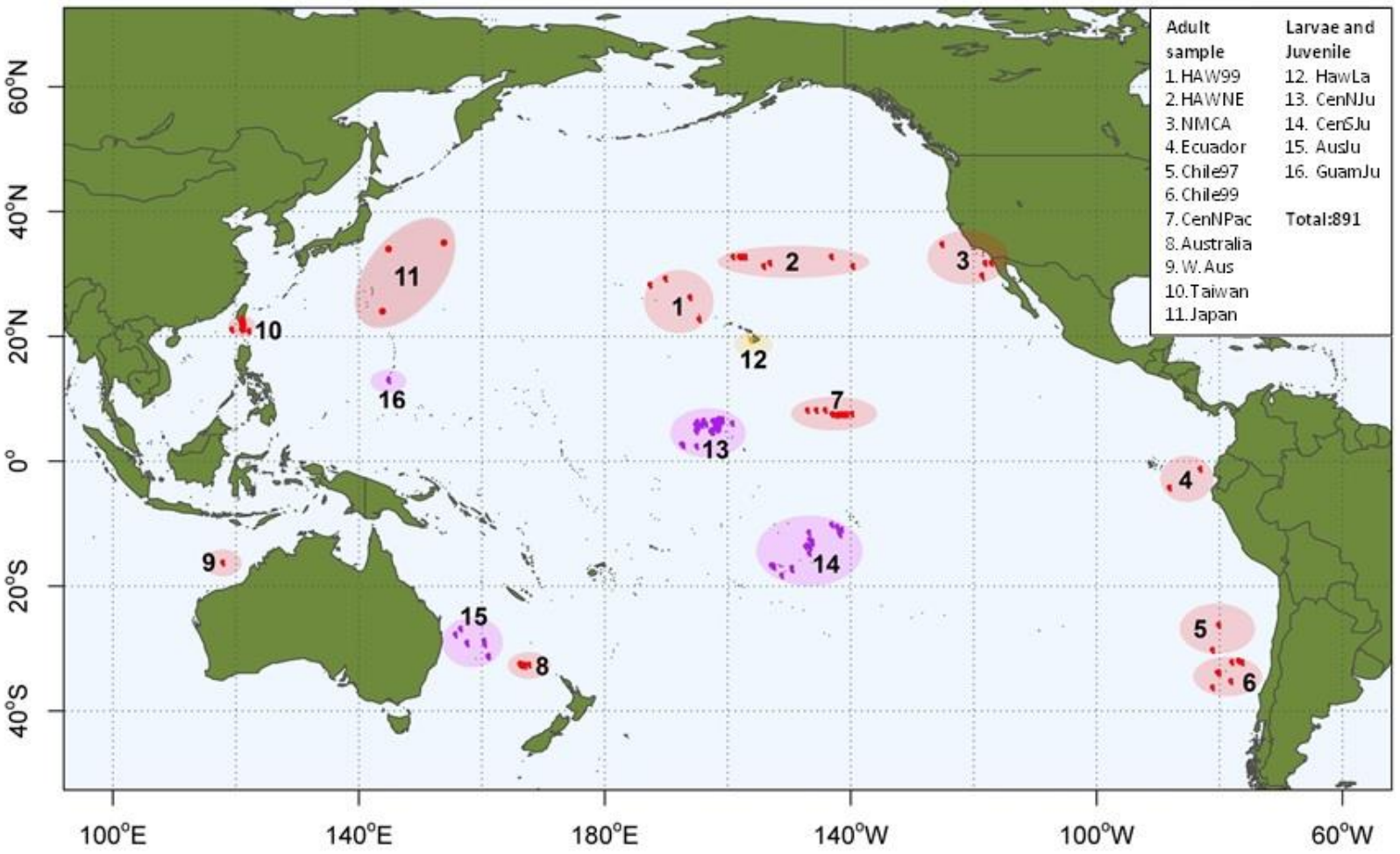


Figure 7. Sampling localities of Pacific swordfish used in this study. Dots presented the sampling localities; Ellipsoids showed the areas of sampling. Adults: red dots within pink ellipsoids 1-11; Larvae: orange dot within yellow ellipsoid 12; Juveniles, purple dots within purple ellipsoids 13-16. Name of sampling localities is shown in brackets.

Materials and methods

Sampling

From 1993 to 2010 swordfish tissue was collected by observers on board of longline fishery vessels coordinated by researchers from different institutions working in the Pacific Ocean. A total of 891 swordfish, including 741 adults, 108 juvenile and 42 larvae were analyzed (**Table 10**). Immediately after collection, all on-board swordfish samples were preserved in 95% ethanol kept at room temperature (RT) until assayed in the laboratory. On the basis of spatial and temporal attributes these samples were assigned into 16 independent localities (**Figure 7**).

DNA template extraction and SNP scoring of ten nuclear loci

Each individual was isolated from a small piece of muscle tissue with a Proteinase K (10 mg/µl) digestion followed by ETOH precipitation without organic extractions (Greig 2000). Of the ten nuclear loci used in this study, seven were single nucleotide polymorphisms (SNPs) characterized via HRMA. Details on the selection of loci and corresponding SNPs, the minimization of ascertainment bias as well as the criteria to design primers and probes that target these, are given in Smith *et al.* (2013). Briefly, ascertainment bias was minimized by selecting potential loci and SNPs after conducting a preliminary characterization with HRMA of 30 swordfish, 10 from each of three geographically disjunctive swordfish populations (NW Atlantic, Mediterranean and Pacific). Those amplicons that generated distinct melting profiles were sequenced, and the electropherograms were inspected to validate for the presence of the SNP that

produce the polymorphic melting curves (see Smith *et al.* 2013 for details). Sequence alignments were also used to evaluate the placement and design of new HRMA-genotyping primers and probes when required. A total of 85 SNPs were initially identified but only differing at frequencies >5% among reference samples were targeted for further HRMA primer and or probe design. PCR conditions of selected potential loci were further optimized and validated using additional samples (n=40) from each of the three reference populations. This process ultimately yielded a total of 26 SNPs among 10 loci that we targeted for genotyping (see Table 2 in Smith *et al.* 2013). In addition, three loci were scored as size polymorphisms through gel electrophoresis. Specifically, one informative SNP was characterized using a restriction enzyme, and scored as a RFLP and two additional loci as short sequence repeats (SSR) (see Smith 2012). Details of the characterization of the rest of the loci are given in Smith *et al.* (2010; 2013). Briefly, the characterization of the ten nuclear loci (the score of each individual were listed in Appendix Table A5) was as follows: 1) Short Amplicon HRMA (SA-HRMA): acidic ribosomal phosphoprotein P0 (ARP), ATP synthase beta-subunit (ATPsβ), and signal recognition particle 54 (SRP54). 2) Unlabeled Probe HRMA (UP-HRMA): adenine nucleotide translocator (ANT), Golgi pH regulator (GpHR), lactose dehydrogenase A (*ldh*A), and myosin light chain (Mlc2) 3) SSR: aldolase-B (AldB) and VBC201 (VBC) 4) RFLP: alpha-skeletal actin (Act2).

Procedures for HRMA genotyping of SNPs followed those described in Smith *et al.* (2013). In addition, the two SSR markers were amplified with the primer sets: 1) AldB: 5'- VIC - TGT GCC CAG TAT AAG AAG GAT GG - 3' and 5' - CTG TGG

AGA ATC AGG GCT CC - 3' (JX042447). 2) VBC201: 5' - 6FAM – GAT GAG TCA TAC TGC CGA CG – 3' and 5' – GAG GCA GGT GAG AGT ATA TTG C – 3' (Louro, *et al.* 2010 (AM961064), Kasapidis, *et al.* 2009). PCR reactions were performed in the total 12.5 µl volume including 10 ng of template DNA, 1X Econotaq Green Master Mix (Lucigen), and with the corresponding primer sets (0.5 µM of each primer) and adjusted ddH_2O volume. Thermocycling consisted of an initial denaturation step at 95℃ for 10 minutes followed by 35 cycles consisting of denaturing at 94℃ for 1 minute, annealing at 54℃ for 1 minute and extension at 72℃ for 1 minute, followed by a final extension step at 72℃ for 30 minutes. For the microsatellite loci analysis, 1µl of PCR product (1:10 dilution product) were loaded in the ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with 0.2 µl GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, California, USA) and 10 µl Hi-Di™ Formamide (Applied Biosystems, Foster City, California, USA). Fragment analyses were performed with GeneMapper® v.4.0 (Applied Biosystems, Foster City, California, USA). Locus Act2α was amplified with primer set: 5'- GTC ACC GGA GTC CAG GAC G-3' and 5'-ATC TGG CAC CAC ACC TTC TAC AA-3' (JX042448, Atarhouch *et al* 2003) using the same thermocycling parameters described above for SSR markers. Amplification success was verified by 1% agarose gel electrophoresis, and amplicons were digested with the restriction endonuclease Hpy8I whose recognition motif includes an informative SNP site. The restriction enzyme digestion of PCR products was conducted in 10µl volumes including 2 µl PCR product, 1X Buffer TANGO (Fermentas), and 0.4U of Hpy8I (Fermentas) for 16 hours at 37℃. The RFLPs

were scored by the 2% agarose gel via gel electrophoresis at 100V for 30min. The RFLPs were visualized on a Gel Doc^{TM} XR+ system (Bio-Rad) and images were captured and scored in Quality One 1-D Analysis software v4.6 (Bio-Rad).

Genetic data analyses

Diversity indices

The number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) were estimated with GenAlEx v6.5 (Peakall and Smouse 2012). Deviations from Hardy-Weinberg equilibrium (HWE) and Linkage disequilibrium between all pairs of loci over all populations were computed with Markov chain parameters: 10,000 dememorisation, 1,000 batches, and 10,000 iterations per batch using GENEPOP version 4.2 (Rousset 2008).

Genetic distance and differentiation between populations

F_{ST} over all loci (Weir and Cockerham, 1984; Michalakis and Excoffier, 1996) and Slatkin's linearized F_{ST} (Slatkin 1993) for all pairwise comparisons were estimated in Arlequin version 3.5 (Excoffier and Lischer, 2010) to evaluate the pattern of population differentiation. Mantel test comparing F_{ST} values and the geographic distances between populations were calculated in GenAlEx v6.5 to test for isolation by distance (IBD). In addition, exact goodness of fit tests (G-tests) of both genic (i.e., allelic) and genotypic differentiation were conducted across ten loci for all pairs of populations with Markov chain parameters: 10,000 dememorisation, 1,000 batches and 10,000 iterations per batch using GENEPOP version 4.2 (Rousset 2008). Allelic tests of

differentiation are particularly appropriate and more powerful than F_{st} and genotypic tests when sampling is unbalanced, as in this study. Corrections for multiple testing are necessary to avoid Type-I statistical error using sequential Bonferroni corrections (Holm, 1979; Rice 1989). However, Bonferroni corrections have been reported to be very conservative and consequently in greatly diminished power to detect differentiation among pairs of sample collections (Narum 2006). Instead, the modified false discovery rate (FDR) method (B-Y method FDR, Benjamini and Yekutieli 2001) which provides the most biologically important critical value for evaluating significance of population differentiation in conservation genetics was adopted here.

Because loci ARP and GpHR have been linked to gender determination in other fishes (Naruse *et al.* 2004; Devlin *et al.* 1994.), we tested for linkage to gender with specimens whose gender was validated by gonadal inspection. Accordingly, swordfish females from Australia (n=27) and Chile (n=30) were pooled (n= 57) compared against a pooled sample (n= 48) of swordfish males from Australia (n=32) and Chile (n=16). Using these two samples, a locus by locus Analysis of Molecular Variance (AMOVA, Excoffier *et al.* 1992) was conducted for these two loci, and for the remaining eight loci, to test for gender-linked bias. None of the tests revealed any difference between allelic or genotypic frequencies for male and female (see Appendix Table A4.).

Genetic population structure cluster analyses

Bayesian cluster analyses of population structure and individual assignment were conducted with STRUCTURE v 2.3.4 (Pritchard, *et al.* 2000; Falush, *et al.* 2003 and 2007; and Hubisz, *et al.* 2009). A no admixture ancestry with correlated allele

frequencies model, as outlined in Falush *et al.* (2003), was adopted. Compared to freshwater and anadromous fishes, marine fishes display weak levels of population structure ($F_{ST} < 0.20$) (Waples 1998) therefore, the LOCPRIOR option was implemented to infer weak population structure using an *a priori* group sampling (Hubisz *et al.* 2009). The number of clusters (K) was estimated using an *ad hoc* approach (Pritchard *et al.* 2000) by obtaining the mean posterior probability of the data (L(K)) and the delta K (ΔK) approach of Evanno *et al.* (2005) with STRUCTURE HARVESTER Web v0.6.93 (Earl and vonHoldt 2012). Twenty independent runs for each K between 1 and 6, were conducted with the Markov Chain Monte Carlo (MCMC) 100,000 iterations and burn in period of 100,000.

Spatial genetic population structure analysis was performed with the geographic and genetic data of individuals using GENELAND (Guillot *et al.* 2005). The geographic data of individuals corresponded to the latitude and longitude of each sampling location. The spatial coordinate's uncertainty value for each individual was defined as 35 decimal degrees, which corresponds to the with movement patterns for Pacific swordfish based on electronic tagging data (Abascal, *et al.* 2010; Dewar, et al 2011; Abecassis, *et al.* 2012; Evans, *et al.* 2012 and 2014). Spatial and correlated allele models were applied for twenty independent runs with 100,000 MCMC iterations and 10,000 thinning for each K value (K=1 to 6). Based on the estimated K value, we conducted twenty independent runs using STRUCTURE and GENELAND, and optimal alignments of replicate cluster analyses were implemented in CLUMPP version 1.1.2 (Jakobsson and

Rosenberg 2007). Results from STRUCTURE and GENELAND were compared to infer population structure and spatial boundaries between clusters.

An F_{ST}-outlier detection method, in which observed locus F_{ST} values are compared to calculated global F_{ST} values expected under neutrality using coalescent simulations (Beaumont and Nichols, 1996) was performed with the program LOSITAN (Antao *et al.* 2008). A total of 50,000 simulations were conducted to detect putative loci under selection running the first simulation with the 'neutral' mean F_{ST} option aimed to remove potential selective loci in the initial mean F_{ST} computation, with a 0.995 confidence interval, a false discovery rate of 0.1, and with sampling localities pooled into the corresponding two clusters (K=2) identified by Bayesian analyses.

Alternative hierarchical arrangements of population subdivision based on the patterns of population differentiation obtained with genetic population clustering analyses and fishery management models (see **Figure 6**) were tested with Analysis of Molecular Variance (AMOVA Excoffier *et al.* 1992) using Arlequin v3.5 (Excoffier and Lischer 2010) with associated significance tests based on 10,100 permutations.

Results

Characterization of variation targeting informative SNPs

A total 20 informative SNPs were genotyped at ten nuclear loci for 891 individuals. None of the linkage disequilibrium tests for each locus pair across all populations were significant after B-Y FDR corrections for multiple testing. The three SA-HRMA assays and the RFLP assay targeted one SNP each and were bi-allelic (**Table**

11). By contrast, four loci scored using UP-HRMA contained between two to five SNPs, and between three to seven alleles each. SSR loci AldB and VBC201, scored as size polymorphisms, yielded 10 and 14 alleles, respectively. Values of observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) for each locus within each population are given in **Table 12**. H_o, H_e and F_{IS} across all loci for each locality ranged from 0.408 to 0.524, 0.442 to 0.504, and -0.049 to 0.059, respectively. Overall mean values of H_o, H_e and F_{IS} across all loci and localities were 0.475, 0.479 and 0.010, respectively, whereas the number of alleles across all loci within populations ranged from 2 to 11.

Significant deviations from HWE using Fisher's probability method after B-Y FDR (P <0.0088) corrections for multiple testing were significant for VBC in Japan (F_{is} = -0.140) which implies heterozygous excess, and for ANT in Chile99 (F_{is} =0.329), indicative of a deficit of heterozygous and thus as potential evidence of a Wahlund effect (**Table 12**). However, none of the global tests across loci and across samples was significant after B-Y FDR correction, suggesting that each sample can be attributed to individuals from the same population with very little evidence of mixing.

Table 11. Information for molecular assays designed of ten nuclear loci used in this study

Locus Name	Gene	Marker Type	A	SNPs (Total:20 SNPs)	Indel
ARP	Acidic ribosomal phosphoprotein P0	SA-HRMA	2	A/G(1)	
ATPsβ	ATP synthase beta-subunit	SA-HRMA	2	A/G(1)	
SRP54	Signal recognition particle 54	SA-HRMA	2	A/G(1)	
Mlc2	Myosin light chain	UP-HRMA	3	G/T(1);C/T(1)	
GpHR	Golgi pH regulator	UP-HRMA	5	A/T(1);C/T(1);A/C(1);C/T(1)	
*ldh*A	Lactose dehydrogenase A	UP-HRMA	6	T/G(1);T/C(1);G/C(1);C/A(1);A/G(1)	1
ANT	Adenine nucleotide translocator	UP-HRMA	7	C/G(1);C/T(1);A/C(1);A/G(1);A/G(1)	2
AldB	Aldolase B	SSR	10	n/a	SSR
VBC201		SSR	14	n/a	SSR
Act2α	Alpha skeletal actin 2	RFLP	2	A/G(1)	

Note: A: allele number, n/a: not applicable.

Table 12. Genetic diversity indices of 10 nuclear loci for 16 sampling localities

Pop		ARP	ATPsβ	SRP54	Mlc2	GpHR	LDHA	ANT	AldB	VBC	Act2α	Mean
HAW99	**Na**	2	2	2	3	4	5	5	7	10	2	4.2
(n=106)	**Ho**	0.481	0.472	0.340	0.340	0.585	0.604	0.519	0.349	0.783	0.236	0.471
	He	0.490	0.442	0.306	0.323	0.599	0.603	0.533	0.358	0.814	0.323	0.479
	F_{IS}	0.018	-0.066	-0.109	-0.053	0.023	-0.001	0.026	0.026	0.038	0.271	0.017
HAWNE	**Na**	2	2	2	3	4	5	4	6	11	2	4.1
(n=112)	**Ho**	0.420	0.375	0.384	0.393	0.598	0.607	0.580	0.366	0.804	0.321	0.485
	He	0.471	0.430	0.388	0.364	0.567	0.581	0.541	0.356	0.787	0.316	0.480
	F_{IS}	0.109	0.127	0.011	-0.079	-0.055	-0.045	-0.073	-0.027	-0.021	-0.018	-0.007
NMCA	**Na**	2	2	2	3	5	6	7	7	10	2	4.6
(n=96)	**Ho**	0.458	0.344	0.292	0.260	0.521	0.677	0.531	0.375	0.802	0.323	0.458
	He	0.486	0.409	0.291	0.305	0.535	0.630	0.550	0.349	0.785	0.298	0.464
	F_{IS}	0.057	0.159	-0.001	0.146	0.027	-0.074	0.034	-0.074	-0.022	-0.083	0.017
Ecuador	**Na**	2	2	2	3	5	5	5	6	10	2	4.2
(n=80)	**Ho**	0.413	0.413	0.500	0.425	0.663	0.600	0.538	0.400	0.800	0.338	0.509
	He	0.472	0.443	0.439	0.410	0.592	0.612	0.505	0.358	0.803	0.393	0.503
	F_{IS}	0.126	0.069	-0.140	-0.036	-0.119	0.020	-0.064	-0.117	0.004	0.141	-0.012
Chile97	**Na**	2	2	2	3	4	4	5	8	11	2	4.3
(n=67)	**Ho**	0.507	0.522	0.269	0.343	0.567	0.567	0.537	0.343	0.746	0.299	0.470
	He	0.495	0.425	0.313	0.320	0.610	0.596	0.482	0.372	0.793	0.348	0.475
	F_{IS}	-0.026	-0.230	0.141	-0.071	0.070	0.048	-0.116	0.078	0.059	0.141	0.010
Chile99	**Na**	2	2	2	3	5	4	4	8	9	2	4.1
(n=53)	**Ho**	0.415	0.509	0.415	0.283	0.623	0.528	0.302	0.396	0.792	0.321	0.458
	He	0.436	0.442	0.422	0.336	0.650	0.588	0.450	0.359	0.806	0.318	0.481
	F_{IS}	0.047	-0.152	0.015	0.158	0.042	0.102	0.329	-0.105	0.017	-0.010	0.044
CenNPac	**Na**	2	2	2	3	4	4	5	7	10	2	4.1
(n=31)	**Ho**	0.484	0.323	0.452	0.258	0.710	0.581	0.645	0.387	0.742	0.323	0.490
	He	0.481	0.412	0.437	0.255	0.672	0.574	0.633	0.387	0.818	0.350	0.502
	F_{IS}	-0.005	0.217	-0.033	-0.010	-0.056	-0.012	-0.019	0.000	0.093	0.077	0.025
Australia	**Na**	2	2	2	3	5	4	4	5	9	2	3.8
(n=65)	**Ho**	0.400	0.385	0.231	0.308	0.585	0.585	0.631	0.385	0.815	0.277	0.460
	He	0.499	0.420	0.291	0.306	0.626	0.592	0.543	0.363	0.776	0.281	0.470
	F_{IS}	0.198	0.084	0.208	-0.005	0.067	0.012	-0.162	-0.060	-0.051	0.015	0.031
W.Aus	**Na**	2	2	2	3	4	4	3	4	10	2	3.6
(n=30)	**Ho**	0.467	0.267	0.400	0.300	0.700	0.600	0.533	0.367	0.833	0.333	0.480
	He	0.491	0.444	0.358	0.346	0.551	0.619	0.443	0.367	0.773	0.320	0.471
	F_{IS}	0.050	0.400	-0.118	0.133	-0.271	0.031	-0.205	0.002	-0.078	-0.042	-0.010
Taiwan	**Na**	2	2	2	3	4	5	4	5	11	2	4.0
(n=63)	**Ho**	0.413	0.508	0.381	0.460	0.714	0.651	0.556	0.397	0.794	0.365	0.524
	He	0.490	0.444	0.363	0.435	0.642	0.532	0.490	0.428	0.819	0.354	0.500
	F_{IS}	0.157	-0.143	-0.050	-0.058	-0.113	-0.222	-0.133	0.072	0.031	-0.030	-0.049
Japan	**Na**	2	2	2	3	4	4	6	5	10	2	4.0
(n=38)	**Ho**	0.500	0.447	0.342	0.316	0.526	0.579	0.368	0.289	0.921	0.158	0.445
	He	0.458	0.441	0.347	0.349	0.511	0.577	0.484	0.257	0.808	0.188	0.442
	F_{IS}	-0.091	-0.013	0.015	0.095	-0.029	-0.003	0.239	-0.127	-0.140	0.162	0.011
HawLa	**Na**	2	2	2	2	4	5	5	6	10	2	4.0
(n=42)	**Ho**	0.238	0.452	0.405	0.381	0.714	0.571	0.643	0.476	0.667	0.452	0.500
	He	0.387	0.452	0.375	0.337	0.651	0.618	0.588	0.411	0.825	0.398	0.504
	F_{IS}	0.384	-0.001	-0.079	-0.131	-0.097	0.075	-0.092	-0.159	0.191	-0.138	-0.005
CenNJu	**Na**	2	2	2	3	4	5	5	4	10	2	3.9
(n=46)	**Ho**	0.500	0.500	0.370	0.217	0.609	0.543	0.500	0.326	0.848	0.413	0.483
	He	0.460	0.481	0.375	0.294	0.642	0.564	0.501	0.362	0.816	0.375	0.487
	F_{IS}	-0.087	-0.040	0.014	0.260	0.051	0.036	0.002	0.100	-0.039	-0.101	0.020
CenSJu	**Na**	2	2	2	3	4	6	6	5	10	2	4.2
(n=36)	**Ho**	0.500	0.361	0.611	0.222	0.556	0.639	0.389	0.333	0.889	0.333	0.483
	He	0.494	0.497	0.475	0.351	0.557	0.554	0.473	0.358	0.829	0.278	0.487
	F_{IS}	-0.013	0.273	-0.286	0.368	0.003	-0.153	0.178	0.069	-0.073	-0.200	0.017
AusJu	**Na**	2	2	2	3	4	5	4	4	5	2	3.3
(n=14)	**Ho**	0.429	0.571	0.357	0.429	0.643	0.500	0.429	0.357	0.714	0.357	0.479
	He	0.490	0.459	0.293	0.472	0.579	0.602	0.474	0.314	0.755	0.375	0.481
	F_{IS}	0.125	-0.244	-0.217	0.092	-0.110	0.169	0.097	-0.138	0.054	0.048	-0.013
GuamJu	**Na**	2	2	2	3	4	3	4	2	6	2	3.0
(n=12)	**Ho**	0.500	0.583	0.250	0.333	0.417	0.333	0.500	0.250	0.583	0.333	0.408
	He	0.444	0.413	0.330	0.288	0.462	0.538	0.503	0.330	0.733	0.375	0.442
	F_{IS}	-0.125	-0.412	0.242	-0.157	0.098	0.381	0.007	0.242	0.204	0.111	0.059

Na: The number of alleles; Ho: Observed Heterozygosity; He: Expected Heterozygosity; F_{IS}:Inbreeding coefficient

Genetic differentiation between populations

Pairwise F_{ST} values over all loci and Slatkin's linearized F_{STs} were very small, ranging from <0.0001 to 0.0149, and from 0.0000 to 0.0151, respectively (**Table 13**), but included several statistically significant comparisons. Four samples, all from tropical areas (mean annual SST>24℃), were significantly different from samples collected in temperate areas of the North Pacific and the South Pacific as follows: 1) Ecuador vs. NMCA and Australia; 2) Taiwan vs. HAW99, NMCA, and Japan; 3) HawLa vs. HAW99, NMCA, Chile97, Australia, and Japan; and 4) CenSJu vs. HAW99, NMCA, and Australia. In addition, the tropical samples HawLa and CenSJu were different from each other. By contrast, no differences were found between any of the temperate samples regardless their hemispheric origin. The results of G-tests for genic and genotypic population differentiation suggest a similar shallow pattern of differentiation between tropical and temperate samples (**Table 14**). Four samples (Ecuador, Taiwan, HawLa and CenSJu) from tropical regions showed differentiation against samples from temperate regions. However, heterogeneity was detected between tropical samples, including differences between Ecuador and Taiwan, Taiwan and HawLa, and HawLa and CenSJu.

Table 13. Population pairwise F_{ST} (lower diagonal) and Slatkin linearized F_{ST} (upper diagonal) for 16 sampling localities.

	HAW99	HAWNE	NMCA	Ecuador	Chile97	Chile99	CenNPac	Australia	W.Aus	Taiwan	Japan	HawLa	CenNJu	CenSJu	AusJu	GuamJu
HAW99		0.0007	0.0000	0.0026	0.0000	0.0026	0.0016	0.0000	0.0000	0.0082	0.0000	0.0063	0.0009	0.0096	0.0000	0.0000
HAWNE	0.0007		0.0000	0.0000	0.0000	0.0003	0.0013	0.0004	0.0000	0.0035	0.0000	0.0049	0.0005	0.0030	0.0000	0.0000
NMCA	-0.0005	-0.0003		0.0050	0.0000	0.0052	0.0035	0.0000	0.0000	0.0081	0.0000	0.0089	0.0050	0.0115	0.0000	0.0000
Ecuador	0.0026	-0.0016	0.0049		0.0023	0.0000	0.0005	0.0053	0.0000	0.0026	0.0039	0.0013	0.0000	0.0013	0.0000	0.0000
Chile97	-0.0014	-0.0006	-0.0021	0.0023		0.0020	0.0016	0.0000	0.0000	0.0039	0.0010	0.0070	0.0006	0.0073	0.0000	0.0000
Chile99	0.0026	0.0003	0.0052	-0.0012	0.0020		0.0002	0.0042	0.0000	0.0026	0.0031	0.0000	0.0000	0.0024	0.0000	0.0000
CenNPac	0.0016	0.0013	0.0035	0.0005	0.0016	0.0002		0.0026	0.0005	0.0048	0.0092	0.0000	0.0000	0.0061	0.0000	0.0000
Australia	-0.0011	0.0004	-0.0019	0.0052	-0.0030	0.0042	0.0026		0.0000	0.0021	0.0040	0.0083	0.0016	0.0110	0.0002	0.0000
W.Aus	-0.0015	-0.0033	-0.0053	-0.0009	-0.0081	-0.0012	0.0005	-0.0042		0.0002	0.0000	0.0038	0.0000	0.0000	0.0000	0.0000
Taiwan	**0.0082***	0.0035	**0.0080***	0.0026	0.0039	0.0026	0.0048	0.0021	0.0002		0.0151	0.0033	0.0000	0.0053	0.0000	0.0003
Japan	-0.0009	-0.0016	-0.0004	0.0039	0.0010	0.0031	0.0091	0.0040	-0.0020	**0.0149***		0.0135	0.0054	0.0060	0.0016	0.0000
HawLa	0.0063	0.0049	0.0089	0.0013	0.0069	-0.0028	-0.0023	0.0082	0.0038	0.0033	0.0133		0.0000	0.0141	0.0012	0.0006
CenNJu	0.0009	0.0005	0.0050	-0.0020	0.0006	-0.0036	-0.0015	0.0016	-0.0004	-0.0003	0.0054	-0.0043		0.0023	0.0000	0.0000
CenSJu	0.0095	0.0030	**0.0114***	0.0013	0.0073	0.0024	0.0061	0.0109	0.0000	0.0053	0.0059	**0.0139***	0.0023		0.0000	0.0014
AusJu	-0.0048	-0.0022	-0.0032	-0.0056	-0.0058	-0.0036	-0.0005	0.0002	-0.0043	-0.0021	0.0016	0.0012	-0.0042	-0.0011		0.0000
GuamJu	-0.0043	-0.0117	-0.0095	-0.0043	-0.0088	-0.0046	-0.0006	-0.0052	-0.0131	0.0003	-0.0092	0.0006	-0.0015	0.0014	-0.0039	

Note: shaded values are with significant p- value (significant level=0.05). *: significant after Benjamini and Yekutieli correction for multiple testing (B-Y FDR= 0.0093).

Table 14. *P*-values associated with G-tests of population differentiation between sample pairs for genic (upper diagonal) and genotypic (lower diagonal) differentiation.

	HAW99	HAWNE	NMCA	Ecuador	Chile97	Chile99	CenNPac	Australia	W.Aus	Taiwan	Japan	HawLa	CenNJu	CenSJu	AusJu	GuamJu
HAW99		0.6271	0.6597	0.1090	0.9131	0.1924	0.6875	0.4271	0.7122	***0.0002****	0.6333	0.0543	0.2035	0.0579	0.8283	0.8508
HAWNE	0.6249		0.3160	0.4987	0.7172	0.3719	0.6881	0.1762	0.5034	***0.0034****	0.5094	***0.0463***	0.2184	0.1811	0.4489	0.9590
NMCA	0.6920	0.3306		***0.0460***	0.9486	0.2303	0.3894	0.9144	0.9739	***0.0001****	0.6788	***0.0217***	0.1161	***0.0380***	0.8795	0.9208
Ecuador	0.0920	0.4769	***0.0438***		0.0518	0.5122	0.6664	***0.0109****	0.3111	***0.0191***	0.1574	0.4888	0.5542	0.1634	0.7714	0.7312
Chile97	0.9238	0.7401	0.9571	0.0828		0.4155	0.5606	0.9364	0.9717	***0.0009****	0.4719	0.0954	0.6661	0.0900	0.8605	0.8879
Chile99	0.2176	0.3919	0.2612	0.5093	0.4625		0.4837	0.0719	0.7108	***0.0135***	0.4453	0.6208	0.7433	0.3774	0.5443	0.8666
CenNPac	0.6841	0.6834	0.4243	0.6370	0.5529	0.5553		0.1378	0.3711	0.0850	0.1870	0.4860	0.4393	0.5125	0.8035	0.7891
Australia	0.4533	0.1988	0.9239	***0.0129***	0.9463	0.1156	0.1641		0.8401	***0.0002****	0.1458	***0.0170***	0.0887	***0.0062****	0.1580	0.9039
W.Aus	0.7175	0.4895	0.9769	0.2833	0.9700	0.7488	0.3450	0.8412		0.0628	0.5810	0.3461	0.5384	0.7108	0.7690	0.7452
Taiwan	***0.0002****	***0.0023****	***0.0001****	***0.0138***	***0.0013****	***0.0182***	0.0657	***0.0002****	***0.0436***		***0.0008****	***0.0171***	0.2629	0.1703	0.3316	0.3646
Japan	0.7759	0.6075	0.7210	0.2234	0.6264	0.5791	0.3094	0.2360	0.7068	***0.0014****		***0.0170***	0.1732	0.2100	0.7068	0.9593
HawLa	0.0713	***0.0453***	***0.0279***	0.4830	0.1258	0.6312	0.5088	***0.0319***	0.3623	***0.0215***	***0.0321***		0.7621	***0.0254***	0.3603	0.3785
CenNJu	0.2265	0.2276	0.1287	0.5309	0.6548	0.7494	0.4524	0.1013	0.5413	0.2520	0.2158	0.7785		0.4138	0.7446	0.5856
CenSJu	***0.0410***	0.2046	***0.0389***	0.1673	0.0834	0.3956	0.5921	***0.0074****	0.6907	0.1427	0.2327	***0.0271***	0.3782		0.6008	0.4351
AusJu	0.8559	0.4386	0.8979	0.7397	0.8689	0.5953	0.8255	0.1652	0.7475	0.3242	0.7871	0.3974	0.7681	0.5274		0.6003
GuamJu	0.8885	0.9591	0.9271	0.7027	0.9309	0.9049	0.8403	0.9321	0.7433	0.3647	0.9794	0.4226	0.6554	0.4483	0.7110	

Note: shaded values are with significant p- value (significant level=0.05). *: significant after Benjamini and Yekutieli correction for multiple testing (B-Y FDR= 0.0093).

Further, the degree of differentiation of these tropical samples with respect to other tropical and also temperate localities varied, with Taiwan, HawLa, CenSJu, and Ecuador differentiating from ten, six, four and three populations, respectively. No significant population genic or genotypic differentiation was detected among any of the temperate populations. The observed pattern of differentiation in Pacific swordfish does not conform to IBD, as the correlation between pairwise F_{ST} values and geographic distances was not significantly different from zero ($R^2 = 0.0094$; P=0.2460) (**Figure 8**).

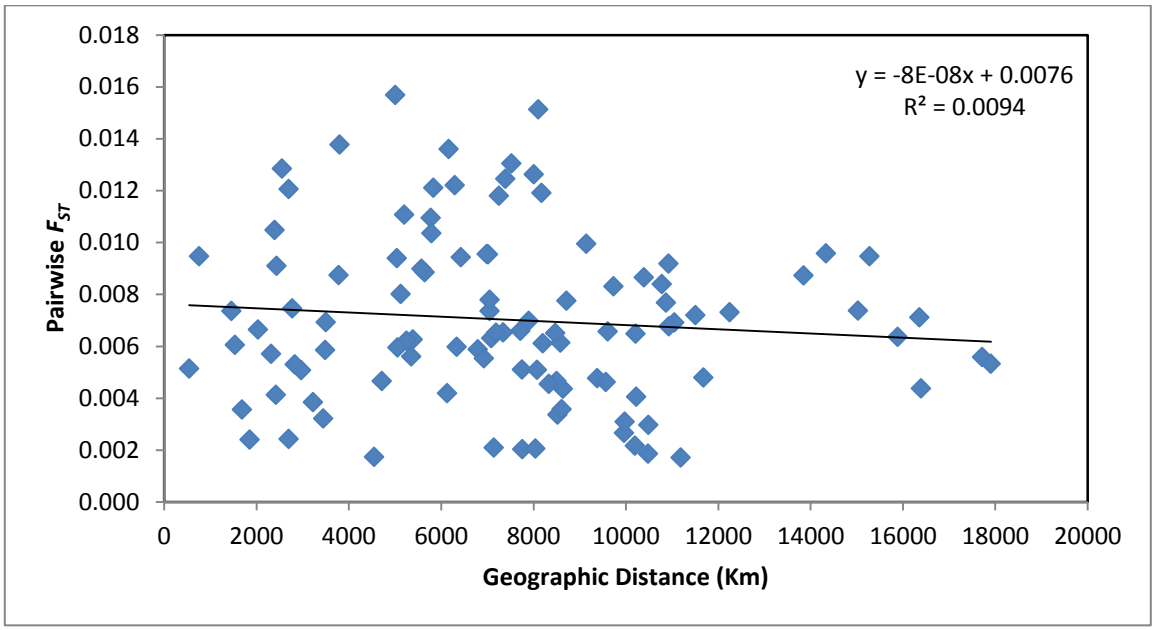


Figure 8. Mantel test of the association of geographic (Km) and genetic (F_{ST}) distances for Pacific swordfish. The Indian Ocean sample (W. Aus) was not included because of the obvious geographic barriers that separate this locality from the Pacific Ocean localities. The correlation was not significantly different from zero ($R^2 = 0.0094$; P=0.2460).

Genetic population structure Bayesian clustering analyses

STRUCTURE analyses

Multiple runs were conducted to determine the number of clusters, by varying the *K* value (1 to 6). The mean Ln P(*K*) with *K*=2 was higher value than those obtained with *K*=3 to 6, although lower than mean Ln P(*K*) with *K=1*. However, the estimate of delta *K*, which more accurately reflects the number of subpopulations identified by STRUCTURE, was highest when *K*=3. Depictions of individual assignments for each sample using different *K* values are shown in **Figure 9**. At *K*=2, the average cluster posterior probability membership $\bar{Q}$ values among samples to belong to two clusters varies (Appendix Table A6). Notably, Taiwan received the highest average posterior probability membership to Cluster 2. In six additional sampling localities the average posterior probability membership for Cluster 2 ($\bar{Q}$> 0.25) was higher than for the rest. Five of these samples came from tropical areas, and one locality (AusJu) was collected in a sub-tropical area immediately adjacent to the 24℃ SST isotherm used to define here the boundary between these two zones. In general, sampling localities from temperate areas received higher $\bar{Q}$ scores to Cluster 1. When *K*=3, the assignment of individuals in all localities to any one of the three clusters was nearly equiprobable. Accordingly, there was no evidence of association of any one cluster to a particular sample or region, suggesting no geographica allele frequency differences. Considering the low levels of differentiation found among Pacific swordfish samples (*Fst* < 0.0141), and seeking to improve the performance of STRUCTURE in individual ancestry assignment, we included data from two reference samples, representing the Northwest Atlantic and

Mediterranean populations. The reference samples provided allele frequency estimates as baseline to compare within the Pacific (**Figure 9C**). The inclusion of reference samples augmented the signal of differentiation between two Pacific swordfish clusters. Largely, the highest $\bar{Q}$ scores to Cluster 1 were among samples found in temperate areas. Conversely, the posterior membership towards Cluster 2 was highest for samples found in tropical areas, with Taiwan scoring the highest ($\bar{Q}$ = 0.9222), followed by HawLa, CenNJu, Ecuador, and CenSJu (Appendix Table A7).

GENELAND analyses

The number of inferred clusters with the highest average posterior probability in GENELAND was *K*=2. Posterior probability contour maps depicting the membership to Cluster 1 are shown in **Figure 10**, where the posterior probability membership to Cluster 2 corresponds to the reciprocal value of belonging to Cluster 1. Six samples (NMCA, Chile97, Chile99, Australia, AusJu, and Japan) from temperate areas and two samples (W.Aus and GuamJu) from tropical areas were assigned to Cluster 1 with high posterior probabilities ($\approx$ 0.75), although HAW99 and HAWNE received lower posterior membership probabilities to Cluster 1. Conversely, six samples from tropical areas (Ecuador, CenNPac, Taiwan, HawLa, CenNJu and CenSJu) scored higher posterior probabilities of membership in Cluster 2. Similar to STRUCTURE, the values of average posterior probability memberships were highest for Taiwan, with lower values towards the tropical central Pacific, and with the lowest values and in the tropical eastern Pacific (Ecuador).

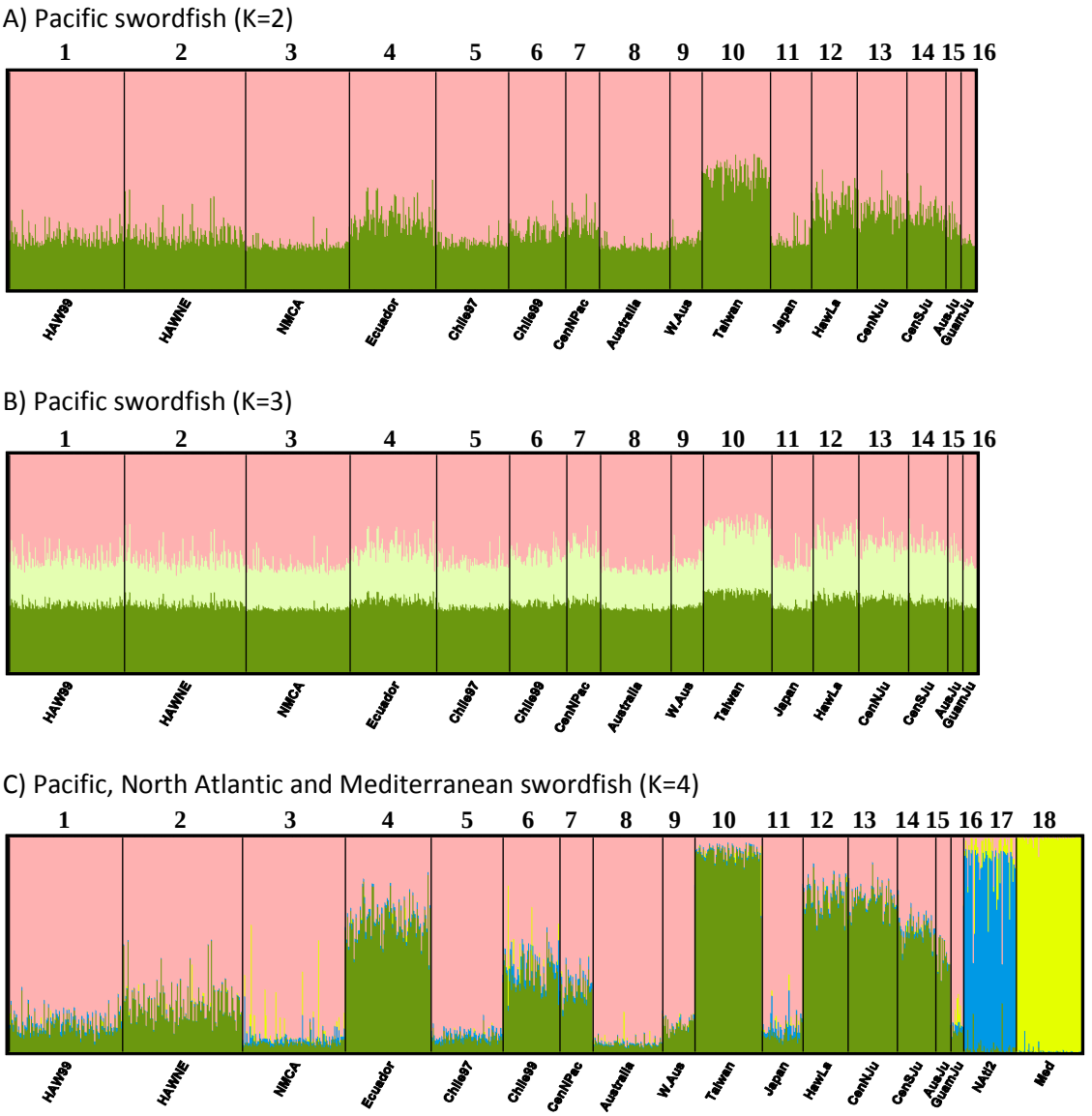


Figure 9. Bayesian individual assignments of Pacific swordfish obtained with STRUCTURE (Pritchard *et al.* 2000). North Atlantic (blue) and Mediterranean (yellow) samples were included as outgroup. Values were estimated with no admixture, correlated alleles and LOCPRIOR models, and were inferred from 50 independent runs with K=4 using 100,000 MCMC iterations and a burn-in period of 100,000. The individual probabilities to belong to the Pacific clusters are depicted as follows: Cluster 1 (pink), Cluster 2 (green), and Cluster 3 (light green).

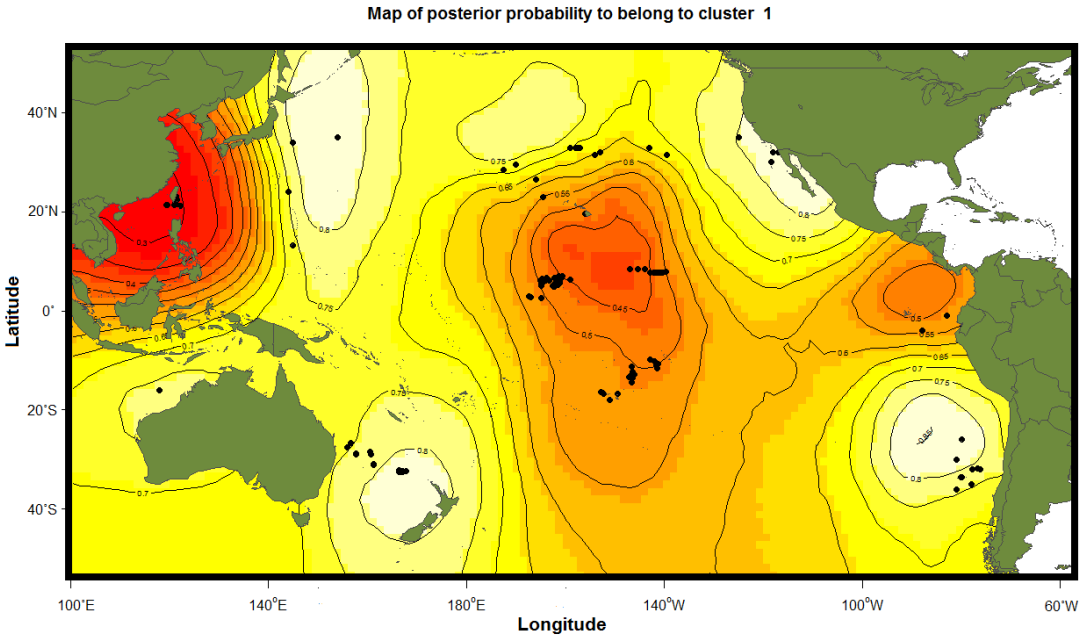


Figure 10. Contour map of posterior probabilities for Pacific swordfish to the temperate Cluster 1 estimated in GENELAND (Guillot *et al.* 2005). Probability contours range from 0.85 (light yellow) to 0.3 (red). Membership to Cluster 2 (tropical) corresponds to the reciprocal contour values. Black dots identify sampling locations and may represent one or more individuals. GENELAND runs were conducted with a coordinate value uncertainty of 35° using correlated allele frequency and spatial models, and 20 independent runs of K = 2 with 100,000 MCMC iterations and a thinning of 100.

Principal Coordinate Analysis (PCoA): The first three axes of a PCoA conducted by pooling 15 of the original samples into nine regional grouping explained 49.87%, 35.75%, and 14.38% of the variation, respectively. The plot of the first two axes separates tropical from temperate samples (**Figure 11**). Among tropical localities, tropical south-central (TSC: CenSJu) and tropical southeastern (TSE: Ecuador) are isolated from both tropical north-central (TNC: CenNPac, HawLa, CenNJu) and tropical northwestern (TNW: Taiwan and GuamJu) which in turn are not separated from each other.

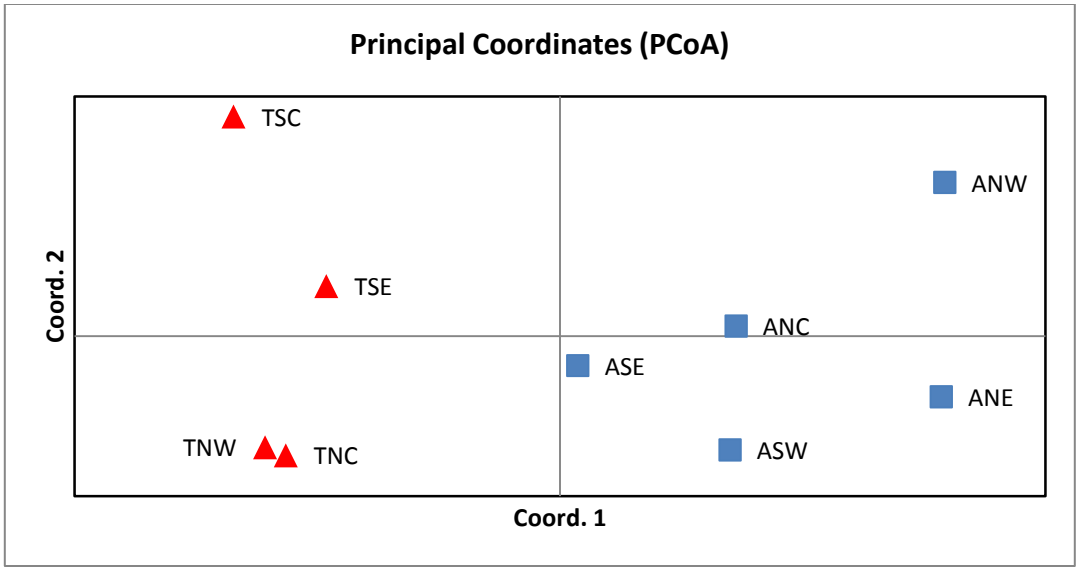


Figure 11. Principal coordinates analysis of nine regions of Pacific swordfish obtained with multilocus nDNA SNP data. PCoA was conducted by pooling 15 of the original samples into nine regional grouping, except W. Aus (see Materials and Methods). The acronyms for the regions correspond to those associated to sampling localities (see **Table 10**). Red triangles identify samples from tropical regions and blue squares samples from temperate regions in the Pacific Ocean.

AMOVA testing

Based on the results of population differentiation, we conducted AMOVA testing the signal of population subdivision based solely on the geographic association to tropical and temperate areas (**Table 15**). Accordingly, the signal was tested by separating all the sampling localities into two groups: G1: Temperate (Eight sampling localities: 1.HAW99, 2.HAWNE, 3.NMCA, 5.Chile97, 6.Chile99, 8.Australia, 11.Japan, and 15.AusJu) and G2: Tropical areas (Eight sampling localities: 4.Ecuador, 7.CenNPac, 9.W.Aus, 10.Taiwan, 12.HawLa, 13.CenNJu, 14.CenSJu, and 16.GuamJu). A very small but significant proportion of variation separates the two groups (F_{CT}=0.0022, P<0.01). To further refine the analysis, a second AMOVA based on the patterns

obtained with STRUCTURE (**Figure 9**) was conducted by assigning eight sampling localities with higher posterior probability membership to belong to Cluster 1 to one group (G1): 1.HAW99, 2.HAWNE, 3.NMCA, 5.Chile97, 8.Australia, 9.W.Aus, 11.Japan, and 16.GuamJu, with the remaining eight samples assigned to a second group (G2): 4.Ecuador, 6.Chile99, 7.CenNPac, 10.Taiwan, 12.HawLa, 13.CenNJu, 14.CenSJu, and 15.AusJu. Significant differentiation between two groups (F_{CT}=0.0039, P<0.001) was obtained. Since Taiwan was identified as the most distinct among the G2 samples, it was assigned into a third group (G3) and compared it against G1 and G2. This AMOVA produced a higher proportion of among-group variation (F_{CT}=0.0044, P<0.001) than the two-group arrangement. Further, an additional AMOVA was conducted on the basis of GENELAND results as follows: The ten sampling localities with highest posterior probability membership to belong to Cluster 1 were assigned to G1 (1. HAW99, 2. HAWNE, 3. NMCA, 5. Chile97, 6. Chile99, 8. Australia, 9. W.Aus, 11. Japan, 15. AusJu, and 16. GuamJu), and the remaining six samples to G2 (4. Ecuador, 7. CenNPac, 10. Taiwan, 12. HawLa, 13. CenNJu, and 14. CenSJu). The AMOVA yielded a significant value of differentiation (F_{CT} = 0.0032; p<0.01) between the two groups. Since GENELAND also identified Taiwan as the most differentiated sample of the Cluster 2, it was assigned into a third group (G3), with this arrangement yielding a higher value of differentiation (F_{CT} = 0.0037; p<0.001) than the two-group hypothesis, but not as high as that in the three-group AMOVA based on STRUCTURE results.

Table 15. AMOVA results of testing alternative hypotheses based on geographic distribution (Tropical versus Temperate areas) and Bayesian clustering analyses (STRUCTURE and GENELAND results) see text for explanation.

Population Structure Grouping:					Source of Variation	Variance Component	% of variance	Fixation Indices
Geographic groups by Temperate and Tropical area								
G1:Temperate area	1.HAW99	2.HAWNE	3.NMCA	5.Chile97	AG	0.00535 Va	0.22	F_{CT} : 0.00220**
(8 sampling locations)	6.Chile99	8.Australia	11.Japan	15.AusJu	AP/WG	0.00084 Vb	0.03	F_{SC} : 0.00035^{ns}
					AI/WP	0.03316 Vc	1.36	F_{IS} : 0.01367**
G2:Tropical area	4.Ecuador	7.CenNPac	9.W.Aus	10.Taiwan	WI	2.39226 Vd	98.38	F_{IT} : 0.01618**
(8 sampling locations)	12.HawLa	13.CenNJu	14.CenSJu	16.GuamJu				
Genetic population subdivision suggested by STRUCTURE: Two-Groups								
G1:Cluster 1	1.HAW99	2.HAWNE	3.NMCA	5.Chile97	AG	0.00949 Va	0.39	F_{CT} : 0.00390***
(8 sampling locations)	8.Australia	9.W.Aus	11.Japan	16.GuamJu	AP/WG	-0.00140 Vb	-0.06	F_{SC} :-0.00058^{ns}
					AI/WP	0.03316 Vc	1.36	F_{IS} : 0.01367^{ns}
G2:Cluster 2	4.Ecuador	6.Chile99	7.CenNPac	10.Taiwan	WI	2.39226 Vd	98.31	F_{IT} : 0.01695^{ns}
(8 sampling locations)	12.HawLa	13.CenNJu	14.CenSJu	15.AusJu				
Genetic population subdivision suggested by STRUCTURE: Three-Groups								
G1:Cluster 1	1.HAW99	2.HAWNE	3.NMCA	5.Chile97	AG	0.01067 Va	0.44	F_{CT} : 0.00438***
(8 sampling locations)	8.Australia	9.W.Aus	11.Japan	16.GuamJu	AP/WG	-0.00257 Vb	-0.11	F_{SC} :-0.00106^{ns}
					AI/WP	0.03316 Vc	1.36	F_{IS} : 0.01367^{ns}
G2:Cluster 2	4.Ecuador	6.Chile99	7.CenNPac	12.HawLa	WI	2.39226 Vd	98.30	F_{IT} : 0.01695^{ns}
(7 sampling locations)	13.CenNJu	14.CenSJu	15.AusJu					
G3:Singal 2 (1 sampling location)	10.Taiwan							
Genetic population subdivision suggested by GENELAND: Two-Groups								
G1:Cluster 1	1.HAW99	2.HAWNE	3.NMCA	5.Chile97	AG	0.00782 Va	0.32	F_{CT} : 0.00321**
(10 sampling locations)	6.Chile99	8.Australia	9.W.Aus	11.Japan	AP/WG	-0.00020 Vb	-0.01	F_{SC} : -0.0008^{ns}
	15.AusJu	16.GuamJu			AI/WP	0.03316 Vc	1.36	F_{IS} : 0.01367^{ns}
					WI	2.39226 Vd	98.32	F_{IT} : 0.01676^{ns}
G2:Cluster 2	4.Ecuador	7.CenNPac	10.Taiwan	12.HawLa				
(6 sampling locations)	13.CenNJu	14.CenSJu						
Genetic population subdivision suggested by GENELAND: Three-Groups								
G1:Cluster 1	1.HAW99	2.HAWNE	3.NMCA	5.Chile97	AG	0.00905 Va	0.37	F_{CT} : 0.00372***
(10 sampling locations)	6.Chile99	8.Australia	9.W.Aus	11.Japan	AP/WG	-0.00115 Vb	-0.05	F_{SC} :-0.00048^{ns}
	15.AusJu	16.GuamJu			AI/WP	0.03316 Vc	1.36	F_{IS} : 0.01367^{ns}
					WI	2.39226 Vd	98.31	F_{IT} : 0.01687^{ns}
G2:Cluster 2	4.Ecuador	7.CenNPac	12.HawLa	13.CenNJu				
(5 sampling locations)	14.CenSJu							
G3:Cluster 2(1 sampling location)	10.Taiwan							

Abbreviations: AG: Among groups; AP/WG: Among populations within groups; AI/WP: Among individuals within populations; WI: Within individuals

ns : P > 0.05; * : P<0.05; ** :P<0.01; *** :P<0.001 with Significance tests(10,100 permutation)

Six additional AMOVAs were conducted to test different models of stock structure based on fisheries data (**Table 16**). Sampling localities were assigned into different groupings corresponding as close as possible to represent the alternative models of Pacific swordfish (see **Figure 6**), including two, three, and four stock hypotheses. None of the AMOVAs tested yielded significant proportions of population subdivision, although the three-stock model of Ichinokawa and Brodziak (2008) explained the largest amount of variance among-groups of any of the fishery models tested. However, the reduced signal of fishery-based models, compared with AMOVA arrangements based upon the signal of genetic differentiation resulting from Bayesian analyses, suggests that such subdivisions may not reflect the biological reality of Pacific swordfish.

Table 16. AMOVA results of testing alternative fishery-based multiple-stock hypotheses of Pacific swordfish.

Population Structure Grouping:						Source of Variation	Variance Component	% of variance	Fixation Indices
Two stocks with boundary at N10˚									
G1:Stock 1:North of boundary	1.HAW99	2.HAWNE	3.NMCA	10.Taiwan	11.Japan	AG	-0.00049 Va	-0.02	F_{CT} : -0.00020^{ns}
(7 sampling locations)	12.HawLa	16.GuamJu				AP/WG	0.00385 Vb	0.16	F_{SC} : 0.00159*
G2: Stock 2:South of boundary	4.Ecuador	5.Chile97	6.Chile99	7.CenNPac	8.Australia	AI/WP	0.03316 Vc	1.37	F_{IS} : 0.01367^{ns}
(9 sampling locations)	9.W.Aus	13.CenNJu	14.CenSJu	15.AusJu		WI	2.39226 Vd	98.50	F_{IT} : 0.01504^{ns}
Two stocks with boundary at Equator									
G1:Stock 1:North of boundary	1.HAW99	2.HAWNE	3.NMCA	7.CenNPac	10.Taiwan	AG	-0.00112 Va	-0.05	F_{CT} :-0.00046^{ns}
(9 sampling locations)	11.Japan	12.HawLa	13.CenNJu	16.GuamJu		AP/WG	0.00417 Vb	0.17	F_{SC} : 0.00172*
G2: Stock 2:South of boundary	4.Ecuador	5.Chile97	6.Chile99	8.Australia	9.W.Aus	AI/WP	0.03316 Vc	1.37	F_{IS} : 0.01367^{ns}
(7 sampling locations)	14.CenSJu	15.AusJu				WI	2.39226 Vd	98.51	F_{IT} : 0.01491^{ns}
Three stocks referenced Bartoo and Coan 1989.									
G1:Stock 1 (5 sampling locations)	1.HAW99	2.HAWNE	10.Taiwan	11.Japan	12.HawLa	AG	-0.00300 Va	-0.12	F_{CT} :-0.00124^{ns}
G2:Stock 2 (5 sampling locations)	8.Australia	9.W.Aus	13.CenNJu	15.AusJu	16.GuamJu	AP/WG	0.00566 Vb	0.23	F_{SC} : 0.00233*
G3:Stock 3 (6 sampling location)	3.NMCA	4.Ecuador	5.Chile97	6.Chile99	7.CenNPac	AI/WP	0.03316 Vc	1.37	F_{IS} : 0.01367^{ns}
	14.CenSJu					WI	2.39226 Vd	98.52	F_{IT} : 0.01475^{ns}
Three stocks referenced Nakano 1998.									
G1:Stock 1 (7 sampling locations)	1.HAW99	2.HAWNE	3.NMCA	10.Taiwan	11.Japan	AG	-0.00143 Va	-0.06	F_{CT} :-0.00059^{ns}
	12.HawLa	16.GuamJu				AP/WG	0.00454 Vb	0.19	F_{SC} : 0.00187*
G2:Stock 2 (5 sampling locations)	8.Australia	9.W.Aus	13.CenNJu	14.CenSJu	15.AusJu	AI/WP	0.03316 Vc	1.37	F_{IS} : 0.01367^{ns}
G3:Stock 3 (4 sampling location)	4.Ecuador	5.Chile97	6.Chile99	7.CenNPac		WI	2.39226 Vd	98.51	F_{IT} : 0.01493^{ns}
Three stocks referenced Ichinokawa and Brodziak 2008.									
G1:Stock 1 (7 sampling locations)	1.HAW99	2.HAWNE	3.NMCA	10.Taiwan	11.Japan	AG	0.00145 Va	0.06	F_{CT} : 0.00060^{ns}
	12.HawLa	16.GuamJu				AP/WG	0.00263 Vb	0.11	F_{SC} : 0.00108^{ns}
G2:Stock 2 (4 sampling locations)	4.Ecuador	7.CenNPac	13.CenNJu	14.CenSJu		AI/WP	0.03316 Vc	1.36	F_{IS} : 0.01367^{ns}
G3:Stock 3 (5 sampling location)	5.Chile97	6.Chile99	8.Australia	9.W.Aus	15.AusJu	WI	2.39226 Vd	98.47	F_{IT} : 0.01533^{ns}
Four stocks.									
G1:Stock 1 (6 sampling locations)	1.HAW99	10.Taiwan	11.Japan	12.HawLa	13.CenNJu	AG	-0.00165 Va	-0.07	F_{CT} : -0.00068^{ns}
	16.GuamJu					AP/WG	0.00486 Vb	0.20	F_{SC} : 0.00200*
G2:Stock 2 (3 sampling locations)	8.Australia	9.W.Aus	15.AusJu			AI/WP	0.03316 Vc	1.37	F_{IS} : 0.01367^{ns}
G3:Stock 3 (4 sampling location)	2.HAWNE	3.NMCA	4.Ecuador	7.CenNPac		WI	2.39226 Vd	98.50	F_{IT} : 0.01497^{ns}
G4:Stock 3 (3 sampling location)	5.Chile97	6.Chile99	14.CenSJu						

AG: Among groups; AP/WG: Among populations within groups; AI/WP: Among individuals within populations; WI: Within individuals

ns : P > 0.05; * : P<0.05; ** :P<0.01; *** :P<0.001 with Significance tests(10100 permutation)

Discussion

The overall mean value of heterozygosity across all loci and localities was 0.475 with number of alleles across all loci within population ranging from 2 to 11. On average, these levels of variation are consistent with the values reported for the same loci (except *CaM* that was not characterized here since it is monomorphic in the Pacific) in Atlantic and Mediterranean swordfish populations (Smith 2012). On average, heterozygosity levels were slightly higher in Pacific swordfish for AldB, GpHR, and *ldh*A than in Atlantic swordfish populations (i.e., North and South). Further, congruent with the results obtained for the Atlantic; the SNP loci characterized in here conformed largely to HWE. However, significant deviations for HWE were observed at two loci in two localities. In Japan, heterozygote excess for VBC201 (F_{is} = -0.140) was observed. Because VBC201 is a microsatellite marker, the possibility of scoring artifacts caused by stuttering cannot be discounted. Alternatively, the excess of heterozygotes can be associated to demographic factors. When the effective number of breeders (N_{eb}) in a population is small, the allele frequencies will (by chance) be different in males and females, causing an excess of heterozygotes in the progeny with respect to Hardy-Weinberg equilibrium expectations (Luikart and Cornuet 1998). This explanation is unlikely for Pacific swordfish given the large effective population size derived from mtDNA CR data (Alvarado-Bremer, Pers. Comm.), and the high levels of heterozygosity in microsatellite loci in Pacific swordfish ($h \geq 0.79$, Kasapidis *et al.* 2008), that are consistent with the average heterozygosity reported for these kind of loci in marine fishes (DeWoody and Avise 2000), and with the comparatively high levels of

heterozygosity reported here with SNPs ($h \geq 0.45$). Conversely, a deficit of heterozygotes was observed in Chile99 for ANT (F_{is} =0.329), that could be interpreted as evidence of a Wahlund effect, and thus of population heterogeneity, or caused by null alleles. However, none of the global tests across loci and across samples was significant after B-Y FDR correction, suggesting that each sample can be attributed to individuals from the same population with very little evidence of mixing.

Pacific swordfish population differentiation

The characterization of genetic variation using SNP data in Pacific swordfish suggest genetic heterogeneity within this basin based on multiple tests, including pairwise F_{ST}, genic and genotypic differentiation, Bayesian cluster analyses using STRUCTURE and GENELAND, and AMOVAs. Pairwise F_{ST} values show significant differentiation between three localities after corrections for multiple testing (**Table 13**). Taiwan is different from Japan and from NEPO (NMCA) and from the northwest Hawaiian sample (but not from HAWNE) (**Table 14**). This pattern is also revealed by STRUCTURE and GENELAND. The central South Pacific sample of juveniles (CenSJu) collected around 20°S is different from the NEPO sample (NMCA), but also from the central Pacific larval sample from Hawaii (HawLa) collected farther north at 20°N. Genic and genotypic differentiation is also significant between some population pairs. In particular, significant genic differentiation was detected in the comparisons of Taiwan against the majority of the temperate samples, including Japan, HAW99, HAWNE, NMCA, Chile97 and Australia. In addition to corroborating the differences

detected with allelic frequencies, genotypic data suggests that Australia and CenSJu are different. It is particularly important to underscore that no differences were detected among the samples collected in temperate areas, regardless of their hemispheric provenance and geographic separation.

Multilocus analyses of SNP data using STRUCTURE identified two clusters in Pacific swordfish with a relative proportion among samples that is largely congruent with their temperate and tropical origin. A very similar pattern of differentiation was obtained with GENELAND, where the posterior probability map identifies two clusters, and provides potential boundaries delimiting the regions of contact. By contrast, no evidence of differentiation among temperate localities was detected, concordant with the tests of population differentiation. Reverely, the tropical sample from Taiwan emerges as the most highly differentiated of all the Pacific swordfish samples characterized in this study, displaying significant differentiation from most of the temperate samples and from some tropical samples.

Alternative AMOVAs were conducted with two and three groups' combinations (**Table 15**). In all instances, the largest amount of among-group variance was explained when Taiwan was treated as third group. This is consistent with the strong signal of differentiation detected with exact tests, and with Bayesian analyses. By contrast, none of the AMOVAs testing fishery models of Pacific swordfish stock structure were significant (**Table 16**). However, the three-stock model of Ichinokawa and Brodziak (2008) explained the largest amount of variance among-stocks of the fishery models tested. That model was used until recently to conduct assessments of Pacific swordfish,

and, further analysis may be required to reject it. In particular, the location of the boundaries separating the three stocks of Ichinokawa and Brodziak (2008) needs to be reexamined given that this study lacked sampling coverage in those areas.

Comparison with previous genetic studies

Most previous genetic studies on Pacific swordfish population structure were exploratory, and consequently had limited sampling coverage often characterizing small sample sizes, and thus potentially had reduced power to detect differentiation (**Table 9**). For example, Grijalva-Chon *et al.* (1994) using RFLP of the entire mtDNA molecule, found no differences between an NEPO sample from waters off the southern portion of the Peninsula of Baja California, Mexico, and a north-central Pacific Ocean (NCPO) sample from Hawaii. Two subsequent PCR-RFLP studies with a more comprehensive sampling coverage of the Pacific Ocean did not help resolve this conflict. Both, the analysis of mtDNA control region (Chow *et al.*, 1997) and the analysis of calmodulin gene intron 4 (*CaM*) (Chow and Takeyama, 2000) revealed no genetic heterogeneity in the Pacific. However, significant differentiation at three allozyme loci was reported between Hawaii and Mexico (Grijalva-Chon *et al.*, 1996). The lack of concordance between the nuclear DNA (nDNA) and the mtDNA data may reflect differences in the mode of inheritance or demographic factors affecting these genomes (Grijalva-Chon *et al.*, 1996). However, Reeb *et al.* (2000) reported very shallow but significant differentiation with mtDNA control region sequence data on a large geographical scale in the Pacific that could not be detected with both RFLP data (i.e., Grijalva-Chon *et al.*

1994; Chow *et al.*, 1997) and with CR data, but using smaller sample sizes and coverage (i.e., Rosel and Block, 1996). Alvarado-Bremer *et al.* (2006) used nuclear sequences of the intron 6 of the lactate dehydrogenase-A (*ldh*-A) gene and found evidence of allelic differentiation within the Pacific: SEPO (Chile) and NCPO (Hawaii) were different from each other and from NEPO (Mexico) and SWPO (Australia), while the two latter samples were not different from each other. Finally, Kasapidis *et al.* (2008) characterized 594 individuals from 6 different regions with 13 microsatellite loci. The results showed very low genetic differentiation among the different geographical areas

In this study, the multilocus analysis of 20 SNPs revealed no difference among the temperate adult samples from Hawaii (HAW99 and HAWNE) and NEPO (NMCA) in opposition with Grijalva-Chon *et al.* (1994). However, genetic heterogeneity between NMCA and HawLa was detected in here, with these two samples corresponding to temperate and tropical clusters, respectively. Accordingly, the region of Hawaiian Archipelago may encompass at least two populations, and in addition those waters may include admixture zones. However, the geographic boundaries separating the two clusters remain uncertain, as no sampling collections between central and eastern North Pacific Ocean were conducted. The Mexican sample characterized by Grijalva-Chon *et al.* (1996) appears to have been collected further south than the Mexico-California sample characterized in here, and thus it may correspond to the tropical cluster. By contrast, their Hawaiian sample appears to come from a temperate location and as such would be equivalent to the adult Hawaiian samples characterized in here. It is therefore possible that the heterogeneity detected with allozymes by Grijalva-Chon *et al.* (1996)

could correspond to the difference in signal between temperate (Hawaii) and tropical (Mexico) clusters. The characterization of swardfish samples from the Mexican tropical areas would be needed to test that hypothesis.

One of the most interesting patterns of differentiation reported in Pacific swordfish is that of IBD conforming to a ⊃-shape corridor of connectivity, proposed by Reeb *et al.* (2000) to account for the differentiation between NWPO (Japan) and SWPO (Australia), and the lack of differentiation of the western Pacific with central and eastern Pacific samples. Based on the analysis of 13 microsatellite loci, Kasapidis *et al.* (2008) reported slight evidence in support of such corridor, but only after samples SWPA1 and SWPA2 were pooled together. By contrast, this study fails to support such corridor in Pacific swordfish, as no differences were detected by any of the tests between the samples from Japan and Australia, although differences between Taiwan and Australia were detected.

Alvarado-Bremer *et al* (2006), reported a pattern of differentiation with pooled samples among four regions of the Pacific based on the characterization of nucleotide sequences for single nuclear locus (*ldh*-A). Accordingly, the Southeastern Pacific Ocean (SEPO: Chile), the Northeastern Pacific Ocean (NEPO: Ecuador to Mexico), the north-central Pacific Ocean (NCPO: Hawaii), and the Southwestern Pacific sample (SWPO: Australia) were different from each other, except no differences between NEPO and SWPO were detected. Accordingly, the authors claimed that that *ldh*-A is an informative marker for the study of population structure in Pacific swordfish, and for that reason, that locus was included among the ten nuclear loci characterized in this

study. The characterization of multiple loci in here was aimed to increase power to detect differentiation, while at the same time reduce the possibility of incurring in a Type I error compared to a single locus analysis. However, the multilocus analysis of nuclear SNP data failed to identify differences among the four temperate regions described by Alvarado-Bremer *et al.* (2006). Specifically, no differences were detected among Hawaii (HAW99 and HAWNE), Mexico, Chile, and Australia. Instead, the tropical adult sample from Taiwan, the larval sample from Hawaii (HawLa) and the adult sample from Ecuador, all members of the tropical cluster, were different from several temperate sampling localities (i.e. HawLa from HAW99, NMCA, Chile97, and Aus; and Ecuador from NMCA and Aus). Accordingly, a general pattern of differentiation emerges based on multilocus data that largely corresponds to a difference between tropical and temperate swordfish. It is important to underscore that the approach used in this study to score variation contained in intron 6 of *ldh*-A gene using UP-HRMA was not capable of characterizing all the SNPs originally described by Greig (2000) and characterized using direct sequencing by Alvarado-Bremer *et al.* (2006). Specifically, the UP-HRMA assay spanned over six of the original SNPs (positions -31, -8,*, +24, and +36), but did not include the SNP (position -51) that defines allele 5 (Alvarado-Bremer *et al.* 2006; Greig, 2000). This is important because the frequency of that allele contributed substantially to the pattern of differentiation reported by Alvarado-Bremer *et al.* (2006). However, attempts to place primers flanking position -51 to characterize allele 5 with SA-HRMA, or by placing a probe over that SNP, both failed. Thus, the lack of concordance of

genetic signal of differentiation among temperate areas in this study compared to Alvarado-Bremer *et al.* (2006) remains unresolved.

There is a substantial difference in sampling coverage between the current study and most previous genetic studies; particularly in reference to the inclusion of more tropical areas, and also ELS stages. For instance, only one sample characterized by Reeb *et al.* (2000) came from a tropical area, whereas only two out of six Pacific samples characterized by Kasapidis *et al.* (2008), and three of 11 samples in Alvarado-Bremer *et al.* (2006) came from tropical areas, and no ELS samples were included in those three studies. Here, most of the tropical sampling localities have a higher proportion of membership to Cluster 2, compared to the temperate localities, and include several ELS samples. The HawLa sample (larvae), a member of the tropical cluster, is different from other Hawaiian samples (adults) that have temperate distribution. Accordingly, the distribution of swordfish in the Hawaiian Archipelago is not homogeneous, and might represent a mixing zone of members of the two clusters (see below). Similarly, the distribution of variation in the eastern Pacific is also not homogeneous, with the sample from NEPO (NMCA) and SEPO (Chile97 and Chile99) corresponding to the temperate Cluster 1, whereas Ecuador is assigned to the tropical Cluster 2.

PCoA conducted on 15 samples pooled into nine regional groupings, also supports the separation of tropical and temperate regions (**Figure 11**). Considering that several previous studies on Pacific swordfish using different genotyping methods and loci have also detected genetic heterogeneity but reached very different conclusions

about the population structure of this species, the questions the validity of the interpretation given here based on multi-locus SNPs. A comparison with the level of concordance (or lack of) among genetic markers employed to study swordfish population structure may shed some light on this question. A surprising concordance among markers has been reported when comparing swordfish populations but only when different basins are compared. Explicitly, differences among Mediterranean, N. Atlantic, S. Atlantic, Indian Ocean, and Pacific Ocean have been reported with mtDNA (Alvarado-Bremer *et al.* 1996; Bradman *et al.* 2011; Lu *et al.* 2006), mtDNA and CaM (Chow and Takeyama 2000), *ldh*-A and aldC (Greig *et al.* 1999; Greig, 2000), microsatellites (Kasapidis *et al.* 2008); and SNPs (Smith *et al.* submitted; this study). On the basis of such concordance, Kasapidis *et al.* (2008) claimed that there is no reason to assume that any of those markers is more powerful in detecting the population structure of swordfish. However, there are substantial differences in the magnitude of the indices of differentiation reported with different types of loci within basins, and specifically within the Pacific Ocean. For instance using mtDNA CR-I sequence data (629 bp) Reeb *et al.* (2000) reported Φ_{ST} =0.032 for the comparison of Japan and the pooled Australian sample. Conversely, that largest *Fst* value reported by Alvarado-Bremer *et al.* (2006) based on the characterization of SNPs contained in 97 bp of sequence of the *ldh*-A gene, was 0.0678, for the comparison the pooled samples of SEPO and SWPO. Here, the largest index of differentiation for ten nuclear loci with 20 SNPs and two SSR was between Japan and Taiwan at 0.0149, and although lower than the two previous examples, it is substantially larger than the index of differentiation

obtained with 13 microsatellite loci by Kasapidis *et al.* (2008), where the largest difference reported was between SWPA1 and SWPA2 (F_{ST}=0.00381, P=0.02051), and with global multilocus F_{ST} of 0.0002. Accordingly, and in agreement with other studies using microsatellites in marine fishes, lower levels of differentiation are obtained compared to other types of markers (DeWoody and Avise 2000). Estimates of F_{ST} obtained microsatellites in marine fishes apparently decline with locus polymorphism, resulting in diminished power to discriminate among samples, with this loss attributed to the effects of size homoplasy (O'Reilly *et al.* 2004).

Comparison with non-genetic evidence of heterogeneity of Pacific swordfish

Several studies aimed to characterize both the reproductive biology and the rates of growth in swordfish have suggested geographic differentiation within the Pacific Ocean. Variations in growth rates have been reported for swordfish off the waters of Taiwan, Australia, Hawaii and Chile. Swordfish from SEPO (Chile) appear to grow at the same rate as those from NCPO (Hawaii), but different from Taiwan and SWPO (Australia) (Cerna, 2009; DeMartini, *et al.* 2007; Sun *et al.* 2002; Young and Drake, 2004). Further, tagging studies conducted on Pacific swordfish using PSATs have provided valuable information of diel behavior, including vertical movements, the association to seamounts and other geological features, the effect of environmental factors such as light, oxygen, and temperature and seasonal displacements particularly valuable for studying the stock structure of Pacific swordfish (Abascal, *et al.* 2010; Abecassis *et al.* 2012; Dewar, et al 2011; Evans, *et al.* 2012; 2014). For instance, Evans

et al. (2014) reported recently distinct patterns of horizontal movements for swordfish tagged of eastern Australia compared to those tagged to the east of New Zealand, and these differences were interpreted as evidence of population subdivision in the SWPO. In addition, these authors reported two very distinct patterns of diving behaviors in different regions South Pacific Ocean. Such behavioral differences could not be associated to any distinctive feature of the water column, and may be indicative of differences among Pacific swordfish populations (Evans *et al.* 2014).

Characterization of ELS samples

In addition to the active movement of adults, population connectivity can be largely facilitated by larval dispersal, with larvae transported regularly between regional and distant populations by oceanic currents (Cowen *et al.*, 2007; Cowen and Sponaugle, 2009). Identification of larval origins and dispersal pathways of swordfish are largely unknown, with spawning occurring over extended temporal and spatial scales with some bias associated with the patchy nature of ichthyoplankton surveys which further precludes determination of these pathways (Grall and De Sylva 1983; Nishikawa *et al.* 1985). However, on the basis of these surveys, together with the characterization of gonadal indices from mature females, a clearer picture of the reproductive biology of swordfish emerges (**Figure 12**). In the northwestern Pacific swordfish larvae are found from Taiwan to about 150˚W between April and June. In the southwestern Pacific, the larvae are mainly distributed near northeastern Australia coast from October to December. In the central south Pacific, larvae are found year-round near 10˚S

(Nishikawa *et al.* 1985). In general, the west to east pattern of larval distribution across the tropical and sub-tropical Pacific is characterized by a wide latitudinal expanse in the western Pacific that begins to contract within the central Pacific and abruptly ends in the eastern Pacific by 108˚W longitude (Mejuto *et al.* 2008; Nishikawa *et al.* 1985). If the absence of swordfish larvae from the eastern Pacific is correct, this suggests that western and central Pacific areas are the only natal grounds for Pacific swordfish and that other conditions beyond surface waters temperature ≥ 24˚C are limiting spawning in the eastern Pacific.

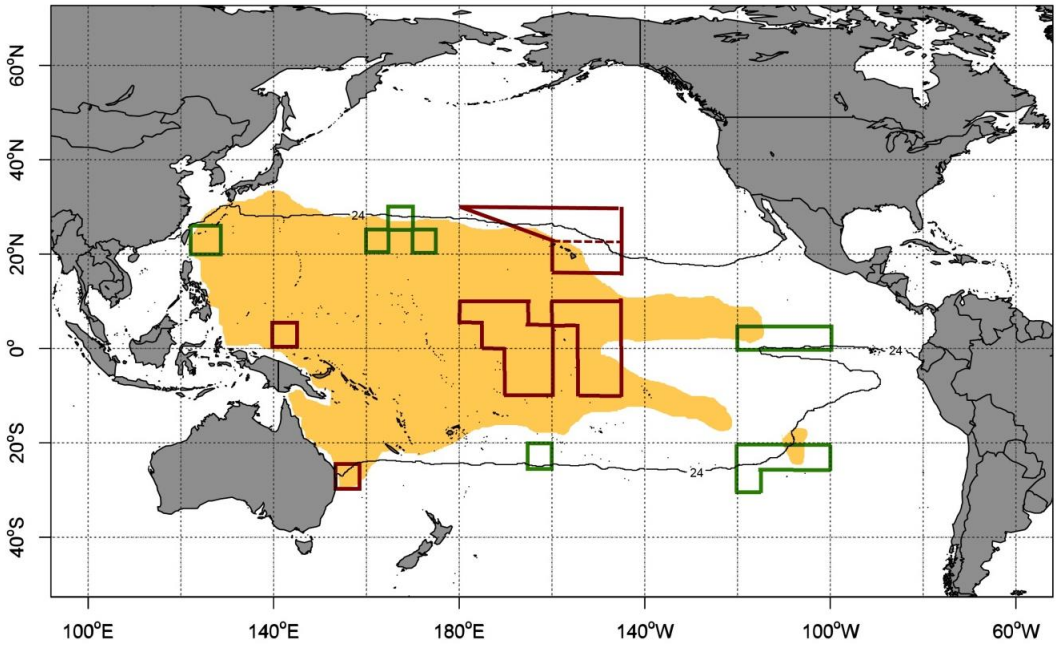


Figure 12. Map of reproductive areas of Pacific swordfish summarized from multiple studies. Shaded in orange are areas where early life history stages have been collected (Grall and de Sylva 1983, Nishikawa *et al.* 1985). Polygons correspond to areas with females with high-gonadal indices (red) and areas with females with smaller GI (green). The dashed line in the red Hawaiian Archipelago polygon indicates the seasonal movement of reproductive females (DeMartini *et al.* 2000; Mejuto *et al.* 2008; Wang *et al.* 2003; Young *et al.* 2003). Mean annual 24 ˚C SST isolines are depicted

Evidence of temporal and geographic discontinuities of spawning areas, together with regional differences in growth rates of adults in the Pacific Ocean, all indicative of potential population subdivision, underscore the relevance of characterizing ELS samples genetically. ELS can be expected to display a lower displacement from their natal sites than adults, and the heterogeneity revealed here with ELS samples appears to support that view. Genetic characterizations of larval and juvenile samples from the central Pacific samples (HawLa, CenNJu and CenSJu) suggest that this region contains swordfish belonging to the tropical cluster based on the membership scores of both STRUCTURE and GENELAND. However, based on allelic differentiation, significant differences were detected between the juvenile sample from the central south Pacific sample of juveniles (CenSJu) collected around 20°S, and the larval sample from Hawaii (HawLa) collected around 20°N. Furthermore, in the waters adjacent to the Hawaiian Archipelago, from the Equator to 35˚ N latitude, an obvious geographic change in otolith elemental fingerprints, corresponding to an increase in Ba and a decrease in Sr concentration, was reported for swordfish juveniles (Humphreys, *et al.* 2005). Although juvenile samples with the exact geographic origin were not characterized in here, a latitudinal genetic difference was detected between larvae (HawLa) and adults (HAW99 and HAWNE) that match the areas characterized by Humphreys *et al.* (2005) with otolith chemistry.

The comparison of the patterns of differentiation revealed by characterizing ELS samples with evidence of connectivity obtained with other non-genetic approaches is relevant. For instance, the results from GENELAND indicate that Australian late

juvenile sample (AusJu) has probability of membership higher than 0.75 to the temperate cluster that contains the adult Australia sample (Aus). According to PSAT experiments conducted in this region (Evans *et al.* 2014), there is a pattern of displacement of tagged swordfish in the SWPO indicative of population structuring. Tracks (up to 365 days) of swordfish tagged to the west of 165° E indicate that fish remain mostly in the area of the Coral and Tasman seas conducting latitudinal movements <10°, and extremely small displacements longitudinally. By contrast, swordfish tagged to the east of New Zealand, around 40°S and 175°W, display displacements towards the central South Pacific, as far north as 20°S and 150°W, whereas swordfish tagged between Fiji and French Polynesia moved predominantly to the north, with movements spanning 20° in latitude and longitude. Further, all fish tagged near Cook Island moved to the SW into waters east of New Zealand. Accordingly at least some swordfish appear to undertake movements between tropical waters extending from around Vanuatu to French Polynesia to waters around New Zealand, indicating greater connectivity than previously thought (Evans *et al.* 2014). Unfortunately, no samples east of New Zealand were characterized in this study, and the characterization of such samples with multi-locus SNPs may reveal a pattern of population structuring in the SWPO concordant with PSAT data. Kasapidis *et al.* (2008) did compare two SWPO samples using microsatellite data, but failed to find differences between the two areas. This lack of concordance may be due to the lower power to detect differentiation in swordfish using microsatellites, but also because the geographic location of their SWPA1 contained a majority of specimens collected to the east of 165°E, the putative boundary of population subdivision proposed by Evans *et al.*

(2014) to separate the two potential stocks inhabiting this region. Similarly, the characterization of the juvenile sample from Guam (GuamJu) yielded a temperate signal with both STRUCTURE and GENELAND that is not consistent with its tropical placement. However, the region neighboring Guam might correspond to the spawning grounds for the temperate NWPO, and thus this association would be consistent with alternative hypotheses advanced below to reconcile the heterogeneity between tropical and temperate regions considering that reproductive behavior of swordfish is not likely to occur in temperate waters (see **Figure 12**). It should be noted, however, that the interpretation of the lack of heterogeneity of the ELS samples of Australia and Guam with respect to the neighboring temperate areas could be due to lack power to detect differentiation, because of the small size of these samples (<20 individuals for both locations). For greater resolution, future studies of early life stage samples should include larger sample sizes and more complete coverage of reproductive areas within the Pacific Ocean.

Alternative hypotheses to the tropical versus temperate model of Pacific swordfish differentiation

Although the multilocus analysis of SNP data appears to identify tropical and temperate clusters, such pattern is not consistent, with reproductive biology of swordfish, including the distribution of mature females with a high gonad index (GI) (Mejuto, *et al.* 2008) and the distribution of ELS which for most part are confined to tropical waters where temperature exceeds 24℃ (**Figure 12**). Further, differences in

growth rates among temperate areas, such as Australia and Chile (Cerna, 2009), identified as members of the same cluster, yet separated by enormous distances (≈11,000 km), urges to consider alternative hypotheses to the simple tropical versus temperate model of differentiation. Whereas long transoceanic migrations have been documented in other billfish, tunas, and sea turtles (Kobayashi *et al.* 2008; Ortiz *et al.* 2003; Whitlock *et al.* 2012), PSAT tagging data from several studies show no evidence of trans-oceanic crossing as have been recorded in swordfish. Explicitly, swordfish tagged the NEPO, SEPO, NWPO and NEPO (Abascal *et al.* 2010; Abecassis, *et al.* 2012; Dewar, et al 2011; Evans, *et al.* 2012; 2014; Takahashi, *et al.*, 2003), all corresponding to the temperate regions characterized in here, for most part maintain a regional association to the release area with no records of trans-Pacific or trans-equatorial crossings (**Figure 13**). As Kasapidis *et al.* (2008) correctly have pointed out, the interpretation of swordfish stock structure using genetic data is not always straightforward. Accordingly, when significant genetic differences between samples are found, and these differences are assumed to result from restricted gene flow and genetic drift rather than local selection, then population differentiation, and thus the allocation to separate stocks, can be inferred. Thus, identifying sample heterogeneity allows more powerful conclusions concerning stock structure, than failing to reject the null hypothesis of no differentiation (Ward, 2000). Moreover, a small amount of gene flow (few migrants per generation) is often sufficient to prevent detectable genetic differentiation between populations (Hartl and Clark, 1997), while in fisheries management, an exchange of as high as 10% between populations may justify their treatment as separate stocks (Kasapidis *et al.*

2008). As such, the discrepancy in gene flow between harvest stock and genetic stocks remains, even when using highly sensitive markers, and genetic information alone may not suffice to identify stocks with small degree of isolation (Hauser and Ward, 1998).

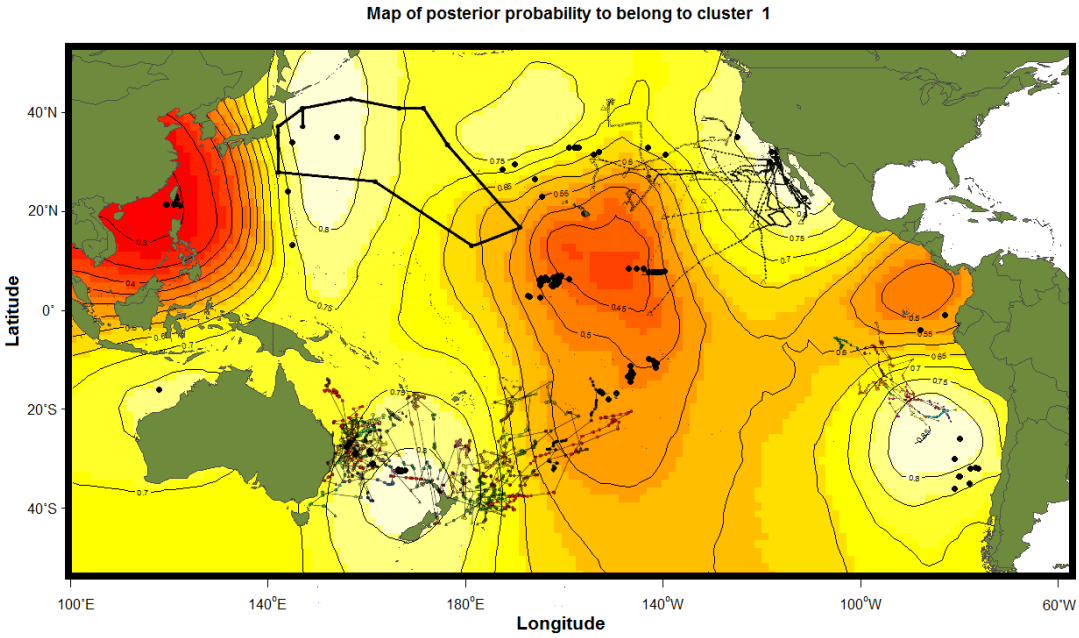


Figure 13. Pacific swordfish PSAT tracks superimposed on the GENELAND map depicting posterior probability membership to Cluster 1. The estimated horizontal tracks come from several studies (Abascal *et al.* 2010; Abecassis, *et al.* 2012; Dewar, et al 2011; Evans, *et al.* 2012; 2014; Takahashi, *et al.*, 2003). Membership to Cluster 2 is corresponds to the reciprocal probability.

Superimposing the tracks of individual PSAT with the patterns obtained with GENELAND reveal patterns that are largely congruent with the differentiation between tropical and temperate areas (although not congruent with the no-difference among temperate regions), since for most part swordfish tagged in temperate regions (Cluster 1)

fail to move into the areas where there is a high probability of belonging to Cluster 2 (Figure 13). Instead, in addition to localized regional displacements, PSAT tags indicate movements into areas where the assignment probability to either cluster is equivocal (≈50%), and thus potential admixture zones. Unfortunately, sampling in these regions was not conducted in this study; as such sampling would be useful to test potential areas of admixture as Wahlund effect would be expected. The concordance between PSAT and GENELAND lends itself to a possible alternative hypothesis, where Pacific swordfish adheres a more complex model of population structure. This would imply that the temperate cluster consists of four separate isolated sub-populations that move towards the tropics to reproduce, but which retain their distinctiveness from tropical populations due to timing and or by avoiding the areas where these populations inhabit. In most instances, PSAT tracks (Figure 13) appear to be consistent with diagonal movements from the four corresponding corners of the Pacific (i.e., NWPO, NEPO, SEPO and SWPO) diagonally towards tropical areas, reaching posterior probability assignment lines to either cluster of about 0.50, thus consistent with admixture zones. This interpretation is also consistent with the mixed signal obtained for most tropical samples with STRUCTURE (**Figure 9C**). The homogeneity among temperate areas detected with Bayesian spatial analyses could be facilitated by gene flow occurring in temperate areas, congruent to some extent with the IBD model suggested by Reeb *et al.* (2000), and with also with the hypothesis of separate stocks in the north and southwest Pacific Ocean (Sakagawa and Bell 1980). Alternatively, the allopatric populations with temperate (e.g. Chile and Japan) and tropical (e.g., Ecuador and Taiwan) signals, may

not have had sufficient time to drift apart due to the large effective population size of each individual deme, as suggested for other highly migratory fishes whose allopatric populations lack clear signals of genetic differentiation (Ely *et al.* 2005). For instance, in Chilean bonito (*Sarda chiliensis*) the northern and the southern populations are allopatric, to the extent that they have been described as subspecies, *S. chiliensis lineolata* and *S. chiliensis chiliensis*, respectively. These two populations show no evidence of genetic differentiation with mtDNA CR and nDNA data (Viñas *et al.* 2010). Although the SNPs characterized here reside in the introns, potential selection cannot be discounted (Gazave *et al.* 2007; Nott *et al.* 2003). However, selection appears not to be operating in any of the 10 markers employed given that none of the replicate runs conducted to identify markers under selection were significant (**Figure 14**). Accordingly, neither selection, nor sex-linkage (see methods), appears to account for the differences between tropical and temperate samples of Pacific swordfish reported here.

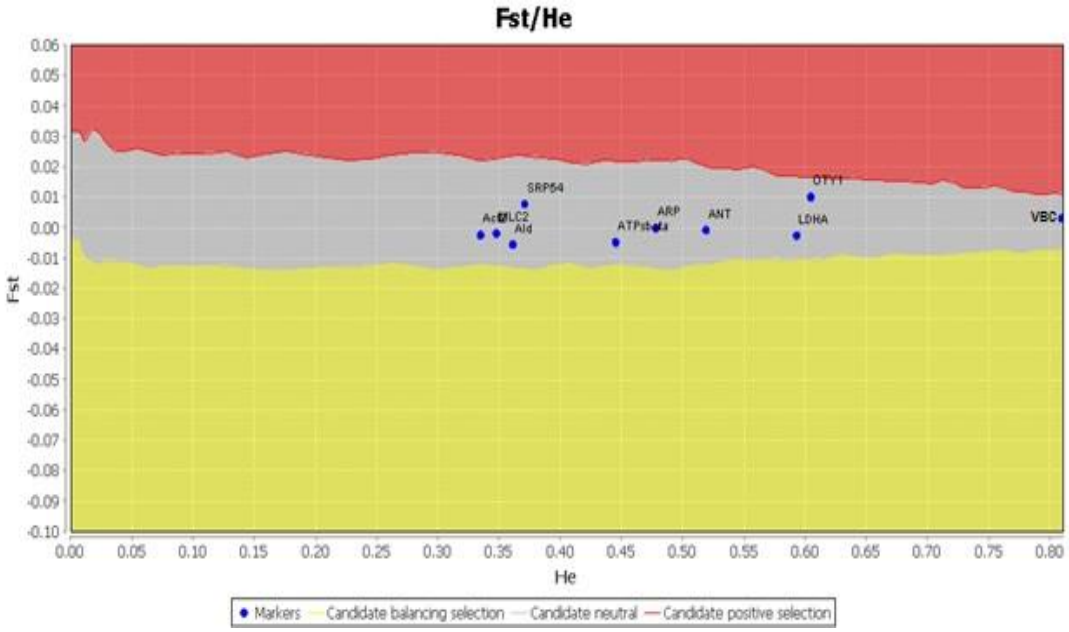


Figure 14. Identification of candidate loci under selection inferred from F_{ST} outlier analysis (Antao *et al.* 2008; Beaumont and Nichols, 1996). Analysis was conducted on all 10 nuclear markers with all the specimens from 16 sampling localities.

Contrasting patterns of intra-oceanic differentiation of Pacific swordfish against other ocean basins

Swordfish is widespread in the tropical and temperate waters of the world (Nakamura, 1985), and genetic studies have revealed both inter- and intra-oceanic differentiation, although both the degree and the patterns of intra-oceanic differentiation differ among basins. Within the Atlantic, swordfish is genetically differentiated into North and South populations (Alvarado-Bremer *et al.* 1996; Alvarado-Bremer *et al.* 2005; Chow and Takeyama 2000) separated by a sharp change in allele frequencies around 25°N with a boundary extending towards 50°W and then south towards the northern coast of Brazil (Smith 2012; Smith *et al.* under review). Areas of admixture

between North and South Atlantic swordfish populations are primarily confined to the northeast Atlantic. By contrast, the pattern of differentiation within the Mediterranean Sea conforms more closely to IBD (Viñas *et al.* 2010). Within the Indian Ocean, the pattern of genetic population structure of swordfish has not been resolved, with several studies assessing variability of mitochondrial and nuclear markers reaching different conclusions, with some reporting heterogeneity, and other failing to identify differences (Bradman *et al.* 2011; Jean *et al.* 2006; Lu *et al.* 2006; Muths *et al.* 2009 and 2013). Similarly, there is no consensus regarding the population structure in Pacific swordfish, with conflicting results obtained with different samples and kinds of markers. This study was intended to resolve those limitations by expanding sample coverage, larger sample sizes, multiple gene markers, and in addition, attempted to improve the resolution by characterizing early life stage individuals with a multilocus analysis based on SNP data. The results of multiple tests reject the hypothesis of panmixia for Pacific swordfish, and suggest a more complex genetic pattern of differentiation compared to the Atlantic and Mediterranean swordfish, with the possibility of a tropical versus temperate pattern of differentiation.

Contrasting Pacific swordfish population differentiation with other pelagic fishes

Genetic population structure studies conducted on other highly migratory species subject to commercial and recreational fisheries have revealed genetic heterogeneity within the Pacific Ocean. For instance, analyses of multilocus microsatellite genotypes and mtDNA CR sequences of the striped marlin (*Kajikia audax*) showed significant

spatial genetic heterogeneity across all samples for microsatellite markers (F_{ST} = 0.013, P < 0.001) (McDowell and Graves 2008). The principal component analysis (PCA) of microsatellite data reveals a very complex pattern of differentiation, with no differences among the North Pacific samples of Japan (multiple years), Taiwan, Hawaii and California, which are different respectively from Mexico, Ecuador and Australia (multiple years), which in turn, are significantly different from each other (but not among the Australian samples). This implies that gene flow maintains genetic homogeneity of striped marlin across the North Pacific (>10,000 Km) with limited latitudinal displacements (McDowell and Graves, 2008; Purcell and Evans 2011), resulting in heterogeneity with respect to the samples of Mexico and Ecuador, but that differences between these two tropical samples, similar to those reported here for tropical swordfish samples (e.g., HawLa versus CenSJu), exist in spite of the shorter geographic distances (≈4,000 Km) that separate these localities. Similarly, genetic heterogeneity has been documented in sailfish (*Istiophorus platypterus*) using microsatellite and mtDNA CR data (Graves and McDowell 1998; McDowell 2002; Lu *et al.* submitted). However, the pattern of differentiation in sailfish appears to be more pronounced than that of Pacific swordfish or striped marlin, consistent with the closer association of this species to coastal environments (Nakamura 1985).

McDowell (2002) and McDowell and Graves (2008) have proposed a basin size hypothesis to account for the absence of genetic differentiation of istiophorid billfish in the Atlantic, and the genetic heterogeneity present in the Pacific Ocean. Accordingly the larger size of the Pacific basin promotes differentiation, whereas in smaller basins, such

as the Atlantic and Indian Ocean, gene flow would be facilitated by the shorter distances in light of the high migratory capabilities and larval dispersal potential. However, the patterns of differentiation that characterize swordfish in different basins do not support the basin size hypothesis. Compared to the Pacific, swordfish displays a much stronger signal of differentiation both within the Atlantic and within the Mediterranean, as well as in the areas of contact shared by Mediterranean and North Atlantic populations to the west of Gibraltar (Smith 2012; Viñas *et al.* 2010; this study and references herein). Instead, these results indicate that migratory potential in swordfish does not translate into gene flow even at reduced spatial scales.

Conclusion and future research

In conclusion, it is clear that a genetic differentiation pattern exists within the Pacific Ocean as revealed by the analysis of SNPs of nuclear loci scored with HRMA. Among all the samples characterized in this study, Taiwan displays the strongest signal of genetic differentiation among all the samples compared. Several samples found in tropical areas were different from samples from temperate areas. Further, samples from tropical areas displayed a certain level of heterogeneity with respect to other tropical samples, whereas samples from temperate regions were homogeneous. However, the findings of this study were not strong enough to identify boundaries separating Pacific swordfish populations. Based on regional differences in biological parameters, including patterns of reproductive biology of adults, larval distribution, and results from tagging experiments, the model of population structure in Pacific swordfish might be

more complex than the simple model of temperate and tropical differentiation. What makes this current study unique is the inclusion of ELS specimens (larvae and young-of-the-year (YOY) longline caught juveniles). The results of this study seem to strongly indicate that the temperate areas (which correspond to feeding grounds and not spawning areas) contain admixtures of swordfish from more than one distinct natal site. Future focus should on obtaining latitudinal sampling of larvae/YOY juveniles within the western and central Pacific in order to test followed hypotheses: 1) Larvae/YOY juveniles from northern and southern sub-tropical (seasonal spawning) sites do not differ genetically from one another and from tropical (year-round spawning) sites along a given longitude within the western and central Pacific. 2.) Larvae/YOY from subtropical and tropical sites in the western Pacific does not differ from subtropical and tropical larvae/YOY in the central Pacific. Finally, future studies should take advantage of double digest RAD sequencing technologies to be able to characterize a much larger number of SNPs for improving the resolution of population structure in Pacific swordfish.

CHAPTER V

GENERAL CONCLUSIONS

This research focused on three lines of work aimed to improve management practices of the Pacific billfish and swordfish using molecular methods (DNA-based): 1) Two HRM-based assays were developed to identify four billfish species in the Pacific Ocean. 2) Potential gender-linked genetic markers for determining the gender in swordfish, blue marlin, and sailfish were screened and surveyed the. 3) Informative SNPs contained in multiple nuclear loci for analyzing population structure of swordfish in the Pacific Ocean using high resolution melting analysis (HRMA) were characterized. The general conclusions and summaries of this study are as followed.

Genetic species identification of Pacific istiophorid billfishes using HRMA

When diagnostic morphological characters are not available or damaged, genetic assays can provide unambiguous species identification to aid in management practices. The aim of this study was to develop fast closed-tube genetic assays based on high resolution melting analysis (HRMA) to identify Pacific billfishes. In Chapter II, two newly designed molecular assays using HRMA were applied successfully to distinguish the four billfish species including black marlin (*Istiompax indica*), blue marlin (*Makaira nigricans*), striped marlin (*Kajikia audax*) and sailfish (*Istiophorus platypterus*) in the Pacific Ocean. These two HRM-based assays were designed based on the SNPs of the mtDNA ND2 gene identified by conducting multiple alignments of billfish sequences. 1)

SA-HRMA: The melting profiles of SA-HRMA assay with a 491 bp fragment were diagnostic for identifying four Pacific billfish species, either by their unique T_m or the shape of the melting curves, or both. The amplicon melts for the four species range from 83.0˚C to 88.0˚C. Each species displayed a very distinct and diagnostic melting curve to identify different species. The melting curve of both sailfish and black marlin were markedly unimodal and leptokurtic shape with melts peaking at 86.0˚C and 86.5˚C, respectively. Conversely, the melting curves of striped marlin and blue marlin differed by one degree, 86.0˚C and 87.0˚C, respectively, but their curves were broader lacking the well-defined peaks displayed by sailfish and black marlin melts. 2) UP-HRMA: This assay employed the variation within a 410 bp fragment using an unlabeled probe (48 nt) for Pacific billfish identification. A total of eight SNPs were identified along the probed segment among the representative haplotypes of the four species of Pacific billfish targeted in this study. The probe matched sailfish ND2 sequence and as expected, the T_m for this species was the highest (78˚C). The range of melting temperatures against the unlabeled probe ranged from 68˚C to 78˚C, with each species revealing distinguish melting temperatures. Striped marlin differs by four SNPs against the sailfish-specific probe equivalent to a 5.5˚C (Tm=72.5 ˚C) difference in Tm. Black marlin and blue marlin both differ by five SNPs from the sailfish-specific probe, but their corresponding melting temperatures are 70˚C and 68˚C, respectively. All four Pacific billfish species can be easily distinguished by UP-HRMA by their respective melting peaks relative to the probed segment. The repeatability and sensitivity test of the two HRMA assay were conducted and validated the individuals that had tentatively been identified as black

marlin generated two very distinct curves that matched those of blue marlin and black marlin, respectively.

Both HRM-based assays developed in this study can be used to identify billfishes from different types of samples including early life stages with similarly shaped, specimens missing diagnostic morphological characters (e.g. dressed fish, or processed products), when high throughput is needed, and when unequivocal species identification (e.g., gender determination) is required.

A survey of potential sex-linked markers in swordfish and istiophorid billfishes

The mechanism of gender determination in fishes is complex, including among others, chromosomal-based (XY and WZ) systems, multiple gene-interactions, environmental factors, and social interactions. Because the manner by which gender is determined in billfish is unknown, in Chapter III, we utilized two types of PCR-based approaches to survey the potential loci linked to gender-determination in swordfish, blue marlin and sailfish. The first involved RAPDs and the second screening of sex-linked genetic markers known to operate in other teleosts. We found that RAPDs experiments were highly sensitive to buffer formulations and selection of *Taq* DNA polymerase. After optimization of RAPDs, a total 100 RAPD assays (10-mer primers for each assay) were conducted for each species. Approximately 13, 12, and 26 percent of the RAPD assays failed to amplify in swordfish, blue marlin, and sailfish, respectively. In swordfish, only one (OPC-10) RAPD appears to generate a banding patterns linked to gender determination, suggesting a ZW sex chromosomal system, but suffienent

evidence is lacking to reach definite conclusion. In blue marlin, one out of six potential sex-linked bands was tested significant (χ^2= 6.74, p=0.009**) suggested that the extra band generated in OPC-13 RAPD in males is sex-linked. Potential gender-related patterns were generated for blue marlin suggesting a XY chromosomal system of gender determination, and in sailfish, the results of polymorphic patterns showed that there were certain bands in females but not in males, suggests a ZW system of gender determination in sailfish. However, these assays need further validated with larger sample sizes.

The second approach targets sex-linked genes that operate in other fishes using both direct sequencing and HRMA to genotype the amplified DNA fragments of swordfish, blue marlin, and sailfish. Not all of the PCR amplifications generated diagnostic characters to be potential markers using a total 12 potential sex-linked markers. A total of 48 primer-sets were employed and generated two kinds of PCR amplification products (multiple bands and single bands) from different loci in the three species. In swordfish, a total of six loci including AMH, ARP, *Znf*, DMRT1, DMRT1 exon3, and OtY1 were sequenced. We designed newly HRMA primers to conduct the experiments to test whether the SNPs in AMH, ARP, and OtY1 were linked to gender determination. Although no gender-linked variants were found in these loci, the SNPs in ARP and OTY1 were deemed useful for population structure studies of Atlantic and Pacific swordfish (Smith *et al.* 2010; 2013; Smith 2012; see Chapter IV.).

In blue marlin, none of the SNPs found in AMH, ARP, and *Znf* were linked to gender. In sailfish, the sequences of these three loci were highly conserved and none of the few SNPs discovered was linked to gender. Similar to swordfish, these SNPs

characterized might be suitable for population structure studies of blue marlin and sailfish, although acertainment bias may be required first.

Genetic differentiation and population structure in Pacific swordfish

The population structure of Pacific swordfish incldues several working hypotheses consisting of two, three, or four stocks based on genetic and fisheries statistics data. Genetic analyses of 20 SNPs using ten nuclear loci in 891 specimens from 16 sampling localities from temperate and tropical regions in the Pacific Ocean that incldued pairwise F_{ST} values, AMOVA tests, PCoA, and Bayesian individual assignments using STRUCTURE and GENELAND suggested that Pacific swordfish is not a single population. Similar pattern of differentiation were obtained with STRUCTURE and GENELAND suggesting a differentiation between temperate and tropical localities. The tropical sample from Taiwan emerges as the most highly differentiated of all the Pacific swordfish samples characterized in this study, displaying significant differentiation from most of the temperate samples and from some tropical samples. Samples from tropical areas displayed a certain level of heterogeneity with respect to other tropical samples, whereas samples from temperate regions were homogeneous. None of the alternative AMOVAs testing fishery-based models were significant. Based on the AMOVA results, we suggested that the boundaries separating the fishery-based model may need to be reexamined.

The patterns of differentiation revealed by characterizing ELS samples were compared with evidence of connectivity obtained with other non-genetic approaches.

The results of this study seem to strongly indicate that the temperate areas (which correspond to feeding grounds and not spawning areas) contain admixtures of swordfish from more than one distinct natal site. For greater resolution, future studies of early life stage samples should include larger sample sizes and more complete coverage of reproductive areas within the Pacific Ocean and the use of next generation sequence techquine to characterize thoundsends of SNPs.

REFERENCES

Abascal, F.J.; Mejuto, J.; Quintans, M.; Ramos-Cartelle, A. 2010. Horizontal and vertical movements of swordfish in the Southeast Pacific. *ICES Journal of Marine Science* 67:466-474.

Abecassis, M.; Dewar, H.; Hawn, D. Polovina, J. 2012. Modeling swordfish daytime vertical habitat in the North Pacific Ocean from pop-up archival tags. *Marine Ecology Progress Series* 452:219-236.

Alfaqih, M.A.; Brunelli, J.P.; Drew, R.E.; Thorgaard, G.H. 2009. Mapping of five candidate sex-determining loci in rainbow trout (*Oncorhynchus mykiss*). *BMC Genetics* 10:2.

Ali, B.; Huang, T.-H.; Qin, D.-N.; Wang, X.-M. 2004. A review of random amplified polymorphic DNA (RAPD) markers in fish research. *Reviews in Fish Biology and Fisheries* 14:443-453.

Alvarado-Bremer, J.R. 1994. Assessment of morphological and genetic variation of the swordfish (*Xiphias gladius* Linnaeus): evolutionary patterns of nucleotide substitution in the mitochondrial genome. *Ph. D. Thesis, Graduate Department of Zoology, University of Toronto, Toronto, Ontario, Canada.*

Alvarado-Bremer, J.R.; Mejuto, J.; Greig, T.W.; Ely, B. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology* 197:295-310.

Alvarado-Bremer, J.R.; Viñas, J.; Mejuto, J.; Ely, B.; Pla, C. 2005. Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Molecular Phylogenetics and Evolution* 36:169-187.

Alvarado-Bremer, J.R.; Hinton, M.G.; Greig, T.W. 2006. Evidence of spatial genetic heterogeneity in Pacific swordfish (*Xiphias gladius*) revealed by the analysis Of *ldh*-A sequences. *Bulletin of Marine Science* 79:493-503.

Alvarado-Bremer, J.R.; Smith, B.L.; Moulton, D.L.; Lu, C-P.; Cornic, M. 2014. Shake and Stew: A non-destructive PCR-Ready DNA isolation method from a single preserved fish larva. *Journal of Fish Biology* 84:267-272

Antao, T.; Lopes, A.; Lopes, R.; Beja-Pereira, A.; Luikart, G. 2008. LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics* 9:323-327.

Artoni, R.F.; Bertollo, LAC. 2002. Evolutionary aspects of the ZZ/ZW sex chromosome system in the Characidae fish, genus *Triportheus.* A monophyletic state and NOR location on the W chromosome. *Heredity* 89:15-19.

Atarhouch, T.; Rami, M.; Cattaneo-Berrebi, G.; Ibanez, C.; Augros, S.; *et al.* 2003. Primers for EPIC amplification of intron sequences for fish and other vertebrate population Genetic Studies. *BioTechniques* 35:676-682.

Bagley, M.; Anderson, S.; May, B. 2001. Choice of methodology for assessing genetic impacts of environmental stressors: polymorphism and reproducibility of RAPD and AFLP fingerprints. *Ecotoxicology* 10:239-244.

Bardakci, F. 2000. The use of random amplified polymorphic DNA (RAPD) markers in sex discrimination in Nile tilapia, *Oreochromis niloticus* (Pisces: Cichlidae). *Turkish Journal of Biology* 24:169-175.

Bartoo, N. W.; Coan, Jr. A. L. 1989. An assessment of the Pacific swordfish resource. *In Stroud, Richard H. (editor), Second International Billfish Symposium (1988), Proc., Part 1: Fishery and Stock Synopses, Data Needs and Management, National Coalition for Marine Conservation, Inc., Savannah, Georgia:*137-151.

Beaumont, M.A.; Nichols, R.A. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* 263:1619-1626.

Benjamini, Y.; Yekutieli, D. 2001. The Control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics* 29:1165-1188.

Berkeley, S. A.; E. D. Houde. 1983. Age determination of broadbill swordfish, *Xiphias gladius*, from the Straits of Florida, using anal fin spine sections. *U.S. Department of Commerce., NOAA Technical Report NMFS* 8:137-143.

Block, B.A.; Finnerty, J.R.; Stewart, A.F.; Kidd, J. 1993. Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science New Series, vol. 260, No. 5105 (Apr. 9, 1993)*:210-214.

Bradman, H.; Grewe, P.; Appleton, B. 2011. Direct comparison of mitochondrial markers for the analysis of swordfish population structure. *Fisheries Research* 109:95-99.

Bréchon, A.L.; Coombs, S.H.; Sims, D.W.; Griffiths, A.M. 2013. Development of a rapid genetic technique for the identification of clupeid larvae in the Western English Channel and investigation of mislabelling in processed fish products. *ICES Journal of Marine Science* 70:399-407

Bruneaux, M.; Johnston, S.E.; Herczeg, G.; Merilä, J.; Primmer, C.R.; *et al.* 2013. Molecular evolutionary and population genomic analysis of the nine-spined stickleback using a modified restriction-site-associated DNA tag approach. *Molecular Ecology* 22:565-582

Caddy, J.F.; Mahon, R. 1995. Reference points for fisheries management: *Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy*.

Carey, F.G. 1982. A brain heater in the swordfish. *Science* 216:1327-1329.

Carey, F.G.; Robinson, B. 1981. Daily patterns in the activities of swordfish, *Xiphias gladius*, observed by acoustic telemetry. *Fishery Bulletin* 79:277-292.

Casas, L.; Sánchez, L.; Orbán, L. 2011. Sex-associated DNA markers from turbot. *Marine Biology Research* 7:378-387.

Cerna, J.F. 2009. Age and growth of the swordfish (*Xiphias gladius* Linnaeus, 1758) in the southeastern Pacific off Chile (2001). *Latin American Journal of Aquatic Research* 37:59-69.

Chapman A.C. 2012. Development of novel high-resolution melting (HRM) assays for gender indentification of Caribbean flamingo (*Phoenicopterus ruber ruber*) and other birds. *Thesis.73pp. Texas A&M University.*

Chen, J.; Wang, Y.; Yue, Y.; Xia, X.; Du, Q.; *et al.* 2009. A novel male-specific DNA sequence in the common carp, *Cyprinus carpio*. *Molecular and Cellular Probes* 23:235-239.

Chiang, W.-C.; Sun, C.-L.; Yeh, S.-Z.; Su, W.-C. 2004. Age and growth of sailfish (*Istiophorus platypterus*) in waters off eastern Taiwan. *Fishery Bulletin* 102:251-263.

Chow, S. 1994. Identification of billfish species using mitochondrial cytochrome b gene fragment amplified by polymerase chain reaction. *SCRS/1992/066 Collect Vol Sci Pap ICCAT* 41:549-556.

Chow, S.; Okamoto, H.; Uozumi, Y.; Takeuchi, Y.; Takeyama, H. 1997. Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Marine Biology* 127:359-367.

Chow, S.; Takeyama, H. 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology* 56:1087-1098.

Cowen, R.K.; Gawarkiewicz, G.; Pineda, J.; Thorrold, S.R.; Werner, F.E. 2007. Population connectivity in marine systems. *Oceanography* 20:14-21.

Cowen, R.K.; Sponaugle, S. 2009. Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science* 1:443-466.

Collette, B.B.; McDowell, J.R.; Graves, J.E. 2006. Phylogeny of recent billfishes (Xiphioidei). *Bulletin of Marine Science* 79:455-468.

DeMartini, E.E.; Uchiyama, J.H.; Williams, H.A. 2000. Sexual maturity, sex ratio, and size composition of swordfish, *Xiphias gladius*, caught by the Hawaii-based pelagic longline fishery. *Fishery Bulletin* 98:489-506.

DeMartini, E.E.; Uchiyama, J.H.; Humphreys Jr, R.L.; Sampaga, J.D.; Williams, H.A. 2007. Age and growth of swordfish (*Xiphias gladius*) caught by the Hawaii-based pelagic longline fishery. *Fishery Bulletin* 105:356-367.

Devlin, R.H., Mcneil, B.k., Solar, I.I., Donaldson, A.M., 1994. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of chinook salmon. *Aquaculture* 128:211-220.

Devlin R. H.; Biagi, C. A.; Smailus, D. E. 2001. Genetic mapping of Y-chromosomal DNA markers in Pacific salmon. *Genetica* 111:43-58.

Devlin R. H.; Nagahama Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208:191-364.

Dewar, H.; Prince, E.D.; Musyl, M.K.; Brill, R.W.; Sepulveda, C.; *et al.* 2011. Movements and behaviors of swordfish in the Atlantic and Pacific Oceans examined using pop-up satellite archival tags. *Fisheries Oceanography* 20:219-241.

DeWoody, .JA.; Avise, J.C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology* 56:461-473.

Dickson, K.A.; Graham, J.B. 2004. Evolution and consequences of endothermy in fishes. *Physiological and Biochemical Zoology* 77:998-1018.

Durán-González, A.L.; Laguarda-Figueras, A. 1992. Cytogenetic characterization of the sailfish *Tetrapturus albidus* Poey, 1860 (Pisces: Istiophoridae) from the Mexican Caribbean Sea. *Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México. Contribución* 19(2):143-150.

Earl, D.; vonHoldt, B. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:359-361.

Ehrhardt, N. M.; R. J. Robbins; F. Arocha. 1996. Age validation and growth of swordfish, *Xiphias gladius*, in the northwest Atlantic. *International Commission for the Conservation of Atlantic Tunas, Collective Volume of Scientific Papers* 45:358-367.

Ely, B.; Vinas, J.; Alvarado-Bremer, J.; Black, D.; Lucas, L.; *et al.* 2005. Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). *BMC Evolutionary Biology* 5:19-27.

Evanno, G.; Regnaut, S.; Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* 14:2611-2620.

Evans, K.; Abascal, F.; Kolody, D.; Sippel, T.; Holdsworth, J.; Maru, P. 2014. The horizontal and vertical dynamics of swordfish in the South Pacific Ocean. *Journal of Experimental Marine Biology and Ecology* 450:55-67.

Evans, K.; Kolody, D.; Abascal, F.; Holdsworth, J.; Maru, P.; Sippel, T. 2012. Spatial dynamics of swordfish in the south Pacific Ocean inferred from tagging data. *Western and Central Pacific Fisheries Commission. Scientific Committee Eighth Regular Session 7-15 August 2012. Busan, Republic of Korea.* WCPFC-SC8-2012/SA-IP-05.

Excoffier, L.; Smouse, P.E.; Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.

Excoffier, L.; Lischer, H.E. L. 2010. Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

Ezaz, T.; Stiglec, R.; Veyrunes, F.; Marshall Graves, J.A. 2006. Relationships between Vertebrate ZW and XY Sex Chromosome Systems. Current Biology 16:R736-R743.

FAO yearbook 2012. Fishery and Aquaculture Statistics 2010. *Statistics and Information Service of the Fisheries and Aquaculture Department. Roma, Italy. FAO*. 78 pp.

Falush, D.; Stephens, M.; Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567-1587.

Falush, D.; Stephens, M.; Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7:574-578.

Fraga, J.; Rodriguez, J.; Fuentes, O.; Fernandez-Calienes, A.; Castex, M. 2005. Optimization of random amplified polymorphic DNA techniques for use in genetic studies of Cuban Triatominae. *Revista do Instituto de Medicina Tropical de São Paulo* 47:295-300.

Galay-Burgos, M.; Llewellyn, L.; Mylonas, C.C.; Canario, A.V.; Zanuy, S.; *et al.* 2004. Analysis of the Sox gene family in the European sea bass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 137:279-284.

Galli, G.L.J.; Shiels, H.A.; Brill, R.W. 2009. Temperature sensitivity of cardiac function in pelagic fishes with different vertical mobilities: yellowfin tuna (*Thunnus albacares*), bigeye tuna (*Thunnus obesus*), mahimahi (*Coryphaena hippurus*), and swordfish (*Xiphias gladius*). *Physiological and Biochemical Zoology* 82:280-290.

Gazave, E.; Marques-Bonet, T.; Fernando, O.; Charlesworth, B.; Navarro, A. 2007. Patterns and rates of intron divergence between humans and chimpanzees. *Genome Biology* 8:R21.

Ghani, N.I.A.; Herczeg, G.; Leinonen, T.; Merilä, J. 2013. Evidence for genetic differentiation in timing of maturation among nine-spined stickleback populations. *Journal of Evolutionary Biology* 26:775-782.

Grall, C.; De Sylva, D.P.; Houde, E.D. 1983. Distribution, relative abundance, and seasonality of swordfish larvae. *Transactions of the American Fisheries Society* 112:235-246.

Graves, J.E.; McDowell, J.R. 1994. Genetic analysis of billfish populations structure. *International Commission for the Conservation of Atlantic Tunas, Collective Volume of Scientific Papers* 41:505-515.

Graves, J.E.; McDowell, J.R. 1998. A genetic perspective on the stock structures of blue marlin and white marlin in the Atlantic Ocean. *International Commission for the Conservation of Atlantic Tunas, Collective Volume of Scientific Papers* 47:329-341.

Greig T.W.; Alvarado-Bremer, J.R.; Ely, B. 1999. Preliminary results from genetic analyses of nuclear markers in swordfish, *Xiphias gladius*, reveals concordance with mitochondrial DNA analyses. *International Commission for the Conservation of Atlantic Tunas, Collective Volume of Scientific Papers* 49:476-482.

Greig, T.W. 2000. Partitioning genetic variation in swordfish (*Xiphias gladius* L.) analysis of sample variance and population structure. *Doctoral Dissertation. University of South Carolina, Columbia.*

Griffiths, R.; Tiwari, B. 1993. The isolation of molecular genetic markers for the identification of sex. *Proceedings of the National Academy of Sciences* 90:8324-8326

Griffiths, R.; Double, M.C., Orr, K.; Dawson, R.J.; 1998. A DNA test to sex most birds. *Molecular Ecology* 7:1071-1075.

Griffiths, R.; Orr, K.J.; Adam, A.; Barber, I. 2000. DNA sex identification in the three-spined stickleback. *Journal of Fish Biology* 57:1331-1334.

Grijalva-Chon, J.M.; Numachi, K.; Sosa-Nishizaki, O.; De la Rosa-Velez, J. 1994. Mitochondrial DNA analysis of North Pacific swordfish *Xiphias gladius* population structure. *Marine Ecology Progress Series* 115:15-19.

Grijalva-Chon, J.M.; De la Rosa-Velez, J.; Sosa-Nishizaki, O. 1996. Allozyme variability in two samples of swordfish, *Xiphias gladius* L., in the North Pacific Ocean. *Fishery Bulletin* 94:589-594.

Gudmundsson, G. 1994. Time-series analysis of catch-at-age observations. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* 43(1):117-126.

Guillot, G.; Mortier, F.; Estoup, A. 2005. Geneland: A computer package for landscape genetics. *Molecular Ecology Notes* 5:712-715.

Gulland, J.A., 1965. Estimation of mortality rates. *Annex to Arctic Fisheries Working Group Report (meeting in Hamburg, January 1965). International Council for the Exploration of the Sea, C.M. 1965, Document 3 (mimeo), Copenhagen.*

Gundry, C.N.; Dobrowolski, S.F.; Martin, Y.R.; Robbins, T.C.; Nay, L.M.; *et al.* 2008. Base-pair neutral homozygotes can be discriminated by calibrated high-resolution melting of small amplicons. *Nucleic Acids Research* 36:3401-3408.

Halm, S., Rocha, A., Miura, T., Prat, F., Zanuy, S., 2007. Anti-Mullerian hormone in the European sea bass: its gene structure, regulatory elements, and the expression of alternatively spliced isoforms. *Gene* 388:148-158.

Hanner, R.; Floyd, R.; Bernard, A.; Collette, B.B.; Shivji, M. 2011. DNA barcoding of billfishes. *Mitochondrial DNA* 22:27-36.

Hartl, D.L.; Clark, A.G. 1997. Principles of population genetics, 3rd edition. *Sinauer Associates Inc., Sunderland, MA.*

Hattori R.S.; Murai Y.; Oura M.; Masuda S.; Majhi S.K; *et al.* 2012. A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination. *Proceedings of the National Academy of Sciences* 109(8):2955-2959.

Hauser L., Ward R.D. 1998. Population identification in pelagic fish: the limits of molecular markers. *In: Carvalho, G.R. (Ed.), Advances in Molecular Ecology, IOS Press*:191-224.

Heino, M.; Baulier, L.; Boukal, D.S.; Ernande, B.; Johnston, F.D.; *et al.* 2013. Can fisheries-induced evolution shift reference points for fisheries management? *ICES Journal of Marine Science* 70:707-721.

Hinton, M.G. 2003. Status of swordfish stocks in the eastern Pacific Ocean estimated using data from Japanese tuna longline fisheries. *Marine and Freshwater Research* 54:393-399.

Hinton, M.G.; Deriso, R.B. 1998. Distribution and stock assessment of swordfish, *Xiphias gladius*, in the eastern Pacific Ocean from catch and effort data standardized on biological and environmental parameters. *US National Marine Fisheries Service, NOAA Techical Report NMFS* 142:161-179.

Holdsworth, J.; Saul, P. 2011. New Zealand Billfish and Gamefish Tagging. *New Zealand Fisheries Assessment Report*: 26.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6:65-70.

Hoolihan, J.P. 2006. Age and growth of Indo-Pacific sailfish, *Istiophorus platypterus*, from the Arabian Gulf. *Fisheries Research* 78:218-226.

Hsieh, H-S.; Chai, T-J.; Hwang, D-F. 2007. Using the PCR-RFLP method to identify the species of different processed products of billfish meats. *Food Control* 18:369-374.

Hubisz, M.J.; Falush, D.; Stephens, M.; Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9:1322-1332.

Humphreys, R.L.; Campana, S.E.; DeMartini, E.E. 2005. Otolith elemental fingerprints of juvenile Pacific swordfish *Xiphias gladius*. *Journal of Fish Biology* 66:1660-1670.

Hyde, J.R.; Lynn, E.; Humphrey Jr., R.; Musyl, M.; West, A.P.; *et al.* 2005. Shipboard identification of fish eggs and larvae by multiplex PCR, and description of

fertilized eggs of blue marlin, shortbill spearfish, and wahoo. *Marine Ecology Progress Series* 286:269-277.

Ichinokawa, M.; Brodziak, J. 2008. Stock boundary between possible swordfish stocks in the northwest and southeast Pacific judged from fisheries data of Japanese longliners. *ISC/08/Special Session on Billfish Stock Structure/04:* 14.

Innes, B.H.; Grewe, P.M.; Ward, R.D. 1998. PCR-based genetic identification of marlin and other billfish. *Marine and Freshwater Research* 49:383-388.

Ito, R.Y.; Coan, A.L. 2005. U. S. swordfish fisheries in the North Pacific Ocean. *Working Document ISC/05/PLENARY/6 Submitted to the Swordfish Working Group for the Fifth Meeting of the Interim Scientific Committee for Tuna and Tuna-Like Species in the North PacificOcean (ISC), March 28–30, 2005, Tokyo, Japan.*

Iturra, P.; Medrano, J.F.; Bagley, M.; Lam, N.; Vergara, N.; *et al.* 1998. Identification of sex chromosome molecular markers using RAPDs and fluorescent in situ hybridization in rainbow trout. *Genetica* 101:209-213.

Jackson, J.B.C.; Kirby, M.X.; Berger, W.H.; Bjorndal, K.A.; Botsford, L.W.; *et al.* 2001. Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science* 293:629-637.

Jakobsson, M.; Rosenberg, N.A. 2007. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801-1806.

Jean, C.; Bourjea, J.; Jouen, E.; Taquet, M. 2006. Stock structure of the swordfish (*Xiphias gladius*) in the Southwest Indian Ocean: a preliminary study. *Bulletin of Marine Science* 79:521-526.

Kasapidis, P.; Magoulas, A.; García-Cortés, B.; Mejuto, J. 2008. Stock structure of swordfish (*Xiphias gladius*) in the Pacific Ocean using microsatellite DNA markers. . *Western and Central Pacific Fisheries Commission. The Scientific Committee Fourth Regular Session 11-22 August 2008 Port Moresby, Papua New Guinea.*WCPFC-SC4-2008/BI-WP-4.

Kasapidis, P.; Pakaki, V.; Kotoulas, G.; Magoulas, A. 2009. Isolation and characterization of 18 new polymorphic microsatellite loci for the swordfish, *Xiphias gladius*. *Molecular Ecology Resources* 9:1383-1386.

Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; *et al.* 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647-1649.

Kikuchi, K.; Hamaguchi, S. 2013. Novel sex-determining genes in fish and sex chromosome evolution. *Developmental Dynamics* 242:339-353.

Kobayashi, D.R.; Polovina, J.J.; Parker, D.M.; Kamezaki, N.; Cheng, I.J.; *et al.* 2008. Pelagic habitat characterization of loggerhead sea turtles, Caretta caretta, in the North Pacific Ocean (1997–2006): Insights from satellite tag tracking and remotely sensed data. *Journal of Experimental Marine Biology and Ecology* 356:96-114.

Kondo, M.; Nanda, I.; Hornung, U.; Schmid, M.; Schartl, M. 2004. Evolutionary Origin of the Medaka Y Chromosome. *Current Biology* 14:1664-1669.

Kopf, K. R.; Drew, K.; Humphreys Jr., R.L. 2010. Age estimation of billfishes (*Kajikia* spp.) using fin spine cross-sections: the need for an international code of practice. *Aquatic Living Resources* 23:13-23.

Kovács, B.; Egedi, S.; Bártfai, R.; Orbán, L. 2001. Male-specific DNA markers from African catfish (*Clarias gariepinus*). *Genetica* 110:267-276.

Liew, M.; Seipp, M.; Durtschi, J.; Margraf, R.L.; Dames, S.; *et al.* 2007. Closed-tube SNP genotyping without labeled probes: A comparison between unlabeled probe and amplicon melting. *American Journal of Clinical Pathology* 127:341-348.

Louro, B.; Passos, A.L.S.; Souche, E.L.; Tsigenopoulos, C.; Beck, A.; *et al.* 2010. Gilthead sea bream (*Sparus auratus*) and European sea bass (*Dicentrarchus labrax*) expressed sequence tags: Characterization, tissue-specific expression and gene markers. *Marine Genomics* 3:179-191.

Lu, C.-P.; Chen, C.A.; Hui, C.-F.; Tzeng, T.-D.; Yeh, S.-Y. 2006. Population genetic structure of the swordfish, *Xiphias gladius* (Linnaeus, 1758), in the Indian Ocean and West Pacific inferred from the complete DNA sequence of the mitochondrial control region. *Zoological Studies* 45:269-279.

Lu, C.-P.; Alvarado-Bremer, J.R.; McKenzie, J.L.; Chiang, W.-C. Contemporary population structure analysis of sailfish (*Istiophorus platypterus*) in the North Pacific Ocean. *Submitted.*

Luikart, G.; J.M. Cornuet 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* 12:228-237.

Mank, J.E.; Promislow, D.E.; Avise, J.C. 2006. Evolution of alternative sex-determining mechanisms in teleost fishes. *Biological Journal of the Linnean Society* 87:83-93.

Matsuda M.; Nagahama Y.; Shinomiya A.; Sato T.; Matsuda C; *et al.* 2002. DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417:559-563.

McDowell, J.R. 2002. Genetic stock structure of the sailfish, *Istiophorus platypterus*, based on nuclear and mitochondrial DNA. *Doctoral Dissertation. Virginia Institute of Marine Science: College of William and Mary.*

McDowell, J.R.; Graves, J.E. 2002. Nuclear and mitochondrial DNA markers for specific identification of istiophorid and xiphiid billfishes. *Fishery Bulletin* 100:537-544.

McDowell, J.R.; Graves, J.E. 2008. Population structure of striped marlin (*Kajikia audax*) in the Pacific Ocean based on analysis of microsatellite and mitochondrial DNA. *Canadian Journal of Fisheries & Aquatic Sciences* 65:1307-1320.

McGlauflin, M.T.; Smith, M.J.; Wang, J.T.; Young, S.F.; Chen, N.; *et al.* 2010. High-resolution melting analysis for the discovery of novel single-nucleotide polymorphisms in rainbow and cutthroat trout for species identification. *Transactions of the American Fisheries Society* 139:676-684.

Mejuto, J.; García-Cortés, B.; Ramos-Cartelle, A. 2008. Reproductive activity of swordfish (*Xiphias gladius*) in the Pacific Ocean on the basis of different macroscopic indicators. *Western and Central Pacific Fisheries Commission. Scientific Committee Fourth Regular Session 11-22 August 2008 Port Moresby, Papua New Guinea.* WCPFC-SC4-2008/BI-WP-6.

Meunier, J. R.; Grimont, P.A.D. 1993. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. *Research in Microbiology* 144:373-379.

Michalakis, Y.; Excoffier, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 142:1061-1064.

Morin, P.A.; Luikart, G.; Wayne, R.K.; the, SNP workshop group 2004. SNPs in ecology, evolution and conservation. Trends in ecology & evolution. 19:208-216

Murphy, G.I. 1965. A solution of the catch equation. *Journal of the Fisheries Research Board of Canada* 22:191-202.

Muths, D.; Grewe, P.; Jean, C.; Bourjea, J. 2009. Genetic population structure of the swordfish (*Xiphias gladius*) in the southwest Indian Ocean: Sex-biased differentiation, congruency between markers and its incidence in a way of stock assessment. *Fisheries Research* 97:263-269.

Muths, D.; Le Couls, S.; Evano, H.; Grewe, P.; Bourjea, J. 2013. Multi-genetic marker approach and spatio-temporal analysis suggest there is a single panmictic

population of swordfish *Xiphias gladius* in the Indian Ocean. *PLoS ONE* 8:e63558.

Myers, R. A.; Mertz, G.; Bridson, J. M. 1997. Spatial scales of interannual recruitment variations of marine, anadromous, and freshwater fish. *Canadian Journal of Fisheries and Aquatic Sciences* 54:1400-1407.

Nakamura, I. 1985. FAO species catalogue. v. 5: Billfishes of the world. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date: *Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy*.

Nakano, H. 1998. Stock status of Pacific swordfish, *Xiphias gladius*, inferred from CPUE of the Japanese longline fleet standardized using general linear models. *US National Marine Fisheries Service, NOAA Techical Report NMFS* 142:195-209.

Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., *et al.* 2002. A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proceedings of the National Academy of Sciences* 99:11778-11783.

Naruse K.; Tanaka, M.; Mita, K.; Shima, A.; Postlethwait, J.; Mitani, H. 2004. A medaka gene map: The trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. *Genome Research* 14:820-828.

Narum, S.R. 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conservation Genetics* 7:783-787.

Nishikawa, Y.; Honma, M.; Ueyanagi, S.; Kikawa, S. 1985. Average distribution of larvae of oceanic species of Scombroid fishes, 1956-1981. *Far Seas Fisheries Research Laboratory, Japan.*

Nott, A.; Meislin S.H.; Moore, M.J. 2003. A quantitative analysis of intron effects on mammalian gene expression. *RNA* 9:607-617.

O'Reilly, P.T.; Canino, M.F.; Bailey, K.M.; Bentzen, P. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology* 13:1799-1814.

Ortega-García, S.; Klett-Traulsen, A.; Rodríguez-Sánchez, R. 2006. Some biological aspects of blue marlin (*Makaira nigricans*) in the recreational fishery at Cabo San Lucas, Baja California Sur, Mexico. *Bulletin of Marine Science* 79:739-746.

Ortiz, M.; Prince, E.D.; Serafy, J.E.; Holts, D.B.; Davy, K.B.; *et al.* 2003. Global overview of the major constituent-based billfish tagging programs and their results since 1954. *Marine and Freshwater Research* 54:489-507.

Ospina-Álvarez, N.; Piferrer, F. 2008. Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. *PLoS ONE* 3:e2837

Palko, B.J.; Beardsley, G.L.; Richards, W.J. 1981. Synopsis of the biology of the swordfish, *Xiphias gladius* Linnaeus. *US National Marine Fisheries Service, NOAA Technical Report. NMFS Circular 441.*

Peakall, R.; Smouse, P.E. GenAlEx 6.5: Genetic analysis in Excel. 2012. Population genetic software for teaching and research—an update. *Bioinformatics* 28:2537-2539.

Peichel, C.L.; Nereng, K.S.; Ohgi, K.A.; Cole, B.L.E.; Colosimo, P.F.; *et al.* 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* 414:901-905.

Peichel, C.L.; Ross, J.A.; Matson, C.K.; Dickson, M.; Grimwood, J.; *et al.* 2004. The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Current Biology* 14:1416-1424.

Penman, D.J.; Piferrer, F. 2008. Fish Gonadogenesis. Part I: Genetic and environmental mechanisms of sex determination. *Reviews in Fisheries Science* 16:16-34.

Penner, G.A.; Bush, A.; Wise, R.; Kim, W.; Domier, L.; *et al.* 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. *Genome Research* 2:341-345.

Piferrer, F.; Guiguen, Y. 2008. Fish Gonadogenesis. Part II: Molecular biology and genomics of sex differentiation. *Reviews in Fisheries Science* 16:35-55.

Poonlaphdecha, S.; Pepey, E.; Huang, S.H.; Canonne, M.; Soler, L.; *et al.* 2011. Elevated amh gene expression in the brain of male tilapia (*Oreochromis niloticus*) during testis differentiation. *Sexual Development* 5:33-47.

Poulson, M.D.; Wittwer, C.T. 2007. Closed-tube genotyping of apolipoprotein E by isolated-probe PCR with multiple unlabeled probes and high-resolution DNA melting analysis. *Biotechniques* 43:87-91.

Pujolar, J.M.; Jacobsen, M.W.; Frydenberg, J.; Als, T.D.; Larsen, P.F.; *et al.* 2013. A resource of genome-wide single-nucleotide polymorphisms generated by RAD tag sequencing in the critically endangered European eel. *Molecular Ecology Resources* 13:706-714.

Purcell, C.M.; Edmands, S.; Moran, P. 2011. Resolving the genetic structure of striped marlin, *Kajikia audax* , in the Pacific Ocean through spatial and temporal sampling of adult and immature fish. *Canadian Journal of Fisheries & Aquatic Sciences* 68:1861-1875.

Pritchard, J.K.; Stephens, M.; Donnelly, P. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetic.* 155:945-959.

Qiu, F.; A. Kitchen, P.; Beerli, M.; Miyamoto, M. 2013. A possible explanation for the population size discrepancy in tuna (genus *Thunnus*) estimated from mitochondrial DNA and microsatellite data. *Molecular Phylogenetics and Evolution* 66 (2):463-468.

Rasmussen, R.S.; Morrissey, M.T. 2008. DNA-based methods for the identification of commercial fish and seafood species. *Comprehensive Reviews in Food Science and Food Safety* 7:280-295.

Reeb, C.A.; Arcangeli, L.; Block, B.A. 2000. Structure and migration corridors in Pacific populations of the swordfish *Xiphius gladius*, as inferred through analyses of mitochondrial DNA. *Marine Biology* 136:1123-1131.

Reed, G.H.; Kent, J.O.; Wittwer, C.T. 2007. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. *Pharmacogenomics* 8:597-608.

Renganayaki, K.; Read, J.C.; Fritz, A.K. 2001. Genetic diversity among Texas bluegrass genotypes (*Poa arachnifera* Torr.) revealed by AFLP and RAPD markers. *Theoretical and Applied Genetics* 102:1037-1045.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.

Rosel, P.E.; Block, B.A. 1996. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. *Marine Biology* 125:11-22.

Rosenberg, A.A.; Restrepo, V.R. 1994. Uncertainty and risk evaluation in stock assessment advice for U.S. marine fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*. 51:2715-2720.

Rousset, F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Rozen, S.; Skaletsky, H. 1999. Primer3 on the WWW for General Users and for Biologist Programmers. in: Misener S., Krawetz S., eds. *Bioinformatics Methods and Protocols: Humana Press.*

Sakagawa, G.T.; Bell, R.R. 1980. Swordfish, *Xiphias gladius*. *U.S. National Marine Fisheries Service, NOAA Techical Memorandum NMFS-SWFC*-5:40-50.

Schnute, J.T.; L.J. Richards 2002. Chapter 6 Surplus Production Models Handbook of Fish Biology and Fisheries. *Volume 2: Fisheries. Blackwell Science Ltd.*

Seeb, J.E.; Pascal, C.E.; Grau, E.D.; Seeb, L.W.; Templin, W.D.; *et al.* 2011. Transcriptome sequencing and high-resolution melt analysis advance single

nucleotide polymorphism discovery in duplicated salmonids. *Molecular Ecology Resources* 11:335-348.

Shepard, S.; Hartmann, J. 1996. Immunologic identification of billfish carcasses. *Molecular Marine Biology and Biotechnology* 5:215-219.

Shivji, M.S.; Magnussen, J.E.; Beerkircher, L.R.; Hinteregger, G.; Lee, D.W.; *et al.* 2006. Validity, identification, and distribution of the roundscale spearfish, *Tetrapturus georgii* (Teleostei: Istiophoridae): morphological and molecular evidence. *Bulletin of Marine Science* 79:483-491.

Skroch, P.; Nienhuis, J. 1995. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. *Theoretical and Applied Genetics* 91:1086-1091.

Slatkin, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. *Evolution* 47:264-279.

Smith, B.L. 2012. Assessment of the contemporary population structure and admixture of Atlantic swordfish (*Xiphias gladius* L.) via mixed stock analysis and Bayesian clustering of multiple nuclear SNPs genotyped through high resolution melting. *Doctoral Dissertation. Texas A&M University.*

Smith, B.L.; Lu, C.-P.; Alvarado-Bremer, J.R. 2010. High-resolution melting analysis (HRMA): a highly sensitive inexpensive genotyping alternative for population studies. *Molecular Ecology Resources* 10:193-196.

Smith, B.L.; Lu, C.-P.; Alvarado-Bremer, J.R. 2013. Methodological streamlining of SNP discovery and genotyping via high-resolution melting analysis (HRMA) in non-model species. *Marine Genomics* 9:39-49.

Smith, B.L.; Lu, C.-P.; García-Cortés, B.; Viñas J.; Yeh, S-Y.; Alvarado-Bremer, J.R. Multilocus Bayesian estimates of intra-oceanic genetic differentiation, connectivity, and admixture in Atlantic swordfish (*Xiphias gladius* L.). S*ubmitted.*

Sosa-Nishizaki, O. 1990. A study on the swordfish *Xiphius gladius* stocks in the Pacific Ocean. *Doctoral Dissertation, Department of Fisheries, Faculty of Agriculture. Tokyo, University of Tokyo: 246 pp. Japan.*

Sosa-Nishizaki, O.; Shimizu, M. 1991. Spatial and temporal CPUE trends and stock unit inferred from them for the Pacific swordfish caught by the Japanese tuna longline fishery. *Bulletin of National Research Institute Far Seas Fisheries* 28:75-89.

Sun, C.-L.; Wang, S.-P.; Yeh, S.-Z. 2002. Age and growth of the swordfish (*Xiphias gladius* L.) in the waters around Taiwan determined from anal-fin rays. *Fishery Bulletin* 100:822-835.

Takahashi, M.; Okamura, H.; Yokawa, K.; Okazaki, M. 2003. Swimming behaviour and migration of a swordfish recorded by an archival tag. *Marine and Freshwater Research* 54:527-534.

Takehana, Y.; Naruse, K.; Hamaguchi, S.; Sakaizumi, M. 2007. Evolution of ZZ/ZW and XX/XY sex-determination systems in the closely related medaka species, *Oryzias hubbsi* and *O. dancena. Chromosoma* 116:463-470.

Thompson, J.D.; Higgins D.G.; Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673-4680.

Tiersch, T.R.; Simco, B.A.; Davis, K.B.; Wachtel, S.S. 1992. Molecular genetics of sex determination in channel catfish: studies on SRY, ZFY, Bkm, and human telomeric repeats. *Biology of Reproduction* 47:185-192.

Vandersteen, J.G., Bayrak-Toydemir, P., Palais, R.A., Wittwer, C.T., 2007. Identifying common genetic variants by high-resolution melting. *Clinical Chemistry* 53:1191-1198.

Viñas, J.; Perez-Serra, A.; Vidal, O.; Alvarado-Bremer, J.R.; Pla, C. 2010. Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region. *ICES Journal of Marine Science* 67: 222-1229.

Volff, J. N.; Kondo, M.; Schartl, M. 2003. Medaka dmY/dmrt1Y is not the universal primary sex-determining gene in fish. *TRENDS in Genetics* 19:196-199.

Volff, J. N. 2005. Genome evolution and biodiversity in teleost fish. *Heredity* 94:280-294.

Vossen, R.H.A.M.; Aten, E.; Roos, A.; den Dunnen, J.T. 2009. High-resolution melting analysis (HRMA)-more than just sequence variant screening. *Human Mutation* 30:860-866.

Waples, R. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* 89:438-450.

Wang, S.-P.; Sun, C.-L.; Yeh, S.-Z. 2003. Sex ratios and sexual maturity of swordfish (*Xiphias gladius* L.) in the waters of Taiwan. *Zoological Studies* 42:529-539.

Wang, S.-P.; Sun, C.-L.; Punt, A.E.; Yeh, S.-Z. 2005. Evaluation of a sex-specific age-structured assessment method for the swordfish, *Xiphias gladius*, in the North Pacific Ocean. *Fisheries Research* 73:79-97.

Wang, S.-P.; Sun, C.-L.; Punt, A.E.; Yeh, S.-Z. 2007. Application of the sex-specific age-structured assessment method for swordfish, *Xiphias gladius*, in the North Pacific Ocean. *Fisheries Research*. 84:282-300.

Ward, R.D. 2000. Genetics in fisheries management. *Hydrobiologia*. 420:191-201.

Ward, R.D.; Reeb, C.A.; Block, B.A. 2001. Population structure of Australian swordfish, *Xiphias gladius*. *Final Report to the Australian Fisheries Management Authority, Canberra*.

Weeden, N.F.; Timmerman, G.M.; Hemmat, M.; Kneen, B.E.; Lodhi, M.A. 1992. Inheritance and reliability of RAPD markers. In: *Proceedings of the Joint Plant Breeding Symposium Series. Applications of RAPD Technology to Plant Breeding.* Crop Science Scociety of America, American Society for Horticultural Science and American Genetic Association. pp. 12–17.

Weir, B.S.; Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.

Wetten, O.F.; Wilson, R.C.; Andersen, Ø. 2012. High-resolution melting analysis of common and recombinant genotypes of the Atlantic cod (*Gadus morhua*) hemoglobin β1 gene in transatlantic populations. *Canadian Journal of Fisheries and Aquatic Sciences* 69:525-531.

Whitlock, R.E.; McAllister, M.K.; Block, B.A. 2012. Estimating fishing and natural mortality rates for Pacific bluefin tuna (*Thunnus orientalis*) using electronic tagging data. *Fisheries Research* 119-120:115-127.

Worm, B.; Barbier, E.B.; Beaumont, N.; Duffy, J.E.; Folke, C.; *et al.* 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* 314:787-790.

Worm, B.; Hilborn, R.; Baum, J.K.; Branch, T.A.; Collie, *et al.* 2009. Rebuilding global fisheries. *Science* 325:578-585.

Young, J.; Drake, A.; Brickhill, M.; Farley, J.; Carter, T. 2003. Reproductive dynamics of broadbill swordfish, *Xiphias gladius*, in the domestic longline fishery off eastern Australia. *Marine and Freshwater Research* 54:315-332.

Young, J.W.; Drake, A.D. 2004. Age and growth of broadbill swordfish (*Xiphias gladius*) from Australian waters. FRDC Protect No 2001/014. *CSIRO Marine Research.* Tasmania, Australia, pp. 13-41.

Yue, G.H.; Ong, D.; Wong, C.C.; Lim, L.C.; Orban, L. 2003. A strain-specific and a sex-associated STS marker for Asian arowana (*Scleropages formosus*, Osteoglossidae). *Aquaculture Research* 34:951-957.

Zhang, J. 2004. Evolution of DMY, a newly emergent male sex-determination gene of medaka fish. *Genetics* 166:1887-1895.

Zhou, L.M., Wang, L., Palais, R., Pryor, R., Wittwer, C.T., 2005. High-resolution DNA melt-ing analysis for simultaneous mutation scanning and genotyping in solution. *Clinical Chemistry* 51:1770-1777.

APPENDIX

Table A1. Results of 100 RAPDs (primer OPA-01 to OPE-20) employed for billfish gender determination.

No.	Primer Name	Primer Sequence	*M.nig*	*I.pla*	*X.gla*		No.	Primer Name	Primer Sequence	*M.nig*	*I.pla*	*X.gla*
1	OPA-01	CAGGCCCTTC	**S**	**S**	N		26	OPB-06	TGCTCTGCCC	N	N	N
2	OPA-02	TGCCGAGCTG	N	N	N		27	OPB-07	GGTGACGCAG	N	N	N
3	OPA-03	AGTCAGCCAC	N	N	N		28	OPB-08	GTCCACACGG	N	N	N
4	OPA-04	AATCGGGCTG	N	X	N		29	OPB-09	TGGGGGACTC	N	**S**	N
5	OPA-05	AGGGGTCTTG	N	N	N		30	OPB-10	CTGCTGGGAC	N	N	X
6	OPA-06	GGTCCCTGAC	X	N	N		31	OPB-11	GTAGACCCGT	X	N	X
7	OPA-07	GAAACGGGTG	N	X	N		32	OPB-12	CCTTGACGCA	X	N	X
8	OPA-08	GTGACGTAGG	N	N	N		33	OPB-13	TTCCCCCGCT	N	N	N
9	OPA-09	GGGTAACGCC	N	N	N		34	OPB-14	TCCGCTCTGG	N	N	N
10	OPA-10	GTGATCGCAG	N	N	N		35	OPB-15	GGAGGGTGTT	N	N	N
11	OPA-11	CAATCGCCGT	**S**	N	N		36	OPB-16	TTTGCCCGGA	X	X	X
12	OPA-12	TCGGCGATAG	N	**S**	N		37	OPB-17	AGGGAACGAG	N	N	N
13	OPA-13	CAGCACCCAC	N	N	N		38	OPB-18	CCACAGCAGT	N	N	N
14	OPA-14	TCTGTGCTGG	N	N	N		39	OPB-19	ACCCCCGAAG	X	N	N
15	OPA-15	TTCCGAACCC	N	N	N		40	OPB-20	GGACCCTTAC	**S**	N	N
16	OPA-16	AGCCAGCGAA	N	**S**	N		41	OPC-01	TTCGAGCCAG	N	N	N
17	OPA-17	GACCGCTTGT	N	X	N		42	OPC-02	GTGAGGCGTC	N	N	N
18	OPA-18	AGGTGACCGT	N	N	N		43	OPC-03	GGGGGTCTTT	N	N	N
19	OPA-19	CAAACGTCGG	N	X	N		44	OPC-04	CCGCATCTAC	N	N	N
20	OPA-20	GTTGCGATCC	N	**S**	N		45	OPC-05	GATGACCGCC	N	X	N
21	OPB-01	GTTTCGCTCC	**S**	N	N		46	OPC-06	GAACGGACTC	N	**S**	N
22	OPB-02	TGATCCCTGG	N	X	N		47	OPC-07	GTCCCGACGA	N	X	N
23	OPB-03	CATCCCCCTG	N	X	N		48	OPC-08	TGGACCGGTG	N	**S**	N
24	OPB-04	GGACTGGAGT	N	N	N		49	OPC-09	CTCACCGTCC	N	X	N
25	OPB-05	TGCGCCCTTC	N	N	N		50	OPC-10	TGTCTGGGTG	N	N	N

Three target species: 1. Blue marlin: *M. nig;* 2. Sailfish: *I. pla;* and 3. Swordfish: *X. gla*

S: Potential Sex-related difference **N** : No polymorphic banding pattern associated gender **X**: No amplification

Table A1. Continued

No.	Primer Name	Primer Sequence	*M.nig*	*I.pla*	*X.gla*		No.	Primer Name	Primer Sequence	*M.nig*	*I.pla*	*X.gla*
51	OPC-11	AAAGCTGCGG	S	S	N		76	OPD-16	AGGGCGTAAG	N	N	N
52	OPC-12	TGTCATCCCC	N	N	X		77	OPD-17	TTTCCCACGG	X	X	X
53	OPC-13	AAGCCTCGTC	S*	N	X		78	OPD-18	GAGAGCCAAC	N	N	N
54	OPC-14	TGCGTGCTTG	N	S	X		79	OPD-19	CTGGGGACTT	N	X	N
55	OPC-15	GACGGATCAG	N	N	X		80	OPD-20	ACCCGGTCAC	N	N	N
56	OPC-16	CACACTCCAG	N	N	X		81	OPE-01	CCCAAGGTCC	N	X	N
57	OPC-17	TTCCCCCCAG	X	X	X		82	OPE-02	GGTGCGGGAA	N	N	N
58	OPC-18	TGAGTGGGTG	N	N	N		83	OPE-03	CCAGATGCAC	N	X	N
59	OPC-19	GTTGCCAGCC	N	N	N		84	OPE-04	GTGACATGCC	N	N	N
60	OPC-20	ACTTCGCCAC	N	N	N		85	OPE-05	TCAGGGAGGT	N	N	N
61	OPD-01	ACCGCGAAGG	N	X	N		86	OPE-06	AAGACCCCTC	N	N	N
62	OPD-02	GGACCCAACC	N	N	N		87	OPE-07	AGATGCAGCC	N	X	N
63	OPD-03	GTCGCCGTCA	N	S	N		88	OPE-08	TCACCACGGT	N	X	N
64	OPD-04	TCTGGTGAGG	N	X	N		89	OPE-09	CTTCACCCGA	X	X	N
65	OPD-05	TGAGCGGACA	N	S	N		90	OPE-10	CACCAGGTGA	X	X	S
66	OPD-06	ACCTGAACGG	N	N	N		91	OPE-11	GAGTCTCAGG	N	N	N
67	OPD-07	TTGGCACGGG	N	N	N		92	OPE-12	TTATCGCCCC	X	X	N
68	OPD-08	GTGTGCCCCA	N	N	N		93	OPE-13	CCCGATTCGG	X	X	X
69	OPD-09	CTCTGGAGAC	N	X	N		94	OPE-14	TGCGGCTGAG	N	N	N
70	OPD-10	GGTCTACACC	N	N	N		95	OPE-15	ACGCACAACC	N	N	N
71	OPD-11	AGCGCCATTG	N	N	S		96	OPE-16	GGTGACTGTG	N	N	N
72	OPD-12	CACCGTATCC	N	N	N		97	OPE-17	CTACTGCCGT	N	X	N
73	OPD-13	GGGGTGACGA	N	N	N		98	OPE-18	GGACTGCAGA	N	N	N
74	OPD-14	CTTCCCCAAG	X	X	X		99	OPE-19	ACGGCGTATG	N	N	N
75	OPD-15	CATCCGTGCT	N	N	N		100	OPE-20	AACGGTGACC	N	N	N

Three target species: 1. Blue marlin: *M. nig;* 2. Sailfish: *I. pla;* and 3. Swordfish: *X. gla*

S: Potential Sex-related difference **N** : No polymorphic banding pattern associated gender **X**: No amplification

Table A2. Sequence details for primers designed by original candidate gene markers and new primers designed for increasing the specificity and reliability of each assay evaluated in this study.

No.	Name	Sequence	Reference
1	IDH-F	5'-GGG ACG AGC AAG ATT TAT TG-3'	Peichel et al 2001
2	IDH-R	5'-TTA TCG TTA GCC AGG AGA TGG-3'	Peichel et al 2001
3	Znf-Ga-F	5'-GAG GAG GAA TTG GAA GAG GC-3'	Peichel et al 2004
4	Znf-Ga-R	5'-GAT CGG TAC CTT AAG GGC G-3'	Peichel et al 2004
5	Znf-Gw-F	5'-CGC TGG AAG TGC CGC ATG TG-3'	Peichel et al 2004
6	Znf-Gw-R	5'-GGA TCT GGA CGA ACT CCA TGC-3'	Peichel et al 2004
7	E-AGG	5'-CGA CTG CGT ACC AAT TCA GG-3'	Griffiths et al 2000
8	M-CAA	5'-GAT GAG TCC TGA GTA ACA A-3'	Griffiths et al 2000
9	AU171840-F	5'-TTC AGC AGT GCC GAC ATC GAG AA-3'	Takehana et al 2007
10	AU171840-R	5'-GTG GCT GCA TCG CTT GTG GCT CC-3'	Takehana et al 2007
11	Green-F	5'-CAA CCC CAT TAT CTA CAT TTT GAT GAA-3'	Takehana et al 2007
12	Green-R	5'-CCA TAA ACA AAC CTC TCC ATT TCA TA-3'	Takehana et al 2007
13	OLb03.10a-F	5'-TAT CCA AAA TGC TTC ATC GTG GGA G -3'	Naruse *et al.*2004
14	OLb03.10a-R	5'-AGC AGC AGA TCC CTG ACT TCA GTC A -3'	Naruse *et al.*2004
15	OLb06.11h-F	5'-CTC CTG AGT CGT TCC ACG AAG ATG C -3'	Naruse *et al.*2004
16	OLb06.11h-R	5'-GTA GGA ACC AAA ACC AGG ACC CGG -3'	Naruse *et al.*2004
17	OLb22.11h-F	5'-ACC AAC TGC GCC CGC TGT GTT CCA A -3'	Naruse *et al.*2004
18	OLb22.11h-R	5'-ATG GGC TTT GGA GGA GCT CTT GGT GCG -3'	Naruse *et al.*2004
19	DMRT1Y-h	5'-TCT GCT GAG CTC CCC GGG-3'	Nanda et al 2002
20	DMRT1Y-i	5'-GCC TCG CAG CTT CTC A-3'	Nanda et al 2002
21	DMRT1(BAC)-k	5'-CAA CTT TGT CCA AAC TCT GA-3'	Nanda et al 2002
22	DMRT1(BAC)-l	5'-AAC TAA TTC ATC CCC ATT CC-3'	Nanda et al 2002
23	DMRT1-GS-m	5'-TCC GGC TCC ACA GCG GTC-3'	Nanda et al 2002
24	DMRT1-GS-n	5'-CAG ACA GAG GGT TGG GGG G-3'	Nanda et al 2002
25	DMRT1Y-GS-a	5'-GGC CGG GTC CCC GGG TG-3'	Nanda et al 2002
26	DMRT1Y-GS-c	5'-CTG GTA CTG CTG GTA GTT GTG-3'	Nanda et al 2002
27	Znf-HRM-reverse	5'-TCA CAA ACG GTG CAC TTG TGA GGT C-3'	New designed
28	B Tubulin-FT-95	5'-GAT CTT CAG ACC CGA CAA CTT T-3'	Greig 2000
29	B Tubulin-FT-96	5'-ACT CTT CTC GGA TTT TGC TGA T-3'	Greig 2000
30	MN32-2F	5'-GTA GCA AGG GGC TGT TGC ATA G-3'	Buonaccorsi *et al.*, 1999
31	MN32-2R	5'-GAG TCA GTG GTT CGG GAT TTT ATC-3'	Buonaccorsi *et al.*, 1999
32	TMO-4C4F	5'-CCT CCG GCC TTC CTA AAA CCT CTC-3	Streelman & Karl 1997
33	TMO-4C4R	5'-CAT CGT GCT CCT GGG TGA CAA AGT-3'	Streelman & Karl 1997
34	ATP6-Universal(A1)	5'-ATG AAC CTA AGC TTC TTC GAC CAA TT-3'	Oliver Haddrath
35	ATP6-Universal(A2)	5'-ATA AAA AGG CTA ATT GTT TCG AT-3'	Oliver Haddrath
36	GH5	5'-AGC CTG GAT GAC AAT GAC TC-3'	Devlin et al 2001
37	GH6	5'-CTA CAG AGT GCA GTT GGC CT -3'	Devlin et al 2001
38	GH28	5'-GTC TGG CTA GGG TAC TCC CA -3'	Devlin et al 2001
39	GH30	5'-TTT CTC TAC GTC TAC ATT CT -3'	Devlin et al 2001
40	OkeGHY-F1	5'-GGC ACA TCA ACA GAT TTC TC -3'	Devlin et al 2001

Table A2. Continued.

No.	Name	Sequence	Reference
41	OkeGHY-R1	5'-GTG TAC AAT TTA AAA CTC CC -3'	Devlin et al 2001
42	OkeGHY-F2	5'-TCA AAC CAG TAC ACA TCA GG -3'	Devlin et al 2001
43	OkeGHY-R2	5'-GCC AAT AGG TGG ACG TTG AC -3'	Devlin et al 2001
44	OTY1-Y1	5'-GAT CTG CTG GCT GGA TTT GG -3'	Devlin et al 1991,1994
45	OTY1-Y2	5'-CCA GCG ATG GTT TGT TTG AG -3'	Devlin et al 1991,1994
46	OTY1-HRM-PROBE	5'-GTT GTA TCT CTG TTT GCC TGG CAG TAC TCA ACA CAC AAG CTC AT/3Phos/ -3'	New designed
47	ZnfS-F	5'-TGA ATC GCC ACC TCT TGG CAG T -3'	Tiersch et al 1992
48	ZnfS-R	5'-TTG TGG TCG CAA TGC AAA CAC T -3'	Tiersch et al 1992
49	ZnfD-F	5'-CCG ACA CCC GTC GGA ACT GAG A -3'	Tiersch et al 1992
50	ZnfD-R	5'-CTC GCA CAT CTC ACA CTT ATG A -3'	Tiersch et al 1992
51	SRYS-F	5'-GGC AAC GTC CAG GAT AGA GTG A -3'	Tiersch et al 1992
52	SRYS-R	5'-CGG CAG CAT CTT CGC CTT CCG A -3'	Tiersch et al 1992
53	SRYL-F	5'-CAG TGT GAA ACG GGA GAA AAC A -3'	Tiersch et al 1992
54	SRYL-R	5'-GTA CAA CCT GTT GTC CAG TTG C -3'	Tiersch et al 1992
55	OTY1-Y1-INT-F	5'-GCT GAG GGT CTG TTA ATC TGA G -3'	New designed
56	OTY1-Y2-INT-R	5'-CTC CAG AGC AAG GAT ATC ACT G -3'	New designed
57	OTY1-HRM-A1-PROBE	5'-GTT GTA TCT TTG TTT GCC TGG CAG TAC TCA ACA CAC AAG CTC AT/3Phos/ -3'	New designed
58	OTY1-HRM-A4-PROBE	5'-GTT GAA TCT CTG TTT GCA TGG CAG TAC TCA ACA CAC AAG TTC AT/3Phos/ -3'	New designed
59	SoxN-F	5'-ATG AAY GCN TTY ATG GTN TGG -3'	Galay-Burgos et al 2004
60	SoxN-R	5'-GGN CGR TAY TTR TAR TCN GG -3'	Galay-Burgos et al 2004
61	Sox9-F	5'-ATG AAY GCS TTY ATG GTI TGG -3'	Galay-Burgos et al 2004
62	Sox-R	5'-GTC IGG GTG RTC YTT CTT RTG YTG -3'	Galay-Burgos et al 2004
63	Ga2-R	5'-ACA GAC GCT GAA TGA CGA AG -3'	Griffith et al 2000
64	Ga2-F	5'-CAC ATT ATT ACA ACA TAC GGA CA -3'	Griffith et al 2000
65	Ga1-R	5'-AGA TGA CGG GTT GAT AAA CAG -3'	Griffith et al 2000
66	Ga1-F	5'-CTT CTT TCC TCT CAC CAT ACT CA -3'	Griffith et al 2000
67	DMRT1-EXON4-Rev	5'-GTA CTG GGA GCT CAT -3'	New designed
68	DMRT1-EXON3-Fwd	5'-GAC TCC ACC TAC TAC AGC A -3'	New designed
69	Dmrt1-Rev	5'-CAA CCT CCT GAC TGG ACA G -3'	Alfaqih et al 2009
70	Dmrt1-Fwd	5'-AGG AAC CAC GGC TAC GTG T -3'	Alfaqih et al 2009
71	Dax-1-Rev2	5'-GTT GTT GCC TTA GCT CAA GC -3'	Alfaqih et al 2009
72	Dax-1-Fwd2	5'-ACC TAC AGC ACC GAA TAT CAC -3'	Alfaqih et al 2009
73	Dax-1-Rev	5'-AGG ATC CGT TGC AAC ATG C -3'	Alfaqih et al 2009
74	Dax-1-Fwd	5'-CTC CGG TCA CCG CAG GTT AC -3'	Alfaqih et al 2009
75	Amh-Rev	5'-TCG GTA CTG CGT CTC ACT G-3'	Alfaqih et al 2009
76	Amh-Fwd	5'-CAT CAC TTT CAC CAG TCA CTC -3'	Alfaqih et al 2009
77	Sox6-Rev	5'-AAC AGC GCT GTG GAG TTC AG -3'	Alfaqih et al 2009
78	Sox6-Fwd	5'-TTC ACA GGC AGC AAG ACC AG -3'	Alfaqih et al 2009
79	DMRT1-EXON4-R-full	5'-CAT CCG GTA CTG GGA GCT CAT -3'	New designed
80	DMRT1-EXON4-R-dc	5'-CAT CCG GTA CCT GGG AGC TCA T -3'	New designed

Table A2. Continued.

No.	Name	Sequence	Reference
81	GCR-F	5’-CTA CAG CAC CAG CAA CAT CAG -3’	New designed
82	GCR-R	5’-GAG CTC ATG CTG CTC TGG AGA C-3’	New designed
83	GCR short-F	5’-TCT GCT CTC CAG CGT GAT GA -3’	New designed
84	GCR short-R	5’-AGT ACG GCT GTG AAT GAA CGA G -3’	New designed
85	AmhExon3_F1	5’-CGT TGA CYT TTG ACC TC-3’	New, Hattori *et al.* and Takashi et al 2012,
86	AmhExon3_F2	5’- CTC AAG CCG AAC CCT GTG CTG CTC-3’	New, Hattori *et al.* and Takashi et al 2012,
87	AmhExon4_F2	5’-TGC TGA CAG GAA AAK CAT-3’	New, Hattori *et al.* and Takashi et al 2012,
88	AmhExon5_F1	5’-AAA TCA GGA AGT AAC ATC-3’	New, Hattori *et al.* and Takashi et al 2012,
89	AmhExon5_F2	5’-CCA CTT CTR CTT TTC TC-3’	New, Hattori *et al.* and Takashi et al 2012,
90	AmhExon6_F3	5’-TAC AGT CYC TGC CTC CCC T-3’	New, Hattori *et al.* and Takashi et al 2012,
91	AmhExon3_R2	5'- YAR GAG CAG CAC AGG GTT-3'	New, Hattori *et al.* and Takashi et al 2012,
92	AmhExon4_R2	5'- ATG MTT TTC CTG TCA GCA -3'	New, Hattori *et al.* and Takashi et al 2012,
93	AmhExon5_R1	5'- GAT GTT ACT TCC TGA TTT -3'	New, Hattori *et al.* and Takashi et al 2012,
94	AmhExon5_R2	5'- GAG AAA AGY AGA AGT GG -3'	New, Hattori *et al.* and Takashi et al 2012,
95	AmhExon6_R3	5'- AGG GGA GGC AGR GAC TGT A -3'	New, Hattori *et al.* and Takashi et al 2012,
96	AmhExon6_R6	5'- AGT TCT TTG AGC CTC CCC AG -3'	New, Hattori *et al.* and Takashi et al 2012,
97	AmhExon7_R3	5’-ATG TRG GAG TTG AGC AGG A-3’	New, Hattori *et al.* and Takashi et al 2012,
98	Ipla_426_HRM_F	5'-CAG ATA CAA GTA AGA TCC-3'	New designed
99	Ipla_499_HRM_R	5'-GCT CTG TGC TAC AAT GAG-3'	New designed
100	XglaAmh371F	5'-ATG TCT CAG GTT CAT CCC CG-3'	New designed
101	XglaAmh390F	5'-GGC CTC TTC ACA CAC CTT CT-3'	New designed
102	XglaAmh450R	5'-CAG AAC GTC ACC CAG GAA CC-3'	New designed
103	XglaAmh457R	5'-TTC CTG GCA GAA CGT CAC C-3'	New designed

Table A3. Gender-linked markers PCR results of three targeted species.

Sex-linked marker		No. of primer set	Blue marlin		Sailfish		Swordfish	
			Amp	Seq	Amp	Seq	Amp	Seq
1.	AMH	11	✓(sb,mb)	✓	✓(sb,mb)	✓	✓(sb,mb)	✓
2.	ARP	1	✓ (sb)	✓	✓ (sb)	✓	✓ (sb)	✓
3.	Dax-1	2	✓ (mb)		✓ (mb)		✓ (mb)	
4.	Autosomal DMRT1	6	✓ (mb)		✓ (mb)		✓ (sb)	✓
5.	DMY	2	✓ (sb)	✓	✓ (mb)		✓ (sb)	✓
6.	GH	2	✓ (mb)		✓ (mb)		✓ (mb)	
7.	GH-Y	2	✓ (sb)		✓ (mb)		✓ (sb)	✓
8.	*Idh*	2	✓ (sb)				✓ (mb)	
9.	OtY	2	✓ (mb)				✓ (sb)	✓
10.	*Sox*	5	✓ (mb)		✓ (mb)		✓ (mb)	
11.	*Znf*	6	✓ (sb)	✓	✓ (sb)	✓	✓ (sb)	✓
12.	ESTs:	7	✓ (mb)		✓ (mb)		✓ (mb)	

Sex-linked marker name are abbreviated from Table3. *Amp*: PCR amplification; *Seq*: nucleotide sequences;
✓: performed, sb: single band; mb: multiple bands.

Table A4. AMOVA results of testing alternative hypotheses using gender-validated swordfish specimens from Chile and Australia in the South Pacific Ocean.

Grouping:	Source of Variation	Variance Component	% of variance	Fixation Indices
Geographic Location Difference				
G1:AusFemales & AusMales	AG	0.00925 Va	0.38	F_{CT} : 0.00385^{ns}
	AP/WG	0.00135 Vb	0.06	F_{SC} : 0.00056^{ns}
G2:ChileFemales & ChileMales	AI/WP	0.09516 Vc	3.96	F_{IS} : 0.03973^{ns}
	WI	2.30000 Vd	95.60	F_{IT} : 0.04396^{ns}
Gender Difference				
G1:AusFemales & ChileFemales	AG	0.00228 Va	0.09	F_{CT} : 0.00095^{ns}
	AP/WG	0.00600 Vb	0.25	F_{SC} : 0.00250^{ns}
G2: AusMales & ChileMales	AI/WP	0.09516 Vc	3.96	F_{IS} : 0.03973^{ns}
	WI	2.30000 Vd	95.70	F_{IT} : 0.04303^{ns}
Gender Mixed and Geographic Location Mixed				
G1:AusFemales & ChileMales	AG	-0.00620 Va	-0.26	F_{CT} : -0.00258^{ns}
	AP/WG	0.01161 Vb	0.48	F_{SC} : 0.00482^{ns}
G2: AusMales & ChileFemales	AI/WP	0.09516 Vc	3.96	F_{IS} : 0.03973^{ns}
	WI	2.30000 Vd	95.81	F_{IT} : 0.04189^{ns}

AG: Among groups; AP/WG: Among populations within groups; AI/WP: Among individuals within populations; WI: Within individuals. ns : P > 0.05; * : P<0.05; ** :P<0.01; *** :P<0.001 with Significance tests(10100 permutation)

Table A5. The score of the ten single-copy nuclear loci using 891 individuals characterized in this study.

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla5379	HAW99	2	2	1	3	2	2	1	1	184	198	1	1	2	3	1	1	1	1	256	256
Xgla5381	HAW99	2	2	1	2	1	2	1	1	184	198	1	1	2	2	1	3	1	1	253	256
Xgla5382	HAW99	2	2	1	2	1	2	1	2	198	198	2	2	2	2	1	2	1	1	241	268
Xgla5383	HAW99	1	2	1	2	2	2	1	1	198	198	1	1	2	3	3	3	1	3	259	262
Xgla5384	HAW99	1	2	1	1	1	1	1	2	198	198	1	1	2	2	1	1	1	1	253	256
Xgla5385	HAW99	1	2	1	1	1	2	1	2	198	204	1	1	2	2	1	3	1	5	256	259
Xgla5386	HAW99	2	2	1	1	1	2	1	1	198	198	1	2	2	3	3	3	1	5	256	259
Xgla5387	HAW99	1	1	1	1	2	2	1	2	198	198	1	1	5	5	1	3	1	1	247	256
Xgla5388	HAW99	2	2	1	1	2	2	1	1	184	198	1	1	2	2	3	3	1	4	250	253
Xgla5389	HAW99	1	2	1	1	1	2	1	1	198	198	1	1	2	5	1	3	1	1	250	253
Xgla5390	HAW99	2	2	1	1	1	2	1	1	184	194	1	1	2	4	1	3	1	1	253	253
Xgla5391	HAW99	1	2	1	1	1	2	1	1	198	198	1	2	2	2	2	3	1	1	253	256
Xgla5392	HAW99	2	2	1	1	2	2	1	2	184	198	1	1	2	5	1	3	1	5	244	268
Xgla5393	HAW99	2	2	1	1	1	2	1	2	198	202	1	1	2	4	1	3	1	1	262	262
Xgla5394	HAW99	1	2	1	1	1	2	1	1	198	198	1	1	2	2	2	3	1	1	250	250
Xgla5395	HAW99	2	2	1	1	2	2	1	1	198	198	1	1	2	3	1	3	1	5	256	259
Xgla5396	HAW99	1	2	1	1	1	2	1	1	198	198	1	1	2	5	1	1	1	1	256	262
Xgla5397	HAW99	2	2	1	1	1	2	1	2	198	198	1	2	2	2	1	1	1	1	250	256
Xgla5398	HAW99	1	1	1	2	1	2	1	1	198	198	1	1	2	3	3	3	5	5	259	268
Xgla5401	HAW99	2	2	1	1	1	2	1	2	198	198	1	1	3	5	1	1	1	3	250	253
Xgla5402	HAW99	1	2	1	1	2	2	1	1	198	198	1	1	2	2	1	1	1	1	256	256
Xgla5403	HAW99	1	2	1	1	2	2	1	2	198	198	1	1	3	3	3	3	1	1	250	256
Xgla5404	HAW99	1	2	1	1	2	2	1	1	198	198	1	1	2	2	3	3	1	5	253	265
Xgla5405	HAW99	1	1	1	1	1	2	1	1	198	198	1	2	2	2	1	2	1	5	256	265
Xgla5406	HAW99	1	2	1	1	2	2	1	1	198	198	1	1	3	3	3	3	1	5	244	253
Xgla5407	HAW99	2	2	1	3	2	2	1	2	198	198	1	1	2	2	1	3	1	5	250	256
Xgla5408	HAW99	1	2	1	1	2	2	1	2	194	198	1	2	2	2	1	7	1	5	250	253
Xgla5409	HAW99	1	2	1	2	1	2	1	2	194	198	1	1	2	4	1	1	1	1	250	253
Xgla5410	HAW99	2	2	1	1	1	1	1	2	198	198	1	1	2	2	1	26	1	1	253	256
Xgla5411	HAW99	2	2	1	2	1	1	1	1	198	198	1	1	2	4	2	3	1	5	250	262
Xgla5412	HAW99	2	2	1	2	1	2	1	1	198	198	1	1	2	2	1	3	1	5	250	256
Xgla5413	HAW99	1	2	1	1	1	2	1	1	198	198	1	1	5	5	1	2	1	5	253	259
Xgla5414	HAW99	1	2	1	1	2	2	1	1	198	198	1	1	2	3	1	3	1	1	250	253
Xgla5415	HAW99	2	2	1	1	1	2	1	2	198	204	1	1	2	3	1	3	1	3	253	256
Xgla5416	HAW99	2	2	1	1	1	2	1	1	194	198	1	1	2	2	1	3	1	6	247	256
Xgla5417	HAW99	2	2	1	1	2	2	1	1	198	202	1	1	2	2	2	3	1	1	259	268
Xgla5418	HAW99	1	2	1	1	2	2	1	2	184	198	1	1	3	4	3	3	5	5	250	256
Xgla5419	HAW99	2	2	1	1	1	2	1	1	184	198	1	1	3	5	1	3	1	5	241	253
Xgla5420	HAW99	1	2	1	2	2	2	1	1	198	198	1	2	2	4	1	3	1	4	259	262
Xgla5421	HAW99	1	2	1	2	1	1	1	1	198	198	1	2	2	2	3	3	1	5	253	256
Xgla5422	HAW99	2	2	1	1	2	2	1	1	198	198	1	1	2	5	3	3	1	5	250	250
Xgla5423	HAW99	1	1	1	1	2	2	1	1	198	198	1	2	2	2	3	7	1	1	247	262
Xgla5424	HAW99	1	1	1	1	1	2	1	1	198	202	1	2	2	3	1	1	1	5	250	259
Xgla5425	HAW99	2	2	2	2	2	2	1	1	184	198	1	1	2	2	3	3	1	5	259	259
Xgla5426	HAW99	2	2	1	2	1	2	1	2	198	198	1	2	2	5	1	1	3	5	256	256
Xgla5427	HAW99	1	2	1	2	2	2	2	2	198	198	1	2	2	5	1	3	1	1	250	253
Xgla5428	HAW99	1	1	1	1	1	2	1	1	198	198	1	1	2	2	3	3	3	3	241	259
Xgla5429	HAW99	2	2	1	1	2	2	1	1	184	198	1	2	2	3	3	3	1	1	247	259
Xgla5430	HAW99	1	2	1	2	2	2	1	1	194	198	1	2	2	4	3	3	1	1	253	253
Xgla5431	HAW99	1	1	1	1	1	2	1	1	198	198	1	2	2	2	1	1	1	1	250	253
Xgla5432	HAW99	1	2	1	1	1	2	1	2	184	184	1	1	3	4	1	26	1	1	253	262
Xgla5433	HAW99	1	2	2	3	2	2	1	2	184	198	2	2	2	5	1	1	5	5	256	256
Xgla5434	HAW99	2	2	1	1	2	2	1	1	194	198	1	1	2	3	1	3	1	1	250	253
Xgla5435	HAW99	2	2	1	1	1	2	1	2	198	198	2	2	2	4	1	3	1	5	259	262
Xgla5436	HAW99	2	2	1	2	2	2	1	1	202	202	1	1	2	3	3	3	1	5	256	259
Xgla5437	HAW99	1	1	1	1	2	2	1	1	184	202	1	1	2	5	3	3	1	3	253	256
Xgla5438	HAW99	2	2	1	2	1	2	1	1	198	198	1	1	2	3	1	3	5	5	253	265
Xgla5439	HAW99	1	2	1	1	1	1	1	1	198	198	1	1	2	2	1	1	1	3	256	256
Xgla5440	HAW99	1	2	1	1	1	2	1	2	198	198	2	2	2	2	1	3	1	1	253	262
Xgla5441	HAW99	1	1	1	3	2	2	1	1	198	198	1	1	2	3	1	3	1	5	253	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla5442	HAW99	1	2	1	2	2	2	1	2	198	198	1	1	2	5	3	3	1	5	250	253
Xgla5443	HAW99	1	2	1	2	2	2	1	2	198	198	1	2	2	3	1	3	1	5	244	259
Xgla5353	HAW99	1	2	1	1	1	2	1	1	198	198	1	1	2	3	1	3	1	3	247	256
Xgla5354	HAW99	1	1	1	2	1	2	1	2	198	206	2	2	2	2	1	1	1	5	253	259
Xgla5355	HAW99	1	2	1	2	1	2	1	1	198	198	1	1	2	3	1	1	1	3	253	259
Xgla5356	HAW99	1	2	1	1	1	2	2	2	198	198	1	1	2	2	1	1	3	5	253	253
Xgla5357	HAW99	1	1	1	1	1	1	1	1	184	202	1	1	2	5	1	2	1	1	250	250
Xgla5358	HAW99	2	2	1	1	1	2	1	1	198	206	2	2	2	5	2	2	1	1	256	256
Xgla5359	HAW99	1	2	1	2	1	2	1	1	198	202	1	1	2	3	1	3	1	3	256	256
Xgla5360	HAW99	1	1	1	1	1	2	1	1	184	198	1	1	2	3	1	3	1	1	250	256
Xgla5361	HAW99	1	2	1	1	1	2	1	2	198	198	1	1	2	3	1	3	1	3	256	256
Xgla5363	HAW99	2	2	1	1	1	2	1	2	198	200	1	1	2	2	1	3	1	4	259	262
Xgla5364	HAW99	1	2	1	1	1	1	1	2	198	198	1	1	2	4	2	3	1	5	253	256
Xgla5365	HAW99	1	1	1	2	2	2	1	1	198	198	2	2	2	3	1	3	1	5	250	262
Xgla5366	HAW99	1	2	1	1	2	2	1	2	184	198	1	1	3	5	1	3	1	5	250	256
Xgla5367	HAW99	1	2	2	3	1	2	1	2	198	198	1	2	2	2	1	2	1	5	253	259
Xgla5368	HAW99	1	2	1	3	1	2	1	2	198	198	1	2	2	2	3	3	1	5	253	259
Xgla5369	HAW99	1	2	1	1	1	2	1	2	198	202	1	2	2	3	1	2	1	1	253	256
Xgla5370	HAW99	1	1	1	1	1	2	1	1	198	198	1	2	2	3	1	7	5	5	250	256
Xgla5371	HAW99	1	2	2	3	1	2	1	2	198	198	1	1	2	3	1	2	1	1	253	259
Xgla5372	HAW99	1	2	1	1	1	2	1	1	198	198	1	2	4	4	1	2	4	6	253	259
Xgla5373	HAW99	1	2	1	1	2	2	1	1	184	198	1	1	2	5	3	7	3	5	253	253
Xgla5374	HAW99	1	1	1	1	2	2	1	1	198	198	1	1	2	5	1	3	1	1	247	259
Xgla5375	HAW99	2	2	1	1	1	2	1	1	198	202	1	2	2	2	1	3	1	4	259	259
Xgla5376	HAW99	1	1	1	1	2	2	1	1	198	198	1	2	2	4	1	1	1	1	250	253
Xgla5377	HAW99	1	2	1	2	1	2	1	1	198	198	1	2	2	4	3	3	5	5	241	259
Xgla5378	HAW99	1	1	1	2	2	2	1	2	184	198	1	1	2	3	1	3	3	5	253	256
Xgla5444	HAW99	1	2	1	2	1	2	1	1	184	198	1	1	2	2	1	3	1	1	253	256
Xgla5445	HAW99	1	2	1	2	2	2	1	1	198	198	1	2	3	3	1	3	1	1	250	259
Xgla5446	HAW99	1	2	1	1	1	2	1	1	198	198	1	1	2	4	1	3	1	1	253	265
Xgla5447	HAW99	1	2	1	1	1	1	1	1	198	198	1	1	2	5	1	7	1	5	253	262
Xgla5448	HAW99	2	2	1	2	2	2	1	1	184	198	1	1	2	3	1	2	1	1	253	259
Xgla5449	HAW99	2	2	1	1	2	2	1	1	198	202	1	1	2	5	2	3	5	5	253	253
Xgla5450	HAW99	1	2	1	2	2	2	1	2	198	198	1	1	5	5	1	3	5	5	244	256
Xgla5451	HAW99	2	2	1	1	2	2	1	1	198	198	2	2	4	4	1	3	1	1	259	259
Xgla5452	HAW99	1	1	1	1	2	2	1	1	198	198	1	1	3	5	3	3	1	1	253	253
Xgla5453	HAW99	1	2	1	1	2	2	1	2	198	198	1	1	2	2	3	3	1	1	256	259
Xgla5454	HAW99	1	2	1	1	2	2	1	1	198	198	2	2	2	5	1	1	1	4	253	259
Xgla5455	HAW99	1	2	1	1	2	2	1	2	198	198	1	1	2	2	2	3	1	5	244	250
Xgla5456	HAW99	2	2	1	2	2	2	1	1	198	198	1	1	5	5	3	3	1	1	250	253
Xgla5457	HAW99	1	2	1	1	2	2	1	1	198	198	1	1	2	2	1	1	1	1	247	253
Xgla5458	HAW99	1	2	1	1	1	1	1	1	198	198	1	1	2	4	1	3	3	5	256	259
Xgla5460	HAW99	2	2	1	2	1	2	1	1	198	204	1	1	3	3	1	3	1	5	253	253
Xgla5461	HAW99	1	2	1	2	1	2	1	2	198	198	1	1	3	5	1	3	1	5	250	253
Xgla5462	HAW99	1	1	1	1	1	1	1	1	198	198	1	1	2	5	1	3	1	5	256	268
Xgla5463	HAW99	1	1	1	1	1	2	1	1	184	198	1	2	2	3	1	1	1	5	253	253
Xgla403	HAWNE	1	2	1	2	1	2	1	2	198	198	1	1	2	5	1	3	1	4	250	250
Xgla404	HAWNE	1	2	1	2	1	2	1	2	184	204	1	1	4	5	3	3	1	1	241	247
Xgla405	HAWNE	2	2	2	2	1	1	1	2	198	202	1	1	2	2	2	3	1	5	253	259
Xgla406	HAWNE	2	2	1	1	1	2	1	2	198	198	1	2	2	2	1	3	3	5	256	256
Xgla407	HAWNE	2	2	1	2	1	2	1	1	184	198	1	2	2	5	1	3	3	5	253	253
Xgla408	HAWNE	1	2	1	2	2	2	1	2	198	202	1	2	3	5	1	11	1	3	256	256
Xgla409	HAWNE	2	2	1	1	2	2	1	1	198	198	1	1	2	2	1	3	1	5	253	262
Xgla410	HAWNE	1	2	1	1	2	2	1	2	198	198	1	1	2	2	3	3	1	5	253	256
Xgla411	HAWNE	1	1	1	1	1	2	1	2	198	198	1	1	2	2	1	2	3	4	241	256
Xgla412	HAWNE	2	2	1	2	1	2	1	1	198	198	1	2	2	2	1	3	1	5	256	265
Xgla413	HAWNE	1	1	1	1	1	2	1	2	184	184	1	2	2	2	3	3	1	1	250	253
Xgla414	HAWNE	2	2	1	2	2	2	1	2	198	202	1	1	2	3	1	3	1	5	253	256
Xgla415	HAWNE	1	2	1	1	2	2	2	2	198	198	1	2	2	4	1	3	1	5	259	265
Xgla416	HAWNE	1	1	1	1	2	2	1	1	198	198	1	1	2	2	1	1	5	5	250	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla417	HAWNE	1	2	1	1	2	2	1	1	198	198	1	1	2	5	3	3	4	5	256	256
Xgla418	HAWNE	2	2	1	1	1	2	1	1	184	198	1	2	2	3	3	3	1	5	253	253
Xgla419	HAWNE	2	2	1	2	1	2	1	2	198	198	1	1	2	3	2	3	1	5	256	256
Xgla420	HAWNE	1	2	1	2	2	2	1	2	184	198	1	1	2	4	1	3	1	5	259	262
Xgla421	HAWNE	1	2	1	2	1	2	1	1	198	198	1	2	2	4	3	3	1	4	241	250
Xgla422	HAWNE	2	2	1	2	1	1	1	2	198	198	1	1	2	4	2	3	1	5	250	256
Xgla423	HAWNE	1	2	1	1	1	2	1	2	198	198	1	1	2	3	3	7	1	5	256	259
Xgla424	HAWNE	1	1	1	1	2	2	1	1	198	198	1	1	2	5	3	3	1	5	256	256
Xgla425	HAWNE	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	3	1	4	250	256
Xgla426	HAWNE	2	2	1	2	2	2	1	2	184	194	1	1	3	4	1	1	1	5	250	253
Xgla427	HAWNE	2	2	1	1	1	2	1	1	198	202	1	1	2	2	1	3	1	5	253	265
Xgla2865	HAWNE	1	2	1	2	1	2	1	2	198	198	1	1	2	3	1	3	1	1	256	262
Xgla2866	HAWNE	1	2	1	2	2	2	1	1	198	198	1	1	2	2	1	3	1	1	256	259
Xgla2867	HAWNE	1	2	1	1	2	2	2	2	184	198	1	2	2	2	1	3	1	3	253	256
Xgla2868	HAWNE	2	2	1	1	2	2	1	2	194	198	1	1	2	2	1	3	1	5	253	256
Xgla2869	HAWNE	1	1	1	1	2	2	1	1	198	198	1	2	2	2	1	2	1	5	253	253
Xgla2870	HAWNE	1	2	1	1	2	2	1	2	198	198	2	2	3	5	1	1	1	1	250	259
Xgla2871	HAWNE	1	1	2	3	2	2	1	1	198	198	1	1	2	4	1	3	1	5	250	253
Xgla2872	HAWNE	2	2	1	1	1	2	1	1	184	184	1	1	2	3	1	3	3	4	256	268
Xgla2873	HAWNE	2	2	1	1	2	2	1	1	184	198	1	2	2	2	3	3	1	1	253	256
Xgla2874	HAWNE	1	2	1	1	1	2	1	2	198	198	1	1	2	5	1	1	1	5	253	262
Xgla2875	HAWNE	1	2	1	2	1	2	1	1	198	198	1	1	2	5	1	3	1	3	244	259
Xgla2876	HAWNE	2	2	1	2	1	2	1	1	198	198	1	1	3	5	3	3	1	3	253	268
Xgla2877	HAWNE	2	2	1	1	2	2	1	1	198	198	1	1	2	3	1	1	1	1	253	256
Xgla2878	HAWNE	2	2	1	2	2	2	1	2	198	198	1	2	2	5	3	3	1	5	256	259
Xgla2879	HAWNE	2	2	1	2	2	2	1	2	198	198	1	2	3	4	3	3	1	1	250	250
Xgla2880	HAWNE	1	1	1	3	2	2	1	1	198	198	1	2	2	4	3	3	1	1	253	262
Xgla2881	HAWNE	2	2	1	1	1	2	1	1	198	198	1	2	2	2	1	1	1	5	256	256
Xgla2882	HAWNE	2	2	1	2	2	2	1	1	198	198	1	1	2	3	1	2	1	5	256	256
Xgla2883	HAWNE	1	1	1	1	2	2	1	2	198	198	1	1	2	5	2	3	1	5	253	256
Xgla2884	HAWNE	1	2	1	1	1	2	1	1	184	198	1	1	2	2	1	7	1	1	256	259
Xgla2885	HAWNE	2	2	1	1	2	2	1	2	198	198	1	1	2	2	3	3	1	5	250	259
Xgla2886	HAWNE	1	2	1	2	1	2	2	2	198	198	1	1	2	5	3	3	1	5	253	256
Xgla4944	HAWNE	1	2	1	2	1	1	1	1	198	198	1	1	2	2	1	3	1	5	250	259
Xgla4945	HAWNE	1	1	1	3	2	2	1	1	184	198	1	1	2	4	1	2	1	1	250	256
Xgla4946	HAWNE	2	2	1	1	1	1	1	1	194	198	1	1	2	2	1	3	1	1	253	259
Xgla4948	HAWNE	2	2	1	2	2	2	1	2	198	198	1	2	2	5	1	1	1	1	256	259
Xgla4949	HAWNE	2	2	1	1	1	1	1	1	198	198	1	2	2	3	1	3	1	1	247	253
Xgla4950	HAWNE	1	2	1	1	2	2	1	2	198	198	1	1	2	2	2	3	1	1	250	256
Xgla4951	HAWNE	1	2	1	2	2	2	1	1	198	198	1	1	2	2	1	1	4	5	247	253
Xgla4952	HAWNE	1	2	1	1	2	2	1	1	194	198	1	1	3	5	1	3	1	1	250	271
Xgla4953	HAWNE	1	2	1	1	2	2	2	2	198	198	1	1	2	3	1	2	1	1	244	250
Xgla4954	HAWNE	1	2	1	1	2	2	1	2	198	198	1	1	2	3	2	3	1	5	256	259
Xgla4955	HAWNE	2	2	1	1	2	2	2	2	198	198	1	2	2	2	1	3	1	5	256	259
Xgla4956	HAWNE	1	1	1	1	2	2	1	1	198	198	1	1	2	2	1	3	1	1	250	265
Xgla4957	HAWNE	2	2	1	1	2	2	1	1	198	202	1	2	2	2	1	1	1	5	253	259
Xgla4958	HAWNE	2	2	1	3	1	2	1	1	198	198	1	1	2	2	1	1	1	5	253	259
Xgla4959	HAWNE	1	2	1	1	1	2	1	1	184	198	1	1	2	3	3	3	1	1	247	256
Xgla4961	HAWNE	1	2	1	1	2	2	1	1	198	202	1	1	2	4	1	3	1	5	256	259
Xgla4962	HAWNE	2	2	1	1	1	2	1	1	184	198	1	2	5	5	1	3	1	5	244	265
Xgla4963	HAWNE	2	2	1	1	2	2	1	1	184	198	1	1	2	5	1	3	5	5	253	256
Xgla4965	HAWNE	2	2	2	2	1	1	1	1	198	198	1	1	2	3	1	3	1	1	253	253
Xgla4966	HAWNE	1	1	1	1	1	2	1	1	198	198	1	1	2	2	1	3	1	5	256	259
Xgla4967	HAWNE	2	2	1	2	1	2	1	1	198	198	1	2	2	2	1	3	1	1	250	256
Xgla4968	HAWNE	1	1	1	1	2	2	1	1	198	198	1	2	2	5	1	1	3	5	244	253
Xgla2805	HAWNE	1	2	1	1	2	2	2	2	184	198	1	1	2	4	1	1	1	4	256	256
Xgla2806	HAWNE	1	1	1	3	1	2	2	2	198	198	1	1	2	2	3	3	1	1	253	256
Xgla2808	HAWNE	1	2	1	2	1	1	1	2	198	198	1	1	2	5	3	3	1	5	256	259
Xgla2809	HAWNE	1	2	1	2	2	2	1	2	184	198	1	2	4	5	1	1	1	3	250	256
Xgla2810	HAWNE	1	2	1	1	1	2	1	2	198	198	1	2	2	3	1	3	1	4	253	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla2811	HAWNE	2	2	1	3	2	2	1	2	198	198	1	1	2	5	1	3	1	1	256	262
Xgla2812	HAWNE	1	2	1	1	1	1	1	1	184	198	1	1	2	3	3	3	1	1	256	256
Xgla2813	HAWNE	2	2	1	2	1	2	1	1	198	198	1	2	2	3	1	1	1	1	253	253
Xgla2814	HAWNE	1	1	1	1	2	2	2	2	194	198	1	1	2	2	3	3	1	1	256	259
Xgla2815	HAWNE	1	1	1	2	2	2	1	1	198	198	1	2	2	2	1	3	1	1	250	259
Xgla2816	HAWNE	1	2	1	1	1	2	1	1	198	200	1	2	2	5	3	3	5	5	250	256
Xgla2817	HAWNE	2	2	1	1	1	1	1	2	198	202	1	2	2	3	3	7	1	5	247	256
Xgla2818	HAWNE	1	2	1	1	1	2	1	1	184	198	1	1	3	5	1	2	3	3	253	262
Xgla2819	HAWNE	1	2	1	1	1	1	1	1	184	198	1	1	2	2	3	3	1	1	253	256
Xgla2820	HAWNE	1	2	1	1	2	2	1	2	198	202	2	2	2	3	3	7	1	5	256	268
Xgla2821	HAWNE	2	2	1	1	1	2	1	1	198	198	1	2	2	5	1	2	4	4	256	256
Xgla2823	HAWNE	2	2	1	1	1	2	1	2	184	198	1	1	2	3	3	3	1	5	250	256
Xgla2824	HAWNE	2	2	2	2	2	2	1	2	198	198	1	2	2	2	3	3	1	1	250	253
Xgla2825	HAWNE	1	1	1	2	2	2	1	2	198	200	1	1	2	2	1	1	1	1	259	262
Xgla2826	HAWNE	2	2	1	1	2	2	1	1	194	198	1	2	2	2	1	3	4	5	253	262
Xgla4970	HAWNE	2	2	1	1	1	1	1	2	198	202	1	1	2	3	1	3	1	1	250	253
Xgla4971	HAWNE	2	2	1	2	2	2	1	1	198	198	1	2	3	5	1	3	1	5	259	262
Xgla4973	HAWNE	1	2	1	1	1	1	1	2	198	198	1	1	2	2	1	3	1	1	256	259
Xgla4974	HAWNE	1	2	1	2	1	2	1	1	198	198	1	2	2	2	1	3	3	5	241	256
Xgla4975	HAWNE	1	2	1	1	1	2	1	2	198	198	1	2	2	3	1	3	1	5	247	253
Xgla4976	HAWNE	1	2	1	2	2	2	1	1	198	198	2	2	2	5	1	1	1	4	253	256
Xgla4977	HAWNE	1	1	1	2	1	2	1	1	184	198	1	1	2	4	3	3	1	1	250	250
Xgla4978	HAWNE	2	2	1	1	1	2	1	2	198	198	1	2	2	5	1	3	1	5	247	253
Xgla4979	HAWNE	1	2	1	2	1	2	1	1	198	198	1	1	3	3	1	3	1	5	256	256
Xgla4980	HAWNE	1	1	1	2	2	2	1	1	184	198	1	1	2	5	3	3	1	5	247	259
Xgla4981	HAWNE	1	2	1	1	1	1	1	2	184	198	1	1	2	2	2	3	1	1	256	256
Xgla4982	HAWNE	1	2	1	1	2	2	1	1	184	198	1	1	2	3	1	3	1	1	256	259
Xgla4983	HAWNE	1	2	1	3	1	2	1	1	198	198	1	1	2	2	1	3	1	1	256	256
Xgla4984	HAWNE	2	2	1	1	2	2	1	2	198	198	1	1	2	5	3	3	1	1	247	253
Xgla4985	HAWNE	1	2	1	1	2	2	1	1	184	198	1	1	2	3	1	7	1	1	253	256
Xgla4986	HAWNE	2	2	1	2	2	2	1	1	184	198	1	1	2	5	1	3	1	4	256	256
Xgla4987	HAWNE	1	1	1	1	2	2	1	2	198	198	1	1	2	2	1	3	1	1	256	259
Xgla4988	HAWNE	2	2	1	2	1	2	1	2	198	198	1	1	4	4	1	2	1	4	253	256
Xgla4989	HAWNE	1	2	1	2	2	2	1	1	198	198	1	2	2	3	3	3	1	3	256	262
Xgla4990	HAWNE	2	2	1	1	1	1	1	1	198	198	1	1	3	3	1	3	1	1	256	259
Xgla4991	HAWNE	1	2	1	1	2	2	1	2	198	198	2	2	2	5	1	1	1	5	256	262
Xgla4992	HAWNE	2	2	1	1	1	2	1	1	198	198	1	1	2	2	1	3	1	1	250	256
Xgla4993	HAWNE	1	2	1	1	1	2	1	1	184	198	1	1	4	5	1	3	1	1	253	256
Xgla1755	NMCA	1	2	1	1	2	2	1	2	198	198	1	2	2	2	1	2	1	1	250	265
Xgla1756	NMCA	1	2	1	1	2	2	1	2	194	198	1	1	2	3	1	2	1	5	250	256
Xgla1757	NMCA	1	2	1	2	1	2	1	1	198	198	1	2	1	5	1	1	1	5	250	253
Xgla1758	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	2	3	3	1	4	256	265
Xgla1759	NMCA	1	2	1	2	2	2	1	2	198	198	1	2	2	2	1	3	1	5	256	256
Xgla1760	NMCA	2	2	1	1	1	2	1	1	198	198	1	2	2	3	2	3	1	1	259	259
Xgla1761	NMCA	2	2	1	2	2	2	1	2	184	198	1	1	2	2	1	1	4	5	259	259
Xgla1762	NMCA	1	2	1	2	1	2	1	1	198	198	1	1	2	2	3	3	1	2	253	253
Xgla1764	NMCA	2	2	1	2	1	2	1	1	184	198	1	2	2	2	1	1	5	5	253	256
Xgla1765	NMCA	1	1	1	1	1	2	1	2	198	198	1	2	2	5	1	1	1	5	253	253
Xgla1766	NMCA	1	1	1	1	2	2	1	1	198	202	1	1	2	3	1	1	3	7	256	259
Xgla1767	NMCA	2	2	1	2	1	2	1	1	198	198	1	1	2	4	1	3	1	5	250	256
Xgla1768	NMCA	1	2	1	3	2	2	1	2	198	200	1	1	3	3	1	1	1	1	241	256
Xgla1769	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	3	2	3	1	1	256	256
Xgla1770	NMCA	1	2	1	1	2	2	1	1	198	198	1	2	2	2	1	3	1	1	250	259
Xgla1771	NMCA	1	2	1	1	2	2	1	1	198	198	1	2	2	2	3	3	1	5	256	259
Xgla1772	NMCA	2	2	1	1	1	1	1	1	198	198	1	2	2	2	1	3	1	3	244	253
Xgla1773	NMCA	1	2	1	1	2	2	1	2	184	198	1	1	5	5	1	3	1	1	253	259
Xgla1774	NMCA	1	2	1	1	2	2	1	1	198	198	1	1	2	2	3	3	3	3	250	259
Xgla1775	NMCA	2	2	1	1	1	2	1	1	198	202	1	2	2	5	3	3	4	5	253	256
Xgla1776	NMCA	1	2	1	3	2	2	1	1	198	198	1	1	2	3	1	7	1	1	253	256
Xgla1777	NMCA	2	2	2	2	2	2	1	1	198	198	1	1	2	3	1	3	1	5	250	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla1778	NMCA	2	2	2	2	2	2	1	1	198	198	1	1	2	2	3	3	1	1	250	250
Xgla1780	NMCA	1	2	1	1	2	2	1	1	184	198	1	2	2	3	1	3	1	5	250	259
Xgla1781	NMCA	1	1	1	1	2	2	1	1	184	198	1	1	2	5	1	3	1	5	253	253
Xgla1782	NMCA	2	2	1	1	1	2	1	1	198	206	1	2	2	5	1	7	1	5	253	256
Xgla1783	NMCA	1	2	1	1	1	1	1	1	198	198	1	1	2	2	1	2	1	5	256	256
Xgla1784	NMCA	2	2	1	1	1	2	1	1	198	198	1	1	2	4	1	3	1	5	253	259
Xgla1785	NMCA	1	2	1	2	2	2	1	1	184	198	1	1	2	5	1	2	1	1	256	256
Xgla1786	NMCA	1	2	1	1	1	1	1	1	198	198	1	1	2	4	1	3	4	5	250	256
Xgla1787	NMCA	1	1	1	3	2	2	2	2	198	202	1	1	2	2	1	3	1	1	250	259
Xgla1788	NMCA	1	2	1	2	1	1	1	1	184	198	1	1	2	2	1	3	1	1	250	253
Xgla1789	NMCA	1	2	1	1	1	2	1	1	198	202	1	2	2	2	3	3	1	5	247	256
Xgla1790	NMCA	1	2	1	1	2	2	1	1	198	198	1	1	4	5	1	3	5	5	247	256
Xgla1791	NMCA	1	1	2	2	2	2	1	1	198	198	1	1	2	2	3	26	4	5	256	256
Xgla1792	NMCA	1	2	1	1	1	2	1	1	198	198	1	1	2	2	3	3	5	5	256	262
Xgla1793	NMCA	1	1	1	1	1	2	1	1	198	198	1	1	2	3	1	3	1	1	250	262
Xgla1794	NMCA	1	2	1	1	2	2	1	2	198	198	1	2	2	2	1	3	1	1	253	256
Xgla1795	NMCA	1	2	1	2	2	2	1	1	198	198	1	1	2	2	1	1	1	1	256	259
Xgla1797	NMCA	2	2	1	1	2	2	1	1	198	198	1	2	2	2	1	3	5	5	253	256
Xgla1798	NMCA	1	1	1	2	2	2	1	2	198	198	1	2	3	5	1	3	1	5	250	253
Xgla1799	NMCA	1	2	1	1	1	1	1	2	198	200	1	1	2	2	3	7	1	5	253	259
Xgla1800	NMCA	2	2	1	1	1	2	1	2	194	202	1	2	2	2	3	3	1	5	253	259
Xgla1801	NMCA	1	2	1	1	2	2	1	1	198	198	1	1	3	3	1	2	1	1	253	256
Xgla1802	NMCA	1	2	1	1	1	2	1	1	198	198	1	2	2	2	1	3	1	5	256	259
Xgla1803	NMCA	1	1	1	1	1	1	1	1	184	198	1	1	2	5	1	3	1	5	250	256
Xgla1804	NMCA	1	1	1	1	2	2	1	1	184	198	1	1	2	2	3	7	1	1	250	256
Xgla1805	NMCA	1	2	1	1	2	2	1	1	198	198	1	2	2	2	1	3	1	6	250	253
Xgla1806	NMCA	2	2	1	1	2	2	1	1	198	204	1	1	2	2	2	3	1	5	256	262
Xgla1807	NMCA	2	2	1	1	2	2	1	2	198	202	1	1	2	2	2	3	1	5	250	259
Xgla1808	NMCA	2	2	1	1	1	2	1	1	184	198	1	1	2	3	1	26	1	5	259	262
Xgla1809	NMCA	1	1	1	1	2	2	1	1	198	198	1	1	2	3	2	3	1	5	259	265
Xgla1810	NMCA	1	1	1	1	2	2	1	1	184	202	1	1	3	5	1	3	1	1	253	256
Xgla1811	NMCA	1	2	1	2	2	2	1	1	198	198	1	2	2	5	2	3	5	5	247	256
Xgla1812	NMCA	1	2	1	1	2	2	1	1	198	198	1	2	3	5	1	3	1	1	250	256
Xgla1813	NMCA	2	2	1	3	1	2	1	2	184	198	1	1	4	4	2	3	1	5	250	253
Xgla1814	NMCA	2	2	1	2	1	1	1	1	198	198	2	2	2	3	1	3	1	1	250	256
Xgla1815	NMCA	1	2	1	2	1	2	1	1	184	198	1	2	2	5	1	1	5	6	253	259
Xgla1816	NMCA	1	1	1	1	1	2	1	2	184	198	1	1	2	3	1	1	1	1	256	259
Xgla3906	NMCA	1	1	1	2	2	2	1	1	198	198	1	1	4	5	3	7	1	1	256	256
Xgla3907	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	5	1	26	1	5	256	259
Xgla3908	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	3	1	3	1	1	253	253
Xgla3909	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	3	1	2	1	5	250	262
Xgla3910	NMCA	1	1	1	1	1	2	1	2	198	202	1	1	2	2	3	11	1	3	256	262
Xgla3911	NMCA	1	2	1	1	2	2	1	2	198	198	1	2	2	2	1	3	5	5	250	262
Xgla3912	NMCA	2	2	1	2	1	1	1	1	198	198	1	1	2	2	2	3	1	5	259	259
Xgla3913	NMCA	1	2	2	2	2	2	1	2	198	198	1	1	1	5	1	3	1	1	250	262
Xgla3914	NMCA	1	2	1	1	1	2	1	1	194	198	1	1	2	5	3	3	1	5	256	259
Xgla3915	NMCA	1	2	1	1	2	2	1	1	198	198	1	1	2	4	1	2	1	1	244	253
Xgla3916	NMCA	2	2	1	1	1	2	1	2	198	202	1	1	2	3	2	3	5	5	256	256
Xgla3917	NMCA	1	2	1	2	1	2	1	2	198	198	1	1	2	5	2	3	5	5	247	253
Xgla3918	NMCA	2	2	2	2	2	2	1	1	184	198	1	1	2	3	1	1	1	3	256	262
Xgla3919	NMCA	1	1	1	1	1	1	1	1	198	198	1	1	2	5	1	3	1	1	244	256
Xgla3920	NMCA	1	2	1	1	2	2	1	2	198	198	1	1	2	2	3	3	1	1	253	256
Xgla3921	NMCA	1	2	1	2	2	2	1	1	198	198	1	1	2	2	2	3	1	5	256	259
Xgla3938	NMCA	1	2	1	1	2	2	1	1	198	198	1	1	2	2	1	3	5	5	250	271
Xgla3939	NMCA	1	1	1	2	1	2	1	1	198	198	1	2	2	2	1	2	1	5	250	253
Xgla3940	NMCA	1	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	1	253	256
Xgla3941	NMCA	1	2	1	1	1	1	1	2	198	198	1	2	2	5	1	3	1	5	256	259
Xgla3942	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	3	3	3	1	1	250	259
Xgla3943	NMCA	2	2	1	1	2	2	1	1	198	198	1	1	2	2	3	3	4	5	259	259
Xgla3944	NMCA	1	2	1	1	2	2	1	2	198	198	1	1	2	2	1	3	1	5	247	250

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla3945	NMCA	2	2	1	2	1	1	1	1	198	198	1	2	2	2	2	2	1	4	256	259
Xgla3946	NMCA	1	2	1	1	1	2	1	2	198	198	1	1	2	5	1	1	1	1	253	256
Xgla3947	NMCA	2	2	1	1	1	2	1	2	198	198	1	1	3	5	1	3	1	1	253	256
Xgla3948	NMCA	1	2	1	1	2	2	2	2	184	198	1	1	2	2	1	3	1	1	253	256
Xgla3949	NMCA	2	2	1	2	1	2	2	2	198	198	1	2	2	3	1	3	1	1	256	256
Xgla3960	NMCA	1	1	1	1	2	2	1	2	184	198	1	2	2	5	1	1	1	4	247	250
Xgla3961	NMCA	1	1	1	1	1	2	1	1	198	198	1	2	2	2	1	1	1	5	256	256
Xgla3962	NMCA	1	2	1	1	2	2	1	2	198	198	1	1	3	5	3	3	1	6	250	256
Xgla3963	NMCA	2	2	1	1	1	2	1	1	198	198	1	2	2	3	1	1	1	1	256	256
Xgla3964	NMCA	1	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	1	256	259
Xgla3965	NMCA	1	2	1	2	1	2	1	2	184	198	1	1	2	3	1	3	1	5	256	259
Xgla3966	NMCA	2	2	1	1	1	2	1	2	184	200	1	2	2	2	1	3	1	1	259	262
Xgla3967	NMCA	2	2	1	1	2	2	1	1	184	198	1	1	3	5	3	3	1	5	250	259
Xgla3968	NMCA	2	2	1	1	1	2	1	1	198	198	2	2	2	3	3	3	1	5	253	256
Xgla3771	Ecuador	1	1	1	1	2	2	1	2	198	198	1	2	2	5	1	3	1	5	253	259
Xgla3772	Ecuador	2	2	1	1	2	2	1	2	184	198	1	1	2	2	2	3	1	1	256	262
Xgla3773	Ecuador	1	2	1	1	1	2	1	1	198	198	1	2	2	5	3	3	5	5	250	250
Xgla3774	Ecuador	1	2	1	1	2	2	1	2	198	198	1	1	2	5	2	26	1	1	256	262
Xgla3775	Ecuador	1	1	1	2	2	2	1	1	198	198	1	2	2	2	3	3	1	1	259	265
Xgla3776	Ecuador	1	2	1	2	1	1	1	2	184	198	1	1	2	5	1	3	1	5	253	259
Xgla3777	Ecuador	2	2	1	2	1	1	1	2	198	198	1	1	2	2	1	1	1	3	253	256
Xgla3778	Ecuador	1	2	1	2	1	2	1	2	184	198	1	1	2	5	3	3	1	6	250	256
Xgla3780	Ecuador	2	2	1	1	2	2	1	1	198	198	1	1	2	4	1	3	1	4	253	259
Xgla3781	Ecuador	1	1	1	1	2	2	1	1	184	198	1	1	2	2	3	3	1	1	250	253
Xgla3782	Ecuador	2	2	1	1	1	1	1	2	198	198	1	1	2	5	1	3	1	1	250	256
Xgla3783	Ecuador	1	1	1	1	1	2	1	2	184	200	1	1	2	3	1	3	1	1	250	253
Xgla3784	Ecuador	1	2	1	1	2	2	1	1	198	198	2	2	2	4	3	3	5	5	259	259
Xgla3785	Ecuador	1	2	1	1	1	2	1	2	198	202	1	1	2	2	1	1	1	5	253	253
Xgla3786	Ecuador	1	1	1	2	1	2	2	2	198	198	1	2	3	5	1	1	1	1	256	259
Xgla3787	Ecuador	1	2	1	1	1	2	1	1	184	198	1	1	2	3	1	3	1	5	250	256
Xgla3788	Ecuador	2	2	1	2	2	2	2	2	198	204	1	1	2	3	1	3	1	5	253	256
Xgla3789	Ecuador	2	2	1	2	2	2	1	2	198	198	1	1	3	5	3	3	1	5	256	268
Xgla3790	Ecuador	2	2	1	2	1	1	1	2	198	198	1	2	3	3	1	1	1	1	253	256
Xgla3791	Ecuador	1	1	1	1	2	2	1	1	198	198	2	2	2	3	1	1	1	1	256	259
Xgla3792	Ecuador	2	2	1	1	2	2	1	1	198	198	1	1	2	2	1	1	1	1	262	268
Xgla3793	Ecuador	2	2	1	1	2	2	1	2	198	198	1	2	2	4	1	3	1	5	247	256
Xgla3794	Ecuador	1	2	1	1	2	2	1	1	184	198	1	1	2	3	1	3	1	5	244	250
Xgla3795	Ecuador	2	2	1	2	2	2	1	2	198	198	1	2	2	3	1	1	5	5	250	253
Xgla3796	Ecuador	1	1	2	2	1	2	1	2	198	198	1	1	2	5	1	2	1	1	253	256
Xgla3797	Ecuador	2	2	1	1	2	2	1	2	184	198	1	1	2	3	3	7	1	5	247	262
Xgla3798	Ecuador	1	2	1	2	1	2	1	2	202	204	1	1	2	3	1	3	1	5	253	262
Xgla3799	Ecuador	2	2	1	2	2	2	1	1	198	202	2	2	2	4	2	3	1	1	247	262
Xgla3800	Ecuador	1	2	1	1	2	2	1	1	198	202	1	1	2	3	1	3	1	5	253	256
Xgla3801	Ecuador	1	1	1	2	1	2	1	1	198	198	1	1	2	3	3	3	3	5	256	259
Xgla3802	Ecuador	2	2	1	2	2	2	1	1	198	198	1	2	5	5	3	3	1	3	250	256
Xgla3803	Ecuador	1	2	1	1	2	2	1	1	198	198	1	2	2	5	3	3	1	5	250	253
Xgla3804	Ecuador	1	2	1	2	2	2	1	2	198	198	1	1	3	4	1	7	1	1	250	253
Xgla3805	Ecuador	2	2	1	1	2	2	1	1	198	198	1	1	2	5	2	3	1	1	253	256
Xgla3806	Ecuador	2	2	1	1	1	2	1	2	184	198	2	2	2	2	3	3	1	1	250	253
Xgla3807	Ecuador	2	2	1	1	1	2	1	1	198	198	1	2	2	2	2	3	1	1	259	259
Xgla3808	Ecuador	1	2	1	1	1	2	1	2	198	202	1	1	2	2	1	1	1	1	250	262
Xgla3809	Ecuador	1	2	1	1	1	1	1	2	198	202	2	2	2	5	1	3	1	5	253	253
Xgla3810	Ecuador	2	2	1	1	1	2	1	1	198	198	1	2	2	4	1	3	1	5	256	268
Xgla5156	Ecuador	1	2	1	1	1	1	1	2	184	198	1	1	3	5	1	3	1	5	256	256
Xgla5157	Ecuador	2	2	1	1	2	2	1	2	198	198	2	2	2	3	3	3	1	5	250	262
Xgla5158	Ecuador	2	2	1	1	1	2	1	2	198	198	1	2	2	2	1	3	5	5	256	256
Xgla5159	Ecuador	1	2	2	2	1	2	1	1	198	198	1	2	2	3	3	3	1	1	256	268
Xgla5160	Ecuador	2	2	1	2	1	2	1	1	198	210	1	1	2	5	1	3	1	1	253	256
Xgla5161	Ecuador	1	2	2	2	2	2	1	1	184	198	1	2	2	2	1	3	1	1	253	256
Xgla5162	Ecuador	1	2	1	1	1	1	1	2	184	198	1	2	2	3	1	3	1	5	253	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla5163	Ecuador	1	2	1	2	1	1	1	2	184	198	1	1	2	5	1	1	1	5	256	256
Xgla5164	Ecuador	2	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	3	247	250
Xgla5165	Ecuador	1	2	1	2	1	2	1	1	198	198	1	2	3	4	1	3	1	4	253	256
Xgla5166	Ecuador	2	2	1	3	1	2	1	1	198	198	1	1	2	2	1	3	1	5	256	259
Xgla5167	Ecuador	1	2	2	3	1	2	1	1	198	202	1	1	2	2	1	3	3	5	256	268
Xgla5168	Ecuador	1	2	1	2	1	2	1	2	198	198	1	1	3	5	1	2	1	1	256	256
Xgla5169	Ecuador	2	2	1	1	1	1	1	2	184	198	1	2	2	5	1	1	1	5	250	256
Xgla5170	Ecuador	1	1	1	1	2	2	1	2	198	202	1	1	2	2	3	3	1	1	253	256
Xgla5171	Ecuador	1	2	2	3	2	2	1	2	198	198	1	1	2	2	1	2	1	1	247	250
Xgla5172	Ecuador	2	2	2	3	1	2	1	2	184	198	1	1	2	3	3	3	1	5	253	256
Xgla5173	Ecuador	2	2	1	2	1	2	1	1	184	198	1	1	1	2	3	7	1	1	253	253
Xgla5174	Ecuador	2	2	1	1	1	1	1	1	184	198	1	1	2	2	2	3	1	5	253	253
Xgla5175	Ecuador	2	2	1	2	2	2	1	1	198	198	1	1	2	2	1	3	1	1	256	256
Xgla5176	Ecuador	1	1	1	3	1	2	1	1	198	198	1	2	2	3	3	3	1	4	244	256
Xgla5177	Ecuador	1	2	1	2	1	2	1	2	198	198	1	1	3	5	1	7	1	5	256	262
Xgla5178	Ecuador	2	2	1	1	2	2	1	2	198	198	1	2	2	2	1	3	1	5	253	256
Xgla5179	Ecuador	1	2	1	1	1	2	1	2	184	198	1	2	2	3	1	3	1	1	250	259
Xgla5180	Ecuador	1	2	1	1	2	2	1	2	198	198	1	2	2	5	3	3	3	5	244	265
Xgla5181	Ecuador	2	2	1	2	1	2	1	2	198	198	1	2	2	2	1	2	1	5	253	268
Xgla5182	Ecuador	1	2	1	2	1	2	1	2	198	198	1	1	2	3	1	3	1	1	253	262
Xgla5183	Ecuador	1	2	1	1	1	2	1	1	198	198	2	2	2	2	1	26	1	5	256	259
Xgla5184	Ecuador	2	2	1	1	1	2	1	1	198	198	2	2	3	3	1	3	1	1	253	256
Xgla5185	Ecuador	1	2	1	1	2	2	1	2	184	198	1	1	2	3	3	3	1	5	253	259
Xgla5186	Ecuador	1	2	1	1	2	2	2	2	198	198	1	1	2	5	2	7	1	1	256	259
Xgla5187	Ecuador	2	2	1	2	2	2	2	2	198	198	1	1	2	2	1	1	1	5	250	259
Xgla5188	Ecuador	1	1	1	1	2	2	1	1	198	198	1	1	2	2	1	3	1	1	253	259
Xgla5189	Ecuador	1	2	1	2	2	2	1	2	184	198	1	2	2	5	1	1	1	4	250	259
Xgla5190	Ecuador	1	1	1	3	1	2	2	2	198	198	1	2	2	5	3	3	4	5	253	253
Xgla5191	Ecuador	2	2	1	2	2	2	1	1	198	198	1	2	2	5	3	3	3	5	256	256
Xgla5193	Ecuador	1	2	1	2	2	2	1	2	198	198	1	1	2	3	3	3	1	1	250	250
Xgla5194	Ecuador	1	1	1	1	1	2	1	1	184	198	1	2	2	5	1	26	1	5	250	256
Xgla5195	Ecuador	2	2	1	2	2	2	1	1	198	198	1	2	3	5	1	7	1	1	253	271
Xgla5196	Ecuador	1	2	1	1	2	2	1	2	198	198	1	1	2	2	1	2	1	4	250	250
Xgla5197	Ecuador	1	1	1	1	1	2	2	2	198	198	1	1	2	5	1	3	1	1	244	256
Xgla2639	Chile97	1	2	1	1	2	2	1	1	198	198	2	2	2	5	1	1	1	1	244	250
Xgla2640	Chile97	2	2	1	1	2	2	1	2	198	198	1	2	2	4	1	3	1	1	256	259
Xgla2641	Chile97	2	2	1	2	1	2	1	1	198	198	1	1	5	5	1	7	1	5	256	259
Xgla2644	Chile97	1	2	1	1	1	2	1	1	194	194	1	1	3	3	3	3	1	4	247	256
Xgla2645	Chile97	1	1	1	1	2	2	1	2	194	194	1	1	2	2	1	3	1	1	256	262
Xgla2646	Chile97	2	2	1	1	1	2	1	1	198	198	1	1	2	2	2	3	1	1	247	274
Xgla2647	Chile97	1	2	1	1	2	2	1	2	198	198	1	2	2	3	1	2	1	4	250	262
Xgla2648	Chile97	1	2	1	1	2	2	1	1	198	198	1	1	2	3	1	1	1	1	256	259
Xgla2649	Chile97	1	1	2	2	2	2	1	1	198	198	1	1	3	3	1	2	1	1	250	256
Xgla2650	Chile97	2	2	1	1	1	2	1	1	198	206	1	2	2	4	1	2	1	5	259	262
Xgla2651	Chile97	2	2	1	2	1	2	1	1	194	198	2	2	2	3	1	1	1	4	256	256
Xgla2652	Chile97	1	2	1	1	2	2	1	1	184	198	1	2	2	2	1	1	1	4	250	253
Xgla2653	Chile97	1	2	1	2	1	2	1	1	184	198	1	1	2	5	2	3	1	5	253	268
Xgla2654	Chile97	1	2	2	3	1	2	1	1	198	198	1	1	2	2	1	1	1	5	259	259
Xgla2655	Chile97	1	1	1	1	1	2	1	2	198	198	1	2	2	3	3	3	1	5	250	250
Xgla2656	Chile97	1	2	1	1	2	2	1	1	198	200	1	1	2	2	1	1	1	3	259	268
Xgla2657	Chile97	1	2	1	2	2	2	1	2	184	198	1	1	2	5	3	3	1	1	250	253
Xgla2658	Chile97	1	2	1	1	1	2	1	1	198	198	1	1	2	3	2	3	1	1	253	256
Xgla2660	Chile97	2	2	1	1	1	2	2	2	184	198	1	2	2	2	3	3	1	1	250	259
Xgla2661	Chile97	1	2	1	1	1	2	1	2	198	198	1	1	2	5	3	3	1	5	259	259
Xgla2663	Chile97	1	1	1	1	2	2	1	2	198	198	1	1	2	4	2	3	1	1	247	262
Xgla2664	Chile97	1	2	1	2	1	2	1	1	198	198	1	1	2	4	1	3	1	1	256	259
Xgla2665	Chile97	2	2	1	2	2	2	1	1	198	198	1	1	3	3	1	3	1	5	250	256
Xgla2666	Chile97	1	2	1	1	1	2	1	1	198	198	1	1	2	3	3	3	1	5	256	256
Xgla2667	Chile97	1	1	1	1	1	2	1	1	198	204	1	1	2	2	3	3	1	5	250	259
Xgla2668	Chile97	1	1	1	1	1	2	1	1	184	198	1	2	2	2	3	3	1	3	259	259

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla2669	Chile97	1	2	1	1	1	1	1	2	194	194	1	2	2	5	3	3	1	1	253	256
Xgla2670	Chile97	2	2	1	1	2	2	1	1	184	198	1	2	2	3	1	7	1	5	253	253
Xgla2671	Chile97	1	2	1	1	2	2	1	1	198	198	1	2	5	5	1	2	1	1	250	256
Xgla2673	Chile97	2	2	1	1	1	1	1	1	194	198	1	1	2	3	3	3	1	1	256	259
Xgla2676	Chile97	1	2	1	1	2	2	1	2	198	198	1	2	2	2	1	2	1	5	250	256
Xgla2680	Chile97	1	1	1	1	1	2	2	2	198	202	1	1	2	5	1	3	1	5	250	262
Xgla2681	Chile97	1	2	1	2	1	2	1	2	198	198	1	1	2	3	1	1	1	1	253	259
Xgla2682	Chile97	1	2	1	1	1	2	1	1	198	202	2	2	2	2	3	3	1	1	253	256
Xgla2683	Chile97	1	1	1	1	2	2	2	2	198	198	1	1	4	5	1	3	5	5	250	259
Xgla2684	Chile97	1	1	1	1	1	2	1	1	184	198	1	1	5	5	3	3	5	5	250	256
Xgla2685	Chile97	2	2	1	2	1	2	1	1	198	198	1	2	2	5	1	1	1	5	250	253
Xgla2686	Chile97	1	2	1	2	1	2	1	1	198	198	1	2	2	2	1	3	1	5	247	256
Xgla2687	Chile97	1	1	1	1	1	2	1	1	184	198	1	1	3	5	1	2	1	1	259	265
Xgla2688	Chile97	2	2	1	1	2	2	1	1	198	198	1	1	2	3	1	3	1	1	253	256
Xgla2689	Chile97	2	2	1	2	2	2	1	1	184	198	1	2	2	2	3	3	1	1	256	256
Xgla2690	Chile97	1	2	1	2	1	2	1	1	198	198	2	2	3	4	1	3	1	1	253	256
Xgla2691	Chile97	2	2	1	1	1	2	1	1	198	198	1	1	2	3	1	3	1	1	256	256
Xgla2692	Chile97	1	2	1	3	1	2	1	1	194	198	1	2	2	3	1	2	1	6	256	256
Xgla2693	Chile97	2	2	1	1	2	2	1	1	198	198	1	1	2	5	1	3	1	5	250	256
Xgla2694	Chile97	1	2	1	1	1	2	1	1	198	198	1	1	5	5	1	3	1	5	253	256
Xgla2696	Chile97	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	3	1	1	256	256
Xgla2697	Chile97	1	1	1	2	2	2	1	2	198	202	1	1	2	5	1	1	1	1	256	256
Xgla2698	Chile97	2	2	1	2	1	2	1	1	198	198	1	1	2	5	1	3	1	5	253	259
Xgla2699	Chile97	1	2	1	1	2	2	1	1	198	198	1	1	2	3	3	3	1	5	238	253
Xgla2700	Chile97	1	2	1	2	2	2	1	1	198	198	1	2	2	2	1	3	1	3	256	262
Xgla2701	Chile97	1	1	1	2	2	2	1	1	184	198	1	1	2	2	1	3	1	5	250	259
Xgla2702	Chile97	2	2	1	1	2	2	1	1	198	198	1	1	2	2	1	2	1	5	247	262
Xgla2703	Chile97	1	2	1	3	2	2	1	1	198	198	1	1	2	3	1	3	1	4	256	262
Xgla2704	Chile97	2	2	1	1	1	2	1	1	198	198	1	2	3	3	1	1	1	1	256	256
Xgla2705	Chile97	1	2	1	2	2	2	1	1	198	198	1	1	2	2	1	2	1	5	256	256
Xgla2706	Chile97	1	2	1	1	2	2	2	2	198	198	1	1	2	4	3	3	1	1	256	259
Xgla2707	Chile97	2	2	1	2	1	1	1	1	184	198	1	1	3	5	1	3	1	5	253	256
Xgla2708	Chile97	1	2	1	1	2	2	1	2	198	208	1	2	2	4	1	3	1	5	259	265
Xgla2709	Chile97	1	1	1	2	2	2	1	1	198	202	1	1	2	3	1	3	1	1	250	253
Xgla2710	Chile97	1	2	1	1	1	2	1	2	198	198	1	1	2	5	1	3	5	5	256	262
Xgla2711	Chile97	2	2	1	1	2	2	1	2	184	198	1	1	2	3	1	1	1	3	247	250
Xgla2712	Chile97	2	2	1	2	1	2	1	1	198	198	1	1	2	2	1	1	4	5	259	259
Xgla2713	Chile97	1	2	1	2	1	2	1	2	198	198	1	2	3	5	1	1	1	4	250	259
Xgla2714	Chile97	1	2	1	1	1	2	1	2	198	198	1	1	2	2	1	3	1	5	253	256
Xgla2715	Chile97	1	2	1	1	1	2	1	2	198	198	2	2	2	2	3	3	1	1	256	256
Xgla2716	Chile97	1	2	1	1	1	2	1	2	198	198	1	2	2	2	1	3	1	1	253	253
Xgla5464	Chile99	1	2	1	1	2	2	2	2	198	198	1	2	3	4	2	3	1	1	250	253
Xgla5469	Chile99	1	2	1	1	1	2	1	1	198	202	1	1	2	5	3	3	1	5	256	262
Xgla5471	Chile99	1	2	1	1	2	2	1	2	198	198	1	1	2	5	1	3	1	1	247	247
Xgla5474	Chile99	1	2	1	2	1	2	1	1	198	202	1	1	2	2	3	3	1	1	253	256
Xgla5475	Chile99	1	2	1	1	2	2	1	1	184	198	1	1	2	5	3	3	3	5	259	259
Xgla5476	Chile99	2	2	2	2	1	1	1	1	184	198	1	1	2	2	1	3	1	1	253	256
Xgla5477	Chile99	2	2	1	1	2	2	1	2	184	198	1	1	2	2	1	1	1	5	250	256
Xgla5478	Chile99	2	2	1	2	2	2	1	2	194	198	1	2	2	5	1	1	1	1	262	268
Xgla5479	Chile99	1	2	1	1	1	2	1	2	198	198	1	2	1	2	3	3	1	1	250	259
Xgla5480	Chile99	1	2	1	1	1	2	1	2	198	198	1	1	2	3	1	1	5	5	253	259
Xgla5482	Chile99	1	2	2	2	2	2	1	1	198	198	2	2	3	4	1	1	1	1	256	262
Xgla5484	Chile99	1	2	1	1	1	2	1	1	198	198	1	1	2	2	3	3	1	1	256	259
Xgla5488	Chile99	1	1	1	2	2	2	1	2	194	198	1	1	2	4	1	3	1	5	253	256
Xgla5489	Chile99	1	1	2	2	1	2	1	2	194	198	1	1	2	4	1	3	1	5	253	256
Xgla5492	Chile99	2	2	1	1	1	2	1	2	198	198	1	1	2	2	1	1	1	1	256	256
Xgla5495	Chile99	1	2	1	1	2	2	2	2	198	198	1	2	3	4	2	3	1	1	253	256
Xgla5497	Chile99	2	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	1	256	262
Xgla5498	Chile99	1	2	1	1	1	2	1	1	198	198	1	2	2	2	3	3	1	1	247	250
Xgla5499	Chile99	1	2	1	1	1	2	1	2	198	198	2	2	2	3	1	3	1	5	244	253

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla5503	Chile99	2	2	1	1	1	2	1	1	198	198	1	2	3	3	1	3	1	1	262	265
Xgla5504	Chile99	1	2	1	1	2	2	1	2	198	198	1	1	1	3	1	3	1	1	250	259
Xgla5505	Chile99	1	2	1	1	2	2	1	2	198	198	1	1	2	4	1	3	1	1	250	250
Xgla5506	Chile99	2	2	1	1	1	2	2	2	198	198	1	1	2	2	3	3	1	1	253	259
Xgla5507	Chile99	2	2	1	1	1	2	1	2	198	198	1	1	3	3	1	3	1	5	250	253
Xgla5508	Chile99	2	2	1	1	1	1	2	2	184	198	1	1	3	5	1	1	1	5	250	253
Xgla5510	Chile99	2	2	1	3	1	2	1	1	198	198	1	1	2	5	2	3	1	5	253	253
Xgla5515	Chile99	2	2	1	1	2	2	1	2	198	198	1	1	3	3	1	7	1	1	256	256
Xgla5516	Chile99	2	2	1	2	1	2	1	1	184	198	1	2	2	3	3	3	5	5	250	253
Xgla5517	Chile99	2	2	1	2	2	2	1	2	198	198	1	2	3	3	1	3	1	1	253	256
Xgla5518	Chile99	2	2	1	2	1	2	1	1	198	198	1	1	2	2	1	1	4	5	259	259
Xgla5519	Chile99	2	2	1	2	1	2	1	2	198	198	1	1	2	5	3	3	1	5	253	259
Xgla5521	Chile99	1	2	1	2	1	2	1	1	198	200	1	2	3	5	1	1	1	1	256	259
Xgla5524	Chile99	2	2	1	1	2	2	1	2	184	198	1	1	2	2	3	3	1	1	247	259
Xgla5526	Chile99	1	2	1	1	2	2	1	1	184	198	1	1	2	2	3	7	1	1	253	256
Xgla5527	Chile99	2	2	1	2	1	2	1	1	198	198	1	1	3	3	1	3	1	1	256	259
Xgla5529	Chile99	1	1	1	1	1	1	1	1	198	198	1	2	2	4	1	3	5	5	253	259
Xgla5533	Chile99	2	2	1	2	2	2	1	1	198	198	1	1	2	4	1	2	1	1	256	259
Xgla5534	Chile99	2	2	1	1	1	2	1	1	198	198	1	2	2	3	2	2	1	1	253	253
Xgla5542	Chile99	2	2	1	1	1	2	1	1	198	198	1	1	2	3	3	3	1	1	253	256
Xgla5543	Chile99	1	2	1	1	2	2	1	2	198	202	1	1	2	2	2	3	1	5	250	259
Xgla5545	Chile99	2	2	1	2	1	2	1	1	194	206	1	1	2	3	3	3	1	3	253	256
Xgla5549	Chile99	2	2	1	1	1	1	1	2	198	204	1	1	2	2	3	7	5	5	256	259
Xgla5552	Chile99	2	2	1	1	2	2	1	1	198	198	1	1	2	5	3	3	1	1	247	250
Xgla5556	Chile99	1	1	1	2	1	2	1	1	196	198	1	2	2	3	1	3	4	5	256	259
Xgla5560	Chile99	1	1	1	1	2	2	1	2	198	198	1	2	5	5	3	3	1	1	250	259
Xgla5905	Chile99	1	2	1	1	1	2	1	2	184	198	1	1	2	4	1	7	5	5	247	250
Xgla5917	Chile99	2	2	1	1	1	2	1	1	198	198	1	2	3	5	1	1	1	1	259	265
Xgla5922	Chile99	1	2	1	1	2	2	2	2	198	198	1	2	2	3	2	3	5	5	256	256
Xgla5925	Chile99	1	2	1	1	2	2	1	2	184	198	1	2	2	2	1	3	1	1	250	256
Xgla5927	Chile99	1	1	1	2	2	2	1	1	198	198	1	1	2	3	1	3	5	5	259	259
Xgla5929	Chile99	1	2	1	1	2	2	1	2	198	198	1	2	2	3	3	3	1	1	256	259
Xgla5936	Chile99	2	2	1	1	1	2	1	1	184	198	1	1	2	3	1	3	1	5	250	259
Xgla5957	Chile99	1	2	2	3	1	2	1	1	198	198	1	1	1	2	1	3	1	5	250	250
Xgla1706	CenNPac	2	2	1	1	1	1	1	1	184	200	1	1	2	5	3	3	4	5	250	256
Xgla1707	CenNPac	2	2	1	1	2	2	1	1	198	198	1	1	2	3	3	7	5	5	250	259
Xgla1708	CenNPac	2	2	1	1	1	2	1	1	198	198	1	2	2	3	3	3	1	1	262	262
Xgla1709	CenNPac	1	1	1	1	2	2	1	1	198	206	1	2	3	4	1	1	1	5	253	256
Xgla1710	CenNPac	2	2	1	1	1	2	1	1	198	198	1	1	4	4	1	3	1	3	259	262
Xgla1711	CenNPac	1	2	1	1	2	2	2	2	184	198	1	1	2	2	1	3	1	5	259	259
Xgla1712	CenNPac	1	2	1	3	1	1	1	2	184	204	1	1	2	5	1	3	1	5	250	256
Xgla1713	CenNPac	1	2	1	1	1	2	1	2	198	198	1	1	2	4	1	3	3	7	253	253
Xgla1714	CenNPac	2	2	1	1	1	1	1	2	194	198	1	1	2	5	1	3	1	3	250	268
Xgla1715	CenNPac	1	2	1	2	2	2	1	2	184	198	1	1	2	5	1	1	5	5	256	262
Xgla1716	CenNPac	2	2	1	1	1	2	1	2	198	198	1	1	2	5	1	3	1	1	247	259
Xgla1717	CenNPac	1	1	1	1	1	2	1	2	198	198	1	1	2	3	3	3	1	3	253	259
Xgla1718	CenNPac	1	2	1	1	2	2	1	1	198	198	1	2	3	3	1	3	5	5	253	256
Xgla1719	CenNPac	1	1	1	1	2	2	1	2	198	198	1	1	4	5	1	3	1	1	250	259
Xgla1720	CenNPac	1	2	1	1	2	2	1	2	198	198	1	1	2	5	1	2	1	3	256	262
Xgla1721	CenNPac	1	2	1	1	2	2	1	1	198	198	1	2	2	3	3	7	1	5	253	256
Xgla1722	CenNPac	1	2	1	2	2	2	1	2	198	202	1	2	2	3	1	2	1	3	259	259
Xgla1723	CenNPac	1	2	2	3	2	2	1	1	194	198	1	1	2	3	3	3	3	5	244	250
Xgla1724	CenNPac	2	2	1	1	2	2	2	2	198	198	1	2	2	5	1	1	1	5	256	259
Xgla1725	CenNPac	2	2	1	2	2	2	1	2	198	202	1	1	5	5	1	3	5	5	241	256
Xgla1726	CenNPac	1	2	1	1	2	2	1	1	198	198	1	1	2	5	1	3	1	1	256	256
Xgla1727	CenNPac	1	1	1	1	2	2	1	1	198	198	1	2	3	5	1	3	4	5	250	250
Xgla1728	CenNPac	2	2	1	2	1	2	2	2	198	198	1	2	2	2	3	3	1	1	253	256
Xgla1729	CenNPac	1	2	1	1	2	2	1	1	184	198	1	1	2	2	1	1	1	5	253	256
Xgla1730	CenNPac	1	2	1	1	1	2	1	2	198	202	1	2	2	5	1	3	1	5	250	256
Xgla1731	CenNPac	1	2	1	1	2	2	1	1	184	198	1	2	2	5	1	2	1	1	259	262

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla1732	CenNPac	2	2	1	1	1	2	1	2	198	198	1	1	2	5	1	1	1	1	253	265
Xgla1733	CenNPac	2	2	1	2	1	1	1	1	198	198	2	2	3	3	1	1	1	3	256	265
Xgla1734	CenNPac	1	2	1	1	1	2	1	2	198	198	1	1	2	2	3	3	1	5	253	253
Xgla1735	CenNPac	1	2	1	1	2	2	1	1	198	198	2	2	2	3	1	3	1	5	250	259
Xgla1736	CenNPac	1	1	1	2	1	2	1	2	198	198	1	1	2	2	3	3	1	5	256	256
Xgla5686	Australia	2	2	1	2	1	2	1	2	184	198	1	1	2	4	3	3	1	5	250	256
Xgla5687	Australia	2	2	1	1	2	2	1	1	198	198	1	1	2	2	1	3	1	5	250	250
Xgla5688	Australia	1	2	1	1	1	1	1	1	198	204	1	1	2	2	1	1	1	5	256	262
Xgla5689	Australia	1	2	1	1	2	2	1	2	198	198	1	1	3	3	2	3	1	5	256	265
Xgla5690	Australia	1	1	1	1	1	2	1	2	184	198	1	1	2	3	1	1	1	1	253	253
Xgla5691	Australia	2	2	1	1	1	2	1	1	198	202	1	1	3	5	1	3	1	5	244	259
Xgla5692	Australia	1	2	1	1	2	2	1	1	198	198	1	2	2	2	1	1	1	5	244	253
Xgla5693	Australia	2	2	1	2	1	2	1	1	184	194	1	1	2	3	3	3	4	5	256	274
Xgla5694	Australia	2	2	1	1	1	2	1	1	198	198	1	2	2	3	1	3	1	1	256	256
Xgla5695	Australia	1	2	1	1	2	2	1	1	198	198	1	2	2	5	1	3	1	1	253	256
Xgla5696	Australia	1	1	1	1	2	2	1	1	184	198	1	2	3	4	1	1	1	5	250	259
Xgla5697	Australia	2	2	1	1	1	2	1	1	198	198	1	1	1	4	1	3	1	1	256	259
Xgla5698	Australia	1	2	1	1	2	2	1	1	198	198	1	1	2	2	3	3	1	1	262	262
Xgla5699	Australia	1	2	1	1	2	2	1	1	198	198	1	1	2	3	1	3	1	5	256	262
Xgla5700	Australia	1	1	1	1	1	2	1	1	184	198	1	1	3	3	1	2	1	3	253	256
Xgla5701	Australia	2	2	1	1	2	2	1	1	198	198	1	1	2	3	3	3	1	5	250	253
Xgla5702	Australia	1	1	1	1	2	2	1	1	198	198	1	2	2	2	1	3	1	5	250	259
Xgla5703	Australia	1	1	1	1	1	1	1	2	198	198	1	1	3	5	2	3	1	5	256	256
Xgla5704	Australia	1	2	1	3	2	2	1	2	198	202	1	2	2	2	3	3	4	4	250	256
Xgla5705	Australia	1	1	1	2	2	2	1	1	184	198	1	1	2	3	2	3	1	5	256	259
Xgla5706	Australia	1	2	1	1	2	2	1	1	184	198	1	2	2	2	1	1	1	5	244	256
Xgla5707	Australia	1	2	1	2	1	2	1	1	198	198	1	1	2	3	3	3	1	5	256	259
Xgla5708	Australia	1	2	1	1	2	2	1	1	184	198	2	2	5	5	2	3	5	5	256	256
Xgla5709	Australia	1	2	1	2	2	2	1	2	198	202	1	1	2	3	1	3	1	5	247	256
Xgla5710	Australia	1	2	1	2	1	1	1	1	198	198	1	1	2	5	3	3	3	4	256	256
Xgla5711	Australia	1	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	5	253	256
Xgla5712	Australia	2	2	1	1	2	2	2	2	198	198	1	1	2	3	3	3	1	4	253	259
Xgla5713	Australia	2	2	1	1	2	2	1	2	194	198	1	2	3	3	2	3	1	5	250	259
Xgla5714	Australia	1	1	1	1	1	2	2	2	198	198	1	2	2	2	1	3	1	5	256	256
Xgla5715	Australia	2	2	2	2	1	2	1	1	198	198	1	2	2	2	1	3	1	1	253	256
Xgla5716	Australia	1	1	1	1	2	2	2	2	198	198	1	1	2	4	1	3	4	5	253	259
Xgla5717	Australia	1	1	1	1	1	2	1	1	198	198	1	1	2	3	1	2	1	1	253	259
Xgla5718	Australia	2	2	1	2	1	1	1	1	198	202	1	1	2	2	1	3	1	5	253	256
Xgla5719	Australia	1	2	1	1	2	2	1	1	198	198	1	1	2	2	1	1	1	1	250	256
Xgla5721	Australia	1	2	1	1	2	2	1	1	198	198	1	2	2	5	1	3	1	1	256	262
Xgla5723	Australia	2	2	1	1	2	2	1	1	198	198	1	2	2	3	1	3	1	1	259	262
Xgla5724	Australia	1	1	1	1	1	2	1	1	198	198	1	2	2	5	1	3	1	1	259	262
Xgla5725	Australia	1	1	1	1	1	2	1	1	184	198	1	1	2	5	3	26	1	1	250	256
Xgla5726	Australia	1	1	1	3	1	2	1	1	198	198	1	1	2	3	1	3	1	5	250	253
Xgla5727	Australia	1	2	1	1	2	2	1	1	194	198	2	2	2	3	1	1	1	5	256	259
Xgla5728	Australia	2	2	1	2	2	2	1	2	198	198	1	1	3	3	3	3	1	5	256	256
Xgla5729	Australia	1	2	1	1	1	2	1	1	198	198	1	2	2	2	1	3	1	5	259	262
Xgla5730	Australia	2	2	1	1	2	2	1	1	198	198	1	1	2	5	3	3	1	5	256	259
Xgla5731	Australia	1	1	1	2	2	2	1	1	198	198	1	1	2	4	1	3	1	5	244	253
Xgla5732	Australia	2	2	1	2	1	2	1	1	198	198	1	1	3	5	2	2	4	5	253	256
Xgla5733	Australia	1	2	1	2	2	2	1	2	198	198	1	1	2	3	1	3	1	1	250	253
Xgla5734	Australia	1	2	1	1	2	2	1	2	184	198	1	2	2	2	2	3	1	1	253	253
Xgla5735	Australia	1	2	1	1	1	2	1	1	198	198	1	2	2	2	2	3	1	4	253	256
Xgla5736	Australia	1	1	1	2	1	2	1	1	184	198	1	1	5	5	3	3	1	5	244	253
Xgla5737	Australia	1	1	1	1	1	2	1	1	184	198	1	1	2	5	1	3	1	1	253	256
Xgla5739	Australia	2	2	1	2	2	2	1	2	194	198	1	1	2	2	3	3	1	1	253	253
Xgla5740	Australia	1	1	1	2	1	1	1	1	198	198	1	1	2	5	1	3	1	1	253	259
Xgla5741	Australia	2	2	1	2	1	2	1	2	198	202	1	1	2	4	1	1	1	5	256	259
Xgla5742	Australia	2	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	1	253	262
Xgla5743	Australia	1	1	1	2	1	2	1	1	198	198	1	1	2	3	1	1	1	4	250	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla5744	Australia	1	2	1	2	1	1	1	1	198	198	1	1	2	4	1	2	1	1	253	256
Xgla5745	Australia	1	2	1	1	1	2	1	1	198	198	1	1	2	3	3	3	1	1	256	259
Xgla5746	Australia	2	2	1	1	2	2	1	1	184	198	1	2	2	2	1	1	1	3	250	253
Xgla5747	Australia	2	2	2	2	2	2	1	2	198	198	1	2	4	5	1	1	1	5	247	253
Xgla5748	Australia	1	1	1	1	2	2	2	2	198	198	1	1	3	4	2	3	5	5	256	256
Xgla5750	Australia	1	2	1	1	2	2	1	1	198	198	1	1	2	2	3	26	1	1	253	256
Xgla5751	Australia	1	2	1	1	2	2	1	1	198	198	1	1	2	2	3	3	1	5	256	259
Xgla5752	Australia	2	2	1	1	1	2	1	2	198	198	1	1	4	5	1	3	1	3	256	259
Xgla5753	Australia	1	2	1	1	1	1	1	1	184	198	1	1	3	3	3	3	1	5	253	259
Xgla5754	Australia	1	2	1	2	2	2	1	2	184	184	1	1	2	3	1	3	1	4	250	256
Xgla966	W.Aus	1	2	1	1	1	1	1	2	184	198	1	2	2	2	1	3	1	5	262	265
Xgla967	W.Aus	1	2	2	2	1	2	1	2	198	198	1	1	2	3	1	3	1	1	250	256
Xgla970	W.Aus	1	2	1	1	2	2	1	1	198	198	1	1	2	5	3	3	3	5	250	256
Xgla971	W.Aus	1	2	1	2	2	2	1	1	198	198	1	2	2	3	1	3	1	1	259	277
Xgla973	W.Aus	1	1	1	2	2	2	1	1	198	202	1	1	2	2	2	3	1	5	256	259
Xgla976	W.Aus	1	2	1	1	1	1	1	1	198	198	1	1	3	5	3	3	1	1	250	256
Xgla977	W.Aus	1	1	1	1	2	2	1	1	184	198	1	1	2	4	2	3	1	1	253	256
Xgla978	W.Aus	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	3	1	5	259	262
Xgla979	W.Aus	1	1	1	1	2	2	1	1	198	202	1	1	2	2	1	3	1	1	256	259
Xgla980	W.Aus	1	1	1	2	2	2	1	2	198	198	1	2	2	3	1	1	1	1	256	259
Xgla981	W.Aus	1	2	1	1	1	1	1	2	198	198	1	2	2	3	1	2	1	1	256	259
Xgla982	W.Aus	2	2	1	1	1	1	1	1	198	198	1	2	2	3	2	2	1	1	253	259
Xgla983	W.Aus	2	2	1	1	1	2	1	1	194	198	1	1	2	3	1	1	1	5	256	262
Xgla984	W.Aus	2	2	1	2	2	2	1	1	198	202	2	2	2	2	1	26	1	1	256	259
Xgla985	W.Aus	1	1	1	2	1	2	1	2	198	198	1	1	2	3	1	3	1	5	253	259
Xgla987	W.Aus	2	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	1	256	256
Xgla988	W.Aus	2	2	1	1	1	2	1	1	198	198	1	2	2	3	1	3	1	5	256	256
Xgla989	W.Aus	2	2	1	1	2	2	1	2	198	198	1	2	2	3	1	3	5	5	250	256
Xgla990	W.Aus	1	2	1	2	2	2	1	1	198	198	1	1	2	2	2	3	1	1	244	256
Xgla991	W.Aus	2	2	1	1	2	2	1	2	198	198	1	1	2	5	1	3	1	1	256	256
Xgla992	W.Aus	2	2	2	2	2	2	1	2	194	198	1	1	3	4	1	1	1	5	250	250
Xgla993	W.Aus	1	2	1	1	2	2	1	1	184	198	1	1	2	5	3	3	1	5	253	262
Xgla994	W.Aus	1	2	1	3	2	2	1	2	198	198	1	1	2	5	2	3	1	5	250	256
Xgla995	W.Aus	1	2	1	1	2	2	1	1	184	184	1	2	2	5	1	1	1	5	253	259
Xgla996	W.Aus	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	1	1	5	256	256
Xgla997	W.Aus	1	2	1	2	1	2	1	2	198	198	1	2	2	3	1	1	1	5	256	259
Xgla998	W.Aus	1	1	1	1	1	1	1	2	184	198	1	1	2	2	1	3	1	1	241	259
Xgla999	W.Aus	2	2	1	1	2	2	1	2	198	202	1	2	2	5	3	3	1	5	259	262
Xgla1000	W.Aus	1	2	1	2	2	2	2	2	198	198	1	1	2	3	2	3	1	5	253	265
Xgla1001	W.Aus	2	2	1	1	1	1	1	1	198	198	1	1	2	3	1	1	1	5	247	256
Xgla7510	Taiwan	2	2	1	2	1	2	1	1	198	198	1	1	2	3	1	3	3	5	256	262
Xgla7511	Taiwan	1	2	1	2	2	2	1	1	198	198	1	1	2	5	3	3	1	1	247	253
Xgla7512	Taiwan	2	2	1	3	1	1	1	1	198	198	1	2	2	5	3	3	1	5	256	256
Xgla7513	Taiwan	1	2	1	1	1	2	1	1	198	198	1	1	2	5	3	3	5	5	256	262
Xgla7514	Taiwan	2	2	2	2	1	2	1	1	198	198	1	2	3	5	1	3	1	5	250	256
Xgla7515	Taiwan	2	2	1	2	2	2	1	2	198	198	1	2	2	3	1	7	1	1	247	253
Xgla7516	Taiwan	1	2	1	1	1	2	1	2	198	198	1	1	2	3	1	3	5	5	253	256
Xgla7517	Taiwan	2	2	1	1	1	2	1	1	198	198	1	1	2	2	1	3	1	5	253	265
Xgla7518	Taiwan	2	2	1	3	1	1	1	2	202	202	1	2	2	3	3	3	1	1	250	253
Xgla7519	Taiwan	2	2	1	1	1	2	1	2	198	198	1	2	2	2	1	1	1	1	259	262
Xgla7520	Taiwan	1	2	1	2	1	2	1	1	198	198	1	2	2	3	1	3	1	5	250	253
Xgla7521	Taiwan	2	2	1	1	2	2	1	1	198	198	1	1	2	5	1	3	1	5	241	262
Xgla7522	Taiwan	1	1	1	2	1	2	1	1	198	198	1	2	2	2	3	11	1	4	256	256
Xgla7523	Taiwan	1	1	1	2	2	2	1	1	198	202	1	1	2	3	1	3	1	5	244	256
Xgla7524	Taiwan	2	2	1	1	1	2	1	2	184	204	1	2	2	2	3	3	1	1	247	253
Xgla7525	Taiwan	1	2	1	2	1	2	1	1	198	198	1	2	2	3	1	3	1	5	244	259
Xgla7526	Taiwan	1	2	1	2	2	2	1	1	198	198	1	1	3	3	1	7	1	1	256	256
Xgla7527	Taiwan	1	2	1	1	1	2	1	2	198	204	1	1	2	3	3	3	1	5	256	259
Xgla7528	Taiwan	1	2	1	1	2	2	1	1	198	204	1	1	2	2	3	3	5	5	250	256
Xgla7529	Taiwan	1	2	1	2	1	2	1	1	198	198	1	2	2	4	3	3	1	4	250	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla7530	Taiwan	2	2	1	1	2	2	1	2	198	198	1	1	2	3	1	3	1	1	253	253
Xgla7531	Taiwan	1	2	1	2	1	2	2	2	184	184	1	2	2	2	1	3	1	5	256	268
Xgla7532	Taiwan	1	2	1	3	1	2	1	2	198	202	2	2	2	5	3	3	1	5	256	256
Xgla7533	Taiwan	1	2	1	2	2	2	1	1	184	198	1	1	2	3	1	3	1	5	247	268
Xgla7534	Taiwan	1	1	1	2	2	2	1	1	198	198	1	2	2	3	1	7	1	5	253	259
Xgla7535	Taiwan	1	2	2	3	1	2	1	1	198	198	1	1	5	5	3	3	1	5	259	265
Xgla7536	Taiwan	2	2	1	1	1	1	1	2	184	198	1	1	5	5	3	3	1	5	250	265
Xgla7537	Taiwan	1	1	1	1	2	2	1	1	194	198	1	1	2	3	3	3	1	1	244	259
Xgla7538	Taiwan	1	2	1	2	2	2	1	1	184	198	2	2	3	5	3	3	1	1	253	256
Xgla7539	Taiwan	1	1	1	1	1	2	1	2	198	198	1	2	2	3	3	3	1	3	253	256
Xgla7540	Taiwan	1	1	1	1	2	2	1	1	198	198	2	2	2	4	1	3	1	5	256	262
Xgla7541	Taiwan	1	2	1	1	2	2	1	2	184	204	1	1	3	3	1	3	1	5	244	262
Xgla7542	Taiwan	2	2	1	1	1	2	1	2	198	198	1	1	2	3	3	7	1	5	259	259
Xgla7543	Taiwan	1	1	1	2	1	2	1	1	198	198	1	1	2	5	1	3	1	1	250	256
Xgla7544	Taiwan	2	2	1	2	2	2	1	2	198	198	1	1	2	2	1	3	1	5	256	259
Xgla7545	Taiwan	2	2	1	1	2	2	1	2	184	198	1	2	3	3	1	3	1	1	256	268
Xgla7546	Taiwan	2	2	2	2	1	2	1	1	184	198	1	2	2	5	1	3	1	1	247	259
Xgla7547	Taiwan	1	2	1	1	1	2	1	1	194	198	1	2	2	3	1	3	5	5	256	256
Xgla7548	Taiwan	2	2	1	1	1	2	1	1	198	198	1	1	2	3	1	3	1	1	253	262
Xgla7549	Taiwan	1	2	1	1	1	2	1	1	184	204	1	2	2	3	1	1	1	1	256	256
Xgla7550	Taiwan	1	1	1	1	2	2	1	1	198	198	1	1	2	2	3	3	1	5	241	271
Xgla7551	Taiwan	1	2	1	1	1	2	2	2	198	202	1	1	2	4	1	3	1	1	253	256
Xgla7553	Taiwan	1	2	1	2	1	2	1	1	184	202	1	1	2	3	3	3	1	5	256	256
Xgla7554	Taiwan	1	2	1	1	1	2	2	2	198	198	1	1	2	3	1	3	1	5	253	256
Xgla7555	Taiwan	1	1	1	1	1	2	1	2	198	198	1	1	2	5	1	3	1	1	250	253
Xgla7556	Taiwan	1	2	1	1	1	1	1	2	184	198	1	2	2	4	1	3	1	1	247	259
Xgla7557	Taiwan	1	1	1	2	1	2	1	1	198	198	1	1	4	5	1	3	1	4	256	259
Xgla7558	Taiwan	2	2	1	2	1	2	1	1	198	198	1	2	5	5	1	1	1	5	256	256
Xgla7559	Taiwan	2	2	1	2	2	2	1	1	184	198	1	1	3	5	1	11	1	1	244	259
Xgla7560	Taiwan	1	2	1	1	1	2	1	2	184	198	1	1	2	4	1	3	1	5	250	262
Xgla7561	Taiwan	1	1	1	2	1	2	1	1	198	198	1	1	2	3	3	3	1	1	256	262
Xgla7562	Taiwan	2	2	1	2	1	2	1	2	184	198	1	2	2	2	3	11	1	5	256	259
Xgla7563	Taiwan	1	1	1	1	2	2	1	2	184	198	1	2	3	5	1	3	1	5	259	271
Xgla7564	Taiwan	1	1	1	1	2	2	1	2	184	198	1	2	3	5	1	3	1	5	259	271
Xgla7565	Taiwan	2	2	1	1	2	2	1	1	198	198	1	1	2	3	1	3	1	5	256	256
Xgla7566	Taiwan	1	2	1	2	2	2	1	2	198	202	1	1	2	3	3	7	1	1	247	256
Xgla7567	Taiwan	1	2	1	1	2	2	1	1	198	198	1	1	2	2	1	3	1	1	244	244
Xgla7568	Taiwan	1	1	1	1	2	2	1	1	198	198	1	1	2	5	1	3	1	5	247	259
Xgla7569	Taiwan	2	2	1	2	2	2	1	2	198	198	1	2	2	3	1	3	5	5	259	265
Xgla7570	Taiwan	2	2	1	1	1	2	1	1	198	198	1	1	2	3	1	3	1	5	241	256
Xgla7571	Taiwan	1	2	1	2	2	2	1	2	198	198	1	1	2	2	3	26	1	5	256	256
Xgla7572	Taiwan	1	2	2	3	2	2	1	2	184	198	1	1	2	2	3	3	1	1	250	256
Xgla7573	Taiwan	2	2	2	2	1	1	1	1	184	198	1	1	2	4	3	3	1	1	256	262
Xgla5641	Japan	2	2	1	2	2	2	1	1	198	198	1	1	2	2	3	3	1	1	250	253
Xgla5642	Japan	1	1	1	2	2	2	1	1	198	198	1	2	2	3	1	3	1	1	244	256
Xgla5643	Japan	2	2	1	2	2	2	1	1	184	198	1	1	2	4	3	3	1	5	253	259
Xgla5644	Japan	2	2	1	1	2	2	1	2	198	198	1	1	2	2	2	3	1	1	253	265
Xgla5645	Japan	2	2	1	1	2	2	1	2	198	198	1	1	2	2	2	3	1	1	253	265
Xgla5646	Japan	2	2	1	1	1	2	1	1	198	198	1	1	4	5	1	3	1	1	250	253
Xgla5647	Japan	1	2	1	2	1	2	1	2	198	198	1	1	2	5	1	1	5	5	256	259
Xgla5649	Japan	1	2	1	1	2	2	1	1	198	202	1	2	2	2	1	1	1	1	253	256
Xgla5650	Japan	1	2	1	1	1	2	2	2	198	198	1	1	2	3	1	3	1	5	247	250
Xgla5651	Japan	1	1	1	1	1	1	1	1	198	198	2	2	2	2	3	3	5	5	250	256
Xgla5652	Japan	2	2	1	1	1	2	1	1	198	204	1	1	2	5	1	1	1	4	253	253
Xgla5653	Japan	1	1	1	1	2	2	1	2	198	198	1	2	2	2	3	3	1	1	256	259
Xgla5654	Japan	2	2	1	3	1	1	1	1	198	198	1	1	2	3	1	3	1	1	250	262
Xgla5655	Japan	1	2	1	2	1	2	1	1	198	198	1	1	2	2	1	3	1	5	256	256
Xgla5656	Japan	2	2	1	1	1	2	1	2	198	198	1	1	2	5	1	2	1	1	253	259
Xgla5657	Japan	2	2	1	3	2	2	1	2	198	198	1	1	2	3	1	3	5	5	250	256
Xgla5658	Japan	2	2	1	1	2	2	1	1	198	206	1	2	2	2	1	3	1	1	253	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla5659	Japan	2	2	1	1	1	2	1	1	198	198	1	2	2	4	1	3	1	1	250	253
Xgla5661	Japan	1	2	1	1	2	2	1	2	198	198	1	1	2	2	1	3	1	1	250	256
Xgla5662	Japan	1	2	1	2	1	2	1	1	198	198	1	1	2	2	2	2	1	3	256	259
Xgla5663	Japan	1	2	1	2	2	2	2	2	184	198	1	1	2	2	1	2	1	1	256	259
Xgla5664	Japan	1	2	1	2	1	2	1	1	198	198	1	1	2	5	3	3	1	1	253	256
Xgla5665	Japan	2	2	1	1	2	2	1	2	198	198	1	1	2	2	1	3	1	3	241	241
Xgla5666	Japan	2	2	1	1	1	2	1	1	198	198	1	1	3	5	3	3	1	5	250	253
Xgla5667	Japan	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	1	1	5	253	256
Xgla5668	Japan	1	2	1	2	1	2	1	1	184	198	1	1	2	4	1	3	2	2	253	256
Xgla5669	Japan	1	2	1	1	1	2	1	2	184	198	1	1	2	2	1	3	1	1	250	262
Xgla5670	Japan	1	2	2	2	2	2	1	2	184	198	1	1	2	2	1	1	1	5	253	256
Xgla5672	Japan	1	2	1	1	1	2	1	1	184	198	1	1	2	2	1	3	1	6	259	262
Xgla5673	Japan	2	2	1	1	1	2	1	2	198	198	1	1	2	2	1	1	1	1	253	256
Xgla5674	Japan	1	2	1	1	1	1	1	1	198	198	1	1	4	5	1	1	1	5	253	256
Xgla5675	Japan	1	2	1	1	1	2	1	1	198	198	1	1	2	3	1	3	1	1	250	256
Xgla5676	Japan	1	1	1	1	2	2	1	2	184	198	1	1	2	3	1	7	1	5	247	265
Xgla5677	Japan	1	2	3	3	2	2	1	1	198	198	1	2	2	2	1	3	4	4	250	256
Xgla5679	Japan	1	2	1	1	1	1	1	1	198	198	1	1	3	4	1	1	1	5	250	256
Xgla5680	Japan	1	2	1	1	1	2	1	1	198	198	1	1	2	3	1	1	1	1	256	259
Xgla5681	Japan	2	2	1	2	2	2	1	2	198	198	1	1	3	5	1	3	1	5	247	250
Xgla5683	Japan	1	2	1	1	2	2	1	1	184	198	1	1	2	3	1	3	1	1	262	268
Xgla6981	HawLa	1	1	2	2	1	2	1	1	184	198	1	1	2	5	2	3	3	5	244	247
Xgla6983	HawLa	2	2	1	1	2	2	1	2	184	198	1	1	5	5	1	3	1	1	253	256
Xgla6984	HawLa	2	2	1	1	2	2	1	1	198	198	1	1	2	2	1	3	1	1	256	256
Xgla6985	HawLa	1	1	1	1	1	1	2	2	198	198	1	1	2	4	1	3	1	1	253	262
Xgla6986	HawLa	1	1	1	2	1	2	1	2	196	198	1	1	2	3	1	1	1	5	247	253
Xgla6987	HawLa	1	2	1	2	1	1	1	1	198	202	1	1	5	5	1	3	1	5	244	253
Xgla6988	HawLa	1	2	1	1	2	2	1	2	198	204	1	2	2	2	1	3	5	5	253	259
Xgla6989	HawLa	2	2	1	1	2	2	1	2	198	198	1	2	2	2	3	3	1	5	247	253
Xgla6990	HawLa	2	2	1	1	2	2	1	2	198	198	1	1	2	3	1	1	1	1	253	253
Xgla6991	HawLa	1	1	1	2	1	2	1	1	198	198	1	1	2	3	1	3	1	1	250	253
Xgla6992	HawLa	2	2	1	1	2	2	1	2	202	204	1	2	5	5	3	7	1	5	250	256
Xgla6993	HawLa	2	2	1	2	1	1	1	1	198	198	1	2	2	5	3	7	1	1	253	259
Xgla6995	HawLa	1	1	1	2	2	2	1	1	184	198	1	2	2	3	2	3	5	5	259	259
Xgla6996	HawLa	2	2	1	2	2	2	1	1	184	198	1	2	2	2	1	2	1	4	253	253
Xgla6997	HawLa	1	2	1	1	1	2	1	2	184	198	1	1	2	3	1	3	1	1	247	259
Xgla6998	HawLa	2	2	1	1	2	2	1	2	198	198	1	2	2	5	1	26	1	3	256	256
Xgla6999	HawLa	1	2	1	1	2	2	1	2	198	198	1	2	2	3	1	2	5	5	259	259
Xgla7000	HawLa	1	1	1	2	2	2	1	1	184	198	1	1	2	2	1	3	1	5	250	259
Xgla7002	HawLa	1	2	1	2	2	2	1	2	184	198	1	2	2	5	2	2	1	5	256	256
Xgla7003	HawLa	2	2	1	1	1	2	1	2	184	198	1	2	2	3	1	3	1	1	244	250
Xgla7004	HawLa	2	2	1	1	2	2	1	2	198	198	1	2	2	3	1	1	1	5	253	256
Xgla7005	HawLa	2	2	1	1	1	2	1	1	184	198	1	1	2	3	3	3	1	5	250	256
Xgla7006	HawLa	1	2	1	2	2	2	1	2	184	198	1	1	2	3	3	3	1	5	250	250
Xgla7007	HawLa	1	2	1	2	1	2	1	1	198	198	1	1	3	5	3	3	1	5	253	253
Xgla7008	HawLa	2	2	1	1	1	2	1	1	184	198	1	1	2	2	1	1	1	5	259	262
Xgla7009	HawLa	2	2	1	1	1	2	1	1	198	198	2	2	3	4	2	3	1	3	259	259
Xgla7010	HawLa	2	2	1	1	1	1	1	2	198	198	1	1	2	3	1	3	1	5	259	259
Xgla7011	HawLa	2	2	1	1	2	2	1	1	198	198	1	1	2	5	3	3	1	5	256	259
Xgla7012	HawLa	2	2	1	2	1	2	1	2	184	198	1	1	2	2	1	7	1	5	256	256
Xgla7013	HawLa	2	2	1	1	1	2	1	1	198	202	2	2	3	5	3	3	1	5	247	250
Xgla7014	HawLa	2	2	1	1	2	2	1	1	198	198	1	2	3	5	1	3	1	5	250	253
Xgla7015	HawLa	1	2	1	1	1	2	1	1	196	198	1	1	2	3	1	1	1	1	256	259
Xgla7016	HawLa	2	2	1	2	2	2	1	1	198	198	1	2	3	5	1	3	1	3	256	256
Xgla7017	HawLa	2	2	1	1	1	1	1	1	194	198	1	2	2	3	1	1	5	5	256	259
Xgla7018	HawLa	2	2	1	2	1	2	1	2	198	198	1	1	2	3	1	3	1	1	256	268
Xgla7019	HawLa	1	2	1	1	1	2	1	2	198	198	1	2	2	3	3	3	1	3	238	262
Xgla7023	HawLa	2	2	1	2	1	2	2	2	198	198	1	1	3	5	1	1	1	4	256	256
Xgla7024	HawLa	1	2	1	2	1	2	1	1	198	198	1	2	3	4	1	3	1	1	250	262
Xgla7027	HawLa	2	2	1	2	2	2	1	1	198	198	1	2	2	3	3	3	3	4	247	253

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla7028	HawLa	2	2	1	1	1	2	1	1	198	198	1	2	2	2	2	3	1	6	256	259
Xgla7029	HawLa	2	2	1	1	1	2	1	1	198	198	1	1	2	3	3	3	3	5	265	268
Xgla7038	HawLa	2	2	1	1	1	2	1	1	184	198	1	2	3	3	3	3	1	5	256	262
Xgla4808	CenNJu	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	1	1	5	253	253
Xgla4809	CenNJu	2	2	1	1	1	1	1	1	184	198	1	1	3	3	1	3	1	5	244	250
Xgla4810	CenNJu	1	1	2	2	2	2	1	2	198	198	1	2	2	2	1	3	1	4	253	256
Xgla4811	CenNJu	1	1	1	2	2	2	1	1	198	198	1	2	2	2	1	3	1	6	253	256
Xgla4812	CenNJu	1	2	1	1	1	2	1	2	184	202	1	2	2	3	3	3	1	1	259	259
Xgla7094	CenNJu	1	2	1	1	1	2	1	1	198	198	1	2	5	5	1	3	1	4	250	262
Xgla7097	CenNJu	1	2	1	1	1	2	2	2	198	198	1	1	2	3	1	1	1	5	253	256
Xgla7102	CenNJu	2	2	1	1	1	2	1	1	184	198	1	2	3	5	1	3	1	1	256	256
Xgla7104	CenNJu	1	2	1	2	2	2	1	1	198	198	1	1	3	5	3	3	4	5	250	253
Xgla7105	CenNJu	2	2	1	1	2	2	1	1	198	198	2	2	2	2	1	3	1	1	262	268
Xgla7106	CenNJu	1	2	1	1	1	2	1	1	198	198	1	2	2	4	1	3	1	5	238	256
Xgla7107	CenNJu	2	2	1	1	1	2	1	1	198	198	1	2	5	5	1	3	1	1	256	262
Xgla7108	CenNJu	1	2	1	1	1	2	1	2	198	198	1	1	3	3	1	7	1	1	256	256
Xgla7109	CenNJu	1	2	1	1	2	2	1	1	184	198	1	2	3	4	1	2	5	5	256	268
Xgla7127	CenNJu	1	2	1	1	1	2	1	1	184	198	1	1	3	5	1	1	1	1	256	256
Xgla7128	CenNJu	1	2	1	1	1	1	1	2	198	198	1	1	2	3	1	3	5	5	250	259
Xgla7136	CenNJu	2	2	1	1	1	2	1	1	198	202	1	1	2	2	3	3	1	5	253	265
Xgla7143	CenNJu	1	2	1	1	2	2	1	1	184	198	1	2	2	5	1	1	1	1	250	253
Xgla7144	CenNJu	2	2	1	1	2	2	1	2	194	198	1	1	3	3	1	2	1	5	247	253
Xgla7145	CenNJu	1	2	1	2	1	2	1	1	198	198	1	2	2	2	3	3	1	5	253	253
Xgla7146	CenNJu	1	1	1	1	2	2	1	1	198	198	1	1	2	3	3	3	1	1	256	265
Xgla7149	CenNJu	2	2	1	1	1	1	1	1	198	198	1	1	2	5	3	3	1	1	250	253
Xgla7150	CenNJu	2	2	1	1	2	2	1	2	184	198	1	1	2	3	1	3	1	5	247	250
Xgla7151	CenNJu	1	2	1	2	2	2	2	2	198	198	1	2	2	3	1	11	1	5	259	268
Xgla7155	CenNJu	1	2	1	1	1	2	1	1	198	198	1	2	2	5	3	3	1	1	244	256
Xgla7156	CenNJu	1	2	1	1	1	2	1	1	184	198	1	2	2	3	3	3	1	5	250	259
Xgla7157	CenNJu	1	2	1	1	2	2	1	1	198	198	1	2	2	5	1	3	5	5	253	256
Xgla7188	CenNJu	2	2	1	1	1	2	1	2	198	198	1	1	2	2	1	1	1	1	247	256
Xgla7191	CenNJu	2	2	1	1	1	2	1	2	198	198	1	2	3	3	1	3	1	1	244	256
Xgla7192	CenNJu	1	2	1	2	1	2	2	2	198	198	1	1	2	5	1	3	1	1	250	259
Xgla7193	CenNJu	2	2	1	1	2	2	1	2	184	202	1	1	3	5	1	3	3	5	250	253
Xgla7198	CenNJu	2	2	1	1	2	2	1	2	198	198	1	2	2	3	1	3	5	5	256	259
Xgla7200	CenNJu	1	2	1	2	1	1	1	2	184	198	1	1	3	3	3	3	1	5	253	259
Xgla7205	CenNJu	1	2	1	1	1	1	1	2	198	198	1	1	2	2	2	3	1	1	250	259
Xgla7206	CenNJu	1	2	1	1	2	2	1	1	194	198	1	1	2	2	3	7	1	5	250	256
Xgla7213	CenNJu	1	1	2	2	1	2	1	1	198	198	1	1	2	2	3	3	1	1	250	256
Xgla7224	CenNJu	2	2	1	1	1	1	1	2	184	184	1	1	2	4	3	3	1	5	256	265
Xgla7236	CenNJu	1	1	1	3	1	2	1	1	198	198	1	1	2	3	3	3	1	5	253	256
Xgla7239	CenNJu	2	2	2	2	1	2	1	2	198	198	1	1	2	5	3	3	1	1	247	250
Xgla7240	CenNJu	1	2	1	1	2	2	1	1	184	194	1	1	2	3	3	3	1	6	253	253
Xgla7241	CenNJu	1	2	1	1	1	2	1	2	198	198	1	1	2	3	1	3	1	1	238	256
Xgla7242	CenNJu	2	2	1	3	1	1	1	1	198	198	1	2	2	5	1	3	1	5	250	253
Xgla7243	CenNJu	2	2	1	1	1	2	1	2	198	198	2	2	2	5	1	2	1	5	256	262
Xgla7244	CenNJu	2	2	1	2	1	2	1	2	198	198	1	2	2	5	1	1	1	1	253	256
Xgla7245	CenNJu	2	2	1	2	2	2	1	1	184	198	1	1	2	2	1	3	1	1	256	259
Xgla7246	CenNJu	1	2	1	1	1	2	1	1	198	198	1	2	2	5	1	1	1	5	253	256
Xgla7298	CenSJu	1	1	1	2	2	2	1	2	198	202	1	1	2	3	1	7	1	5	247	259
Xgla7299	CenSJu	1	2	1	1	2	2	1	2	198	202	1	1	2	5	3	11	1	5	244	253
Xgla7300	CenSJu	1	2	1	1	2	2	1	2	198	198	1	2	2	5	3	3	1	1	241	250
Xgla7301	CenSJu	2	2	1	2	2	2	1	2	198	198	1	1	2	3	1	1	1	5	256	259
Xgla7302	CenSJu	1	2	1	1	2	2	1	2	198	198	1	2	2	2	3	3	1	1	250	256
Xgla7303	CenSJu	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	1	5	7	253	256
Xgla7304	CenSJu	1	2	1	2	1	2	1	2	198	198	1	1	2	2	1	3	1	1	250	253
Xgla7305	CenSJu	1	1	1	1	1	1	1	2	194	198	1	2	2	2	1	3	1	4	256	265
Xgla7306	CenSJu	1	2	2	2	1	1	1	2	198	198	1	2	2	4	1	3	1	3	256	259
Xgla7307	CenSJu	2	2	1	1	1	1	1	1	198	198	1	1	2	3	1	3	1	1	253	262
Xgla7308	CenSJu	2	2	1	3	1	1	1	2	184	198	1	1	2	2	1	3	1	5	256	256

Table A5. Continued

Sample	Pop	ARP		MLC2		ATPsbeta		SRP54		Ald		Act2		OTY1		LDHA		ANT		VBC	
Xgla7309	CenSJu	1	2	1	1	1	1	1	2	198	198	1	2	2	4	1	3	1	5	250	259
Xgla7310	CenSJu	2	2	1	1	2	2	1	2	184	198	1	1	2	3	3	3	1	1	253	265
Xgla7311	CenSJu	2	2	1	2	1	1	1	2	198	198	1	1	5	5	1	1	1	1	241	247
Xgla7312	CenSJu	1	2	1	1	1	2	1	2	198	198	1	1	2	2	3	26	1	5	247	253
Xgla7313	CenSJu	1	1	1	1	1	1	1	1	198	198	1	2	2	5	3	3	1	5	256	265
Xgla7314	CenSJu	2	2	2	2	2	2	1	1	198	198	1	1	3	3	1	2	1	1	250	256
Xgla7315	CenSJu	1	2	2	2	2	2	1	2	198	198	1	1	2	2	1	3	1	1	253	259
Xgla7316	CenSJu	2	2	1	1	2	2	1	2	198	198	1	1	2	2	1	3	5	5	247	259
Xgla7317	CenSJu	2	2	1	1	1	2	1	1	198	198	1	1	2	2	3	3	1	1	256	256
Xgla7318	CenSJu	1	2	2	2	2	2	1	2	184	198	1	1	2	4	1	3	1	1	250	268
Xgla7319	CenSJu	1	1	1	1	1	2	1	1	184	198	1	2	2	5	1	3	1	1	250	256
Xgla7320	CenSJu	2	2	1	1	2	2	1	2	200	202	1	1	2	3	1	3	1	5	262	265
Xgla7321	CenSJu	1	2	1	2	1	2	1	1	198	198	1	1	2	3	1	3	1	1	253	262
Xgla7322	CenSJu	1	2	1	1	1	2	1	2	198	198	1	2	2	4	1	3	1	1	256	259
Xgla7323	CenSJu	1	2	1	1	1	1	1	2	198	198	1	1	3	3	1	3	1	5	250	262
Xgla7324	CenSJu	1	2	1	1	1	2	1	1	198	198	1	1	2	2	1	1	5	5	241	265
Xgla7325	CenSJu	2	2	1	2	1	2	1	1	184	202	1	2	2	5	3	3	1	3	250	256
Xgla7326	CenSJu	1	2	1	1	1	1	2	2	184	198	1	1	2	2	3	3	1	1	256	259
Xgla7327	CenSJu	1	2	1	1	2	2	2	2	198	198	1	1	2	2	3	3	1	1	244	250
Xgla7328	CenSJu	1	2	1	2	1	2	2	2	198	198	1	1	2	3	2	3	5	5	250	259
Xgla7329	CenSJu	1	1	1	1	1	2	1	1	198	198	1	1	2	5	3	3	1	1	250	256
Xgla7330	CenSJu	2	2	1	1	1	2	1	2	198	198	1	2	2	5	1	3	5	5	256	256
Xgla7331	CenSJu	1	1	1	1	1	2	1	2	194	198	1	2	4	5	1	3	1	6	256	259
Xgla7332	CenSJu	1	1	1	1	2	2	1	2	198	198	1	2	2	3	1	3	1	1	256	256
Xgla7333	CenSJu	1	2	1	1	1	1	1	1	184	202	1	1	2	2	1	3	1	1	250	256
Xgla7274	AusJu	1	2	2	3	1	2	1	2	198	198	1	1	2	3	1	3	1	1	250	250
Xgla7275	AusJu	2	2	1	2	2	2	1	1	198	202	1	1	2	2	3	3	5	5	250	259
Xgla7276	AusJu	1	2	1	1	1	2	1	1	198	198	1	1	2	4	1	3	1	5	250	256
Xgla7277	AusJu	1	2	1	1	1	2	1	1	198	204	1	1	3	5	3	3	1	1	250	259
Xgla7278	AusJu	1	2	2	2	1	2	1	2	198	198	1	2	5	5	3	3	1	1	250	256
Xgla7279	AusJu	1	1	1	1	1	2	1	1	198	202	1	2	2	5	1	2	1	6	256	259
Xgla7280	AusJu	1	2	1	1	2	2	1	1	198	198	1	1	2	5	1	11	1	1	256	256
Xgla7281	AusJu	2	2	1	1	2	2	1	2	198	198	2	2	2	2	1	3	1	1	259	259
Xgla7282	AusJu	2	2	1	2	2	2	1	1	198	198	1	2	2	3	3	7	1	5	250	253
Xgla7283	AusJu	1	1	1	1	1	1	1	1	198	198	1	2	2	5	3	3	1	1	253	256
Xgla7284	AusJu	1	1	1	2	1	2	1	2	198	198	1	1	2	5	1	1	1	5	250	265
Xgla7285	AusJu	2	2	1	3	1	2	1	1	198	198	1	2	2	2	1	1	1	5	250	259
Xgla7286	AusJu	1	2	1	2	1	2	1	2	184	198	1	1	2	5	2	3	1	1	253	253
Xgla7287	AusJu	2	2	1	1	2	2	1	1	184	198	1	1	2	2	3	3	3	5	253	259
Xgla7255	GuamJu	2	2	1	1	2	2	1	2	198	198	2	2	2	3	3	3	1	4	259	259
Xgla7256	GuamJu	1	2	1	2	1	2	1	1	198	198	1	1	4	4	2	3	1	1	253	259
Xgla7257	GuamJu	1	2	1	3	1	2	1	2	198	198	1	1	2	2	1	3	1	1	244	253
Xgla7258	GuamJu	1	2	1	1	2	2	1	1	184	184	1	1	2	4	3	3	1	1	259	259
Xgla7259	GuamJu	1	2	1	2	1	2	1	1	198	198	1	1	2	3	1	3	1	3	250	256
Xgla7260	GuamJu	2	2	1	1	2	2	1	2	198	198	1	1	2	2	1	1	1	5	253	256
Xgla7261	GuamJu	1	2	1	1	1	2	1	1	184	198	1	2	2	4	1	1	1	5	256	262
Xgla7262	GuamJu	2	2	1	1	1	2	1	1	184	198	1	2	2	2	1	3	1	1	256	256
Xgla7263	GuamJu	2	2	1	2	2	2	1	1	198	198	1	1	2	2	1	1	5	5	244	256
Xgla7264	GuamJu	2	2	1	1	1	2	1	1	198	198	1	1	2	2	3	3	1	5	256	256
Xgla7265	GuamJu	1	2	1	1	2	2	1	1	198	198	1	2	2	5	1	1	1	1	256	256
Xgla7266	GuamJu	1	1	1	1	1	2	2	2	184	198	1	2	2	2	3	3	1	4	250	259

Table A6. Average cluster posterior probability membership ($\bar{Q}$) of 16 sampling localities in the Pacific Ocean calculated by STRUCTURE with *K*=2.

Sampling locality	Cluster 1	Cluster 2	Sample size
HAW99	0.7986	0.2014	106
HAWNE	0.7920	0.2081	112
NMCA	0.8358	0.1642	96
Ecuador	0.7194	0.2806	80
Chile97	0.8121	0.1878	67
Chile99	0.7682	0.2317	53
CenNPac	0.7379	0.2622	31
Australia	0.8406	0.1594	65
W.Aus	0.8091	0.1909	30
Taiwan	0.5496	0.4505	63
Japan	0.8116	0.1884	38
HawLa	0.6549	0.3451	42
CenNJu	0.6845	0.3155	46
CenSJu	0.6945	0.3055	36
AusJu	0.7424	0.2577	14
GuamJu	0.8183	0.1817	12

Table A7. Average cluster posterior probability membership ($\bar{Q}$) of 16 sampling localities in the Pacific Ocean and two reference samples from North Atlantic and Mediterranean calculated by STRUCTURE.

Sampling locality	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Sample size
HAW99	0.8529	0.1255	0.0196	0.0020	106
HAWNE	0.7645	0.2265	0.0049	0.0042	112
NMCA	0.9051	0.0430	0.0244	0.0276	96
Ecuador	0.3685	0.6110	0.0178	0.0028	80
Chile97	0.9143	0.0616	0.0227	0.0014	67
Chile99	0.5635	0.3643	0.0458	0.0265	53
CenNPac	0.6742	0.2935	0.0305	0.0018	31
Australia	0.9500	0.0369	0.0075	0.0056	65
W.Aus	0.8649	0.1253	0.0073	0.0025	30
Taiwan	0.0572	0.9222	0.0100	0.0106	63
Japan	0.8687	0.0811	0.0418	0.0084	38
HawLa	0.2550	0.7220	0.0204	0.0026	42
CenNJu	0.2743	0.7112	0.0131	0.0013	46
CenSJu	0.4030	0.5695	0.0242	0.0033	36
AusJu	0.5453	0.4455	0.0080	0.0012	14
GuamJu	0.8386	0.0963	0.0244	0.0406	12
NAtl	0.0791	0.0229	0.8225	0.0754	49
Med	0.0042	0.0012	0.0043	0.9902	60

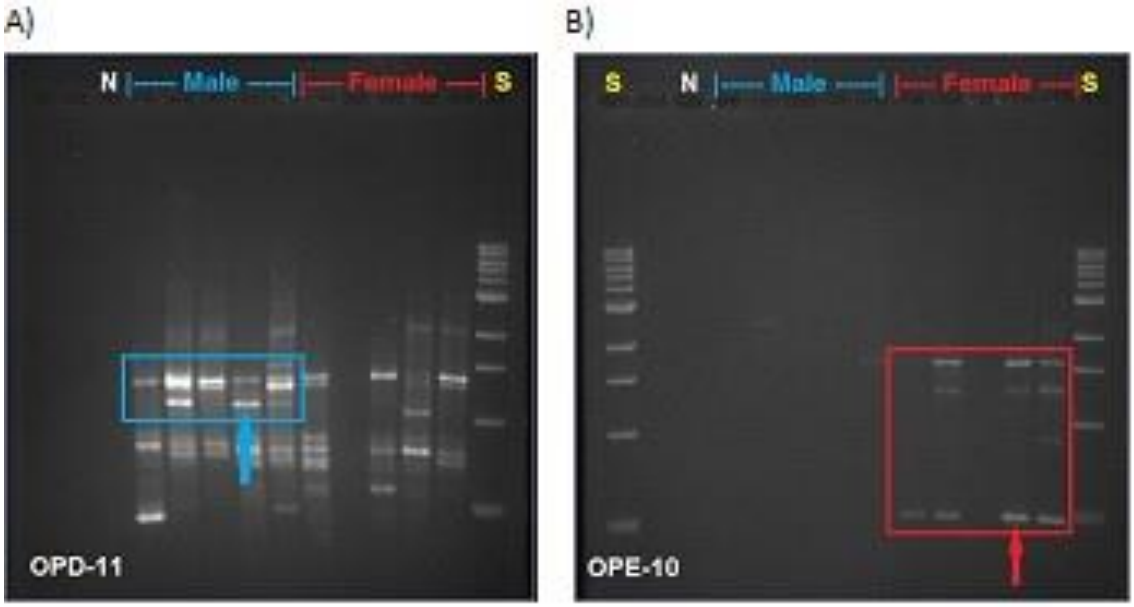


Figure A1. TA 2% agarose gels displaying potential gender-related band (arrow) in swordfish (*Xiphias gladius*) with two RAPD primers: A) OPD-11 and B) OPE-10. Size markers (S) correspond to a 1 kb DNA Ladder (New England BioLabs) with the following fragment sizes (10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, 0.5 kb) from top to bottom. Each gel was loaded with five males (M) and six females (F), plus a negative control (N).

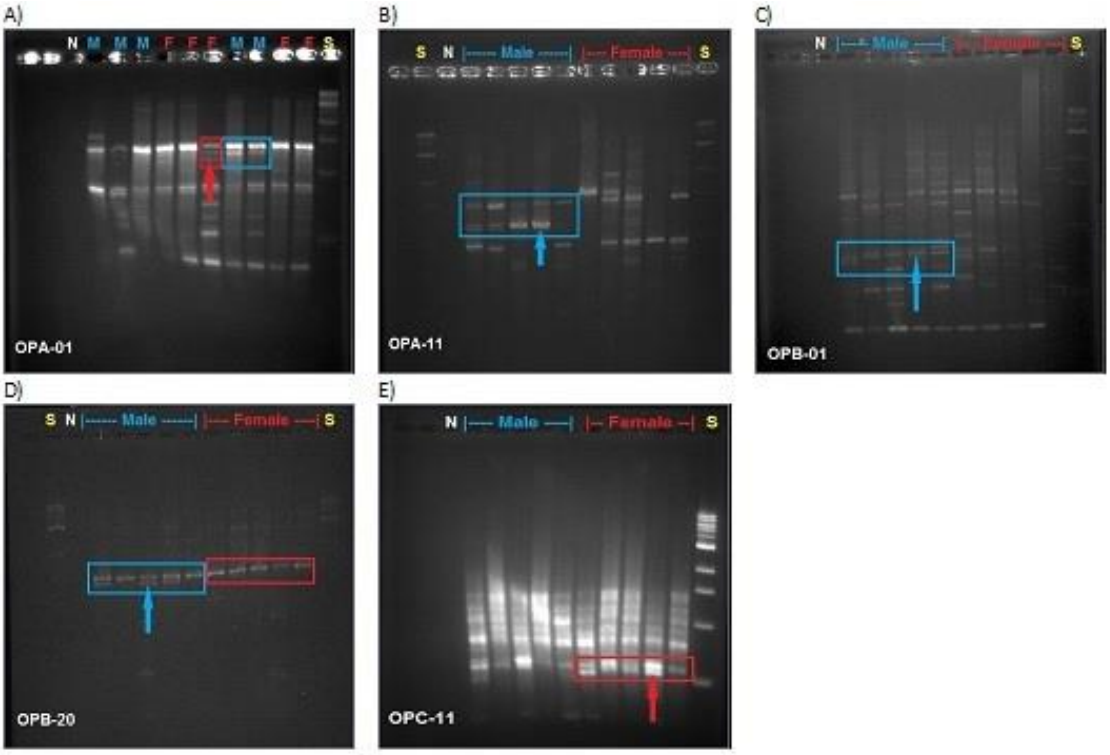


Figure A2. TA 2% agarose gels displaying potential gender-related band (arrow) in blue marlin (*Makaira nigricans*) with six RAPD primers: A) OPA-01, B) OPA-11, C) OPB-01, D) OPB-20, and E) OPC-11. Primer names are listed below left. Size markers (S) correspond to a 1 kb DNA Ladder (New England BioLabs) with the following fragment sizes (10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, 0.5 kb) from top to bottom. Each gel was loaded with five males (M) and five females (F), plus a negative control (N).

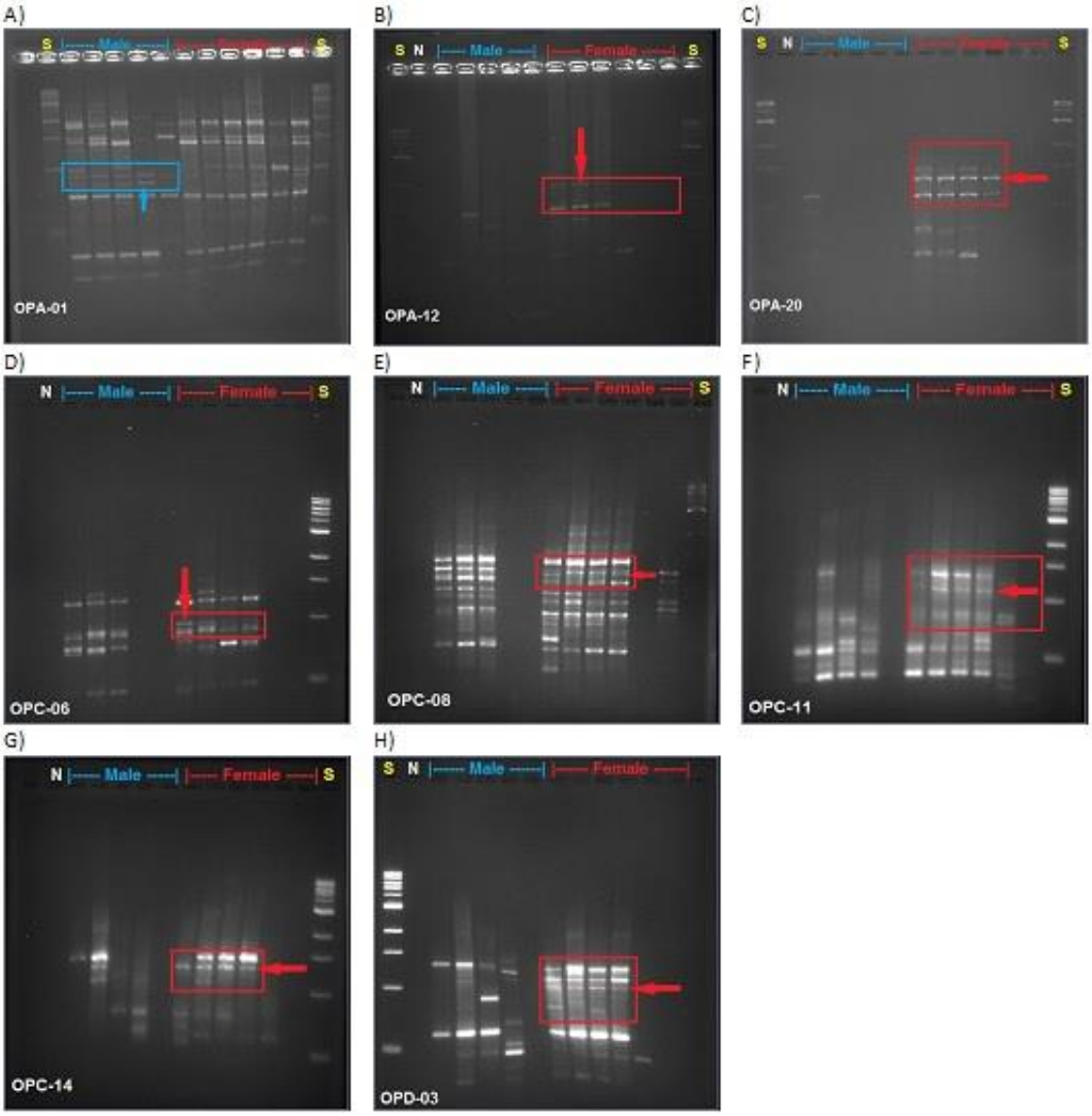


Figure A3. TA 2% agarose gels displaying potential gender-related band (arrow) in sailfish (*Istiophorus platypterus*) with eight RAPD primers: A) OPA-01, B) OPA-12, C) OPA-20, D) OPC-06, E) OPC-08, F) OPC-11, G) OPC-14, and H) OPD-03. Size markers (S) correspond to a 1 kb DNA Ladder (New England BioLabs) with the following fragment sizes (10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, 0.5 kb) from top to bottom. Each gel was loaded with five males (M) and six females (F), plus a negative control (N).

```
           1
Xgla1072F  ATAAGAGTAA GATCCCATCT TCTCATGTTT TAGTTTTTCT TGTTTCATGT GCACTCTCCT GACATTTCCT CTCATTGTAG AGCAGAGCCT
Xgla1073M  .......... .......... .......... .......... .......... .......... .......... ..M....... ..........
Xgla1074M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           91
Xgla1072F  AAAAGACGTC CTCATTGGTG AAAAATCAGG AAGTAACATC AGCATAAATC CACTTCTGCT TTTCTCTTGG GAAACAGGAA CTGATACAAG
Xgla1073M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1074M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           181
Xgla1072F  GTGGTTTTAG TGTTCATTTT CCCCACTGAA ATTTTCAGCA CACTATTATT GTCAGAGGAG TGCTCATTGA TGTCTGTTTT TTACTCTCTT
Xgla1073M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1074M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           271
Xgla1072F  TCTCAGATAT AGAAATGTCT CAGGTTCATC CCCGGCCTCT TCACACACCT TCTCCTTCCT MTGTGAGCTG AAGCGGTTCC TGGGTGACGT
Xgla1073M  .......... .......... .......... .......... .......... .......... A......... .......... ..........
Xgla1074M  .......... .......... .......... .......... .......... .......... A......... .......... ..........

           361
Xgla1072F  TCTGCCAGGA ACCGCTAGCC GGACGTCAGG GGTTCCTGAA TCCCCTCCGC TCAAGCTGGA CTCTTTACAG TCCATGCCTC CCCTGTCACT
Xgla1073M  .......... ..T....... .......... .......... .......... .......... .......... .......... ..........
Xgla1074M  .......... ..Y....... .......... .......... .......... .......R.. .......... .......... ..........

           451
Xgla1072F  GGGCTTATCC TCCAGCGAGA CCCTGCTGGC AGGACTGATC AACTCATCGT CCCCCGTCGT CTTTTCCTTC AATAGAATGG GCTCCATGTT
Xgla1073M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1074M  .......... .......... .......... .......... .......... .....R.... .......... .......... ..........

           541
Xgla1072F  TCAGGCGCAT CATGGACAGT TGGCCCTGTC TCCTGCACTG TTGGAGGAGC TCAGGCAGAG GTTGGAGCAG ATTGTGAAGC AGATAATGGA
Xgla1073M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1074M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           631
Xgla1072F  GGTAATAAGG GAGGGGGAGG TGGGGCACAG GGCCACAGAG AGGCTGGGGA G
Xgla1073M  .......... .......... .......... .......... .......... .
Xgla1074M  .......... .......... .......... .......... .......... .
```

Figure A4. A) Multiple sequence alignments of 681 bp of anti-Müllerian (AMH) gene for 3 specimens of swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (M=A or C; R=A or G; Y=C or T), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence.

```
           1
Xgla0034M  AAAACACCAT GATGCGCAAA GCCATCCGTG GCCACCTGGA GAACAATCCG GCCCTGGAGA AGTGAGTTTA TTGCCCCCCT CCACTTTTGC
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .........G ..........

           91
Xgla0034M  TACCCTGAAC CATCAGTTTT TATATGGCAG GATAAGTTAC AGTGCAGCTT GTGTGGTGCT GTGTTGTCGT TCCCCCTGCA AACAGTAATC
Xgla0656M  .......... .......... .......... .......... ......R... .......... ........S. .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           181
Xgla0034M  TGTCTCCAGC TATCCCTGTC TGTCTAAATC RCTTCCGGCA GCTAGGGACG CAAGGTCTCA GACACTTTAT GACCTTTTTG TCTTTGTAGA
Xgla0656M  .......... .......... .......... A......... .......... .......... N......... .......... ..........
Xgla5804M  .......... .......... .......... G......... .......... .......... .......... .......... ..........

           271
Xgla0034M  AATGATGGTC TGGCTGCATA ATTAACAGCT GATCCCACGA TTTGACAGGC TCCTGCCCCA CATTAAAGGA AATGTGGGCT TTGTCTTCAC
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           361
Xgla0034M  CAAGGAGGA
Xgla0656M  .........
Xgla5804M  .........
```

Figure A4. B) Multiple sequence alignment of 369 bp of Acidic ribosomal phosphoprotein P0 (ARP) gene for 3 specimens of swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (R=A or G; S= C or G; N=unknown), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence

```
           1
Xgla0108F  GTGGAAAGCT CTTCAAGCAG CCGAGCCACC TCCAGACTCA CCTGCTCACM CACCAGGGAA CCCGACCTCA CAAGTGCACC GTTTGTGAGA
Xgla0113F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla0655F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla1153F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla1166M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5755M  .......... .......... .......... .......... .........C .......... ..S....... .......... ..........
Xgla5797M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .........C .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .........C .......... .......... .......... ..........

           91
Xgla0108F  AGGCCTTCAC GCAGACTAGC CACCTGAAGA GGCATATGCT GCAGCATTCA GATGTCAAGC CCTACAGCTG TCGCTTCTGT GGCCGTGGCT
Xgla0113F  .......... ..W....... .......... .......... .......... .......... .......... .......... ..........
Xgla0655F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1166M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5755M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           181
Xgla0108F  TCGCCTATCC CAGTGAGCTG AGGACCCACG AGAACAAACA CGAGAATGGT CAGTGCCACG TCTGCACACA GTGTGGTCTG GAGTTCCCAA
Xgla0113F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0655F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .....Y.... .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .......... .......... .......... .......... ......Y... ..........
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1166M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5755M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
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Figure A4. C) Multiple sequence alignments of 420 bp of of Zinc finger (Znf) gene for 19 specimens of swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (K=G or T; M=A or C; R=A or G; S=C or G; W=A or T; Y=C or T), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence

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           271
Xgla0108F  CCTACGCGCA CCTGAAGCGC CACCTGACCA GCCATCAGGG CCCCACCACG TACCAGTGCA CAGAGTGCCA CAAGTCCTTC GCTTACCGCA
Xgla0113F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0655F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .S........ .......... .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1166M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5755M  .......... .......... .......... .......... .......... .......... .......... .......... ........S.
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           361
Xgla0108F  GCCAGCTGCA GAACCACTTG ATGAAGCACC AGAACGTGCG GCCCTACGTT TGCCCCGAGT
Xgla0113F  .......... .......... .......... .......... .......... ..........
Xgla0655F  .......... .......... .......... .......... .......... ..........
Xgla1153F  .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .......... ..........
Xgla1166M  .......... .......... .......... .......... .......... ..........
Xgla5755M  .......... .......... .......... .......... .......... ..........
Xgla5797M  .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... ..........
```

Figure A4. C) continued.

```
          1
Xgla0108F  TGCTGAACAT GTCTTTGGCC TACAGTATTT GAGCTTATGG AAGAGGGCAT GTGTAGAAAT ATAGACATTG TGAAGAGCCT TATAGGACCA
Xgla0113F  .......... .......... .......... .R........ ......R... .......... .......... .......... ..........
Xgla1488F  .......... .......... .......... .G........ .......... .......... .......... .......... ..........
Xgla1490F  .......... .......... .......... .G........ .......... .......... .......... ....R..... ..........
Xgla1492F  .......... .......... .......... .G........ .......... .......... .......... .......... ..........
Xgla5706F  .......... .......... .......... .G........ .......... .......... .......... .......... ..........
Xgla5709F  .......... .......... .......... .R........ .......... .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .......... .......W.. .......... .......... ..........
Xgla1482M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5707M  .......... .......... .......... .R........ .......... .......... .......... .......... ..........
Xgla5708M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5711M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5755M  .......... .......... .......... .R........ R......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .G........ .......... .......... .......... .......... ..........

          91
Xgla0108F  TACCAGTATT TTTGYAAATT ATTATGTAAA WCACACAAAM TTTATAGTAT TTTTTTAAAT ATTTTTTTGA CATTCCAGTC AACCGTTGGT
Xgla0113F  .......... .......... .......... T...R....C .W........ .......... .......... .......... ..........
Xgla1488F  .......... ....T..... .......... T...R....C .......... .......... .......... .......... ...Y......
Xgla1490F  .......... ....T..... .......... T...R....C .......... .......... .......... .......... ...Y......
Xgla1492F  .......... ....T..... .......... T........C .......... .......... .......... .......... M.........
Xgla5706F  .......... ....T..... .......... T........C .......... .......... .......... .......... C.........
Xgla5709F  .......... ....C..... .......... T........C .......... .......... .......... .......... ..........
Xgla1160M  .......... ....C..... .......... T........C .......... .......... .......... .......... ..........
Xgla1482M  .......... ....C..... .......... T........C .......... .......... .......... .......... ..........
Xgla5707M  .......... .......... .......... T........C .......... .......... .......... .......... ...Y......
Xgla5708M  .......... ....C..... .......... T........C .......... .....W.... .......... .......... ..........
Xgla5711M  .......... .......... .......... T........C .......... .......... .......... .......... ...Y......
Xgla5755M  .......... .......... .......... T........C .......... .....W.... .......... .......... M.........
Xgla5804M  .......... ....T..... .......... T...R..... .......... .....W.... .......... .......... ..........

          181
Xgla0108F  TTTCATTTAT TTCCATAGAS TATAAAAATA STGCATTTGT GTCCAAGTTA CATTATCATT ACGAGGTAAT GCAGTGCAGA CAGTTTACAG
Xgla0113F  .......... .........C .......... .......... .......... .......... .......... .......... ..........
Xgla1488F  .......... .......... .......... C......... .......... .......... ..R....... .......... ..........
Xgla1490F  .......... .......... .......... C......... .......... .......... .......... .......... ..........
Xgla1492F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5706F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5709F  .......... .........C .......... G......... .......... .......... .......... .......... ..........
Xgla1160M  .......... .........C .......R.. G......... .......... .......... .......... .......... ..........
Xgla1482M  .......... .........C .......... G......... .......... .......... ..R....... .......... ..........
Xgla5707M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5708M  .......... .........C .......... G......... .......... .......... .......... .......... ..........
Xgla5711M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5755M  .......... .........C .......... G......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .........G .......... C......... .......... .......... .Y........ .......... ..........

          271
Xgla0108F  TAGATGAGTC TTAACAATAA TGTAACTGCT AAAAATGAAT GCAGGCACAA CTACCAGCAG TACCAG
Xgla0113F  .......... .......... .......... .......... .......... .......... ......
Xgla1488F  .......... .......... .......... .......... .......... .......... ......
Xgla1490F  .......... .......... .......... .......... .......... .......... ......
Xgla1492F  .......... .......... .......... .......... .......... .......... ......
Xgla5706F  .......... .......... .......... .......... .......... .......... ......
Xgla5709F  .......... .......... .......R.. .......... .......... .......... ......
Xgla1160M  .......... .......... .......... .......... .......... .......... ......
Xgla1482M  .......... .......... .......... .......... .......... .......... ......
Xgla5707M  .......... .......... .......... .......... .......... .......... ......
Xgla5708M  .......... .......... .......... .......... .......... .......... ......
Xgla5711M  .......... .......... .......... .......... .......... .......... ......
Xgla5755M  .......... .......... .......... .......... .......... .......... ......
Xgla5804M  .......... .......... .......... .......... .......... .......... ......
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Figure A4. D) Multiple sequence alignments of 336 bp of DM related transcription factor 1 (DMRT1) gene for 14 specimens of swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (R=A or G; Y=C or T; M=A or C; W=A or T) and identical (.) relative to the reference sequence. Number above reference sequence shows the position in the sequence.

```
           1
Xgla0108F  GACTCCACCT ACTACAGCAC CAGCAACATC AGCTTCACCA TCAACATCAG CAGCCTCCAT CTCTGCTCTC CAGCGTGATG ATCAAGGAAG
Xgla0113F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5504F  ........Y. .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5641F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1160M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           91
Xgla0108F  AGAAAGATYC CGACGACTCG TTCATTCACA GCCGTACTCC TGGTGTGGTC AAACTGGAGA AGCAGGACAG CACTGGTTTC TGCCAGTCGC
Xgla0113F  ........C. .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5504F  ........C. .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5641F  ........C. .......... .......... .......... .......... R......... .......... .......... ..........
Xgla5795F  ........C. .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0034M  ........C. .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  ........C. .......... .......M.. .......... .......... .......... .......... .......... ..........
Xgla1160M  ........C. .......... K......... .......... .......... .......... .......... .......... ...S......

           181
Xgla0108F  ACTGTCTCCA GAGCAGCATG AGCTCCCAGT ACCGGAT
Xgla0113F  .......... .......... .......... .......
Xgla5504F  .......... .......... .......... .S.....
Xgla5641F  .......... .......... .......... .......
Xgla5795F  .......... .......... .......... .......
Xgla0034M  .......... .......... .......... .......
Xgla1160M  .......... .......... .......... .......
Xgla0656M  .......... .......... .......... .......
```

Figure A4. E) Multiple sequence alignments of 217 bp of of DMRT1 gene Exon3 for 8 specimens of swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (K=G or T; R=A or G; Y=C or T; M=A or C; S=C or G) and identical (.) relative to the reference sequence. Number above reference sequence shows the position in the sequence

```
           1
Xgla0113F  GATCTGCTGG CTGGATTTGG TGCTGTTAAC TGTCCCTACA CGTACATGTC CTATTTCCTC AGGTAAGATC AGCTCTGCAT TTTCTCTGAC
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5756F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           91
Xgla0113F  ATCACCCTCA ACAGCTGAGG GTCTGTTAAT CTGAGTATGC TCATGCATCA GTAATTGTCA TACTCAGTTG TATCTYTGTT TGCCTGGCAG
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... W....C.... ...M......
Xgla1171F  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........
Xgla5756F  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........
Xgla0033M  .......... .......... .......... .......... .......... .......... .......... W....C.... ...M......
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .....T.... ..........
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .....T.... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .....C.... ..........

           181
Xgla0113F  TACTCAACAC ACAAGCTCAT GTTGTTTTAT GCTTCTTGCT TTTGTTATGT TCACAGAAAT GTAACAGACA GTGATATCCT TGCTCTGGAG
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5756F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .....Y.... .......... .......... .......... .......... .......... .......... ..........
Xgla0034M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           271
Xgla0113F  AGACGGCTGC TCCAAACTAT GGACATGATT GTGAGCAAGA AGAAACGGTG AGCAAAGAAA AAAAATATTT TAAAGGAAAT TACTCAAACA
Xgla1153F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla1171F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5756F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5757F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5795F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5803F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5811F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5814F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla0033M  .......... .......... .......... .......... .......... .......... .......... .......... Y.........
Xgla0034M  .......... .......... .......... ..S....... .......... .......... .......... .......... K.........
Xgla0656M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5797M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5804M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Xgla5805M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
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Figure A4. F) Multiple sequence alignments of 372 bp of of Golgi pH regulator (GpHR) gene (OtY1) for 15 specimens of swordfish (*Xiphias gladius*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (K=G or T; M=A or C; S=C or G; W=A or T; Y=C or T) and identical (.) relative to the reference sequence. Number above reference sequence shows the position in the sequence

```
            361
Xgla0113F   AACCATCGCT GG
Xgla1153F   .......... ..
Xgla1171F   .......... ..
Xgla5756F   .......... ..
Xgla5757F   .......... ..
Xgla5795F   .......... ..
Xgla5803F   .......... ..
Xgla5811F   .......... ..
Xgla5814F   .......... ..
Xgla0033M   .......... ..
Xgla0034M   .......... ..
Xgla0656M   .......... ..
Xgla5797M   .......... ..
Xgla5804M   .......... ..
Xgla5805M   .......... ..
```

Figure A4 F) Continued.

```
           1
Mnig201F   TTTTTTCCAG ACTATTATTC TCAGAGTGGT GCTCACAAAT GTTTTTTTTC TTTTAATGCT CTCTCTCCTT CTTTCTCAGA AATTTATCAG
Mnig277F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig278F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig281F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig284F   .......... .......... .......... .......... .......... .......KY. .......... .......... ..........
Mnig285F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig279M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig280M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig282M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig283M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig287M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           91
Mnig201F   GTTCGTCCTC GGCCTCTTCA GAGACTTTTT CATTCCTCTG TGAGCTGAAG CGGTTCCTGG GTGATGTCCT GCCAGGAACC ACGGGCCGGA
Mnig277F   .......... K......... .......... .......... .......... .......... .......... .......... ..R.......
Mnig278F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig281F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig284F   .......... .......... .......... .......... .......... .......... .......... .......... ..R.......
Mnig285F   .......... K......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..R.......
Mnig279M   .......... K......... .......... .......... .......... .......... .......... .......... ..R.......
Mnig280M   .......... .......... .......... .......... .......... .......... .......... .......... ..R.......
Mnig282M   .......... .......... .......... .......... .......... .......... .......... .......... ..R.......
Mnig283M   .......... K......... .......... .......... .......... .......... .......... .......... ..........
Mnig287M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           181
Mnig201F   CGTCTGTGGT CCCCGAGTCC CCTCCGCTCC AGCTGGACTC CTTACAGTCC ATGCCTCCCC TGTCACTGGG ATTATCCTCC AGTGAGACCC
Mnig277F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig278F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig281F   .R........ ....R..... .......... .......... .......... .......... .......... .......... ..........
Mnig284F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig285F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig279M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig280M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig282M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig283M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig287M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           271
Mnig201F   TGCTGGCAGG ACTGATCAAC TCCTCATCCC CCACTGTCCT CTCCTTTACT AGAATGGGCT CCATGTTTCA CGTGCATCAT GGACAGTTGG
Mnig277F   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig278F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig281F   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig284F   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig285F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig279M   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig280M   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig282M   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........
Mnig283M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig287M   .......... .......... .......... ........Y. .......... .......... .......... .......... ..........

           361
Mnig201F   CCTTGTCTCC TGCACTGTTG GAGGAGCTCA GACAGAGGTT GGAGCAAATT GTGATGCAGA TAA
Mnig277F   .......... .......... .......... .......... .......... .......... ...
Mnig278F   .......... .......... .......... .......... .......... .......... ...
Mnig281F   .......... .......... .......... .......... .......... .......... ...
Mnig284F   .......... .......... .......... .......... .......... .......... ...
Mnig285F   .......... .......... .......... .......... .......... .......... ...
Mnig211M   .......... .......... .......... .......... .......... .......... ...
Mnig279M   .......... .......... .......... .......... .......... .......... ...
Mnig280M   .......... .......... .......... .......... .......... .......... ...
Mnig282M   .......... .......... .......... .......... .......... .......... ...
Mnig283M   .......... .......... .......... .......... .......... .......... ...
Mnig287M   .......... .......... .......... .......... .......... .......... ...
```

Figure A5. A) Multiple sequence alignments of 423 bp of anti-Müllerian (AMH) gene for 12 specimens of blue marlin (*Makaira nigricans*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (R=A or G; Y=C or T; K=G or T), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence.

```
           1
Mnig201F   AAAACACCAT GATGCGCAAA GCCATCCGTG GCCATCTGGA GAACAATCCA GCCCTGGAGA AGTGAGTTGT TTTTGTTTTT TGCCCCCTCT
Mnig203F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig204M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig202M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig207M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           91
Mnig201F   ACTTCTGCTA CCTTGAGCCC TCAGTTTTTA TATAGCAGGA TAAGTTACAG TGCAGCGTGT GTGGTGCTGT GCTGTCGTTC CCCCTGCAAA
Mnig203F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig204M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig202M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig207M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           181
Mnig201F   CAAAAATCTG TCTCCAGCTA TCCCTGTCTA TCTAAATCTC TTCTGGTAGC CAGGGACGCA AGGTCTCAGA CAATTTTATA YTCTTTCTGT
Mnig203F   .......... .......... .......... .......... .......... .......... .......... .......... C.........
Mnig204M   .......... .......... .......... .......... .......... ......Y... .......W.. .......... C.........
Mnig202M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig207M   .......... .......... .......... .........Y .......... .......... .......... .......... T.........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           271
Mnig201F   TTCTCTGTGG AAATG
Mnig203F   .......... .....
Mnig204M   .......... .....
Mnig202M   .......... .....
Mnig207M   .......... .....
Mnig211M   .......... .....
```

Figure A5. B) Multiple sequence alignments of 285 bp of Acidic ribosomal phosphoprotein P0 (ARP) gene for 6 specimens of blue marlin (*Makaira nigricans*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (Y=C or T; K=G or T; W=A or T), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence

```
           1
Mnig201F   GCGGAAAGCT CTTCAAGCAG CCAAGCCACC TCCAGACTCA CCTGCTCACC CACCAGGGAA CCCGACCTCA CAAGTGCACC GTTTGTGAGA
Mnig203F   .......... .......... .......... .......... .........Y .......... .......... .......... ..........
Mnig212F   .......... .......... ..M....... .......... .........Y .......... .......... .......... ..........
Mnig202M   .......... .......... .......... .......... .........Y .......... .......... .......... ..........
Mnig204M   .......... .......... ..M....... .......... .........Y .......... .......... .......... ..........
Mnig207M   .......... .......... .......... .......... .........T .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           91
Mnig201F   AGGCCTTCAC GCAGACTAGC CATCTGAAGA GGCACATGCT GCAGCACTYR GACGTCAAGC CCTACAGCTG TCGCTTCTGC GGCCGCGGCT
Mnig203F   .......... .......... .......... .......... .........A .......... .......... .......... ..........
Mnig212F   .......... .......... .......... .......... ........CA .......... .......... .......... ..........
Mnig202M   .......... .......... .......... .......... ........C. .......... .......... .......... ..........
Mnig204M   .......... .......... .......... .......... ...S....CA .......... .......... .......... ..........
Mnig207M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           181
Mnig201F   TCGCCTACCC CAGCGAGCTG AGGACCCACG AGAACAAACA CGAGAATGGT CAGTGCCACG TCTGCACCCA GTGTGGCCTG GAGTTCCCAA
Mnig203F   .......... .......... .......... .......... .......... .......... .......... .......... ........R.
Mnig212F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig202M   .Y........ .......... .......... .......... .......... .......... .M........ .......... ..........
Mnig204M   .......... .......... .......... .......... .......... .......... .......... .......... .......Y..
Mnig207M   .......... .......... .......... .......... .......... .......... .....W.... .......... ........R.
Mnig211M   .......... .......... .......... .......... .......... .......... .......... .......... ..........

           271
Mnig201F   CCWACSMGCA CCTGAAGCGA CACCTGACTA GCCATCAGGG CCCCACCACG TACCAGTGCA CCGAGTGCCA CAAGTCCTTC GCATACCGCA
Mnig203F   .......... .......... .......... .......... .......... .......... .......... .........Y ..........
Mnig212F   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig202M   ..T....... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig204M   .......... .......... .......... .......... .......... .......... .......... .......... ..........
Mnig207M   ..T....... .......... ..S....... .......... .......... .......... .......... .......... ..........
Mnig211M   ..T....... .......... .......... ....C..... .......... .......... .......... .......... ..........

           361
Mnig201F   GCCAGCTTCA GAACCACCTG ATGAAGCACC AGAACGTGCG CCCCTACGTG TGCCCCGAGT
Mnig203F   .......... .......... .......... .......... .......... ..........
Mnig212F   .......... .......... .......... .......... .......... ..........
Mnig202M   .......... .......... .......... .......... .......... ..........
Mnig204M   .......... .......... .......... .......... .......... ..........
Mnig207M   .......... .......... .......... .......... .......... ..........
Mnig211M   .......... .......... .......... .......... .......... ..........
```

Figure A5. C) Multiple sequence alignment of 420 bp of Zinc finger (Znf) gene for 7 specimens of blue marlin (*Makaira nigricans*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (R=A or G; Y=C or T; M=A or C; S=C or G; W=A or T), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence

```
          1
Ipla0031F AATGCTCTCT CTCCTTCTTT CTCAGAAATT TATCAGGTTC GTCCTCGGCC TCTTCAGAGA CTTTTTCATT CCTCTGTGAG CTGAAGCGGT
Ipla304F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla305F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0041M .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla324M  .......... .......... ....R..... .......... .......... .......... .......... .......... ..........
Ipla325M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

          91
Ipla0031F TCCTGGGTGA TGTCCTGCCA GGAACCACGG GCCGGACGTC TGTGGTCCCC GAGTCCCCTC CGCTCCAGCT GGACTCYTTA CAGTCCATGC
Ipla304F  .......... .......... .......... .......... .......... .......... .......... ......C... ..........
Ipla305F  .......... .......... .......... .......... .......... .......... .......... ......C... ..........
Ipla0041M .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla324M  .......... .......... .......... .......... .......... .......... .......... ......C... ..........
Ipla325M  .......... .......... .......... .......... .......... .......... .......... ......C... ..........

          181
Ipla0031F CTCCCCTGTC ACTGGGATTA TCCTCCAGTG AGACCCTGCT GGCAGGACTG ATCAACTCCT CATCCCCCAC TGTCTTCTCC TTTACTAGAA
Ipla304F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla305F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0041M .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla324M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla325M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

          271
Ipla0031F TGGGCTCCAT GTTTCACGTG CATCATGGAC AGTTGGCCTT GTCTCCTGCA CTGTTGGAGG AGCTC
Ipla304F  .......... .......... .......... .......... .......... .......... .....
Ipla305F  .......... .......... .......... .......... .......... .......... .....
Ipla0041M .......... .......... .......... .......... .......... .......... .....
Ipla324M  .......... .......... .......... .......... .......... .......... .....
Ipla325M  .......... .......... .......... .......... .......... .......... .....
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Figure A6. A) Multiple sequence alignments of 335 bp of anti-Müllerian (AMH) gene for 6 specimens of sailfish (*Istiophorus platypterus*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (R=A or G), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence.

```
           1
Ipla0031F  AAAACACCAT GATGCGCAAA GCCATCCGTG GCCATCTGGA GAACAATCCA GCCCTGGAGA AGTGAGTTGT TTTTGTTTTT TGCTCCCTCT

           91
Ipla0031F  ACTTCTGCTA CCTTGAGCCC TCAATTTTAA TATAGCAGGA TAAGTTACAG TGCAGCGTGT GTGGTGCTGT GCTGTCGTTC CCCCTGCAAA

           181
Ipla0031F  CAAAAATCTG TCTCCAGCTA TCCCTGTCTA TCTAAATCTC TTCTGGTAGC CAGGGACGCA AGGTCTCAGA CAATTTTATA CTTTCTGTTT

           271
Ipla0031F  CTCTGTGGAA ATGATGGCCC GTCTGCATAA TTAACAGTTA ATCCTGATTC GACAGGCTCC TGCCCCACAT TAAAGGAAAT GTGGGTTTTG

           361
Ipla0031F  TCTTCACCAA GGAGGATCTG ACTGAAGTCA GGGATCTGCT G
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Figure A6. B) Single sequence of 401 bp of Acidic ribosomal phosphoprotein P0 (ARP) gene for 1 specimens of sailfish (*Istiophorus platypterus*). Gender for females (F) is included at the end of specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T). Number above reference sequence shows the position in the sequence

```
           1
Ipla0002F  GCGGAAAGCT CTTCAAGCAG CCMAGCCACC TCCAGACTCA CCTGCTCACC CACCAGGGAA CCCGACCTCA CAAGTGCACC GTTTGTGAGA
Ipla0031F  .......... .......... ..A....... .......... .......... .......... .......... .......... ..........
Ipla0038F  .......... .......... ..A....... .......... .......... .......... .......... .......... ..........
Ipla0039F  .......... .......... ..A....... .......... .......... .......... .......... .......... ..........
Ipla0028M  .......... .......... ..A....... .......... .......... .......... .......... .......... ..........
Ipla0041M  .......... .......... ..A....... .......... .......... .......... .......... .......... ..........

           91
Ipla0002F  AGGCCTTCAC GCAGACTAGC CATCTGAAGA GGCACATGCT GCAGCACTCA GACGTCAAGC CCTACAGCTG TCGCTTCTGC GGCCGCGGCT
Ipla0031F  .......... .......... .......... .......... .........R .......... .......... .......... ..........
Ipla0038F  .......... .......... .......... .......... .........R .......... .......... .......... ..........
Ipla0039F  .......... .......... .......... .......... .........G .......... .......... .......... ..........
Ipla0028M  .......... .......... .......... .......... .........R .......... .......... .......... ..........
Ipla0041M  .......... .......... .......... .......... .........R .......... .......... .......... ..........

           181
Ipla0002F  TCGCCTACCC CAGCGAGCTG AGGACCCACG AGAACAAACA CGAGAATGGT CAGTGCCACG TCTGCACCCA GTGTGGCCTG GAGTTCCCAA
Ipla0031F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0038F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0039F  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0028M  .......... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0041M  .......... .......... .......... .......... .......... .......... .......... .......... ..........

           271
Ipla0002F  CCWACSMGCA CCTGAAGCGA CACCTGACTA GCCATCAGGG CCCCACCACG TACCAGTGCA CCGAGTGCCA CAAGTCCTTC GCATACCGCA
Ipla0031F  ..T..GC... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0038F  ..T..G.... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0039F  ..T....... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0028M  ..T..GC... .......... .......... .......... .......... .......... .......... .......... ..........
Ipla0041M  ..T..G.... .......... .......... .......... .......... .......... .......... .......... ..........

           361
Ipla0002F  GCCAGCTTCA GAACCACCTG ATGAAGCACC AGAACGTGCG CCCCTACGTG TGCCCCGAGT
Ipla0031F  .......... .......... .......... .......... .......... ..........
Ipla0038F  .......... .......... .......... .......... .......... ..........
Ipla0039F  .......... .......... .......... .......... .......... ..........
Ipla0028M  .......... .......... .......... .......... .......... ..........
Ipla0041M  .......... .......... .......... .......... .......... ..........
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Figure A6. C) Multiple sequence alignments of 420 bp of of Zinc finger (Znf) gene for 6 specimens of sailfish (*Istiophorus platypterus*). Gender for males (M) and females (F) is included at the end of each specimen's acronym. Sequence nucleotides are symbolized by IUPAC notions: adenine (A), cytosine (C), guanine (G) and thymine (T), ambiguities (M=A or C; R=A or G; S=C or G; W=A or T), and identical (**.**) relative to the reference sequence. Number above reference sequence shows the position in the sequence